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► To cite this version:

Marine Pacé, Anne Bonis, Didier Alard, Cian Blaix, Maxime Burst, et al.. Identifying generic proxies for assessing ecosystem properties and conservation status of semi-natural grasslands. *Ecological Indicators*, 2024, 158, pp.111586. 10.1016/j.ecolind.2024.111586 . hal-04412391

HAL Id: hal-04412391

<https://hal.univ-lorraine.fr/hal-04412391v1>

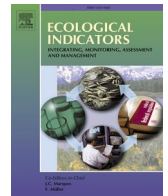
Submitted on 23 Jan 2024

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Original Articles

Identifying generic proxies for assessing ecosystem properties and conservation status of semi-natural grasslands

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ARTICLE INFO

Keywords:

Conservation status
Ecosystem functioning
Genericity
Indicator of function
Natura 2000
Permanent grasslands

ABSTRACT

Despite the recommendations of the 1992 European Habitat Directive regarding the evaluation of the conservation status of ecosystems, information on ecosystem functioning is still poorly integrated in long-term monitoring of European semi-natural grasslands. We investigated how to evaluate several ecosystem properties in relation to services provided by grasslands - plant and litter biomass and chemical composition, soil C and N content - by identifying non-destructive proxies involving little or no additional field sampling effort, or calculated from vegetation species composition. These proxies need to be i) informative, i.e. that can be used to approach one or more ecosystem properties based on linear or non-linear relationship models, and ii) generic, i.e. applicable across a wide range of semi-natural grassland habitats and environmental conditions. Based on a large set of field data collected in a variety of semi-natural grasslands in France, we showed that good proxies for ecosystem properties can be identified when the range of climate or soil moisture conditions was reduced. This suggests that these environmental factors influenced the functional pattern of semi-natural grassland ecosystems. The majority of proxies identified so far, including the community weighted mean of leaf dry matter content (LDMC_{CWM}), the height of the herb layer, and litter thickness, are linked to ecosystem properties through non-linear relationships. They are generic in that they are applicable to several grassland habitat types. The height of the herb layer reflects standing plant biomass relatively well, but different non-linear models have to be used depending on climate and soil moisture conditions. Calculating LDMC_{CWM} from species composition data provides a good approximation of the digestibility and P content of plant biomass. Similarly, litter thickness can be used to approach the digestibility and K content of plant biomass in wet grasslands. No generic proxy was found to approximate litter and soil characteristics across the wide range of grasslands habitats under study. By identifying generic and low-constraint proxies for several ecosystem properties, this work provides guidelines for assessing the functioning of semi-natural grasslands - a key criterion of their conservation status - with limited additional field effort and cost compared to plant community monitoring programs.

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1. Introduction

Semi-natural grasslands represent 42.8 % of agricultural lands in Western Europe (Boch et al., 2020), and are of particular interest for conservation as biodiversity reservoirs (Hoekstra et al., 2005; Dengler et al., 2020). They are also an essential part of the cultural landscape, as they are the result of centuries or even millennia of extensive land use (Dengler et al., 2014; Bardgett et al., 2021). However, they are threatened by multiple drivers of degradation, including overgrazing, eutrophication, or abandonment (Henle et al., 2008; Bardgett et al., 2021).

The conservation status of grassland habitats has to reflect three primary attributes of ecosystems i.e., their composition, their structure, and their functioning (Noss, 1990; Dale and Beyeler, 2001). In application of the European Habitats Directive (Council Directive 92/43/EEC 1992), the assessment and reporting of the conservation status of grassland habitats in Europe focus primarily on the species composition of plant communities, and occasionally on the presence and/or abundance of other taxa (e.g., fauna, fungi). Although less often implemented in the routine monitoring of grasslands of conservation interest (de Bello et al., 2010b), the characterization of grassland structure and functioning is also essential to evaluate the capacity of ecosystems to sustain and regulate the processes necessary to ensure their long-term maintenance (Dale and Beyeler, 2001; Cabello et al., 2012). Ecosystem structure is “the physical organization or pattern of an ecosystem, from habitat complexity as measured within communities to the pattern of patches and other elements at the landscape scale” (Noss, 1990) and the functioning of an ecosystem encompasses all the ecological processes resulting from the interaction between its different compartments, mainly in the form of fluxes of matter and energy (Hooper et al., 2005; Cabello et al., 2012). These key attributes are to be considered when defining management practices that guarantee a good conservation status of semi-natural grasslands. Forage quality, as a provisioning service, is particularly relevant in a conservation objective as the conservation of these grasslands involves maintaining a farming activity, grazing or mowing, to produce forage.

Habitat degradation can be defined as a decline in the capacity of the ecosystem to provide multiple services (Bardgett et al., 2021), including forage production, maintenance of soil fertility, and carbon sequestration (de Bello et al., 2010a; Bengtsson et al., 2019). The provision of these services can be determined on the basis of several characteristics of the plant, litter and soil compartments, giving a snapshot of ecosystem functioning. Among these characteristics, hereafter referred to as ecosystem properties (*sensu* Violle et al., 2007), aboveground plant biomass and chemical composition provide information on the forage value of grasslands (Blaix et al., 2023). The carbon (C) and nitrogen (N) contents of plant biomass reflect forage digestibility (Baumont et al., 2009), while phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) are essential mineral macro-elements for animal health (Suttle, 2022). Litter biomass and chemical composition, as well as the soil C and N contents, are directly related to the rate of nutrient cycling in the ecosystem, providing information on regulating services such as maintenance of soil fertility and C sequestration (De Deyn et al., 2008).

One of the main challenges for better tackling ecosystem functioning in the assessment of the conservation status of grassland habitats consists in identifying parameters that are not only informative and relevant but also simple enough to be easily and routinely monitored (Dale and Beyeler, 2001). Ecosystem properties like plant and litter biomass/chemical composition, and the soil C and N contents, are difficult to integrate into routine monitoring of grassland ecosystems because their systematic acquisition can be destructive, tedious and expensive, especially for the local stakeholders in charge of monitoring who do not always have access to laboratory facilities. Conversely, some simple components of the ecosystem structure are easy to measure in the field and non-destructive. For instance, proxies such as the height of the herb layer are easier to integrate in routine monitoring than measurements of plant biomass or primary production.

The functional characteristics of plant communities also have the advantage of being accessible at a reasonable cost, from existing species composition surveys. The functional composition of plant communities can be characterized by assessing the proportion of different functional groups (e.g., growth forms, N-fixers or not), and/or determining plant functional trait values at the plant community level, e.g., community-weighted mean (CWM) traits (de Bello et al., 2010a). Specific leaf area (SLA) and leaf dry matter content (LDMC) are two functional traits related to the C and N metabolisms at the plant level (Wright et al., 2001; Hodgson et al., 2011). SLA is positively correlated to the plant photosynthetic capacity (Wright et al., 2001; Garnier et al., 2004) and its relative growth rate (Cornelissen et al., 2003), while LDMC is negatively related to the rate of litter decomposition (Kazakou et al., 2006; Quedet et al., 2007; Fortunel et al., 2009). Both traits are well documented, and mean species trait values can be obtained from open-access databases (e.g., TRY plant trait database, Kattge et al., 2011).

While species have restricted geographic distributions, the ecosystem properties and functional characteristics of plant communities can be compared whatever the geographic context if acquired with a common protocol (Diaz et al., 2004; Cabello et al., 2012). Previous research conducted at relatively large geographic scales, and involving heterogeneous environmental and/or management conditions, has evidenced a few generic relationships among several functional characteristics of the plant community (Diaz et al., 2004; Gardarin et al., 2014). These results are promising for the search for generic relationships between easy-to-obtain proxies and relevant ecosystem properties to assess and monitor ecosystem processes and services in a variety of semi-natural grassland habitats for large-scale conservation purposes.

