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# Interactions des espèces d'arbres en peuplements mélangés : effets sur la réponse à la sécheresse des arbres et rôle des mécanismes de partage des ressources en eau et en lumière

Soline Martin-Blangy

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## **Thèse**

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Mention « Biologie et écologie des forêts et des agrosystèmes »

par  
**Soline MARTIN-BLANGY**

Sous la direction de Damien BONAL et Marie CHARRU

# **Interactions des espèces d'arbres en peuplements mélangés : effets sur la réponse à la sécheresse des arbres et rôle des mécanismes de partage des ressources en eau et en lumière**

Soutenance le 01/12/2021

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# Interactions des espèces d'arbres en peuplements mélangés : effets sur la réponse à la sécheresse des arbres et rôle des mécanismes de partage des ressources en eau et en lumière

Tree-species interactions in mixed forests: effects on tree drought response and role of water and light resource partitioning mechanisms

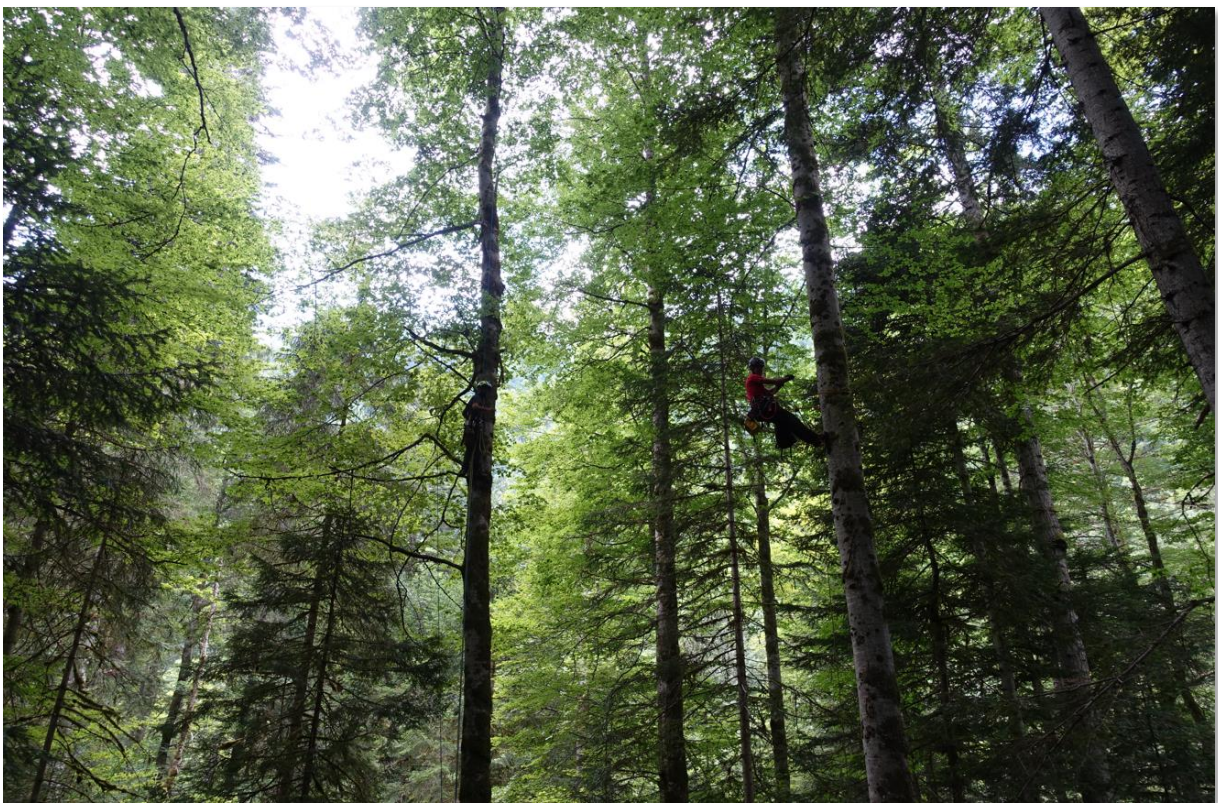


Photo : Soline Martin-Blangy. Bauges, France.

“Examine each question in terms of what is ethically and esthetically right, as well as what is economically expedient. A thing is right when it tends to preserve the integrity, stability, and beauty of the biotic community. It is wrong when it tends otherwise.”

Aldo Leopold, 1949, “The Land Ethic”, *A Sand County Almanac*.

*A Maurice Blangy*



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# Contributions scientifiques issues du travail de thèse

## *Articles dans des revues internationales à comité de lecture*

**Martin-Blangy, S.**, Charru, M., Gérard, S., Jactel, H., Jourdan, M., Morin, X., Bonal D. (2021). Mixing beech with fir or pubescent oak does not help mitigate drought exposure at the limit of its climatic range. *For. Ecol. Manage.*, 482, 118840. <https://doi.org/10.1016/j.foreco.2020.118840> [Chapitre I]

**Martin-Blangy, S.**, Meredieu, C., Jactel, H., Bonal, D., Charru, M. (*Submitted*). Mixture effects on crown dimensions and canopy packing in a pine-birch plantation in interaction with tree density and irrigation. *Submitted to "Trees – Structure and function" on April 6<sup>th</sup>, 2021 – Under Review* [Chapitre II]

Toïgo, M., Ledoux, G., **Martin-Blangy, S.**, Coste, L. & Morin, X. (2021). Mixture effect on radial stem and shoot growth differs and varies with temperature. *For. Ecol. Manage.*, 488, 119046. <https://doi.org/10.1016/j.foreco.2021.119046> [co-auteure, travail issu de la campagne de terrain que j'ai organisée en 2018]

## *Communication orale dans un colloque international*

**Martin-Blangy, S.**, Gérard, S., Charru, M., Morin, X., Jourdan, M., Angeli, N., Hossann, C., Jactel, H., Bonal, D. (Dec. 2018). Mechanisms leading to tree species diversity effects: a test on water and light acquisition in mixed-forests. *Functional Ecology Conference - JEF AnaEE France*. Nancy, France.

## *Communication orale préparée pour un colloque international, mais non-présentée à cause de l'épidémie de covid-19.*

**Martin-Blangy, S.**, Charru, M., Gérard, S., Angeli, N., Hossann, C., Louis, L., Jactel, H., Jourdan, M., Morin, X., Bonal, D. (March 2020). Interactive effects of drought and species mixture on European beech (*Fagus sylvatica*) in Southeastern France. *Mixed Species Forests: Risks, Resilience and Management*. Lund, Sweden.

## *Communication orale dans un colloque national*

**Martin-Blangy, S.**, Charru, M., Bonal, D. (Juin 2018). Effets des interactions entre espèces d'arbres sur l'acquisition des ressources hydriques et lumineuses en peuplements mélangés. *Les forêts mélangées : quel état des connaissances scientifiques ?* Nancy, France.

## *Communication sous forme de poster*

**Martin-Blangy, S.**, Charru, M., Morin, X., Jourdan, M., Bonal, D. (Oct. 2018). Effects of tree species interactions and climate on water use efficiency of trees. *Sfécologie 2018, Société Française d'Ecologie et d'Evolution (SFE2)*. Rennes, France.



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# INTRODUCTION

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Photo : Soline Martin-Blangy. Forêt de Hanau, Vosges, France.



## **1. Etat des lieux de la diversité des écosystèmes forestiers**

Les forêts sont les écosystèmes les plus représentés sur terre : elles couvrent 30.6 % du territoire mondial (FAO 2020) et 31 % du territoire français (IGN 2020). Elles sont sources de nombreux services écosystémiques car elles contribuent à la régulation du climat, participent à la régulation du cycle de l'eau, sont une source de bien être pour les humains, et sont une ressource économique importante (Brockerhoff et al. 2017) (voir **Encadré 1**). Aujourd'hui, elles sont au cœur de nombreux enjeux socio-économiques. D'une part, la demande pour les produits bois est en augmentation constante en réponse à la nécessité de favoriser des ressources renouvelables et d'atteindre les objectifs de réduction des émissions de CO<sub>2</sub> dans le cadre de l'Accord de Paris de 2015 (FAO 2020). D'autre part, les forêts sont menacées par de nombreux dégâts liés aux changements climatiques (sécheresses, tempêtes, incendies, attaques d'insectes et de pathogènes...) (IPCC 2014; Lehner et al. 2017), mais aussi par une perte de biodiversité croissante engendrée par les activités humaines (Millennium Ecosystem Assessment 2005). Il est donc essentiel de réfléchir à des systèmes de gestion des forêts qui permettent de réduire leur vulnérabilité et qui assurent la durabilité de la couverture forestière et de sa productivité. Les forêts plurispécifiques semblent être une bonne orientation pour répondre à ces enjeux.

En effet, il existe de plus en plus d'études qui démontrent l'importance de la biodiversité dans les écosystèmes forestiers pour l'apport de services écosystémiques (Balvanera et al. 2006; Brockerhoff et al. 2017). Les forêts plus diversifiées en espèces d'arbres permettent une meilleure conservation de la biodiversité : du fait de la présence d'un plus grand nombre de types d'habitats elles hébergent une plus grande diversité d'espèces d'invertébrés, d'oiseaux et de mammifères (Brockerhoff et al. 2017; Messier et al. 2021). Ces espèces peuvent, en retour, jouer un rôle important de pollinisation et de dispersion des graines. De plus, la diversité en forêt peut jouer un rôle important de régulation face à des perturbations telles que les tempêtes, les incendies ou les attaques d'insectes (Jactel et al. 2017; Messier et al. 2021).

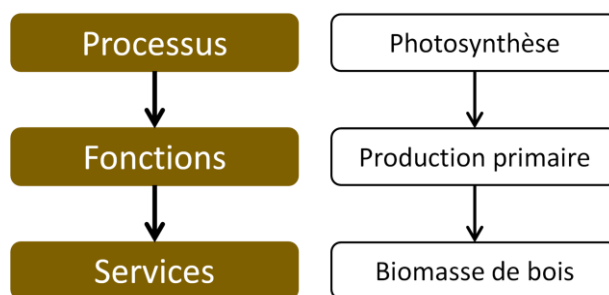
Cependant, historiquement, la gestion des forêts a entraîné une perte de diversité en forêt en favorisant des forêts secondaires en monoculture ou avec une plus faible diversité spécifique, afin de maximiser la production de quelques essences cibles (Puettmann et al. 2009; Pretzsch et al. 2017). Actuellement, en Europe, on assiste à un retour vers la gestion de peuplements mixtes, mais ceux-ci restent minoritaires dans le monde (Pretzsch et al. 2017), en particulier dans les forêts de plantation (Kelty 2006; Nichols et al. 2006). C'est pourquoi il est devenu indispensable de réfléchir à une gestion des forêts qui permettrait d'assurer une plus grande biodiversité de l'écosystème afin d'améliorer leur durabilité et leur multifonctionnalité. Ceci implique nécessairement de ne pas se focaliser sur la seule fonction de production des forêts (Bravo-Oviedo 2018).

**Encadré 1** Définitions autour du fonctionnement de l'écosystème adaptées de Brockerhoff et al. (2017).<sup>1</sup>

**Processus écologiques (Ecological processes)** : ce concept est « **centré sur les organismes** » ; les processus peuvent être physiologiques (ex. : photosynthèse, respiration), biologiques (ex. : dispersion) et/ou évolutifs (ex. : sélection ou mutation). Ils représentent des interactions complexes entre les éléments biotiques et abiotiques des écosystèmes qui sous-tendent les flux d'informations (ex. : les stimuli), d'énergie (ex. : la lumière du soleil) et de matière (ex. : les nutriments, les gaz, l'eau).

**Fonctions écosystémiques (Ecosystem functions)** : mécanismes écologiques (biologiques, chimiques et physiques) qui soutiennent l'intégrité ou le maintien des écosystèmes. Ce concept est « **centré sur l'écosystème** ». Les fonctions des écosystèmes, telles que la production primaire ou la décomposition, résultent des interactions entre les structures et les processus des écosystèmes. Elles n'apportent pas forcément de services pour les humains. Elles sont parfois considérées comme des "services de soutien" des écosystèmes.

**Services écosystémiques (Ecosystem services)** : contributions des écosystèmes au bien-être humain. Ce concept est donc « **centré sur l'homme** ». Ces services sont définis en fonction de leurs avantages spécifiques pour les individus ou la société. Ils sont considérés comme des services écosystémiques car ils conservent un lien avec les fonctions écosystémiques sous-jacentes qui les génèrent. Ils sont généralement subdivisés en services d'approvisionnement, de régulation et culturels.



*Exemples pour les différentes catégories*

**Encadré 2** Définitions des types de forêts.

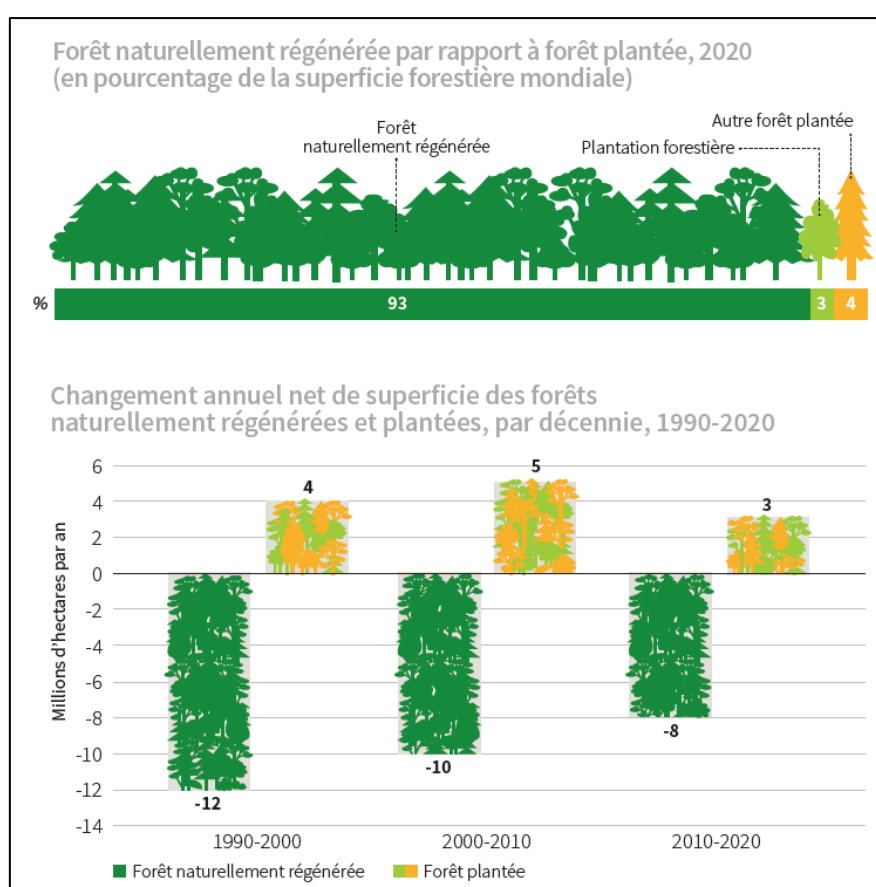
La FAO (2020) définit la typologie des forêts suivante :

- **Forêts naturellement régénérées (Naturally regenerating forest)**. Également appelées forêts naturelles. Forêt régénérée par le cycle naturel de reproduction des peuplements en place.
- **Forêts plantées (Planted forest)**. Forêt composée principalement d'arbres établis par plantations et/ou par semis délibérés. Elles incluent les plantations forestières et les autres forêts plantées.
  - **Plantations forestières (Plantation forest)**. Les plantations forestières sont gérées de manière intensive, composées d'une ou deux essences, équiennes, plantées à des intervalles réguliers, et établies principalement à des fins productives.
  - **Autres forêts plantées (Other planted forest)**. Elles incluent les plantations forestières et les autres forêts plantées. Les autres forêts plantées, ne sont pas gérées de manière intensive et, lorsque le peuplement arrive à maturité, elles ressemblent plutôt à des forêts naturelles.

<sup>1</sup> La traduction en anglais des mots définis dans cette introduction sont indiqués en bleu dans le texte

## 1.1. Diversité des forêts au niveau global et européen

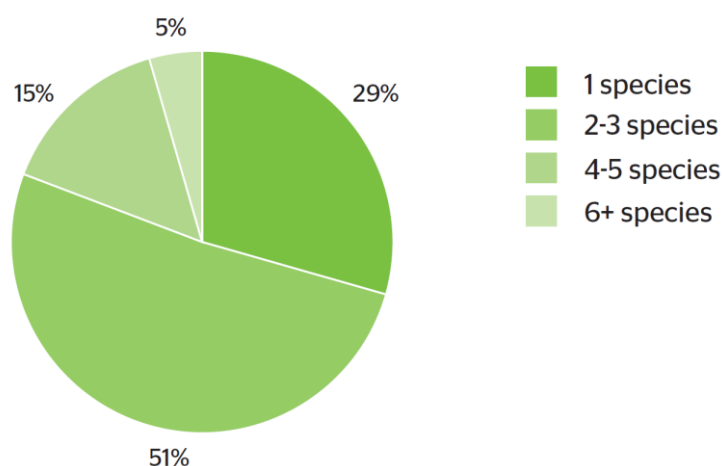
Les forêts hébergent une très grande partie de la biodiversité terrestre et aquatique et sont l'écosystème le plus représenté sur terre, recouvrant 30.6 % du territoire mondial. A l'échelle globale, les pressions dues aux activités humaines engendrent chaque année une perte et une fragmentation de la surface forestière mondiale, ainsi qu'une perte de biodiversité (FAO 2020) (**Fig. 1**). La perte de surface forestière, via la déforestation, continue de manière significative en particulier dans les écosystèmes forestiers les plus diversifiés sous les tropiques, malgré un ralentissement (FAO 2020). Par ailleurs, une augmentation des surfaces forestières est observée dans certaines régions du monde (Europe, Asie), dont en France, mais celle-ci ne compense pas les pertes au niveau global (Millennium Ecosystem Assessment 2005; FAO 2020). De plus, les surfaces de forêts plantées (voir **Encadré 2**), majoritairement monospécifiques, sont en augmentation et représentent actuellement environ 7 % de la surface forestière mondiale (Payn et al. 2015; FAO 2020) (**Fig. 1**). Ces dynamiques de couverture forestière sont préoccupantes, parce qu'elles engendrent une perte générale de biodiversité et une dégradation de l'apport de services écosystémiques pour les populations humaines.



**Fig. 1** Proportion des types de forêts au niveau mondial et dynamique décennale des superficies. Extrait de l'évaluation des ressources forestières mondiales 2020 (FAO 2020).

La plupart des forêts naturelles sont constituées d'un mélange d'espèces d'arbres qui peuvent atteindre un nombre d'espèces élevé (jusqu'à 300 espèces d'arbres par hectare en forêts tropicales) (Gentry 1988). D'un point de vue global, la richesse et la diversité des espèces d'arbres dans les forêts ne sont pas réparties de manière uniforme. La richesse en espèces d'arbres augmente généralement depuis les régions froides et boréales jusqu'aux régions chaudes et tropicales (Pretzsch et al. 2017). Ce schéma biogéographique mondial de la richesse spécifique en général, y compris celle des plantes et des arbres, a été un sujet d'étude central en écologie au cours du XX<sup>ème</sup> siècle (May 1986; Currie and Paquin 1987; Loreau et al. 2001). Il s'explique par de nombreux facteurs (quantité d'énergie disponible, complexité de l'habitat, régimes de perturbations, stress environnementaux, stabilité environnementale, interactions biotiques, événements climatiques passés...) (Hansen and Rotella 1999). Par exemple, les forêts naturelles tempérées européennes sont moins riches en espèces d'arbres que les forêts tempérées d'Asie de l'Est et d'Amérique du Nord, ce qui s'explique par une extinction d'espèces plus importante en Europe, à la suite des dernières périodes glaciaires du Quaternaire (Hewitt 1999). Globalement, la plupart des forêts naturelles ou semi-naturelles sont constituées de plusieurs espèces d'arbres, en Europe et dans le monde, même si des exemples de peuplements monospécifiques existent également en conditions naturelles.

La diversité en composition spécifique peut être considérablement modifiée dans les forêts gérées par l'homme (Puettmann et al. 2009). Les trois quarts de la superficie actuellement couverte par les forêts en Europe sont constitués de peuplements ne comptant pas plus de trois espèces d'arbres dans l'étage dominant (**Fig. 2**) (Forest Europe et al. 2011), ce qui est le résultat de la composition naturelle des forêts européennes combiné aux effets de la gestion des forêts par l'homme. Les modifications apportées par l'homme aux forêts, par les boisements à grande échelle avec des peuplements monospécifiques mais également par les choix de gestion qui ciblent préférentiellement certaines essences, ont aussi contribué à l'appauvrissement en nombre d'espèces d'arbres en Europe (Pretzsch et al. 2017). Dans les forêts de plantations, par exemple, les processus de succession d'espèces d'arbres qui auraient lieu dans une forêt naturelle sont souvent limités aux phases de début et de milieu de succession. De plus la composition en espèces y est fortement contrôlée, au point qu'une seule espèce y soit cultivée dans tout le peuplement (Pretzsch et al. 2017), et parfois même dans tout le paysage d'une région (par exemple, le pin maritime (*Pinus pinaster* Ait.) dans les Landes de Gascogne en France) (Mason and Meredieu 2011). En effet, les espèces d'arbres ciblées dans les programmes de plantation sont celles qui ont la capacité de bien se développer (forte croissance et bonne qualité du bois) dans une large gamme de conditions de sols et de sites. Les monocultures sont donc généralement favorisées dans la gestion et la plantation des forêts (Pretzsch et al. 2017).

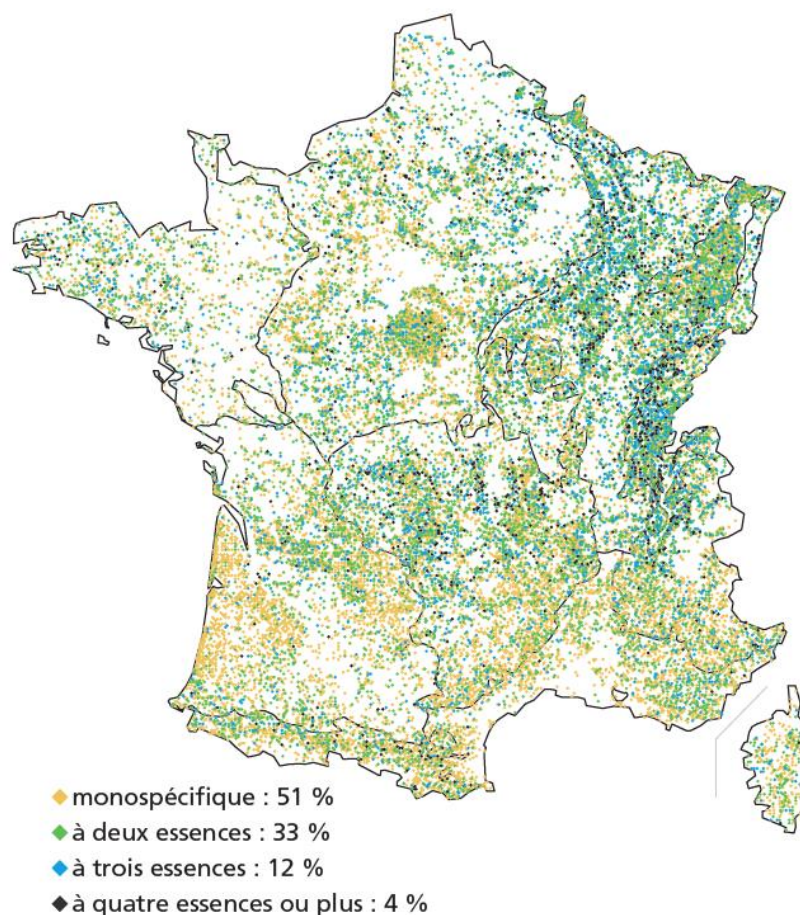


**Fig. 2** Proportion de forêts en Europe présentant différents niveaux de richesse en espèces. Extrait du rapport « State of Europe's Forests 2011 » (Forest Europe et al. 2011).

## 1.2. Caractéristiques des forêts françaises

La forêt en France métropolitaine couvre 16,9 millions d'hectares, soit 31 % du territoire (IGN 2020). Les forêts dans les DOM-TOM couvrent par ailleurs environ 9,0 millions d'hectares. C'est l'occupation du sol la plus importante après l'agriculture, qui couvre plus de la moitié de la France métropolitaine. En termes de diversité en essences, la moitié de la forêt française est constituée de peuplements monospécifiques (c.-à-d. les peuplements pour lesquels une essence représente plus des trois quarts du couvert des arbres), soit 7,3 millions d'hectares. Les peuplements à deux essences représentent un tiers des peuplements, tandis que ceux à plus de deux essences en représentent 17 %. Les forêts du nord-est de la France et du Massif central sont les plus diversifiées (**Fig. 3**). À l'opposé, le massif des Landes de Gascogne est un grand massif d'environ un million d'hectares de peuplements monospécifiques de pin maritime.

En termes de composition en essences, la forêt française métropolitaine est composée majoritairement de feuillus, qui représentent 67 % de la superficie forestière (9,9 millions d'hectares). Les peuplements de feuillus se situent surtout dans les plaines ou à moyenne altitude. Les peuplements de conifères se trouvent essentiellement en zone montagneuse, dans le massif landais et dans les plantations assez récentes de l'ouest de la France. Les peuplements mixtes feuillus-conifères se rencontrent souvent en moyenne montagne ou dans les massifs forestiers accueillant à la fois des peuplements de feuillus et de résineux (Sologne, Dordogne, Bretagne) (IGN 2020).



**Fig. 3** Répartition de la diversité des peuplements en France métropolitaine. Les informations relatives à la composition et à la diversité des peuplements ne sont prises que dans les peuplements ayant un couvert par des arbres de plus de 7,5 cm de diamètre supérieur à 15 %. Ils couvrent une superficie de 14,8 millions d’hectares. ©IGN 2020

Les forêts plantées représentent 2.1 millions d’hectares, soit 13 % de la surface forestière de France métropolitaine. Elles sont constituées à 80 % d’essences résineuses : les trois principales essences de plantation sont le pin maritime, le douglas (*Pseudotsuga menziesii* (Mirb.) Franco) et l’épicéa commun (*Picea abies* (L.) H.Karst). Sur l’ensemble des forêts plantées, seulement 16 % (330 000 hectares) sont des plantations mélangées à deux espèces ou plus, et la majeure partie de ces mélanges en plantation sont des mélanges de deux espèces de résineux (par exemple : sapin pectiné (*Abies alba* Mill.) et épicéa commun, douglas et épicéa commun...) (IGN 2017).



Au vu de l'importance des forêts diversifiées pour assurer un nombre de services écosystémiques, il semble important d'adapter les pratiques de gestions afin de favoriser à nouveau la biodiversité, par exemple avec des peuplements mélangés. Pour cela il est indispensable de comprendre comment la diversité en espèces d'arbres peut influencer le fonctionnement des espèces d'arbres dans les écosystèmes forestiers et les services écosystémiques de ces peuplements, afin d'apporter des connaissances qui pourront guider la gestion forestière des peuplements mélangés (voir **Encadré 3**).

**Encadré 3** Définition d'une forêt mélangée adaptée de Bravo-Oviedo (2018).

**Qu'est-ce qu'une forêt mélangée ?**

Une forêt mélangée se définit en opposition à une forêt monospécifique (ou pure). Une forêt mélangée comporte deux espèces ou plus, qui doivent dépasser une certaine proportion en nombre de tiges ou surface terrière, souvent défini entre 10 et 20 %. Ainsi, si une seule espèce constitue plus de 80 % de l'étage dominant d'un peuplement, celui-ci est considéré comme pur.

Bravo-Oviedo et al. (2014) ont proposé la définition suivante (traduit) : « *Un peuplement mélangé est une unité forestière, à l'exclusion des formations linéaires, où au moins deux espèces d'arbres coexistent à n'importe quel stade de développement, partageant des ressources communes (lumière, eau et/ou nutriments du sol). La présence de chacune des espèces composantes est généralement quantifiée en tant que proportion du nombre de tiges ou de la surface terrière, bien que le volume, la biomasse ou la couverture du couvert ainsi que les proportions de la surface occupée du peuplement puissent être utilisés pour des objectifs spécifiques. Une variété de structures et de modèles de mélanges sont possibles, et les interactions entre les espèces qui les composent et leurs proportions respectives peuvent changer avec le temps.* »

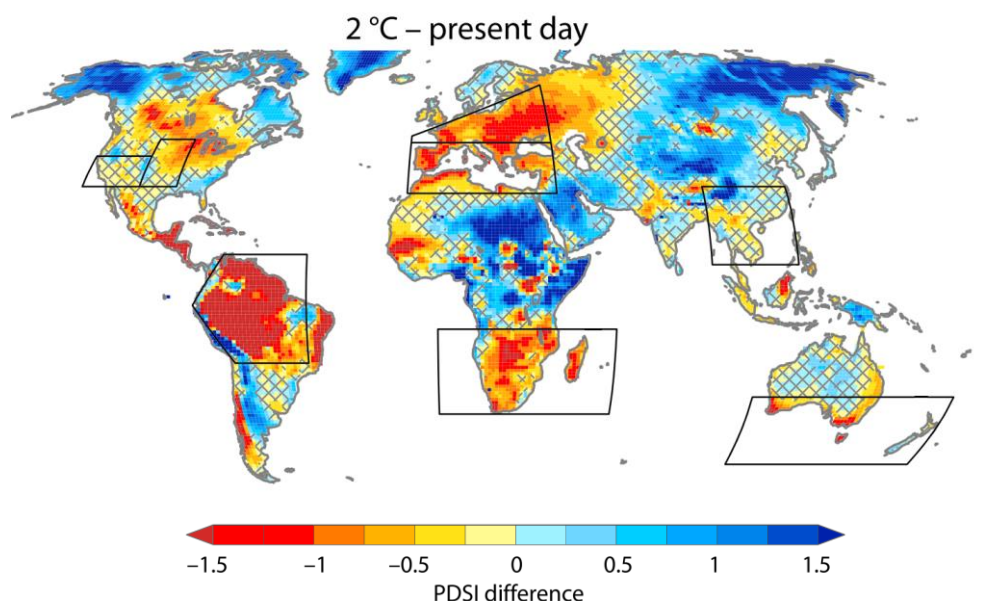
*Attention* : Au niveau européen, une forêt mélangée a parfois également été définie comme une forêt constituée d'un mélange d'espèces feuillues et de conifères (Forest Europe et al. 2011).

## 2. Conséquences des changements climatiques sur les écosystèmes forestiers

### 2.1. Conséquences à l'échelle globale

Depuis le début de l'ère industrielle au 19<sup>ème</sup> siècle, les activités humaines ont entraîné une augmentation continue de la consommation d'énergie fossile, entraînant une augmentation des émissions de gaz à effet de serre, notamment du CO<sub>2</sub>, dans l'atmosphère. L'augmentation de la concentration en gaz à effet de serre a engendré une augmentation des températures à l'échelle du globe depuis les années 1950 (IPCC 2014). En 2017 l'augmentation des températures était de +1.0 [+0.8 to +1.2]°C au niveau global par rapport à la période préindustrielle de 1850 à 1900 (IPCC 2018). Cette augmentation varie en fonction des régions du globe : elle est plus forte au niveau des continents que des océans et est notamment supérieure à la moyenne globale dans l'hémisphère nord pour les températures hivernales (IPCC 2018). L'augmentation globale des températures, et donc de l'énergie disponible dans l'atmosphère, s'accompagne d'autres changements au niveau de l'atmosphère. En particulier, cela modifie les patrons de précipitations, ce qui entraîne l'augmentation de la fréquence et de l'intensité des épisodes de sécheresse en été, ou des épisodes de fortes pluies en hiver, qui ont des effets majeurs sur le fonctionnement des écosystèmes forestiers (Allen et al. 2010; IPCC 2014; Lehner et al. 2017). Les scénarios climatiques prévoient que les régions méditerranéennes et d'Europe centrale, incluant le territoire français, seront particulièrement sensibles à l'accentuation des risques de sécheresses dans les prochaines 50-100 années (Lindner et al. 2010; Hanewinkel et al. 2013; Lehner et al. 2017) (**Fig. 4**).

Les changements climatiques actuels ont également pour conséquence l'augmentation de la fréquence de nombreux événements extrêmes (incendies, tempêtes, invasions de pathogènes...). Ces événements engendrent aussi de fortes perturbations pour le fonctionnement des écosystèmes forestiers, mais je ne les développerai pas dans cette introduction, sachant que mon sujet de recherche a porté spécifiquement sur l'effet des sécheresses. Cependant, il est à noter que ces différentes perturbations peuvent interagir, avec notamment une plus forte probabilité d'occurrence des incendies ou d'attaques d'insectes dans des peuplements affectés par les sécheresses (Lindner et al. 2010).



**Fig. 4** Variations de l'indice de sécheresse (Palmer Drought Severity Index, PDSI) moyen entre la période actuelle (1967-2016) et le scénario +2°C (2051- 2100) tel que modélisée par le Community Earth System Model (CESM). Les hachures indiquent les différences qui ne sont pas significatives selon un test-t bilatéral (intervalle de confiance à 95 %). Extrait de Lehner et al. (2017).

Par conséquent, la combinaison des différents effets des changements climatiques sur le fonctionnement des arbres va très probablement avoir des conséquences sur l'aire de répartition des différentes espèces d'arbres (Cheaib et al. 2012). En effet, les individus d'une espèce donnée peuvent se retrouver exposés à des conditions climatiques qui ne correspondent plus à leur niche écologique (voir **Encadré 4**), c'est-à-dire beaucoup moins propices à leur développement ou leur survie. Des changements environnementaux importants, prolongés ou répétés peuvent ainsi entraîner sur le long terme la disparition d'espèces moins adaptées dans un milieu donné, en raison d'une diminution des ressources, des blocages phénologiques ou d'autres facteurs (Saxe et al. 2001). Ces changements d'aire de répartition seront différents selon les espèces, et peuvent modifier la composition des communautés forestières (Thuiller et al. 2006). Pour cela il est important de mieux comprendre les mécanismes d'interactions des espèces d'arbres afin de prévoir comment ces changements de composition pourraient impacter le fonctionnement des écosystèmes.

**Encadré 4** La notion de niche écologique.**Qu'est-ce que la niche écologique ?**

La **niche écologique** (*ecological niche*) est un concept théorique, central en écologie, qui a été défini de plusieurs manières historiquement. La niche écologique a été définie d'une part par son rôle ou sa fonction dans l'environnement (Elton 1927) et d'autre part par les ressources, biotiques et abiotiques, nécessaires au maintien d'une population viable de cette espèce (Grinnell 1917; Hutchinson 1957). Je me concentrerai ici sur la seconde définition, qui décompose « l'habitat » nécessaire à une espèce en de multiples ressources.

Dans ce cadre-là, la niche écologique d'une espèce peut être visualisée comme un hypervolume à n dimensions dans lequel chaque ressource représente un axe indépendant. Cette définition prend en compte l'espace physique qu'une espèce occupe, les conditions de température et d'humidité de cet espace, et la saisonnalité des conditions abiotiques et biotiques dans cet espace, ainsi que les besoins alimentaires et les interactions qu'une espèce entretient avec d'autres espèces. L'espace que chaque espèce occupe dans cet hypervolume est défini par ses besoins en ressources de base (c'est-à-dire la **niche fondamentale**) et est réduit par les interactions antagonistes avec d'autres organismes (c'est-à-dire la **niche réalisée**) (Hutchinson 1957).

Deux espèces ne peuvent occuper, sur un même territoire géographique, la même niche écologique, une espèce finissant toujours par dominer les autres et par l'exclure de la niche. C'est le **principe d'exclusion compétitive** (*competitive exclusion principle*) (Gause 1934). On dit de deux espèces qu'elles ont la même niche si elles utilisent la même ressource de la même manière. Dans ce cas, la théorie prédit qu'elles ne peuvent cohabiter. En effet, à court terme, la compétition va favoriser celle qui est la mieux adaptée à la niche (c'est-à-dire celle qui dans ces conditions peut se reproduire le plus efficacement). Cependant, deux espèces peuvent occuper un même territoire, mais dans des niches écologiques différentes.

Deux espèces peuvent aussi occuper des niches écologiques chevauchantes dans l'espace et/ou dans le temps, c'est-à-dire que seulement une partie des dimensions de l'hypervolume de Hutchinson sont occupées de manière simultanée. On parle alors de recouvrement. Les deux espèces peuvent cohabiter si ce chevauchement n'est pas trop important. Il en résulte une compétition interspécifique plus ou moins forte. Cette compétition peut produire une **différentiation de niche** (*niche differentiation*), c'est-à-dire qu'une ou les deux espèces vont modifier leur niche réalisée afin de coexister. Le **partitionnement des ressources** (*resource partitioning*) est un exemple de différenciation de niche.

## 2.2. Impact de la sécheresse sur le fonctionnement des arbres

Les épisodes de sécheresse affectent les processus physiologiques des arbres tels que la photosynthèse, la transpiration et l'allocation du carbone, et peuvent entraîner une réduction des taux de croissance (Saxe et al. 2001; Bréda et al. 2006). Le stress induit par la sécheresse peut conduire à une perte de vitalité et à terme à la mortalité des arbres (McDowell et al. 2008; Hartmann et al. 2018). En effet, des effets du changement climatique sur la mortalité des arbres ont déjà été observés à l'échelle globale (Allen et al. 2010). De plus, le sujet a été particulièrement d'actualité ces dernières années (2018 à 2020) en Europe, avec de fortes sécheresses estivales successives et des conséquences déjà observables sur la mortalité des arbres (Schuldt et al. 2020). Cette partie a pour objectif de résumer quelques concepts sur la réponse des arbres à la sécheresse. La compréhension des réponses physiologiques fines à la sécheresse, et notamment des processus de mortalité des arbres, est un vaste domaine scientifique, en progression permanente et particulièrement actif dans le contexte actuel.

### 2.2.1. Définition d'une sécheresse

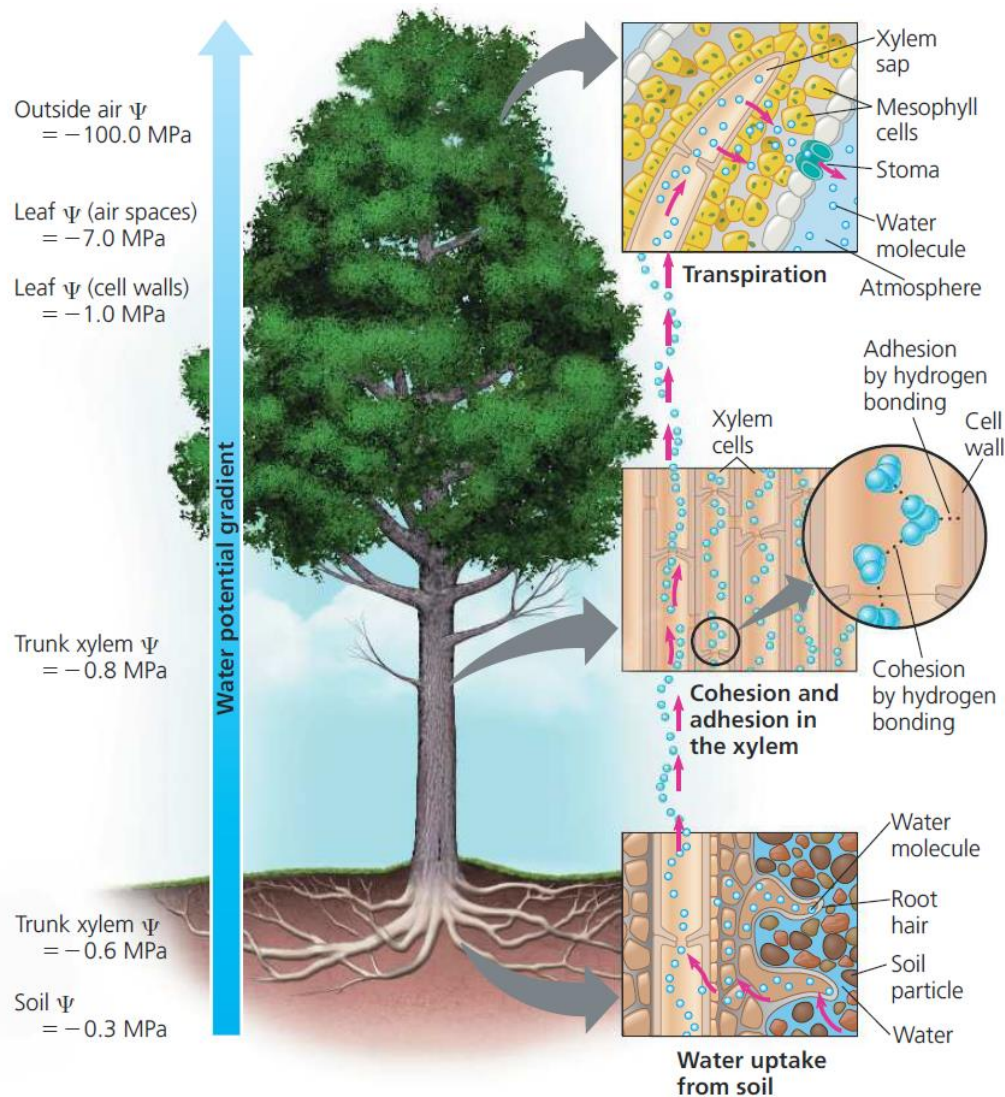
Une sécheresse peut être définie, du point de vue d'un arbre, comme une période prolongée de stress hydrique résultant d'une demande atmosphérique accrue (c'est-à-dire une sécheresse atmosphérique) et/ou d'une faible disponibilité en eau du sol autour des racines de cet arbre (c'est-à-dire une sécheresse édaphique) (Gutierrez Lopez et al. 2021).

La sécheresse atmosphérique, engendrée par de fortes températures, une faible humidité de l'air, et généralement un fort rayonnement, engendre une demande évaporative élevée au niveau des surfaces d'échange (aiguilles ou feuilles), du fait d'une forte différence de potentiel hydrique ( $\Psi$ , en MPa) entre l'atmosphère et l'arbre (**Fig. 5**).

La sécheresse édaphique correspond à une diminution de la teneur en eau du sol qui engendre un déficit d'eau disponible pour les racines des arbres. L'intensité de la sécheresse édaphique peut être caractérisée par la mesure de la teneur en eau du sol à différentes profondeurs, à l'aide de capteurs de type TDR (« Time Domain Reflectometry ») ou sondes à neutrons. L'intensité de la sécheresse édaphique peut être exprimée également via le potentiel hydrique du sol : plus il est négatif, plus le sol est sec. Cette diminution du potentiel hydrique du sol affecte ensuite le statut hydrique de l'arbre, car le potentiel hydrique dans les vaisseaux du xylème s'équilibre avec celui du sol en absence de transpiration (typiquement, de nuit).

Ainsi, les conditions atmosphériques et édaphiques affectent toutes deux le statut hydrique d'un arbre du fait d'un continuum hydraulique sol-plante-atmosphère. En effet, le mécanisme de transport de l'eau sur de longues distances dans les arbres, entre les racines qui absorbent l'eau libre du sol et les feuilles où l'eau est transpirée, résulte en un continuum hydraulique entre ces différents compartiments. Le transport de l'eau dans les plantes s'explique par la théorie de cohésion-tension (Dixon and Joly 1895;

Steudle 2001) : du fait de la nature polaire et cohésive des molécules d'eau, elles sont fortement liées entre elles par des liaisons hydrogène et forment une « colonne » ininterrompue dans l'arbre, du sol jusqu'à l'atmosphère (**Fig. 5**). Ainsi, lors d'une période de sécheresse, le potentiel hydrique va diminuer tout au long de la trajectoire de l'eau dans l'arbre.



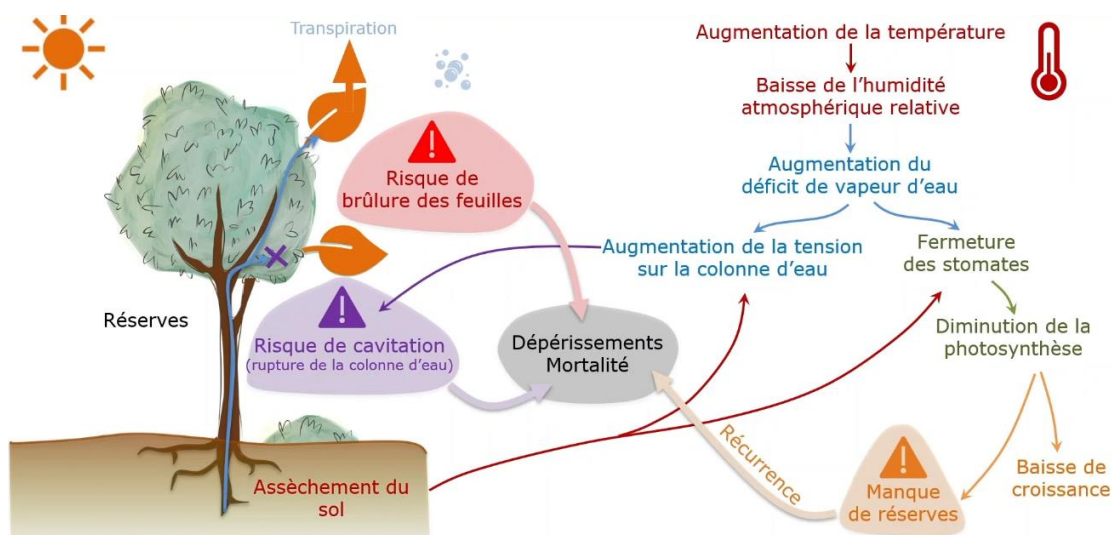
**Fig. 5** Transport de l'eau (sève brute) dans le xylème, du sol à l'atmosphère, selon la théorie de la cohésion-tension. Les liaisons hydrogènes forment une chaîne ininterrompue de molécules d'eau qui s'étend des feuilles au sol. La force qui entraîne la montée de la sève du xylème est un gradient de potentiel hydrique ( $\Psi$ ). Sur une longue distance, le gradient  $\Psi$  est principalement dû à un gradient du potentiel de pression ( $\Psi_P$ ). La transpiration fait que, dans le xylème, le  $\Psi_P$  à l'extrémité foliaire est plus faible que le  $\Psi_P$  à l'extrémité racinaire. Les valeurs de  $\Psi_P$  indiquées à gauche sont un exemple pour un instant donné. Elles peuvent varier pendant la journée, mais la direction du gradient  $\Psi$  reste la même. Extrait de *Campbell Biology*, 12<sup>th</sup> Edition, © 2021 Pearson Education.

La disponibilité en eau dans le sol pour les arbres dépend de nombreux facteurs en plus des conditions climatiques (Bréda et al. 2006). En effet les propriétés physiques du sol rendent l'eau présente plus ou moins facile à extraire par les racines. De plus, les propriétés d'exploration des racines en profondeur déterminent aussi l'exposition à la sécheresse d'un arbre. Enfin, la présence d'une nappe phréatique peu profonde implique que l'eau disponible ne vient pas uniquement de la percolation des précipitations mais aussi de l'eau de la nappe, qui est soit directement accessible par les racines ou soit rendue disponible par des remontées capillaires dans le sol (Bréda et al. 2002; Vincke and Thiry 2008).

### 2.2.2. Réponse physiologique de l'arbre à la sécheresse

Le stress hydrique, selon sa rapidité de mise en place, son intensité et sa durée, provoque des réponses complexes chez les arbres, qui ont souvent pour conséquence *in fine* de limiter leur croissance (**Fig. 6**).

La fermeture des stomates, ainsi que l'inhibition de la croissance des feuilles, sont parmi les premières étapes de la réponse à la sécheresse, protégeant les arbres d'une perte d'eau importante, qui pourrait entraîner la déshydratation des cellules, la cavitation du xylème et la mort de l'arbre. La perception et la signalisation du stress hydrique qui va déclencher la fermeture des stomates est complexe et multifactorielle. Les principaux mécanismes qui y participent sont : le potentiel hydrique des feuilles, l'intensité du rayonnement, la concentration en CO<sub>2</sub>, mais aussi des signaux hormonaux émis lors de la déshydratation des racines (Chaves et al. 2003). La signalisation d'une sécheresse, par signaux hydrauliques ou biochimiques, va provoquer une fermeture des stomates pour la plupart des espèces d'arbres. Cette fermeture des stomates va limiter les pertes en eau par transpiration, au détriment de l'assimilation en CO<sub>2</sub> par l'arbre (Chaves et al. 2003; Bréda et al. 2006). Cependant, la baisse de la conductance stomatique est généralement plus rapide que la baisse de l'assimilation du CO<sub>2</sub>, ce qui engendre une augmentation de l'efficacité d'utilisation de l'eau intrinsèque (« intrinsic water use efficiency », WUE<sub>i</sub>). WUE<sub>i</sub> est défini comme le ratio entre l'assimilation nette de CO<sub>2</sub> (A) et la conductance stomatique pour la vapeur d'eau (g<sub>s</sub>) (Farquhar et al. 1982). Ce trait est souvent évalué par la mesure de la composition isotopique du <sup>13</sup>C dans les feuilles ou le tronc, car la discrimination isotopique du carbone au cours de la photosynthèse dépend fortement de la conductance stomatique (Farquhar et al. 1989; Saurer et al. 1995). La fermeture stomatique contribue à maintenir le potentiel hydrique au-dessus d'un seuil critique pour l'arbre en dessous duquel le taux d'embolie des vaisseaux du xylème augmente fortement (Cruziat et al. 2002). Ce seuil est spécifique à chaque espèce. Hormis la fermeture des stomates, la perception d'une sécheresse par l'arbre va également déclencher de nombreux mécanismes de protection métaboliques, pour éviter les effets délétères de la déshydratation et du stress oxydatif sur les tissus végétaux (Chaves et al. 2003).



**Fig. 6** Schéma simplifié des conséquences de la sécheresse pour un arbre à court terme et à long terme. © RMT AFORCE

Par ailleurs, on peut aussi noter que les fortes températures qui accompagnent souvent une période de sécheresse peuvent créer un stress thermique qui va également impacter le fonctionnement physiologique de l'arbre (Teskey et al. 2015). En effet, le stress thermique peut induire une réduction de l'activité photosynthétique des feuilles au-delà d'un certain seuil de température, entraînant une diminution de l'assimilation de carbone par l'arbre. De plus, lors de vagues de chaleur extrêmes, le stress thermique peut aussi entraîner l'augmentation du stress oxydatif, et l'abscission des tissus foliaires.

### 2.2.3. Stratégies de réponse à la sécheresse

Afin de maintenir l'intégrité de leur système hydraulique, les arbres répondent donc à la sécheresse par des mécanismes à **court terme** de régulation dynamiques et réversibles, comme une réduction de la transpiration par la fermeture des stomates, qui peuvent avoir l'avantage supplémentaire de retarder l'épuisement de l'eau du sol. Par ailleurs, des réponses **plastiques** et à **long terme** sont également possibles. Par exemple : (i) le développement d'un xylème plus résistant à la cavitation, capable de supporter un potentiel hydrique plus faible ; (ii) la réduction de la surface foliaire transpirante, soit par une modification de l'allocation de la biomasse aux racines par rapport aux feuilles, soit par une perte foliaire plus ou moins importante ; (iii) une amélioration de la conductance hydraulique dans le continuum sol-feuilles par l'augmentation de la biomasse allouée aux racines par exemple (Bréda et al. 2006).

Le niveau de réponse des arbres à la sécheresse varie entre espèces. En effet, la perception et la signalisation du stress, des variations de croissance et de l'allocation de croissance, l'augmentation de l'efficacité d'utilisation de l'eau, etc. ne sont pas identiques entre les espèces. Les stratégies de réponse à la sécheresse des espèces sont souvent regroupées en trois catégories : l'échappement (*escape*),



l'évitement (*avoidance*) et la tolérance (*tolerance*) (Delzon 2015; Volaire 2018). L'échappement, qui consiste à réaliser un cycle de vie complet avant une éventuelle saison sèche, ne s'applique pas au cas des forêts mais plutôt à des espèces de plantes annuelles. L'évitement consiste, par exemple, à une fermeture stomatique précoce et/ou à la capacité à extraire de l'eau en profondeur afin de maintenir un potentiel hydrique élevé. La tolérance s'exprime par la mise en place de mécanismes à travers l'arbre qui « prend le risque » de supporter des niveaux de stress hydrique élevés, par exemple par une fermeture stomatique tardive ou une forte capacité de résistance à l'embolie. Les réponses liées à ces deux stratégies ne sont pas nécessairement mutuellement exclusives et tout un gradient de stratégies existent (Volaire 2018).

#### 2.2.4. Conséquences à long terme de la sécheresse

Le stress induit par la sécheresse peut conduire à une perte de vitalité et à terme à la mortalité des arbres, causée par une défaillance hydraulique et/ou une famine carbonée (McDowell et al. 2008; Hartmann et al. 2018). Lorsque l'intensité de la sécheresse augmente, les conditions d'équilibre du transfert d'eau (principalement dans les tissus du xylème) peuvent être irréversiblement perturbées, en raison de la rupture de la cohésion de l'eau et de l'embolie massive des vaisseaux. Cela peut entraîner une mortalité prématurée des racines ou des rameaux, et finalement conduire à la mort de l'arbre (**Fig. 6**) (Bréda et al. 2006; McDowell et al. 2008; Choat et al. 2018). De plus, la réduction de l'assimilation du carbone au cours d'une sécheresse prolongée peut mener à une famine carbonée. En effet, les réserves en glucides non structuraux dont l'arbre a besoin pour maintenir son fonctionnement (respiration, croissance) deviennent insuffisantes, entraînant à terme la mort de l'arbre (McDowell et al. 2008). Selon le cadre théorique développé par McDowell et al. (2008), la part relative de ces différents processus de mortalité dépendrait théoriquement de l'intensité et de la durée de la sécheresse. La défaillance hydraulique surviendrait plutôt lors d'épisodes de stress hydrique de forte intensité et faible durée, tandis que la famine carbonée surviendrait lors d'épisodes prolongés et de plus faible intensité. En réalité, les deux processus sont interdépendants et les processus de mortalité des arbres sont probablement liés à une combinaison des deux processus (Sevanto et al. 2014; Adams et al. 2017). Néanmoins, la cause finale de mortalité est probablement souvent la défaillance hydraulique (Adams et al. 2017; Choat et al. 2018).

### 2.3. Comment adapter les forêts face aux sécheresses ?

Au vu des conséquences physiologiques que peuvent avoir l'augmentation de la fréquence et de l'intensité des sécheresses sur les arbres, il semble donc important de réfléchir à des solutions pour limiter ces effets. Différentes approches sont considérées pour limiter l'impact majeur des sécheresses sur les arbres.

La première est de privilégier, lors du renouvellement des peuplements forestiers, la plantation ou l'installation par régénération d'espèces résistantes à la sécheresse. En effet, la migration assistée de certaines espèces qui poussent dans des climats plus secs pourrait être une solution en se basant sur les caractéristiques physiologiques des différentes espèces (Niinemets and Valladares 2006), mais celle-ci comporte beaucoup d'incertitudes liées à la difficulté de prédire le climat futur (Williams and Dumroese 2013; Lindner et al. 2014; Albert et al. 2017).

La deuxième est de mettre au point des modes de gestion des forêts qui pourraient diminuer l'exposition à la sécheresse des arbres dans ces peuplements dans le contexte climatique actuel et futur. Une des pistes de gestion envisagées est de diminuer la densité des peuplements. En effet, il a été montré que le fait de diminuer la densité du peuplement pouvait améliorer sa résistance à la sécheresse (Bottero et al. 2017; Gavinet et al. 2020). En réduisant la surface foliaire du peuplement, l'éclaircie devrait réduire l'interception des pluies et la transpiration du peuplement, améliorant ainsi la disponibilité de l'eau pour les arbres restants (Bréda et al. 1995). Cependant, l'ouverture du peuplement peut exposer le sol à d'avantage d'évaporation et pourrait aussi favoriser le développement de la végétation de sous-bois (Gebhardt et al. 2014). De plus, l'avantage obtenu lors de l'éclaircie pourrait être temporaire et disparaître avec le temps par l'augmentation de la surface foliaire des arbres restants (Bréda et al. 1995). Dans certaines conditions, des éclaircies modérées répétées peuvent donc représenter une mesure sylvicole viable pour anticiper les possibles limitations d'eau dues au changement climatique.

Enfin, une autre piste de gestion mise en avant serait de favoriser le mélange d'espèces d'arbres présentant des complémentarités fonctionnelles du point de vue de l'utilisation de l'eau et de la réponse à la sécheresse, en supposant que cela pourrait atténuer les niveaux de contrainte hydrique au sein d'un peuplement (Pretzsch et al. 2013b). Cependant, les résultats concernant cette question restent à ce jour assez controversés (Ammer 2019; Grossiord 2019). C'est à cette option d'adaptation des peuplements au changement climatique que je me suis particulièrement intéressée au cours de ma thèse et j'apporterai plus de détail sur la question dans la **section 4.2.3**.

### 3. Effets de la biodiversité sur le fonctionnement des écosystèmes (B-EF)

#### 3.1. Historique de l'étude des relations B-EF

Dans un contexte de perte de biodiversité liée aux activités humaines (Cardinale et al. 2012; Naeem et al. 2012), la compréhension du rôle de la biodiversité, et de la perte d'espèces en particulier, dans le fonctionnement des écosystèmes est devenue un sujet majeur en sciences écologiques au cours des dernières décennies (Loreau 2010; Tilman et al. 2014). Les premières publications établissant un lien expérimental entre la biodiversité et le fonctionnement des écosystèmes (e.g., Naeem et al. 1994)<sup>2</sup> ont vu le jour peu après le Sommet de la Terre de 1992 à Rio de Janeiro. Ces études ont constitué un changement de paradigme majeur dans la recherche en écologie (Schulze and Mooney 1993; Naeem 2002). Jusqu'à cette époque, la biodiversité était principalement étudiée comme un facteur qui répond aux changements environnementaux et au fonctionnement des écosystèmes (voir **section 1.1**), mais pas, à l'inverse, comme quelque chose qui détermine le fonctionnement des écosystèmes (Loreau et al. 2001; Tilman et al. 2014). Ainsi est née une nouvelle sous-discipline scientifique en écologie intitulée « biodiversité et fonctionnement des écosystèmes » (« *biodiversity – ecosystem functioning* », B-EF). Cet intérêt pour les relations B-EF a permis de réunir des concepts issus de deux disciplines écologiques : l'écologie des communautés et l'écologie des écosystèmes (Naeem 2002; Loreau 2010). Dans le cadre des relations B-EF, le fonctionnement des écosystèmes se définit comme « les effets combinés de tous les processus naturels qui assurent la pérennité d'un écosystème, c'est-à-dire les effets combinés des fonctions individuelles, le fonctionnement global étant régi par l'interaction de facteurs abiotiques (physiques et chimiques) et/ou biotiques » (Reiss et al. 2009) (voir aussi **Encadré 1**). Le thème central de la recherche B-EF est donc la modification des processus écologiques (abiotiques ou biotiques) par les organismes eux-mêmes. On peut citer comme exemples : la production de carbone, la consommation de ressources, ou encore l'absorption de nutriments (Reiss et al. 2009).

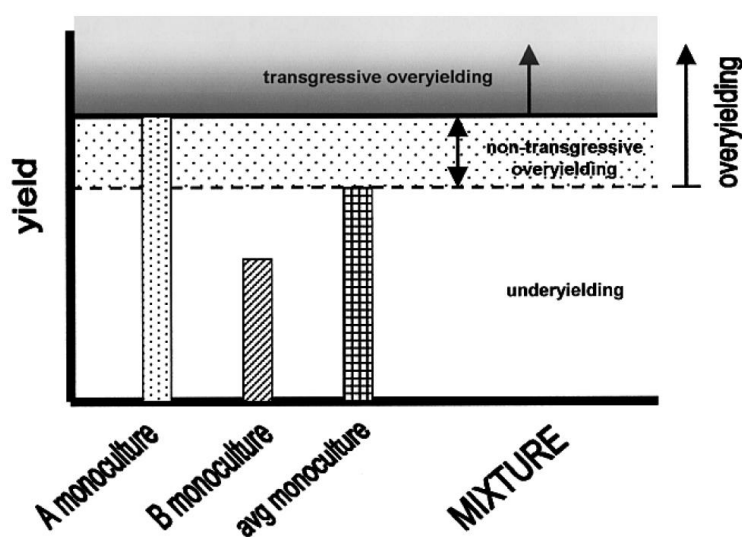
Les relations B-EF ont été largement étudiées depuis près de trois décennies (Tilman et al. 2014; van der Plas 2019), principalement via des systèmes expérimentaux à courte durée de vie comme des microcosmes, des mésocosmes, ou des communautés de plantes herbacées (Naeem et al. 1994; Hooper et al. 2005). Les expériences sur les communautés de plantes herbacées ont joué un rôle particulièrement prépondérant dans le domaine. Des expériences à long terme et de grande ampleur se sont basées sur des écosystèmes expérimentaux de prairie, où la richesse en espèces ou la diversité fonctionnelle étaient manipulées. Quelques exemples particulièrement marquants sont les expériences de Cedar Creek dans le Minnesota aux Etats-Unis (Tilman et al. 2006) et BIODDEPTH sur un gradient à travers l'Europe

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<sup>2</sup> Voir l'article de blog scientifique suivant, pour une perspective historique intéressante concernant cet article et les débuts de la recherche sur les relations entre biodiversité et fonctionnement de l'écosystème (<https://reflectionsonpaperspast.wordpress.com/2017/12/30/revisiting-naeem-et-al-1994/>)

(Hector et al. 1999) qui démarrèrent toutes deux en 1996, ainsi que l'expérience menée à Jena (Allemagne) qui démarra en 2002 (Weisser et al. 2017). Ces écosystèmes expérimentaux ont permis de démontrer un résultat central dans les relations B-EF : une augmentation de la production en biomasse avec la richesse spécifique (Tilman et al. 2014).

Cette augmentation de la production de biomasse, dans le cadre des relations B-EF, est déterminée en comparant le mélange par rapport à la moyenne des monocultures. Ainsi, on obtient un « **bonus de productivité** » (*overyielding*) lorsque la production de biomasse du mélange est supérieure à la moyenne des monocultures des espèces présentes dans le mélange (**Fig. 7**). C'est donc un bonus relatif à la moyenne, et non pas absolu par rapport à la monoculture la plus productive. On obtient un bonus absolu (*transgressive overyielding*) lorsque la production de biomasse du mélange est supérieure à la monoculture de l'espèce la plus productive (Fridley 2001; Schmid et al. 2008). Le résultat de l'effet du mélange, qu'il représente une meilleure ou moins bonne productivité par rapport à la moyenne du mélange, a aussi été appelé « **effet net de la biodiversité** » (*net biodiversity effect*).



**Fig. 7** Illustration des types de bonus de productivité (*overyielding*). Deux monocultures de différents avec différents niveaux de productivité moyen sont représentées, A et B. Si un mélange de A et B (à même densité totale) donne une productivité comprise entre celle de la monoculture la plus productive (ici, la monoculture A) et le rendement moyen des monocultures, il y a un bonus de productivité relatif (*non-transgressive overyielding*, souvent appelé simplement *overyielding*). Si la productivité du mélange est supérieure à celle de la monoculture la plus productive, il y a un bonus de productivité absolu (*transgressive overyielding*). Un malus de productivité (*underyielding*) est également possible. Extrait de Fridley (2001).

Ces résultats de l'effet de la biodiversité sur la productivité dans les écosystèmes de prairies ont généré de nombreux débats à la fin des années 1990 et au début des années 2000 pour savoir si ces effets étaient réellement causés par des effets de complémentarité de niche entre espèces, ou simplement liés à des effets d'échantillonnage des espèces (voir **section 5.1**) (Loreau and Hector 2001; Loreau 2010). Par la suite ces expériences ont également démontré que de nombreuses autres variables de fonctionnement de l'écosystème prairial bénéficiaient de l'augmentation de la diversité en espèces et en groupes fonctionnels (ex., herbacées, légumineuses) de plantes : la perte d'azote, le stockage de carbone, et la diminution des effets des pathogènes en sont quelques exemples (Weisser et al. 2017).

Progressivement, dans une volonté d'extrapolation de ces résultats à des systèmes naturels et à des espèces végétales pérennes, un pan de recherche sur les relations B-EF s'est développé autour des écosystèmes forestiers, autant pour les forêts naturellement régénérées que pour les forêts plantées (Scherer-Lorenzen et al. 2005; Kelty 2006). Au début des années 2000, les premières expériences ont été mises en place pour étudier l'importance de la diversité des espèces d'arbres sur le fonctionnement des écosystèmes forestiers. Cet essor de plantations expérimentales a donné naissance à TreeDivNet<sup>3</sup>, un réseau mondial d'expériences sur le mélange d'espèces d'arbres, qui rassemble aujourd'hui environ 25 expériences réparties sur tous les continents (Verheyen et al. 2016; Paquette et al. 2018). Dans le cadre de cette thèse, j'ai moi-même pu travailler dans l'un de ces dispositifs (plantation ORPHEE à Bordeaux, voir **section 5.3.2**) (Castagneyrol et al. 2013). De nombreux progrès dans la compréhension du fonctionnement des forêts mélangées ont été réalisés au cours des dix dernières années, grâce à la maturation de ces plantations expérimentales (Grossman et al. 2018; Huang et al. 2018), mais aussi grâce au développement de plateformes exploratoires dans des forêts matures (Baeten et al. 2013; Pretzsch et al. 2015; Jourdan et al. 2019b) et à l'utilisation de données d'inventaire forestier à différentes échelles (Paquette and Messier 2011; Toigo et al. 2015a; Liang et al. 2016). J'ai également pu bénéficier de l'un de ces réseaux d'observations de terrain dans le cadre de ma thèse (réseau GMAP dans les pré-Alpes françaises, voir **section 5.3.1**) (Jourdan et al. 2019b). Les résultats de ces études ont pu souligner l'importance fondamentale de la diversité en espèces d'arbres pour de nombreuses fonctions et services écosystémiques, de la production de bois à la résistance et à la résilience aux extrêmes climatiques, et à d'autres perturbations (Vilà et al. 2013; Brockerhoff et al. 2017; Pardos et al. 2021). Plus récemment, les questions de recherche se sont aussi tournées vers la compréhension des mécanismes d'interactions des espèces qui pourraient expliquer les relations B-EF, en particulier pour la relation entre diversité et productivité (**diversity-productivity relationship, DPR**) (voir les synthèses suivantes : Forrester 2014; Tilman et al. 2014; Forrester and Bauhus 2016; Ammer 2019; Trogisch et al. 2021) (voir **section 5.1**). Ces recherches ont permis de poser de nombreuses hypothèses concernant les mécanismes d'interactions derrière les relations B-EF mais les démonstrations expérimentales restent encore rares.

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<sup>3</sup> <https://treedivnet.ugent.be/index.html>

## 3.2. Relations B-EF dans les forêts mélangées

Dans cette partie, nous allons nous concentrer sur les relations B-EF qui ont un lien avec la productivité, le fonctionnement hydrique et carboné et la réponse à la sécheresse. J'ai évoqué en début d'introduction l'importance de la biodiversité pour la résistance aux perturbations et aux attaques biotiques ainsi que pour la qualité et la quantité des services écosystémiques fournis (Jactel et al. 2017; Brockerhoff et al. 2017). Ces aspects-là ne seront pas développés ici car j'ai fait le choix de me focaliser sur les variables de fonctionnement physiologique des arbres, qui sont celles qui seront au centre de mon travail de thèse.

### 3.2.1. Productivité

Comme évoqué précédemment, la variable de fonctionnement de l'écosystème qui a été indéniablement la plus étudiée dans les études sur les relations B-EF est la productivité, c'est-à-dire la production de biomasse (van der Plas 2019). Bien que la majorité des études sur les communautés végétales de prairies montrent un effet positif du mélange sur la croissance (Tilman et al. 2014), ce n'est pas forcément toujours le cas pour les écosystèmes forestiers. Une relation positive entre diversité et productivité a été démontrée pour les forêts à une échelle globale dans de nombreux types de peuplements et de climats (Paquette and Messier 2011; Morin et al. 2011; Zhang et al. 2012; Vilà et al. 2013; Liang et al. 2016; Jactel et al. 2018). Par exemple, dans une méta-analyse à l'échelle globale, Jactel et al. (2018) ont trouvé que la productivité des forêts mélangées était 15 % plus élevée que la moyenne attendue à partir des monocultures. Deux autres méta-analyses globales ont trouvé une augmentation de 24 % de la productivité dans les mélanges (Zhang et al. 2012; Vilà et al. 2013). Cependant, ces résultats, souvent à l'échelle globale, sont à mettre en perspective avec d'autres études, à échelle plus locale, qui ne trouvent pas toujours un effet positif du mélange sur la productivité du peuplement ou bien trouvent des effets contrastés entre les espèces étudiées (Pretzsch et al. 2013a; Toïgo et al. 2015a). De plus, lorsqu'il y a un effet, l'amplitude de l'effet de la diversité sur la productivité peut dépendre des conditions environnementales des sites (Toïgo et al. 2015a; Mina et al. 2018).

La relation diversité-productivité dans les peuplements forestiers n'est donc pas facile à généraliser, car elle dépend fortement de la composition en espèces d'arbres, de la diversité fonctionnelle des espèces concernées (et donc de l'identité des espèces), des conditions du site, du climat, du stade de développement des peuplements, de l'échelle spatiale considérée et, enfin, dans les forêts gérées, de l'intervention humaine qui affecte la densité et la composition des peuplements (Vilà et al. 2013; Forrester and Bauhus 2016; Ammer 2019).

En lien avec ces questions de productivité annuelle du peuplement, la question de l'effet du mélange sur la variabilité temporelle de la productivité a aussi été explorée. La variabilité temporelle de la productivité diminue avec l'augmentation de la richesse en espèces et de la diversité fonctionnelle (Jucker et al. 2014b; Morin et al. 2014; Jourdan et al. 2020). Grâce à des réponses asynchrones aux

conditions climatiques entre espèces, l'effet stabilisateur permet aux forêts mixtes de maintenir une productivité plus constante par rapport aux monocultures (Jucker et al. 2014b).

### 3.2.2. Fonctionnement hydrique et carboné

Afin d'améliorer la compréhension de la relation diversité-productivité, il semble essentiel de s'intéresser aux effets du mélange sur le fonctionnement hydrique et carboné des arbres. En effet, la productivité étant un résultat intégré de nombreux processus, ce point de vue écophysiological sur les relations B-EF nous permet d'avoir une compréhension plus fine des effets du mélange sur le fonctionnement des arbres. La transpiration et l'efficacité d'utilisation de l'eau sont deux fonctions de l'écosystème essentielles liées à la productivité du peuplement. De plus, la transpiration est l'un des principaux processus susceptibles d'influencer la disponibilité en eau du sol dans les mélanges par rapport aux monocultures, et donc la sensibilité des écosystèmes forestiers mixtes à la sécheresse (Forrester 2015).

Par rapport aux travaux portant sur la productivité, comparativement peu d'études se sont intéressées à la transpiration ou à l'efficacité de l'utilisation de l'eau et leur saisonnalité dans les mélanges et les monocultures (voir synthèse de Forrester 2015). Une utilisation plus importante de l'eau (mesurée par la transpiration) a été observée dans les peuplements mélangés par rapport aux monocultures, surtout s'il y avait des effets du mélange sur la croissance (Forrester et al. 2010; Kunert et al. 2012; Gebauer et al. 2012). A l'échelle du peuplement et de l'espèce, lorsqu'il n'y avait pas d'effets du mélange sur la croissance, il n'y avait généralement pas d'effets du mélange sur la transpiration ou l'efficacité d'utilisation de l'eau (Grossiord et al. 2013a; Forrester 2015). Cette plus forte utilisation de l'eau dans les mélanges pose la question de l'exposition à la sécheresse : elle pourrait rendre les mélanges plus sensibles à la sécheresse que les monocultures et réduire la disponibilité de l'eau du sol en conditions de sécheresse (Forrester 2015).

### 3.2.3. Réponse à la sécheresse

Malgré les nombreuses preuves de l'importance de la diversité des espèces pour de multiples fonctions forestières, le rôle de la diversité des espèces d'arbres sur la réponse des forêts à la sécheresse est largement absent des recherches plus anciennes. Depuis une dizaine d'années, l'atténuation potentielle des effets de la sécheresse grâce à la diversité en essences a suscité un intérêt grandissant dans le contexte de l'augmentation de la fréquence et de la sévérité des sécheresses. Ammer (2019) et Grossiord (2019) proposent des synthèses bibliographiques sur l'effet de la diversité sur la réponse à la sécheresse. Ils concluent tous deux sur l'absence d'un consensus sur les effets attendus. Selon le type de peuplement et de climat, la diversité peut avoir un effet positif (Lebourgeois et al. 2013), négatif ou neutre (Grossiord et al. 2014d; Bonal et al. 2017) sur la résistance à la sécheresse du peuplement. Pardos et al. (2021) ont rassemblé les résultats de 30 études à travers le continent européen. Ils montrent que, en moyenne, les

peuplements mixtes sont plus résistants et résilients face aux événements de sécheresse que les peuplements monospécifiques. Cependant, l'effet bénéfique des mélanges ne peut pas être généralisé, et est fortement modulé par le type de mélange et l'identité des espèces d'arbres, et dépendant de la disponibilité en eau du site et des caractéristiques du peuplement, telles que la surface terrière et l'âge. Ils notent tout de même que l'augmentation de la résilience dans les mélanges par rapport aux monocultures est plus importante dans les mélanges conifères-feuillus, que dans les mélanges feuillus-feuillus, soulignant l'importance des caractéristiques fonctionnelles des espèces.

Parmi les études rassemblées dans Grossiord (2019), environ la moitié ont étudié la réponse à la sécheresse via des variations de croissance annuelle des arbres. L'autre moitié a utilisé des variables traduisant plus directement le fonctionnement hydrique, telles la transpiration et l'efficacité d'utilisation de l'eau. Ces variables permettent une détection plus directe et fonctionnelle des conséquences de l'impact de la sécheresse par rapport à la croissance, qui est une variable intégrative de très nombreux processus (Jucker et al. 2017).

Comme évoqué précédemment, les effets du mélange d'essences sur le fonctionnement hydrique et la réponse à la sécheresse sont difficiles à prédire du fait de l'interaction de nombreux facteurs. D'un côté, une complémentarité dans l'utilisation de la ressource en eau entre espèces pourrait atténuer l'exposition à la sécheresse des arbres (Grossiord 2019) ; d'un autre côté, si le mélange est plus productif et a une plus grande consommation en eau que les monocultures, cela pourrait mener à un épuisement plus rapide de la ressource en eau (Forrester 2015). Le chapitre suivant a pour objectif de détailler ces différents mécanismes.

## 4. Les mécanismes à l'origine des relations B-EF

### 4.1. Mécanismes d'interaction des espèces végétales dans les relations B-EF

#### 4.1.1. Les types d'interactions des espèces végétales dans le cadre des forêts mélangées

Afin de comprendre les relations entre biodiversité et fonctionnement des écosystèmes abordées dans la partie précédente, il faut s'intéresser aux mécanismes d'interactions des espèces végétales de manière générale. Il existe différents types d'interactions, positives ou négatives, qui peuvent avoir des effets opposés sur le fonctionnement des arbres, et de l'écosystème. Pour les décrire, nous faisons appel à la notion de **niche écologique** d'une espèce (voir **Encadré 4**).

Les interactions **négatives** sont la conséquence de la **compétition** (*competition*) entre espèces pour les ressources (eau, lumière, nutriments). Elle a lieu lorsque des individus d'espèces différentes occupent des niches écologiques proches. Ils utilisent alors les mêmes ressources, ce qui peut avoir un effet négatif



sur l'acquisition de ces ressources et sur la croissance de l'espèce. Cependant, en monoculture, il y a également une forte compétition intraspécifique, puisque les arbres en interaction utilisent à priori les mêmes ressources. Par conséquent, en peuplement mélangé et à densité égale, la compétition entre individus peut être accentuée ou réduite selon les caractéristiques de l'autre espèce (cf. paragraphe suivant). Des interactions négatives peuvent aussi venir d'interférences physiques ou chimiques d'une espèce sur l'autre (Loreau and Hector 2001).

Il a été défini deux types d'interactions **positives** : la réduction de compétition et la facilitation (Forrester 2014). La **réduction de compétition** (*competitive reduction*) correspond à une **complémentarité de niche** qui résulte en un **partitionnement des ressources** entre les deux espèces (voir **Encadré 4**) : alors qu'elles co-existent, soit elles utilisent des ressources différentes, soit elles utilisent les mêmes ressources mais de manière différente dans l'espace ou dans le temps. La compétition pour les ressources ayant aussi lieu entre les individus d'une même espèce, la présence de plusieurs espèces réduit la compétition intra-spécifique. La présence de plusieurs espèces peut alors avoir un effet positif sur le fonctionnement de l'écosystème (exemples : profondeurs d'enracinement différentes, stratification des houppiers dans la canopée...). À noter que les termes « partitionnement des ressources », « différenciation de niche », « complémentarité de niche », et « réduction de compétition » sont utilisés de manière plus ou moins synonymes dans la littérature sur les forêts mélangées (Loreau and Hector 2001; Forrester and Bauhus 2016; Grossiord 2019). La **facilitation** (*facilitation*), elle, se traduit par un effet positif d'une espèce sur une autre, lui permettant par exemple d'avoir accès à des ressources qui ne lui étaient pas disponibles (Bertness and Callaway 1994) (exemples : espèce fixatrice d'azote, redistribution hydraulique par les racines...). Du fait que les effets de réduction de compétition et de facilitation sont difficiles à séparer ils sont souvent regroupés ensemble sous le terme de **processus de complémentarité** (*complementarity processes*) dans la recherche sur les relations B-EF (Loreau and Hector 2001; Grossiord 2019).

#### 4.1.2. Effet d'échantillonnage et effet de complémentarité

Afin de comprendre les mécanismes à l'origine des effets de la diversité, Loreau et Hector (2001) ont initialement mis l'accent sur la séparation statistique de l'**effet net de la biodiversité** (*net biodiversity effect, NBE*) entre des **effets d'échantillonnage** (*selection effect, sampling effect*) et de **complémentarité** entre espèces (*complementarity effect*) (Loreau and Hector 2001; Tilman et al. 2014) (**Fig. 8**). Prenons par exemple le cas de la relation positive entre la diversité (nombre d'espèces) et la productivité observée dans les systèmes herbacés ou les forêts. L'**effet d'échantillonnage** correspond au fait que lorsque le nombre d'espèces augmente, la probabilité d'inclure une espèce productive augmente également. L'effet d'échantillonnage n'implique donc pas d'interactions entre les espèces. L'**effet de complémentarité** correspond à une interaction positive entre espèces, qui est donc liée soit à une complémentarité de niche qui permet une réduction de la compétition, soit à de la facilitation.

Ainsi, dans la littérature sur les relations B-EF, le terme de complémentarité est souvent utilisé pour englober ces deux types d'interactions positives définies plus haut (Loreau and Hector 2001; Forrester 2014). Les effets liés à l'échantillonnage et à la complémentarité ne s'excluent pas mutuellement et peuvent tous deux affecter le fonctionnement de l'écosystème. Dans une revue sur ces relations, Cardinale et al. (2012) ont démontré que les deux mécanismes contribuaient à environ 50 % des relations diversité-productivité.

Define, for any mixture,  
 $M_i$  = yield of species  $i$  in monoculture  
 $Y_{O_j}$  = observed yield of species  $i$  in the mixture  
 $Y_O = \sum_i Y_{O_j}$  = total observed yield of the mixture  
 $RY_{E_j}$  = expected relative yield of species  $i$  in the mixture, which is simply its proportion seeded or planted  
 $RY_{O_j} = Y_{O_j}/M_i$  = observed relative yield of species  $i$  in the mixture  
 $Y_{E_j} = RY_{E_j}M_i$  = expected yield of species  $i$  in the mixture  
 $Y_E = \sum_i Y_{E_j}$  = total expected yield of the mixture  
 $\Delta Y = Y_O - Y_E$  = deviation from total expected yield in the mixture  
 $\Delta RY_i = RY_{O_j} - RY_{E_j}$  = deviation from expected relative yield of species  $i$  in the mixture  
 $N$  = number of species in the mixture

It then follows that

$$\begin{aligned}\Delta Y &= Y_O - Y_E = \sum_i RY_{O_j}M_i - \sum_i RY_{E_j}M_i = \sum_i \Delta RY_i M_i \\ &= N \overline{\Delta RY} \overline{M} + N \text{cov}(\Delta RY, M)\end{aligned}$$

In this equation,  $N \overline{\Delta RY} \overline{M}$  measures the complementarity effect, and  $N \text{cov}(\Delta RY, M)$  measures the selection effect.

**Fig. 8** Détails du calcul de l'effet net de la biodiversité sur la productivité ( $\Delta Y$ ), définie dans ce cas-là à partir du rendement (*yield*), et de la séparation statistique entre effet de complémentarité et effet d'échantillonnage selon la méthode de Loreau et Hector (2001). Extrait de Loreau et Hector (2001).

Dans la littérature sur les forêts mélangées, des notions similaires sont parfois abordées sous d'autres termes, évoqués ici en anglais (Pretzsch 2014; Jourdan et al. 2019b). Pour l'effet d'échantillonnage on trouve les termes de : *selection effect* et *additive effect*. Pour l'effet de complémentarité, on trouve les termes de : *true mixing effect*, *non-additive effect*, *multiplicative effect*, *interaction effect*. Même si ces définitions ne se rapportent pas à un calcul de séparation statistique entre effets d'échantillonnage et complémentarité (Loreau and Hector 2001), elles sont similaires dans le sens où elles se rapportent au fait qu'une partie des effets du mélange est due aux traits intrinsèques des espèces, sans modification des valeurs de ces traits entre peuplement pur et peuplement mélangé, tandis qu'une autre partie des effets est due à une plasticité des traits des espèces avec une modification de ces traits entre les différents

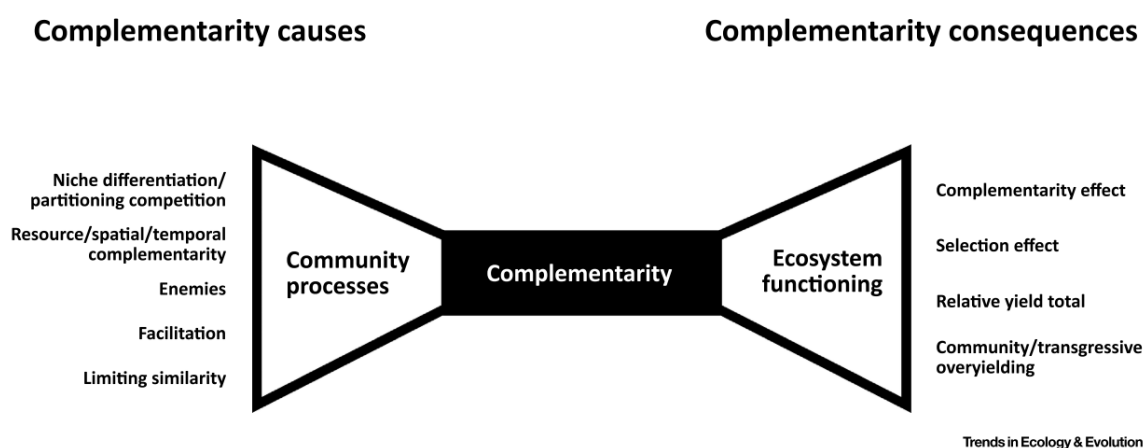
niveaux de diversité en espèces. De plus, Forrester (2014) propose aussi un calcul de « complémentarité » qui est différent de celui de Loreau et Hector (2001) :

$$\text{Complémentarité (\%)} = \left( \frac{\text{Croissance}_{\text{mélange}}}{\text{Croissance}_{\text{monoculture}} \times \text{Proportion de l'espèce}_{\text{mélange}}} - 1 \right) \times 100$$

Cette diversité dans la sémantique et dans les types de statistiques utilisées rend difficile la comparaison des résultats de la littérature.

#### 4.1.3. Conflits entre les différentes définitions du terme de complémentarité

Barry et al. (2019) ont proposé une synthèse qui rassemble les différentes utilisations du terme de complémentarité. Ils proposent l'idée que ce terme a été utilisé d'une part comme une cause et d'autre part comme une conséquence des relations B-EF (**Fig. 9**) (Barry et al. 2019). Pour éviter la confusion entre le calcul statistique de « l'effet de complémentarité » et l'observation d'un « processus de complémentarité », Ammer (2019) a même proposé d'utiliser pour ce dernier le terme de « [relaxation](#) » dans le sens de la détente du niveau de compétition.



**Fig. 9** L'intérieur de la boîte noire de la complémentarité, extrait de Barry et al. (2019). Les auteurs ont constaté que dans la littérature B-EF, les utilisations du terme de complémentarité se répartissent généralement en deux catégories : comme des causes (à gauche) et des conséquences (à droite) du couplage entre la diversité et le fonctionnement des écosystèmes en lien avec la complémentarité.

Dans le cadre de ma thèse, comme je m'intéresse plus particulièrement aux mécanismes d'utilisation des ressources en eau et en lumière, j'ai choisi d'utiliser le terme de « complémentarité » comme une cause explicative des relations B-EF. De plus, puisque dans mon travail de thèse je ne m'intéresse qu'à des mélanges de deux espèces, la séparation statistique des effets d'échantillonnage et de complémentarité ne présente qu'un intérêt limité.

## 4.2. Les mécanismes d'interactions dans le cadre de l'acquisition des ressources

Les effets du mélange sur la productivité en forêt peuvent être expliqués par des mécanismes liés à l'acquisition des ressources (lumière, eau, nutriments) (Forrester and Bauhus 2016; Pretzsch et al. 2017). Comme évoqué précédemment, le partitionnement des ressources peut se produire lorsque la dissimilarité des traits fonctionnels des espèces réduit la compétition pour les ressources dans l'espace et/ou dans le temps. Dans le cadre de ma thèse je m'intéresse plus particulièrement au partitionnement des ressources en eau et en lumière ; j'ai donc choisi ici de détailler les résultats existants concernant ces deux ressources seulement. Cependant, il est important de noter que les interactions pour l'acquisition des nutriments est également très importante pour expliquer ces effets (Richards et al. 2010; Maxwell et al. 2020; de Stree et al. 2021). De façon très synthétique, sur la base de ces travaux, les mécanismes expliquant une complémentarité liée aux nutriments peuvent être : une amélioration de la disponibilité des nutriments, de la minéralisation des nutriments, de la vitesse du cycle des nutriments, ou de l'acquisition des nutriments par les arbres (Richards et al. 2010; Forrester and Bauhus 2016).

### 4.2.1. Interception de la lumière

L'interception de la lumière peut être plus importante dans les peuplements mélangés que dans les monocultures (Forrester and Albrecht 2014; Pretzsch 2014; Sapijanskas et al. 2014). Par exemple, dans une étude incluant 21 triplets de peuplements purs et mixtes de pin sylvestre (*Pinus sylvestris*) et de hêtre (*Fagus sylvatica*, L.) à travers l'Europe, la proportion de rayonnement photosynthétiquement actif interceptée par le couvert (fiPAR) était en moyenne 14 % plus importante dans les peuplements mélangés que dans les monocultures des deux espèces (Forrester et al. 2017).

Cette augmentation peut être expliquée par des mécanismes de complémentarité spatiale et/ou temporelles. La complémentarité spatiale peut s'observer par une meilleure utilisation de l'espace de la canopée (Jucker et al. 2015; Williams et al. 2017), liée à : (i) une stratification verticale des houppiers entre espèces (Pretzsch et al. 2017) ou (ii) une plasticité de la forme des houppiers (Ishii et al. 2013; Jucker et al. 2015; Barbeito et al. 2017). La complémentarité temporelle de l'interception de la lumière peut être liée à des différences de phénologie des espèces dans leur cycle annuel (Sapijanskas et al. 2014).

Un nombre important de travaux de recherche se sont intéressés à l'interception de la lumière dans les peuplements mélangés ces cinq dernières années, et en particulier sur la complémentarité spatiale des houppiers. Il a été démontré que les peuplements mélangés utilisent l'espace de la canopée de manière plus efficace que les monocultures dans les forêts tempérées (Pretzsch 2014; Jucker et al. 2015; Williams et al. 2017) ainsi que dans les forêts tropicales (Sapjanskas et al. 2014). Cette meilleure occupation de l'espace de la canopée en mélange a été qualifiée de « *canopy packing* » (Jucker et al. 2015). Ce *canopy packing* a été identifié comme un mécanisme potentiellement majeur pour expliquer les effets du mélange sur la productivité forestière (Morin et al. 2011; Pretzsch 2014; Sapjanskas et al. 2014; Williams et al. 2017), via l'augmentation de l'interception de la lumière à l'échelle du peuplement (Rissanen et al. 2019; Williams et al. 2021; Duarte et al. 2021).

Par ailleurs, une meilleure interception de la lumière, en plus d'être un mécanisme explicatif d'une meilleure productivité du peuplement, peut aussi être une conséquence de cette productivité. En effet, une meilleure croissance implique aussi un développement des houppiers plus important (Sapjanskas et al. 2014; Pretzsch et al. 2017; Ammer 2019).

#### *Le cas particulier de la réponse à la sécheresse*

Enfin, ces modifications d'interception de la lumière en peuplements mélangés peuvent aussi entraîner des conséquences sur la réponse des espèces à la sécheresse (Grossiord 2019). En effet, du fait des complémentarités spatiales entre espèces, la structure de la canopée est plus complexe en mélange et les conditions microclimatiques (température et humidité) sont modifiées (Kovács et al. 2017). L'ombrage plus ou moins important des houppiers ou les conditions microclimatiques peuvent alors modifier l'exposition à la sécheresse ou aux fortes températures d'une espèce en mélange par rapport aux monocultures. Ces hypothèses ont été proposées pour expliquer des effets du mélange observés sur la transpiration ou la résistance à la sécheresse (Jonard et al. 2011; Forrester 2015; Fichtner et al. 2020a).

#### 4.2.2. Utilisation de l'eau

Le partage des ressources en eau entre espèces a souvent été discuté comme un effet possible pour expliquer l'augmentation de la productivité dans divers mélanges (Forrester 2015; Ammer 2019). En particulier, une différence de profondeur d'extraction de l'eau par les racines entre espèces a été identifiée comme un mécanisme potentiel de partage des ressources (Schume et al. 2004; Grossiord et al. 2014a), mais a encore très peu été démontrée (Schwendenmann et al. 2015). En effet, les patrons d'enracinement des espèces pourraient avoir des caractéristiques complémentaires qui permettraient une plus grande absorption d'eau dans le même volume de sol dans les peuplements mixtes, ou l'exploration d'un plus grand volume de sol par rapport aux monocultures. Par conséquent, il a également été suggéré que ce partage des ressources souterraines pourrait contribuer à une moindre vulnérabilité des arbres à la sécheresse du sol dans les forêts mixtes (Grossiord 2019). Mais cette hypothèse est controversée car une utilisation plus importante et plus complète de l'eau du sol pourrait également conduire à un

épuisement plus rapide des ressources du sol et donc à une plus grande exposition des arbres à la sécheresse (Forrester 2015; Ammer 2019).

La présence ou l'absence de stratification verticale des racines dans les peuplements mélangés dépend de plusieurs facteurs. Premièrement, deux espèces peuvent avoir des profondeurs d'enracinement différentes dans les monocultures, ce qui entraînerait une réduction de la compétition intraspécifique dans les peuplements mélangés (c'est-à-dire une complémentarité de niche) (Grossiord et al. 2014a; Schwendenmann et al. 2015). Deuxièmement, un partage des ressources en eau dans les peuplements mélangés pourrait également se produire si les deux espèces ont des profondeurs d'enracinement similaires dans les monocultures, mais que la compétition interspécifique dans les peuplements mélangés entraîne une différence intraspécifique de la profondeur racinaire pour l'une ou les deux espèces (c'est-à-dire une différenciation de niche) (Bolte and Villanueva 2006). Dans les deux cas, le résultat dans les peuplements mélangés serait une stratification verticale des racines entre les espèces permettant une plus grande absorption d'eau au niveau du peuplement par rapport aux monocultures.

Plusieurs études se sont intéressées à la distribution verticale des racines dans les forêts mélangées en examinant soit la biomasse des racines fines, soit la composition isotopique de l'eau extraite par l'arbre pour estimer sa profondeur d'origine. Des résultats contrastés ont été trouvés : une complémentarité pour l'absorption de l'eau entre espèces dans les peuplements mélangés a été observée dans certaines études (Schmid and Kazda 2001; Bolte and Villanueva 2006; Grossiord et al. 2014a; Schwendenmann et al. 2015; Goisser et al. 2016; Bello et al. 2019) confirmant une contribution potentielle de ce mécanisme aux relations B-EF. En revanche, la ségrégation verticale des racines était absente dans d'autres cas (Meinen et al. 2009; Fruleux et al. 2018; Altinalmazis-Kondylis et al. 2020).

La stratification verticale de l'absorption de l'eau, lorsqu'elle était présente, était liée soit à une complémentarité de niche (Grossiord et al. 2014a; Schwendenmann et al. 2015), soit à une différenciation de niche (Goisser et al. 2016; Bello et al. 2019). Lorsqu'aucune différence n'a été trouvée, cela a été expliqué par une similarité fonctionnelle entre les espèces étudiées (Fruleux et al. 2018). En effet, les différences les plus fortes ont souvent été observées lorsque les espèces de conifères et de feuillus étaient mélangées (Bolte and Villanueva 2006; Grossiord et al. 2014a; Goisser et al. 2016).

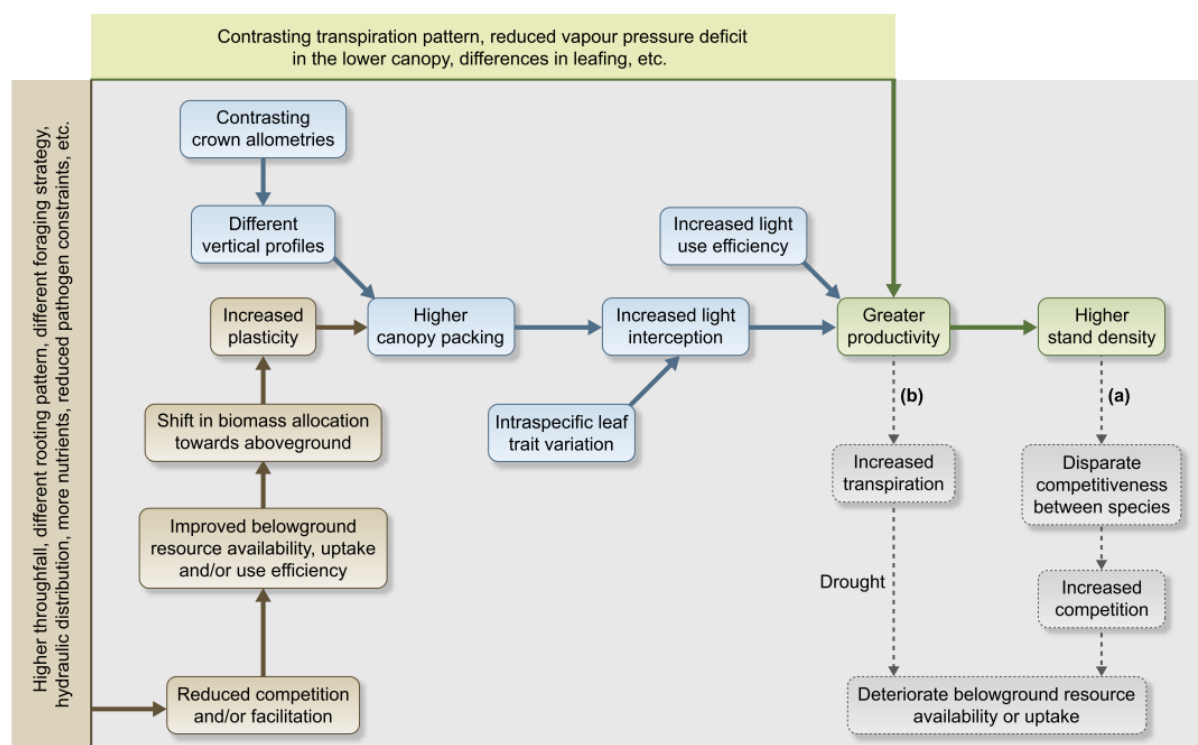
#### *Le cas particulier de la réponse à la sécheresse*

Dans le cas de la réponse à la sécheresse, la complémentarité entre espèces pour l'acquisition de l'eau pourrait aussi augmenter le risque d'exposition à la sécheresse (Pretzsch et al. 2017). En effet, d'un côté, une stratification des profondeurs racinaires des espèces peut entraîner un partage des ressources qui améliorerait l'état hydrique de chaque espèce en réduisant la compétition pour l'eau entre les espèces (par exemple, Schwendenmann et al. 2015). Il en résulterait une moindre exposition à la diminution de la teneur en eau du sol dans les peuplements mélangés lors d'événements de sécheresse (Forrester 2014). En revanche, le fait d'aller chercher l'eau à des profondeurs différentes pourrait aussi mener à un

épuisement plus rapide du stock (Schume et al. 2004) qui pourrait mener à plus grande exposition à la sécheresse pour toutes les espèces des peuplements mixtes (Forrester 2015). Ces deux situations peuvent être rencontrées conjointement ou non dans les écosystèmes forestiers et rendent difficiles la compréhension précise des mécanismes fins à l'origine des effets dans les peuplements mélangés.

#### 4.2.3. Interactions entre les différents mécanismes

Les différents mécanismes de complémentarité pour l'acquisition des ressources ne sont pas indépendants. En effet, dans le cadre de la relation diversité-productivité par exemple, tous ces mécanismes peuvent interagir entre eux, et c'est le résultat de ces interactions qui va donner les effets observés sur la croissance des arbres et leur productivité. Dans la synthèse de Ammer (2019), un schéma conceptuel a été proposé afin de visualiser comment les mécanismes de complémentarité peuvent interagir entre eux et promouvoir un effet positif de la diversité sur la productivité du peuplement, mais aussi éventuellement augmenter l'exposition à la sécheresse (**Fig. 10**). Dans ce schéma, Ammer (2019) fait par exemple l'hypothèse qu'un *canopy packing* plus important est la conséquence de complémentarités aériennes, mais aussi d'une plus grande disponibilité des ressources en eau à l'échelle du peuplement. La comparaison des effets du mélange entre les compartiments du mélange est un aspect important pour la suite des recherches dans le domaine des relations B-EF. Martin-Guay et al. (2020) ont notamment démontré que les effets du mélange sur la productivité aérienne ne se retrouvaient pas nécessairement sur la productivité du compartiment souterrain, du fait que les espèces dominantes étaient celles qui avaient une plus faible allocation au compartiment souterrain. Cependant, l'importance relative des mécanismes aériens et souterrains n'a pas encore pu être évaluée directement au sein d'un même dispositif (mais voir Jing et al. 2021 pour une estimation indirecte).



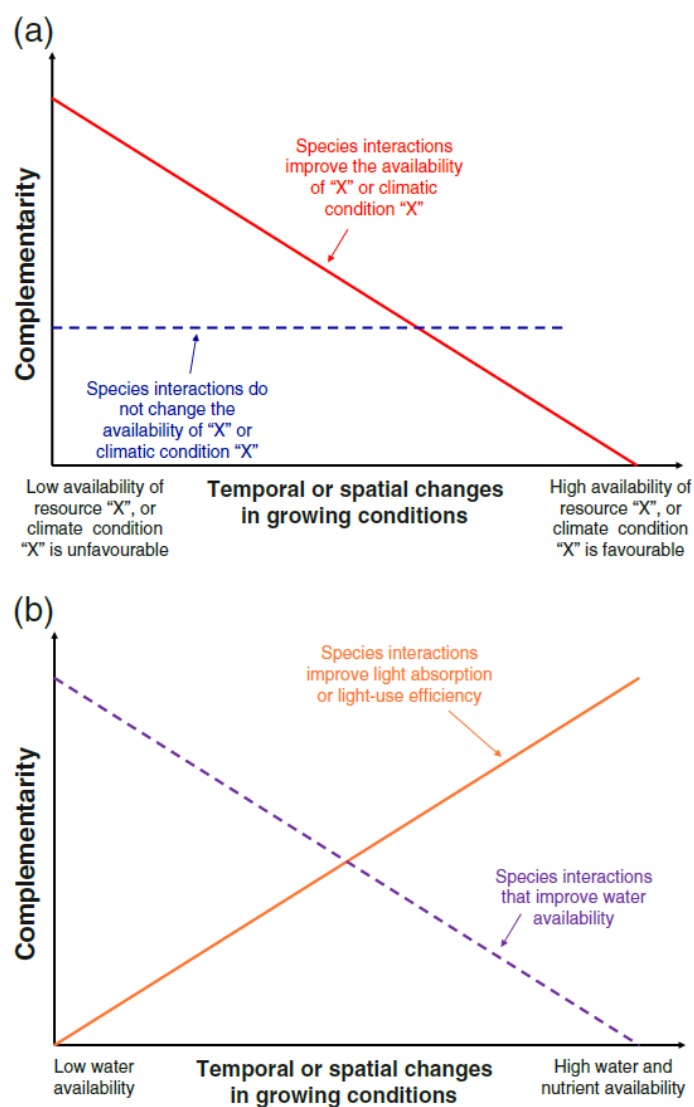
**Fig. 10** Diagramme conceptuel illustrant comment la complémentarité souterraine et aérienne peut favoriser les effets positifs de la diversité des espèces d'arbres sur la productivité du peuplement. Les facteurs influençant les processus souterrains sont énumérés du côté gauche ; les facteurs favorisant un gain photosynthétique plus élevé sont énumérés au-dessus de la ligne horizontale. Les flèches pointillées représentent les éventuelles réactions négatives qui pourraient résulter du changement climatique. Extrait de Ammer (2019).



### 4.3. Variabilité des mécanismes d'interaction en fonction des conditions environnementales

Les conditions environnementales peuvent également influencer la présence et l'importance des effets du mélange sur la productivité ou l'exposition à la sécheresse (Forrester 2014; Forrester and Bauhus 2016). En effet, les mécanismes de complémentarité à l'origine de ces effets jouent un rôle plus ou moins important en fonction des conditions environnementales. Si la ressource concernée par un mécanisme de complémentarité n'est pas limitante, alors les effets possibles du mélange sont limités. Forrester et Bauhus (2016) ont proposé un cadre théorique, qui généralise l'hypothèse du gradient de stress (« *stress gradient hypothesis* », SGH) (Bertness and Callaway 1994; Maestre et al. 2009). La SGH suggère que lorsque les conditions environnementales deviennent plus stressantes, la facilitation augmente et la compétition diminue. Cependant, le concept de la SGH ne peut s'appliquer aux forêts mélangées que dans un cadre théorique plus large, car les postulats initiaux du SGH impliquent uniquement des relations de facilitation (Forrester and Bauhus 2016). Je n'utiliserai donc pas le terme de SGH dans le cadre de ma thèse, mais je me baserai sur le cadre théorique évoqué dans Forrester et Bauhus (2016). Ce dernier suppose une relation négative entre les effets de complémentarité en peuplements mélangés et la disponibilité d'une ressource donnée (**Fig. 11a**). Par exemple le long d'un gradient de précipitations, les effets de complémentarité sont plus importants lorsque les ressources en eau sont limitantes.

Outre les variations spatiales, les effets du mélange peuvent également évoluer dans le temps sur un site donné en raison des variations climatiques interannuelles et/ou du développement du peuplement (Forrester 2014). Par exemple, aux premiers stades du développement du peuplement, la concurrence pour les ressources augmente, ce qui peut également accroître les effets de complémentarité potentiels dans les mélanges (Huang et al. 2018). Les variations temporelles des effets des mélanges peuvent également provenir de changements dans l'équilibre entre les processus de complémentarité et de compétition sur un site donné. Par exemple, une croissance plus élevée due à la complémentarité dans des conditions hydriques non limitantes peut conduire à une concurrence accrue pendant les années de sécheresse en raison d'une utilisation d'eau plus importante dans les peuplements mixtes (Jacobs et al. 2021). Il est donc important, lorsqu'on étudie un assemblage d'espèces spécifique, de l'évaluer dans différentes conditions climatiques et de le suivre au long de différents stades de développement.

