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## Differential effects of tree species identity on rhizospheric bacterial and fungal community richness and composition across multiple trace element-contaminated sites

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1           **Differential effects of tree species identity on rhizospheric bacterial and fungal**  
2           **community richness and composition across multiple trace element-contaminated sites**

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26 **Abstract:**

27           Soil microbial communities play a key role in plant nutrition and stress tolerance. This  
28 is particularly true in sites contaminated by trace metals, which often have low fertility and  
29 stressful conditions for woody plants in particular. However, we have limited knowledge of  
30 the abiotic and biotic factors affecting the richness and composition of microbial communities  
31 inhabiting the rhizosphere of plants in contaminated sites. Using high-throughput amplicon  
32 sequencing, we studied the rhizospheric bacterial and fungal community structures of 14  
33 woody plant families planted in three contrasting sites contaminated by metals (Pb, Cd, Zn,  
34 Mn, Fe, S). The rhizospheric bacterial communities in the given sites showed no significant  
35 difference between the various woody species but did differ significantly between sites. The  
36 Proteobacteria phylum was dominant, accounting for over 25% of the overall relative  
37 abundance, followed by Actinobacteria, Bacteroidetes and Gemmatimonadetes. Site was also  
38 the main driver of fungal community composition, yet unlike bacteria, tree species identity  
39 significantly affected fungal communities. The Betulaceae, Salicaceae and Fagaceae families  
40 had a high proportion of Basidiomycota, particularly ectomycorrhizal fungi, and the lowest  
41 diversity and richness. The other tree families and the unplanted soil harboured a greater  
42 abundance of Ascomycota and Mucoromycota. Consequently, for both bacteria and fungi, the  
43 site effect significantly impacted their community richness and composition, while the  
44 influence of plants on the richness and composition of rhizospheric microbial communities  
45 stayed consistent across sites and was dependent on the microbial kingdom. Finally, we  
46 highlighted the importance of considering this contrasting response of plant rhizospheric  
47 microbial communities in relation to their host identity, particularly to improve assisted  
48 revegetation efforts at contaminated sites.

49

50 **Keywords:** Phytomanagement, Metabarcoding, Contaminated sites, Microbial communities

## 51 **1. Introduction**

52 In the last two centuries, soil pollution in European countries has increased, and the  
53 number of polluted sites has reached approximately three million (Europe's environment -  
54 The fourth assessment). Among soil contaminants, mineral oil, trace elements (TEs) and  
55 polycyclic aromatic hydrocarbons are the most common (Mench et al., 2010; Quintela-  
56 Sabarís et al., 2017). In addition, soil contaminants may be transferred or dispersed to other  
57 environmental compartments by several drivers, such as wind (Quintela-Sabarís et al., 2017).  
58 Conventional engineering technologies for mitigating soil pollution have often been proven  
59 economically prohibitive and ecologically unfriendly (Adriano et al., 2004). Consequently, it  
60 is necessary to find new strategies to mitigate and remediate soil pollution (Hullot et al.,  
61 2021). New soil management strategies have emerged in recent decades, prominently  
62 featuring plant-based alternatives such as phytoremediation (Kidd et al., 2015). This  
63 development has led to significant growth in the literature about using native pioneer trees for  
64 revegetation efforts on degraded lands (Asensio et al., 2018; Mleczek et al., 2017).  
65 Importantly, most studies have focused on contaminated sites planted with a single tree  
66 species (Beauchamp et al., 2018; Gonzalez et al., 2018) or various genotypes of the same  
67 species, such as poplar (Di Baccio et al., 2003; Laureysens et al., 2004; Pottier et al., 2015;  
68 Robinson et al., 2007; Sebastiani et al., 2004). In some other studies, a combination of trees  
69 (Al Souki et al., 2022; Hullot et al., 2021) or trees and herbaceous plants (Desjardins et al.,  
70 2018) has been investigated, and in some of these studies, plant-associated microorganisms  
71 were studied. Plant microbiomes are very diverse and contribute to plant fitness; thus, they  
72 deserve attention because they might help plants deal with the often harsh conditions of  
73 polluted sites.

74 Soil contamination, as well as plant-based management, induces changes in fungal and  
75 bacterial community structures. It was reported that TEs have several deleterious effects on

76 microorganisms and play a substantial role as an environmental filter in structuring soil  
77 communities (Song et al., 2021). Several authors have used the phytoremediation strategy  
78 combined with bacterial or fungal inoculation (Ciadamidaro et al., 2019; Phanthavongsa et al.,  
79 2017; Pogrzeba et al., 2017) to enhance plant resistance to soil contamination and improve  
80 biomass production. Among the mechanisms involved, microorganisms can enhance plant  
81 growth and health by producing phytohormones, cytokinins and gibberellins (Taghavi et al.,  
82 2009; Tanimoto, 2005). They can also improve nutrient availability through the production of  
83 organic acids and siderophores (Álvarez-López et al., 2020). Furthermore, a large range of  
84 woody species are known to select their microbial communities (Zhang et al., 2021) to  
85 improve plant resistance to stress (*e.g.*, through production of 1-aminocyclopropane-1-  
86 carboxylic acid deaminase), such as drought, insects, diseases, or TEs (Glick, 2014; Meyer et  
87 al., 2023, 2023; Taghavi et al., 2009)

88 Numerous authors have assessed the microbial communities in noncontaminated  
89 rhizospheric soils, such as forestry fields (Baldrian, 2016; de Gannes et al., 2016). Most of  
90 these studies focused on woody species of biomass interest or noble woods (*e.g.*, oaks,  
91 poplars, pines). At contaminated sites, the majority of studies have assessed the rhizospheric  
92 fungal (Foulon et al., 2016; Gonzalez et al., 2018) and bacterial communities (Álvarez-López  
93 et al., 2020; Durand et al., 2018; Girardot et al., 2020; Zappelini et al., 2018) in monoculture  
94 and/or in several species from the same genus/genotype (*e.g.*, *Populus*, *Salix*, *Betula*).  
95 However, as contaminants (*e.g.*, TEs) are known to impact microbial communities, it is  
96 important to understand the structure of rhizospheric fungi and bacteria. Moreover, the  
97 microbial communities from trees growing in several types of soil exhibiting different  
98 agronomical and contamination parameters have been poorly studied.

99 In Leforest, Carrières and Thann, three studied contaminated sites in France, the  
100 growth of cultured woody species does not appear to be restricted by the presence of TEs in

101 the soil (Ciadamidaro et al., 2019). However, at the three sites, the effect of these trace  
102 elements on the rhizospheric microbial communities that likely drive plant primary  
103 productivity is unknown. It is thus important to determine how site and tree species identity  
104 shape the richness and composition of the rhizospheric microbial communities, as it may help  
105 in understanding the revegetation processes of contaminated areas. With regard to our  
106 previous data (Foulon et al., 2016), we hypothesized that the site characteristics would  
107 intensely shape bacterial and fungal communities. In a second hypothesis, we predicted that  
108 tree species would be an additional source of variation in microbial communities. We also  
109 aimed to determine which of the two factors (soil parameters vs. tree species identity) is the  
110 main microbial community driver. To address these hypotheses, the rhizospheric bacterial and  
111 fungal communities of twenty-five woody species growing at three phytomanaged sites with  
112 contaminants of various origins were assessed and compared using high-throughput amplicon  
113 sequencing. In this study, we also address the question of the influence of site  
114 physicochemical parameters versus tree selection on microbial communities.

115

## 116 **2. Materials and Methods**

### 117 *2.1. Site description*

118 We conducted our study at three experimental sites in France, namely, Carrières  
119 (48°57'39.7"N 2°02'09.6"E), Leforest ((50°25'40.5"N 3°02'40.5"E) and Thann (47°47'48.4"N  
120 7°08'20.8"E) (Ciadamidaro et al., 2019). Carrières and Leforest have agricultural soils with  
121 high levels of lead (Pb), cadmium (Cd) and zinc (Zn) due to wastewater and atmospheric  
122 deposition, respectively. Thann is an industrial landfill mainly contaminated by manganese  
123 (Mn), sulfur (S) and iron (Fe). They were initially planted in autumn 2013 with 38 tree  
124 varieties belonging to 14 families (Sapindaceae, Betulaceae, Bignoniaceae, Meliaceae,  
125 Moraceae, Elaeagnaceae, Paulowniaceae, Platanaceae, Juglandaceae, Fagaceae,

126 Anacardiaceae, Salicaceae, Oleaceae, Ulmaceae). Each of the three sites was divided into four  
127 plots that were planted with 16 (in Carrières and Leforest) or 18 (in Thann) tree species (31  
128 replicates per tree species in each plot). For the present study, 12 tree species were selected  
129 per site based on their initial growth (Table S1). *Pterocarya stenoptera* (PTS, Fagaceae), *Salix*  
130 *aquatica grandis* (SAQ, Salicaceae) and *Ostrya carpinifolia* (OCA, Betulaceae) were the  
131 common species at all three sites. The species that were planted on one or two sites are ABU  
132 (*Acer buergerianum*), ALS (*Alnus subcordata*), ASA (*Acer saccharinum*), CON (Control -  
133 unplanted soil), CSP (*Catalpa speciosa*), EUM (*Eleagnus umbellata*), FOR (*Fraxinus ornus*),  
134 FOX (*Fraxinus oxyphylla*), HRH (*Hippophae rhamnoides*), MPO (*Maclura pomifera*), OCA  
135 (*Ostrya carpinifolia*), PLO (*Platanus orientalis*), POX (*Populus maximowiczii* x *P.*  
136 *berolinensis* (Oxford clone)), PTS (*Pterocarya stenoptera*), QAC (*Quercus acutissima*), QCE  
137 (*Quercus cerris*), QPD (*Quercus pedunculata*), QPT (*Quercus petraea*), RCO (*Rhus*  
138 *copallina*), SAC (*Salix acutifolia*), SAL (*Salix alba*), SAQ (*Salix aquatica grandis*), SCA  
139 (*Salix caprea*), SFR (*Salix fragilis*), UPA (*Ulmus pumila*) and ZSE (*Zelkova serrata*). The  
140 initial sampling campaign (Ciadamidaro et al., 2019) was carried out in 2015, *i.e.*, two  
141 growing seasons after plantation establishment. For the present study, the sampling campaign  
142 was carried out in early spring 2018, *i.e.*, four growing seasons after planting.

