Towards the assessment of biodiversity and management practices in mountain pastures using diagnostic species?

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ABSTRACT

Diversity of plant communities in semi-natural grasslands is strongly dependent on management practices. To characterize community diversity and agricultural practices, we need easy-to-use indicators. The diagnostic species are used to characterize and differentiate vegetation types, but are they useful to assess the various facets of community diversity and the agricultural practices applied to managed grasslands?

Based on the fidelity analysis of 311 phytosociological relevés, considered as references for mesic grassland classification in the Franche-Comté region (eastern France), we identified eight diagnostic species of mesic pastures (*Cynosurion cristati*). We then compared the number of diagnostic species observed in 45 mountain pastures with taxonomic, phylogenetic and multi-trait functional diversity indices, as well as with agronomic variables describing stocking rate and fertilization. To account for the role of diagnostic species identity, we compared Spearman rank correlations obtained for these diagnostic species with correlations calculated for 1000 random samples of eight species from the regional species pool of mesic grasslands.

The number of diagnostic species in the grassland plot was significantly positively correlated with most taxonomic, phylogenetic and functional diversity metrics. However, only the positive correlations with Simpson taxonomic diversity and Rao functional diversity were significantly different from those obtained from random samples. Moreover, the number of diagnostic species was significantly negatively correlated with stocking rate and fertilization intensity, although only correlations with industrial fertilizer input were significantly different from results obtained from random samples.

These results show that the number of diagnostic species observed in a pasture may be a good indicator of a high taxonomic and functional diversity and of a low fertilization. Thereby, a special attention should be given to diagnostic species to assess the status of grassland biodiversity, including functional aspects.

1. Introduction

Oligotrophic to mesotrophic semi-natural grasslands with a long history of management through grazing or mowing are among the most species-rich ecosystems in the world and display very high small-scale plant species richness (Cousins and Eriksson, 2002; Wilson et al., 2012). Their high biodiversity, resulting from a long evolution through

extensive management (Mašková et al., 2009; Schermer et al., 2016), provides high levels of many provisioning ecosystem services (Mauchamp et al., 2013; Schirpke et al., 2017) and support their stability. It is recognized that plant diversity reduces inter-annual variability of biomass production (Hector et al., 2010; Cadotte et al., 2012), species richness improves resistance of grassland productivity to climatic extreme events (Isbell et al., 2015), and the presence of

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subordinate species increases the adaptability of the grassland ecosystem to climatic hazard (Mariotte et al., 2013). Beyond conservation concerns, maintaining or enhancing grassland biodiversity is therefore of first interest to promote resilience and maintain ecosystem functions in a context of global change (Craine et al., 2011; Oliver et al., 2015). However, temperate grasslands are among the most endangered ecosystems by land-use changes (Hoekstra et al., 2005), which are considered as one of the primary drivers of biodiversity loss (Foley et al., 2005; Flynn et al., 2009).

In Europe, significant changes in grassland management occurred in the last decades, aiming at improving grassland productivity (Wesche et al., 2012). Two main opposite tendencies affect grassland vegetation (Plantureux et al., 2005; Buttler et al., 2009): less productive parcels that are located far away from the farms and not easily accessible are progressively neglected and tend to be afforested; most productive parcels, with an easy access for machines, are intensified, often with an associating increase in defoliation and nutrient inputs (Gaujour et al., 2012). In relation to changes in grassland management, recent studies recorded a decrease in plant diversity and a shift in species composition over the last decades in different grassland types across European regions. In central Europe, an important decrease of species richness and functional diversity was observed in resampled plots compared to historical ones (Wesche et al., 2012). In the French Jura Mountains, resampled plots showed a lower phylogenetic and functional diversity and became dominated by nitrogen-demanding competitive grasses (Gillet et al., 2016). Similar studies in Scottish grasslands highlighted a loss of functional richness (Pakeman et al., 2017) and taxonomic diversity, associated with an increased cover of nitrogen-demanding species (Mitchell et al., 2017). Research in the Swiss Alps indicated a recent homogenization of plant diversity at a regional scale (Homburger and Hofer, 2012).

In response to the current loss of biodiversity in Europe, the Habitat Directive was adopted by the European Union to protect the most seriously threatened habitats and to ensure their resilience to global changes (OJEC, 1992; Gaston et al., 2008; Louette et al., 2015). Each EU member state is required by the Habitat Directive to ensure the favorable conservation status of natural habitats belonging to the Natura 2000 network through conservation measures (Ostermann, 1998). However, the Habitat Directive does not give a precise definition of the conservation status, nor indicators to evaluate it (Kovač et al., 2016). Consequently, although the concept of favorable conservation status is still subject to intense discussion and research (Velázquez et al., 2010; Louette et al., 2015), recent research was conducted to identify cost-effective indicators to evaluate it, but mainly for forest habitats (Cantarello and Newton, 2008; Hernando et al., 2010; Kovač et al., 2016).

In this context, we stress the need to determine easy-to-use indicators in the field, allowing the monitoring of plant diversity and agricultural practices in mountain pastures. Specifically, the large traditionally pastoral region of the French Jura Mountains, devoted to dairy farming and the production of protected designation of origin (PDO) cheese, was subject to a decline in grassland biodiversity over the past decades due to management intensification (Gillet et al., 2016). Thus, the PDO cheese sector requires indicators to monitor the trends of biodiversity and management practices in agricultural grasslands to be able to prevent further decline in grassland biodiversity. Indeed, in addition to the provision and the maintenance of high levels of many ecosystem services, high grassland biodiversity is one of the key components of the "terroir", the basis of the specificity of PDO products. In this study, we investigated the use of diagnostic species as indicators of biodiversity and management practices for pastures grazed by dairy cows in the French Jura Mountains. As it is generally possible to detect diagnostic species by non-destructive an inexpensive means, it could be possible to use them to assess the conservation status of a local grassland habitat under the Habitat Directive, provided they are good indicators of biodiversity and management practices of this habitat.