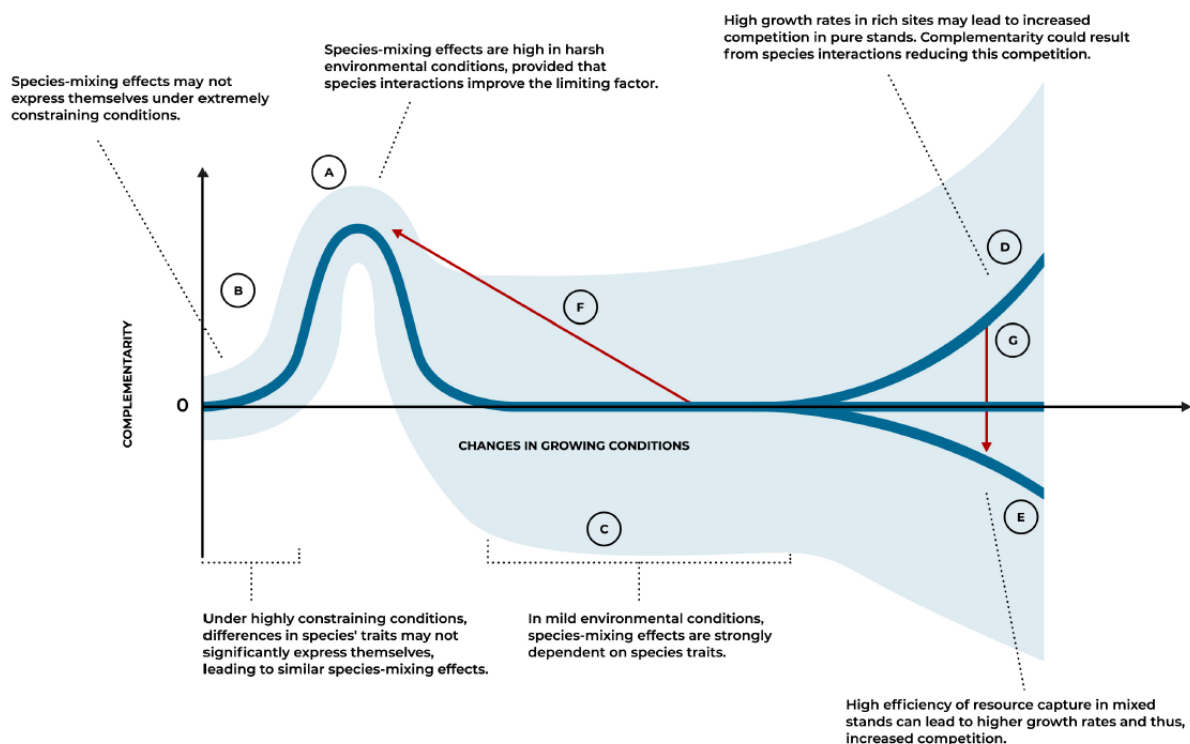


**Fig. 11** (a) Schéma conceptuel montrant l'évolution possible des effets du mélange pour deux espèces d'arbres mélangées en fonction de modifications spatiales ou temporelles des conditions de croissance. Les lignes représentent une situation où les interactions des espèces améliorent (rouge) ou n'améliorent pas (bleu) la condition de croissance limitante. La partie (b) montre comment différents types d'interactions peuvent conduire à des patrons de complémentarité contrastés le long d'un même gradient (ici, la disponibilité en eau). La ligne pleine représente une communauté pour laquelle le mélange améliore l'interception de la lumière ou l'efficacité d'utilisation de la lumière. Dans ce cas, la complémentarité augmente avec l'amélioration des conditions de disponibilité en eau car celles-ci entraînent une croissance élevée et une plus grande surface foliaire qui ont pour conséquence une plus forte compétition pour la lumière. Extrait de Forrester et Bauhus (2016).

Différents gradients de ressources ont été étudiés pour les forêts mélangées: la disponibilité en azote ou la fertilité des sites (Toïgo et al. 2015a), les conditions climatiques (Vilà et al. 2013), la disponibilité en eau (Grossiord et al. 2014d) qui vont dans le sens de ce cadre théorique. Concernant les effets de l'augmentation de l'interception de la lumière, ils varient le long des gradients climatiques de manière inverse à celle supposée pour les autres ressources en eau ou en nutriments (**Fig. 11b**). Forrester et Albrecht (2014) ont observé que l'effet de la complémentarité pour l'interception de la lumière augmentait lorsque la productivité des sites s'améliorait dans des mélanges de *Abies alba* et *Picea abies*, ce qui peut s'expliquer par le fait que dans les sites productifs c'est en fait la ressource en lumière qui devient limitante. La part relative des différents mécanismes d'acquisition des ressources liés à l'eau ou la lumière peut donc varier selon le niveau de stress, dans l'espace et dans le temps. Peu d'études ont étudié simultanément ces deux mécanismes, et il est donc essentiel de réaliser des études qui regardent les effets de complémentarité pour l'acquisition de différentes ressources dans les mêmes dispositifs expérimentaux.

Dans le cadre des effets du mélange sur la résistance à la sécheresse, le cadre théorique de Forrester et Bauhus (2016) pourrait expliquer en partie la grande variabilité des effets observés dans la littérature. Ainsi, le mélange serait plus résistant à la sécheresse que les monocultures dans les sites où l'eau est un facteur limitant (Lebourgeois et al. 2013; Forrester 2014; Grossiord et al. 2014d). Par exemple, Grossiord et al. (2014c, d) ont trouvé des effets négatifs ou neutres du mélange sur l'exposition à la sécheresse dans les sites plus humides et des effets positifs dans les sites plus secs le long d'un gradient européen de placettes forestières.

Cependant, cette relation entre les effets du mélange et un gradient de ressources pourrait ne pas être linéaire et pourrait plutôt dépendre de seuils de disponibilité des ressources (de Streel et al. 2020). Les travaux de thèse de Géraud de Streel, qui a travaillé sur un gradient européen de forêts de hêtre et pin sylvestre, montrent en effet que les effets du mélange sur l'exposition à la sécheresse peuvent être neutres dans des conditions humides, positifs dans des conditions modérément sèches et revenir à des effets neutres au-delà d'un certain seuil, dans des conditions très sèches (de Streel 2021). Géraud de Streel a représenté ces idées sous la forme d'un schéma dérivé de celui de Forrester et Bauhus (2016) dans la discussion de sa thèse (Fig. 5.2 dans de Streel 2021, p.145) (**Fig. 12**). Un tel seuil pourrait s'expliquer par le fait que les effets positifs du mélange ne sont pas assez forts pour compenser les contraintes environnementales croissantes dans les sites extrêmement secs. En effet, lors d'un fort stress hydrique, les bénéfices de la complémentarité entre espèces pour l'extraction de l'eau ne peuvent plus se manifester si l'eau n'est plus disponible.



**Fig. 12** Schéma conceptuel montrant l'évolution possible des effets du mélange pour deux espèces d'arbres mélangées le long de gradients spatiaux ou temporels de conditions de croissance. Les espèces d'arbres associées sont supposées avoir des caractéristiques qui font que leurs interactions influencent les mécanismes associés au facteur limitant. Les lignes bleues montrent l'évolution attendue des effets du mélange le long du gradient tandis que la zone ombrée indique l'incertitude autour des effets du mélange en fonction des traits fonctionnels spécifiques aux deux espèces. Les flèches rouges indiquent comment les variations temporelles des conditions de croissance, telles que les épisodes de sécheresse, peuvent influencer les résultats des interactions entre espèces. Extrait du document de la discussion de thèse de Géraud de Streel, Fig. 5.2, p.145 (de Streel 2021).

## 5. Objectifs et méthodologies

### 5.1. Objectifs

Au cours de cette introduction, nous avons pu voir que la diversité des espèces d'arbres, via leurs interactions, peut favoriser un grand nombre de fonctions et de services des écosystèmes forestiers. Cependant, les impacts des interactions des espèces sur l'utilisation de l'eau et sur l'interception de la lumière restent encore mal connus dans ces écosystèmes et ont souvent été étudiés séparément. De plus, dans le contexte actuel de changement climatique, l'influence de ces interactions sur la réponse des écosystèmes forestiers à des événements climatiques extrêmes tels que la sécheresse est encore en débat. Enfin, les mécanismes d'interaction aériens et souterrains sous-jacents pouvant expliquer ces relations entre diversité et fonctionnement de l'écosystème forestier restent encore à déterminer.

L'objectif de ce travail a donc été premièrement de déterminer l'impact des interactions entre espèces d'arbres en peuplements mélangés sur des variables de fonctionnement des arbres en lien avec l'utilisation de l'eau et la réponse à la sécheresse. Deuxièmement, j'ai cherché à mettre en évidence les mécanismes d'interaction souterrains ou aériens pouvant expliquer ces effets sur le fonctionnement, tels que la profondeur d'extraction de l'eau ou l'occupation de l'espace de la canopée. Ces différentes mesures ont été réalisées dans des conditions contrastées de disponibilité en eau du sol afin de mettre en évidence la variation des effets et des mécanismes en fonction du stress hydrique.

Afin de mieux comprendre l'influence des interactions entre espèces sur le fonctionnement hydrique et les mécanismes aériens et souterrains sous-jacents, ce travail de thèse avait donc pour objectif de répondre aux questions suivantes :

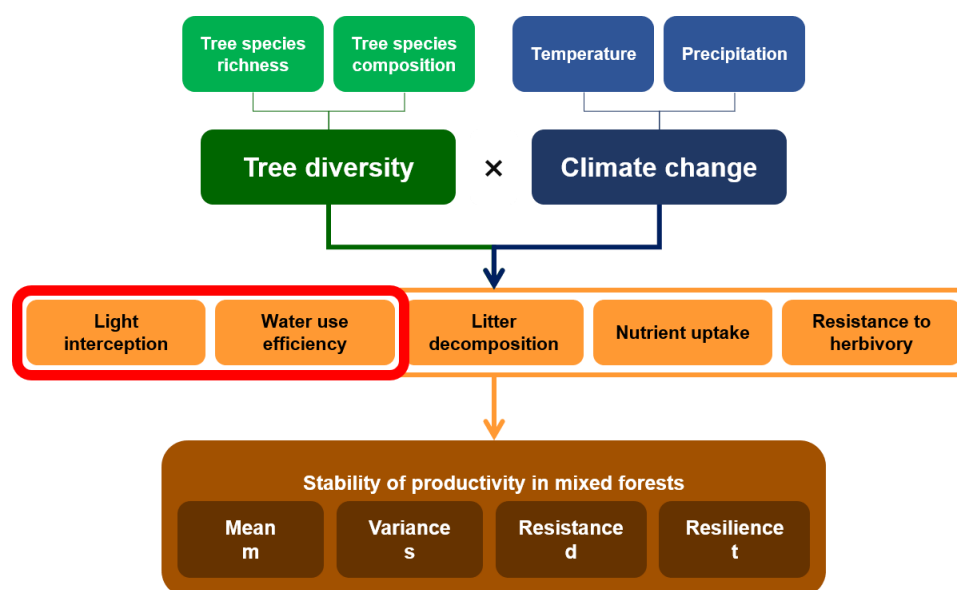
1. Quels sont les effets du mélange d'espèces sur le fonctionnement hydrique des arbres, notamment en condition de sécheresse ?
2. Quels sont les effets du mélange d'espèces d'arbres sur le partage des ressources en eau et en lumière entre espèces ?
3. Est-ce que les mécanismes de partage des ressources en eau et en lumière peuvent expliquer les effets du mélange sur le fonctionnement hydrique ?

Mes travaux de thèse se sont concentrés sur des espèces de forêts tempérées françaises. Mes recherches se sont focalisées sur deux dispositifs expérimentaux : un dispositif d'observation de terrain en forêts naturelles gérées dans le Sud-Est de la France (**GMAP**, mélanges hêtre/sapin et hêtre/chêne pubescent) et une plantation expérimentale proche de Bordeaux (**ORPHEE**, mélange bouleau/pin maritime). Dans chaque dispositif le design expérimental se base sur un système de triplets : une placette avec un mélange de deux espèces et deux placettes pures avec une seule espèce. Nous avons choisi de comparer des peuplements purs et des peuplements mélangés à deux espèces. Cela m'a permis d'observer les effets

du mélange sur différents couples d'espèces qui ont une importance économique dans les forêts françaises.

Pour étudier ces questions, je me suis basée sur des mesures expérimentales dans ces deux dispositifs en lien avec : les processus d'utilisation de l'eau (flux de sève, composition isotopique du carbone dans le bois), l'extraction de l'eau par les racines (composition isotopique de l'eau dans le xylème) et l'interception de la lumière (volume de houppier, photos hémisphériques).

Ce travail s'insère dans le cadre d'un projet scientifique financé par l'ANR intitulé DiPTICC (Diversité et Productivité des forêts Impactées par le Changement Climatique), porté par Hervé Jactel (UMR Biogeco), en collaboration avec des chercheurs travaillant à Bordeaux (UMR BIOGECO, UMR ISPA, et Unité Expérimentale Forêt - Pierroton), à Montpellier (UMR CEFÉ) et à Nancy (UMR Silva). Le but du projet était de mieux prédire et quantifier les effets de la diversité en essences sur la productivité des forêts dans un contexte de changement climatique en apportant une meilleure compréhension des différents mécanismes d'interaction (**Fig. 13**). Mon projet de thèse portait plus particulièrement sur les aspects en lien avec les ressources en eau et en lumière.



**Fig. 13** Cadre de travail initial du projet ANR DiPTICC (2017-2021). L'objectif initial du projet était de tester les effets interactifs de la diversité des arbres et du changement climatique sur des processus aériens et souterrains clés de l'écosystème qui déterminent la croissance des arbres et expliquent ainsi la stabilité de la productivité dans les forêts mélangées.

## 5.2. Structure du manuscrit de thèse

Ce manuscrit de thèse est composé de trois chapitres de résultats :

- Le **Chapitre I** (sous format de manuscrit d'article, publié dans la revue *Forest Ecology and Management* en décembre 2020), dans lequel je me suis focalisée sur le dispositif GMAP afin de caractériser le fonctionnement hydrique et carboné pendant des années sèches dans deux types de mélange incluant le hêtre (hêtre-sapin et hêtre-chêne pubescent), et de déterminer si une complémentarité de profondeur d'extraction de l'eau était possible entre ces espèces.
- Le **Chapitre II** (sous format de manuscrit d'article, soumis à la revue *Trees - Structure and Function* en avril 2021), dans lequel je me suis focalisée sur la plantation d'ORPHEE afin de caractériser l'influence du mélange sur l'interception de la lumière du peuplement via les volumes des houppiers.
- Le **Chapitre III** (constitué de plusieurs sous-chapitres, écrits en anglais en vue de la publication d'articles) dans lequel j'ai cherché à caractériser le fonctionnement hydrique dans la plantation ORPHEE. Dans ce chapitre, j'ai étudié le fonctionnement hydrique journalier (**Chapitre III.3**) et saisonnier (**Chapitre III.4**) en l'absence et en présence de stress hydrique et j'ai également caractérisé l'extraction de l'eau par les racines en saison sèche (**Chapitre III.5**). Une discussion générale sur les différents sous-chapitres vient clore ce chapitre.

Enfin, je termine ce manuscrit par un chapitre de conclusions générales dans lequel j'ai essayé de prendre du recul vis-à-vis de l'ensemble des résultats de ces différents chapitres et j'y discute ainsi la portée générale de mon travail de thèse.

## 5.3. Description des dispositifs expérimentaux

### 5.3.1. GMAP

Le dispositif « Gradient Méditerranéo-Alpin de Placettes forestières » (GMAP<sup>4</sup>) a été mis en place par Xavier Morin, chercheur au Centre d'Ecologie Fonctionnelle et Evolutive à Montpellier, depuis 2012, dans des forêts naturelles publiques gérées. Le réseau GMAP est constitué de six sites situés sur un gradient nord-sud dans les massifs des pré-Alpes dans le sud-est de la France (**Fig. 14a**). Le choix des sites a été fait de manière à avoir toujours le même type de roche mère calcaire. Le dispositif est centré autour du hêtre (*Fagus sylvatica*) en mélange avec le sapin (*Abies alba*) dans les trois sites du nord et en mélange avec le chêne pubescent (*Quercus pubescens*) dans les trois sites du sud.

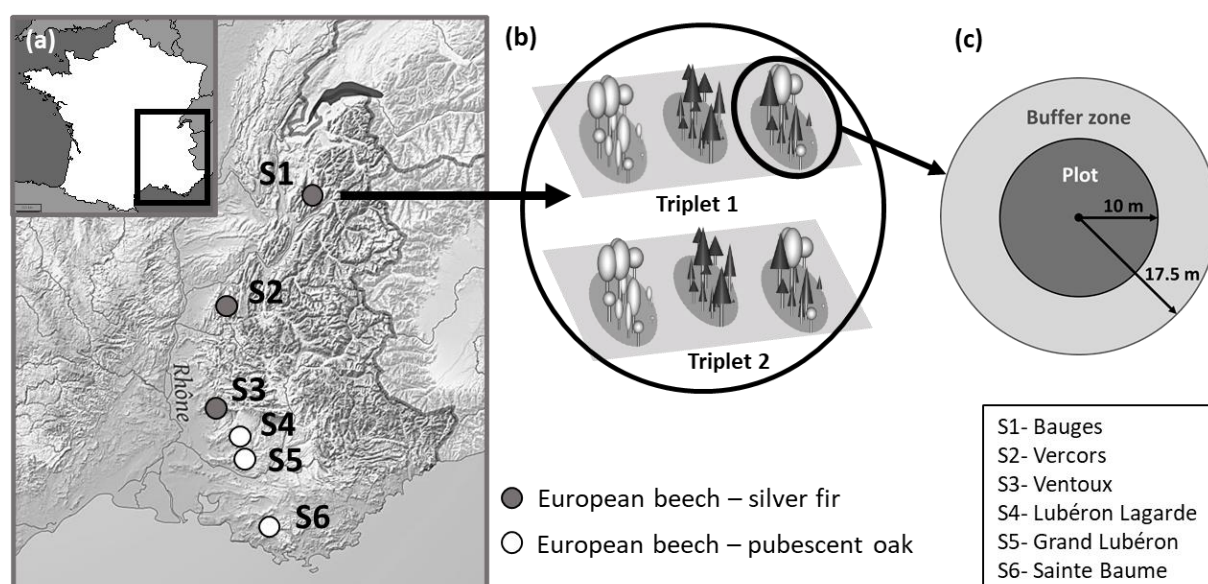
Au sein de chaque site, nous avons utilisé une approche par triplet, c'est-à-dire un ensemble de trois placettes : une placette dans des peuplements purs de chaque espèce étudiée et une placette dans un

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<sup>4</sup> <https://oreme.org/observation/foret/gmap/>

peuplement mélangé des deux mêmes espèces (**Fig. 14b**). Tous les triplets étaient situés sur des pentes orientées au nord. Les trois placettes de chaque triplet étaient aussi proches que possible les unes des autres et avec une pente similaire. Les textures de sol et les teneurs en gravier étaient similaires au sein d'un même triplet. Sur chaque site, deux ensembles de tels triplets ont été sélectionnés afin de couvrir une certaine variabilité dans les conditions environnementales locales et dans la structure des peuplements, résultant en six placettes par site (**Fig. 14b**). L'altitude des placettes variait de 700 m à 1400 m.

Les placettes sont circulaires et font 962 m<sup>2</sup> (17.5 m de rayon) avec un cœur de placette de 314 m<sup>2</sup> (10 m de rayon) (**Fig. 14c**). Dans le cadre de la thèse de Marion Jourdan, Xavier Morin et ses collègues ont effectué des relevés dendrométriques (hauteur, diamètre à 1m30, positionnement) entre 2013 et 2015 et prélevé des carottes dans le tronc pour les arbres ayant un diamètre supérieur à 7.5 cm dans le cœur de placette.



**Fig. 14** Design expérimental du réseau GMAP. (a) Zone d'étude et localisation des six sites dans le sud-est de la France. Les points sombres (sites S1 à S3) représentent les sites du sud composés de forêts de hêtres (*Fagus sylvatica*) et de sapins (*Abies alba*). Les points blancs (sites S4 à S6) représentent des sites du nord composés de forêts de hêtre et de chêne pubescent (*Quercus pubescens*). Les différents sites sont situés dans les chaînes de montagnes suivantes des Préalpes françaises : Bauges (S1), Vercors (S2), Ventoux (S3), Lubéron Lagarde (S4), Grand Lubéron (S5), Sainte-Baume (S6). (b) Représentation schématique d'un site, avec les deux triplets par site. Chaque triplet est composé d'une placette monospécifique pour chaque espèce et d'une placette du mélange des deux espèces. (c) Représentation d'une placette individuelle. Les placettes sont circulaires avec une zone centrale (10 m de rayon) et une zone tampon (7,5 m de rayon). Tous les arbres de la placette centrale ayant un diamètre > 7,5 cm ont été échantillonnés.



### 5.3.2. ORPHEE

ORPHEE<sup>5</sup> est une plantation expérimentale mise en place par l'Institut National de Recherche Agronomique sur le centre de Cestas-Pierroton (proche de Bordeaux) en 2008 pour tester l'effet de la diversité en espèces d'arbres et de la disponibilité en eau (irrigation) sur différentes variables de fonctionnement de l'écosystème forestier (Castagneyrol et al. 2013) (**Fig. 15**).

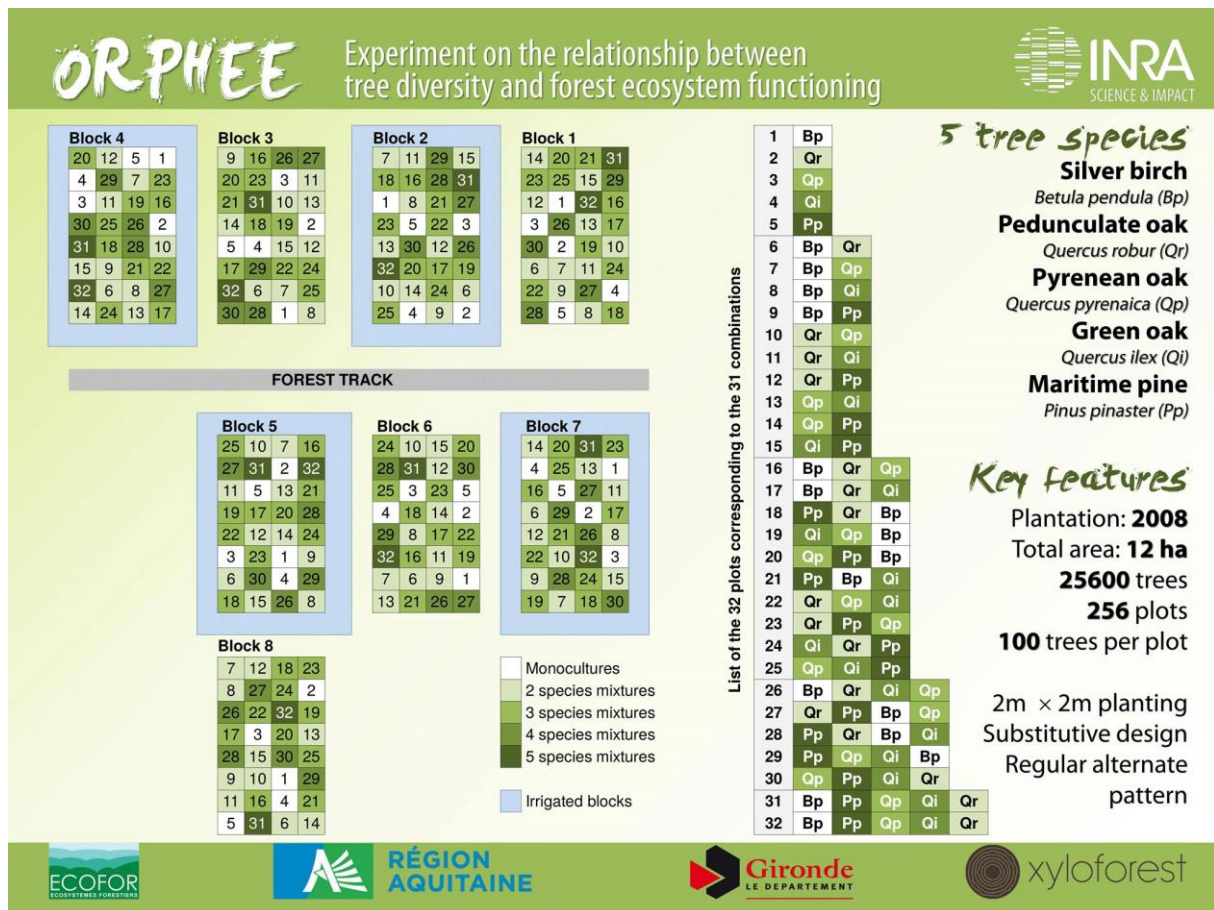
Le dispositif est composé de huit blocs, mais je n'ai travaillé que dans les blocs 1 à 6 (les blocs 7 et 8 n'ont pas été utilisés dans le cadre du projet DiTPICC). Chaque bloc mesure 100 m sur 175 m et comprend 32 parcelles de 20 m sur 20 m, qui correspondent à toutes les combinaisons possibles entre cinq essences (**Fig. 15**) : le bouleau verruqueux (*Betula pendula*), le chêne pédonculé (*Quercus robur*), le chêne tauzin (*Quercus pyrenaica*), le chêne vert (*Quercus ilex*) et le pin maritime (*Pinus pinaster*).

Un bloc sur deux est irrigué (blocs 2,4,5,7) depuis 2015 pour diminuer les effets éventuels de la contrainte hydrique estivale (**Fig. 15**). Dans un même bloc, les parcelles sont séparées les unes des autres d'une distance de 3 m. Chaque parcelle est carrée et est composée de 10 lignes de 10 arbres, avec un espacement de 2 m entre les arbres. Les mesures sont réalisées sur les 36 arbres centraux (6 × 6 arbres) afin d'éviter les effets de bordure.

Dans le cadre de ma thèse, je m'intéresse uniquement à deux espèces parmi les cinq initialement plantées : le bouleau verruqueux (*Betula pendula*) et le pin maritime (*Pinus pinaster*). La raison de ce choix correspond à mon objectif de me focaliser sur des mélanges à deux espèces dans ma thèse. De plus, la croissance primaire des trois espèces de chênes était encore très faible au début de ma thèse (en moyenne 1.5 m de haut en 2017) et ces arbres n'étaient pas assez grands pour être en compétition pour la lumière avec les bouleaux ou les pins (en moyenne 7.1 m de haut en 2017). Dans chaque bloc, j'étudie donc un triplet de parcelles : bouleau, pin et bouleau + pin. De plus, dans le Chapitre II, j'ai également inclus dans mon étude certaines des parcelles contenant des chênes en mélange avec des bouleaux et/ou des pins, en considérant les chênes comme des trouées dans une canopée constituée uniquement de bouleaux et/ou de pins, afin d'étudier une densité réduite de houppiers de bouleaux et/ou pins. Pour chacune de ces modalités j'ai trois répétitions de blocs irrigués (blocs 1,3 et 6) ou non-irrigués (blocs 2,4 et 5).

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<sup>5</sup> <https://sites.google.com/view/orpheexperiment/home>



**Fig. 15** Design expérimental de la plantation ORPHEE. Chaque bloc est composé de toutes les combinaisons possibles parmi les cinq essences du dispositif, réparties de manière aléatoire. La moitié des blocs sont irrigués depuis 2015.

# CHAPITRE I

## Effets du mélange sur le fonctionnement hydrique du hêtre en forêts naturelles de montagne pendant des années sèches



Photo : Soline Martin-Blangy. Sainte-Baume, France.

**Préambule :** Ce chapitre a pour objectif de caractériser l'effet du mélange sur le fonctionnement hydrique des arbres pendant des années sèches et sur l'origine d'extraction de l'eau par les racines dans des forêts naturelles. Pour cela nous avons utilisé le réseau expérimental GMAP, composé de six sites le long d'un gradient nord-sud dans les pré-Alpes françaises et nous nous sommes focalisés sur le fonctionnement hydrique du hêtre (*Fagus sylvatica*) dans des peuplements purs ainsi qu'en peuplements mélangés avec le sapin (*Abies alba*) ou le chêne pubescent (*Quercus pubescens*). L'efficacité d'utilisation de l'eau pendant les années sèches a été mesurée grâce à la composition isotopique du carbone dans le bois ( $\delta^{13}\text{C}$ ), et l'origine d'extraction de l'eau a été estimée à partir de la composition isotopique de l'eau du xylème ( $\delta^2\text{H}$ ). Ce chapitre a été écrit sous forme d'article scientifique et publié dans la revue *Forest Ecology and Management* en décembre 2020. J'ai repris le format du manuscrit accepté dans la revue afin de l'inclure dans ce document de thèse.




## Title: Mixing beech with fir or pubescent oak does not help mitigate drought exposure at the limit of its climatic range

**Authors:** Soline Martin-Blangy<sup>1\*</sup>, Marie Charru<sup>2, 3†</sup>, Sylvain Gérard<sup>1,4</sup>, Hervé Jactel<sup>5</sup>, Marion Jourdan<sup>6</sup>, Xavier Morin<sup>7</sup>, Damien Bonal<sup>1†</sup>

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### Mixing beech with fir or pubescent oak does not help mitigate drought exposure at the limit of its climatic range

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## Abstract

In the context of climate change, it remains unclear whether mixed-species forests will help mitigate the impacts of future droughts and, if so, through which processes. As European beech (*Fagus sylvatica* L.) is one of the major European species, it is crucial to evaluate its response to drought when mixed with species with contrasted functional traits and in contrasted climatic conditions, particularly at the limit of its climatic range. This study aimed to (i) characterize the effects of tree species interactions on the drought exposure of beech in south-eastern France, and (ii) determine whether belowground water uptake complementarity underlies these effects. We focused on beech-silver fir (*Abies alba* Mill.) and beech-pubescent oak (*Quercus pubescens* Willd.) forests across six sites in the French pre-Alps, a region at the limit of the climatic range for beech. We used a triplet approach to compare the tree-ring carbon isotope composition ( $\delta^{13}\text{C}$ ) of these species in pure and two-species mixed stands during a period of dry years, and used water hydrogen isotope composition ( $\delta^2\text{H}$ ) in the xylem to identify water uptake sources. Overall, we found no clear mixture effect pattern on beech physiological functioning among sites and triplets. In beech-fir sites, mixing beech with fir had no effect on beech  $\delta^{13}\text{C}$  values during dry years. In beech-oak sites, mixture effects on beech were mostly neutral, although sometimes beech suffered from a stronger exposure to drought in mixed stands. Our study emphasizes the impact of the tree sampling design on the outcome of studies on forest biodiversity-ecosystem functioning relationships. Limiting tree sampling to dominant trees when analyzing stand-level relationships may bias these outcomes. We evidenced differences in water uptake sources between beech and fir, but not between beech and oak during a dry summer. However, these patterns did not help explain the lack of species mixture effects, or existence thereof, at the triplet scale. Our study demonstrates that managing beech in mixed stands with silver fir or pubescent oak at the limit of beech climatic range does not buffer drought impacts on beech during dry years. In the long term, with more frequent extreme droughts, promoting beech-fir mixtures will not be detrimental to beech drought response, while beech may suffer in mixtures with pubescent oak.

## Highlights

- Species mixing effects on beech functioning during drought assessed with  $\delta^{13}\text{C}$
- No general pattern of mixture effect was observed across or within sites
- No mixture effects for beech mixed with fir, small effects for beech with pubescent oak
- Complementary water uptake between beech and fir but not between beech and pubescent oak
- No mitigation of drought effects on beech by promoting mixed forests in this region

## 1. Introduction

European forest ecosystems are expected to be highly impacted by ongoing climate change (Hanewinkel et al. 2013) through an increase in temperatures and more frequent and intense droughts (IPCC 2014; Lehner et al. 2017). Drought-induced stress can lead to a loss of vitality and to mortality for trees, caused by hydraulic failure and carbon starvation (McDowell et al. 2008; Hartmann et al. 2018). This will be especially true in southern parts of the European temperate and Mediterranean regions (Lindner et al. 2010). There is therefore a strong need for new management strategies to help forest owners mitigate current and future drought impacts (Bolte et al. 2009).

European beech (*Fagus sylvatica* L.) is an essential species in European forests: its natural distribution covers most of continental Europe and it is the most abundant broad-leaved forest tree in Central Europe (Dittmar et al. 2003). Beech is a drought-sensitive species and is especially vulnerable when the soil water deficit is severe and prolonged (Gessler et al. 2007). Model-based projections predict that climate change will likely induce a reduction in beech-dominated forests in southern Europe (Cheaib et al. 2012; Hanewinkel et al. 2013). Growth declines for beech have already been reported in recent decades (Charru et al. 2017), especially at its southern range limits (Jump et al. 2006).

Managing temperate forests with higher tree species diversity has been identified as one management strategy that can improve forest productivity (e.g. Toïgo et al., 2015; Liang et al., 2016; Ammer, 2019) and other ecosystem functions (Brockerhoff et al. 2017), such as resistance to disturbances (Jactel et al. 2017). In particular, it has been shown that beech productivity improves in mixed forests compared to pure ones, particularly when beech is admixed with silver fir (*Abies alba* Mill.) or other coniferous species (e.g. Toïgo et al., 2015; Pretzsch and Forrester, 2017). However, it is still not clear whether mixed-species forests also contribute to mitigating drought impacts (Grossiord 2019), which in turn would have an effect on forest vitality and productivity. In the case of beech, contrasted effects of species interactions have been found under drought conditions. Some studies demonstrate lower drought exposure of beech in mixed stands (e.g. Pretzsch et al., 2013; Metz et al., 2016), but others conclude that the mixture effect is neutral (e.g. Schwarz and Bauhus, 2019). Furthermore, contrasted results were found when comparing the effects of the same species mixture, including beech, among different sites (Schäfer et al. 2017; González de Andrés et al. 2018; Jourdan et al. 2019b).

Two factors have been identified as major drivers for the variability in the outcome of mixture effects on the mitigation of drought impacts: the importance of functional variability among species and the effect of site climatic conditions (Forrester 2014; Forrester and Bauhus 2016). First, the outcomes are species-dependent: species with more contrasted functional traits in terms of physiology, phenology or morphology (e.g. root depth, stomatal control, physical traits involved in xylem resistance to embolism), are more likely to show complementarity effects, especially for drought responses (Grossiord 2019). Secondly, even if the given species mixture potentially mitigates drought impacts, the degree of the

effect may vary with site-level environmental conditions (Forrester and Bauhus 2016). Forrester and Bauhus (2016) suggested a theoretical framework, as a generalization of the stress gradient hypothesis (Bertness and Callaway 1994), in which positive mixture effects are expected to be stronger when resources are scarce, for example along a declining precipitation gradient or during severe drought events. In accordance with this framework, Grossiord et al. (Grossiord et al. 2014c, d) found negative or neutral effects in more humid sites and positive effects in drier ones (Grossiord et al. 2014c, d). However, the relationship between mixture effects and a resource gradient may not be linear but may rather emerge from threshold points of resource availability (de Streel et al. 2020). Such thresholds could be explained by the fact that positive mixture effects may not be strong enough to compensate for the increasing environmental constraints in extremely dry sites and that positive mixture effects on drought response could disappear with strong water stress (e.g. de Streel et al., 2020).

The outcome of studies on the effects of species mixing may also depend on how the trees were sampled. To date the typical approach has been to limit the sampling design to dominant trees when examining mixture effects on the response of trees to drought, particularly as measured by carbon isotope composition ( $\delta^{13}\text{C}$ ) (Grossiord et al., 2014c; Metz et al., 2016; Schwarz and Bauhus, 2019). However, not including light-suppressed trees in these analyses could bias the overall patterns of mixture effects, as the drought sensitivity of suppressed and dominant trees may differ (Mérian and Lebourgeois 2011). Including all trees for a more complete representation of the forest stand could help understanding the variability of mixture effects on drought responses.

The processes behind mixture effects on drought exposure are linked to spatial or temporal resource partitioning among species due to competition, facilitation, or niche differentiation for resource acquisition and use (Ammer 2019; Grossiord 2019). At aboveground level, this may result in, for example, differences in canopy packing and shading effects (Ishii and Asano, 2010; Jucker et al., 2015; Grossiord 2019). At belowground level, complementarity for water uptake and niche differentiation among species could result in a lesser exposure to decreased soil water content during extreme drought events (Forrester 2014), but these processes have rarely been experimentally tested in the field (Grossiord et al. 2014a; Goisser et al. 2016).

Further studies for species diversity effects on beech drought exposure are therefore necessary to identify whether or not tree-species mixing might mitigate drought impacts and maintain beech productivity, particularly along the southern margins of beech distribution in Europe where climate change is occurring the fastest. To address these questions, we studied the effect of species mixture on beech drought exposure during a period with severe summer droughts, in forest plots of different species compositions distributed across six sites in south-eastern France with contrasting climate conditions. We studied two widespread species mixtures with beech in mountainous areas of south-eastern France: beech associated with silver fir (*Abies alba* Mill.) in the northern part of the region, and beech associated with pubescent oak (*Quercus pubescens* Willd.) in the southern part. These two accompanying species



are likely to show complementarity effects with beech as they have contrasted physiological functioning, i.e. contrasted water transport systems and phenology between beech (deciduous species) and fir (evergreen conifer), and contrasted drought and shade tolerance between beech and pubescent oak (Niinemets and Valladares 2006). We sampled all trees in the stands, including both dominant and light-suppressed trees. We used stable carbon isotope composition ( $\delta^{13}\text{C}$ ) in tree rings from dry years to examine the effect of species composition on tree exposition to drought (Grossiord et al. 2014d; Metz et al. 2016). Tree ring analysis provided us with an integrative measurement of past drought effects on tree functioning (McCarroll and Loader 2004). Tree-ring  $\delta^{13}\text{C}$  is expressed as a negative value that increases (less negative values) when a tree is exposed to increased water stress (Saurer et al. 1995). It indirectly reflects the compromise between carbon and water acquisition and use at leaf and plant level (Farquhar et al. 1982). Finally, to assess potential water-use complementarity mechanisms, we looked at differences in water uptake sources between species. To do so, we measured the stable hydrogen isotope composition ( $\delta^2\text{H}$ ) in branch xylem water (Dawson 1993; West et al. 2006).

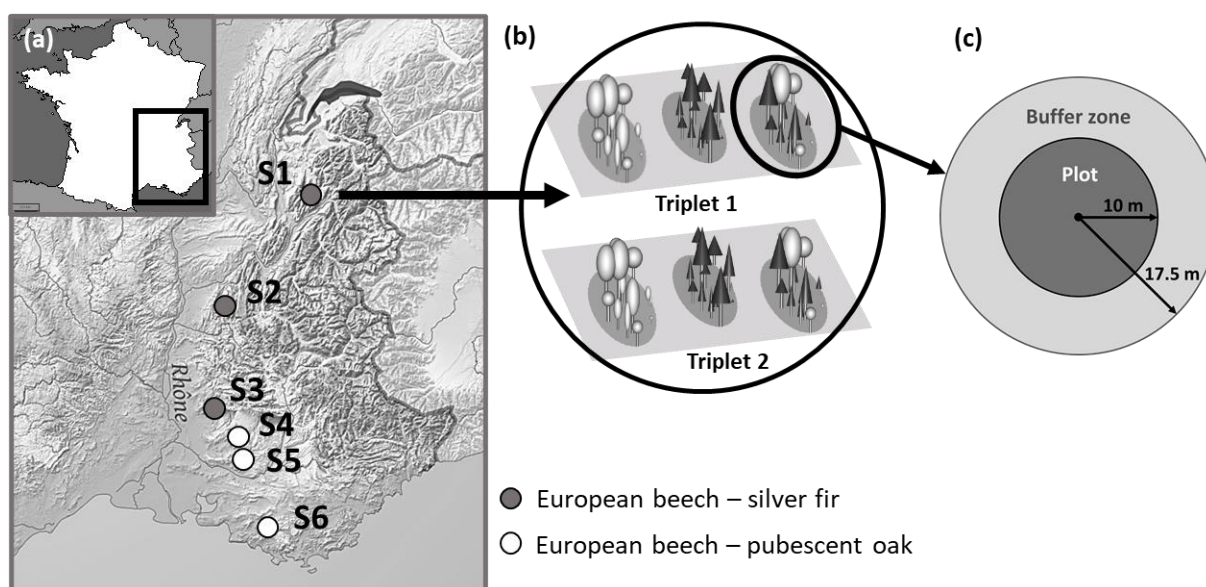
We specifically addressed the following questions:

1. Is beech physiological functioning ( $\delta^{13}\text{C}$ ) during dry years influenced by site-specific environmental conditions?
2. Is beech  $\delta^{13}\text{C}$  during dry years different between pure and mixed stands?
3. Can potential mixture effects be explained by complementarity in species water uptake sources?

## 2. Material and Methods

### 2.1. Field design

We selected six sites in the French pre-Alps on limestone bedrock (**Fig. I. 1a**, see also Jourdan et al., 2019b) spanning a wide range of climatic conditions (**Fig. I. 2**). At each site, we used a triplet approach, i.e. an ensemble of three plots: one plot in pure stands of each studied species and one plot in a mixed stand of the same two species (**Fig. I. 1b**). All the triplets were located on north-facing slopes. The three plots within each triplet were as close to each other as possible and with a similar slope (**Table I.A 1**). The soil textures and gravel contents were similar within a triplet. At each site, two sets of such triplets were selected in order to cover local variability in environmental conditions and stand structure, resulting in six plots per site (**Fig. I. 1b**). Plot elevations ranged from 700 m to 1400 m (**Table I.A 1**).



**Fig. I. 1** Field design of the GMAP network (Mediterranean and Alpine Plot Gradient, set up by X. Morin in 2013). (a) Study area and location of the six sites in south-eastern France. Dark points (sites S1 to S3) represent northern sites with European beech (*Fagus sylvatica*) and silver fir (*Abies alba*) forests. White points (sites S4 to S6) represent southern sites with European beech and pubescent oak (*Quercus pubescens*) forests. The sites are located in the following mountain ranges of the French pre-Alps: Bauges (S1), Vercors (S2), Ventoux (S3), Lubéron Lagarde (S4), Grand Lubéron (S5), Sainte-Baume (S6). (b) Schematic representation of a site, with the two triplets per site. Each triplet is made up of two pure plots in monospecific stands and one mixed plot in a mixed-species stand. (c) Representation of an individual plot. Plots are circular with a central plot area (10 m radius) and a buffer zone (7.5 m radius). All the trees in the central plot with a DBH > 7.5 cm were sampled.

European beech (*Fagus sylvatica* L.) was present at all six sites and was mixed with either silver fir (*Abies alba* Mill.) in the three northern sites (S1 to S3) or with pubescent oak (*Quercus pubescens* Willd.) in the three southern ones (S4 to S6) (**Fig. I. 1a**). In pure plots, the focal species accounted for at least 80 % of the total basal area and in the mixed plots each focal species accounted for at least 40 % of the total basal area (**Table I.A 2**). Other tree species present in the plots represented less than 20 % of the total basal area and were mostly in the understory (**Table I. 1, Table I.A 2**). The trees from both focal species in the mixed stands were mixed on a stemwise intermingling pattern (del Río et al. 2018). Sites S1 to S4 were located in forests with continuous-cover management whereas sites S5 and S6 had limited management. To limit direct management effects, we only worked in stands where no thinning operations had been conducted for at least the last two decades. All the stands were located in mature forests with an uneven-aged high forest structure, except for S5, which was in a coppice forest. Individual plots were circular with a radius of 17.5 m (area = 962 m<sup>2</sup>) and consisted of a central area (10 m radius, area = 314 m<sup>2</sup>) where the measurements were conducted, plus a buffer zone (7.5 m radius) to ensure the plot was surrounded by homogeneous conditions (**Fig. I. 1c**).

## 2.2. Climate data

Climatic variables were extracted from the mesoscale SAFRAN reanalysis of the Météo France national observation network data (Quintana-Seguí et al. 2008). As SAFRAN was initially designed for mountainous areas, it was the best-estimated source of daily climatic data for our study region. The precision of the climate data was relevant at the site scale, but not at the triplet scale, as the SAFRAN analysis covers France with an 8 km × 8 km grid and is calculated for the average elevation of the corresponding grid. We used the SAFRAN long-term climate data to estimate the average cumulative annual precipitation and the mean annual temperature per site over a 20-year period (1994-2013) (**Fig. I. 2; Table I.A 3**).

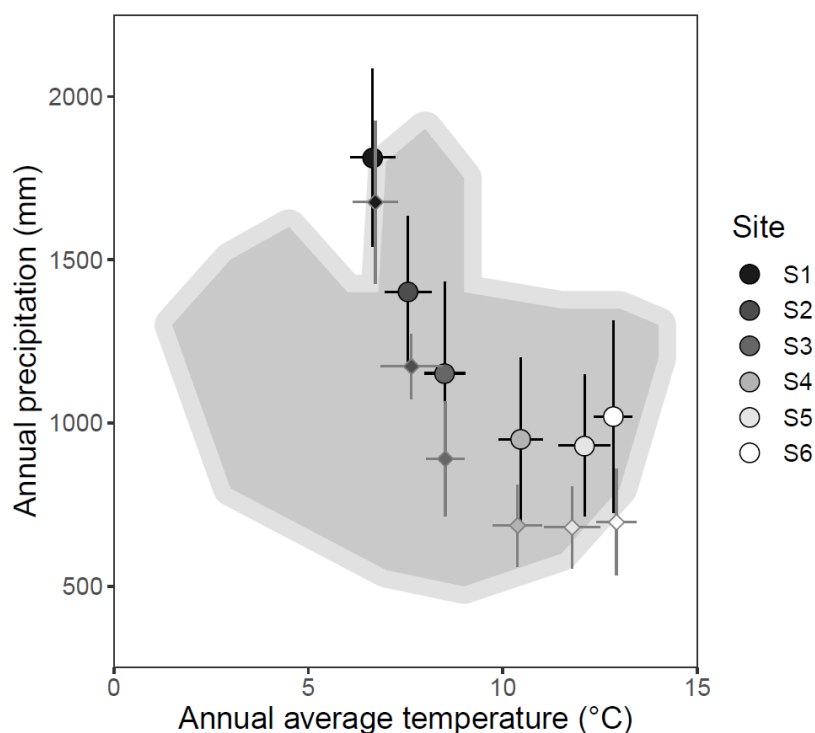
## 2.3. Choice and characterization of dry years

Jourdan et al. (2019b) calculated the standardized precipitation evapotranspiration index (SPEI, Vicente-Serrano et al., 2010) for each of the six sites for the months of February to July, i.e. the growth period, of each year from 1994 to 2013 (see Fig. 2 in Jourdan et al., 2019b). SPEI is a standardized index that makes it possible to identify dry years for a given site, but not to compare drought intensity among sites. This enabled us to choose the period of dry years relative to each site within the 1994-2013 period for tree-ring carbon isotope composition ( $\delta^{13}\text{C}$ ) analyses. We targeted the 2003-2007 period because it was representative of a series of severe dry years for all sites. Indeed, this period was on average much drier in terms of precipitation across sites than the 20-year average for 1994-2013 (**Fig. I. 2**), but was also hotter during the summer months (**Table I.A 3**).

**Table I. 1** Plot characteristics: mean DBH (cm) and range by species for all trees in each site, triplet, and plot.

Site	Triplet	Plot	DBH (cm)			
			Beech	Fir	Oak	Other sp.
S1	1	Pure beech	41.9 (8-105)	–	–	19.1 (13.4-24.2)
S1	1	Pure fir	8.3 (8.3-8.3)	37 (8.6-92.9)	–	21.5 (10.5-49.3)
S1	1	Mixed	39 (28.3-49.7)	22.9 (7.6-38.2)	–	18.5 (9.2-34.4)
S1	2	Pure beech	47 (28.3-59.8)	14.1 (5.7-28.6)	–	–
S1	2	Pure fir	–	32.6 (8-79.9)	–	26.7 (13.1-50)
S1	2	Mixed	42.4 (9.5-76.4)	28.3 (7.3-91.7)	–	45.2 (45.2-45.2)
S2	1	Pure beech	16.5 (8.3-46.8)	–	–	19.4 (14.3-26.7)
S2	1	Pure fir	–	24.2 (8-42)	–	23.6 (23.6-23.6)
S2	1	Mixed	14.2 (7.3-40.1)	19.6 (7.6-57.3)	–	10.2 (8.6-14.3)
S2	2	Pure beech	47.4 (35.3-58.3)	17.3 (12.7-23.9)	–	–
S2	2	Pure fir	15.3 (15.3-15.3)	42.4 (15.6-57.3)	–	–
S2	2	Mixed	36.8 (30.2-44.2)	27.7 (12.7-57.6)	–	–
S3	1	Pure beech	25.3 (9.2-38.8)	–	–	–
S3	1	Pure fir	18.8 (18.1-19.4)	35.9 (15-47.7)	–	–
S3	1	Mixed	14.9 (7.6-23.9)	44.8 (9.5-59.8)	–	–
S3	2	Pure beech	29.9 (16.6-53.5)	20.4 (20.4-20.4)	–	–
S3	2	Pure fir	19.2 (8-30.2)	17.1 (7.3-41.7)	–	15 (10.8-19.1)
S3	2	Mixed	24 (16.2-32.1)	15.2 (7.3-60.5)	–	30.6 (28.6-32.5)
S4	1	Pure beech	24.9 (7.6-68.1)	–	–	13.3 (11.8-14.3)
S4	1	Pure oak	11.4 (7.6-15.3)	–	18.3 (7.6-42.7)	10.7 (7.6-15.9)
S4	1	Mixed	16.1 (8.6-24.5)	–	20.7 (11.1-27.4)	18.9 (8.3-39.8)
S4	2	Pure beech	22.6 (8.6-64.3)	–	11.1 (11.1-11.1)	–
S4	2	Pure oak	13.4 (13.4-13.4)	–	15.2 (8.9-24.8)	9.7 (9.2-10.2)
S4	2	Mixed	14.4 (7-27.7)	–	17.1 (12.1-22.9)	13.8 (12.4-15.3)
S5	1	Pure beech	17.1 (7.3-28.6)	–	12.9 (8.9-16.9)	13.7 (8-19.4)
S5	1	Pure oak	–	–	18.7 (10.5-32.5)	18.6 (9.2-29)
S5	1	Mixed	14.5 (8-22)	–	14.7 (7.6-21)	10.4 (7.3-16.2)
S5	2	Pure beech	16.6 (7-27.1)	–	–	13.8 (8.3-23.6)
S5	2	Pure oak	–	–	15.6 (10.8-22.9)	10.1 (7-13.4)
S5	2	Mixed	13.7 (7.3-23.6)	–	15.9 (7.6-20.7)	9.2 (7-15.3)
S6	1	Pure beech	40.4 (29.6-53.2)	–	–	16 (9.9-29)
S6	1	Pure oak	–	–	36.1 (28-45.5)	12.8 (7-22.3)
S6	1	Mixed	30.6 (9.5-45.2)	–	34.6 (20.4-42.3)	15.5 (7.3-34.4)
S6	2	Pure beech	19.6 (7.6-35.7)	–	17.2 (14-19.4)	13.3 (7.6-25.5)
S6	2	Pure oak	–	–	19 (9.9-38.2)	8.2 (7-9.9)
S6	2	Mixed	21.3 (8.6-31.8)	–	18 (8.3-26.7)	11.3 (7.6-15.9)

Note: Other tree species represent less than 20 % of the total basal area of the plots (12 % on average). Most common species are *Acer pseudoplatanus*, *Acer campestre*, *Ilex aquifolium*, *Sorbus torminalis* and *Taxus baccata*.



**Fig. I. 2** Climate-space diagram of presence for beech in Europe, study site annual average temperature and annual sum of precipitation 20-year averages. The shaded area represents observed beech presence in Europe in all national forest inventories in terms of annual climatic conditions for temperature and precipitation (adapted from the European Atlas of Forest Tree Species, Houston Durrant et al., 2016). Large circular points represent the 20-year averages (1994-2013) from the SAFRAN weather data for each of our study sites. Small diamond points represent the 5-year averages of the selected dry years (2003-2007) for each site. The x-axis is the annual average of daily average temperatures. The y-axis is the annual sum of daily precipitation. Error bars represent  $\pm$  one standard deviation around the mean.

To characterize the water stress during these dry years, we used the daily forest water balance model BILJOU© (Granier et al. 1999). We estimated the daily relative extractable water (REW, unitless) for each site based on daily climate data over the 1994-2013 period. REW is the ratio between available soil water and the maximum extractable water (water holding capacity) and varies from field capacity (REW=1) to permanent wilting point (REW=0). Water stress is assumed to occur when REW drops below a threshold of 0.4 under which stomatal conductance, and therefore leaf gas exchange, is gradually reduced (Granier et al. 1999). The BILJOU© model has a daily time step and requires daily climatic input variables (mean air temperature, precipitation, wind speed, global radiation, and air humidity) as well as soil water holding capacity and leaf area index (LAI). We used SAFRAN daily climatic variables at the site scale. We fixed soil water holding capacity at 100 mm since we were not able to characterize the plot soil profiles precisely due to very rocky soils. Nevertheless, we tested the BILJOU© model with different soil water holding capacity values but this did not change the ranking of the sites in terms of water stress (data not shown). Similarly, we used a LAI value of  $7.0 \text{ m}^2 \text{ m}^{-2}$  in northern sites and  $6.0 \text{ m}^2$

m<sup>-2</sup> in southern sites as standard values for mature forests (Bréda 2003). Adapting this value to the different sites did not change the ranking of the sites. For each year at each site, an annual number of days when REW is below 0.4 was calculated, which allowed to quantify water stress levels (**Table I.A 3**).

## 2.4. Tree measurements

### 2.4.1. Dendrometry

In 2014, in the central area of each of the 36 plots, Jourdan et al. (2019b) surveyed all of the trees with a diameter at breast height (DBH, cm) greater than 7.5 cm and recorded their DBH and total height. They also cored the trunk of all of these trees at a height of 1.3 m with a Pressler increment borer (Haglöf, Sweden) in 2014-2015. In coppice stands, they only cored the largest stem of each clump of coppice shoots (individual tree). Some cores were too difficult to date accurately and were discarded from further analyses, leaving a total of 792 trees sampled (368 beech, 222 fir and 202 pubescent oak trees), representing all the diameter classes and different strata in the canopy. On these cores, Jourdan et al. (2019b) measured tree-ring width for the 1994-2013 period and performed cross-dating for each species according to specific pointer years.

### 2.4.2. Carbon isotope composition ( $\delta^{13}\text{C}$ )

We used these tree cores to analyse the stable carbon isotope composition ( $\delta^{13}\text{C}$ ) of each tree in the tree-rings of the selected period (2003-2007). Using a scalpel under a stereomicroscope, we extracted the tree-rings (including earlywood and latewood) that corresponded to the target period (2003-2007) as one pooled block for all five years. We used whole wood tissue for our analyses as it shows the same trends as cellulose-only isotopic measurements and allowed us to analyze a larger number of trees (McCarroll and Loader 2004). For each tree, the tree-rings of the 2003-2007 period were kept as one sample. We ground them into a fine powder, then weighed around 1 mg of powder for each sample in tin capsules for the isotopic analysis. We used a different technique when the dry mass of the 2003-2007 tree-ring sample was less than 6 mg (15 samples out of 792) to avoid any loss of matter in the grinding process. These 15 samples were cut into four quarters perpendicularly to the tree-ring axis with a scalpel instead of being ground. For each of these samples, we then analyzed separately three out of four subsamples for isotopic composition and used the average of these three  $\delta^{13}\text{C}$  values. The fourth subsample was kept as a spare in case further analyses should be needed on these samples.

The  $^{13}\text{C}/^{12}\text{C}$  ratios of each wood sample were analyzed with an isotope-ratio mass spectrometer (IRMS, Isoprime 100, Isoprime Ltd., Cheadle Hulme, UK) coupled with an elemental analyzer (EA, Elementar vario, ISOTOPE cube, Elementar Analysen Systeme GmbH, Hanau, Germany) at the SILVATECH platform (SILVATECH, INRAE, 2018. Structural and Functional Analysis of Tree and Wood Facility,

Nancy, France). The results were expressed as carbon isotope composition  $\delta^{13}\text{C}$  (‰) relative to the international standard Vienna Pee Dee Bee Belemnite (V-PDB) (IAEA 1995) following Eq. 1:

$$\delta^{13}\text{C} (\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 \quad (1)$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  represent the  $^{13}\text{C}/^{12}\text{C}$  ratios of the sample and the V-PDB standard, respectively (Farquhar et al. 1982). The mass spectrometer had a precision of  $\pm 0.2$  ‰ for  $\delta^{13}\text{C}$  values.

In this study, we used two sets of  $\delta^{13}\text{C}$  data for our statistical analyses. First, we used the dataset containing all the  $\delta^{13}\text{C}$  values to characterize the response of all the trees, individually or at the stand level, hereafter called “all trees”. Second, we used a subsample dataset of pre-selected dominant trees in each plot, hereafter called “dominant-tree subsample”, to analyze the effect of mixture on the  $\delta^{13}\text{C}$  of the trees without any confounding factor related to light availability in the canopy (McCarroll and Loader 2004). These dominant trees were selected according to the following criteria: (i) they were in the biggest diameter class in each plot, (ii) they had a crown illumination index (Dawkins index) of 4 or 5, meaning that they received direct vertical light (Clark and Clark 1992), (iii) they were well mixed with the other species on a stem-to-stem basis in the mixed stands, and (iv) they were suitable for branch sampling by climbers (see  $\delta^2\text{H}$  section). We then randomly chose between four and seven of these dominant trees per species in each plot, leading to a total of 240 dominant trees (beech = 121, fir = 57, pubescent oak = 62) out of a total of 792 trees measured for  $\delta^{13}\text{C}$ .

One caveat should be noted concerning our  $\delta^{13}\text{C}$  measurements: we were able to compare  $\delta^{13}\text{C}$  functioning during dry years between pure and mixed plots, but we were not able to use the difference in  $\delta^{13}\text{C}$  between a reference wet year and a dry year to estimate tree drought resistance, as some studies have done to detect mixture effects (e.g., Grossiord et al., 2014c; Schwarz and Bauhus, 2019). Indeed, during the period from 1994 to 2013, we were not able to find any years that were wet enough to serve as a reference across all sites. Moreover, as growth is very slow at some of the sites with a Mediterranean influence, we would not have been able to cut out single yearly tree-rings precisely enough. Other studies have also used absolute values of  $\delta^{13}\text{C}$ , as we did, albeit usually over a longer-term chronology (González de Andrés et al. 2018; de Streel et al. 2020). Moreover, in a study looking at tree diversity effects on drought responses using  $\delta^{13}\text{C}$ , the same trends were found with differences in  $\delta^{13}\text{C}$  between a wet and a dry year (Grossiord et al. 2014d) than with absolute  $\delta^{13}\text{C}$  values for the dry years only (Grossiord, 2014, p.48). We therefore focused our analyses on mixture effects on tree functioning during dry years by comparing values between pure and mixed stands, expecting more negative  $\delta^{13}\text{C}$  values for beech in mixed stands as compared to pure ones if the presence of oak or fir in the mixture induced less competition for water resources.

### 2.4.3. Xylem water $\delta^2\text{H}$

To estimate among-tree differences in water uptake sources, we used a method that relies on the natural abundance of stable hydrogen isotopes in the xylem water from branches (Dawson 1993). During dry spells, an isotopic gradient for hydrogen is established in the top layers of the soil (Clark and Fritz 1997). The hydrogen isotope composition ( $\delta^2\text{H}$ , ‰) of water flowing in the xylem, which is the raw sap coming up from the roots, represents the mean signature of all the water sources for the tree's rooting system. Due to very rocky shallow soils, we were not able to obtain a full soil profile at all of our sites. To address niche partitioning in water uptake sources, we therefore analyzed differences in  $\delta^2\text{H}$  values within triplets, but we were not able to quantitatively estimate a depth of water uptake by trees.

We sampled the same trees selected for the dominant-tree subsample of  $\delta^{13}\text{C}$  analyses. To determine the  $\delta^2\text{H}$  of the xylem water, we sampled a 30 to 40-cm-long branch from each dominant tree in the summer of 2018 during a dry spell at five sites, for a total of 200 samples. We could not visit S4 that year for technical reasons, and therefore we do not have  $\delta^2\text{H}$  values for that site. Professional climbers cut the branches out of the upper third of the tree crown. We removed bark tissue immediately with a knife to prevent phloem sap from mixing with xylem water. Five-centimeter-long samples were then cut from the branches and placed into closed airtight glass vials and stored in cool conditions.

Once in the lab, we stored the branch samples in cool chambers at 4°C to reduce the risk of evaporation. We then extracted water from the branch samples through cold trapping with a custom-made static vacuum cryogenic distillation system (West et al. 2006). The water from the branch samples was evaporated by heating the sampling tube in a water bath (65°C), then condensed with liquid nitrogen for 90 minutes in a collection tube. The extracted water was analyzed for  $^2\text{H}/^1\text{H}$  ratios with an elemental analyzer (EA, EuroPyrOH; EuroVector, Milano, Italy) coupled to an isotope ratio mass spectrometer (Isoprime IRMS; Elementar, Manchester, UK) at the SILVATECH platform (SILVATECH, INRAE, 2018. Structural and Functional Analysis of Tree and Wood Facility, Nancy, France). The  $^2\text{H}/^1\text{H}$  ratio of each water sample was determined from six subsamples of 0.2  $\mu\text{L}$  of extracted water and only the last three measurements were kept, then averaged for each sample. The first three measurements were discarded to avoid any bias related to a potential “memory effect”, meaning a contamination from the previous sample analyzed. The results were expressed as  $\delta^2\text{H}$  relatively to the international Vienna-standard mean ocean water standard (V-SMOW) following Eq. 2:

$$\delta^2\text{H} (\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 \quad (2)$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  represent the  $^2\text{H}/^1\text{H}$  ratios of the sample and of the V-SMOW standard respectively (IAEA 1995). The mass spectrometer used in this study had a precision of  $\pm 2.0$  ‰ for  $\delta^2\text{H}$  values.



We weighed the branch samples after cryo-distillation and placed them in a drying oven for 48 h at 65°C to check if all the water had been extracted. If part of the water had not been completely extracted, we applied a correction to the  $\delta^2\text{H}$  values since the extracted water had less of the heavier isotope ( $^2\text{H}$ ) than the water remaining in the branch. We calculated the theoretical fractionation of the hydrogen isotopes with Eq. 3 to obtain a corrected  $\delta^2\text{H}$  value (Araguás-Araguás et al. 1995):

$$\delta^2H_{corr} = \delta^2H_{measured} - \left( \frac{F^{(1/\alpha)} - F}{F - 1} * 1000 \right) \quad (3)$$

where  $\delta^2H_{measured}$  is the known measured value,  $\delta^2H_{corr}$  the expected real value if there had been no fractionation during extraction,  $F$  the fraction of water remaining in the branch, and  $\alpha$  a fixed factor specific to the isotope measured ( $^2\text{H}$ ) and to the extraction temperature (65°C). Factor  $\alpha$  is calculated from Eq. 4 (Majoube 1971):

$$10^3 * \ln(\alpha) = a * \left( \frac{10^6}{T_K^2} \right) + b * \left( \frac{10^3}{T_K} \right) + c \quad (4)$$

where  $T_K$  is the extraction temperature in degrees Kelvin, and  $a$ ,  $b$ , and  $c$  are fixed factors for  $^2\text{H}$  defined in Majoube (1971) with  $a = 24.844$ ,  $b = -76.248$ , and  $c = 52.612$  (Clark and Fritz 1997). We applied this correction to all the samples with a water-extraction percentage below 100 %.

## 2.5. Data analysis

All data analyses were conducted including either all trees or those in the dominant-tree subsample.

### 2.5.1. Variability of $\delta^{13}\text{C}$ values in pure stands

To assess the variability of  $\delta^{13}\text{C}$  values among sites and species, we compared  $\delta^{13}\text{C}$  values in the pure stands only. First, for site effects, we studied the differences in  $\delta^{13}\text{C}$  values among the six pure beech stands, and among the three stands of pure fir and pure pubescent oak, following the model:

$$\delta^{13}\text{C} \sim \text{Site} + \text{Triplet} + \text{Site: Triplet} \quad (5)$$

We used a linear model for each species with *Site* (S1 to S6) and *Triplet* (1 or 2) as factor variables. We further tested the interaction between *Site* and *Triplet* to reveal the differences between the two triplets at each site.

Second, we tested for differences in  $\delta^{13}\text{C}$  values between the two species (beech and fir, or beech and oak) in pure stands, following the model:

$$\delta^{13}\text{C} \sim \log(\text{DBH}) + \text{Species} + \log(\text{DBH}) : \text{Species} + 1|\text{Triplet ID} \quad (6)$$

We used a linear mixed model with *Species* as a two-level factor (beech vs. fir, or beech vs. oak) and  $\log(\text{DBH})$  of the individual tree as a continuous variable. The interaction between  $\log(\text{DBH})$  and *Species*

was also included in the model. *Triplet ID* was used as a random factor (*Triplet ID* is a six-level factor for each type of mixture, identified by site and triplet number, e.g. S1:2 for site 1, triplet 2). The introduction of individual tree DBH as a covariate was used as a proxy for light availability, as the biggest trees within triplets are the ones with the most access to light while smaller trees represent the understory trees. This approach allowed us to take into account the confounding factor related to light availability in the canopy and its effect on  $\delta^{13}\text{C}$  (McCarroll and Loader 2004). We used the log transformation of DBH to obtain a linear relationship between  $\log(\text{DBH})$  and  $\delta^{13}\text{C}$  values .

### 2.5.2. Mixture effects on $\delta^{13}\text{C}$

We analyzed the effect of species mixture on  $\delta^{13}\text{C}$  values separately by type of mixture (beech-fir or beech-oak) with three different types of analyses that made it possible to test for a mixture effect at different levels. First, we tested the mixture effect on individual  $\delta^{13}\text{C}$  values by species separately for the beech-fir and beech-oak sites with all six triplets together following the linear mixed-effect model:

$$\delta^{13}\text{C} \sim \log(\text{DBH}) + \text{Mixture} + \log(\text{DBH}) : \text{Mixture} + 1|\text{Triplet ID} \quad (7)$$

with *Mixture* (pure vs. mixed stands) as a fixed two-level factor,  $\log(\text{DBH})$  of each tree as a continuous variable, and *Triplet ID* as a random factor (*Triplet ID* is a six-level factor for each type of mixture, identified by site and triplet number, e.g. S1:2 for site 1, triplet 2). The interaction between  $\log(\text{DBH})$  and *Mixture* was also included in the model.

Second, we tested the same effect separately for each triplet with the following linear model for each species and triplet:

$$\delta^{13}\text{C} \sim \log(\text{DBH}) + \text{Mixture} + \log(\text{DBH}) : \text{Mixture} \quad (8)$$

with *Mixture* as a two-level factor (pure vs. mixed stands),  $\log(\text{DBH})$  as a covariate. The interaction between  $\log(\text{DBH})$  and *Mixture* was also included in the model.

Finally, to evaluate any effect of mixture on stand-level functioning, we used a metric called “Net Biodiversity Effect” (NBE, ‰), adapted for complex traits such as  $\delta^{13}\text{C}$  by Grossiord et al. (2013) from Loreau and Hector (2001). We calculated NBE for the dominant-tree subsample only (NBE<sub>dom</sub>, ‰) because with all trees we would not have been able to separate the effects of light availability vs. water availability.

NBE<sub>dom</sub> on the average  $\delta^{13}\text{C}$  of a given mixture of species is the difference between the observed averaged  $\delta^{13}\text{C}$  in the mixture ( $\delta^{13}\text{C}_O$ , ‰) and the expected averaged  $\delta^{13}\text{C}$  in the mixture ( $\delta^{13}\text{C}_E$ , ‰) calculated with species values in pure stands:

$$\text{NBE}_{dom} = \delta^{13}\text{C}_O - \delta^{13}\text{C}_E = \sum_{i=1}^2 (\delta^{13}\text{C}_{Oi} * P_{Oi}) + \sum_{i=1}^2 (\delta^{13}\text{C}_{Ei} * P_{Oi}) \quad (9)$$

where  $\delta^{13}\text{C}_{\text{O}_i}$  is the observed average  $\delta^{13}\text{C}$  value for dominant trees of species  $i$  in mixed stands,  $\delta^{13}\text{C}_{\text{E}_i}$  is the average  $\delta^{13}\text{C}$  value for dominant trees of species  $i$  in its respective pure stand and  $P_{\text{O}_i}$  is the proportion of species  $i$  in the mixed plot in terms of basal area.

We calculated  $\text{NBE}_{\text{dom}}$  values for each triplet. Based on the precision of the mass spectrometer used for the isotopic measurements in this study ( $\pm 0.2 \text{ ‰}$ ),  $\text{NBE}_{\text{dom}}$  values were considered different from  $0.0 \text{ ‰}$  when the difference between observed and expected mixed stand values was greater than  $\pm 0.4 \text{ ‰}$ . A positive difference means that the observed mixed stand had a higher  $\delta^{13}\text{C}$  value than expected based on values from the corresponding pure stands. A negative difference means that the observed mixed stand had a lower  $\delta^{13}\text{C}$  value than expected from pure-stand values. Since there was only one value per triplet, no statistical analyses were possible for this variable, although we were able to compare results across triplets.

### 2.5.3. $\delta^2\text{H}$ analysis

To test for differences in water uptake sources between species and between pure and mixed stands, we analyzed  $\delta^2\text{H}$  data separately by type of mixture (beech-fir or beech-oak). We used the following linear mixed model to test for differences between species and pure or mixed stands:

$$\delta^2\text{H} \sim \text{Species} + \text{Mixture} + \text{Species:Mixture} + 1|\text{Triplet ID} \quad (10)$$

with *Species* a two-level factor (beech and fir, or beech and oak), *Mixture* a two-level factor (pure or mixed), and *Triplet ID* a random factor (*Triplet ID* is a six-level factor for each type of mixture, identified by site and triplet number, e.g. S1:2 for site 1, triplet 2).

### 2.5.4. Statistical analyses

All analyses were performed with R software version 3.6.2 (R Core Team 2019). Linear mixed effect models were conducted with the `lme` function of the NLME package (Pinheiro et al. 2019). Analyses of variance following linear models or linear mixed models were conducted with the `Anova` function (Type II SS) from the CAR package (Fox and Weisberg 2019) and post-hoc multiple comparisons were made with the `glht` function (Tukey contrasts) in the MULTCOMP package (Hothorn et al. 2008). We graphically assessed normality and homoscedasticity of all model residuals. For mixed effect models, we used a pseudo R-squared in the R package MUMIN (Barton 2019) to determine the variance explained by both the fixed terms only (marginal coefficient of determination,  $R^2_{\text{m}}$ ) and the entire model including the random terms (conditional coefficient of determination,  $R^2_{\text{c}}$ ).

### 3. Results

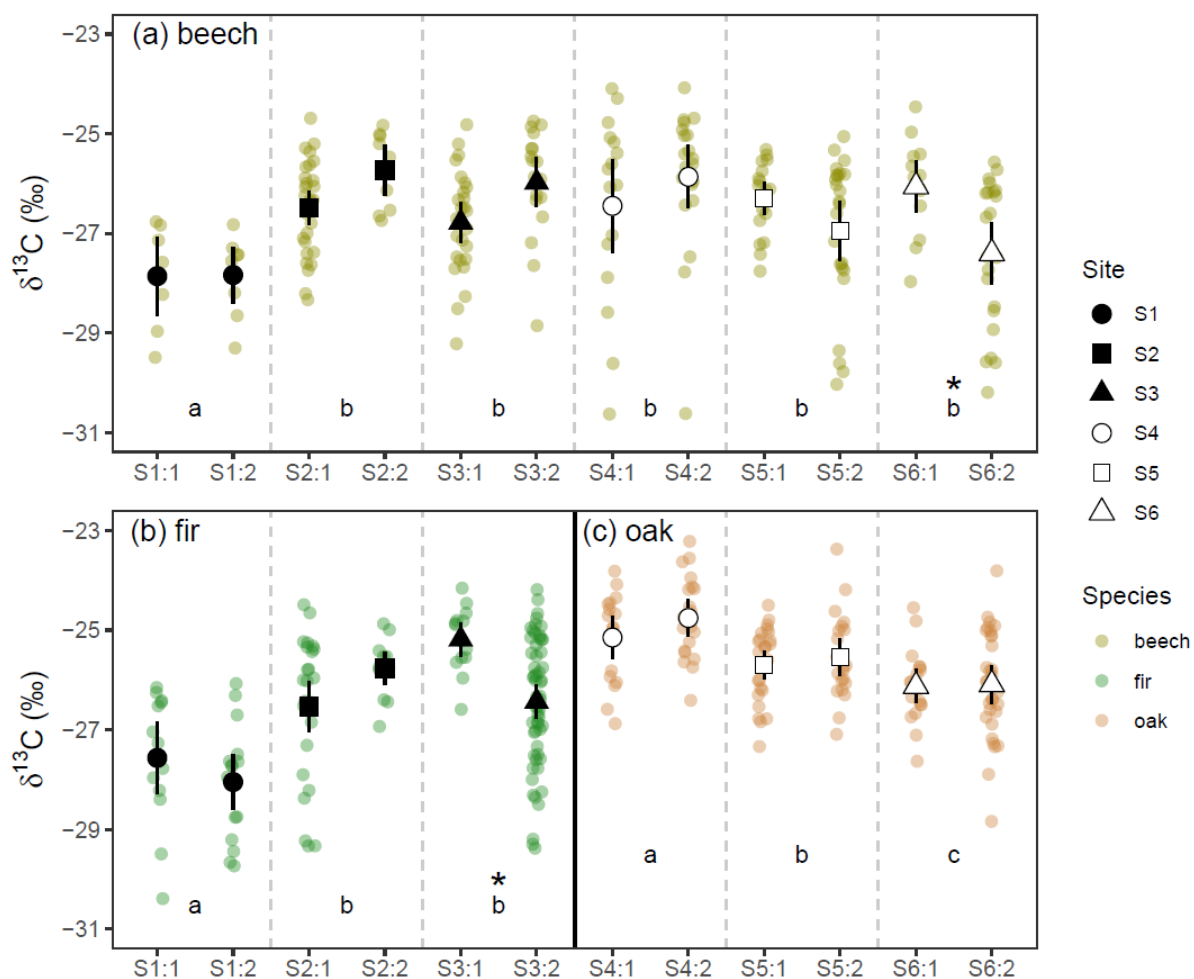
#### 3.1. Site and species patterns of $\delta^{13}\text{C}$ in pure stands

##### 3.1.1. Site and triplet effects on $\delta^{13}\text{C}$ within species

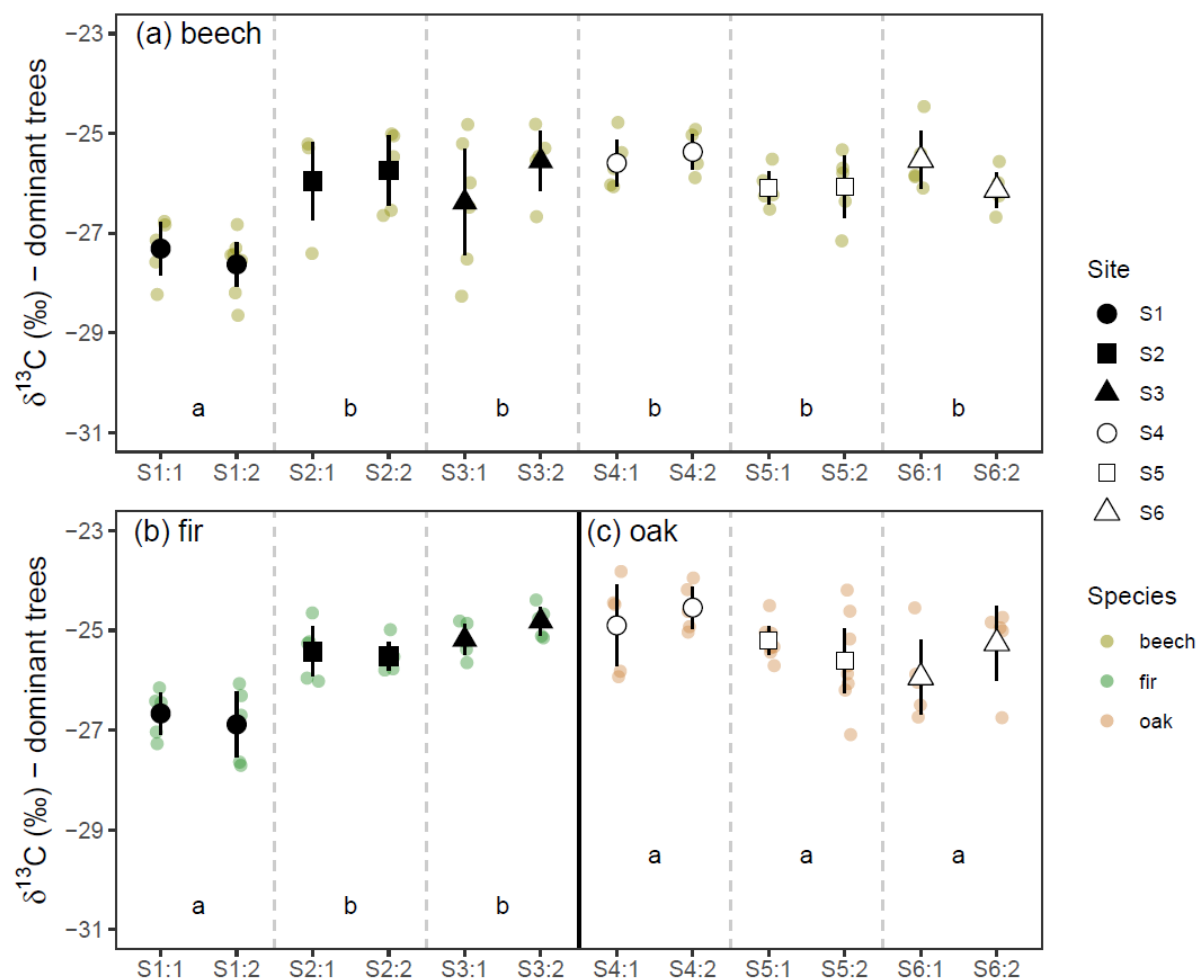
In pure beech stands, mean  $\delta^{13}\text{C}$  values for the selected dry years for all sampled trees ranged from  $-27.9 \pm 0.8 \text{ ‰}$  at S1:1 to  $-25.7 \pm 0.5 \text{ ‰}$  at S2:2 (**Fig. I. 3a**). There was a significant site effect on  $\delta^{13}\text{C}$  values (Eq. 5,  $P < 0.001$ ): the trees at S1 had significantly lower  $\delta^{13}\text{C}$  values than at the other five sites (**Fig. I. 3a**). Mean  $\delta^{13}\text{C}$  values differed significantly between the two triplets of a site at S6 only (**Fig. I. 3a**). The range of mean values for the dominant-tree subsample was similar to the range for all trees, but the  $\delta^{13}\text{C}$  values were higher, with beech mean  $\delta^{13}\text{C}$  values ranging from  $-27.6 \pm 0.4 \text{ ‰}$  at S1:2 to  $-25.4 \pm 0.4 \text{ ‰}$  at S4:2 (**Fig. I. 4a**). As for all trees, there was a significant site effect on  $\delta^{13}\text{C}$  values (Eq. 5,  $P < 0.001$ ): the trees at S1 had significantly lower  $\delta^{13}\text{C}$  values than the trees at the other five sites (**Fig. I. 4a**).

In pure fir stands, mean  $\delta^{13}\text{C}$  values for all sampled trees ranged from  $-28.0 \pm 0.6 \text{ ‰}$  at S1:2 to  $-25.2 \pm 0.3 \text{ ‰}$  at S3:1 (**Fig. I. 3b**). There was a significant site effect on  $\delta^{13}\text{C}$  values (Eq. 5,  $P < 0.001$ ): the trees at S1 had significantly lower  $\delta^{13}\text{C}$  values than the trees at S2 and S3 (**Fig. I. 3b**). Mean  $\delta^{13}\text{C}$  values differed significantly between the two triplets of a site at S3 only (**Fig. I. 3b**). For the dominant-tree subsample, the range of mean values was narrower and the  $\delta^{13}\text{C}$  values were higher, with fir mean  $\delta^{13}\text{C}$  values ranging from  $-26.9 \pm 0.7 \text{ ‰}$  at S1:2 to  $-24.8 \pm 0.3 \text{ ‰}$  at S3:2 (**Fig. I. 4b**). As for all trees, there was a significant site effect on  $\delta^{13}\text{C}$  values (Eq. 5,  $P < 0.001$ ): the trees at S1 had significantly lower  $\delta^{13}\text{C}$  values than the trees at the other five sites (**Fig. I. 4b**).

In pure pubescent oak stands, mean  $\delta^{13}\text{C}$  values for all sampled trees ranged from  $-26.1 \pm 0.3 \text{ ‰}$  at S6:1 to  $-24.8 \pm 0.4 \text{ ‰}$  at S4:2 (**Fig. I. 3c**). There was a significant site effect on  $\delta^{13}\text{C}$  values (Eq. 6,  $P < 0.001$ ): all three sites had significantly different  $\delta^{13}\text{C}$  values (**Fig. I. 3c**). For the dominant-tree subsample, the range of mean values was narrower, but the mean  $\delta^{13}\text{C}$  values were similar to all trees: oak mean  $\delta^{13}\text{C}$  values ranged from  $-25.9 \pm 0.7 \text{ ‰}$  at S6:1 to  $-24.5 \pm 0.4 \text{ ‰}$  at S4:2 (**Fig. I. 4c**). We observed no significant differences among the three sites or between triplets within sites (**Fig. I. 4c**).



**Fig. I. 3** Tree-ring  $\delta^{13}\text{C}$  values for all the trees sampled in pure stands, by site and triplet, for (a) beech, (b) fir, and (c) oak. Closed and open symbols with error bars (95 % confidence intervals) show mean plot values. Letters at the bottom of each graph indicate results for differences among sites by species (Eq. 5). The pure plots of the two triplets within each site are shown separately (i.e., S1:1 is triplet 1 in site S1). Stars indicate significant differences between the triplets of a site in pure stands of the same species (Eq. 5). Dots in the background represent individual tree data. For individual points, a jitter function is used to add a small amount of random noise on the x-axis to show overlapping points.



**Fig. I. 4** Tree-ring  $\delta^{13}\text{C}$  values for all the trees in the dominant-tree subsample for pure stands, by site and triplet for (a) beech, (b) fir, and (c) oak. Closed and open symbols with error bars (95 % confidence intervals) show mean plot values. Letters at the bottom of each graph indicate results for differences among sites by species (Eq. 5). The pure plots of the two triplets within each site are shown separately (i.e., S1:1 is triplet 1 in site S1). Stars indicate significant differences between the two triplets of a site in pure stands of the same species (none here) (Eq. 5). Dots in the background represent individual tree data. For individual points, a jitter function is used to add a small amount of random noise on the x-axis to show overlapping points.

### 3.1.2. Species differences in $\delta^{13}\text{C}$

For pure stands including data for all the trees, species effect was not significant between beech and fir in the more northern sites ( $P = 0.38$ ), but was significant between beech and oak in the more southern sites ( $P < 0.001$ ). When including  $\log(\text{DBH})$  as a covariate in the model (Eq. 6),  $\log(\text{DBH})$  was always significant. Beech had significantly lower  $\delta^{13}\text{C}$  values than fir in the northern sites or than oak in the southern sites (**Table I.B 1**). The interaction between  $\log(\text{DBH})$  and  $\delta^{13}\text{C}$  was significant with all trees (**Table I.B 1**), meaning that the slope of the relationship between  $\log(\text{DBH})$  and  $\delta^{13}\text{C}$  values differed between species, although it was always positive. In the northern sites, fir had a stronger positive slope ( $b=1.76$ ) than beech ( $b=0.85$ ). In the southern sites, beech had a stronger positive slope ( $b=2.23$ ) than oak ( $b=0.85$ ).

For pure stands with only the dominant-tree subsample, species effect was significant for beech-fir sites ( $P < 0.001$ ) and for beech-oak sites ( $P < 0.01$ ). When including  $\log(\text{DBH})$  in the models (Eq. 6), the species effect was still significant but neither  $\log(\text{DBH})$  nor the interaction between *Species* and  $\log(\text{DBH})$  were significant (**Table I.B 1**). Beech had significantly lower  $\delta^{13}\text{C}$  values than fir in the northern sites or than oak in the southern sites (**Table I.B 1**). Mean species  $\delta^{13}\text{C}$  values of the dominant-tree subsample was  $-26.5 \pm 0.4$  ‰ for beech and  $-25.8 \pm 0.3$  ‰ for fir at the northern sites, whereas it was  $-25.8 \pm 0.2$  ‰ for beech and  $-25.3 \pm 0.3$  ‰ for oak at the southern sites.

## 3.2. Mixture effects on $\delta^{13}\text{C}$

### 3.2.1. Effect of dbh

Before analyzing the mixture effects on  $\delta^{13}\text{C}$  values, we assessed the effect of  $\log(\text{DBH})$  in both the global (Eq. 7) and triplet-level (Eq. 8) models for each species. The effect of  $\log(\text{DBH})$  was significant for all four global models when all the sampled trees were included (**Table I. 2**). At the triplet-level,  $\log(\text{DBH})$  was significant for beech, except at S1:2 and S2:2. It was significant for all fir triplets and for half of the oak triplets (**Table I. 3**). The positive relationships between  $\delta^{13}\text{C}$  values and  $\log(\text{DBH})$  are consistent with expectations from the literature and with the impact of canopy light gradients on leaf or needle  $\delta^{13}\text{C}$  (McCarroll and Loader 2004). When only the dominant-tree subsample was used in the models, the effect of  $\log(\text{DBH})$  on  $\delta^{13}\text{C}$  was not significant in the global models nor in most triplets, except at S4:2 for beech and S2:2 for fir (**Table I. 2, Table I. 3**). The absence of significant effects for the dominant-tree subsample was the consequence of our sampling design: this subsample only included trees that received direct vertical light and for which the possible impact of canopy light gradients on  $\delta^{13}\text{C}$  (McCarroll and Loader 2004) was not expected.

**Table I. 2** Linear mixed model output for *Mixture* and  $\log(DBH)$  effects on  $\delta^{13}C$  by type of mixture and species (Eq. 7).

	Beech-fir sites						Beech-oak sites					
	Beech			Fir			Beech			Oak		
	$\chi^2$	<i>df</i>	<i>P</i>	$\chi^2$	<i>df</i>	<i>P</i>	$\chi^2$	<i>df</i>	<i>P</i>	$\chi^2$	<i>df</i>	<i>P</i>
<b>All trees</b>	154			213			191			189		
$\log(DBH)$	27	1	<.001***	268	1	<.001***	185	1	<.001***	21	1	<.001***
<i>Mixture</i>	0	1	0.77	2	1	0.13	26	1	<.001*** $\delta^{13}C_m > \delta^{13}C_p$	1	1	0.35
$\log(DBH):Mixture$	0	1	0.52	4	1	0.04*	1	1	0.24	1	1	0.30
$R_m^2 (R_c^2)$	0.16 (0.65)			0.40 (0.76)			0.41 (0.68)			0.10 (0.46)		
<b>Dominant-tree subsample</b>	52			48			50			58		
$\log(DBH)$	0	1	0.58	3	1	0.07	0	1	0.90	1	1	0.27
<i>Mixture</i>	2	1	0.21	1	1	0.25	0	1	0.49	2	1	0.15
$\log(DBH):Mixture$	2	1	0.14	5	1	0.02*	4	1	0.05	1	1	0.35
$R_m^2 (R_c^2)$	0.03 (0.52)			0.10 (0.62)			0.06 (0.26)			0.05 (0.32)		

*Note:* Stars indicate significant  $\log(DBH)$ , *Mixture* and interaction effects. *Mixture* is a two-level factor (pure vs. mixed). For significant *Mixture* effects, the direction of the estimate is indicated by  $\delta^{13}C_p > \delta^{13}C_m$  if pure stands have a higher  $\delta^{13}C$  estimate than mixed stands (i.e., mixture improves response to water stress compared to pure stands), and  $\delta^{13}C_m > \delta^{13}C_p$  if mixed stands have a higher  $\delta^{13}C$  estimate than pure stands.  $\chi^2$ : chi square test results for type “II” ANOVA. *df*: degrees of freedom. *P*: p-value for fixed terms. *Triplet ID* is used as a random factor in all eight models. A pseudo R-squared for mixed effect models was used to determine the variance explained by the two fixed terms only (marginal coefficient of determination,  $R_m^2$ ) and the entire model including the random terms (conditional coefficient of determination,  $R_c^2$ ).

### 3.2.2. Beech-fir sites

At the tree level for the global model, there were no significant mixture effects on  $\delta^{13}C$  values during the selected dry years, either for all trees or the dominant-tree subsample (Table I. 2). This result indicates that beech and fir trees in the mixed stands had statistically similar  $\delta^{13}C$  values to their values in pure stands for a given DBH (Table I. 2). The interaction between  $\log(DBH)$  and *Mixture* was significant for fir with all trees: both pure and mixed stands had a positive relationship between  $\log(DBH)$  and  $\delta^{13}C$  values, but pure stands had a stronger slope ( $b=1.69$ ) than mixed stands ( $b=1.35$ ). Despite this difference in the slopes of the relationship, it did not result in a statistical difference between pure and mixed stands. We also included the interaction term for the dominant-tree subsample, although we did not expect any interaction between  $\log(DBH)$  and *Mixture* as (i) trees of this subsample should receive the same levels of light and (ii) because none of the triplets, except one for fir, had a significant  $\log(DBH)$  effect. There was a small interaction effect for fir in the global model with dominant trees (Table I. 2).



**Table I. 3** Model p-values for Mixture and log(DBH) effects on  $\delta^{13}\text{C}$  by triplet and species (Eq. 8).

Site+Triplet	Beech			Fir			Oak		
	<i>log(DBH)</i>	<i>Mixture</i>	<i>log(DBH): Mixture</i>	<i>log(DBH)</i>	<i>Mixture</i>	<i>log(DBH): Mixture</i>	<i>log(DBH)</i>	<i>Mixture</i>	<i>log(DBH): Mixture</i>
<b>All trees</b>									
S1:1	0.03*	0.13	0.67	<0.001***	<0.01** $\delta^{13}\text{C}_m > \delta^{13}\text{C}_p$	0.7	–	–	–
S1:2	0.18	0.24	0.31	<0.001***	0.94	0.15	–	–	–
S2:1	<0.001***	0.62	0.45	<0.001***	<0.01** $\delta^{13}\text{C}_m > \delta^{13}\text{C}_p$	0.35	–	–	–
S2:2	0.42	0.85	0.61	<0.001***	0.66	0.09	–	–	–
S3:1	0.03*	0.44	0.62	<0.01**	0.09	0.77	–	–	–
S3:2	<0.01**	0.54	0.82	<0.001***	0.87	0.04*	–	–	–
S4:1	<0.001***	0.06	0.6	–	–	–	0.04*	0.47	0.49
S4:2	<0.001***	<0.001*** $\delta^{13}\text{C}_m > \delta^{13}\text{C}_p$	<0.01**	–	–	–	0.02*	0.02* $\delta^{13}\text{C}_p > \delta^{13}\text{C}_m$	0.75
S5:1	<0.001***	0.32	0.39	–	–	–	0.06	<0.01** $\delta^{13}\text{C}_m > \delta^{13}\text{C}_p$	0.83
S5:2	<0.001***	<0.001*** $\delta^{13}\text{C}_m > \delta^{13}\text{C}_p$	0.15	–	–	–	0.81	0.09	0.67
S6:1	<0.01**	0.12	0.19	–	–	–	0.21	0.53	0.56
S6:2	<0.001***	0.22	0.95	–	–	–	<0.01**	0.55	0.88
<b>Dominant-tree subsample</b>									
S1:1	0.88	0.97	0.53	0.48	0.93	0.02*	–	–	–
S1:2	0.17	0.09	0.17	0.09	0.68	0.46	–	–	–
S2:1	0.95	0.54	0.59	0.10	0.57	0.61	–	–	–
S2:2	0.51	0.52	0.51	<0.01**	0.04* $\delta^{13}\text{C}_p > \delta^{13}\text{C}_m$	0.05	–	–	–
S3:1	0.45	0.48	0.86	0.40	0.34	0.28	–	–	–
S3:2	0.30	0.98	0.07	0.87	0.06	0.24	–	–	–
S4:1	0.76	0.47	0.55	–	–	–	0.22	0.37	0.65
S4:2	<0.001***	<0.001*** $\delta^{13}\text{C}_m > \delta^{13}\text{C}_p$	<0.001***	–	–	–	0.63	0.18	0.47
S5:1	0.62	0.53	0.78	–	–	–	0.48	0.62	0.43
S5:2	0.51	0.06	0.7	–	–	–	0.36	0.21	0.43
S6:1	0.19	0.19	0.93	–	–	–	0.31	0.96	0.09
S6:2	0.98	0.18	0.42	–	–	–	0.81	0.44	0.44

Note: Stars indicate significant *log(DBH)*, *Mixture* or interaction effects. *Mixture* is a two-level factor (pure vs. mixed). For significant *Mixture* effects, the direction of the estimate is indicated by  $\delta^{13}\text{C}_p > \delta^{13}\text{C}_m$  if pure stands have a higher  $\delta^{13}\text{C}$  estimate than mixed stands (i.e., mixture improves response to water stress compared to pure stands), and  $\delta^{13}\text{C}_m > \delta^{13}\text{C}_p$  if mixed stands have a higher  $\delta^{13}\text{C}$  estimate than pure stands.

At the triplet level (**Table I. 3**, **Fig. I. 5a**, **Fig. I. 6a**), there were no significant mixture effects for beech in any of the triplets, either for all trees or the dominant-tree subsample. There were no significant interactions between  $\log(DBH)$  and *Mixture* for beech. For fir, at the triplet level for all trees, a significant mixture effect was found for triplets S1:1 and S2:1 only. In both these triplets, the models predicted higher  $\delta^{13}C$  values in mixed stands than in pure stands (**Table I. 3**). For fir with all trees, only triplet S3:2 had a significant interaction between  $\log(DBH)$  and *Mixture*: the pure stand had a steeper slope ( $b=2.22$ ) than the mixed stand ( $b=1.33$ ). There was no mixture effect for fir with the dominant-tree subsample except for triplet S2:2 (**Table I. 3**). Triplet S1:1 for fir with the dominant-tree subsample had a significant interaction effect when including all trees (**Table I. 3**), but this was probably driven by the differences in DBH of the dominant trees between stands (**Table I. 1**).

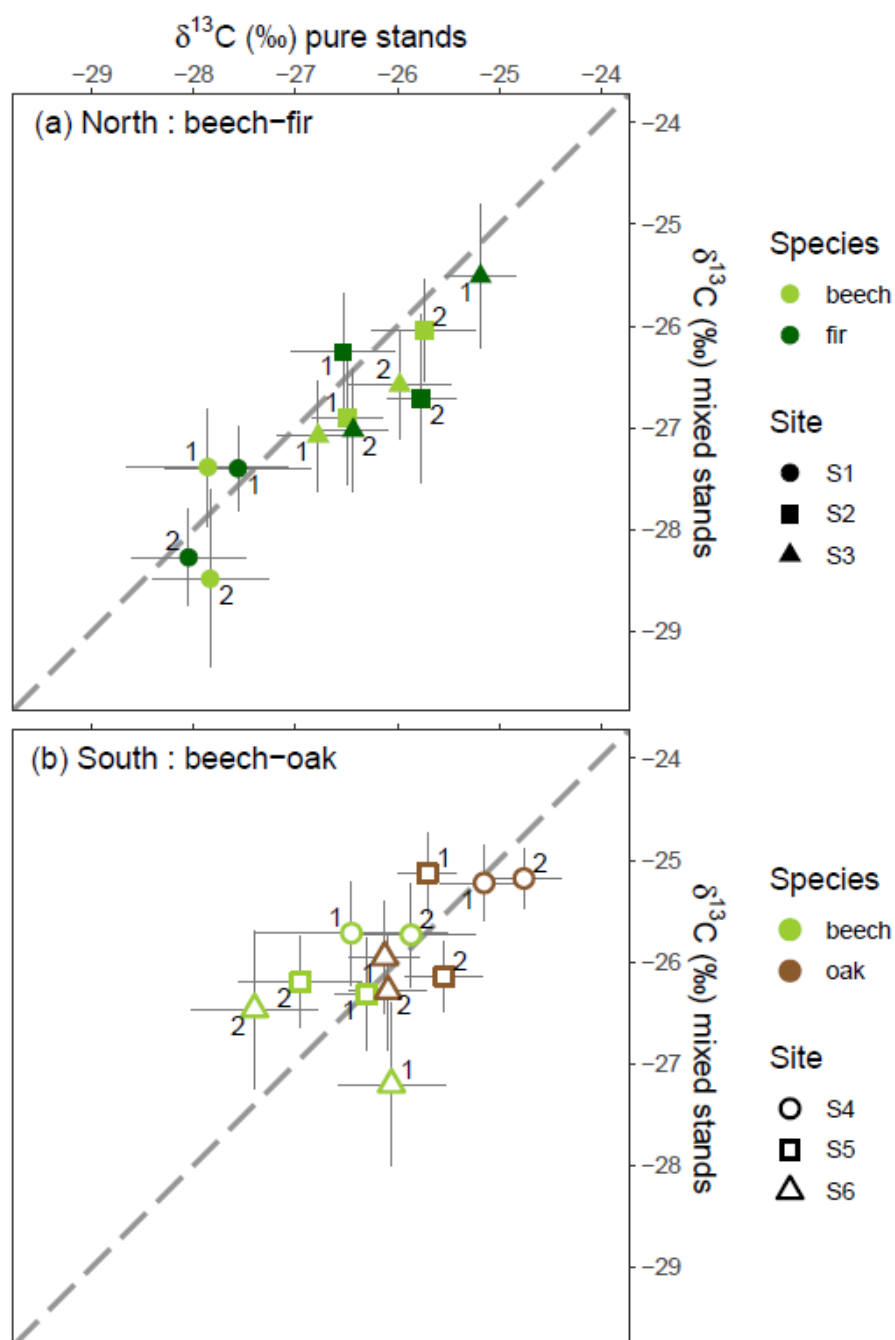
Three triplets had  $NBE_{dom}$  values close to 0.0 ‰ and the three other triplets had values below -0.4 ‰ (**Fig. I. 7**).

### 3.2.3. Beech-oak sites

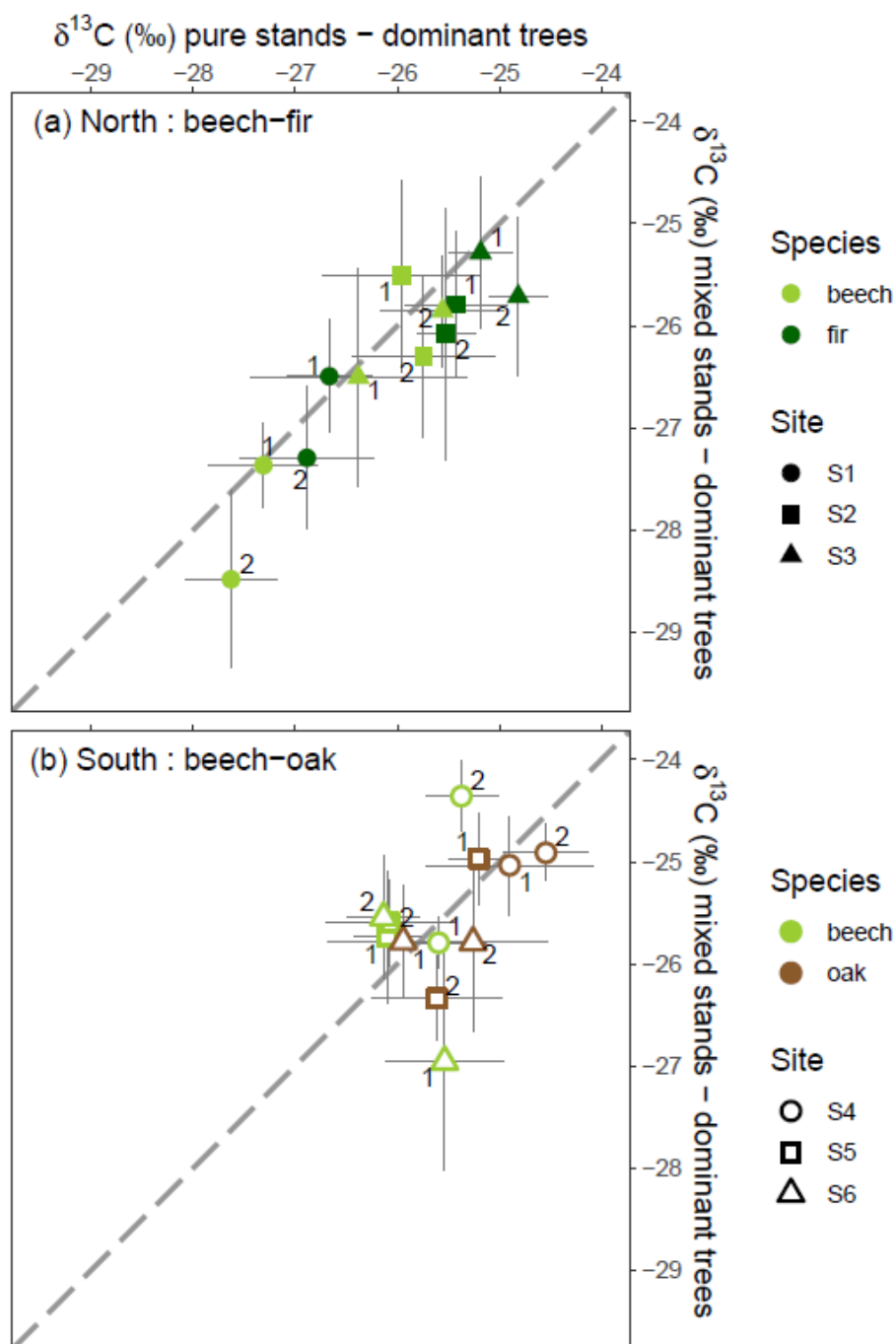
When including all trees in the global model, we found a significant mixture effect on beech  $\delta^{13}C$ , with higher  $\delta^{13}C$  values in mixed stands compared to pure ones (**Fig. I. 5b**, **Table I. 2**). There was no significant mixture effect for oak (**Fig. I. 5b**, **Table I. 2**). We no longer observed the significant effect for beech when we restricted the analysis to the dominant-tree subsample (**Fig. I. 6b**, **Table I. 2**). The interaction between  $\log(DBH)$  and *Mixture* was not significant for either species with all trees and with the dominant-tree subsample (**Table I. 2**).

At the triplet level, we observed significant mixture effects for both beech and oak (**Table I. 3**). For beech, when including all the trees,  $\delta^{13}C$  values were higher in mixed stands than in pure stands in triplets S4:2 and S5:2 (**Table I. 3**). This result held true for the beech dominant-tree subsample at S4:2, but not at S5:2 (**Table I. 3**). S4:2 was the only triplet with a significant interaction effect between  $\log(DBH)$  and *Mixture* for beech (**Table I. 3**), with a steeper slope for mixed stands ( $b= 3.78$ ) than pure stands ( $b=1.69$ ). However, this did not change the mixture effect for this triplet. For oak, when all the trees were included,  $\delta^{13}C$  values were significantly lower in mixed stands at S4:2 and significantly higher at S5:1 (**Table I. 3**). We did not observe these effects for oak when testing the dominant-tree subsample only (**Table I. 3**). There were no interaction effects between  $\log(DBH)$  and *Mixture* for oak (**Table I. 3**) with all trees and with the dominant-tree subsample.

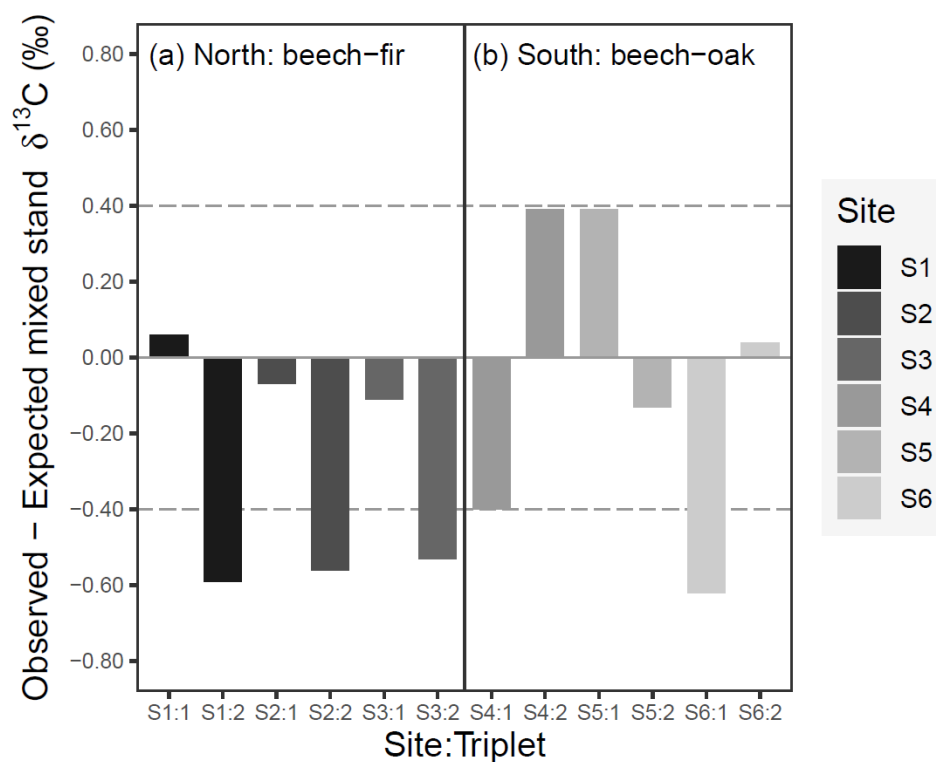
In this region, there was a trend toward the lack of a net biodiversity effect: most  $NBE_{dom}$  values were close to 0.0 ‰ (**Fig. I. 7**), except in triplet S6:1 where it was below -0.4 ‰.



**Fig. I. 5** Mean tree-ring  $\delta^{13}\text{C}$  values of all the sampled trees in pure versus mixed stands by species, site and triplet for (a) beech-fir sites (S1 to S3, closed symbols) and (b) beech-oak sites (S4 to S6, open symbols). Symbols with error bars (95 % confidence intervals) show mean plot values for pure (x-axis) vs. mixed (y-axis) stands. Numbers 1 and 2 represent the triplet number within the site. The dashed lines are the identity lines of slope 1 and intercept 0.



**Fig. I. 6** Mean tree-ring  $\delta^{13}\text{C}$  values of the dominant-tree subsample for pure versus mixed stands by species, site and triplet for (a) beech-fir sites (S1 to S3, closed symbols) and (b) beech-oak sites (S4 to S6, open symbols). Symbols with error bars (95 % confidence intervals) show mean plot values for pure (x-axis) vs. mixed (y-axis) stands. Numbers 1 and 2 represent the triplet number within the site. The dashed lines are the identity lines of slope 1 and intercept 0.