143

## 144 2.2. Soil parameters and sampling

145 For each of the four plots at each site, we randomly selected three trees per species  
146 (*i.e.*, 12 replicates/tree species/site) that were uprooted using a mechanical shovel. Directly  
147 after uprooting, approximately 2 L of non-rhizospheric soil (*i.e.* unplanted soil corresponding  
148 to the control soil = CON) and rhizospheric soils were collected in sterile plastic bags and  
149 kept at 4 °C for further soil analysis of CaCl<sub>2</sub>-extractable concentrations of 12 TEs. For  
150 community analysis, 16 g of the rhizospheric soil of each tree was sampled as described and

151 placed in 250 mL plastic tubes with four silica gel bags as described in Taberlet et al. (2012).  
152 In total, 432 rhizospheric soil samples were collected (3 sites x 4 plots/site x 12 species x 3  
153 replicates) and shipped to the Laboratoire d'Écologie Alpine (Grenoble, France) for DNA  
154 extraction, PCR amplification and high-throughput amplicon sequencing.

155 The soil physico-chemical characteristics were analysed in 2013 at the plantation  
156 (agronomical characteristics and TE concentrations), and in 2017 at the sampling campaign  
157 (TE concentrations). The initial physico-chemical characteristics of the soils were presented  
158 in a previous study (Ciadamidaro et al., 2019). The soil samples were dried at 40 °C in a  
159 forced air oven to a constant weight and ground with a grinder (agate mortar, Retsch RM100).  
160 The samples were sent to a certified laboratory (SADEF, France) for the following analyses:  
161 particle size distribution, exchangeable Ca (CaO), Mg (MgO), K (K<sub>2</sub>O) and Na (Na<sub>2</sub>O),  
162 cation exchange capacity (CEC) (NF X 31-130), organic matter (OM) (NF ISO 14235), total  
163 CaCO<sub>3</sub> (NF ISO 10693), total nitrogen (N<sub>t</sub>) (NF ISO 13878), total phosphorus (Joret-Hebert  
164 method) (NF X 31-161) and soluble boron (B) (NF X 31-122). The available element  
165 concentrations in the soil were assessed in 2013 and 2017 using a 0.01 M CaCl<sub>2</sub>-extraction  
166 with a sample extraction ratio of 1:10 via ICP-AES. Briefly, in 2013, the soil pH ranged from  
167 5.4 at Leforest to 7.1 at Thann, with Carrières presenting an intermediate pH (pH= 6.8). Other  
168 parameters, such as the organic matter (37, 68 and 29 g kg<sup>-1</sup> dry weight (DW) in Leforest,  
169 Carrières and Thann, respectively), total nitrogen (1.9, 3.8 and 1.3 g kg<sup>-1</sup> DW) and organic  
170 carbon (21, 39 and 16 g kg<sup>-1</sup> DW) concentrations, were higher in Carrières than in the two  
171 other sites. The main chemical elements characterizing the soil of Thann were Mn (total Mn  
172 concentrations [Mn]<sub>tot</sub> = 1472 µg g<sup>-1</sup>), Fe ([Fe]<sub>tot</sub> = 28267 µg g<sup>-1</sup>) and S ([S]<sub>tot</sub> = 85712 µg  
173 g<sup>-1</sup>), while Pb ([Pb]<sub>tot</sub> = 311 and 195 µg g<sup>-1</sup>), Zn ([Zn]<sub>tot</sub> = 685 and 250 µg g<sup>-1</sup>) and Cd ([Cd]  
174 <sub>tot</sub> = 6 and 3.7 µg g<sup>-1</sup>) were the main metallic contaminants in Carrières and Leforest at the  
175 time of planting.

176

### 177 *2.3. DNA extraction*

178 Extracellular DNA was extracted from the dried soil following a modified version of  
179 the phosphate buffer protocol described by Taberlet et al. (2012). Briefly, each soil sample  
180 was combined with 20 ml of saturated phosphate buffer ( $\text{Na}_2\text{HPO}_4$ ; 0.12 M; pH 8). The  
181 resulting mixture was shaken gently (45 rpm) for 15 min and centrifuged at 11,000 g for 10  
182 min. The following DNA extraction steps were carried out using the NucleoSpin Soil Kit  
183 (Macherey–Nagel, Düren, Germany) according to the manufacturer’s instructions and after  
184 skipping the lysis steps. Four hundred microlitres of supernatant was added to 250  $\mu\text{L}$  of SB  
185 buffer, loaded onto the extraction column, and washed once with SB buffer, once with SW1  
186 buffer, and twice with SW2 buffer. The final elution was performed with 100  $\mu\text{L}$  of SE buffer.

187

### 188 *2.4. Molecular methods and sequencing*

189 Microbial diversity was surveyed using the Bact02 (GCCAGCMGCCGCGGTAA and  
190 GGACTACCMGGGTATCTAA) and Fung02 (GGAAGTAAAAGTCGTAACAAGG and  
191 CAAGAGATCCGTTGYTGAAAGTK) primer pair reported in Taberlet et al. (2018). These  
192 markers target the V4 region of the bacterial 16S rDNA and the fungal ITS1 region,  
193 respectively. Four independent PCR replicates were carried out for each DNA extract and  
194 each marker. To allow bioinformatic discrimination of PCR replicates after sequencing, eight-  
195 nucleotide tags were added to the 5’ end of both the forwards and reverse primers so that each  
196 PCR replicate was represented by a unique combination of forwards and reverse tags. Tags  
197 had at least five nucleotide differences among them (Coissac, 2012). Two to four random  
198 nucleotides were also added on the 5’ end of the tags to ensure sufficient sequence diversity  
199 and efficient detection of clusters during sequencing.

200 PCR amplifications were performed in a 20  $\mu\text{L}$  volume containing 10  $\mu\text{L}$  of AmpliTaq  
201 Gold 360 Master Mix (Applied Biosystems, Foster City, CA, USA), each primer at 0.2  $\mu\text{M}$  ,  
202 0.16  $\mu\text{L}$  of 20  $\text{mg}\cdot\text{ml}^{-1}$  bovine serum albumin (BSA; Roche Diagnostics, Basel, Switzerland),  
203 and 2  $\mu\text{L}$  of DNA extract. Thermocycling conditions included an initial DNA polymerase  
204 activation step of 10 min at 95  $^{\circ}\text{C}$ , 40 cycles with a 30 s denaturation at 95  $^{\circ}\text{C}$ , a 30 s  
205 annealing step at 53  $^{\circ}\text{C}$  for Bact02 and 56  $^{\circ}\text{C}$  for Fung02, and a 60 s elongation step at 72  $^{\circ}\text{C}$ ,  
206 followed by a final extension at 72  $^{\circ}\text{C}$  for 7 min.

207 After amplification, PCR products were combined in equal volumes and purified with  
208 the MinElute PCR purification kit (Qiagen, Hilden, Germany) following the manufacturer's  
209 instructions. Library preparation and sequencing were outsourced to Fasteris SA (Geneva,  
210 Switzerland). Four sequencing libraries (two per marker) were prepared according to the  
211 PCR-free MetaFast protocol ([www.fasteris.com](http://www.fasteris.com)) that limits the formation of chimaeras. All  
212 amplicons were sequenced on a HiSeq 2500 sequencing platform (Illumina, San Diego, CA,  
213 USA) using the rapid run mode generating 250 bp paired-end sequences.

214 To monitor potential contaminants and false positives caused by tag jumps (Schnell et  
215 al. 2015), the experiment included extraction and PCR negatives, as well as unused tag  
216 combinations. Soil extracts from another experiment were also used as positive controls.

