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1 **Title: Post-glacial determinants of regional species pools in alpine grasslands**

2

3 Borja Jiménez-Alfaro^{1,*}, Sylvain Abdulhak², Fabio Attorre³, Ariel Bergamini⁴, Maria Laura Carranza⁵,
4 Alessandro Chiarucci⁶, Renata Čušterevska⁷, Stefan Dullinger⁸, Rosario G. Gavilán⁹, Gianpietro Giusso del
5 Galdo¹⁰, Nevena Kuzmmanović¹¹, Paola Laiolo¹, Javier Loidi¹², George P. Malanson¹³, Corrado Marcenó¹⁴,
6 Đorđije Milanović¹⁵, Elizabeth R. Pansing¹³, José V. Rocas-Díaz¹⁶, Eszter Ruprecht¹⁷, Jozef Šibik¹⁸, Angela
7 Stanisci¹⁹, Riccardo Testolin³, Jean-Paul Theurillat^{20,21}, Kiril Vassilev²², Wolfgang Willner⁸, Manuela
8 Winkler^{23,24}

9

10 ¹Research Unit of Biodiversity Research (CSIC/UO/PA), University of Oviedo, Mieres, Spain

11 ²National Alpine Botanical Conservatory, Gap, France

12 ³Department of Environmental Biology, Sapienza University of Rome, Rome, Italy

13 ⁴WSL Swiss Federal Research Institute, Birmensdorf, Switzerland

14 ⁵Department of Bioscience and Territory, University of Molise, Pesche, Italy

15 ⁶Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum - University of
16 Bologna, Bologna, Italy

17 ⁷Institute of Biology, Faculty of Natural Sciences and Mathematics, University of Ss. Cyril and Methodius,
18 Skopje, Republic of North Macedonia

19 ⁸Department of Botany and Biodiversity Research, University Vienna, Vienna, Austria

20 ⁹Department of Pharmacology, Pharmacognosy and Botany, Complutense University, Madrid, Spain

21 ¹⁰Department of Biological, Geological and Environmental Sciences, University of Catania, Catania, Italy

22 ¹¹Faculty of Biology, Institute of Botany and Botanical Garden Jevremovac, University of Belgrade,

23 Belgrade, Serbia 12Department of Plant Biology and Ecology, University of the Basque Country

24 (UPV/EHU), Bilbao, Spain ¹³Department of Geographical & Sustainability Sciences, University of Iowa,
25 Iowa City, IA, USA

26 ¹⁴Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

27 ¹⁵Faculty of Forestry, University of Banja Luka, Banja Luka, Bosnia and Herzegovina

28 ¹⁶Department of Geography, College of Science, Swansea University, Swansea, UK

29 ¹⁷Hungarian Department of Biology and Ecology, Babeş-Bolyai University, Cluj-Napoca, Romania

30 ¹⁸Institute of Botany, Plant Science and Biodiversity Center Slovak Academy of Sciences, Bratislava,
31 Slovakia

32 ¹⁹Department of Bioscience and Territory, University of Molise, Termoli, Italy

33 ²⁰Fondation J.-M. Aubert, Champex-Lac, Switzerland

34 ²¹Department of Botany and Plant Biology, University of Geneva, Chambésy, Switzerland

35 ²²Institute of Biodiversity and Ecosystem Research, Department of Plant and Fungal Diversity and
36 Resources, Bulgarian Academy of Sciences, Sofia, Bulgaria

37 ²³Institute for Interdisciplinary Mountain Research, Austrian Academy of Sciences, Vienna, Austria

38 ²⁴Department of Integrative Biology and Biodiversity Research, University of Natural Resources and Life
39 Sciences, Vienna, Austria

40

41 *Corresponding author: Borja Jiménez-Alfaro

42 Email: jimenezalfaro@uniovi.es

43

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50

51 **Conflict of interest**

52 The authors declare no conflict of interest.

53

54 **Author contributions**

55 B.J.-A. conceived the idea, analysed the data and led the writing. Co- authors contributed either with vegetation
56 data, the assessment of regional floras, or the delineation of regions and tree lines from local literature. J.V.R.-
57 D. prepared the spatial layers for current and LGM areas. R.T. prepared the map for dispersal dispersion. All
58 authors contributed to the manuscript and to the interpretation of results.

59 **Abstract**

60 **Aim:** Alpine habitats support unique biodiversity confined to high-elevation areas in the current interglacial.
61 Plant diversity in these habitats may respond to area, environment, connectivity and isolation, yet these
62 factors have been rarely evaluated in concert. Here we investigate major determinants of regional species
63 pools in alpine grasslands, and the responses of their constituent species groups.

64 **Location:** European mountains below 50° N.

65 **Time period:** Between 1928 and 2019.

