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3

4 **Exploring the drivers of vascular plant richness at very fine spatial scale in sub-Mediterranean limestone**  
5 **grasslands (Central Apennines, Italy)**

6

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12

13 **Abstract**

14 Secondary dry grasslands in Europe can host high levels of vascular plant richness at small spatial scales.  
15 However, in Southern Europe their biodiversity patterns are largely unexplored. In this work, we aim at: *i)*  
16 estimating plant species richness patterns at very fine scales in montane dry grasslands, on limestone  
17 bedrock, in Abruzzo Lazio & Molise National Park (Central Apennines, Italy); *ii)* assessing the most  
18 important physical and edaphic drivers of biodiversity patterns at multiple plot sizes. We used randomly  
19 placed nested-plot series where we measured alpha-diversity at three different plot sizes (1 m<sup>2</sup>, 0.1 m<sup>2</sup> and  
20 0.01 m<sup>2</sup>) and within-plot beta-diversity (as expressed by the slope of the species-area curve across plot  
21 sizes). Variable selection was performed by means of Random Forests. Relationships between selected  
22 variables and diversity measures were then assessed using Regression Trees, Linear and Generalized Linear  
23 Models. Overall, results pointed to topographically-controlled edaphic factors (soil pH and silt fraction) as  
24 the main drivers positively influencing alpha-diversity at all spatial scales, with a positive effect of rock  
25 cover and slope inclination at smaller spatial grains. Beta-diversity was positively influenced by rock cover.  
26 We suggest that high-pH, steep and/or rocky sites feature higher species richness because they lack

27 competitive grass species. Our results are in agreement with previous works underlining the importance of  
28 less productive habitats for the conservation of secondary grassland biodiversity.

29

### 30 **Keywords**

31 Dry grasslands, Diversity metrics, Multi-scale approach, Soil properties, Species–area relationship,  
32 Topographical gradients

33

### 34 **Introduction**

35 Fine-scale species richness (species density or alpha-diversity) of vascular plants, i.e. the number of species  
36 occurring in fixed-area plots of small size (usually  $\leq 100 \text{ m}^2$ ), and the quest for its environmental  
37 determinants have been the subject of important studies and considerable discussion by plant ecologists  
38 (reviewed e.g. by Grace 1999). A considerable number of such works focused on European secondary  
39 grasslands (e.g. Grime 1973; Al-Mufti et al. 1977; van der Maarel and Sykes, 1993; Pärtel and Zobel 1999;  
40 Bennie et al. 2006; Löbel et al. 2006; Turtureanu et al. 2014; Reitalu et al. 2014; Palpurina et al. 2015; Güler  
41 et al. 2016). These are semi-natural habitats originated and maintained by disturbance such as livestock  
42 grazing, in areas that would be otherwise covered by forest vegetation; however, they are not man-made  
43 ecosystems, as livestock disturbance replaces the grazing and browsing by wild prehistoric megaherbivores  
44 and by extant ungulates (Pärtel et al. 2005; Feurdean et al. 2018). European secondary grasslands, and  
45 especially “dry” grasslands (i.e. those growing on free-draining substrates such as limestone), are of  
46 outstanding ecological value since they are among the most diverse plant communities in the world at plot  
47 sizes  $<100 \text{ m}^2$  (Pärtel et al. 2005; Wilson et al. 2012; Chytrý et al. 2015; Roleček et al. 2014). Moreover, they  
48 are an essential part of the cultural landscape of the continent, often resulting from millennia of low-  
49 intensity land-use (Poschlod and Wallis DeVries, 2002; Dengler et al., 2014; Feurdean et al. 2018); they host  
50 a high proportion of Europe’s endemic plants (Habel et al. 2013); and act as a network of “interglacial  
51 refugia” for Pleistocene (ice-age) steppic species (Hájková et al. 2011; Kajtoch et al. 2016; Cancellieri et al.  
52 2017b). European secondary grasslands are currently threatened by grazing abandonment, conversion into  
53 cropland, afforestation and transformation into “improved” grasslands (Dolek and Geyer 2002; Habel et al.

54 2013; Dengler et al. 2014; Burrascano et al. 2016; Feurdean et al. 2018). For these reasons, some types of  
55 secondary grasslands are the subject of protection schemes at national (e.g. Kleijn and Sutherland 2003;  
56 Knop et al. 2006) and EU level (European Union, 1992).

57 In this framework, understanding the relationships between variability in species richness and  
58 environmental gradients is essential in order to develop management strategies in semi-natural habitats  
59 and limit biodiversity losses (Pärtel et al. 2005; Palpurina et al. 2015). However, such relationships are still  
60 not well known (Habel et al. 2013; Dengler et al. 2014; Palpurina et al. 2015), and different studies often  
61 found different strength and sign of a given environmental factor. In some instances, this can be due to  
62 non-linear relationships, as demonstrated by Chytrý et al. (2007) and Palpurina et al. (2017) for pH and by  
63 Fraser et al. (2015) for productivity. At the same time, scale-related issues may frequently explain the wide  
64 range of richness-environment relationships reported in the literature for semi-natural grasslands (Auestad  
65 et al. 2008): the effect and importance of any environmental factor can be different between very fine  
66 scales (e.g.  $\leq 1 \text{ m}^2$ ) and larger plot sizes (e.g.  $100 \text{ m}^2$ ), or between alpha- and beta-diversity (de Bello et al.  
67 2007; Auestad et al. 2008; Polyakova et al. 2016). Indeed, biodiversity has many different facets that carry  
68 complementary information, so it is important to take into account different diversity metrics, or several  
69 spatial scales of the same measurement (e.g. Chiarucci et al. 2011; Pavoine and Bonsall 2011; Dengler et al.  
70 2014). Moreover, from many geographic regions even descriptive information on grassland biodiversity  
71 patterns, such as the mean species richness or the most frequent taxa, are still lacking - especially for very  
72 fine spatial scales such as  $1 \text{ m}^2$  or smaller (Dengler 2009). This is even more so in Southern European  
73 landscapes, as they are less studied than their Central European counterparts (Apostolova et al. 2014).

74 Although richness patterns at very fine spatial scales ( $\leq 1 \text{ m}^2$ ) have to be collected and interpreted with  
75 caution because of many potential pitfalls (Gotelli and Colwell 2001; Palmer et al. 2008), these are the  
76 scales at which – in herbaceous vegetation – plant individuals interact with each other and build up  
77 ecosystem functioning and services (Grime 1998; Wellstein et al. 2014; Borer et al. 2014). In addition, using  
78 plots  $\leq 1 \text{ m}^2$  ensures that the sampling units are small in relation to the spatial scale of variation in  
79 topography and soil heterogeneity (Grime 1973).

80 In the Italian Peninsula, extensive surveys of dry grassland biodiversity patterns and their edaphic drivers at  
81 spatial grains  $\leq 1 \text{ m}^2$  are lacking: the only paper we are aware of, that analyzed richness patterns and  
82 edaphic variables at fine scales (Maccherini 2006), was focused on a very small study area (cf. also  
83 Wellstein et al. 2014; Chelli et al. 2017). Nevertheless, the large areas of secondary grasslands in the  
84 Apennines chain (the mountain range forming the backbone of the Italian peninsula) are extremely  
85 interesting, because of considerable climatic and edaphic gradients (Primi et al. 2016), biogeographical  
86 position (e.g. Rosati et al. 2010; Blasi et al. 2012), conservation values (e.g. Manzi 2012; Primi et al. 2016;  
87 Cancellieri et al. 2017b) and a long history of sheep grazing and transhumance (Barker et al. 1991; Brown et  
88 al. 2013).

89 The present paper thus aims at analysing fine-scale diversity patterns and drivers from a network of  
90 secondary dry grasslands within the Central Apennine Mountains. We focused on the karst plateaux of the  
91 upper-montane bioclimatic belt: these habitats are characterized by a high topographical heterogeneity at  
92 micro- and meso-scale, thus featuring steep gradients of the potential controlling variables for species  
93 richness. We used randomized nested plots of three different sizes (0.01-0.1-1  $\text{m}^2$ ) to answer the following  
94 questions: *i*) what are the levels and patterns of very fine-scale species richness at multiple spatial grains  
95 (alpha-diversity)? *ii*) what are the patterns of spatial turnover of species across the considered spatial grains  
96 (within-plot beta-diversity)? *iii*) what are the main environmental drivers of these diversity metrics, and do  
97 they vary between spatial scales?

98

## 99 **Methods**

### 100 **Study area**

101 Data were gathered within the “Abruzzo Lazio e Molise” National Park, in the Central Apennine mountains,  
102 Italy (41°48’N, 13°50’E). This protected area was designated in 1922 because it was the last refuge for both  
103 the Marsican brown bear (*Ursus arctos* ssp. *marsicanus*) and the Apennine chamois (*Rupicapra pyrenaica*  
104 ssp. *ornata*); at the same time, this area was until the 1950’s one of the most important centers in Italy for  
105 transhumant shepherding (Sievert 1999). The Park’s flora comprises >2,000 species, including >30 taxa  
106 endemic to the Central Apennine mountains (Conti and Bartolucci 2015); most of the Park’s grasslands

107 correspond to ecosystem types protected under the EU Habitats Directive (Primi et al. 2016). Five patches  
108 of secondary dry grassland were chosen as study sites, for a total area of c. 500 ha (**Fig. 1**; additional  
109 information are given in **Online Resource 1**). Their elevation ranges between c. 1350-1750 m a.s.l. and they  
110 thus belong to the upper-montane phytoclimatic belt of the Apennines (Gerdol et al. 2008). All the studied  
111 grassland patches are very similar: they lie on Mesozoic limestone bedrock (Bigi et al. 1986) and are located  
112 in large bowl-shaped depressions, hosting in turn a system of different smaller landforms (see photos in  
113 **Online Resource 1**) originated by complex interactions between karst, tectonic and glacial processes (Bigi et  
114 al., 1986; Galadini and Messina 1990; Cinque et al. 1990). Such micro- and meso-scale landforms include  
115 shallow dolines, sinkholes, hillocks, rocky outcrops, karren, etc. Because of karst hydrology, no surface  
116 water bodies occur. The grassland patches are surrounded by beech (*Fagus sylvatica*) forest, which is  
117 considered the potential vegetation of the area (Filibeck et al. 2015). Within the considered elevation belt,  
118 dominant species in the Park's grasslands include *Avenula praetutiana*, *Bromus erectus*, *Festuca*  
119 *circummediterranea*, *Koeleria lobata* and *Phleum ambiguum* on shallow to medium-depth soils; while on  
120 deep soils (e.g. in karst hollows) *Agrostis capillaris*, *Festuca microphylla* and *Nardus stricta* often prevail  
121 (Bruno and Bazzichelli, 1966). No phytosociological studies targeted the study sites; however, relatively  
122 similar habitats in neighbouring areas were described by Lucchese et al. (1995), Blasi et al. (1998) and Di  
123 Pietro et al. (2005).

124 The local climate can be defined as sub-Mediterranean (Filibeck et al., 2015): it features high annual  
125 precipitation (c. 1,200-1,500 mm/yr), with a rainfall maximum in autumn and a very sharp drop in summer.  
126 This precipitation regime leads to significant drought stress, especially for grassland plants, during July and  
127 August (Catorci et al. 2012; Primi et al. 2016). Average annual temperature is between 6-8 °C; winters are  
128 characterized by marked frost and abundant snowfall (see Filibeck et al. 2015, for climate diagrams and  
129 details).

130 In the study area, secondary grasslands are maintained by grazing. Transhumant sheep grazing was widely  
131 practiced until the 1950's, when ovine farming started to decrease for socio-economic reasons; sheep  
132 stocking rate is nowadays drastically lower than in the early twentieth century (Manzi 2012). The decrease  
133 of sheep husbandry has resulted in a steep increase in cattle and horse farming; such animals are usually

134 raised for meat production only and left free-ranging night and day in the wild (Primi et al. 2016). Because  
135 of summer drought, mowing has never been a large-scale management system in the region (Manzi 1990),  
136 and is currently not practiced at any of our study sites.