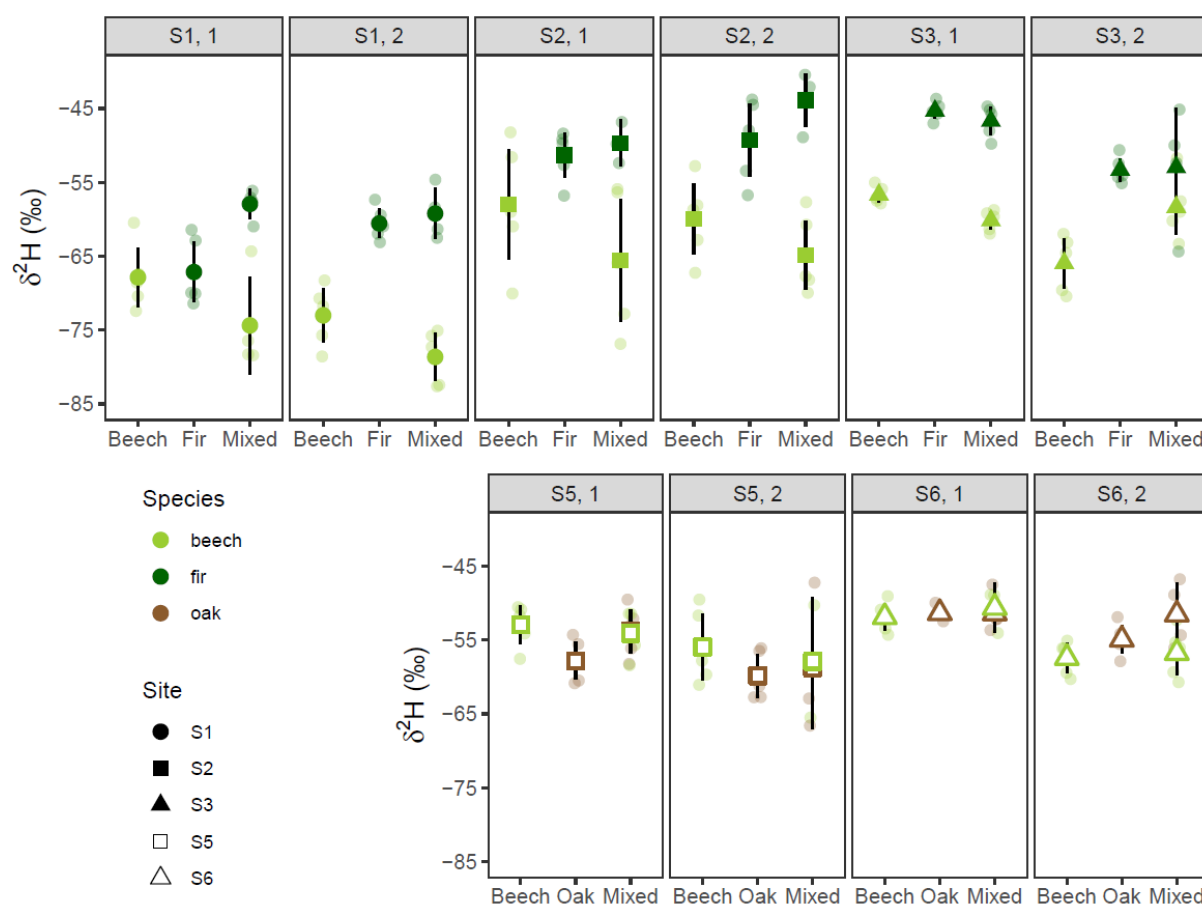


**Fig. I. 7** Net Biodiversity Effect (NBE) for  $\delta^{13}\text{C}$  values at the stand level (see **Eq. 9**) for the dominant-trees subsample. Differences under 0.4 ‰ are considered as not different from 0.0 ‰. A positive NBE means that the observed mixed stand had a higher (less negative)  $\delta^{13}\text{C}$  value than expected based on data from pure stands. A negative NBE means that the observed mixed stand had a lower (more negative)  $\delta^{13}\text{C}$  value than expected based on data from pure stands.

### 3.3. Xylem-water $\delta^2\text{H}$

At the beech-fir sites, we observed a significant interaction effect between *Species* and *Mixture* ( $P=0.002$ ) (**Table I.B 2**). There was a significant mixture effect on the  $\delta^2\text{H}$  values for beech, but not for fir. More specifically, beech  $\delta^2\text{H}$  values were lower in mixed stands than in pure stands, except for triplet S3:2 (**Fig. I. 8**). There was also a significant difference between species for  $\delta^2\text{H}$  values in both pure and mixed stands ( $P<0.001$ ) (**Table I.B 2**): beech  $\delta^2\text{H}$  values were lower than those of fir, except in triplet S1:1 (**Fig. I. 8**). The difference between beech and fir was larger in mixed stands (estimate:  $15.3 \pm 1.4$  ‰) than in pure stands (estimate:  $9.1 \pm 1.3$  ‰).

In beech-oak sites, we observed no mixture effect on  $\delta^2\text{H}$  values for either beech or oak ( $P=0.33$ ) (**Fig. I. 8**), no differences between species ( $P=0.71$ ), and no interaction effect ( $P=0.16$ ) (**Table I.B 2**).



**Fig. I. 8** Xylem-water  $\delta^2\text{H}$  values of the dominant-tree subsample by triplet and species. Each box represents a triplet with values for both species (beech-fir in the top line and beech-oak in the bottom line). The x-axis represents the different plots within a triplet (two pure plots and one mixed plot). Closed and open symbols with error bars (95 % confidence intervals) show mean plot values. Dots in the background represent individual tree data. For site S5:1 in the mixed plot, the average for beech and oak overlap. No data could be recorded at site S4.

## 4. Discussion

### 4.1. Variability of $\delta^{13}\text{C}$ values in pure stands

#### 4.1.1. Site effect for beech

Our range of mean  $\delta^{13}\text{C}$  values in beech tree-rings for pure stands was close to those reported in the literature (**Fig. I. 3a**, **Fig. I. 4a**). For instance, in Germany, at the heart of beech's distribution range, mean beech  $\delta^{13}\text{C}$  value was  $-26.7\text{‰}$  during the very dry year of 2003 (Schwarz and Bauhus 2019). In northern Spain, the southern-most area of beech distribution, mean  $\delta^{13}\text{C}$  value was  $-26.5\text{‰}$  at a drier lower-elevation site and  $-27.5\text{‰}$  at a wetter higher-elevation site for the period 2000 to 2003 (Peñuelas et al. 2008). We therefore recorded one of the least negative mean  $\delta^{13}\text{C}$  values for beech published to date, i.e.  $-25.4 \pm 0.4\text{‰}$  at S4:2 for the dominant-tree subsample (**Fig. I. 4a**). The origin of the high  $\delta^{13}\text{C}$  values observed in our study can be explained (i) by our choice to select, by design, a range of years characterized by severe summer droughts (2003-2007), which undoubtedly triggered high tree water use efficiency (Saurer et al. 1995), and (ii) by the fact that some of our sites were at the range limit of precipitation and temperature conditions for beech in Europe (**Fig. I. 2**).

We found that the mean  $\delta^{13}\text{C}$  values for beech in pure stands did not differ among sites except at S1, either for all trees or for the dominant-tree subsample (**Fig. I. 3a**, **Fig. I. 4a**), though we had expected stronger differences due to the wide range of climatic conditions across our sites (**Fig. I. 2**). Plant tissue  $\delta^{13}\text{C}$  is a functional trait that is highly sensitive to differences in environmental conditions (Farquhar et al. 1989). However, different environmental conditions can have opposite effects on  $\delta^{13}\text{C}$  values, and the lack of difference in  $\delta^{13}\text{C}$  among the five sites (S2 to S6) could be explained by a combination of conditions acting in opposite directions. We explain the significantly lower mean  $\delta^{13}\text{C}$  values for beech at S1 (**Fig. I. 3a**, **Fig. I. 4b**) by the fact that S1 has the wettest and coolest climate among the six sites (**Fig. I. 2**). Site S1 also had relatively low water stress and was the least affected by water deficit during the period 2003-2007 covered by our study (**Table I.A 3**).

#### 4.1.2. Species differences

The pure beech stands had significantly lower  $\delta^{13}\text{C}$  values than did fir or oak in the respective sites (**Table I.B 1**). This indicated lower mean water use efficiency for beech, even though the magnitude of the differences between species remained low. For beech and fir, lower  $\delta^{13}\text{C}$  values for beech are consistent with the study by Schwarz and Bauhus (2019) during the 2003 drought in Germany and Croatia. For beech and pubescent oak, to our knowledge, no studies have compared  $\delta^{13}\text{C}$  values of mature pubescent oak with other species. Differences in  $\delta^{13}\text{C}$  values among species can be explained by physiological and morphological characteristics (McCarroll and Loader 2004) or by differences in canopy structure and light availability in pure stands. Yet, we included a dendrometric variable

( $\log(DBH)$ ) in the statistical tests as a proxy for light availability when testing for species differences. Species effects remained significant in the models with the dominant-tree subsample while the effect of  $\log(DBH)$  did not (**Table I.B 1**). We can therefore conclude that species differences in  $\delta^{13}C$  were mainly related to intrinsic differences among species in water use efficiency, and not just to canopy structure factors.

As expected, a large part of the variability in  $\delta^{13}C$  values was explained by the  $\log(DBH)$  variable when we included all trees (**Table I.B 1**). The relationship between  $\delta^{13}C$  values and  $\log(DBH)$  was always positive. This is consistent with the fact that gradients in light availability in the canopy strongly influence  $\delta^{13}C$  values (McCarroll and Loader 2004). The slopes of this relationship differed between species: fir always had a stronger slope than beech in northern sites, indicating that small fir trees were more suppressed for light than small beech trees in their respective pure stands. As both species have high shade tolerance (Niinemets and Valladares 2006), this could simply be due to the canopy structure of fir trees leading to lower light availability for dominated fir trees. Beech had a stronger slope than oak in the southern sites, indicating that small beech trees were more suppressed for light than small oak trees in pure stands. As beech is more shade tolerant than oak (Niinemets and Valladares 2006), this could be explained by the fact that small beech trees are growing in light-suppressed environments whereas oak trees, even when small, seem to only grow in environments with a higher light availability leading to less variability in  $\delta^{13}C$  values. These results show that in addition to intrinsic species differences in water use efficiency, canopy structure and light interception also influenced water and carbon functioning in pure stands.

## 4.2. Mixture effects on $\delta^{13}C$

### 4.2.1. Beech-fir sites

For beech, there was no mixture effect on  $\delta^{13}C$  values during dry years when mixed with fir (**Table I. 2, Table I. 3, Fig. I. 5a, Fig. I. 6a**), despite strong differences in functional traits between these two species. The lack of mixture effect is consistent with several studies on beech-fir mixtures. For instance, at the same sites, Jourdan et al. (2019a) reported no effect of the proportion of fir on the drought resistance of beech, estimated with growth data at the neighborhood tree-to-tree level. In another study in Romania, no mixture effect was observed for beech on  $\delta^{13}C$  differences between a wet and a dry year, either at the species level (Forrester et al. 2016) or at the stand level (Grossiord et al. 2014d). Recently, Schwarz & Bauhus (2019) looked at the resistance to a drought event for four sites with beech-fir mixtures in Germany and Croatia and found no mixture effect on  $\delta^{13}C$ , even though they observed long-term positive effects of mixture on radial growth.

For fir, the influence of mixture on  $\delta^{13}C$  values during dry years was more variable (**Table I. 2, Table I. 3, Fig. I. 5a, Fig. I. 6a**). We observed that mixture effects for fir differed among sites, between triplets



within a given site, or according to the type of dataset we used. This suggests that local environmental conditions and stand structure strongly influenced the outcome of the tests for fir and that mixture effects may play a minor role in explaining observed differences. Consequently, we cannot conclude that including fir in mixtures with beech will mitigate fir exposure to drought. The existing literature has highlighted contrasted patterns for fir in mixtures with beech. Some studies observed that the sensitivity of fir to drought was reduced when mixed with beech in drier sites (Lebourgeois et al. 2013; Gazol and Camarero 2016), while other studies found no effect (Forrester et al. 2016; Schwarz and Bauhus 2019), or even a negative effect (Jourdan et al. 2019a). Differences in site conditions could explain discrepancies in the observed patterns between our results and previous studies, as mixture effects may change along resource gradients (Forrester and Bauhus 2016). The interaction effects detected for fir, with all trees, between  $\log(DBH)$  and stand composition suggest that there was less variability in  $\delta^{13}C$  values due to light availability in the mixed stands. In pure stands, small fir trees had lower light availability (more negative  $\delta^{13}C$  values) than in mixed ones. This could be explained by the fact that fir trees in mixed stands received more light than in pure stands due to canopy opening with the presence of beech and to phenological differences between fir and beech. However, these interactions did not impact the detected mixture effects.

At the stand level with both species, there was no single mixture effect pattern among sites and triplets for beech-fir sites (**Fig. I. 7**).  $NBE_{dom}$  was either neutral or negative, meaning that mixtures had more negative  $\delta^{13}C$  values, and potentially lower water stress, than expected from pure stands. Within each site, triplet  $NBE_{dom}$  values were often neutral for one triplet and negative for the other one. As a reminder, these triplets were not selected as replicates, but were designed to cover local variations in environmental conditions and stand structure; within-site variability was therefore not surprising. The neutral or negative  $NBE_{dom}$  values could be explained by differences in above-ground features, such as stand structure, proportion of shaded subcanopy or canopy packing levels, or in below-ground processes, such as complementarity or facilitation for water acquisition (Grossiord 2019). The species-interaction effects between beech and fir for some triplets may be attenuated or even masked by local differences in environmental conditions, which could not be controlled for in these stands (Metz et al. 2016). Furthermore, opposite processes that may strongly influence photosynthesis (e.g. light gradients) or water use (e.g. depth of water uptake) in mixed forests may actually cancel each other out and result in the absence of overall mixture effects (Forrester and Bauhus 2016). However, the overall result of  $NBE_{dom}$  for mixed beech-fir stands is that  $\delta^{13}C$  values were never less negative than expected, suggesting a potentially lower water stress in mixtures. These results differ from the mixture effects tested for each species separately. Differences between species-level and stand-level results have been observed before (Forrester et al. 2016) and can result from opposite trends of mixture effects between the species (Toïgo et al. 2015a). For the overall stand  $\delta^{13}C$  values, beech-fir mixtures were not detrimental in terms of functioning during dry years. We will discuss potential differences in water sources among species and

stand composition below; however, we were not able to characterize above-ground influences in the current study.

#### 4.2.2. Beech-oak sites

The presence of pubescent oak did not decrease beech exposure to drought. In fact, with all trees in the global model (**Table I. 2**) and for two individual triplets (**Table I. 3**), the interaction with pubescent oak even led to higher beech  $\delta^{13}\text{C}$  values, indicating a higher drought exposure of beech in mixed stands compared to pure ones. In the global model (**Table I. 2**) and in one of these two triplets (S5:2, **Table I. 3**), the absence of any mixture effect for the dominant-tree subsample suggested that these effects might be related to the social status of the trees, even though we were not able to demonstrate this. The interaction effect between  $\log(\text{DBH})$  and stand composition on  $\delta^{13}\text{C}$  values in S4:2 for beech in beech-oak sites (**Table I. 3**) was opposite to what was observed for fir in beech-fir sites: there was less variability in  $\delta^{13}\text{C}$  values due to light availability in the pure stand than in the mixed one. However, this effect was mostly driven by a few bigger trees in the pure stand (**Table I. 1**) and did not affect the observed mixture effect. Comparing these results with previous work on beech-pubescent oak mixtures is impossible as this is the first study to look at beech  $\delta^{13}\text{C}$  response in interaction with pubescent oak. Beech  $\delta^{13}\text{C}$  responses when mixed with another oak species (sessile oak, *Quercus petraea* (Matt.) Liebl.) in German forests indicated that it was less exposed to drought than in pure stands (Forrester et al. 2016). Compared to sessile oak, pubescent oak is more drought tolerant (Ninemets and Valladares 2006) and could be more competitive than sessile oak when mixed with beech. Furthermore, when looking at growth response patterns to drought in mixture with different oak species, beech showed very inconsistent results so far, with positive (Pretzsch et al., 2013; *Quercus petraea*), negative (Vanhellemont et al., 2019; *Quercus robur* L.) or neutral (Jourdan et al., 2019a; *Quercus pubescens*) patterns. Our results underline the current uncertainty on the choice of silvicultural practices that forest managers must make today to mitigate drought impact on beech productivity along its southern margins under future climate conditions.

The presence of beech did not influence the physiological functioning of pubescent oak during dry summers in this region (**Fig. I. 5b**, **Fig. I. 6b**, **Table I. 2**, **Table I. 3**). As with beech, we were not able to reveal any general trend for beech-oak interactions under severe drought conditions. Our study suggests that, at least in this region, managing pubescent oak with beech does not lead to clear mitigation of climate change impacts on oak response to drought.

#### 4.2.3. Impact of sampling design and use of absolute $\delta^{13}\text{C}$ values

Our results emphasize how the sampling design (all trees or dominant ones only) and level of analysis (tree or stand) of diversity-ecosystem functioning studies may influence the outcome of mixture effect tests. Limiting the sampling design to dominant trees when looking at mixture effects on the  $\delta^{13}\text{C}$

response of trees to drought has been the typical approach to date (Grossiord et al., 2014c; Metz et al., 2016; Schwarz and Bauhus, 2019). However, we show that this choice may bias the outcome of these mixture effect tests. Selecting dominant trees only is, indeed, pertinent with regards to  $\delta^{13}\text{C}$ , as dominant trees are those with the greatest sunlit leaf areas and therefore potential carbon (photosynthesis) and water (transpiration) exchange with the atmosphere. However, not including suppressed trees in these analyses may bias the stand-level patterns of mixture effects, as the sensitivity to drought of suppressed and dominant trees in mixed stands may differ (Mérian and Lebourgeois 2011). This highlights the need for a standardization of protocols in mixed-species forest studies in accordance with the goal of the study (e.g. ecophysiological or forest management questions).

A limitation of this study, as mentioned in the methods, was the use of absolute  $\delta^{13}\text{C}$  values for dry years instead of the difference in  $\delta^{13}\text{C}$  between wet and dry years. Calculating a difference between the  $\delta^{13}\text{C}$  of a wet and a dry year would allow analyzing a direct response of trees to drought and compare this response in pure and mixed stands independently of the micro-environmental variability among stands within a given triplet. However, in a study looking at diversity effects on drought responses of  $\delta^{13}\text{C}$ , the same trends were found with  $\delta^{13}\text{C}$  differences between wet and dry years (Grossiord et al. 2014d) than with absolute  $\delta^{13}\text{C}$  values for the dry years only (Grossiord, 2014, p.48). Then, this methodological caveat should not prevent us from interpreting mixture effects, especially since we took precautions to avoid over-interpreting these effects at the triplet scale.

### 4.3. Complementarity in water uptake sources

#### 4.3.1. Beech-fir sites

At the more northern beech-fir sites, during the 2018 summer drought, we observed a variability in  $\delta^2\text{H}$  values suggesting a plasticity in the depth of water uptake for beech at these sites between pure and mixed stands (**Fig. I. 8**). This is consistent with the known plasticity of the species in terms of rooting traits and depth of water uptake (Brinkmann et al. 2019). When competing with fir, beech seemed to have a deeper mean depth of water uptake than when competing with beech only. These results are in accordance with previous studies which show a shift of beech fine roots down to deeper soil horizons when competing with coniferous tree species (Bolte and Villanueva 2006; Grossiord et al. 2014a). Plasticity in functional traits can be the result of abiotic or biotic interactions (Valladares et al. 2007). Therefore, the shift in beech water uptake depth could be explained either by a response to the competition with fir roots in the same soil horizons, or by a response to a depletion in soil water availability in the topsoil (Brinkmann et al. 2019).

In the mixed stands, xylem  $\delta^2\text{H}$  values showed that beech tended to uptake water from deeper soil layers than fir did (**Table I.B 2, Fig. I. 8**), suggesting niche complementarity for water uptake between the two species. This kind of complementarity between species in water use is assumed to partly explain positive

biodiversity-ecosystem functioning relationships (Grossiord 2019). However, it has rarely been linked to mixture effects on plant growth (Mueller et al. 2013; Bachmann et al. 2015) or functional traits under drought (Verheyen et al. 2008; Goisser et al. 2016). In our study, the absence of a clear mixture effect on  $\delta^{13}\text{C}$  for both beech and fir, despite a complementarity in water uptake, may be explained by several factors. First, our results did not allow us to quantify the real gain in soil water availability during these dry periods for beech. Indeed, the shift in  $\delta^2\text{H}$  between pure and mixed stands was slight (10 ‰ on average) and may indicate only a small difference in water uptake depth and soil water content. Second, below-ground processes may not be the main drivers of the mixture effects for these species in the study region. Above-ground processes could well be the major drivers, but they were not tested in this study. Third, as discussed by Goisser et al. (2016), the impact of species mixture on soil water availability may vary across seasons. The time scale of the processes associated with our  $\delta^2\text{H}$  or  $\delta^{13}\text{C}$  approaches were different:  $\delta^2\text{H}$  gave a one-shot image of the mean signature of soil water extracted during the summer, whereas the  $\delta^{13}\text{C}$  values gave an integrated measurement of carbon and water acquisition and use over five years with dry summers.

#### 4.3.2. Beech-oak sites

For the more southern beech-oak sites, there were no signs of complementarity or niche differentiation in water acquisition for either species (**Table I.B 2, Fig. I. 8**). This result is consistent with the absence of mixture effects on  $\delta^{13}\text{C}$  for beech and pubescent oak in most triplets. The absence of plasticity in water uptake for beech when mixed with pubescent oak is interesting to note in comparison to the beech-fir sites. It suggests that the presence of pubescent oak did not induce any changes in beech water uptake compared to pure beech stands. The shallow soils in our sites (especially S5) might be one reason for the absence of differences in rooting depth between the species. We did not find data in the literature on rooting depth of pubescent oak. However, Lebourgeois and Jabiol (2002) have shown that beech rooting depth, compared to two other oak species (*Quercus petraea* and *Quercus robur*), is more sensitive to constraints but also that in the absence of strong constraints, beech and oak species rooting profiles are similar. In this part of the beech distribution area, during extreme dry summers, beech and pubescent oak displayed a functional redundancy in their below-ground water uptake. Mixing these two species to improve beech access to soil water in southern France may not be an appropriate forest management strategy.

## 5. Conclusion

Our study was designed to help decision-making for beech forest management strategies adapted to more frequent and extreme droughts in the future, particularly at the limits of the distribution area of beech. Overall, we found no major mixture effect on drought exposure for beech, although there was a high variability in the outcome of mixture effects at the triplet scale. In the northern part of our study, mixing beech with fir had no effect on beech  $\delta^{13}\text{C}$  values during dry years. This result is in accordance with a growing body of literature showing the lack of a species-interaction effect on beech drought resistance. Managing mixed stands of beech and fir does not seem to help mitigate drought impacts for either of the two species, despite an observed complementarity in water uptake. In the southern part of our study, mixture effects on beech were mostly neutral, although beech seemed to be more exposed to drought in mixed stands with pubescent oak at some sites. In a region at the limits of the distribution area of beech, this study allowed us to conclude that managing beech in mixed stands with silver fir or pubescent oak does not buffer drought impacts during dry years. Yet, in the long term, as extreme droughts will become more frequent, beech-fir mixtures should not be detrimental to beech response to drought, while it might be in mixtures with pubescent oak. In the southernmost distribution regions of beech, an overall assessment of multi-criteria potential benefits for each type of mixture should then be conducted in order to take management decisions.

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## CRedit authorship contribution statement

**Soline Martin-Blangy:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft. **Marie Charru:** Conceptualization, Methodology, Investigation, Writing - original draft, Supervision. **Sylvain Gérard:** Investigation, Writing - review & editing. **Hervé Jactel:** Conceptualization, Resources, Project administration, Funding acquisition, Writing - review & editing. **Marion Jourdan:** Resources, Investigation, Writing - review & editing. **Xavier Morin:** Conceptualization, Investigation, Resources, Writing - review & editing. **Damien Bonal:** Conceptualization, Methodology, Investigation, Writing - original draft, Supervision.

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# Appendices

## 6. Appendices

### 6.1. Appendix I.A: Additional plot characteristics

**Table I.A 1** Environmental characteristics of the triplets.

Site	Triplet	Triplet ID	Lat. (°N)	Lon. (°E)	Mean Elevation (m)	Mean Slope (°)
S1	1	S1:1	45.70548	6.2192	1198	21
S1	2	S1:2	45.71188	6.21625	1025	18
S2	1	S2:1	44.90231	5.3513	1390	20
S2	2	S2:2	44.9411	5.32774	1137	11
S3	1	S3:1	44.17788	5.24168	1354	27
S3	2	S3:2	44.18707	5.25234	1328	27
S4	1	S4:1	43.97376	5.48	1094	13
S4	2	S4:2	43.97661	5.47961	1082	12
S5	1	S5:1	43.8203	5.53343	1005	26
S5	2	S5:2	43.82269	5.53423	889	34
S6	1	S6:1	43.33197	5.77038	736	8
S6	2	S6:2	43.33281	5.77328	746	12

**Table I.A 2** Stand structure for each plot, triplet and site. Plot dendrometric variables include: the dominant height of the plot ( $H_{dom}$ , in m) and the basal area of all stems by plot and species (in  $m^2 ha^{-1}$ ).

Site	Triplet	Plot	$H_{dom}$ (m)	Basal area ( $m^2 ha^{-1}$ )				Total
				Beech	Fir	Oak	Other sp.	
S1	1	Pure beech	37	80.3	–	–	4.5	84.8
S1	1	Pure fir	30	0.2	81.0	–	8.5	89.7
S1	1	Mixed	28	27.4	29.6	–	4.3	61.4
S1	2	Pure beech	34	46.0	5.5	–	–	51.5
S1	2	Pure fir	34	–	62.5	–	7.4	69.9
S1	2	Mixed	33	32.7	45.5	–	5.1	83.3
S2	1	Pure beech	16	24.9	–	–	3.0	27.9
S2	1	Pure fir	22	–	50.5	–	1.4	51.9
S2	1	Mixed	18	16.0	18.9	–	1.4	36.2
S2	2	Pure beech	33	63.0	2.4	–	–	65.5
S2	2	Pure fir	30	0.6	64.4	–	–	65.0
S2	2	Mixed	26	27.5	19.2	–	–	46.7
S3	1	Pure beech	22	62.0	0.2	–	–	62.2
S3	1	Pure fir	16	0.9	47.5	–	0.8	49.3
S3	1	Mixed	18	17.6	39.4	–	–	57.0
S3	2	Pure beech	20	58.5	1.0	–	–	59.6
S3	2	Pure fir	17	14.1	52.1	–	1.2	67.5
S3	2	Mixed	20	32.0	20.5	–	4.7	57.3
S4	1	Pure beech	20	42.5	–	–	1.3	43.8
S4	1	Pure oak	12	1.3	–	25.3	2.0	28.6
S4	1	Mixed	16	12.5	–	24.2	9.8	46.5
S4	2	Pure beech	19	41.9	–	0.3	–	42.2
S4	2	Pure oak	13	1.0	–	19.0	0.7	20.6
S4	2	Mixed	15	35.5	–	11.9	1.7	49.1
S5	1	Pure beech	13	24.7	–	0.9	11.9	37.5
S5	1	Pure oak	11	–	–	34.4	9.7	44.1
S5	1	Mixed	11	15.2	–	13.0	8.0	36.2
S5	2	Pure beech	16	30.5	–	–	8.0	38.5
S5	2	Pure oak	10	–	–	24.3	4.2	28.5
S5	2	Mixed	12	11.9	–	12.0	5.4	29.3
S6	1	Pure beech	30	54.8	–	–	13.3	68.1
S6	1	Pure oak	19	–	–	61.6	18.2	79.8
S6	1	Mixed	21	23.3	–	24.2	22.5	70.0
S6	2	Pure beech	20	32.5	–	3.0	7.7	43.2
S6	2	Pure oak	16	–	–	33.4	0.5	33.9
S6	2	Mixed	19	22.3	–	21.2	8.1	51.7



**Table I.A 3** Average summer rainfall and summer temperature for 4-month summer periods (June to September) at each site calculated over two time periods and mean number of days with water stress ( $REW < 0.4$ ) over 2003-2007 from BILJOU© model.

Site	Name of region	Summer Rain (mm)	Summer Rain (mm)	Mean summer temp. (°C)	Mean summer temp. (°C)	Mean number of days with water stress
		1994-2013	2003-2007	1994-2013	2003-2007	2003-2007
<b>S1</b>	<b>Bauges</b>	619 ( $\pm 50$ )	581 ( $\pm 158$ )	13.7 ( $\pm 0.41$ )	14.3 ( $\pm 1.34$ )	1
<b>S2</b>	<b>Vercors</b>	429 ( $\pm 48$ )	332 ( $\pm 46$ )	14.5 ( $\pm 0.41$ )	15.1 ( $\pm 0.93$ )	52
<b>S3</b>	<b>Ventoux</b>	293 ( $\pm 39$ )	221 ( $\pm 35$ )	15.6 ( $\pm 0.37$ )	16.2 ( $\pm 1.01$ )	94
<b>S4</b>	<b>Lubéron Lagarde</b>	246 ( $\pm 38$ )	172 ( $\pm 36$ )	17.8 ( $\pm 0.37$ )	18.2 ( $\pm 1.08$ )	105
<b>S5</b>	<b>Grand Lubéron</b>	227 ( $\pm 30$ )	169 ( $\pm 40$ )	19.8 ( $\pm 0.35$ )	20.2 ( $\pm 1.16$ )	107
<b>S6</b>	<b>Sainte-Baume</b>	212 ( $\pm 39$ )	158 ( $\pm 56$ )	20.1 ( $\pm 0.37$ )	20.7 ( $\pm 0.93$ )	108

## 6.2. Appendix I.B: Additional model results

**Table I.B 1** Linear mixed model output for *Species* effect,  $\log(DBH)$  and their interaction on  $\delta^{13}C$  values in pure stands by type of mixture (Eq. 6).

	Beech-fir sites				Beech-oak sites			
	$\chi^2$	<i>df</i>	<i>P</i>	post-hoc	$\chi^2$	<i>df</i>	<i>P</i>	post-hoc
<b>All trees</b>		228				238		
log(DBH)	183	1	<.001***		113	1	<.001***	
Species	7	1	<.01**	beech<fir	92	1	<.001***	beech<oak
log(DBH):Species	17	1	<.001***		24	1	<.001***	
<i>R2m (R2c)</i>	0.30 (0.72)				0.40 (0.61)			
<b>Dominant-tree subsample</b>		54				56		
log(DBH)	0	1	0.61		0	1	0.48	
Species	11	1	<.001***	beech<fir	7	1	<.01**	beech<oak
log(DBH):Species	0	1	0.72		4	1	0.06	
<i>R2m (R2c)</i>	0.09 (0.63)				0.18 (0.28)			

*Note:* Stars indicate significant  $\log(DBH)$ , *Species* and interaction effects. *Species* is a two-level factor (beech-fir or beech-oak). For significant *Species* effects, the direction of the estimate is indicated by beech<fir (beech<oak) because fir (oak) trees have a higher  $\delta^{13}C$  estimate than beech stands.  $\chi^2$ : chi square test results for type “II” ANOVA. *df*: degrees of freedom. *P*: p-value for fixed terms. *Triplet ID* is used as a random factor in all four models. A pseudo R-squared for mixed effect models was used to determine the variance explained by the two fixed terms only (marginal coefficient of determination,  $R^2_m$ ) and the entire model including the random terms (conditional coefficient of determination,  $R^2_c$ ).

**Table I.B 2** Linear mixed model output for *Species* effect, *Mixture* and their interaction on  $\delta^2\text{H}$  of the dominant-tree subsample by type of mixture (Eq. 10).

	Beech-fir sites				Beech-oak sites		
	$\chi^2$	<i>df</i>	<i>P</i>	post-hoc	$\chi^2$	<i>df</i>	<i>P</i>
<b>Dominant-tree subsample</b>		104				65	
Species	149	1	<0.001***	beech<fir	0	1	0.71
Mixture	0	1	0.61		1	1	0.33
Species:Mixture	10	1	0.002**	beech: m<p fir: m=p pure: beech<fir mixed: beech<fir	2	1	0.16
<i>R</i> <sup>2</sup> <sub>m</sub> ( <i>R</i> <sup>2</sup> <sub>c</sub> )	0.36 (0.75)				0.03 (0.32)		

*Note:* Stars indicate significant *Species*, *Mixture* and interaction effects. *Species* is a two-level factor (beech-fir or beech-oak); *Mixture* is also a two-level factor (pure vs. mixed). For significant *Species* effects, the direction of the estimate is indicated by beech<fir because fir trees have a higher  $\delta^2\text{H}$  estimate than beech. Post-hoc results for the *Species:Mixture* interaction are similarly indicated.  $\chi^2$ : chi square test results for type “II” ANOVA. *df*: degrees of freedom. *P*: p-value for fixed terms. *Triplet ID* is used as a random factor in both models. A pseudo R-squared for mixed effect models was used to determine the variance explained by the two fixed terms only (marginal coefficient of determination,  $R^2_{\text{m}}$ ) and the entire model including the random terms (conditional coefficient of determination,  $R^2_{\text{c}}$ ).



## CHAPITRE II

# Effet du mélange sur l'interception de la lumière en plantation mélangée de pin et de bouleau



Photo : Soline Martin-Blangy. Plantation ORPHEE, Cestas, France.

**Préambule :** Ce chapitre a pour objectif de tester si le mélange augmente l'interception de la lumière à l'échelle du peuplement, via une modification de la forme et du volume des houppiers qui engendrerait une plus forte occupation de l'espace de la canopée (*canopy packing*). De plus, nous avons cherché à savoir comment les effets du mélange variaient avec la densité du peuplement et l'irrigation. Nous avons réalisé ce travail dans le cadre de la plantation expérimentale d'ORPHEE, dans des placettes pures et mélangées de bouleau (*Betula pendula*) et de pin maritime (*Pinus pinaster*). Nous avons estimé le volume des houppiers à partir de mesures sur le terrain, puis calculé l'occupation totale de l'espace de la canopée. Ce chapitre a été écrit sous forme d'article scientifique et soumis à la revue *Trees - Structure and Function* en avril 2021. J'ai repris le format du manuscrit soumis à la revue afin de l'inclure dans ce document de thèse.



**Title: Stand density and irrigation modulate mixing effects on crown dimensions and canopy packing in a young pine-birch plantation.**

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## Abstract

Mixed-species plantation forests are of high interest due to their simultaneous potentially superior productivity and multi-functionality benefits compared to monocultures. However, how trees of different species interact at the canopy level in mixed forests remains unclear, even at young growth stages. We tested whether crown shape and size and stand-level canopy packing were affected by stand composition and how mixture effects varied with stand density and irrigation. We measured crown attributes in pure and mixed plots of two light-demanding species, silver birch (*Betula pendula* Roth) and maritime pine (*Pinus pinaster* Ait.), in a 10-year-old tree diversity experiment (ORPHEE). This allowed us to estimate tree-level crown volumes and stand-level canopy packing. We found that (i) at the tree level, stand composition influenced crown-stem allometric relationships in pine but not in birch, (ii) mixture led to greater crown and tree dimensions in pine, but the opposite was true for birch, (iii) the changes in crown volume resulted in higher canopy packing in high-density mixed stands without irrigation, and finally (iv) stand density and irrigation modulated mixture effects on crown dimensions and canopy packing. This study sheds light on the effects of water constraints on the aboveground mechanistic processes that explain greater productivity in young mixed plantations, and improves our understanding of canopy packing in mixed stands.

**Key message:** Higher canopy packing in mixed stands occurs in a plantation of two light-demanding species, but only at high planting density and with no irrigation.

**Keywords:** biodiversity-ecosystem functioning; mixed-species plantation; canopy space filling; crown plasticity; *Pinus pinaster*; *Betula pendula*



## 1. Introduction

In the context of a global biodiversity crisis, researchers have been trying to understand how the loss of species diversity could affect the functioning of natural ecosystems (Cardinale et al. 2012; Tilman et al. 2014). Special attention has been given to forest ecosystems, and the major role of forest biodiversity in the provision of many ecosystem services, including climate change mitigation, has been recognized (Brockerhoff et al. 2017). The functioning of mixed-species forests is under strong scrutiny, with a major focus on ecosystem productivity (Liang et al. 2016; Jactel et al. 2018; Ammer 2019) and on resistance to disturbances (Jactel et al. 2017, 2021). These questions are of even greater importance in plantation forests, which have nearly all been established as tree monocultures for production purposes (Kelty 2006; Nichols et al. 2006). There is, however, a growing interest in mixed-species plantations as a potential management strategy to adapt these planted forests to climate change and to increasing rates of disturbances (Liu et al. 2018). Despite an increasing number of studies on the productivity of mixed-species forests and plantations (Huang et al. 2018; Ammer 2019), a better understanding of the complex ecological mechanisms involved when different species interact is necessary to identify which species should be associated (Kelty 2006; Ammer 2019).

Mixed-species forests have been shown to use canopy space more efficiently (i.e., they have higher canopy packing) than monocultures in temperate (Pretzsch 2014; Jucker et al. 2015; Williams et al. 2017) as well as tropical forests (Sapijanskas et al. 2014). Canopy packing has been identified as a major mechanism explaining the effects of species diversity on forest productivity (Morin et al. 2011; Pretzsch 2014; Sapijanskas et al. 2014; Williams et al. 2017), probably by increasing light interception at the stand scale (Rissanen et al. 2019; Williams et al. 2021). However, empirical demonstrations of higher canopy packing in mixed plantation forests remain scarce (but see Sapijanskas et al. 2014; Williams et al. 2017).

Species interactions may improve canopy packing at the stand level through two types of processes: (i) niche complementarity between interacting species that have different light requirements and/or crown architectures (inter-specific differences or “selection effects” according to Pretzsch (2014)), or (ii) an increase in crown volume for at least one of the mixed species (“true mixing effects” according to Pretzsch (2014)). An increase in crown volume can occur because of simple tree-size effects, with bigger crown volumes correlated to enhanced tree growth. It can also be the result of changes in crown allometry (i.e., changes in tree-crown metrics for a given tree diameter) (Jucker et al. 2014a; Barbeito et al. 2017; Guillemot et al. 2020). In this case, it is tree species plasticity that is at play. However, because crown shape and volume are part of a feedback loop between genetically-controlled tree architecture, local environmental conditions and tree growth, it is difficult to properly disentangle the effects of inherent species-specific differences, tree-size effects and crown plasticity (Pretzsch 2014; Kunz et al. 2019). Therefore, it is important to go back to a simpler mechanistic understanding of mixing

effects, by investigating individual tree-level variations in crown shape and size, in order to better interpret stand-level outcomes.

The effects of tree diversity on canopy packing through changes in crown shape and size may also depend on resource availability, similarly to what has been shown for other ecosystem functions (Forrester and Bauhus 2016). Crown shape and size may respond to a variety of external factors other than species diversity, such as water or nutrient availability (Dieler and Pretzsch 2013; Lübbe et al. 2016), or stand density (Barbeito et al. 2014) which are key parameters for tree plantations. Consequently, the outcome of mixture effects on tree crowns can be modulated by these environmental variations (Pretzsch et al. 2016; Barbeito et al. 2017). Analyses studying the combined effects of density and species diversity on crown variables are very scarce (Barbeito et al. 2014) and the two parameters have never been studied in interaction with water availability. For canopy packing in particular, very little is known on how it may vary with water availability.

In this study, we took advantage of a 10-year-old tree-diversity experiment, ORPHEE, where tree species composition, stand density and water availability (through irrigation) are all manipulated to evaluate their interactive effects on forest ecosystem functioning. The experimental plantation is located on a site in the Landes de Gascogne forest with poor sandy soil and a warm temperate climate (SW France) (Augusto et al. 2010). Most of the forests in the Landes de Gascogne are cultivated as monocultures of maritime pine (*Pinus pinaster* Ait.) and are intensively managed as production plantations in even-aged stands with rotation times of 35-60 years (Mason and Meredieu 2011). Yet, such monocultures are at high risk in terms of resistance to biotic disturbances (Jactel et al. 2017) and more resilient, alternative management options must be found (Morin et al. 2020). An interesting option there would be to associate maritime pine with silver birch (*Betula pendula* Roth), a native species able to grow on poor sandy soils and which has the potential to reduce processionary moth and pine stem borer damage on maritime pine (Castagneyrol et al. 2019; Jactel et al. 2019). Moreover, a stronger potential niche complementarity is expected in mixed stands composed of species with contrasted functional traits (Kelty 1992; Niklaus et al. 2017). Pine and birch are functionally quite different (evergreen vs. deciduous), and this could potentially result in aboveground complementarities, but the two species also have some functional similarities as they are both light-demanding pioneer species (Abad Viñas et al. 2016; Beck et al. 2016).

We focused this study on pure vs. mixed plots of these two species, maritime pine and silver birch, for which we measured tree crown dimensions to estimate crown volumes and stand-level canopy packing. Our main objective was to understand the aboveground mechanistic processes involved in tree species interactions and how these interactions varied with stand density and irrigation treatment. We asked the following specific questions:

- (1) Are there any mixture effects at the species level on crown shape and volume?
- (2) Is canopy packing (i.e., canopy space filling) higher in mixed than in pure stands and is this measure correlated to higher canopy closure (i.e., light interception)?
- (3) How do these effects vary with stand density and irrigation?

## 2. Methods

### 2.1. Study site

This study was conducted in the ORPHEE experimental plantation (<https://sites.google.com/view/orpheeexperiment/home>) located in southwestern France, 40 km southwest of Bordeaux (44°44.35 N, 00°47.9' W, altitude of 60 m asl). ORPHEE is included in the global Tree Diversity Network (<http://www.treedivnet.ugent.be/>). The site is located on a flat area and podzol soils are predominant (Augusto et al. 2010). Mean annual temperature is 12.5°C and mean average precipitation is 870 mm (1993–2018). Most of the rainfall occurs from fall to spring, and summers are rather dry. The water table is shallow in wintertime, generally ranging from 40 to 80 cm below the soil surface. The design of the plantation is described in Castagneyrol et al. (2013, 2017); here we will only provide a brief overview and the details specific to our study.

Our study focused on maritime pine (*Pinus pinaster*, hereafter pine) and silver birch (*Betula pendula*, hereafter birch) but is part of a broader tree diversity experiment that includes five tree species (birch, pine and three oak species: *Quercus robur* L., *Quercus pyrenaica* Willd. and *Quercus ilex* L.). One-year-old tree seedlings were planted at the site in January 2008 after clear-cutting of a mature pine plantation. Eight blocks were established, with plots in each block, planted at a density of 2500 trees ha<sup>-1</sup> per plot, corresponding to all the possible combinations of from one to five of the tree species mentioned above; each plot has 10 rows of 10 trees planted 2 m apart (100 trees per plot, 20 m-by-20 m plot area). The mixed plots were established according to a substitutive design, with the initial plantation density kept constant across the plots. Within each mixed plot, individual trees from different species were planted in a regular alternate pattern such that a tree of a given species had at least one neighbor from each of the other species within a 2-m radius.

In 2015, an irrigation system was added to half of the blocks, resulting in four irrigated blocks and four non-irrigated blocks. The irrigated plots are watered seasonally from early May to late September to alleviate summer stress. A 2-m-high sprinkler installed in the center of each plot sprays the equivalent of 3 mm day<sup>-1</sup> of precipitation. Maxwell et al. (2020) showed that the non-irrigated blocks were subjected to more water stress than the irrigated blocks and Castagneyrol et al. (2017) demonstrated that the irrigation treatment reduced soil water limitation during the summer of 2015 in the irrigated blocks by about 50 % compared to non-irrigated blocks.

In 2017, the average tree height for birch and pine was 7.1 m, while the three oak species had an average height of only 1.5 m (**Fig. II.A 1**). The rest of the understory layer is mostly composed of bracken, moor grass, gorse, and ericaceous shrubs. It represents less than 1 % of the total aboveground biomass and its diversity is similar across all combinations of treatments, with an average of six to seven vascular species per plot (Corcket et al. 2020).

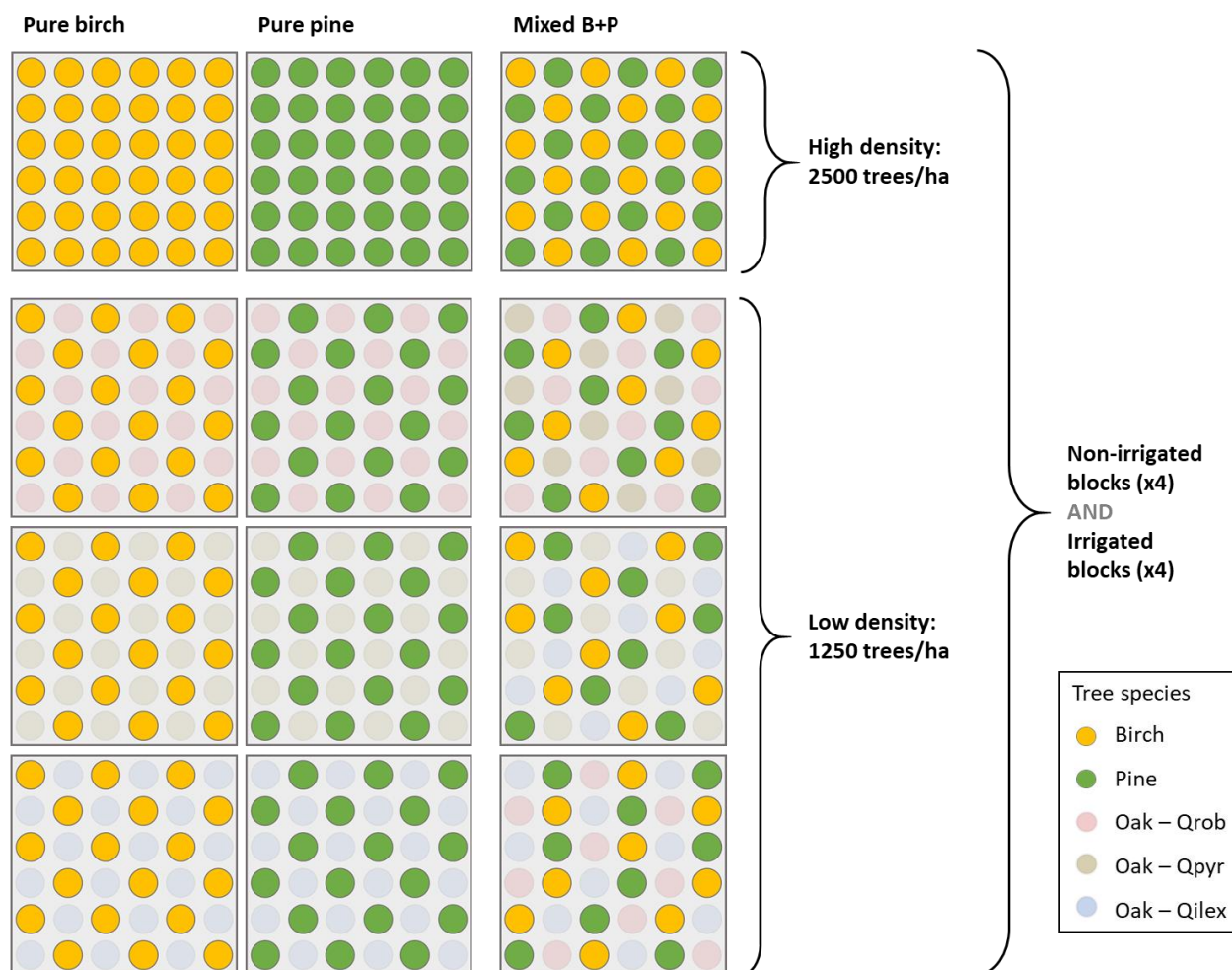
## 2.2. Experimental design and sampling

In this study, we focused on birch and pine, both in monospecific and two-species mixed plots resulting in three plot composition treatments: “pure pine”, “pure birch” and “mixed B+P”.

We were able to cross each of these composition treatments with two levels of stand density by including the plots with oaks. Indeed, due to their small size, the oak saplings did not reach the canopy layer and therefore did not affect light interception by the birch and pine tree crowns in 2017 (**Fig. II.A 1**). We therefore assimilated oak-sapling locations to regular gaps in the canopy layer. These gaps resulted in lower plot density and lower crown interaction for birch in birch + oak plots, for pine in pine + oak plots, and for the birch-pine mixture in birch + pine + oak plots. We were therefore able to test two density levels for the trees in the tree canopy layer: (i) a “high density” level in plots with birch alone, pine alone or birch + pine planted at 2500 trees ha<sup>-1</sup>, and (ii) a “low density” treatment with the oak saplings creating gaps in the main canopy layer resulting in a density of 1250 trees ha<sup>-1</sup> for birch alone, pine alone or birch + pine (**Fig. II. 1**). For the high-density treatment, there were three plots in each block (pure birch, pure pine, and mixed B+P). For the low-density treatment, there were nine plots in each block: three pure birch and three pure pine plots, one with each species of oak, and three mixed B+P plots, one for each combination of two oak species in each block (**Fig. II. 1**). Therefore, within each block, there was a total of 12 plots (3 at high density, 9 at low density) in which we carried out measurements on the birch and pine trees.

Finally, as mentioned above, there were two irrigation treatments. The “irrigated” treatment comprised the plots in the four irrigated blocks. The “non-irrigated” treatment comprised the plots in the four non-irrigated blocks (**Fig. II. 1**).

As a result, our full experimental design included 12 treatment combinations ( $3 \times 2 \times 2$ ): three species-composition treatments (pure birch, pure pine, and mixed B+P), 2 planting-density treatments (high, low) and 2 irrigation treatments (irrigated, non-irrigated). At the species level, this gave 16 treatment combinations ( $4 \times 2 \times 2$ ), because mixed B+P plots included both species.



**Fig. II. 1** Schematic representation of the 12-plot selection in each block of the ORPHEE experimental plantation. The low-density planting level for the target species (birch and pine) was created by taking into account oak sapling locations (Qilex = holm oak, Qpyr = Pyrenean oak, Qrob = pedunculate oak). Only the 6 x 6 rows in the center of the plots, where tree measurements were taken, are represented here. The two outer rows on all sides of plots were considered buffer zones.

## 2.3. Tree measurements

Our study was focused only on the innermost trees of each plot ( $6 \times 6$  rows) to avoid potential edge effects in the two outermost rows. This resulted in a total of 36 trees (birch and/or pine) per plot in the high-density treatments and 18 trees per plot in the low-density treatments (**Fig. II. 1**). Hereafter, the terms “all trees” or “stand level” refer to all 36 (or 18) trees.

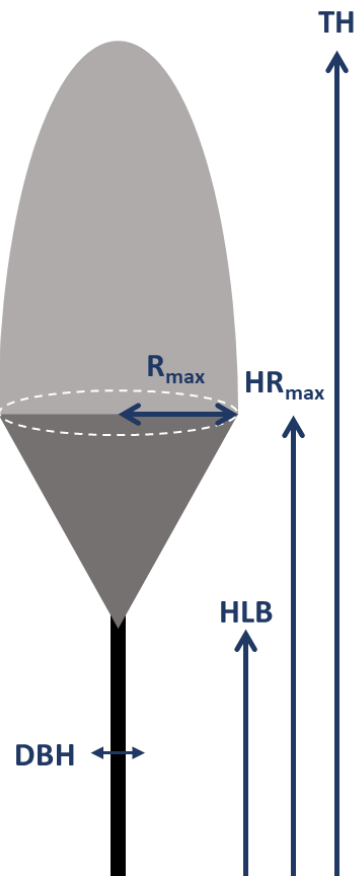
The diameter at breast height (DBH, cm) of all trees was measured in February 2017, for a total of 2160 trees. A subset of these trees, between seven and 11 trees per plot and per species, was selected for total tree height (TH, m) and crown measurements, resulting in a total of 1105 crowns. The sampled trees were representative of the distribution of tree diameters for birch and pine in each plot (**Table II.A 1**). We measured the following crown characteristics in June 2017 with a telescoping pole (**Fig. II. 2**): (i) maximum crown radius ( $R_{\max}$ , m) measured on both a south-east and a north-west axis, and retaining the widest radius of the two, (ii) height of maximum crown radius ( $HR_{\max}$ , m), and (iii) height of insertion of the lowest living branch on the stem (HLB, m). We then calculated (i) the live crown ratio (LCR, %) as the ratio between crown length (TH-HLB) and tree height (TH), and (ii) the slenderness of the trunk (H/D ratio, unitless) as the ratio between TH and DBH.

## 2.4. Estimating individual crown volume

### 2.4.1. Calculation of crown volume for measured trees

We used the field measurements of the tree crowns to estimate individual crown volume (CV,  $\text{m}^3$ ) for the measured trees. Simple geometric shapes can give a good approximation in young plantations since the trees have relatively regular shapes (Zhu et al. 2020). Tree crown shape has often been approximated by a single parabolic cone or half-elliptical shape (Jucker et al. 2015; Forrester et al. 2017) when only crown length data are available. However, our crown measurement data allowed us to refine our crown shape estimates to better represent our two species. To avoid overestimating the volume of the bottom part of the crown with a single half-ellipse, we divided the crown into two parts: above and below the maximum crown radius; then, we applied two geometrical shapes adapted from Pretzsch (2009). We therefore estimated CV for each tree as the sum of the volume of a half-ellipsoid shape (top part of the crown, above  $HR_{\max}$ ) and a cone (bottom part of the crown, below  $HR_{\max}$ ) (**Fig. II. 2**) according to the following equation:

$$CV = \frac{1}{2} \frac{4\pi}{3} R_{\max}^2 (TH - HR_{\max}) + \frac{\pi}{3} R_{\max}^2 (HR_{\max} - HLB). \quad (1)$$



**Fig. II. 2** Schematic representation of field tree and crown measurements. The following variables were measured: diameter at breast height (DBH), tree height (TH), height of maximum radius of the crown ( $HR_{max}$ ), maximum radius of the crown ( $R_{max}$ ) and height of insertion of the first live branch (HLB).



### 2.4.2. Predicting crown volume for unmeasured trees

As only a subsample of the trees was measured for tree height and crown dimensions in each plot, we estimated the crown volume of the unmeasured trees through a modeling approach. We fitted log-log allometric models with DBH and CV of measured trees by species and for each composition, density, and irrigation treatment combination. This resulted in 16 models (see section 2.2) (**Fig. II.A 2**) following:

$$\log CV = a_i + b_i \log DBH + \varepsilon, \quad (2)$$

where “i” is the treatment  $\times$  species combination from 1 to 16.

For the trees that had not been measured in the field for total height and crown dimensions, we used the regression coefficients from the model corresponding to their treatment  $\times$  species combination plus their measured DBH to predict their CV, as follows:

$$CV_p = \exp\left(a_i + b_i \log DBH + \frac{\sigma^2}{2}\right), \quad (3)$$

where  $CV_p$  is the predicted crown volume and  $a_i$  and  $b_i$  are the linear regression coefficients fitted for a given treatment  $\times$  species combination, with “i” from 1 to 16 (Eq. 2). Because the models were fitted on a logarithmic scale, a correction factor ( $\exp(\sigma^2/2)$ ) was applied to account for log-normally distributed errors and to avoid systematic underestimations of the predicted CV (Baskerville 1972).

## 2.5. Stand-level canopy packing

We calculated stand-level canopy packing (CP, %) for each plot. CP percentage is the proportion of the above-ground space potentially available to tree crown growth which is already occupied by tree crowns, as defined by Jucker et al. (2015):

$$CP = \frac{\sum CV}{Plot\ area \times (TH_{max} - HLB_{min})} \times 100, \quad (4)$$

where  $\sum CV$  is the sum of the crown volumes for all trees (either measured or predicted) within a plot, plot area is  $144\text{ m}^2$  ( $12 \times 12\text{ m}$ ), and  $TH_{max}$  and  $HLB_{min}$  are respectively the maximum TH and minimum HLB measured over all the plots (constant values). Canopy packing values can theoretically range from 0 to 100 %, with 0 representing no canopy in the potential canopy space and 100% representing a fully occupied canopy volume.

To estimate the net biodiversity effect, that is non-additive effect of mixing birch and pine, on canopy packing, we used a method derived from Loreau and Hector (2001) to compare the observed canopy packing ( $CP_{obs.}$ ) in the two-species mixed plots with the expected canopy packing ( $CP_{exp.}$ ) that would be obtained from the average CP values of the respective monocultures ( $\Delta CP$ ):

$$\Delta CP_j = CP_{obs.j} - CP_{exp.j}. \quad (5)$$

where  $j$ , corresponding to the triplets of plots considered, ranges from 1 to 32. A triplet is made up of one mixed plot and two pure plots of the corresponding species; each pure plot is matched to its corresponding mixture within the same block. We therefore had four triplets of plots inside each of the eight blocks: one triplet of high-density plots and three triplets of low-density plots (i.e. plots with oak species, see **Fig. II. 1**).

$CP_{exp.}$  was obtained with the following equation:

$$CP_{exp.j} = \frac{CP_{pure Bp_j} + CP_{pure Pp_j}}{2}, \quad (6)$$

where  $j$  is the same as in Eq (5).

## 2.6. Canopy closure

To study how canopy packing translated into stand-level light interception, we used digital hemispherical photographs. This ground-based method is related to the interception of radiation by all the canopy elements (foliage and woody elements) (Bréda 2003). It measures the directional distribution of the gap fraction, from 0 to 360° in azimuth angle and from 0 up to ~90° in zenith angle, and makes it possible to estimate the stand-level tree cover fraction (i.e., the opposite of gap fraction). The photographs were taken in July 2017 on a subset of the initial plot selection: in six out of eight blocks (3 irrigated and 3 non-irrigated) and in three out of nine low-density plots (the ones with *Q. robur*). We took four hemispherical photographs in each plot at 1.3 m above the ground with the camera lens looking upwards. The photographs were taken from four points, four to five meters away from each other, in the central zone of each plot in order to capture the canopy structure diversity within the plots.

The photographs, taken with a Nikon CoolPix 995 camera equipped with a FC-E8 fisheye lens under automatic exposure to prevent saturation, were recorded in jpeg format at the highest possible resolution (1536\*2048 pixels) (**Fig. II.A 3**). The optical axis was maintained in a vertical position. The photographs were taken on days when the sky was as most evenly overcast as possible for a homogeneous contrast between leaves and gaps (sky) and to avoid direct sunlight on the leaves, i.e., to minimize local overexposures and therefore the chance of misestimating the cover fraction.

The photographs were processed with the CAN-EYE free software (Demarez et al. 2008; Weiss and Baret 2017) to extract the vegetation cover fraction over a 0-60° zenith angle. CAN-EYE makes it possible to classify a set of images interactively and concurrently, which allowed us to process all four photographs from a given plot together. Once the pixels were classified either as sky or vegetation (leaves and branches), the vegetation cover fraction was computed for every 10° of azimuth angle and every 2.5° of zenith angle. To avoid including the tops of trees from neighboring plots that appeared in the field of view of some photographs, we did not keep the vegetation cover fraction data for the outer

ring (zenith angle from 50° to 60°). We averaged the vegetation cover fraction values over all angle sectors from 0 to 50° zenith angles to obtain one value for each photograph, then averaged this value over the four photographs taken in each plot. This averaged value per plot is hereafter called “canopy closure” (%). We calculated a net biodiversity effect on canopy closure ( $\Delta CC$ ) as we did for canopy packing (see section 2.5).

## 2.7. Statistical analyses

Tests for mixture effects on tree and crown dimensions (DBH, TH,  $R_{\max}$ ,  $HR_{\max}$ , HLB, LCR, H/D ratio) and crown volumes (CV) in interaction with stand density and irrigation treatment were done on individual tree-level data, while tests on canopy packing (CP and  $\Delta CP$ ) and canopy closure were done on stand-level data. The predictor variable for mixture effects used for tree-level data was called “*Mixture*” (two-level factor: mixed vs. pure), while for stand-level data, it was called “*Composition*” (three-level factor: pure birch vs. pure pine vs. mixed B+P).

For tree-level data, we used linear mixed-effects models (LMMs) to test the effects of *Mixture*, *Density* (two-level factor: low density vs. high density), *Irrigation* (two-level factor: non-irrigated vs. irrigated) and all two- and three-way interactions on the response variables (tree and crown dimensions and CV). We also repeated the same tests on tree and crown dimensions with DBH as a covariate to check for effects that were independent of changes in tree size and for changes in allometric relationships. Since the irrigation treatment is applied at the block level, the ORPHEE experiment is a split-plot experimental design. We therefore used *Block* as a random factor to account for potential environmental heterogeneity among blocks and for the non-independence of different plots within the same block. Non-independence of individual trees of a given species within plots was accounted for by defining *Plot* as a random factor, nested within *Block*. For the tests on crown volume, we modelled the variance structure with a power variance function to correct for heteroscedasticity of the residuals.

For stand-level data, we tested the effect of *Composition*, *Density*, *Irrigation* and all two- and three-way interactions on canopy packing (CP) and on canopy closure (CC) as fixed effects in the LMMs. We used *Block* as a random factor to account for heterogeneity among blocks and for the non-independence of different plots within the same block.

To test if  $\Delta CP$  and  $\Delta CC$  were different from 0, we used a paired t-test on  $CP_{\text{obs}}$  and  $CP_{\text{exp}}$  values and on  $CC_{\text{obs}}$  and  $CC_{\text{exp}}$  values, respectively.

R language programming (R Core Team 2019) with the Rstudio interface was used for all the analyses with the following packages: nlme, MuMIn and multcomp (Hothorn et al. 2008; Barton 2019; Pinheiro et al. 2019).

### 3. Results

#### 3.1. Variations in tree and crown dimensions with treatments

We observed differences between species independently of the different treatments: on average, birch trees had a smaller diameter at breast height (DBH) (**Table II.A 1, Fig. II.B 1**) and smaller crown dimensions (**Table II.A 2, Fig. II. 3, Fig. II. 5**) than did pine trees.

##### 3.1.1. Birch

We found that birch trees had smaller diameters at breast height (DBH) in mixed plots than in pure plots (**Table II.B 1, Fig. II.B 1**). Birch crowns in mixed plots were narrower ( $R_{\max}$ ), the lowest living branch was higher on the stem (HLB) and the crown occupied a shorter proportion of the tree height (LCR) than in pure plots (**Table II. 1, Fig. II. 3**). Even though there was no mixture effect on total height (TH), birch trees in mixed plots were more slender (H/D ratio) than in pure plots (**Table II. 1**) due to the lower DBH in the mixed plots. These mixture effects on tree metrics disappeared when DBH was introduced as a covariate in the models (**Table II. 2**). The effect of  $\log(DBH)$  was significant in all the models, indicating that the previously identified effects were mainly due to size effects. No significant interaction of  $\log(DBH)$  with *Mixture* was found (**Table II. 2**).

A decrease in stand density had a positive effect on birch DBH (**Table II.B 1, Fig. II.B 1**), with significantly larger diameters at the low density. We also found that a lower density resulted in wider crowns ( $R_{\max}$ ), a higher live crown ratio (LCR), a downward shift of the lowest living branch (HLB) and a higher maximum radius ( $HR_{\max}$ ) (**Table II. 1, Fig. II. 3**). Even though there was no density effect on total height (TH), birch trees at low density were less slender (H/D ratio) than those at high density (**Table II. 1**) due to their larger DBH. These density effects on tree metrics also disappeared when DBH was introduced as a covariate in the models (**Table II. 2**). The effect of  $\log(DBH)$  was significant in all the models, indicating that the previously identified effects were mainly due to size effects, with larger trees at low density. For  $HR_{\max}$ , we found an additional interaction between  $\log(DBH)$  and *Density* (**Table II. 2**), indicating that the trees with a small DBH had similar  $HR_{\max}$  values irrespective of the density treatment, but that the trees with a larger DBH had lower  $HR_{\max}$  values at low density than at high density. Similarly, we found a significant interaction between  $\log(DBH)$  and *Density* for  $R_{\max}$  (**Table II. 2**):  $R_{\max}$  was higher for trees with a higher DBH at low density.

Finally, we did not find any significant irrigation effects on birch DBH or on the other tree and crown metrics, except for LCR, which was higher in the irrigated plots (**Table II.B 1, Table II. 1**). When DBH was introduced as a covariate, the positive effect of irrigation on LCR remained true (**Table II. 2**). No interaction of  $\log(DBH)$  with *Irrigation* was found (**Table II. 2**).

### 3.1.2. Pine

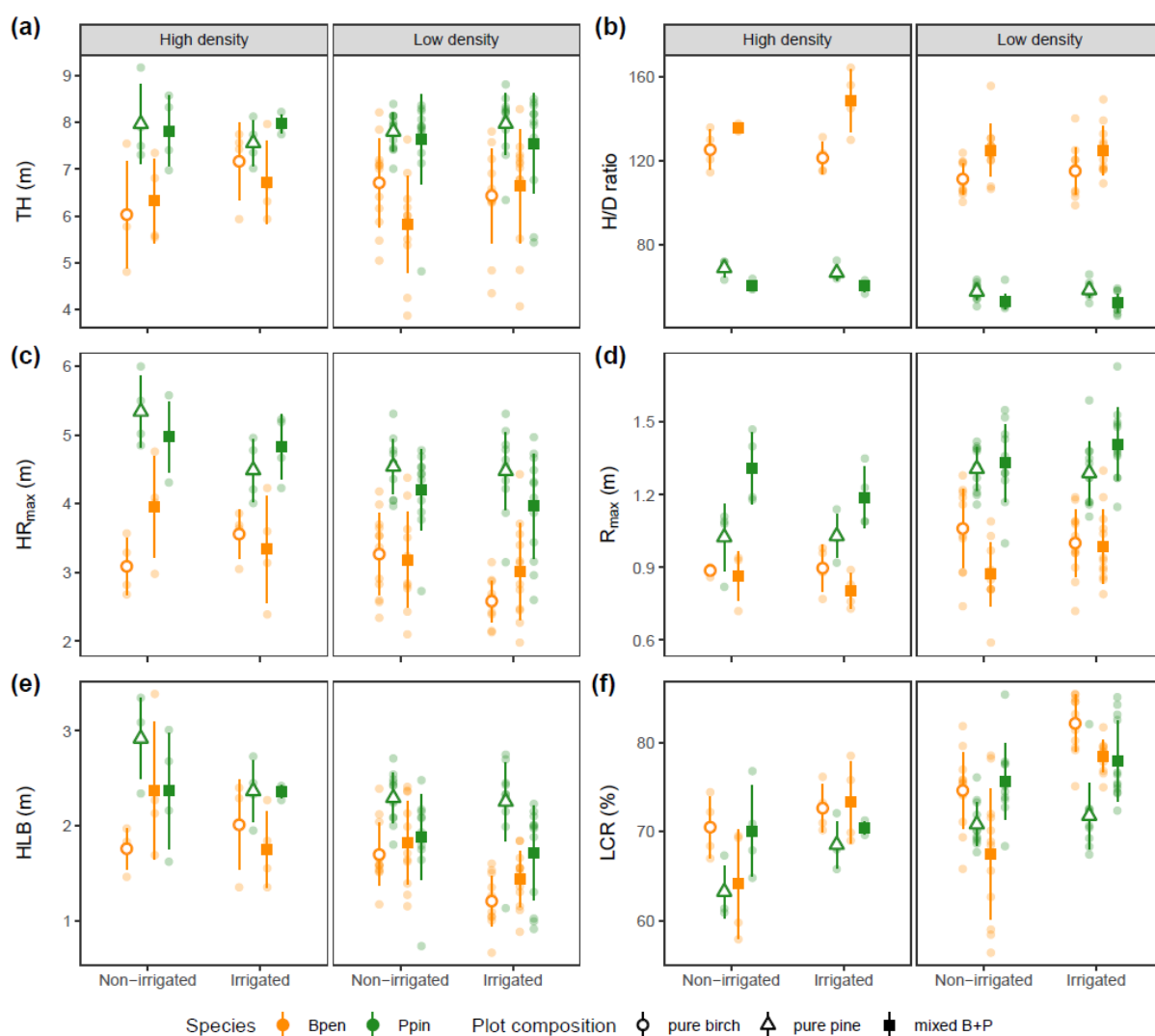
We found that pine trees had larger diameters at breast height (DBH) in mixed plots than in pure plots (**Table II.B 1, Fig. II.B 1**). Contrary to birch, pine crowns in mixed plots were wider ( $R_{\max}$ ), the lowest living branch was lower on the stem (HLB) and the crown occupied a larger proportion of the tree (LCR) than in pure plots (**Table II. 1, Fig. II. 3**). Even though there was no mixture effect on total height (TH), the pines in mixed plots had lower H/D ratios than in pure plots due to their larger DBH. The effect of  $\log(DBH)$  was significant in all the models (except for LCR), but the mixture effects on pine tree metrics were maintained independently of size effects (**Table II. 2**), contrary to what was observed for birch trees. Moreover, the introduction of DBH as a covariate revealed that for a given diameter, pine trees were shorter (TH) in mixed plots than in pure plots. We found additional  $\log(DBH) \times Mixture$  interactions for HLB, LCR, TH and H/D ratio, meaning that the slopes of the allometric relationships between DBH and these variables were different between pure and mixed plots. Therefore, the magnitude of the mixture effect was modulated by tree size: it was stronger for smaller pine trees than for larger ones.

As with birch, a decrease in density had a positive effect on pine DBH (**Table II.B 1, Fig. II.B 1**), with larger diameters at low density. We also found that a lower density resulted in wider crowns ( $R_{\max}$ ), a higher live crown ratio (LCR), a downward shift of the lowest living branch (HLB) and a lower maximum radius ( $HR_{\max}$ ) (**Table II. 1, Fig. II. 3**). Even though there was no density effect on total height (TH), trees at low density were less slender (H/D ratio) than those at high density due to larger DBH. The density effects on  $R_{\max}$ , HLB and HD disappeared when DBH was introduced as a covariate in the models, indicating that they were mainly due to size effects; however, the density effect remained for  $HR_{\max}$  and LCR (**Table II. 2**).

Finally, we did not find any significant irrigation effects on DBH or on the other tree and crown metrics for pine (**Table II.B 1, Table II. 1**). When DBH was introduced as a covariate, there were no independent effects of irrigation (**Table II. 2**). There was, however, a significant interaction between  $\log(DBH)$  and *Irrigation* for  $HR_{\max}$ , indicating that a downward shift in the height of the maximum crown radius occurred in irrigated plots, though only for the largest trees.

## 3.2. Average crown volumes (CV)

The two species differed in crown volume, with a lower mean CV for birch than for pine, regardless of the density treatment. Mean CV (at the plot level) ranged from 3.2 to 23.4 m<sup>3</sup> for birch and from 7.1 to 31.6 m<sup>3</sup> for pine (**Fig. II. 4**). First, we found a significant mixture effect on crown volume for both species (**Fig. II. 4, Table II. 3**) and the effect was opposite for the two species.



**Fig. II. 3** Tree measurement averages by species, plot and treatment for all measured trees. (a) total tree height (TH, m), (b) height to diameter ratio (H/D, m m<sup>-1</sup>), (c) height of maximum crown radius (HR<sub>max</sub>, m), (d) maximum crown radius (R<sub>max</sub>, m), (e) height of first live branch (HLB, m), and (f) live crown ratio (LCR, %). The points in the background represent average plot values for each plot. Large symbols represent the mean of the plot averages; error bars represent standard deviations.

**Table II. 1** Results for LMMs testing the effects of *Mixture*, *Density*, *Irrigation* and their interactions on birch and pine crown dimensions, for measured trees only. The variables are total tree height (TH, m), height to diameter ratio (H/D ratio, m m<sup>-1</sup>), height of maximum crown radius (HR<sub>max</sub>, m), maximum crown radius (R<sub>max</sub>, m), height of first live branch (HLB, m) and live crown ratio (LCR, %). For further details on the statistical results see **Table II.B 1** for DBH and **Table II.B 2** for the other variables. The number of measured trees varies between DBH (birch: n=1003; pine: n=1043) and the other measured variables (birch: n=527; pine: n=578).

	Predictors	Response variables						
		DBH	TH	H/D ratio	HR <sub>max</sub>	R <sub>max</sub>	HLB	LCR
<b>Birch</b>	Mixture (M)	** ( <i>m&lt;p</i> )	<i>ns</i>	*** ( <i>m&gt;p</i> )	<i>ns</i>	* ( <i>m&lt;p</i> )	** ( <i>m&gt;p</i> )	*** ( <i>m&lt;p</i> )
	Density (D)	* ( <i>l&gt;h</i> )	<i>ns</i>	*** ( <i>l&lt;h</i> )	** ( <i>l&lt;h</i> )	*** ( <i>l&gt;h</i> )	*** ( <i>l&lt;h</i> )	*** ( <i>l&gt;h</i> )
	Irrigation (I)	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	** ( <i>i&gt;ni</i> )
	M x D	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	M x I	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	*
	D x I	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	*	<i>ns</i>
	M x D x I	*	*	<i>ns</i>	**	<i>ns</i>	<i>ns</i>	<i>ns</i>
<b>Pine</b>	Mixture (M)	** ( <i>m&gt;p</i> )	<i>ns</i>	*** ( <i>m&lt;p</i> )	<i>ns</i>	*** ( <i>m&gt;p</i> )	*** ( <i>m&lt;p</i> )	*** ( <i>m&gt;p</i> )
	Density (D)	*** ( <i>l&gt;h</i> )	<i>ns</i>	*** ( <i>l&lt;h</i> )	*** ( <i>l&lt;h</i> )	*** ( <i>l&gt;h</i> )	*** ( <i>l&lt;h</i> )	*** ( <i>l&gt;h</i> )
	Irrigation (I)	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	M x D	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	**	<i>ns</i>	<i>ns</i>
	M x I	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	D x I	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	M x D x I	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	*	<i>ns</i>	<i>ns</i>

*Note:* Significant effects are indicated by asterisks: p-value ≤ 0.05\*, p-value ≤ 0.01\*\*, p-value ≤ 0.001\*\*\*, ns for non-significant. For single-term response variables (not interactions), the sign of the effect is indicated next to the p-value (for *Mixture* p=pure, m=mixed; for *Density* h=high density, l=low density; for *Irrigation* ni=non-irrigated, i=irrigated).

**Table II. 2** Results for LMMs testing the effects of  $\log(DBH)$ , *Mixture*, *Density*, *Irrigation* and their interactions on birch and pine crown dimensions, for measured trees only. The variables are total tree height (TH, m), height to diameter ratio (H/D ratio,  $m\ m^{-1}$ ), height of maximum crown radius ( $HR_{max}$ , m), maximum crown radius ( $R_{max}$ , m), height of first live branch (HLB, m) and live crown ratio (LCR, %). For further details on the statistical results see **Table II.B 3**.

	Predictors	Response variables					
		$\log(TH)$	$\log(HD)$	$\log(HR_{max})$	$\log(R_{max})$	$\log(HLB)$	$\log(LCR)$
<b>Birch</b> (n=527)	$\log(DBH)$	*** (+)	*** (-)	*** (+)	*** (+)	** (+)	*** (+)
	Mixture (M)	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	Density (D)	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	Irrigation (I)	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	* ( <i>i&gt;ni</i> )
	$\log(DBH) \times M$	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	$\log(DBH) \times D$	<i>ns</i>	<i>ns</i>	*	*	<i>ns</i>	<i>ns</i>
	$\log(DBH) \times I$	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	M x D	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	M x I	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	D x I	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	**	*
<b>Pine</b> (n=578)	$\log(DBH)$	*** (+)	*** (-)	*** (+)	*** (+)	*** (+)	<i>ns</i>
	Mixture (M)	*** ( <i>m&lt;p</i> )	*** ( <i>m&lt;p</i> )	<i>ns</i>	* ( <i>m&gt;p</i> )	*** ( <i>m&lt;p</i> )	*** ( <i>m&gt;p</i> )
	Density (D)	<i>ns</i>	<i>ns</i>	* ( <i>l&lt;h</i> )	<i>ns</i>	<i>ns</i>	* ( <i>l&gt;h</i> )
	Irrigation (I)	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	$\log(DBH) \times M$	***	***	<i>ns</i>	<i>ns</i>	**	***
	$\log(DBH) \times D$	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	$\log(DBH) \times I$	<i>ns</i>	<i>ns</i>	*	<i>ns</i>	<i>ns</i>	<i>ns</i>
	M x D	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	M x I	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
	D x I	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

*Note:* Significant effects are indicated by asterisks: p-value  $\leq 0.05^*$ , p-value  $\leq 0.01^{**}$ , p-value  $\leq 0.001^{***}$ , *ns* for non-significant. For single-term response variables (not interactions), the sign of the effect is indicated next to the p-value (for  $\log(DBH)$  “+” = positive slope, “-” = negative slope; for *Mixture* p=pure, m=mixed; for *Density* h=high density, l=low density; for *Irrigation* ni=non-irrigated, i=irrigated).



Birch had smaller crown volumes in mixed plots than in pure plots, while pine had larger crown volumes in mixed plots than in pure plots. Second, both species had larger crown volumes in the low-density treatment (**Fig. II. 4, Table II. 3**). Finally, there were no significant individual effect of irrigation on CV for birch or pine.

For pine there was a significant interaction between *Mixture* and *Density*, with a stronger mixture effect on crown volume at high density. The three-way interaction term was significant for pine because this stronger mixture effect at high density was also stronger in non-irrigated plots than in irrigated ones.

### 3.3. Canopy packing (CP)

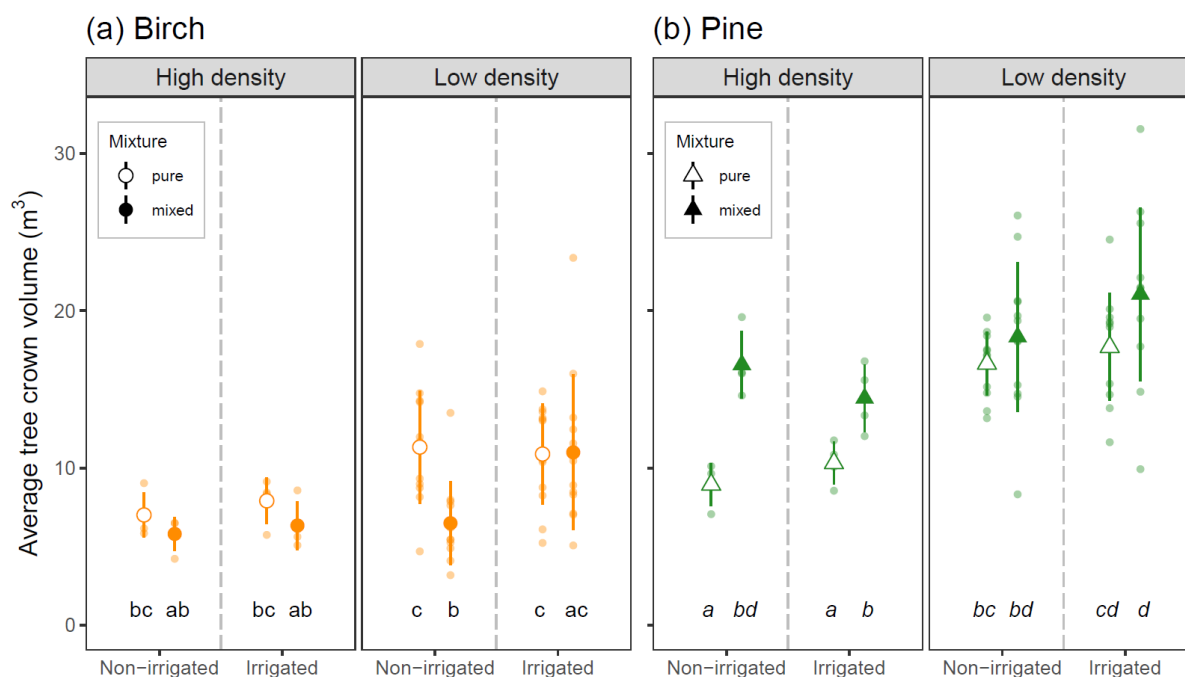
At the stand level, canopy packing (CP) values were highly variable with an average of 13.8 % (4.1 – 21.6 %) for birch, 20.8 % (13.3 – 29.6 %) for pine, and 18.4 % (5.0 – 30.4 %) for mixed plots (**Fig. II. 6a**). We found that plot species composition influenced CP values (**Table II. 4**). CP values were lower for pure birch than for pure pine in all treatment combinations. Mixed plots at high density reached higher CP values than pure pine in the non-irrigated plots, and similar CP values to pure pine in the irrigated plots. At low density, mixed plots had intermediate CP values between pure birch and pure pine; these values were closer to pure birch in the non-irrigated plots and closer to pure pine in the irrigated plots (**Fig. II. 6a**). Moreover, regardless of plot composition there was a strong density effect on CP, with higher CP at high density (**Table II. 4**). This density effect was stronger for mixed plots than for pure birch or pure pine plots. Finally, we found no irrigation effect on CP (**Table II. 4**).

Only high-density non-irrigated plots showed a positive net biodiversity effect on CP. These plots had  $\Delta$ CP values significantly differing from 0, with 7.3 % higher canopy packing in mixed plots than expected from pure plots (**Fig. II. 6b**).

### 3.4. Canopy closure (CC)

Canopy closure at high density was on average 56 % (33-75 %) for birch, 74 % (69-78 %) for pine, and 72 % (65-75 %) for mixed plots (**Fig. II. 7a**). We observed a strong irrigation effect in the pure birch plots. In non-irrigated plots pure birch plots had significantly lower canopy closure than pure pine plots. The values were lower and more variable at low density, due to the presence of gaps in the canopy.

The trends for the net biodiversity effect on canopy closure ( $\Delta$ CC) were similar to those for  $\Delta$ CP: both revealed positive differences in high-density non-irrigated plots. However, because of the smaller sampling for  $\Delta$ CC, the paired t-test was only marginally significant (**Fig. II. 7b**).



**Fig. II. 4** Plot averages for tree crown volumes by density, irrigation and plot composition treatments for (a) birch and (b) pine. The points in the background represent average plot values for each block. Large symbols represent the mean of the plot averages; error bars represent standard deviations. Letters represent the results of post-hoc multiple comparisons by species over all treatments.

**Table II. 3** Results for LMMs testing the effects of *Mixture*, *Density*, *Irrigation*, and interactions on tree crown volumes (CV, m<sup>3</sup>) by species. The test included crown volumes for all trees, i.e. both measured and predicted.

Predictors	Birch (n=1003)		Pine (n=1043)	
	<i>F(df)</i>	<i>p-value</i>	<i>F(df)</i>	<i>p-value</i>
Mixture (M)	8.12 (1;50)	<b>&lt;0.01</b>	67.81 (1;50)	<b>&lt;0.001</b>
Density (D)	15.13 (1;50)	<b>&lt;0.001</b>	127.17 (1;50)	<b>&lt;0.001</b>
Irrigation (I)	2.39 (1;6)	0.17	0.44 (1;6)	0.53
M x D	0.65 (1;50)	0.42	9.04 (1;50)	<b>&lt;0.01</b>
M x I	1.91 (1;50)	0.17	1.09 (1;50)	0.30
D x I	0.65 (1;50)	0.42	4.03 (1;50)	0.05
M x D x I	2.84 (1;50)	0.10	5.36 (1;50)	<b>0.02</b>

*Note:* Estimations of pseudo-R<sup>2</sup> were not included for these two models because we used a power function to model the variance structure, which invalidates the method for the pseudo-R<sup>2</sup>. Bold values indicate significant effects (p-value < 0.05).

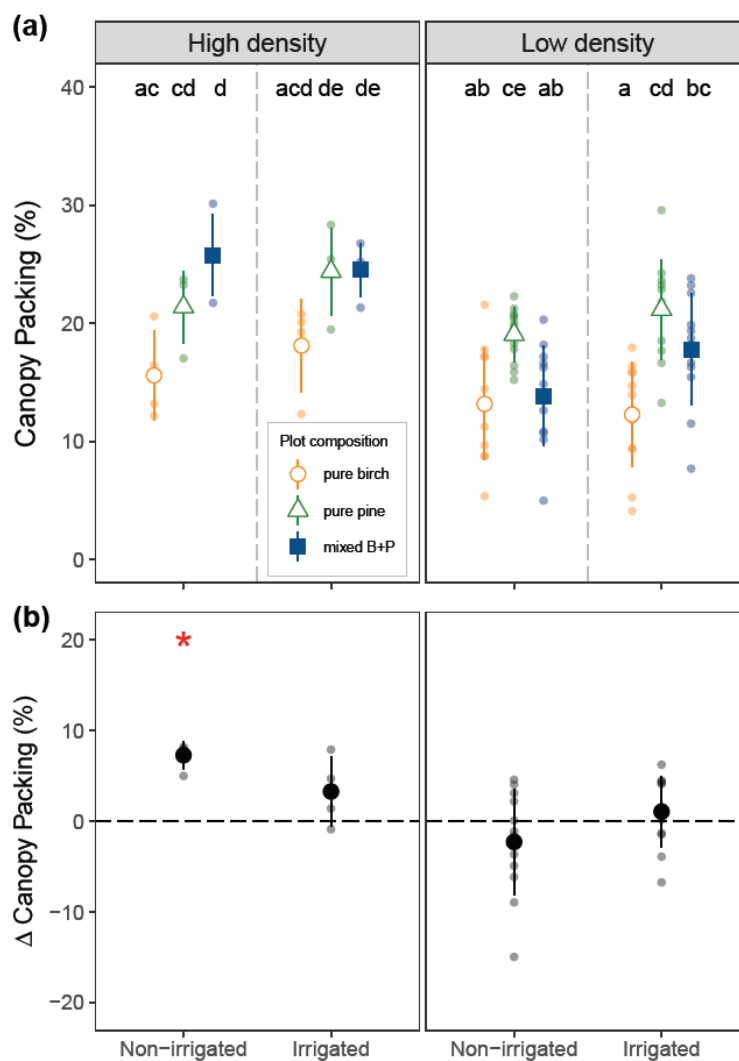


**Fig. II. 5** Schematic representation of crown measurements and volume averaged by type of treatment. All the parameters (DBH, TH,  $HR_{max}$ ,  $R_{max}$ , HLB) are represented to scale. For average DBH values see **Table II.A 1**, for average crown metrics see **Table II.A 2**. Birch trees are represented in orange and pine in green.

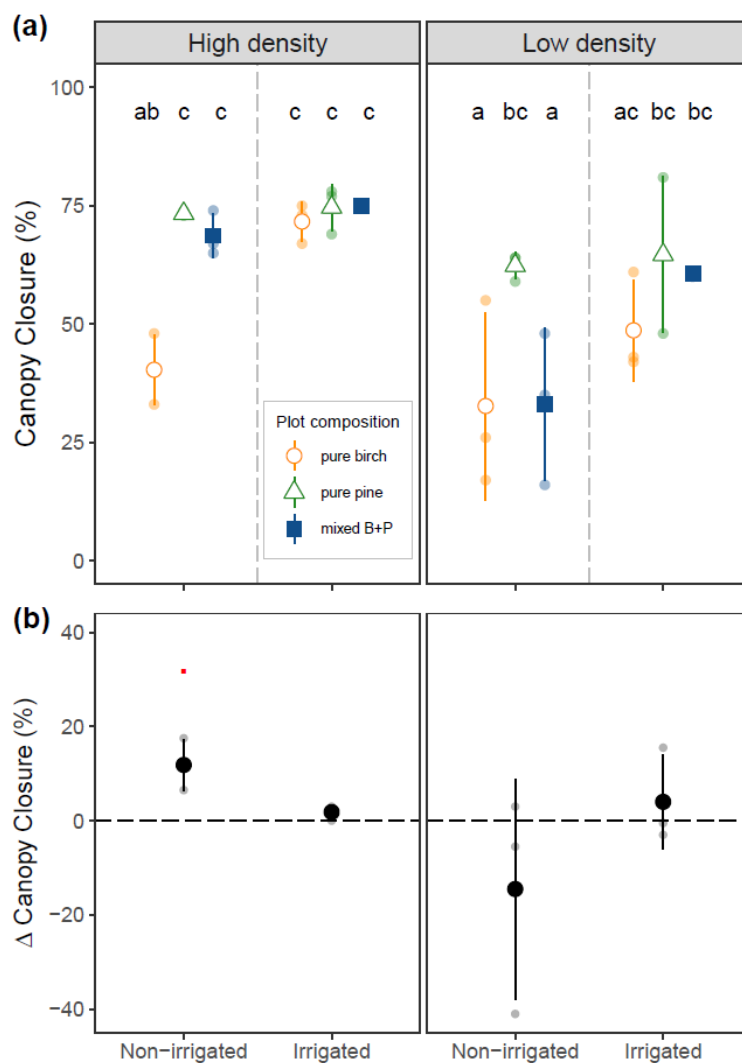
**Table II. 4** Results for LMM testing the effects of *Composition*, *Density*, *Irrigation* and their interactions on stand-level canopy packing (%).

Predictors	Canopy Packing (n=96)		
	<i>F(df)</i>	<i>p-value</i>	$R^2_m (R^2_c)$
Composition (C)	23.32 (2;78)	< <b>0.001</b>	0.55 (0.63)
Density (D)	39.22 (1;78)	< <b>0.001</b>	
Irrigation (I)	1.15 (1;6)	0.32	
C x D	5.35 (2;78)	< <b>0.01</b>	
C x I	0.36 (2;78)	0.70	
D x I	0.03 (1;78)	0.87	
C x D x I	2.18 (2;78)	0.12	

*Note:* Marginal ( $R^2_m$ ) and conditional ( $R^2_c$ )  $R^2$  values were calculated for the model. They correspond to the variance explained by fixed and fixed plus random factors, respectively. Bold values indicate significant effects ( $p$ -value < 0.05).



**Fig. II. 6** Stand-level canopy packing **(a)** and net biodiversity effect on canopy packing **(b)**. **(a)** Stand-level canopy packing by plot composition, irrigation and density. The points in the background represent individual plot values for each block. Large symbols represent the mean of the plot values; error bars represent standard deviations. Letters represent the results of post-hoc multiple comparisons for all treatment combinations together. **(b)** Mean difference between observed and expected canopy packing ( $\Delta$ CP) by plot composition, irrigation and density. The points in the background represent individual triplet values for each block. Large symbols represent the mean of the  $\Delta$ CP triplet values; error bars represent standard deviations. The star indicates that values differed significantly from 0.



**Fig. II. 7** Stand-level canopy closure **(a)** and net biodiversity effect on canopy closure **(b)**. **(a)** Stand-level canopy closure by plot composition, irrigation and density. The points in the background represent individual plot values for each block. Large symbols represent the mean of the plot values; error bars represent standard deviations. Letters represent the results of post-hoc multiple comparisons for all treatment combinations together. **(b)** Mean difference between observed and expected canopy closure ( $\Delta CC$ ) by plot composition, irrigation, and density. The points in the background represent individual triplet values for each block. Large symbols represent the mean of the  $\Delta CP$  triplet values; error bars represent standard deviations. The dot indicates a marginally significant difference ( $p=0.06$ ).

## 4. Discussion

In this study, we looked at mixture effects on crown shape and size at the tree level, and on canopy packing at the stand level, in interaction with stand density and irrigation. Below, we first discuss mixture effects at the tree and stand level for the high-density non-irrigated plots. We later discuss how mixture effects vary with density and irrigation levels.

### 4.1. Mixture effects at high density and without irrigation

#### 4.1.1. Tree-level crown shape and size

At high density and without irrigation, crown shape and size for both species responded with opposite effects to mixing. When mixed with pine, birch had shorter and narrower crowns (**Fig. II. 3**), which resulted in crown volumes on average 17 % smaller than in pure plots (**Fig. II. 4, Fig. II. 5**). In contrast, when mixed with birch, pine had longer and wider crowns (**Fig. II. 3**), which resulted in crown volumes 85 % larger than in pure plots (**Fig. II. 4, Fig. II. 5**). The effects on birch crowns indicate that birch suffered from a more competitive environment when mixed with pines than in monocultures, similarly to what was reported by Lintunen and Kaitaniemi (2010) for birch in mixtures with Scots pine (*Pinus sylvestris* L.). Conversely, pines in mixed plots benefited from a release in competitive inhibition of crown growth compared to monocultures. Jucker et al. (2014a) reported similar results for crown volume with other pine species, especially in mixtures with broadleaved species. This highlights that the outcome of mixture effects on each species is highly dependent on the choice of the interacting species.

Our study focused on two species that have similar light acquisition strategies: they are both fast-growing in height, light-demanding pioneer species (Abad Viñas et al. 2016; Beck et al. 2016) and have similarly low shade tolerance, although birch is slightly more shade tolerant than pine (Niinemets and Valladares 2006; Poorter et al. 2012). We might therefore have expected limited niche complementarity between these two species (Kelty 1992; Niklaus et al. 2017), although it has been shown that mixing species with similar traits can also have positive effects on productivity and can influence crown architecture (Lintunen and Kaitaniemi 2010; Riofrío et al. 2017; Cattaneo et al. 2020). In our case, despite the similarities in light requirements, the two studied species showed strong differences in crown architecture. In their corresponding pure plots, the pine trees were taller than the birch trees. They also had a higher crown volume and a lower proportion of their crown above the maximum crown radius than did birch (**Fig. II. 5**). Finally, the pines in pure plots also had larger diameters at breast height than did birch in pure plots (DBH, **Fig. II.B 1**). The mixture effects we observed may therefore stem from these inherent species differences in crown architecture and diameter growth rates (Williams et al. 2017), which reflect above-ground niche complementarity, as confirmed by the net biodiversity effect on canopy packing in the high density plots with no irrigation. Differences in crown architecture may have therefore led to differences in light availability for the two species in the mixed plots, with birch

suffering from lower light availability in the mixtures because of the surrounding taller and larger pines. The pines, in turn, benefited from the greater growing space and higher light availability in the mixtures with smaller birch trees. The downward displacement of pine crowns in mixed plots, suggestive of a slower crown recession process, supports this hypothesis (Mäkelä 1997; Cattaneo et al. 2020). Furthermore, the increase in crown volume for the pines in mixtures might also be explained by an asynchrony in the phenology and growth patterns of the two species, which could have created a temporal complementarity (Sapijanskas et al. 2014). Pine, as an evergreen species, starts growing early in the spring before birch starts leafing. For similar reasons, pine may achieve additional growth in the fall after birch sheds its leaves (Aldea et al. 2021).

In this study, the mixture effects on crown shape for birch and pine were driven both by changes in tree size (changes in DBH) and by crown plasticity, as suggested by Guillemot et al. (2020). It is well known that diameter growth is correlated, through allometric relationships, with greater crown development (Pretzsch 2014). For birch, mixture effects disappeared when we controlled for the effect of DBH on crown metrics (**Table II. 2**). This indicated that the effects we found were mostly due to the smaller tree size of birch in mixtures compared to birch in pure plots. Our results therefore indicate that mixing with pine did not allow any expression of crown plasticity for birch. Crown plasticity is more likely to be expressed when the tree has favorable conditions for crown expansion into the space that becomes available (Cattaneo et al. 2020). Moreover, according to Pretzsch (2014), silver birch has the least lateral crown plasticity among 14 common European species. The pine trees, on the other hand, showed mixture effects for all tree and crown metrics (except  $HR_{max}$ ) that were independent of changes in DBH (**Table II. 2**). This indicates that, in addition to a tree-size effect, pine also expressed crown plasticity in response to mixtures with birch. The pines, for a given diameter, invested less in tree height, had a wider crown which started lower in the tree, and occupied a higher proportion of tree height in mixtures than in pure plots. This was especially true for smaller pine trees, which benefited the most from an increase in light availability in mixtures with birch, as Jucker et al. (2014a) found for pines mixed with oak species. Moreover, a lower height-diameter ratio is a recognized plastic response to lower competition for light (Harja et al. 2012; Jucker et al. 2014a). In addition, Maritime pine has shown good plasticity in crown architecture in response to light-related changes in its environment (Riofrío et al. 2017; Condés et al. 2020; Cattaneo et al. 2020). This plasticity in crown structure probably further enhanced the ability of pine in our study to intercept light, thus reinforcing the mixture effect on pine productivity (Jucker et al. 2014a).

#### 4.1.2. Stand-level canopy packing

Mixture effects on individual tree crown shape and volume can modify canopy packing at the stand level, which can influence stand light interception processes and productivity (Pretzsch 2014; Jucker et al. 2015). A major result of our study was that we found a higher canopy packing in the mixed plots



than expected from the pure plots ( $\Delta CP$ ), indicating a more efficient use of canopy space in mixtures at high density and without irrigation (**Fig. II. 5, Fig. II. 6, Table II. 4**). A similar diversity effect has been observed over a wide range of mature European forests (Jucker et al. 2015). Nevertheless, contrasting results have also been found (Seidel et al. 2013) and diversity effects may vary with species assemblage.

The positive mixture effect we observed on canopy packing was the result of both vertical and horizontal complementarities between the crowns of the two species. Vertical complementarity refers to the fact that species show inherent vertical profile differences in pure plots, and these differences can be enhanced or hindered in mixed plots through crown plastic responses (Schmid and Niklaus 2017). In our study, vertical crown overlap between birch and pine in their corresponding pure plots was 51 % on average (**Fig. II. 3, Table II.B 4**), indicating that only 51 % of the crowns were in the same horizontal layer. This vertical overlap increased to 66 % in mixed plots, revealing less vertical complementarity between species. This was due to a combination of higher birch crowns and a slower crown recession for pine (**Table II. 1**). This is consistent with Vallet and Perot (2016) who mention a leveling process between the taller and shorter species in mixed plots. The leveling process can be explained in our study by light interception, which is the main driver of height growth and crown development for these two light-demanding species. We also observed horizontal complementarity: the pine trees were able to widen their crowns in the space not occupied by birch trees (**Table II. 1, Table II. 2**), in part because the pine trees were taller than the birch. This resulted in a horizontal overlap of birch-pine crown radii in these plots (**Table II.A 2**, the sum of  $R_{max}$  of both species increased in the mixed plots). These results underline the necessity to study both the vertical and horizontal dimensions to understand changes in canopy packing (Forrester and Bauhus 2016; Kunz et al. 2019; Guillemot et al. 2020).

Species mixing can therefore increase canopy packing even with limited vertical stratification if the coexisting species show at least some degree of niche partitioning (e.g., in terms of crown dimensions). Still, some vertical stratification between the species in mixtures is necessary to allow a plastic crown response in pine, similarly to what was described by Jucker et al. (2014a, 2015). We also showed that, in the case of two light-demanding species, although the net mixture effect was positive at the stand level, this resulted from one species overcoming the other (+85 % crown volume for pine, -17 % crown volume for birch), indicating asymmetric repartition of the light resources between the two species. Similar results have been found when comparing total stand overyielding with productivity at the species levels (Pretzsch et al. 2015; Trogisch et al. 2021). However, because of the low shade-tolerance of birch, our result questions the stability of birch-pine mixtures over time (Morin et al. 2020) since the asymmetric pattern observed here may limit birch growth and survival in the coming years.

#### 4.1.3. Relating canopy packing and light interception

We demonstrated higher canopy packing in mixed plots, but more efficient use of canopy space is not necessarily directly linked to higher canopy closure and higher light interception at the stand level.

Indeed, there could be changes in leaf area or branching density in mixtures, independently of the mixture effect on crown volume (Binkley et al. 2013; Guillemot et al. 2020; Hildebrand et al. 2021). We found that canopy closure in mixed plots was higher than expected from the average of the pure plots only in high-density, non-irrigated plots (**Fig. II. 7b**), a similar trend to what was found for canopy packing (**Fig. II. 6b**). These results suggest that higher canopy packing does in fact result in higher light interception in mixed plots. However, the two measures were not strongly correlated because of the differences in light interception between the species in non-irrigated plots. Nevertheless, this result is an important step towards linking canopy packing to overyielding as a higher light interception in mixed plots may contribute to higher productivity, as hypothesized in several studies (Williams et al. 2017; Guillemot et al. 2020).

## 4.2. Variations in mixture effects at lower density and with irrigation

The design of our study allowed us to test how the mixture effects previously discussed varied with stand density (high density vs. low density) or when trees were irrigated to determine the sensitivity of these effects to changes in environmental conditions.

### 4.2.1. Density x Mixture

Our objective here was to test whether mixture effects were different when stand density was reduced by half (**Fig. II.A 1**). We hypothesized that this would release the trees from the competition for light and that complementarity effects between crowns would then be lower in mixed plots (Forrester 2014). Our results confirm this hypothesis; the net biodiversity effect on canopy packing was no longer significant at low density where canopy packing values in mixed plots were equivalent to the simple average of the values in pure plots (**Fig. II. 5, Fig. II. 6**).

At the tree-level, the competitive release for the pines in mixtures at low density was not as strong as at high density, probably because the lateral growing space for the pines was already large enough in pure plots. The mixture effect on the vertical dimension of pine was not affected by density (**Table II. 1**), suggesting that the main constraint for pine at high density concerned the lateral expansion of the crown ( $R_{\max}$ ). Consequently, the negative mixture effect on birch and the positive mixture effect on pine compensated each other at low density, resulting in a null net effect on canopy packing. This is in accordance with the theoretical framework of Forrester and Bauhus (2016) who suggest that as light availability per tree decreases, mixture effects that improve light interception may increase. Our results demonstrate that a given level of competition between crowns must be achieved before one can expect to observe net biodiversity effects on canopy packing. Based on our results for the high-density plots in this plantation, one might expect a positive mixture effect on canopy packing at the low density in the future as the canopy closes and the crowns become big enough to induce strong interaction effects (Williams et al. 2021).

### 4.2.2. Irrigation x Mixture

The plots were irrigated to relieve summer drought stress, at least partially, and we therefore expected larger diameters, greater crown growth and stronger competition for light among crowns in irrigated plots (Belluau et al. 2021). We hypothesized that there would be stronger aboveground mixture effects in the irrigated plots compared to the non-irrigated ones at similar density levels (Forrester and Albrecht 2014; Forrester and Bauhus 2016).

In contrast to these expectations, irrigation had no significant overall effect on crown volume for either species, nor on canopy packing (**Table II. 3**, **Table II. 4**). However, irrigation indirectly modulated the results of the mixture effects on pine and  $\Delta CP$ . Indeed, the increase in crown radii (**Table II. 1**, **Fig. II. 3**) and crown volumes (**Table II. 3**, **Fig. II. 4**) for pine in mixed plots was lower in the irrigated plots than in the non-irrigated ones, though at high density only. This could be an indirect consequence of an irrigation effect on birch, which is less drought tolerant than pine (Niinemets and Valladares 2006; Andivia et al. 2020). Indeed, we observed that the birch trees in the irrigated plots had a higher live crown ratio, especially in the mixed plots (**Table II. 1**, **Fig. II. 3f**). This resulted in more vertical crown overlap in the mixed irrigated than in the mixed non-irrigated plots (**Table II.B 4**). Moreover, we observed a strong irrigation effect on birch canopy closure in the pure plots (**Fig. II. 7a**), conversely to canopy packing (**Fig. II. 6a**), indicating that birch intercepted more light with irrigation than without it. We therefore suppose that birch was able to compete better with pine in the mixed irrigated plots at high density, meaning that the pines could not benefit as much from crown expansion as in the non-irrigated plots. Consequently, the canopy packing in the irrigated mixed plots was not significantly higher than the expected average from the corresponding pure plots (**Fig. II. 6b**). Contrary to our hypothesis, at high density, mixture effects were not stronger with irrigation. The fact that no mixture effects were observed at low density suggests that light was the limiting factor at high density, similarly to what has been suggested by Belluau et al. (2021). The interaction between light and water resources for tree and crown growth make it difficult to study the effect of either resource independently on mixture effects (Forrester 2014).

### 4.3. Implications for forest management

This study brings to light some interesting applications in terms of forest plantation management. In southwestern France, maritime pine is the target species in terms of wood production, with a planting density usually around 1250 trees ha<sup>-1</sup> (Mason and Meredieu 2011). An interesting perspective for these plantations arises when comparing the results obtained in this experiment for pure pine plots at low density (1250 trees ha<sup>-1</sup>) and mixed birch-pine plots at high density (1250 pine + 1250 birch trees ha<sup>-1</sup>). Adding birch to a maritime pine stand with a standard operational density did not induce any decrease in pine height, crown volume or diameter growth (**Fig. II. 3a**, **Fig. II. 4b**, **Fig. II.B 1**). That is, pine productivity was not reduced, at least during the first 10 years (Morin et al. 2020). We therefore believe

that mixing pine and birch in plantations would be relevant for several reasons in this region. Firstly, adding birch would likely result in a more closed canopy in the early years after planting, thereby limiting the growth of understory vegetation and improving the shape of the pines through natural pruning (Valkonen and Ruuska 2003). Secondly, birch could help improve soil fertility in mixed plantations through litter deposits or microorganism activity (Dubois et al. 2020; Maxwell et al. 2020). Thirdly, it has been shown that the presence of birch in pine plantations leads to associational resistance against insect herbivores such as the pine processionary moth and the pine stem borer (Castagneyrol et al. 2019; Jactel et al. 2019). Finally, the birch biomass could be harvested at the first pine thinning and be marketed as a supplementary product (Dubois et al. 2020) to compensate for the extra-cost of planting the birch seedlings. However, this possible multifunctionality must be verified over the entire rotation time and further evaluated in terms of costs and benefits. In addition, the site conditions, especially the soil water availability, under which these two species can be planted as a mixture have yet to be determined on a regional scale.

## **5. Conclusion**

The primary objective of this study was to understand the processes of canopy interaction between tree species in mixed stands and to investigate how these processes vary with stand density and water availability in a young plantation of pine and birch. We found that, at the tree level, stand composition influenced crown allometric relationships for pine but not for birch. Species interactions allowed increased crown and tree diameter dimensions for pine, but the opposite was found for birch. In addition, changes in crown volume resulted in higher canopy packing in mixed plots only for high-density plots with no irrigation, i.e., under highest constraints on light and soil water availability. Stand density and irrigation modulated the mixture effects on crown dimensions and canopy packing in this young tree plantation, confirming that water resources influence the potential complementarity between species for light interception in mixed forests as compared to monocultures.