Environmental constraints such as climate and soil moisture conditions influence ecosystem functioning (Gardarin et al., 2014), and may limit the genericity of the relationships between proxies and ecosystem properties. Taking these factors into account may improve the quality of proxies in some cases. Saturation and threshold effects are also quite common in trait-environment relationships, especially when considering ecosystems under very heterogeneous environmental conditions (Saatkamp et al., 2010). While most large-scale studies have investigated linear relationships between ecosystem properties and/or functional characteristics of plant communities (Diaz et al., 2004; Fortunel et al., 2009; Gardarin et al., 2014), the existence of non-linear patterns has been little explored until now.

The objective of this study was to identify informative proxies, i.e. proxies that can be used to approximate one or more ecosystem properties difficult or expensive to measure, based on a pool of non-destructive variables that are easy to measure in the field, or can be derived from the species composition of the plant community. Two additional criteria were used to determine the relevance of candidate proxies to assess ecosystem properties: (i) only proxies sensitive enough to detect variations in ecosystem properties under relatively homogeneous floristic conditions, i.e. within the same habitat, were considered; (ii) proxies were considered generic if their relationship to ecosystem properties did not depend on specific floristic conditions, so that they can be used and compared across various semi-natural grassland habitats. To explore proxy genericity, we considered a wide range of grassland habitats of conservation interest throughout France, with various floristic compositions, and determined whether the relationships between proxies and ecosystem properties were influenced by climate and soil moisture. The most relevant proxies, their range of application in terms of climate and soil moisture conditions, as well as the shape of their relationship to ecosystem properties were determined.

2. Materials and methods

2.1. Semi-natural grassland habitats

This study included a set of semi-natural grassland habitats with different floristic compositions and subjected to diverse climate and soil

conditions. Grasslands were sampled in or near nine sites located in mainland France and subject to conservation management as part of the European Natura 2000 network (min–max latitude: N 43°22'59" - N 49°55'23"; min–max longitude: O 1°13'48" - E 7°5'25", Fig. 1). These sites included dry grasslands located in Aquitaine (AQU), dry grasslands and saline meadows in Camargue (CAM), marshes in Cotentin (COT), dry grasslands in the Mediterranean Crau plain (CRA), mesic and wet grasslands in Lorraine (LOR), wet grasslands in the Poitevin marshland (POI), mesic and dry grasslands in the volcanic belt “Chaîne des Puys” (PUY), dry grasslands in Somme (SOM), and mesic and dry grasslands in the Vercors mountains (VER). The sampled grasslands corresponded to the local expression of 20 elementary habitats included in 11 generic habitats as referred in Annex I of the European Habitats Directive (Council Directive 92/43/EEC 199, see Table S1 for details).

2.2. Sampling design

Sampling was designed according to various gradients and/or factors, depending on the specificities and main conservation issues of each study site: management intensity/type, including grazing and/or mowing regime (AQU, CAM, CRA, COT, LOR, PUY, VER), water regime (AQU, CAM, LOR, POI), soil salinity (CAM), soil pH (VER), substrate type (COT), nutrient availability (PUY, SOM, VER), orientation (SOM). The design maximized intra-site variability, in line with the objective of defining generic proxies. Three to twelve vegetation units were sampled per site, each unit being characterized by similar species composition (same phytosociological association, hence same European generic and elementary habitat types), geographic features, soil conditions and management type. Three plots were randomly distributed per vegetation unit (at least 500 m apart from each other) and sampled (N = 204).

2.2.1. Vegetation survey

Each plot consisted of a homogenous 10 m × 10 m area. Plant species

composition was determined based on visual estimation of the species cover (%) in a 4 m × 4 m quadrat located within the plot. All sites were sampled in 2019, except CRA that was sampled in 2020.

2.2.2. Standing plant height and biomass

Sampling dates were set to coincide with the beginning of the grazing season at all sites. This period corresponds to 500–600 growing degree days (GDD) in most sites. For CAM, CRA and COT, biomass production and the start of the grazing period were not only controlled by temperature, but also directly dependent on the water regime (drought in CRA and flooding in CAM and COT). Therefore, the sampling dates were adjusted for these three sites, and set at 210–220 GDD in CRA, 1100–1200 GDD in CAM, and 1000–1100 GDD in COT. The height of the herb layer (cm) was determined at three random points within five 20 cm × 20 cm quadrats (15 measurements per plot). The standing aboveground plant biomass (g/m²) was measured from each 20 cm × 20 cm quadrat. Plant material was oven-dried at 60 °C for 48 h and weighed to obtain standing plant biomass at the time of sampling.

2.2.3. Chemical analysis of plant material

Plant material from the five 20 cm × 20 cm quadrats per plot was collected along with additional samples when necessary to obtain a minimum of 40 g of dry plant material per plot for the chemical analyses. Plant material was ground to 1 mm. The N content of plant biomass (g/kg) was measured according to the Kjeldahl method (NF ISO 5983-1), and used to estimate the protein content of plant biomass (g/kg). The crude fiber content (g/kg) was measured according to the Weende method (NF V03-040). Plant biomass digestibility (%) was estimated from *in vitro* dry matter digestibility by pepsine cellulase (Aufreere et al., 2007; Noziere et al., 2018). The plant biomass P, K, Mg and Ca contents (g/kg) were assessed by inductively coupled plasma optical emission spectrometry (NF EN 15510). Chemical analyses were conducted by the agronomic laboratory of Normandie (LANO, Saint-Lô,

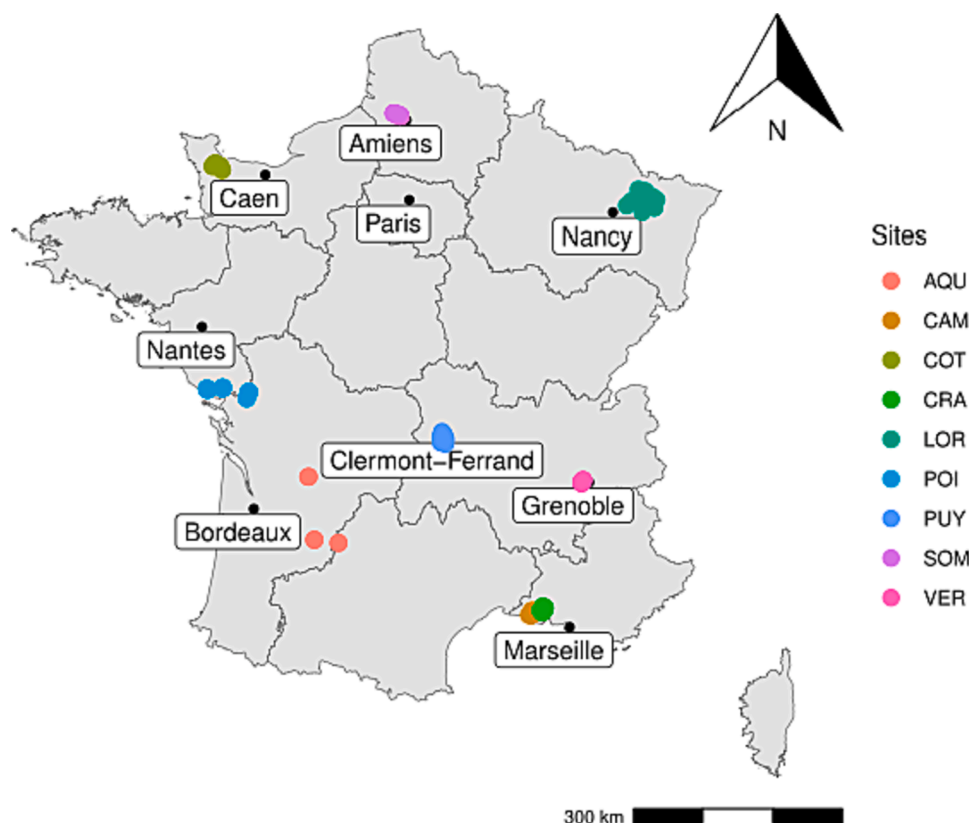


Fig. 1. Localization of the study sites in France.