137

### 138 **Data collection**

139 Data were collected in early summer in 2013 and 2014. Sampling design was based on a grid of 300 x 300 m  
140 cells, overlaid on the National Park area, with one random point selected within each cell (tessellation  
141 stratified sampling: see e.g. Barabesi et al. 2012; Chiarucci et al. 2012). The five grassland clearings to be  
142 investigated were chosen *a priori*, following field reconnaissance and inspection of detailed geological maps  
143 that showed they had comparable features. All the random points falling within the five selected grassland  
144 patches were retained, resulting in a total of 50 points (**Fig. 1**). At each GPS-located point (hereafter  
145 “sampling point”), vascular plants were surveyed using a nested-plot series based on the design proposed  
146 by Dengler (2009), as modified by Cancellieri et al. (2017a): one 1 m<sup>2</sup> squared plot was centred on the  
147 random point, and oriented N-S; two 0.1 m<sup>2</sup> subplots were placed at the NE and SW corners of the 1 m<sup>2</sup>  
148 plot, and two 0.01 m<sup>2</sup> subplots were placed within each of the 0.1 m<sup>2</sup> plots (i.e., there are four 0.01 m<sup>2</sup>  
149 subplots at each sampling point) (**Fig. 2** and **Online Resource 1**). The rationale for sampling a higher  
150 number of subplots of smaller areas within each nested-plot series is twofold (Dengler 2009): i) it allows to  
151 better account for the micro-environmental heterogeneity contained within the largest plot; ii) it allows the  
152 use of mean species richness values within each nested series, thus liberating the resulting species-area  
153 curve from stochasticity. Moreover, while species counts often do not follow a normal distribution,  
154 averaging alpha-diversity values among sub-plots leads to normally distributed data (e.g. Quinn and Keough  
155 2002).

156 Each nested-plot series was precisely delimited in the field by means of a plastic frame, and was surveyed  
157 by at least two people to reduce inter-observer variability (e.g. Morrison 2015). For each plot and subplot,  
158 we listed all vascular plant species (presence/absence data only), following the “shoot presence” criterion:  
159 that is, a species is recorded as present if the vertical projection of any above-ground part of an individual  
160 falls within the plot (Cancellieri et al. 2017a). Plant specimens that could not be identified in the field were

161 collected and determined in the laboratory. Identification was mostly based on Pignatti (1982); plant names  
162 were subsequently standardized following Conti et al. (2005). Given the still poorly-known taxonomy and  
163 distribution of the genus *Festuca* in Italy, and considering that this taxon is one of the main builders of the  
164 local plant communities, all *Festuca* “species” within each nested-plot series were collected for  
165 identification in the laboratory through leaf section, following Foggi and Rossi (1996) and Foggi et al.  
166 (1999).

167 For each nested-plot series, the following environmental variables were measured at 1-m<sup>2</sup> spatial scale  
168 (**Tab. 1**). *Elevation* was measured with a GPS receiver. *Slope inclination* was measured by a clinometer.  
169 Slope aspect was measured with a compass and used to calculate *heat-load index* (based on latitude,  
170 aspect and inclination) following eq. 2 in McCune and Keon (2002). *Soil depth* was measured at 5 random  
171 points with a 65-cm long iron rod (soils deeper than rod length were arbitrarily given a value of 65 cm); the  
172 median of the 5 measurements was used in the analyses. Percentage of *stone and rock cover* was visually  
173 estimated. *Land-form position* was visually assessed as a categorical variable as follows: “hollow”  
174 (indicating the flat or gently sloping position in concave morphologies such as the bottom of dolines, other  
175 karst depressions or gullies), “hillside” (for the sloping sides of both meso- and macro-scale landforms), and  
176 “hump” (for convex landforms such as hillocks, ridges or rocky outcrops). Soil samples were collected with a  
177 hand auger (Eijkelkamp, NL) from the upper 10-cm layer as a mixed sample from 3 randomly placed points  
178 within each 1 m<sup>2</sup> plot, and subsequently air-dried and crushed to pass a 2-mm sieve. *Soil pH* was measured  
179 in water (soil-solution suspension of 1:2.5). Soil texture (*clay*, *silt* and *sand* fraction) was determined  
180 through the standard sedimentation techniques (Gee and Bauder 1986) adopting the ISSS dimensional  
181 limits, without pre-treatment with H<sub>2</sub>O<sub>2</sub>. *Soil organic matter* was determined using Walkley-Black method.  
182 The plot data are available in GrassPlot, the Database of Scale-Dependent Phytodiversity Patterns in  
183 Palaeartic Grasslands (GIVD ID EU-00-003) (Dengler et al. 2018).

184

### 185 **Data analysis**

186 For each nested-plot series, the following diversity metrics were calculated from the collected data, to be  
187 used as response variables in the statistical analysis: species richness of the whole 1 m<sup>2</sup> plot (hereafter, “1

188  $m^2$  alpha-diversity"); mean species richness of the two 0.1  $m^2$  subplots (hereafter, "0.1  $m^2$  alpha-diversity");  
189 mean species richness of the four 0.01 $m^2$  subplots (hereafter, "0.01 $m^2$  alpha-diversity"); and "z-value", i.e.  
190 the slope of the species-area relationship calculated from the three spatial scales at each nested-plot  
191 series.

192 We assumed for the species-area curve the Arrhenius (1921) power function:

$$193 \quad S = c A^z$$

194 and we calculated the z-values with linear regression in double-log space (e.g. Chiarucci et al. 2006), i.e.  
195 using the equation:

$$196 \quad \log S = \log c + z \log A$$

197 (where S = number of species, A = plot size; c and z are the estimated parameters, i.e. the intercept at A=1  
198 and the slope of the regression line, respectively).

199 Fitting of the species-area function was performed for each nested-plot series, using for the 0.1  $m^2$  size the  
200 mean richness values of the two subplots, and for the 0.01  $m^2$  size the mean richness values of the four  
201 subplots. The z-values of nested-plot series are useful for comparing the degree of spatial species turnover  
202 between different ecological situations (e.g. Chiarucci et al. 2006; de Bello et al. 2007; Dengler 2008;  
203 Turtureanu et al. 2014; Polyakova et al. 2016). They are a measure of beta-diversity (i.e. of the variation in  
204 the identities of species between plots) at fine spatial scale, as the shape of the species area-curve reflects  
205 the level of spatial homogeneity of species distribution and community composition (Storch 2016).

206 Collinearity between the predictor variables, as well as the strength of the univariate relationships between  
207 each predictor variable and the diversity metrics, was preliminarily checked using pairwise Spearman  
208 correlations. We then excluded the percentage of sand from further analyses because by definition the  
209 three soil texture fractions sum up to 100 and, of the three texture components, sand exhibited the highest  
210 degree of correlation with other predictor variables. Since the study area had been deliberately defined  
211 within a specific elevation belt, and the range of altitudinal variation among plots was thus small, elevation  
212 was not included in the analyses. One sampling point presented outlying values from soil analysis, leading  
213 to suspect an error occurred in laboratory processing, and was excluded from statistical analysis on  
214 diversity-environment relationships, that are thus based on  $n=49$ .

215 We performed our main statistical analysis through Recursive Partitioning approaches (“Regression Trees”  
216 and “Random Forests”: Strobl et al. 2009a). Recursive Partitioning methods have many desirable properties  
217 that make them attractive for the ecologist (De'ath and Fabricius 2000): they can successfully handle very  
218 high number of predictors, are robust to multicollinearity, independent from any assumptions on the  
219 distribution of errors and can successfully handle and reveal complex interactions and non-linear  
220 relationships (Sandri and Zuccolotto 2006; Hapfelmeier and Ulm 2013).

221 As a first step, in order to identify the most relevant explanatory variables we used Random Forests (i.e. an  
222 ensemble method combining several Classification or Regression Trees), which several studies highlighted  
223 as an effective variable selection approach (Sandri and Zuccolotto 2006; Hapfelmeier and Ulm 2013; Svitok  
224 et al. 2016). Random Forests were run using R package “party” (function “cforest”: Hothorn et al., 2006b;  
225 Strobl et al. 2007; Strobl et al. 2008), as it implements conditional inference trees which are designed to  
226 reduce the bias that such tools otherwise have towards highly correlated variables (Strobl et al. 2008,  
227 2009b). The number of “trees” in the “forests” was set to 1000, while the number of randomly preselected  
228 predictor variables for each split was set to 3, following Strobl et al. (2009a). As Random Forests are, by  
229 definition, “random”, repeating the process (i.e. re-running the algorithm) is recommended in order to  
230 ensure the robustness and stability of the model (Strobl et al. 2009a; Shih 2011). For this reason, for each of  
231 the four response variables a Random Forest was run 10 times. For each Random Forest, a conditional  
232 measure of variable importance was computed for all predictors using function “varimp”. We defined as  
233 “important” and retained for further analysis only those environmental variables which, in at least six out  
234 of the 10 Random Forest tries, featured positive values exceeding the absolute value of the most negative  
235 score (cf. Strobl et al. 2009a).

236 As a second step of analysis, individual Regression Trees were built for each response variable, including as  
237 predictors those environmental variables identified by the Random Forests as important for each  
238 biodiversity measure. Regression Trees have recently proven to be very effective in studying plant diversity  
239 drivers (e.g. Chytrý et al. 2007; Palpurina et al. 2015; Svitok et al. 2016). We used them to identify the  
240 environmental characteristics most strongly associated with each response variable, assess the sign of the  
241 relationship, detect interactions among predictors and estimate the environmental thresholds for species

242 richness (De'ath and Fabricius 2000; Strobl et al. 2009a). We performed Regression Tree analysis with  
243 package “party” in R (function “ctree”, Hothorn et al. 2006a), implementing “Conditional Inference” Trees:  
244 these were preferred to the more classical approach, based on the “CART” algorithm, as the latter may be  
245 biased towards continuous predictors measured over large scales or categorical predictors with many  
246 categories (Hothorn et al. 2006a; Strobl et al. 2009a). Besides, “Conditional Inference” Trees, implementing  
247 a statistically robust stopping criterion, do not suffer from overfitting (Strobl et al. 2009a). The algorithm  
248 tests the global null hypothesis of independence between any of the input variables and the response; it  
249 stops if this hypothesis cannot be rejected, otherwise selects the input variable with strongest association  
250 to the response. This association is measured by a p-value corresponding to a test for the partial null  
251 hypothesis of a single input variable and the response. The stop criterion is by default based on multiplicity  
252 adjusted p-values (Bonferroni correction), but univariate p-values can be alternatively used. This approach  
253 ensures that the right sized “tree” is grown and no “pruning” or cross-validation is needed (Hothorn et al.  
254 2006a).

255 Additionally, we assessed the strength of the relationship between the response variables and the selected  
256 predictors through Generalized Linear Models (GLM) and multiple Linear Models. Whenever two predictors  
257 (among those selected through the Random Forest output) were highly correlated with each other (i.e. had  
258 a Spearman correlation coefficient > 0.6), we retained only the most biologically informative one. Since the  
259 explanatory variables are measured in different units, standardized regression coefficients (obtained by  
260 subtracting, from each observation, the variable mean and dividing by the standard deviation: e.g. Quinn  
261 and Keough 2002) were computed to allow comparison of the effect sizes on the response variable ; for  
262 Linear Models we standardized both explanatory and response variables, while for Generalized Linear  
263 Models we only transformed predictors. For 1 m<sup>2</sup> alpha-diversity, a Poisson Generalized Linear Model was  
264 fitted, setting “log” as link function. Overdispersion was tested using R package “AER” (function  
265 “dispersiontest”: Kleiber and Zeileis 2008). We used an adjusted Pseudo-R<sup>2</sup> (Guisan and Zimmermann 2000)  
266 to quantify the improvement in the deviance explained by the model including the selected predictors over  
267 the intercept-only model (R package “modEvA”, function “Dsquared”: Barbosa et al. 2016). For 0.1 m<sup>2</sup> and  
268 0.01 m<sup>2</sup> alpha-diversity, as they were obtained averaging the species counts among subplots of the same

269 size within each nested series, we assumed a Gaussian distribution of errors: thus multiple Linear Models  
270 were fitted and adjusted-R<sup>2</sup> was used to quantify explained variability. Z-values were log-transformed  
271 before model fitting in order to meet linear-model assumptions.

272 Finally, to help ecological interpretation of the richness patterns and elucidate their relationship with  
273 floristic composition, a PCA (based on a correlation matrix) of the plot × species matrix was run, adding the  
274 1 m<sup>2</sup> alpha-diversity as a covariate, in order to underline which taxa were associated with different levels of  
275 species richness.

276 Note that in the results section we shall use p-values as a continuous measure of fit of the null hypothesis  
277 to the data, instead of using them for a “significant/non-significant” dichotomy (Wassertein and Lazar 2016;  
278 Greenland et al. 2016).

279

## 280 **Results**

### 281 ***Overall diversity***

282 Across all 50 sampling points, a total of 198 vascular plant species were recorded; 64 species (32% of the  
283 total recorded flora) were found at only one nested series (singletons). Mean species richness per plot was  
284 10.3, 18.5 and 28.0 for 0.01 m<sup>2</sup>, 0.1 m<sup>2</sup> and 1 m<sup>2</sup>, respectively (**Tab.2**). The mean slope (z) of the species-  
285 area relationship was 0.238 (**Tab.2**); i.e., richness increased by c. 1.7 times, on average, with a ten-fold  
286 increase in plot area. The most frequent species (in terms of proportion of sampling plots where the taxon  
287 was recorded) are listed in **Tab. 3**. The full 1m<sup>2</sup>-plot × species matrix (including the frequency values of all  
288 species) is provided as **Online Resource 3**.