## Declarations

**Authors' contribution statement.** **SMB:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft. **CM:** Conceptualization, Methodology, Investigation, Writing - review & editing. **HJ:** Conceptualization, Resources, Project administration, Funding acquisition, Writing - review & editing. **DB:** Conceptualization, Methodology, Investigation, Writing - original draft, Supervision. **MC:** Conceptualization, Methodology, Investigation, Writing - original draft, Supervision.

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**Conflicts of interest/Competing interests.** The authors have no conflicts of interest.

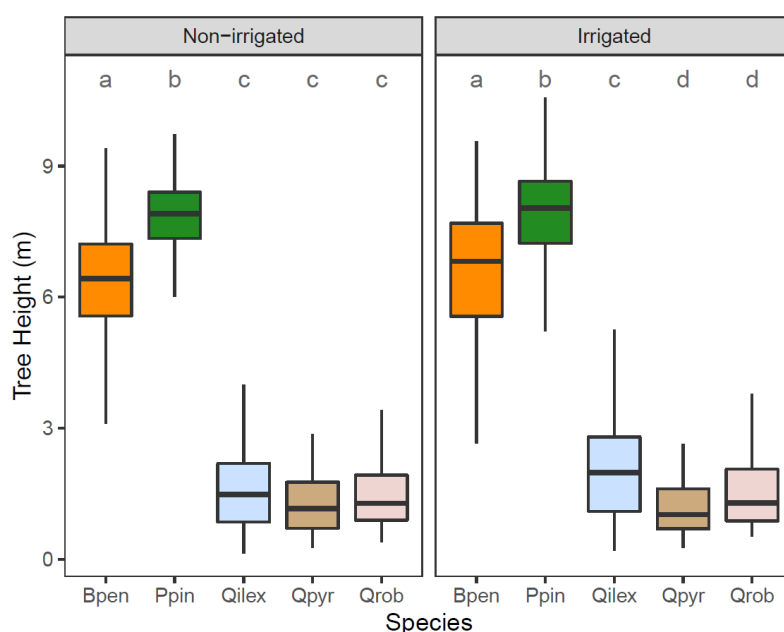
**Data availability.** The dataset analyzed during the current study are available from the corresponding author on reasonable request.



# Appendices

## 6. Appendices

### 6.1. Appendix II.A: Supplementary figures and tables for methods



**Fig. II.A 1** Mean tree height for the five tree species in the ORPHEE plantation by irrigation treatment (Bpen = birch, Ppin = pine, Qilex = holm oak, Qpyr = Pyrenean oak, Qrob = pedunculate oak). Pine and birch trees from the plots without oak species (high density) were not included in this graph. The mean height of the oak species is much lower than that of birch or pine ( $p < 0.001$ ), thus the upper canopy of these plots is constituted of birch and pine trees only. The oaks were therefore considered as gaps in the main canopy layer and were assumed not to interact with the birch or pine crowns. This allowed us to define these plots as low-density plots for pine and birch.

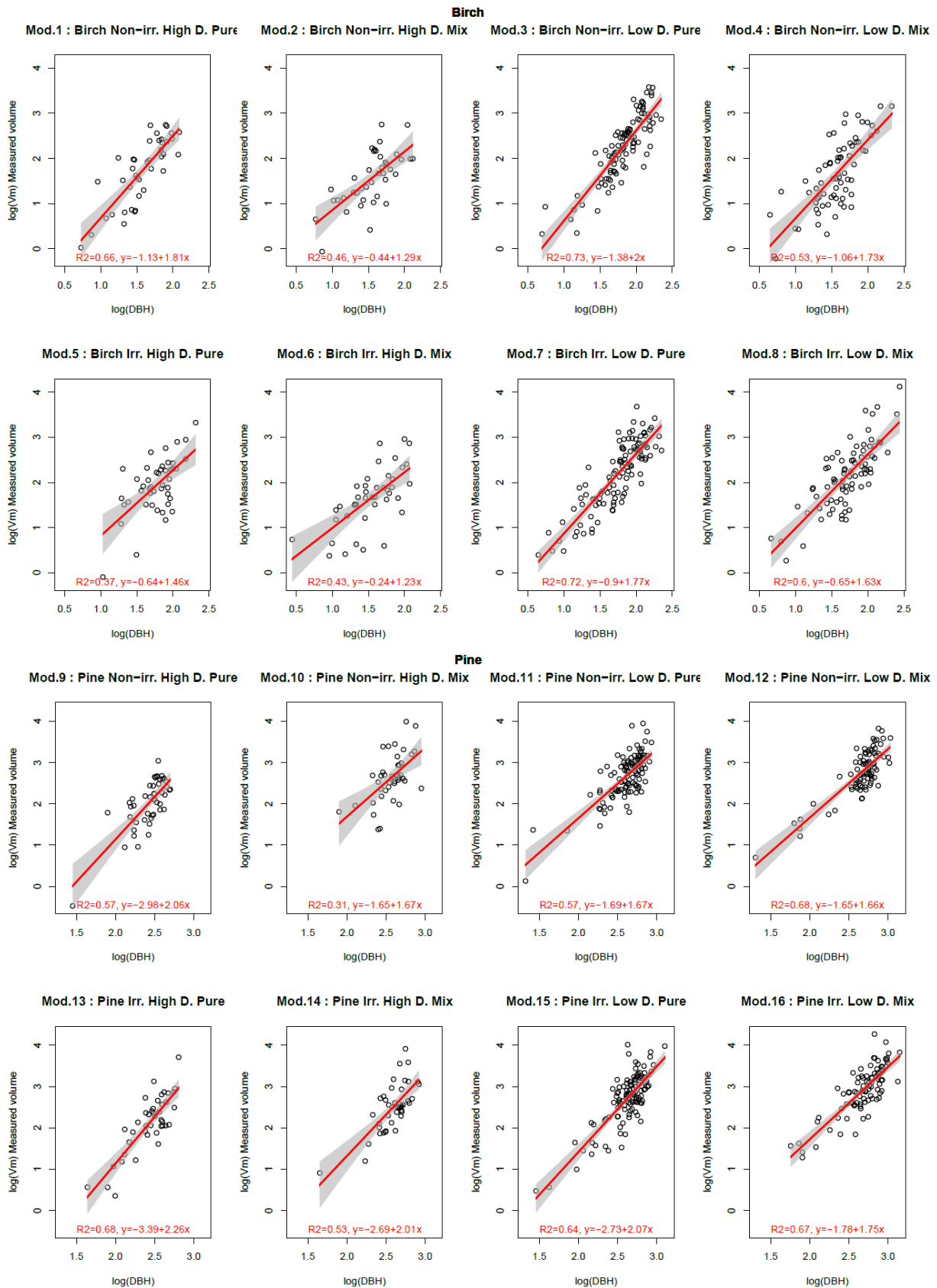
**Table II.A 1** Summary of the 16 treatment combinations with: number of selected plots, potential number of target birch and pine trees in each plot center (12 m x 12 m), average percentage of dead trees, total number of live trees measured for DBH, mean DBH of all living trees (cm), total number of trees selected for crown measurements (between 7 and 11 for each plot and species), mean DBH for the selected trees (cm). Numbers in parentheses are standard deviations.

Density	Irrigation	Composition	Species	Nb of plots	Nb of trees in each plot center	Average mortality	Total nb of live trees	Mean DBH all trees	Total nb of selected trees	Mean DBH of selected trees
High density	Non-irrigated	pure birch	Birch	4	36	8.33 % (5.07)	132	5.04 (1.55)	41	5.09 (1.51)
		pure pine	Pine	4	36	0.70 % (1.39)	143	11.64 (2.25)	47	11.55 (2.15)
		mixed B+P	Birch	4	18	1.39 % (2.78)	71	5.02 (1.35)	40	4.88 (1.47)
			Pine	4	18	5.56 % (0.00)	68	13.19 (3.01)	41	13.21 (2.47)
	Irrigated	pure birch	Birch	4	36	5.56 % (4.54)	136	5.86 (1.53)	45	6.05 (1.51)
		pure pine	Pine	4	36	2.08 % (2.66)	141	11.60 (3.14)	42	11.70 (2.68)
		mixed B+P	Birch	4	18	4.17 % (5.32)	69	4.89 (1.55)	42	4.91 (1.59)
			Pine	4	18	1.39 % (2.78)	71	13.64 (2.50)	43	13.58 (2.49)
Low density	Non-irrigated	pure birch	Birch	12	18	4.63 % (5.72)	206	6.29 (1.72)	100	6.43 (1.73)
		pure pine	Pine	12	18	4.63 % (8.15)	206	14.02 (2.69)	112	13.99 (2.74)
		mixed B+P	Birch	12	9	11.11 % (15.71)	96	4.92 (1.52)	69	5.05 (1.56)
			Pine	12	9	8.33 % (11.72)	99	14.99 (3.05)	87	14.86 (3.11)
	Irrigated	pure birch	Birch	12	18	8.80 % (12.64)	197	6.03 (1.82)	110	6.08 (1.91)
		pure pine	Pine	12	18	0.93 % (2.16)	214	14.11 (2.93)	114	14.13 (2.91)
		mixed B+P	Birch	12	9	11.11 % (14.98)	96	5.82 (1.94)	80	5.85 (1.86)
			Pine	12	9	6.48 % (8.81)	101	14.81 (3.87)	92	15.17 (3.55)

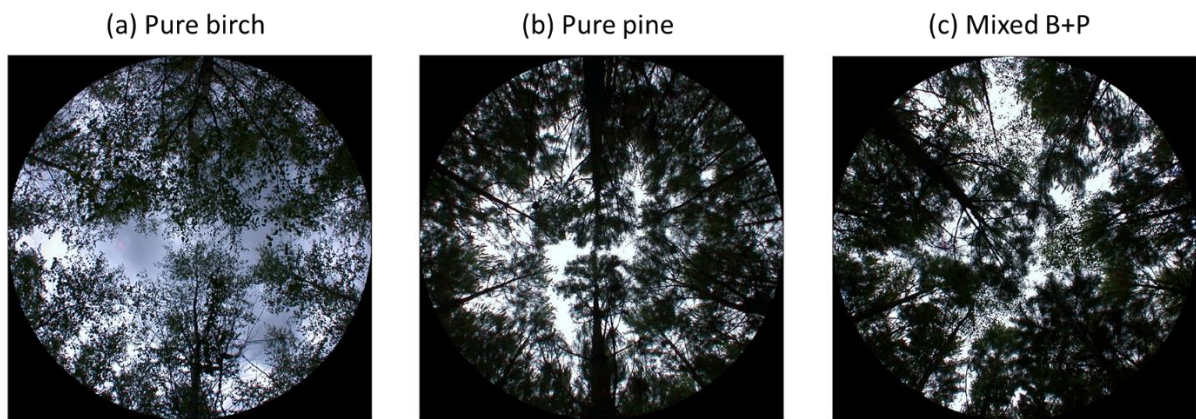


**Table II.A 2** Mean and standard deviations of the tree and crown metrics for each type of treatment combination. The variables are: total tree height (TH), height to diameter ratio (H/D ratio,  $\text{m m}^{-1}$ ), height of maximum crown radius ( $\text{HR}_{\text{max}}$ ), maximum crown radius ( $\text{R}_{\text{max}}$ ), height of first live branch (HLB), live crown ratio (LCR) and crown volume (CV). Here CV is the crown volume averaged over the measured trees only.

Density	Irrigation	Composition	Species	TH (m)	$\text{HR}_{\text{max}}$ (m)	$\text{R}_{\text{max}}$ (m)	HLB (m)	LCR (%)	H/D ratio	CV (m <sup>3</sup> )
High density	Non-irrigated	pure birch	Birch	6.03 (1.38)	3.10 (0.67)	0.89 (0.22)	1.8 (0.5)	70.49 (9.13)	125.39 (22.70)	7.03 (4.23)
		pure pine	Pine	7.73 (0.95)	5.30 (0.91)	1.01 (0.22)	2.9 (0.6)	63.60 (6.48)	69.78 (11.75)	8.65 (4.11)
		mixed B+P	Birch	6.36 (1.22)	3.94 (1.02)	0.87 (0.18)	2.4 (0.9)	64.22 (9.64)	135.76 (20.99)	5.59 (3.34)
			Pine	7.84 (0.99)	4.97 (1.14)	1.32 (0.35)	2.4 (0.7)	70.07 (7.34)	60.36 (7.09)	16.46 (10.70)
	Irrigated	pure birch	Birch	7.17 (1.29)	3.56 (0.93)	0.90 (0.19)	2.0 (0.7)	72.67 (6.80)	121.56 (17.99)	8.20 (4.96)
		pure pine	Pine	7.54 (1.04)	4.46 (0.99)	1.03 (0.26)	2.4 (0.5)	68.60 (5.75)	66.38 (10.70)	10.24 (7.01)
		mixed B+P	Birch	6.70 (1.50)	3.35 (1.29)	0.80 (0.19)	1.8 (0.7)	73.32 (9.66)	148.89 (35.98)	6.36 (4.26)
			Pine	7.97 (0.88)	4.82 (0.83)	1.19 (0.32)	2.4 (0.4)	70.42 (4.83)	60.21 (9.04)	14.38 (9.10)
Low density	Non-irrigated	pure birch	Birch	6.68 (1.46)	3.34 (0.99)	1.07 (0.26)	1.7 (0.7)	74.46 (9.71)	111.46 (19.80)	11.95 (7.85)
		pure pine	Pine	7.79 (1.04)	4.55 (0.94)	1.30 (0.29)	2.3 (0.5)	70.83 (5.40)	57.66 (10.93)	16.53 (8.44)
		mixed B+P	Birch	5.89 (1.35)	3.20 (1.00)	0.89 (0.25)	1.9 (0.7)	67.62 (11.52)	124.60 (19.80)	6.97 (5.41)
			Pine	7.69 (1.14)	4.23 (0.99)	1.34 (0.27)	1.9 (0.7)	75.57 (7.26)	52.59 (7.46)	18.34 (8.85)
	Irrigated	pure birch	Birch	6.40 (1.54)	2.59 (0.69)	1.00 (0.26)	1.2 (0.5)	82.21 (7.17)	114.73 (20.40)	11.36 (7.35)
		pure pine	Pine	7.98 (1.03)	4.48 (0.94)	1.30 (0.32)	2.2 (0.6)	71.86 (6.14)	58.22 (10.46)	17.73 (9.98)
		mixed B+P	Birch	6.64 (1.66)	3.07 (1.08)	0.99 (0.27)	1.5 (0.6)	78.51 (7.10)	124.85 (24.26)	11.07 (9.44)
			Pine	7.62 (1.40)	4.02 (1.03)	1.42 (0.32)	1.8 (0.6)	77.51 (6.59)	52.12 (8.13)	21.76 (11.81)

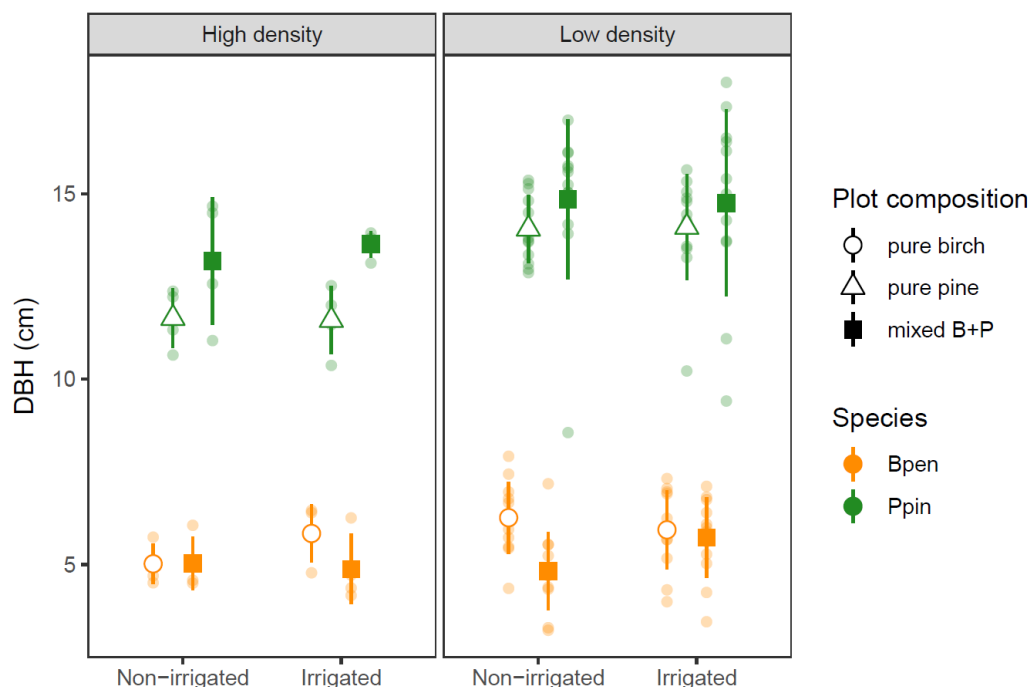


**Fig. II.A 2** Allometric relationships between  $\log(CV)$  and  $\log(DBH)$  by treatment type for measured trees. These relationships were used to predict crown volume (CV) from diameter at breast height (DBH) for the unmeasured trees in the plot centers for canopy packing analysis.



**Fig. II.A 3** Examples of hemispherical photographs for high-density non-irrigated birch, pine and mixed plots. Photos show zenith angles from 0° to 60°.

## 6.2. Appendix II.B: Supplementary analyses on tree and crown dimensions



**Fig. II.B 1** Average diameters at breast height (DBH, cm) by species, plot and treatment for all the trees in the plot center.

**Table II.B 1** Results of the LMMs for *Mixture*, *Density*, and *Irrigation* effects on DBH for all the trees in the center of the plots.

Predictors	Birch (n=1003)			Pine (n=1043)		
	<i>F</i> ( <i>df</i> )	<i>p</i> -value	<i>R</i> <sup>2</sup> <sub><i>m</i></sub> ( <i>R</i> <sup>2</sup> <sub><i>c</i></sub> )	<i>F</i> ( <i>df</i> )	<i>p</i> -value	<i>R</i> <sup>2</sup> <sub><i>m</i></sub> ( <i>R</i> <sup>2</sup> <sub><i>c</i></sub> )
Mixture (M)	7.39 (1;50)	<b>0.01</b>	0.09 (0.29)	11.61 (1;50)	< <b>0.01</b>	0.14 (0.30)
Density (D)	4.78 (1;50)	<b>0.03</b>		26.61 (1;50)	< <b>0.001</b>	
Irrigation (I)	0.99 (1;6)	0.36		0.02 (1;6)	0.89	
M x D	0.47 (1;50)	0.49		1.87 (1;50)	0.18	
M x I	0.06 (1;50)	0.81		0.04 (1;50)	0.84	
D x I	0.01 (1;50)	0.92		0.10 (1;50)	0.75	
M x D x I	5.29 (1;50)	<b>0.03</b>		0.22 (1;50)	0.64	

**Table II.B 2** Detailed results of the LMMs testing the effects of *Mixture*, *Density*, *Irrigation* and their interactions on birch and pine crown dimensions, for measured trees only. The variables are total tree height (TH, m), height to diameter ratio (H/D ratio, m m<sup>-1</sup>), height of maximum crown radius (HR<sub>max</sub>, m), maximum crown radius (R<sub>max</sub>, m), height of first live branch (HLB, m), and live crown ratio (LCR, %). For the summarized results see **Table II. 1**.

Response variable	Predictors	Birch (n=527)			Pine (n=578)		
		<i>F</i> ( <i>df</i> )	<i>p</i> -value	<i>R</i> <sup>2</sup> <sub><i>m</i></sub> ( <i>R</i> <sup>2</sup> <sub><i>c</i></sub> )	<i>F</i> ( <i>df</i> )	<i>p</i> -value	<i>R</i> <sup>2</sup> <sub><i>m</i></sub> ( <i>R</i> <sup>2</sup> <sub><i>c</i></sub> )
TH	Mixture (M)	1.24 (1;49)	0.27	0.08 (0.37)	0.17 (1;49)	0.68	0.02 (0.36)
	Density (D)	0.11 (1;49)	0.74		0.2 (1;49)	0.66	
	Irrigation (I)	2.21 (1;6)	0.19		0.00 (1;6)	0.98	
	M x D	0.51 (1;49)	0.48		1.70 (1;49)	0.20	
	M x I	0.51 (1;49)	0.48		0.36 (1;49)	0.55	
	D x I	1.07 (1;49)	0.31		0.34 (1;49)	0.56	
	M x D x I	5.18 (1;49)	<b>0.03</b>		0.77 (1;49)	0.38	
H/D ratio	Mixture (M)	30.15 (1;49)	<b>&lt;0.001</b>	0.18 (0.24)	51.73 (1;49)	<b>&lt;0.001</b>	0.23 (0.25)
	Density (D)	31.69 (1;49)	<b>&lt;0.001</b>		93.95 (1;49)	<b>&lt;0.001</b>	
	Irrigation (I)	0.79 (1;6)	0.41		0.48 (1;6)	0.52	
	M x D	1.71 (1;49)	0.20		1.33 (1;49)	0.25	
	M x I	1.64 (1;49)	0.21		0.23 (1;49)	0.63	
	D x I	0.15 (1;49)	0.70		0.67 (1;49)	0.42	
	M x D x I	2.86 (1;49)	0.10		1.06 (1;49)	0.31	
HR <sub>max</sub>	Mixture (M)	2.85 (1;49)	0.10	0.14 (0.33)	2.48 (1;49)	0.12	0.13 (0.31)
	Density (D)	10.53 (1;49)	<b>&lt;0.01</b>		20.09 (1;49)	<b>&lt;0.001</b>	
	Irrigation (I)	1.71 (1;6)	0.24		2.74 (1;6)	0.15	
	M x D	0.25 (1;49)	0.62		2.60 (1;49)	0.11	
	M x I	1.24 (1;49)	0.27		0.53 (1;49)	0.47	
	D x I	2.03 (1;49)	0.16		1.69 (1;49)	0.20	
	M x D x I	11.38 (1;49)	<b>&lt;0.01</b>		1.83 (1;49)	0.18	
R <sub>max</sub>	Mixture (M)	4.71 (1;49)	<b>0.03</b>	0.10 (0.24)	29.51 (1;49)	<b>&lt;0.001</b>	0.14 (0.20)
	Density (D)	13.16 (1;49)	<b>&lt;0.001</b>		49.53 (1;49)	<b>&lt;0.001</b>	
	Irrigation (I)	0.16 (1;6)	0.71		0.02 (1;6)	0.91	
	M x D	0.09 (1;49)	0.76		7.63 (1;49)	<b>&lt;0.01</b>	
	M x I	0.36 (1;49)	0.55		0.14 (1;49)	0.71	
	D x I	0.34 (1;49)	0.56		3.01 (1;49)	0.09	
	M x D x I	2.41 (1;49)	0.13		3.87 (1;49)	<b>0.05</b>	
HLB	Mixture (M)	6.61 (1;49)	<b>0.01</b>	0.23 (0.41)	13.31 (1;49)	<b>&lt;0.001</b>	0.24 (0.52)
	Density (D)	20.82 (1;49)	<b>&lt;0.001</b>		22.07 (1;49)	<b>&lt;0.001</b>	
	Irrigation (I)	5.21 (1;6)	0.06		1.72 (1;6)	0.24	
	M x D	0.00 (1;49)	0.99		1.08 (1;49)	0.30	
	M x I	2.01 (1;49)	0.16		0.91 (1;49)	0.34	
	D x I	4.59 (1;49)	<b>0.04</b>		0.8 (1;49)	0.37	
	M x D x I	5.00 (1;49)	<b>0.03</b>		2.19 (1;49)	0.15	

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	Mixture (M)	17.63 (1;49)	<b>&lt;0.001</b>	0.30 (0.41)	28.43 (1;49)	<b>&lt;0.001</b>	0.29 (0.43)
	Density (D)	30.97 (1;49)	<b>&lt;0.001</b>		43.52 (1;49)	<b>&lt;0.001</b>	
	Irrigation (I)	15.60 (1;6)	<b>&lt;0.01</b>		3.66 (1;6)	0.10	
LCR	M x D	0.74 (1;49)	0.39		0.41 (1;49)	0.53	
	M x I	5.77 (1;49)	<b>0.02</b>		0.81 (1;49)	0.37	
	D x I	3.70 (1;49)	0.06		0.41 (1;49)	0.52	
	M x D x I	0.68 (1;49)	0.41		2.06 (1;49)	0.16	

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**Note:** Marginal ( $R^2_m$ ) and conditional ( $R^2_c$ )  $R^2$  values were calculated for the model. They correspond to the variance explained by fixed and fixed plus random factors, respectively. Bold values indicate significant effects ( $P < 0.05$ ).

**Table II.B 3** Detailed results of the LMMs testing the effects of  $\log(\text{DBH})$ , *Mixture*, *Density*, *Irrigation* and their interactions on birch and pine crown dimensions, for measured trees only. The variables are total tree height (TH, m), height to diameter ratio (H/D ratio,  $\text{m m}^{-1}$ ), height of maximum crown radius ( $\text{HR}_{\max}$ , m), maximum crown radius ( $\text{R}_{\max}$ , m), height of first live branch (HLB, m), and live crown ratio (LCR, %). For the summarized results see **Table II. 2**.

Response variable	Predictors	Birch (n=527)			Pine (n=578)		
		<i>F</i> ( <i>df</i> )	<i>p</i> -value	$R^2_m$ ( $R^2_c$ )	<i>F</i> ( <i>df</i> )	<i>p</i> -value	$R^2_m$ ( $R^2_c$ )
log(TH)	log(DBH)	1992.86 (1;460)	<b>&lt;0.001</b>	0.81 (0.87)	1020.15 (1;511)	<b>&lt;0.001</b>	0.67 (0.79)
	Mixture (M)	1.46 (1;50)	0.23		16.62 (1;50)	<b>&lt;0.001</b>	
	Density (D)	3.97 (1;50)	0.05		1.61 (1;50)	0.21	
	Irrigation (I)	1.17 (1;6)	0.32		0.03 (1;6)	0.87	
	log(DBH) x M	0.02 (1;460)	0.90		11.09 (1;511)	<b>&lt;0.001</b>	
	log(DBH) x D	0.27 (1;460)	0.60		0.05 (1;511)	0.82	
	log(DBH) x I	0.02 (1;460)	0.90		0 (1;511)	0.95	
	D x M	1.92 (1;50)	0.17		2.06 (1;50)	0.16	
	I x M	3.89 (1;50)	0.05		0 (1;50)	0.98	
	D x I	1.74 (1;50)	0.19		0.78 (1;50)	0.38	
log(HD)	log(DBH)	1282.18 (1;460)	<b>&lt;0.001</b>	0.76 (0.83)	1434.5 (1;511)	<b>&lt;0.001</b>	0.79 (0.87)
	Mixture (M)	1.46 (1;50)	0.23		16.62 (1;50)	<b>&lt;0.001</b>	
	Density (D)	3.97 (1;50)	0.05		1.61 (1;50)	0.21	
	Irrigation (I)	1.17 (1;6)	0.32		0.03 (1;6)	0.87	
	log(DBH) x M	0.02 (1;460)	0.90		11.09 (1;511)	<b>&lt;0.001</b>	
	log(DBH) x D	0.27 (1;460)	0.60		0.05 (1;511)	0.82	
	log(DBH) x I	0.02 (1;460)	0.90		0 (1;511)	0.95	
	D x M	1.92 (1;50)	0.17		2.06 (1;50)	0.16	
	I x M	3.89 (1;50)	0.05		0 (1;50)	0.98	
	D x I	1.74 (1;50)	0.19		0.78 (1;50)	0.38	
log( $\text{HR}_{\max}$ )	log(DBH)	75.89 (1;460)	<b>&lt;0.001</b>	0.22 (0.37)	111.42 (1;511)	<b>&lt;0.001</b>	0.32 (0.43)
	Mixture (M)	1.02 (1;50)	0.32		3.12 (1;50)	0.08	
	Density (D)	0.95 (1;50)	0.34		5.26 (1;50)	<b>0.03</b>	
	Irrigation (I)	0.32 (1;6)	0.59		3.18 (1;6)	0.12	
	log(DBH) x M	3.57 (1;460)	0.06		1.58 (1;511)	0.21	
	log(DBH) x D	5.31 (1;460)	<b>0.02</b>		1.65 (1;511)	0.20	
	log(DBH) x I	0.2 (1;460)	0.66		5.11 (1;511)	<b>0.02</b>	
	D x M	0.32 (1;50)	0.57		3.71 (1;50)	0.06	
	I x M	0 (1;50)	0.95		0.01 (1;50)	0.94	
	D x I	2.06 (1;50)	0.16		2.42 (1;50)	0.13	
log( $\text{R}_{\max}$ )	log(DBH)	230.9 (1;460)	<b>&lt;0.001</b>	0.45 (0.48)	396.61 (1;511)	<b>&lt;0.001</b>	0.56 (0.60)
	Mixture (M)	0.86 (1;50)	0.36		4.08 (1;50)	<b>0.05</b>	
	Density (D)	1.7 (1;50)	0.20		2.31 (1;50)	0.13	
	Irrigation (I)	0.06 (1;6)	0.82		2.87 (1;6)	0.14	
	log(DBH) x M	1.33 (1;460)	0.25		2.69 (1;511)	0.10	
	log(DBH) x D	4.74 (1;460)	<b>0.03</b>		1.25 (1;511)	0.26	
	log(DBH) x I	0.56 (1;460)	0.46		2.53 (1;511)	0.11	
	D x M	0.17 (1;50)	0.69		2.06 (1;50)	0.16	

	I x M	0.22 (1;50)	0.64		0 (1;50)	0.96	
	D x I	2.34 (1;50)	0.13		1.74 (1;50)	0.19	
log(HLB)	log(DBH)	6.84 (1;460)	<b>0.01</b>	0.23 (0.35)	61.31 (1;511)	<b>&lt;0.001</b>	0.31 (0.55)
	Mixture (M)	0.08 (1;50)	0.77		16.84 (1;50)	<b>&lt;0.001</b>	
	Density (D)	0.43 (1;50)	0.51		2.62 (1;50)	0.11	
	Irrigation (I)	2.07 (1;6)	0.20		0.09 (1;6)	0.78	
	log(DBH) x M	1.4 (1;460)	0.24		10.21 (1;511)	<b>&lt;0.01</b>	
	log(DBH) x D	0.68 (1;460)	0.41		0.25 (1;511)	0.62	
	log(DBH) x I	0.15 (1;460)	0.70		0.41 (1;511)	0.52	
	D x M	0.24 (1;50)	0.63		3 (1;50)	0.09	
	I x M	0.01 (1;50)	0.93		0.01 (1;50)	0.91	
	D x I	7.71 (1;50)	<b>0.01</b>		0.16 (1;50)	0.69	
log(LCR)	log(DBH)	57.69 (1;460)	<b>&lt;0.001</b>	0.37 (0.50)	2.65 (1;511)	0.10	0.30 (0.44)
	Mixture (M)	0.02 (1;50)	0.90		18.21 (1;50)	<b>&lt;0.001</b>	
	Density (D)	2.73 (1;50)	0.10		5.02 (1;50)	<b>0.03</b>	
	Irrigation (I)	9.37 (1;6)	<b>0.02</b>		0 (1;6)	0.98	
	log(DBH) x M	1.09 (1;460)	0.30		11.8 (1;511)	<b>&lt;0.001</b>	
	log(DBH) x D	0.3 (1;460)	0.59		1.6 (1;511)	0.21	
	log(DBH) x I	2.46 (1;460)	0.12		0.23 (1;511)	0.63	
	D x M	0.2 (1;50)	0.66		1.36 (1;50)	0.25	
	I x M	3.53 (1;50)	0.07		0.15 (1;50)	0.70	
	D x I	4.57 (1;50)	<b>0.04</b>		1.09 (1;50)	0.30	

**Note:** Marginal ( $R^2_m$ ) and conditional ( $R^2_c$ )  $R^2$  values were calculated for the model. They correspond to the variance explained by fixed and fixed plus random factors, respectively. Bold values indicate significant effects ( $P < 0.05$ ).

**Table II.B 4** Average crown vertical overlap between species in pure and mixed plots. The vertical overlap is calculated for each block and type of plot as the difference between the average vertical profile of each species in pure and mixed plots. The average vertical profile of each species was calculated with the average height of the first live branch (HLB) and average total height (TH) for each plot. The values in the table are the mean values (standard deviation) across blocks and plot type.

Density	Irrigation	Pure plots	Mixed plots
High density	Non-irrigated	51 % ( $\pm 18$ )	66 % ( $\pm 6$ )
	Irrigated	78 % ( $\pm 14$ )	71 % ( $\pm 18$ )
Low density	Non-irrigated	71 % ( $\pm 15$ )	64 % ( $\pm 14$ )
	Irrigated	62 % ( $\pm 18$ )	79 % ( $\pm 15$ )



## CHAPITRE III

# Effet du mélange sur le fonctionnement hydrique et l'acquisition de l'eau des arbres en plantation mélangée de pin et de bouleau



Photo : Soline Martin-Blangy. Plantation ORPHEE, Cestas, France.

**Préambule :** Ce chapitre a pour objectif de comprendre les effets du mélange sur l'utilisation de l'eau en absence de stress hydrique et sur la réponse à la sécheresse dans une jeune plantation. Nous avons réalisé ce travail dans le cadre de la plantation expérimentale d'ORPHEE, dans des placettes pures et mélangées de bouleau (*Betula pendula*) et de pin maritime (*Pinus pinaster*). Les questions abordées dans ce chapitre sont similaires à celles abordées dans le Chapitre I, mais les conditions expérimentales contrôlées et l'accessibilité du site ont permis de faire des mesures plus poussées et d'aller plus loin dans les interprétations. Nous avons donc pu étudier le fonctionnement hydrique journalier et saisonnier en l'absence et en présence de stress hydrique et avons également caractérisé l'origine d'extraction de l'eau par les racines en saison sèche. Ce chapitre a été écrit sous un format qui rassemble plusieurs sous-chapitres écrits en anglais : une introduction, quatre sous-chapitres de résultats et une discussion générale. Les sous-chapitres ont été écrits en anglais afin de faciliter la publication future d'articles.



## Chapter III.1

### General introduction



# 1. General Introduction

## 1.1. Context & objectives

After two decades of a strong focus on the relationship between diversity and forest productivity (Ammer 2019), one of the main goals today for the field of biodiversity - ecosystem functioning (B-EF) research is to understand how diversity can impact water use and drought resistance in mixed forests, but also what are the underlying mechanisms behind B-EF relationships. In the context of climate change, these questions are of particular importance because of an increasingly limited water supply and more frequent droughts (Lehner et al. 2017). As of today, it remains controversial whether mixed-species forests will help mitigate the impacts of future droughts and, if so, through which processes (Forrester and Bauhus 2016; Ammer 2019; Grossiord 2019). On one hand, mixing species with complementary traits for water acquisition might increase the availability of water resources at the ecosystem level, on the other hand since mixed stands can be more productive than monocultures they may use more water, and therefore could be more susceptible to droughts (Forrester 2015). Moreover, these effects might vary in space and time for a given species combination (Forrester 2014).

Tree water use and drought response can be studied through a wide range of measures that can be either representative of a single point in time, study the evolution of a process through time, or give an integrated measure of water use over a longer period. In the context of my PhD, I was interested in using different approaches to investigate water use and drought response for silver birch (*Betula pendula*) and maritime pine (*Pinus pinaster*) in pure and mixed plots at the ORPHEE experimental plantation. In this chapter, I investigated several functional traits related to water use and drought response that allowed me to explore the following questions:

- i. What are the differences between the two species in terms of water use and drought response?
- ii. Do mixtures result in a higher water use at the species and plot levels in the absence of water stress?
- iii. Are there mixture effects on the drought exposure and drought response at the species and plot levels?
- iv. What are the effects of summer irrigation on tree functioning in pure stands?
- v. Are the mixture effects mitigated by the irrigation treatment?
- vi. Can the mixture effects be explained by water uptake complementarities?

This last chapter of results (**Chapter III**) from my PhD work is written in English as a preparation for the publication of future articles. The structure of the chapter, however, is divided into sub-chapters corresponding to different experiments that I conducted at ORPHEE over the course of my PhD. Some might act as a stand-alone for future published articles, but some of the experiments will be combined around a central question and reorganized before being published. I included more detailed context-

dependent introductions in the different sub-chapters, except for the short chapter on predawn water potentials.

I investigated the following traits, which make the outline of the different sub-chapters of Chapter III:

- *Chapter III.2, p. 139*: **Predawn leaf water potential ( $\Psi_{pd}$ )** to evaluate the water status of the trees in the different treatments during summer drought conditions.
- *Chapter III.3, p. 149*: **Sap flux density of trees ( $F_d$ ) and total plot transpiration ( $T_{plot}$ )** before and during summer drought, to look at mixture effects on water acquisition in non-limiting soil water conditions and on the drought response of the trees and whole plots.
- *Chapter III.4, p. 195*: **Stable carbon isotope composition in tree-rings ( $\delta^{13}C$ )** for years with wet or dry summers, to evaluate mixture and irrigation effects on drought exposure and resistance to drought.
- *Chapter III.5, p. 229*: **Stable water isotope composition ( $\delta^{18}O$  and  $\delta^2H$ ) in branches and soil** to estimate mixture effects on the proportion of root water uptake from different depths.

Moreover, data on the **resistance to cavitation (P50) and maximum conductivity ( $K_{max}$ )** to detect potential acclimations to mixture and irrigation effects on water stress conditions are also included in *Appendix III.A, p. 275*.

Finally, I close this chapter by a general discussion, bringing together the results from the different sub-chapters (*Chapter III.6, p. 265*).

## 1.2. Description of the experimental site

I summarize here the principal information about the experimental site to avoid repetitions in the sub-chapters. The experiments in this chapter were conducted at the ORPHEE experimental plantation located in southwestern France, 40 km southwest of Bordeaux (44°44.4' N, 00°47.80' W, altitude of 60 m asl). ORPHEE is included in the global Tree Diversity Network (<http://www.treedivnet.ugent.be/>). The site is located on a flat area with typical podzol soils with a coarse texture (95 % sand) (Augusto et al. 2010). This soil is characterized by a discontinuous cemented spodic horizon (hardpan) at around 50 cm depth (Altinmazis-Kondylis et al. 2020). Mean annual temperature is 12.5°C and mean average precipitation is 870 mm (1993–2018). Most of the rainfall occurs from fall to spring, and summers are rather dry. The water table is shallow in wintertime, generally ranging from 40 to 80 cm below the soil surface but drops down to between 150 and 180 cm during the summer months (**Fig. III. 3.3**) (Altinmazis-Kondylis et al. 2020).

The design of the plantation is described in Castagneyrol et al. (2013, 2017); here I will only provide a brief overview and the details specific to my work. My experiments were focused on silver birch (*Betula pendula*, hereafter birch) and maritime pine (*Pinus pinaster*, hereafter pine), both in monospecific and two-species mixed plots, resulting in three plot composition treatments: “pure pine”, “pure birch” and “mixed birch and pine”. One-year-old tree seedlings were planted at the site in January 2008 after clear-cutting of a mature pine plantation. Eight blocks were established, with plots in each block, planted at a density of 2500 trees ha<sup>-1</sup> per plot. Each plot has 10 rows of 10 trees planted 2 m apart (100 trees per plot, 20 m-by-20 m plot area). The mixed plots were established according to a substitutive design, with the initial plantation density kept constant across one or two-species plots.

In 2015, an irrigation system was added to half of the blocks, resulting in four irrigated blocks and four non-irrigated blocks. The irrigated plots are watered seasonally from early May to late September to alleviate summer stress. A 2-m high sprinkler installed in the center of each plot sprays the equivalent of about 3 mm day<sup>-1</sup> of precipitations. The irrigation takes place during the night to avoid evaporation. The irrigation water is pumped from the water table with a pump system that is nearby to the site. The volume of irrigation (3 mm day<sup>-1</sup>) was calculated based on regional climatic data (evapotranspiration). It is assumed to avoid any soil water deficit in the irrigated blocks during the entire growing season (Castagneyrol et al. 2017), although this assumption will be questioned in the sub-chapter on predawn leaf water potential.

The understory layer is mostly composed of bracken, moor grass, gorse, and ericaceous shrubs. It represents less than 1 % of the total aboveground biomass and its diversity is similar across all combinations of treatments, with six to seven vascular species per plot on average (Corcket et al. 2020).

*The sampling of trees and replicate blocks varies among the experiments: it will be detailed in the different Methods sections of each sub-chapter.*





## Chapter III.2

# Predawn leaf water potential during a summer drought



## 2. Predawn leaf water potential during a summer drought

### 2.1. Introduction

#### 2.1.1. Context

This short sub-chapter serves as a preliminary analysis of the potential mixture and irrigation effects on tree water availability during a drought period. In the following chapters, I will go more in depth into the state of the art on how species interactions can influence tree water use and drought exposure at different scales in space (tree and stand level) and time (daily measurements or integrated over a whole season).

Leaf water potentials are considered as reliable indicators of tree water status (Choné et al. 2001; Williams and Araujo 2002; Remorini and Massai 2003). Specifically, predawn leaf water potential ( $\Psi_{pd}$ ) expresses the maximum value of water potential in the plant during the whole daily cycle. At dawn, the tree is at equilibrium with soil conditions and  $\Psi_{pd}$  represents the average water status of the soil to which the tree has access with its root system (Schulze and Hall 1982). Predawn leaf water potential is therefore considered a reliable indicator of tree water status and soil water availability (Correia et al. 1995). Predawn leaf water potential is expressed in MPa, with more negative values indicating a stronger water deficit. Values close to zero indicate that there is no water deficit, as the soil water is easily available to the plant. As an example, for vine (*Vitis vinifera*), a  $\Psi_{pd}$  above -0.2 MPa was defined as an absence of water deficit (Van Leeuwen et al. 2009) and for *Eucalyptus tetradonta*, Prior et al. (1997) values below -0.5 MPa were used as a threshold for the end of the wet season and the start of the dry season.

Predawn base water potential is independent of the demand for water imposed by the atmosphere to the tree during the day and only reflects belowground water availability. It can therefore serve as a baseline for my expectations of drought response in the following chapters, independently of aboveground conditions such as light availability and canopy structure.

#### 2.1.2. Objectives

We measured predawn leaf water potentials ( $\Psi_{pd}$ ) to assess soil water availability for individual trees during a drought episode in 2019 at ORPHEE. I wanted to investigate the following questions:

- i. What is the water availability for the two species during summer drought in non-irrigated pure plots?
- ii. What is the effect of summer irrigation on water status for both species in pure plots?
- iii. Are birch and pine less stressed in mixed plots compared to pure plots in non-irrigated and irrigated blocks respectively?

## 2.2. Methods

### 2.2.1. Study site

For this section, please refer to the description of the experimental site at the beginning of this chapter (**section 1.2**).

### 2.2.2. Predawn leaf water potential ( $\Psi_{pd}$ ) measurements

We measured predawn leaf water potential ( $\Psi_{pd}$ , MPa) in blocks 1 to 6 at ORPHEE: three non-irrigated blocks (1, 3 and 6) and three irrigated blocks (2, 4 and 5). In each block, I selected the three following plots: one pure birch, one pure pine and one mixed birch and pine. In each of these plots, I selected six trees per species for a total of 144 trees. The individual trees were selected based on previous sampling protocols in 2019. In blocks 1 and 2 these were the same trees as the sap flux experiment that had been selected earlier in the year (March 2019) (**Chapter III.3, p. 149**). In blocks 3 to 6 we selected six trees among those that had been selected to measure vulnerability to embolism ( $\Psi_{50}$ ) in February and March 2019 (**Appendix III.A, p. 275**).

$\Psi_{pd}$  was measured on July 18<sup>th</sup> and 19<sup>th</sup>, 2019 during a dry period (see **Fig. III. 3.3**). We collected small branches from the upper part of the crowns with a pole pruner before sunrise (between 4:30 and 7:30 a.m.). The samples were immediately measured in the field with a Scholander-type pressure chamber (PMS Instruments, Corvallis, Oregon, USA) (Scholander et al. 1965). For birch we used the extremity of a branch with 2-3 leaves in the pressure chamber. For pine we used a paired needle that had been cut at the base of the sheath in the pressure chamber.

### 2.2.3. Statistical analysis

I first tested *Species* and *Irrigation* effects on  $\Psi_{pd}$  in **pure plots** with the following mixed model:

$$\Psi_{pd} \sim \text{Species} + \text{Irrigation} + \text{Species:Irrigation} + 1|\text{Block} \quad (1)$$

where *Species* is a fixed factor with two levels (birch and pine), *Irrigation* is a fixed factor with two levels (non-irrigated and irrigated) and *Block* is a random factor with six levels. The result from the mixed model is reported in **Table III. 2.1**. I used post-hoc multiple comparison tests to identify which pairs of treatment combinations are statistically different from each other.

I further tested *Species* and *Mixture* effects on  $\Psi_{pd}$  for non-irrigated and irrigated blocks separately with the following mixed model:

$$\Psi_{pd} \sim \text{Species} + \text{Mixture} + \text{Species:Mixture} + 1|\text{Block} \quad (2)$$

where *Species* is a fixed factor with two levels (birch and pine), *Mixture* is a fixed factor with two levels (pure vs. mixed) and *Block* is a random factor with six levels. The result from the mixed models for each

species are reported in **Table III. 2.2**. I used post-hoc multiple comparison tests to identify if pure and mixed plots were statistically different from each other within each model and for each species.

R 3.6.2 language programming (R Core Team 2019) with the Rstudio interface was used for all the analyses with the following packages: nlme (“lme” function), MuMIn and emmeans (“lsmeans” function). Type “III” ANOVAs were used after linear mixed models.

## 2.3. Results

### 2.3.1. Species effects in non-irrigated pure plots

In the non-irrigated pure plots, there was a strong difference between species  $\Psi_{pd}$  (est.  $\pm$  SE:  $0.40 \pm 0.04$  MPa,  $p < 0.001$ ), with less negative  $\Psi_{pd}$  for birch (mean  $\pm$  SE:  $-0.7 \pm 0.05$  MPa) than pine trees ( $-1.1 \pm 0.05$  MPa) (**Fig. III. 2.1, Table III. 2.1**).

### 2.3.2. Irrigation effects in pure plots

In pure plots, the irrigation effect on  $\Psi_{pd}$  was strong and of a similar magnitude for both birch (est.  $\pm$  SE:  $0.48 \pm 0.08$  MPa,  $p < 0.01$ ) and pine ( $0.44 \pm 0.08$  MPa,  $p < 0.01$ ) (**Fig. III. 2.1, Table III. 2.1**).

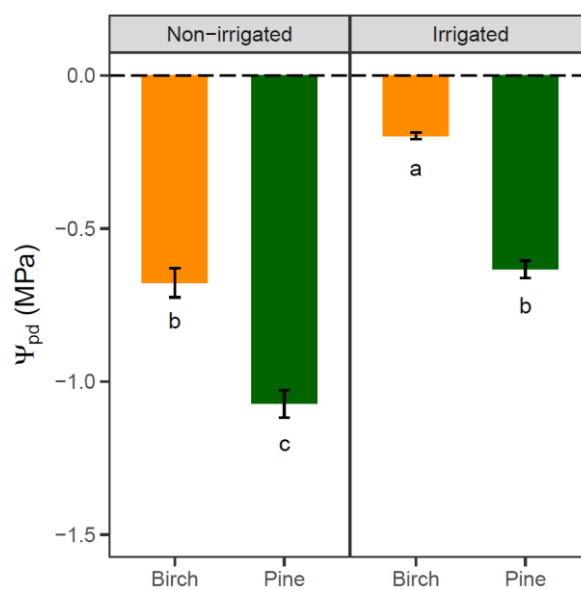
Consequently, the size of the species effect was similar in irrigated than in non-irrigated pure plots (est.  $\pm$  SE:  $0.44 \pm 0.04$  MPa,  $p < 0.001$ ) and  $\Psi_{pd}$  for birch trees (mean  $\pm$  SE:  $-0.2 \pm 0.01$  MPa) remained less negative than for pine trees ( $-0.6 \pm 0.03$  MPa) (**Fig. III. 2.1, Table III. 2.1**).

### 2.3.3. Mixture effects

There were no significant mixture effects on  $\Psi_{pd}$  in the non-irrigated blocks ( $p = 0.61$ , **Table III. 2.2**) for either species (post-hoc test for birch:  $p = 0.61$ , pine:  $p = 0.05$ ). Although the p-value was close to being significant for pine, the magnitude of the effect was small (est.  $\pm$  SE:  $0.11 \pm 0.05$  MPa) (**Fig. III. 2.2**).

Similarly, there were no mixture effects on  $\Psi_{pd}$  in the irrigated blocks ( $p = 0.06$ , **Table III. 2.2**) for either species (post-hoc test for birch:  $p = 0.06$ , pine:  $p = 0.83$ ). Although the p-value was close to being significant for birch, the magnitude of the effect was small (est.  $\pm$  SE:  $0.05 \pm 0.03$  MPa) (**Fig. III. 2.2**).

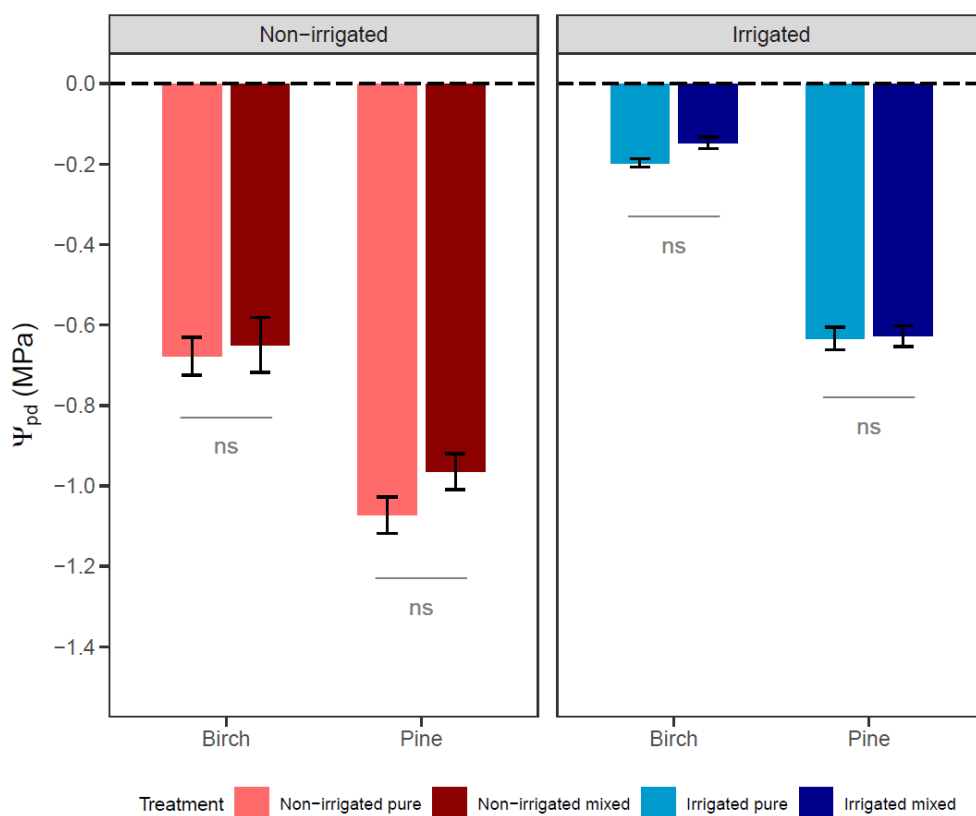
Consequently, the magnitude of the species and irrigation effects on  $\Psi_{pd}$  remained similar in the mixed plots compared to the pure plots.



**Fig. III. 2.1**  $\Psi_{pd}$  averaged by species and irrigation treatment for all blocks in pure plots only. The error bars represent the standard error of the mean. The letters represent results of post-hoc tests after linear mixed model testing for species and irrigation effects on  $\Psi_{pd}$  in pure plots. See **Table III. 2.1** for linear mixed model results.

**Table III. 2.1** ANOVA table for linear mixed model for species and irrigation effects on  $\Psi_{pd}$  in pure plots.

Predictor	$F(df)$	$p$ -values
Species (S)	83.1 (1;64)	<b>&lt;0.001</b>
Irrigation (I)	37.5 (1;4)	<b>&lt;0.01</b>
S x I	0.5 (1;64)	0.50
$R^2_m (R^2_c)$		0.82 (0.87)



**Fig. III. 2.2**  $\Psi_{pd}$  averaged by irrigation treatment, species and mixture for all blocks (data for pure plots are the same as in **Fig. III. 2.1**). The error bars represent the standard error of the mean. Text with horizontal lines indicate the results of post-hoc tests after linear mixed model testing for mixture and irrigation effects for each species. Significance at a level of 5 % are indicated by stars (ns: non-significant; (.) p-values < 0.1; \* p-values < 0.05; \*\* p-values < 0.01; \*\*\* p-values < 0.001; ns no significant effect). See **Table III. 2.2** for linear mixed model results.

**Table III. 2.2** ANOVA table for linear mixed models for species and mixture effects on  $\Psi_{pd}$  for non-irrigated and irrigated blocks separately.

Predictors	Non-irrigated		Irrigated	
	<i>F(df)</i>	<i>p-value</i>	<i>F(df)</i>	<i>p-value</i>
Species (S)	53.6 (1;66)	<0.001	288.8 (1;66)	<0.001
Mixture (M)	0.3 (1;66)	0.61	3.8 (1;66)	0.06
S x M	1.1 (1;66)	0.50	1.5 (1;66)	0.22
$R^2_m$ ( $R^2_c$ )		0.42 (0.69)		0.88 (0.91)

## 2.4. Discussion

### 2.4.1. Species effects in non-irrigated pure plots

As expected, in the middle of the dry summer of 2019, both species experienced significant water deficit in the non-irrigated pure plots. Birch had a higher  $\Psi_{pd}$  (mean  $\pm$  SE:  $-0.7 \pm 0.05$  MPa) than pine trees ( $-1.1 \pm 0.05$  MPa) (**Fig. III. 2.1, Table III. 2.1**), indicating a higher water availability for the roots of birch than those of pine in pure plots (Scholander et al. 1965).

The difference between the two species can be explained by different factors. First, if one considers that superficial soils are drier than deep ones in summer (see **Fig. III. 3.3**), this difference suggests that pine trees have a shallower rooting depth than birch (see **Chapter III.5, p. 229**). This result would be surprising, even though it cannot be excluded, because pine is thought to have a deeper rooting system than birch (Mauer and Palátová 2003; Curt and Prévosto 2003; Bakker et al. 2006) and Altinalmazis-Kondylis (2020) actually found no difference in fine root biomass vertical distribution between the two species at ORPHEE in 2018. Second, these results suggest for birch that these trees may have access to the groundwater. This cannot be excluded and will be tested in **Chapter III.5, p. 229**, to determine the contributions of soil water from different depths to root water uptake. Finally, this difference could be explained by a difference in canopy structure in the pure plots of the two species, particularly by a higher LAI and therefore a higher evaporative demand in the pine plots. Would this be true, this would induce a higher water demand and then a faster depletion of soil water resources in pure pine plots than in pure birch plots, and therefore lower  $\Psi_{pd}$  values. I will discuss this in detail in **Chapter III.3, p. 149**.

Birch  $\Psi_{pd}$  represented a value of a moderate water deficit, while pine  $\Psi_{pd}$  was representative of a stronger water deficit, even though not very severe (Van Leeuwen et al. 2009). Pine  $\Psi_{pd}$  was in the common range compared to other measures during summer drought in the same region: for example, Loustau et al. (1998) found a  $\Psi_{pd}$  of  $-0.96$  MPa for maritime pine at the beginning of August 1995.

We cannot say if drought levels were strong enough to cause embolisms in the xylem vessels based on predawn leaf water potentials, because water potentials will reach much more negative values during the day. Actually, I did measure midday leaf water potentials in 2019 on the same trees but did not yet have time to analyze the data. In the non-irrigated pure plots, these midday leaf water potentials reached an average of  $-1.5$  MPa for birch and  $-1.9$  MPa for pine (data not shown) during days with a high VPD (July 22<sup>nd</sup> and 23<sup>rd</sup>, 2019). Cavitation will happen only during extreme drought events, at lower potentials than the ones inducing maximal stomatal closure (Choat et al. 2018). Colleagues at Bordeaux (S. Delzon and colleagues) collected data for us to determine xylem vulnerability to embolism ( $\Psi_{50}$ ) in the spring of 2019. The results are summarized in **Appendix III.A, p. 275**: the average  $\Psi_{50}$  in non-irrigated pure plots was  $-2.3$  MPa for birch and  $-3.6$  MPa for pine (**Fig. III.A 3**). These values do indeed show that the midday leaf potentials we measured are not negative enough to induce strong losses in conductivity. However, the vulnerability curves (**Fig. III.A 2**) show that it could be possible that a small



loss of conductivity due to embolisms would start to appear around -1.5 MPa for birch, while there would be no risks of embolisms for pine at -1.9 MPa.

#### 2.4.2. Irrigation effects in pure plots

Birch  $\Psi_{pd}$  was 3.5 times higher (i.e., lower water deficit) in irrigated than in non-irrigated pure plots, while pine  $\Psi_{pd}$  was 1.8 times higher (**Fig. III. 2.1**). The irrigation treatment was therefore sufficient to strongly influence soil water conditions and remove any water deficit for birch. On the other hand, for pine, the irrigation treatment was not sufficient to remove all water deficit. When the irrigation treatment was set up, the amount of irrigation was designed to remove all water stress (H. Jactel, pers. comm.), but this illustrates how difficult it is managing such an experiment in the field at this scale (several hectares of irrigated plantation). In fact, pine trees in the irrigated blocks were as stressed as birch in the non-irrigated blocks. It was previously demonstrated that the irrigation treatment relieved water deficit stress at ORPHEE for oak and birch trees (Castagneyrol et al. 2017, 2018). However, my results evidence for the first time that the irrigation treatment is not sufficient to completely relieve summer stress for pine. This is probably due to a higher evaporative demand in pure pine plots compared to pure birch plots (see **Chapter III.3, p. 149**), as observed with the lower  $\Psi_{pd}$  for pine in non-irrigated pure plots.

The consequence of these results, particularly for pine, are important for my study. It requires that in the following chapters, I consider the irrigated blocks as a treatment with lower water deficit during the summer than in the non-irrigated ones, but not as a treatment with a complete absence of water deficit.

#### 2.4.3. Mixture effects

Surprisingly, we found no significant mixture effects for either species and in either non-irrigated or irrigated blocks (**Fig. III. 2.2**). This means that mixing birch and pine did not impact their respective water deficit levels. In contrast with our results, Bello et al. (2019) showed that a partial niche complementarity for limited water resources between Scots pine (*Pinus sylvestris*) and sessile oak (*Quercus petraea*) resulted in less water constraint (i.e., less negative  $\Psi_{pd}$ ) for oak in mixed stands than in pure stands. On the other hand, Grossiord et al. (2014a) did not find any effect of species diversity on  $\Psi_{pd}$  despite a wider range of species. Finally, Didion-Gency et al. (Didion-Gency et al. 2021) found that for a given species (beech, *Fagus sylvatica*)  $\Psi_{pd}$  was either higher (i.e., lower water deficit) in mixed stands with fir (*Abies alba*) or lower (i.e., higher water deficit) in mixed stands with pubescent oak (*Quercus pubescens*) than in pure stands.

The difference in  $\Psi_{pd}$  between the two species remained the same in mixed stands. The presence of conspecific or heterospecific neighbors resulted in the same  $\Psi_{pd}$ , meaning that  $\Psi_{pd}$  was mostly dependent on the species-specific water use and water uptake but was not dependent on neighbor identity. This suggests that we might not find complementarity mechanisms in the rooting patterns of both species (see **Chapter III.5, p. 229**). Independently of complementarities in water uptake depth, I had expected

that the potentially lower water use of birch would allow pine to be less water stressed than in pure plots, since more water could be available in the direct surroundings of a given pine tree with birch as neighbors. However, in mixed stands pine trees were also bigger and therefore had a higher water use than in pure stands (**Chapter III.3, p. 149**).

#### 2.4.4. Reliability of $\Psi_{pd}$ as an indicator of water deficit

It must be noted that this experiment was conducted only once during the summer. However, the water use and mean depth of water uptake of these trees may vary during the growing season when soil water availability and evaporative demand change (Brinkmann et al. 2019).

Moreover,  $\Psi_{pd}$  does not always represent the average water availability for the roots of a tree. In heterogeneous soil conditions, such as with the presence of a water table or with the irrigation input,  $\Psi_{pd}$  might align with the water potential of the most humid zone (Améglio et al. 1999).  $\Psi_{pd}$  might not always be the most reliable indicator of water status, and sap flux rate might bring useful insight into the trees drought response (Remorini and Massai 2003).

## 2.5. Conclusions

There were strong differences in  $\Psi_{pd}$  between birch and pine in pure plots, indicating that pine trees were more stressed than birch. The irrigation treatment was sufficient to remove water deficit for birch, but not for pine: pine trees in the irrigated blocks had a similar water deficit than birch trees in the non-irrigated blocks. Finally, contrary to our expectations, there were no mixture effects for either species or in either irrigation treatment which suggests a probable absence of complementarity in water uptake depth.

## Chapter III.3

# Sap flux density and plot transpiration before and during a summer drought



### 3. Sap flux density and plot transpiration before and during a summer drought

#### 3.1. Introduction

##### 3.1.1. Context

Transpiration is a critical process for trees since water availability is frequently a limiting factor in ecosystems and droughts are forecasted to increase in frequency and severity under future climate scenarios (Lehner et al. 2017). The major physiological adjustment trees undergo to preserve leaf and whole tree water status during drought, is related to stomatal closure (Bréda et al. 2006). Among the main factors, leaf stomatal conductance is driven by atmospheric vapor pressure deficit and soil water supply. By closing their stomata during drought, trees reduce leaf transpiration and therefore total water-use. Yet, stomatal closure also induces a restriction in CO<sub>2</sub> uptake and assimilation rates (Farquhar and Sharkey 1982). As a reminder, the decrease in CO<sub>2</sub> fluxes is usually smaller than the decrease in H<sub>2</sub>O fluxes, which induces an increase in intrinsic water use efficiency (WUE<sub>i</sub>) and in carbon isotope composition ( $\delta^{13}\text{C}$ ) in the leaf (Farquhar et al. 1982) (see **Chapter III.3, p. 149**).

In mixed stands, transpiration is one of the main processes that could influence soil water availability compared with monocultures, and hence the susceptibility of mixed forest ecosystems to drought or their influence on local water supplies (Forrester 2015). Several studies have found a higher water use in mixed stands compared to monocultures, i.e. higher ecosystem transpiration in mixed stands, especially if there were mixture effects on growth (Forrester et al. 2010; Kunert et al. 2012; Gebauer et al. 2012). At the stand and species levels, when there were no mixture effects on growth, there were usually no mixture effects on transpiration or water use efficiency (Grossiord et al. 2013a; Forrester 2015).

There is no consensus so far on the effects of mixtures on tree and stand-level response to drought (see the following reviews: Forrester 2015; Ammer 2019; Grossiord 2019). This is because the overall pattern can be influenced by several factors. (i) The response to drought in mixtures could be facilitated by water uptake complementarities among species, which would result in a higher water availability at the ecosystem level. However, when a higher productivity is observed in mixtures, it may increase the total water demand compared to pure stands (if there are no major changes in WUE) and result in mixtures that are more susceptible to drought. This higher drought exposure could be especially true if it is not compensated by a higher soil water availability obtained thanks to complementary water uptake (Forrester 2015; Ammer 2019). (ii) Moreover, since mixtures also influence canopy structure and light interception, they could impact canopy microclimatic conditions that will in turn affect the species' response to drought (Baldocchi 2005; Kunert et al. 2012; Forrester 2015). (iii) Consequently, the outcome is highly dependent on the choice of interacting species and their species-specific below and

aboveground traits (Forrester et al. 2016). (iv) Finally, these effects are also climate dependent and can vary depending on the intensity of the drought (Forrester 2015).

To quantify the temporal variability of plant transpiration, and to understand the plants' response to the environment, sap flux density measurements can be used. Sap flux density quantifies the flow of water for a given conducting area in the tree for a given time (sap flux, kg per m<sup>2</sup> of sapwood per s).

### 3.1.2. Objectives

To further elucidate how species mixture influence tree and stand water use and resistance to drought, I monitored sap flux density of trees in pure birch, pure pine and mixed birch and pine plots at ORPHEE during the entire growing season of 2019, which was characterized by a wet spring and a severe summer drought. I first looked at potential mixture effects on water use in the absence of drought (late-spring/early-summer conditions) and in a second time during summer drought conditions. Additionally, I was able to compare plots in similar climatic conditions but with varying soil water conditions thanks to the irrigation treatment in half of the blocks at ORPHEE. I first focused on daily sap flux density of individual trees, which are independent of mixture effects on tree growth and sapwood area, to observe functional differences between treatments. I further focused on stand level transpiration to provide an estimation of total water use and mixture effects on plot transpiration.

In this sub-chapter, I thus asked the following questions: (i) are there mixture effects on sap flux density in the spring before summer drought, (ii) are sap flux density losses in response to summer drought different between pure and mixed stands, (iii) does the irrigation treatment modulate mixture effects on the response to drought of sap flux density, and (iv) how do species interactions and soil water availability influence stand-level transpiration ?

More specifically, during the spring (May-June period) in the absence of water stress, I hypothesized that:

- (i) there would be differences in sap flux density between the species, with higher values for birch,
- (ii) there would be no differences in sap flux density with mixture,
- (iii) there would be no irrigation effects on sap flux density of both species.

During the summer drought (July), I hypothesized that:

- (i) there would be an important drop in sap flux density that would vary between species,
- (ii) this loss in sap flux density would be faster and more important in stands with pine (pure pine and mixed B+P) due to a potentially higher water use based on observed plot basal areas,
- (iii) in the irrigated plots there would be a lower loss of sap flux density and a smaller mixture effect than in the non-irrigated plots.

Scaling up to stand-level transpiration, in the absence of stress (May-June) I hypothesized that, based on plot basal areas:

- (i) mixed stands would have an intermediate plot transpiration rate between pure pine and pure birch,
- (ii) there would be a higher transpiration in mixed plots than expected from pure plots (positive net biodiversity effect),
- (iii) with irrigation the transpiration rates would be higher than in the non-irrigated plots.

Finally, during summer stress (July), I expected that:

- (i) without irrigation mixed stands would have a higher loss of transpiration than expected from pure stands due to a faster depletion of water resources,
- (ii) with irrigation there would be no mixture effects on the transpiration rates.

## 3.2. Methods

### 3.2.1. Experimental design

#### 3.2.1.1. Study site

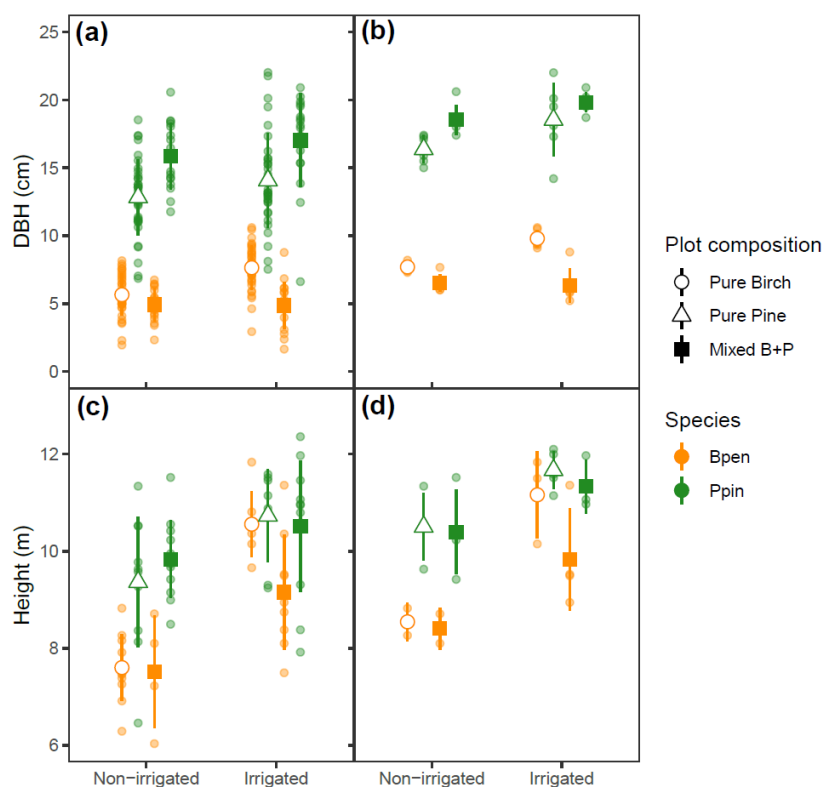
For this section, please refer to the description of the experimental site at the beginning of this chapter (**section 1.2**).

#### 3.2.1.2. Sampling

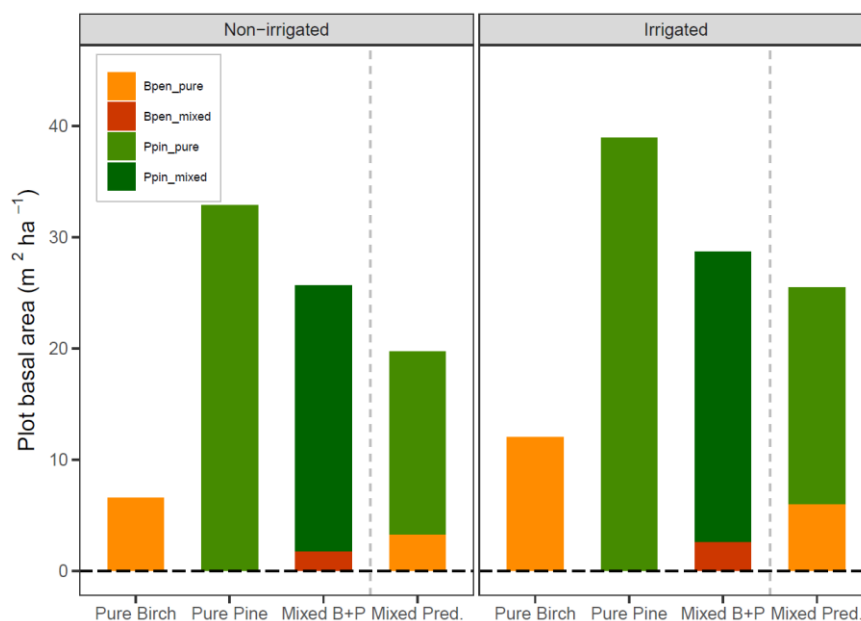
In this study I focused on two blocks only, one non-irrigated (block 1) and one irrigated (block 2). I could not replicate our measures in other blocks because sap flux sensors are costly to install, and the monitoring is very time consuming. In each block, I selected three plots: pure birch, pure pine and mixed birch and pine plots. In each of these plots, I selected six trees per species and plot to be equipped with sap flux sensors, for a total of 48 trees. I defined this sampling size based on Granier et al. (1996) who estimated that a set of 5-10 sap flow sensors could provide an accurate estimate of stand transpiration, especially in an even-aged plantation like ORPHEE. The aim was to select trees that would best represent the average transpiration of the trees in these plots. Since all trees had the same age, I chose trees that had vigorous crowns and a high crown illumination percentage, and I avoided trees that had a gap from a dead tree in their direct neighborhood. The trees were selected among the trees from the six inner rows of each plot to avoid potential edge effects in the two outermost rows. However, with regards to this criterion, two birch trees in the non-irrigated mixed plot and one birch tree in the irrigated mixed plot had to be selected from the second to last rows because of birch mortality in the center of the plot.

The DBH of all trees in the selected plots was measured before (February 2019) and after (February 2020) the 2019 growing season (**Fig. III. 3.1a**). Total tree heights were also measured on a subset of trees (7 to 11 per species and plot) (**Fig. III. 3.1c**). I used an average value between these two years to represent tree characteristics in the summer of 2019 (**Fig. III. 3.1**) and to calculate plot basal area (**Fig. III. 3.2**) for plot-level transpiration calculations (**section 3.2.5**).





**Fig. III. 3.1** Diameter at breast height (DBH, cm) and height (m) of birch (Bpen) and pine (Ppin) in 2019 in the selected plots for all measured trees (a, c) and for the trees monitored with sap flux sensors only (b, d). DBH was measured on all the 36 trees in the plot centers (a), while height was only measured for a subset of 7 to 11 trees per plots (c), and hence also a subset of the monitored trees (d).



**Fig. III. 3.2** Plot basal area (BA) in 2019 by species and plot composition for the chosen plots in blocks 1 and 2. The predicted values for the mixture (*Mixed Pred.*) are the weighted average from the respective monocultures with the proportion of basal area of each species in the observed mixed plots.

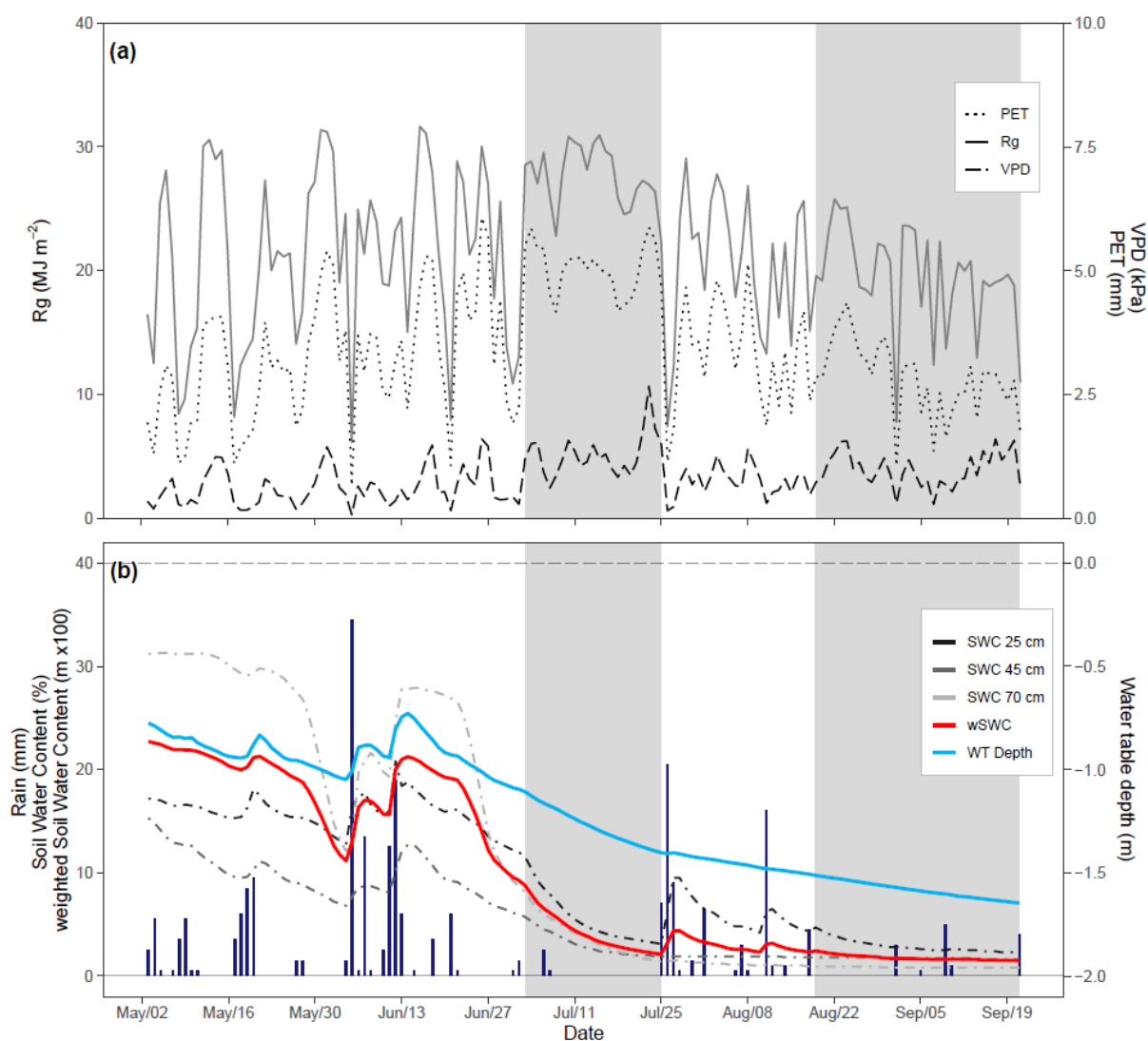
### 3.2.2. Climate and soil variables

I used nearby weather and soil water content monitoring stations to characterize the seasonal dynamics in climate and soil water content in the ORPHEE plantation in 2019. Daily climatic data were obtained from the INRAE weather station of Cestas-Pierroton (44°44.52' N, 00°46.92' W, 1.2 km away from ORPHEE) (**Fig. III. 3.3**) made available by the INRAE CLIMATIK platform (<https://intranet.inrae.fr/climatik/>, in French) managed by the AgroClim laboratory of Avignon, France. Daily water table depth and soil water content (SWC, %) data were recorded in the Castillonville Gis-Coop pure pine plantation next to the ORPHEE plantation (44° 44.08' N, 00°47.74' W, 500 m away). Water table depth was measured with a pressure transducer (CS450, Campbell Scientific). SWC was measured with volumetric water content sensors (CS650, Campbell Scientific) set-up at three different depths: 25 cm, 45 cm and 70 cm (**Fig. III. 3.3b**). With the data from these three depths, I calculated a daily weighted SWC (wSWC, m) according to Bonal et al. (2008), an index which is representative of the soil water content from the surface down to 1 m depth, according to the following equation:

$$\text{wSWC} = 0.350 \times \text{SWC}_{25} + 0.225 \times \text{SWC}_{45} + 0.425 \times \text{SWC}_{70} ,$$

where 0.350, 0.225 and 0.425 are the weighting coefficients that were divided to add up to 1 m and represent a range of depths for each probe: 0-35 cm for  $\text{SWC}_{25}$ , 35-57.5 cm for  $\text{SWC}_{45}$ , and 57.5-100 cm for  $\text{SWC}_{70}$ . The intrinsic values of wSWC should not be interpreted in terms of raw values, as they depend on the total depth selected to calculate wSWC, but rather as relative values to observe seasonal change. In the case of my study this index is interesting because (i) it takes into account the different values of SWC measured at the different depths, which may vary differently over the season (e.g. when the soil dries up or gets rewetted), and (ii) it is useful to use as a proxy of soil water content over a given depth (here, I chose 1.0 m depth) and to test whether seasonal variations in these different SWC values influence transpiration.

The irrigation treatment, which was set-up in spring 2015 (**section 1.2**), adds a constant amount of water in the irrigated plots every night from early May to late September, equivalent to 3.0 mm of precipitations (3.0 liters per m<sup>2</sup>) (Castagneyrol et al. 2017). Unfortunately, I was not able to monitor the SWC within the irrigated block and then could not infer the soil water conditions for this block from the available data.



**Fig. III. 3.3** Seasonal pattern of daily environmental conditions at ORPHEE in 2019 over the period of sap flux density measurements. **(a)** Daily sum of global radiation ( $R_g$ , MJ m<sup>-2</sup>), daily mean vapor pressure deficit (VPD, kPa), and daily potential evapotranspiration (PET, mm). **(b)** Daily sum of precipitation (Rain, mm), daily soil water content at three depths: 25 cm, 45 cm and 70 cm (SWC, %), weighted soil water content over 100 cm using the three depths (wSWC, m, multiplied by 100 to fit on the same scale), and mean daily water table depth (WT Depth, m). The grey areas show two periods of strong water stress.

### 3.2.3. Sap flux density

#### 3.2.3.1. Field measurements

Sap flux density ( $F_d$ , kg of sap per  $\text{dm}^2$  of sapwood per hour,  $\text{kg dm}^{-2} \text{h}^{-1}$ ) measurements were made with thermal dissipation probes (TDP) sensors according to the thermal dissipation method (Granier 1987; Lu et al. 2004). Each sensor consists of two probes, one heating probe and one without a heating system, that are inserted into a plant's sapwood, with the heating probe above the other one.

We installed the two 20-mm-long probes on each sap flux sensors (SFS2, UPSystem, Germany) after removing the bark and the cambium at about 70 cm above ground level with a 10-cm vertical spacing between the probes. We placed all sap flux density sensors on the North facing side of the tree to reduce the risk of direct sunlight causing thermal gradients and distorting the measurements. The probes were protected with a reflective insulation cover for temperature regulation, to minimize potential bias caused by direct radiation or rain runoff on the trunk. The heating probe of each sensor was supplied with 0.048 A (at about 12 V) of constant power. A CR1000 data logger (Campbell Scientific Inc, Leicestershire, UK) collected data every 10 s and recorded an average value every 30 min. Car batteries (75 Ah) powered with solar panels supplied sap flux sensors and data loggers with power. We monitored sap flux density continuously between May 3<sup>rd</sup>, 2019, and September 21<sup>st</sup>, 2019.

#### 3.2.3.2. Data processing

I calculated sap flux density from the temperature gradient between the two probes  $\Delta T$  ( $^{\circ}\text{C}$  or K, or recorded in mV by the probes) following the equation from Granier (1987):

$$F_d = 119 \times 10^{-6} \left( \frac{\Delta T_{max} - \Delta T}{\Delta T} \right)^{1.23} \times 10 \times 3600,$$

where  $\Delta T_{max}$  represents the maximum temperature difference in the absence of sap flow during the nighttime of the given measurement day, when  $F_d = 0$ .  $\Delta T_{max}$  for each day was selected as the local maximum value within a 24-hour period from midnight to midnight. Calibration constants  $119 \times 10^{-6}$  and 1.23 are empirically derived for woody plants (Granier 1987). As the constants were measured in  $\text{m s}^{-1}$  the multiplicative factor  $10 \times 3600$  is used to obtain  $\text{dm}^{-2} \text{h}^{-1}$ .

I checked half-hourly measurements to remove any obvious errors in the raw data (i.e., before and after a power supply cut, or sometimes after heavy rain events) to produce a clean signal before sap flux density calculations.  $\Delta T_{max}$  values were also checked for their evolution in time for each tree to detect any outlier or a drift in the values with time. I had to remove two trees following this analysis because of unexplained variations in  $\Delta T_{max}$  and  $F_d$  and drift in the data with time: unfortunately, both were birch trees in the mixture of the non-irrigated block. This reduced our sampling effort in this plot to 4 birch trees only (instead of 6).

### 3.2.3.3. Daily sap flux density

To obtain daily sums of sap flux density ( $F_d$ ,  $\text{kg dm}^{-2} \text{ day}^{-1}$ ), I summed the 48 half-hourly measurements and divided the total by two to have the result for one day (24 hours). If there was only one missing data point among the 48, the point was gap filled with the mean of the values before and after this half-hour. When more than one half-hour data per day was missing for a given tree, the whole day was discarded for the given tree.

Final data checks were done by comparing the seasonal trends among trees of the same species and within the same plots, and by comparing the relationship between daily sum of radiation ( $R_g$ ) and  $F_d$ . We used this relationship because  $R_g$  (and therefore PET), is the main driver of seasonal variations in  $F_d$ . Gap-filling of missing daily data was applied on days for which other trees of the same species and in the same plot had data. A cubic relationship was fitted between the data from the tree with missing data and a reference tree over a period of 10-20 days. When the  $R^2$  of this relationship was greater than 0.95, this relationship was used to gap-fill the missing data.

### 3.2.3.4. Analysis of drought response

Seasonal variations in daily potential evapotranspiration (PET, mm) during the year are mainly due to seasonal variations in global radiation ( $R_g$ ) (**Fig. III. 3.3**). Moreover, since  $F_d$  is highly dependent on PET (Granier et al. 1996), it is expected that  $F_d$  varies for all trees on a daily basis, independently of variations in soil water content. This means that if we compare summer  $F_d$  with spring, there should be a confounding factor inducing a loss of  $F_d$  simply because of decreasing daily  $R_g$  (and PET) after the summer solstice (21<sup>st</sup> of June). Therefore, to avoid this confounding factor in the interpretation of the seasonal change in  $F_d$ , I calculated the potential value of the daily sum of sap flow density ( $F_{d \text{ potential}}$ ,  $\text{kg dm}^{-2} \text{ d}^{-1}$ ) that I should observe if there was no change in  $R_g$  during the summer, based on the linear relationship between  $F_d$  and PET in the absence of water stress (May 3<sup>rd</sup> to July 2<sup>nd</sup>) (**Fig. III.S 3.1**). The best relationship (higher  $R^2$ ) between  $F_d$  and PET was obtained by using the relationship between  $F_d$  and the square root of PET. One relationship per tree was defined and then used to predict  $F_{d \text{ potential}}$  over the whole measurement period for each tree. I could then use  $F_{d \text{ potential}}$  as a reference of what trees should transpire in the summer in the absence of soil water deficit and stomatal closure.

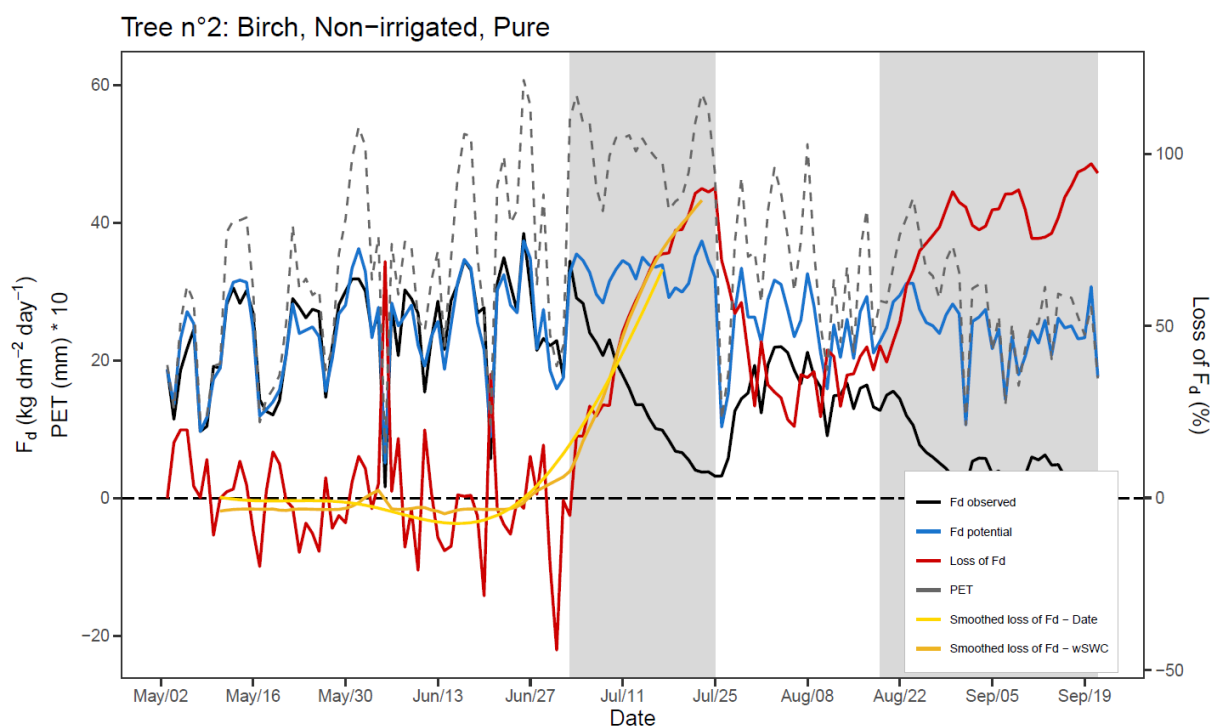
I then calculated  $F_{d \text{ loss}}$  (%) as the daily relative difference between  $F_{d \text{ potential}}$  and  $F_d$ :

$$F_{d \text{ loss}} = \frac{F_{d \text{ potential}} - F_d}{F_{d \text{ potential}}} \times 100 ,$$

which represented the loss of  $F_d$  that was only as a direct consequence of soil water content variations. High  $F_{d \text{ loss}}$  represents a high percentage loss of the potential  $F_d$ . An example of the different variables for one tree is represented in **Fig. III. 3.4**.

Finally, to analyze the treatment effects on the seasonal dynamics and onset of  $F_{d\ loss}$  focusing on the first drought episode (July 3<sup>rd</sup> to July 24<sup>th</sup>), I identified the starting date of drought response and the slope of  $F_{d\ loss}$  after this breakpoint. To do this, I first used two different methods to obtain a smoothed version of the daily variations of  $F_{d\ loss}$ . I decided to use two methods, because the one that seemed the most relevant (using wSWC) was only applicable for the non-irrigated block since we did not have SWC measurements for the irrigated block. The two methods were the following: (i)  $F_{d\ loss}$  as a function of the relative wSWC across the season, and (ii)  $F_{d\ loss}$  as a function of the date. I defined two separate smoothing spline functions for each tree:  $F_{d\ loss} \sim \text{wSWC}_{\text{relative}}$  and  $F_{d\ loss} \sim \text{Date}$  (with the function “smooth.spline” in R). Since the first method (with wSWC) was only applicable to the non-irrigated block, I also used the “Date” method to have predictions for both blocks to compare them. The spline functions were then used to predict a smoothed version of  $F_{d\ loss}$  as a function of the date for both methods (**Fig. III. 3.4**).

In a second time, using the smoothed  $F_{d\ loss}$  defined for each day for both methods, I performed breakpoint analyses (with the package “segmented” in R) to define two periods before and after the onset of drought. The breakpoint analysis is a segmented regression, a method in regression analysis in which the independent variable is partitioned into intervals, and a separate line segment is fitted to each interval. A segmented linear regression with two segments separated by one breakpoint can be useful to quantify an abrupt change of the response function (here,  $F_d$ ) of a varying influential factor (here, the Date linked to a critical soil water deficit for the tree). The breakpoint can be interpreted as a threshold value beyond which an undesired effect occurs (here, the increase in  $F_{d\ loss}$ ). In this study, I parametrized my analysis to define two slopes and one breakpoint, which represented one period with a relatively flat slope before the onset of drought response, a breakpoint value at the date of onset of the drought response, and a second slope representing the speed of  $F_{d\ loss}$ .



**Fig. III. 3.4** Example for Tree n°2 of the calculations for  $F_{d \text{ loss}}$  (%) and the smoothing methods of  $F_{d \text{ loss}}$  with Date and variations of wSWC across the spring and first dry spell.  $F_{d \text{ observed}}$  (black line,  $\text{kg dm}^{-2} \text{ day}^{-1}$ ) is the initial measured daily sap flux density.  $F_{d \text{ potential}}$  (blue line,  $\text{kg dm}^{-2} \text{ day}^{-1}$ ) is the theoretical sap flux density in the absence of water stress, defined from the relationship between  $F_{d \text{ observed}}$  and the square root of PET in the spring (May 3<sup>rd</sup> to July 2<sup>nd</sup>).  $F_{d \text{ loss}}$  (red line, %) is the relative difference between  $F_{d \text{ potential}}$  and  $F_{d \text{ observed}}$ , note that its scale is on a separate axis on the right. The yellow and orange lines represent two different smoothed versions of  $F_{d \text{ loss}}$ . PET (in grey, mm) is left as a reference to daily climatic conditions; the values were multiplied by 10 to be visible on the  $F_d$  scale.

### 3.2.4. Plot transpiration

#### 3.2.4.1. Calculations

I calculated daily plot-level transpiration ( $T_{plot}$ , mm day<sup>-1</sup>) with the following equation:

$$T_{plot} = \frac{\sum_{i=1}^n \overline{F_{d_i}} \times BA_{sum_i}}{A_{plot}},$$

where  $i$  is the species number (either one or two species per plot),  $\overline{F_{d_i}}$  is the mean daily sap flux density for species  $i$ , and  $BA_{sum_i}$  is the sum of the basal area (m<sup>2</sup>) of the trees for species  $i$  in the plot. Plot transpiration was converted from kg of sap per day to mm per day by dividing the values by the plot surface area ( $A_{plot}$ , 144 m<sup>2</sup>). This allowed me to compare daily plot transpiration to daily precipitation or PET.

To obtain  $\overline{F_{d_i}}$ , I used a simple average of the values from all measured trees in a plot. Some studies in mature forests advocate for separate averages by diameter classes. However, in our case, all trees had the same age and there were no major differences in diameter that would justify defining diameter classes. Moreover, for the measured trees there was no relationship between the size of the tree (DBH) or its social status (data not shown) and sap flux density. A similar absence of relationship has been previously reported by Loustau et al. (1998) and Gartner et al. (2009).

I used tree basal area as a proxy for the real sapwood area of the trees. Indeed, it has been reported that there is no heartwood in young pine trees (Pinto et al. 2004; Delzon et al. 2004b). Furthermore, I also considered that there was no heartwood in these young birch trees. Birch is known to have a higher proportion of conducting sapwood than pine, even in older trees (Baumgarten et al. 2019). I did not correct for potential radial variations in sap flux density in these trees because Delzon et al. (2004b) estimated that such a correction would lead to an overestimation of stand transpiration of only about 4 % only in 10-year old maritime pine stands.

#### 3.2.4.2. Predicted values for mixed plots

I compared the observed transpiration values in the mixed plots to predicted values with two different methods adapted from Loreau and Hector (2001). First, I calculated a predicted plot transpiration for mixed plots ( $T_{mixed\ pred. BA_{pred.}}$ ) based on the observed average  $F_d$  from pure plots for each species and on the predicted basal area from the pure plots ( $BA_{pred\ i}$ ) with the following equation:

$$T_{mixed\ pred. BA_{pred.}} = \frac{\sum_{i=1}^2 \overline{F_{d_{pure\ i}}} \times \frac{BA_{pure\ i}}{2}}{A_{plot}}.$$

$BA_{pred\ i} = \frac{BA_{pure\ i}}{2}$ , is the basal area in pure plots divided by the proportion planted in mixed stands (50 %) (Fig. III. 3.12, Fig. III. 3.13). With this first approach, we expected to see an effect of mixture on plot



transpiration due to the difference between the predicted basal area ( $BA_{pred\ i}$ ) and the actual realized basal area of both species in the mixed stands. In order to isolate the effect of differences in  $F_d$ , I used another calculation based on the actual observed proportion of both species in the mixed stands.

Second, I calculated a predicted plot transpiration for mixed plots ( $T_{mixed\ pred. BA_{obs.}}$ ) based on the observed average  $F_d$  from pure plots for each species and this time using the observed BA of the species in the mixed plot as a weighting factor ( $BA_{mixed\ i}$ ) with the following equation:

$$T_{mixed\ pred. BA_{obs.}} = \frac{\sum_{i=1}^2 \overline{F_{d\ pure\ i}} \times BA_{mixed\ i}}{A_{plot}}$$

This second formula allowed us to isolate the mixture effects that originated from differences in  $F_d$  and exclude mixture effects on stand BA (**Fig. III. 3.14, Fig. III. 3.15**).

To estimate the net biodiversity effect (NBE) on plot transpiration, I then compared the observed plot transpiration in the two-species mixed plots ( $T_{mixed\ obs.}$ ) with both estimations of the predicted plot transpiration ( $T_{mixed\ pred. BA_{pred.}}$  and  $T_{mixed\ pred. BA_{obs.}}$ ).

$$\Delta T_{plot} = T_{mixed\ obs.} - T_{mixed\ pred.}$$

### 3.2.5. Statistical analysis

After data processing, I conducted the analyses on the 46 remaining trees (two birches removed because of a drift in  $F_d$  data, **section 3.2.3**). To answer my initial questions, I divided the data into two periods:

- (i) the May-June period (May 3<sup>rd</sup> to July 2<sup>nd</sup>) which represented the late-spring season with non-limiting soil water conditions in both the non-irrigated and the irrigated blocks,
- (ii) the summer period (July 4<sup>th</sup> to September 21<sup>st</sup>) during which we could observe the response of trees to severe drought conditions.

I analyzed species, mixture, and irrigation effects for these two periods on  $F_d$  of individual trees in a first part and on  $T_{plot}$  in a second part.

All statistical analysis were conducted in R language programming (R Core Team 2019) with the Rstudio interface. Linear mixed models were done using “nlme” package, analyses of variance were done with type III ANOVAs from the “car” package, post-hoc tests for multiple comparisons were done with least-square means method (“lsmeans” package with “tukey” adjustments).

#### 3.2.5.1. Sap flux density ( $F_d$ )

May-June period: During days with low radiation and precipitations, the trees had significant drops in sap flux density. Therefore, to test for species, mixture, and irrigation effects on  $F_d$ , I removed the days with precipitation events to test for the effects only on the days with a high level of transpiration (**Fig.**

**III. 3.6a).** I used separate models for the non-irrigated and irrigated blocks. I used a linear mixed model with the tree ID as a random factor to take into account the repeated measures for the trees.

During summer drought: I analyzed the species, mixture and irrigation effects on  $F_{d\text{ loss}}$  at three different dates with high PET conditions. The dates were chosen to compare the resistance and resilience of  $F_d$  to drought among treatments. Here, *resistance* is defined as the inverse of  $F_d$  reduction during the drought episode (i.e., inverse of  $F_{d\text{ loss}}$ ) and *resilience* as the capacity to reach pre-drought episode  $F_d$  levels (i.e.,  $F_{d\text{ loss}}$  after drought episode) (adapted from Lloret et al. 2011). The following dates were used for the analysis:

- at the end of the first dry spell (July 24<sup>th</sup>), in the middle of a rainy episode (*resistance*),
- on a sunny day, after the first dry spell (August 4<sup>th</sup>) (*resilience*),
- at the end of the second dry spell (September 20<sup>th</sup>) (*resistance*) (**Fig. III. 3.8**).

I analyzed the treatment effects at the chosen dates on  $F_{d\text{ loss}}$  and on the breakpoint date and the slope after the breakpoint with simple linear models and analysis of variance.

### 3.2.5.2. Plot transpiration ( $T_{plot}$ )

To analyze the treatment effects on plot transpiration before and during the drought period, I selected two periods:

- (i) 20 days for the spring period (DOY between 123-183, days without rain only and with no missing data),
- (ii) 9 days for the first dry spell (DOY between 196-205 and with no missing data).

I used a mixed model with the tree ID as a random factor to take into account the repeated measures for the trees.

I then used the daily difference in plot transpiration between the observed and predicted mixed plots to calculate a net biodiversity effect on transpiration. I tested if those differences were different from 0 with a paired t-test.

### 3.3. Results

#### 3.3.1. Seasonal variations in climate and soil water content

A strong seasonality was observed during the spring and summer of 2019 (**Fig. III. 3.3**). These climatic variations influenced  $F_d$  and allowed me to study the impact of species mixture under non-limiting and limiting soil water conditions in the two irrigation treatments. May and June were relatively wet months but with periods of sunny days during which I could analyze the transpiration of the trees under high solar radiation levels and non-limiting soil water conditions. During this period, the weighted soil water content (wSWC, m) varied from 0.23 m on May 3<sup>rd</sup> to 0.09 m on July 2<sup>nd</sup> and water table depth went down from -0.8 m to -1.1 m (**Fig. III. 3.3**).

Reduced precipitation during the summer resulted in a decrease in wSWC down to 0.02 m on July 24<sup>th</sup> and the water table depth dropped to -1.4 m. The lack of precipitations during several weeks in the summer resulted in two strong dry spells: from July 3<sup>rd</sup> to July 24<sup>th</sup> and from August 20<sup>th</sup> to September 21<sup>st</sup>. In between, precipitations induced an increase in SWC (**Fig. III. 3.3**, July 25<sup>th</sup> to August 19<sup>th</sup>). Potential evapotranspiration (PET) and vapor pressure deficit (VPD) were highest during the first dry spell due to high temperatures, low air humidity, and high global radiation.

#### 3.3.2. Daily sap flux density at the tree level

##### 3.3.2.1. Sap flux density in the May-June period

Inter- and intra-specific variations: There were strong variations in  $F_d$  among trees (**Fig. III. 3.5**, **Fig. III. 3.6**), both between and within species. In the pure plots of the non-irrigated block, birch had an average  $F_d$  ( $15.6 \pm 6.8 \text{ kg dm}^{-2} \text{ day}^{-1}$ ) that was 59 % higher than for pine ( $9.8 \pm 3.0 \text{ kg dm}^{-2} \text{ day}^{-1}$ ) (post-hoc test for species effect in pure plots:  $p=0.04$ ). This significant difference between species in non-irrigated pure plots remained true and in the same magnitude order for the other treatment combinations (**Fig. III. 3.6b**, **Table III. 3.1**). The intra-specific variation of sap flux density was higher for non-irrigated birch trees than for the irrigated ones and for all pine trees (**Fig. III. 3.6b**). The coefficient of variation (CV) of the values in **Fig. III. 3.6b** for the non-irrigated birch trees (44 % and 38 % for pure and mixed plots respectively) was twice as high as the CV for irrigated birch trees (18 % and 21 % for pure and mixed plots respectively). For pine, the CV ranged from 19 % (irrigated pure) to 30 % (non-irrigated pure).

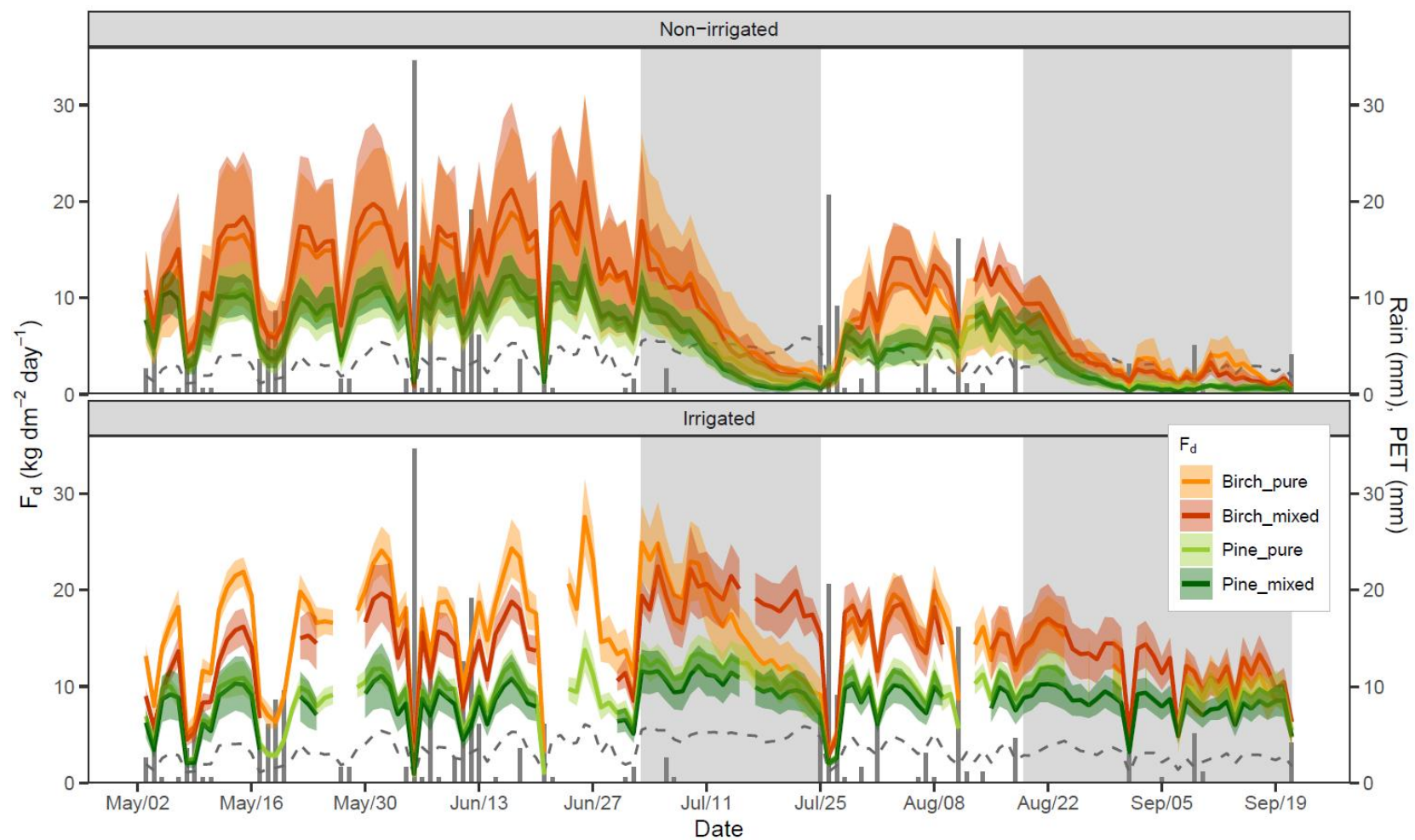
Mixture effects: There were no significant differences in  $F_d$  between pure and mixed plots for either species in the non-irrigated block ( $p = 0.64$ , **Table III. 3.1**).

In the irrigated block, however, the mixture effect was significant ( $p < 0.001$ , **Table III. 3.1**): birch  $F_d$  was on average 27 % higher in the pure plot ( $19.5 \pm 3.4 \text{ kg dm}^{-2} \text{ day}^{-1}$ ) than in the mixed plot ( $15.3 \pm 3.2 \text{ kg dm}^{-2} \text{ day}^{-1}$ ) (**Fig. III. 3.6b**). For pine, there was no mixture effect in the irrigated block ( $p=0.26$ ).

