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

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21 **Abstract**

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

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

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

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

45 **Key-words:** diagnostic species; grasslands; mountain pastures; biodiversity assessment;  
46 management.

## 47 1. Introduction

48 Oligotrophic to mesotrophic semi-natural grasslands with a long history of management through  
49 grazing or mowing are among the most species-rich ecosystems in the world and display very high  
50 small-scale plant species richness (Cousins and Eriksson 2002; Wilson *et al.* 2012). Their high  
51 biodiversity, resulting from a long evolution through extensive management (Mašková *et al.*, 2009;  
52 Schermer *et al.*, 2016), provides high levels of many provisioning ecosystem services (Mauchamp *et al.*  
53 *et al.*, 2013; Schirpke *et al.*, 2017) and support their stability. It is recognized that plant diversity reduces  
54 inter-annual variability of biomass production (Hector *et al.*, 2010; Cadotte *et al.*, 2012), species  
55 richness improves resistance of grassland productivity to climatic extreme events (Isbell *et al.*, 2015),  
56 and the presence of subordinate species increases the adaptability of the grassland ecosystem to  
57 climatic hazard (Mariotte *et al.*, 2013). Beyond conservation concerns, maintaining or enhancing  
58 grassland biodiversity is therefore of first interest to promote resilience and maintain ecosystem  
59 functions in a context of global change (Craine *et al.*, 2011; Oliver *et al.*, 2015). However, temperate  
60 grasslands are among the most endangered ecosystems by land-use changes (Hoekstra *et al.*, 2005),  
61 which are considered as one of the primary drivers of biodiversity loss (Foley *et al.*, 2005; Flynn *et al.*,  
62 2009).

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

78 In response to the current loss of biodiversity in Europe, the Habitat Directive was adopted by the  
79 European Union to protect the most seriously threatened habitats and to ensure their resilience to  
80 global changes (OJEC, 1992; Gaston *et al.*, 2008; Louette *et al.*, 2015). Each EU member state is  
81 required by the Habitat Directive to ensure the favorable conservation status of natural habitats  
82 belonging to the Natura 2000 network through conservation measures (Ostermann *et al.*, 1998).  
83 However, the Habitat Directive does not give a precise definition of the conservation status, nor  
84 indicators to evaluate it (Kovač *et al.*, 2016). Consequently, although the concept of favorable  
85 conservation status is still subject to intense discussion and research (Velázquez *et al.*, 2010; Louette

86 *et al.*, 2015), recent research was conducted to identify cost-effective indicators to evaluate it, but  
87 mainly for forest habitats (Cantarello and Newton, 2008; Hernando *et al.*, 2010; Kovač *et al.*, 2016).

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

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

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

## 121 2. Material and methods

### 122 2.1. Study area and vegetation sampling

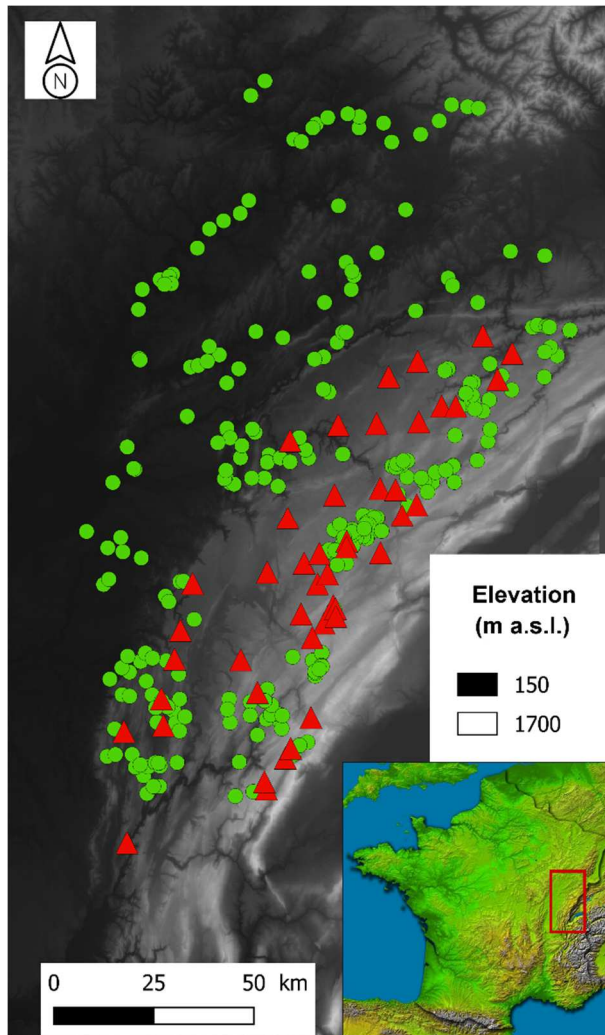
123 Our study was carried out in the French Jura Mountains. This mountain range is composed of three  
124 main structural units across an elevation gradient: first plateau (500-800 m a.s.l.), second plateau  
125 (800-950 m a.s.l.) and high range (950-1700 m a.s.l.). Climate is suboceanic (fully humid, warm  
126 summer), ranging from warm temperate (Cfb) to boreal (Dfb, at the highest elevations) according to  
127 Köppen-Geiger climate classification (Kottek *et al.*, 2006; Rubel *et al.*, 2017). Predominant soils are  
128 cambisols developed on limestone with a variable superficial cover of silt. In the French Jura  
129 Mountains, permanent grasslands represent 22% of the area and support dairy farming and PDO  
130 cheese production, mainly Comté. Such production implies constraining specifications for agricultural