217

## 218 *2.5. Bioinformatic analysis of metabarcoding data*

219 In total, 81,589,286 sequencing clusters were paired-end sequenced. Raw reads were  
220 analysed using the OBITools software suite (Boyer et al., 2016), specifically dedicated to  
221 handling metabarcoding data. Paired-end reads were first assembled with the program  
222 *illuminapairedend*, keeping only sequences with an alignment score higher than 40. Aligned  
223 sequences were assigned to the corresponding sample using the program *ngsfilter* by allowing  
224 two and zero mismatches for primers and tags, respectively. After sequence dereplication

225 using *obiuniq*, poor-quality sequences (*i.e.*, containing “N”), sequences whose length fell  
226 outside the expected size interval (shorter than 40 bp for Bact02 or 66 bp for Fung02) and  
227 sequences observed less than ten times in at least one PCR replicate were filtered out. The  
228 remaining unique sequences were clustered into molecular operational taxonomic units  
229 (MOTUs) as follows. First, the Sumatra algorithm (Mercier et al., 2013) was run to compute  
230 pairwise similarities between sequences based on the length of the longest common  
231 subsequence (LCS). Then, pairwise similarities were used to cluster the sequences with a  
232 similarity threshold of 97% using Sumacust (Mercier et al., 2013). Abundances of sequences  
233 belonging to the same cluster (*i.e.*, MOTU) were summed, and the cluster centre was kept as  
234 the MOTU representative of the cluster. The final datasets contained 2,440 MOTUs and  
235 7,130,286 sequences for Bact02 and 8,119 MOTUs and 25,772,129 sequences for Fung02.  
236 Finally, the taxonomic assignment of each MOTU was performed using the program *ecotag*  
237 of OBITools, which compares the MOTU sequence to a database of full-length reference  
238 metabarcodes. The reference database was built for each marker by running the *ecoPCR*  
239 program (Ficetola et al., 2010). More specifically, *ecoPCR in-silico* PCR was carried out on  
240 the EMBL public database (release 133) with the primer pair used for the experiment and  
241 allowing three mismatches per primer. The obtained reference databases were further curated  
242 by keeping only sequences assigned at the species, genus and family levels.

243

## 244 2.7. Statistical analysis of metabarcoding data

245 The community composition of all three sites was first analysed separately for  
246 bacterial and fungal OTUs. A Bray–Curtis dissimilarity matrix was built for both fungal and  
247 bacterial community compositions and graphically represented by principal coordinate  
248 analysis (PCoA) (Borcard et al., 2018). Significant differences in community composition  
249 were assessed by permutational multivariate analysis of variance (PERMANOVA) between

250 the three sites (Anderson, 2001). The numbers of OTUs observed in more than one site  
251 (“shared OTUs”) and those in only one site (“specific OTUs”) were represented by Venn  
252 diagrams. The same approach (PCoA followed by PERMANOVA and Venn diagram  
253 construction) was used for the OTUs observed in the rhizosphere of the three common woody  
254 species (PTS, SAQ and OCA).

255 Richness, abundance and community composition were then separately analysed per  
256 site for both bacterial and fungal OTUs. Richness was estimated by the number of different  
257 OTUs, whereas abundance was estimated by the number of OTUs per unique OTU or per  
258 phylum. Richness was compared between planted woody species per site using the  
259 nonparametric Kruskal–Wallis test. When the Kruskal–Wallis test showed a significant  
260 difference, multiple comparisons by Fisher's least significant difference with the Bonferroni  
261 correction were also carried out. The numbers of shared and specific OTUs between planted  
262 woody species were visualized by a graphic composed of a matrix encoding all set  
263 intersections and bar plots representing the number of OTUs in each intersection (Lex and  
264 Gehlenborg, 2014). Abundance was estimated by the number of OTUs in different phyla.  
265 Compositions at the phylum level were graphically represented by PCoA and tested by  
266 PERMANOVA.

267 Redundancy analysis (RDA) was carried out per site to assess the effects of the  
268 environmental conditions on the microbial communities (Borcard et al., 2018). Fungal OTU  
269 composition was considered the response matrix. A Hellinger transformation was applied to  
270 each of the bacterial and fungal community compositions before carrying out RDA. CaCl<sub>2</sub>-  
271 extractable concentrations of 10 TEs (Al, Cd, Fe, K, Mg, Mn, P, Pb, S, Zn) were  
272 logarithmically transformed and applied to RDA as explanatory variables. Significant  
273 explanatory variables were determined by forward selection (Borcard et al., 2018). Each of all  
274 variables was added to the null model and tested its significance by permutation. When there

275 were several significant variables, the most significant one was added. Then, another selection  
276 was carried out to check the significance of the remaining variables. This process was  
277 repeated until there was no significant variable. The proportion of variance explained by the  
278 selected explanatory variables was indicated by an adjusted  $R^2$  (Borcard et al., 2018).

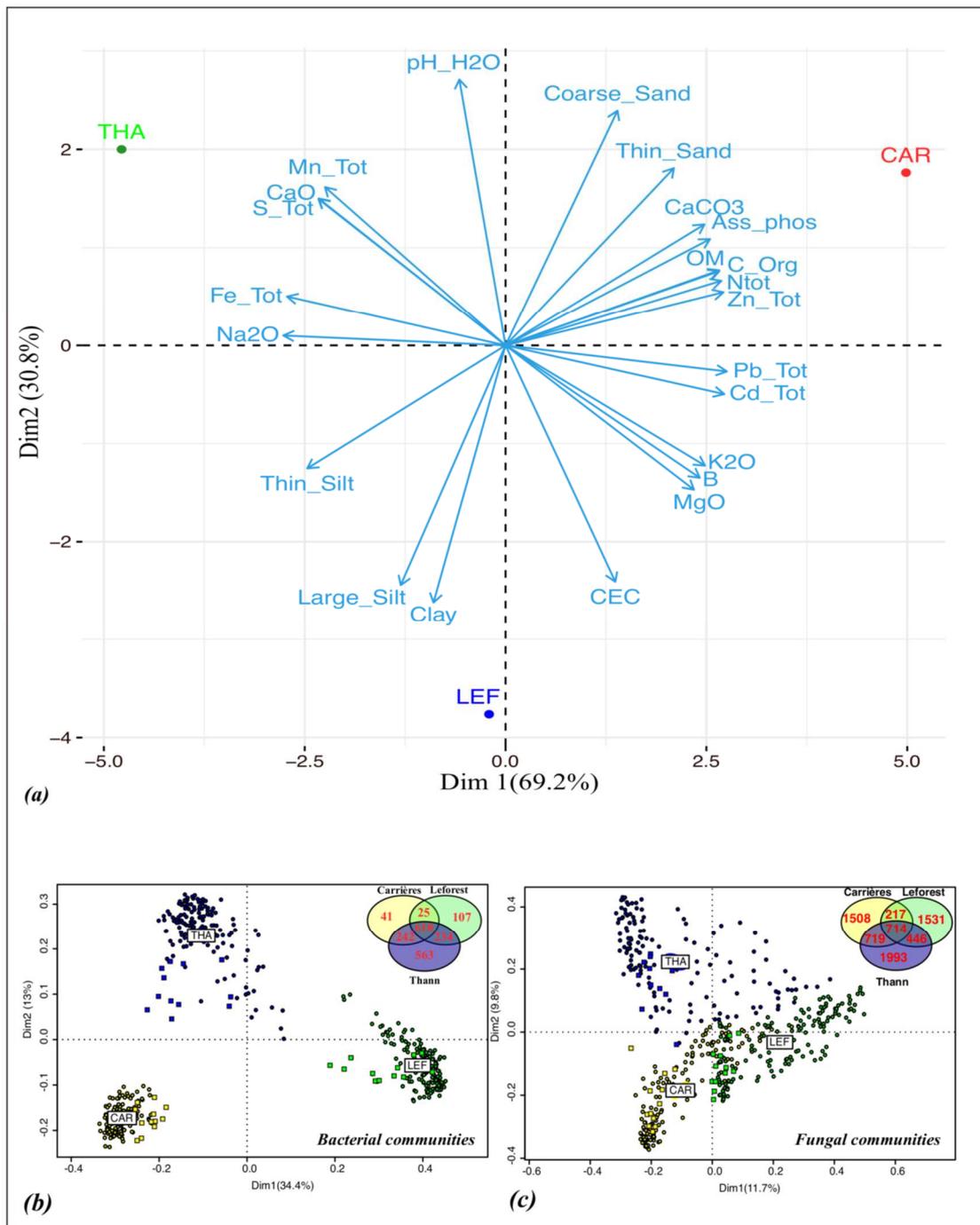
279 All statistical analyses were performed using R software (version 4.1.0; R  
280 Development Core Team). The Kruskal–Wallis tests and multiple comparisons were  
281 performed by the “kruskal” function in the “agricolae” package. Set intersections were  
282 visualized by the “upset” function in the “UpSetR” package. PERMANOVA and tests for  
283 multivariate homogeneity were carried out by the “adonis” and the “betadisper” functions in  
284 the “vegan” package, respectively. The “ordiplot” and “ordiellipse” functions in the “vegan”  
285 package were used to carry out PCoA and draw confidence ellipses, respectively. RDA and  
286 forward selection were carried out by the “rda” and “ordistep” functions in the “vegan”  
287 package.