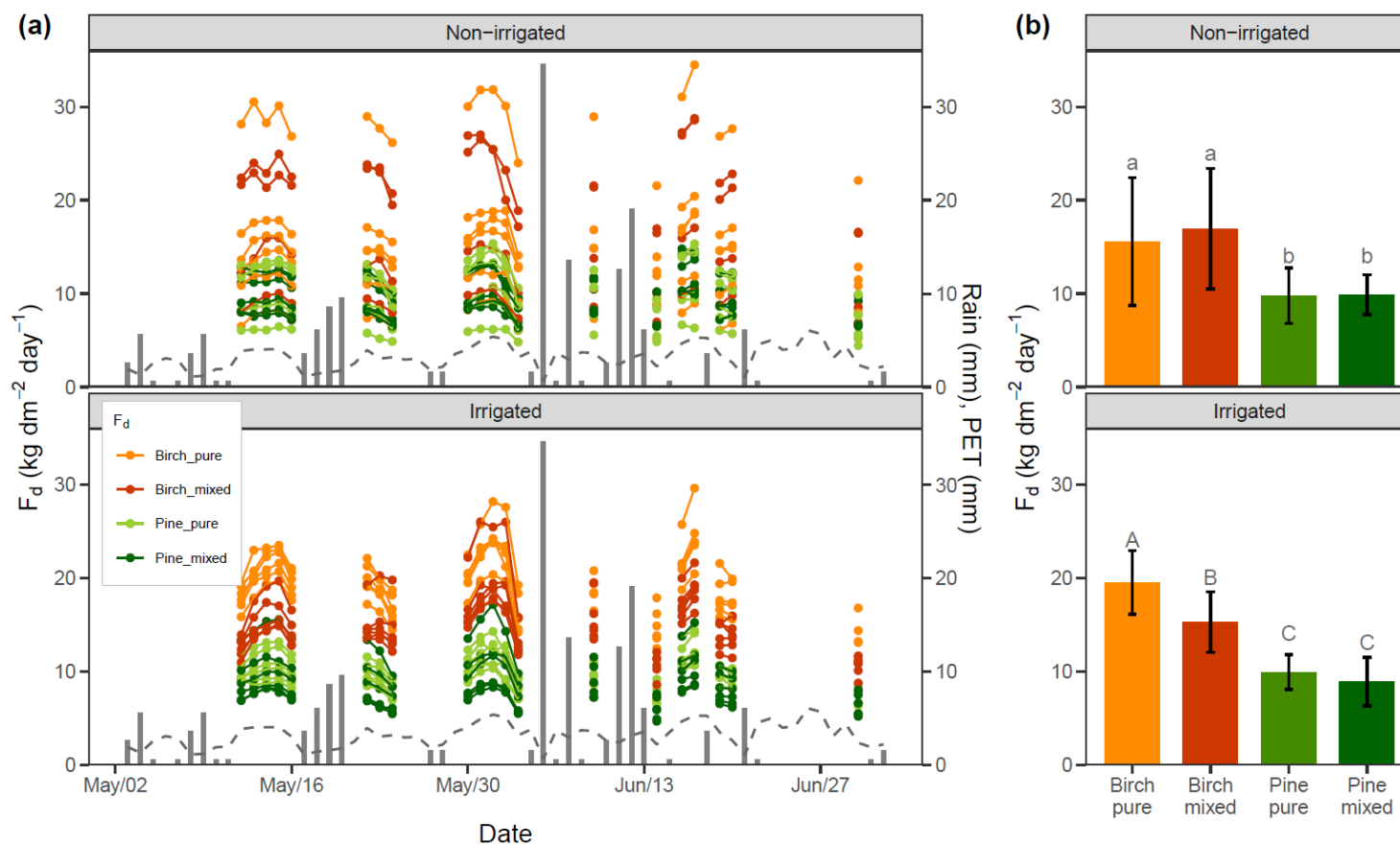
**Irrigation effect:** For this subset of days, in the absence of water stress, there were no significant differences in  $F_d$  between irrigated and non-irrigated plots, either for pure birch plots ( $p=0.17$ ) or pure pine plots ( $p=0.86$ ).

**Table III. 3.1** Linear mixed model results for *Species* and *Mixture* effects on  $F_d$  during the May-June period (see **Fig. III. 3.6** for post-hoc test results).

<b>Predictors</b>	<b>Non-irrigated</b>		<b>Irrigated</b>	
	<i>F(df)</i>	<i>p-value</i>	<i>F(df)</i>	<i>p-value</i>
Species (SP)	4.9 (1;18)	<b>0.04</b>	105.3 (1;20)	<b>&lt;0.001</b>
Mixture (M)	0.2 (1;18)	0.64	19.9 (1;20)	<b>&lt;0.001</b>
SP x M	0.1 (1;18)	0.75	6.1 (1;20)	<b>0.02</b>



**Fig. III. 3.5** Seasonal dynamics of daily sap flux density ( $F_d$ ) averaged per plot and species in the two different blocks (left axis), with Rain and PET (right axis). The grey bars correspond to daily Rain and the grey dashed line corresponds to the daily PET. Gaps in  $F_d$  correspond to periods where there were interruptions in the power supply of the sensors.



**Fig. III. 3.6** Daily sap flux density ( $F_d$ ) during the May-June period for days without rainfall and no missing data (total of 19 days analyzed). **(a)** Daily sap flux density data for each measured tree (left axis), with Rain and PET (right axis). The grey bars correspond to daily Rain and the grey dashed line corresponds to the daily PET. **(b)** Averaged data over spring days without rain. The letters represent results for each block of post-hoc tests after linear mixed model testing for species and mixture effects. See **Table III. 3.1** for linear mixed model results.

### 3.3.2.2. Influence of drought on sap flux density

Over the course of both dry spells, we observed a strong decrease in  $F_d$  for the trees of both species in the non-irrigated block (**Fig. III. 3.5**).

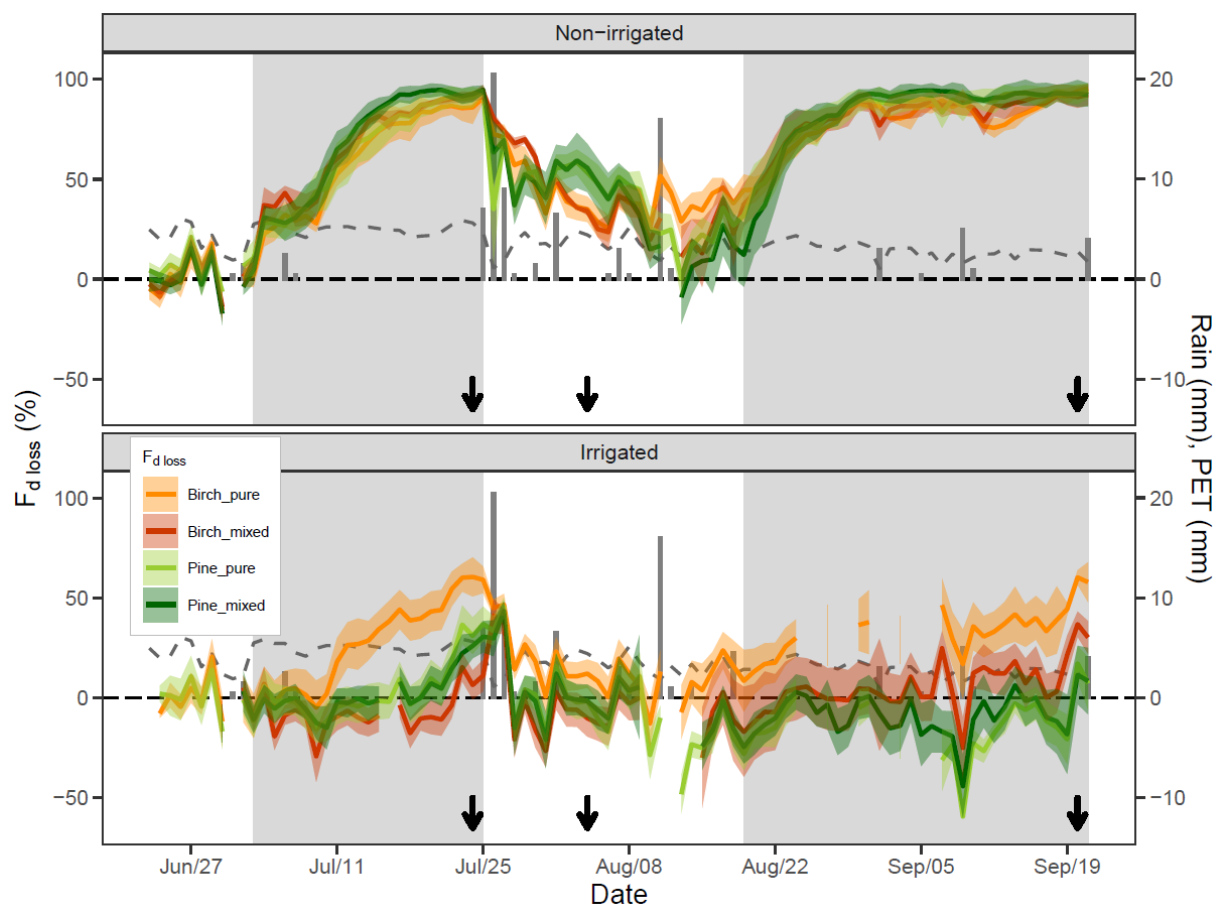
In the irrigated block, the decrease was delayed and much less pronounced than in the non-irrigated one (**Fig. III. 3.5**). To analyze these patterns in more details (see below), I used  $F_{d\text{ loss}}$  (**Fig. III. 3.7**) which is the percentage loss in  $F_d$  for a given tree relatively to  $F_{d\text{ potential}}$  (calculated to eliminate the effect of the decrease in  $R_g$  after the 21<sup>st</sup> of June on seasonal variations in sap flow density, see **section 3.2.3.4**).

**Non-irrigated block:** In the non-irrigated block,  $F_{d\text{ loss}}$  at the end of the first drought spell (July 24<sup>th</sup>) was on average the same for both species, and the same for the pure or the mixed plots for each species (**Table III. 3.2, Fig. III. 3.8a**). The average  $F_{d\text{ loss}}$  per plot and species ranged between 86 % and 93 % (**Fig. III. 3.8a**), meaning that they had reached sap flux densities close to zero. Moreover, the breakpoint date of  $F_{d\text{ loss}}$  was similar for all the treatments (**Fig. III. 3.9**). The breakpoint date estimated from wSWC was DOY 181 (June 30<sup>th</sup>, 2019), which corresponded to an average SWC of 12 % at 25 cm, 6 % at 45 cm, 10 % at 70 cm (wSWC of 10 m) (**Fig. III. 3.3**). The slope of  $F_{d\text{ loss}}$  after the breakpoint date was significantly steeper for pine trees in mixed plots compared to pine trees in pure plots and to birch (**Fig. III. 3.9**), indicating a faster rate of  $F_{d\text{ loss}}$  for these trees.

During the precipitation events at the beginning of August,  $F_{d\text{ loss}}$  was smaller for birch than for pine (**Table III. 3.2, Fig. III. 3.8b**): birch then showed a better resilience than pine and was closer to the  $F_{d\text{ potential}}$  after the rain events. The response of the non-irrigated plots to the second dry spell was similar to the first one and both species in pure and mixed plots reached on average 93 % of  $F_{d\text{ loss}}$  on September 20<sup>th</sup> (**Table III. 3.2, Fig. III. 3.8c**).

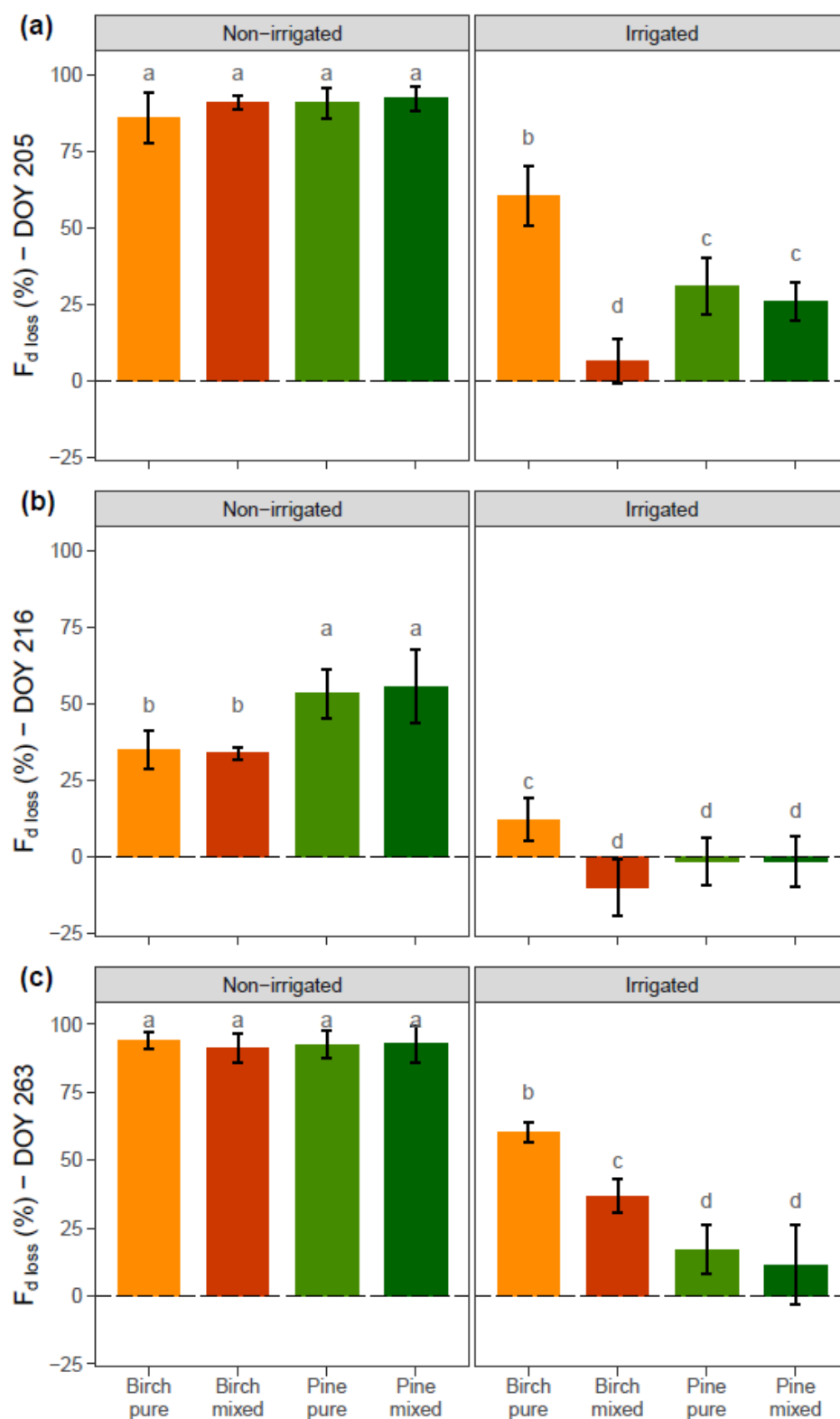
**Irrigated block:** At all three dates selected to analyze these effects, there was a strong irrigation effect on  $F_{d\text{ loss}}$ , with smaller loss in sap flow density in the irrigated block (**Table III. 3.2, Fig. III. 3.8**). This was probably due to the daily input of irrigation water and the resulting potentially higher soil water content (unfortunately not measured in the irrigated blocks). Moreover, the breakpoint date of drought response came later and the slope of  $F_{d\text{ loss}}$  was more gradual in irrigated plots (**Fig. III. 3.10**). Seasonal dynamics in  $F_d$  were more contrasted across species and pure or mixed plots than in the non-irrigated block. At the end of the first drought episode (July 24<sup>th</sup>), there was a significant mixture effect on birch  $F_{d\text{ loss}}$  while pine did not differ between pure and mixed plots. Birch  $F_{d\text{ loss}}$  was higher for pure birch trees than mixed birch trees (**Table III. 3.2, Fig. III. 3.8a**). Similarly, pure birch trees were the ones with the earliest breakpoint date and the steepest slope of  $F_{d\text{ loss}}$  within the irrigated block (**Fig. III. 3.10**). During the precipitation events at the beginning of August,  $F_{d\text{ loss}}$  of all trees went back to an  $F_{d\text{ loss}}$  around 0 %, which meant that  $F_d$  could reach  $F_{d\text{ potential}}$  (**Table III. 3.2, Fig. III. 3.8b**). The response of the irrigated plots to the second dry spell was slightly different from the first one, with a lower  $F_{d\text{ loss}}$  for pine trees

and an increased  $F_{d\text{ loss}}$  for birch trees in the mixed plot. However, birch trees in the pure plot remained the one with the highest  $F_{d\text{ loss}}$  on September 20<sup>th</sup> (Table III. 3.2, Fig. III. 3.8c).



**Fig. III. 3.7** Seasonal dynamics of the average  $F_{d\text{ loss}}$  by species and plot composition during the summer of 2019 (left axis), with Rain and PET (right axis). The grey bars correspond to daily Rain and the grey dashed line corresponds to the daily PET. The black arrows represent the three dates used to analyze the response to drought in Fig. III. 3.8 (July 24<sup>th</sup>, August 4<sup>th</sup> and September 20<sup>th</sup>).

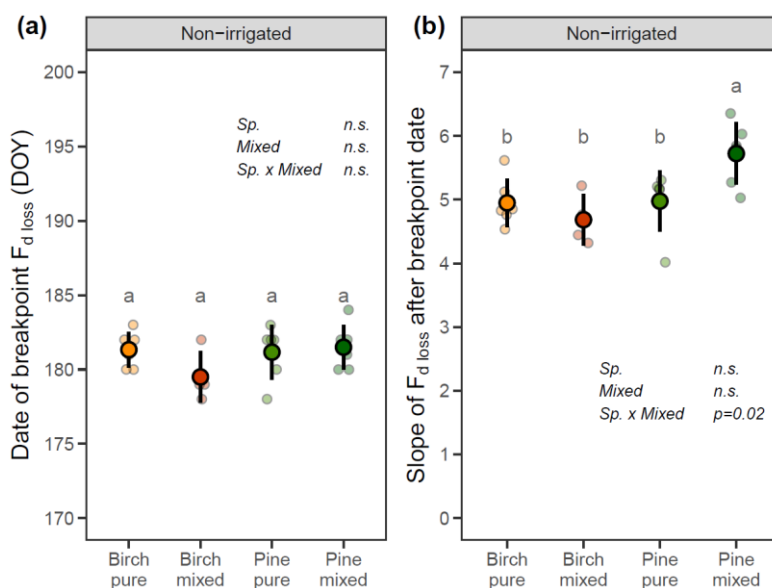




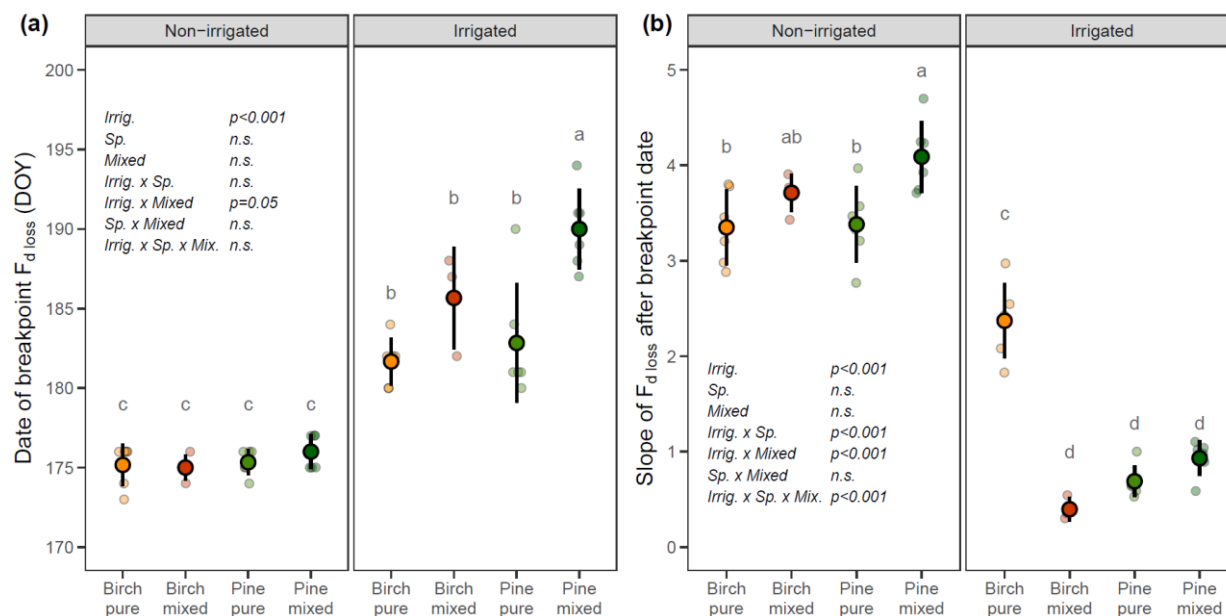
**Fig. III. 3.8** Average  $F_{d\ loss}$  by species and plot composition for (a) the end of the first dry spell (day of year (DOY) 205, July 24<sup>th</sup>), (b) the middle of a rainy episode after the first dry spell (DOY 216, August 4<sup>th</sup>) and (c) the end of the second dry spell (DOY 263, September 20<sup>th</sup>). The letters represent the results of post-hoc tests after the linear mixed models. See **Table III. 3.2** for linear model results.

**Table III. 3.2** Linear model p-values for *Irrigation*, *Species* and *Mixture* effects on  $F_d$  at three dates: (i) the end of the first dry spell (day of year (DOY) 205, July 24<sup>th</sup>), (ii) the middle of a rainy episode after the first dry spell (DOY 216, August 4<sup>th</sup>) and (iii) the end of the second dry spell (DOY 263, September 20<sup>th</sup>). See **Fig. III. 3.8** for post-hoc test results.

<b>Predictors</b>	<b>DOY 205</b>	<b>DOY 216</b>	<b>DOY 263</b>
Irrigation (I)	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>
Species (SP)	0.23	< <b>0.001</b>	0.79
Mixture (M)	0.26	0.86	0.65
I x SP	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>
I x M	< <b>0.001</b>	< <b>0.01</b>	<b>0.01</b>
SP x M	0.56	0.66	0.68
I x SP x M	< <b>0.001</b>	0.07	0.17



**Fig. III. 3.9** Breakpoint dates (a) and slopes (b) of drought response, modelled with wSWC variations but back transformed to dates, during the first dry spell in the non-irrigated block. The points in the background represent individual tree values. Large symbols represent the average values, and error bars represent standard deviations. The text in both panels are the results of linear models testing for species and mixture effects on the breakpoint date and the slope respectively. The letters represent the results of post-hoc tests.



**Fig. III. 3.10** Breakpoint dates (a) and slopes (b) of drought response, modelled with “Date”, during the first dry spell in both blocks. The points in the background represent individual tree values. Large symbols represent the average values, and error bars represent standard deviations. The text in the left-side panels are the results of linear models testing for irrigation, species and mixture effects on the breakpoint date and the slope respectively. The letters represent the results of post-hoc tests.

### 3.3.3. Plot-level transpiration

#### 3.3.3.1. Plot transpiration in the May-June period

Non-irrigated block: On days without rain, the average plot transpiration ( $T_{\text{plot}}$ ) in the non-irrigated block was  $1.0 \pm 0.2 \text{ mm day}^{-1}$  in the pure birch plot,  $3.2 \pm 0.5 \text{ mm day}^{-1}$  in the pure pine plot, and  $2.7 \pm 0.4 \text{ mm day}^{-1}$  in the mixed plot (**Fig. III. 3.11**, **Fig. III. 3.13a**). The mixture plot then had intermediate  $T_{\text{plot}}$  compared to the pure birch and pure pine plots. Note that the variability given here represents the variability among sunny days in the May-June period, and it is therefore a variability due to climatic conditions. The statistical tests presented in the figures represent tests on this variability, as there were no repetitions of measures in other blocks.

In the non-irrigated block, using the method with  $BA_{\text{pred}}$  for net biodiversity effects (NBE) calculations on  $T_{\text{plot}}$ , the daily  $T_{\text{plot}}$  in mixed plots was higher than the predicted value from the pure plots. This result showed that the mixed plot transpired more than what could be expected from pure plots (**Fig. III. 3.12a**). The difference between observed and predicted values for each day yielded significant positive NBE on transpiration (**Fig. III. 3.13b**). At the species level, the effect was explained by a higher transpiration for pine than expected, while birch had a lower transpiration than expected (**Fig. III. 3.12b**, **Fig. III. 3.13a**). However, this positive NBE effect was mainly due to mixture effects on plot BA (**Fig. III. 3.2**). Indeed, as demonstrated by the second method of calculation of NBE, with  $BA_{\text{mixed}}$ , we observed that the daily  $T_{\text{plot}}$  in the non-irrigated mixed plot was identical to what could be expected from the weighted average of the pure plots (**Fig. III. 3.14a**), with NBE not significantly different from zero (**Fig. III. 3.15b**).

Irrigated block: Compared to the non-irrigated block, daily  $T_{\text{plot}}$  in the irrigated block was higher for both pure plots, with an average of  $2.3 \pm 0.4 \text{ mm day}^{-1}$  in the pure birch plot and  $3.8 \pm 0.6 \text{ mm day}^{-1}$  in the pure pine plot (**Fig. III. 3.11**). In contrast, daily  $T_{\text{plot}}$  of the mixed plot in the irrigated block was similar than  $T_{\text{plot}}$  in the non-irrigated block, with an average of  $2.7 \pm 0.4 \text{ mm day}^{-1}$ . The mixture plot had an intermediate  $T_{\text{plot}}$  compared to that of the pure birch and pure pine plots.

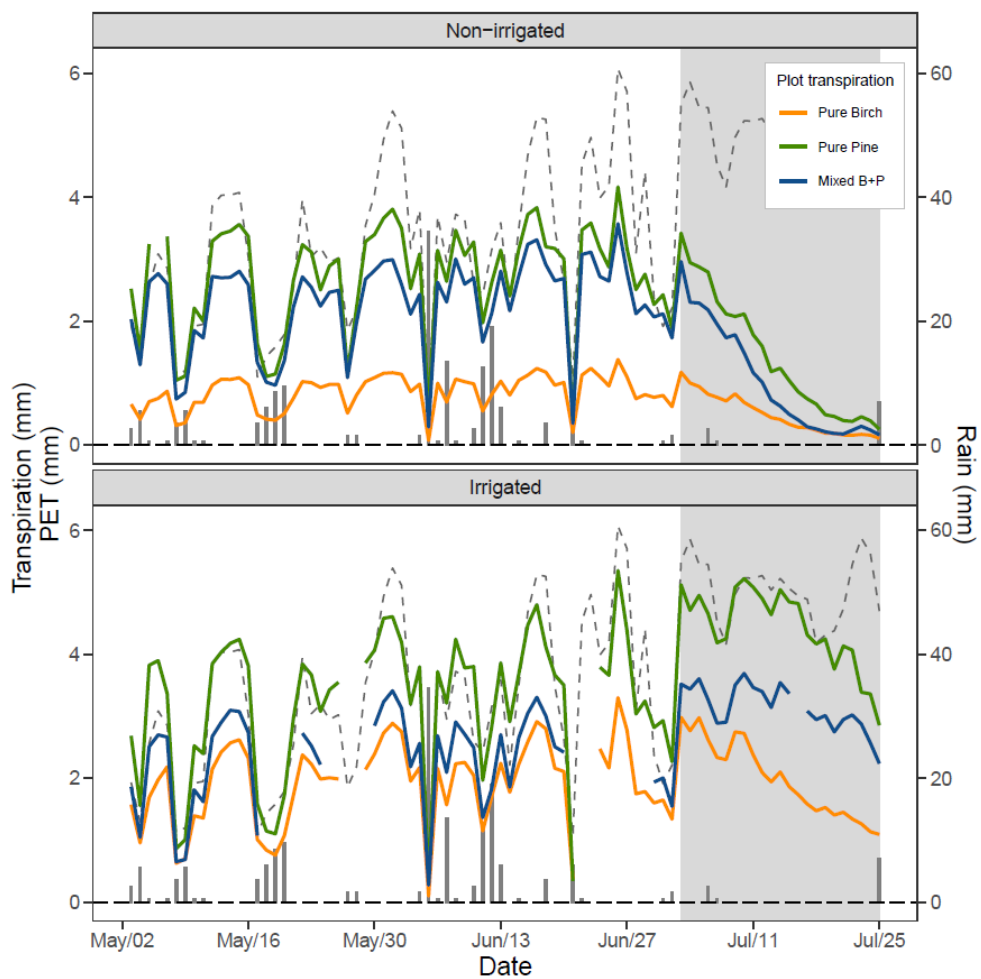
Contrary to the non-irrigated block, the observed transpiration in the mixed stand was lower than expected, independently of the method used to obtain the predicted values (**Fig. III. 3.12**, **Fig. III. 3.14**). There was a significantly negative NBE (**Fig. III. 3.13b**, **Fig. III. 3.15b**), indicating that the observed mixed plot transpired less than what could be expected from the respective pure plots. However, depending on the method, it originated from a different combination of effects at the species level. When testing with the  $BA_{\text{pred}}$  method, the effect was explained by a lower transpiration for birch than expected, while pine had a higher transpiration than expected but in a smaller degree (**Fig. III. 3.12b**, **Fig. III. 3.13a**). Contrarily, when testing with the  $BA_{\text{mixed}}$  method, it originated from a slightly lower transpiration rate than expected for both studied species (**Fig. III. 3.14b**, **Fig. III. 3.15a**).

### 3.3.3.2. Influence of drought on plot transpiration

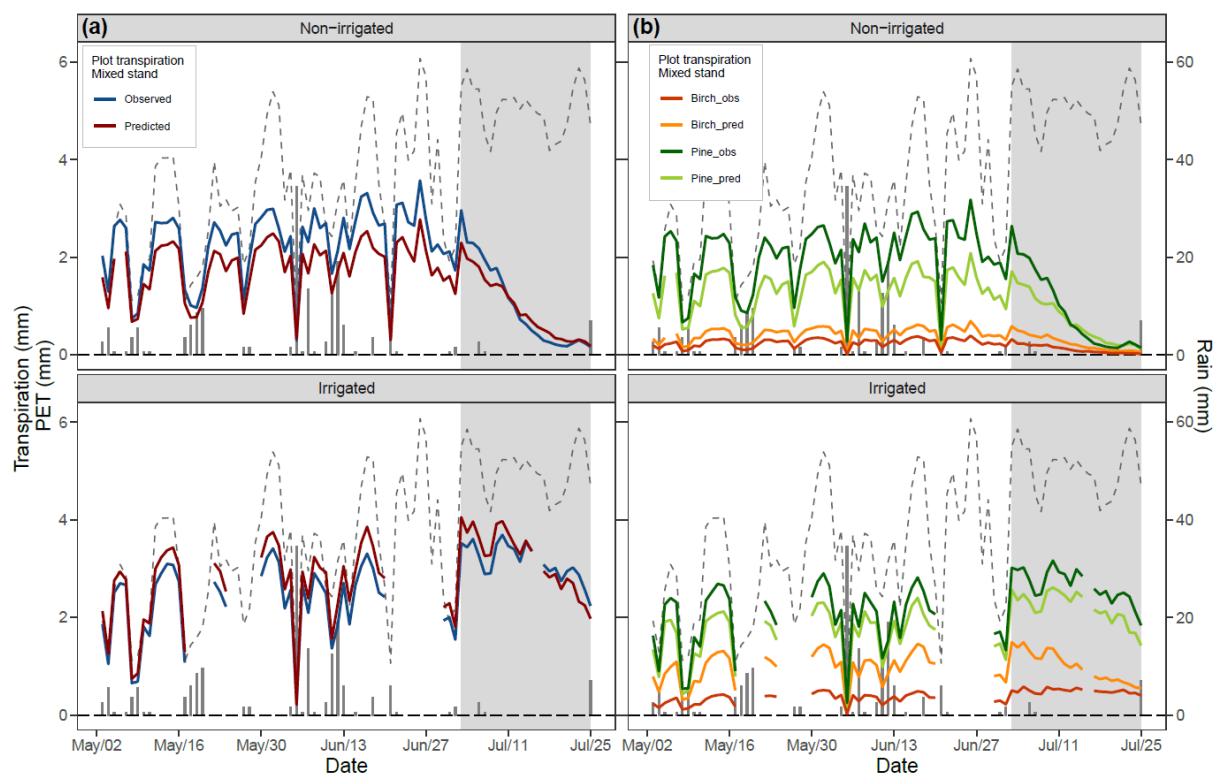
I focused the analysis on the first drought episode of July to avoid the confounding factors of decreasing PET for the drought spell at the end of the summer. During this drought episode, I observed a strong effect of water stress on  $T_{\text{plot}}$  in the non-irrigated block, while  $T_{\text{plot}}$  remained high for a longer period in the irrigated block (**Fig. III. 3.11**).

In the non-irrigated block, the pure pine plot lost a similar proportion of  $T_{\text{plot}}$  (81 %) than the pure birch plot (78 %) between the spring and drought episode average values, while the mixed plot lost a slightly higher proportion (89 %) (**Fig. III. 3.13**). On the other hand, in the irrigated block, the only significant loss in transpiration due to the drought episode compared to the spring was in the pure birch plot (35 %).

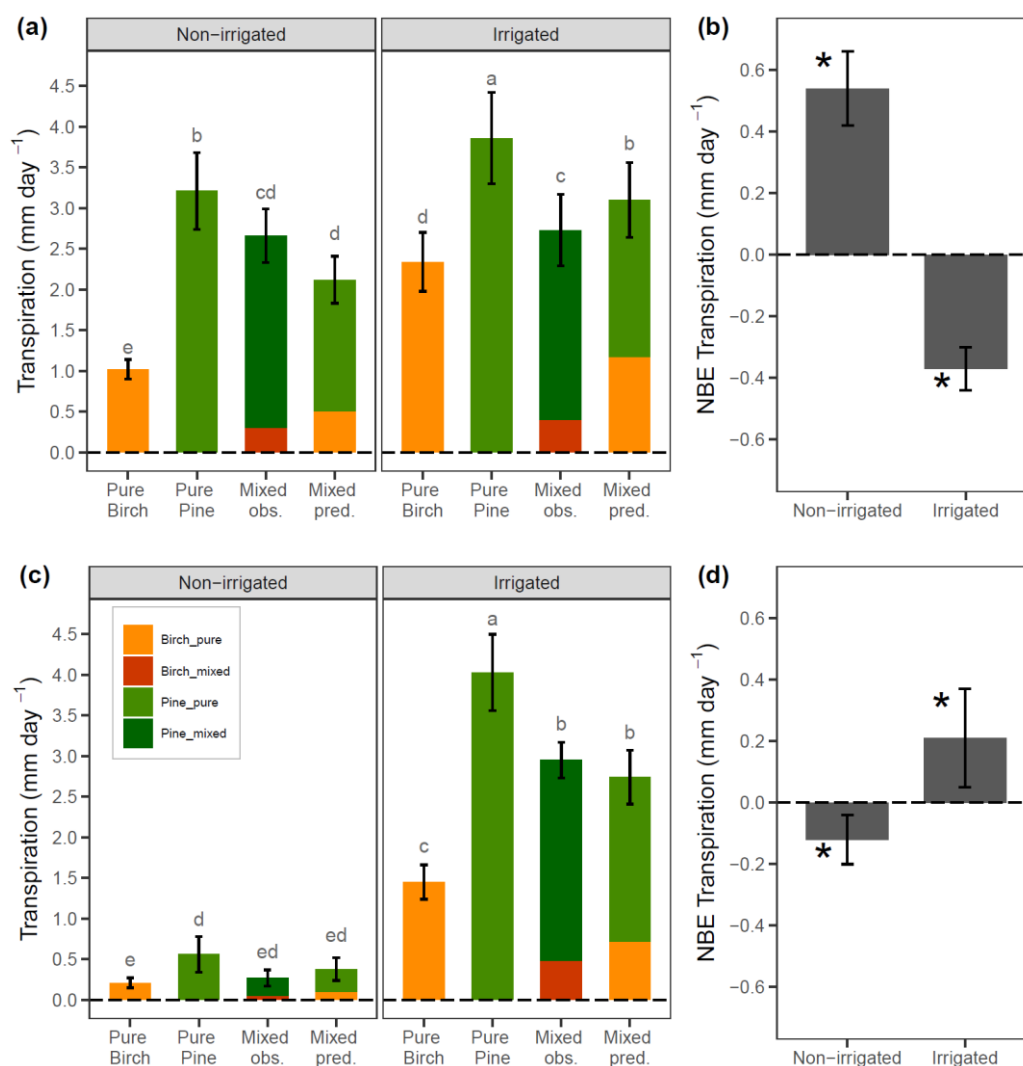
Mixture effects were reversed during the dry spell compared to the effects in the absence of soil water limitations. In the non-irrigated mixed plot, the NBE shifted from positive to negative with  $BA_{\text{pred}}$  (**Fig. III. 3.13bd**) (respectively, from neutral to negative with  $BA_{\text{mixed}}$ , **Fig. III. 3.15bd**), while in the irrigated plot they shifted from negative to positive with  $BA_{\text{pred}}$  (**Fig. III. 3.13bd**) (respectively from negative to neutral with  $BA_{\text{mixed}}$ , **Fig. III. 3.15bd**).



**Fig. III. 3.11** Plot-level transpiration ( $T_{plot}$ ) for each plot composition and irrigation treatment, with PET (left axis) and Rain (right axis). The grey bars correspond to daily Rain values and the grey dashed line corresponds to the daily PET.

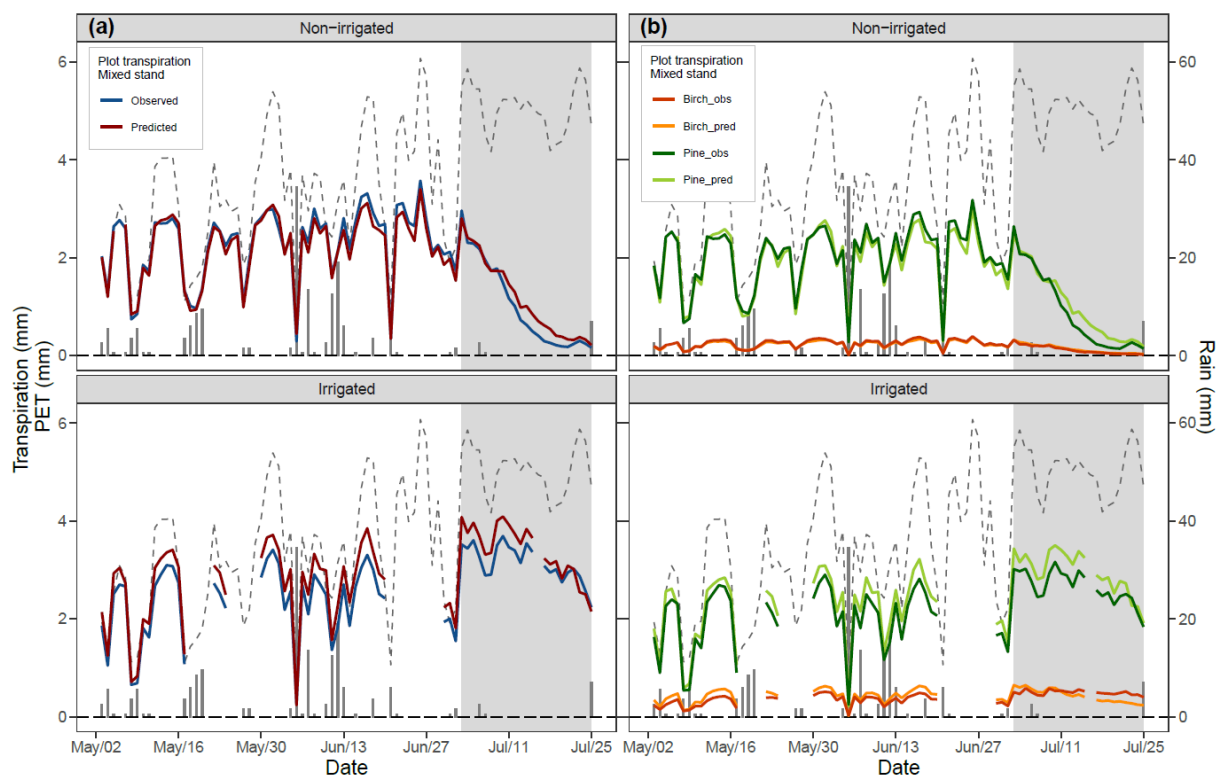


**Fig. III. 3.12** Mixed plot observed and predicted  $T_{\text{mixed}}$  using  $BA_{\text{pred}}$ , represented by plot (a) and by species (b), with PET (left axis) and Rain (right axis). The grey bars correspond to daily Rain values and the grey dashed line corresponds to the daily PET.

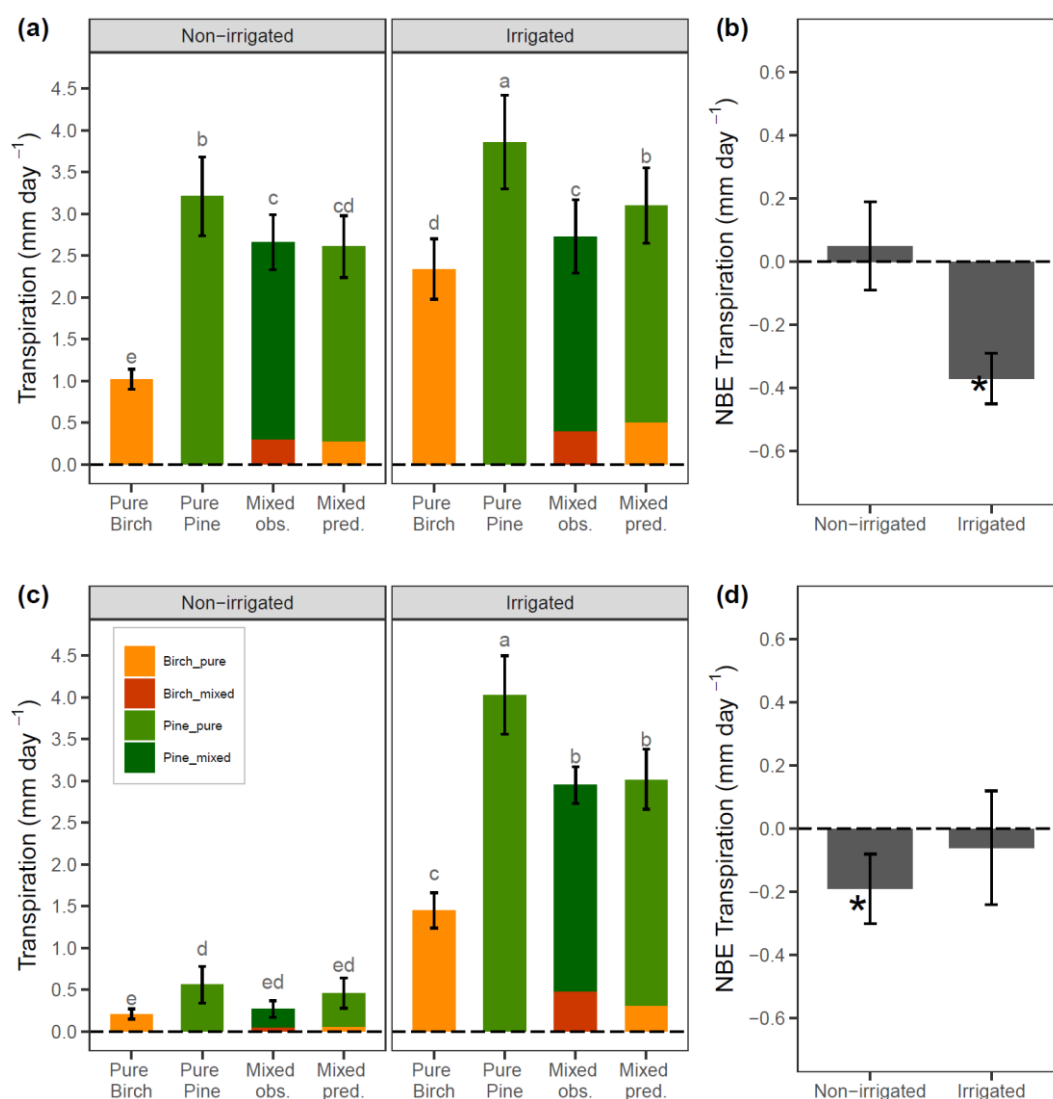


**Fig. III. 3.13** Average  $T_{plot}$  for each observed plot composition and for the predicted mixed plots (using  $BA_{pred}$ ), with species contribution represented separately, during the May-June period (a) and the first dry spell (c) and respectively NBE on transpiration during the May-June period (b) and the first dry spell (d). The error bars represent the variability of the values over the period of days kept for the analysis: 20 days for the May-June period (DOY between 123-183, days without rain only and with no missing data), 9 days for the first dry spell (DOY between 196-205 and with no missing data).





**Fig. III. 3.14** Mixed plot observed and predicted  $T_{\text{mixed}}$  using  $BA_{\text{mixed}}$ , represented by plot (a) and by species (b), with PET (left axis) and Rain (right axis). The grey bars correspond to daily Rain values and the grey dashed line corresponds to the daily PET.



**Fig. III. 3.15** Average  $T_{plot}$  for each observed plot composition and for the predicted mixed plots (using  $BA_{mixed}$ ), with species contribution represented separately, during the May-June period (a) and the first dry spell (c) and respectively NBE on transpiration during the May-June period (b) and the first dry spell (d). The error bars represent the variability of the values over the period of days kept for the analysis: 20 days for the May-June period (DOY between 123-183, days without rain only and with no missing data), 9 days for the first dry spell (DOY between 196-205 and with no missing data). **Note:** the only difference with Fig. III. 3.13 in panels (a) and (c) are the values for “Mixed pred.”

## 3.4. Discussion

### 3.4.1. Daily sap flux density at the tree level

#### 3.4.1.1. Sap flux density in the May-June period

##### Inter- and intra-specific differences in $F_d$

The average  $F_d$  was of  $15.6 \pm 6.8$  for birch and  $9.8 \pm 3.0$  kg dm<sup>-2</sup> day<sup>-1</sup> for pine under non-limiting soil water conditions (**Fig. III. 3.6b**). Most studies on tree sap flow refer to sap flux density data per hour, so I could not directly compare my daily results with those. I found daily sums of sap flux density only in the following two examples. Magh et al. (2019) found an  $F_d$  of  $\sim 23$  kg dm<sup>-2</sup> day<sup>-1</sup> for beech and  $\sim 15$  kg dm<sup>-2</sup> day<sup>-1</sup> for fir on sunny days in May 2016 for a temperate forest in Southwest Germany. Moore et al. (2011) found an average  $F_d$  of  $\sim 35$  kg dm<sup>-2</sup> day<sup>-1</sup> for red alder and  $\sim 20$  kg dm<sup>-2</sup> day<sup>-1</sup> for Douglas-fir on sunny days in May 2001 in a young plantation in North-western USA. Loustau et al. (1998) and Delzon and Loustau (2005) provided detailed hourly sap flux measurements for young maritime pines for sunny days in the spring. These experiments were performed in plantations in the same region as our experiment. Based on these data, I estimated a daily sap flux density of about 10-20 kg dm<sup>-2</sup> day<sup>-1</sup>. The  $F_d$  for birch and pine found in our experiment were then in the lower range compared to these studies. These studies differ by a large range of factors (species, climate, management, stand age...) which all may contribute to explain these differences. Yet, Delzon and Loustau (2005) found no age-specific trends for  $F_d$  in maritime pine. These low  $F_d$  values might rather be related to the high density of the plantation, which could result in lower  $F_d$  than at lower densities (Gebhardt et al. 2014).

In our study, sap flux density was 59 % higher for birch than for pine in the pure non-irrigated plots, indicating species-specific differences in functioning (**Fig. III. 3.6**). In the previously cited examples (Moore et al. 2011; Magh et al. 2019), the sap flux density was always higher for the broadleaved species than for the coniferous species. This is in accordance with previous results which showed that sap flux density is usually lower in conifers than in broadleaves (Catovsky et al. 2002; Gartner et al. 2009). The origin of this difference is probably the intrinsic difference in the xylem water transport system among these species, with tracheids in conifers that leads to a lower capacity to transport water than vessels in broadleaves (Meinzer et al. 2005). Comparing this result with the maximum hydraulic conductivity for pine and birch might help confirm this hypothesis (see **Appendix III.A, p. 275**). The differences between these two species in water transport systems and functioning could lead to potential differences in the response to drought and to potential mixture effects. It is interesting to note that this species effect was significant despite a high intraspecific variability among trees. Species differences also prevailed in mixed plots and in the irrigated block in pure and mixed plots.

For birch, intra-specific variations in  $F_d$  were about twice as high in the non-irrigated block than in the irrigated block in both pure and mixed plots (**Fig. III. 3.6b**). This difference could not be the result of

different light availability among trees since the effect was the same in mixed plots where birch trees were more dominated due to taller pine trees surrounding them (**Fig. III. 3.1b**). It could not be either a result of genetic differences among trees between the non-irrigated and the irrigated block, because all planted trees came from the same nursery. I hypothesized that it might be a result of more frequent embolism events in the previous years which could have led to this variability in the non-irrigated block (Schenk et al. 2013). Meanwhile, the birch trees in the irrigated block were not as exposed to summer droughts and therefore less likely to be exposed to climatic conditions which could lead to embolism. On the other hand, the intra-specific variability of pine was the same in all the treatments. Birch might be more sensitive to cavitation than pine (Choat et al. 2012) (see **Appendix III.A, p. 275**), which could have led to this variability in  $F_d$  in the non-irrigated block.

#### Mixture effect on $F_d$

In the non-irrigated block, in accordance with my initial hypothesis, there were no mixture effects on  $F_d$  during the May-June period for birch and pine (**Fig. III. 3.6b**). Forrester (2015) and Magh et al. (2019) reported a higher  $F_d$  in mixed plots for at least one species out of two, which they explained by physiological adaptations to a less competitive environment. On the other hand, Grossiord et al. (2014b) reported higher  $F_d$  in pure plots for *Quercus cerris* while the other species showed no mixture effects in the absence of water stress. They explained this effect with a higher belowground competition for belowground resources for *Quercus cerris* in mixed stands. Moreover, a large number of studies found an absence of mixture effects on sap flux density in non-limiting soil water conditions (Jonard et al. 2011; Moore et al. 2011; Kunert et al. 2012; Grossiord et al. 2013a), and the results from this study at ORPHEE follow this trend. In our study, the absence of a significant mixture effect can be explained (i) by the large variability in  $F_d$  within the different plots (pure and mixed ones) which could hide a smaller mixture effect for which our sample size was not enough to detect, or (ii) by the fact that during this time of the year, aboveground and belowground resources are not limited and therefore potential complementarity in water or nutrient do not impact tree water flux.

In the irrigated block, surprisingly, while pine showed no mixture effects, birch trees in the pure plot had 27 % higher  $F_d$  than in the mixed plot (**Fig. III. 3.6b**). The irrigated pure birch plot was also higher on average than both pure and mixed plots in the non-irrigated block, although this difference was not significant because of the between-tree variability in the non-irrigated plots. This effect was not expected and could be explained by a higher competitive environment either for water or light for birch in the mixed irrigated plot. Grossiord et al. (2014b) reported a detrimental effect of interspecific competition for water resources on *Quercus cerris* when mixed with another oak species. Here, however, we did not see the same effect in the non-irrigated block, indicating that the effect is not directly related to water availability. It might rather be an indirect effect of irrigation on birch leaf area. Indeed, changes in crown in leaf area index (LAI) can affect  $F_d$ , with higher values for higher LAI (Oren et al. 1999). Colleagues from the ANR Diptic project collected LAI data in 2019 at ORPHEE (**Fig. III.S 3.2**). The data for the

plots we monitored show that irrigation had a high impact on the LAI in pure birch plots, with an increase from 2.0 to 4.0  $\text{m}^2 \text{m}^{-2}$  between the non-irrigated and the irrigated pure plots (**Fig. III.S 3.2**). In mixed plots however, the irrigation effect on birch growth and LAI was much lower, because of the competition for light with pine. Therefore, the higher  $F_d$  values in the pure birch plot could be the result of this increase in LAI, while birch in the mixed plot could not benefit in the same way because of the competition with pine.

#### Irrigation effect

In accordance with my initial hypothesis, there were no significant irrigation effects on sap flux density ( $F_d$ ) during the May-June period. However, as described in the previous paragraphs, irrigation did have indirect effects on birch, with a higher variability in  $F_d$  in the non-irrigated plots than in the irrigated ones and a higher  $F_d$  in the pure birch irrigated plot than in the mixed one. During this time of the year when water resources were not limiting, the  $F_d$  of all the trees was close to its maximum with or without irrigation inputs, which is why the general absence of irrigation effects was not surprising. The indirect effects of irrigation on birch are probably the consequence of the response of the irrigated trees to moister soil water conditions during the four previous years, with a long-term positive impact of irrigation on tree growth (**Fig. III. 3.1**) and LAI (**Fig. III.S 3.2**). However, at the tree level, there was no relationship between  $F_d$  and DBH (data not shown) and  $F_d$  is the ratio of kg of water per sapwood area: there would be therefore no reason to expect an irrigation effect on  $F_d$ . The positive irrigation effect on tree growth, and therefore on sapwood area, might however affect the results at the plot level (plot transpiration), which I will discuss in **section 3.4.2**. For pine, the physiological and morphological responses of the trees to the irrigated treatment did not influence their baseline sap flux density on sunny days, which appeared as a relatively stable functional trait in the absence of water stress.

#### Take-home message

During the May-June period, in non-limiting soil water conditions, we found strong differences in species sap flux density ( $F_d$ ) and a high tree-to-tree variability for birch in non-irrigated plots. There was an overall absence of mixture effects on  $F_d$ , except for birch trees in the irrigated block. Irrigation did not significantly influence  $F_d$  of pine or birch.

#### *3.4.1.2. Influence of drought on sap flux density*

##### Non-irrigated block

##### *Species effect*

For all trees, the response of  $F_d$  to drought was strong and highly synchronized across species and plots. The breakpoint date indicating the start of a decline in  $F_d$  was on average at DOY 181 (June 30<sup>th</sup>, 2019)

(**Fig. III. 3.9**), which corresponded to a SWC of 12 % at 25 cm, 6 % at 45 cm, 10 % at 70 cm. In a Scot's pine stand it was found that transpiration was strongly restricted below a SWC of 12 % (Irvine et al. 1998). These thresholds are probably different for maritime pine and birch, but it is interesting to note that transpiration can be maintained for Scot's pine at much lower thresholds of SWC thanks to the presence of a water table and to the capillary rise of water above the limit of the water table (Vincke and Thiry 2008). The water table in our study probably played an important role in maintaining a minimum level of sap flow, even when the upper soil layers reached very low SWC at the peak of the drought.

On July 24<sup>th</sup>, at the end of the first drought period,  $F_{d\ loss}$  was on average 90 % across plots (**Fig. III. 3.7**, **Fig. III. 3.8a**). This meant that, compared to the potential  $F_d$  that could have been realized in this period in the absence of water stress ( $F_{d\ potential}$ , **Fig. III. 3.4**), only 10 % of  $F_d$  was realized. Since I used a standardization approach with PET to calculate  $F_{d\ loss}$ , this decrease was not due to the seasonal decrease in PET due to a decrease of global radiation and day length. This loss was therefore mainly due to the response of trees to drought and therefore to strong stomatal closure (Bréda et al. 2006). Such a strong stomatal regulation in response to soil water deficit has been previously reported for birch (Gartner et al. 2009; Sellin et al. 2014) and pine (Delzon et al. 2004a). However, we cannot totally exclude a loss of  $F_d$  due to high VPD effects on stomatal closure (independently of soil drought). To detect these fine VPD effects, I could have used hourly  $F_d$  variations to detect patterns of stomatal regulation around midday on days with high VPD values. Gartner et al. (2009) or Sullivan et al. (2021) also used these diurnal variations to detect species-specific patterns to drought response as the drought stress progressed. However, I did not have the time to do this during my PhD, but it would be an interesting perspective. Finally, a high loss of  $F_d$  in response to drought could also be the result of reduced transpiring leaf area due to leaf shedding (Bréda et al. 2006). At ORPHEE, leaf discoloration and loss were reported for birch between July 19<sup>th</sup> and 30<sup>th</sup> 2019 in the non-irrigated block (data not shown for leaf discoloration, see **Appendix A, p. 321** for leaf loss). The fact that birch trees in both pure and mixed plots of the non-irrigated block never go back to a  $F_{d\ loss}$  of zero, while pine does in mid-August (**Fig. III. 3.7**), shows that this leaf loss probably played a role in the regulation of transpiration for birch.

The percentage of loss I observed for birch and pine was surprisingly high compared to previously reported reductions in  $F_d$  with drought. For example, Gutierrez Lopez et al. (2021) reported a loss of 30 % in tree transpiration during the severe summer drought of 2018 in boreal forests. In Mediterranean forests, Grossiord et al. (2014b) reported a loss of 56 % for *Quercus cerris* at the maximum of the drought while in Grossiord et al. (2015), the mean decrease in sap flux density ranged from 30 % for *Quercus faginea* to 85 % for *Pinus sylvestris*. Of course, these experiments were conducted in different environmental conditions and with different species, so these comparisons are limited. In a site similar to ours, Delzon and Loustau (2005) reported transpiration losses of about 50-60 % (Note that I estimated this loss from the figures provided in these papers, on days with high VPD values and no rain) for maritime pine at the end of a dry period compared to spring levels for two consecutive years (2001 and

2002). These examples illustrate how sensitive the 12-year-old trees at ORPHEE are to drought and how severe soil drought was in this plantation during the summer 2019.

The similarity in  $F_{d \text{ loss}}$  between species in pure plots was surprising. We expected species-specific responses to drought in terms of sap flux density (Gutierrez Lopez et al. 2021). Birch is indeed considered much less drought tolerant than maritime pine (Niinemets and Valladares 2006; Andivia et al. 2020) and is supposed to be more sensitive to cavitation (Choat et al. 2012) (also, see **Appendix III.A, p. 275**). I thus expected a greater decrease in  $F_d$  for birch than pine trees. On the other hand, both species have a similar drought avoidance strategy, as isohydric species. The synchronization in the response of  $F_{d \text{ loss}}$  could therefore indicate a similar stomatal regulation in response to water deficit for both species. This is consistent with the results from Gartner et al. (2009) who studied the drought response of spruce and birch in Austria during the extreme drought of 2003. They reported that the drought response started at the same time for both species, at a critical soil water content. In our study, the similarity in drought sensitivity between the species could also be due to an age effect: these species might have a similar sensitivity to drought in their young stages only, while it would be different between older trees. Delzon et al. (2005) reported that maritime pine can be more sensitive to drought in young stands than older stands. This potential ontogenic change in drought tolerance could differ between birch and pine. For example, Zang et al. (2014) noted that resistance to drought, in terms of tree ring growth, was lower for older beech trees than for younger ones, while it did not change with age for spruce and fir.

#### *Mixture effect*

We did not find strong mixture effects on  $F_{d \text{ loss}}$  at the end of the drought and on the dynamics of  $F_{d \text{ loss}}$ , contrary to my expectations (**Fig. III. 3.8, Fig. III. 3.9**). Even though pine trees in mixed stand reached the same total loss of  $F_d$  by the end of the dry spell, we found a small mixture effect on pine drought response: pines in the mixed plot showed a steeper slope of  $F_{d \text{ loss}}$  than pines in the pure plots (**Fig. III. 3.9**). The absence of a strong mixture effect on drought response was surprising because we expected a higher total water use in the stands with pine that could lead to a faster and higher total loss of water. I did not observe this potential pattern. The reasons for this lack of difference among treatments might be a combination of processes in each plot acting in opposite ways: (i) the rate of soil water depletion due to the total plot transpiration and to water uptake depths, (ii) species-specific drought tolerances, and (iii) mixture effects on the canopy structure that could lead to different microclimatic conditions. As a result, a different combination of these factors in each plot could lead to an overall similar response to drought: a faster depletion of soil water in pure pine but with more drought tolerant trees, a slower depletion of soil water in pure birch but with more drought sensitive trees, and an intermediate situation in the mixed plot. In the mixed plot birch might have less water in than in pure ones because of the presence of pine, but birch crowns are also more protected among pines in mixed plots and might have a delayed response to drought. On the other hand, pines in mixed plots might have more water than in

pure plots with the presence of smaller birch trees that use less water, but their crowns might be more exposed to light leading to stronger stomatal regulation. Indeed, pine trees were taller than birch in the non-irrigated block (**Fig. III. 3.1**) and might have had a higher crown exposure to high temperatures,  $R_g$  and VPD than in pure plots, leading to an stronger stomatal closure (Grossiord 2019). In the same line of thought, the faster drought response of pine in mixed plots could be due to this microclimatic effect on pine crowns, with a faster stomatal regulation. A similar mechanism has been suggested by Forrester (2015) to explain higher  $F_d$  for *Eucalyptus globulus* when mixed with *Acacia mearnsii*. Furthermore, Jonard et al. (2011) pointed out that the sensitivity of  $F_d$  to VPD seemed to be dependent on crown exposure to atmospheric conditions which varied according to the competition level encountered by the different species by stand type populations. Other experiments may help us understand this homogeneity in the drought response among species and plot composition: during my PhD, I also measured the response to drought using wood  $\delta^{13}C$  and estimated water uptake depths during drought. Both measurements will help me to interpret these effects in a broader view (see **section 6**: general discussion of this chapter).

#### *Resilience after a drought event*

After July's rain events, we observed an increase in  $F_d$  for trees of both species and both in pure and mixed plots (**Fig. III. 3.5**). However, the level of this increase differed between species. Birch had a stronger resilience (i.e., capacity to reach pre-drought  $F_d$  levels) as it had lower  $F_{d,loss}$  than pine in pure plots after the first drought episode (**Fig. III. 3.8b**). The same species difference was observed in the mixed plots. This could be the result of potential differences in fine root vertical distribution at that time of the year and in water uptake depths between the species. If birch trees had a higher proportion of water uptake near the surface than pine, it could make them able to take better advantage of recent precipitation events. A premature leaf fall during the drought spell could also explain a lower resilience after a drought event (Bréda et al. 2006). However, in our case, we observed a stronger leaf fall for birch than pine in July 2019 (pers. comm.: data obtained from field observations and PAR sensors, see **Appendix A, p. 321**), which cannot explain the lower resilience for pine. Moreover, I can see that this weaker resilience for pine is temporary as pine reaches similar values of  $F_{d,loss}$  by the middle of August (**Fig. III. 3.7**). Little is known about potential species and mixture effects on the resilience after drought events at this short time scale. In the literature, the question of resilience in mixed forests has rather been observed through annual growth trends before and after drought events (Pardos et al. 2021).

#### Irrigated block

As expected, the irrigation treatment significantly relieved the water limitations during the summer, but the daily additional volume was not enough to exclude water deficit completely. This can be seen by the decrease in  $F_d$  at the end of July (**Fig. III. 3.5, Fig. III. 3.7**). The input of around 3 mm of water day<sup>-1</sup>



by irrigation clearly delayed the beginning of the loss in  $F_d$  by about 10 days (**Fig. III. 3.10a**) and strongly reduced the speed of the decrease (**Fig. III. 3.10b**).

However, this impact differed between species and mixture treatments. For birch, the difference between pure mixed plots for  $F_{d\text{ loss}}$  was surprising as pure plots had a more important total loss and a faster loss than mixed birch (**Fig. III. 3.8**, **Fig. III. 3.10**). I had expected that birch would have a higher loss in mixed stand due to higher basal area in the plots with pine (**Fig. III. 3.2**) which could mean a higher total water use. I hypothesized that this higher loss in pure plots could be an indirect effect of the irrigation coupled to the openness of pure birch plots resulting in a higher understory vegetation biomass. However, Altinalmazis-Kondylis et al. (2020) showed that the proportion of fine root biomass of the understory compared to birch and/or pine trees was similar among the different plots at ORPHEE. Another hypothesis is that the effective irrigation input was lower for pure birch because of the openness of the canopy which allowed for more evaporation of the irrigation water during the day. These are just hypotheses, as I was not able to precisely measure these local microclimate conditions. For pine, there was no difference between pure and mixed plots and  $F_{d\text{ loss}}$  was intermediate between pure birch and mixed birch (**Fig. III. 3.8**). This was different from what I observed in the non-irrigated block as we did not observe a faster loss for pine in the mixed plot, this could be because of a denser canopy than in the non-irrigated mixed plot (higher LAI, **Fig. III.S 3.2**) that prevented us from seeing a similar microclimatic effect on pine crowns.

#### Take-home message

During summer drought, we observed a strong loss of sap flux density ( $F_d$ ) that was highly synchronized across species and plot composition in the non-irrigated block, contrary to our expectations. However, pine trees in mixed plots had a slightly faster loss of  $F_d$  than in pure plots which could be explained by modifications of the canopy structure in mixed plots.

The drought effects on  $F_d$  in the irrigated block was delayed and much less important. In this block, there was no mixture effect on pine, and a strong mixture effect on birch drought response. Birch trees in pure plots had a much stronger  $F_{d\text{ loss}}$  than the ones in the mixed plot. This could be the result of a lower effective input of irrigation due to the openness of the canopy in the birch pure plot.

### 3.4.2. Plot-level transpiration

#### 3.4.2.1. Basal area of the plots in 2019

As presented in the description of the experimental design (section 3.2.1.1), there were strong differences in plot basal area (BA) among the different plot compositions (Fig. III. 3.2). In the non-irrigated block, in pure plots, pine had a plot BA more than three times higher than birch, and the mixed plot had an intermediate value close to the pine plot. The same difference among plot compositions was found in the irrigated block. Irrigation resulted in higher BA values for all plots than in the non-irrigated ones, although the increase was small for the mixed plot. This increase in BA is the result of the irrigation effect on individual tree growth (Fig. III. 3.1). The transpiration data that we will discuss in the following paragraphs is dependent on both on these results on BA and on the results for  $F_d$  that we discussed in the previous section (3.4.1).

#### 3.4.2.2. Plot transpiration in the May-June period

##### Non-irrigated block

##### *Species effect*

On days without rain, the average plot transpiration ( $T_{\text{plot}}$ ) in the non-irrigated block was  $1.0 \pm 0.2$  mm day<sup>-1</sup> in the pure birch plot,  $3.2 \pm 0.5$  mm day<sup>-1</sup> in the pure pine plot, and  $2.7 \pm 0.4$  mm day<sup>-1</sup> in the mixed plot (Fig. III. 3.11, Fig. III. 3.13a). Plot transpiration for pine was close, but still inferior, to PET at this site (Fig. III. 3.11). These values were within the range of expected plot transpiration, which can range between 0.5 to 4 mm day<sup>-1</sup> on a sunny day in the absence of water stress depending on the forest type, age and stand density (see for example: Kunert et al. 2012; Gebauer et al. 2012; Grossiord et al. 2013). Grossiord et al. (2013a) reported similar  $T_{\text{plot}}$  values for a pure birch stand (1.7 mm day<sup>-1</sup>) in a 12-year-old boreal plantation. Delzon and Loustau (2005) found values that ranged around 3.0 mm day<sup>-1</sup> on sunny days of a wet year for a 10 year-old pure maritime pine plantation also located in Southwest France. Despite the higher  $F_d$  we found for birch than for pine, plot transpiration in pure birch plots was much lower than in pure pine plots (Fig. III. 3.11), due to lower basal area of birch trees (Fig. III. 3.2).

##### *Mixture effect*

In absolute values, the mixed plot had intermediate  $T_{\text{plot}}$  compared to the pure birch and pure pine plots (Fig. III. 3.11, Fig. III. 3.13a). However, the difference between observed and predicted values (with the net biodiversity effect calculated with  $BA_{\text{pred.}}$ ) yielded a significant positive NBE on transpiration (Fig. III. 3.13b), meaning that the mixed plot transpired more than what could be expected from pure plots (Fig. III. 3.12a). A higher plot transpiration in mixed stands has been reported in various types of forests: in a temperate old-growth forest (Gebauer et al. 2012), in a Eucalyptus-Acacia plantation in Australia (Forrester et al. 2010; Forrester 2015) and in a tropical plantation (Kunert et al. 2012). In these studies, they found values in mixed stands that were higher than all monocultures (i.e., for growth it

would be called “transgressive overyielding”), while in our study the mixed stand was intermediate to monocultures but higher than the expected average (i.e., “overyielding”). A higher transpiration in mixed stands can result in a higher productivity in the absence of water stress, but might be detrimental during droughts, making the trees in mixed stands more susceptible to drought.

Since there were no mixture effects on  $F_d$ , and that when using  $BA_{\text{mixed}}$  the NBE was equal to zero (**Fig. III. 3.15b**), this initial positive NBE was only due to mixture effects on plot BA. In other words, higher plot-level transpiration in the mixed plot simply reflected the larger mean diameter of pine in the mixed plot as compared to the pure one, which is consistent with my initial hypothesis and with the findings of Forrester (2015). Indeed, only pine benefited from the mixture in terms of basal area, while it was not the case for birch. This might be due to a competitive reduction in aboveground competition for pine in mixtures at the expense of birch (see **Chapter II**). It was also previously reported that the relative contribution of each species to stand transpiration is driven largely by the proportion of species sapwood area rather than to species-specific differences in daily water use (Wullschleger et al. 2001; Jonard et al. 2011).

### Irrigated block

#### *Irrigation effects on $T_{\text{plot}}$*

Compared to the non-irrigated block, daily  $T_{\text{plot}}$  in the irrigated block were higher for both pure plots, with an average of  $2.3 \pm 0.4 \text{ mm day}^{-1}$  in the pure birch plot,  $3.8 \pm 0.6 \text{ mm day}^{-1}$  in the pure pine plot and similar for the mixed plot with an average of  $2.7 \pm 0.4 \text{ mm day}^{-1}$ . Transpiration for the pure pine plot was almost equal to PET during the spring (**Fig. III. 3.11**). The increase in plot transpiration with the irrigation treatment was especially high for birch, with an increase of 130 % in daily transpiration while pine had an increase of 18 % only. The higher increase for birch indicated that birch was more limited by water availability than pine in the non-irrigated plots, as birch is less drought tolerant than pine (Niinemets and Valladares 2006; Andivia et al. 2020). For both species, the higher plot transpiration in pure plots of the irrigated block compared to the non-irrigated block was mainly a consequence of a long-term positive impact of irrigation on tree growth (**Fig. III. 3.1**) and LAI (**Fig. III.S 3.2**). The irrigation effect on growth resulted in a higher plot basal area (BA) in the irrigated block (**Fig. III. 3.2**): the irrigated pure birch plot had an 83 % higher BA than the non-irrigated one while pine had an 18 % increase in BA with irrigation in the pure plots. Indeed, irrigation is known to improve plant productivity if water is a limiting resource (e.g., Hubbard et al. 2010). For birch, the increase in BA (83 %) was not as strong as the increase in transpiration (130 %). This discrepancy might be due to an increase in leaf area proportionally bigger than the increase in productivity for birch. Indeed, birch trees in the non-irrigated blocks, which are more exposed to drought, might have a reduced leaf area index due to drought effects from the previous year (Bréda et al. 2006), while this is not the case in the irrigated block. The rate of transpiration per day was similar in the mixed plots of both the non-irrigated and the irrigated

block due to a combination of slightly higher BA (**Fig. III. 3.2**) but slightly lower  $F_d$  (**Fig. III. 3.6b**) in the irrigated mixed plot compared to the non-irrigated one.

#### *Mixture effects in the irrigated block*

The mixture plot still had intermediate  $T_{plot}$  compared to that of the pure birch and pure pine plots (**Fig. III. 3.11**). However, contrary to the non-irrigated block, the observed transpiration in the mixed stand was lower than expected with both calculation methods ( $BA_{pred.}$  and  $BA_{mixed}$ ) (**Fig. III. 3.12**, **Fig. III. 3.14**), with a negative NBE, indicating that the observed mixed plot transpired less than what could be from the respective pure plots. This is an interesting perspective compared to previous studies: modifying the water supply during several years influenced the overall mixture effect on plot transpiration. Therefore, when looking at the effect of species diversity on forest stand water use, in future studies it will be crucial to include a wider range of climatic conditions for the same type of species assemblages as advocated by Forrester (2015).

The negative NBE was the consequence of mixture effects on both plot BA and species  $F_d$ . The difference between the observed and the predicted BA for the mixed stand was smaller than in the non-irrigated block (**Fig. III. 3.2**): pine did not benefit from the mixture as much in the irrigated block (+34 % when comparing observed and predicted values) than in the non-irrigated block (+45 %) and birch had a bigger drop in BA compared to expected values in the irrigated block (-57 %) than in the non-irrigated block (-45 %). This explains in part the negative difference between observed and predicted  $T_{plot}$  for birch (**Fig. III. 3.12b**). The sum of these effects for both species results in a lower plot transpiration than expected (**Fig. III. 3.12a**). The fact that  $T_{plot}$  was not higher than expected in this irrigated block where mixture effect on BA were closer to neutral values is in accordance with the idea that  $T_{plot}$  usually only increases in the mixtures when growth also increases (Forrester 2015). The irrigation might have resulted in higher growth rates earlier in the developmental stages because of the irrigation treatment. Therefore, this may have resulted in a more closed canopy and a lower light availability in the irrigated mixed plot (**Fig. III.S 3.2**), which may have in turn limited the positive mixture effects in this plot in recent years compared to the non-irrigated block. The negative NBE was also likely due to the small differences in the average  $F_d$ , with lower values in the mixed plots than in the pure plots for birch (**Fig. III. 3.6b**) resulting in lower values in the observed mixed stands even when removing the mixture effect on BA (**Fig. III. 3.14b**). As discussed in **section 3.4.1.1**, this higher  $F_d$  in the pure birch plot could be due to a higher LAI (**Fig. III.S 3.2**).

#### Potential mechanisms

In both blocks, my analyses suggest that the observed NBE effects are the result of mixture effects on growth and on canopy structure rather than on belowground complementarities. I will further discuss this hypothesis in the general discussion of this PhD thesis. This is consistent with previous studies that reported mixture effect on canopy structure as potential mechanisms influencing on species and stand-

level transpiration (Jonard et al. 2011; Forrester 2015). Crown exposure to atmospheric conditions might be modified in mixed stands (Baldocchi 2005). However, these effects may still vary with changes in environmental conditions and influence the response to drought. Because the non-irrigated mixed plot uses more water than expected from the pure plots we can expect that it might be more susceptible to droughts (Forrester 2015), which is the question we will explore in the following section.

#### Take-home message

In non-limiting soil water conditions in both blocks, the absolute values of plot transpiration ( $T_{\text{plot}}$ ) for the mixed stand were intermediate to pure pine and birch plots. I found that the non-irrigated mixed plot used more water than expected from the pure plots, which I explained by a positive mixture effect on the basal area of pine in mixtures, but not by changes in sap flux density ( $F_d$ ).

In the irrigated block, I found the opposite effect, with a lower water use than expected from pure stands. This was mainly due to a smaller positive mixture effect on BA for pine and a stronger negative mixture effect on BA of birch.

### *3.4.2.3. Influence of drought on plot transpiration*

#### Non-irrigated block

During the drought episode of July, the strong decrease in  $F_d$  in the pure plots induced an 81 % and 78 % loss of transpiration, for pine and birch, respectively, while the mixed plot lost a slightly higher proportion (89 %) (**Fig. III. 3.13a, c**). Mixture effects were reversed during the dry spell compared to the effects in the absence of soil water limitations (**Fig. III. 3.12a**). The shift from positive to negative NBE in the non-irrigated mixed plot (**Fig. III. 3.13d**) during the dry spell indicated that the observed mixed plot transpired less than expected from the weighted average of the pure plots, contrarily to what was observed during the spring season. This is in accordance with my initial hypothesis: I expected that the NBE would shift from positive to neutral or negative due to a faster depletion of water resources in mixed stands. A similar shift has been observed by Magh et al. (2019) from a higher than expected transpiration in spring to a lower than expected transpiration during summer drought for beech when mixed with fir. Forrester et al. (2010) observed that eucalyptus trees transpired more in mixed stands than in monocultures for the total over the year, but during the dry season this effect was reversed with a sharper decrease in transpiration. Seasonal variability (intra-annual) of mixture effects has been observed in other studies (Schume et al. 2004; Kunert et al. 2012; Goisser et al. 2016). Consequently, studies that consider only a small portion of the growing season may not provide a good indication of the annual mixture effects.

The shift from positive to negative NBE was a consequence of a faster loss of  $F_d$  for mixed pine trees than for pure pine trees (**Fig. III. 3.9**), which resulted in a faster loss than expected at the plot-level

transpiration for pine (**Fig. III. 3.12b**) that could also be observed when using  $BA_{\text{mixed}}$  to calculate the predicted transpiration (**Fig. III. 3.14b**). I did not observe the same effect for birch, which indicated as mentioned before (**section 3.4.1.2**) that this was not the consequence of soil water conditions, but rather the consequence of aboveground mixture effects on tree crown exposure of pine. Moreover, Forrester (2015) suggests that, if there are belowground complementarities, transpiration rates could potentially be maintained longer in the mixed plots despite a higher water use. I will further discuss the relationship between the effect of crown exposure and transpiration in the general discussion of this chapter and of the thesis.

#### Irrigated block

As expected from  $F_d$  results, high plot transpiration rates were maintained longer in the irrigated block (**Fig. III. 3.11**). The only significant loss in transpiration due to the drought episode compared to the spring was in the pure birch plot (35 %). NBE shifted from negative to positive with  $BA_{\text{pred}}$  (**Fig. III. 3.12a, Fig. III. 3.13bd**). This confirmed the variability of mixture effects observed in the non-irrigated block and emphasized the importance of comparing mixture effects in different climatic conditions (Forrester 2015). This shift from negative to neutral NBE in the irrigated mixed plot during the dry spell was a consequence of a faster loss of  $F_d$  for birch in the pure plot (**Fig. III. 3.9, Fig. III. 3.12b**) which, as discussed in **section 3.4.1.2** might be due to a lower effective irrigation input.

#### Take-home message

During summer drought, we observed that the mixture effect could change compared to non-limiting soil water conditions. For the non-irrigated block, a positive net biodiversity effect (NBE) in the spring became negative and the opposite trend was found for the irrigated block. The shift from positive to a negative NBE in the non-irrigated plot was not due to a faster depletion of water resources in mixed stands, contrary to my initial hypothesis, but rather to a faster loss of sap flux density ( $F_d$ ) for pine due to a higher crown exposure to radiation and temperatures than in pure plots.

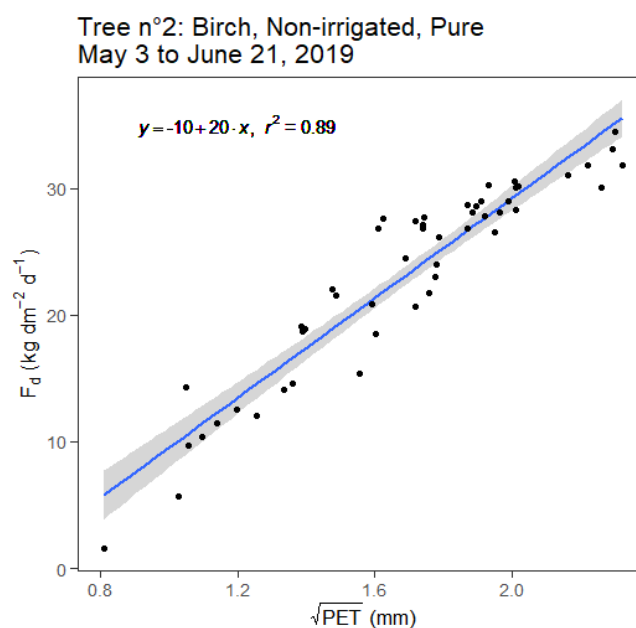
### 3.5. Conclusion

In conclusion, we observed small and contrasted mixture effects on sap flux densities and plot transpiration in the absence and in the presence of water stress. The few observed effects did not appear to be linked to potential belowground complementarities or to a faster soil water depletion. For  $F_d$  they were more likely due to aboveground mixture effects on the canopy structure leading to differences in microclimatic conditions. At the plot transpiration level, they were strongly linked to mixture effects on growth from the previous years. Although transpiration was higher than expected from the average value of the pure plots, managing mixed stands of birch and pine in this region will still lead to an intermediate total water use between the one of pure birch and pure pine stands. Therefore, compared to the monoculture of maritime pine commonly used in this region, the mixture of birch and pine will lead to a lower total water use.

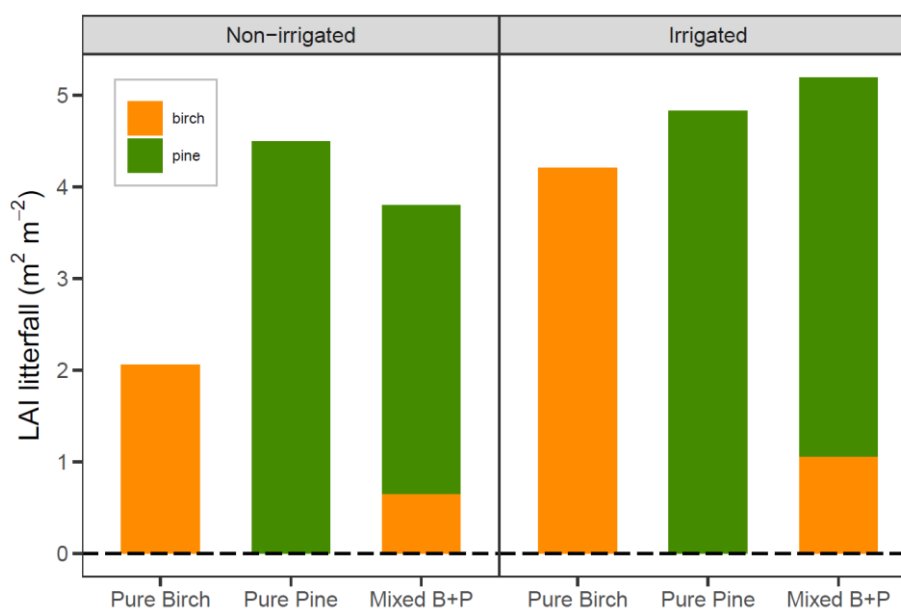
Mixing birch and pine in these climatic conditions did not provide major benefits in terms of mitigating drought response. It seems that when the mixture leads to a higher transpiration than expected from the monocultures in the absence of water stress, this could result in a faster response to drought for pine. However, these differences remain small and the percentage loss of sap flux density at the end of the dry season was similar for all plots.

With irrigation, the stand transpiration was more important, and the response to drought was delayed compared to the non-irrigated treatment. The irrigation treatment allowed to document how the trees functioned with a higher water availability and to compare these results to the non-irrigated block. Mixture effects on water use and drought response in the irrigated block were different than in the non-irrigated block. Indeed, the size of the mixture effect between a given pair of species is dynamic (Forrester 2015). It changes as resource availability or climatic conditions change along spatial and temporal gradients or as stands develop and trees age (Forrester 2014). The interactions that influence water availability or use are thought to be more useful on dry sites or during dry periods if they improve the availability of the resource. It was not the case in our study, mainly as a result of indirect effects of irrigation on growth.

## 3.6. Supplementary figures Chapter III.3



**Fig. III.S 3.1** Example of a linear relationship between  $F_d$  and the square root of PET during the May-June period (all days) for Tree n°2.



**Fig. III.S 3.2** Plot leaf area index (LAI) in 2019 by species and plot composition for the chosen plots in blocks 1 and 2. LAI was estimated from litterfall collection in litter traps by Laurent Augusto and colleagues (unpublished data).



## Chapter III.4

# Stable carbon isotope composition in summer wood



## 4. Stable carbon isotope composition in summer wood

### 4.1. Introduction

#### 4.1.1. Context

##### *More frequent droughts threaten European forests*

European forest ecosystems are expected to be highly impacted by ongoing climate change (Hanewinkel et al. 2013) through an increase in temperatures and more frequent and intense droughts (IPCC 2014; Lehner et al. 2017). Drought episodes affect physiological processes in trees such as photosynthesis, transpiration, and carbon allocation, which can result in reduced growth rates (Bréda et al. 2006). Drought-induced stress can lead to a loss of vitality and to mortality for trees, caused by hydraulic failure and/or carbon starvation (McDowell et al. 2008; Hartmann et al. 2018). This will be especially true in southern parts of the European temperate and Mediterranean regions (Lindner et al. 2010). There is therefore a strong need for new management strategies to help forest owners mitigate current and future drought impacts (Bolte et al. 2009).

##### *Mixed forests as a solution to mitigate drought impacts*

Favoring mixed species stands has been proposed as a strategy to adapt forests to the increasing risk of water stress. Indeed, it was hypothesized that this may reduce the overall competition for limiting resources, such as water, by a more efficient partitioning through spatial or temporal resource-use complementarity (Loreau and Hector 2001; Forrester 2014). Although it has been discussed for more than a decade, it is still not clear whether managing forests with coexisting tree species can contribute to mitigating drought impacts on these forests (Grossiord 2019). So far, contrasted effects of species interactions on drought exposure in mixed forests have been observed under drought conditions (see the following reviews for a list of studies and effects: Ammer 2019; Grossiord 2019). Even for a single species, results of mixture effects can be variable. For example, some studies demonstrated lower drought exposure for beech in mixed stands (e.g. Pretzsch et al., 2013; Metz et al., 2016), while others concluded that the mixture effect was neutral (e.g. Schwarz and Bauhus, 2019). Furthermore, contrasted results were also found when comparing the effects of the same species mixture, but among different sites (Schäfer et al. 2017; González de Andrés et al. 2018; Jourdan et al. 2019b; de Streel et al. 2020). Finally, within a given mixed stand, mixture effects on drought exposure can lead to opposite outcomes among the coexisting species, with one species benefiting from the mixture while another does not (Grossiord et al. 2014d; Forrester et al. 2016; Jacobs et al. 2021).

##### *Mechanisms underlying mixture effects on drought*

Tree species diversity can contribute to a reduced vulnerability to drought because resource partitioning and facilitation processes may occur in the mixed stands (Loreau and Hector 2001; Grossiord 2019).

However, these mechanisms are often difficult to separate and are usually described together as the “complementarity processes” (Loreau and Hector 2001; Grossiord 2019) (see the **Introduction** chapter of this PhD for more details). The concept of resource partitioning has also been referred to as “niche differentiation” by Loreau and Hector (2001) or as “competition reduction” by Forrester and Bauhus (2016). In addition to complementarity, a “species selection” effect (Loreau and Hector 2001) can also play a role in mixture effects, but is less relevant in two-species mixtures, and especially when looking at species-specific responses (Grossiord 2019). Here, complementarity effects refer to biological mechanisms that modify the environment and the degree of stress experienced by trees. Resource partitioning may happen when dissimilarities in functional traits of species reduces competition for resources in space and time. For instance, root stratification of species may result in resource partitioning that can improve species-specific soil water status by reducing competition for water among species (e.g., Schwendenmann et al. 2015). This would result in a lesser exposure to decreased soil water content in mixed stands during extreme drought events (Forrester 2014). In contrast, because mixing can also improve tree growth and stand productivity (Ammer 2019), species-diverse forests may also use more water than pure ones (assuming that water use efficiency of these trees does not increase). This would lead to a faster depletion of soil water resources and higher drought exposure for all species in mixed stands (Forrester 2015).

#### *Species functional identity as the main driver for mixture effects*

Consequently, the main driver of the variability in the outcome of mixture effects on drought exposure is the functional variability among species. Tree species are known to vary widely in the ecological strategies they use to deal with drought stress (Niinemets and Valladares 2006). Species with more contrasted functional traits in terms of physiology, phenology or morphology (e.g. root depth, stomatal control, physical traits involved in xylem resistance to embolism), are more likely to show complementarity effects, especially for drought responses (Grossiord 2019). For example, Pardos et al. (2021) found that beneficial effects of mixtures on drought exposure were greater in conifer-broadleaved admixtures, which have strong functional differences, than in broadleaved-broadleaved combinations. Further studies on species diversity effects on drought exposure are necessary to identify which species assemblages are susceptible to show complementarity effects towards drought responses and thus which ones should be favored in forest management programs. In Southwest France, large areas are cultivated as monocultures of maritime pine (*Pinus pinaster* Ait.) and are intensively managed as production plantations (Mason and Meredieu 2011). An interesting option there to mitigate climate change risks would be to grow maritime pine with silver birch (*Betula pendula* Roth), another tree species that is also able to grow on the poor sandy soils of this region. These two species have contrasted functional traits which could result in complementarity effects.

#### *Spatial and temporal variations of mixture effects*

In addition to species functional identity, local environmental conditions also play an important role in shaping mixture effects on drought exposure (Forrester 2014; Forrester and Bauhus 2016). Even if species interactions may improve the availability of limiting resources, the degree of the mixture effect may still vary with site-level climatic conditions (Forrester and Bauhus 2016). Forrester and Bauhus (2016) suggested a theoretical framework, as a generalization of the stress gradient hypothesis (SGH) to forest ecosystems (Bertness and Callaway 1994), in which positive mixture effects are expected to be stronger when resources are scarce, for example along a declining precipitation gradient. In accordance with this framework, Grossiord et al. (2014c, d) found negative or neutral effects in more humid sites and positive effects in drier ones. However, this relationship between mixture effects and a resource gradient may not be linear and may rather emerge from threshold points of resource availability (de Streel et al. 2020). Mixture effects on drought exposure may be neutral in wet conditions, positive in moderately dry conditions and revert back to neutral effects in very dry conditions (de Streel 2021, p.145, Fig. 5.2). Such thresholds could be explained by the fact that positive mixture effects may not be strong enough to compensate for the increasing environmental constraints in extremely dry sites and that positive mixture effects on drought response could disappear with strong water stress (de Streel et al. 2020). In addition to spatial variations, mixture effects can also change with time at a given site due to inter-annual climatic variations and/or to stand development. For example, in the early stages of stand development, competition for resources increases which may also increase potential complementarity effects in mixtures (Huang et al. 2018). Temporal variations in mixture effects can also arise from changes in the balance between complementarity and competition processes at a given site. For instance, a higher growth due to complementarity in non-limiting soil water conditions can lead to an increased competition during drought years due to a higher water use in mixed stands (Jacobs et al. 2021). It is therefore important to study specific species assemblages in different climatic conditions and to follow them throughout different developmental stages.

#### *Measuring drought impacts on trees at the inter-annual scale*

Two types of retrospective measures have been commonly used to detect a higher drought exposure for trees across different years: (i) a reduction of the annual radial growth and (ii) carbon isotope composition ( $\delta^{13}\text{C}$ , ‰) in tree rings. The reduction in radial growth due to limited water availability is often used to detect drought impacts on trees (e.g., Pardos et al. 2021). However,  $\delta^{13}\text{C}$  are thought to detect drought stress signals more precisely than radial stem growth (Jucker et al. 2017). Tree-ring  $\delta^{13}\text{C}$  provides a time-integrated record of the ratio of intercellular to atmospheric  $\text{CO}_2$  concentrations during the period when the carbon used to build this ring was fixed (Farquhar et al. 1989). It reflects the balance between net  $\text{CO}_2$  assimilation and stomatal conductance during this period (i.e., water use efficiency, Farquhar et al. 1989). As a response to drought stress, trees close their stomata and reduce carbon assimilation rates. However, leaf stomatal conductance is affected to a greater extent than carbon assimilation, resulting in greater intrinsic water use efficiency and an increase in  $\delta^{13}\text{C}$  (Farquhar et al.

1989; Saurer et al. 1995; McCarroll and Loader 2004). Therefore, the difference in tree-ring  $\delta^{13}\text{C}$  between values during a wet summer and a dry summer can be used as an indicator of the intensity of drought exposure in trees (Grossiord et al. 2014d). Comparing this indicator for various species in pure and mixed stands helps understanding whether mixing tree species induce more, less, or similar drought exposure in these stands. Indeed, if complementarity for water use is occurring among species,  $\delta^{13}\text{C}$  values should increase less between wet and dry soil conditions in mixed stands than in pure stands. In our study the response to summer drought was assessed as the difference in wood  $\delta^{13}\text{C}$  between a wet and a dry year, in order to detect changes in drought exposure independently of species and plot specific initial conditions (Grossiord et al. 2014c).

#### *Influence of light availability on $\delta^{13}\text{C}$*

Variation in  $\delta^{13}\text{C}$  is not only influenced by water availability, but also by fluctuations in other environmental factors such as light and nutrients (Farquhar et al. 1989; Ferrio et al. 2003). Light intensity has a positive relationship with  $\delta^{13}\text{C}$  (Ferrio et al. 2003). For example, the vertical profile of leaf tissue  $\delta^{13}\text{C}$  within a forest canopy varies following irradiance gradients. As tree-ring  $\delta^{13}\text{C}$  is an integrated measure of what happens for the whole crown of a tree, trees that are more shaded will have lower (more negative)  $\delta^{13}\text{C}$  than trees that receive more radiation (Warren et al. 2001; Ponton et al. 2002). This is the case because a higher radiation will induce higher photosynthetic rates, which will in turn increase  $\delta^{13}\text{C}$  (Farquhar et al. 1989). Above a certain threshold of water availability, the effect of light availability on  $\delta^{13}\text{C}$  probably influences  $\delta^{13}\text{C}$  variations more than water availability (Warren et al. 2001). As a result  $\delta^{13}\text{C}$  is a complex trait to analyze, but it remains a useful indicator of water availability or drought stress in seasonally dry climates when variations in other environmental factors can be accounted for (Warren et al. 2001; McCarroll and Loader 2004). In this study, because it is a young plantation with a fast growth dynamic (Toigo et al. 2021a), changes in canopy closure and in social status of trees may influence  $\delta^{13}\text{C}$ , which will be discussed throughout this sub-chapter.

#### 4.1.2. Objectives

I measured tree-ring  $\delta^{13}\text{C}$  of trees in pure birch, pure pine and mixed birch and pine plots at ORPHEE during a wet year (2013) and dry years (2016 and 2018) in both irrigated and non-irrigated blocks and both before and after the start of the irrigation treatment in 2015. This allowed me to test the effect of species-mixture and irrigation on  $\delta^{13}\text{C}$  and to analyze drought exposure levels in these different conditions. The two studied species are functionally different both aboveground and belowground with a potential for complementarity effects. I therefore expected that the species would respond with lower drought exposure in mixed stands than in pure stands. I included two dry years (2016 and 2018) to detect potential changes in canopy structure with time that could also affect  $\delta^{13}\text{C}$ .

In this sub-chapter I asked the following general questions:

1. Are there species and mixture effects on  $\delta^{13}\text{C}$  in a wet year before the start of irrigation?
2. Is  $\delta^{13}\text{C}$  higher during dry years due to drought exposure in pure plots of the non-irrigated blocks?
3. Are there mixture effects on  $\delta^{13}\text{C}$  of dry years in the non-irrigated blocks?
4. Are these mixture effects due to changes in drought exposure or light availability in mixed plots compared to pure plots?
5. What are the effects of summer irrigation on tree  $\delta^{13}\text{C}$  in pure and mixed plots?

For the non-irrigated blocks, I hypothesized that (**Fig. III. 4.1, Fig. III. 4.2**):

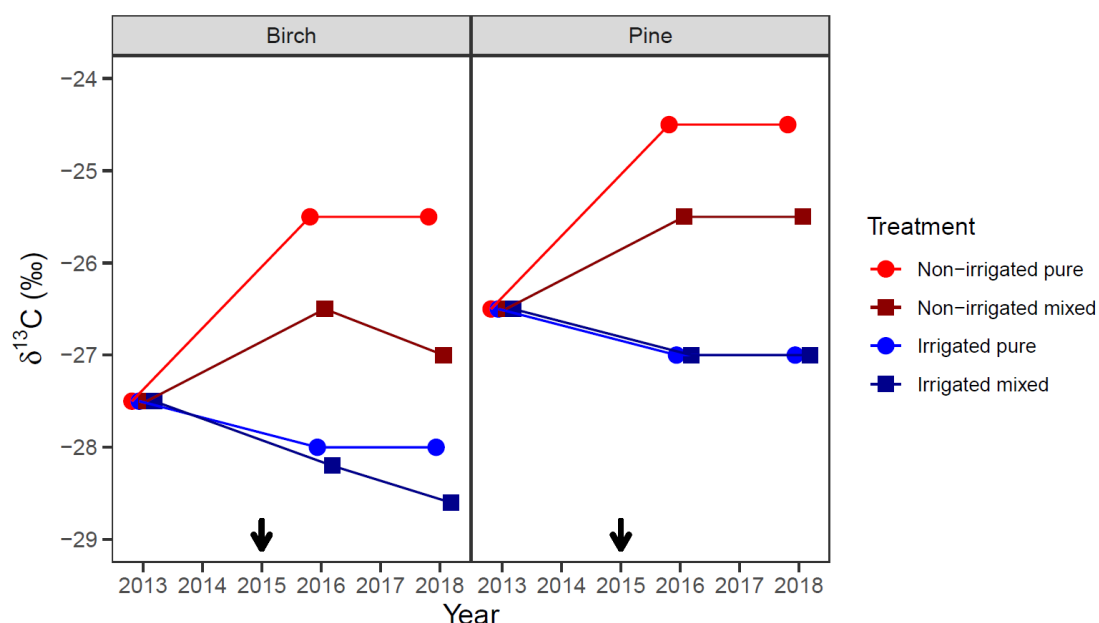
- (i) In 2013,  $\delta^{13}\text{C}$  would be different between species in pure stands due to species-specific functional characteristics. I expected less negative values for pine, as conifer species usually have less negative  $\delta^{13}\text{C}$  than broadleaved species (Heaton 1999).
- (ii) In 2013,  $\delta^{13}\text{C}$  would be the same between pure and mixed stands for a given species. I based this hypothesis on the fact that in 2013, the plantation was still young (5 years old) and that potential aboveground and/or belowground complementarity effects were not yet expected. Moreover, Toïgo et al. (2021a) did not find any significantoveryielding in mixed stands compared to pure stands in this plantation in 2014.
- (iii)  $\delta^{13}\text{C}$  would be less negative in 2016 and 2018 compared to 2013 for both species, because of the strong limiting soil water conditions in 2016 and 2018.
- (iv) The increase in  $\delta^{13}\text{C}$  between the wet and the dry years ( $\Delta\delta^{13}\text{C}$ ) would be smaller in mixed stands than in pure stands due to a complementarity between species.
- (v) The increase in  $\delta^{13}\text{C}$  between the wet and the dry years ( $\Delta\delta^{13}\text{C}$ ) would be smaller in 2018 compared to 2016 for birch in mixed stands because of a progressive domination of birch by pine in mixed stands ( $\delta^{13}\text{C}$  is more negative when light is limiting).

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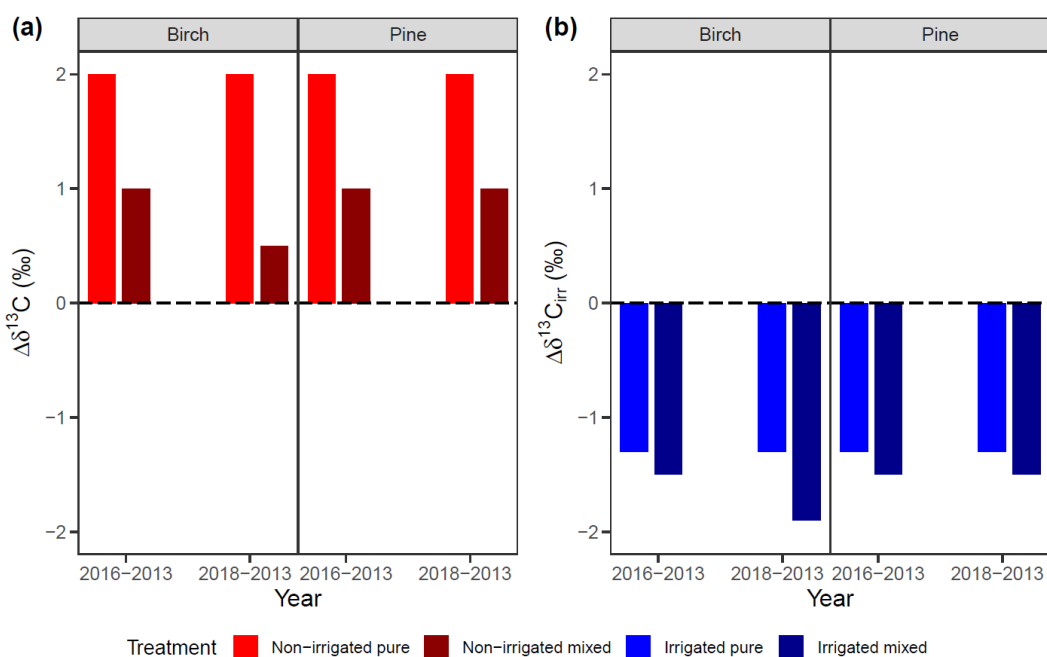
For the irrigated blocks, I hypothesized that (**Fig. III. 4.1, Fig. III. 4.2**):

- (i) In 2013 (before the irrigation started), the initial species effect and absence of mixture effects on  $\delta^{13}\text{C}$  would be the same as in the non-irrigated blocks.
- (ii)  $\delta^{13}\text{C}$  values would be slightly more negative (i.e., negative  $\Delta\delta^{13}\text{C}$ ) in 2016 and 2018 as compared to the reference year before irrigation (2013), due to the irrigation input which should release any drought effect. Yet, as the summer 2013 was wet and that of 2016 and 2018 were very dry, the irrigation effect in 2016 and 2018 might just induce  $\delta^{13}\text{C}$  values in 2016 and 2018 to be rather close to that of 2013.
- (iii)  $\delta^{13}\text{C}$  values in 2016 and 2018 in the irrigated blocks would be much more negative than in the non-irrigated ones (irrigation effect that almost completely removes any limiting soil water conditions).
- (iv) The decrease in  $\delta^{13}\text{C}$  between the reference and irrigated years ( $\Delta\delta^{13}\text{C}$ ) would be the same in mixed stands and in pure stands if only water-use processes are at stake. A mixture effect on  $\Delta\delta^{13}\text{C}$  in the irrigated blocks would be the result of light-related changes.





**Fig. III. 4.1** Schematic representation of the hypotheses I state on the change in tree-ring  $\delta^{13}\text{C}$  with drought and irrigation effects for both species, with an interaction with light availability effects on birch crowns because of domination by pine in 2018. The black arrows indicate the year when the summer irrigation treatment started at ORPHEE.



**Fig. III. 4.2** Schematic representation of the hypotheses on  $\Delta\delta^{13}\text{C}$  (a) and  $\Delta\delta^{13}\text{C}_{\text{irr}}$  (b) for 2016 and 2018 compared to 2013 by species.  $\Delta\delta^{13}\text{C}$  is the difference between the reference and the dry years calculated as DRY-REF.  $\Delta\delta^{13}\text{C}_{\text{irr}}$  is the difference between the reference and the irrigated years calculated as IRR-REF. Positive values indicate a lower water availability and/or a higher light availability than during the reference year (2013) and vice-versa. I do not expect species differences in  $\Delta\delta^{13}\text{C}$  in pure stands as there is no evidence for species-specific  $\Delta\delta^{13}\text{C}$  at a given site (Forrester et al. 2016).

## 4.2. Methods

### 4.2.1. Experimental design

#### 4.2.1.1. Study site

For this section, please refer to the description of the experimental site at the beginning of this chapter (**section 1.2**).

#### 4.2.1.2. Sampling

In this study, I focused on blocks 1 to 6 at ORPHEE: three non-irrigated (blocks 1, 3 and 6) and three irrigated (blocks 2, 4 and 5). In each block, I selected three plots: one pure birch, one pure pine and one mixed birch and pine. In each of these plots, I selected at least five trees per species (six in block 1 and 2 to sample the same trees as the sap flux experiment, see **Chapter III.3, p. 149**) for a total of 136 trees that were sampled for wood cores.

The aim was to select trees that would best represent the optimal functioning and average environmental conditions of the trees in these plots. Since all trees had the same age, I chose the trees that had vigorous crowns and a high crown illumination percentage, and I avoided trees that had a gap from a dead tree in their direct neighborhood. The trees were selected among the trees from the six inner rows of each plot to avoid potential edge effects in the two outermost rows. However, with regards to this criterion, 9 out of 33 birch trees in the non-irrigated blocks and 6 out of 37 birch trees in the irrigated blocks had to be selected from the second to last rows because of birch mortality or birches that were too small to be cored in the center of the plots (inner six rows).

In February 2019, all the targeted trees were cored at a height of 70 cm with a 5-mm-diameter Pressler increment borer (Haglöf, Sweden) on the South-facing side of the trunk. The trees were cored lower than 1.30 m to avoid disturbing the yearly diameter at breast height (DBH) measurements. These cores were dried and scanned at the Pierroton Forest experimental unit (UEFP). They were analyzed with WinDENDRO™ (Regent Instruments Inc.) for the identification of annual growth rings by Frédéric Lagane. The samples were then sent to Nancy for isotopic analyses.

For a subset of birch trees (20 out of 70) that had a DBH under 7 cm (but with a minimum DBH of 5.5 cm), we did not use the same coring technique. Indeed, the Pressler increment borer might have led to damages on such small trees. For these trees, we collected micro-cores with a Trephor, a tool specifically designed for extracting small cylindrical wood fragments (15 mm in length and 2 mm in diameter) from living trees (Rossi et al. 2006). These micro-cores were collected in March 2019. They were not measured with the WinDENDRO™ software for annual growth to help reading the tree-rings. Instead, the tree rings were directly counted under a stereomicroscope.

#### 4.2.1.3. Hegyi competition index

I calculated a competition index for each tree based on the Hegyi index to evaluate the strength of neighboring competition (Hegyi 1974). This index is typically used to characterize local competition and was calculated with the equation from Prevosto (2005) as:

$$H_i = \sum_{\substack{j=1 \\ j \neq i}}^n \frac{d_j}{d_i L_{ij}}$$

where  $d_i$  and  $d_j$  are respectively the diameter at breast height of the target tree  $i$  and competitor  $j$ ,  $L_{ij}$  is the distance between  $i$  and  $j$ , and  $n$  is the number of competitors in a radius of 3 m which in the plantation corresponds to 8 neighbors.

