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

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Abstract

Secondary dry grasslands in Europe can host high levels of vascular plant richness at small spatial scales. However, in Southern Europe their biodiversity patterns are largely unexplored. In this work, we aim at: *i*) estimating plant species richness patterns at very fine scales in montane dry grasslands, on limestone bedrock, in Abruzzo Lazio & Molise National Park (Central Apennines, Italy); *ii*) assessing the most important physical and edaphic drivers of biodiversity patterns at multiple plot sizes. We used randomly placed nested-plot series where we measured alpha-diversity at three different plot sizes (1 m², 0.1 m² and 0.01 m²) and within-plot beta-diversity (as expressed by the slope of the species-area curve across plot sizes). Variable selection was performed by means of Random Forests. Relationships between selected variables and diversity measures were then assessed using Regression Trees, Linear and Generalized Linear Models. Overall, results pointed to topographically-controlled edaphic factors (soil pH and silt fraction) as the main drivers positively influencing alpha-diversity at all spatial scales, with a positive effect of rock cover and slope inclination at smaller spatial grains. Beta-diversity was positively influenced by rock cover. We suggest that high-pH, steep and/or rocky sites feature higher species richness because they lack

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

Keywords

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

Introduction

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

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

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

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

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

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

Methods

Study area

Data were gathered within the “Abruzzo Lazio e Molise” National Park, in the Central Apennine mountains, Italy ($41^{\circ}48'N$, $13^{\circ}50'E$). This protected area was designated in 1922 because it was the last refuge for both the Marsican brown bear (*Ursus arctos* ssp. *marsicanus*) and the Apennine chamois (*Rupicapra pyrenaica* ssp. *ornata*); at the same time, this area was until the 1950's one of the most important centers in Italy for transhumant shepherding (Sievert 1999). The Park's flora comprises >2,000 species, including >30 taxa 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

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

Data collection

Data were collected in early summer in 2013 and 2014. Sampling design was based on a grid of 300 x 300 m cells, overlaid on the National Park area, with one random point selected within each cell (tessellation stratified sampling: see e.g. Barabesi et al. 2012; Chiarucci et al. 2012). The five grassland clearings to be investigated were chosen *a priori*, following field reconnaissance and inspection of detailed geological maps that showed they had comparable features. All the random points falling within the five selected grassland patches were retained, resulting in a total of 50 points (**Fig. 1**). At each GPS-located point (hereafter “sampling point”), vascular plants were surveyed using a nested-plot series based on the design proposed by Dengler (2009), as modified by Cancellieri et al. (2017a): one 1 m² squared plot was centred on the random point, and oriented N-S; two 0.1 m² subplots were placed at the NE and SW corners of the 1 m² plot, and two 0.01 m² subplots were placed within each of the 0.1 m² plots (i.e., there are four 0.01 m² subplots at each sampling point) (**Fig. 2** and **Online Resource 1**). The rationale for sampling a higher number of subplots of smaller areas within each nested-plot series is twofold (Dengler 2009): i) it allows to better account for the micro-environmental heterogeneity contained within the largest plot; ii) it allows the use of mean species richness values within each nested series, thus liberating the resulting species-area curve from stochasticity. Moreover, while species counts often do not follow a normal distribution, averaging alpha-diversity values among sub-plots leads to normally distributed data (e.g. Quinn and Keough 2002).