288

### 289 **3. Results and discussion**

#### 290 *3.1. Site is the main factor affecting the composition of rhizospheric bacterial and fungal* 291 *communities*

292 The soil properties of the three study sites (Carrières, Leforest and Thann) were  
293 significantly different at the planting time (Fig. 1), as shown in Ciadamidaro et al. (2019).  
294 Here, we further analysed the TE bioavailable fractions, as well as pH (Table S2). In general,  
295 the extractable TE concentrations in rhizospheric soils were lower than the total TE  
296 concentrations. Additionally, the bioavailable TE concentrations were slightly higher than the  
297 extractable TE concentrations in the bulk soil measured in a previous experiment conducted at  
298 the same sites in 2015 (Ciadamidaro et al. 2019).



299

300 **Figure 1.** Soil parameters and microbial features of the three sites. (a) Principal component  
 301 analysis (PCA) of agronomic parameters measured at the Carrières, Leforest and Thann sites  
 302 at the plantation (in 2013). All parameters were standardized before PCA because of their  
 303 different units. Principal coordinate analysis (PCoA) of the bacterial (b) and fungal (c)  
 304 communities from rhizospheric and non-rhizospheric soils sampled at the three sites, Leforest  
 305 (LEF), Carrières (CAR) and Thann (THA) in 2017. The numbers of specific and shared

306 *bacterial and fungal OTUs are shown in Venn diagrams. Abbreviations: CEC (cation*  
307 *exchange capacity); OM organic matter); Tot (total); Ass\_phos (assimilable phosphorus).*  
308 *Squares: bulk soils; circles: rhizospheric soils.*

309

310 At the three study sites, the CaCl<sub>2</sub> extractable concentrations of Cd, Pb, Zn and Mn in  
311 the rhizospheric soils were compared between the 12 tree species and the control soil. No  
312 significant difference was observed for Cd, Pb and Mn, while Zn showed few differences.  
313 Significantly higher concentrations of Zn were measured for the three *Quercus* species at  
314 Carrières, but the control soil showed a significantly lower Zn concentration (p value < 0.05)  
315 in comparison to all the rhizospheric soils. In general, the pH values at the three sites slightly  
316 decreased from those measured in our previous experiment in 2015: from 5.4 to 5.0 at  
317 Leforest and from 6.8 and 7.1 to 6.7 at Carrières and Thann, respectively. Given the ability of  
318 certain woody species to accumulate TE in their aerial tissues, changes in total TE  
319 concentrations may have occurred between 2013 and 2017, but these were not measured,  
320 given the large number of samples. A new sampling campaign is planned for 2024, which will  
321 provide a more detailed answer to this very pertinent question.

322 The bacterial (p value < 0.01, Fig. 1b) and fungal (p value < 0.01, Fig. 1c)  
323 communities significantly differed between the three study sites. For bacterial communities,  
324 the Venn diagram shows that the Leforest and Carrières communities shared more OTUs with  
325 each other than with the Thann community, which had more specific OTUs (563 specific  
326 OTUs compared with 41 and 107 OTUs at Carrières and Leforest, respectively). For fungal  
327 communities, twice as many specific OTUs (1508, 1531 and 1993 OTUs in Carrières,  
328 Leforest and Thann, respectively) were found at each site compared with their shared OTUs  
329 (714 OTUs).

330 The differences in soil structure and physico-chemical composition may explain the  
331 difference in microbial community composition. Several studies have reported that soil  
332 chemistry strongly influences microbial communities (Van Horn et al., 2013; Urbanová et al.,  
333 2015; Pinot, 2016; Xue et al., 2018). For instance, Dequiedt et al. (2009) showed a highly  
334 significant correlation between microbial communities and soil physico-chemical parameters  
335 such as soil texture, pH, CEC, C<sub>org</sub>, N, CaCO<sub>3</sub>, P, Ca, Mg and K. It is also known that one of  
336 the most prominent factors affecting the microbial community in the soil is pH (Foulon et al.,  
337 2016; Rousk et al., 2009), which controls numerous biotic factors, such as soil bacterial  
338 biomass and composition (Fierer and Jackson, 2006a). However, pH seemed to be less  
339 important for soil fungi (Lauber et al., 2009; Rousk et al., 2009; Urbanová et al., 2015). The  
340 impact of tree species is further discussed in Section 3.2 below.

341 Figure 2 represents the number of fungal (Fig. 2 a, b and c) and bacterial (Fig. 2 d, e  
342 and f) OTUs that were specific to one soil sample (red bars) or shared (green bars) by all soil  
343 samples at the three sites. At the Leforest site, 48% of the total fungal OTUs (1399/2910)  
344 recorded were specific to one soil sample, *i.e.*, to one tree species (Fig. 2a), whereas 52%  
345 (1511/2910) of the OTUs were shared by at least two tree species (not shown). In detail, *Salix*  
346 *fragilis* (SFR) had the fewest specific OTUs (39/2910 = 1.3%), while *Platanus orientalis*  
347 (PLO) presented the most specific OTUs (7.4%). The number of fungal OTUs shared by all  
348 the tree species accounted for 5.4% (156/2910), whereas the control soil (unplanted soil)  
349 presented 7.9% of the total OTUs in the whole Leforest soil (229/2910). Similarly, at the  
350 Carrières site, 46.4% of the total fungal OTUs (1465/3158) were specific to a single tree  
351 species sample (Fig. 2b), whereas 53.6% (1693/3158) of the OTUs were shared by at least  
352 two tree species (not shown). The *Quercus*, *Ostrya*, *Salix* and *Poplar* species showed the  
353 fewest specific OTUs. A total of 4.8% of the total OTUs (152/3158) were shared by all tree  
354 species. Finally, 38% of the total fungal OTUs (1471/3872) recorded at the Thann site were

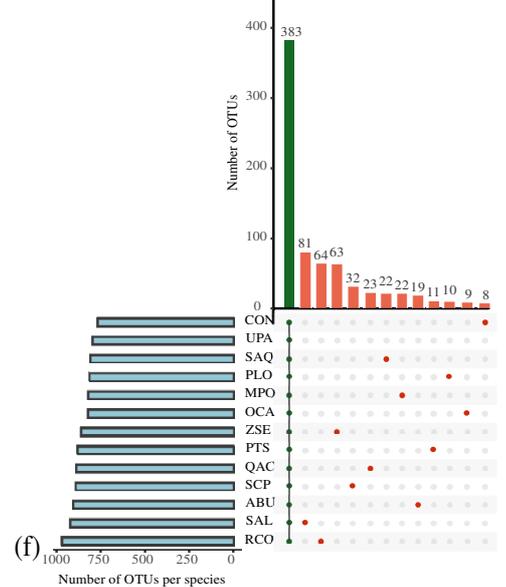
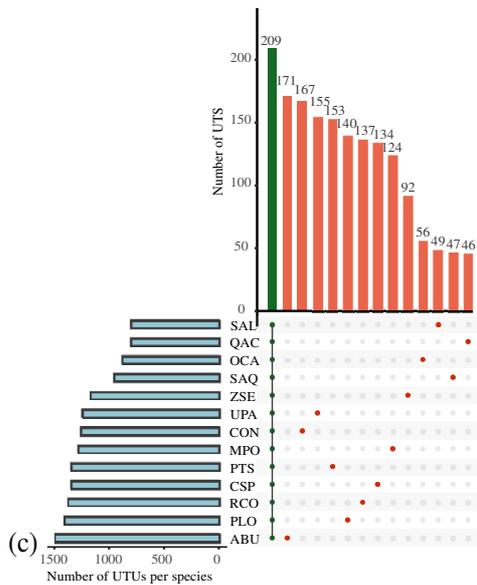
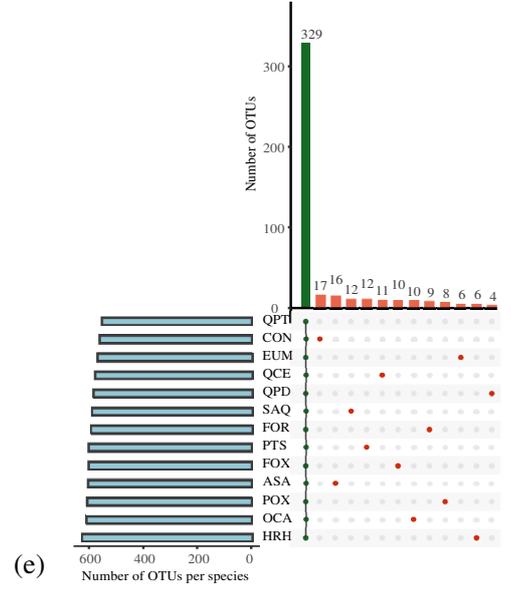
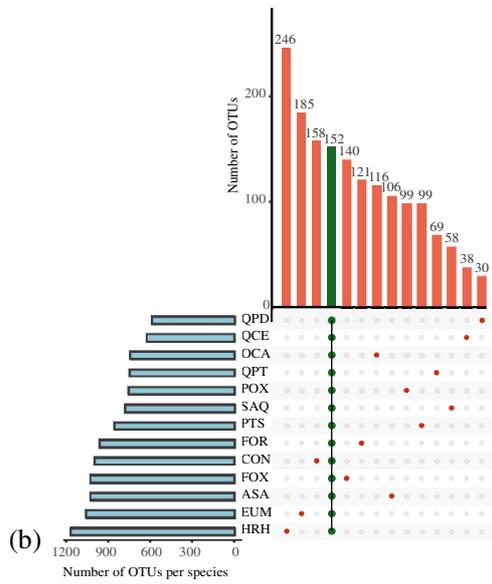
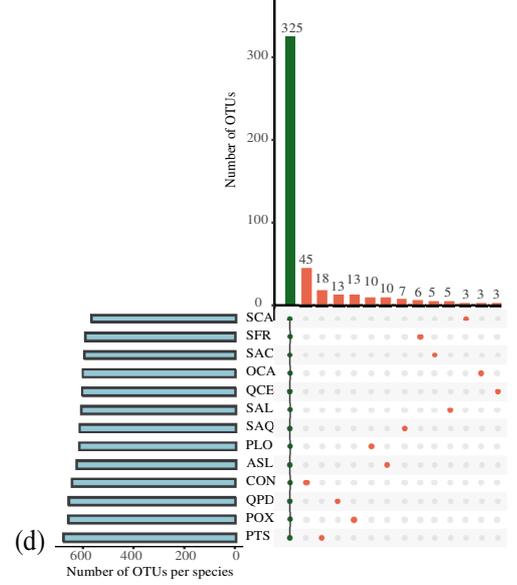
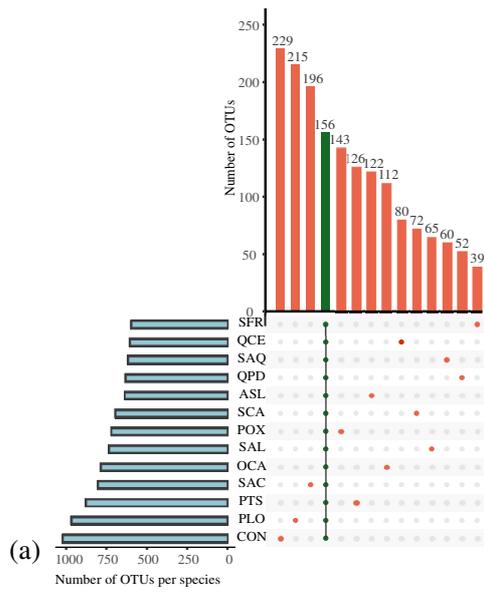
355 specific to one soil sample (Fig. 2c), whereas 62% (2401/3872) of the OTUs were shared by  
356 at least two tree species (not shown). As at the Leforest and Carrières sites, the same tree  
357 species (*Salix*, *Ostrya*, *Quercus*) accounted for the fewest specific OTUs, whereas 5.4% of  
358 OTUs were shared by all the tree species. These results are in agreement with those of Buée et  
359 al. (2011), who demonstrated that more than one-third of the dominant fungal species were  
360 restricted to one or two tree species.