131 practices; e.g. in the PDO Comté area, the average nutrient inputs must not exceed 120 kg N ha<sup>-1</sup> yr<sup>-1</sup>  
132 and the stocking rate is limited to 1.3 livestock units per hectare (one livestock or adult bovine unit LU  
133 is equivalent to one dairy cow).

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

142 45 permanent grasslands were then chosen in the French Jura Mountains across an elevation  
143 gradient, from which both vegetation relevés and detailed agricultural data were available (Fig. 1).  
144 They cover a wide range of agricultural practices but are all primarily used as pastures for dairy cows.  
145 According to the regional phytosociological classification (Ferrez *et al.*, 2011), the plant communities in  
146 these 45 grasslands belong to the *Cynosurion cristati* Tüxen 1947 (mesic pastures).

147 It has been shown that, for a same plot size, sampling units with more elongated shapes present  
148 higher species richness than squares or circles (Heegaard *et al.*, 2007; Güler *et al.*, 2016). Thus, to  
149 account for a maximum of plant species, the vegetation of each grassland was sampled from May  
150 2017 to July 2017 in a rectangular plot of 120 m<sup>2</sup> (60 m × 2 m), on the flattest area located inside the  
151 grassland and far from parcel margin. The choice of a plot size close to 100 m<sup>2</sup> is common in  
152 phytosociology (Chytrý and Otýpková, 2003) and is consequently widely applied to permanent  
153 grasslands (Hejcman *et al.*, 2010; Homburger and Hofer 2012; Gillet *et al.*, 2016), because it is more  
154 efficient than a square plot of 16 m<sup>2</sup> to account for most of the species present. All observed vascular  
155 plant species were listed and the cover of each species was estimated using the seven degrees of the  
156 dominance Braun-Blanquet scale (r, +, 1, 2, 3, 4, 5). These codes were converted into absolute  
157 percentage cover (van der Maarel, 1979) and then adjusted to relative percentage cover by summing  
158 to 100% for each plot. We considered relative cover because its estimation is more reliable than the  
159 one of absolute cover, and because only relative dominance was considered for the computation of  
160 diversity metrics.



161

162 Fig. 1: Location of the 311 phytosociological relevés used to compute the diagnostic species list of  
 163 mesic pastures (green circles) and of the 45 grassland plots sampled in 2017 (red triangles).

164 **2.2. Establishment of the list of diagnostic species**

165 The geographical area, the number of phytosociological relevés and the vegetation units taken into  
 166 account strongly influence the selection of diagnostic species (Chytrý *et al.*, 2002; Tichý and Chytrý,  
 167 2006; De Cáceres and Legendre, 2009). To determine diagnostic species of a given vegetation unit,  
 168 the most appropriate dataset should be selected in relation with the objectives of the study, as there is  
 169 no better way to proceed (De Cáceres and Legendre, 2009). As this study focused on mesic pastures  
 170 in the French Jura Mountains, we used only phytosociological relevés of mesic grasslands realized in  
 171 the study area. This choice is supported by the fact that extracting a common list of diagnostic species  
 172 of larger geographical area is difficult due to the absence of shared species in remote areas (De  
 173 Cáceres *et al.*, 2015).