#### 4.2.2. Climate and soil variables

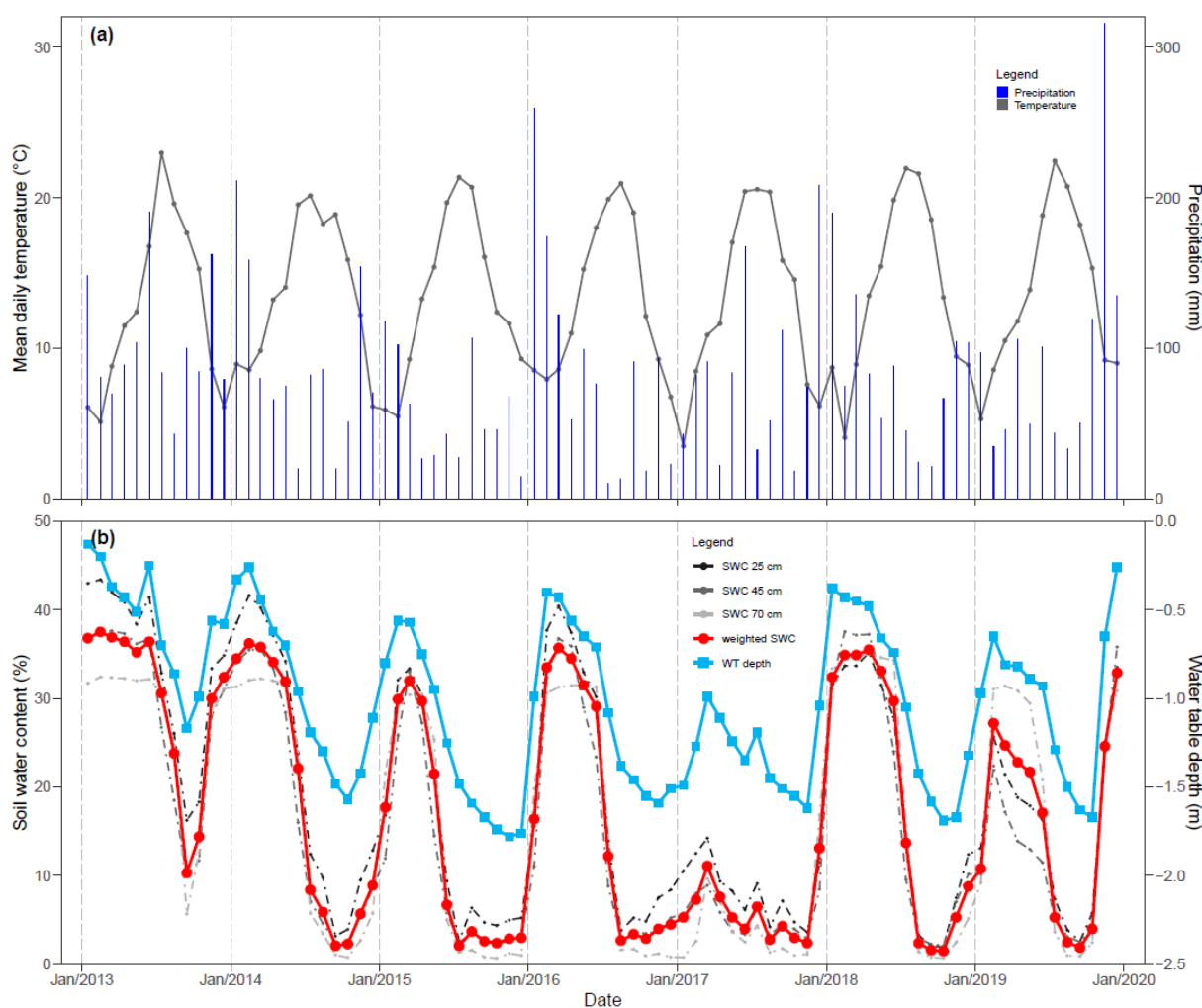
##### 4.2.2.1. Available data

I used nearby weather and soil water content monitoring stations to characterize the seasonal dynamics in climate and soil water content in the ORPHEE plantation for 2013-2018. Monthly climatic data were obtained from the INRAE weather station of Cestas-Pierroton (44°44.52' N, 00°46.92' W, 1.2 km away from ORPHEE) (**Fig. III. 4.3**) made available by the INRAE CLIMATIK platform (<https://intranet.inrae.fr/climatik/>, in French) managed by the AgroClim laboratory of Avignon, France. Monthly water table depth and soil water content (SWC, %) data were recorded in the Castillonville Gis-Coop pure pine plantation next to the ORPHEE plantation (44° 44.08' N, 00°47.74' W, 500 m away). Water table depth was measured with a pressure transducer (CS450, Campbell Scientific). SWC was measured with volumetric water content sensors (CS650, Campbell Scientific) set-up at three different depths: 25 cm, 45 cm and 70 cm (**Fig. III. 4.3b**). With the data from these three depths, I calculated a daily weighted SWC (wSWC, m) according to Bonal et al. (2008), an index which is representative of the soil water content from the surface down to 1 m depth, according to the following equation:

$$\text{wSWC} = 0.350 \times \text{SWC}_{25} + 0.225 \times \text{SWC}_{45} + 0.425 \times \text{SWC}_{70},$$

where 0.350, 0.225 and 0.425 are the weighting coefficients that were divided to add up to 1 m and represent a range of depths for each probe: 0-35 cm for  $\text{SWC}_{25}$ , 35-57.5 cm for  $\text{SWC}_{45}$ , and 57.5-100 cm for  $\text{SWC}_{70}$ . The intrinsic values of wSWC should not be interpreted in terms of raw values, as they depend on the total depth selected to calculate wSWC, but rather as relative values to observe seasonal change. In the case of my study this index is interesting because (i) it takes into account the different values of SWC measured at the different depths, which may vary differently over the season (e.g. when the soil dries up or gets rewetted), and (ii) it is useful to use as a proxy of soil water content over a given depth (here, I chose 1.0 m depth).

The irrigation treatment, which was set-up in spring 2015 (**section 1.2**), adds a constant amount of water in the irrigated plots every night from early May to late September, equivalent to about 3.0 mm of precipitations (3.0 liters per m<sup>2</sup>) (Castagneyrol et al. 2017). Unfortunately, I was not able to monitor the SWC within the irrigated blocks and then could not infer the soil water conditions for these blocks from the available data.



**Fig. III. 4.3** Seasonal patterns of monthly environmental conditions at ORPHEE from 2013 to 2019. (a) Monthly average of the mean daily temperature (°C), and monthly sum of precipitation (mm), (b) Monthly average of soil water content at three depths: 25 cm, 45 cm and 70 cm (SWC, %), weighted soil water content over 100 cm using the three depths (wSWC, %, multiplied by 100 to fit on the same scale), and mean monthly water table depth (WT Depth, m).

#### 4.2.2.2. Characterization and choice of targeted years

To select years with contrasting drought conditions (wet vs. dry) and to characterize the drought intensity experienced by the trees during the summer, I first intended to use the BILJOU© water balance model (Granier et al. 1999, <https://appgeodb.nancy.inra.fr/biljou/>). However, BILJOU© has not been implemented so far with a module that includes the presence of a shallow water table when computing the daily water balance. Yet, at ORPHEE, the presence of such a water table is well-known (see **Fig. III. 4.3** for seasonal variations in water table depths) and it plays a very important role in the water balance at this site, and potentially in the water uptake by trees (**Chapter III.5, p. 229**) (Fan et al. 2017). Therefore, I was not able to simulate water balance at this site with BILJOU ©.

Consequently, I rather used the sum of precipitation during the summer months as well as SWC and water table depth to select years with contrasted drought conditions (**Fig. III. 4.3**). Based on these soil and climate data, I identified 2013 as a year with a wet summer, with a high soil water content in the summer and shallow water table (**Fig. III. 4.3**). No other wet year could be identified after the start of the irrigation (2015): the only candidate was 2017, with significant summer precipitations. However, the low precipitations during the previous winter (2016-2017) resulted in an absence of recharge of the water table before the summer, making this year also prone to soil drought.

I chose 2016 and 2018 as the dry years, because of strong summer droughts starting in July (**Fig. III. 4.3**). 2015 was discarded as a potential candidate year, although it was also a very dry year, because it was the year when the irrigation treatment was first set up. I was interested in keeping two dry years (2016 and 2018) to test the potential effect of canopy closure with time and domination of birch trees by pine trees under drought conditions.

#### 4.2.3. Carbon isotope composition ( $\delta^{13}\text{C}$ )

##### 4.2.3.1. Wood Sample Preparation

I identified the targeted years (2013, 2016 and 2018) in the tree cores with the ring width measurements from the WinDENDRO™ scans, but also with the information from a stereomicroscope for birch that had less defined tree-ring limits (diffuse porous species). For birch micro-cores, I only used the visual identification under the stereomicroscope. Using a scalpel under the stereomicroscope, I cut out the selected years for each tree core, which resulted in a total of 408 samples (136 trees  $\times$  3 years). I then carefully cut out the last third of each tree ring (latewood) with a scalpel and retained this part only for analyses. This ensured that the carbon isotope signature I obtained for each sample and each given year characterized the functioning of the selected trees during the second part of the growing season, i.e., under high soil water conditions during the wet years and under limited soil water conditions during the dry years. Indeed, due to the potential remobilization of photosynthates from the last growing season when wood is produced in the spring, analyzing the  $\delta^{13}\text{C}$  (‰) of bulk wood may lead to potential

misinterpretation of the effect of tree species diversity during the dry year. I used whole wood tissue for the analyses as it shows the same trends as cellulose-only isotopic measurements and allowed us to analyze a larger number of trees (McCarroll and Loader 2004).

Each wood sample was then ground into a fine powder, and I weighed around 1 mg of powder for each sample in tin capsules for the isotopic analysis. I used a different technique when the dry mass of the tree-ring sample was less than 6 mg (three samples out of 402) to avoid any loss of matter in the grinding process. Instead of being ground, these samples were cut into small pieces with a scalpel. A subsample of around 1 mg was then randomly selected among the pieces and put into the tin capsules. Finally, for the birch microcore samples, the extracted wood was directly weighed in the tin capsule. Using micro-cores instead of normal cores did not impact  $\delta^{13}\text{C}$  measurements:  $\delta^{13}\text{C}$  values were not significantly different between the two groups in 2013 ( $p=0.27$ ).

#### 4.2.3.2. Isotopic analysis

The  $^{13}\text{C}/^{12}\text{C}$  ratios of each wood sample were analyzed with an isotope-ratio mass spectrometer (IRMS, Isoprime 100, Isoprime Ltd., Cheadle Hulme, UK) coupled with an elemental analyzer (EA, Elementar vario, ISOTOPE cube, Elementar Analysen Systeme GmbH, Hanau, Germany) at the SILVATECH platform (Silvatech, INRAE, 2018. Structural and functional analysis of tree and wood Facility, Nancy, France). The results were expressed as carbon isotope composition  $\delta^{13}\text{C}$  (‰) relative to the international standard Vienna Pee Dee Bee Belemnite (V-PDB) (IAEA 1995) following Eq. 1:

$$\delta^{13}\text{C} (\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  represent the  $^{13}\text{C}/^{12}\text{C}$  ratios of the sample and the V-PDB standard, respectively (Farquhar et al. 1982). The mass spectrometer had a precision of  $\pm 0.2$  ‰ for  $\delta^{13}\text{C}$  values.

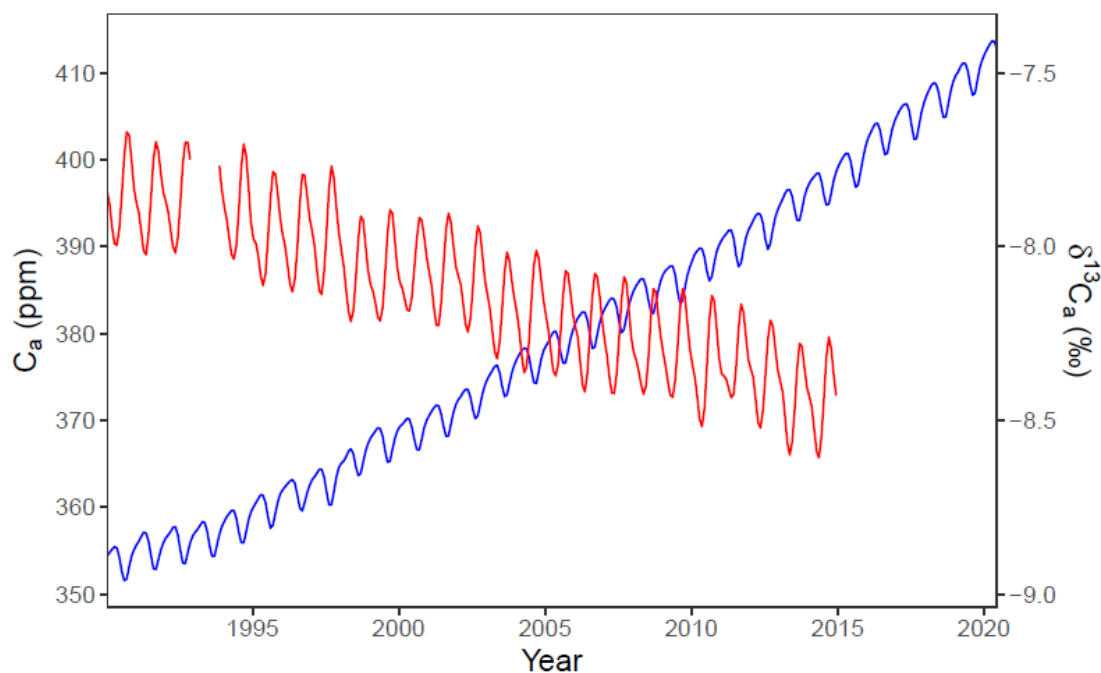
#### 4.2.3.3. Data processing

Out of the 136 trees sampled, 8 trees were removed from the data analyses because 2013 could not be sampled on their micro-cores; the collected core indeed did not go far enough within the trunk. Furthermore, 7 trees were removed from the analyses because I detected  $\delta^{13}\text{C}$  values that were unusually high or low for one of the three years sampled, indicating a possible error in the identification of the year during the cutting process or in the isotope analyses. As a result, the number of trees analyzed dropped down to 121, with three samples instead of 5 for birch in three plots (block 5 mixed, block 6 pure, block 6 mixed); all other plot and species combinations had at least five samples.

#### 4.2.3.4. Corrections on $\delta^{13}\text{C}$

Because of global industrialization and the release of burned fossil fuel (which is made from plant material that is depleted in  $^{13}\text{C}$ ) in the atmosphere, the isotopic composition of atmospheric  $\text{CO}_2$  ( $\delta^{13}\text{C}_a$ )

and the atmospheric CO<sub>2</sub> concentration ( $C_a$ ) are not constant.  $\delta^{13}C_a$  is getting more and more negative with time. On the other hand,  $C_a$  has been rising since 1850 (reference period), with an exponential increase since the 1950's. These yearly changes in  $\delta^{13}C_a$  and  $C_a$  impact the isotope signature of the CO<sub>2</sub> that is absorbed by plants during the photosynthesis and thus tree ring  $\delta^{13}C$ . When the objective of a study is to compare the  $\delta^{13}C$  of tree-ring of different years, it is then necessary to take this long-term change into account. I therefore corrected the  $\delta^{13}C$  values in 2016 and 2018 with the atmospheric conditions in 2013, taken as the reference year, in order to compare them without any bias.



**Fig. III. 4.4** Mauna Loa Observatory monthly average  $C_a$  and  $\delta^{13}C_a$  trends from 1990 to 2020.  $C_a$  (ppm, blue line) is mean annual atmospheric CO<sub>2</sub> concentration,  $\delta^{13}C_a$  (‰, red line) is the mean annual isotopic composition of atmosphere.  $C_a$  and  $\delta^{13}C_a$  were downloaded from the NOAA Mauna Loa Observatory database (<https://gml.noaa.gov/dv/data/index.php?site=MLO>).  $\delta^{13}C_a$  data for Mauna Loa after 2014 was not publicly available, I predicted  $\delta^{13}C_a$  values for 2016 and 2018 with a linear model between  $\delta^{13}C_a$  and the year with the available data.

I based the correcting method on the Farquhar model (Farquhar et al. 1982) and hypothesized that the response of  $C_i$  to  $C_a$  and  $\delta^{13}C_a$  is linear and independent of any other factor. **Eq. 2** and **Eq. 3** are used as starting points (Farquhar et al. 1982):

$$\Delta^{13}C = a + (b - a) \frac{C_i}{C_a}, \quad (2)$$

$$\Delta^{13}C = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + 0.001 \delta^{13}C_p}, \quad (3)$$

where  $\Delta^{13}\text{C}$  is the carbon isotope discrimination,  $C_a$  is the mean annual atmospheric  $\text{CO}_2$  concentration (ppm),  $C_i$  is the leaf internal  $\text{CO}_2$  concentration,  $a$  is the fractionation during  $\text{CO}_2$  diffusion ( $a = 4.4\%$ ),  $b$  is the fractionation during carboxylation ( $b = 27\%$ ),  $\delta^{13}\text{C}_a$  is the mean annual carbon isotope composition of the atmosphere and  $\delta^{13}\text{C}_p$  is the carbon isotope composition of plant tissue (here, tree-ring samples).

Then, combining **Eq. 2** and **Eq. 3**, I recalculated  $C_i$  for year  $y$  using the measured  $\delta^{13}\text{C}$  and this year  $y$ 's atmospheric conditions with **Eq. 4**:

$$C_{i_y} = \frac{C_{a_y}}{b - a} \left[ \frac{\delta^{13}\text{C}_{a_y} - \delta^{13}\text{C}_{p_y}}{1 + 0.001 \delta^{13}\text{C}_{p_y}} - a \right]. \quad (4)$$

Finally, combining **Eq. 2** and **Eq. 3**, I calculated a  $\delta^{13}\text{C}_{\text{cor}}$  for 2016 and 2018 using the recalculated  $C_i$  and the atmospheric conditions from 2013 with Eq. 5:

$$\delta^{13}\text{C}_{p_{y_{\text{cor}}}} = \frac{\delta^{13}\text{C}_{p_{2013}} - \left[ a + (b - a) \frac{C_{i_y}}{C_{a_{2013}}} \right]}{1 + 0.001 \left[ a + (b - a) \frac{C_{i_y}}{C_{a_{2013}}} \right]}. \quad (5)$$

$C_a$  and  $\delta^{13}\text{C}_a$  for 2013, 2016 and 2018 were downloaded from Mauna Loa Observatory database (<https://gml.noaa.gov/dv/data/?site=mlo>). The corrected values for 2016 and 2018 will be used in all the following analyses. As the years were corrected compared to 2013, the raw and corrected  $\delta^{13}\text{C}$  values are the same for 2013. Corrected  $\delta^{13}\text{C}$  values are hereafter referred to as  $\delta^{13}\text{C}_{\text{cor}}$ . The uncorrected data can be found in supplementary figures (**Fig. III.S 4.1**).

#### 4.2.3.5. Difference in $\delta^{13}\text{C}$ between the 2013 and 2016 or 2018 ( $\Delta\delta^{13}\text{C}$ )

In the non-irrigated blocks, for each tree, I calculated the difference in  $\delta^{13}\text{C}_{\text{cor}}$  between the dry years (2016, 2018) and the reference year (2013), as  $\Delta\delta^{13}\text{C} = \text{DRY } \delta^{13}\text{C}_{\text{cor}} - \text{REF } \delta^{13}\text{C}_{\text{cor}}$ . I named these differences  $\Delta\delta^{13}\text{C}_{2016}$  and  $\Delta\delta^{13}\text{C}_{2018}$ . This standardizes the values for each tree, in order to isolate variations in  $\delta^{13}\text{C}$  due to the response to changes in environmental conditions between 2013 and 2016 or 2018 from other sources of between-tree variation already present in 2013 (inter-tree genetic variability, block effects...). It is then usually assumed that the main factor that varies between the wet and dry years is the water-related stress (see Grossiord et al. 2014b; Forrester et al. 2016). If this is the case, a higher  $\Delta\delta^{13}\text{C}$ , and hence a greater increase in  $\delta^{13}\text{C}$  in the dry year, indicates a stronger stomatal response and thus a higher level of drought stress; a  $\Delta\delta^{13}\text{C}$  of zero would indicate that there was no change in  $\delta^{13}\text{C}$  in response to drought and hence no drought-induced stress. However, in our case, because this is a young plantation with a fast-growing dynamic, we cannot rule out that  $\delta^{13}\text{C}$  variations may also be due to changes in light availability for the trees with time, in association with the expected slow canopy closure occurring in such a young plantation.



In the irrigated blocks, for each tree, I calculated the difference in  $\delta^{13}\text{C}$  between the irrigated years (2016, 2018) and the reference year (2013), as  $\Delta\delta^{13}\text{C}_{\text{irr}} = \text{IRR } \delta^{13}\text{C}_{\text{cor}} - \text{REF } \delta^{13}\text{C}_{\text{cor}}$ . I named these differences  $\Delta\delta^{13}\text{C}_{\text{irr } 2016}$  and  $\Delta\delta^{13}\text{C}_{\text{irr } 2018}$ . I expected the  $\Delta\delta^{13}\text{C}_{\text{irr}}$  values to be either slightly negative (i.e., a decrease in water use efficiency compared to 2013) or neutral, as 2013 was a wet year. Strong negative values would indicate changes due to a lower light availability compared to 2013.

#### 4.2.4. Statistical analysis

I used the following mixed model to test for *Species*, *Irrigation* and *Mixture* effects on  $\delta^{13}\text{C}$  for the reference year (2013):

$$\delta^{13}\text{C}_{2013} \sim \text{Species} + \text{Irrigation} + \text{Mixture} + \text{Species:Irrigation} + \text{Species:Mixture} + \text{Irrigation:Mixture} + \text{Species:Irrigation:Mixture} + 1|\text{Block} \quad (6)$$

where *Species* is a fixed factor with two levels (birch and pine), *Irrigation* is a fixed factor with two levels (non-irrigated and irrigated), *Mixture* is a fixed factor with two levels (pure vs. mixed) and *Block* is a random factor with six levels. The result from the mixed model is reported in **Table III. 4.1**. I used post-hoc multiple comparison tests to identify which pairs of treatment combinations are statistically different from each other.

I used mixed models to test *Year* and *Mixture* effects on  $\delta^{13}\text{C}_{\text{cor}}$  for each irrigation treatment and each species separately (4 separate models) with *Block* as a random factor. The models were written as:

$$\delta^{13}\text{C}_{\text{cor}} \sim \text{Year} + \text{Mixture} + \text{Year:Mixture} + 1|\text{Block} \quad (7)$$

where *Year* is a fixed factor with three levels (2013, 2016 and 2018), *Mixture* is a fixed factor with two levels (pure vs. mixed) and *Block* is a random factor with three levels for each model. The result from the mixed models is reported in **Table III. 4.2**. The results of post-hoc multiple comparison tests between years for 2016-2013 and 2018-2013 as well as mixture effects is reported in **Fig. III. 4.7**.

R 3.6.2 language programming (R Core Team 2019) with the Rstudio interface was used for all the analyses with the following packages: nlme (“lme” function), MuMIn and emmeans (“lsmeans” function).

## 4.3. Results

*Reminder: Corrected values in 2016 and 2018 ( $\delta^{13}C_{cor}$ ) were used in all the following analyses. As the years were corrected compared to 2013, raw and corrected  $\delta^{13}C$  values are the same for 2013.*

### 4.3.1. Differences in $\delta^{13}C$ in 2013

In the global statistical model for 2013 (**Eq. 6**), there was a significant difference in  $\delta^{13}C$  between species ( $p < 0.001$ , **Table III. 4.1**), with an average of  $-26.8 \pm 0.4$  ‰ (average  $\pm$  standard deviation) for birch and  $-27.9 \pm 0.6$  ‰ for pine in pure plots (**Fig. III. 4.5**, **Fig. III. 4.6**). As expected, there was no irrigation effect (as irrigation started in 2015) ( $p = 0.67$ , **Table III. 4.1**). In pure plots, the non-irrigated and irrigated blocks were not different for either species (post-hoc test for birch:  $p = 0.68$ , and pine:  $p = 0.60$ ). Finally, there were no significant mixture effects in 2013 for either species (non-irrigated birch:  $p = 0.12$ , non-irrigated pine:  $p = 0.11$ , irrigated birch:  $p = 0.78$ , irrigated pine:  $p = 0.51$ ).

### 4.3.2. Response to drought in non-irrigated blocks

#### 4.3.2.1. Response to drought in pure plots

Contrary to our expectations, birch did not have significantly positive  $\Delta\delta^{13}C_{2016}$  (post-hoc test for the difference between  $\delta^{13}C_{cor}$  in 2013 and 2016 in pure plots:  $p = 0.98$ ) and  $\Delta\delta^{13}C_{2018}$  ( $p = 0.69$ ) in pure plots (**Fig. III. 4.7a**, **Table III. 4.2**). On the contrary, for pine, there was a significant  $\delta^{13}C_{cor}$  difference among years (**Table III. 4.2**):  $\Delta\delta^{13}C_{2016}$  ( $p < 0.01$ ) and  $\Delta\delta^{13}C_{2018}$  ( $p < 0.001$ ) were both positive in pure plots. The increase in  $\delta^{13}C_{cor}$  compared to 2013 was on average of  $+0.6$  ‰ in 2016 and  $+0.9$  ‰ in 2018 (**Fig. III. 4.7a**).

There were no significant differences in  $\delta^{13}C_{cor}$  between the year 2016 and 2018 for birch ( $p = 0.58$ ) nor for pine ( $p = 0.22$ ).

#### 4.3.2.2. Mixture effects on $\Delta\delta^{13}C$ across the dry years

For birch, there was no mixture effect on  $\delta^{13}C_{cor}$  in 2016 (post-hoc test for mixture effect:  $p = 0.76$ ), but a significant mixture effect in 2018 ( $p = 0.02$ ) with a significantly negative  $\Delta\delta^{13}C_{2018}$  ( $-0.4$  ‰) in the mixed plots (**Fig. III. 4.7a**, **Table III. 4.2**). For pine, in mixed plots,  $\Delta\delta^{13}C_{2016}$  and  $\Delta\delta^{13}C_{2018}$  were both positive with values close to the ones in pure plots. Indeed, there was no significant mixture effect on  $\delta^{13}C_{cor}$  for 2016 ( $p = 0.12$ ) or 2018 ( $p = 0.09$ ).

### 4.3.3. Response to irrigation in irrigated blocks

#### 4.3.3.1. Irrigation effects in pure plots

Surprisingly, birch had highly significantly negative  $\Delta\delta^{13}C_{irr2016}$  (post-hoc test for the difference between  $\delta^{13}C_{cor}$  in 2013 and 2016 in pure plots:  $p < 0.01$ ) and  $\Delta\delta^{13}C_{irr2018}$  ( $p < 0.01$ ) in pure plots (**Fig. III. 4.7b**,

**Fig. III. 4.8b**), indicating a strong change in  $\delta^{13}\text{C}_{\text{cor}}$  in the irrigated blocks in 2016 and 2018 compared to 2013 (**Table III. 4.2**). The decrease in  $\delta^{13}\text{C}_{\text{cor}}$  compared to 2013 in pure plots was on average  $-0.7\text{‰}$  in 2016 and 2018 (**Fig. III. 4.7**). There was no significant difference in  $\delta^{13}\text{C}_{\text{cor}}$  between 2016 and 2018 in birch pure plots ( $p=0.99$ ).

For pine,  $\Delta\delta^{13}\text{C}_{\text{irr } 2016}$  ( $p=0.28$ ) and  $\Delta\delta^{13}\text{C}_{\text{irr } 2018}$  ( $p=0.43$ ) was on average not significantly different from zero in pure plots (**Fig. III. 4.7b**), but results were surprisingly variable across blocks with  $\Delta\delta^{13}\text{C}_{\text{irr}}$  sometimes negative and neutral for both  $\Delta\delta^{13}\text{C}_{\text{irr } 2016}$  and  $\Delta\delta^{13}\text{C}_{\text{irr } 2018}$ , and also sometimes positive for  $\Delta\delta^{13}\text{C}_{\text{irr } 2018}$  (**Fig. III. 4.8b**). In the irrigated blocks, the variability in  $\delta^{13}\text{C}_{\text{cor}}$ , and therefore in  $\Delta\delta^{13}\text{C}_{\text{irr}}$  among blocks, was high (**Fig. III. 4.6b**, **Fig. III. 4.8b**). The results for block 4 especially stood out compared to blocks 2 and 5. However, removing block 4 from the mixed model did not change the outcome of the global statistical tests. There was a significant difference in  $\delta^{13}\text{C}_{\text{cor}}$  between 2016 and 2018 for the irrigated pure pine plots only ( $p=0.02$ ):  $\delta^{13}\text{C}_{\text{cor}}$  was less negative in 2018 than in 2016 (**Fig. III. 4.7**).

#### 4.3.3.2. Mixture effects on $\Delta\delta^{13}\text{C}$

For birch, there were significant mixture effects on  $\delta^{13}\text{C}_{\text{cor}}$  in the irrigated blocks for both 2016 ( $p<0.001$ ) and 2018 ( $p<0.001$ ).  $\Delta\delta^{13}\text{C}_{\text{irr } 2016}$  and  $\Delta\delta^{13}\text{C}_{\text{irr } 2018}$  were more negative in the mixed plots than in the pure plots, indicating a stronger decrease in  $\delta^{13}\text{C}_{\text{cor}}$  values in the irrigated blocks compared to 2013 in mixed plots than in pure ones (**Fig. III. 4.7b**, **Fig. III. 4.8b**). The decrease in  $\delta^{13}\text{C}_{\text{cor}}$  compared to 2013 in mixed plots was on average  $-1.4\text{‰}$  in 2016 and  $-1.6\text{‰}$  in 2018, which meant that compared to pure plots there was a decrease in  $\delta^{13}\text{C}_{\text{cor}}$  of  $-0.8\text{‰}$  in 2016 and  $-1.0\text{‰}$  in 2018 (**Fig. III. 4.7b**).

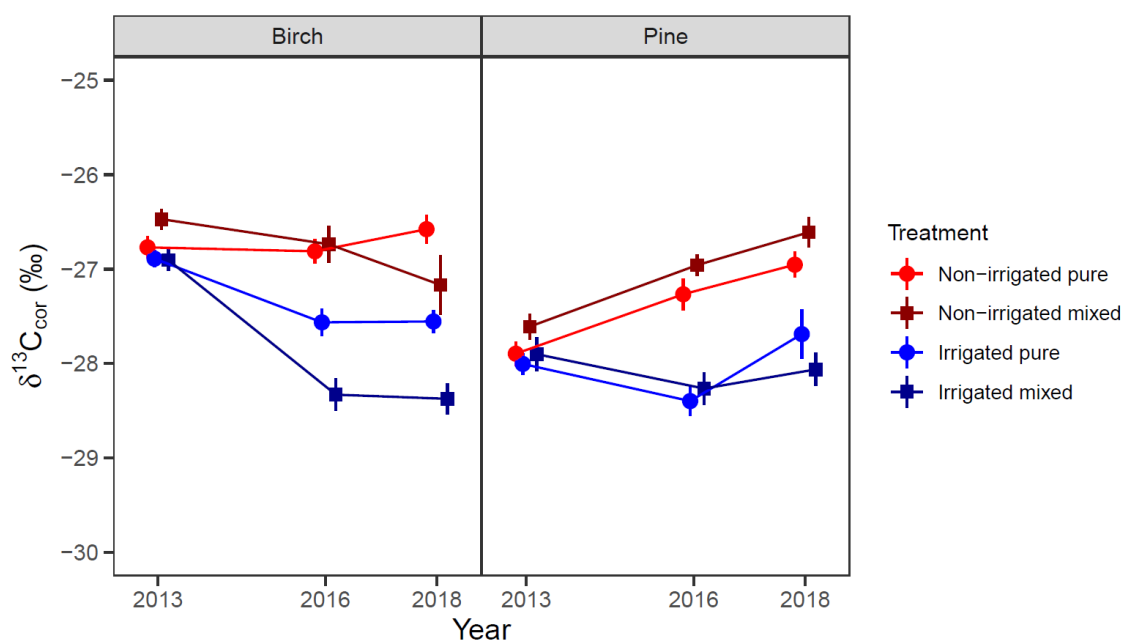
For pine, there were no mixture effects on  $\Delta\delta^{13}\text{C}$  in the irrigated blocks (**Fig. III. 4.7b**).  $\delta^{13}\text{C}_{\text{cor}}$  was not significantly different between mixed and pure plots in 2016 ( $p=0.59$ ) as well as in 2018 ( $p=0.14$ ).

**Table III. 4.1** Mixed model results for *Species*, *Irrigation* and *Mixture* effects and their interactions on  $\delta^{13}\text{C}_{\text{cor}}$  for the year 2013. Block identity was used as a random factor in the model.

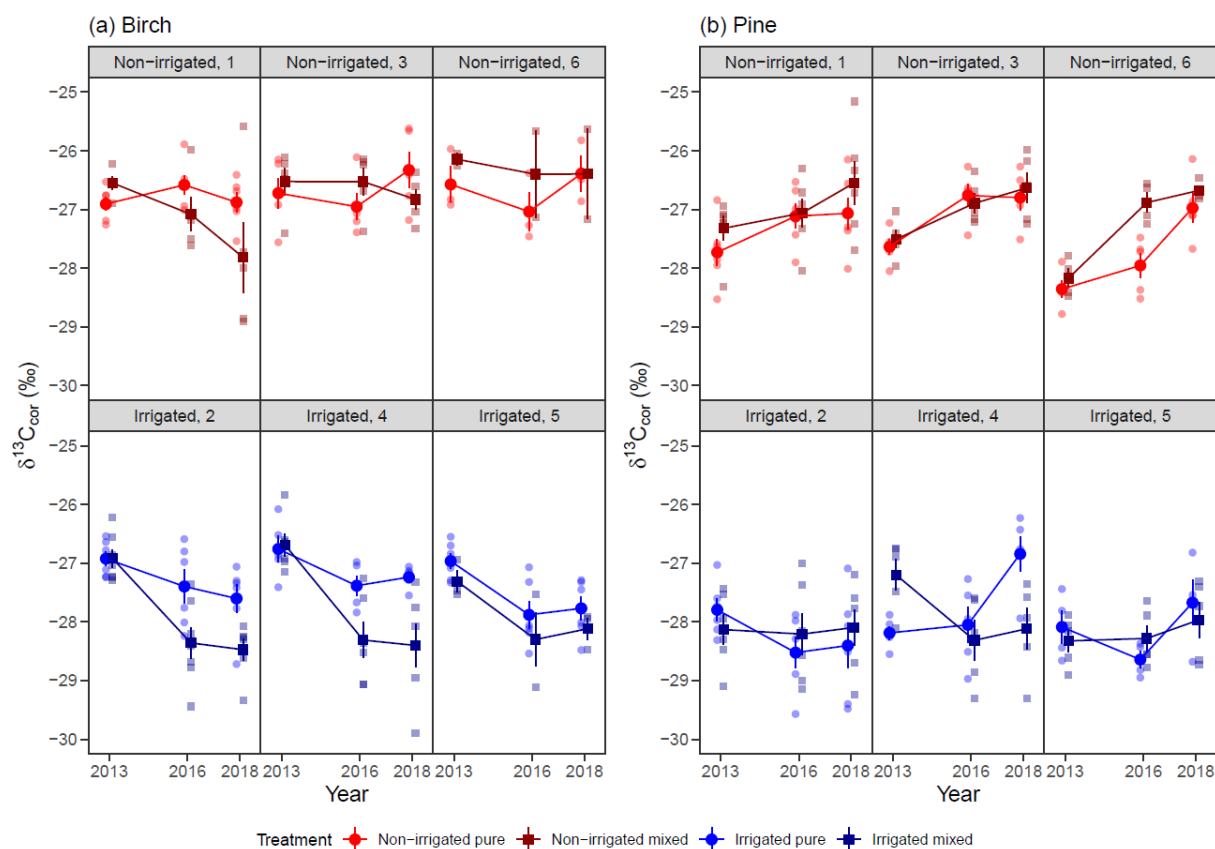
Predictor	p-values
Species (S)	<b>&lt;0.001</b>
Irrigation (I)	0.67
Mixture (M)	0.12
S x I	0.93
S x M	0.97
I x M	0.18
S x I x M	0.63
$R^2_m (R^2_c)$	0.58 (0.60)

**Table III. 4.2** Mixed model results for the four separate models for *Year* and *Mixture* effects and their interaction on  $\delta^{13}\text{C}_{\text{cor}}$  for all three years. Block identity was used as a random factor in the models.

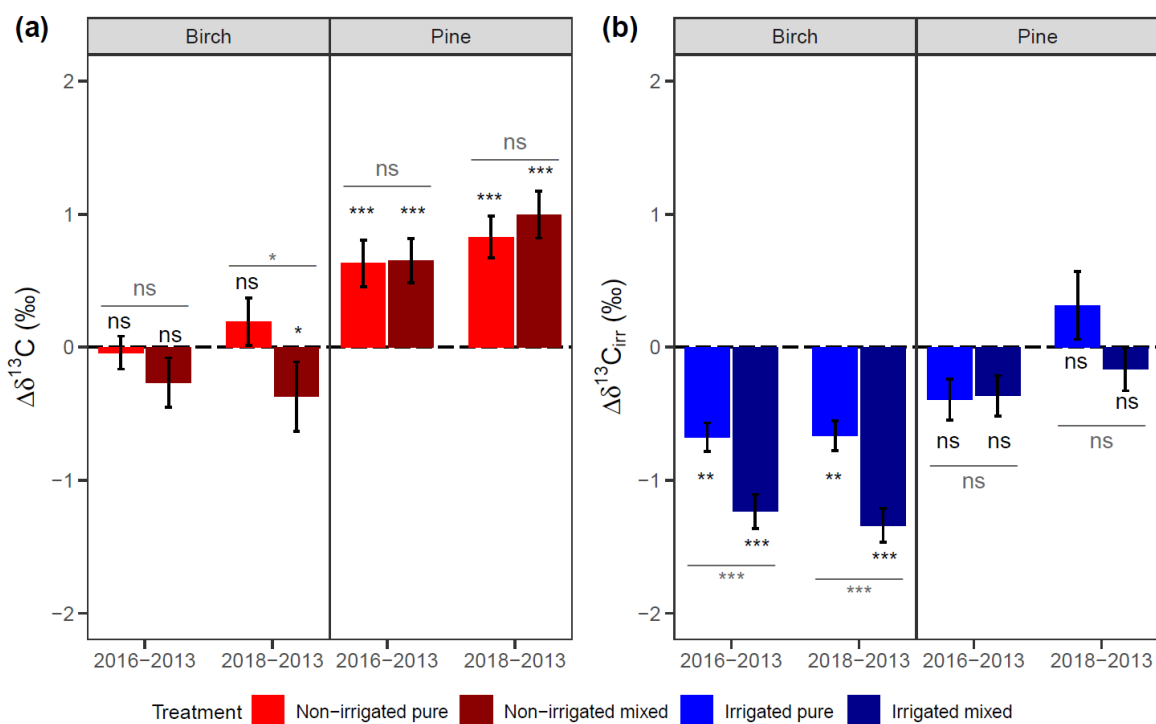
	Non-irrigated		Irrigated	
	Birch	Pine	Birch	Pine
Year	0.57	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.01</b>
Mixture	0.23	0.15	0.90	0.67
Year x Mixture	<b>0.03</b>	0.98	<b>&lt;0.01</b>	0.28
$R^2_m (R^2_c)$	0.11 (0.15)	0.39 (0.46)	0.55 (0.56)	0.10 (0.14)



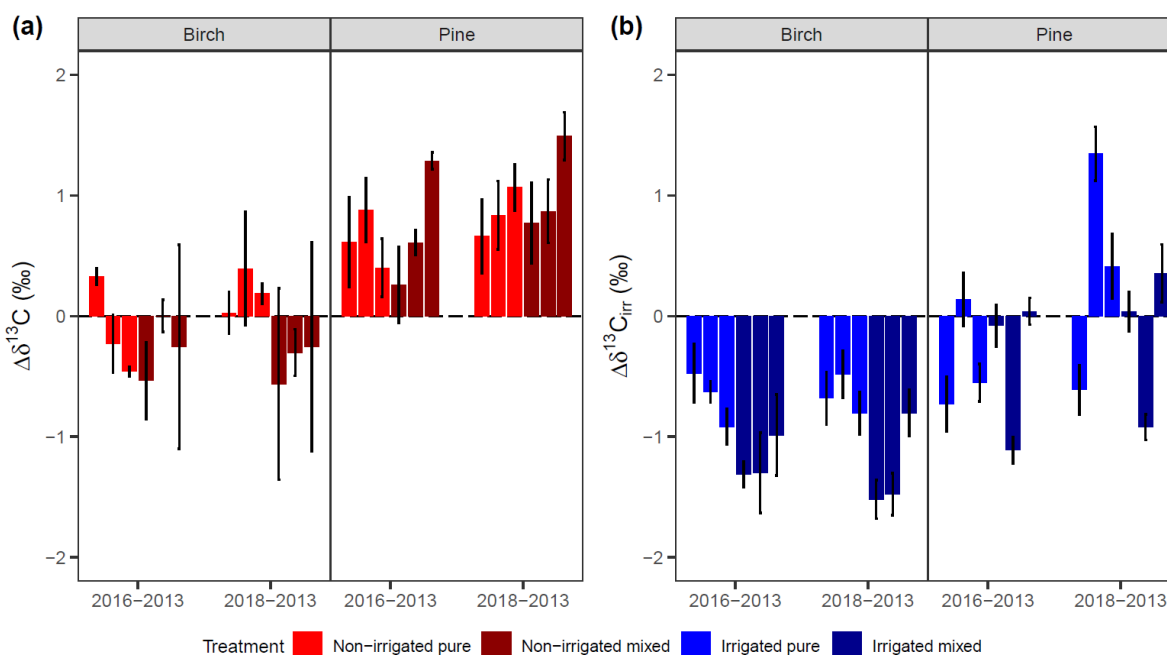
**Fig. III. 4.5** Corrected  $\delta^{13}\text{C}_{\text{cor}}$  values ( $\delta^{13}\text{C}_{\text{cor}}$ ) averaged by species and treatment for all blocks. Symbols represent the average for all blocks, and the error bars represent the standard error of the mean.



**Fig. III. 4.6**  $\delta^{13}\text{C}_{\text{cor}}$  averaged by species and treatment for each block separately. Small points in the background represent individual tree values. Large symbols represent the average, and the error bars represent the standard error of the mean.



**Fig. III. 4.7**  $\Delta\delta^{13}\text{C}$  (a) and  $\Delta\delta^{13}\text{C}_{\text{irr}}$  (b) averaged by species and treatment for each block separately. Error bars represent the standard error of the mean. Statistics represent the post-hoc results of mixed models on  $\delta^{13}\text{C}_{\text{cor}}$  data (one for each irrigation and species combination). Black stars above each bar represent the *Year* effect compared to 2013, stars with horizontal lines indicate the results of *Mixture* effects. Significance at a level of 5 % are indicated by stars (\* p-values < 0.05; \*\* p-values < 0.01; \*\*\* p-values < 0.001; ns no significant effect).



**Fig. III. 4.8**  $\Delta\delta^{13}\text{C}$  (a) and  $\Delta\delta^{13}\text{C}_{\text{irr}}$  (b) averaged by species and treatment for each block separately. Error bars represent the standard error of the mean.

## 4.4. Discussion

### 4.4.1. Initial differences in $\delta^{13}\text{C}$ in 2013

#### 4.4.1.1. Species differences in $\delta^{13}\text{C}$

##### *Comparison of $\delta^{13}\text{C}$ by species with other published data*

In 2013, the average  $\delta^{13}\text{C}$  was  $-26.8 \pm 0.4$  ‰ for birch and  $-27.9 \pm 0.6$  ‰ for pine in pure plots (**Fig. III. 4.5**). The average for birch was similar to what Grossiord et al. (2014c) found for this species for a wet year in pure plots of a boreal forest ( $-27.1 \pm 0.3$  ‰).

For pine, however, the values were more negative than what can be found in the literature for this species. For example, Camarero et al. (2018) found a value of about  $-25.0$  ‰ for 50-years-old trees during the period 2010-2015 in southern Spain (dry site). Fernandez et al. (2005) found an average of  $-26.6$  ‰ over the period 1999-2003 for trees from a wide range of ages (10 to 45 years old) in northwest Spain. Finally, De Micco et al. (2007) found an average of  $-26.5$  ‰ for wood formed during the summer in 1999 for 55-years-old trees in a sandy site and coastal climate in Italy. I therefore found more negative values for maritime pine at our site compared to the literature. Yet, one must always be cautious when comparing such raw data among studies conducted across different environmental conditions. The differences between the values in this study and those from the literature might be explained by the fact that I am comparing  $\delta^{13}\text{C}$  measured during a wet year and with a water table that remained shallow in 2013, but also in a generally wetter climate than in the studies previously cited. I did not find any published studies with  $\delta^{13}\text{C}$  values for maritime pine that were recorded during wet years. The cited studies were conducted either under a much drier climate (in Spain), or during drier years (in Italy) than in my study. It is well-known that trees adapt to drier conditions by closing stomata and increasing WUE, which results in less negative  $\delta^{13}\text{C}$  (Farquhar et al. 1989). Conversely, a wetter climate, similarly to a wet year, then results in more negative  $\delta^{13}\text{C}$  for the same species (e.g., González de Andrés et al. 2018). A study looking at genetic variations in maritime pine  $\delta^{13}\text{C}$  in the same region and therefore the same climate, also found values that were less negative (on average  $-26.1$  ‰) than our values in tree-rings of 12 years-old trees (Marguerit et al. 2014). Therefore, I can hypothesize that the more  $\delta^{13}\text{C}$  negative values observed here can be explained mostly by the local climate (wet year).

##### *Comparison of $\delta^{13}\text{C}$ between species*

The higher values for birch than pine points to a higher water use efficiency for birch, which is surprising as coniferous species are usually expected to have a higher  $\delta^{13}\text{C}$  (i.e., a higher WUE) compared to broadleaved species (Garten and Taylor 1992; Heaton 1999). For example, higher values were found for Scots pine (*Pinus sylvestris*) than for beech (*Fagus sylvatica*) (Daux et al. 2018; de Streel et al. 2020). Differences in  $\delta^{13}\text{C}$  values among species can be explained by physiological and morphological characteristics (McCarroll and Loader, 2004) or by differences in canopy structure and light availability

in pure stands. However, this difference between broadleaved and coniferous species might not always be consistent because variation between species can also depend on site water status (i.e., interactions between species and site effects). For example, Garten and Taylor (1992) only found significant differences between pine species and broadleaved species at a dry site and none at a humid site. I found a similar species effect trend when I measured foliar  $\delta^{13}\text{C}$  in leaves and needles in formed in spring 2019, so at least I know that this trend was not due to any measurement errors in wood  $\delta^{13}\text{C}$  (**Fig. III.S 4.2**). Although the direction of the difference is not what was expected, the magnitude of the mean difference in  $\delta^{13}\text{C}$  between the two studied species (1.2 ‰) is within the range of the one found in the literature between tree species (i.e., Daux et al. 2018); indeed a difference up to 3.0 ‰ between coniferous and broadleaved tree species has been found (Heaton 1999). These differences are consistent with previous studies that have shown a wide range of  $\delta^{13}\text{C}$  among temperate tree species (Heaton 1999; Ponton et al. 2001).

#### 4.4.1.2. Mixture effects on $\delta^{13}\text{C}$ in 2013

In accordance with our hypothesis, there were no mixture effects on  $\delta^{13}\text{C}$  for either species in 2013 (**Fig. III. 4.5**). This absence of mixture effects at that time can be explained by (i) the wet climatic conditions of 2013, and (ii) the early stage of the plantation (trees were 5 years old). Potential mixture effects that would be the result of complementarity effects on water acquisition and use are not expected to be observed in the absence of water stress, i.e., when the resource is non-limiting (Grossiord et al. 2013a; Forrester 2014). The year 2013 was characterized by wet conditions without drought stress, which is why I chose it as the reference year to analyze the drought effect. On the other hand, there could have been mixture effects on  $\delta^{13}\text{C}$  in 2013 due to differences in light availability and canopy structure between pure and mixed plots. However, in 2013, both species had similar mean tree heights in mixed plots (**Fig. III.S 4.3**) and the crowns of the different trees were not yet touching each other, thus making differences in light availability for individual trees between pure and mixed plots unlikely. Then, because the plantation was still young in 2013, potential complementarity effects between trees (both belowground and aboveground) might not yet be expressed as the roots or the branches of these trees were not yet directly interacting. Recently published results on mixture effects on productivity at ORPHEE support this hypothesis. Indeed, Toïgo et al. (2021a) found that mixture effects on growth were not yet significant in 2014 at ORPHEE, although a trend was starting to appear, with a lower stand volume increment (SVI) for birch in mixtures and a higher one for pine in mixtures as compared to monocultures. In addition, Morin et al. (2020) simulated potential mixture effects in this plantation with a forest gap model and predicted the existence of a potential overyielding in these mixed plots only after 7 or 8 years of growth. After this period, they predicted an overall overyielding in mixed plots, resulting from a small underyielding for birch and a strong overyielding for pine. All these information put together, I may therefore expect mixture effects to appear after 2015, and then potentially for  $\delta^{13}\text{C}$  in 2016 and 2018.



## 4.4.2. Drought response in non-irrigated blocks

### 4.4.2.1. Drought response in pure plots

#### *Birch trees*

Surprisingly, birch trees did not show an increase in  $\delta^{13}\text{C}_{\text{cor}}$  between 2013 and 2016 or 2018 (**Fig. III. 4.7a**). I clearly did not expect this result (see Hypotheses **Fig. III. 4.1**, **Fig. III. 4.2**), as birch is known to show a strong response to drought. For instance, pure birch plots showed an increase in  $\delta^{13}\text{C}$  between a wet and a dry year of +1.5 ‰ in a boreal forest and of +1.0 ‰ in a hemiboreal forest (Grossiord et al. 2014c; Forrester et al. 2016; Jucker et al. 2017). Therefore, there is no reason to believe that birch functioning did not acclimate to the much drier conditions in 2016 and 2018 as compared to 2013 and that an increase in WUE did not occur: I formulate several hypotheses to explain this pattern.

First, there is no reason to consider a methodological or technical issue to explain this pattern. Similar care was taken to select the rings of the different years. Isotope analyses of the two species and the three years were conducted in a random way and a problem with the mass spectrometer cannot then be the cause.

Second, the lack of an increase in  $\delta^{13}\text{C}_{\text{cor}}$  between a wet (2013) and the dry years (2016 and 2018) could be the result of a strong effect of canopy closure on birch  $\delta^{13}\text{C}$  from 2013 to 2016 and 2018, acting in the opposite direction of the effect of drought on  $\delta^{13}\text{C}_{\text{cor}}$ . Indeed, lower light levels and interception are known to induce a decrease in  $\delta^{13}\text{C}_{\text{cor}}$  (more negative values) (Warren et al. 2001), whereas drought effect induce less negative  $\delta^{13}\text{C}_{\text{cor}}$ . However, this hypothesis cannot be the major driver of this pattern for birch in the pure plots, as the leaf area index (LAI) in these pure plots in 2019 was still low (2.0 m<sup>2</sup> m<sup>-2</sup> on average) (see **Fig. III.S 3.2**). Such a low value clearly still represents an open canopy, which cannot explain a change in  $\delta^{13}\text{C}_{\text{cor}}$  of around 1-2 ‰ that would be related to important shade effects.

Third, the lack of response of birch  $\delta^{13}\text{C}_{\text{cor}}$  to drought in pure plots could be explained by a drought avoidance strategy of birch at this site. Indeed, we noticed that birch trees started having yellow leaves as early as June 21<sup>st</sup> in 2018 and had a strong leaf fall between July 13<sup>th</sup> and 30<sup>th</sup> 2018 (field observations from monitoring photographs that were taken bi-weekly at the location of the PAR sensors: data not shown; see also **Appendix A, p. 321** and **Appendix B, p. 327** for 2018, no data for 2016). This early leaf fall was a response to strong drought conditions (Bréda et al. 2006) and induced a strong decrease in canopy-level photosynthesis, and thus carbohydrate production and tree-ring formation for the rest of the growing season. Therefore, I hypothesize that the last third of the tree-ring that I used for  $\delta^{13}\text{C}_{\text{cor}}$  analyses might be constituted mostly of wood formed before any leaf fall and any decrease in photosynthesis, i.e., before a potential effect of limiting soil water conditions on birch gas exchange (photosynthesis and transpiration). Would this hypothesis be true, then this would result in a reduction or absence of new ring cells formed when the WUE of these trees increased because of the drought conditions, and therefore in the absence of any detection of a response of birch to drought with tree-ring

$\delta^{13}\text{C}_{\text{cor}}$  values. I was not able to confirm this hypothesis, but it seems to be to me the most probable origin of the  $\delta^{13}\text{C}_{\text{cor}}$  pattern observed for birch in pure plots.

#### *Pine trees*

As expected, pine trees showed significantly higher  $\delta^{13}\text{C}_{\text{cor}}$  in 2016 and 2018 compared to 2013, with an average increase in  $\delta^{13}\text{C}_{\text{cor}}$  of +0.6 ‰ in 2016 and +0.9 ‰ in 2018 (**Fig. III. 4.7a**). Yet, the results were not significantly different between 2016 and 2018. This increase in  $\delta^{13}\text{C}_{\text{cor}}$  was in the same range as other studies reporting the response to drought in different temperate and Mediterranean forest types (Grossiord et al. 2014d; Forrester et al. 2016; Bonal et al. 2017; Jacobs et al. 2021). As mentioned before, in general, an increase in  $\delta^{13}\text{C}$  can either be the result of a response to a lower water availability or to a higher light availability compared to the reference year. The change in  $C_a$  and  $\delta^{13}\text{C}_a$  with time (see **section 4.2.3.4**) cannot explain this trend as I here analyzed the corrected data, not the raw  $\delta^{13}\text{C}$  ones. In our case, it is unlikely that the increase in  $\delta^{13}\text{C}_{\text{cor}}$  was due to a higher exposition to light as all pine trees have been growing in height and crown volume since 2013 and the canopy in these plots was then more closed in 2016 and 2018 compared to 2013. It is therefore more likely that the increase in  $\delta^{13}\text{C}_{\text{cor}}$  for pine trees between 2013 and 2016 or 2018 was, as expected, a functional response to the limiting soil water conditions in 2016 and 2018. Nevertheless, a canopy closure effect, which would induce more negative  $\delta^{13}\text{C}_{\text{cor}}$  values, cannot be ruled out. If this effect exists, then it would minimize the drought effects we detected. In any case, pine clearly responded to an increase in drought exposure during the summers of 2016 and 2018 with less negative  $\delta^{13}\text{C}_{\text{cor}}$  (i.e., a lower discrimination towards  $^{13}\text{C}$ ). This result is consistent with a long-list of previous literature (e.g., Saurer et al. 1995) and shows that in dry years, the decrease in photosynthesis is less than the decrease in stomatal conductance, resulting in higher water use efficiency (Farquhar et al. 1989).

#### *4.4.2.2. Mixture effects on drought response*

The main objective of this study was to test for interacting effects of drought and species mixture on birch and pine  $\delta^{13}\text{C}_{\text{cor}}$  in the non-irrigated blocks.

#### *Birch trees*

For birch, there was no significant difference between pure and mixed plots in 2016, whereas in 2018,  $\delta^{13}\text{C}_{\text{cor}}$  was more negative in the mixed plots than in the pure ones (**Fig. III. 4.7a**). As a result,  $\Delta\delta^{13}\text{C}_{2018}$  was significantly negative for birch in mixed plots. In different temperate, drought-prone forest types, Grossiord et al. (2014c, d) found that  $\Delta\delta^{13}\text{C}$  was smaller in stands with a higher species richness than in monocultures, suggesting that species interactions in these forests diminished the drought exposure of the ecosystem. However, in these studies,  $\Delta\delta^{13}\text{C}$  remained positive, which is consistent with expectations based on the direct effect of drought on  $\delta^{13}\text{C}_{\text{cor}}$  (Saurer et al. 1995). Our results for birch thus differ from these previous ones. A more negative  $\delta^{13}\text{C}_{\text{cor}}$  in mixed plots can be the result of a lower drought exposure in mixed plots as compared to pure ones (Saurer et al. 1995). However, it can also be

the result of a lower light interception and availability of leaf or needles (Warren et al. 2001; Ponton et al. 2002) compared to the pure plots. In our study, because the mixture effect was significant only in 2018, and not in 2016, while these years show similar drought conditions, it is likely that the difference in  $\delta^{13}\text{C}_{\text{cor}}$  between pure and mixed plots is the consequence of a lower light availability for birch in mixed plots. This could be the consequence of the fact that birch trees have been progressively dominated by pine since 2014 in mixed plots (**Fig. III.S 4.3**). This was potentially not the case yet in 2016. Indeed, I found that Hegyi's competition index was higher in mixed plots than in pure plots for birch in 2016 and 2018, but also that the  $\delta^{13}\text{C}_{\text{cor}}$  of birch trees was better correlated to the competition index in 2018 than in 2016 (**Fig. III.S 4.4**). In addition to being responsible for this mixture effect, the lower light availability for birch in mixed plots in 2016 and 2018 might also have contributed to minimizing the detection of a drought effect in these plots.

#### *Pine trees*

In the case of pine, for which I had observed a clear response to drought in pure plots, there was no significant difference in  $\Delta\delta^{13}\text{C}$  between pure and mixed plots (**Fig. III. 4.7a**). Pine trees were therefore not less or more exposed to drought than in pure plots. I cannot compare mixture effects on maritime pine with other studies as there are none to my knowledge. However, Scots pine (*Pinus sylvestris*) has been shown to have a higher drought exposure in mixed stands with birch in a boreal forest, and did not show any trend with increasing species richness in a hemiboreal forest (Grossiord et al. 2014c; Forrester et al. 2016). Bonal et al. (2017) also found an absence of mixture effect on Scots pine when mixed with sessile oak (*Quercus petraea*). Finally, across a wide range of climatic conditions in Europe, Scots pine mixed with beech (*Fagus sylvatica*) was sometimes more, sometimes less, and sometimes similarly exposed to drought in mixed stands than in pure stands depending on site climatic conditions (de Streel et al. 2020). Therefore, at least for Scots pine, it is not surprising to observe an absence of mixture effect on drought exposure.

#### *Ecological implications*

In conclusion, contrary to our hypothesis, there was no evidence that mixing these two species significantly altered their functional response to drought, even under severe soil drought conditions. Our results are consistent with the increasing body of literature indicating that a higher tree species diversity in temperate forests does not systematically improve tree and forest ecosystem resistance to drought (Grossiord et al. 2014c; Bonal et al. 2017; Schwarz and Bauhus 2019).

This absence of mixture effect suggested that there was no complementarity for water use between the two species in mixed plots under drought conditions. Even though these two species strongly differ in ecological and functional characteristics (phenology, root systems, growth patterns...), species interactions did not modify species response to drought, or perhaps light-related factors that would influence tree functioning counterbalanced them. In our case, even though the studied species had a

potential for different rooting patterns, these were probably not expressed due to strong soil constraints. Indeed, the presence of a shallow water table in winter and a shallow hardpan layer (~50 cm) probably limited root development towards deeper soil layers (Altınalmazis-Kondylis et al. 2020) (see **Chapter III.5, p. 229**).

Even though there were no beneficial mixture effects, it can be noted that the response to drought of the two species in our study was not worse in mixed plots than in pure ones. An absence of negative effects (either positive or neutral effects) has been observed in many studies when looking at mixture effects on drought exposure (Grossiord et al. 2014d; Toïgo et al. 2015b; Bonal et al. 2017), although other studies did find negative mixture effects for at least one of the studied species (Grossiord et al. 2014c; González de Andrés et al. 2018). In the case of maritime pine and silver birch, mixing the two species can be beneficial for other ecosystem services (Castagneyrol et al. 2019). My results bring new insights into management strategies of these two species that are useful for forest managers: mixed stand will not result in a higher drought exposure for either species.

### 4.4.3. Acclimation to irrigation in the irrigated blocks

#### 4.4.3.1. Irrigation effects in pure plots

I expected that a constant irrigation input during the summer since 2015 should result in more negative  $\delta^{13}\text{C}_{\text{cor}}$  values in 2016 and 2018 compared to 2013, because of the potential morphological and functional acclimation of trees to the absence of water stress during the summer in the irrigated blocks. However, as 2013 was a relatively wet year, I did not expect this adaptation to irrigation to be strong (see hypotheses in **Fig. III. 4.1** and **Fig. III. 4.2**). The result of the irrigation treatment on  $\Delta\delta^{13}\text{C}$  was different between the two species, with a negative  $\Delta\delta^{13}\text{C}$  (i.e., a decrease in  $\delta^{13}\text{C}_{\text{cor}}$  in 2016 and 2018 compared to 2013) for birch and a  $\Delta\delta^{13}\text{C}$  centered around zero for pine (**Fig. III. 4.7b**).

For birch, the negative  $\Delta\delta^{13}\text{C}$  was stronger than expected and it was homogeneous across blocks (**Fig. III. 4.8**). It suggested that birch functioning (i.e., carbon and water assimilation and use) was at least partly limited by water resources during the summer of 2013 and that summer irrigation removed this limitation. However, this hypothesis is not very likely as the spring and summer of 2013 had high precipitation levels and soil water content did not reach low values in the summer (**Fig. III. 4.3**). Therefore, at least part of this effect (negative  $\Delta\delta^{13}\text{C}$ ) was probably related to a canopy closure in the pure plots, especially since irrigation also induced higher crown volume and tree growth compared to non-irrigated blocks (see **Chapter II**).

For pine, the  $\Delta\delta^{13}\text{C}$  were centered around zero, indicating a functioning in 2016 and 2018 that was similar to the one in 2013. We expected these values to be more negative as a response to the irrigation treatment but also due to the canopy closure of the plots compared to 2013. The absence of negative values might be due to an insufficient irrigation input to completely remove water stress for pine,

contrarily to birch, and this small drought effect might have compensated the canopy closure effect on  $\delta^{13}\text{C}_{\text{cor}}$ . There was also a significant increase in  $\delta^{13}\text{C}_{\text{cor}}$  between 2016 and 2018 in pure plots. This suggested that in pure plots, pine was more exposed to drought in 2018 than in 2016. It could be that the irrigation treatment was even more deficient in 2018 compared to 2016 to remove all water stress for pine, due to an increasing stand basal area with time which in turn increased water use (see **Chapter III.2, p. 139** and **Chapter III.3, p. 149**). The strong variability in results across blocks for pine was hard to explain, especially since birch had a very homogeneous response in the same blocks.

#### 4.4.3.2. Mixture effects on $\Delta\delta^{13}\text{C}$

For birch, there was a strong mixture effect on  $\Delta\delta^{13}\text{C}_{2016}$  and  $\Delta\delta^{13}\text{C}_{2018}$ : both were more negative in the mixed plots than in the pure plots (**Fig. III. 4.7b**). Since water stress was likely absent in these plots due to the irrigation, the mixture effect was clearly related to light availability effects on  $\delta^{13}\text{C}_{\text{cor}}$  (de Streel et al. 2020). Birch trees were more shaded in the mixed plots compared to pure plots as they were dominated in height by pine (**Fig. III.S 4.3**). Indeed, I observed that birch trees were subject to a more intense competition (measured by Hegyi's competition index) in mixed plots than in pure plots (**Fig. III.S 4.4**). Trees with a higher competition index had a lower  $\delta^{13}\text{C}_{\text{cor}}$  (**Fig. III.S 4.4**), which is thought to reflect an increased shading (Mölder et al. 2011). This effect was the same in both 2016 and 2018, probably because the irrigation accelerated the canopy closure compared to the non-irrigated blocks. These results for mixture effects in the irrigated blocks also bring new insights for the hypothesis I made in the non-irrigated blocks. Indeed, this confirms that the mixture effect on  $\Delta\delta^{13}\text{C}_{2018}$  for birch in the non-irrigated blocks was probably due to canopy closure effects and not differences in water availability.

For pine, there were no mixture effects on  $\Delta\delta^{13}\text{C}$ . This can be explained by the fact that in irrigated blocks, the canopy closure was similar between pure pine and mixed plots (see **Chapter II** and **Fig. III.S 3.2**) and that pine was the dominant species (**Fig. III.S 4.3**), making light related mixture effects less likely.

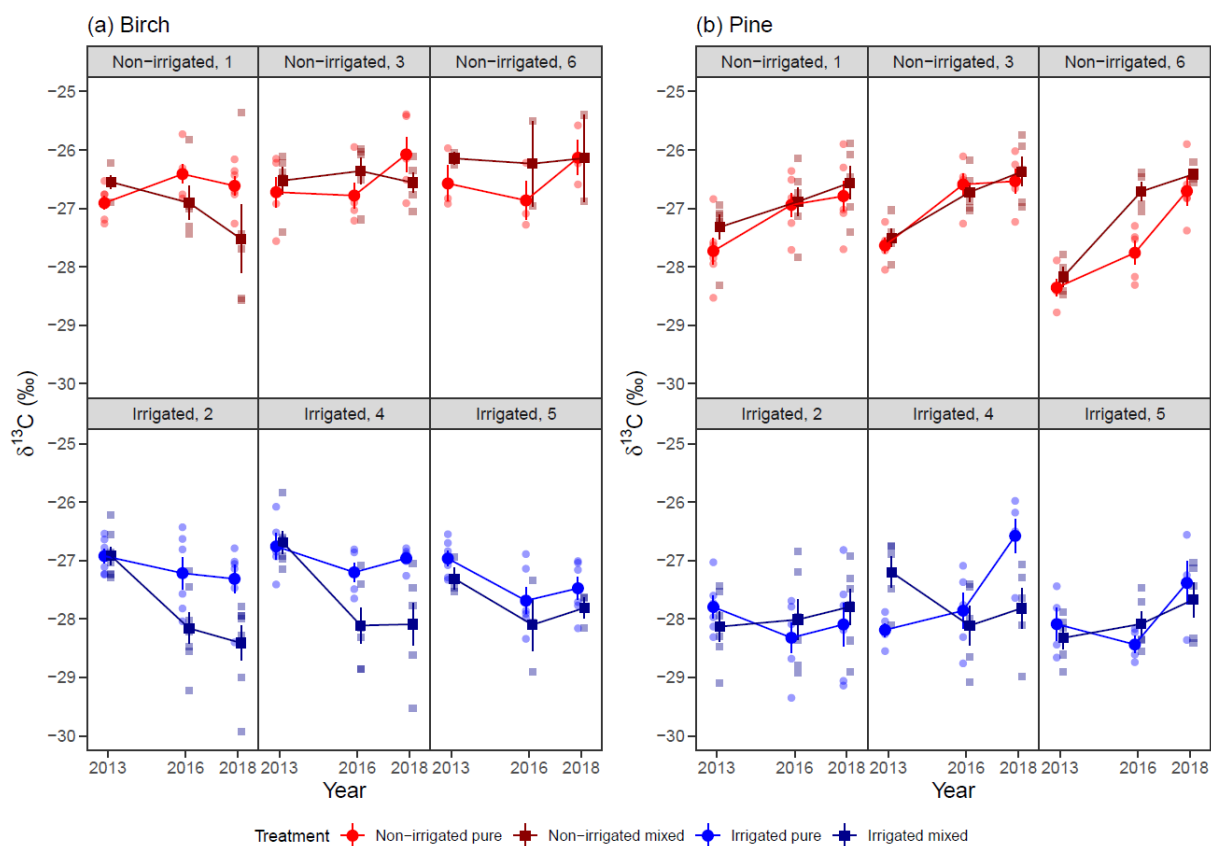
## 4.5. Conclusion

I found no evidence for water-use related mixture effects on drought exposure in the non-irrigated blocks for either species, while there were some effects of an increased shading for birch in mixed plots. In the irrigated blocks, I found that birch responded strongly to the water input while pine did not. Moreover, there was a stronger mixture effect on birch in the irrigated blocks due to the shading of birch crowns by pine in mixed plots. The irrigation treatment allowed me to confirm the importance of the canopy closure effects on birch  $\delta^{13}\text{C}$  in both non-irrigated and irrigated blocks.

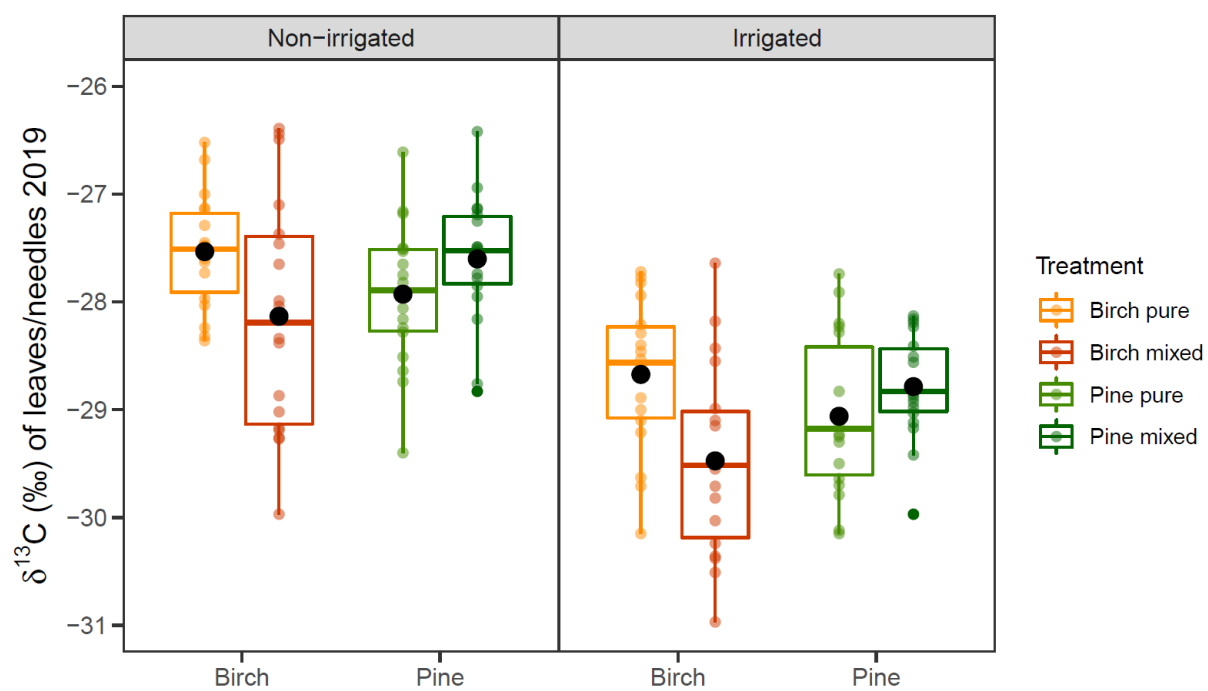
Studying mixture effects on drought exposure in a retrospective manner with tree-ring  $\delta^{13}\text{C}$  in such young plantations is made difficult by the dynamic effects of crown growth and canopy closure on  $\delta^{13}\text{C}$ . The presence of a wet year right before or after the studied dried years would have helped with this difficulty but was unfortunately not possible with my dataset because of a succession of dry years after the start of the irrigation treatment (2015 to 2020). It would be interesting to repeat the experiment in a few years, after the succession of a wet and a dry summer, now that the canopy closure will evolve more slowly from year to year.

I conclude from this sub-chapter that, at this stage of development of such a plantation (10 years), mixing birch and pine in equivalent proportions did not improve drought exposure of either species, but it did not worsen it either. Therefore, this makes it a viable strategy to increase biodiversity in forest stands in this region. However, birch did show a functional response to mixing due to a progressive shading by pine trees in mixed stands. This progressive shading of birch might lead, in the next years, to very low growth rates and ultimately to the death of birch trees in mixed stands. If this proves to be true, then birch should be harvested earlier than pine in these plantations. This progressive domination of birch will have to be considered in potential management strategies for this specific species assemblage.

### 4.6. Supplementary figures Chapter III.4

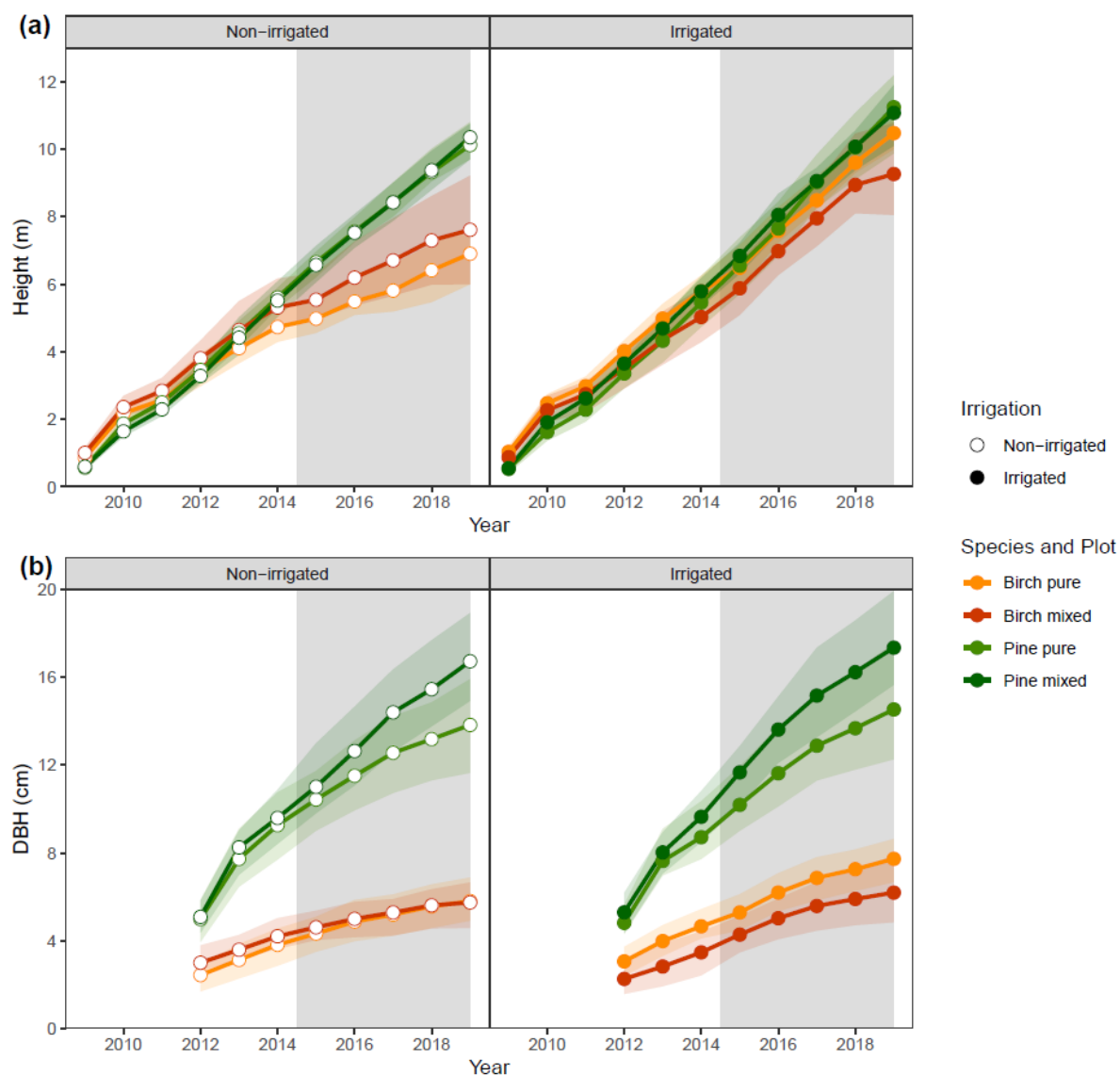


**Fig. III.S 4.1** Raw  $\delta^{13}\text{C}$  data before correction represented by species and for each block. Small points in the background represent individual tree values. Large symbols represent the average, and the error bars represent the standard error of the mean.

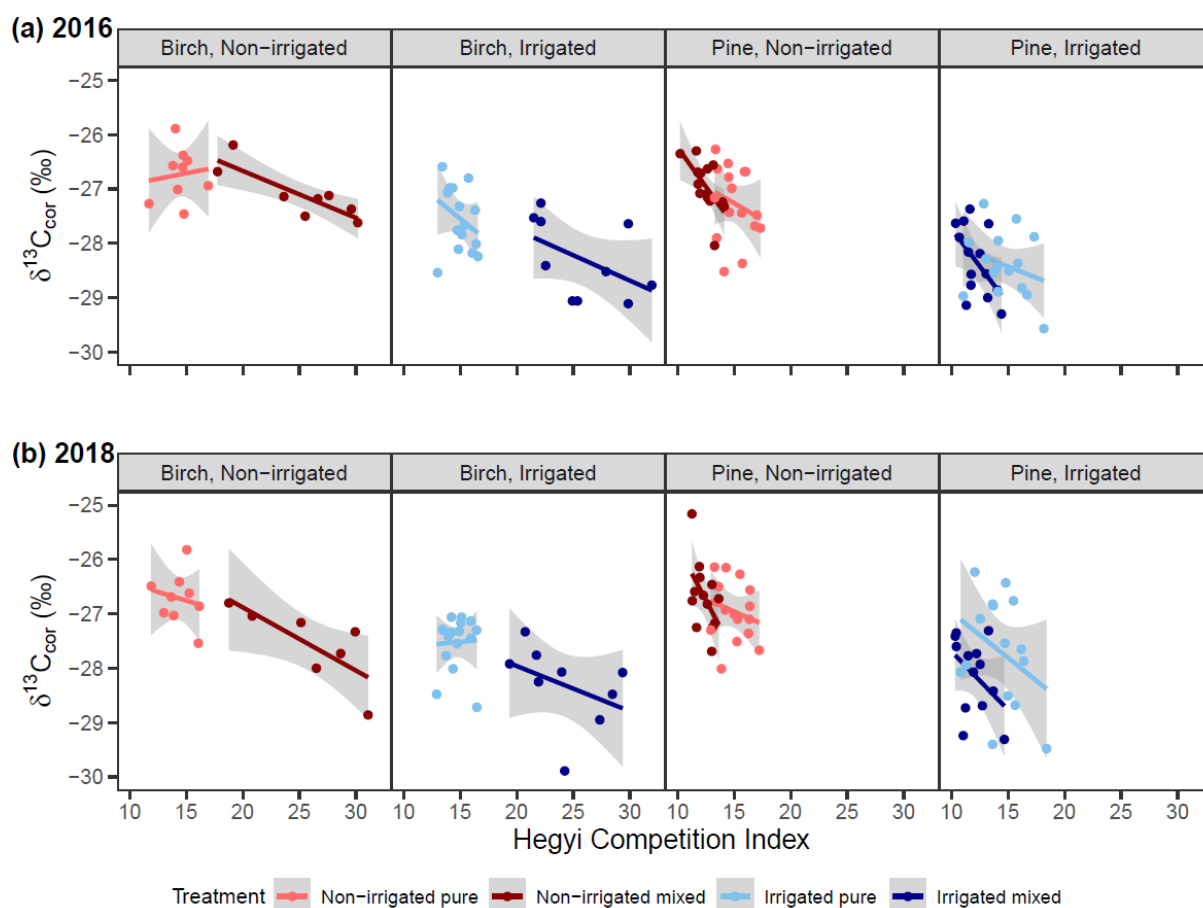


**Fig. III.S 4.2** Leaf and needle  $\delta^{13}\text{C}$  measured in 2019. The leaves and needles were sampled in the south-facing upper third of the crown at the end of July 2019 for five to six trees per species and plot (blocks 1 to 6).





**Fig. III.4.3** Evolution of height (a) and diameter at breast height (DBH) (b) with time at the ORPHEE experimental plantation. This figure represents the data for the plots that I used in my  $\delta^{13}\text{C}$  experiment (blocks 1 to 6: pure birch, pure pine and mixed birch and pine plots). A subset of 7 to 11 trees per species and plot were monitored each year since the plantation for height, and since 2012 for DBH.



**Fig. III.S 4.4** Relationship between  $\delta^{13}\text{C}_{\text{cor}}$  and Hegyi competition index by species and irrigation treatment for 2016 (a) and 2018 (b). Hegyi index was calculated for the 8 direct neighbors of each tree (3 m radius around a tree). Small points in the background represent individual tree values. Large symbols represent the average, and the error bars represent the standard error of the mean.

## Chapter III.5

# Root water uptake during drought conditions



## 5. Root water uptake during drought conditions

### 5.1. Introduction

Resource partitioning for water use among species has often been discussed as a possible effect to explain increased productivity in diverse tree species mixtures (Forrester 2015; Ammer 2019). Belowground vertical stratification of water uptake by roots among species has been identified as a potential resource partitioning mechanism (Grossiord 2019). Indeed, rooting patterns of species could have complementary characteristics in mixed stands that would allow a higher water uptake in the same volume of soil, or the exploration of a bigger volume of soil compared to monocultures. Consequently, it was also suggested that this belowground resource partitioning could contribute to a lower vulnerability of trees to soil drought in mixed forests (Grossiord 2019). But this hypothesis is controversial as a higher and more complete soil water utilization could also lead to a faster depletion of soil resources and therefore a higher exposure of trees to drought (Forrester 2015; Ammer 2019). Moreover, the role of this belowground complementarity in explaining mixture effects on ecosystem functioning – even though it has often been hypothesized (Ammer 2019) – has rarely been directly associated to mixture effects on growth and/or resistance to drought in forests (Schwendenmann et al. 2015) or even in grassland experiments (Mueller et al. 2013).

A presence or absence of vertical stratification of roots in mixed stands is dependent on several factors. First, two species could have different rooting depths in monocultures which would result in a reduction of the intra-specific competition in mixed stands (i.e., niche complementarity) (Grossiord et al. 2014a; Schwendenmann et al. 2015). Second, the resource partitioning in mixed stands could also happen if both species have similar rooting patterns in monocultures, but the inter-specific competition in mixed stands results in an intra-specific plasticity of the rooting pattern for one or both species (i.e., niche differentiation) (Bolte and Villanueva 2006). In both cases, the result in mixed stands would be a vertical stratification of roots between the species allowing for a higher water uptake at the stand level compared to monocultures. Finally, the presence of a vertical stratification of roots is also dependent on the environmental context such as precipitation, soil fertility, or soil structure, which can all drive root distribution (Zanetti et al. 2015) and affect potential mixture effects.

Several studies investigated vertical stratification by looking at the vertical distribution in fine root biomass in pure vs. species diverse ecosystems. Contrasted results were found: vertical root segregation along the soil profile was indeed observed in some studies (Schmid and Kazda 2001; Bolte and Villanueva 2006), confirming a potential contribution of such mechanism to biodiversity-ecosystem functioning (B-EF) relationships. In contrast, vertical root segregation was not observed in other studies (Meinen et al. 2009; Fruleux et al. 2018; Altinalmazis-Kondylis et al. 2020).

Another approach to study complementarities in root water uptake is based on stable isotopes in soil and xylem water (Grossiord et al. 2014a; Schwendenmann et al. 2015; Fruleux et al. 2018). Stable isotopes allow the direct testing of resource partitioning by considering root activity, instead of relying on indirect methods such as comparing fine root biomass production (Trogisch et al. 2016). Indeed, fine root vertical distribution is not necessarily correlated to root water uptake activity, especially under limiting soil water conditions (Fruleux et al. 2020). The stable isotope approach allows to characterize the water sources used by plants at a given time based on the isotope signature of water in the soil and the xylem sap in trees (Ehleringer and Dawson 1992). During dry spells, the natural abundance of water isotopes can be used because an isotopic gradient for O and H isotopes is established through evaporation in the top layers of the soil (Clark and Fritz 1997) with an enrichment in heavy isotopes ( $^2\text{H}$  and  $^{18}\text{O}$ ) near the soil surface that commonly decreases exponentially with soil depth. An absence of isotopic fractionation is assumed during root water uptake (Dawson 1993) although this assumption has been challenged recently (Barbeta et al. 2020b; Freyberg et al. 2020; De Deurwaerder et al. 2021). Following this assumption, the isotopic signature of the xylem water should reflect the mean signature of all the water sources for the tree's rooting system.

As with the fine root biomass method, contrasted results were found when using stable isotopes to identify complementarity in water uptake of species in mixed stands. A vertical stratification of water uptake among species was found in mixed stands in some cases (Grossiord et al. 2014a; Schwendenmann et al. 2015; Goisser et al. 2016; Bello et al. 2019), whereas it was not in others (Fruleux et al. 2018). The vertical stratification, when present, was linked either to niche complementarity (Grossiord et al. 2014a; Schwendenmann et al. 2015) or niche differentiation (Goisser et al. 2016; Bello et al. 2019). When no differences were found, it was explained by a high functional similarity between the studied species (Fruleux et al. 2018). Indeed, the strongest differences were often observed when coniferous and broadleaved species were mixed (Grossiord et al. 2014a; Goisser et al. 2016).

In this study, I was particularly interested in testing whether such mechanisms do exist in the ORPHEE tree diversity experiment, particularly during drought conditions. A previous study at this site explored fine root biomass production and distribution in the spring of 2018 and demonstrated that there was no vertical root segregation between birch and pine in mixed plots (Altinalmazis-Kondylis et al. 2020). However, one does not necessarily expect to find the same results during the summer, when soil water is limiting. Indeed, during drought stress, root activity has to adapt to limited water availability and belowground interactions might be modified compared to non-limiting soil water conditions (Grossiord et al. 2019; Brinkmann et al. 2019). As an example, a shift of water uptake towards deeper water sources in dry conditions has been observed for drought tolerant species (Bakker et al. 2006; Brinkmann et al. 2019). Few studies have looked at root water uptake complementarities among species in mixed stands during drought conditions (Fruleux et al. 2018; Bello et al. 2019; Martin-Blangy et al. 2021). It is

particularly important to study belowground complementarities in these conditions to bring a mechanistic understanding to mixture effects on tree functioning during drought.

The aim of this study was to investigate potential differences in water uptake sources by roots during dry conditions in pure and mixed stands of birch (*Betula pendula* Roth) and pine (*Pinus pinaster* Ait.) in the ORPHEE plantation during the dry summer of 2018. To do so, I used the isotopic method previously described. Because the isotopic method based on the natural abundance of isotopes can only be used in dry conditions when a gradient is established in the soil, I focused this study on the non-irrigated blocks at ORPHEE and was not able to characterize water uptake in the irrigated blocks. I did however sample branches in one of the irrigated blocks to compare the values of branches of trees in the irrigated block with those in the non-irrigated one.

Within this sub-chapter, I asked the following questions:

- (i) Do birch and pine differ in isotopic signatures ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ) in xylem water in pure plots?
- (ii) Do birch and pine show a shift in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of xylem water in mixed compared to pure plots, because of potential interaction between the roots of these species?
- (iii) What are the relative contributions from the different soil water depths to the total root water uptake?

I hypothesized that:

- (i) There would be differences in the isotopic signature ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ) in xylem water of the two species in the pure plots, indicating a potential for complementarity in mixed plots.
- (ii) There would be a shift in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of xylem water for birch in mixed plots compared to pure plots, in response to the competition with pine, but potentially no such shift for pine. I based this hypothesis on the existing evidence that birch has more plastic rooting traits than pine (Curt and Prévosto 2003; Altınalmazis-Kondylis et al. 2020).
- (iii) The differences between species in xylem water isotopic signature translated into differences in the pattern of water uptake from the different soil depths. Based on the existing knowledge on the root architecture and vertical distribution of these two species (see review in Altınalmazis-Kondylis (2020)), I expected that pine trees would extract water from deeper soil sources, as it is supposed to be a deeper-rooted species than birch.

## 5.2. Methods

### 5.2.1. Study site

For this section, please refer to the description of the experimental site at the beginning of this chapter (**section 1.2**).

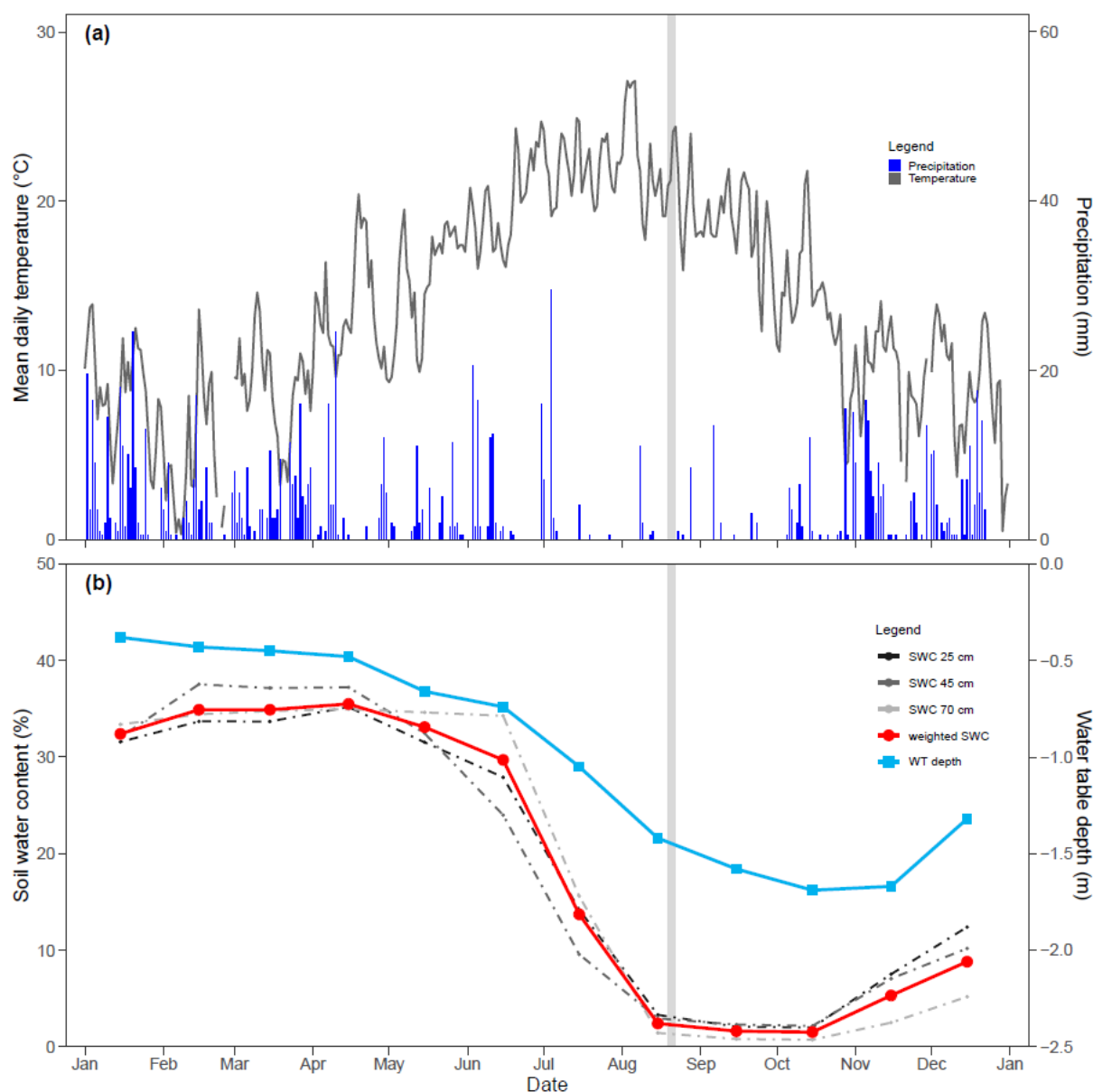
### 5.2.2. Climate and soil variables

I used nearby weather and soil water content monitoring stations to characterize the best period for sampling. Daily climatic data were obtained from the INRAE weather station of Cestas-Pierroton (44°44.52' N, 00°46.92' W, 1.2 km away from ORPHEE) (**Fig. III. 3.3**) made available by the INRAE CLIMATIK platform (<https://intranet.inrae.fr/climatik/>, in French) managed by the AgroClim laboratory of Avignon, France. Monthly water table depth and soil water content (SWC, %) data were recorded in the Castillonville Gis-Coop pure pine plantation next to the ORPHEE plantation (44° 44.08' N, 00°47.74' W, 500 m away). Water table depth was measured with a pressure transducer (CS450, Campbell Scientific). SWC was measured with volumetric water content sensors (CS650, Campbell Scientific) set-up at three different depths: 25 cm, 45 cm and 70 cm (**Fig. III. 3.3**). With the data from these three depths, I calculated a daily weighted SWC (wSWC, m) according to Bonal et al. (2008), an index which is representative of the soil water content from the surface down to 1 m depth, according to the following equation:

$$\text{wSWC} = 0.350 \times \text{SWC}_{25} + 0.225 \times \text{SWC}_{45} + 0.425 \times \text{SWC}_{70}, \quad (1)$$

where 0.350, 0.225 and 0.425 are the weighting coefficients that were divided to add up to 1 m and represent a range of depths for each probe: 0-35 cm for  $\text{SWC}_{25}$ , 35-57.5 cm for  $\text{SWC}_{45}$ , and 57.5-100 cm for  $\text{SWC}_{70}$ . The intrinsic values of wSWC should not be interpreted in terms of raw values, as they depend on the total depth selected to calculate wSWC, but rather as relative values to observe seasonal change. In the case of my study, this index is interesting because (i) it takes into account the different values of SWC measured at the different depths, which may vary differently over the season (e.g. when the soil dries up or gets rewetted), and (ii) it is useful to use as a proxy of soil water content over a given depth (here, I chose 1.0 m depth) and to observe seasonal variations in SWC over the soil profile.





**Fig. III. 5.1** 2018 seasonal pattern of (a) mean daily temperature (°C), daily sum of precipitation (mm), (b) mean monthly soil water content at different depths and weighted over the three depths (SWC, %) and mean monthly water table depth (m) for ORPHEE. The grey vertical bar shows the date of sampling for branches and soil (August 20-21, 2018).

### 5.2.3. Field sampling

#### 5.2.3.1. Sampling dates

As our method relies on a natural isotopic gradient for O and H with soil depth established through evaporation in the top layers of the soil (Clark and Fritz 1997), it was therefore essential for my study to obtain measures during a strong drought spell in order to have an established gradient. Consequently, we sampled branches on August 20<sup>th</sup>, 2018, and soil cores on August 21<sup>st</sup>, 2018, a period indicated with the gray bar in **Fig. III. 3.3**. At that date, there had been little recent precipitations, the soil was very dry (on average 2 % SWC for soil between 0 and 70 cm deep), and the water table depth was around -1.5 m.

#### 5.2.3.2. Branches

We sampled branches in three out of four non-irrigated blocks (blocks 1, 3 and 6), resulting in nine plots (six pure and three mixed). We also sampled branches in one irrigated block (block 2) to test the impact of the irrigation treatment on xylem water isotope composition. We sampled a branch from five individual trees per species and plot, resulting in a total of 80 trees sampled. The five trees per species were randomly selected among the trees from the six inner rows of each plot to avoid potential edge effects in the two outermost rows.

To determine isotope composition of xylem water, for each selected tree, we sampled a 30 to 40 cm long branch with a pole pruner. We selected branches in the crown with live leaves to ensure that there was active transpiration and water flow in the xylem. We kept only the lower segments of the branch that were at a distance from the leaves to avoid the back-diffusion of evaporatively enriched foliar water (i.e., Péclet effect) (Farquhar and Lloyd 1993). Bark tissue was immediately removed with a knife to prevent phloem sap from contaminating the sample. The samples (5 cm long segments) were then placed into closed glass airtight vials and stored at 4°C to prevent evaporation before further analysis.

#### 5.2.3.3. Soil cores

To characterize the vertical soil water isotope profile in the non-irrigated plots, one soil core was sampled in each plot (pure birch, pure pine and mixed) of blocks 1 and 3 (non-irrigated), resulting in a total of six soil cores sampled. Block 6 was not sampled for technical reasons; indeed, the soil borer broke down in block 3 because of the presence of a hardpan zone and could not be repaired in time to allow the sampling of block 6. Block 2 could not be sampled either; nevertheless, as this block receives about 3 mm of water from the irrigation system every day, the existence of a vertical gradient of  $\delta^2\text{H}$  or  $\delta^{18}\text{O}$  in soil water is very improbable. This amount of water is not enough to balance daily plot evapotranspiration during a sunny day during the summer, and isotope fractionation in soil water may then occur in these plots. However, we cannot expect that such fractionation results in any clear vertical gradient in soil water isotope signature in these blocks.

For each soil core sample, we first removed the forest floor litter within a rectangular frame of 10 × 20 cm. Then, the top 0–15 cm of soil was collected manually with a soil corer (8 cm Ø). The following layer of soil (15–100 cm) was collected with an electrical percussion hammer (Makita®, HM1400), attached onto a percussion gouge (6 cm Ø). The last layer of soil (100–200 cm) was collected with a narrower percussion gouge (4 cm Ø). We aimed at drilling and collecting soil cores down to 200 cm, but we could not always sample that deep. The goal was to reach at least the depth of the upper limit of the groundwater (around 150 cm depth at this time of the year (**Fig. III. 3.3**)) (Barbeta et al. 2018). After collection, the soil cores were carefully separated into subsamples according to the following depth categories (in cm): 0-5, 5-10, 10-15, 15-25, 25-35, 35-45, 45-55, 55-65, 65-75, 75-85, 85-100, 100-120, 120-140, 140-160, 160-180, 180-200. We placed each soil subsample in a hermetically sealed plastic bag and stored them at 4°C to prevent evaporation before further analysis.

#### 5.2.3.4. Groundwater

To estimate groundwater isotope signature, we sampled water from the water table from a water bore hole located at the center of the ORPHEE plantation, which is used to get the irrigation water for irrigated blocks (C. Meredieu, pers. comm). Irrigation water is extracted at a depth of 18 m below the surface. We analyzed this water for both  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  signatures. Groundwater isotopic signature is the result of the average of all percolating waters over time. At the time of our sampling, groundwater probably became the major source of water in the soil profile below a depth of 1.5 m. We thus expect the isotope signature of the groundwater to be close to that of the soil layers below 1.5 m depth. Groundwater probably also influenced the isotopic signature of the soil water in the layers above 1.5 m depth with water going up by capillary rise. The isotopic signatures in the layers above the water table level would then be the result of a mixture of water from the water table and precipitation water percolating through the soil from the surface.

#### 5.2.4. Sample processing and isotope analysis

##### 5.2.4.1. Cryogenic vacuum distillation

To extract water from the branch and the soil samples, I used the “cold trapping” method with a custom-made static vacuum cryogenic distillation system (West et al., 2006) at the SILVATECH platform (Silvatech, INRAE, 2018. Structural and functional analysis of tree and wood Facility, Nancy, France). The water from the branch and soil samples was evaporated by heating the sampling tube in a water bath (65°C), then condensed with liquid nitrogen for 90 minutes in a collection tube.

##### 5.2.4.2. Isotopic analysis

The extracted water from branch and soil samples was analyzed for isotopic composition with an elemental analyzer (EA, EuroPyrOH; EuroVector, Milano, Italy) coupled to an isotope ratio mass

spectrometer (Isoprime IRMS; Elementar, Manchester, UK) at the SILVATECH platform. Each sample was measured six times with subsamples of 0.2  $\mu\text{L}$  of extracted water, of which only the last three measurements were kept and averaged for each sample. The first three measurements were discarded to avoid any bias related to a potential “memory effect”, meaning a contamination from the previous sample analyzed (C. Hossann, pers. comm.).

Isotopic composition, expressed in terms of  $^{18}\text{O}/^{16}\text{O}$  and  $^2\text{H}/^1\text{H}$  ratios, is represented by  $\delta$ -values. The results are expressed as  $\delta$ -values relatively to the international Vienna-standard mean ocean water standard (V-SMOW) following Eq. 2:

$$\delta_{\text{sample}}(\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000, \quad (2)$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  represent the  $^{18}\text{O}/^{16}\text{O}$  or  $^2\text{H}/^1\text{H}$  ratios of the sample and of the V-SMOW standard (IAEA 1995). The mass spectrometer used in this study had a precision of  $\pm 0.5 \text{‰}$  for  $\delta^{18}\text{O}$  and of  $\pm 2.0 \text{‰}$  for  $\delta^2\text{H}$ .

#### 5.2.4.3. Isotope data corrections

If the extraction of the water from a given sample is incomplete, then the extracted water can be depleted in heavy isotopes compared to the initial sample, because of a faster evaporation of the lighter isotopes (Araguás-Araguás et al. 1995). To avoid any bias due to potentially incomplete extractions, I weighed the branch samples after cryo-distillation and placed them in a drying oven for 48 h at  $65^\circ\text{C}$  to check if all the water had been extracted. If part of the water had not been completely extracted, I applied a correction to  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  since the extracted water had less of the heavier isotope ( $^{18}\text{O}$  or  $^2\text{H}$ ) than the water remaining in the branch. To do so, I calculated the theoretical fractionation of the hydrogen isotopes with Eq. 3 to obtain a corrected  $\delta$ -value (Araguás-Araguás et al. 1995):

$$\delta_{\text{corr}} = \delta_{\text{measured}} - \left( \frac{F^{(1/\alpha)} - F}{F - 1} * 1000 \right), \quad (3)$$

where  $\delta_{\text{measured}}$  is the known measured  $\delta$ -value ( $\delta^{18}\text{O}$  or  $\delta^2\text{H}$ ),  $\delta_{\text{corr}}$  is the expected real  $\delta$ -value if there had been no fractionation during extraction,  $F$  the fraction of water remaining in the branch, and  $\alpha$  a fixed factor specific to the isotope measured and to the extraction temperature ( $65^\circ\text{C}$ ). Factor  $\alpha$  is calculated from Eq. 4 (Majoube 1971):

$$10^3 * \ln(\alpha) = a * \left( \frac{10^6}{T_K^2} \right) + b * \left( \frac{10^3}{T_K} \right) + c, \quad (4)$$

where  $T_K$  is the extraction temperature in degrees Kelvin, and  $a$ ,  $b$ , and  $c$  are fixed factors defined in Majoube (1971) with  $a = 1.137$ ,  $b = -0.4156$ , and  $c = -2.0667$  for  $^{18}\text{O}$  and  $a = 24.844$ ,  $b = -76.248$ , and  $c = 52.612$  for  $^2\text{H}$  (Clark and Fritz 1997), resulting in  $\alpha=1.006669405$  for  $^{18}\text{O}$  and  $\alpha=1.045391623$  for  $^2\text{H}$ .

I applied this correction on both isotopes to all the samples with a water-extraction percentage less than 100 %.

## 5.2.5. Data analysis

### 5.2.5.1. Raw isotopic data from branch xylem water

A simple linear model was used on branch raw data for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  to test for the effects of species, plot composition, and block (**Fig. III. 5.2**, **Table III. 5.1**) with the following model:

$$\delta \sim \text{Species} \times \text{Composition} \times \text{Block} , \quad (5)$$

where  $\delta$  is either  $\delta^{18}\text{O}$  or  $\delta^2\text{H}$ , *Species* is a two-level factor (birch or pine), *Composition* is a two-level factor (pure or mixed plots), and *Block* is a three-level factor (three non-irrigated blocks: n°1, n°3 and n°6). I used the block identity as a fixed factor in this analysis, instead of as a random factor, because I wanted to see how it interacted with species and composition effects. The multiplicative sign indicates that I included all two and three-way interactions in the model.

### 5.2.5.2. Contribution of water sources to xylem water

Graphical inference can be used to make a preliminary identification of the mean soil water acquisition depth of each tree by comparing soil and xylem water isotope composition; to do so, the method assumes that the mean depth of soil water being used by a plant is the depth at which the soil water has a similar isotope composition to the xylem water (Rothfuss and Javaux 2017). This method only uses one isotope at a time and can give biased results if the xylem water isotopes are the result of a mixture of isotopically distinct water sources. Moreover, there can be multiple solutions if a given branch isotope signature corresponds to two different depths in the isotope soil profile (**Fig. III. 5.3**).

Measuring both water isotopes ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ) can help distinguish isotopic signatures of soil water and xylem water on a dual isotope space (**Fig. III. 5.4**). The meteoric water lines on the dual isotope plots represent the relationship between  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  found in precipitation water, at a global and a local scale. The global meteoric water line (GMWL) is defined with the following equation:  $\delta^2\text{H} = 8 \delta^{18}\text{O} + 10$  (Craig 1961). The local meteoric water line (LMWL) is defined from the precipitation data for 2007-2016 at the Cestas weather station with the following equation:  $\delta^2\text{H} = 6.7 \delta^{18}\text{O} + 2.8$  (J. Ogée, pers. comm., following the IAEA/GNIP precipitation sampling guide), giving a more precise portrayal of local precipitation waters. These meteoric water lines are useful to check the distribution of soil water samples. Indeed, the physical process of evaporation results in a situation in which water samples that have undergone some evaporation plot 'below' the LMWL of precipitation isotopic data (Barbeta et al. 2018). The dual isotope plot does not however give an estimation of water uptake depth or of the contribution of different water sources for the xylem water.

To quantify the contribution of different potential water sources to that of xylem water a Bayesian mixing model can be used, especially if both isotopes were measured. Consequently, in addition to using the graphical inference approach, I used a Bayesian mixing model with the package MixSIAR in R (Stock et al. 2018). MixSIAR is a flexible framework to create mixing models based on the Bayesian theory: it uses Markov Chain Monte Carlo (MCMC) methods to determine proportions and probability distributions of “sources” contribution to a “mixture” of these different sources. In our case, the “sources” are the potential soil water sources and the “mixture” (also called the “consumer” in the MixSIAR package) is the xylem water from the trees. To determine the contribution of the sources, biological tracers are needed (e.g., stable isotopes). I used the two isotopologues ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ) as individual tracers in the mixing models (Freyberg et al. 2020).