361 For the bacterial communities, 33% of the OTUs were shared by all tree species at the  
362 Leforest site, whereas 12.2% of bacterial OTUs were specific, with a maximum of 1.6% for  
363 *Pterocarya stenoptera* (PTS) and a minimum of 0.3% for *Salix caprea* (SCA), *Ostrya*  
364 *carpinifolia* (OCA) and *Quercus cerris* (QCE; Fig. 2d). The same trends were observed at the  
365 Carrières site (maximum 2.5% for *Populus* “Oxford” (POX) and minimum 0.5% for *Acer*  
366 *saccharinum* (ASA), Fig. 2e) and the Thann site (maximum 2.1% for *Salix alba* (SAL) and  
367 minimum 0.2% for OCA and PLO, Fig. 2f). The results obtained for bacterial communities  
368 show a different pattern in comparison to those of the fungal communities, especially because  
369 of the high number of bacterial OTUs that are shared by all the tree species and the low  
370 number of specific OTUs.

371

372

373



375 **Figure 2.** Shared and specific fungal (a, b and c) and bacterial (d, e and f) OTUs recorded  
376 from the rhizosphere of different tree species at the Leforest (a and d), Carrières (b and e)  
377 and Thann (c and f) sites. The horizontal blue bars correspond to the number of OTUs  
378 recorded for each tree species. The green bars indicate the number of OTUs common to all  
379 tree species designated with green dots (e.g., in Fig. 2 (a) there are 156 OTUs that are shared  
380 by all the tree species). The red bars represent the number of OTUs specific to a tree species  
381 designated by the red dots (e.g., in Fig. 2 (a) there are 215 OTUs specific to the PLO  
382 rhizospheric soil). The OTUs shared by more than 2 sampled trees (and less than 12) are not  
383 shown for clarity.

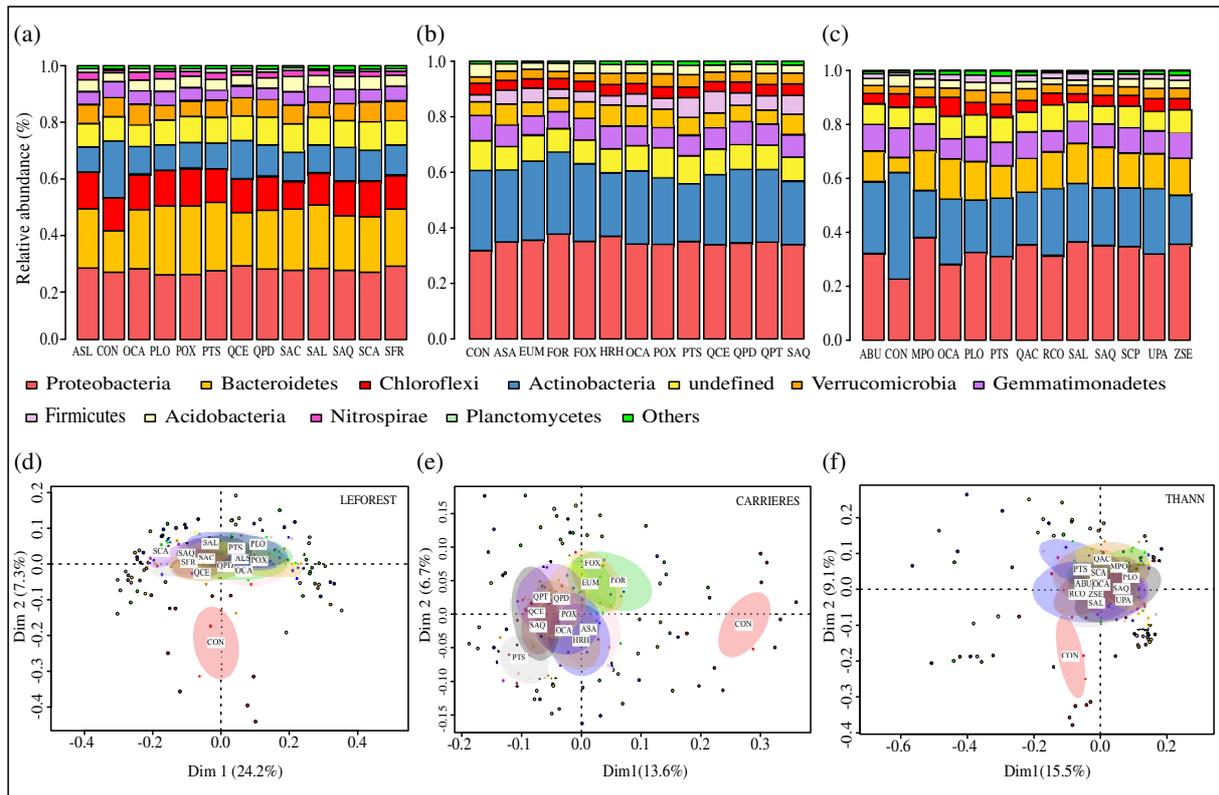
384

### 385 3.2. Tree species and soil characteristics have different impacts on bacterial and 386 fungal communities

387 At each of the three sites, the Proteobacteria phylum was dominant, accounting for  
388 more than 25% of the total abundance (Fig. 3). The Bacteroidetes and Chloroflexi phyla  
389 further dominated at the Leforest site (Fig. 3a), whereas Actinobacteria dominated at the  
390 Carrières (Fig. 3b) and Thann (Fig. 3c) sites. The obtained results allow us to extend our  
391 knowledge for several tree species that have not yet been reported. For tree species that have  
392 already been studied (e.g., *Populus*, *Salix*), the present results are in agreement with those  
393 obtained in several other studies. Fierer (2017) reported that Bacteroidetes, Proteobacteria,  
394 and Actinobacteria are usually dominant in soils. Álvarez-López et al. (2020) found a  
395 predominance of Actinobacteria and Proteobacteria in the *Betula pendula* rhizosphere at the  
396 Thann site. These two phyla also dominated under poplar trees at the Pierrelaye site (Ile-de-  
397 France, France), of which the contamination features were similar to those of the Carrières  
398 site (Foulon et al., 2016). The Proteobacteria and Actinobacteria phyla were also predominant  
399 in ultramafic rhizosphere soils (Lopez et al., 2022), in an Indian Mn-contaminated mine

400 tailing (Ghosh and Das, 2018) and in a Cu-polluted mining soil in China (Jia et al., 2022).  
401 Actinobacteria exhibit diverse physiological and metabolic features, such as the production of  
402 extracellular enzymes and the formation of a wide variety of secondary metabolites, which  
403 makes them relevant microbial resources for bioremediation (Narsing Rao et al., 2022).  
404 Proteobacteria isolated from a highly polluted coal mine drainage showed capacities for toxic  
405 metal sequestration that render them useful for bioremediation (Oyetibo et al., 2021).  
406 Additionally, the PCoA of the composition of the bacterial communities highlights significant  
407 differences between communities inhabiting the rhizospheric soil of planted trees compared to  
408 unplanted controls at the Leforest (Fig. 3d), Carrières (Fig. 3e) and Thann (Fig. 3f) sites. Tree  
409 species greatly affect the composition, diversity and activity of soil bacterial communities  
410 (Dukunde et al., 2019; Pfeiffer et al., 2013). However, bacterial richness did not differ  
411 between planted and unplanted soils in the present study (Table 1).