174 The 311 selected phytosociological relevés were assigned to four vegetation units (alliances)  
 175 according to the mesic grassland classification of the Franche-Comté region proposed in Ferrez *et al.*  
 176 (2007): 29% (88/311) belonged to the *Arrhenatherion elatioris* W. Koch 1926 (lowland mesic hay  
 177 meadows), 35% (108/311) to the *Trisetum flavescens-Polygonum bistortae* Braun-Blanquet & Tüxen ex  
 178 Marschall 1947 (mountain mesic hay meadows), 33% (105/311) to the *Cynosurion cristati* Tüxen 1947  
 179 (mesic pastures), and 3% (10/311) to the *Lolium perennis-Plantaginion majoris* G. Sissingh 1969 (mesic  
 180 trampled grasslands).

181 To compute a diagnostic species list of mesic pastures, we used the phi coefficient of association  
182 (Chytrý *et al.*, 2002; De Cáceres and Legendre, 2009), a measure of species fidelity to vegetation  
183 types. Applied to species cover data, this index is the Pearson correlation computed between a  
184 quantitative vector (*i.e.*, the vector containing the species cover values in the various phytosociological  
185 relevés) and a binary vector (*i.e.*, “1” for phytosociological relevés belonging to the alliance of interest  
186 and “0” for phytosociological relevés belonging to other alliances). The calculation was performed  
187 using the `multipatt()` function from the `indicspecies` package (De Cáceres and Legendre, 2009) in R  
188 3.4.2 (<http://www.R-project.org>), setting 5000 permutations. Because differences in the size of the  
189 target vegetation units are known to influence the determination of diagnostic species (Tichý and  
190 Chytrý, 2006) and the variation in cover of different species can be an important source of  
191 heterogeneity among vegetation units (Willner *et al.*, 2009), we used a group-equalized phi coefficient  
192 of association and considered species cover values instead of presence-absence data (see De  
193 Cáceres and Legendre, 2009). We retained only the species that showed significant fidelity ( $P < 0.05$ )  
194 for 10 runs of the `multipatt()` function, to avoid selecting species close to the threshold of significance.

### 195 *2.3. Diversity metrics*

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

205 Multi-trait Rao quadratic diversity was computed from a standardized Euclidean distance matrix using  
206 four selected functional traits, weighted by the relative cover of individual plant species: leaf dry matter  
207 content (LDMC), leaf nitrogen content per dry mass (LNC), specific leaf area (SLA) and maximum  
208 plant height (MH). These traits are widely used in functional ecology of grasslands (Louault *et al.*,  
209 2005; Ansquer *et al.*, 2009; Garnier and Navas, 2012; Lososová *et al.*, 2016; Mauchamp *et al.*, 2016).  
210 Maximum plant height is an important morphological factor determining plant competitive ability  
211 (Tilman, 1988; Westoby *et al.*, 2002). LDMC, LNC and SLA are functional traits linked to resource  
212 acquisition and growing strategy (Garnier *et al.*, 2004). These traits were extracted from two  
213 databases: LEDA Traitbase for LDMC and SLA (Kleyer *et al.*, 2008) and TRY database for LNC and  
214 MH (Kattge *et al.*, 2011). Species with missing information were removed, so that multi-trait Rao  
215 quadratic diversity was sometimes computed with a bit less than 90% of the relative percentage cover.  
216 All computations were performed using R v 3.4.2 (<http://www.R-project.org>), `vegan` package (Oksanen  
217 *et al.*, 2018), and `Rao()` function (de Bello *et al.*, 2010).

218 To compute phylogenetic diversity, we constructed a phylogenetic tree composed of the vascular plant  
219 species recorded during the field work. The phylogenetic tree was built following the same procedure  
220 described in different studies conducted in the French Jura Mountains (Perronne *et al.*, 2014;  
221 Mauchamp *et al.*, 2014 and Mauchamp *et al.*, 2016). The sequences of two genes encoding  
222 chloroplast proteins (*rbcL* and *matK*) were obtained using the GenBank database (Benson *et al.*,  
223 2006). Their complementarity allows a good resolution of the tree for the recorded species (Hilu *et al.*,  
224 2003). We replaced species not yet sequenced for the selected genes by their closest available  
225 relative, according to the APG III classification (APG 2009). All sequences were first aligned using  
226 ClustalX 2.1 software (Larkin *et al.*, 2007) and then adjusted manually for both genes. A combined

