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

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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^2, 0.1 m^2 and

20   0.01 m^2) 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

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 ≤ 100 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 m² (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.

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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. ≤1 m^2) and larger plot sizes (e.g. 100 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 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 (≤1 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 ≤1 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).

In the Italian Peninsula, extensive surveys of dry grassland biodiversity patterns and their edaphic drivers at spatial grains ≤1 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 (0.01-0.1-1 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°48'N, 13°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

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

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

In the study area, secondary grasslands are maintained by grazing. Transhumant sheep grazing was widely practiced until the 1950's, when ovine farming started to decrease for socio-economic reasons; sheep stocking rate is nowadays drastically lower than in the early twentieth century (Manzi 2012). The decrease 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^2 squared plot was centred on the

147   random point, and oriented N-S; two 0.1 m^2 subplots were placed at the NE and SW corners of the 1 m^2

148   plot, and two 0.01 m^2 subplots were placed within each of the 0.1 m^2 plots (i.e., there are four 0.01 m^2

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

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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^2 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^2 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_2O_2. *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 Palaearctic 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^2 plot (hereafter, "*1*

188 *m² 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.

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

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

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

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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^2 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^2 (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^2 and

268 0.01 m^2 alpha-diversity, as they were obtained averaging the species counts among subplots of the same

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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 × species matrix was run, adding the 1 m^2 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 m^2, 0.1 m^2 and 1 m^2, 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 × 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

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

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

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

For 0.1 m^2 and 0.01 m^2 alpha-diversity, Regression Tree analysis performed with "ctree" default rules didn't yield any statistically significant partition. If univariate p-values were used (see Methods) instead of the stop criterion based on Bonferroni correction (cf. Nakagawa 2004), then the resulting tree for the 0.1-m^2 sub-plots highlighted the role of slope inclination and soil pH (and their interaction). Steeper slopes (indicating also stony soils, as explained above) showed greater species richness at 0.1 m^2 spatial scale. The lowest richness was found when a low slope inclination was combined with a pH value lower than 6.2, indicating flat bottoms of concave landforms with deep, sub-acid soils **(fig. 4).** As for the 0.01 m^2 alpha-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^2 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^2 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^2 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^2 of

338 the model was 0.53 (i.e. the predictors explained 53% of the deviance in 1 m^2 alpha-diversity).

339 As for 0.1 m^2 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^2 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^2 model **(Tab. 6)**. The model explained 31% of variability.

345 For 0.01 m^2 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.

13

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^2 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^2 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^2 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.

14

376

Diversity-environment relationship: alpha-diversity

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

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

However, since many of our environmental variables showed correlated gradients in the field (cf. Bennie et al. 2006; Baumann et al. 2016), it is difficult to separate the role of pH per se from that of other environmental variables. Sites with steep and rocky or shallow soils have necessarily high pH values because of the surfacing limestone material (e.g. Palpurina et al. 2017); on the other hand, because of the widespread calcareous bedrocks, in our study area low pH values are usually confined to concave land-forms (hollows, dolines, bases of slopes), in turn typically associated with deep soils, low rock cover and low slope inclination. Such land-forms can host acid soils, in spite of the base-rich bedrock, mainly because they accumulate clayey and decarbonated soil through colluvial deposition and karstic processes (e.g. Calandra 1999); in addition, they may trap huge amounts of wind-blown siliceous materials such as Saharan quartz silt and volcanic ashes (Giraudi 1995; Sauro 2004), and/or host a thick snow cover, favoring podzolization (Hiller et al. 2005). At the same time, dolines and hollows will receive more water and nutrients than convex land-forms or steep slopes (Araya et al. 2011; Batori et al. 2017), host soil types with higher clay fraction and higher available water content (Di Pietro et al. 2005), and undergo lower levels of natural 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^2 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

17

457 obtained without the prior destruction of soil organic matter (SOM) with H_2O_2, 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^2.