289

### 290 ***Diversity-environment relationships***

291 Descriptive statistics of the environmental variables are shown in **Tab.1**. Pairwise Spearman’s correlation  
292 matrix (**Tab.4**) highlighted that many predictor variables were correlated with each other: most notably,  
293 soil pH was positively correlated with stone and rock cover ( $r_s=0.64$ ) and with slope inclination ( $r_s=0.48$ ),  
294 and negatively correlated with soil depth ( $r_s= -0.61$ ); stone and rock cover was positively related to slope  
295 inclination ( $r_s=0.54$ ). As for the relationships between predictor and dependent variables, species richness

296 at 1 m<sup>2</sup> spatial scale (1 m<sup>2</sup> alpha-diversity) was positively correlated with silt % ( $r_s=0.38$ ), soil pH ( $r_s=0.37$ )  
297 and stone and rock cover ( $r_s=0.30$ ). However, all these relationships became weaker moving to 0.1 and 0.01  
298 m<sup>2</sup> spatial scales. The z-values were positively correlated with stone and rock cover ( $r_s=0.58$ ); in addition,  
299 they showed a positive correlation with slope inclination ( $r_s=0.37$ ) and a negative relationship with soil  
300 depth ( $r_s=-0.48$ ).

301 In **Tab. 5** it is reported, for each of the four response variables, how many times, out of the 10 Random  
302 Forest tries, each predictor variable turned out to be significant. Both soil pH and silt % were found to be  
303 significant predictors across all 10 runs for all three alpha-diversity spatial scales. Stone and rock cover and  
304 slope inclination were stable outcomes for 1 m<sup>2</sup> and 0.1 m<sup>2</sup>; soil depth and land-form position were  
305 constantly significant only at 1 m<sup>2</sup> spatial scale. As for z-values, soil pH was never a significant predictor,  
306 while land form, stone and rock cover, inclination and soil depth were significant in all runs. Heat-load  
307 index was never significant for any of the dependent variables.

308 The Regression Tree for 1 m<sup>2</sup> alpha-diversity identified a significant threshold value for pH at 6.4, i.e. the  
309 plots with soil pH smaller than this value hosted, on average, significantly lower richness. The model further  
310 partitioned the group of high-pH sites based on soil texture, showing that the maximum species richness is  
311 attained at sites featuring simultaneously high pH values and high silt % (**fig. 3**). However, as shown above,  
312 within the study area soil pH is positively correlated with slope inclination and stone and rock cover, and  
313 negatively correlated with soil depth; thus, high pH values can also indicate steep slopes or rocky humps  
314 with shallow soil.

315 For 0.1 m<sup>2</sup> and 0.01 m<sup>2</sup> alpha-diversity, Regression Tree analysis performed with “ctree” default rules didn’t  
316 yield any statistically significant partition. If univariate p-values were used (see Methods) instead of the  
317 stop criterion based on Bonferroni correction (cf. Nakagawa 2004), then the resulting tree for the 0.1-m<sup>2</sup>  
318 sub-plots highlighted the role of slope inclination and soil pH (and their interaction). Steeper slopes  
319 (indicating also stony soils, as explained above) showed greater species richness at 0.1 m<sup>2</sup> spatial scale. The  
320 lowest richness was found when a low slope inclination was combined with a pH value lower than 6.2,  
321 indicating flat bottoms of concave landforms with deep, sub-acid soils (**fig. 4**). As for the 0.01 m<sup>2</sup> alpha-  
322 diversity, no significant partitions were detected even after the Bonferroni correction was removed.

323 In the Regression Tree for z-values, stone and rock cover was identified as a strongly significant predictor,  
324 with two subsequent partitions based on the same variable (**fig. 5**), thus underlining a regular increase of  
325 the slope of the species-area curve with increasing soil rockiness, and showing that low z-values were  
326 consistently found when stone and rock cover was less than 3%.

327 For the 1 m<sup>2</sup> alpha-diversity GLM, the predictor variables selected on the basis of Random Forest output  
328 were soil pH, land-form, silt %, slope inclination, soil depth and stone and rock cover. However, as both soil  
329 depth and stone and rock cover were highly correlated with soil pH (Spearman's  $r_s > 0.6$ ), they were not  
330 included in the model. The inclusion of pH as quadratic term (based on a preliminary graphical exploration,  
331 that showed a slightly hump-shaped trend) did not improve significantly the fit of the models as checked  
332 via likelihood ratio test for the GLM and ANOVA for the Linear Models; therefore we retained only the first-  
333 order term for pH in the final models. GLM results (**Tab. 6**) showed that 1 m<sup>2</sup> alpha-diversity was positively  
334 associated with silt % (standardized coefficient=0.13,  $p < 0.001$ ), and was on average higher in "hump" land-  
335 forms than in hollows and hillsides. Slope inclination and pH were positively, but very weakly, associated  
336 with 1 m<sup>2</sup> alpha-diversity (stand. coeff.=0.05,  $p = 0.1$ , for both predictors); however, as soil pH was markedly  
337 correlated with two omitted variables, its parameter estimate may be biased. The adjusted Pseudo-R<sup>2</sup> of  
338 the model was 0.53 (i.e. the predictors explained 53% of the deviance in 1 m<sup>2</sup> alpha-diversity).

339 As for 0.1 m<sup>2</sup> alpha-diversity, the predictor variables selected for fitting the Linear Model were: soil pH,  
340 slope inclination, silt %, clay % and stone and rock cover. As stone and rock cover was highly correlated  
341 with soil pH ( $r_s > 0.6$ ), it was removed from the model. Silt fraction showed a positive relationship with 0.1  
342 m<sup>2</sup> alpha-diversity (stand. coeff.=0.47,  $p < 0.001$ ); a smaller positive effect, with a high error, was showed by  
343 slope inclination (stand. coeff.=0.23,  $p = 0.1$ ) and soil pH (stand. coeff.=0.22,  $p = 0.1$ ); for this latter, the same  
344 caveats hold as in the 1m<sup>2</sup> model (**Tab. 6**). The model explained 31% of variability.

345 For 0.01 m<sup>2</sup> alpha-diversity, variable selection based on Random Forests led to include only silt % and soil  
346 pH. Species richness at this spatial scale showed a positive relationship with silt % (stand. coeff.=0.37;  
347  $p < 0.01$ ); a smaller positive effect was showed also by soil pH, but with a high standard error and a large p-  
348 value (stand. coeff.=0.18;  $p = 0.2$ ) (**Tab. 6**). The model explained only 13% of variability.

349 Finally, z-values were modelled as a function of land-form position, stone and rock cover, slope inclination,  
350 and soil depth. Model results (**Tab. 6**) showed a strong positive effect (stand. coeff.=0.63, p<0.001) of stone  
351 and rock cover. The model explained 47% of variability.

352 In the PCA of the 1-m<sup>2</sup> plots × species matrix, species richness was correlated with PC2 (loading = -0.57).  
353 The correlation coefficients of the species on this factor, allowing to highlight the taxa that were  
354 consistently associated with either the richest or the poorest plots, are listed in **Online Resource 2**.

355

## 356 **Discussion**

### 357 **Overall diversity**

358 Especially at the finest spatial scales, mean and maximum species richness per plot ranked quite high within  
359 the context of Palaearctic grasslands, supporting earlier hypothesis that S-European grasslands can host  
360 high levels of alpha-diversity (Apostolova et al. 2014): for instance, our maximum of 20 vascular plant  
361 species in 0.01 m<sup>2</sup> quadrats is the second highest among all the Eurasian datasets reported by Dengler et al.  
362 (2016) (cf. also Chytrý et al. 2015). At 1 m<sup>2</sup> scale, the maximum richness value (41 species) was not  
363 exceptionally high (cf. Merunková et al. 2012; Wilson et al. 2012), although still within the “upper range” at  
364 European scale (Chytrý et al. 2015; Dengler et al. 2016). However, our data stem from a randomized  
365 sampling design, while most datasets that contain or approach the European “richness records” originate  
366 from plots that were placed subjectively with the aim to record the most species-rich sites (e.g. Merunková  
367 et al. 2012). Beta-diversity as expressed by z-values was within the range typically reported for dry  
368 grasslands in Europe, and our mean z-value (0.24) was very close to what found in many previous studies  
369 (see e.g. Baumann et al. 2016, and references therein).

370 The probabilistic sampling used in this study allowed to quantitatively define (Chiarucci 2007) the most  
371 common species within the landscapes studied (**Tab. 3**). Among grasses, the most frequent taxon was  
372 *Festuca circummediterranea*, followed by *Poa alpina* and *Koeleria lobata*, pointing to an interesting co-  
373 dominance of Mediterranean and Boreal geographic elements. Similarly, among forbs, the Apennine  
374 endemic *Viola eugeniae* turned out to be present in 2/3 of the samples, but the most common species was  
375 *Hieracium pilosella* (found in 80% of the quadrats), a typical example of the European geographic element.

376

377 ***Diversity-environment relationship: alpha-diversity***

378 Soil pH and silt fraction were positively correlated with species richness at all spatial scales. Slope  
379 inclination, stone and rock cover and “hump” land-form were positively influencing species richness as well,  
380 but only at 1 m<sup>2</sup> and/or 0.1 m<sup>2</sup> spatial grains, and with weaker effect sizes.

381 There is a huge body of works on the positive (or unimodal) relationship between soil pH and plot-scale  
382 diversity in Eurasian grasslands (see references in: Chytrý et al. 2007; Palpurina et al. 2017), although in  
383 most studies the quadrat size was larger (10-100 m<sup>2</sup>) than in the present work (but see Grime 1973). This  
384 relationship has been explained assuming that during the glacial phases the acidophilous pool of species  
385 underwent a much higher extinction rate than the acidifuge contingent because of the unbalanced  
386 distribution of soil types across the ice-age biomes), thus leading to a smaller pool of acidophilous than  
387 calcicolous species in the present-day regional floras (Grime 1973; Grace 1999; Ewald 2003; Tyler 2003;  
388 Chytrý et al. 2003).

389 However, since many of our environmental variables showed correlated gradients in the field (cf. Bennie et  
390 al. 2006; Baumann et al. 2016), it is difficult to separate the role of pH per se from that of other  
391 environmental variables. Sites with steep and rocky or shallow soils have necessarily high pH values  
392 because of the surfacing limestone material (e.g. Palpurina et al. 2017); on the other hand, because of the  
393 widespread calcareous bedrocks, in our study area low pH values are usually confined to concave land-  
394 forms (hollows, dolines, bases of slopes), in turn typically associated with deep soils, low rock cover and low  
395 slope inclination. Such land-forms can host acid soils, in spite of the base-rich bedrock, mainly because they  
396 accumulate clayey and decarbonated soil through colluvial deposition and karstic processes (e.g. Calandra  
397 1999); in addition, they may trap huge amounts of wind-blown siliceous materials such as Saharan quartz  
398 silt and volcanic ashes (Giraudi 1995; Sauro 2004), and/or host a thick snow cover, favoring podzolization  
399 (Hiller et al. 2005). At the same time, dolines and hollows will receive more water and nutrients than  
400 convex land-forms or steep slopes (Araya et al. 2011; Batori et al. 2017), host soil types with higher clay  
401 fraction and higher available water content (Di Pietro et al. 2005), and undergo lower levels of natural  
402 disturbance at micro-scale via summer drought, water run-off and erosion (Bennie et al. 2006).

403 A large number of studies found that grassland species richness in Europe and N-America was reduced on  
404 soils with high moisture and nutrients, while attained maximum values at sites with relatively dry, nutrient-  
405 poor and shallow soils (reviews by Grace 1999 and by Keddy 2005; see also Bennie et al. 2006; Bonanomi et  
406 al. 2006; Maccherini 2006; Moeslund et al. 2013b; Baumann et al. 2016; Filibeck et al. 2016; Kleinebecker et  
407 al. 2018; Palpurina et al. 2019). Even in a different floristic kingdom, Cantero et al. (2003) found that in  
408 mountain grasslands in Argentina, species richness of 1-m<sup>2</sup> quadrats had a negative relationship with soil  
409 depth and available water, and a positive correlation with stone cover. This is consistent with the classical  
410 model of humped-back relationship between productivity and richness, usually explained assuming that in  
411 very productive sites a few strong competitors will create closed canopies and accumulate litter, reducing  
412 the amount of light reaching soil surface and outcompeting stress-tolerant species that have lower growth  
413 rates (e.g. Al-Mufti et al. 1977; Janssens et al. 1998; Grytnes 2000; Stevens and Carson 2002; Pärtel et al.  
414 2005; Kleinebecker et al. 2018; but see Šímová et al. 2013 for a review and empirical testing of some  
415 alternative or concurrent explanations).