227 analysis of these two datasets was then performed following the Bayesian Monte Carlo Markov Chain  
228 (MCMC) approach under BEAST 1.8.4 software (Drummond and Rambaut, 2007). We used  
229 MrModeltest2 software (v. 2.0, Evolutionary Biology Center, Uppsala University, SE) to choose the  
230 model of nucleotides substitution that best fits the data according to Akaike's information criterion. The  
231 general time reversible model (GTR) with among-site substitution rate heterogeneity described by a  
232 gamma distribution (Yang, 1994) was then selected. To calibrate the rates of molecular evolution of  
233 each lineage for angiosperms clades (Bell *et al.*, 2005), several family relationships were constrained  
234 in BEAST following the APG III phylogeny (APG 2009). An uncorrelated relaxed molecular clock that  
235 takes into account evolutionary heterogeneity between lineages was used for branch length  
236 calculation (Drummond *et al.*, 2006). Finally, the ultrametric tree topology was saved in a newick  
237 format. We used the Rao quadratic entropy, weighted by the ultrametric distances of the phylogenetic  
238 tree, to compute phylogenetic diversity (RaoPD), utilizing Rao() function (de Bello *et al.*, 2010).

#### 239 2.4. Management practices

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

248 The stocking rate in 2017, traducing the animal pressure on the pasture and expressed in LU day ha<sup>-1</sup>,  
249 was calculated by multiplying the number of dairy cows with the time they spent on the parcel, divided  
250 by the parcel area. To account for variations in grazing intensity along the year, we calculated the  
251 stocking rate in spring, summer and autumn separately. The studied pastures were located close to  
252 the farm buildings and aimed to be grazed by dairy cows according to a same rotation plan applied by  
253 the farmers each year. Since the dairy cows herd was not expected to change significantly in recent  
254 years, the values calculated for 2017 were assumed to be similar to those of previous years.

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

$$258 F_{man} = \frac{T}{12} N Q$$

259  $T$  is the time spent by the cattle inside the cowshed, expressed in month.  $N$  is the number of dairy  
260 cows constituting the herd (LU) and  $Q$  is the farmyard manure amount produced by a dairy cow  
261 present 12 months in a cowshed (expressed in m<sup>3</sup> LU<sup>-1</sup> yr<sup>-1</sup> or t LU<sup>-1</sup> yr<sup>-1</sup>). The  $Q$  value depends on the  
262 type of building and the kind of produced manure. According to the Institut de l'Elevage (2012), the  
263 retained values were 13.5 t LU<sup>-1</sup> yr<sup>-1</sup> for solid manure production and 23 m<sup>3</sup> LU<sup>-1</sup> yr<sup>-1</sup> for liquid manure  
264 production. The calculated total manure amount produced in the farm per year  $F_{man}$  was then divided  
265 by the total surface of spreading in order to obtain an estimate per hectare and per year. This quantity  
266 was multiplied by the mean nitrogen content of each manure type: 5 kg N t<sup>-1</sup> for solid manure, 5 kg N  
267 m<sup>-3</sup> for liquid manure and 3 kg N m<sup>-3</sup> for diluted liquid manure (Institut de l'Elevage, 2012). The given  
268 result was the estimated N input spread on the parcel from farmyard manure, expressed in kg N ha<sup>-1</sup>  
269 yr<sup>-1</sup>. As the organic part of the nitrogen present in farmyard manure is not directly available for plants  
270 because it requires a post-spreading mineralization by soil microorganisms (Kirkham *et al.*, 2008;



271 Gaujour *et al.*, 2012), a last calculation was done to consider only the N input available for plant  
272 growth during the field work in 2017. Its aim was to exclude the part of the N input not yet mineralized.  
273 To do so, we multiplied the N input by the proportion of available nitrogen depending of the time  
274 elapsed from the manure spread to the vegetation sampling period. The utilized values were 0.5 for  
275 liquid manure spread during the sampling year, 0.2 for liquid manure spread one year before the field  
276 work, 0 for liquid manure spread two years before the field work, 0.15 for solid manure spread during  
277 the sampling year, 0.1 for solid manure spread one year before the field work and 0.05 for solid  
278 manure spread two years before the field work (Institut de l'Élevage, 2012). Thereby, the calculated  
279 available N input from farmyard manure (N<sub>man</sub>), expressed in kg N ha<sup>-1</sup> yr<sup>-1</sup>, relied on the fertilization  
280 practices of the past three years.