Only a limited number of “sources” can be used in a Bayesian mixing model for it to be able to converge. Moreover, these sources must be as isotopically distinct as possible (Freyberg et al. 2020). Our extensive sampling of the vertical isotopic profile of the root zone allowed us to characterize the vertical variations across depths until the groundwater was reached. I then chose to group all soil profiles sampled in the different plots into a single dataset to take into account the horizontal variability of isotopic values. I then used both isotopes (on the dual isotope plot, **Fig. III. 5.5**) to visually define breakpoints in the distribution of soil isotope values in this two-dimension space (Rothfuss and Javaux 2017). With this approach, I visually identified four categories of water sources data. To characterize these categories and name them, I calculated the mean depth of the corresponding data in each category. These categories are then defined as soil layers at different depths: “Shallow”, “Sub-shallow”, “Intermediate”, “Deep”. All the individual points in each of these categories were used as raw data input within the MixSIAR model to consider the variability of the isotopic signature of the category. This translated into a mean and standard deviation for each of these categories in the MixSIAR model.

The purpose of the model was to examine water uptake proportions from different sources for the investigated plant species (not individual plants) in mixed or pure plots. Hence, the analysis was performed on a species level. I grouped together trees of the same plot and species with one fixed factor with four levels (“Birch\_pure”, “Birch\_mixed”, “Pine\_pure”, “Pine\_mixed”), and included block identity as another fixed factor.

The following parameters were set for the model. I kept the default process  $\times$  residual error term in the mixing models as it seemed the most appropriate for our data (Stock and Semmens 2016). I set discrimination data to zero because I assumed no isotopic fractionation during water uptake (Dawson 1993; but see Barbeta et al. 2020). I used a generalist prior ( $\alpha=1$ ) assuming water uptake from all potential sources is equally likely. For each model, the number of Markov Chain Monte Carlo iterations was increased manually (by trial and error) until convergence was reached and until the results for the Gelman and Geweke diagnostics (implemented into the MixSIAR package in R) were acceptable.

The results of a Bayesian mixing model are probability distributions of source contributions (soil water sources) to a mixture (xylem water) that are accounting for the uncertainty of the sources. The median source contribution values (50 % quartiles [25 % and 75 % Bayesian credibility intervals]) were taken as the final predictions for each factor.

## 5.3. Results

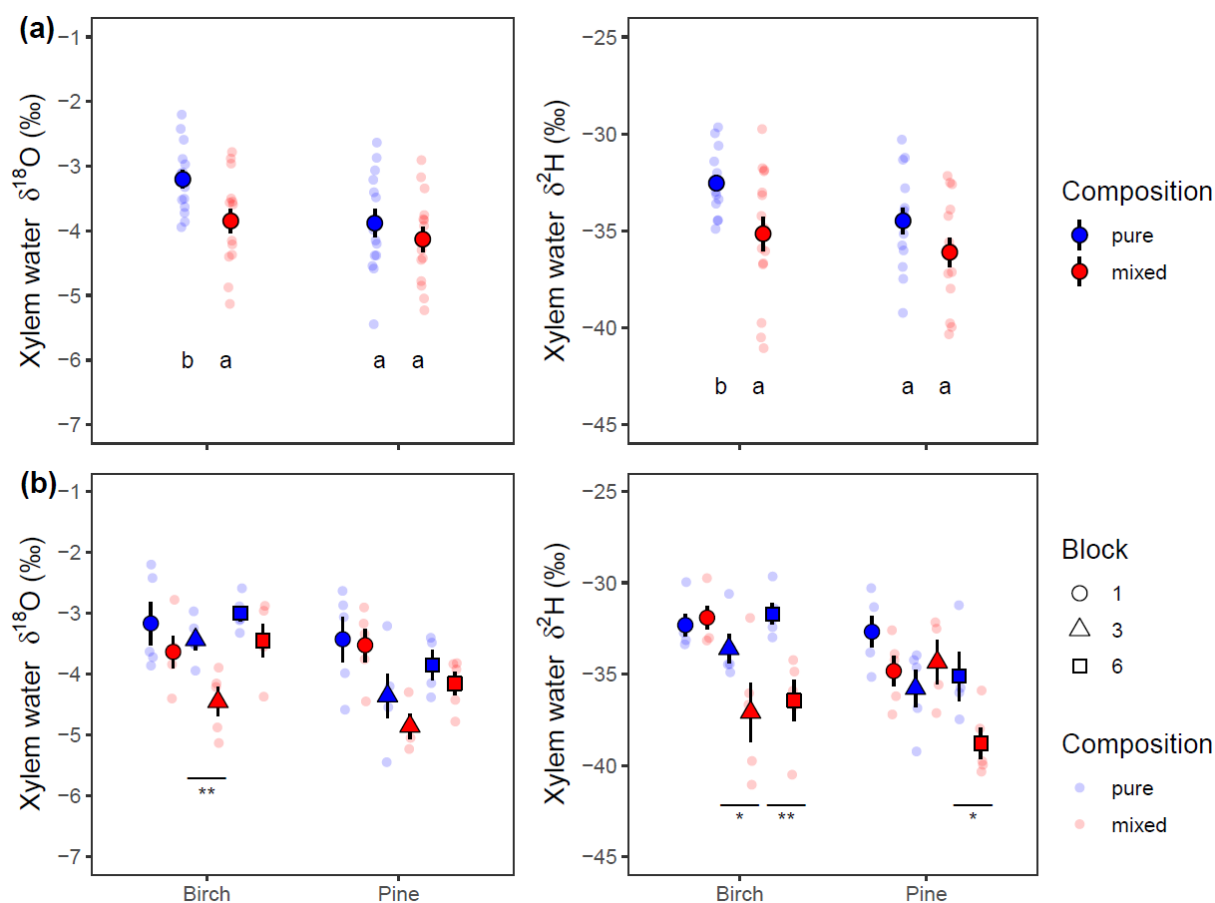
### 5.3.1. Isotopic signature of xylem and soil water

#### 5.3.1.1. Xylem water

**Non-irrigated blocks:** The isotopic signature of xylem water ranged from  $-5.1$  ‰ to  $-2.2$  ‰ for  $\delta^{18}\text{O}$  and from  $-41.1$  ‰ to  $-29.7$  ‰ for  $\delta^2\text{H}$  for birch. For pine, it ranged from  $-5.5$  ‰ to  $-2.6$  ‰ for  $\delta^{18}\text{O}$  and from  $-40.3$  ‰ to  $-30.3$  ‰ for  $\delta^2\text{H}$  (**Fig. III. 5.2**). In pure plots, birch had an average of  $-3.2 \pm 0.1$  ‰ for  $\delta^{18}\text{O}$  (average  $\pm$  standard error) and  $-32.5 \pm 0.4$  ‰ for  $\delta^2\text{H}$  while pine had an average of  $-3.9 \pm 0.2$  ‰ for  $\delta^{18}\text{O}$  and  $-34.5 \pm 0.7$  ‰ for  $\delta^2\text{H}$ . There were significant *Species*, *Composition*, and *Block* effects on both isotopes in xylem water (**Fig. III. 5.2, Table III. 5.1**). In pure plots, birch had less negative  $\delta^{18}\text{O}$  (post-hoc test estimate  $\pm$  standard error:  $+0.7 \pm 0.2$ ,  $p < 0.01$ ) and  $\delta^2\text{H}$  ( $+2.0 \pm 0.8$ ,  $p = 0.02$ ) than pine. In mixed plots, both species had similar values for  $\delta^{18}\text{O}$  ( $p = 0.14$ ) and  $\delta^2\text{H}$  ( $p = 0.30$ ). There was a shift towards more negative values for birch from pure to mixed plots  $\delta^{18}\text{O}$  ( $-0.6 \pm 0.2$ ,  $p < 0.01$ ) and  $\delta^2\text{H}$  ( $-2.6 \pm 0.8$ ,  $p < 0.01$ ), while pine did not show significant mixture effects for  $\delta^{18}\text{O}$  ( $p = 0.19$ ) or  $\delta^2\text{H}$  ( $p = 0.08$ ) (**Fig. III. 5.2a**).

The *Block* effect was significant for both isotopes: for example, Block 3 had lower  $\delta^{18}\text{O}$  values for both species and all plot compositions. However, this *Block* effect did not change the direction of *Species* and *Composition* effects for  $\delta^{18}\text{O}$ , as there were no significant interaction terms (**Table III. 5.1**). Some of the interaction terms were significant for  $\delta^2\text{H}$ : mixed plots did not have more negative values than pure ones for birch in Block 1 and for pine in Block 3 (**Fig. III. 5.2b**).

**Irrigated block:** In the irrigated block (Block 2), I found no *Species* or *Composition* effects on  $\delta^{18}\text{O}$ , but there was a significant *Composition* effect on  $\delta^2\text{H}$  ( $p < 0.001$ ), with more negative values in mixed plots for both species (**Fig. III.S 5.1**). For this block, we did not go any further towards the inference of root water uptake since we did not sample soil cores as the irrigation input prevented the establishment of an isotope gradient with soil depth.



**Fig. III. 5.2** Raw xylem isotope data for both  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , with all blocks pooled together (a) and by block (b). In (a) the letters represent post-hoc test results from for *Species* and *Composition* effects (Eq. 5). In (b) stars indicate post-hoc results for *Mixture* effects within blocks (i.e., significant differences between pure and mixed plots) (Eq. 5). The points in the background represent individual tree values. Large symbols represent the average values, and error bars represent the standard error of the mean.

**Table III. 5.1** Linear model results for *Species*, *Composition* and *Block* effects on xylem water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  (Eq. 5).

Predictors	$\delta^{18}\text{O}$			$\delta^2\text{H}$		
	$F(df; n)$	$p$ -value	$R^2$	$F(df; n)$	$p$ -value	$R^2$
<b>Species (SP)</b>	10.63 (1;46)	<0.01	0.51	6.28 (1;46)	<b>0.02</b>	0.56
<b>Composition (C)</b>	9.3 (1;46)	<0.01		13.03 (1;46)	<0.001	
<b>Block (B)</b>	10.81 (2;46)	<0.001		8.5 (2;46)	<0.001	
<b>SP x C</b>	1.24 (1;46)	0.27		1.04 (1;46)	0.31	
<b>SP x B</b>	2.01 (2;46)	0.15		2.61 (2;46)	0.08	
<b>C x B</b>	0.88 (2;46)	0.42		3.69 (2;46)	<b>0.03</b>	
<b>SP x C x B</b>	0.12 (2;46)	0.89		3.7 (2;46)	<b>0.03</b>	



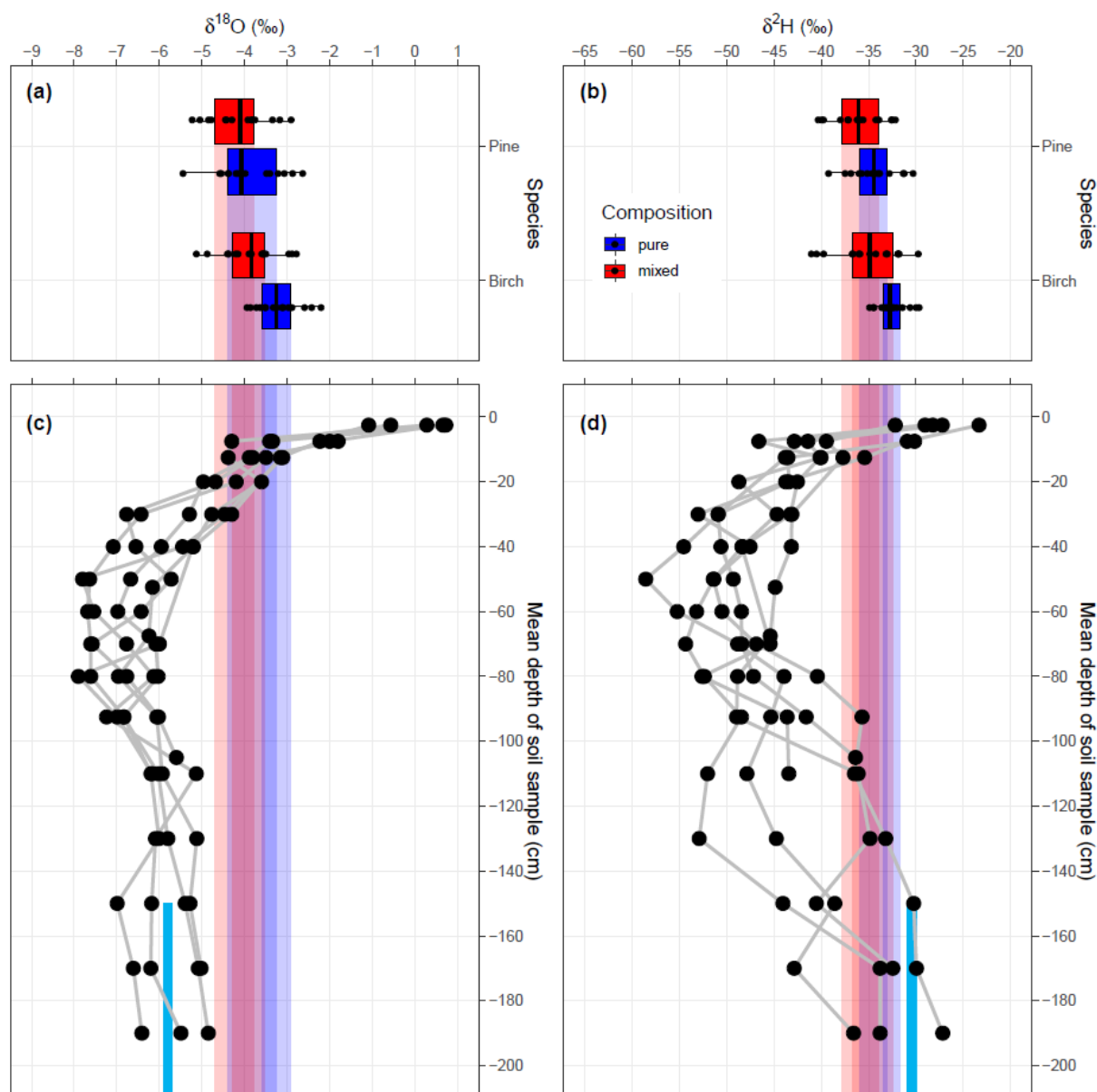
### 5.3.1.2. Soil depth profiles

In soil water samples  $\delta^{18}\text{O}$  ranged from  $-7.9\text{‰}$  to  $+0.7\text{‰}$  and  $\delta^2\text{H}$  ranged from  $-58.5\text{‰}$  to  $-23.3\text{‰}$ . For both isotopes, I observed a clear depletion in the heavy isotopes ( $^{18}\text{O}$  and  $^2\text{H}$ ) with depth until about 70 cm (**Fig. III. 5.3**). Evaporation in the superficial soil layers resulted, as expected, in an enrichment of the remaining water in the heavy isotopes. Below 70 cm, the isotopic depletion with depth disappeared. Below 150 cm, the soil was probably saturated with water from the water table. This was confirmed by the measured isotopic signature from the water table at 18 m below ground:  $\delta^{18}\text{O} = -30.4\text{‰}$  and  $\delta^2\text{H} = -5.8\text{‰}$ , which was in the range of the isotopic data of the deepest soil layers that we sampled (**Fig. III. 5.3**).

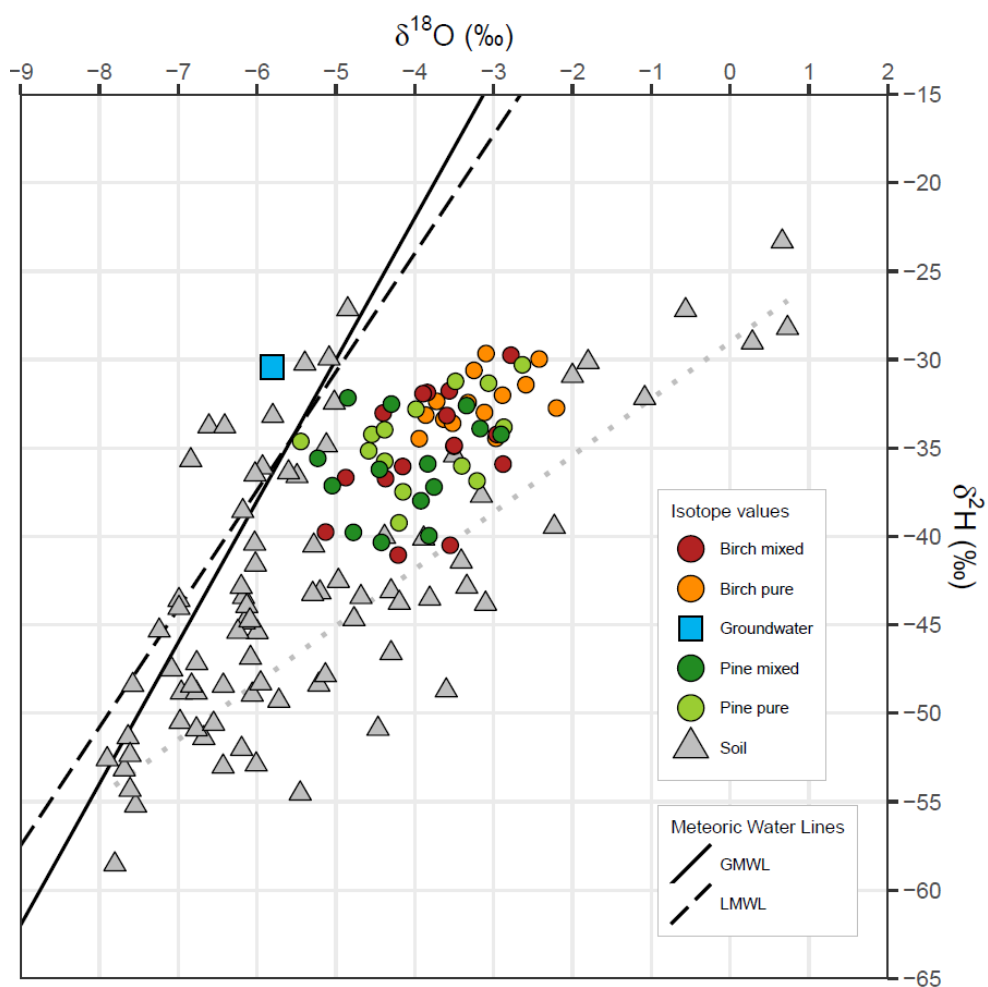
The meteoric water lines represent the relationship between  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in precipitation water (at a global and a local scale, both represented in **Fig. III. 5.4**). Soil water samples that fall along that line are samples that did not undergo evaporation after the last precipitation events. Soil water samples that do not fall on that line are the ones that were subject to evaporation processes after the precipitation events. The dual isotope approach (**Fig. III. 5.4**) shows that the soil data in our study were grouped along two different slopes: (i) along an evaporation line for the soil water from 0 to 70 cm, and (ii) along the meteoric water line for deeper soil waters.

### 5.3.1.3. Branch and soil together

Looking at branch data and soil data together for a graphical inference, I found that  $\delta^{18}\text{O}$  for xylem water corresponded on average to the  $\delta^{18}\text{O}$  values of the 10-15 cm soil layer (**Fig. III. 5.3**). However, for  $\delta^2\text{H}$ , I could see that a simple graphical inference could not be used to infer root water uptake depth: one  $\delta^2\text{H}$  value for branches was representative of two different soil depths. This was confirmed by the dual isotope plot (**Fig. III. 5.4**): the isotope values of xylem water did not correspond to any single soil sample and were intermediate between the shallowest and deepest soil values. This clearly indicated that branch xylem water isotopic signature was a result of the mixture of water sources from different soil layers, and that only a statistical method based on the contribution of both isotopes could allow me to infer these depths. To me, the Bayesian mixing model was the best method to analyze branch and soil data together. However, it is important to recall that this method only allows to identify the proportion of the contribution of different water sources; it does not give a “mean” depth value for water uptake.



**Fig. III. 5.3** Raw  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values for xylem (a,b) and soil (c,d) water. Shaded zones indicate 25 % and 75 % quantiles of branch data by species and plot composition for all three blocks pooled together. In (a) and (b) the points represent individual tree values. Soil data points are connected by gray lines for data from a given plot. Soil data points are represented at the average depth of the subsample (e.g. for subsample 180-200 cm, the average depth represented is 190 cm). The light blue rectangle starting at -150 cm indicates isotope signature of the groundwater from the central well.



**Fig. III. 5.4** Dual isotope plot with branch and soil data for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ . The circles represent xylem water isotope values of each tree. The gray triangles represent all the soil water samples in the different plots and at different depths. The blue square represents the isotopic signature of the groundwater. The gray dotted line is the estimated soil evaporation line (linear regression for soil data from 0 to 70 cm). The black lines are the meteoric water lines for isotope values of global precipitations (GMWL) and local precipitations (LMWL).

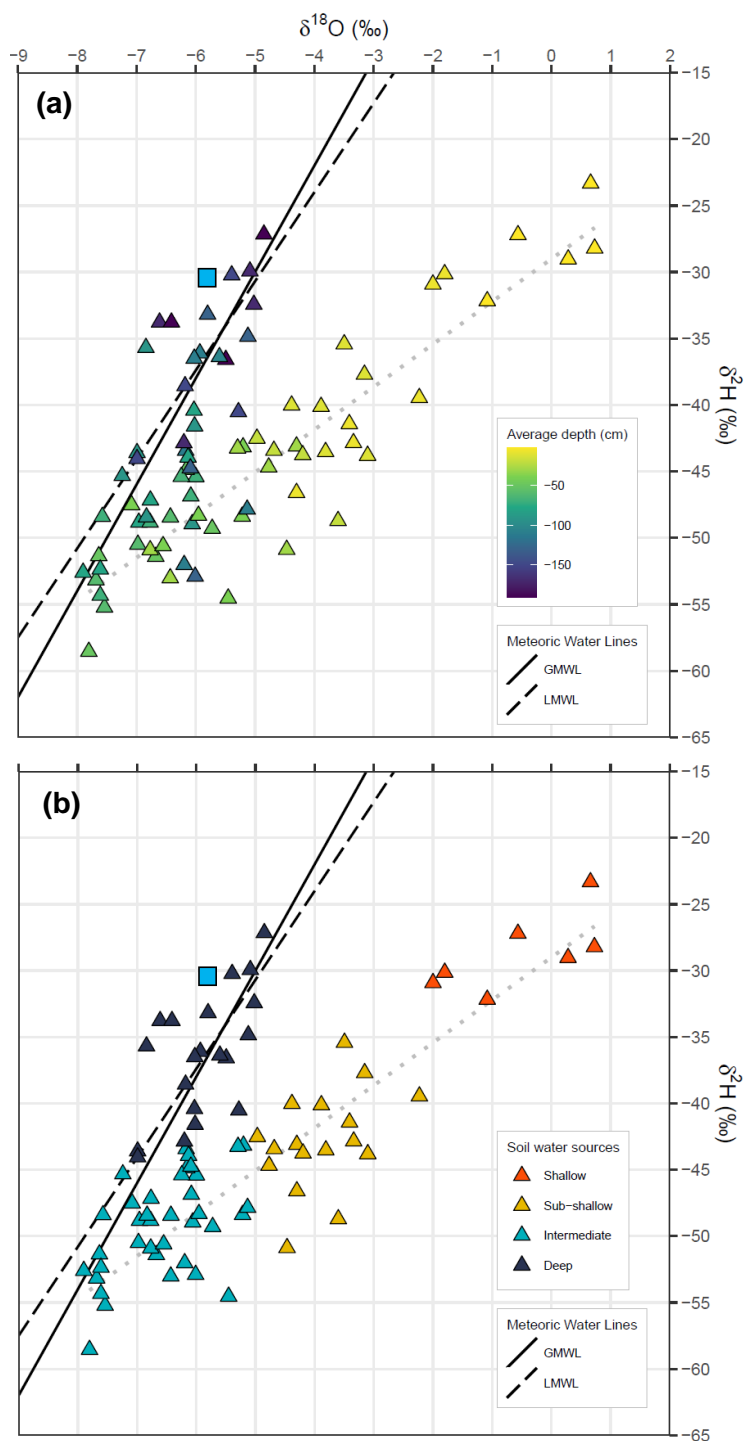
### 5.3.2. Contribution of water sources to root water uptake (MixSIAR results)

The four water sources defined for the MixSIAR model based on the variations of both isotopes (**Fig. III. 5.5**) corresponded to the following depth zones within the soil profile: “Shallow” (mean depth = 4 cm [range = 2.5-7.5 cm]), “Sub-shallow” (16 cm [7.5-30 cm]), “Intermediate” (68 cm [30-130 cm]), “Deep” (140 cm [80-190 cm]). The mean, the standard deviation for each of these categories for both isotopes, and the individual xylem water points are represented on the *isospace* plot from the MixSIAR model (**Fig. III. 5.6**). As observed before, the values for xylem water were intermediate between the water source categories.

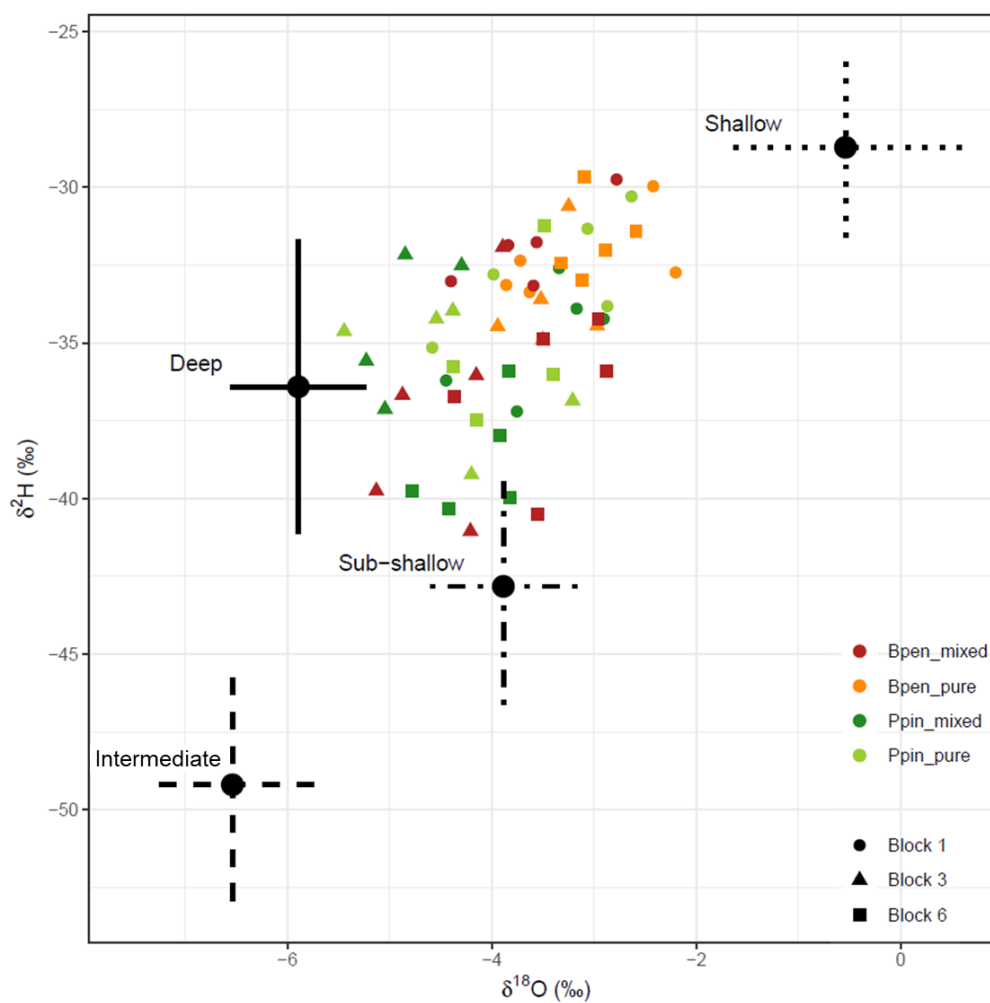
Results from the MixSIAR model show that for most trees, a major part of the water in the branch xylem was extracted from both the “Shallow” (average and range of the median contribution: 39 % [22-62 %]) and the “Deep” (45 % [31-63 %]) soil sources. Only a small proportion of water was detected by the mixing model to have been extracted from the “Sub-shallow” (10 % [1-36 %]) and “Intermediate” (3 % [1-4 %]) soil sources (**Fig. III. 5.7, Fig. III.S 5.2, Fig. III.S 5.3a**).

In pure plots across blocks, birch had on average a higher contribution from the “Shallow” water source (54 %) than pine (38 %) (difference of relative contribution: 16 %), and birch had a smaller contribution of the “Deep” water source (39 %) than pine (49 %) (difference of relative contribution: 10 %) (**Fig. III. 5.7**). This difference between species was smaller in the mixed plot for “Shallow” soil water (difference of contribution: 9 %), and absent for “Deep” soil water (**Fig. III. 5.7**).

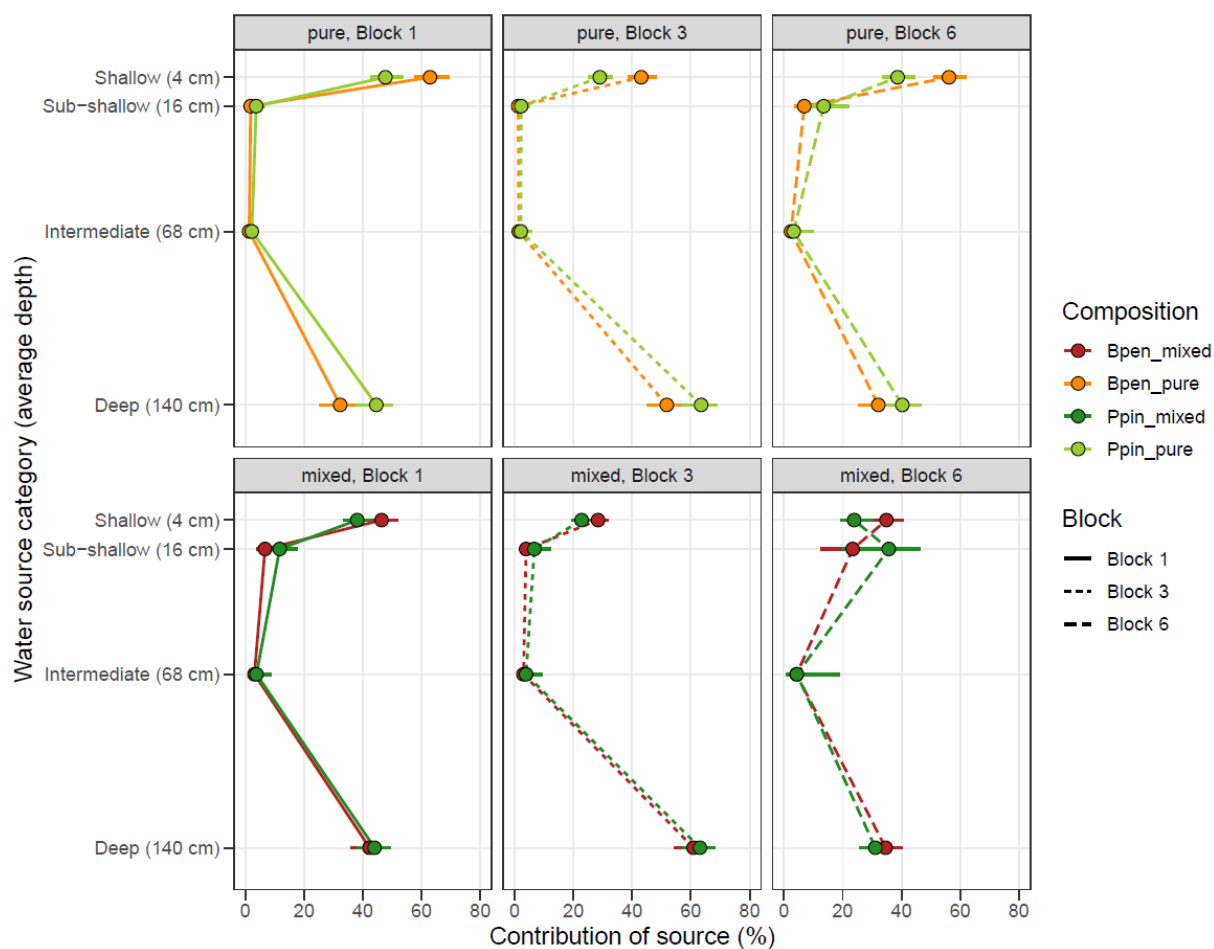
Consequently, there was a shift in contributions with plot composition, especially for birch, with a smaller proportion of water coming from the “Shallow” water sources in mixed (36 %) than in pure plots (54 %) (difference of relative contribution: 18 %), and a bigger proportion coming from the “Deep” water sources in mixed plots (46 %) compared to pure ones (39 %) (difference of relative contribution: 7 %) (**Fig. III. 5.8, Fig. III.S 5.3b**). For pine, the effect of plot composition on the respective contributions was less important than for birch: a smaller proportion of water came from the Shallow soil water in mixed plots (28 %) compared to pure ones (38 %) (difference of relative contribution: 10 %), and the contribution from the Deep soil water was almost the same in mixed plots (46 %) than in pure plots (49 %). Block 1 and 3 showed similar patterns while block 6 showed a different pattern in the mixed plot, with a shift from the Shallow to the Sub-shallow water sources.



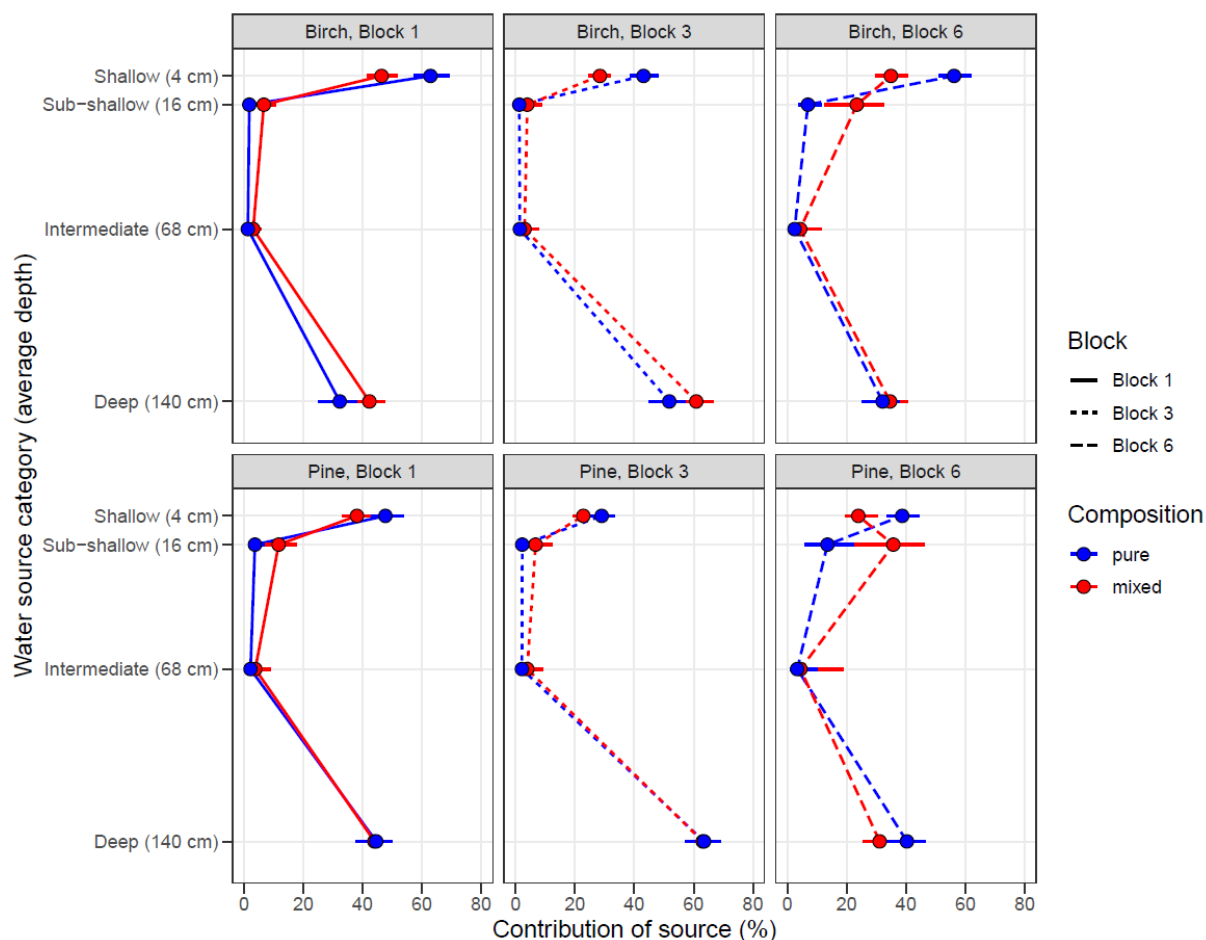
**Fig. III. 5.5** Dual isotope plot with soil data only, with (a) a continuous color scale according to the sample's average depth and (b) divided into four source zones for the MixSIAR model. The triangles represent all the soil water samples in the different plots and at different depths. The blue square represents the isotopic signature of the groundwater. The gray dotted line is the estimated soil evaporation line (linear regression for soil data from 0 to 70 cm). The black lines are the meteoric water lines for isotope values of global precipitations (GMWL) and local precipitations (LMWL).



**Fig. III. 5.6** “Isospace plot” from MixSIAR model outputs, with soil sources grouped into four categories (black symbols and error bars) and individual xylem water values of the sampled trees. Error bars represent standard deviations of the data in each soil source category.



**Fig. III. 5.7** Summary of MixSIAR results by plot composition and block to observe *Species* effects. At each depth category, the points represent the median source contribution values (50 % quartile) of the probability distribution. Error bars represent the 25 % and 75 % Bayesian credibility intervals. See **Fig. III.S 5.2** for the full probability distributions.



**Fig. III. 5.8** Summary of MixSIAR results by species and block to observe *Plot composition* effects. At each depth category, the points represent the median source contribution values (50 % quartile) of the probability distribution. Error bars represent the 25 % and 75 % Bayesian credibility intervals. See **Fig. III.S 5.2** for the full probability distributions.



## 5.4. Discussion

### 5.4.1. Raw xylem and soil isotope data

#### 5.4.1.1. Variability in xylem isotopic values

The isotopic signature of xylem water for all samples ranged from -5.5 ‰ to -2.2 ‰ for  $\delta^{18}\text{O}$  and from -41.1 ‰ to -29.7 ‰ for  $\delta^2\text{H}$  (**Fig. III. 5.2**). Water isotopes in plant xylem water can span a wide range of values, which are dependent on the signature of the local precipitations, of the groundwater, and on evapotranspiration levels. In a global analysis over all continents, Evaristo et al. (2015) found that plant xylem water can range from -20 ‰ to +8 ‰ for  $\delta^{18}\text{O}$  and from -170 ‰ to +20 ‰ for  $\delta^2\text{H}$ . The values that we found in our study are in the typical range of values that can be found for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in Southwest France. For example, Barbeta et al. (2019) found average values of -5 ‰ for  $\delta^{18}\text{O}$  and -45 ‰ for  $\delta^2\text{H}$  in the same region for *Fagus sylvatica* (L.) and *Quercus robur* (L.) xylem water in August 2017.

In pure plots, the two species showed differences in isotope xylem values, with less negative values for birch than pine for both isotopes, but with a high intra-specific variability (**Fig. III. 5.2a**). The intra-specific variability in xylem isotopic signature for both species and both isotopes indicates that for trees of a same species, there was a wide range of possible water sources extracted by these trees. This suggests that the root system of these trees extend over various soil layers, potentially down to the groundwater table, but not just down a precise and limited one for each species. Moreover, it could also be the result of a variability in the distribution of fine root biomass among trees despite similar rooting depths. Indeed, the soil water content in the different soil layer might vary locally within and among plots. This intra-specific variability will then make complex a precise identification of differences in the mean depth of soil water uptake among species and treatments. Part of this intra-specific variability was explained by the variability among blocks, as (i) there were evident block effects on the isotope values of xylem water, and (ii) we could see that the intra-specific variability was reduced within some of the blocks (**Fig. III. 5.2b**). The intra-specific variability with blocks might be due to the presence of a discontinuous hardpan in these podzol soils which, when present, started at a depth of about 50 cm and was about 17 cm thick (Altınalmazis-Kondylis et al. 2020). Soil characteristics can strongly drive the rooting profiles (Zanetti et al. 2015) and limit the expression of potential species and mixture effects (Altınalmazis-Kondylis et al. 2020). Because of this variability, we kept “Block” as a fixed effect within the MixSIAR model to test for differences among blocks (see **section 5.4.2**). The Bayesian mixing model outputs will give one probability distribution for all five trees of each species/plot/block combination.

This significant difference between species in pure plots was absent in mixed plots because of a shift of birch values towards isotope values similar to those of pine (**Fig. III. 5.2a**). The size of the species difference in pure plots and of the shift between pure and mixed plots for birch were not much higher than the precision of the spectrometer for both isotopes ( $\pm 0.5$  ‰ for  $\delta^{18}\text{O}$  and of  $\pm 2.0$  ‰ for  $\delta^2\text{H}$ ).

Therefore, these differences, although significant, might not represent a very big difference in water uptake patterns. Other studies have shown bigger species differences and shifts in response to mixtures in isotope values. For example, in my first chapter (Martin-Blangy et al. 2021), I found a difference in  $\delta^2\text{H}$  of  $9.1 \pm 1.3$  ‰ between beech and fir in pure stands. Bello et al. (2019) found an average shift in  $\delta^{18}\text{O}$  of  $-1.4$  ‰ between pure and mixed stands for sessile oak (*Quercus petraea* (Matt.) Liebl.) trees mixed with Scot's pine (*Pinus sylvestris* L.). Since these differences in isotope values represent mixtures from different soil water sources, I cannot make assumptions here on which species has a deeper water uptake or how the shift for birch translated in water uptake depth (see **section 5.4.2**).

#### 5.4.1.2. Soil water isotopic variations with depth

Soil water isotopic signature ranged from  $-7.9$  ‰ to  $+0.7$  ‰ for  $\delta^{18}\text{O}$  and from  $-58.5$  ‰ to  $-23.3$  ‰ for  $\delta^2\text{H}$ , showing a wider range than xylem water values. The range of values is not unusual compared to the global range for soil water samples (Evaristo et al. 2015) and similar to other soil data from the same region (Barbeta et al. 2019).

The shape of the soil water profiles for both isotopes confirmed that we had a clear evaporation pattern in the isotope values of the water from the upper soil layers (**Fig. III. 5.3**). Moreover, the dual isotope approach (**Fig. III. 5.4**) showed that the soil data in our study were grouped along two different slopes: (i) along an evaporation line for the soil water from 0 to 70 cm, and (ii) along the meteoric water line for deeper soil waters, which matches the relationship between  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in local precipitation waters (Barbeta et al. 2018). The water from the intermediate soil layers from 70 cm to 150 cm was probably a mix of water from different origins in different proportions: (i) a mix precipitation water from different seasons that percolated through the soil layers, (ii) water enriched due to evaporation in the upper layers and later mixed with precipitation water in the summer, and (iii) water from the groundwater table coming up by capillary rise (Sánchez-Pérez et al. 2008; Vincke and Thiry 2008). Thanks to these different processes (seasonal variations in distinct isotopic composition of meteoric waters, enrichment in heavy isotopes with evaporation, mixing of all meteoric waters in the groundwater table) the presence of distinct isotopic composition in water pools allows to trace the origin of plant water (Barbeta et al. 2018).

For the deepest soil water samples, isotope values were in the same range as the groundwater ones (**Fig. III. 5.3**), which confirmed that I was able to characterize all possible sources of water for these trees. Sampling all potential plant water sources is a very important step to be able to identify where plants take up water (Barbeta et al. 2018) and for the use of the Bayesian mixing model in the following section (Freyberg et al. 2020). It must be noted that even though we characterized all potential sources vertically, our soil core sampling was probably not sufficient to estimate horizontal variations in soil water. As seen with the block effects on branch xylem water values, these horizontal variations can have an important effect.

### 5.4.1.3. *Limits of isotopic methods to study root water uptake*

Using stable water isotope composition is a relatively straightforward technique to relate relative root water uptake with soil depth. Several shortcomings in the isotopic methods must however be kept in mind. The variability among trees and in the horizontal variability of soil properties, and the time-consuming water extractions, make it difficult to have an exhaustive sampling. Moreover, an increasing number of studies discuss a potential mismatch between available water sources and isotopically depleted compositions in xylem water (Barbeta et al. 2020b; Freyberg et al. 2020; De Deurwaerder et al. 2021). These could result from: (i) water isotope heterogeneities within the soil pore and stem tissues (Barbeta et al. 2020b), (ii) a potential isotopic discrimination at root-level (Vargas et al. 2017) (ii) an internal mixing of xylem water with isotopically depleted water reserves stored in stem tissue (Barbeta et al. 2020a) and (iii) a water-transport lag between roots and xylem water (De Deurwaerder et al. 2021). Although we did not detect offsets that seemed suspicious in our study, these limitations that have recently started to be underlined must be kept in mind for further work.

### 5.4.2. Water sources contributions to xylem water in dry conditions

In this sub-chapter, I was interested in looking at species and mixture effects on water uptake patterns of trees during drought conditions. For this reason, for sampling, we chose a period with a low soil water content and an absence of recent rain events (**Fig. III. 5.1**). In August 2018, the soil water content was about 2 % in the soil profile up to at least -70 cm (**Fig. III.S 5.4a**). Therefore, we must keep in mind that in these very dry conditions, the daily amount of water transpired by the trees was small (see **Fig. III. 3.5** for sap flux density in similar conditions at the end of August 2019). Therefore, our estimation of water uptake in this season concerns only a low quantity of water extracted by the roots.

#### 5.4.2.1. *Species effects on water uptake patterns in pure plots*

In a context of low water availability and dry climatic conditions, I found that for birch in pure plots, most of the water uptake (93 %) originated from two distinct soil layers, out of the four I identified. The Shallow (average depth of -4 cm) and Deep (-140 cm) water sources had the highest contributions to the water uptake (on average 54 % and 39 % respectively), while the Sub-shallow (-16 cm) and Intermediate (-68 cm) layers had very low contributions (on average 3 % and 2 % respectively) (**Fig. III. 5.7, Fig. III.S 5.3**). For pines in pure plots, the contributions were 38 % for the Shallow soil category, 6 % for Sub-shallow, 2 % for Intermediate and 49 % for Deep.

Therefore, contrary to my initial hypothesis, there was no strong evidence of a vertical stratification between the water uptake patterns of birch and pine in pure plots (i.e., one species taking up a high proportion of water from one soil depth category while the other species is taking up water in another) (**Fig. III. 5.7**). There was however a small difference between birch and pine in the median contributions from the Shallow and Deep water source categories, which explains the differences detected between

the species for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in xylem water. In pure plots, pine had a lower contribution from the Shallow water source than birch, and a higher contribution from the Deep water source (**Fig. III. 5.7**). The fact that pine had a higher proportion of water that came from the zone with available groundwater might be the result of a higher depletion of soil water in the top soil layers in pure pine plots compared to pure birch plots. This hypothesis is supported by the fact that we found that pure pine plots had a higher total transpiration during the following summer (see **Chapter III.3, p. 149**). Although many studies have reported rooting depth differences among species (Zapater et al. 2011; Stahl et al. 2013; Schwendenmann et al. 2015), other studies carried out during summer droughts in temperate forests also reported no major differences among species in pure plots (Fruleux et al. 2018; Bello et al. 2019). In these last two studies, the selected species primarily extracted soil water near the surface. The absence of strong species differences in our study might be due to constraining soil properties with a shallow water table in winter, but also a very low soil water content during the dry season in the upper layers. Indeed, belowground functional traits such as water uptake depth can reduce differences among species when there are strong soil constraints (Zanetti et al. 2015; Fan et al. 2017). The results of my study are also in accordance with the findings of Altinalmazis-Kondylis et al. (2020) who found an absence of species-specific fine root biomass distributions at ORPHEE in March 2018. In conclusion, in these specific soil and climatic conditions, there were no major functional differences in the water uptake of the birch and pine species that could lead to a potential complementarity for soil water uptake in mixed plots.

The general water uptake pattern of these two species in pure plots was surprising because it was discontinuous, with a very low contribution to water uptake from the intermediate layers. Both species had similar water uptake patterns in pure plots: high contributions from the Shallow and Deep water categories and small contributions from the Sub-shallow and Intermediate categories.

The high contribution of the Shallow soil layer for water uptake is in agreement with the fine root distribution reported at this site in March 2018 (Altinalmazis-Kondylis et al. 2020): about 83 % of the fine root biomass in these plots is present in the first 15 cm of soils (**Fig. III.S 5.4b**). Moreover, Bakker et al. (2006) found the highest fine root densities for *Pinus pinaster* in the 0 to 5 cm soil layer for similar sandy soils, and reported a fast decrease in the following soil layers.

As a reminder, our data only allows us to infer the proportion of the contribution of different water sources, but not the depth of the actual roots. The small contribution of Sub-shallow and Intermediate sources does not signify the absence of fine roots in these layers, but rather that they represent a small proportion of the origin of water present in the xylem water of branches. Moreover, a profile of water uptake is not necessarily continuous (Barbeta et al. 2018): for example, a dimorphic root system can result in an uptake of water at two very different soil depths (upper soil water vs. water table) (Dawson and Pate 1996). In our case we know that there was a presence of roots in the intermediate soil layers, although fine root biomass was decreasing strongly with depth after a depth of 15 cm (**Fig. III.S 5.4b**)

(Altinalmazis-Kondylis et al. 2020). The absence of contribution from the Sub-shallow and Intermediate soil layers is therefore still surprising and challenging. Perhaps the Shallow soil layer contributed more than the Sub-shallow because of a higher organic matter content in the first 5 cm (**Fig. III.S 5.5**) and because it retained the small precipitation events of the summer. The Intermediate layer probably did not contribute to water uptake because the soil was dry, and the presence of fine roots is already scarce at this depth (**Fig. III.S 5.4**). The presence of a dry gap at intermediate soil layers, located between upper soil layers containing infiltrated precipitation water and deeper soils layers containing groundwater coming up by capillary rise, has been described by Fan et al. (2017). The presence of the hardpan within this part of the soil might also play a role in a limited presence of fine roots in this layer of soil (Danjon et al. 2005; Altinalmazis-Kondylis et al. 2020).

The high contribution of the Deep soil water was surprising. Indeed, Altinalmazis-Kondylis et al. (2020) reported very low fine root biomass densities in the 60-90 cm soil layers for both species at this site in March 2018 (**Fig. III.S 5.4b**). However, they were not able to characterize the whole profile as they did not sample the soil below 90 cm. When comparing our results with pure pine plantations in the same region, the presence of roots below 90 cm that could access the groundwater seems very likely. Bakker et al. (2006) found a maximum rooting depth for pine of 0.75–1.3 m at a humid site and of 2.5–3.0 m at a dry site. Danjon et al. (2005) reported a maximum tree rooting depth that ranged from 0.74 to 1.28 m for coarse roots of pine. The presence of deep roots for pine is limited to the depth of the water table in summer, but these roots can grow in the layers above despite the presence of the water table in winter (Danjon et al. 2005). On the other hand, the presence of birch roots in the Deep soil layer was more surprising than for pine. Birch has been described as a shallow rooted species (50 – 75 cm) with a very dense fine root biomass in the upper horizon (Mauer and Palátová 2003; Curt and Prévosto 2003). However, birch roots were reported to be able to go down to 1.5 m depth in sandy soils in Germany (Mauer and Palátová 2003). In conclusion, even though deep roots below 90 cm were not characterized at this site, their presence is very likely above the summer water table depth (~ about 1.5 m at this site). The Deep soil layer that we characterized for isotopic analysis included samples that ranged from 80 cm to 190 cm deep, but the presence of roots was probably limited to a depth of 1.5 m in the unsaturated soil zone with groundwater coming up by capillary rise (Fan et al. 2017). The Deep soil water had a surprisingly high contribution for both species, knowing that in these soil layers fine root biomass density was probably very low. However, a small number of fine roots might be able to uptake a high proportion of water, especially if the higher soil horizons are very dry and with the presence of a water table (Stahl et al. 2013). The water from the Deep soil category was likely mostly made of water from the groundwater coming up by capillary rise above the water table depth. In a meta-analysis of 35 studies combining data for trees and other plant species, Barbeta and Peñuelas (2017) found that the use of groundwater was more likely at sites with a pronounced dry season and represented on average 49 % of transpired water in dry seasons and 28 % in wet seasons. Vincke and Thiry (2008) also reported that the

groundwater was the most important source of water during prolonged periods with no rain, and was used through capillary rise from the groundwater up to the rooted soil layers. The activity of deep roots was therefore essential in these studies during dry conditions. It has been shown that water uptake can shift to lower soil layers in dry conditions (Bakker et al. 2006; Schwendenmann et al. 2015; Brinkmann et al. 2019). The combination of dry conditions and the presence of a relatively shallow water table in our site may explain this high contribution of the Deep water category.

#### Take-home message

We did not find major differences in water uptake patterns of birch and pine in pure plots. Pine had a slightly higher contribution from Deep soil water than birch, which might be due to a faster depletion of water from the upper horizons than birch. The absence of a vertical differentiation between species might be primarily driven by the soil constraints at this site with a shallow water table limiting root growth.

Both species had a very high contribution of the Shallow and Deep soil water categories but a low contribution from the Sub-shallow and Intermediate soil water. The high contribution of the Shallow and Deep soil water categories for both species can be explained by (i) a high fine root biomass in the upper soil horizon taking up water from a soil with a low SWC and (ii) by small number of fine roots taking up water from the unsaturated zone above the water table with water coming up by capillary rise. The low contribution of the Sub-shallow and Intermediate soil categories may be explained by a fast-decreasing fine root density with depth and a low SWC because of sandy soil with low water retention capacity, but also to the presence of a hardpan in the Intermediate soil category.

#### *5.4.2.2. Shifts in water uptake between pure and mixed plots*

In mixed plots the contribution from the Shallow soil category dropped for both species, slightly more strongly for birch (18 %) than for pine (10 %) (**Fig. III. 5.8**). The contribution of the Deep soil layer increased by 7 % for birch in mixed plots than in pure plots, while it remained the same for pine. In total, this shift was significant only for birch, and only for the Shallow soil water category (see the overlap of the credibility intervals in **Fig. III. 5.8**). Therefore, the main consequence of the mixture effect was that birch trees had a lower contribution from the shallowest soil water in mixed plots, while pine did not. This resulted in a homogenization of the source contributions between the two species, resulting in even lower evidence for vertical stratification in mixed plots. Fruleux et al. (2018) and Bello et al. (2019) both carried out similar experiments during summer drought periods in France with two-species mixtures of beech (*Fagus sylvatica* L.) and maple (*Acer pseudoplatanus* L.) and of Scot's pine (*Pinus sylvestris* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.) respectively. In both studies they showed deeper water extraction by trees in mixtures compared to the pure stands for all species and an

absence of difference between the species in mixed stands. Fruleux et al. (2018) explained this shift by higher productivity and transpiration in the mixed stands resulting in the drying of the shallow layers may induce the trees to shift the depth of their soil water uptake. A similar process could help explain the shift for birch in our study. The lower contribution from the Shallow water source in mixed plots could be the result of a lower SWC in mixed plots compared to pure birch plots. Pine trees depleted the soil water faster than birch because of a higher transpiration, which could cause birch trees in mixed plots to have access to less shallow soil water than in pure plots. However, it does not explain the lower contribution from shallow soil water for pine in mixed plots compared to pure plots, as the total plot transpiration for mixed plots is lower than in the pure pine plots (see **Chapter III.3, p. 149**). The shift towards a lower contribution of shallow soil water for pine might be the result of a stronger competition with birch fine roots in mixed plots since birch has a higher fine root density than pine in superficial soil layers (Curt and Prévosto 2003; Altınalmazis-Kondylis et al. 2020). This hypothesis is strengthened by the fact that Sub-shallow contributions are higher for pine in mixed plots than in pure plots, indicating that the shift had a different origin than for birch.

Moreover, birch probably has a higher plasticity in fine-root architecture than pine (Curt and Prévosto 2003; Altınalmazis-Kondylis et al. 2020). Similarly, a high plasticity for beech water uptake was observed in beech-conifer stands (Bolte and Villanueva 2006; Grossiord et al. 2014a; Martin-Blangy et al. 2021). Although Altınalmazis-Kondylis et al. (2020) did not find significant differences in fine root biomass distributions between species in the mixed plots in March 2018, there might have been shifts between the spring and the summer, and we cannot rule out this hypothesis.

Finally, despite strong block effects, all three blocks showed similar trends for species and mixture effects. The mixed plot in Block 6 also had similar trends, even though there was a higher contribution of the Sub-shallow category compared to the Shallow category. I hypothesized that this could be the result of particular soil conditions in the topsoil of this plot that could have induced this shift, for example a thinner litter layer resulting in a drier topsoil. But a precise vertical and horizontal characterization of soil conditions has not been conducted there so far.

#### Take-home message

In mixed plots there was a shift towards a lower contribution of water from the shallow soil water for birch, resulting in a homogenization in water sources between the two species compared to pure plots. The evidence for the absence of a vertical differentiation of water uptake between species was therefore even stronger than in pure plots.

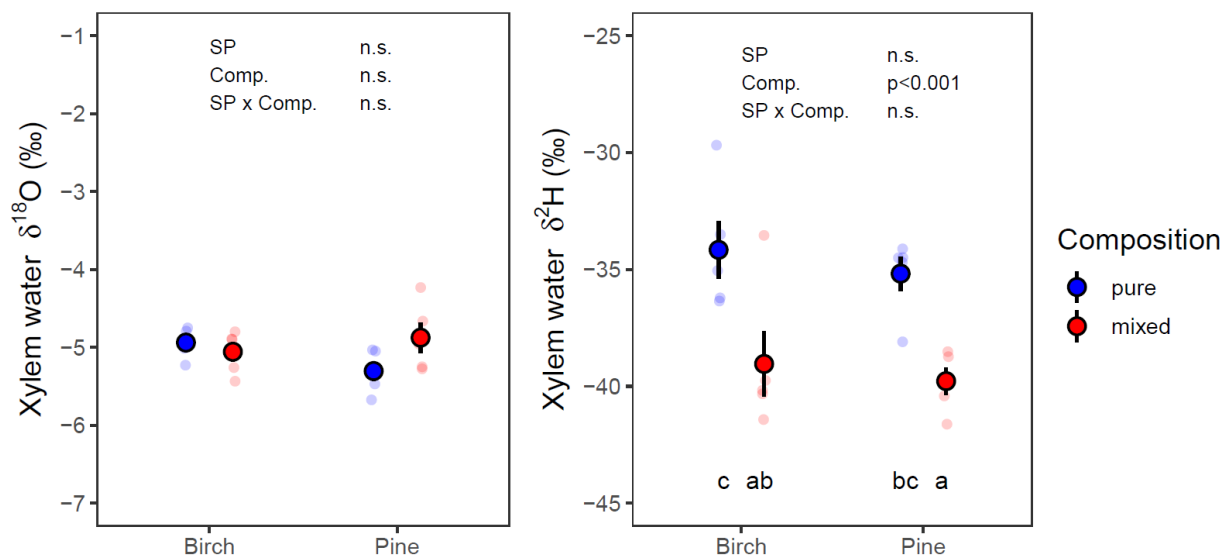
The lower contribution from the shallow waters for birch was probably due to a higher transpiration in mixed plots than in pure birch plots resulting in a lower soil water content.

## 5.5. Conclusion

In conclusion, contrary to our hypothesis, there was no evidence for a vertical differentiation of water uptake between the two species in mixed plots and this absence of difference was even more evident than in pure plots. This suggests that, at least during dry summer conditions, we should not expect mixture effects on water use or response to drought that can be explained by belowground complementarities. Overall, this is in accordance with what we have found in the previous sub-chapters, but I will further develop the links between mixture effects on ecosystem functioning at ORPHEE and the potential underlying mechanisms in the general discussion of this chapter (see general discussion of this chapter: **Chapter III.6, p. 265**).



## 5.6. Supplementary figures Chapter III.5



**Fig. III.S 5.1** Raw xylem isotope data for both  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  for the sampled irrigated block (Block 2). The text at the top of the panels represents results from linear models for *Species* and *Composition* effects. The letters at the bottom of the panels represent post-hoc test results. The points in the background represent individual tree values. Large symbols represent the average values, and error bars represent the standard error of the mean.

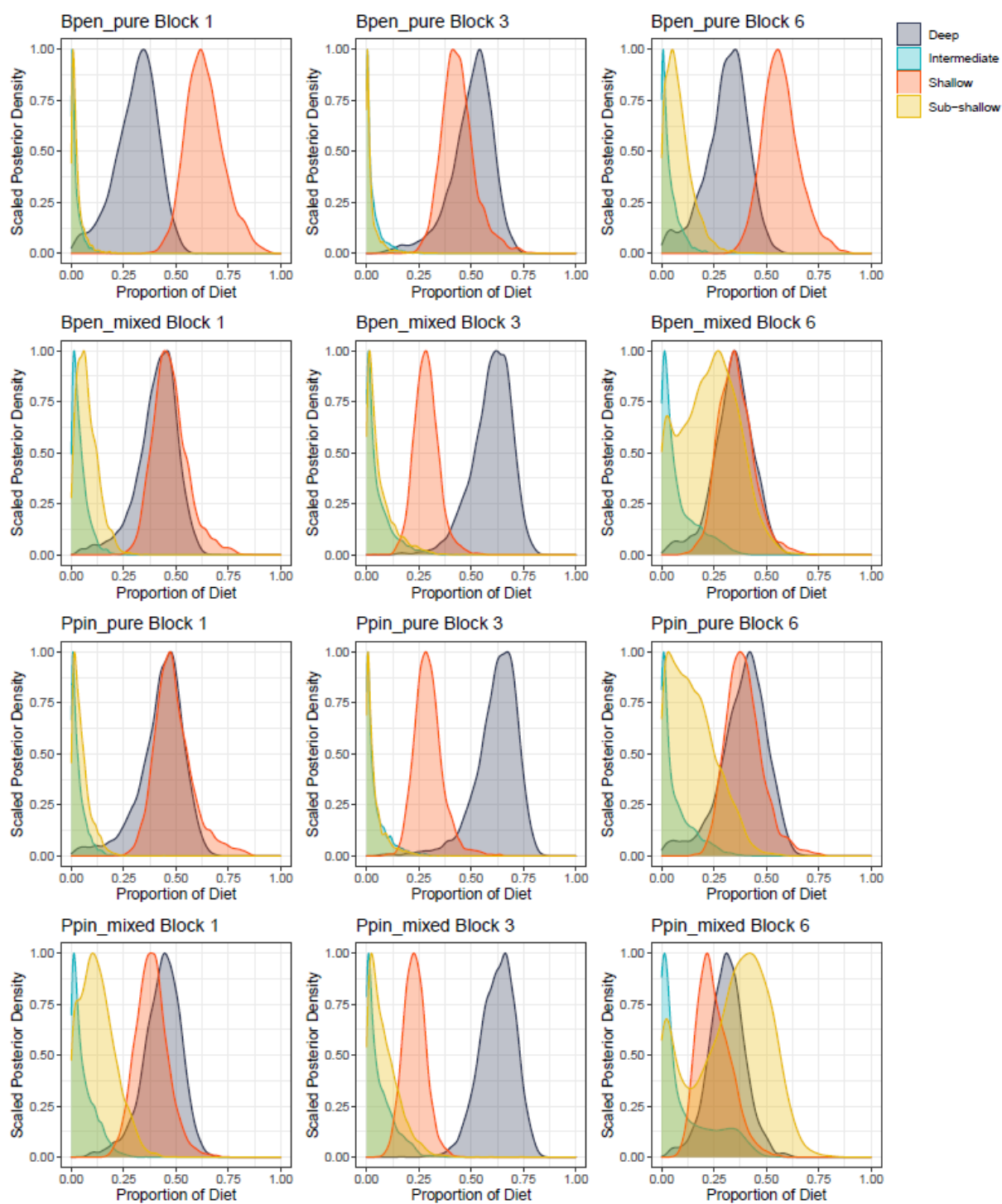
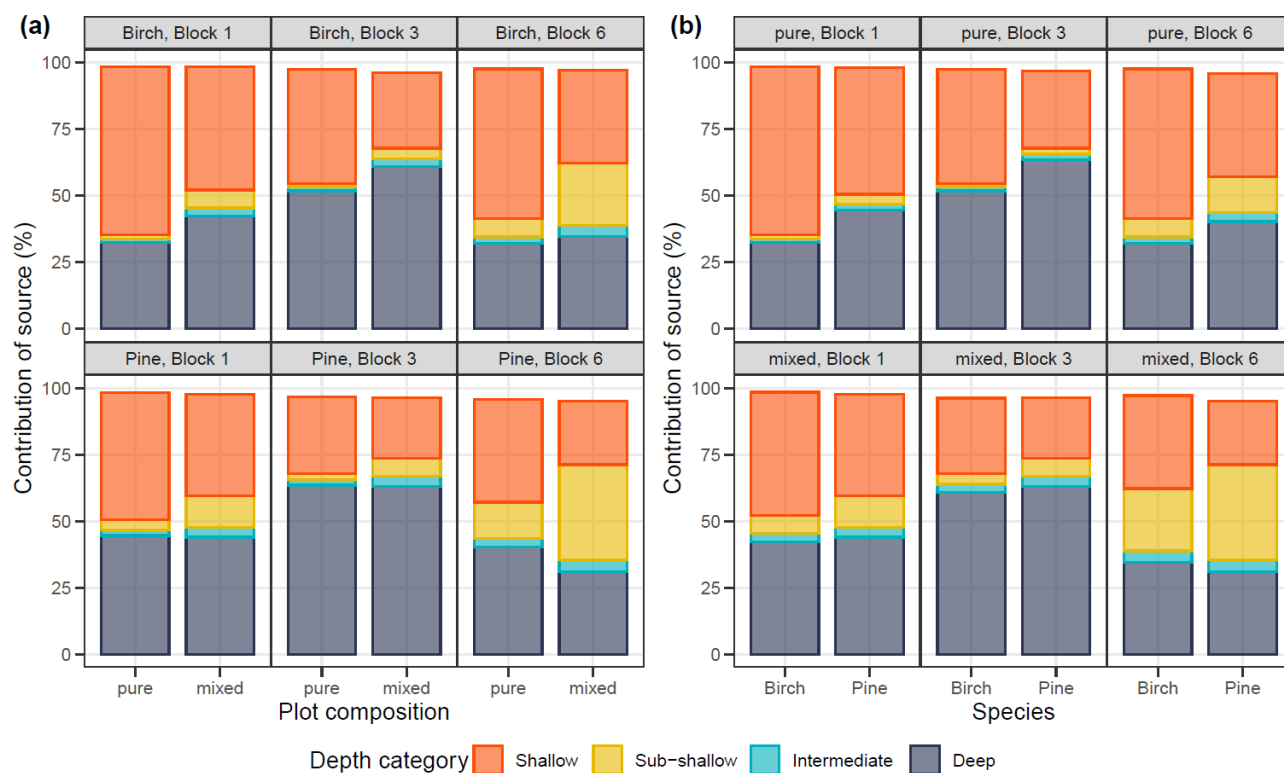
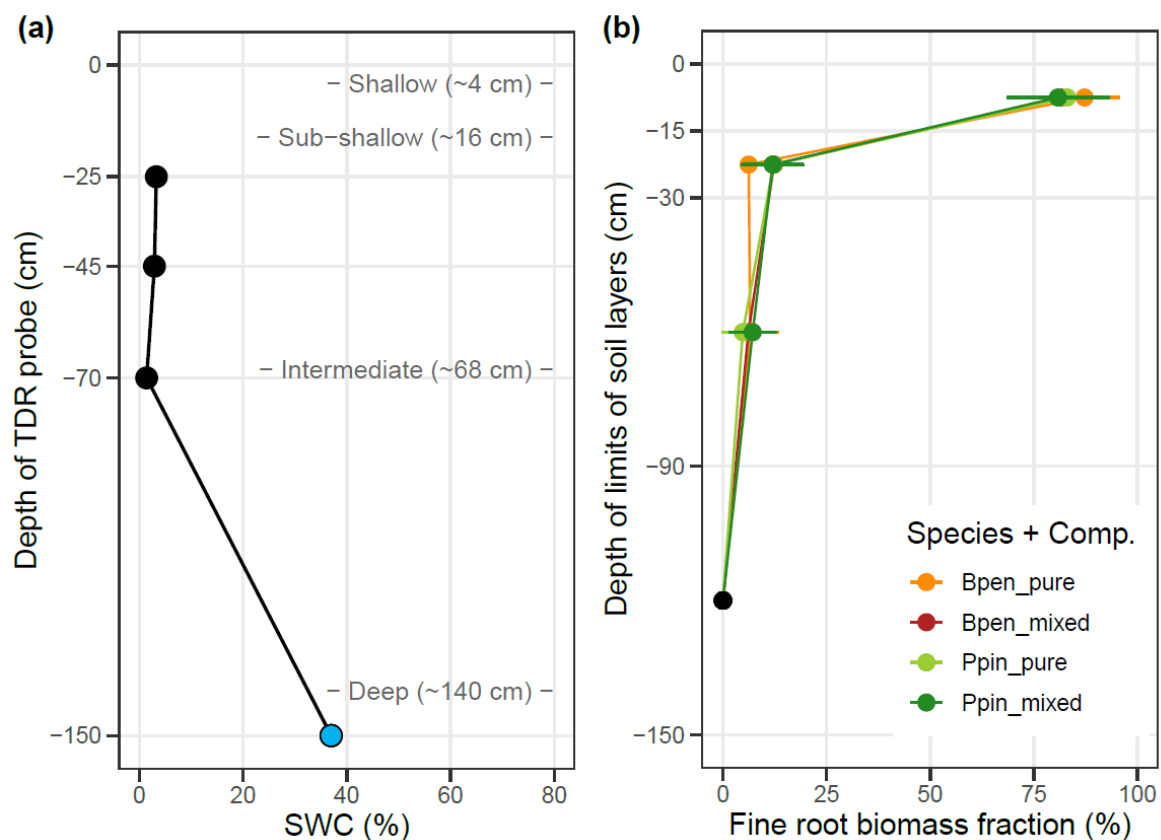


Fig. III.S 5.2 Output posterior plots of the probability distributions from the Bayesian mixing model with “SP\_stand” and “Block” as fixed factors.



**Fig. III.S 5.3** Summary of MixSIAR results for each block represented either (a) by plot composition to observer *Species* effects or (b) by species to observe *Plot composition* effects. Each stacked section in a bar represents the median source contribution value (50 % quartile) of one depth category. See Fig. III.S 5.2 for the full probability distributions. This is an alternative representation for the same data as in Fig. III. 5.7 and Fig. III. 5.8.



**Fig. III.S 5.4** Soil water content and fine root biomass fraction complementary data. **(a)** Average soil water content at Castillonville GIS Coop plantation for **August 2018** for probes at a depth of 25, 45 and 70 cm. The SWC at 150 cm is estimated at 37 % for a saturated soil at groundwater table depth: this value was the maximum observed in SWC in winter at this site, and is in the range for a sandy soil at saturation. The average depth of the soil water categories identified for the isotope mixing model are indicated in grey text. **(b)** Fine root biomass fraction in **March 2018** at ORPHEE estimated for three depth categories (0-15 cm, 15-30 cm and 60-90cm). The value for 90-150 cm is estimated to be close to 0 % but was not measured in the field. This data was adapted from Altinalmazis-Kondylis (2020).



**Fig. III.S 5.5** Example of soil color evolution with depth for Block 2, Plot 1 (pure birch) in July 2019. The top of the picture represents the topsoil (0-5 cm) and each following box the following depths (5-10, 10-15, 15-25, 25-35, 35-45, 45-55, 55-65, 65-75, 75-85, 85-100, 100-115, 115-125). We can observe here that the top horizons are the ones with the most organic matter.



## Chapter III.6

### General discussion





## 6. General discussion

In this general discussion of **Chapter III**, I present an overview of the results obtained in the different sub-chapters and how they relate to each other. The goal here is to discuss what I learned from the different sub-chapters (i) on the differences in functioning between the two studied species, (ii) on how they responded to drought and/or irrigation, and (iii) on how they interacted in mixed stands. Finally, I will discuss the underlying mechanisms behind the presence or absence of mixture effects.

*Note: The figures from the sub-chapters of Chapter III are numbered with the chapter, sub-chapter, and figure number. For example, “Fig. III.3.6” is Figure 6 in the sub-chapter 3 (sap flux density) of Chapter III, and “Fig. III.S 3.1” is Figure 1 in the supplementary figures of sub-chapter 3.*

### 6.1. Functional differences between species in pure plots of the non-irrigated blocks

#### 6.1.1. Water use in the absence of water stress

Birch and pine showed different strategies in terms of water use in pure plots at ORPHEE. Indeed, in non-limiting soil water conditions, pine had a higher transpiration than birch at the tree and stand scale ( $T_{\text{plot}}$ , **Fig. III. 3.11**) despite a lower sap flux density ( $F_d$ , **Fig. III. 3.6**) and maximum hydraulic conductivity compared to birch ( $K_{\text{max}}$ , **Fig. III.A 3**). This was the result of a higher sapwood area for pine trees compared to birch, due to a faster diameter growth for pine (**Fig. III.S 4.3**) and which resulted in a higher stand basal area (and stand sapwood area) in pure pine plots (**Fig. III. 3.2**). Finally, birch had higher tree-ring  $\delta^{13}\text{C}$  (i.e., a higher water use efficiency) than pine during wet summer conditions. In conclusion, in the absence of water stress, the two species showed strong differences in water use and physiological functioning, which I expected could translate into differences in the response to summer drought and into potential mixture effects.

#### 6.1.2. Response to drought

The different results concerning the response of birch and pine to drought in pure plots seemed contradictory at first. Indeed, birch trees had a higher predawn leaf water potential ( $\Psi_{\text{pd}}$ ) than pine in pure plots (**Fig. III. 2.1**), indicating that there was a higher water availability for birch than for pine trees at the end of July 2019. I hypothesized that this was the result of the higher water use in pure pine plots compared to pure birch (**Fig. III. 3.11**), which could have led to a faster depletion of soil water resources. In line with these results, I also found an absence of increase in  $\delta^{13}\text{C}$  between a wet year and two dry years for birch, while pine  $\delta^{13}\text{C}$  had increased during dry years (**Fig. III. 4.7**). This led me to think that

while pine suffered from a strong water deficit, birch did not. However, during the summer drought of 2019, I did observe a strong loss of sap flux density for both species that was highly synchronized between the two species in the non-irrigated block: birch lost the same proportion of sap flux density than pine with time during the summer drought of July (**Fig. III. 3.7, Fig. III. 3.8**). This seemed contradictory, as data from the  $\Psi_{pd}$  and  $\delta^{13}C$  indicated that birch would have a lower or delayed exposure to water deficit than pine during drought events.

From the literature, I expected birch to be less drought tolerant than pine (Niinemets and Valladares 2006; Andivia et al. 2020). Moreover, it must be noted that in this region, silver birch is at the southern limit of its distribution range, close to the highest mean annual temperature in which it can grow in Europe (Beck et al. 2016), whereas maritime pine is at the heart of its climatic range (Abad Viñas et al. 2016). Therefore, it seems likely that birch would be experiencing water deficit during a dry summer in this climate. Because of this lower drought tolerance, birch might respond to drought with stomatal closure at less negative  $\Psi_{pd}$  values than pine. Precise soil water content (SWC) data from each plot at different depths would have been needed to answer this question and could be an interesting perspective for future research. Moreover, we know that birch suffered from drought as we observed leaf loss in July 2018 and 2019 (**Appendix A, p. 321** and **Appendix B, p. 327**), indicating a drought avoidance strategy for birch (Wendler and Millard 1996). This can also be linked to the higher xylem vulnerability to cavitation of birch (**Fig. III.A 3**), which is a good indicator of drought tolerance (Maherali et al. 2004; Awad et al. 2010). Therefore, the seemingly contradictory results from  $\Psi_{pd}$  and loss of sap flux density during drought were probably the result of different factors acting in opposite directions: on the one hand, birch had a slower growth in pure plots, and the evaporative demand in the pure birch plots was smaller than in the pure pine plots. This resulted in a higher soil water availability at a given point in time. On the other hand, birch was more sensitive to drought than pine and therefore responded to drought at a lower threshold of water deficit than pine. Overall, this resulted in a synchronized response of sap flux density to drought for the two species. It was then interesting to test if these different interacting factors would result in a change in drought exposure for one or both species when grown in mixed stands.

## 6.2. Irrigation effects on tree functioning in pure plots

### 6.2.1. Acclimation to irrigation

I was interested to test how the summer irrigation treatment set up in 2015 could impact birch and pine functioning in pure plots on the mid-term, and how these trees acclimate to these conditions. Indeed, the irrigation treatment gave me interesting insights into the origin of the different responses in non-irrigated plots and whether they were indeed due to differences in water availability. However, one major limit of this approach is that, as irrigation also impacted the growth dynamics of the trees, there are

interactions between long-term irrigation effects on growth and light interception and short-term irrigation effects on drought response. Indeed, irrigation resulted in a higher growth (in height and DBH) for both birch and pine (**Fig. III.S 4.3**). This, in turn, resulted in a higher basal area in irrigated pure plots compared to non-irrigated ones (**Fig. III. 3.2**) and in an increase in plot level transpiration (**Fig. III. 3.11**). Birch and pine also had lower leaf and needle  $\delta^{13}\text{C}$  in 2019 in the irrigated pure plots compared to the non-irrigated pure plots (**Fig. III.S 4.2**), which indicated a strong physiological acclimation to a higher water availability in these plots, or, if looked the other way, it indicated that the trees in the non-irrigated plots do suffer every year from drought stress.

Irrigation effects on growth and transpiration were stronger for birch than for pine (**Fig. III.S 4.3**). Consequently, irrigation more strongly impacted the canopy closure of pure birch plots: the leaf area index (LAI) in birch plots in 2019 was two times higher in the irrigated plots than in the non-irrigated ones (**Fig. III.S 3.2**). Moreover, there was a decrease in  $\delta^{13}\text{C}$  between before and after the onset of the irrigation treatment (i.e., between 2013 and 2016 or 2018) for birch but not for pine (**Fig. III. 4.7**). For birch, this could indicate a higher water availability in these plots and/or a lower light availability for their crowns compared to 2013. The second hypothesis seemed more likely as 2013 was a wet year and seemed confirmed by the absence of a change for pine. Finally, I observed long-term physiological acclimations to the irrigation treatment for birch, but not for pine. Indeed, in the irrigated blocks birch was more vulnerable to embolism (higher  $\Psi_{50}$ , **Fig. III.A 3**). The combination of these different irrigation effects on birch indicated a stronger water limitation for birch than pine in non-irrigated blocks due to a lower drought tolerance for birch, as hypothesized in the previous section. However, because of the irrigation effect on canopy closure, I am not certain of the magnitude of the effects, as some of them ( $\delta^{13}\text{C}$ ,  $\Psi_{50}$ ) can also be explained by a acclimation to a lower irradiance (Warren et al. 2001; Barigah et al. 2006).

### 6.2.2. Summer drought exposure in irrigated blocks

With the irrigation treatment, exposition to summer drought was relieved but not completely removed for the trees in this experimental plantation. Indeed,  $\Psi_{\text{pd}}$  values in July 2019 (**Fig. III. 2.1**) indicated that pine trees still were under a water deficit that can be qualified as “moderate”. In addition, I observed that sap flux density of both species decreased during the summer drought of 2019 even in the irrigated blocks (**Fig. III. 3.7**, **Fig. III. 3.8**), albeit later and in a lower proportion than in the non-irrigated blocks. Surprisingly, the birch trees in the irrigated pure plots had an earlier and stronger loss of sap flux density than the pines in the irrigated pure plots (**Fig. III. 3.7**, **Fig. III. 3.8**, **Fig. III. 3.9**), although it had a less negative  $\Psi_{\text{pd}}$  than pine during this period. In the sub-chapter on sap flux density (**Chapter III.3, p. 149**), I had hypothesized that this could be due to a lower efficiency of the irrigation input in the pure birch plot, because of a stronger evaporation in the more open birch plots compared to the pine ones. This suggests that in this plantation,  $\Psi_{\text{pd}}$  may not be the best indicator of drought stress. Indeed, in

heterogeneous soil conditions, such as with irrigation,  $\Psi_{pd}$  might align with the water potential of the most humid zone (Améglio et al. 1999). In this case, the variations of sap flux density seem to provide a better indicator of drought stress for birch (Remorini and Massai 2003). Therefore, contrary to what was expected at the time of the setup of the summer irrigation treatment (H. Jactel, pers. comm.), I found that the irrigation input was not sufficient to remove water stress in this plantation.

## 6.3. Mixture effects

### 6.3.1. Mixture effects on growth dynamics

In terms of growth, mixing birch and pine resulted in a higher productivity than the one expected from the average of the two pure plots after 2014 (Morin et al. 2020; Toïgo et al. 2021a) (see also **Fig. III.S 4.3**). This is typically a pattern that can be called “overyielding” (Loreau and Hector 2001). However, the mixed plots did not have a higher productivity than the most productive species (here, pine), suggesting the absence of “transgressive overyielding” in this plantation (Morin et al. 2020; Toïgo et al. 2021a). This is also what I observed and discussed in **Chapter II** with crown volumes and canopy packing in 2017. These stand-level observations were the results of an overyielding for pine when mixed with birch and of an underyielding for birch when mixed with pine that was not strong enough to counterbalance the overyielding effect of pine. Indeed, in mixed plots, pine crowns benefited from the competition release of smaller birch crowns and then expanded their crowns laterally (**Chapter II**), but also pine trees grew faster in height than birch after 2014 (**Fig. III.S 4.3**). These changes in canopy closure and light availability for trees are of primary importance as they also impacted mixture effects on water use and drought response.

In irrigated blocks, birch trees were able to maintain a speed in height growth that was similar to pine trees in mixed plots until 2018, but diameter growth was always lower for birch in mixed plots compared to pure plots (**Fig. III.S 4.3**). The leaf area index (LAI) in 2019 was higher in the mixed plots than both pure plots (**Fig. III.S 3.2**), showing a positive mixture effect on LAI that was stronger with irrigation than in non-irrigated plots. Unfortunately, the overyielding was not yet studied in the irrigated blocks, as the currently published papers focused on data before the onset of the irrigation treatment (Morin et al. 2020; Toïgo et al. 2021a).

### 6.3.2. Mixture effects on water use in the absence of water stress

In the absence of water stress, there were no mixture effects on individual tree functioning and water use. I expected this result, as potential belowground complementarities for water acquisition are less likely to be expressed in the absence of water stress (Grossiord et al. 2013a; Forrester 2014). This was observed for example with sap flux density in the spring period which was similar in both pure and mixed plots for both species (**Fig. III. 3.6**). Similarly, there were no mixture effects on tree-ring  $\delta^{13}\text{C}$  in

2013, a wet year in the early stages of the plantation (**Fig. III. 4.5**). However, in 2019, leaf and needle  $\delta^{13}\text{C}$  was lower, but also more variable, in mixed plots than in pure plots for birch (**Fig. III.S 4.2**). Since this was true in both non-irrigated and irrigated blocks, it was more likely to be an effect of a lower light availability for birches in mixed plots with the shading from pine trees. Overall, except for leaf and needle  $\delta^{13}\text{C}$ , there was no sign of mixture effects at the tree level in the absence of water stress.

At the stand scale, water use in mixed plots was higher than in pure birch plots and lower than in pure pine plots ( $T_{\text{plot}}$ , **Fig. III. 3.11**). However, it was higher than the expected average from the two pure plots (**Fig. III. 3.13**). This difference from the expected average shows that there were interactions between the two species when planted together that modified their functioning compared to the monocultures. These results were the consequence of mixture effects on growth and therefore total sapwood area in a plot as there were no mixture effects on sap flux density at the individual tree scale. In the irrigated blocks, the irrigation effects on growth affected the results of mixture effects on water use compared to non-irrigated blocks ( $T_{\text{plot}}$ , **Fig. III. 3.11**). Indeed, while the water use was still intermediate between pure birch and pure pine, it was this time lower than the expected average from the two pure plots (**Fig. III. 3.13**). This was the consequence of a stronger negative mixture effect on birch basal in the irrigated blocks compared to the non-irrigated blocks (**Fig. III. 3.2**). A higher transpiration in mixed stands can be related to a higher productivity in the absence of water stress (Forrester et al. 2010; Kunert et al. 2012; Gebauer et al. 2012), but might be detrimental during droughts, making the trees in mixed stands more susceptible to drought (Jacobs et al. 2021). Therefore, from these stand-level results, one might have expected that birch would be more stressed in mixed plots because of the higher water use of neighboring pine trees.

### 6.3.3. Mixture effects on drought response

Contrary to my initial expectations, there were very few mixture effects on the drought response of birch and pine in the non-irrigated blocks. Birch trees showed no mixture effect on the loss of sap flux density during the summer of 2019 (**Fig. III. 3.8**, **Fig. III. 3.9**), which was consistent with an absence of mixture effect on predawn leaf water potential on July 19<sup>th</sup>, 2019 (**Fig. III. 2.2**) and on the vulnerability to cavitation (**Fig. III.A 4**). On the other hand, although birch trees had a lower  $\Delta\delta^{13}\text{C}$  between 2013 and 2018 in mixed stands than in pure stands, which might indicate a lower drought exposure, the fact that the  $\Delta\delta^{13}\text{C}$  value in mixed stands was negative suggested instead a mixture effect due to a lower light availability because of the domination of birch crowns by pine trees in mixed plots (**Fig. III. 4.7**). Similarly, for pine trees, there were no mixture effects on predawn leaf water potential on July 19<sup>th</sup>, 2019 (**Fig. III. 2.2**) and on the vulnerability to cavitation (**Fig. III.A 4**), but also on drought exposure measured with  $\Delta\delta^{13}\text{C}$  (**Fig. III. 4.7**). There was however a weak mixture effect on the loss of sap flux density during the summer of 2019 (**Fig. III. 3.8**, **Fig. III. 3.9**). The loss of sap flux was slightly faster for pine in mixed plots than in pure plots, which I explained by a higher exposure of pine crowns to

radiation when mixed with birch, because of the lower light interception of birch crowns, but also because they reached smaller heights than pine. This hypothesis is confirmed by the absence of mixture effect on predawn leaf water potential, indicating that the effect did not originate from belowground soil water availability. Because of this faster loss of sap flux density for pine, there was a shift at the plot level from a higher water use than expected from the average of the pure plots in the absence of water stress to a lower water use than expected from the pure plots at the end of the drought event (**Fig. III. 3.12, Fig. III. 3.13**).

In the irrigated blocks, I observed a few mixture effects, especially for birch. There were no mixture effects on  $\Psi_{pd}$  for either species in the irrigated blocks. However, birch trees had a higher and faster loss of sap flux density in the pure birch plot compared to the mixed one (**Fig. III. 3.8, Fig. III. 3.10**). There was also a strong mixture effect on  $\Delta\delta^{13}C$  which indicated a lower drought exposure or a lower light availability in mixed plots compared to pure plots (**Fig. III. 4.7**). Finally, there was a small mixture effect on birch vulnerability to cavitation (**Fig. III.A 4**), with a higher vulnerability in mixed plots. The fact that most of these effects for birch were not significant in the non-irrigated blocks is interesting because, as most water deficit was removed in the irrigated blocks, it confirms that these effects are more likely due to aboveground species interactions. For pine, there were no mixture effects in the irrigated blocks for all variables ( $\Psi_{pd}$ ,  $F_{d\ loss}$ ,  $\Delta\delta^{13}C$  and  $\Psi_{50}$ ). At the stand scale, the net mixture effect on plot-level transpiration shifted from a lower water use than expected from the pure plots in the non-irrigated block to a higher water use than expected in the irrigated block. I had not expected to find mixture effects on drought exposure in irrigated blocks, as I thought that mixture effects would be absent when water was not a limiting factor (Grossiord et al. 2013a; Forrester 2014). However, as noted in the previous section, the irrigation did not completely remove water stress. But the mixture effects observed in the irrigated blocks were more likely to come from canopy structure modifications rather than from a higher water availability in mixed plots. Indeed, since all these mixture effects were observed for birch and not for pine, and that no mixture effects were observed on  $\Psi_{pd}$ , this indicated that the mixture effects were not related to belowground water availability, but rather due to aboveground effects with lower light availability for birch in mixed plots.

The absence of clear mixture effects on drought response is consistent with a growing body of literature that shows that the beneficial effect of mixtures cannot be generalized, being greatly modulated by the type of admixture and tree species identity, and depending on site water supply, soil characteristics and stand characteristics, such as basal area and age (Ammer 2019; Grossiord 2019; Pardos et al. 2021). In our case, we had expected a lower drought exposure in mixed stands for this specific species combination due to belowground complementarities in rooting depths between the species, so the absence of strong mixture effects on drought exposure suggests an absence of belowground complementarity effects. Also, the degree of the water stress might affect the presence or absence of mixture effects: mixture effects could be expected at intermediate drought levels, while absent during

extreme drought events (de Streel et al. 2020). However, in this case we were not in extreme drought conditions so we should have been able to see potential mixture effects in these conditions.

## 6.4. Underlying mechanisms

### 6.4.1. Belowground complementarity for water uptake

Overall, the few mixture effects that were found, both in non-irrigated and irrigated blocks, were hypothesized to come from canopy closure and shading of birch by pine in mixed plots compared to pure plots. There was no strong evidence for a belowground complementarity in water use between the two species that could result in a lower drought exposure in mixed stands and a better resistance to drought. Because sap flow and  $\delta^{13}\text{C}$  are also directly influenced by light related processes it makes it difficult to tease apart belowground and aboveground effects.

In the last section of the chapter (**Chapter III.5, p. 229**), I directly estimated the relative contribution to root water uptake of soil water sources at different depths, without confounding factors related to light. To test whether there was a belowground complementarity in water acquisition between birch and pine in mixed plots, I compared water isotopes in the soil at different depths with water isotopes in the xylem water of branches to estimate the relative contribution of soil water from different depths.

Contrary to my initial expectations, there was no evidence for a vertical stratification of water uptake between the two species either in pure plots or in mixed plots (**Fig. III. 5.2, Fig. III. 5.7, Fig. III. 5.8**). Both species extracted water mainly from the first few centimeters of soil and from the deeper soil layers below 80 cm. Only a very small proportion of the extracted water came from the intermediate soil layers. These contributions from the different soil water categories did not vary much between species and between pure and mixed plots. The high contribution from the deep soil water category for both species was explained by a small number of fine roots taking up water from the unsaturated zone above the water table with water coming up by capillary rise.

The absence of mixture effects was consistent with the results from the other sub-chapters on water use and response to drought in which I saw little evidence for a potential belowground complementarity, but also with the results from Altinalmazis-Kondylis (2020) on fine root biomass production at ORPHEE in March 2018. As mentioned in **Chapter III.5**, despite potentially contrasted rooting patterns between the two species, these were under strong constraints of the soil profile at ORPHEE with a shallow fluctuating water table and a hardpan layer in the soil, that both prevented the roots to grow towards deeper soil horizons (Danjon et al. 2005; Altinalmazis-Kondylis et al. 2020).

Although belowground water uptake differentiation between species is often hypothesized as a potential mechanism behind mixture effects on productivity or drought resistance, there is actually very little evidence of this hypothesis. Two examples come to my mind in which they found species-specific water

uptakes in experiments that had also evidenced aboveground overyielding (Mueller et al. 2013; Schwendenmann et al. 2015). The other studies that have evidenced vertical stratification of roots between species did not have companion papers measuring mixture effects on stand productivity or other ecosystem functions (Schmid and Kazda 2001; Bolte and Villanueva 2006). Some studies that did associate ecosystem functions and processes were not able to link belowground complementarity to mixture effects on ecosystem functions. For example, in Martin-Blangy et al. (2021) (see **Chapter I**), the presence of a vertical stratification between beech and fir did not result in a lower drought exposure in mixed stands. In Goisser et al. (2016), mixture induced a shift in spruce rooting patterns but there were no consequences on growth in mixed stands. On the contrary, some studies that did find positive mixture effects on productivity did not find belowground complementarity (Fruleux et al. 2018, 2020). Based on my results and this literature, there is little evidence so far that belowground complementarity plays a direct role in positive mixture effects on productivity. However, mixture effects on productivity are measured over an integrated period (i.e., one year) while experiments measuring fine root biomass distribution or water uptake are representative of a single point in time. In my case, the results that I obtained with the water isotope experiment are representative of the dry season conditions only, but not for the whole growing season.

#### 6.4.2. Aboveground mechanisms

In this chapter, I have shown that canopy structure in pure versus mixed plots can influence water relations and drought response in many ways and should not be underestimated when interpreting mixture effects. Indeed, mixing birch and pine resulted in higher crown volumes for pine but also a higher canopy packing (**Chapter II**) and a stratification of the two species with birch being dominated by pine (**Fig. III.S 4.3**). This then probably impacted the light availability of birch and pine in opposite ways compared to pure plots, with lower light availability for birch and higher for pine in mixed plots. Moreover, this is a dynamic process, especially in such a young plantation, and therefore mixture effects will change with the evolution of the canopy structure. Such effects of canopy structure on mixture effects have been observed in the literature before. For example, Fichtner et al. (2020b) observed that microclimate amelioration via facilitative neighborhood interactions might act as a further mechanism, on top of resource partitioning, by which the water demand of a focal tree growing in diverse neighborhoods is decreased. These effects are possible to interpret only if we study the response of each species rather than whole stand response and emphasizes the importance to look at both scales when looking to understand mixture effects (Trogisch et al. 2021).



## 6.5. Conclusion

In this chapter, I found no evidence for belowground complementarity in water use for these two species in mixed plots. The absence of belowground complementarity, which is in contradiction with my initial expectations based on the functional characteristics of the two species, reflects the outcome of the realized interactions between these two coexisting species in this plantation. This absence might be related to the specific soil conditions at ORPHEE and could be expressed differently in other site conditions. However, the few mixture effects that I found on water use and drought response were related to the impact of species interactions on canopy structure. Overall, this species assemblage does not have a higher or lower water availability than when planted in monocultures. However, since pine was able to grow faster than birch in mixed plots, birch was slightly less exposed to drought because of a lower light availability. This also impacted birch growth and might result in a complete domination of birch by pine in mixed stands in a few years, and perhaps to birch mortality, as this species is a light-demanding, shade-intolerant species (Hynynen et al. 2010; Beck et al. 2016). These results highlight the importance of considering the species' functional characteristics (rooting pattern and shade tolerance) but also their growth dynamics when the choice of species assemblage in mixed plantations is discussed by forest managers. Moreover, my results highlight the importance of the impact of (i) climatic conditions, with the irrigation treatment that modified the mixture effects compared to non-irrigated blocks, and (ii) site soil conditions, with the presence of a shallow water table which impacted rooting patterns, on the expression of potential mixture effects.



# Appendix III.A

## 7. Appendix III.A: Xylem embolism resistance

### 7.1. Context

When xylem sap is exposed to large negative pressures, cavitation can occur and air embolism can form (Cochard 2002). Widespread cavitation or embolism in xylem (under severe water stress) reduces a plant's capacity to transport water from soil to leaves. This reduction in xylem's hydraulic conductivity can impair the rate of carbon fixation by inducing stomatal closure to prevent further cavitation and desiccation of leaf tissues. This may result in premature mortality of roots or twigs and could ultimately lead to tree death (Bréda et al. 2006). Vulnerability to drought-induced embolism is therefore a key component for drought tolerance of trees. It can be estimated by the xylem tension at which a certain percentage loss of hydraulic conductivity occurs, typically a threshold of 50 % loss of conductivity is used ( $\Psi_{50}$ ).

We investigated whether species interactions and the irrigation treatment impacted the maximum conductivity and  $\Psi_{50}$  of branches at ORPHEE. This study was conducted by Bordeaux colleagues (S. Delzon, H. Jactel, M. Cambon and members of the Delzon lab) in the context of the ANR-DiPTiCC project. As this dataset revealed to be interesting to be analyzed in this chapter, I asked the authorization to use it and include it as an Appendix in this chapter. I am very thankful to all of them to allow me to include this dataset here.

### 7.2. Methods

#### 7.2.1. Study site

For this section, please refer to the description of the experimental site at the beginning of this chapter (**section 1.2**).

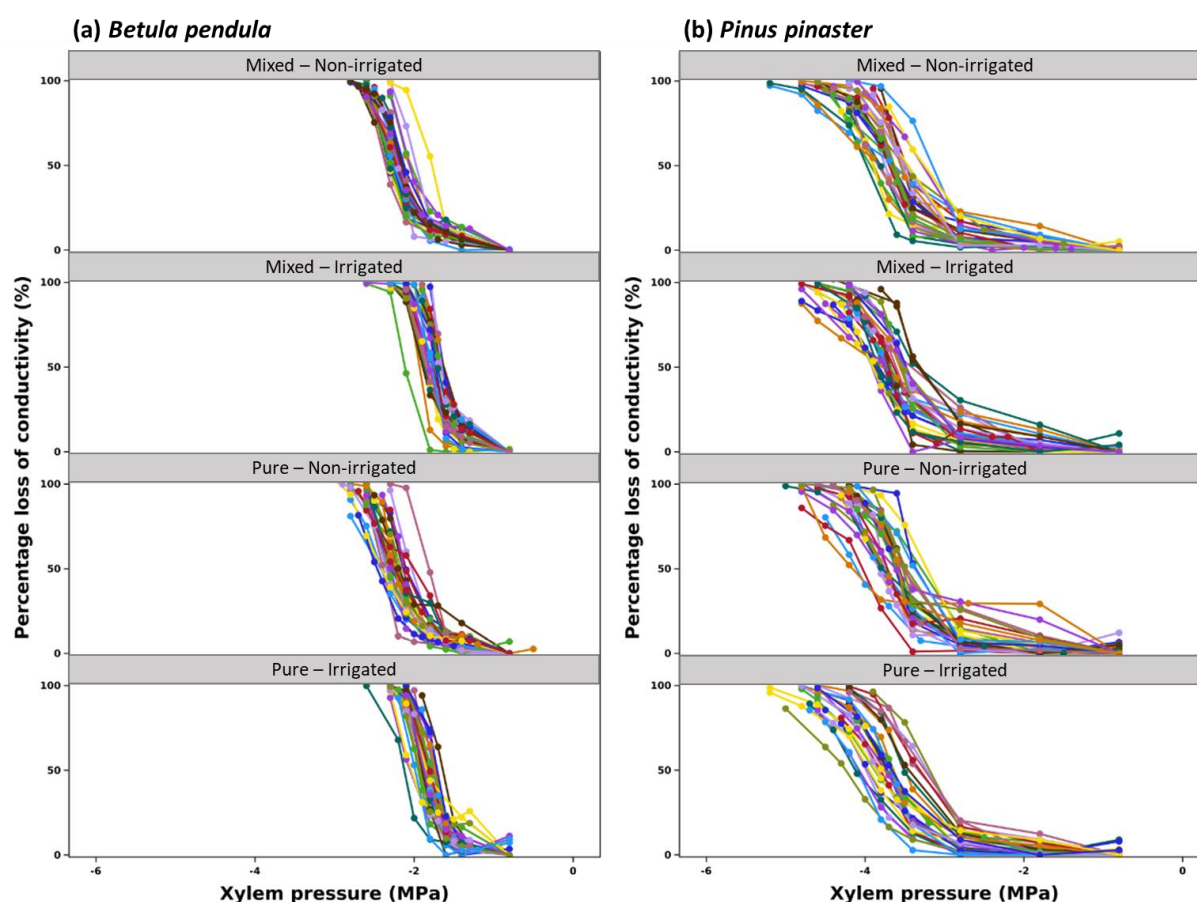
#### 7.2.2. Sampling

For this experiment, a 40-cm long branch was sampled on eight to ten trees per species and per plot. Trees were sampled in blocks 1 to 6 (three non-irrigated and three irrigated blocks), in pure birch, pure pine and mixed birch and pine plots. Branches were collected in February 2019 for blocks 1 and 2 and in March 2019 for blocks 3 to 6. After sampling, the branches were immediately defoliated to prevent transpiration loss, conditioned in wet cloth, and sealed in plastic bags to halt moisture loss. They were

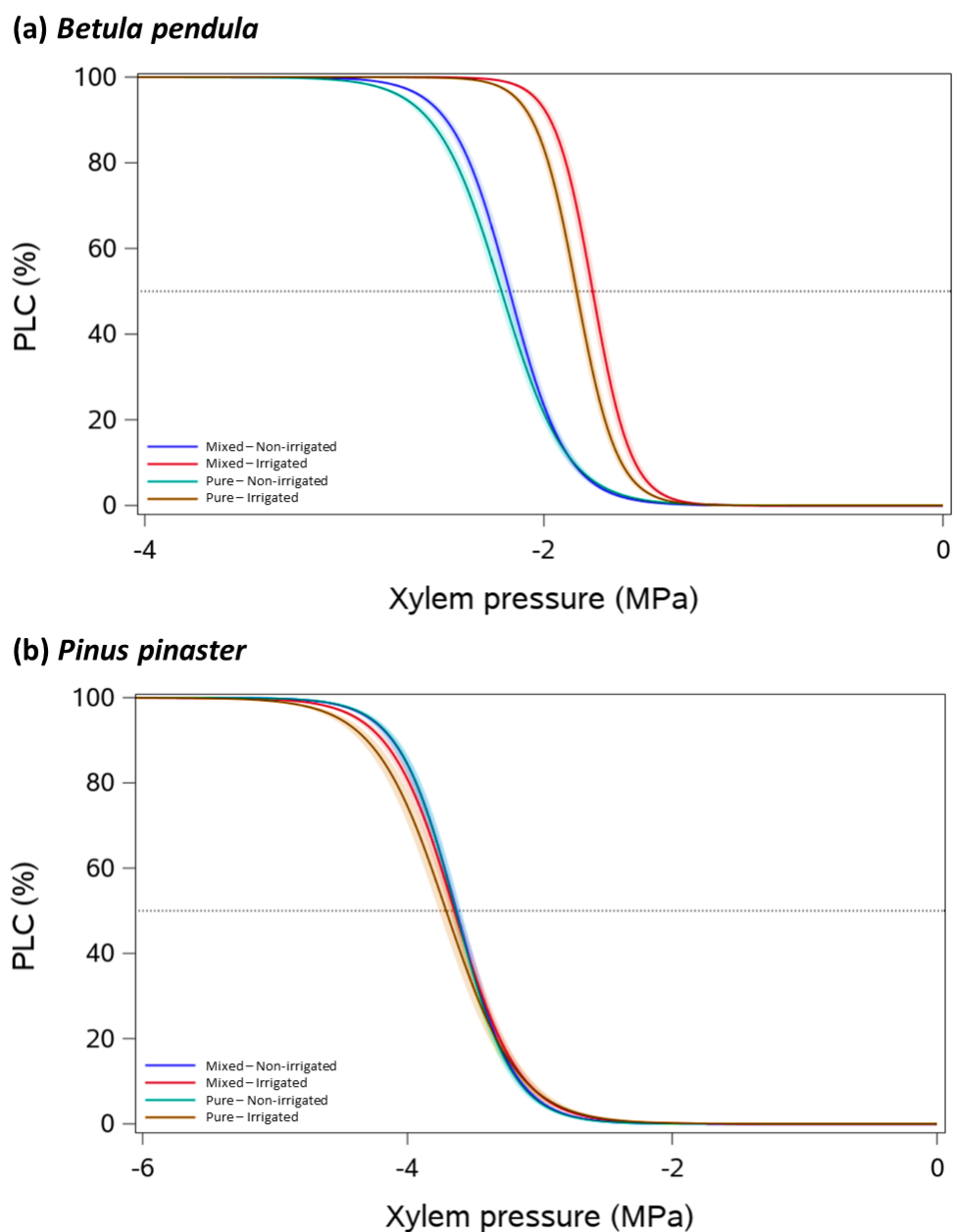
immediately taken to the Caviplace phenotyping platform (<http://sylvain-delzon.com/caviplace/>, Univ. of Bordeaux, Pessac, France) and kept refrigerated until measurements.

### 7.2.3. Vulnerability curves

Xylem cavitation was assessed with the CAVITRON, a centrifuge technique following the procedure described by Cochard (Cochard 2002; Cochard et al. 2005). Centrifugal force was used to generate negative pressure into the xylem and induce cavitation. Initially, the maximum xylem-specific hydraulic conductivity ( $K_{\max}$  in  $\text{m}^2 \text{s}^{-1} \text{MPa}^{-1}$ ) was calculated under low xylem pressures (close to zero). Then, rotation speed of the centrifuge was gradually increased by 0.5 or 1 MPa, to lower xylem pressure. The percentage loss of conductivity (PLC) of the stem was determined at each pressure step (see detailed method in Delzon et al. 2010). Vulnerability curves (VC) were obtained by plotting increasing PLC with decreasing xylem pressure (Fig. III.A 1, Fig. III.A 2). S. Delzon fitted the VC with a sigmoid function following the NLIN procedure in SAS (SAS 9.4, SAS Institute, Cary, NC, USA).  $\Psi_{50}$  (MPa), the xylem pressure inducing 50 % loss of branch hydraulic conductivity, is a widely used metric representing the steepest point of the VC (Fig. III.A 2), meaning that even a small decrease in water potential will cause a substantial reduction in hydraulic conductivity (Choat et al. 2012).



**Fig. III.A 1** Raw percentage loss of conductivity data at the different xylem pressure steps. This data was used by S. Delzon to model the vulnerability curves for each tree.



**Fig. III.A 2** Average vulnerability curves calculated by S. Delzon for each species, mixture and irrigation treatment combination. Note: the color scale is reversed compared to my usual graphs, the non-irrigated blocks are in blue while the irrigated blocks are in red.

#### 7.2.4. Statistical analysis

I tested whether there were differences in  $K_{\max}$  and  $\Psi_{50}$  between species, with or without irrigation, and between pure and mixed plots.

I first tested *Species* and *Irrigation* effects on  $\Psi_{50}$  and  $K_{\max}$  in **non-irrigated pure plots** with the following linear mixed model:

$$Y \sim \text{Species} + 1|\text{Block} \quad (1)$$

where  $Y$  is either  $\Psi_{50}$  or  $K_{\max}$ , *Species* is a fixed factor with two levels (birch and pine), and *Block* is a random factor with three levels.