Diagnostic species can be defined as species that show a preference for a given habitat or biotic community type (De Cáceres et al., 2008). Thus, they are commonly used in field survey for the identification of community types. It is acknowledged that diagnostic species are taxa that indicate habitat conditions where they are found because they are particularly responsive to habitat modifications (Chiarucci et al., 2008; Santoro et al., 2012). In pastures, the intensification of management practices leads to a decrease of taxonomic, functional and phylogenetic diversity (Carmona et al., 2012; Mauchamp et al., 2014; Dainese et al., 2015). As the condition of the habitat is a limiting factor controlling the population of specialist species (Evans and Arvela, 2011), diagnostic species are supposed to be among the first species to disappear in response to the intensification of management practices. Thus, it may be possible to use diagnostic species to assess the trends of biodiversity and management practices in pastures. Indeed, the disappearance of diagnostic species should indicate a change in management practices towards an intensification in association with a decrease in plant diversity. Based on the hypothesis that species restricted to one or a few habitats are potentially better indicators of environmental change than generalist species (De Cáceres and Legendre, 2009), we addressed the following questions:

1) Are diagnostic species good indicators of biodiversity and management practices of the vegetation type they characterize?
2) Are diagnostic species better indicators of biodiversity and management practices than other grassland species?

2. Material and methods

2.1. Study area and vegetation sampling

Our study was carried out in the French Jura Mountains. This mountain range is composed of three main structural units across an elevation gradient: first plateau (500–800 m a.s.l.), second plateau (800–950 m a.s.l.) and high range (950–1700 m a.s.l.). Climate is suboceanic (fully humid, warm summer), ranging from warm temperate (Cfb) to boreal (Dfb, at the highest elevations) according to Köppen-Geiger climate classification (Kottek et al., 2006; Rubel et al., 2017). Predominant soils are cambisols developed on limestone with a variable superficial cover of silt. In the French Jura Mountains, permanent grasslands represent 22% of the area and support dairy farming and PDO cheese production, mainly Comté. Such production implies constraining specifications for agricultural practices; e.g. in the PDO Comté area, the average nutrient inputs must not exceed $120\,kg\,N\,ha^{-1}\,yr^{-1}$ and the stocking rate is limited to 1.3 livestock units per hectare (one livestock or adult bovine unit LU is equivalent to one dairy cow).

In Ferrez (2007), 525 phytosociological relevés, realized between 1973 and 2007 in eastern France, were analyzed to elaborate a typology for mesic grassland classification. To obtain homogeneous groups of relevés for the typology, the authors excluded the phytosociological relevés not well discriminated by cluster analysis, thus retaining 371 relevés. In our study, we selected 311 among the 371 phytosociological relevés presented in Ferrez (2007) to compute a list of diagnostic species of mesic pastures (*Cynosurion cristati* Tüxen 1947). When selecting these relevés, we considered only those belonging to phytosociological associations present in the French Jura Mountains, which explains the exclusion of 60 phytosociological relevés.

45 permanent grasslands were then chosen in the French Jura Mountains across an elevation gradient, from which both vegetation relevés and detailed agricultural data were available (Fig. 1). They cover a wide range of agricultural practices but are all primarily used as pastures for dairy cows. According to the regional phytosociological classification (Ferrez et al., 2011), the plant communities in these 45 grasslands belong to the *Cynosurion cristati* Tüxen 1947 (mesic pastures).

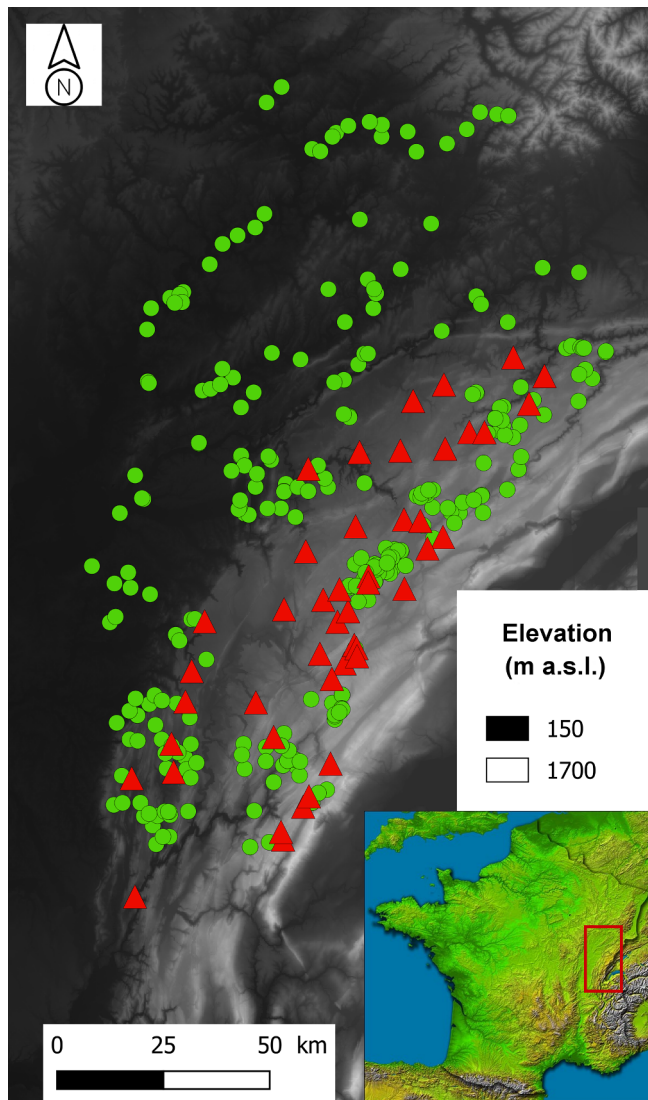It has been shown that, for a same plot size, sampling units with