281 As nutrients present in industrial fertilizers are directly available for plants (Cavalli *et al.*, 2014), we  
282 considered the values given by farmer, expressed in kg N ha<sup>-1</sup> yr<sup>-1</sup>, as the available N input on the  
283 parcel from industrial fertilizers (N<sub>ind</sub>).

## 284 2.5. Statistical analyses

285 We first performed a principal component analysis (PCA) on the Hellinger-transformed species-cover  
286 matrix of the 45 grassland plots (Borcard *et al.*, 2018). To get an overview of the relationships between  
287 all computed variables and the number of observed diagnostic species, we fitted these variables on  
288 the PCA plot using the `envfit()` function from the `vegan` R package (Oksanen *et al.*, 2018). To address  
289 the question of whether diagnostic species are reliable biodiversity indicators in mountain pastures, we  
290 tested Spearman rank correlation between all diversity metrics and the number of diagnostic species  
291 observed in each plot. We also tested Spearman rank correlation between agronomic variables and  
292 the number of diagnostic species to address of whether diagnostic species are good management  
293 indicators or not. It is a major difference, especially in field surveys, whether a species is diagnostic  
294 *per se* or only when it exceeds a certain cover value (Willner *et al.*, 2009). Here, we decided to  
295 consider all diagnostic species present in the grassland plots regardless of their cover. Indeed,  
296 defining a cover threshold to take into account or not a diagnostic species would have been subjective  
297 and different for each species, as some species naturally show low or high cover values.

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

$$307 \quad P = 1 - \frac{|A - B|}{1000}$$

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

## 310 3. Results

### 311 3.1. Diagnostic species

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

318 A significant positive phi coefficient indicated that the cover of the species in phytosociological relevés  
 319 belonging to the *Cynosurion cristati* Tüxen 1947 (mesic pastures) was higher than its cover in  
 320 phytosociological relevés not belonging to it. Larger values (close to 1) indicated a greater degree of  
 321 joint fidelity between the species and the group of relevés belonging to the *Cynosurion cristati* Tüxen  
 322 1947. As the phi values were rather low (close to 0) for the eight diagnostic species, it indicated a low  
 323 degree of joint fidelity.

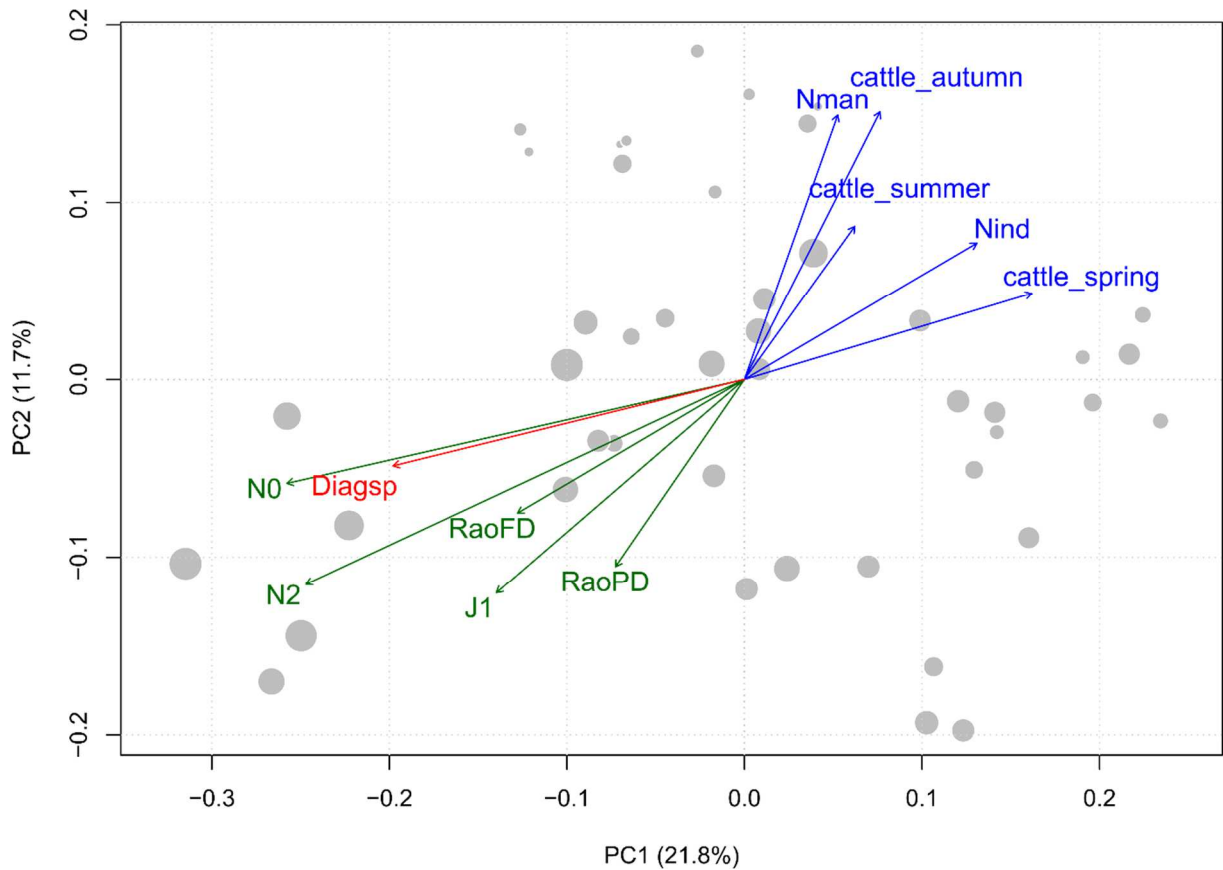
324 Table 1. Degree of joint fidelity of the eight diagnostic species of mesic pastures (*Cynosurion cristati*  
 325 Tüxen 1947), obtained after 5000 permutations on the 311 phytosociological relevés. The last column  
 326 presents the frequency of these diagnostic species in the 45 grassland plots.