Each nested-plot series was precisely delimited in the field by means of a plastic frame, and was surveyed by at least two people to reduce inter-observer variability (e.g. Morrison 2015). For each plot and subplot, we listed all vascular plant species (presence/absence data only), following the “shoot presence” criterion: that is, a species is recorded as present if the vertical projection of any above-ground part of an individual 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-m² 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 m² 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 H₂O₂. *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 m² plot (hereafter, “1

188 m^2 alpha-diversity"); mean species richness of the two 0.1 m² subplots (hereafter, "0.1 m² alpha-diversity");
189 mean species richness of the four 0.01m² subplots (hereafter, "0.01m² 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
$$S = c A^z$$

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

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 m² size the
200 mean richness values of the two subplots, and for the 0.01 m² 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

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

Additionally, we assessed the strength of the relationship between the response variables and the selected predictors through Generalized Linear Models (GLM) and multiple Linear Models. Whenever two predictors (among those selected through the Random Forest output) were highly correlated with each other (i.e. had a Spearman correlation coefficient > 0.6), we retained only the most biologically informative one. Since the explanatory variables are measured in different units, standardized regression coefficients (obtained by subtracting, from each observation, the variable mean and dividing by the standard deviation: e.g. Quinn and Keough 2002) were computed to allow comparison of the effect sizes on the response variable ; for Linear Models we standardized both explanatory and response variables, while for Generalized Linear Models we only transformed predictors. For 1 m² alpha-diversity, a Poisson Generalized Linear Model was fitted, setting “log” as link function. Overdispersion was tested using R package “AER” (function “dispersiontest”: Kleiber and Zeileis 2008). We used an adjusted Pseudo-R² (Guisan and Zimmermann 2000) to quantify the improvement in the deviance explained by the model including the selected predictors over the intercept-only model (R package “modEvA”, function “Dsquared”: Barbosa et al. 2016). For 0.1 m² and 0.01 m² alpha-diversity, as they were obtained averaging the species counts among subplots of the same

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

Finally, to help ecological interpretation of the richness patterns and elucidate their relationship with floristic composition, a PCA (based on a correlation matrix) of the plot \times species matrix was run, adding the 1 m² alpha-diversity as a covariate, in order to underline which taxa were associated with different levels of species richness.

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

Results

Overall diversity

Across all 50 sampling points, a total of 198 vascular plant species were recorded; 64 species (32% of the total recorded flora) were found at only one nested series (singletons). Mean species richness per plot was 10.3, 18.5 and 28.0 for 0.01 m², 0.1 m² and 1 m², respectively (**Tab.2**). The mean slope (z) of the species-area relationship was 0.238 (**Tab.2**); i.e., richness increased by c. 1.7 times, on average, with a ten-fold increase in plot area. The most frequent species (in terms of proportion of sampling plots where the taxon was recorded) are listed in **Tab. 3**. The full 1m²-plot \times species matrix (including the frequency values of all species) is provided as **Online Resource 3**.

Diversity-environment relationships

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

296 at 1 m² spatial scale (1 m² 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 m² 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 m² and 0.1 m²; soil depth and land-form position were
 305 constantly significant only at 1 m² 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 m² 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 m² and 0.01 m² 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-m²
 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 m² 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 m² 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 m² 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 m² 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 m² 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-R² of
 338 the model was 0.53 (i.e. the predictors explained 53% of the deviance in 1 m² alpha-diversity).

339 As for 0.1 m² 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 m² 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 1m² model (**Tab. 6**). The model explained 31% of variability.

345 For 0.01 m² 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-m² 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 m² 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 m² 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 m² and/or 0.1 m² 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 m²) 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-m² 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^2) – 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^2 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

obtained without the prior destruction of soil organic matter (SOM) with H_2O_2 , leading to possible overestimation of silt and underestimation of clay fraction because of silt-sized micro-aggregates made up of SOM-clay complexes (Jensen et al. 2017). However, silty soils are generally better drained than clayey soils, and extremely silt-rich soils may be scarcely fertile as they do not show any structural arrangement because of lack of the binding properties provided by clay (e.g. Thompson 1952). In a phytosociological study performed in a neighboring area, silt fraction was found to be higher in the xerophytic, sparse grasslands of steep slopes, than in the mesic, dense communities of karst hollows (Di Pietro et al. 2005). Thus, the effect of silt might point once again to a negative correlation – within the study region – between species richness and biomass or competition, in turn controlled by topographic-hydrological gradients.