Fig. 1. Location of the 311 phytosociological relevés used to compute the diagnostic species list of mesic pastures (green circles) and of the 45 grassland plots sampled in 2017 (red triangles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

more elongated shapes present higher species richness than squares or circles (Heegaard et al., 2007; Güler et al., 2016). Thus, to account for a maximum of plant species, the vegetation of each grassland was sampled from May 2017 to July 2017 in a rectangular plot of $120\,m^2$ ($60\,m \times 2\,m$), on the flattest area located inside the grassland and far from parcel margins. The choice of a plot size close to $100\,m^2$ is common in phytosociology (Chytrý and Otýpková, 2003) and is consequently widely applied to permanent grasslands (Hejcman et al., 2010; Homburger and Hofer 2012; Gillet et al., 2016), because it is more efficient than a square plot of $16\,m^2$ to account for most of the species present. All observed vascular plant species were listed and the cover of each species was estimated using the seven degrees of the dominance Braun-Blanquet scale (r, +, 1, 2, 3, 4, 5). These codes were converted into absolute percentage cover (van der Maarel, 1979) and then adjusted to relative percentage cover by summing to 100% for each plot. We considered relative cover because its estimation is more reliable than the one of absolute cover, and because only relative dominance was considered for the computation of diversity metrics.

2.2. Establishment of the list of diagnostic species

The geographical area, the number of phytosociological relevés and the vegetation units taken into account strongly influence the selection of diagnostic species (Chytrý et al., 2002; Tichý and Chytrý, 2006; De Cáceres and Legendre, 2009). To determine diagnostic species of a given vegetation unit, the most appropriate dataset should be selected in relation with the objectives of the study, as there is no better way to proceed (De Cáceres and Legendre, 2009). As this study focused on mesic pastures in the French Jura Mountains, we used only phytosociological relevés of mesic grasslands realized in the study area. This choice is supported by the fact that extracting a common list of diagnostic species of larger geographical area is difficult due to the absence of shared species in remote areas (De Cáceres et al., 2015).

The 311 selected phytosociological relevés were assigned to four vegetation units (alliances) according to the mesic grassland classification of the Franche-Comté region proposed in Ferrez (2007): 29% (88/311) belonged to the *Arrhenatherion elatioris* W. Koch 1926 (lowland mesic hay meadows), 35% (108/311) to the *Triseto flavescentis-Polygonion bistortae* Braun-Blanquet & Tüxen ex Marschall 1947 (mountain mesic hay meadows), 33% (105/311) to the *Cynosurion cristati* Tüxen 1947 (mesic pastures), and 3% (10/311) to the *Lolio perennis-Plantaginion majoris* G. Sissingh 1969 (mesic trampled grasslands).

To compute a diagnostic species list of mesic pastures, we used the phi coefficient of association (Chytrý et al., 2002; De Cáceres and Legendre, 2009), a measure of species fidelity to vegetation types. Applied to species cover data, this index is the Pearson correlation computed between a quantitative vector (*i.e.*, the vector containing the species cover values in the various phytosociological relevés) and a binary vector (*i.e.*, "1" for phytosociological relevés belonging to the alliance of interest and "0" for phytosociological relevés belonging to other alliances). The calculation was performed using the multipatt() function from the indicspecies package (De Cáceres and Legendre, 2009) in R 3.4.2 (http://www.R-project.org), setting 5000 permutations. Because differences in the size of the target vegetation units are known to influence the determination of diagnostic species (Tichý and Chytrý, 2006) and the variation in cover of different species can be an important source of heterogeneity among vegetation units (Willner et al., 2009), we used a group-equalized phi coefficient of association and considered species cover values instead of presence-absence data (see De Cáceres and Legendre, 2009). We retained only the species that showed significant fidelity ($P < 0.05$) for 10 runs of the multipatt() function, to avoid selecting species close to the threshold of significance.

2.3. Diversity metrics

The taxonomic, functional and phylogenetic facets of community diversity were acknowledged to be complementary aspects of community structure, responding differently to environmental changes and not always positively correlated (Cadotte et al., 2013; Pavoine et al., 2013). Thus, to measure community diversity, we computed taxonomic, functional and phylogenetic alpha diversities at the plot level (Devictor et al., 2010; Cadotte et al., 2013; Dainese et al., 2015). Taxonomic diversity was measured through species richness (N0), inverse Simpson diversity (N2) based on Rényi general entropy (Hill, 1973), and Pielou evenness (J1) according to Jost (2010). Functional and phylogenetic facets of diversity were computed using the Rao quadratic diversity with Jost correction, as it corresponds to a generalization of the inverse Simpson index (Jost, 2007; de Bello et al., 2010).