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

327

### 328 3.2. Relationships between diversity, management and number of diagnostic species

329 The fitted variables on the PCA first axes showed positive correlations between all agronomic  
 330 variables, as well as positive correlations between all diversity metrics (Fig. 2). Axes 1 and 2  
 331 represented 33.5% of the variance of the Hellinger-transformed species matrix. The number of  
 332 diagnostic species observed in the grassland plot (Diagsp) was positively correlated with all displayed  
 333 diversity metrics and negatively correlated with all displayed agronomic variables. This means that  
 334 when a high number of diagnostic species was found in a given grassland, it indicated a high species  
 335 richness (N0), inverse Simpson diversity (N2), Pielou evenness (J1), functional diversity (RaoFD) and  
 336 phylogenetic diversity (RaoPD). At the opposite, when a low number of diagnostic species was found  
 337 in a given grassland, it indicated a high fertilization intensity through the use of farmyard manure  
 338 (Nman) and industrial fertilizers (Nind), and a high stocking rate during spring (cattle\_spring), autumn  
 339 (cattle\_autumn), and summer in a lesser extent (cattle\_summer).



340

341

342 Fig. 2: Principal component analysis of the Hellinger-transformed species composition of the 45  
 343 grasslands plots, with fitted diversity metrics (green arrows), agronomic variables (blue arrows) and  
 344 number of diagnostic species observed in the grassland plot (Diagsp, red arrow). Diversity metrics: N0  
 345 species Richness, N2 inverse Simpson Diversity, J1 Pielou evenness, RaoPD Rao phylogenetic  
 346 diversity, RaoFD Rao functional diversity. Agronomic variables: cattle\_spring stocking rate during  
 347 spring, cattle\_summer stocking rate during summer, cattle\_autumn stocking rate during autumn,  
 348 Nman available N input from farmyard manures, Nind available N input from industrial fertilizers. Sites  
 349 are represented by grey point whose size is proportional to elevation. Species are not shown.

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

359 Table 2: Spearman rank correlations between diversity metrics or agronomic variables and the number  
 360 of diagnostic species observed in the grassland plot.

		<i>rho</i>	<i>P</i>
<b>Diversity metrics</b>			
N0	Species richness	0.741	< 0.001 ***

N2	Inverse Simpson diversity	0.689	< <b>0.001</b> ***
J1	Pielou evenness	0.302	<b>0.044</b> *
RaoPD	Rao phylogenetic diversity	0.395	<b>0.007</b> **
RaoFD	Rao functional diversity	0.540	< <b>0.001</b> ***
<b>Agronomic variables</b>			
cattle_spring	stocking rate (LU·day·ha <sup>-1</sup> during spring)	- 0.521	< <b>0.001</b> ***
cattle_summer	stocking rate (LU·day·ha <sup>-1</sup> during summer)	- 0.168	0.269
cattle_autumn	stocking rate (LU·day·ha <sup>-1</sup> during autumn)	- 0.371	<b>0.012</b> *
Nman	Available N on the parcel from farmyard manures (kg N·ha <sup>-1</sup> ·yr <sup>-1</sup> )	- 0.328	<b>0.028</b> **
Nind	Available N on the parcel from industrial fertilizers (kg N·ha <sup>-1</sup> ·yr <sup>-1</sup> )	- 0.599	< <b>0.001</b> ***

361

### 362 3.3. Diagnostic species in comparison to randomly selected species

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

372 Table 3: Number of greater and lower Spearman rank correlations between diversity metrics or  
 373 agronomic variables and the number of species in 1000 random samples, as compared to the  
 374 correlations calculated using the set of eight diagnostic species.