416 Thus, we surmise that, in our study area, the positive effect by soil pH on richness may partly be explained  
417 with the different levels of stress controlled by the same topographical factors that influence soil pH  
418 (Moeslund et al. 2013a): dry and steep land-forms may promote a higher species density because they  
419 favor the coexistence of many small-sized species that colonize “gaps” and are not able to grow in more  
420 productive sites dominated by large, mat-forming and litter-accumulating species (Grubb 1976; Bennie et  
421 al. 2006; Wellstein et al. 2013, 2014).

422 This hypothesis is supported by the PCA of the plot × species matrix (**Online Resource 2**), revealing that the  
423 poorer plots were characterized by perennial grasses known for their typical “competitive” characteristics  
424 (Grime 1973) such as *Agrostis capillaris* and *Festuca microphylla*. The former is a patch-forming species,  
425 capable of establishing a dense leaf canopy and assuming dominance due to its ability for rapid lateral  
426 spread (Grime et al. 2007); the latter belongs to the group of fescues with extra-vaginal branching strategy  
427 (Pignatti 1982; for a definition of this trait see e.g. Stace et al. 1992), a character leading to the  
428 development of expanded tussocks and mats. Notably, both *A. capillaris* and *F. microphylla* are also well  
429 known for being bound to acidic to sub-acid soils throughout their geographic range (e.g. Pignatti 1982;

430 Grime et al. 2007) and, within the pedo-climatic context of the Apennines, to fine-earth deep soils (e.g.  
431 Bruno and Bazzichelli 1966; Pignatti 1982; Blasi et al. 1998). Thus, in our study area, the observed effect of  
432 pH on species richness might actually be caused by vegetation structure patterns led by these two grasses,  
433 unable to establish at high-pH and dry sites. Consistently, among the species significantly associated (in the  
434 PCA) with the poorer plots, there were some herbs characterized by creeping growth-forms (*Potentilla*  
435 *rigoana*, *Veronica serpyllifolia*), a strategy that allows to exploit the shaded micro-habitat under the grass  
436 canopy or to colonize the surface of the thick litter (Grime 1998). On the other hand, the perennial grass  
437 most significantly associated in the PCA with the high-richness plots was *Festuca circummediterranea*, a  
438 fescue that can only develop into isolated tufts with a reduced lateral spread, because of intra-vaginal  
439 shoot system (e.g. Pignatti 1982). Indeed, the other species correlated with the richer plots included either  
440 opportunistic annuals (e.g. *Arenaria serpyllifolia*, *Cynosurus echinatus*, *Geranium molle*, *Medicago lupulina*,  
441 *Trifolium scabrum*) that may fill the “gaps” between the perennial grasses, or typically stress- resistant,  
442 slow-growing plants (e.g. the CAM species *Sedum acre*).

443 Somewhat similar conclusions were drawn by a study on plant traits and assembly rules in another area of  
444 the Central Apennines (Wellstein et al. 2013, 2014), showing that *Sesleria nitida*, a grass capable of forming  
445 high-density carpets, was the main factor suppressing species coexistence at extremely fine spatial scale  
446 (0.0025 m<sup>2</sup>) – but only on N-facing slopes, because on the dryer S-facing slopes it built single, isolated  
447 tussocks leaving large spaces available to other species. However, since in the present study we didn’t  
448 collect data on species cover or traits, further work is necessary to confirm our hypotheses.

449 Finally, an interesting problem concerns silt fraction: this predictor showed a positive effect on species  
450 richness, even at the 0.01 m<sup>2</sup> grain, and it was the only significant environmental variable to be  
451 uncorrelated with the pH gradient. The strong role of this predictor was quite unexpected and is not easy to  
452 explain. Only a small number of studies on grassland diversity analyzed soil texture, and even these often  
453 failed to find a correlation with species richness (e.g. Turtureanu et al. 2014). The results by Stohlgreen et  
454 al. (1999) and Cousins and Eriksson (2002), showing a positive effect of silty soils on species richness, can’t  
455 be compared with our observations because of their plot sizes being much larger and their pedo-climatic  
456 context very different. Moreover, we acknowledge that the soil texture data used in our study were

457 obtained without the prior destruction of soil organic matter (SOM) with H<sub>2</sub>O<sub>2</sub>, leading to possible  
458 overestimation of silt and underestimation of clay fraction because of silt-sized micro-aggregates made up  
459 of SOM-clay complexes (Jensen et al. 2017). However, silty soils are generally better drained than clayey  
460 soils, and extremely silt-rich soils may be scarcely fertile as they do not show any structural arrangement  
461 because of lack of the binding properties provided by clay (e.g. Thompson 1952). In a phytosociological  
462 study performed in a neighboring area, silt fraction was found to be higher in the xerophytic, sparse  
463 grasslands of steep slopes, than in the mesic, dense communities of karst hollows (Di Pietro et al. 2005).  
464 Thus, the effect of silt might point once again to a negative correlation – within the study region – between  
465 species richness and biomass or competition, in turn controlled by topographic-hydrological gradients.

466

#### 467 ***Diversity-environment relationship: spatial scales***

468 We found that the relative importance of some predictors varied with plot size. This was already shown by  
469 previous studies dealing with multiple-scale sampling in grasslands (e.g. Auestad et al. 2008; Polyakova et  
470 al. 2016). It has been suggested that at extremely fine scales the availability of nutrients will influence the  
471 identity of the dominant species, in turn influencing richness; while at larger plot sizes, factors controlling  
472 richness via habitat heterogeneity will prevail (Auestad et al. 2008). Although we only had a small range of  
473 plot sizes, this may explain while stone and rock cover (a proxy of habitat heterogeneity) had a relevant  
474 effect only at 1 m<sup>2</sup>.

475 The overall explanatory power of the predictor variables became weaker with decreasing plot size. This is  
476 not unexpected, because very small spatial scales are unavoidably associated with the increasing effects of  
477 factors other than the physical variables, such as plant individual number and size (Palmer et al. 2008;  
478 Storch 2016), stochastic disturbance and diaspore availability (Zobel et al. 2000), and fine-scale plant  
479 interactions (Wellstein et al. 2014). Moreover, environmental variables were measured at the scale of the  
480 largest plot, so they might not be representative of the micro-habitat variability between the subplots.

481

#### 482 ***Diversity-environment relationship: z-values***

483 A high z-value (slope) of the species-area curve implies that there is a low mean number of individuals per  
484 species (Storch 2016), i.e. each species occurs with scattered individuals leading to a high spatial  
485 heterogeneity of the vegetation. This can be in some cases a result of physical habitat heterogeneity.  
486 Indeed, we found that z-values were positively correlated with stone and rock cover and with “humps”, and  
487 negatively correlated with soil depth: this can be explained as rocky and shallow soils give rise to a “rough”  
488 microtopography; while the flat, deep soils of the doline bottoms are rather homogeneous. Although stone  
489 and rock cover can also influence z-values via a trivial mechanism (i.e., on very rocky soils there is a higher  
490 chance that the subplots of the smallest size will fall on a completely bare surface, thus inflating the  
491 steepness of the species-area curve), in our study area rock-free soils are usually found only in concave  
492 land-forms, i.e. at sites with high pH and deep soils: hence, there is some overlapping between sites that  
493 curtail species richness (alpha-diversity) and sites featuring a low turnover of species between spatial scales  
494 (beta-diversity). Our results are thus consistent with Chiarucci et al. (2006), who found a negative  
495 relationship between z-values and productivity in Tuscany and in Germany. Polyakova et al. (2016), in  
496 primary steppe grasslands in S-Siberia, found a strong positive effect on z-values of soil roughness at micro-  
497 scale. In contrast, both Turtureanu et al. (2014) (in secondary grasslands in Romania) and Baumann et al.  
498 (2016) (in high-elevation grasslands in the Italian Alps) found that their measured environmental predictors  
499 explained little of the variability in z-values. However, comparisons of z-values between different studies  
500 have to be interpreted very cautiously, as the results will depend on the size of the plots used (Chiarucci et  
501 al. 2006).

502

### 503 **Conclusions**

504 The importance ranking and effect size of the environmental variables were partly different across alpha-  
505 diversity spatial scales or between alpha- and beta-diversity; nevertheless, the sites with acid and deep soils  
506 had on average lower levels of all the diversity measures, while maximum alpha- and beta-diversity were  
507 usually attained at sites with neutro-basic and shallow soils, often on steep or rocky land-forms. These  
508 relationships seem to be mediated by competition: at the low-diversity sites, species coexistence is  
509 probably suppressed by a small number of carpet-forming species, in turn bound to topographically-

510 controlled “islands” of acidic or deep soils. Further studies will be necessary in the Apennine grasslands to  
511 confirm this explanation.

512 However, the observed patterns point to the importance of the less productive and dryer sites for the  
513 conservation of secondary grassland plant diversity, as underlined by many previous works (e.g. Grime  
514 1973; Moore et al. 1989; Wassen et al. 2005; Bennie et al. 2006; Cingolani et al. 2010; Filibeck et al. 2016;  
515 Kleinebecker et al. 2018; Palpurina et al. 2019). In the Apennine mountains, these oligotrophic habitats  
516 seem to be not (yet) significantly threatened by atmospheric nitrogen deposition (Bonanomi et al. 2006;  
517 Nogueira et al. 2017; but see Ochoa-Hueso et al. 2017), a factor heavily impacting some C- and N-European  
518 grasslands (e.g. Stevens et al. 2010). They may be nevertheless at risk because of grazing abandonment  
519 (Bonanomi et al. 2006): many sectors of the Apennines have witnessed the development of a closed  
520 woodland in a very short time (Bracchetti et al. 2012; Amici et al. 2013). Moreover, in many European and  
521 Mediterranean landscapes, shrub and tree colonization seems to be hindered in land-forms with deep  
522 clayey soils and to take place preferentially on shallow or rocky soils, probably because of reduced  
523 competition by grasses and larger availability of suitable microsites (Grove and Rackham 2001: 57; Rackham  
524 2006: 201-202; Pärtel and Helm 2007). This might lead to a proportionally larger loss of those grassland  
525 types that host the highest levels of alpha- and beta-diversity.

526

#### 527 **Authors' contribution**

528 G.F. and L.C. conceived the study and the sampling methodology; L.C. coordinated field work; L.D.M.,  
529 M.G.S. and G.F. participated in field sampling; L.C. revised critical vascular plants; F.R. supervised soil  
530 analysis; M.G.S., M.B. and G.F. performed data analysis; G.F. supervised the overall work and led the  
531 writing; all authors discussed the results and contributed to text editing.

532

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540

541 **Online resource 1:** Geographic features and photographs of the study sites

542 **Online resource 2:** Results of the correlation PCA from the plots × species matrix

543 **Online resource 3:** Matrix of 1m<sup>2</sup> plots × species matrix

544

#### 545 **References**

546 Al-Mufti MM, Sydes CL, Furness SB, Grime JP, Band SR (1977) Quantitative analysis of shoot phenology and  
547 dominance in herbaceous vegetation. *J Ecol* 65:759–791

548 Amici V, Santi E, Filibeck G, Diekmann M, Geri F, Landi S, Scoppola A, Chiarucci A (2013) Influence of  
549 secondary forest succession on plant diversity patterns in a Mediterranean landscape. *J Biogeogr* 40:  
550 2335-2347.