412         Regarding the richness (Table 1) and abundance (data not shown) of bacterial OTUs,  
413 our analyses revealed no significant difference between the different tree species, regardless  
414 of the site. Similar trends were observed for the bacterial communities of various poplar  
415 cultivars grown on contaminated soils, with little variation in bacterial richness and  
416 abundance (Foulon et al., 2016). No difference in richness was found between the planted and  
417 unplanted soils at our sites. Foulon et al. (2016) also found that bacterial species richness and  
418 diversity in soil habitats were not different between planted and unplanted plots in a poplar  
419 plantation in Pierrelaye (France). The lack of a significant relationship between plant diversity  
420 and soil bacterial diversity was observed by (Fierer and Jackson, 2006b) in a field study.  
421 Previous studies on grassland and agricultural sites (Buckley and Schmidt, 2001; Felske et al.,  
422 2000; Kowalchuk et al., 2002) also yielded similar conclusions. Different tree species (*Acer*  
423 *pseudoplatanus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Carpinus betulus*, *Picea abies* and *Tilia*  
424 *cordata*) also hosted similar bacterial communities (Pfeiffer et al., 2013; Uroz et al., 2016).



425

426 **Figure 3.** Composition of the bacterial communities at the Leforest (a), Carrières (b) and  
 427 Thann (c) sites at the phylum level based on 16S rRNA gene sequencing. Bacterial  
 428 communities were further analysed using PCoA at Leforest (d), Carrières (e) and Thann (f).

429

430 **Table 1.** Bacterial and fungal richness at the three sites Leforest, Carrières and Thann for all  
 431 25 planted woody species (n=12). Significant differences are indicated in brackets. “CON”  
 432 corresponds to the control (unplanted plot).

	Carrières		Leforest		Thann			
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi		
	926	3158	984 OTUs	2910	1657 OTUs	3872 OTUs		
	OTUs	OTUs		OTUs				
<b>CO</b>								
<b>N</b>	235 (n.s)	226 (a)	CON	239 (n.s)	241 (a)	CON	267 (n.s)	287 (a)

<b>ASA</b>	251 (n.s)	239 (a)	<b>PLO</b>	225 (n.s)	191 (ab)	<b>ABU</b>	297 (n.s)	302 (a)
<b>EU</b>	245 (n.s)	227 (a)	<b>PTS</b>	241 (n.s)	188 (b)	<b>MPO</b>	271 (n.s)	292 (a)
<b>M</b>								
<b>FO</b>	247 (n.s)	229 (a)	<b>OCA</b>	217 (n.s)	165 (bc)	<b>PLO</b>	292 (n.s)	322 (a)
<b>X</b>								
<b>HR</b>	262 (n.s)	243 (a)	<b>SAC</b>	220 (n.s)	165 (bcd)	<b>PTS</b>	278 (n.s)	269 (a)
<b>H</b>								
<b>FO</b>	250 (n.s)	223 (ab)	<b>SCA</b>	234 (n.s)	162 (bcde)	<b>UPA</b>	284 (n.s)	284 (a)
<b>R</b>								
<b>PTS</b>	252 (n.s)	185 (bc)	<b>SAL</b>	236 (n.s)	155 (cdef)	<b>ZSE</b>	272 (n.s)	297 (a)
<b>SAQ</b>	246 (n.s)	166 (cd)	<b>SAQ</b>	244 (n.s)	142 (cdefg)	<b>RCO</b>	280 (n.s)	317 (a)
<b>OC</b>	258 (n.s)	135 (de)	<b>SFR</b>	236 (n.s)	143 (cdefg)	<b>CSP</b>	285 (n.s)	274 (a)
<b>A</b>								
<b>PO</b>	256 (n.s)	147 (de)	<b>QPD</b>	235 (n.s)	136 (defg)	<b>OCA</b>	287 (n.s)	170 (b)
<b>X</b>								
<b>QPT</b>	244 (n.s)	152 (de)	<b>POX</b>	232 (n.s)	135 (efg)	<b>QAC</b>	263 (n.s)	144 (b)
<b>QC</b>	248 (n.s)	126 (e)	<b>QCE</b>	231 (n.s)	128 (fg)	<b>SAL</b>	273 (n.s)	130 (b)
<b>E</b>								
<b>QP</b>	249 (n.s)	120 (e)	<b>ASL</b>	229 (n.s)	117 (g)	<b>SAQ</b>	263 (n.s)	196 (b)
<b>D</b>								

433

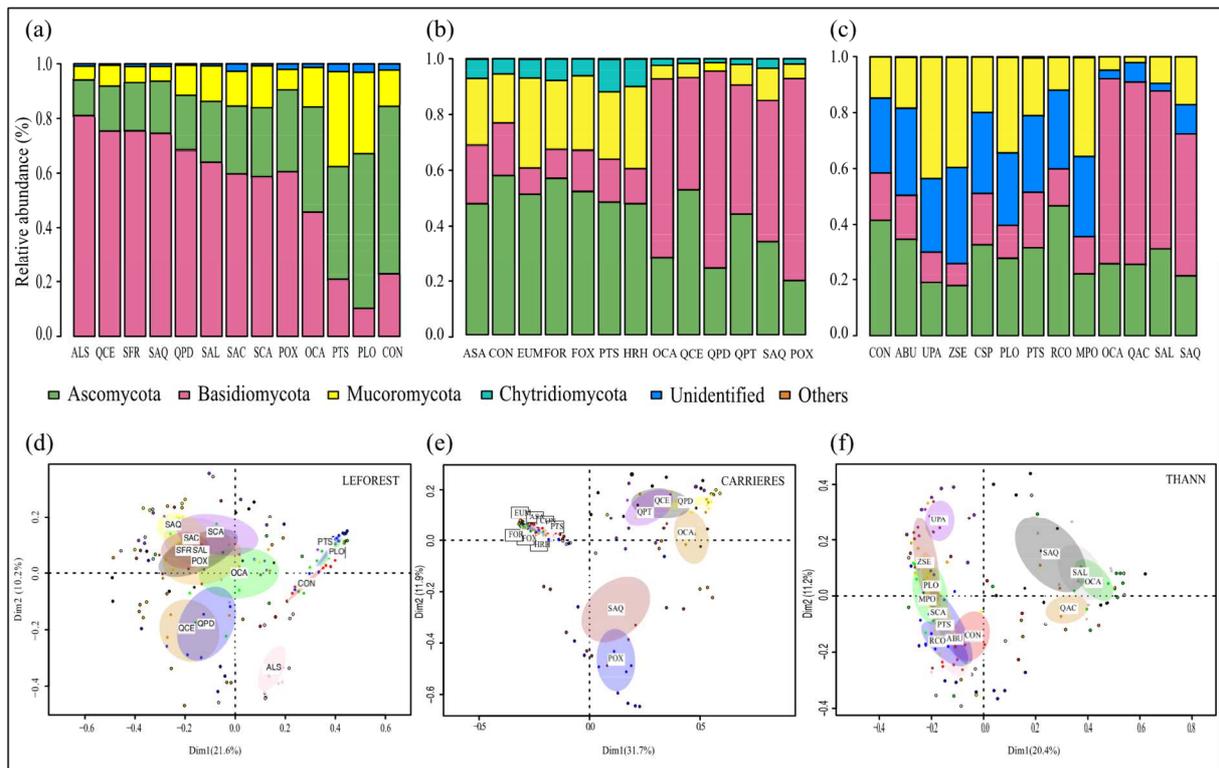
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436

In contrast to bacterial communities, fungal communities showed contrasting patterns among sites and tree species. Ascomycota, Basidiomycota and Mucoromycota sequences dominated at the Leforest (Fig. 4a), Carrières (Fig. 4b) and Thann (Fig. 4c) sites. The