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

375

## 376 4. Discussion

### 377 4.1. Establishment of the list of diagnostic species

378 In this study, we identified a set of eight diagnostic species of mesic pastures (*Cynosurion cristati*  
379 Tüxen 1947) based on 311 phytosociological relevés realized in eastern France. Our results revealed  
380 that the degree of joint fidelity between each diagnostic species and the group of relevés belonging to  
381 the alliance of mesic pastures was low. Indeed, the diagnostic species of pastures were sometimes  
382 found with low cover in relevés belonging to other alliances of mesic grasslands (Ferrez *et al.*, 2007).  
383 These results were not surprising, regarding the fact that it is unattainable to find species with absolute  
384 fidelity to a given vegetation type (Willner *et al.*, 2009). Moreover, we considered only the class of  
385 mesic grasslands (*Arrhenatheretea elatioris* Braun-Blanquet 1949) for the establishment of the list of  
386 diagnostic species. Thus, we ignored the affinity of our diagnostic species for vegetation units  
387 belonging to other classes, such as the dry grasslands (*Festuco valesiacaе-Brometea erecti* Braun-  
388 Blanquet & Tüxen ex Braun-Blanquet 1949). Consequently, the diagnostic species *Pimpinella*  
389 *saxifraga*, commonly found in dry grasslands, was not present in the 45 grassland plots that we  
390 analysed. In the study area, this species is present in lowland pastures whereas it occurs only in dry  
391 grasslands and fringes in mountain areas.

#### 392 4.2. Diagnostic species as biodiversity and management indicators

393 The number of diagnostic species present in a grassland revealed to be a good indicator of  
394 biodiversity and management practices, as almost all tested variables showed significant correlations  
395 with it. The combined presence of several diagnostic species of the *Cynosurion cristati* Tüxen 1947  
396 (mesic pastures) in a grassland belonging to this vegetation type was associated with a high plant  
397 taxonomic, functional and phylogenetic diversity and an extensive management (low stocking rate and  
398 low fertilization). These findings were predictable as an increase in the number of plant species is  
399 often associated with an increase of plant diversity (Cadotte *et al.*, 2011; Pavoine and Bonsall, 2011),  
400 and an extensive management in grassland is necessary to maintain plant diversity (Mašková *et al.*,  
401 2009; Wilson *et al.*, 2012; Mauchamp *et al.*, 2014).

402 The nitrogen input from industrial fertilizers showed to be significantly negatively correlated with the  
403 number of observed diagnostic species. Moreover, the correlation obtained for the diagnostic species  
404 was higher than correlations obtained for randomly selected species. These results have to be  
405 considered regarding the fact that industrial fertilizers are the most detrimental source of nutrients for  
406 plant diversity in mountain grasslands (Mauchamp *et al.*, 2016), because nutrients are directly  
407 available for plants (Cavalli *et al.*, 2014). Diagnostic species are able to react to modification of the  
408 environmental conditions of their habitat (Bazzichetto *et al.*, 2016). A study in coastal dunes  
409 ecosystems showed that diagnostic species can be indicators of soil conditions and provide useful  
410 indications for habitat monitoring, because they have narrow edaphic requirement (Angiolini *et al.*,  
411 2018). These previous results are similar to our observation concerning the nitrogen input from  
412 industrial fertilizers. Our results support the idea that diagnostic species are the first species to  
413 disappear when the nitrogen input increases through the use of industrial fertilizers, because they  
414 have narrow requirement concerning the nitrogen supply (Online Appendix A). In comparison to  
415 industrial fertilizers, the impact of the application of farmyard manures on mountain grassland plant  
416 diversity is less pronounced (Mauchamp *et al.*, 2016). Indeed, the organic forms of the nitrogen  
417 present in farmyard manure are not directly available for plants and requires a post-spreading  
418 mineralization by soil microorganisms (Kirkham *et al.*, 2008; Gaujour *et al.*, 2012). The fact that the  
419 impact of this type of fertilizer on plant diversity is less important compared to industrial fertilizers could  
420 explain why the response of diagnostic species was not different from the response of other grassland  
421 species. The number of diagnostic species present in a grassland showed to be negatively correlated  
422 with the stocking rate, particularly with the stocking rate in spring. This can be explained by the fact  
423 that an important stocking rate in spring is the most detrimental for plant diversity (Critchley *et al.*,  
424 2007). However, the response of the eight diagnostic species to the stocking rate was not different