France).

2.2.4. Litter analysis

Litter thickness was measured in autumn (between September and the beginning of October) in five locations within the plot. When present, litter material was harvested from five 20 cm × 20 cm quadrats and used to determine litter biomass and chemical composition (N = 168). Samples were dried at 80 °C for 48 h, weighed and ground to 1 mm before chemical analysis. The lignin content was determined by the Van Soest method (NF V18-122), and total N by the Kjeldahl method (NF ISO 5983-1).

2.2.5. Soil analysis

Three 10-cm deep topsoil samples were harvested in each plot. They were dried at room temperature (18 °C) for 3 to 5 weeks and sieved to 2 mm. Soil texture was determined by the pipette method (NF X 31-107). Soil total N was assessed by dry combustion (NF ISO 13878, Flash EA 1112, Thermo Electron, Germany) and soil organic C by HCl decarbonation followed by dry combustion (NF ISO 694). Litter and soil analyses were conducted by the soil analysis laboratory of Arras (Arras, France).

2.3. Determination of candidate proxies among plant, litter and soil characteristics

Several characteristics of the plant, litter and soil compartments provide information on ecosystem services such as forage production, maintenance of soil fertility, and carbon sequestration in grasslands. Among these characteristics, we set up a list of easy-to-obtain candidate

Table 1

Characteristics of the plant, litter and soil compartments sorted by ease of acquisition.

| Compartment | Type | Characteristics | Acquisition |
|-------------|---|---|---|
| Plant | Structure | Standing plant biomass (g/m ²) | Relatively difficult to obtain or destructive (referred to as ecosystem property) |
| | | Chemistry | |
| | | Protein content (g/kg) | |
| | | P content (g/kg) | |
| | | Base content (K, Mg, Ca, g/kg) | |
| Litter | Structure | Digestibility (%) | |
| | | Litter biomass (g/m ²) | |
| | Chemistry | Lignin content (g/kg) | |
| Soil | Chemistry | Protein content (g/kg) | |
| | | Lignin / N content | |
| Plant | Structure | Soil organic C (g/kg) | Easy-to-obtain (pool of candidate proxies) |
| | | Soil total N (g/kg) | |
| | Functional composition of the plant community (calculated from the species composition) | Height of the herb layer (cm) | |
| | | Proportion of grasses (%) | |
| | | Proportion of N-fixing forbs (%) | |
| | | Proportion of non N-fixing forbs (%) | |
| | | Proportion of sedges and rushes (%) | |
| | | CWM of the specific leaf area | |
| | | CWM of the leaf dry matter content (sedges and rushes excluded) | |
| | | Litter | |

proxies, i.e. requiring little or no additional sampling effort in the field (Table 1). They were either based on complementary, non-destructive measurements easy to carry out in the field (vegetation height and litter thickness), or deduced from the species composition of the plant community. For ease of discussion, references to “ecosystem properties” in the subsequent sections of this paper will exclusively refer to the characteristics that are relatively difficult to obtain and/or those that require destructive measurements, in order to distinguish them from the candidate proxies.

Candidate proxies describing the functional composition of the plant community were calculated based on the species that collectively represented at least 80 % of the total vegetation cover in the plot (Pakeman and Quested, 2007). Functional groups were established based on growth forms (grasses, forbs, sedges and rushes) and physiological features (functional effect types *sensu* Díaz and Cabido, 2001, N-fixing and non-N-fixing forbs). Community weighted means (CWM) of SLA and LDMC (Garnier et al., 2004; Lavorel et al., 2008) were used to scale up the two quantitative leaf traits from the species level to the plant community level.

Mean species trait values and information on the membership of species to the different functional groups were extracted from local databases when they existed, or from the TRY database (Kattge et al., 2011). We were able to obtain SLA values for 86 % of the overall species sampled and LDMC values for 83 % of sampled species. The species for which we were unable to obtain trait data were for the most part subordinate species which would not have contributed much to the community-weighted mean values. Functional group cover was determined for each plot. Sedges and rushes differ from other functional groups in that they display large variation in leaf thickness, which distorts the relationship between LDMC and ecosystem functioning (Tasset et al., 2019). To avoid a spurious effect of these species' groups occurring in high abundance (>20 %) in 33 out of 204 plots, they were excluded from LDMC_{CWM} calculations.

2.4. Statistical analyses

To keep the approach simple and user-friendly, candidate proxies - including traits - were studied separately to judge their predictive potential for different ecosystem properties, i.e., we chose not to use complex models involving multiple proxies for a single ecosystem property. We also chose not to include random factors describing the nested organization of the experimental design in the models to determine the most generic relationships possible. Indeed, complex models including site as a random factor introduce a site effect that is not known for locations other than those included in the study. This choice is likely to reduce the predictive power of the models, but the models have the advantage of being widely applicable, and this property is of utmost importance in a large-scale evaluation framework. Non-linear modeling manages the possible information gaps due to the simplicity and genericity of the approach, by permitting, for example, the existence of plateaus in the modeled relationships. Given the unbalanced design, the choice not to introduce a site effect implies that the most sampled sites have a greater weight in the relationships highlighted than the less sampled sites.

A three-step procedure was applied to identify the best proxies for ecosystem properties.

Step 1: Use of non-parametric methods to detect the strongest monotonic relationships between candidate proxies and ecosystem properties while controlling for habitat contrasts.

To select proxies that are both generic and sensitive, the first step was to identify relationships between candidate proxies and ecosystem properties that exist not only among habitats but also within a same habitat. Most of the candidate proxies and ecosystem properties had multimodal distributions that did not allow a classical parametric approach for correlation analysis. Accordingly, the Spearman rank correlation coefficient was chosen to highlight the strongest monotonic

(positive or negative) relationships.

Spearman correlation coefficients between candidate proxies and ecosystem properties were assessed according to two different schemes. (i) A first analysis was conducted on the original data set, without controlling for variations related to habitat type. (ii) In a second analysis, grasslands that met the same N2000 generic habitat description were grouped together to calculate the habitat mean for each candidate proxy and ecosystem property. Spearman correlation coefficients were calculated between candidate proxies and ecosystem properties after accounting for variations related to N2000 generic habitat type, i.e. using the distances to the mean of the N2000 generic habitat.

Step 2: Measurement and comparison of the influence of climate and soil moisture on the relationships between candidate proxies and ecosystem properties

The aim of the second step of the analysis was to determine whether the links between candidate proxies and ecosystem properties were influenced by climate and soil moisture, and if so, to assess which of these two factors was most relevant to consider when modeling the relationships. For this intermediate step, we used a linear approach insofar as the models compared include two explanatory variables and their interaction.

Step 2a: Grouping of plots according to their climate and soil moisture conditions

The analysis of the plant species composition across the study sites revealed the predominance of two main structuring pressure factors: (i) climate and (ii) soil moisture (see Fig. S3 for additional information).

- (i) Three ‘climate’ groups were distinguished using k-means clustering based on 9-year average annual temperature (2010–2018), average daily minimum and maximum temperatures (2010–2018), as well as an aridity index calculated as the ratio of precipitations over evapotranspiration, corresponding to the year of sampling (2019 for all sites except CRA sampled in 2020). Overall, climate parameters were obtained from the WorldClim database. Evapotranspiration and precipitation data were obtained from Météo France and recorded in the weather stations nearest to the sampling sites. The optimal number of climate groups for k-means clustering was determined by analyzing within-cluster sums of squares. The first climate group (N = 105) included the continental and Atlantic sites AQU, COT, LOR, POI and SOM; the second group (N = 42) included the Mediterranean sites CAM and CRA, and the third group (N = 57) included the mountain to sub-mountain sites VER and PUY (Table 2).
- (ii) Two ‘soil moisture’ groups were established using k-means clustering based on the grain size of the soil mineral component (% clay, % sand) measured from field samples, topography (slope and altitude) and precipitations (average daily precipitations in 2019). Information on topography was extracted from local georeferenced databases. The first group (N = 75) corresponded to wet grasslands and included all the plots of COT, LOR, and POI, as well as 9 plots sampled in CAM; the second group (N = 129)

corresponded to mesophilic to xerophilic grasslands and included all the plots of AQU, CRA, PUY, SOM and VER, as well as 6 plots sampled in CAM (Table 2). The cation exchange capacity was closely related to the soil moisture group (Table 2), suggesting that the wet grasslands investigated in this study (COT, LOR, POI) were also the most fertile ones.