551 Apostolova I, Dengler J, Di Pietro R, Gavilán RG, Tsiripidis I (2014) Dry grasslands of Southern Europe:  
552 syntaxonomy, management and conservation. *Hacquetia* 13:1–14

553 Araya YN, Silvertown J, Gowing DJ, McConway KJ, Peter Linder H, Midgley G (2011) A fundamental, eco-  
554 hydrological basis for niche segregation in plant communities. *New Phytologist* 189:253–258

555 Arrhenius O (1921) Species and area. *J Ecol* 9:95-99

556 Auestad I, Rydgren K, Økland RH (2008) Scale-dependence of vegetation-environment relationships in semi-  
557 natural grasslands. *J Veg Sci* 19:139–148

558 Barabesi L, Franceschi S, Marcheselli M (2012) Properties of design-based estimation under stratified  
559 spatial sampling with application to canopy coverage estimation. *The Annals of Applied Statistics* 6:210–  
560 228

561 Barbosa AM, Brown JA, Jimenez-Valverde A, Real R (2016) modEvA: Model Evaluation and Analysis. R  
562 package version 1.3.2. <https://CRAN.R-project.org/package=modEvA>

563 Barker G, Grant A, Beavitt P, Christie N, Giorgi J, Hoare P, Leggio T, Migliavacca M (1991) Ancient and  
564 modern pastoralism in central Italy: an interdisciplinary study in the Cicolano mountains. *Papers of the*  
565 *British School at Rome* 59:15–88

566 Bátori Z, Vojtkó A, Farkas T, Szabó A, Havadtóti K, Vojtkó AE, Tölgyesi C, Cseh V, Erdős L, Maák I, Keppel G  
567 (2017) Large- and small -scale environmental factors drive distributions of cool-adapted plants in karstic  
568 microrefugia. *Ann Bot* 119:301–30

569 Baumann E, Weiser F, Chiarucci A, Jentsch A, Dengler J (2016) Diversity and functional composition of  
570 alpine grasslands along an elevational transect in the Gran Paradiso National Park (NW Italy). *Tuexenia*  
571 36:337–358

572 Bennie J, Hill MO, Baxter R, Huntley B (2006) Influence of slope and aspect on long-term vegetation change  
573 in British chalk grasslands. *Journal of Ecology* 94:355–368

574 Bigi G, Colacicchi R, Damiani AV, D'Andrea M, Pannuzi L, Parotto M, Praturlon A, Sirna G (1986) Carta  
575 Geologica del Parco Nazionale d'Abruzzo. Ente Autonomo Parco Nazionale d'Abruzzo, Pescasseroli.

576 Blasi C, Capotorti G, Fortini P (1998) On the vegetation series in the northern sector of the Simbruini  
577 mountains (Central Apennines). *Fitosociologia* 35:85-102

578 Blasi C, Facioni L, Burrascano S, Del Vico E, Tilia A, Rosati L (2012) Submediterranean dry grasslands along  
579 the Tyrrhenian sector of central Italy: Synecology, syndynamics and syntaxonomy. *Plant Biosystems* 146:  
580 266-290

581 Bonanomi G, Caporaso S, Allegranza M (2006) Short-term effects of nitrogen enrichment, litter removal and  
582 cutting on a Mediterranean grassland. *Acta Oecologica* 30:419-425

583 Borer ET, Seabloom EW, Gruner DS, Harpole WS, Hillebrand H et al (2014) Herbivores and nutrients control  
584 grassland plant diversity via light limitation. *Nature* 508:517–520

- 585 Bracchetti L, Carotenuto L, Catorci A (2012) Land-cover changes in a remote area of central Apennines  
586 (Italy) and management directions. *Landsc Urb Plann* 104:157-170
- 587 Brown AG, Hatton J, Selby KA, Leng M, Christie N (2013) Multi-proxy study of Holocene environmental  
588 change and human activity in the Central Apennine Mountains, Italy. *J Quatern Sci* 28:71–82
- 589 Bruno F, Bazzichelli G (1966) Note illustrative alla carta della vegetazione del Parco Nazionale d’Abruzzo.  
590 *Annali di Botanica (Roma)* 28:739–778
- 591 Burrascano S, Chytrý M, Kuemmerle T, Giarrizzo E, Luysaert S, Sabatini FM, Blasi C (2016) Current  
592 European policies are unlikely to jointly foster carbon sequestration and protect biodiversity. *Biol Conserv*  
593 201:370-376
- 594 Calandra R (1999). I suoli di Campo Imperatore. *Braun-Blanquetia* 16:21-32
- 595 Cancellieri L, Mancini LD, Sperandii MG, Filibeck G (2017a) In and out: effects of shoot- vs. rooted-presence  
596 sampling methods on plant diversity measures in mountain grasslands. *Ecological Indicators* 72:315–321
- 597 Cancellieri L, Sperandii MG, Filibeck G (2017b) First record of the steppic relict *Astragalus exscapus* L.  
598 subsp. *exscapus* in the Apennines (Abruzzo, Italy), and biogeographic implications. *Plant Biosystems*  
599 151:944–948
- 600 Cantero JJ, Liira J, Cisneros JM, Gonzalez J, Nuñez C, Petryna L, Cholaky C, Zobel M (2003) Species richness,  
601 alien species and plant traits in Central Argentine mountain grasslands. *J Veg Sci* 14:129–136
- 602 Catorci A, Ottaviani G, Kosić IV, Cesaretti S. (2012). Effect of spatial and temporal patterns of stress and  
603 disturbance intensities in a sub-Mediterranean grassland. *Plant Biosystems* 146:352-367
- 604 Chelli S, Wellstein C, Campetella G, Canullo R, Tonin R, Zerbe S, Gerdol R (2017) Climate change response of  
605 vegetation across climatic zones in Italy. *Climate Research* 71:249-262
- 606 Chiarucci A, Viciani D, Winter C, Diekmann M (2006) Effects of productivity on species–area curves in  
607 herbaceous vegetation: evidence from experimental and observational data. *Oikos* 115:475–483

- 608 Chiarucci, A. (2007) To sample or not to sample? That is the question... for the vegetation scientist. *Folia*  
609 *Geobotanica* 42:209-216
- 610 Chiarucci A, Bacaro G, Scheiner SM (2011) Old and new challenges in using species diversity for assessing  
611 biodiversity. *Phil Trans R Soc B* 366:2426–2437
- 612 Chiarucci A, Bacaro G, Filibeck G, Landi S, Maccherini S, Scoppola A (2012) Scale dependence of plant  
613 species richness in a network of protected areas. *Biodivers Conserv* 21:503–516
- 614 Chytrý M, Tichý L, Roleček J (2003) Local and regional patterns of species richness in central European  
615 vegetation types along the pH/calcium gradient. *Folia Geobotanica* 38:429–442.
- 616 Chytrý M, Danihelka J, Ermakov N, Hájek M, Hájková P, Kočí M, Kubešová S, Lustyk P, Otýpková Z, Popov D,  
617 Roleček J, Řezníčková M, Šmarda P, Valachovič M (2007) Plant species richness in continental southern  
618 Siberia: effects of pH and climate in the context of the species pool hypothesis. *Global Ecol Biogeogr*  
619 16:668–678
- 620 Chytrý M, Dražil T, Hájek M, Kalníková V, Preislerová Z, Šibík J, Ujházy K, Axmanová I, Bernátová D, Blanař D,  
621 Dančák M, Dřevojan P, Fajmon K, Galvanek D, Hajkova P, Herben T, Hrivnak R, Janeček S, Janišova M,  
622 Jiraska Š, Kliment J, Kochjarova , Lepš J, Leskovjanska A, Merunkova K, Mladek J, Slezak M, Šeffler J,  
623 Šefflerova V, Škodova I, Uhliřova J, Ujhazyova M, Vymazalova M (2015) The most species-rich plant  
624 communities in the Czech Republic and Slovakia (with new world records). *Preslia* 87:217–278
- 625 Cingolani AM, Vaieretti MV, Gurvich DE, Giorgis MA, Cabido M (2010) Predicting alpha, beta and gamma  
626 plant diversity from physiognomic and physical indicators as a tool for ecosystem monitoring. *Biological*  
627 *Conservation* 143:2570–2577
- 628 Cinque A, Liccardo C, Palma B, Pappalardo L, Roszkopf C, Sepe C (1990) Le tracce glaciali nel Parco  
629 Nazionale d’Abruzzo (Appennino centrale): nota preliminare. *Geografia Fisica Dinamica Quaternaria*  
630 13:121–133
- 631 Conti F, Abbate G, Alessandrini A, Blasi C (2005) An Annotated Checklist of the Italian Vascular Flora.  
632 Palombi, Roma

- 633 Conti F, Bartolucci F (2015) The vascular flora of the National Park of Abruzzo, Lazio and Molise (Central  
634 Italy). Springer, CH.
- 635 Cousins SA, Eriksson O (2002) The influence of management history and habitat on plant species richness in  
636 a rural hemiboreal landscape, Sweden. *Landscape Ecology* 17:517-529
- 637 De'ath G, Fabricius KE (2000) Classification and regression trees: a powerful yet simple technique for  
638 ecological data analysis. *Ecology* 81:3178–3192
- 639 de Bello F, Lepš J, Sebastià MT (2007) Grazing effects on the species–area relationship: variation along a  
640 climatic gradient in NE Spain. *J Veg Sci* 18:25–34
- 641 Dengler J, (2008) Pitfalls in small-scale species-area sampling and analysis. *Folia Geobotanica* 43:269–287
- 642 Dengler J (2009) A flexible multi-scale approach for standardised recording of plant species richness  
643 patterns. *Ecological Indicators* 9:1169–1178
- 644 Dengler J, Janišová M, Török P, Wellstein C (2014) Biodiversity of Palaeartic grasslands: a synthesis. *Agric  
645 Ecosyst Environ* 182:1–14
- 646 Dengler J, Biurrun I, Apostolova I, Baumann E, Becker T et al (2016) Scale-dependent plant diversity in  
647 Palaeartic grasslands: a comparative overview. *Bulletin of the Eurasian Dry Grassland Group* 31:12–26
- 648 Dengler J, Wagner V, Dembicz I, García-Mijangos I, Naqinezhad A et al (2018) GrassPlot—a database of  
649 multi-scale plant diversity in Palaeartic grasslands. *Phytocoenologia* 48:331–347
- 650 Di Pietro R, De Santis A, Fortini P (2005) A geobotanical survey on acidophilous grasslands in the Abruzzo,  
651 Lazio and Molise National Park (Central Italy). *Lazaroa* 26:115–137
- 652 Dolek M, Geyer A (2002) Conserving biodiversity on calcareous grasslands in the Franconian Jura by grazing:  
653 a comprehensive approach. *Biological Conservation* 104:351-360
- 654 European Union (1992) Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats  
655 and of wild fauna and flora. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31992L0043>.  
656 Accessed 15 September 2018

- 657 Ewald J (2003) The calcareous riddle: why are there so many calciphilous species in the Central European  
658 flora? *Folia Geobotanica* 38:357–366
- 659 Feurdean A, Ruprecht E, Molnár Z, Hutchinson SM, Hickler T (2018): Biodiversity-rich European grasslands:  
660 Ancient, forgotten ecosystems. *Biological Conservation* 228:224-232
- 661 Filibeck G, Adams J, Brunetti M, Di Filippo A, Rosati L, Scoppola A, Piovesan G (2015) Tree ring ecological  
662 signal is consistent with floristic composition and plant indicator values in Mediterranean *Fagus sylvatica*  
663 forests. *Journal of Ecology* 103:1580–1593
- 664 Filibeck G, Petrella P, Cornelini P (2016) All ecosystems look messy, but some more so than others: a case-  
665 study on the management and acceptance of Mediterranean urban grasslands. *Urban Forestry & Urban*  
666 *Greening* 15:32–39
- 667 Foggi B, Rossi G (1996) A survey of the genus *Festuca* L. (Poaceae) in Italy. I. The species of the summit flora  
668 in the Tuscan-Emilian Apennines and Apuan Alps. *Willdenowia* 26:1–33
- 669 Foggi B, Rossi G, Signorini MA (1999): The *Festuca violacea* aggregate (Poaceae) in the Alps and Apennines  
670 (central-southern Europe). *Can J Bot* 77:989–1013
- 671 Fraser LH, Pither J, Jentsch A, Sternberg M, Zobel M et al (2015) Worldwide evidence of a unimodal  
672 relationship between productivity and plant species richness. *Science* 349:302–305
- 673 Galadini F, Messina P (1990) Osservazioni su un deposito a blocchi al margine SW della conca di  
674 Pescasseroli (Italia centrale). *Il Quaternario* 3:23–30
- 675 Gee GW, Bauder JW (1986) Particle-size Analysis. In: *Methods of Soil Analysis, Part 1*. Soil Science Society of  
676 America, Madison:383–411
- 677 Gerdol R, Stanisci A, Tomaselli M, Fazzini M (2008) *La vegetazione delle montagne italiane*. Club Alpino  
678 Italiano, Roma
- 679 Giraudi C (1995) Sedimenti eolici, variazioni climatiche ed influenza antropica: considerazioni su alcune  
680 piane intermontane dell'Appennino Abruzzese. *Il Quaternario* 8:211–216

681 Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and  
682 comparison of species richness. *Ecology Letters* 4:379–391

683 Grace JB (1999) The factors controlling species density in herbaceous plant communities: an assessment.  
684 *Perspectives in Plant Ecology, Evolution and Systematics* 2:1–28