425 from the response of randomly selected species. Diagnostic species of pastures are species that are  
426 well adapted to the disturbances induced by cattle activity. Thus, it is likely that diagnostic species of  
427 pastures do not react particularly to variations in stocking rate in comparison to other species.

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

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

453 Our list of diagnostic species was constituted of species from different lineages. Thus, the combined  
454 presence of many diagnostic species revealed that different species lineages were present in the plant  
455 community, which can explain the significant positive correlation obtained between the number of  
456 observed diagnostic species and phylogenetic diversity. Moreover, according to Villalobos and Vamosi  
457 (2016), the alteration in grassland plant community composition increases the prevalence of  
458 zygomorphic flower species lineages. Thus, a plant indicator list equilibrated in actinomorphic and  
459 zygomorphic lineages like our list of diagnostic species, consequently representative of a large  
460 phylogenetic diversity, reinforces the potential for the assessment of phylogenetic diversity. However,  
461 results obtained for phylogenetic diversity were not different between diagnostic species and randomly  
462 selected species. Indeed, it is likely that many random species subsets were also equilibrated in  
463 species from different lineages.

#### 464 4.3. Future direction for the use of diagnostic species in European grasslands

465 In this study, we have shown that we can use diagnostic species of the *Cynosurion cristati* to assess  
466 biodiversity and management practices in mesic pastures of the French Jura Mountains. Given the  
467 fact that it is easy to count for a number of diagnostic species in a local grassland, the use of  
468 diagnostic species to assess biodiversity and management practices in agricultural grasslands should  
469 be further investigated. In Europe, permanent grasslands are ecosystems whose floristic composition  
470 is strongly dependent on the agricultural practices used for their management (Královec *et al.*, 2009).

471 Moreover, permanent grasslands belonging to the alliance of the *Cynosurion cristati* Tüxen 1947 are  
472 widely represented across temperate Europe at low and mid-elevations (Mucina *et al.*, 2016).  
473 Consequently, we believe that the use of diagnostic species as biodiversity and management  
474 practices indicators in pastures of the French Jura Mountains belonging to the *Cynosurion cristati*  
475 Tüxen 1947 can be applied to other European grasslands belonging to this vegetation type. More  
476 globally, we believe that the methods that we used in the French Jura Mountains can be extended to  
477 other alliances of anthropogenic managed grasslands in Europe, because their high biodiversity is the  
478 result of a relatively similar long evolution through extensive management (Mašková *et al.*, 2009). In  
479 addition, the results of a recent study conducted in the Jura Mountains support the potential use of  
480 diagnostic species as biodiversity and management practices indicators in other habitat types. Indeed,  
481 in relation to land-use abandonment and nitrogen deposition in wetlands, Rion *et al.* (2018) reported a  
482 decrease of taxonomic diversity associated with a decrease in frequency and cover of diagnostic  
483 species. However, the fact that we only investigated the use of diagnostic species as biodiversity and  
484 management practices indicators in pastures for dairy cows have to be considered regarding the  
485 possible generalization of the results. Moreover, our results seem difficult to generalize to grassland  
486 ecosystems that are not managed, such as alpine grasslands, continental steppes or tallgrass prairie.

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

## 505 5. Conclusion

506 This study provides a first insight about the use of diagnostic species as biodiversity and management  
507 indicators. In the study area, the number of diagnostic species in a pasture revealed to be a good  
508 indicator of taxonomic and functional diversity of the plant community and of management practices,  
509 especially fertilization. As it is easy to count a number of diagnostic species in a grassland plot, a  
510 special attention should be given to diagnostic species to assess the status of grassland biodiversity,  
511 including functional aspects. However, future research is needed to show if the results of this study  
512 can be extended to others grassland types such as hayfields, dry grasslands or wet grasslands, and to  
513 other geographical areas.

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