Step 2b: Determination of the influence of climate and soil moisture on the relationships between candidate proxies and ecosystem properties

Almost all the wet grasslands sampled in this study were part of the continental and Atlantic climate group (Table 2). Since climate and soil moisture groups provided partially redundant information, their respective influences on the links between proxies and ecosystem properties were compared to determine which of these two factors was the most relevant to consider when modeling the relationships.

We used a set of linear models with ecosystem properties as response variables to detect and compare the influence of climate and soil moisture. Models were compared based on their Akaike information criterion (AIC).

- Climate model: Ecosystem property ~ Proxy * Climate group
- Soil moisture model: Ecosystem property ~ Proxy * Soil moisture group
- Null model: Ecosystem property ~ Proxy

Square root and log- transformations of the response variables were used to improve the normality of the residuals and the homogeneity of the residual variance. In the absence of homoscedasticity for one of the two environmental factors (climate and/or soil moisture groups were still not comparable in terms of variance), supplementary parameters were used to estimate the variance associated with each level of the corresponding factor. Model comparison accounted for these additional parameters and their impact on AIC. The most parsimonious model was retained.

Step 3: Identification of the proxies that best reflect variations in ecosystem properties according to climate or the soil moisture context

Considering the ecosystem properties as the response variables, the relationships between proxies and ecosystem properties were first modeled for all sampled grasslands without considering the climate or the soil moisture context. Where climate or soil moisture had a significant influence, the relationships between proxies and ecosystem properties were also modeled within subsamples according to the most relevant grouping, i.e., by distinguishing climate or soil moisture groups.

For each proxy-ecosystem property pair, the shape of the relationship was investigated by comparing the fit of different models, including linear and non-linear regressions. The fit of simple linear regression was compared to the fits obtained from a set of other functions (see Table S4 for detailed equations) including exponential (2 and 3 parameters), asymptotic (2 and 3 parameters), log-logistic (3 and 4 parameters), and Weibull type 1 (3 and 4 parameters) equations. These functions were

Table 2

Range of environmental characteristics (minimum–maximum) of the nine sites sampled in the study and distribution of the sites within the climate and soil moisture groups.

| Site (number of plots) | 9-year average annual temperature (°C) | Average daily precipitations 2019 (mm) | Soil clay (% of the mineral part) | Soil cation exchange capacity (mEq/100 g) | Climate group | Soil moisture group |
|------------------------|--|--|-----------------------------------|---|---------------|---------------------|
| AQU (9) | 12.28–12.62 | 2.09–2.46 | 5.50–33.20 | 16–27.5 | 2 | 2 |
| CAM (15) | 14.94–15.07 | 1.02–1.02 | 6.40–45.40 | 5.70–24.50 | 1 | 1–2 |
| COT (24) | 11.27–11.30 | 2.47–2.47 | 53.30–78.60 | 25.70–76.20 | 2 | 1 |
| CRA (27) | 14.98–15.17 | 1.02–1.02 | 17.00–23.10 | 7.90–13.30 | 1 | 2 |
| LOR (15) | 9.43–9.83 | 1.69–2.04 | 21.50–78.20 | 13.80–49.00 | 2 | 1 |
| POI (27) | 12.38–12.72 | 2.21–2.62 | 24.80–65.70 | 18.50–58.40 | 2 | 1 |
| PUY (36) | 6.74–8.94 | 2.44–2.60 | 3.10–32.10 | 13.00–44.10 | 3 | 2 |
| SOM (30) | 10.27–10.41 | 2.07–2.07 | 13.10–30.50 | 2.20–27.40 | 2 | 2 |
| VER (21) | 5.33–7.24 | 3.78–3.78 | 9.20–56.00 | 8.60–43.90 | 3 | 2 |

chosen to locate any threshold effect, tipping point and/or saturation point. Log-based sigmoidal curves (log-logistic and Weibull) were preferred to the non-log-based ones (e.g., logistic) because they were better suited for strictly positive values of the explanatory variable. The most parsimonious model was selected based on AIC and residual variance.

When several models were equivalent, several criteria were used to select the most appropriate model for easy routine monitoring. The level of model usability was evaluated according to the number of parameters and the complexity of the shape (linear relationship was preferred to convex/concave curves, which were preferred to sigmoidal curves). Linear relationships were preferred because the predictive power of the proxy is stable regardless of its value, whereas the predictive power offered by non-linear relationships varies according to the value of the proxy.

The residuals of the best-fit models were analyzed. When necessary, log or square-root transformation of the response variable was used to stabilize the variance of the residuals. The confidence interval around the parameters of each best-fit model was estimated by bootstrapping (999 iterations).

All statistical analyses were performed using R software (R Development Core Team, 2022) with the following packages: FD (Laliberté et al., 2014), factoextra (Kassambara and Mundt, 2020), nlme (Pinheiro et al., 2022), drc (Ritz et al., 2015), car (Fox and Weisberg, 2019), ggplot2 (Wickham, 2016), and gridExtra (Auguie and Antonov, 2017).

3. Results

3.1. Pre-selection of generic relationships sensitive enough to be detected within the same generic habitat (Step 1)

Eighteen generic relationships between candidate proxies and ecosystem properties were found highly significant after controlling for the variation related to the type of N2000 generic habitat (Table 3). None of the ecosystem properties describing the soil compartment was retained at this first stage. Several significant correlations between candidate proxies and soil properties across habitats were driven by inter-habitat contrasts and not sensitive enough to be detected within the same N2000 generic habitat (see Table S5 for details).

3.2. Consideration of climate or soil moisture for more accurate modeling of relationships (Step 2)

Null, climate and soil moisture models were established and compared for each candidate proxy / ecosystem property pair identified in Table 3 (see Table S6 for detailed results). All the best-fit models included one of the two environmental factors (Table 4), indicating that the parameters of the linear relationships (slope and intercept) between candidate proxies and ecosystem properties varied depending on the climate or soil moisture group. In all cases, considering climate or soil moisture conditions yielded a more accurate modeling of the relationship, and a more reliable approximation of the ecosystem property from the candidate proxy.

Grouping according to soil moisture yielded more accurate modeling of the relationships between ecosystem properties and candidate proxies in 9 cases out of 18, while climate groups were most relevant for six pairs of variables (Table 4). Models including climate or soil moisture factors were associated with similar AIC values ($\Delta AIC < 2$) for the relationships between the protein and K contents of plant biomass and $LDMC_{CWM}$, as well as for the relationship between the plant biomass Mg content and the proportion of legumes (AIC were not significantly different, Table S6). In these two cases, grouping according to soil moisture was preferred for the next step because more generic relationships can be investigated using larger groups.