I further tested *Irrigation* and *Mixture* effects on  $\Psi_{pd}$  for each species separately with the following linear mixed model:

$$Y \sim \text{Irrigation} + \text{Mixture} + \text{Irrigation:Mixture} + 1|\text{Block} \quad (2)$$

where  $Y$  is either  $\Psi_{50}$  or  $K_{\max}$ , *Irrigation* is a fixed factor with two levels (non-irrigated and irrigated), *Mixture* is a fixed factor with two levels (pure vs. mixed) and *Block* is a random factor with six levels. I used post-hoc multiple comparison tests to identify if pure and mixed plots were statistically different from each other within each model and for each species.

R 3.6.2 language programming (R Core Team 2019) with the Rstudio interface was used for all the analyses with the following packages: nlme (“lme” function), MuMIn and emmeans (“lsmeans” function). Type “III” ANOVAs were used after linear mixed models.

## 7.3. Results and discussion

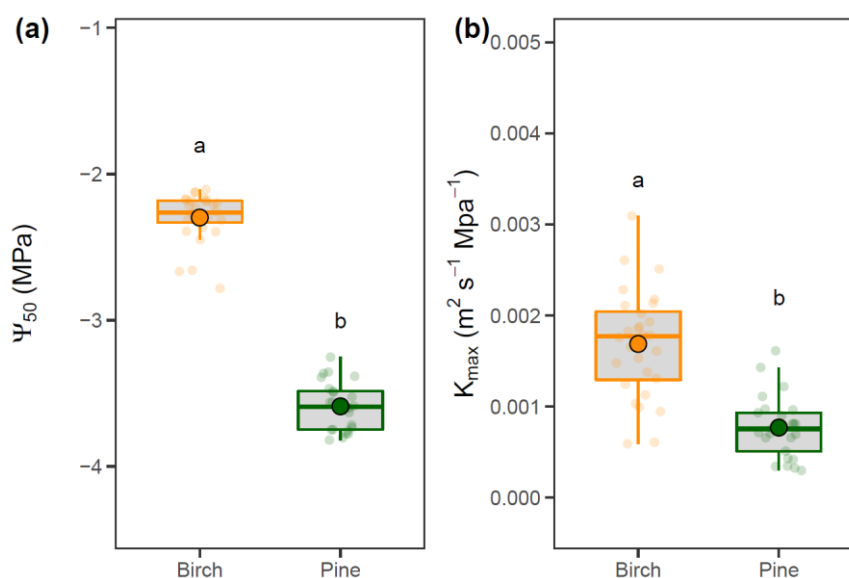
### 7.3.1. Species effect in non-irrigated pure plots

There was a strong species effect on xylem vulnerability to embolism ( $\Psi_{50}$ ) (**Fig. III.A 3a**) and on maximum hydraulic conductivity ( $K_{\max}$ ) (**Fig. III.A 3b**). For  $\Psi_{50}$  the average in non-irrigated pure plots was  $-2.3 \pm 0.03$  MPa (mean  $\pm$  standard error of the mean) for birch and  $-3.6 \pm 0.03$  MPa for pine. For  $K_{\max}$  the average in non-irrigated pure plots was  $1.7 \cdot 10^{-3} \pm 0.1 \cdot 10^{-3} \text{ m}^2 \text{ s}^{-1} \text{ MPa}^{-1}$  for birch and  $0.8 \cdot 10^{-3} \pm 0.1 \cdot 10^{-3} \text{ m}^2 \text{ s}^{-1} \text{ MPa}^{-1}$  for pine.

The differences between species are consistent with  $\Psi_{50}$  values that have been previously reported for birch and pine (Martínez-Vilalta and Piñol 2002; Herbette and Cochard 2010; Choat et al. 2012; Lamy et al. 2014; González-Muñoz et al. 2018). In fact, in general, angiosperms are known to have a lower tolerance to embolism and a smaller hydraulic safety margin than gymnosperms (Choat et al. 2012). Furthermore, birch is a species that has a particularly high vulnerability to cavitation compared to other angiosperms (Barigah et al. 2006).

The higher hydraulic conductivity for birch compared to pine can be explained by anatomical differences for water transport between the species, with vessel-bearing wood for birch and tracheids for pine (Becker et al. 1999).

For birch, variability levels were much higher for  $K_{\max}$  than for  $\Psi_{50}$  which has been previously reported (González-Muñoz et al. 2018).  $\Psi_{50}$  is thought to be a trait under a uniform evolutionary selection leading to trait conservatism, while hydraulic conductivity could be more strongly influenced by interactions between genotype and environment but also vary with sampling position (González-Muñoz et al. 2018).



**Fig. III.A 3** Species effect on  $\Psi_{50}$  and  $K_{\max}$  in non-irrigated pure plots. Letters represent the results of linear mixed models for  $\Psi_{50}$  and  $K_{\max}$  separately.

### 7.3.2. Irrigation and mixture effects

For birch, we observed a significant irrigation effect on  $\Psi_{50}$  in pure plots, with less negative values (i.e., a higher vulnerability to embolism) in irrigated blocks compared to non-irrigated blocks:  $\Psi_{50}$  went up from  $-2.3 \pm 0.03$  MPa to  $-1.9 \pm 0.03$  MPa with irrigation (an increase of +0.4 MPa) (**Fig. III.A 4**). Similarly, there was a significant increase in  $\Psi_{50}$  with mixture for birch, but only in irrigated plots ( $-1.7 \pm 0.02$  MPa in irrigated mixed plots). However, the size of this effect was small (+0.2 MPa).  $K_{\max}$  did not increase significantly with irrigation in pure plots, but did increase significantly in mixed plots from  $1.6 \cdot 10^{-3} \pm 0.1 \cdot 10^{-3} \text{ m}^2 \text{ s}^{-1} \text{ MPa}^{-1}$  in non-irrigated mixed to  $2.9 \cdot 10^{-3} \pm 0.2 \cdot 10^{-3} \text{ m}^2 \text{ s}^{-1} \text{ MPa}^{-1}$  in irrigated mixed.

For pine, there were no significant irrigation or mixture effects on  $\Psi_{50}$  or  $K_{\max}$ .

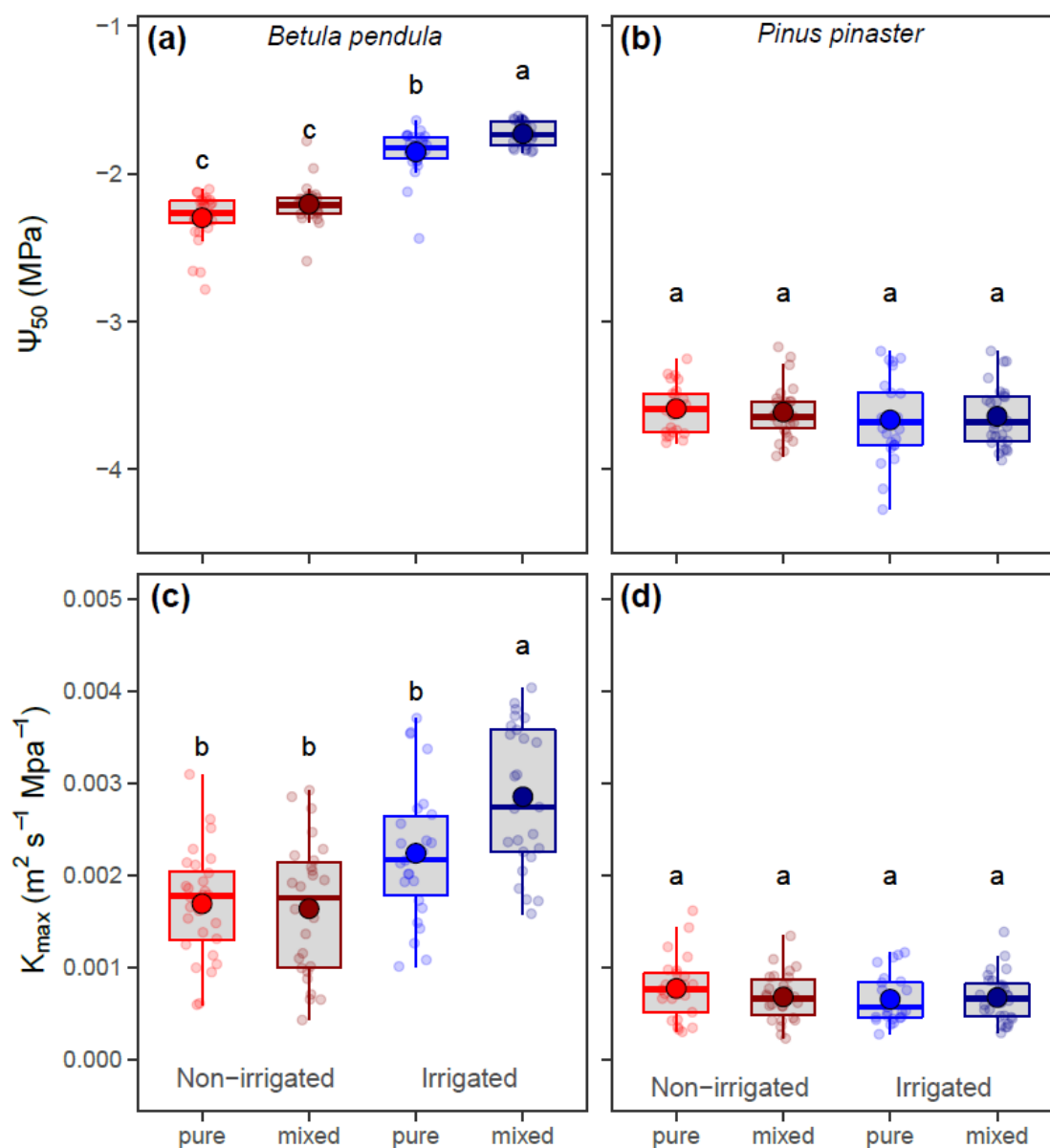
Therefore, birch showed a higher plasticity for a drought-tolerance related trait ( $\Psi_{50}$ ) than pine. The low variability for  $\Psi_{50}$  was not surprising. In fact, gymnosperms in general, and maritime pine in particular, are known to have a lower phenotypic plasticity and acclimation potential for the vulnerability to embolism (Lamy et al. 2014; Choat et al. 2018). Angiosperm species, including birch, have a higher potential for phenotypic plasticity, although the observed shifts in vulnerability are often small in magnitude (Awad et al. 2010; Schuldt et al. 2016; Stojnić et al. 2018; Choat et al. 2018).

The adaptation of birch  $\Psi_{50}$  to the irrigation treatment is consistent with what we expected: a higher water availability in irrigated blocks resulted in the acclimation in the vulnerability to embolism in the absence of a water constraint. A decrease in vulnerability to cavitation with increasing drought conditions has been observed before for a hybrid poplar (*Populus tremula*  $\times$  *Populus alba*), and was correlated with anatomical changes such as decreased vessel diameter and increased vessel wall thickness in drier conditions (Awad et al. 2010). Similar anatomical adaptations could explain the observed irrigation effect on  $\Psi_{50}$  (i.e., wider xylem vessels in the irrigation treatment).

The slightly higher  $\Psi_{50}$  in mixed irrigated plots compared to pure ones for birch could indicate a higher water availability for pure birch. However, xylem vulnerability to cavitation has also been shown to acclimate to other environmental conditions such as irradiance (Barigah et al. 2006), including for birch, with a higher vulnerability (i.e., less negative  $\Psi_{50}$ ) with decreasing irradiance. Therefore, a higher canopy closure and shading of birch trees by pine trees in mixed plots could be the source of this mixture effect on  $\Psi_{50}$ .

Increasing  $K_{\max}$  with irrigation and mixture for birch indicate that the acclimation of  $\Psi_{50}$  to water availability and irradiance levels were partly explained by anatomical changes in xylem vessels that would also result in a higher conductivity (vessel diameter, pit membrane structure...) (Lens et al. 2011).





**Fig. III.A 4** Irrigation and mixture effects on  $\Psi_{50}$  and  $K_{max}$  by species. Letters represent the results of post-hoc tests on linear mixed models testing for irrigation and mixture effects on  $\Psi_{50}$  and  $K_{max}$  respectively for each species.



# CONCLUSIONS ET PERSPECTIVES

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Photo : Soline Martin-Blangy. Bauges, France.



## 1. Cheminement de la thèse

Afin de mieux comprendre l'influence des interactions entre espèces sur le fonctionnement hydrique des arbres et les mécanismes aériens et souterrains sous-jacents, cette thèse avait pour objectif de répondre aux questions suivantes :

1. Quels sont les effets du mélange d'espèces sur le fonctionnement hydrique des arbres, notamment en conditions de sécheresse ?
2. Quels sont les effets du mélange d'espèces d'arbres sur le partage des ressources en eau et en lumière entre espèces ?
3. Est-ce que les mécanismes de partage des ressources en eau et en lumière peuvent expliquer les effets du mélange sur le fonctionnement hydrique ?

Pour étudier ces questions, je me suis basée sur des mesures expérimentales en lien avec : les processus d'utilisation de l'eau (flux de sève, composition isotopique du carbone dans le bois), l'extraction de l'eau par les racines (composition isotopique de l'eau dans le xylème) et l'interception de la lumière (volume de houppier, photos hémisphériques). Mes recherches se sont focalisées sur deux dispositifs expérimentaux : un dispositif d'observation de terrain en forêts naturelles gérées dans le Sud-Est de la France (GMAP) et une plantation expérimentale proche de Bordeaux (ORPHEE).

Dans le **Chapitre I** (Martin-Blangy et al. 2021), je me suis focalisée sur le dispositif GMAP afin de caractériser le fonctionnement hydrique et carboné pendant des années sèches dans deux types de mélange incluant le hêtre (hêtre-sapin et hêtre-chêne pubescent), et de déterminer si une complémentarité de profondeur d'extraction de l'eau était possible entre ces espèces.

Les **Chapitres II et III** sont centrés sur la plantation expérimentale d'ORPHEE (mélange bouleau-pin maritime) qui permettait une caractérisation plus fine des processus du fait du contrôle de nombreux facteurs confondants qui rendaient les interprétations plus compliquées dans le Chapitre I (sol, climat, topographie, âge des arbres et gestion...). Dans le **Chapitre II** (Martin-Blangy et al., *under review*), j'ai cherché à caractériser l'influence du mélange sur les volumes des houppiers et l'interception de la lumière du peuplement. Le **Chapitre III** fait en partie écho aux mesures du Chapitre I réalisées dans le réseau GMAP, en réalisant des mesures similaires dans la plantation ORPHEE. Dans ce chapitre, j'ai étudié le fonctionnement hydrique journalier (**Chapitre III.3**) et saisonnier (**Chapitre III.4**) en l'absence et en présence de stress hydrique et j'ai également caractérisé l'origine d'extraction de l'eau par les racines en saison sèche (**Chapitre III.5**).

Dans ce chapitre de conclusions, je synthétise et discute les résultats majeurs de mon travail et propose également des perspectives de recherche sur les suites possibles de ces travaux en recherche ainsi que sur les applications possibles à la gestion forestière.

## 2. Effets du mélange sur le fonctionnement hydrique des arbres

### 2.1. En absence de stress hydrique

A l'échelle de l'individu, en conditions hydriques non-limitantes, aucun effet du mélange n'a été observé sur le fonctionnement hydrique, que ce soit avec les mesures de densité de flux ( $F_d$ ) journalières (**Chapitre III.3**) ou avec les mesures de composition isotopique du carbone ( $\delta^{13}C$ ) du bois d'été, qui traduit l'efficacité d'utilisation de l'eau de l'arbre intrinsèque (« intrinsic water use efficiency »,  $WUE_i$ ) (**Chapitre III.4**). Ces résultats sont conformes avec mes hypothèses de départ et avec des résultats d'études précédentes qui soulignent l'absence d'effets de complémentarité sur le fonctionnement hydrique et carboné des arbres en absence de stress hydrique (Grossiord et al. 2013, 2014b; Lebourgeois et al. 2013; Grossiord 2014 p.48). En effet, selon le cadre théorique de Forrester et Bauhus (2016), lorsque la réserve en eau du sol est non-limitante, la complémentarité entre espèces pour l'utilisation de l'eau est peu susceptible d'avoir un effet sur le fonctionnement hydrique des arbres. Ce cadre théorique est dérivé de l'hypothèse du gradient de stress (« stress gradient hypothesis », SGH), selon laquelle les interactions compétitives seraient remplacées par de la facilitation lorsque les conditions deviennent plus stressantes (Bertness and Callaway 1994). En absence de stress hydrique, dans notre cas, l'absence d'effets de complémentarité pour l'utilisation de l'eau est donc conforme avec ce cadre théorique. Mon étude complète donc les connaissances acquises sur les écosystèmes forestiers dans ce type de situation non-stressante. Elle suggère qu'à l'avenir, il est peu intéressant de mettre en place des expérimentations testant ce type d'effet sur le fonctionnement hydrique individuel des arbres sans contrainte environnementale, sachant que la probabilité d'observer des effets du mélange est très faible. Malgré tout, ces mesures en absence de stress restent importantes afin de servir de référence pour observer les effets d'une sécheresse.

A l'échelle du peuplement, on peut s'attendre à des effets du mélange sur l'utilisation de l'eau même en conditions hydriques non-limitantes. En effet, la consommation en eau dépend non-seulement du fonctionnement hydrique de l'arbre au moment de la prise de mesure – fonctionnement hydrique qui, dans le cas d'ORPHEE, n'était pas influencé par le mélange (cf. paragraphe précédent) – mais aussi des effets globaux du mélange, accumulés au cours des années précédentes, sur l'extraction de l'eau, l'interception de la lumière, etc... *et in fine* la croissance primaire (hauteur) et secondaire (diamètre) des arbres. Ainsi, au moment de la mesure des densités de flux de sève, la surface d'aubier totale du peuplement est affectée par l'effet du mélange sur la croissance secondaire des années précédentes, qui impacte donc la transpiration totale du peuplement. Dans le cas d'ORPHEE, j'ai en effet observé une transpiration totale dans les mélanges qui était intermédiaire entre les peuplements purs de pins et de bouleaux, mais supérieure à la moyenne attendue à partir des deux peuplements purs (**Chapitre III.3**). L'explication principale que j'ai pu donner à ce résultat est donc la différence de surface d'aubier entre

ces différents peuplements, en lien avec les différences de croissance secondaire. Ce résultat semblait bien refléter les effets du mélange sur la croissance lors des années précédentes à ORPHEE (**Fig. III.S 3.2, Fig. III.S 4.3**) (Toïgo et al. 2021a). Cependant, il faut noter que ces observations sur la transpiration à l'échelle du peuplement n'ont pas pu donner lieu à des analyses statistiques du fait que nous n'avons pu équiper des arbres avec des capteurs de flux de sève que dans un seul bloc non-irrigué. Il faudrait donc pouvoir renouveler l'expérience sur plusieurs blocs pour pouvoir confirmer ces observations. De manière similaire, plusieurs études ont montré qu'une plus grande diversité des espèces d'arbres (Kunert et al. 2012; Gebauer et al. 2012) ou que l'interaction de deux espèces aux traits fonctionnels très contrastés (Forrester et al. 2010) augmentait la transpiration annuelle des peuplements. Dans ces exemples, les auteurs ont à chaque fois observé une plus grande productivité ou surface terrière dans les mélanges.

Il est important de noter que, si la transpiration totale du peuplement était supérieure à la moyenne attendue à ORPHEE, c'était le résultat d'une augmentation de la transpiration pour les pins qui était plus forte que la diminution de la transpiration subie par les bouleaux dans les peuplements mélangés. Ce résultat confirme une tendance souvent observée dans les études sur les effets du mélange, à savoir le fait que ces effets sont le résultat d'un effet positif pour une ou quelques espèces seulement, et non pour l'ensemble des espèces dans le peuplement. Par exemple, Forrester et al. (2010) ont observé des réponses contrastées entre les deux espèces présentes dans le mélange eucalyptus/acacia étudié. Les auteurs ont constaté que le fait de mélanger des eucalyptus héliophiles avec des acacias tolérants à l'ombre, améliorait la transpiration des eucalyptus mais semblait diminuer celle des acacias. Ces tendances confirment la complexité de l'origine de ces effets des interactions des espèces dans les peuplements mélangés, quand ils existent. De plus, cela souligne le fait qu'il n'est pas envisageable de tirer des conclusions *a priori* sur les effets du mélange pour chaque espèce à partir des résultats à l'échelle peuplement, mais qu'il faut étudier précisément et individuellement la réponse de chaque espèce d'arbre.

Le résultat à l'échelle du peuplement est donc la conséquence d'un équilibre entre les effets des interactions sur les différentes espèces. De plus, cet équilibre peut être modifié par les conditions environnementales de croissance de ces arbres. En effet, j'ai observé dans les blocs irrigués à ORPHEE que l'effet net du mélange sur la transpiration (c.à.d., la comparaison avec la moyenne des deux monocultures) n'était pas positif (**Chapitre III.3**). Contrairement aux blocs non-irrigués, ces résultats étaient dus à une combinaison des effets du mélange sur la croissance (qui étaient plus proches de la moyenne attendue que dans les blocs non-irrigués, du fait d'un plus faible effet positif sur les pins et un plus fort effet négatif sur les bouleaux), mais aussi à une plus faible densité de flux de sève ( $F_d$ ) dans le mélange pour les bouleaux (lié à un effet positif de l'irrigation sur le LAI des bouleaux en monoculture uniquement). Ainsi, les effets du mélange sur la transpiration ont été modifiés par le traitement d'irrigation via des effets indirects de l'irrigation sur l'interception de la lumière. Ces résultats soulignent à nouveau la variabilité des effets du mélange, qui est la conséquence d'un équilibre entre les

effets d'interactions des espèces sur l'acquisition des différentes ressources (eau, lumière...). Il est à noter aussi que cette tendance va compliquer la comparaison entre blocs irrigués et non irrigués en période de sécheresse, car les résultats observés seront dus à la levée de contrainte hydrique au moment de la mesure, mais également dus aux effets de l'irrigation lors des années précédentes sur la croissance et la structure du peuplement.

Enfin, à la lumière de ces résultats, on peut se demander si, en conditions de sécheresse, dans les peuplements mélangés, une transpiration plus élevée que la moyenne des peuplements purs pourrait être préjudiciable, en rendant les arbres des peuplements mélangés plus sensibles à la sécheresse (Jacobs et al. 2021). Ainsi, à ORPHEE, on pourrait s'attendre à ce que les bouleaux soient plus exposés à la sécheresse dans les parcelles mélangées en raison de la plus grande utilisation d'eau par les pins avec lesquels ils coexistent en mélange. Ces interrogations font partie des points que je discute dans la **section 2.2.**

## 2.2. En conditions de stress hydrique

En conditions de stress hydrique, mes travaux ont montré une importante variabilité des effets du mélange à l'échelle de l'arbre et du peuplement sur l'exposition et la réponse à la sécheresse à travers les différents types de mélanges, ainsi qu'au sein d'un même mélange selon les conditions climatiques. J'ai étudié la réponse à la sécheresse grâce à des mesures journalières de densité de flux de sève (ORPHEE), des mesures annuelles de  $\delta^{13}\text{C}$  dans le bois d'été (ORPHEE), et des mesures pluriannuelles de  $\delta^{13}\text{C}$  dans le bois pendant des années sèches (GMAP).

Pour le dispositif GMAP (**Chapitre I**), nous nous sommes focalisés sur le hêtre et avons observé qu'il pouvait présenter des réponses contrastées aux interactions selon l'espèce avec laquelle il était mélangé. En effet, dans les sites hêtre-sapin, pour le hêtre le mélange avec le sapin n'avait aucun effet sur les valeurs de  $\delta^{13}\text{C}$  pendant les années sèches. Cependant, à l'échelle du peuplement, l'effet du mélange suggérait une moins grande exposition à la sécheresse dans la moitié des triplets hêtre-sapin (**Fig. I. 7**). Dans les sites hêtres-chênes, les effets du mélange sur le hêtre étaient généralement neutres, mais dans quelques sites le hêtre souffrait d'une légèrement plus forte exposition à la sécheresse dans les peuplements mélangés quand on considérait les arbres dominés et dominants. Quelques études récentes dans le même dispositif viennent confirmer le contraste de résultats entre les deux types de mélange. Par exemple, Didion-Gency et al. (2021) ont constaté que, pour le hêtre, le potentiel de base mesuré pendant une sécheresse estivale était plus élevé (c'est-à-dire un déficit hydrique plus faible) dans les peuplements mélangés avec du sapin, et plus faible (c'est-à-dire un déficit hydrique plus élevé) dans les peuplements mélangés avec du chêne pubescent que dans les peuplements purs. Toujours dans le dispositif GMAP, il a été démontré un effet positif pour le mélange hêtre-sapin – et pas d'effet pour le mélange hêtre-chêne – sur la résistance à la sécheresse (Jourdan et al. 2019b) et sur la stabilité de la productivité à l'échelle du peuplement (Jourdan et al. 2020) à l'échelle du peuplement.



Par ailleurs, mes résultats à GMAP ont aussi montré que, sur un site donné, les effets du mélange pouvaient différer entre espèces. Par exemple, dans un site du nord on observe un effet positif du mélange sur les sapins dominants alors qu'il n'y a pas d'effet pour les hêtres dominants, et inversement pour un site du sud, où on observe un effet négatif du mélange sur les hêtres dominants et pas d'effet pour les chênes dominants. Ces résultats indiquent une variabilité dans les réponses aux interactions pendant un stress hydrique selon l'espèce considérée. Ce sont des résultats qui ont également été observés dans le dispositif de FunDivEurope, un réseau de six sites dans des forêts naturelles tempérées à travers l'Europe, pour lequel les tendances de l'effet de la diversité en espèces sur l'exposition à la sécheresse à l'échelle du peuplement ne se retrouvaient pas nécessairement au niveau espèce et variaient fortement entre espèces d'un même site (Grossiord et al. 2014d; Forrester et al. 2016). Comme évoqué dans la partie précédente, on retrouve donc l'importance d'évaluer les réponses des différentes espèces avant de tirer des conclusions à l'échelle du peuplement.

Dans la plantation ORPHEE (**Chapitre III**), il y avait très peu d'effets du mélange sur la réponse à la sécheresse des bouleaux et des pins dans les blocs non irrigués, peu importe la façon dont elle a été mesurée (flux de sève,  $\delta^{13}\text{C}$ ). Je n'ai pas non plus observé d'acclimatation aux conditions de mélange pour la résistance à la cavitation ( $\Psi_{50}$ ). Les quelques effets du mélange détectés sur la réponse à la sécheresse n'étaient pas non plus identiques entre espèces, avec par exemple une perte de densité flux de sève légèrement plus rapide en mélange pour les pins mais pas pour les bouleaux (**Chapitre III.3**).

L'absence d'effets clairs du mélange sur la réponse à la sécheresse ainsi que leur variabilité est cohérente avec un nombre croissant de travaux qui montrent que les effets du mélange sur la réponse à la sécheresse ne peuvent pas être généralisés. Ils sont fortement modulés par le type de mélange et l'identité des espèces d'arbres, et dépendent de l'approvisionnement en eau du site, des caractéristiques du sol et des caractéristiques du peuplement, telles que la surface terrière et l'âge. Cette variabilité des résultats est vraie pour la réponse à la sécheresse mesurée par des variations de  $\delta^{13}\text{C}$  du bois (Grossiord et al. 2014d; Bonal et al. 2017; Schwarz and Bauhus 2019; de Stree et al. 2020), par la perte de transpiration (Grossiord et al. 2014b, 2015), mais aussi par les variations de croissance annuelle (Schwarz and Bauhus 2019; Steckel et al. 2020; Pardos et al. 2021).

Cependant, à partir de mes résultats et de récents travaux de synthèse (Ammer 2019; Grossiord 2019; Pardos et al. 2021) on peut commencer à dégager quelques grandes tendances et orienter les recherches futures autour de ces questions. Premièrement, il a été constaté qu'une majorité des effets du mélange sur la réponse à la sécheresse sont plutôt soit positifs soit neutres et plus rarement négatifs à l'échelle du peuplement (Grossiord 2019). Un effet négatif, c.à.d. une plus grande exposition à la sécheresse en mélange, est moins souvent observé, et concerne le plus souvent seulement une partie des espèces présentes dans le mélange (par ex., Jacobs et al. 2021). En effet, dans le cadre de mon travail de thèse j'ai observé très peu d'effets négatifs du mélange sur l'exposition à la sécheresse, mis à part pour le hêtre (arbres non-dominants) en mélange avec le chêne pubescent à GMAP (**Chapitre I**) et une perte

légèrement plus rapide de flux de sève pour les pins en mélange avec les bouleaux à ORPHEE (**Chapitre III.3**). Deuxièmement, la résistance et la résilience à la sécheresse semblent plus souvent bénéfiques dans les mélanges entre des espèces fonctionnellement très contrastées, par exemple entre un feuillu et un conifère (Pardos et al. 2021). Ce n'est pas ce qui a été observé dans le cadre de ma thèse, où nous avons plutôt observé une absence d'effets pour les mélanges feuillus-conifères à l'échelle de l'espèce (bouleau-pin et hêtre-sapin), mais cela pouvait s'expliquer par les caractéristiques des sols (pour le mélange bouleau-pin) ainsi que par le degré de stress sur la période étudiée (pour le mélange hêtre-sapin). En effet, pour le mélange hêtre-sapin, d'autres études sur le même dispositif ont observé des effets positifs du mélange sur la croissance ou le potentiel de base (Jourdan et al. 2019b; Didion-Gency et al. 2021). L'absence d'effets dans mon étude, à l'échelle de l'espèce, s'explique peut-être par un degré de stress insuffisant ou trop élevé, selon les sites, pour détecter un effet du mélange sur les années que j'ai étudiées. Dans le cadre théorique de Forrester et Bauhus (2016), dérivé du SGH, une prévalence des effets bénéfiques du mélange, lorsqu'il y a des complémentarités entre les espèces, est attendue dans des conditions de stress plus fortes. Cependant, de Streel et al. (2020) proposent d'affiner ce cadre théorique en supposant qu'au-delà d'un certain seuil de sécheresse il n'y ait à nouveau plus d'effets bénéfiques du mélange. Ces hypothèses pourraient expliquer en partie l'absence d'effets observés dans le dispositif GMAP pour le mélange hêtre-sapin alors qu'il présente des complémentarités pour la profondeur d'extraction de l'eau (**voir section 3.1**). Pour étudier cela il faudrait étudier des années avec différents degrés de contrainte hydrique dans des mélanges pour lesquels on s'attend à une complémentarité entre espèces.

Un point important à noter parmi les résultats que j'ai obtenus dans la plantation ORPHEE, au cours d'étés très secs, est la présence d'effets du mélange sur le fonctionnement hydrique des bouleaux dans les blocs *irrigués* (sur la perte de densité de flux de sève : **Fig. III. 3.8**, les valeurs de  $\Delta\delta^{13}\text{C}$  : **Fig. III. 4.7**, les valeurs de  $\Psi_{50}$  : **Fig. III.A 4**), alors qu'il n'y avait pas d'effets du mélange dans les blocs non-irrigués. Ces résultats allaient dans le sens d'une moins forte exposition à la sécheresse pour les bouleaux des blocs irrigués en mélange par rapport aux peuplements purs, mais étaient principalement liés au fait que les houppiers des bouleaux étaient moins exposés à la sécheresse du fait d'une plus forte fermeture de la canopée que dans les blocs non-irrigués (**Fig. III.S 3.2**). Ce résultat n'était pas attendu, car nous observons dans ce cas-là des résultats contraires aux cadres théoriques développés dans la littérature, que ce soit pour des effets de complémentarité qui améliorent l'acquisition de l'eau ou de la lumière. En effet, Forrester et Bauhus (2016) indiquent que le long d'un gradient de disponibilité en eau on s'attend à trouver des effets de complémentarités aériennes plus fortes lorsque la disponibilité en eau est plus forte (**voir Fig. 12 dans l'Introduction**). On pourrait supposer que dans le cadre de la complémentarité pour l'interception de la lumière, on assiste aussi ici à un effet de seuil. En effet, l'apport en eau a entraîné une fermeture du couvert, dans une plantation déjà très dense (**Chapitre II : Fig. II. 7, Chapitre III : Fig. III.S 3.2, Appendix A p. 321**), qui ne permet plus l'expression d'une complémentarité entre

espèces et où les interactions compétitives prennent le dessus. Ainsi, les effets du mélange observés dans les blocs irrigués pour les bouleaux indiquent plutôt une domination progressive des houppiers de bouleaux par les pins, donc une interaction négative, qu'un réel effet de complémentarité (**Chapitre III.4**).

Nous aurions souhaité pouvoir utiliser les sites du réseau de GMAP comme un gradient climatique afin de tester ce type d'hypothèses, cependant il y avait trop de facteurs confondants pour l'utiliser à ces fins. En effet, nous n'avons que trois sites avec la même composition en espèces puisqu'il y avait un changement de composition de l'espèce en mélange avec le hêtre entre les sites du sud (chêne pubescent) et les sites du nord (sapin), ce qui reflète la variation naturelle de la composition de ces peuplements des pré-Alpes. Parmi les sites, et les triplets, au sein de chaque type de mélange, les résultats de l'effet du mélange étaient très variables. Cela a été observé non seulement dans mon étude (**Chapitre I**) mais aussi dans les autres études sur le même dispositif (Jourdan et al. 2019b; Toïgo et al. 2021b). En effet, il semble difficile d'observer uniquement un effet du climat sur ce type de gradient de sites alors que de nombreux autres facteurs varient entre ces sites. De plus, même si les placettes pures ou mélangées au sein d'un même triplet sont sélectionnées proches les unes des autres, la présence d'une ou des deux espèces n'est pas due au hasard mais plutôt le résultat de filtres biotiques et abiotiques, et de choix de gestion, qui ont influencé cette composition spécifique locale. Hagan et al. (2021) soutiennent que l'on ne devrait pas s'attendre à des relations positives entre biodiversité et fonctionnement de l'écosystème dans les observations de terrain et que les recherches devraient plus se concentrer sur des plantations expérimentales. Pour pouvoir tester les hypothèses de gradient de stress il faudrait donc répéter un certain nombre de plantations expérimentales avec les mêmes compositions en essences le long d'un gradient climatique, en évitant au maximum des changements de topographie et de type de sol.

### **3. Mécanismes de partage des ressources en eau et en lumière**

Dans le cadre de mon travail de thèse, je me suis également particulièrement intéressée aux effets du mélange sur les mécanismes en lien avec le partage des ressources aériennes et souterraines. Il s'agissait d'évaluer si des mécanismes dans ces deux parties d'un écosystème sont en jeu pour expliquer, quand ils existent, l'origine des effets des interactions des espèces d'arbres. L'eau et la lumière sont les deux ressources qui impactent le plus le fonctionnement hydrique et la réponse des arbres à la sécheresse. L'acquisition et l'utilisation des nutriments jouent également un rôle important dans le fonctionnement de l'écosystème mais n'ont pas été étudiés dans le cadre de mon travail de thèse. D'autres travaux approfondis ont été menés ces dernières années sur la disponibilité des nutriments et la décomposition dans les peuplements mélangés (par ex., de Streel et al. 2021), et notamment pour ORPHEE dans le cadre du travail de thèse de Tania Maxwell (Maxwell et al. 2020) et d'Andreas Altinalmazis-Kondylis (2021). La mise en regard de ces différents mécanismes de partage des ressources sera l'objectif de la

synthèse générale du projet ANR DiPTICC dans lequel s'inscrit mon travail de thèse. La mesure directe de ces mécanismes de partitionnement des ressources est essentielle afin de progresser dans notre compréhension des relations B-EF.

### 3.1. Utilisation de l'eau

Parmi les mécanismes qui peuvent avoir un effet sur la disponibilité de l'eau dans les mélanges, la profondeur d'extraction de l'eau est un des facteurs couramment évoqués dans les hypothèses explicatives des effets du mélange (Lebourgeois et al. 2013; Grossiord 2019). En effet, une complémentarité de profondeur d'extraction de l'eau (par complémentarité de niche, ou différenciation de niche) pourrait expliquer une réduction de la compétition pour l'eau dans le peuplement mélangé par rapport aux peuplements purs et une meilleure productivité ou une moins grande exposition à la sécheresse.

Je me suis intéressée à ce mécanisme dans les différents dispositifs de recherche de mon travail de thèse. J'ai observé des différences d'origine d'extraction de l'eau entre le hêtre et le sapin, mais pas de différence entre le hêtre et le chêne pubescent dans les sites de forêts semi-naturelles des pré-Alpes (GMAP, **Chapitre I**). Dans les sites hêtre-sapin, non seulement le hêtre semblait extraire de l'eau plus profondément que le sapin dans les peuplements purs (complémentarité de niche), mais la présence du sapin a aussi engendré un changement d'extraction de l'eau pour le hêtre, avec une plus grande proportion d'eau venant de profondeur dans les mélanges (différenciation de niche). La différence de résultat entre les deux types de mélange pourrait s'expliquer par les caractéristiques fonctionnelles de l'espèce accompagnatrice du hêtre. En effet, le hêtre et le sapin ont probablement des caractéristiques racinaires plus contrastées que le hêtre et le chêne pubescent. Il a d'ailleurs déjà été démontré que le hêtre avait une profondeur racinaire plus importante que des conifères en peuplements mélangés (Bolte and Villanueva 2006; Grossiord et al. 2014a). Pour le mélange hêtre-chêne, les deux espèces étaient probablement en compétition dans les mêmes horizons de sol du fait de profils racinaires similaires, d'autant plus que les sols des sites concernés étaient très peu profonds.

Pour la plantation ORPHEE, dans les blocs non-irrigués, (**Chapitre III.5**), les bouleaux et les pins allaient puiser l'eau dans les mêmes horizons de sol, et le fait d'être en peuplement mélangé n'apportait pas de modification majeure à la contribution des différents horizons pour l'extraction de l'eau des deux espèces. Ce résultat se démarquait nettement de nos attentes, à partir des connaissances sur les systèmes racinaires de ces deux espèces, qui nous laissaient supposer que les pins pourraient puiser l'eau plus profondément que les bouleaux. Cependant, nos résultats sur l'origine de l'extraction de l'eau, que nous avons obtenus pendant une période de sécheresse estivale, concordent avec les résultats d'Altinalmazis-Kondylis et al. (2020) dans le même dispositif au printemps 2018. En effet, ils n'observent pas de différences de distribution de la biomasse des racines fines entre les deux espèces et en fonction du mélange. Cette absence de complémentarité entre pins et bouleaux est peut-être liée aux contraintes

fortes du sol à ORPHEE (nappe peu profonde) qui réduit le volume de sol qui peut être exploré par les racines dans ce type de sol (Danjon et al. 2005).

A partir des résultats de ces deux dispositifs, nous pouvons en déduire que l'absence de complémentarités racinaires rend la probabilité d'effets du mélange sur la réponse à la sécheresse faible (hêtre-chêne à GMAP et bouleau-pin à ORPHEE), mais qu'en revanche, lorsqu'il y a une complémentarité pour la profondeur d'extraction de l'eau, cela n'engendre pas nécessairement un effet du mélange observable (hêtre-sapin à GMAP). En effet, l'existence de complémentarités pour l'extraction de l'eau entre le hêtre et le sapin ne s'est pas traduite par un effet du mélange sur l'exposition à la sécheresse mesurée avec le  $\delta^{13}\text{C}$  à l'échelle de l'espèce dans le dispositif GMAP. Cependant, à l'échelle du peuplement, l'effet du mélange suggérait une moins grande exposition à la sécheresse dans la moitié des triplets hêtre-sapin (**Fig. I. 7**). La comparaison des résultats concernant l'extraction de l'eau avec ceux concernant le  $\delta^{13}\text{C}$  du bois est limitée : avec le  $\delta^{13}\text{C}$  du bois, nous avons mesuré un fonctionnement intégré sur cinq années sèches à GMAP ; il est donc difficile de le comparer avec une profondeur d'extraction de l'eau à un temps donné pendant une sécheresse estivale. Cette profondeur racinaire peut en effet évoluer en fonction des conditions de sécheresse dans le sol (Brinkmann et al. 2019). La période de cinq ans, utilisée pour le  $\delta^{13}\text{C}$ , intègre des périodes avec une moins forte contrainte hydrique en dehors des sécheresses estivales et on pourrait donc s'attendre à ce que l'effet pendant les périodes de sécheresse soit modéré par des effets tantôt positifs, tantôt négatifs ou neutres selon les années et les saisons malgré des sécheresses estivales. De plus, l'absence de cet effet peut aussi s'expliquer par des facteurs confondants, qui auraient masqué les effets de la disponibilité en eau sur le  $\delta^{13}\text{C}$  des arbres dans les mélanges hêtre-sapin, tels que des effets liés à la disponibilité de la lumière ou des nutriments (Warren et al. 2001). Les résultats d'autres études dans le même dispositif confirment un potentiel effet positif de la différence d'origine d'extraction de l'eau entre les racines des hêtres et des sapins. En effet, Didion-Gency (2021), qui ont fait des mesures de potentiels de base en 2019, l'été suivant nos mesures de profondeur d'extraction de l'eau, montrent une moins forte exposition à la sécheresse pour le hêtre en mélange avec le sapin par rapport aux peuplements purs. Ces résultats, établis à un moment « t » durant une sécheresse estivale sont sans doute plus comparables à nos données d'extraction de l'eau. De plus, Toïgo et al. (Toïgo et al. 2021b) et Jourdan et al. (Jourdan et al. 2019b) ont observé une plus grande fréquence d'effets positifs sur la productivité et la résistance à la sécheresse dans les mélanges hêtre-sapin que dans les mélanges hêtre-chêne. Pour confirmer les différences de profondeur d'extraction de l'eau que suggèrent mes résultats, il faudrait pouvoir reproduire l'expérience en utilisant un marquage isotopique de l'eau.

### 3.2. Interception de la lumière

Les interactions des espèces d'arbre peuvent modifier l'interception de la lumière pour les différentes espèces en mélange, mais aussi augmenter le niveau d'interception de la lumière à l'échelle du peuplement (Pretzsch 2014). Une augmentation de l'interception de la lumière peut se faire par une complémentarité spatiale entre espèces qui résulte en une plus grande occupation de l'espace par les houppiers (*canopy packing*) (Jucker et al. 2015). De manière similaire à la complémentarité pour l'utilisation de l'eau par les racines, le *canopy packing* peut être le résultat d'une stratification verticale entre houppiers (complémentarité de niche) ou d'une plasticité de l'architecture des houppiers (différentiation de niche). Ces différences d'interception de la lumière pourraient être un des mécanismes principaux pour expliquer les effets du mélange sur le fonctionnement hydrique et la productivité en absence de stress hydrique (Forrester and Bauhus 2016), mais aussi sur la réponse à la sécheresse en l'absence de complémentarités souterraines entre les espèces (**Chapitre III**).

A ORPHEE (**Chapitre II**), j'ai pu caractériser le *canopy packing* dans les blocs irrigués et non-irrigués, mais aussi à deux niveaux de densité contrastés (dans le **Chapitre III**, seul le niveau de « forte densité » a été étudié). Au niveau de forte densité et sans irrigation, nous avons observé un accroissement de volume de houppier pour les pins en mélange par rapport aux monocultures et une diminution du volume de houppier pour les bouleaux. On retrouve donc un effet du mélange symétrique à ce qui était observé pour la surface terrière des arbres dans ces placettes. Cependant, j'ai aussi observé que l'augmentation du volume des pins était en partie indépendante de la croissance en diamètre, avec un changement de relation allométrique entre le diamètre et le volume des arbres qui démontrait une plasticité de l'architecture des houppiers de pins. J'ai donc observé un effet positif du mélange pour les pins grâce à une réduction de compétition pour la lumière avec la présence de bouleaux voisins. A l'échelle du peuplement, cela s'est traduit par un *canopy packing* supérieur à la moyenne attendue à partir des deux peuplements purs. Ainsi, le mélange bouleau-pin présentait une complémentarité et une différenciation de niche dans le compartiment aérien dans les parcelles à forte densité et sans irrigation. Cet effet positif du mélange sur le *canopy packing* ne s'observait plus dans les blocs irrigués (qui avaient une canopée plus fermée), ni dans les peuplements à plus faible densité (donc avec une canopée plus ouverte), traduisant un effet de seuil de disponibilité de la lumière pour les effets bénéfiques pour la complémentarité aérienne entre ces deux espèces. Cet effet de seuil en lien avec la lumière est intéressant à mettre en parallèle avec l'effet de seuil de disponibilité en eau identifié par de Stree et al. (2020) pour la réponse à la sécheresse.

Un effet positif du mélange sur le *canopy packing* a pu être observé précédemment dans d'autres types de forêts et de climats (Jucker et al. 2015; Williams et al. 2017; Duarte et al. 2021). Cependant, mes travaux montrent pour la première fois que ce résultat peut être dépendant des conditions climatiques et de leur influence sur la croissance du peuplement. Mes résultats soulignent l'importance du stade de développement du peuplement sur la détection de ces effets. En effet, on a observé une absence d'effet

positif sur le *canopy packing* soit quand le peuplement était encore trop ouvert (modalité à faible densité) ou lorsque l'irrigation avait engendré une fermeture de la canopée plus précoce. Il serait donc intéressant de réaliser un suivi temporel des volumes de houppiers, à partir d'un stade avant la fermeture de la canopée du peuplement, sur plusieurs années afin de caractériser l'évolution de l'effet du mélange sur le *canopy packing* au cours du temps.

Cet effet net positif du mélange sur l'occupation de l'espace de la canopée à forte densité et sans irrigation, s'est accompagné d'une interception de la lumière plus importante en mélange qu'attendu à partir des peuplements purs (**Chapitre II : Fig. II. 6, Fig. II. 7**). On peut donc supposer que c'est cet effet-là qui est un des mécanismes principaux à l'origine des effets du mélange sur la productivité et la transpiration à l'échelle du peuplement. En effet, pour la transpiration, une augmentation de la surface transpirante peut expliquer une consommation en eau plus forte dans les mélanges (Kunert et al. 2012). Dans les peuplements irrigués à ORPHEE, l'effet net du mélange n'est plus positif pour la transpiration (**Chapitre III.3**). Ce résultat est similaire à celui observé dans le **Chapitre II**, où l'effet net du mélange sur le *canopy packing* était significativement positif uniquement dans les blocs non-irrigués à forte densité. Ces résultats viennent donc conforter l'idée que dans ce dispositif, ce sont plutôt les interactions pour l'interception de la lumière qui déterminent les effets du mélange sur le fonctionnement du peuplement.

Dans le **Chapitre III**, j'ai pu observer que les effets du mélange sur la structure de la canopée, et en particulier la domination des bouleaux par les pins, ont pu influencer les relations hydriques et la réponse à la sécheresse de plusieurs façons et ne doivent pas être sous-estimés lors de l'interprétation des effets du mélange sur la réponse à la sécheresse. L'effet principal pour les bouleaux était un effet indirect lié à une plus faible disponibilité de la lumière, qui a pu constituer un facteur confondant à la réponse à la sécheresse pour certaines mesures ( $\delta^{13}\text{C}$ ). Mais, par ailleurs, les changements de structure de la canopée par rapport aux peuplements purs pourraient aussi jouer un rôle direct sur la réponse à la sécheresse, via des effets sur le microclimat subi par les houppiers en période de sécheresse : ainsi les houppiers de pins pourraient être un peu plus exposés à des températures plus élevées et à un taux d'humidité plus faible en mélange, et vice versa pour les bouleaux. Cela pouvait s'observer également par une chute des feuilles plus tardive pour les bouleaux dans les mélanges (**Appendix A p. 321** et **Appendix B p. 327**). Ces hypothèses ont été proposées dans des études similaires pour expliquer des effets du mélange sur la transpiration ou la résistance à la sécheresse (Jonard et al. 2011; Forrester 2015; Fichtner et al. 2020a). Pour confirmer ces hypothèses il faudrait réaliser, de manière simultanée aux mesures de flux de sève pendant une période de sécheresse, des mesures d'humidité du sol à différentes profondeurs et de microclimat à différents niveaux de la canopée des peuplements purs et mélangés.

J'ai pu étudier ces questions dans la plantation d'ORPHEE, mais malheureusement pas dans les sites du réseau GMAP. En effet, les différentes mesures de houppiers réalisées à ORPHEE étaient initialement également prévues à GMAP, mais elles se sont révélées trop compliquées à réaliser dans des

peuplements irréguliers. En effet, avec une espèce comme le hêtre qui présente des houppiers asymétriques, il était difficile d'envisager l'estimation du volume de houppier de manière simple comme sur ORPHEE. Nous n'avons pas eu le temps ni les moyens d'utiliser des méthodes plus élaborées qui auraient permis d'obtenir ces volumes (par ex. des mesures de rayons dans plusieurs directions, des scans par LiDAR terrestre...). J'ai cependant pu estimer des indices d'éclairement des houppiers à GMAP, similaires à l'indice de Dawkins (Clark and Clark 1992), qui montraient une plus forte proportion de houppiers qui avaient un indice plus bas en mélange, soit un accès à la lumière plus restreint (données non présentées). En effet, la stratification des houppiers était plus importante en mélange, avec une plus forte proportion d'arbres dominés. Mais il est difficile de dire si cela dépendait de l'interception de la lumière par les houppiers ou de stratégies de gestion sylvicoles différentes dans les peuplements mélangés. Une plus forte hétérogénéité de structure du peuplement est un facteur important à prendre en compte lors de l'étude de forêts matures, naturelles ou gérées (Forrester and Bauhus 2016). En effet, la structure du peuplement peut être différente du fait de la régénération naturelle du peuplement ou de la gestion forestière. En peuplements mélangés la densité du peuplement, ou bien la proportion d'arbres dominés peut être différente, ce qui va exercer une influence sur le fonctionnement à l'échelle du peuplement. Ce sont donc des facteurs importants à contrôler ou prendre en compte lors de la mise en place de dispositifs d'observation de terrain.

### 3.3. Quel rôle des mécanismes lumière vs. eau

L'originalité de ma thèse repose sur l'étude de mécanismes aériens et souterrains dans le même dispositif. Les effets de la complémentarité dans les forêts mélangées sont généralement dus à plusieurs mécanismes simultanés. Cependant, la contribution de chaque mécanisme à l'effet de complémentarité total est très difficile à quantifier et cette démarche est rarement tentée (Forrester and Bauhus 2016). Dans le cadre de ma thèse, je n'ai pas pu mettre en évidence des liens directs entre complémentarité racinaire et fonctionnement hydrique, même lorsqu'une complémentarité racinaire était effectivement présente. Il existe en réalité peu d'études qui ont pu faire un lien direct entre une stratification verticale des racines et un effet positif du mélange sur la productivité ou le fonctionnement hydrique (Mueller et al. 2013; Schwendenmann et al. 2015). Ce n'est peut-être donc pas un mécanisme aussi important que le laisse sous-entendre le nombre de fois où il est évoqué comme hypothèse explicative des effets observés. En effet, cette complémentarité racinaire va être fortement dépendante des contraintes sur le développement des racines dans le sol. A contrario, j'ai pu souligner l'importance des mécanismes de complémentarité aérienne pour l'interception mais aussi les conditions microclimatiques subies par les houppiers. Les études faisant un lien direct entre les complémentarités entre houppiers et effets du mélange sur la productivité sont plus nombreuses (Jucker et al. 2014a; Sapjanskas et al. 2014; Forrester et al. 2017; Williams et al. 2017; Cattaneo et al. 2020).



La comparaison des contributions relatives des différents types de mécanisme reste difficile et très peu abordée jusqu'à présent. Quelques articles ont cherché à comparer les effets du mélange entre compartiments aériens et souterrains (Martin-Guay et al. 2020; Jing et al. 2021), même si les effets aériens et souterrains du mélange restent interdépendants et peuvent tous deux être le résultat de mécanismes aériens ou souterrains, ce qui rend l'estimation des contributions relatives des différents mécanismes d'autant plus difficiles (Ammer 2019). Une hypothèse serait que les mécanismes aériens serait prédominants en absence de stress hydrique (del Río et al. 2014; Forrester and Bauhus 2016). Cependant, j'ai pu observer un effet positif du mélange sur l'interception de la lumière, qui était justement plus important en absence d'irrigation à ORPHEE. Ce résultat ne va donc pas dans le sens de ces précédentes hypothèses. La question de la part relative des mécanismes aériens et souterrains pour expliquer les effets observés dans les mélanges reste donc ouverte et mon travail de thèse a apporté une petite contribution à cette connaissance. Afin de progresser dans notre compréhension de ces mécanismes, il faudrait réussir à étudier simultanément mécanismes aériens et souterrains et à comparer leur importance relative sur le fonctionnement en absence et présence de stress hydrique. Dans le cadre de ma thèse, nous n'avons pas forcément réussi à réaliser les mesures sur les différents compartiments aériens ou souterrains au cours de la même année de croissance, ce qui a limité les comparaisons possibles. Pour cela, il faudra également bien caractériser les conditions de stress hydrique du sol, mais aussi les conditions microclimatiques au niveau des houppiers.

## **4. Applications à la gestion forestière**

Malgré le nombre limité de cas de figures étudiés, les différentes espèces étudiées au cours de ma thèse représentent des essences très importantes économiquement pour les forêts d'Europe, pour lesquelles il est essentiel d'évaluer si les peuplements mélangés peuvent présenter un avantage en termes de fonctionnement et de réponse à la sécheresse. Ainsi, mes résultats peuvent apporter quelques pistes afin de répondre aux questions de gestion forestière qui se posent actuellement autour du sujet des forêts mélangées (Coll et al. 2018). Le fait d'avoir étudié des mélanges à deux essences, un nombre limité en termes de diversité, permet d'être assez proche d'applications possibles en gestion forestière.

Nous avons pu observer que globalement, le mélange hêtre-sapin ne résultait pas en une plus, ou une moins grande exposition à la sécheresse pour le hêtre, mais que potentiellement l'effet pouvait être bénéfique dans certains sites à l'échelle du peuplement (**Chapitre I**). Le hêtre et le sapin présentaient des différences de d'origine d'extraction de l'eau qui pourraient être bénéfiques pendant des conditions de stress hydrique intermédiaires. Ainsi, la gestion du hêtre et du sapin en peuplements mélangés dans les forêts de montagnes subalpine, qui se pratique d'ailleurs déjà dans ces régions-là (Gauquelin and Courbaud 2006), semble être une piste intéressante à poursuivre dans des conditions climatiques futures. Dans le cas du mélange hêtre-chêne pubescent, il n'y avait pas d'avantage évident à privilégier le mélange en termes de réponse à la sécheresse. Comme les sites étudiés se situent en limite de l'aire de

répartition du hêtre sur des stations sèches ou chaudes (Houston Durrant et al. 2016), mes résultats suggèrent qu'il est probablement souhaitable de privilégier la présence du chêne pubescent en mélange avec le hêtre dans ces régions. En effet, le chêne pubescent étant plus tolérant à la sécheresse que le hêtre (Niinemets and Valladares 2006), il a de meilleures chances de faire face à une augmentation de la fréquence des épisodes de sécheresse dans ces sites (Ladier et al. 2011). Cependant, le maintien de ces peuplements mélangés nécessite des interventions de gestion, car sinon, à terme, le peuplement pourrait évoluer naturellement vers une hêtraie pure (Ladier et al. 2011).

L'exemple de la plantation d'ORPHEE (**Chapitre II et III**) est particulièrement intéressant d'un point de vue de la gestion, car elle se situe dans la plus vaste région d'Europe de plantations monospécifiques. En effet, les plantations de pin maritime dans les Landes de Gascogne sont des plantations à but de production rapide sur des temps de rotation courts (Mason and Meredieu 2011). La question de l'utilisation de mélange d'essences est d'autant plus importante pour les plantations industrielles de ce type car elles sont vulnérables aux perturbations, climatiques comme biotiques (Kelty 2006; Nichols et al. 2006; Messier et al. 2021). Ainsi, la réflexion sur l'introduction de diversité en essence dans ces plantations semble importante, même si difficile à appliquer dans ce type de système intensif.

Nos résultats du **Chapitre II** ont montré que la présence de bouleaux avait un effet bénéfique sur le développement individuel du diamètre et des houppiers des pins, dans la mesure où les houppiers plus étroits des bouleaux libèrent des espaces à coloniser pour les houppiers de pins. Par ailleurs, les pins ne subissaient pas de stress hydrique plus fort en mélange, même si j'ai observé une légère perte plus rapide de la densité de flux de sève en été (**Chapitre III**). Il semble donc que la présence de bouleaux en mélange avec les pins maritimes n'affectera pas le fonctionnement hydrique des pins, qui sont l'essence objectif dans cette région. De plus, la densité de plantation maximale pour laquelle nous avons fait des mesures (2500 tiges ha<sup>-1</sup>) est deux fois plus importante que celle habituellement utilisée dans les Landes (1250 tiges ha<sup>-1</sup>) (Mason and Meredieu 2011). On pourrait alors envisager, plutôt que de substituer des pins par des bouleaux, d'ajouter des bouleaux entre les pins plantés à 1250 tiges ha<sup>-1</sup>. En effet, nous avons montré dans le **Chapitre II** que l'ajout de bouleaux à cette densité-là n'affectait pas le développement des volumes de houppiers des pins. Cette solution pourrait donc être intéressante, car elle permettrait de ne pas perdre en rendement pour l'essence objectif (le pin), tout en introduisant une essence d'accompagnement (le bouleau) qui permettrait d'avoir une plus grande diversité dans ce type de plantation. Cette diversité en essences, si elle n'apporte pas d'avantage en termes de fonctionnement hydrique ou de réponse à la sécheresse, pourrait alors apporter d'autres types de bénéfices. En effet, le bouleau pourrait contribuer à améliorer la fertilité des sols dans les plantations mélangées, grâce aux chutes de litière ou de l'activité des micro-organismes (Dubois et al. 2020; Maxwell et al. 2020). De plus, il a été démontré que la présence de bouleau dans les plantations de pins entraîne une plus grande résistance des pins contre les insectes herbivores tels que la processionnaire du pin et le foreur de la tige du pin (Castagneyrol et al. 2019; Jactel et al. 2019). Enfin, la domination des bouleaux par les pins après

quelques années de plantation laisse penser que le maintien des bouleaux ne serait peut-être pas possible sur toute la durée de rotation de la plantation. Cependant, la biomasse des bouleaux pourrait être récoltée lors de la première éclaircie de pin et être commercialisée comme produit d'appoint (Dubois et al. 2020) pour compenser le surcoût de la plantation des bouleaux. Ainsi, ces différents bénéfices du mélange doivent être vérifiés sur toute la durée de la rotation (Morin et al. 2020) et évalués en termes de coûts et de bénéfices pour les gestionnaires. Une évaluation qui n'était pas l'objectif de ce travail, mais qui pourrait être une perspective intéressante. En outre, les conditions de site, notamment la disponibilité en eau du sol, dans lesquelles ces deux espèces peuvent être plantées en mélange doivent encore être déterminées à l'échelle régionale.

## **5. Conclusion générale**

Les recherches dans le domaine des forêts mélangées, et plus largement sur le rôle de la biodiversité pour le fonctionnement des écosystèmes, ont fortement progressé pendant la dernière décennie, particulièrement sur la question de la productivité des communautés végétales (Ammer 2019). Cependant, il reste encore de nombreuses questions afin de comprendre les sources de variabilité des relations biodiversité-fonctionnement de l'écosystème, mais aussi les mécanismes sous-jacents à ces relations (Forrester and Bauhus 2016; Coll et al. 2018). La question du rôle de la diversité pour la résistance à la sécheresse est particulièrement d'actualité, dans le contexte actuel de changement climatique et d'augmentation de la fréquence des épisodes de sécheresse (Grossiord 2019). Au fil de mon travail de thèse, j'ai cherché à répondre à ces questions à travers des exemples d'assemblage d'espèces importantes à l'échelle européenne. J'ai pu montrer que le fonctionnement hydrique et la réponse à la sécheresse des espèces étudiées étaient peu impactés par les interactions entre espèces. J'ai pu étudier dans ces mêmes dispositifs des mécanismes aériens et souterrains qui pourraient expliquer des complémentarités entre espèces. Les différences de profondeur racinaire pour l'acquisition de l'eau entre les espèces, quand elles étaient présentes, n'ont pas nécessairement engendré une meilleure disponibilité de l'eau pour les espèces en mélange en condition de sécheresse. En revanche, les mécanismes de complémentarité des houppiers ont engendré des modifications de structure du peuplement et d'interception de la lumière et sont apparus comme ayant un fort impact sur le fonctionnement du mélange. Les effets du mélange d'espèces sur le fonctionnement hydrique des arbres étaient fortement dépendants du contexte et pouvaient être modifiés par les conditions environnementales locales et les conditions climatiques pour un même assemblage d'espèces d'arbres. L'application de ces connaissances à la gestion forestière est donc dépendante du contexte et ne peut pas être généralisée de manière simple.

Il reste encore beaucoup de travail avant de bien comprendre les mécanismes sous-jacents aux relations entre biodiversité et fonctionnement de l'écosystème, les interactions entre ces mécanismes, et comment ils varient dans le temps et dans l'espace. Pour la suite des recherches dans ce domaine, il serait

intéressant de chercher à caractériser de manière simultanée plusieurs des mécanismes de complémentarité aériens et souterrains afin d'évaluer leurs interactions. Ce domaine de recherche est très dynamique actuellement, et la maturation de nombreuses plantations expérimentales (cf. réseau TreeDivNet, et le projet EU-Biodiversa « MixForChange » 2020-2024) devrait permettre d'éclaircir ces questions dans les années à venir. Il est donc essentiel de maintenir ce type de réseau sur le long terme pour pouvoir progresser dans notre compréhension des questions abordées au cours de cette thèse.

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# ANNEXES

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## Annexe A

### 1. Annexe A : Dynamique annuelle de la fermeture de la canopée à ORPHEE

#### 1.1. Objectif

L'objectif de cette expérimentation était de suivre la dynamique saisonnière et annuelle de la surface foliaire du couvert forestier. Pour cela on mesure la quantité de rayonnement photosynthétiquement actif (PAR, *photosynthetically active radiation*) qui arrive sous la canopée (Guyon et al. 2014). Le PAR est un rayonnement dont les longueurs d'onde s'étendent de 400 à 700 nm, qui sont celles que les plantes peuvent utiliser dans le processus de photosynthèse. Les mesures de fiPAR (fraction de PAR interceptée) directionnel permettent d'estimer la proportion de PAR interceptée par le couvert par rapport au PAR incident à ciel ouvert. Nous nous sommes intéressés à cette variable afin de voir l'effet du mélange et de l'irrigation sur ces dynamiques d'interception de la lumière.

#### 1.2. Méthodes

Un capteur de PAR par placette et par bloc (3 x 6 = 18) a été installé en février 2017 par Sylvia Dayau, Dominique Guyon et Alain Kruszewski. Chaque capteur est directionnel (azimut de visée Nord ~-2°, angle zénithal= 45°, Champ de visée = ±23°). Deux capteurs incidents ont été installés à ciel ouvert au bord de la piste qui traverse la plantation. Le suivi des capteurs a été assuré par Sylvia Dayau pendant les 3 années 2017 à 2019.

Le fiPAR (*fraction of intercepted photosynthetically active radiation*) est calculé à partir des données des capteurs dans chaque placette par rapport aux capteurs incidents (ici avec le capteur incident n°1) tel que  $fiPAR = 1 - (PAR_{transmis}/PAR_{incident})$ . Une valeur moyenne de fiPAR est obtenue pour chaque jour de l'année dans chaque placette à l'aide d'un protocole de traitement des données établi et exécuté par Sylvia Dayau et Dominique Guyon (UMR ISPA, Bordeaux) (**Fig. A 1**). Ces données journalières ont ensuite été modélisées par Sylvia Dayau, qui a utilisé une fonction de lissage Spline pour observer la dynamique annuelle.

### 1.3. Principaux résultats

Dans cette annexe j'ai rassemblé des figures de synthèse des résultats de modélisation de la dynamique annuelle du fiPAR pour les placettes de bouleau pur (**Fig. A 2**), pin pur (**Fig. A 3**) et mélange bouleau + pin (**Fig. A 4**) des blocs 1 à 6 dans la plantation à ORPHEE. Pour rappel les blocs non-irrigués sont les blocs 1, 3 et 6 et les blocs irrigués sont les blocs 2, 4 et 5. L'axe des ordonnées pour la **Fig. A 3** et la **Fig. A 4** est différent de la **Fig. A 2**, il commence à 0.5 pour pouvoir distinguer les courbes.

Dans les placettes de bouleau pur (**Fig. A 2**), la saisonnalité (débourrement : augmentation du fiPAR vers le mois de mars, chute des feuilles : diminution du fiPAR en milieu d'été et automne) s'observe clairement pour chaque année. On observe également un effet de l'irrigation avec un fiPAR plus important dans les blocs irrigués. Ceci est lié à l'effet de l'irrigation sur la croissance et le LAI des bouleaux. Le bloc 3 a des valeurs plus faibles que les autres courbes du fait de la mortalité d'un arbre dans le champ de mesure du capteur, créant une grande ouverture.

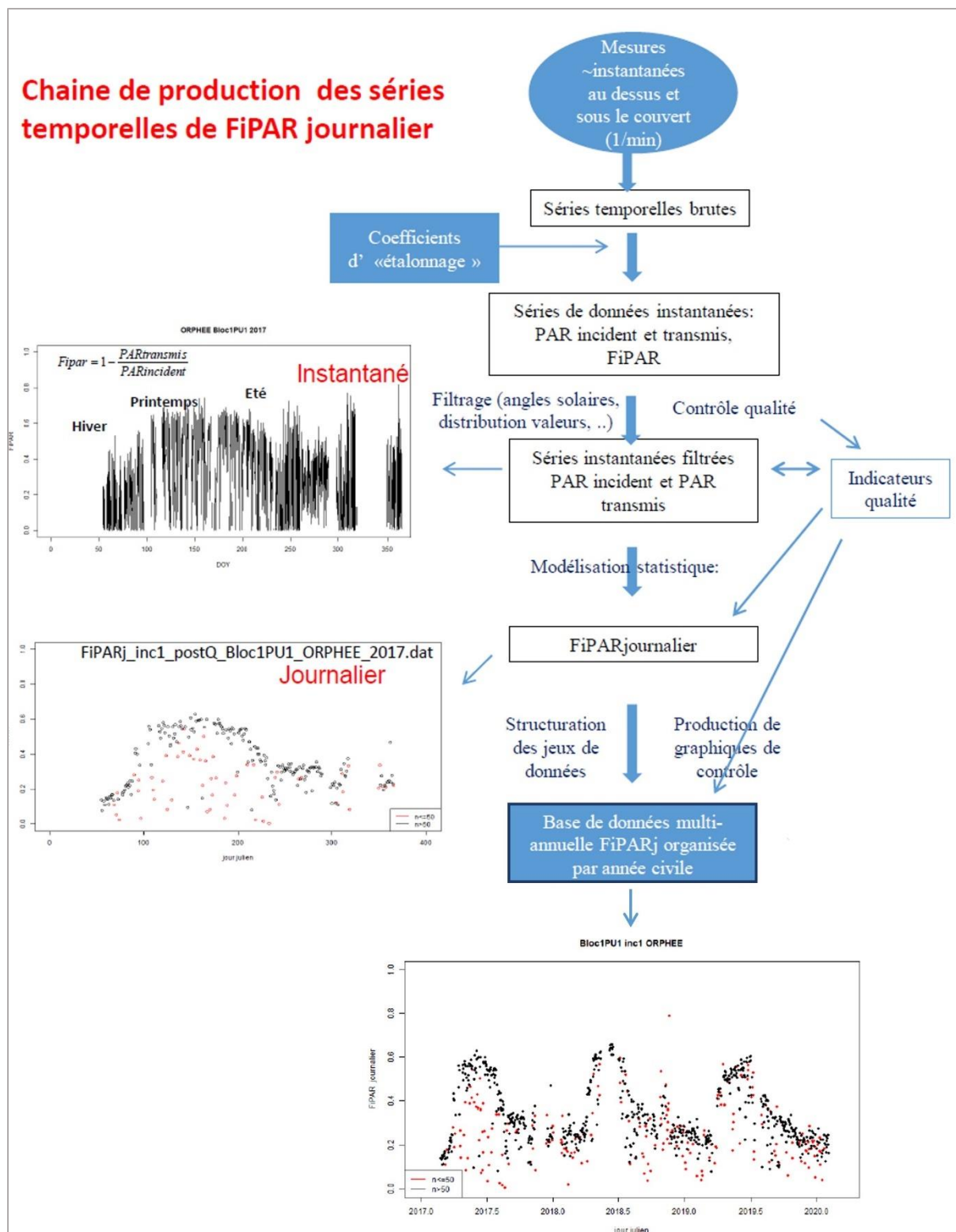
Dans les placettes de pin pur (**Fig. A 3**), peu de variations annuelles sont observées. Le couvert est très fermé avec un fiPAR entre 0.8 et 1. Les blocs irrigués 2 et 5 ont des valeurs de fiPAR un peu plus élevées que les blocs non-irrigués. Cependant le bloc irrigué n°4 a des valeurs similaires aux blocs non-irrigués.

Dans les placettes mélangées (**Fig. A 4**), la situation est intermédiaire aux deux peuplements purs avec une saisonnalité marquée en 2017 et 2018 mais absente en 2019.

### 1.4. Discussion

Ces données nous ont permis d'observer une forte fermeture de la canopée sur toute l'année dans les placettes de pin purs et de mélange à partir de 2018. Elles permettent également de mettre en évidence le fort impact de l'irrigation sur les bouleaux en peuplements purs. Enfin, on peut y observer la précocité de la chute des feuilles de bouleaux qui démarre en juillet en 2017 et 2018

Nous n'avons cependant pas pu analyser ces données plus en détail du fait d'un manque de temps, mais aussi de l'impossibilité de séparer les effets liés à la réponse des bouleaux ou des pins dans les placettes mélangées. En effet, l'objectif était aussi de comparer les dates de point d'inflexion sur les courbes de fiPAR avec les dates de suivi phénologiques observées sur le terrain. Mais cette analyse se serait donc limitée aux placettes de bouleau pur.



**Fig. A 1** Résumé du protocole de traitement des données des capteurs de PAR (rayonnement photosynthétiquement actif) afin d'estimer le fiPAR directionnel journalier (fraction de PAR intercepté). Chaîne de traitement développée et réalisée par Sylvia Dayau et Dominique Guyon (UMR ISPA, Bordeaux).

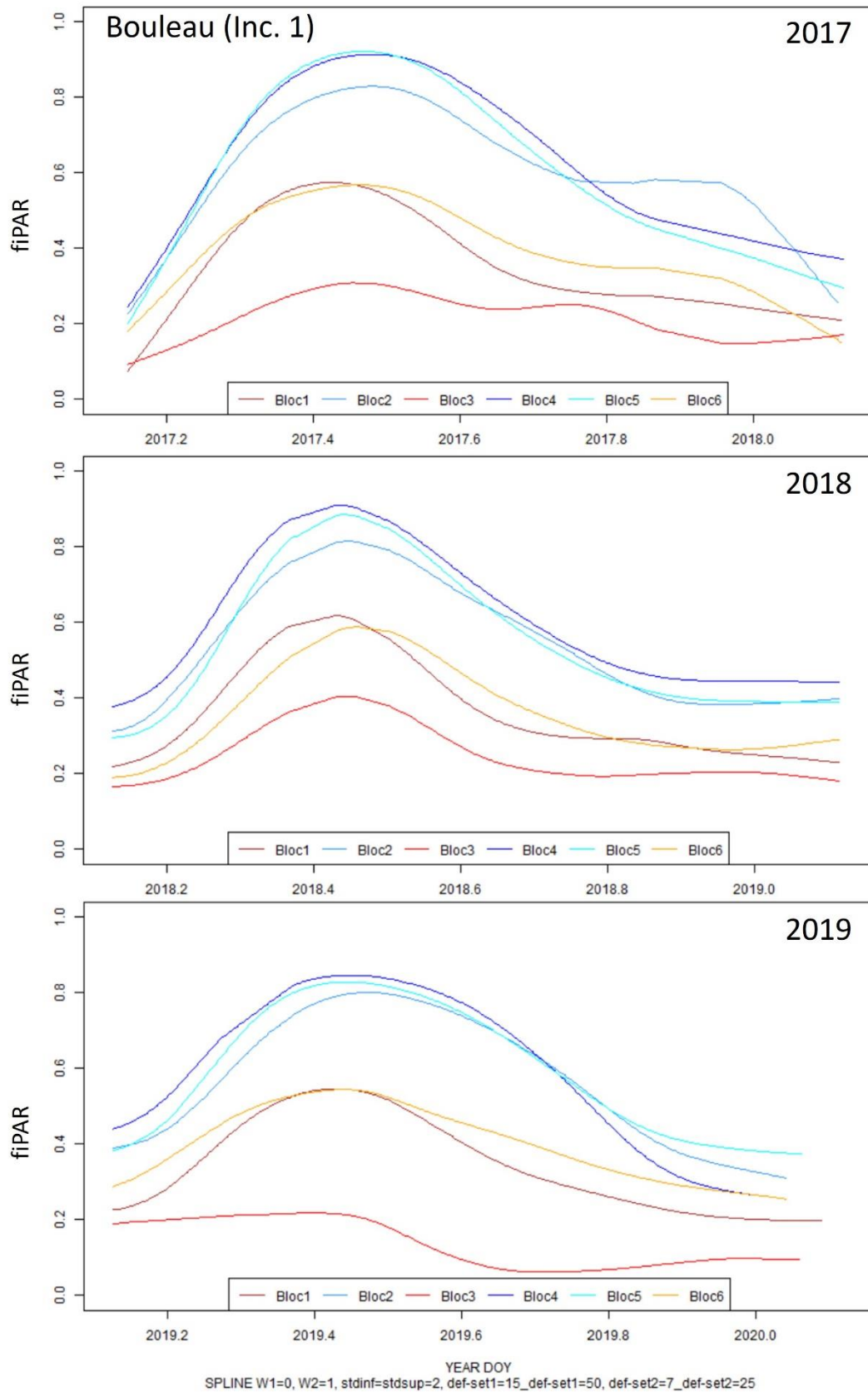


Fig. A 2 Dynamique annuelle du fIPAR pour les placettes de bouleaux purs calculés à partir du capteur incident 1.

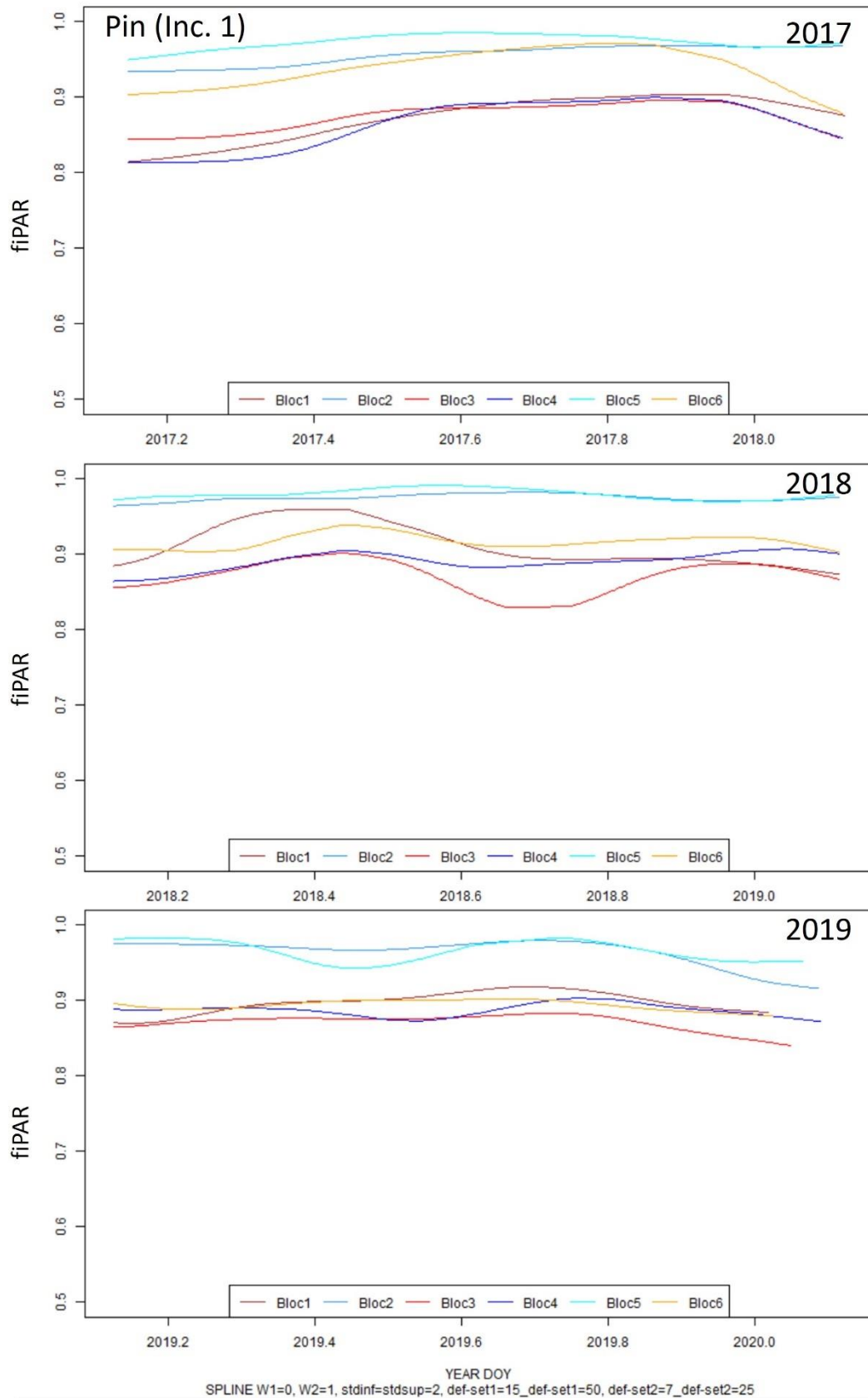
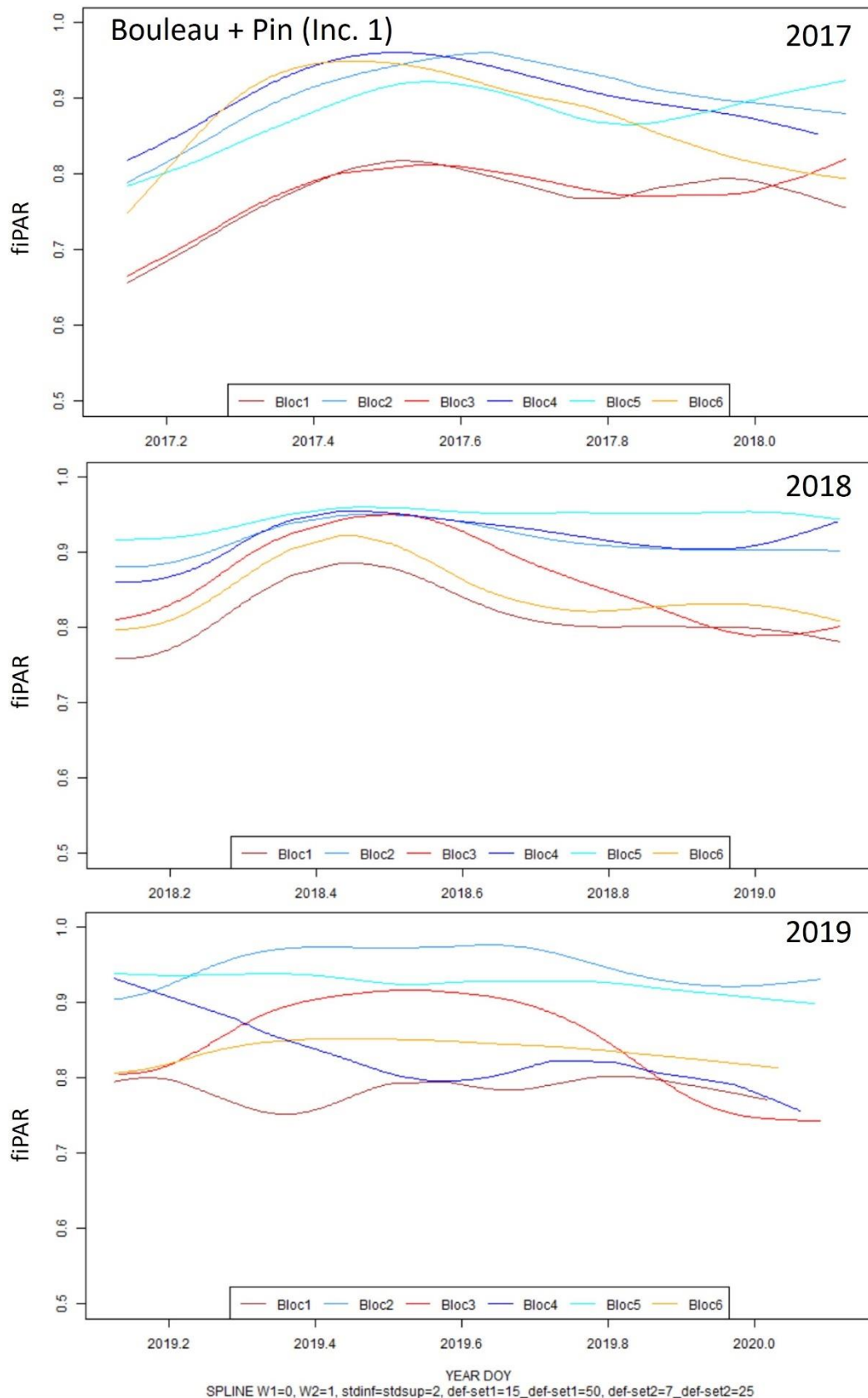


Fig. A 3 Dynamique annuelle du fIPAR pour les placettes de pins purs calculés à partir du capteur incident 1



**Fig. A 4** Dynamique annuelle du fiPAR pour les placettes de pins purs calculés à partir du capteur incident 1

# Annexe B

## 2. Annexe B : Phénologie du développement et de la chute des feuilles à ORPHEE

### 2.1. Objectif

L'objectif de cette expérimentation était d'observer les effets éventuels du mélange et de l'irrigation sur la phénologie foliaire des bouleaux et des pins, afin de tester l'hypothèse d'une meilleure interception de la lumière en mélange liée à un décalage temporel de la phénologie entre les deux espèces. Pour cela nous avons suivi les dates de débourrement des feuilles de bouleaux et des aiguilles de pins au printemps et la date de chute des feuilles de bouleaux à l'automne en 2017 et 2018.

### 2.2. Méthodes

Les suivis de phénologie ont été réalisés pour cinq arbres par espèce dans les placettes bouleau pur, pin pur et mélange bouleau + pin des blocs 1 à 6 à ORPHEE par l'Unité Expérimentale Forêt Pierroton (principalement par Arnaud Lafitte, Audrey Albet et Frédéric Bernier). Les suivis de débourrement ont démarré en février 2017 et ont été effectués une fois par semaine pendant la période de débourrement, puis espacés toutes les deux semaines jusqu'en été.

A chaque passage le suivi des arbres individuel était fait selon le protocole suivant. L'opérateur observe globalement le stade phénologique de l'arbre, puis en le comparant aux échelles de notation (**Fig. B 1**), le note sur l'appareil de saisie. Il faut que l'arbre ait atteint un stade sur au moins 25% du houppier pour que celui-ci lui soit délivré. Seul le stade 33 du pin maritime est donné dès qu'on observe la présence de la seconde pousse à la cime de l'arbre.

A partir de la moitié du mois de juillet, une autre variable était mesurée : la défoliation (uniquement chez le bouleau). L'opérateur observe l'arbre et estime (de 5 en 5) un pourcentage de feuilles tombées sur le total des feuilles présentes sur l'arbre. (Ex : 0%, 5%, 10%, 15%...). En 2017, ces mesures ont débuté fin août seulement et nous avons donc raté le début de la chute des feuilles de bouleaux (**Fig. B 3**).

### 2.3. Principaux résultats

#### 2.3.1. Débourrement des feuilles et des aiguilles

Plusieurs stades phénologiques de débourrement ont été suivis (**Fig. B 1**), mais j'ai choisi ici de ne représenter les résultats que pour un seul stade représentatif des résultats pour le bouleau et pour le pin. Les stades phénologiques représentés dans la **Fig. B 2** sont : le stade 19 pour le bouleau (feuilles étalées,

pas d'élongation) et le stade 31 pour le pin (élongation des aiguilles, taille supérieure au brachyblaste, vert tendre). Que ce soit en 2017 ou 2018 nous n'observons pas d'effet de l'irrigation ni du mélange sur les stades phénologiques de débourrement du bouleau et du pin.

### 2.3.2. Senescence des feuilles (bouleau uniquement)

La chute des feuilles de bouleaux démarre de manière très précoce, dès juillet. Cela implique que nous avons manqué le début de cette chute des feuilles en 2017. Nous observons un effet de l'irrigation et du mélange sur la chute des feuilles de bouleau, qui est similaire entre 2017 et 2018. L'irrigation retarde la chute des feuilles : les peuplements irrigués (en bleu) ont une chute de feuille plus tardive que les non-irrigués (en rouge) (**Fig. B 3, Fig. B 4**). Les bouleaux en mélange ont une chute de feuille plus tardive que les bouleaux en peuplement purs, dans les blocs non-irrigués comme irrigués (**Fig. B 3, Fig. B 4**).

## 2.4. Discussion

L'absence d'effet de l'irrigation et du mélange sur les dates de débourrement n'est pas surprenante car il n'y a pas de stress hydrique à cette période-là de l'année. Nous aurions cependant pu observer des effets pour les bouleaux qui auraient été liés à une moins forte exposition à la lumière dans les placettes mélangées par exemple.

La chute des feuilles précoce pour le bouleau indique une réponse à des conditions de sécheresse dès le mois de juillet au cours des deux années étudiées. Cette chute des feuilles est plus précoce dans les blocs non-irrigué et en peuplements purs. Ces effets indiquent que les bouleaux sont moins exposés à la sécheresse (ou à des fortes températures qui provoquent la chute des feuilles) dans les blocs irrigués et dans les placettes en mélange avec les pins.

Ces données n'ont pas pu être analysées de manière plus approfondies du fait d'un manque de temps, mais aussi car nous n'avions pas la totalité de la dynamique de chute des feuilles en 2017.




















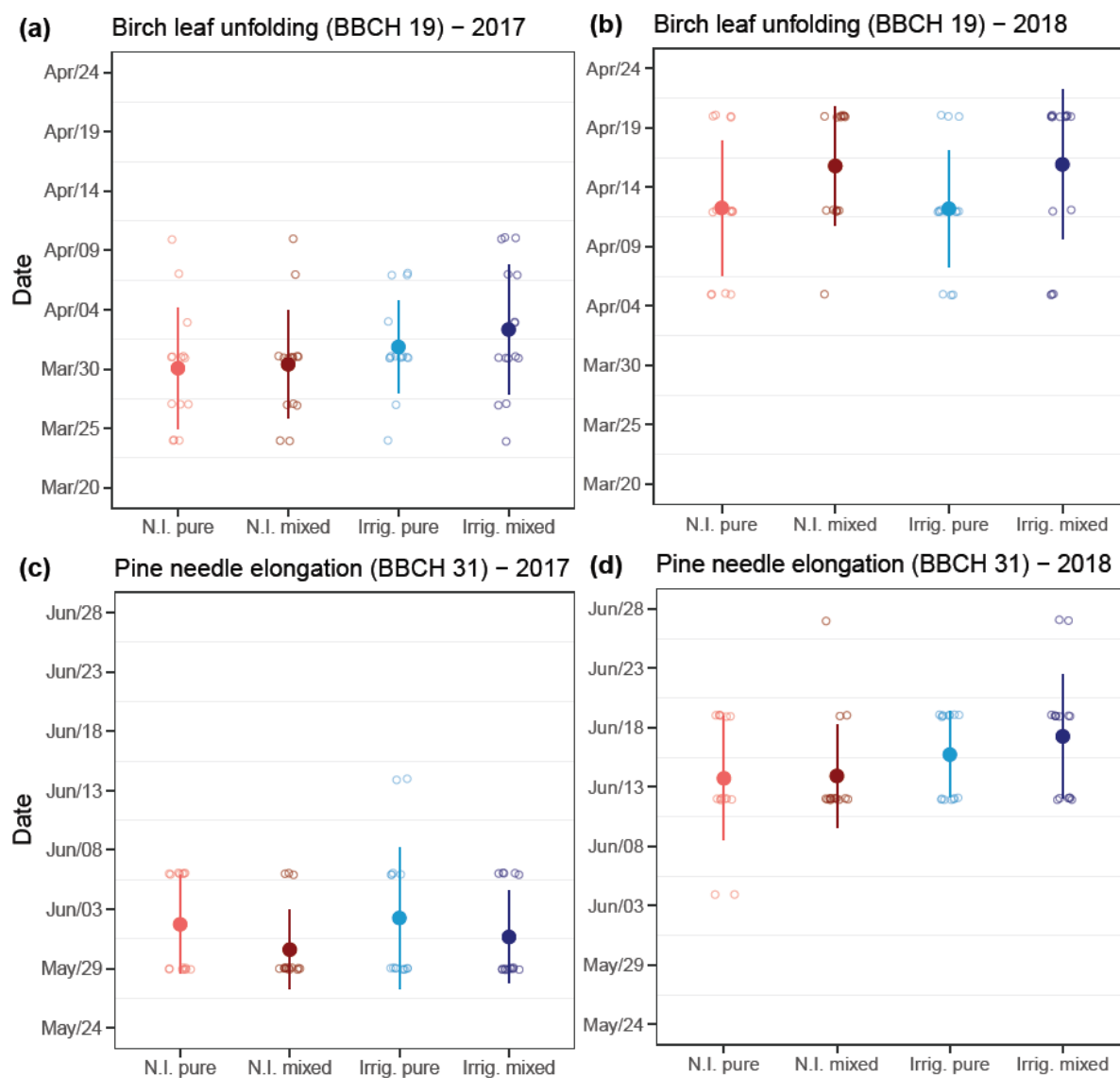
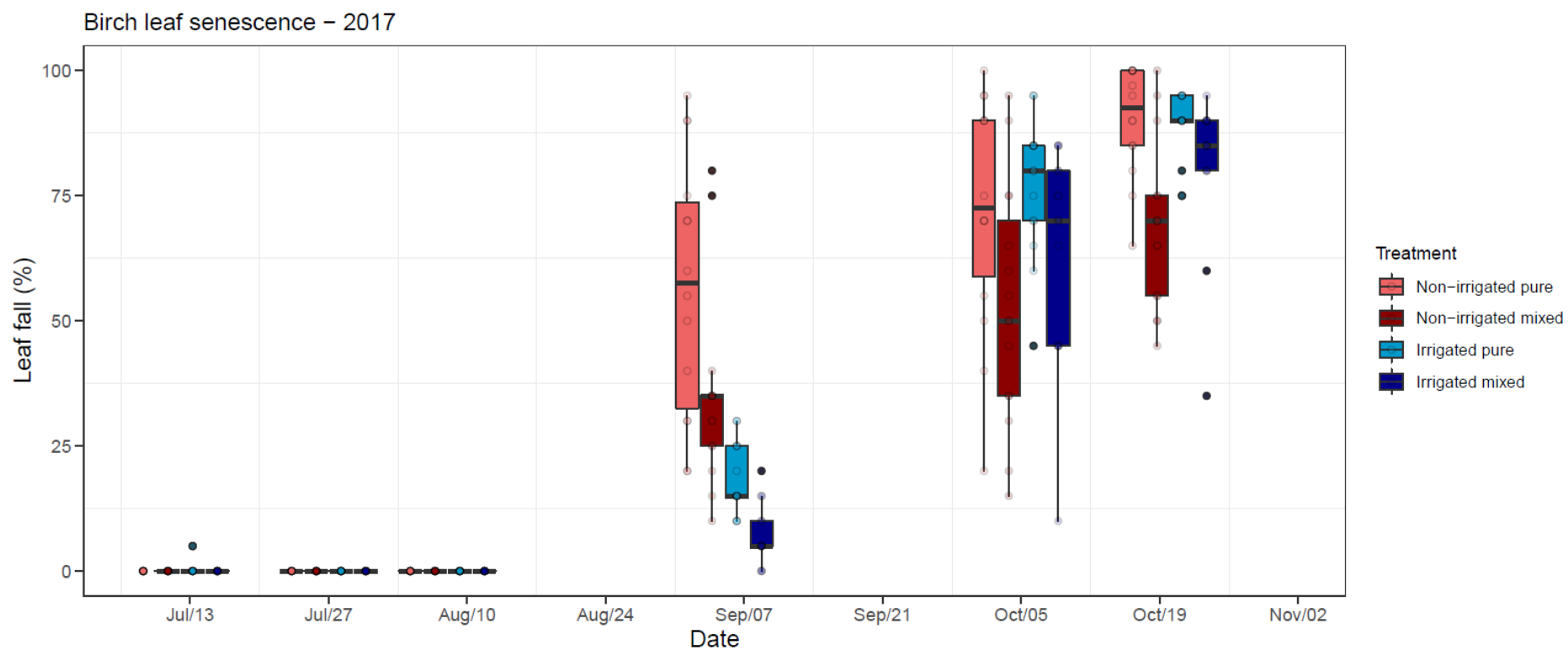
Essence	DEBOURREMENT				FEUILLAISSON				ELONGATION			
	stade 0	stade 1	Stade 5	stade 9	stade 11	stade 13	stade 15	stade 19	stade 30	stade 31	stade 32	stade 33
Pinus Pinaster	Bourgeon sombre de plus ou moins grande taille	Changement de couleur du bourgeon et élongation de ce dernier. Ce stade peut durer assez longtemps selon la taille de l'élongation	La gaine protectrice se désagrège et laisse apparaître les brachyblastes	Apparition de la partie chlorophyllienne des aiguilles à l'extrémité des brachyblastes. La longueur des futures aiguilles n'excède pas celle des brachyblastes					Au stade 30 les aiguilles sont séparées à l'extrémité, de longueur égale ou légèrement supérieure aux brachyblastes	Au stade 31, les aiguilles développées sont d'une longueur nettement supérieure à celle du brachyblaste. Elles sont de couleurs vertes tendres et la terminalson de la pousse est constituée d'un "amas" d'aiguilles	Au stade 32 les aiguilles n'ont pas forcément toutes atteintes leur taille finale. On observe un bourgeon en fin de pousse	Au stade 33, on observe une seconde pousse après la première
												
	(Échelle8)											
Essence	DEBOURREMENT				FEUILLAISSON				ELONGATION			
	stade 0	stade 1	Stade 7	stade 9	stade 11	stade 15	stade 19	stade 31	stade 32			
Betula Pendula	bourgeon sombre, écaille bien visible	Le bourgeon s'ouvre vert bien visible mais impossible de deviner les feuilles	Le bourgeon continue de s'ouvrir. On commence à deviner les futures feuilles. Attention les bourgeons fleurissent débourent au même moment.	La base du bourgeon est toujours visible. On distingue bien les feuilles mais elles restent regroupées	La feuille est complètement sortie du bourgeon mais reste recroquevillée sur elle-même. Pas d'élongation de la tige observée	La feuille n'est pas encore complètement étalée, très petite, tendre et encore pâle. Pas d'élongation de la tige observée	A ce stade la feuille est complètement étalée. Pas d'élongation de la tige observée	A ce stade les entre nœuds sont visibles et plusieurs feuilles sont sorties et étalées sur l'élongation. La tige n'est pas lignifiée	Au stade 32 on peut facilement observer les bourgeons qui se forment sur l'élongation de l'année			
												
	(Échelle7)											

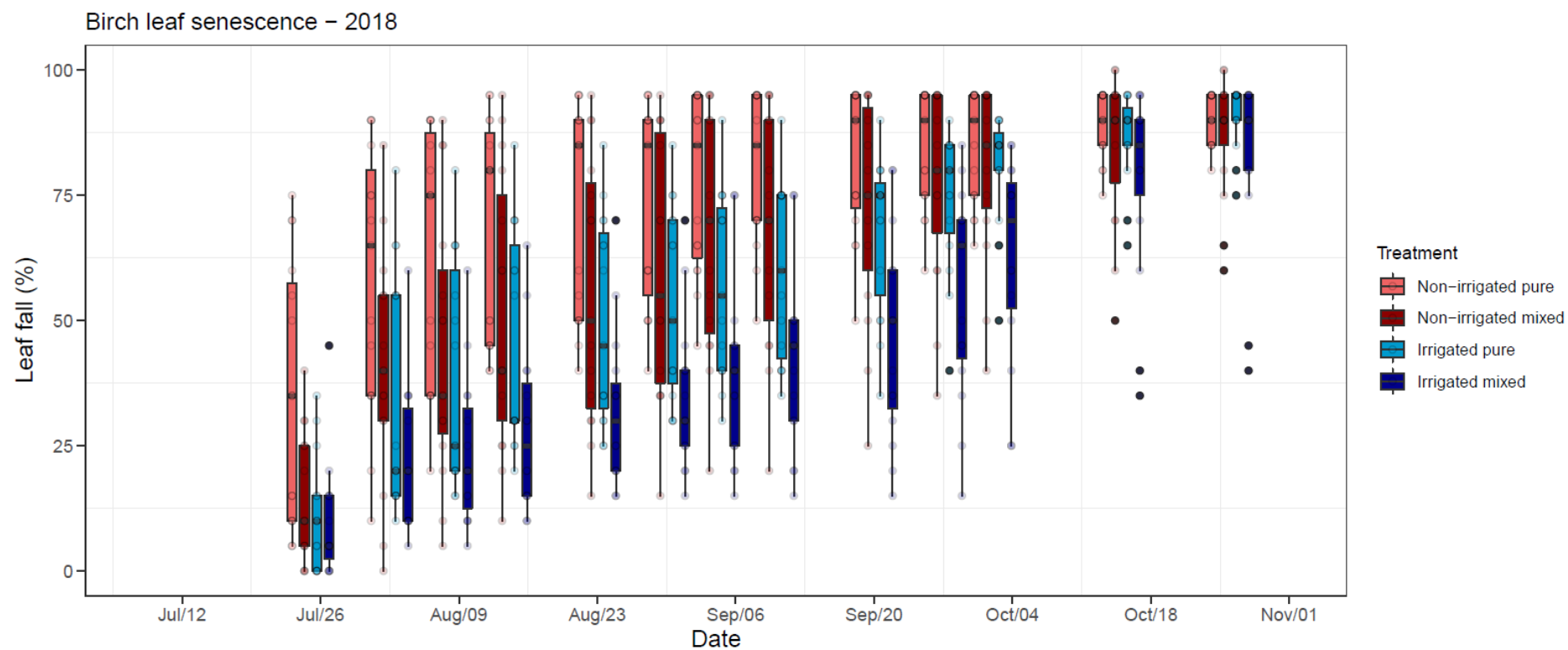
Fig. B 1 Protocole de suivi des stades de développement des feuilles et aiguilles au printemps (Issu du rapport de stage d'Arnaud Lafitte)



**Fig. B 2** Phénologie du développement foliaire des bouleaux (a, b) et des pins (c, d) en 2017 (a,c) et 2018 (b,d). Les stades phénologiques représentés ici sont : le stade 19 pour le bouleau (feuilles étalées, pas d'élongation) et le stade 31 pour le pin (élongation des aiguilles, taille supérieure au brachyblaste, vert tendre).



**Fig. B 3** Phénologie de la senescence des feuilles pour le bouleau en 2017 : pourcentage de feuilles mortes en fonction de la date de suivi. Il y a eu peu de dates de suivi en 2017 pour la chute des feuilles.



**Fig. B 4** Phénologie de la senescence des feuilles pour le bouleau en 2018 : pourcentage de feuilles mortes en fonction de la date de suivi. Les dates de suivi ont été beaucoup plus fréquentes en 2018 qu'en 2017, cependant le début de la chute des feuilles a été manqué, du fait d'une perte précoce liée à la sécheresse.

## Tree-species interactions in mixed forests: effects on tree drought response and role of water and light resource partitioning mechanisms

### Abstract:

Biodiversity, through tree species interactions, can support many ecosystem functions and services in forest ecosystems. However, in the current context of climate change, the influence of these interactions on the response of forest ecosystems to extreme climatic events, such as drought, is still under debate. Moreover, evidence for the underlying above-ground and below-ground interaction mechanisms that can explain these relationships between diversity and forest ecosystem functioning is still rare.

The first objective of my thesis work was to determine the effect of tree species interactions in mixed stands on tree water relations and drought response. My second objective was to identify below-ground or above-ground mechanisms related to resource partitioning that could explain mixture effects on functioning, such as depth of water uptake by roots or canopy space filling by tree crowns.

This work was carried out, within the framework of the ANR DiPTICC project, in two distinct experimental systems: an observational network in mature natural forests in the south-east of France (GMAP) and a young experimental plantation near Bordeaux (ORPHEE). I worked on three types of two-species mixtures: beech (*Fagus sylvatica*) - fir (*Abies alba*) and beech - downy oak (*Quercus pubescens*) along the GMAP network and birch (*Betula pendula*) - maritime pine (*Pinus pinaster*) at ORPHEE. My work is based on an empirical approach, with ecophysiological and light interception measurements, under contrasting conditions of soil water availability. Functions such as transpiration or water use efficiency (estimated by measuring carbon isotopic composition) were studied.

My work shows that in young and old stands, the water relations and drought response of the species studied are little impacted by species interactions. Differences among species in water extraction depths, when present, did not necessarily result in higher water availability for species in mixed stands under drought conditions. Contrary to hypotheses often made in the literature, this suggests that belowground mechanisms would seldom drive the effects of species interactions. In contrast, I observed that the mechanisms of crown complementarity generated changes in stand structure and light interception and appeared to have a strong impact on the functioning of mixed stands.

My work confirms that, for the same combination of tree species, the species-mixing effects on tree functioning are highly dependent on local environmental conditions. It is therefore essential for forest managers to take these local conditions into account when making choices about the species composition of future forests. Overall, we found no negative mixture effects on water relations, suggesting that managing forests in favor of mixed stands remains an interesting option for managers to maintain many other forest ecosystem functions and services under climate change.

**Keywords:** functional ecology, community ecology, mixed forests, species interactions, biodiversity, climate change, drought, water use, stable isotopes, light interception

## Interactions des espèces d'arbres en peuplements mélangés : effets sur la réponse à la sécheresse des arbres et rôle des mécanismes de partage des ressources en eau et en lumière

### Résumé :

La biodiversité, via les interactions entre espèces d'arbres, peut favoriser un grand nombre de fonctions et de services écosystémiques dans les écosystèmes forestiers. Cependant, dans le contexte actuel de changement climatique, l'influence de ces interactions sur la réponse des écosystèmes forestiers à des événements climatiques extrêmes, tels que la sécheresse, fait encore débat. De plus, la mise en évidence des mécanismes d'interaction aériens et souterrains pouvant expliquer ces relations entre diversité et fonctionnement de l'écosystème forestier reste encore rare.

Le premier objectif de mon travail de thèse était de déterminer l'effet des interactions des espèces d'arbres en peuplements mélangés sur le fonctionnement hydrique et la réponse à la sécheresse des arbres. Mon deuxième objectif était de mettre en évidence les mécanismes d'interaction souterrains ou aériens de partage des ressources pouvant expliquer ces effets sur le fonctionnement, tels que la profondeur d'extraction de l'eau par les racines ou l'occupation de l'espace de la canopée par les houppiers.

Ce travail a été réalisé, dans le cadre du projet ANR DiPTICC, dans deux dispositifs expérimentaux distincts : un réseau de forêts naturelles matures dans le sud-est de la France (GMAP) et une jeune plantation expérimentale proche de Bordeaux (ORPHEE). J'ai travaillé sur trois types de mélanges à deux espèces : hêtre (*Fagus sylvatica*) – sapin (*Abies alba*) et hêtre - chêne pubescent (*Quercus pubescens*) le long du réseau GMAP et bouleau (*Betula pendula*) - pin maritime (*Pinus pinaster*) à ORPHEE. Mon travail est fondé sur une approche empirique avec des mesures expérimentales d'écophysiologie et d'interception de la lumière, dans des conditions contrastées de disponibilité en eau du sol. Des fonctions telles que la transpiration ou l'efficacité d'utilisation de l'eau (estimée par la mesure de la composition isotopique du carbone) ont été étudiées.

Mes travaux montrent que dans ces peuplements jeunes ou âgés, le fonctionnement hydrique et la réponse à la sécheresse des espèces étudiées sont peu impactés par les interactions des espèces. Les différences de profondeur d'extraction de l'eau entre les espèces, quand elles étaient présentes, n'ont pas nécessairement engendré une meilleure disponibilité de l'eau pour les espèces en mélange en conditions de sécheresse. Contrairement à des hypothèses souvent formulées dans la littérature, cela suggère que les mécanismes souterrains seraient peu souvent à l'origine des effets des interactions des espèces. En revanche, j'ai observé que les mécanismes de complémentarité des houppiers ont engendré des modifications de structure du peuplement et d'interception de la lumière et sont apparus comme ayant un fort impact sur le fonctionnement du mélange.

Mes travaux confirment que, pour une même combinaison d'espèces d'arbres, les effets du mélange d'espèces sur le fonctionnement des arbres dépendent fortement des conditions environnementales locales. Il est donc primordial pour les gestionnaires forestiers de prendre en compte ces conditions locales dans les choix sur la composition en espèces des forêts du futur. Globalement, nous n'avons pas trouvé d'effets négatifs du mélange sur le fonctionnement hydrique, ce qui suggère que la gestion des forêts au profit de peuplements mélangés reste une option intéressante pour les gestionnaires afin de maintenir de nombreuses autres fonctions et services écosystémiques des forêts dans le contexte du changement climatique.

**Mots clés :** écologie fonctionnelle, écologie des communautés, forêts mélangées, interactions des espèces, biodiversité, changement climatique, sécheresse, utilisation de l'eau, isotopes stables, interception de la lumière