437 proportion of Basidiomycota sequences ( $\geq 50\%$ ) was significantly higher in tree species of the  
438 Fagaceae, Salicaceae and Betulaceae (referred to as ‘tree species Group 1’), together with  
439 lower proportions of Ascomycota ( $< 35\%$ ) and Mucoromycota ( $< 15\%$ ) sequences, than in  
440 tree species from the Sapindaceae, Bignoniaceae, Meliaceae, Moraceae, Elaeagnaceae,  
441 Paulowniaceae, Platanaceae, Juglandaceae, Anacardiaceae, Oleaceae, and Ulmaceae families  
442 (referred to as ‘tree species Group 2’). Soils collected under the latter group of tree species, as  
443 well as the control soils, showed higher proportions of Ascomycota ( $\geq 50\%$ ) and  
444 Mucoromycota ( $> 20\%$ ), contrasting with a much lower abundance of Basidiomycota  
445 sequences ( $<20\%$ ), than those collected under Group 1. Mucoromycota sequences from the  
446 Thann site even reached 45% of the sequenced dataset for some tree species of these Group 2  
447 families (*e.g.*, UPA). Regarding the fungal richness index (Table 1), the woody species could  
448 also be clustered into the two groups described above. Group 1 exhibited the lowest fungal  
449 richness, while the highest fungal richness occurred in Group 2. The low fungal richness  
450 observed for Group 1 may be explained by the tree species-specific nature of ectomycorrhizal  
451 fungi (ECMFs), to which the majority of Basidiomycota fungi belong. In fact, Urbanová et al.  
452 (2015) reported the complex nature of plant–fungal symbiosis of ECMFs, which explained  
453 the stronger effects of trees on the fungal community than on the bacterial community.



454

455 **Figure 4.** Composition of the fungal communities at the Leforest (a), Carrières (b) and Thann  
 456 (c) sites at the phylum level based on ITS rRNA gene sequencing. Fungal communities were  
 457 further analysed using PCoA at Leforest (d), Carrières (e) and Thann (f).

458

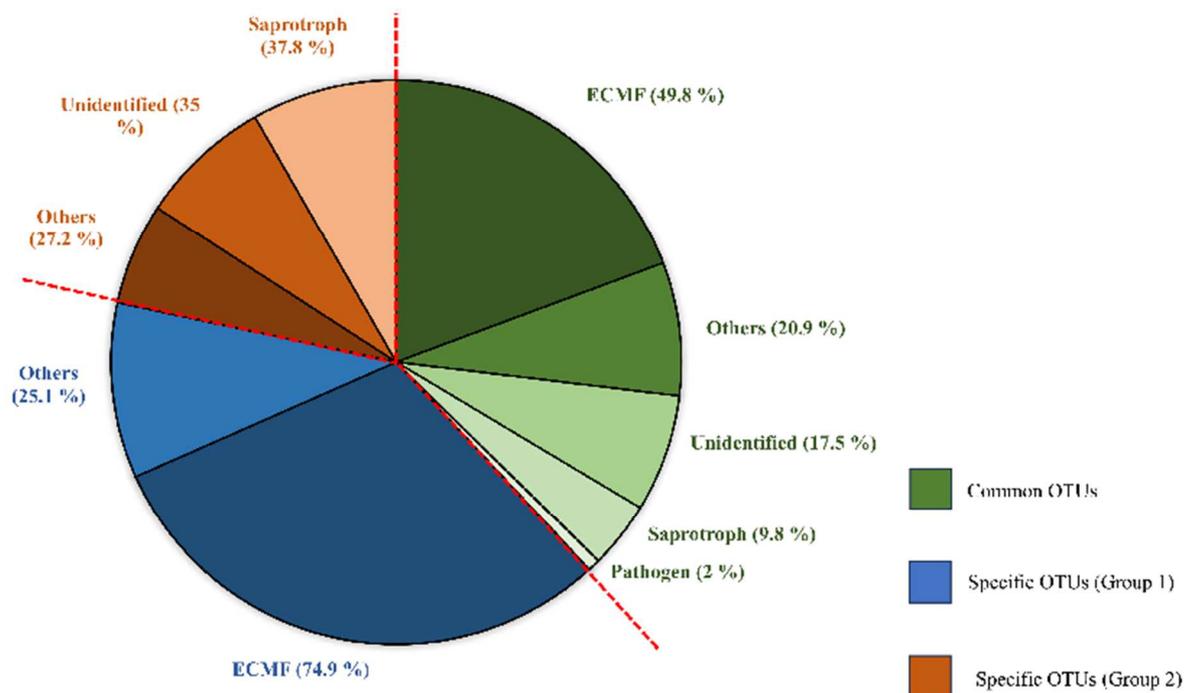
459 The PCoA revealed that tree species might be classified into the two groups described  
 460 above based on their fungal communities. For instance, at the Leforest site, the Betulaceae  
 461 and Salicaceae families (Group 1) did not show significantly different fungal communities,  
 462 which were similar to the communities under the Fagaceae family (Group 1) (Fig. 4d). The  
 463 unplanted soil, PTS and PLO species (Group 2) exhibited similar communities. Finally,  
 464 specific fungal communities for the ALS species were recorded at the Leforest site. Clear  
 465 clusters of tree species (Group 1 versus Group 2) were also noticed for the Carrières (Fig. 4e)  
 466 and Thann (Fig. 4f) sites. In agreement with the present results, Lindhe et al. (2004) showed  
 467 that the highest similarities for fungal communities were found between a *Betula* sp. and a  
 468 *Populus* sp., followed by a *Quercus* sp., compared to spruce. It has been hypothesized that the

469 richness and diversity of natural fungal communities are affected by tree species (*Buée et al.*,  
470 *2011; Tedersoo et al., 2015*).

471

### 472 *3.3. Trees from the two tree species groups host distinct fungal communities*

473 The previous datasets described above highlighted the occurrence of two tree family  
474 groups, namely, Group 1 (Basidiomycota-enriched samples from the Fagaceae, the Salicaceae  
475 and the Betulaceae) and Group 2 (Ascomycota-enriched samples from all the other tree  
476 families). The ecological functions of the fungal species in these two groups were further  
477 assessed using FunGuild (Nguyen et al., 2016), and the abundance of DNA reads was  
478 calculated and expressed as a percentage of the total reads. Our analysis indicated that 74.9%,  
479 72.9% and 49.2% of the specific fungal reads of Group 1 tree families were assigned to the  
480 ECMF guild for the Leforest, Carrières and Thann samples, respectively (Fig. 5, Fig. S1 and  
481 Fig. S2). In a previous study at a set of post-mining sites recultivated with six different trees,  
482 80% of the fungal sequences were composed of only 23 to 28 OTUs, which led to the  
483 conclusion that the fungal taxa showed a greater tendency to be tree-specific (*Urbanová et al.*,  
484 *2015*). It can be hypothesized that tree species with few specific OTUs are selecting their  
485 symbionts in a more restrictive way and thus exhibit lower microbial richness.



486

487 **Figure 5.** *Distribution of shared and specific OTUs for the two groups of trees, as well as the*  
 488 *main fungal guilds on the Leforest site. The common OTUs, the specific OTUs (Group 1) and*  
 489 *the specific OTUs (Group 2) represented 1,116 OTUs (38.4 %), 1,161 OTUs (39.9 %) and*  
 490 *631 OTUs (21.7 %), respectively.*

491

492 The dominant ECMFs associated with Group 1 tree species in the present work  
 493 (*Tuber*, *Tomentella*, *Hymenogaster*, *Geopora*, and *Hebeloma*) were also identified in *Salix*  
 494 roots in a previous study carried out at a TE- and PCB-contaminated site (Fortin Faubert et  
 495 al., 2022) and in some *Quercus* species in other studies in tropical forests and in the Taihang  
 496 Mountains (China) (Morris et al., 2009; Wei et al., 2021). Members of the *Hebeloma*  
 497 mycorrhizal genus (e.g., *H. mesophaeum*) were frequently detected within forest soils, and  
 498 their presence was correlated with a high growth rate of host trees in soils contaminated with  
 499 metals (Durand et al., 2017; Foulon et al., 2016). Moreover, a peculiar trend was noticed for

500 *Alnus subcordata* planted at the Leforest site, of which 76% of dominant OTUs belonged to  
501 the *Tomentella* and *Alnicola* genera. *Alnus* species are known to contract specific symbiotic  
502 relationships with a few fungal genera (Schwob et al., 2017).

503 In contrast, only 0.32%, 0.21% and 4.8% of reads of Group 2 tree families were  
504 related to the ECMF guild at Leforest, Carrières and Thann, respectively, while saprotrophs  
505 and unidentified OTUs were dominant at these sites. The OTUs shared between the two  
506 groups that were identified in Leforest, Carrières and Thann samples were distributed within  
507 various fungal guilds (e.g., orchid mycorrhizal, plant pathogen, wood saprotroph,  
508 ectomycorrhizal, fungal parasite, soil saprotroph, undefined saprotroph). However, none of  
509 these guilds were dominant in terms of read abundance.