66 **Major taxa studied:** Vascular plants.

67 **Methods:** We compiled species pools from alpine grasslands in 23 regions, including 794 alpine species and
68 2,094 non-alpines. We used species–area relationships to test the influence of the extent of alpine areas on
69 regional richness, and mixed-effects models to compare the effects of 12 spatial and environmental
70 predictors. Variation in species composition was addressed by generalized dissimilarity models and by a
71 coefficient of dispersal direction to assess historical links among regions.

72 **Results:** Pool sizes were partially explained by current alpine areas, but the other predictors largely
73 contributed to regional differences. The number of alpine species was influenced by area, calcareous
74 bedrock, topographic heterogeneity and regional isolation, while non-alpines responded better to
75 connectivity and climate. Regional dissimilarity of alpine species was explained by isolation and
76 precipitation, but non-alpines only responded to isolation. Past dispersal routes were correlated with latitude,
77 with alpine species showing stronger connections among regions.

78 **Main conclusions:** Besides area effects, edaphic, topographic and spatio-temporal determinants are
79 important to understand the organization of regional species pools in alpine habitats. The number of alpine
80 species is especially linked to refugia and isolation, but their composition is explained by past dispersal and
81 post-glacial environmental filtering, while non-alpines are generally influenced by regional floras. New
82 research on the dynamics of alpine biodiversity should contextualize the determinants of regional species
83 pools and the responses of species with different ecological profiles.

85 **Keywords:** alpine grasslands, area effects, Europe, glaciations, island biogeography, neutral theory, species

86 pools, species richness, species–area relationships

87

88 **Introduction**

89 Alpine habitats occur globally in mountain areas between the climatic tree line and the snowline (if present),
90 yet they are mainly distributed in extratropical regions (Körner et al., 2011; Testolin et al., 2020). Climatic
91 history has had a lasting imprint on the biodiversity of these habitats (Kadereit et al., 2004; Schmitt et al.,
92 2010) due to the cycles of glaciation and deglaciation that occurred during the Pleistocene (2.6 Ma – 12 ka
93 BP). These cycles alternated long glacial ages with short and warm interglacial periods that resulted in the
94 expansion and contraction of alpine-like habitats, respectively (Birks & Willis, 2008; Flantua et al., 2019). In
95 temperate mountains, glacial cold periods are associated with the extinction of cold-sensitive species, while
96 post-glacial warming produced local extinctions (extirpations or exterminations, Birks, 2019) of cold-
97 adapted species due to physiological constraints and the upward expansion of forests and competitive species
98 (Birks, 2008). After the Last Glacial Maximum (LGM, 21 ka BP), climate warming triggered upslope shifts
99 of cold-adapted species for the last time, and alpine habitats reached their current extent at the beginning of
100 the Holocene, c. 11 ka BP (Tinner & Theurillat, 2003). The biodiversity we observe today in alpine habitats
101 is thus the outcome of the post-glacial reorganization of cold-adapted species during the present interglacial
102 (Hewitt, 1999).

103 The geographical extent of current alpine habitats has been recognized as a major determinant of alpine
104 diversity (Hadley, 1987; Riebesell, 1982), following the ecological principle of species–area relationships
105 applied to island-like habitats, that is, island species–area relationships (ISARs; Matthews et al., 2016). Since
106 alpine habitats are generally located around mountaintops and surrounded by a matrix of non-alpine habitats
107 (Itescu, 2019; Schmitt, 2009), they look like habitat islands (also known as sky islands) aggregated along
108 mountain ranges in networks or archipelagos (Flantua et al., 2020). This spatial configuration has prompted
109 the investigation of alpine habitats under the theory of island biogeography, with a focus on the influence of
110 area and isolation on species richness under an equilibrium between immigration and extinction (MacArthur
111 & Wilson, 1967). However, the foundational theory developed for oceanic islands has evolved into a
112 complex geodynamic system driven by evolutionary and ecological drivers (Whittaker et al., 2017). Using
113 mountaintops of North America as a study system, Brown (1971) originally suggested a non-equilibrium
114 scenario with few extinctions and low colonization rates, making high-elevation relict faunas relatively
115 stable over the last 8,000 years. Intriguingly, the analysis of regional alpine floras has shown that area and

116 isolation explain species richness and composition better in tropical (Gehrke & Linder, 2014; Sklenář et al.,
117 2014) than in temperate regions (Hadley, 1987; Virtanen et al., 2002). It has been further suggested that other
118 determinants (e.g. geology, habitat heterogeneity, or climate) should be taken into account when analysing
119 alpine plant diversity, assuming a common biogeographical history among the compared units (Nagy &
120 Grabherr, 2009).