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-

19

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

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 $1m^2$ plots × species matrix

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860

FIGURES

862
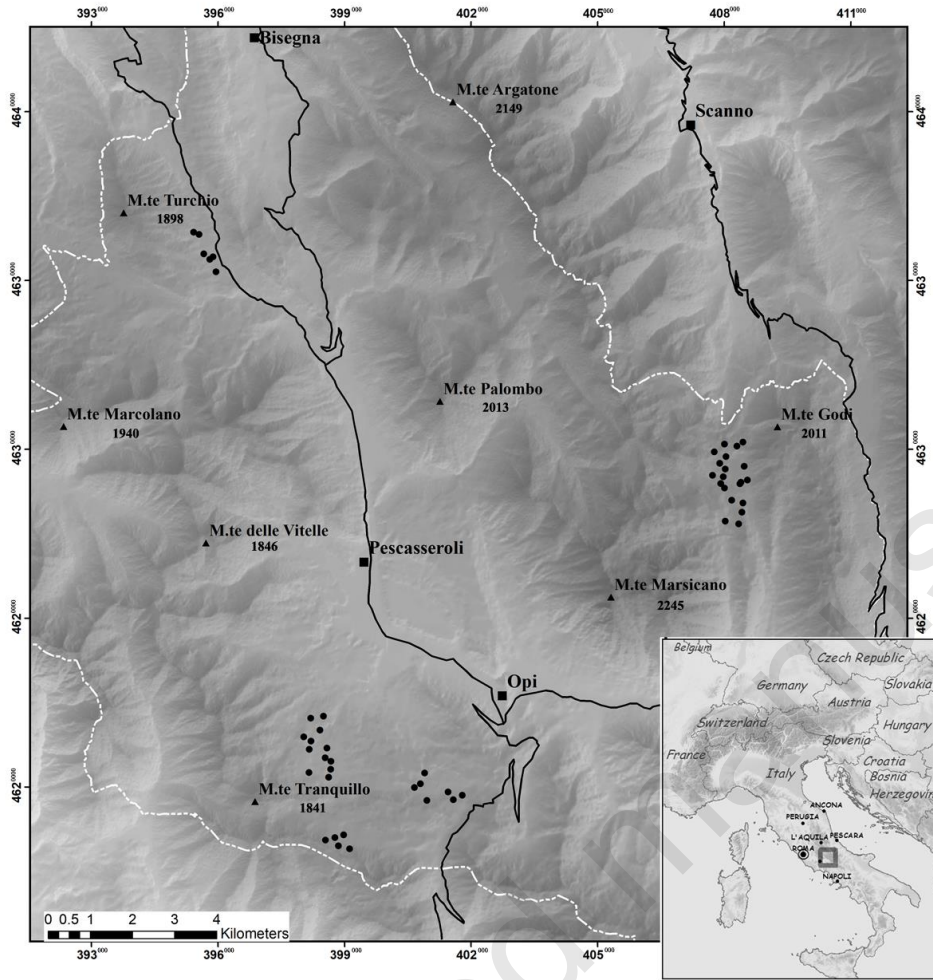
Fig. 1 Study area position (empty square in the inset) and distribution of sampling points (black dots in the

main map). Legend of main map: black lines: roads; white broken line: borders of Abruzzo Lazio & Molise

National Park; filled squares: towns; filled triangles: main mountain peaks (with elevation, m a.s.l.)

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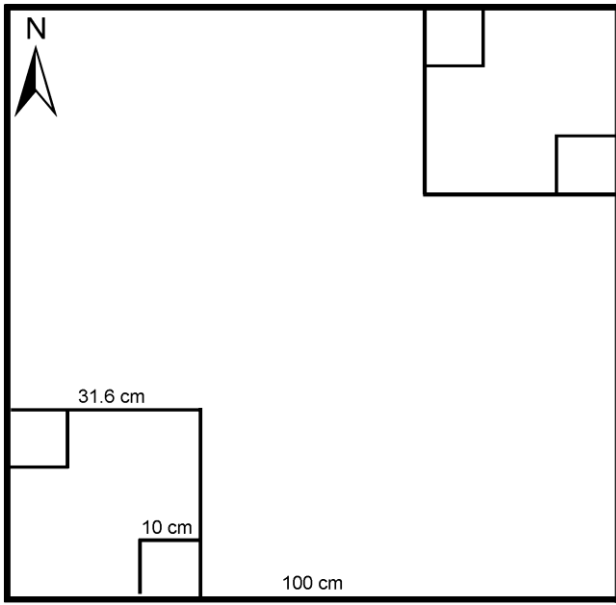
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868 **Fig. 2** Layout of the nested-plot series surveyed at each random sampling point.