Diversity-environment relationship: spatial scales

We found that the relative importance of some predictors varied with plot size. This was already shown by previous studies dealing with multiple-scale sampling in grasslands (e.g. Auestad et al. 2008; Polyakova et al. 2016). It has been suggested that at extremely fine scales the availability of nutrients will influence the identity of the dominant species, in turn influencing richness; while at larger plot sizes, factors controlling richness via habitat heterogeneity will prevail (Auestad et al. 2008). Although we only had a small range of plot sizes, this may explain while stone and rock cover (a proxy of habitat heterogeneity) had a relevant effect only at 1 m².

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

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

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

Online resource 3: Matrix of 1m² plots × species matrix

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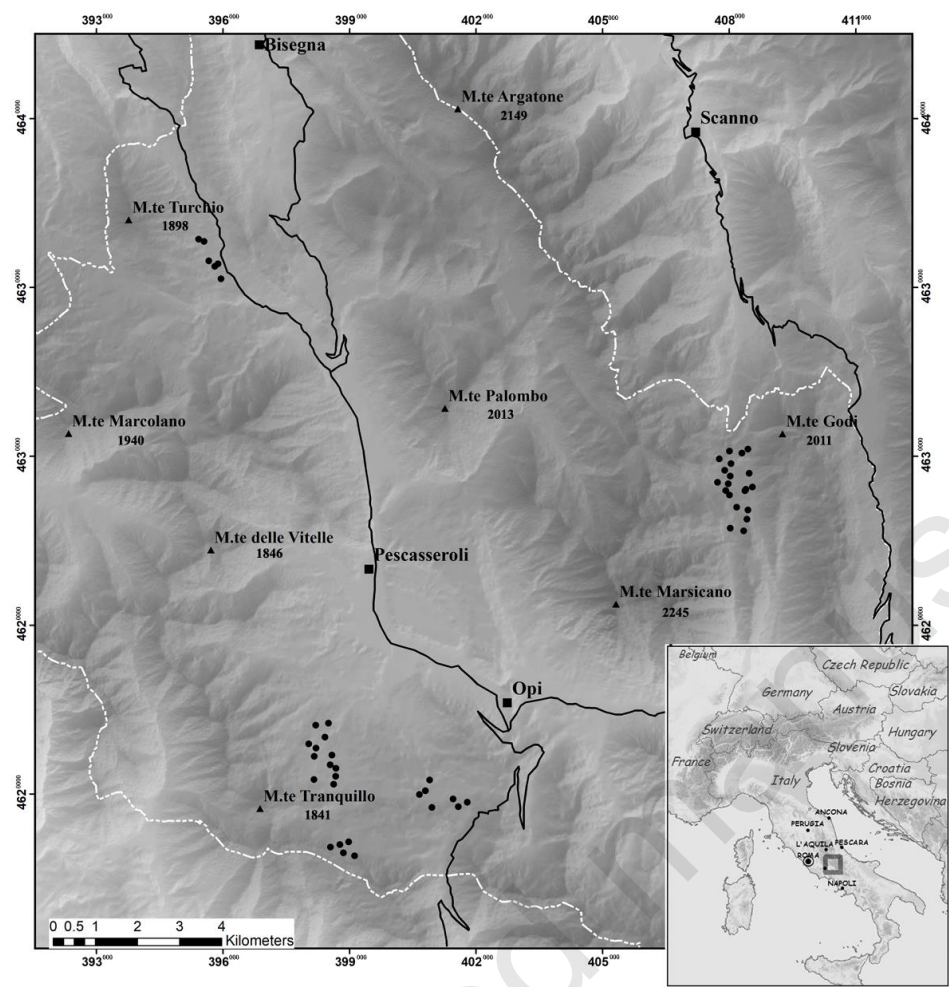
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862
863 **Fig. 1** Study area position (empty square in the inset) and distribution of sampling points (black dots in the
864 main map). Legend of main map: black lines: roads; white broken line: borders of Abruzzo Lazio & Molise
865 National Park; filled squares: towns; filled triangles: main mountain peaks (with elevation, m a.s.l.)