Table 3

Significant Spearman correlations between candidate proxies and ecosystem properties across habitat types and within N2000 generic habitats (deviation from the generic habitat mean). Only highly significant relationships (p -value < 0.0001) after controlling for variation related to N2000 generic habitat types are presented.

| Ecosystem property | Candidate proxy | Spearman correlation | |
|------------------------------------|----------------------------------|----------------------|--|
| | | Across habitat types | Within N2000 generic habitats ^a |
| Dry plant biomass | Height of the herb layer | 0.54 | 0.67 |
| | Proportion of grasses | 0.38 | 0.39 |
| Digestibility of plant biomass | $LDMC_{CWM}$ ^b | -0.59 | -0.50 |
| Cellulose content of plant biomass | Litter thickness | -0.42 | -0.35 |
| | $LDMC_{CWM}$ ^b | 0.46 | 0.46 |
| Protein content of plant biomass | Proportion of grasses | 0.34 | 0.42 |
| | $LDMC_{CWM}$ ^b | -0.42 | -0.39 |
| P content of plant biomass | $LDMC_{CWM}$ ^b | -0.64 | -0.42 |
| | Proportion of legumes | 0.36 | 0.30 |
| K content of plant biomass | $LDMC_{CWM}$ ^b | -0.61 | -0.42 |
| | Litter thickness | -0.47 | -0.33 |
| Mg content of plant biomass | Proportion of non-N-fixing forbs | 0.40 | 0.40 |
| | Proportion of grasses | -0.47 | -0.50 |
| Ca content of plant biomass | Proportion of legumes | 0.31 | 0.34 |
| | $LDMC_{CWM}$ ^b | -0.51 | -0.49 |
| Protein content of litter | Proportion of non-N-fixing forbs | 0.50 | 0.31 |
| | SLA_{CWM} | 0.38 | 0.30 |
| Lignin / N content of litter | SLA_{CWM} | -0.38 | -0.32 |

For all ecosystem properties characterizing the plant and soil compartments, Spearman correlations > 0.27 corresponded to highly significant relationships ($N = 204$; p -value < 0.0001).

For the two ecosystem properties characterizing the litter compartment, Spearman correlations > 0.29 corresponded to highly significant relationships ($N = 168$; p -value < 0.0001).

See also Table S5 for a list of the relationships characterized by p -values above the significance threshold.

^a Each variable was transformed prior to correlation analysis: plots that met the same N2000 generic habitat description were grouped to calculate the habitat mean for that variable. Then, the mean was subtracted from the original data to obtain the deviation from the habitat mean.

^b Sedges and rushes were excluded from the $LDMC_{CWM}$ calculations.

3.3. Determination of the most relevant models to approximate ecosystem properties from proxies (step 3)

Model comparison revealed that most relationships between candidate proxies and ecosystem properties were significantly better reflected by non-linear functions than by linear ones (see Table S7 for detailed results). Residual errors were often reduced, and model fit increased when climate or soil moisture groups were considered separately based on the most influential environmental factor identified in Table 4. The parameter estimates associated with each of the models reported in Table 5 are listed in Table S8.

Three ecosystem properties showed generic relationships with one or two proxies, with at least 40 % of their variation reflected by the models (Table 5). The proxies approached characteristics of the plant compartment, including plant biomass and chemistry. The height of the herb layer captured 41 % to 58 % of the variation in the aboveground plant biomass, with the shape of the relationship varying with soil moisture (Table 5, Fig. 2). $LDMC_{CWM}$ reflected 42 % of the variation in

Table 4
Determination of the most relevant environmental factor when modeling the relationships between candidate proxies and ecosystem properties.

| Ecosystem property [transformation] | Candidate proxy | Environmental factor of the best-fit model |
|---|----------------------------------|--|
| Dry plant biomass [sqrt] | Height of the herb layer | Soil moisture |
| | Proportion of grasses | Climate |
| Digestibility of plant biomass [log] | LDMC _{CWM} | Soil moisture |
| | Litter thickness | Soil moisture |
| Cellulose content of plant biomass | LDMC _{CWM} | Climate |
| | Proportion of grasses | Climate |
| Protein content of plant biomass [sqrt] | LDMC _{CWM} | Soil moisture |
| P content of plant biomass [log] | LDMC _{CWM} | Climate |
| K content of plant biomass [log] | Proportion of legumes | Soil moisture |
| | LDMC _{CWM} | Soil moisture |
| Mg content of plant biomass [log] | Litter thickness | Soil moisture |
| | Proportion of non-N-fixing forbs | Soil moisture |
| | Proportion of grasses | Soil moisture |
| | Proportion of legumes | Soil moisture |
| | LDMC _{CWM} | Climate |
| Ca content of plant biomass [log] | Proportion of non-N-fixing forbs | Soil moisture |
| | SLA _{CWM} | Soil moisture |
| Protein content of litter [log] | SLA _{CWM} | Soil moisture |
| Lignin / N content of litter [log] | SLA _{CWM} | Climate |

the digestibility of plant tissues in wet grasslands (Table 5, Fig. 3). Besides, LDMC_{CWM} captured 42 % of the variation in the P content of plant biomass when all grassland types were considered in the model, and this proportion rose to 63 % when the model was restricted to continental and Atlantic grasslands. The plant biomass digestibility and K content were also related to litter thickness in wet grasslands (Table 5, Fig. 4). SLA_{CWM}, which was identified as a potential proxy for litter characteristics (steps 1 and 2), did not account for a sufficient proportion of variance to be retained in any of the groups.

All the non-linear relationships meet a threshold, and all the transformed variables are associated with variation in the prediction intervals.

Due to the existence of asymptotes, some non-linear models explained a substantial part of the variance by revealing threshold effects in the proxy-ecosystem property relationship, as in the asymptotic

Table 5

Shape and goodness of fit of the best models (reflected variation* > 0.40) reflecting the relationships between proxies and ecosystem properties for all sampled grasslands or for subsets belonging to the same climate or soil moisture group. W1.3, W1.4 correspond to the Weibull functions listed in Table S4, EXD2 and EXD3 to exponential 2 and 3, AR3 to asymptotic 3, Lin. to linear models.

| Ecosystem property [transformation] | Proxy | Climate or soil moisture group covered by the model** | Range of the ecosystem property (min-max) | Shape of the relationship (function) | Residual standard error | Variation reflected by models* |
|--|-------------------------------|---|---|--------------------------------------|-------------------------|--------------------------------|
| Dry plant biomass (g/m ²) [sqrt] | Height of the herb layer (cm) | Wet grasslands | 91.8–594.0 | LL.4 | +/- 2.43 | 0.41 |
| | | Meso/dry grasslands | 11.4–800.4 | LL.3 | +/- 3.46 | 0.58 |
| Digestibility of plant biomass (%) | LDMC _{CWM} | Wet grasslands | 32.40–79.90 | Lin. | +/- 8.52 | 0.42 |
| | Litter thickness (cm) | Wet grasslands | 32.40–79.90 | AR3 | +/- 8.15 | 0.47 |
| P content of plant biomass (g/kg) [log] | LDMC _{CWM} | All grasslands | 0.48–8.19 | W1.3 | +/- 0.41 | 0.42 |
| | | Continental and Atlantic grasslands | 0.52–3.42 | W1.4 | +/- 0.32 | 0.63 |
| K content of plant biomass (g/kg) [log] | Litter thickness (cm) | Wet grasslands | 3.77–29.63 | Lin. | +/- 0.30 | 0.54 |

*The variation in the ecosystem property reflected by the models was estimated by applying this calculation: Variation reflected by the models = 1 - (Residual variance / Total variance).

**The model can be applied to all the grassland types sampled in the study, or it can be restricted to a single climate or soil moisture group.

equation that relates litter thickness to the plant biomass digestibility in wet grasslands (Table 5, Fig. 4). In addition, the transformation of the response variable to stabilize the variance of the residuals induced visible trends in model prediction intervals (e.g., Figs. 2, 3b and 4b). In these particular cases, the precision of the model prediction depended on the proxy value. For example, the prediction of the plant biomass P content from LDMC_{CWM} offered more confidence for high values of LDMC_{CWM} than for low values (Fig. 3b).

3.4. Overview of the three-step analysis results

This work identifies several easy-to-obtain proxies for multiple ecosystem properties across a wide range of semi-natural grassland plant communities, climate and soil conditions. These proxies have proven sensitive enough to be used within the same generic habitat, i.e. they can detect the variations in ecosystem properties operating at this level of floristic homogeneity. The range of semi-natural grassland habitat types covered by the present study was broad, including continental to coastal locations, sea-level to mountains habitats, dry to wet conditions, and oligotrophic to mesotrophic conditions, which is consistent with the needs of a national-scale monitoring program.