Multi-trait Rao quadratic diversity was computed from a standardized Euclidean distance matrix using four selected functional traits, weighted by the relative cover of individual plant species: leaf dry matter content (LDMC), leaf nitrogen content per dry mass (LNC), specific leaf area (SLA) and maximum plant height (MH). These traits

are widely used in functional ecology of grasslands (Louault et al., 2005; Ansquer et al., 2009; Garnier and Navas, 2012; Lososová et al., 2016; Mauchamp et al., 2016). Maximum plant height is an important morphological factor determining plant competitive ability (Tilman, 1988; Westoby et al., 2002). LDMC, LNC and SLA are functional traits linked to resource acquisition and growing strategy (Garnier et al., 2004). These traits were extracted from two databases: LEDA Traitbase for LDMC and SLA (Kleyer et al., 2008) and TRY database for LNC and MH (Kattge et al., 2011). Species with missing information were removed, so that multi-trait Rao quadratic diversity was sometimes computed with a bit less than 90% of the relative percentage cover. All computations were performed using R v 3.4.2 (http://www.R-project.org), vegan package (Oksanen et al., 2018), and Rao() function (de Bello et al., 2010).

To compute phylogenetic diversity, we constructed a phylogenetic tree composed of the vascular plant species recorded during the field work. The phylogenetic tree was built following the same procedure described in different studies conducted in the French Jura Mountains (Perronne et al., 2014; Mauchamp et al., 2014; Mauchamp et al., 2016). The sequences of two genes encoding chloroplast proteins (*rbcL* and *matK*) were obtained using the GenBank database (Benson et al., 2006). Their complementarity allows a good resolution of the tree for the recorded species (Hilu et al., 2003). We replaced species not yet sequenced for the selected genes by their closest available relative, according to the APG III classification (APG, 2009). All sequences were first aligned using ClustalX 2.1 software (Larkin et al., 2007) and then adjusted manually for both genes. A combined analysis of these two datasets was then performed following the Bayesian Monte Carlo Markov Chain (MCMC) approach under BEAST 1.8.4 software (Drummond and Rambaut, 2007). We used MrModeltest2 software (v. 2.0, Evolutionary Biology Center, Uppsala University, SE) to choose the model of nucleotides substitution that best fits the data according to Akaike's information criterion. The general time reversible model (GTR) with among-site substitution rate heterogeneity described by a gamma distribution (Yang, 1994) was then selected. To calibrate the rates of molecular evolution of each lineage for angiosperms clades (Bell et al., 2005), several family relationships were constrained in BEAST following the APG III phylogeny (APG, 2009). An uncorrelated relaxed molecular clock that takes into account evolutionary heterogeneity between lineages was used for branch length calculation (Drummond et al., 2006). Finally, the ultrametric tree topology was saved in a newick format. We used the Rao quadratic entropy, weighted by the ultrametric distances of the phylogenetic tree, to compute phylogenetic diversity (RaoPD), utilizing Rao() function (de Bello et al., 2010).

2.4. Management practices

For each of the 45 selected pastures, farmers provided detailed information about grazing management and fertilization level. For the grazing management, the obtained information was the number of dairy cows or livestock units (LU) grazing the parcel, their time of presence at each rotation and the time elapsed between two rotations in 2017. For the fertilization practices, the collected data were the type, the quantity and the spreading date of the different fertilizers applied (farmyard manures and industrial fertilizers). Moreover, general characteristics of the farm, building type, herd performance, manure and slurry production, and spreading management of fertilizers were collected at the farm level. Based on this survey, we computed different agronomic variables.

The stocking rate in 2017, traducing the animal pressure on the pasture and expressed in $LU\,day\,ha^{-1}$, was calculated by multiplying the number of dairy cows with the time they spent on the parcel, divided by the parcel area. To account for variations in grazing intensity along the year, we calculated the stocking rate in spring, summer and autumn separately. The studied pastures were located close to the farm

buildings and aimed to be grazed by dairy cows according to a same rotation plan applied by the farmers each year. Since the dairy cows herd was not expected to change significantly in recent years, the values calculated for 2017 were assumed to be similar to those of previous years.

The data given by farmers relative to the N input from farmyard manures were sometimes imprecise. Thus, we decided to estimate the total manure amount produced in the farm per year F_{man}. To do so, we used the following formula:

$$F_{man} = \frac{T}{12}NQ$$

T is the time spent by the cattle inside the cowshed, expressed in month. N is the number of dairy cows constituting the herd (LU) and Q is the farmyard manure amount produced by a dairy cow present 12 months in a cowshed (expressed in $m^3\,LU^{-1}\,yr^{-1}$ or $t\,LU^{-1}\,yr^{-1}$). The Q value depends on the type of building and the kind of produced manure. According to the Institut de l'Elevage (2012), the retained values were $13.5\,t\,LU^{-1}\,yr^{-1}$ for solid manure production and $23\,m^3\,LU^{-1}\,yr^{-1}$ for liquid manure production. The calculated total manure amount produced in the farm per year F_{man} was then divided by the total surface of spreading in order to obtain an estimate per hectare and per year. This quantity was multiplied by the mean nitrogen content of each manure type: $5\,kg\,N\,t^{-1}$ for solid manure, $5\,kg\,N\,m^{-3}$ for liquid manure and $3\,kg\,N\,m^{-3}$ for diluted liquid manure (Institut de l'Elevage, 2012). The given result was the estimated N input spread on the parcel from farmyard manure, expressed in $kg\,N\,ha^{-1}\,yr^{-1}$. As the organic part of the nitrogen present in farmyard manure is not directly available for plants because it requires a post-spreading mineralization by soil microorganisms (Kirkham et al., 2008; Gaujour et al., 2012), a last calculation was done to consider only the N input available for plant growth during the field work in 2017. Its aim was to exclude the part of the N input not yet mineralized. To do so, we multiplied the N input by the proportion of available nitrogen depending of the time elapsed from the manure spread to the vegetation sampling period. The utilized values were 0.5 for liquid manure spread during the sampling year, 0.2 for liquid manure spread one year before the field work, 0 for liquid manure spread two years before the field work, 0.15 for solid manure spread during the sampling year, 0.1 for solid manure spread one year before the field work and 0.05 for solid manure spread two years before the field work (Institut de l'Elevage, 2012). Thereby, the calculated available N input from farmyard manure (Nman), expressed in $kg\,N\,ha^{-1}\,yr^{-1}$, relied on the fertilization practices of the past three years.