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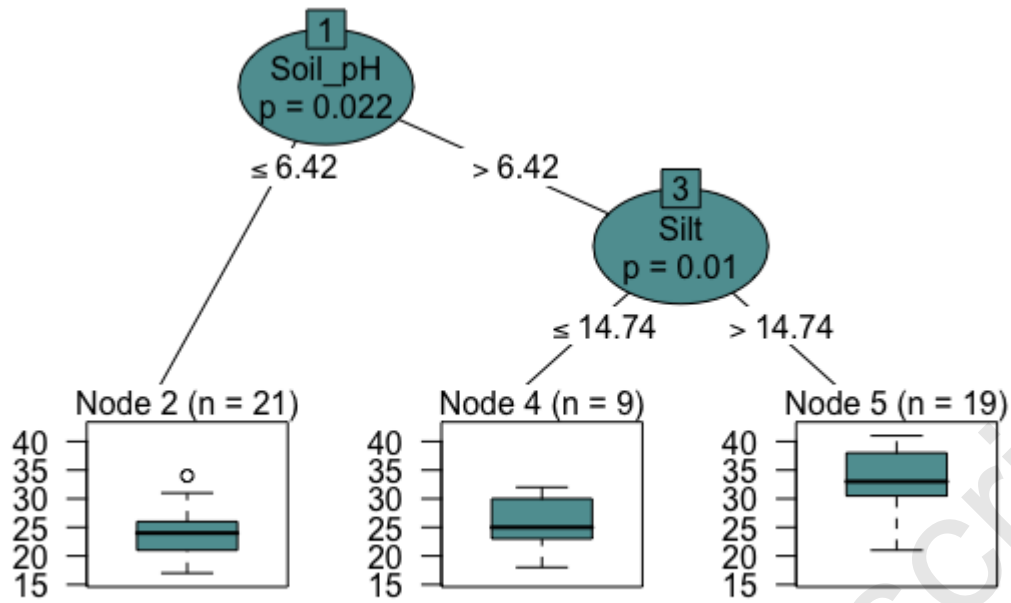
870

871   **Fig. 3** Regression Tree for 1 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

876

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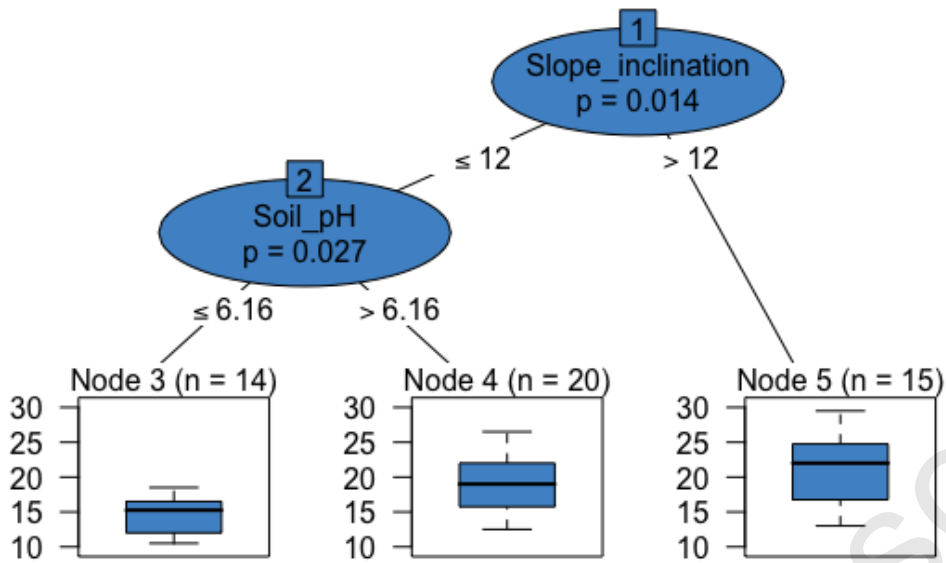
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879 **Fig. 4** Regression Tree for 0. 1 m^2 alpha-diversity as response variable. See caption in Fig. 3 for explanations.