We showed that some aspects of forage quality in semi-natural grasslands can be approximated by proxies based on easy and non-destructive field measurements or derived *a posteriori* from plant species composition data. While the height of the herb layer provides a cost- and time- efficient means of approximating aboveground dry plant biomass, LDMC_{CWM}, and litter thickness may be used to assess two important and complementary dimensions of forage quality: its digestibility and mineral macro-elements content (P and K).

This study also reveals certain limitations in the search for generic proxies at the studied scale. Indeed, the predictive power of the models was greater when the data were considered for specific climate or soil moisture conditions, while remaining relatively limited (the variations reflected by the models were between 41 and 63 %). Furthermore, given the modest size of our dataset, we were unable to split the data into a training set and a validation set to test the quality of model prediction. This validation can be carried out by collecting additional data in the future, which will help to refine the models.

4. Discussion

4.1. Estimating ecosystem properties from proxies: A trade-off between genericity and accuracy

In line with previous research (Diaz et al., 2004; Gardarin et al., 2014; Tasset et al., 2019), this work demonstrates that generic

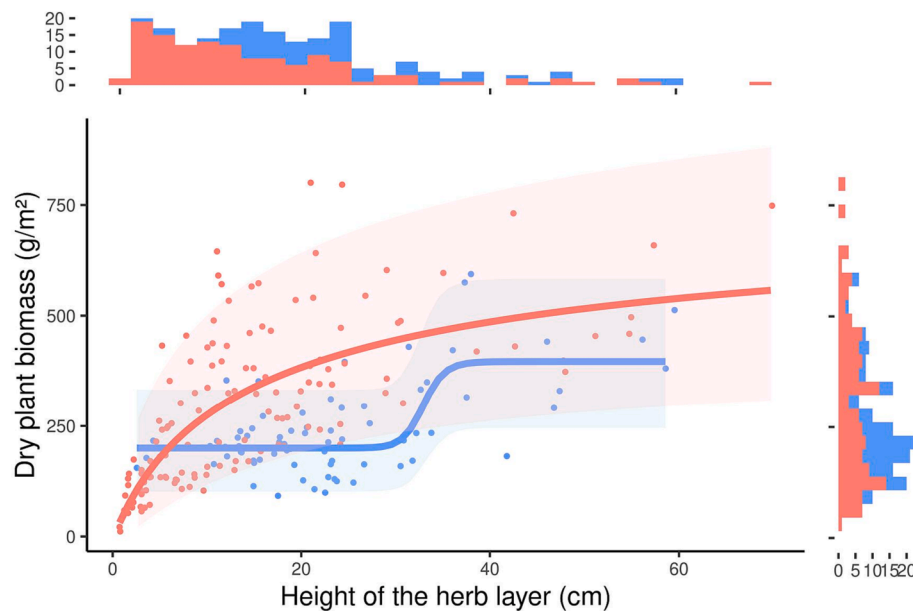


Fig. 2. Height of the herb layer as a proxy for the dry plant biomass according to the soil moisture group (blue, wet grasslands; red, mesophilous/dry grasslands). The solid lines correspond to the average predictions of the models for the two moisture groups (reflected variation > 0.40). The light-colored area delimits the model's prediction interval containing 90 % of the points. The histograms at the top and right of the figures represent the distribution of observations for each variable. See also Table 5 for more details on the models. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

relationships between grassland ecosystem properties are found even across floristically and environmentally variable ecosystems. The identification of proxies for ecosystem properties across a wide range of environmental and management conditions is an interesting input for large-scale functional biogeography studies (Violle et al., 2014; Violle et al., 2015; Borge et al., 2017) and national-scale monitoring programs (Cabello et al., 2012). Nevertheless, large environmental and management gradients reduced the accuracy of the approximation compared to models based on more specific studies (e.g., Tasset et al., 2019 in wet grasslands). In this work, the formation of homogenous climate or soil moisture groups was preferred to the inclusion of the sampling site as a random factor in the models (e.g., Gardarin et al., 2014) to investigate which of climate or soil moisture was the most determinant factor in the proxy-ecosystem property relationships. Grouping improved model accuracy but did not entirely encompass the effect of the sampling site, as evidenced by the variable quality of the approximation across sites.

All the models fitting the relationships between proxies and ecosystem properties were improved by including climate or soil moisture as covariates, illustrating the trade-off between proxy genericity and accuracy. These results suggest a change in the functional pattern of semi-natural grassland ecosystems depending on climate and soil moisture conditions, as previously observed by Gardarin et al. (2014) in a similar range of conditions.

Management type and/or intensity have been shown to influence grassland functioning, as assessed by a trait-based approach (Garnier et al., 2007; Bonanomi et al., 2013; Bardgett et al., 2021 for a review), especially in the case of under-utilization or abandonment (Garnier et al., 2004; Kazakou et al., 2006; Fortunel et al., 2009). In the studied grasslands, management intensity was globally low and practices such as fertilization very infrequent as most of them were protected within the Natura 2000 framework. Further research on more intensively used grasslands would be useful and interesting; nevertheless, the consistency of the relationships between proxies and ecosystem properties given the range of management conditions explored in this study is an encouraging result for monitoring the functioning of semi-natural grasslands. Indeed, this suggests that management is likely to impact ecosystem properties without altering the relationships between proxies and

ecosystem properties.

Non-linear modeling is an asset for determining generic relationships in that it allows some variation in the predictive power of proxies along their range of values. However, when the predictive power of a proxy is low over a large part of its range of variation, the model may show a low residual variance without being very informative. For instance, $LDMC_{CWM}$ and litter thickness were identified as two potential proxies for plant biomass digestibility in wet grasslands, although $LDMC_{CWM}$ is likely to constitute a more reliable proxy than litter thickness: the range of proxy values returning the same approximation of the ecosystem property (presence of a plateau) and providing low predictive power was narrower for $LDMC_{CWM}$ than for litter thickness (Figs. 3 and 4).

4.2. Three generic proxies to help approximate forage quantity and quality

Among the set of candidate proxies, three enabled the establishment of models capturing at least 40 % of the variation in the targeted ecosystem properties: the height of the herb layer, $LDMC_{CWM}$ and litter thickness. Together, they made it possible to approximate the quantity of biomass produced, as well as certain aspects of the forage quality.

Estimating dry plant biomass from the height of the herb layer was more reliable when wet grasslands were distinguished from mesophilic and dry grasslands. Nevertheless, the predictive power of this proxy was limited in wet grasslands, where the non-linear relationship between the two variables showed strong threshold effects. Our results suggest that dry plant biomass increased faster with the height of the herb layer in mesophilic to dry grasslands than in wet grasslands. This seems consistent given the influence of water availability on the mechanical strength of stems and leaves and its impact on the amount of structural tissue (Read and Stokes, 2006).