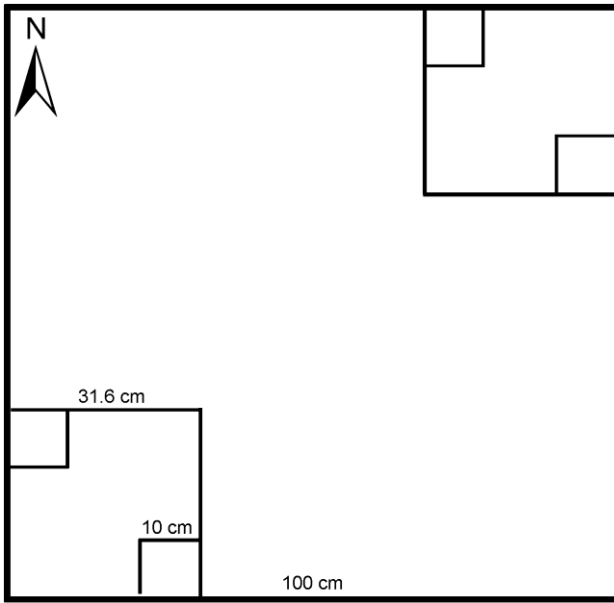
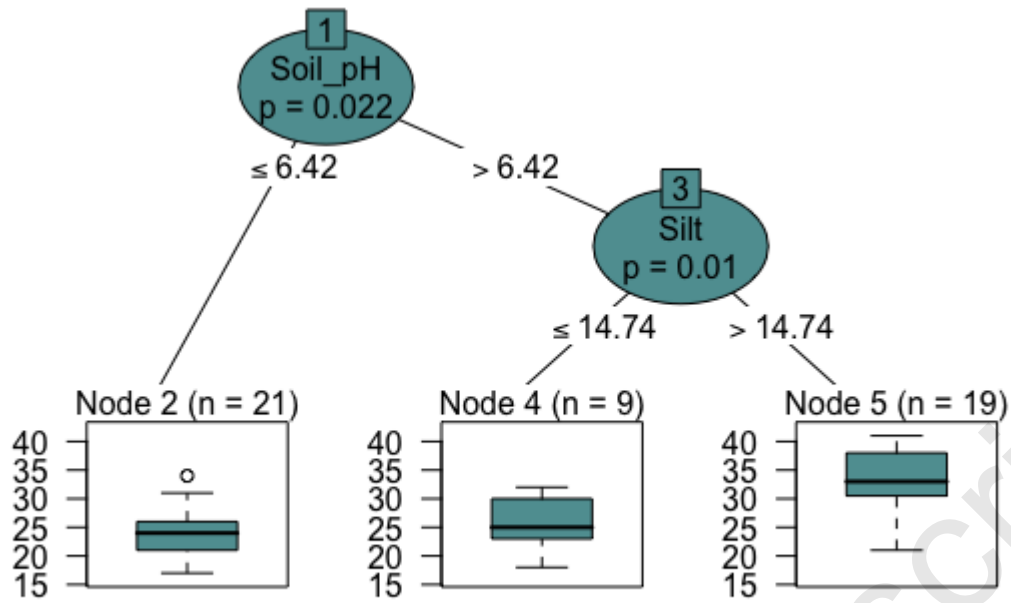


Fig. 2 Layout of the nested-plot series surveyed at each random sampling point.