As nutrients present in industrial fertilizers are directly available for plants (Cavalli et al., 2014), we considered the values given by farmer, expressed in $kg\,N\,ha^{-1}\,yr^{-1}$, as the available N input on the parcel from industrial fertilizers (Nind).

2.5. Statistical analyses

We first performed a principal component analysis (PCA) on the Hellinger-transformed species-cover matrix of the 45 grassland plots (Borcard et al., 2018). To get an overview of the relationships between all computed variables and the number of observed diagnostic species, we fitted these variables on the PCA plot using the envfit() function from the vegan R package (Oksanen et al., 2018). To address the question of whether diagnostic species are reliable biodiversity indicators in mountain pastures, we tested Spearman rank correlation between all diversity metrics and the number of diagnostic species observed in each plot. We also tested Spearman rank correlation between agronomic variables and the number of diagnostic species to address of whether diagnostic species are good management indicators or not. It is a major difference, especially in field surveys, whether a species is diagnostic *per se* or only when it exceeds a certain cover value (Willner et al., 2009). Here, we decided to consider all diagnostic

species present in the grassland plots regardless of their cover. Indeed, defining a cover threshold to take into account or not a diagnostic species would have been subjective and different for each species, as some species naturally show low or high cover values.

To address the question of whether the selected diagnostic species are better indicators compared to any other grassland species, we generated 1000 samples of the same number of randomly selected species. The species taken into account to do so were all the species that occurred in more than 5% of the 311 phytosociological relevés describing mesic grasslands in the study area, so that casual taxa were avoided. We then compared Spearman rank correlation calculated using the diagnostic species with correlations calculated using the randomly selected species samples, for all diversity and agronomic variables. We set the correlation value obtained for the diagnostic species as a reference and computed a non-parametric *P*-value based on the number of greater and lower correlations obtained for the 1000 random samples:

$$P = 1 - \frac{|A - B|}{1000}$$

A is the number of higher correlation values and *B* the number of lower correlation values. All analyses were performed using R v 3.4.2 (http://www.R-project.org).

3. Results

3.1. Diagnostic species

The set of diagnostic species was composed of eight species (Table 1): *Cynosurus cristatus* L., *Prunella vulgaris* L., *Achillea millefolium* L., *Agrostis capillaris* L., *Plantago media* L., *Veronica serpyllifolia* L., *Scorzoneroides autumnalis* (L.) Moench, and *Pimpinella saxifraga* L. The phi values ranged from 0.241 to 0.422 (after 5000 permutations). Six of these species were frequent in the 45 grassland plots whereas *Scorzoneroides autumnalis* (L.) Moench was present at low frequency and *Pimpinella saxifraga* L. was absent.

A significant positive phi coefficient indicated that the cover of the species in phytosociological relevés belonging to the *Cynosurion cristati* Tüxen 1947 (mesic pastures) was higher than its cover in phytosociological relevés not belonging to it. Larger values (close to 1) indicated a greater degree of joint fidelity between the species and the group of relevés belonging to the *Cynosurion cristati* Tüxen 1947. As the phi values were rather low (close to 0) for the eight diagnostic species, it indicated a low degree of joint fidelity.

3.2. Relationships between diversity, management and number of diagnostic species

The fitted variables on the PCA first axes showed positive correlations between all agronomic variables, as well as positive correlations between all diversity metrics (Fig. 2). Axes 1 and 2 represented 33.5%

Table 1
Degree of joint fidelity of the eight diagnostic species of mesic pastures (*Cynosurion cristati* Tüxen 1947), obtained after 5000 permutations on the 311 phytosociological relevés. The last column presents the frequency of these diagnostic species in the 45 grassland plots.

Species name	phi	*P*	Frequency
Cynosurus cristatus L.	0.422	< 0.001	0.60
Prunella vulgaris L.	0.383	0.002	0.58
Achillea millefolium L.	0.324	0.007	0.78
Agrostis capillaris L.	0.324	0.007	0.76
Plantago media L.	0.298	0.007	0.44
Veronica serpyllifolia L.	0.268	0.022	0.62
Scorzoneroides autumnalis (L.) Moench	0.261	0.016	0.09
Pimpinella saxifraga L.	0.241	0.033	0.00

of the variance of the Hellinger-transformed species matrix. The number of diagnostic species observed in the grassland plot (Diagsp) was positively correlated with all displayed diversity metrics and negatively correlated with all displayed agronomic variables. This means that when a high number of diagnostic species was found in a given grassland, it indicated a high species richness (N0), inverse Simpson diversity (N2), Pielou evenness (J1), functional diversity (RaoFD) and phylogenetic diversity (RaoPD). At the opposite, when a low number of diagnostic species was found in a given grassland, it indicated a high fertilization intensity through the use of farmyard manure (Nman) and industrial fertilizers (Nind), and a high stocking rate during spring (cattle_spring), autumn (cattle_autumn), and summer in a lesser extent (cattle_summer).