In wet grasslands, $LDMC_{CWM}$ and litter thickness captured 42 and 47 % of variations in plant biomass digestibility, respectively. This result is in accordance with previous research that established a negative relationship between $LDMC$ and digestibility at the plant level (Khaled et al., 2006; da Pontes et al., 2007; Tasset et al., 2019) and at the community level (Gardarin et al., 2014). Considering wet grasslands separately from

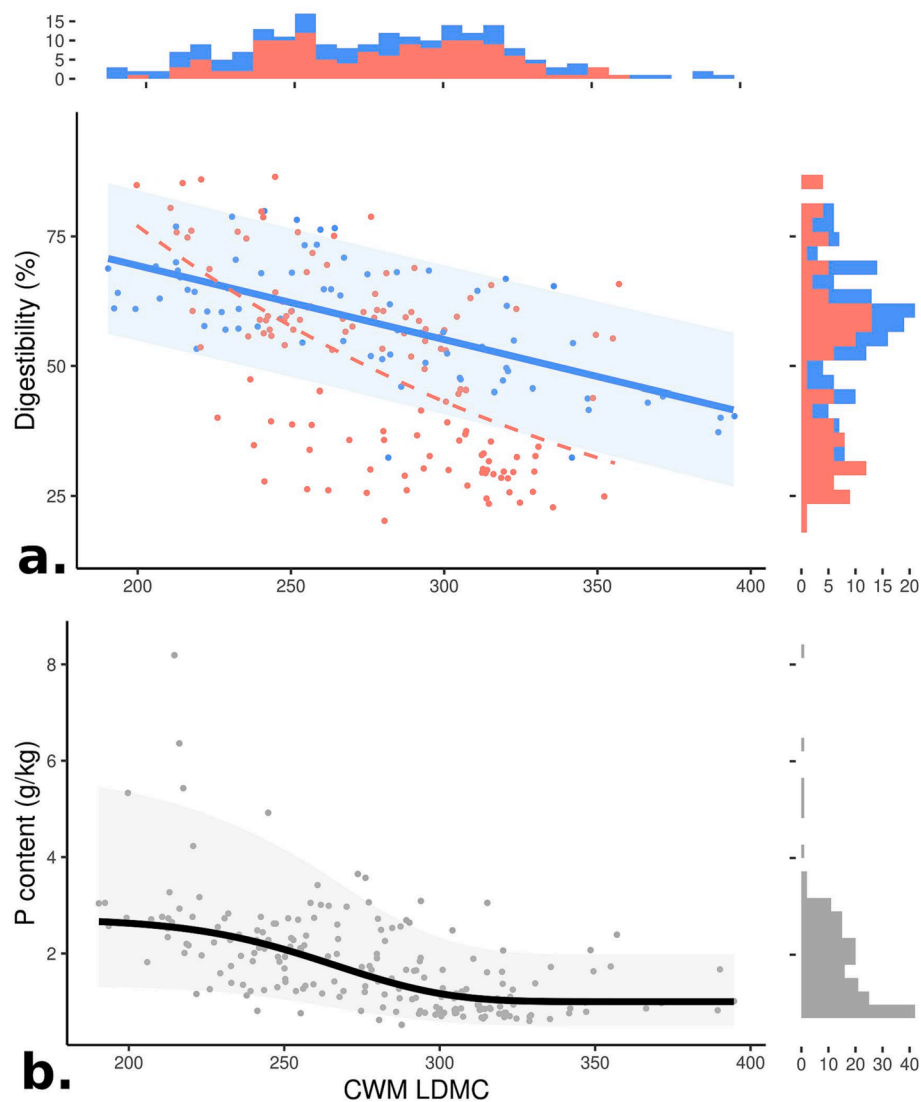


Fig. 3. $LDMC_{CWM}$ as a proxy for (a) the plant biomass digestibility according to the soil moisture group (blue, wet grasslands; red, mesophilous/dry grasslands), and (b) the plant biomass P content in all grasslands (black, all grasslands). The lines correspond to the average predictions of the models (solid, reflected variation > 0.40; dashed, reflected variation < 0.40). The light-colored area delimits the model's prediction interval containing 90 % of the points. The histograms at the top and right of the figures represent the distribution of observations for each variable. Details on the models reflecting more than 40 % of the variation in ecosystem property are provided in Table 5. The model that best reflected the variation in plant biomass digestibility as a function of $LDMC_{CWM}$ in mesophilous to dry grasslands (reflected variation = 0.34) is based on a two-parameter exponential function. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mesophilous to dry grasslands improved the accuracy of the model predictions, in line with the suggestion of Gardarin et al. (2014) that the relationship linking digestibility to $LDMC_{CWM}$ partly depends on the level of potential evapotranspiration. Our results indicate that overall, digestibility decreased with increasing $LDMC_{CWM}$, but the slope was steeper in mesophilous to dry grasslands than in wet grasslands (Fig. 3).

$LDMC$ has been described as a functional marker of litter decomposition at the plant level (Kazakou et al., 2006), and community-aggregated $LDMC$ has been shown to correlate negatively with litter decomposability (Fortunel et al., 2009; Pakeman et al., 2011), and positively with aboveground total dead biomass (Garnier et al., 2007) across a wide range of grasslands. The relationship found between litter thickness and biomass digestibility in wet grasslands is probably related to the link between plant dry matter content (including $LDMC$) and two degradation processes: the digestibility of plant tissues by ruminants and the decomposition of litter by soil microorganisms (Bumb et al., 2018).

Another aspect of forage quality reflected by $LDMC_{CWM}$ and litter thickness was the concentration of P and K in plant biomass. Previous

research has shown that $LDMC_{CWM}$ tends to be negatively correlated with leaf P content (Kazakou et al., 2006). Our results indicate that this relationship is non-linear, and show a threshold for high values of $LDMC_{CWM}$. The linear relationship we found between litter thickness and the K content of plant biomass in wet grasslands is consistent with the results of Cornelissen and Thompson (1997), who observed a positive relationship between litter decomposition and the concentration of this macroelement in the leaf. Since K plays a role in the uptake of nitrate and phosphate, the authors suggested that this relationship is a consequence of the plant's investment in compounds associated with fast growth rather than compounds promoting toughness and low palatability.

Besides being a generic proxy for plant digestibility and P content, $LDMC_{CWM}$ has been shown to respond to pressures such as N inputs (e.g., fertilization, Borgy et al., 2017) and management intensity (e.g., grassland under-utilization or abandonment, Garnier et al., 2004; Garnier et al., 2007; Fortunel et al., 2009). These characteristics suggest that this parameter may also be used as a 'state indicator' (*sensu* Niemeijer