685 Greenland S, Senn SJ, Rothman KJ, Carlin JC, Poole C, Goodman SN, Altman DG (2016) Statistical tests,  
686 confidence intervals, and power: A guide to misinterpretations. *European Journal of Epidemiology*  
687 31:337–50

688 Grime JP (1973) Control of species density in herbaceous vegetation. *Journal of Environmental*  
689 *Management* 1:151–167

690 Grime JP (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of*  
691 *Ecology* 86:902–910

692 Grime JP, Hodgson JG, Hunt R (2007) *Comparative plant ecology: a functional approach to common British*  
693 *species*. Springer

694 Grove AT, Rackham O (2001) *The nature of Mediterranean Europe: an ecological history*. Yale University  
695 Press, Yale

696 Grubb PJ (1976) A theoretical background to the conservation of ecologically distinct groups of annuals and  
697 biennials in the chalk grassland ecosystem. *Biological Conservation* 10(1):53–76

698 Grytnes JA (2000) Fine-scale vascular plant species richness in different alpine vegetation types:  
699 relationships with biomass and cover. *J Veg Sci* 11:87–92

700 Guisan A, Zimmermann NE (2000) Predictive habitat distribution models in ecology. *Ecological*  
701 *Modelling* 135:147–186

702 Güler B, Jentsch A, Bartha S, Bloor JMG, Competella G, Canullo R, Házi J, Kreyling J, Pottier J, Szabó G,  
703 Terziyska T, Ugurlu E, Wellstein C, Zimmermann Z, Dengler J (2016) How plot shape and dispersion affect

704 plant species richness counts: implications for sampling design and rarefaction analyses. *J Veg Sci* 27:692-  
705 703

706 Habel JC, Dengler J, Janišová M, Török P, Wellstein C, Wiezik M (2013) European grassland ecosystems:  
707 threatened hotspots of biodiversity. *Biodivers Conserv* 22:2131–2138

708 Hájková P, Roleček J, Hájek M, Horsák M, Fajmon K, Polák M, Jamrichová E (2011) Prehistoric origin of the  
709 extremely species-rich semi-dry grasslands in the Bílé Karpaty Mts (Czech Republic and Slovakia). *Preslia*  
710 83:185-204

711 Hapfelmeier A, Ulm K (2013) A new variable selection approach using random forests. *Computational*  
712 *Statistics & Data Analysis* 60:50–69

713 Hiller B, Nuebel A, Broll G, Holtmeier FK (2005) Snowbeds on silicate rocks in the Upper Engadine (Central  
714 Alps, Switzerland)—pedogenesis and interactions among soil, vegetation, and snow cover. *Arctic,*  
715 *Antarctic, and Alpine Research* 37(4):465-476

716 Hothorn T, Hornik K, Zeileis A (2006a) Unbiased Recursive Partitioning: A Conditional Inference Framework.  
717 *Journal of Computational and Graphical Statistics* 15:651–674

718 Hothorn T, Buehlmann P, Dudoit S, Molinaro A, Van Der Laan M (2006b) Survival Ensembles. *Biostatistics*  
719 7:355–373

720 Janssens F, Peeters A, Tallowin JRB, Bakker JP, Bekker RM, Fillat F, Oomes MJM (1998) Relationship  
721 between soil chemical factors and grassland diversity. *Plant and Soil* 202:69–78

722 Jensen JL, Schjønning P, Watts CW, Christensen BT, Munkholm LJ (2017) Soil texture analysis revisited:  
723 Removal of organic matter matters more than ever. *PLoS ONE* 12(5): e0178039

724 Kajtoch Ł, Cieślak E, Varga Z, Paul W, Mazur MA, Sramkó G, Kubisz D (2016) Phylogeographic patterns of  
725 steppe species in Eastern Central Europe: a review and the implications for conservation. *Biodiv Conserv*  
726 25:2309-2339

727 Keddy P (2005) Putting the Plants Back into Plant Ecology: Six Pragmatic Models for Understanding and  
728 Conserving Plant Diversity. *Annals of Botany* 96:177–189

729 Kleijn D, Sutherland WJ (2003) How effective are European agri-environment schemes in conserving and  
730 promoting biodiversity? *J Appl Ecol* 40:947-969

731 Kleiber C, Zeileis A (2008) *Applied Econometrics with R*. New York: Springer-Verlag.

732 Kleinebecker T, Busch V, Hölzel N, Hamer U, Schäfer D, Prati D, Fischer M, Hemp A, Lauterbach R, Klaus VH  
733 (2018) And the winner is....! A test of simple predictors of plant species richness in agricultural grasslands.  
734 *Ecological Indicators* 87:296–301

735 Knop EVA, Kleijn D, Herzog F, Schmid B (2006) Effectiveness of the Swiss agri-environment scheme in  
736 promoting biodiversity. *J Appl Ecol* 43:120-127

737 Kuzemko AA, Steinbauer MJ, Becker T, Didukh YP, Dolnik C, Jeschke M, Naqinezhad A, Ugurlu E, Vassilev K,  
738 Dengler J (2016) Patterns and drivers of phytodiversity in steppe grasslands of Central Podolia (Ukraine).  
739 *Biodivers Conserv* 25:2233–2250

740 Löbel S, Dengler J, Hobohm C (2006) Species richness of vascular plants, bryophytes and lichens in dry  
741 grasslands: The effects of environment, landscape structure and competition. *Folia Geobotanica* 41:377–  
742 393

743 Lucchese F, Persia G, Pignatti S (1995) I prati a *Bromus erectus* Hudson dell'Appennino Laziale.  
744 *Fitosociologia* 30:145–180

745 Maccherini S (2006) Factors associated with species richness in a remnant calcareous grassland. *Grassland*  
746 *Science* 52:181–184

747 Manzi A (1990) La gestione dei pascoli montani in Abruzzo e la Società delle Erbe Seconde di Pescasseroli  
748 ed Opi. *Archivio Botanico* 66:129–142

749 Manzi A (2012) Storia dell'ambiente nell'Appennino centrale: la trasformazione della natura in Abruzzo  
750 dall'ultima glaciazione ai giorni nostri. Chieti: Meta.

- 751 McCune B, Keon D (2002) Equations for potential annual direct incident radiation and heat load. *J Veg Sci*  
752 13:603–606
- 753 Merunková K, Preislerová Z, Chytrý M (2012) White Carpathian grasslands: can local ecological factors  
754 explain their extraordinary species richness? *Preslia* 84: 311-325
- 755 Moeslund JE, Arge L, Bøcher PK, Dalgaard T, Svenning JC (2013a) Topography as a driver of local terrestrial  
756 vascular plant diversity patterns. *Nord J Bot* 31:129–144
- 757 Moeslund JE, Arge L, Bøcher PK, Dalgaard T, Odgaard MV et al (2013b) Topographically controlled soil  
758 moisture is the primary driver of local vegetation patterns across a lowland region. *Ecosphere* 4:1–26
- 759 Moore DRJ, Keddy PA, Gaudet CL, Wisheu IC (1989) Conservation of wetlands: do infertile wetlands deserve  
760 a higher priority? *Biological Conservation* 47:203–217
- 761 Morrison LW (2015) Observer error in vegetation surveys: a review. *Journal of Plant Ecology* 9: 367-379.
- 762 Nakagawa S (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias.  
763 *Behavioral Ecology* 15:1044–1045
- 764 Nogueira C, Bugalho MN, Pereira JS, Caldeira MC (2017) Extended autumn drought, but not nitrogen  
765 deposition, affects the diversity and productivity of a Mediterranean grassland. *Environmental and*  
766 *Experimental Botany* 138:99-108
- 767 Ochoa-Hueso R., Munzi S, Alonso R, Arróniz-Crespo M, Avila A, et al. (2017) Ecological impacts of  
768 atmospheric pollution and interactions with climate change in terrestrial ecosystems of the  
769 Mediterranean Basin: Current research and future directions. *Environmental Pollution* 227:194-206
- 770 Palmer MW, McGlenn DJ, Fridley JD (2008) Artifacts and artificions in biodiversity research. *Folia*  
771 *Geobotanica* 43:245–257
- 772 Palpurina S, Chytrý M, Tzonev R, Danihelka J, Axmanová I, Merunková K, Duchoň M, Karakiev T (2015)  
773 Patterns of fine-scale plant species richness in dry grasslands across the eastern Balkan Peninsula. *Acta*  
774 *Oecologica* 63:36–46

775 Palpurina S, Wagner V, von Wehrden H, Hájek M, Horsák M et al (2017) The relationship between plant  
776 species richness and soil pH vanishes with increasing aridity across Eurasian dry grasslands. *Global Ecology*  
777 *and Biogeography* 26:425–434

778 Palpurina S, Chytrý M, Hölzel N, Tichý L, Wagner V et al 2019 The type of nutrient limitation affects the  
779 plant species richness–productivity relationship: Evidence from dry grasslands across Eurasia. *Journal of*  
780 *Ecology*, in press. doi.org/10.1111/1365-2745.13084

781 Pärtel M, Bruun HH, Sammuli M (2005) Biodiversity in temperate European grasslands: origin and  
782 conservation. *Grassland Science in Europe* 10:1-14

783 Pärtel M, Helm A (2007) Invasion of woody species into temperate grasslands: relationship with abiotic and  
784 biotic soil resource heterogeneity. *Journal of Vegetation Science* 18:63-70

785 Pärtel M, Zobel M (1999) Small-scale plant species richness in calcareous grasslands determined by the  
786 species pool, community age and shoot density. *Ecography* 22:153–159

787 Pavoine S, Bonsall MB (2011) Measuring biodiversity to explain community assembly: a unified approach.  
788 *Biol. Rev.* 86:792–812

789 Pignatti S (1982) *Flora d'Italia*. Edagricole, Bologna.

790 Polyakova MA, Dembicz I, Becker T, Becker U, Demina ON, Ermakov N, Filibeck G, Guarino R, Janišová M,  
791 Jaunatre R, Kozub L, Steinbauer MJ, Suzuki K, Dengler J (2016) Scale- and taxon-dependent patterns of  
792 plant diversity in steppes of Khakassia, South Siberia (Russia). *Biodivers Conserv* 25:2251–2273

793 Poschlod P, WallisDeVries MF (2002) The historical and socioeconomic perspective of calcareous grasslands  
794 – lessons from the distant and recent past. *Biological Conservation* 104:361–376

795 Primi R, Filibeck G, Amici A, Bückle C, Cancellieri L, Di Filippo A, Gentile C, Guglielmino A, Latini R, Mancini  
796 LD, Mensing SA, Rossi CM, Rossini F, Scoppola A, Sulli C, Venanzi R, Ronchi B, Piovesan G (2016) From  
797 Landsat to leafhoppers: a multidisciplinary approach for sustainable stocking assessment and ecological  
798 monitoring in mountain grasslands. *Agric Ecosyst Environm* 234:118–133

799 Quinn GP, Keough MJ (2002) *Experimental Design and data Analysis for Biologists*. Cambridge University  
800 Press, Cambridge

801 Rackham O (2006) *Woodlands*. Harper Collins, London

802 Reitalu T, Helm A, Pärtel M, Bengtsson K, Gerhold P, Rosén E, Takkis , Znamenskiy S, Prentice HC (2014)  
803 Determinants of fine-scale plant diversity in dry calcareous grasslands within the Baltic Sea region. *Agric  
804 Ecosyst Environ* 182:59–68

805 Roleček J, Čornej II, Tokarjuk AI (2014) Understanding the extreme species richness of semi-dry grasslands  
806 in east-central Europe: a comparative approach. *Preslia* 86:13-34

807 Rosati L, Filibeck G, De Lorenzis A, Lattanzi E, Surbera F, Fascetti S, Blasi C. (2010) The forest vegetation of  
808 the Alburni Mts. (Cilento N. P., Campania, Southern Italy): Syntaxonomy and phytogeography  
809 *Fitosociologia*, 47:17-55

810 Sandri M, Zuccolotto P (2006) Variable selection using random forests. In: Zani S, Cerioli A, Riani M, Vichi M  
811 (Eds.) *Data Analysis, Classification and the Forward Search*. In: *Studies in Classification, Data Analysis, and  
812 Knowledge Organization*. Springer, Berlin, Heidelberg

813 Sauro U (2004) Altopiani carsici. In: IGM, “Atlante dei Tipi Geografici”, pp. 306–310

814 Sievert J (1999) Abruzzo National Park: Land of Dreams. *Environment and History* 5: 293-307.

815 Šímová I, Li YM, Storch D (2013) Relationship between species richness and productivity in plants: the role  
816 of sampling effect, heterogeneity and species pool. *Journal of Ecology* 101:161–170