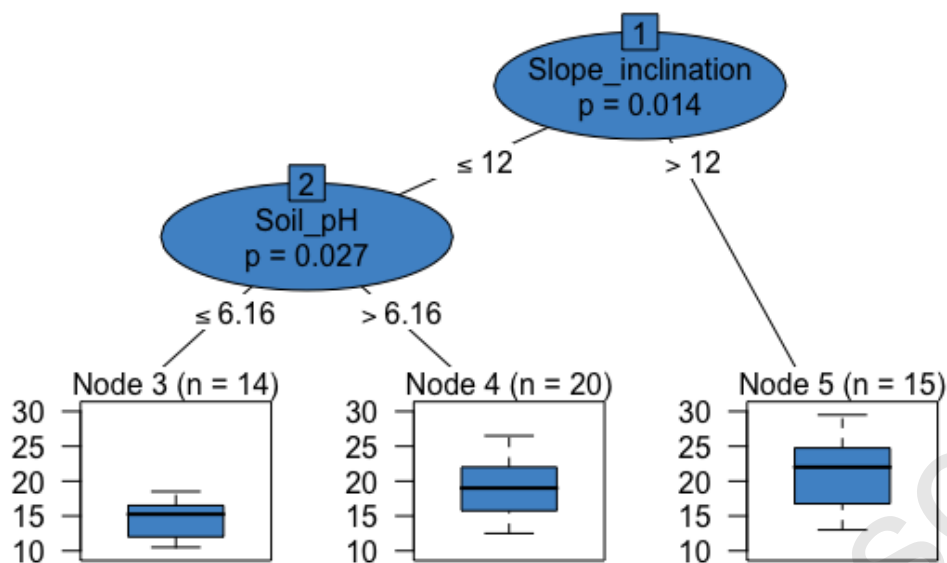


870

871 **Fig. 3** Regression Tree for 1 m² 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 m² alpha-diversity as response variable. See caption in Fig. 3 for explanations.

880 Unlike the tree for 1 m², here “univariate p-values” (instead of Bonferroni-corrected) was set as stopping

881 rule

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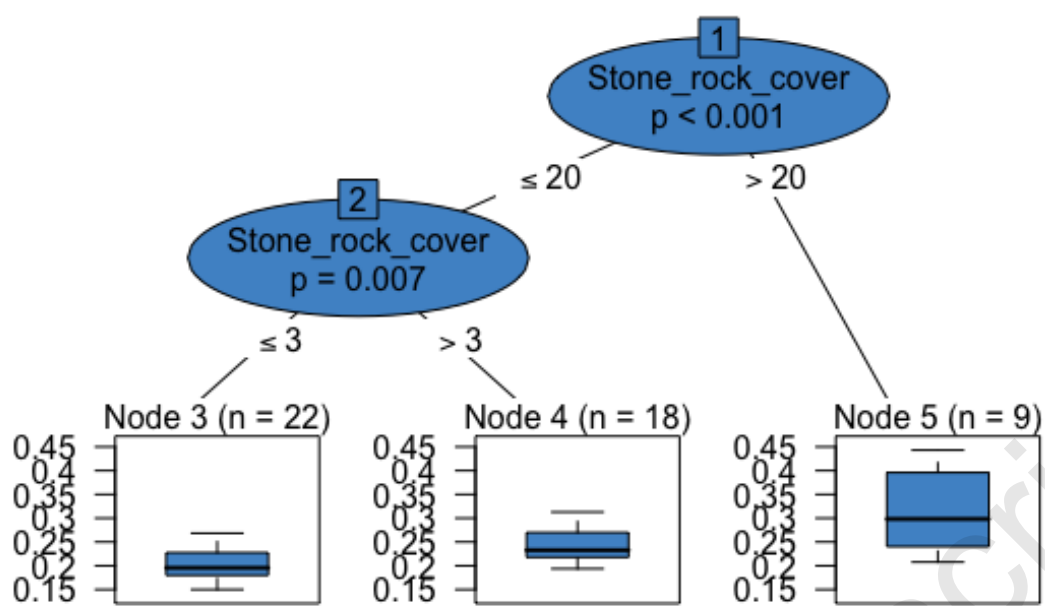