These results were confirmed by Spearman rank correlations (Table 2). Indeed, we found significant positive correlations between the number of observed diagnostic species in the grassland plot and all diversity metrics (N0, N2 and J1 for taxonomic diversity, RaoPD for phylogenetic diversity and RaoFD for functional diversity). Significant negative correlations were also found between the number of observed diagnostic species and almost all agronomic variables (cattle_spring and cattle_autumn for stocking rate, and Nman and Nind for fertilization). Only the negative correlation with stocking rate during summer (cattle_summer) was not significant. Thereby, the number of diagnostic species in a pasture revealed to be a good indicator of high plant diversity and extensive management practices (low stocking rate and low fertilization).

3.3. Diagnostic species in comparison to randomly selected species

The comparison of the Spearman rank correlations calculated using the diagnostic species list with correlations calculated using the 1000 randomly selected species lists (Table 3) revealed that diagnostic species were better indicators than other grasslands species for inverse Simpson diversity (N2), Rao functional diversity (RaoFD), and the available N input from industrial fertilizers (Nind). Although results obtained for stocking rate during spring (cattle_spring) showed *P* = 0.058, the eight diagnostic species were not better indicators than randomly selected species for the other variables. In particular, species richness (N0), which presented a high correlation with the number of diagnostic species (rho = 0.741), was also highly correlated to the number of species issued from random samples.

4. Discussion

4.1. Establishment of the list of diagnostic species

In this study, we identified a set of eight diagnostic species of mesic pastures (*Cynosurion cristati* Tüxen 1947) based on 311 phytosociological relevés realized in eastern France. Our results revealed that the degree of joint fidelity between each diagnostic species and the group of relevés belonging to the alliance of mesic pastures was low. Indeed, the diagnostic species of pastures were sometimes found with low cover in relevés belonging to other alliances of mesic grasslands (Ferrez, 2007). These results were not surprising, regarding the fact that it is unattainable to find species with absolute fidelity to a given vegetation type (Willner et al., 2009). Moreover, we considered only the class of mesic grasslands (*Arrhenatheretea elatioris* Braun-Blanquet 1949) for the establishment of the list of diagnostic species. Thus, we ignored the affinity of our diagnostic species for vegetation units belonging to other classes, such as the dry grasslands (*Festuco valesiacae-Brometea erecti* Braun-Blanquet & Tüxen ex Braun-Blanquet 1949). Consequently, the diagnostic species *Pimpinella saxifraga*, commonly found in dry grasslands, was not present in the 45 grassland plots that we analysed. In the study area, this species is present in lowland pastures whereas it occurs only in dry grasslands and fringes in mountain areas.
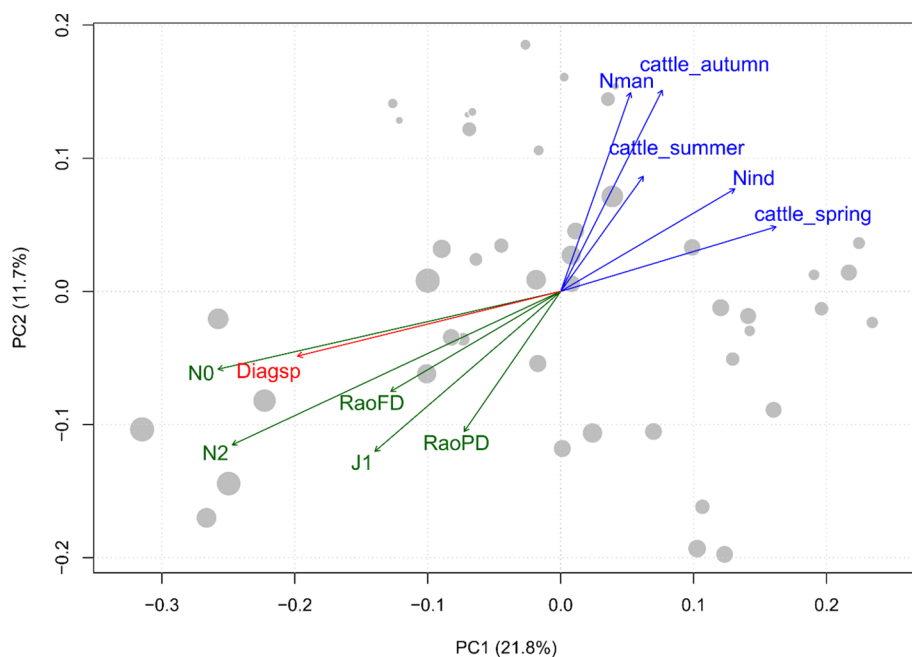
Fig. 2. Principal component analysis of the Hellinger-transformed species composition of the 45 grasslands plots, with fitted diversity metrics (green arrows), agronomic variables (blue arrows) and number of diagnostic species observed in the grassland plot (Diagsp, red arrow). Diversity metrics: N0 species Richness, N2 inverse Simpson Diversity, J1 Pielou evenness, RaoPD Rao phylogenetic diversity, RaoFD Rao functional diversity. Agronomic variables: cattle_spring stocking rate during spring, cattle_summer stocking rate during summer, cattle_autumn stocking rate during autumn, Nman available N input from farmyard manures, Nind available N input from industrial fertilizers. Sites are represented by grey point whose size is proportional to elevation. Species are not shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