880 Unlike the tree for 1 m^2, here "univariate p-values" (instead of Bonferroni-corrected) was set as stopping

881 rule

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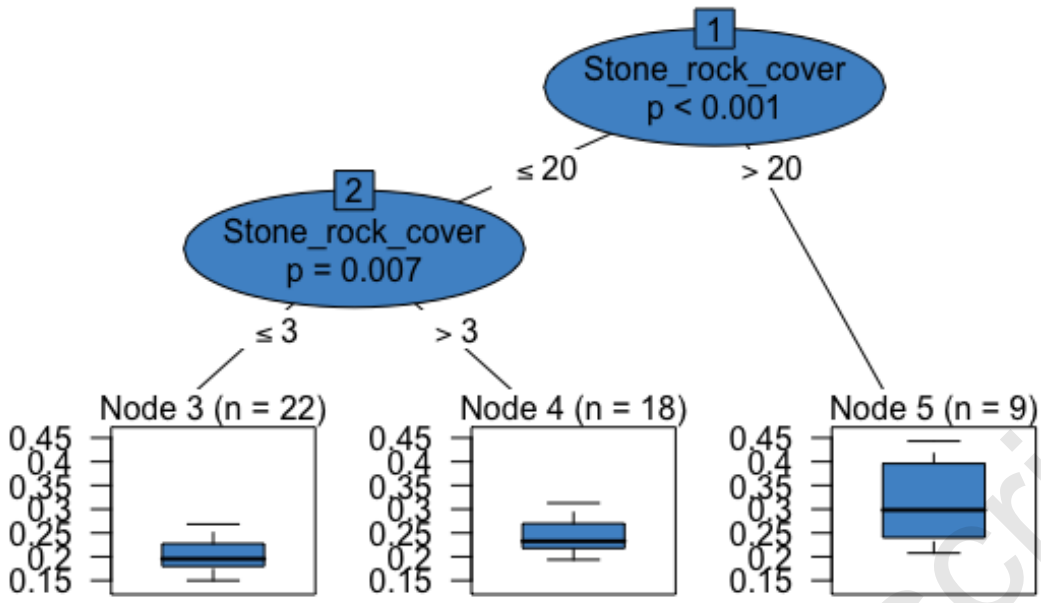
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884   **Fig. 5** Regression Tree for z-value as response variable. See caption in Fig. 3 for explanations

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886

Continuous variables	*Mean*	*SD*	*Min.*	*Max.*
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

Species richness values					
Area (m²)	n	Mean ±SD	Median	Min	Max
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
Diversity metrics					
Name	n	Mean ±SD	Median	Min	Max
1 m² alpha-diversity	50	28.0 ±6.38	27	17	41
0.1 m² average alpha-diversity	50	18.46 ±4.79	17	10.5	29.5
0.01 m² 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)
Hieracium pilosella	80%
Cerastium tomentosum	76%
Festuca circummediterranea	76%
Veronica arvensis	74%
Euphrasia stricta	72%
Trifolium repens	68%
Achillea millefolium subsp. millefolium	64%
Viola eugeniae	64%
Bunium bulbocastanum	62%
Poa alpina subsp. alpina	62%
Medicago lupulina	60%
Arenaria serpyllifolia subsp. serpyllifolia	58%
Bellis perennis	56%
Koeleria lobata	56%
Avenula praetutiana	50%
Dactylis glomerata	50%
Potentilla rigoana	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 m² alpha-diversity	0.1 m² alpha-diversity	0.01 m² 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 m² 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 m² 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 m² 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**	

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

916

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

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

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

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

Tab. 6 Results of the Poisson GLM (for 1 m² alpha-diversity) and of the Linear Models (for the remainder of the response variables), after variable selection based on Random Forest outputs. Within each model,

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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