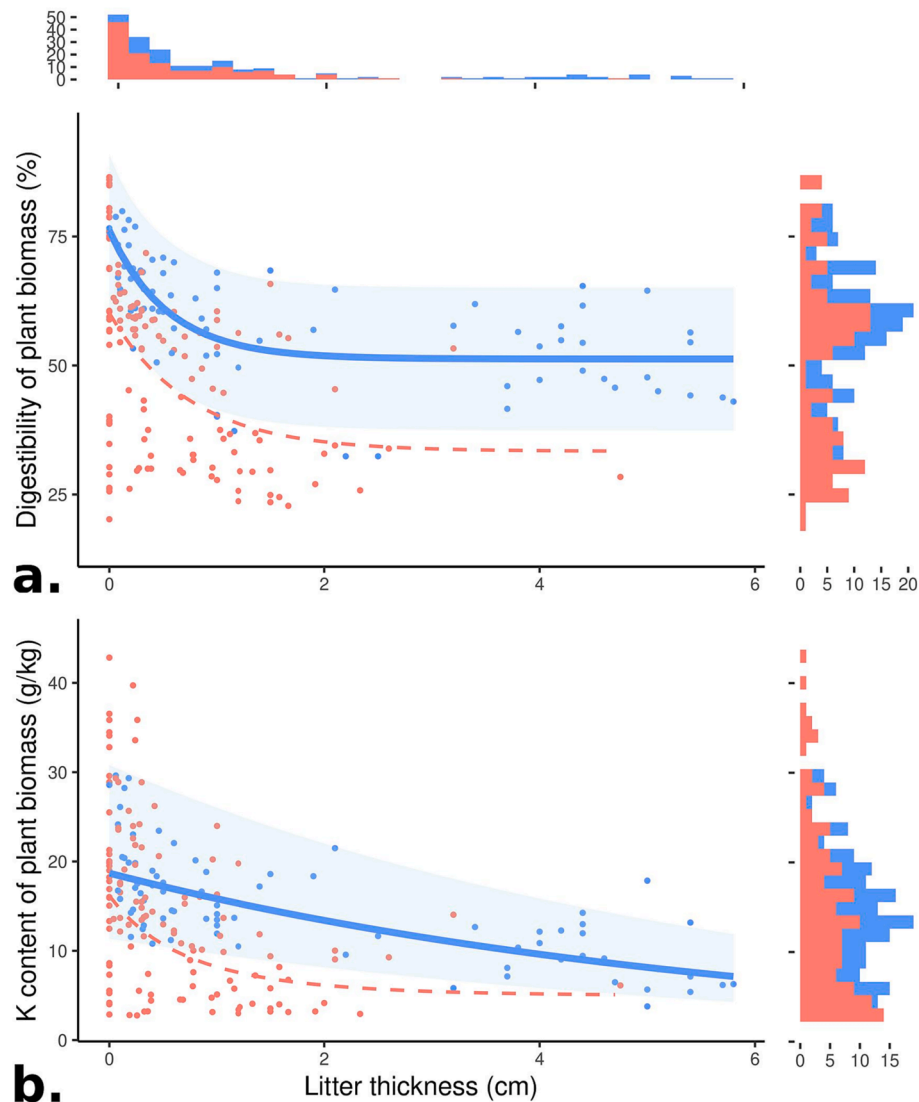


Fig. 4. Litter thickness as a proxy for the plant biomass (a) digestibility, and (b) K content according the soil moisture group (blue, wet grasslands; red, mesophilic/dry grasslands). The lines correspond to the average predictions of the models (solid, reflected variation > 0.40; dashed, reflected variation < 0.40). The light-colored area delimits the model's prediction interval containing 90 % of the points. The histograms at the top and right of the figures represent the distribution of observations for each variable. Details on the models reflecting more than 40 % of the variation in ecosystem property are provided in Table 5. The model that best reflected the variation in plant biomass digestibility as a function of litter thickness in mesophilic to dry grasslands (reflected variation = 0.26) is based on a three-parameter asymptotic function. The model that best reflected the variation in plant biomass K content as a function of litter thickness in mesophilic to dry grasslands (reflected variation = 0.18) is based on a two-parameter exponential function. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and de Groot, 2008) of ecosystem functioning in response to pressures.

4.3. None of the investigated proxies was effective in approximating soil and litter characteristics

Plant community characteristics are considered as key determinants of primary production and litter decomposition, two ecological processes whose balance modulates the aboveground accumulation of dead plant biomass (Chabrierie et al., 2003; Garnier et al., 2007) and the mean residence time of C and N in the litter and soil compartments (De Deyn et al., 2008; Soussana and Lemaire, 2014; Kohler et al., 2020). Although aboveground vegetation attributes have proven to be good proxies for assessing the belowground carbon stock under relatively homogenous environmental conditions (Garnier et al., 2004; Lopatin et al., 2019; Kohler et al., 2020), this was not the case at the scale of our study, which encompassed a wide range of soil and climate conditions. None of the proxies investigated performed well in assessing the structure and

chemistry of the soil and litter compartments, probably due to the influence of climate and geology. In addition, the weakness of the detected relationships may be partly related to the organization of our sampling design, which focused on replicating vegetation units without considering soil variability within those entities.

5. Conclusions

The identification of easy-to-obtain proxies such as the height of the herb layer, $LDMC_{CWM}$, and litter thickness to approach plant biomass and chemical composition is an asset to support appropriate and adaptive management of semi-natural grassland habitats:

- (i) Implementing these proxies will allow for low-cost approximation of the quantity and quality of available forage. These proxies do not allow a perfect prediction of the parameters but represent an interesting compromise between feasibility (cost-time

expertise) and data quality. The information about the agronomic value of semi-natural grasslands may support continued agricultural use, or justify subsidizing the maintenance of grasslands of low agronomic value for biodiversity conservation.

- (ii) Using proxies is a reasonable and affordable way to complete the routine assessment of the conservation status of protected semi-natural grasslands by integrating information on their functional state. Generic proxies can be implemented in large-scale monitoring programs to approach ecosystem properties across grassland habitat types and/or management regimes, and draw comparisons among them.

This work provides tools to approach grassland ecosystem functioning with low sampling and data acquisition effort. Measuring the height of the herb layer and litter thickness is quick and easy in the field. However, we should note that while the use of CWM leaf traits is a common practice in academic works, calculating this parameter from databases available online can be limiting and not easily accessible to site managers. Three actions can be implemented to address this challenge: (i) inform managers on the interest of $LDMC_{CWM}$ as an informative proxy for ecosystem properties to evaluate the conservation status and guide the management of a grassland ecosystem; (ii) provide easy access to trait data through information on existing databases and stimulate efforts to complete and improve them through existing coordinated academic initiatives (e.g., TRY); and (iii) develop simple tools to facilitate the calculation of CWM traits from species abundance data collected in the field.

Credit authorship contribution statement

Marine Pacé: Writing & editing, Conceptualization, Methodology, Formal analysis. **Anne Bonis:** Writing – review & editing, Investigation, Conceptualization. **Didier Alard:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Cian Blaix:** Writing – review & editing, Validation, Methodology, Data curation. **Maxime Burst:** Writing – review & editing, Investigation, Data curation. **Manuella Catterou:** Writing – review & editing, Validation, Methodology, Investigation. **Sylvain Diquélou:** Writing – review & editing, Conceptualization. **Thierry Dutoit:** Writing – review & editing, Investigation. **Hugo Fontès:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Servane Lemauiel-Lavenant:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **Grégory Loucougaray:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **François Mesleard:** Writing – review & editing, Investigation. **Alice Michelot-Antalik:** Writing – review & editing, Supervision, Methodology, Investigation. **Sylvain Plantureux:** Writing – review & editing, Conceptualization. **Olivier Chabrierie:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank the three anonymous referees for their valuable comments, which helped to improve our manuscript, as well as the Associate editor who handled the submitted paper. They

would also like to thank the following people for their help in field work and data acquisition: Benjamin Allard, Pauline Boubert, Méghan Boulembert, Lucie Chauvel, Clémence Helleu, Nathan Daumergue, Françoise Dubois, Frédéric Dubois, Adeline François, Claude Gallois, Olivier Gore, Marion Girardier, Jérôme Lacoux, Loan Madej, Chloé Malik, Daniel Pavon, Lisa Puisse, Hervé Ramone, Emmanuelle Revardel, Antoine Tardif, Adelaïde Theraroz, Romain Ulmer, and Christel Vidaller. We thank the laboratory of Normandie and the laboratory of Arras for the management of chemical analyses. We thank Lise Maciejewski and Margaux Mizarz from PatriNat for useful discussions all along the SurPas project, Christian Damgaard for his helpful comments on the manuscript, and Annie Buchwalter for the linguistic review. We are grateful to the farmers who provided access to their fields and to the following french organizations that provided basic information and facilities for working in the Natura 2000 sites: Parc naturel régional (PNR) des Marais du Cotentin et du Bessin, PNR Volcans d'Auvergne, PNR du Vercors, Conservatoire des Espaces Naturels (CEN) Aquitaine, CEN Lorraine, Pôle Lorrain du Futur Conservatoire Botanique National Nord-Est, CEN de Provence-Alpes-Côte-d'Azur, Réserve Naturelle Nationale des Coussouls de Crau, the "Coussouls sentinelles" program of the Conseil Départemental des Bouches-du-Rhône, Chambre d'Agriculture des Bouches-du-Rhône, CEN des Hauts-de-France, Etablissement Public du Marais poitevin et le PNR du Marais poitevin, Société Nationale de Protection de la Nature, Réserve Naturelle Nationale de Camargue, and Réserve Naturelle Régionale de la Tour du Valat.

This work is an outcome of the SURPAS ("SURveillance de l'état de conservation des habitats agro-PASToraux pelousaires et prairiaux") project funded by the Ministère de la Transition Ecologique et Solidaire (MTES, France) and driven by UMS PatriNat for the Office Français de la Biodiversité (OFB, France), within the framework of the "Habitats, Fauna, Flora" directive, Natura 2000 Project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2024.111586>.

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