Spearman rank correlations between diversity metrics or agronomic variables and the number of diagnostic species observed in the grassland plot. Significant *P* are displayed in bold.

		rho	*P*
Diversity metrics			
N0	Species richness	0.741	**< 0.001**
N2	Inverse Simpson diversity	0.689	**< 0.001**
J1	Pielou evenness	0.302	**0.044**
RaoPD	Rao phylogenetic diversity	0.395	**0.007**
RaoFD	Rao functional diversity	0.540	**< 0.001**
Agronomic variables			
cattle_spring	stocking rate (LU·day·ha^{-1} during spring)	−0.521	**< 0.001**
cattle_summer	stocking rate (LU·day·ha^{-1} during summer)	−0.168	0.269
cattle_autumn	stocking rate (LU·day·ha^{-1} during autumn)	−0.371	**0.012**
Nman	Available N on the parcel from farmyard manures (kg N·ha^{-1}·yr^{-1})	−0.328	**0.028**
Nind	Available N on the parcel from industrial fertilizers (kg N·ha^{-1}·yr^{-1})	−0.599	**< 0.001**

Table 3

Number of greater and lower Spearman rank correlations between diversity metrics or agronomic variables and the number of species in 1000 random samples, as compared to the correlations calculated using the set of eight diagnostic species. Significant *P* are displayed in bold.

	Diagnostic species	1000 random samples		
	rho	*Number of greater rho*	*Number of lower rho*	*P*
Diversity metrics				
N0	0.741	105	895	0.210
N2	0.689	15	985	**0.030**
J1	0.302	133	867	0.266
RaoPD	0.395	58	942	0.116
RaoFD	0.540	10	990	**0.020**
Agronomic variables				
cattle_spring	−0.521	973	27	0.054
cattle_summer	−0.168	695	305	0.610
cattle_autumn	−0.371	849	151	0.302
Nman	−0.328	911	89	0.178
Nind	−0.599	993	7	**0.014**

4.2. Diagnostic species as biodiversity and management indicators

The number of diagnostic species present in a grassland revealed to be a good indicator of biodiversity and management practices, as almost all tested variables showed significant correlations with it. The combined presence of several diagnostic species of the *Cynosurion cristati* Tüxen 1947 (mesic pastures) in a grassland belonging to this vegetation type was associated with a high plant taxonomic, functional and phylogenetic diversity and an extensive management (low stocking rate and low fertilization). These findings were predictable as an increase in the number of plant species is often associated with an increase of plant diversity (Cadotte et al., 2011; Pavoine and Bonsall, 2011), and an extensive management in grassland is necessary to maintain plant diversity (Mašková et al., 2009; Wilson et al., 2012; Mauchamp et al., 2014).

The nitrogen input from industrial fertilizers showed to be significantly negatively correlated with the number of observed diagnostic species. Moreover, the correlation obtained for the diagnostic species was higher than correlations obtained for randomly selected species. These results have to be considered regarding the fact that industrial

fertilizers are the most detrimental source of nutrients for plant diversity in mountain grasslands (Mauchamp et al., 2016), because nutrients are directly available for plants (Cavalli et al., 2014). Diagnostic species are able to react to modification of the environmental conditions of their habitat (Bazzichetto et al., 2016). A study in coastal dunes ecosystems showed that diagnostic species can be indicators of soil conditions and provide useful indications for habitat monitoring, because they have narrow edaphic requirement (Angiolini et al., 2018). These previous results are similar to our observation concerning the nitrogen input from industrial fertilizers. Our results support the idea that diagnostic species are the first species to disappear when the nitrogen input increases through the use of industrial fertilizers, because they have narrow requirement concerning the nitrogen supply (Online Appendix A). In comparison to industrial fertilizers, the impact of the application of farmyard manures on mountain grassland plant diversity is less pronounced (Mauchamp et al., 2016). Indeed, the organic forms of the nitrogen present in farmyard manure are not directly available for plants and requires a post-spreading mineralization by soil

microorganisms (Kirkham et al., 2008; Gaujour et al., 2012). The fact that the impact of this type of fertilizer on plant diversity is less important compared to industrial fertilizers could explain why the response of diagnostic species was not different from the response of other grassland species. The number of diagnostic species present in a grassland showed to be negatively correlated with the stocking rate, particularly with the stocking rate in spring. This can be explained by the fact that an important stocking rate in spring is the most detrimental for plant diversity (Critchley et al., 2007). However, the response of the eight diagnostic species to the stocking rate was not different from the response of randomly selected species. Diagnostic species of pastures are species that are well adapted to the disturbances induced by cattle activity. Thus, it is likely that diagnostic species of pastures do not react particularly to variations in stocking rate in comparison to other species.

The significant positive correlation obtained between the number of observed diagnostic species and species richness is rather tautological, as the number of diagnostic species is not independent from species richness. This also explains why the correlation obtained using the set of eight diagnostic species was not different from correlations obtained using randomly selected species. Indeed, relationships between these two indices are positive regardless of the identity of the concerned species. Although Pielou evenness showed significant positive correlation with the number of diagnostic species, indicating that cover differences between species in the grassland plot were smaller when a high number of diagnostic species was observed, diagnostic species were not better indicators than randomly selected species for this diversity metric. However, in comparison to randomly selected species, diagnostic species were more reliable to assess inverse Simpson index. The relationships between these taxonomic diversity metrics and the number of observed diagnostic species could be related to the nitrogen input from industrial fertilizers. Indeed, in the French Jura Mountains, Mauchamp et al. (2016) recorded that the nitrogen input from industrial fertilizer had a negative impact on inverse Simpson index, but not on species evenness. As diagnostic species revealed to be better indicators than randomly selected species to assess the nitrogen input from industrial fertilizers, they were also indirectly better indicators to assess inverse Simpson index.