Fig. 5 Regression Tree for z-value as response variable. See caption in Fig. 3 for explanations

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
<i>Categorical variables</i>				
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 (m²)</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 ² alpha-diversity	50	28.0 ±6.38	27	17	41
0.1 m ² average alpha-diversity	50	18.46 ±4.79	17	10.5	29.5
0.01 m ² 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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Taxon	Frequency (n=50)
<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%

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

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	Elevation	Slope inclination	Heat-Load index	Stone and rock cover	Soil depth	Clay	Silt	Sand	Soil organic matter	Soil pH	1 m ² alpha-diversity	0.1 m ² alpha-diversity	0.01 m ² 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	0.425		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	-0.291	-0.380		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	0.537	0.562	-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	-0.464		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	-0.295	-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	0.288	-0.115	0.118	-0.068	-0.530	-0.868		0.0207	0.1899	0.2058	0.2712	0.2725	0.6742
Soil organic matter	0.229	0.258	-0.206	0.303	-0.133	-0.246	-0.305	0.326		0.0016	0.3513	0.2896	0.5206	0.6732
Soil pH	0.590	0.481	-0.111	0.642	-0.611	-0.113	-0.095	0.189	0.435		0.0063	0.0309	0.4272	0.0314
1 m ² alpha-diversity	0.207	0.236	-0.141	0.337	-0.228	-0.177	0.302	-0.182	0.135	0.381		<0.0001	<0.0001	0.1323
0.1 m ² alpha-diversity	0.232	0.268	-0.088	0.239	-0.048	-0.135	0.256	-0.159	0.153	0.306	0.877		<0.0001	0.6193
0.01 m ² alpha-diversity	0.070	0.049	0.016	-0.081	0.133	-0.101	0.252	-0.158	0.093	0.115	0.702	0.844		0.0002
z-values	0.117	0.321	-0.206	0.579	-0.477	-0.193	-0.016	0.061	0.061	0.305	0.216	-0.072	-0.502	

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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 <0.05 are in bold

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	1 m ² alpha-diversity	0.1 m ² alpha-diversity	0.01 m ² 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

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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

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1 m² alpha-diversity

	Stand. Coef.	Std. Error	z	p
Land-form: hollow vs. hump	0.161	0.076	2.12	0.034
Silt (%)	0.128	0.028	4.61	<0.0001
Soil pH	0.054	0.033	1.63	0.104
Slope inclination	0.052	0.037	1.42	0.155
Clay (%)	-0.040	0.029	-1.39	0.166
Land-form: hollow vs. hillside	-0.011	0.087	-0.13	0.897
Adjusted Pseudo-R² = 0.53				

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0.1 m² alpha-diversity

	Stand. Coef.	Std. Error	t	p
Silt (%)	0.471	0.124	3.81	<0.001
Slope inclination	0.226	0.140	1.62	0.113
Soil pH	0.220	0.134	1.64	0.109
Clay (%)	-0.156	0.129	-1.21	0.233
Adjusted R² = 0.31				

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0.01 m² alpha-diversity

	Stand. Coef.	Std. Error	t	p
Silt (%)	0.370	0.135	2.75	0.009
Soil pH	0.176	0.135	1.31	0.198
Adjusted R² = 0.13				

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Z-values

	Stand. Coef.	Std. Error	t	p
Stone and rock cover	0.627	0.136	4.62	<0.0001
Land-form: hollow vs. hillside	0.503	0.328	1.53	0.133
Slope inclination	-0.268	0.147	-1.82	0.076
Soil depth	-0.247	0.120	-2.06	0.045
Land-form: hollow vs. hump	0.047	0.300	0.16	0.877
Adjusted R² = 0.47				

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Tab. 6 Results of the Poisson GLM (for 1 m² alpha-diversity) and of the Linear Models (for the remainder of

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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
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