510

### 511 3.4. Effect of environmental conditions on the microbial communities

512 At the Leforest site, no TE significantly explained fungal community composition.  
513 However, the first two principal axes of the RDA represented 11.0 % and 6.5% of the fungal  
514 community variation, respectively (Fig. S3a). A group of fungi associated with Group 1 tree  
515 species (SAC, SAL, SAQ, SCA, SFR, QCE, QPD, OCA), mainly composed of *Hymenogaster*  
516 *griseus* and *H. vulgaris* (ectomycorrhiza), was positively correlated with soil Zn, Cd, Pb, Fe  
517 and Al, and to a lesser extent, with Mn and K. Moreover, three OTUs belonging to the  
518 *Tomentella* and *Alnicola* genera, which were associated with ASL, were positively correlated  
519 with Mn. As already discussed above, *Tomentella* and *Alnicola* represented more than two-  
520 thirds of the *Alnus* community in terms of abundance. *Alnus* species are known to establish  
521 symbiotic relations with these ectomycorrhizal fungi. For instance, (Tedersoo et al., 2009)  
522 demonstrated that *Tomentella*, *Alnicola* spp. and the *Lactarius cyathuliformis* groups were  
523 among the most frequent taxa in *Alnus* samples collected from various forests. *Alnus* is the  
524 only member of the Betulaceae family that has evolved root symbiotic relationships with

525 nitrogen-fixing actinobacteria from the genus *Frankia* (Benson & Clawson, 2000; Pujic et al.,  
526 2022) that were recorded in the present study. Nitrogen fixation requires abundant sources of  
527 carbon and phosphorus, and mycorrhizal fungi provide mineral nutrition to plants and are a  
528 supplier of phosphorus for both leguminous and actinorhizal plants (Benson & Clawson,  
529 2000; Tedersoo et al., 2009).

530 At the Carrières site (Fig. S3b), the first two principal RDA axes represent 24.1 % and  
531 8.0 % of the variation of fungal communities. The results showed that the communities are  
532 significantly explained by the presence of Zn (adjusted  $R^2= 0.15$ ). Thus, fungal communities  
533 associated with certain tree species of Group 1, such as QPD, QCE, and QPT, were correlated  
534 with Cd and Zn. Fungi associated with OCA were correlated with Al and Fe, and fungi  
535 associated with SAQ are correlated with Pb. In contrast, the communities associated with  
536 Group 2 tree species (PTS, FOR, FOX, HRH, ASA and EUM) were correlated with soil Mg  
537 and K contents. A large proportion of these OTUs belongs to Ascomycota-rich group. For  
538 example, the OTU 4 corresponding to *Hebeloma mesophaeum* is a Zn-accumulating  
539 ectomycorrhizal (ECM) species (Sácký et al., 2014) and is frequently associated with metal-  
540 disturbed sites (Foulon et al., 2016; Lacercat-Didier et al., 2016). The association of roots  
541 with metal-resistant ECM fungi would enhance plant fitness in metal-contaminated  
542 environments by improving plant nutrition, reducing the transfer of TEs to the plant and  
543 stimulating the response of trees to abiotic stress (de Moura et al., 2022; Dowarah et al.,  
544 2022).

545 Similarly to Leforest site, no TE significantly explained the fungal communities at  
546 Thann site. The first two principal axes of RDA represent 20.3 % and 3.6 % of the variation  
547 of fungal communities, respectively (Fig. S3c). The fungal OTUs associated with Group 1  
548 tree species (SAQ, SAL, OCA and QAC) corresponded to ectomycorrhizal fungi and were  
549 positively correlated with K and Mg. The same fungal OTUs, composed by various fungal

550 guilds, were shared by the tree species of the Group 2 and were positively correlated with the  
551 S content in the soil.

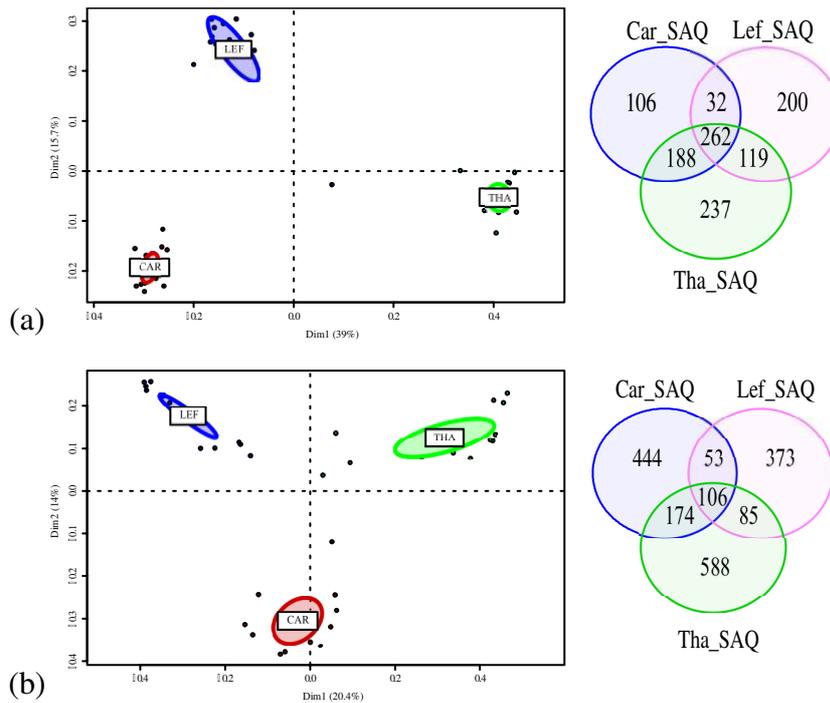
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### 553 *3.5. Microbial communities of OCA, PTS and SAQ change as a function of the study site*

554 As described in the Materials and Methods section, OCA, PTS and SAQ were planted  
555 at the three sites, which allowed us to assess the impact of site on microbial communities. The  
556 results indicated that the three tree species exhibited different bacterial communities,  
557 depending on the site (Fig. 6, Fig. S4 and Fig. S5). The Venn diagram indicates that 262  
558 OTUs (22.9%) were common at Leforest, Carrieres and Thann for SAQ. For OCA, 291 OTUs  
559 (25.8%) were recorded, and 293 OTUs (24.2%) were recorded for PTS. Similarly, the dataset  
560 for SAQ (Fig. 6), PTS (Fig. S4) and OCA (Fig. S5) shows that the fungal communities were  
561 specific to the site where the trees were grown. Only 6% of the fungal OTUs were shared by  
562 all three sites (106 OTUs for SAQ, 115 OTUs for PTS and 144 OTUs for OCA).

563 The ten most abundant bacterial (Table S3) and fungal (Table S4) OTUs of the three  
564 common tree species OCA, PTS and SAQ and the unplanted soil (CON) were compared at  
565 the three study sites. For OCA, only one fungal OTU identified at the species level (*Tuber*  
566 *maculatum*) was present at the three sites. For SAQ, PTS and control soils, no fungal OTUs  
567 were shared among the three sites (Table S4). For bacterial communities, one OTU was  
568 recorded for the unplanted soil, PTS and OCA (Sphingomonadaceae for the unplanted soil  
569 and *Gemmatimonas* sp. for PTS and OCA), while SAQ did not share any OTUs (Table S3).

570



571

572

573

574 **Figure 6.** PCoA comparing the bacterial (a) and fungal (b) OTU communities inhabiting the  
 575 SAQ rhizosphere at the three study sites. The numbers of specific and common OTUs are  
 576 shown by Venn diagrams.

577

## 578 5. Conclusions

579 This study aimed to compare the rhizospheric microbiomes of 25 woody species that  
 580 were planted at an industrial landfill (Thann), an agricultural soil contaminated by wastewater  
 581 (Carrières) and an agricultural soil contaminated by atmospheric deposition (Leforest) in  
 582 France. Despite the strong anthropization of the study sites, especially at Thann (*industrial  
 583 landfill-technosoil*), microbial colonization of tree roots after four years of phytomanagement  
 584 occurred, but strong variations were observed between the sites and tree species. The bacterial  
 585 communities associated with plants differed from those in the unplanted soil, but no  
 586 difference was observed between the different woody species families. In contrast, the data

587 obtained from the sequencing of fungal DNA showed significant differences between groups  
588 of trees, highlighting the selection of fungal communities by some tree species. Indeed, the  
589 Betulaceae, Salicaceae and Fagaceae families had the highest proportion of Basidiomycota,  
590 especially ECMFs, and the lowest diversity and richness. The other tree families, as well as  
591 the unplanted soils, exhibited larger Ascomycota and Mucoromycota communities. Overall,  
592 site (and hence soil) and tree species were the main drivers of the microbial communities. The  
593 potential for root colonization by microbes was high and led to successful revegetation,  
594 improving our phytomanagement strategy. Further studies on the functionality of microbial  
595 communities would provide a complete view of these plant–soil–microorganism systems.

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597

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611 **References**

612

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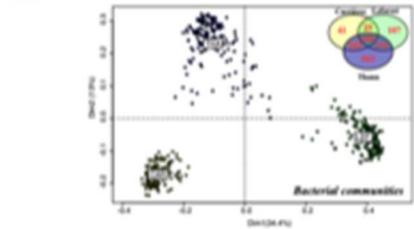
858

Graphical abstract

Assessment of fungal and bacterial communities on 468 soil samples



Three sites, three distinguished bacterial and fungal communities



Microbial communities is driven by soil parameters and tree species