817 Stace CA, Al-Bermani AKKA, Wilkinson MJ (1992) The distinction between the *Festuca ovina* L. and *Festuca  
818 rubra* L. aggregates in the British Isles. *Watsonia* 19:107–112

819 Stevens MHH, Carson WP (2002) Resource quantity, not resource heterogeneity, maintains plant diversity.  
820 *Ecology Letters* 5:420–426

821 Stevens CJ, Duprè C, Dorland E, Gaudnik C, Gowing DJ, et al. (2010). Nitrogen deposition threatens species  
822 richness of grasslands across Europe. *Environmental Pollution* 158:2940-2945

823 Stohlgren TJ, Schell LD, Heuvel BV (1999). How grazing and soil quality affect native and exotic plant  
824 diversity in Rocky Mountain grasslands. *Ecological Applications* 9:45-64

825 Storch D (2016) The theory of the nested species–area relationship: geometric foundations of biodiversity  
826 scaling. *J Veg Sci* 27:880–891

827 Strobl C, Boulesteix AL, Zeileis A, Hothorn T (2007) Bias in Random Forest Variable Importance Measures:  
828 Illustrations, Sources and a Solution. *BMC Bioinformatics* 8:25

829 Strobl C, Boulesteix AL, Kneib T, Augustin T, Zeileis A (2008) Conditional variable importance for random  
830 forests. *BMC Bioinformatics* 9:307

831 Strobl C, Malley J, Tutz G (2009a) An introduction to recursive partitioning: rationale, application, and  
832 characteristics of classification and regression trees, bagging, and random forests. *Psychological Methods*  
833 14:323–348

834 Strobl C, Hothorn T, Zeileis A (2009b) Party on! A new, conditional variable importance measure for random  
835 forests available in party package. *The R Journal*. 1/2:14–17

836 Svitok M, Hrivnák R, Kochjarová J, Ořáhelová H, Paľove-Balang P (2016) Environmental thresholds and  
837 predictors of macrophyte species richness in aquatic habitats in central Europe. *Folia Geobotanica*  
838 51:227–238

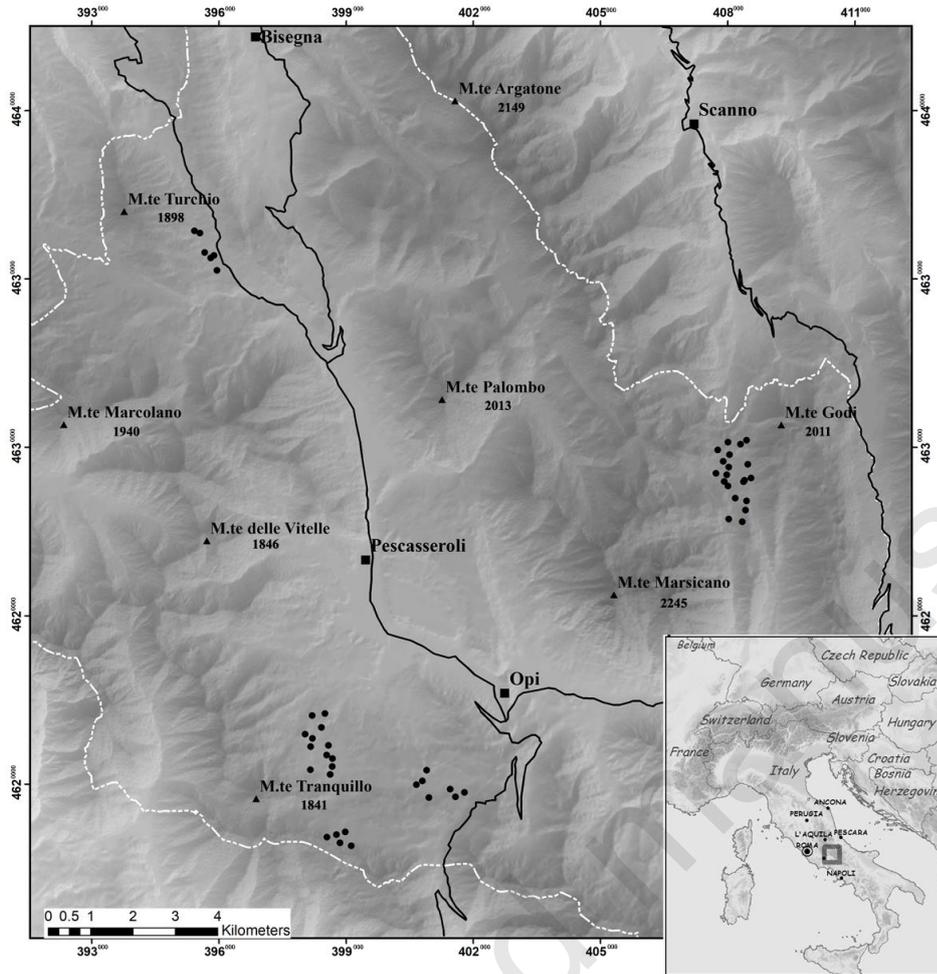
839 Thompson LM (1952) *Soils And Soil Fertility*. McGraw-Hill, New York

840 Turtureanu PD, Palpurina S, Becker T, Dolnik C, Ruprecht E, Sutcliffe LME, Szabó A, Dengler J (2014) Scale-  
841 and taxon-dependent biodiversity patterns of dry grassland vegetation in Transylvania. *Agric Ecosyst*  
842 *Environ* 182:15–24

843 Tyler G (2003) Some ecophysiological and historical approaches to species richness and calcicole/calcifuge  
844 behaviour - contribution to a debate. *Folia Geobotanica* 38:419–428

845 van der Maarel E, Sykes MT (1993) Small-scale plant species turnover in a limestone grassland: the carousel  
846 model and some comments on the niche concept. *J Veg Sci* 4:179-188

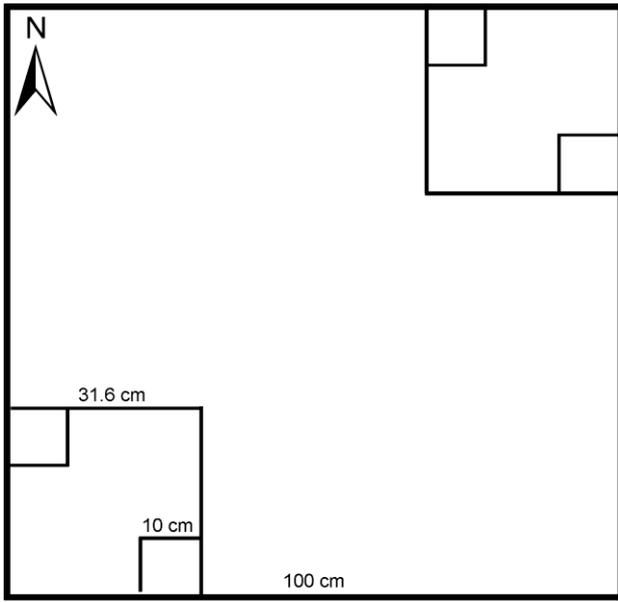
- 847 Wassen MJ, Venterink HO, Lapshina ED, Tanneberger F (2005) Endangered plants persist under phosphorus  
848 limitation. *Nature* 437:547-550
- 849 Wasserstein RL, Lazar NA (2016) The ASA's Statement on p-Values: Context, Process, and Purpose. *The*  
850 *American Statistician* 70:129–133
- 851 Wellstein C, Chelli S, Competella G, Bartha S, Galiè M, Spada F, Canullo R (2013) Intraspecific phenotypic  
852 variability of plant functional traits in contrasting mountain grasslands habitats. *Biodiv Cons* 22:2353-2374
- 853 Wellstein C, Competella G, Spada F, Chelli S, Mucina L, Canullo R, Bartha S (2014) Context-dependent  
854 assembly rules and the role of dominating grasses in semi-natural abandoned sub-Mediterranean  
855 grasslands. *Agric Ecosyst Environ* 182:113–122
- 856 Wilson JB, Peet RK, Dengler J, Pärtel M (2012) Plant species richness: the world records. *J Veg Sci* 23:796–  
857 802
- 858 Zobel M, Otsus M, Liira J, Moora M, Möls T (2000) Is small-scale species richness limited by seed availability  
859 or microsite availability? *Ecology* 81:3274–3282
- 860



862

863 **Fig. 1** Study area position (empty square in the inset) and distribution of sampling points (black dots in the  
 864 main map). Legend of main map: black lines: roads; white broken line: borders of Abruzzo Lazio & Molise  
 865 National Park; filled squares: towns; filled triangles: main mountain peaks (with elevation, m a.s.l.)

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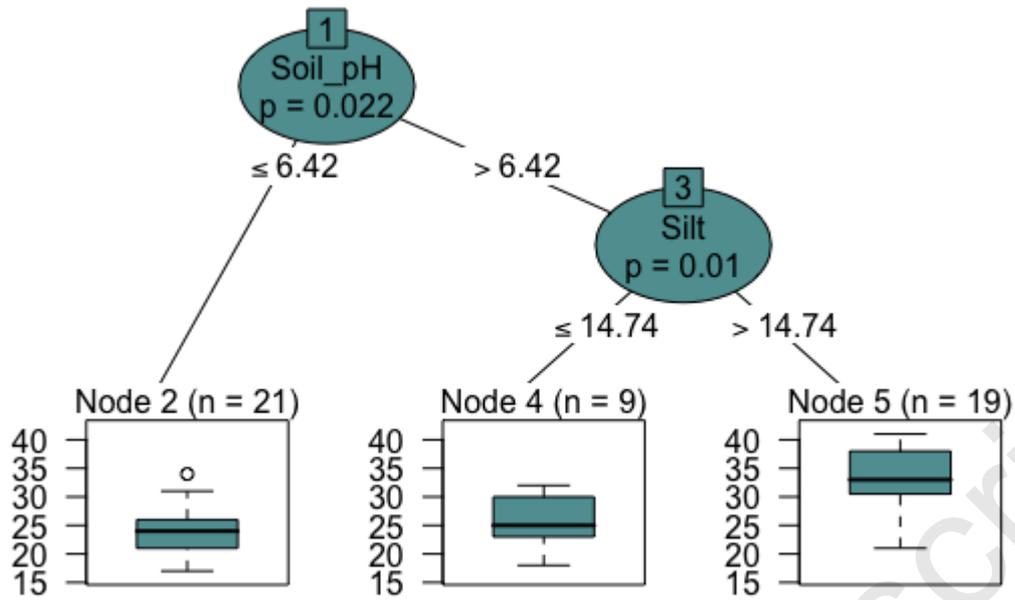


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868 **Fig. 2** Layout of the nested-plot series surveyed at each random sampling point.

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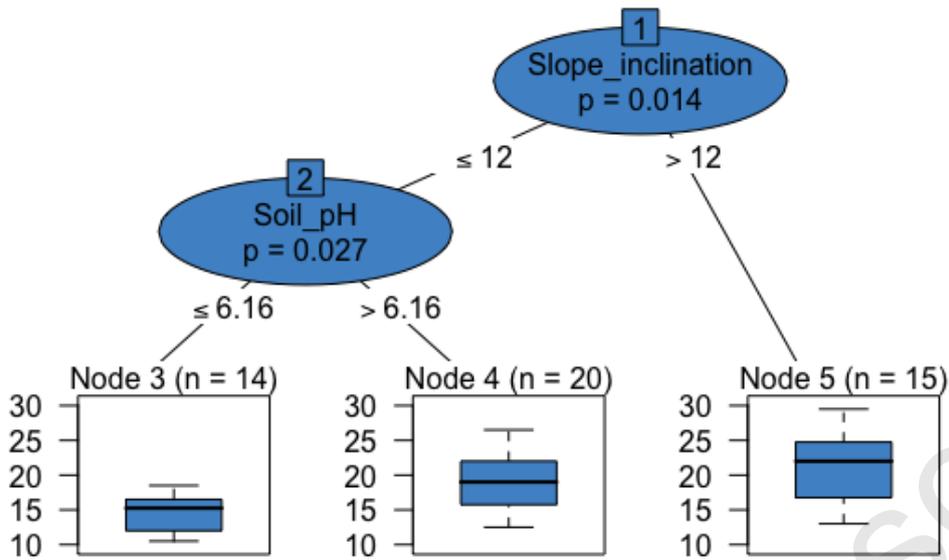


870

871 **Fig. 3** Regression Tree for 1 m<sup>2</sup> alpha-diversity as response variable. Variables responsible for tree splitting  
 872 are indicated in circles, with associated p-values. For each node, threshold values at which the observations  
 873 are split in groups are reported on the tree branches. For each terminal node (cluster of observations), the  
 874 boxplot describes the distribution of the response variable. On top of each boxplot, the number of  
 875 observations (n) in the group is reported