According to our results, diagnostic species were better indicators than randomly selected species for functional diversity. This can be explained by the fact that the range of the trait values of the diagnostic species matched the range of the trait values of the species pool of the 45 grassland plots, at least for SLA and LDMC (Online Appendix B). Functional traits are known to be particularly responsive to environmental changes (Díaz et al., 2013). Therefore, in relation to a change in environmental conditions, a loss of species with particular values for SLA or LDMC in the plant community is likely to be followed by a loss of the diagnostic species with the same traits values. Thus, the combined presence of many diagnostic species in a given grassland indicated a wide range of the trait values for SLA and LDMC, consequently a high functional diversity.

Our list of diagnostic species was constituted of species from different lineages. Thus, the combined presence of many diagnostic species revealed that different species lineages were present in the plant community, which can explain the significant positive correlation obtained between the number of observed diagnostic species and phylogenetic diversity. Moreover, according to Villalobos and Vamosi (2016), the alteration in grassland plant community composition increases the prevalence of zygomorphic flower species lineages. Thus, a plant indicator list equilibrated in actinomorphic and zygomorphic lineages like our list of diagnostic species, consequently representative of a large phylogenetic diversity, reinforces the potential for the assessment of phylogenetic diversity. However, results obtained for phylogenetic diversity were not different between diagnostic species and randomly selected species. Indeed, it is likely that many random species subsets were also equilibrated in species from different lineages.

4.3. Future direction for the use of diagnostic species in European grasslands

In this study, we have shown that we can use diagnostic species of the *Cynosurion cristati* to assess biodiversity and management practices in mesic pastures of the French Jura Mountains. Given the fact that it is easy to count for a number of diagnostic species in a local grassland, the use of diagnostic species to assess biodiversity and management practices in agricultural grasslands should be further investigated. In Europe, permanent grasslands are ecosystems whose floristic composition is strongly dependent on the agricultural practices used for their management (Královec et al., 2009). Moreover, permanent grasslands belonging to the alliance of the *Cynosurion cristati* Tüxen 1947 are widely represented across temperate Europe at low and mid-elevations (Mucina et al., 2016). Consequently, we believe that the use of diagnostic species as biodiversity and management practices indicators in pastures of the French Jura Mountains belonging to the *Cynosurion cristati* Tüxen 1947 can be applied to other European grasslands belonging to this vegetation type. More globally, we believe that the methods that we used in the French Jura Mountains can be extended to other alliances of anthropogenic managed grasslands in Europe, because their high biodiversity is the result of a relatively similar long evolution through extensive management (Mašková et al., 2009). In addition, the results of a recent study conducted in the Jura Mountains support the potential use of diagnostic species as biodiversity and management practices indicators in other habitat types. Indeed, in relation to land-use abandonment and nitrogen deposition in wetlands, Rion et al. (2018) reported a decrease of taxonomic diversity associated with a decrease in frequency and cover of diagnostic species. However, the fact that we only investigated the use of diagnostic species as biodiversity and management practices indicators in pastures for dairy cows have to be considered regarding the possible generalization of the results. Moreover, our results seem difficult to generalize to grassland ecosystems that are not managed, such as alpine grasslands, continental steppes or tallgrass prairie.

Our results support the idea that the concept of diagnostic species matches the concept of typical species mentioned, but not defined, in the Habitat Directive (OJEC, 1992). According to Evans and Arvela (2011), typical species should be species only found in a habitat and sensitive to changes in the condition of this habitat. They should also be selected to reflect favorable structure and functions of the habitat. In our study, we showed that diagnostic species were good indicator of management practices in pastures of the Jura Mountains, especially fertilization. Our results also revealed that the combined presence of many diagnostic species in a grassland habitat was associated with a high plant diversity, mainly taxonomic and functional diversity. Thereby, we argue that the number of diagnostic species in a given grassland could be used as one of the indicators of the favorable conservation status of the local grassland habitat. This statement is supported by a study realized in Mediterranean coastal dunes, which highlighted that diagnostic species can provide helpful indications for conservation, monitoring and restoration of these habitats (Angiolini et al., 2018). However, despite the fact they can present a high plant diversity (Mauchamp et al., 2014), pastures belonging to the *Cynosurion cristati* Tüxen 1947 are not integrated in the habitat directive (OJEC, 1992). In order to use the number of diagnostic species in the evaluation of the conservation status of a local grassland habitat, future research have to be conducted, focusing on the assessment of biodiversity using diagnostic species in grassland types integrated in the Natura 2000 network, such as mesic hayfields, dry grasslands or wet grasslands.

5. Conclusion

This study provides a first insight about the use of diagnostic species as biodiversity and management indicators. In the study area, the number of diagnostic species in a pasture revealed to be a good

indicator of taxonomic and functional diversity of the plant community and of management practices, especially fertilization. As it is easy to count a number of diagnostic species in a grassland plot, a special attention should be given to diagnostic species to assess the status of grassland biodiversity, including functional aspects. However, future research is needed to show if the results of this study can be extended to others grassland types such as hayfields, dry grasslands or wet grasslands, and to other geographical areas.

Acknowledgment

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2019.105584.

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