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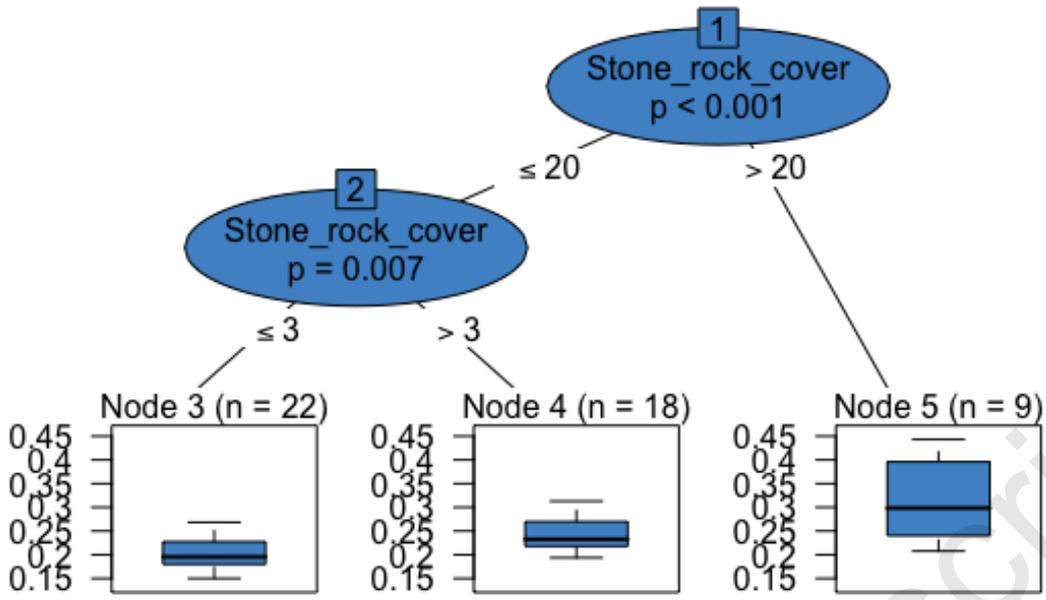
878

879 **Fig. 4** Regression Tree for 0.1 m<sup>2</sup> alpha-diversity as response variable. See caption in Fig. 3 for explanations.

880 Unlike the tree for 1 m<sup>2</sup>, here “univariate p-values” (instead of Bonferroni-corrected) was set as stopping

881 rule

882



883

884 **Fig. 5** Regression Tree for z-value as response variable. See caption in Fig. 3 for explanations

885

886

887 **TABLES**

888

<i>Continuous variables</i>	<i>Mean</i>	<i>SD</i>	<i>Min.</i>	<i>Max.</i>
Elevation (m)	1523	138	1331	1767
Slope inclination (°)	10.3	8.5	0	35
Heat-load index	0.9	0.1	0.57	1.04
Soil depth (median) (cm)	20.7	13.4	4	60
Stone and rock cover (%)	10.3	11.9	0	40
Soil pH	6.5	0.7	4.9	7.6
Silt (%)	20.2	12.1	0	68.5
Clay (%)	14	7.7	0.06	41
Sand (%)	65.9	16.5	3.3	89.8
Soil organic matter (%)	12.08	5.4	0.21	21.5

***Categorical variables***

Land-form position (number of cases in each category)

Hollow (16), Hillside (19), Hump (15)

889

890 **Tab. 1** Summary statistics of the environmental variables measured at each nested-plot series and used as

891 predictor variables in the statistical analysis

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893

<i>Species richness values</i>					
<i>Area (m<sup>2</sup>)</i>	<i>n</i>	<i>Mean ±SD</i>	<i>Median</i>	<i>Min</i>	<i>Max</i>
1	50	28.0 ±6.38	27	17	41
0.1	100	18.46 ±5.03	17	9	31
0.01	200	10.26±3.48	10	0	20
<i>Diversity metrics</i>					
<i>Name</i>	<i>n</i>	<i>Mean ±SD</i>	<i>Median</i>	<i>Min</i>	<i>Max</i>
1 m <sup>2</sup> alpha-diversity	50	28.0 ±6.38	27	17	41
0.1 m <sup>2</sup> average alpha-diversity	50	18.46 ±4.79	17	10.5	29.5
0.01 m <sup>2</sup> average alpha-diversity	50	10.26±2.87	10	4.5	16.25
z-value	50	0.238± 0.063	0.224	0.150	0.443

894

895 **Tab.2** Summary statistics of the vascular plant richness encountered in all plots at different scales, and of  
 896 the derived biodiversity metrics used as dependent variables in the statistical analysis

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<b>Taxon</b>	<b>Frequency (n=50)</b>
<i>Hieracium pilosella</i>	80%
<i>Cerastium tomentosum</i>	76%
<i>Festuca circummediterranea</i>	76%
<i>Veronica arvensis</i>	74%
<i>Euphrasia stricta</i>	72%
<i>Trifolium repens</i>	68%
<i>Achillea millefolium</i> subsp. <i>millefolium</i>	64%
<i>Viola eugeniae</i>	64%
<i>Bunium bulbocastanum</i>	62%
<i>Poa alpina</i> subsp. <i>alpina</i>	62%
<i>Medicago lupulina</i>	60%
<i>Arenaria serpyllifolia</i> subsp. <i>serpyllifolia</i>	58%
<i>Bellis perennis</i>	56%
<i>Koeleria lobata</i>	56%
<i>Avenula praetutiana</i>	50%
<i>Dactylis glomerata</i>	50%
<i>Potentilla rigoana</i>	50%

900

901 **Tab.3** Most common species in the sample, and their frequency expressed as percentage of plots. Only the  
902 species occurring in at least 50% of the plots are shown; see Online Resource 3 for full species list and  
903 frequency values

904

	Elevation	Slope inclination	Heat-Load index	Stone and rock cover	Soil depth	Clay	Silt	Sand	Soil organic matter	Soil pH	1 m <sup>2</sup> alpha-diversity	0.1 m <sup>2</sup> alpha-diversity	0.01 m <sup>2</sup> alpha-diversity	z-values
Elevation		0.002	0.0406	0.0001	0.3229	0.5566	0.8269	0.7875	0.1089	<0.0001	0.1496	0.1043	0.6268	0.4198
Slope inclination	<b>0.425</b>		0.0064	<0.0001	0.0838	0.1252	0.0553	0.0424	0.0700	0.0004	0.0987	0.0600	0.7375	0.0231
Heat-Load index	<b>-0.291</b>	<b>-0.380</b>		0.0888	0.7618	0.2853	0.4740	0.4275	0.1519	0.4418	0.3290	0.5415	0.9119	0.1512
Stone and rock cover	<b>0.537</b>	<b>0.562</b>	-0.243		0.0007	0.0375	0.8169	0.4132	0.0325	<0.0001	0.0167	0.0947	0.5773	<0.0001
Soil depth	-0.143	-0.247	0.044	<b>-0.464</b>		0.8117	0.6150	0.6368	0.3589	<0.0001	0.1114	0.7386	0.3567	0.0005
Clay	-0.085	-0.220	0.154	<b>-0.295</b>	-0.035		0.4055	0.0001	0.0849	0.4342	0.2178	0.3512	0.4836	0.1790
Silt	0.032	-0.273	0.104	-0.034	0.073	0.120		<0.0001	0.0310	0.5116	0.0329	0.0729	0.0775	0.9096
Sand	0.039	<b>0.288</b>	-0.115	0.118	-0.068	<b>-0.530</b>	<b>-0.868</b>		0.0207	0.1899	0.2058	0.2712	0.2725	0.6742
Soil organic matter	0.229	0.258	-0.206	<b>0.303</b>	-0.133	-0.246	<b>-0.305</b>	<b>0.326</b>		0.0016	0.3513	0.2896	0.5206	0.6732
Soil pH	<b>0.590</b>	<b>0.481</b>	-0.111	<b>0.642</b>	<b>-0.611</b>	-0.113	-0.095	0.189	<b>0.435</b>		0.0063	0.0309	0.4272	0.0314
1 m <sup>2</sup> alpha-diversity	0.207	0.236	-0.141	<b>0.337</b>	-0.228	-0.177	<b>0.302</b>	-0.182	0.135	<b>0.381</b>		<0.0001	<0.0001	0.1323
0.1 m <sup>2</sup> alpha-diversity	0.232	0.268	-0.088	0.239	-0.048	-0.135	0.256	-0.159	0.153	<b>0.306</b>	<b>0.877</b>		<0.0001	0.6193
0.01 m <sup>2</sup> alpha-diversity	0.070	0.049	0.016	-0.081	0.133	-0.101	0.252	-0.158	0.093	0.115	<b>0.702</b>	<b>0.844</b>		0.0002
z-values	0.117	<b>0.321</b>	-0.206	<b>0.579</b>	<b>-0.477</b>	-0.193	-0.016	0.061	0.061	<b>0.305</b>	0.216	-0.072	<b>-0.502</b>	

906

907 **Tab.4** Pair-wise correlation table between all predictor and dependent variables. Below the diagonal:908 Spearman's correlation coefficients ( $r_s$ ). Above the diagonal (in italic): p-values. Note that these are not

909 corrected for multiple testing. Correlation coefficients corresponding to a p-value &lt;0.05 are in bold

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	1 m <sup>2</sup> alpha- diversity	0.1 m <sup>2</sup> alpha- diversity	0.01 m <sup>2</sup> alpha- diversity	z-values
Soil pH	10	10	10	0
Silt	10	10	10	0
Stone and rock cover	10	10	0	10
Slope inclination	9	10	0	10
Soil depth	10	0	2	10
Land-form position	10	0	0	10
Clay	8	9	0	0
Soil organic matter	0	1	1	0
Heat-load index	0	0	0	0

912

913 **Tab. 5** Number of times, out of 10 Random Forest tries, that each predictor variable turned out to be  
914 important for each dependent variable, i.e. featured positive values exceeding the absolute value of the  
915 most negative score. Variables are listed in decreasing order of number of significant results

916

917 **1 m<sup>2</sup> alpha-diversity**

	<b>Stand. Coef.</b>	<b>Std. Error</b>	<b>z</b>	<b>p</b>
<b>Land-form: hollow vs. hump</b>	0.161	0.076	2.12	0.034
<b>Silt (%)</b>	0.128	0.028	4.61	<0.0001
<b>Soil pH</b>	0.054	0.033	1.63	0.104
<b>Slope inclination</b>	0.052	0.037	1.42	0.155
<b>Clay (%)</b>	-0.040	0.029	-1.39	0.166
<b>Land-form: hollow vs. hillside</b>	-0.011	0.087	-0.13	0.897
<b>Adjusted Pseudo-R<sup>2</sup> = 0.53</b>				

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**0.1 m<sup>2</sup> alpha-diversity**

	<b>Stand. Coef.</b>	<b>Std. Error</b>	<b>t</b>	<b>p</b>
<b>Silt (%)</b>	0.471	0.124	3.81	<0.001
<b>Slope inclination</b>	0.226	0.140	1.62	0.113
<b>Soil pH</b>	0.220	0.134	1.64	0.109
<b>Clay (%)</b>	-0.156	0.129	-1.21	0.233
<b>Adjusted R<sup>2</sup> = 0.31</b>				

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**0.01 m<sup>2</sup> alpha-diversity**

	<b>Stand. Coef.</b>	<b>Std. Error</b>	<b>t</b>	<b>p</b>
<b>Silt (%)</b>	0.370	0.135	2.75	0.009
<b>Soil pH</b>	0.176	0.135	1.31	0.198
<b>Adjusted R<sup>2</sup> = 0.13</b>				

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**Z-values**

	<b>Stand. Coef.</b>	<b>Std. Error</b>	<b>t</b>	<b>p</b>
<b>Stone and rock cover</b>	0.627	0.136	4.62	<0.0001
<b>Land-form: hollow vs. hillside</b>	0.503	0.328	1.53	0.133
<b>Slope inclination</b>	-0.268	0.147	-1.82	0.076
<b>Soil depth</b>	-0.247	0.120	-2.06	0.045
<b>Land-form: hollow vs. hump</b>	0.047	0.300	0.16	0.877
<b>Adjusted R<sup>2</sup> = 0.47</b>				

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930

931 **Tab. 6** Results of the Poisson GLM (for 1 m<sup>2</sup> alpha-diversity) and of the Linear Models (for the remainder of

932 the response variables), after variable selection based on Random Forest outputs. Within each model,

933 predictor variables are listed in order of decreasing (standardized) effect size. Stand. Coef. = Standardized

934 Coefficient; Std. Error = Standard Error of the coefficients

935

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