121 Besides area, topographic heterogeneity is a complementary determinant of mountain biodiversity (Jiménez-
122 Alfaro, Girardello et al., 2018; Rahbek et al., 2019), increasing the number of microhabitats in alpine
123 landscapes (Scherrer & Körner, 2011). It is broadly recognized that some species survived in LGM glaciated
124 areas in suitable habitats found in steep slopes, deep canyons and ice-free glacier margins or nunataks (Graae
125 et al., 2018; Hewitt, 1999; Pan et al., 2020). These species further contributed to the formation of the species
126 pools, that is, all vascular plants known to occur in the region (Cornell & Harrison, 2014). In addition, the
127 geographical position of a mountain region determines isolation as a function of overall distance to other
128 mountain regions, affecting the chances of cold-adapted species dispersing and colonizing other areas. The
129 spatial configuration of alpine habitats within a mountain range also may affect their functional connectivity,
130 influencing dispersal and extirpation (Flantua et al., 2020; Hadley, 1987). The post-glacial reorganization of
131 species pools has been further determined by species responses to soil and climatic conditions, influencing
132 the richness and composition of alpine plants at different scales (Nagy & Grabherr, 2009; Virtanen et al.,
133 2002). The composition of species pools in alpine habitats is also linked to long dispersal routes and
134 allopatric speciation events that mostly occurred in the Pleistocene (Muellner-Riehl et al., 2019), and the
135 environmental constraints that ultimately shaped floristic similarity across regions in the Holocene (Hadley,
136 1987; Malanson et al., 2020).

137 Altogether, the aforementioned factors may explain the size and composition of regional pools that have
138 persisted in alpine habitats during the current interglacial, and will likely play a role in their response to
139 ongoing climate change (Malanson et al., 2019). For a better understanding of the main determinants of
140 regional pools, we should consider that species inhabiting alpine habitats are heterogeneous in their
141 ecological requirements (Jiménez-Alfaro, Marcenó et al., 2014; Klanderud, 2008). Many cold-adapted
142 species are specialists with physiological adaptations to cold conditions typical of low-temperature alpine
143 habitats, or with low competitive abilities (Körner, 2003; Ross et al., 2010). Other cold-adapted species

144 occurring in alpine habitats frequently have wide elevation ranges and a distribution centre below the tree
145 line (Jiménez-Alfaro, Gavilán et al., 2014; Nagy & Grabherr, 2009). Such ecological differences may have
146 shaped the diversity of regional species pools in response to their post-glacial spatial reorganization, leading
147 to either elevation shifts (Dullinger et al., 2012) or extirpations (Jiménez-Alfaro et al., 2016). It is, therefore,
148 expected that the determinants of species pools will differ between alpine species (those whose populations
149 are mainly distributed above the tree line) and non-alpines (those that mainly occur at lower elevation but
150 also can thrive in alpine habitats).

151 Here, we investigate the role of area and other spatial and environmental determinants in explaining the size
152 and composition of regional species pools in alpine grasslands of central and southern Europe (Figure 1). We
153 hypothesize that, besides area, other factors related to topography, environment, connectivity of alpine habitats
154 and regional isolation are important to understand the size and composition of regional pools. Specifically, we
155 test a series of predictions for 12 variables expected to influence regional species pools (Table 1). We also
156 hypothesize that alpine species and non-alpines will respond differently, with stronger effects on alpine species
157 for those determinants linked to alpine area, topography, historical isolation of alpine habitats and
158 macroclimatic gradients. To further interpret the historical determinants of species pool composition, we also
159 evaluated past species migrations by estimating post-glacial dispersal directions among regions.

160

161 **Methods**

162 **Study system**

163 We defined 23 geographical regions (Figure 1) as distinct biogeographical features (see Supporting
164 Information Appendix S1). These regions cover the mid-latitude mountains of Europe (between 37° and 49°
165 N), which have a comparable post-glacial history and related floras (Kadereit et al., 2004; Ozenda, 1985;
166 Schmitt, 2009). We focused on alpine grasslands as the dominant habitats that occur above the climatic tree
167 line (Testolin et al., 2020). These habitats are dominated by graminoids, typically grasses (Poaceae), sedges
168 (Cyperaceae), rushes (Juncaceae), and several chamaephytes and forbs. They occupy relatively large areas in
169 the Alps but small patches in the Iberian, Italian and Balkan peninsulas (Figure 1). According to the European
170 vegetation classification (Mucina et al., 2016), the study system includes zonal vegetation related to

171 acidophilous alpine grasslands (*Juncetea trifidi*), alpine or subalpine calcicolous swards (*Elyno-Seslerietea*),
172 Mediterranean silicicolous fescue grasslands (*Festucetea indigestae*), dry calcicolous grasslands (*Festuco-*
173 *Poetalia ligulatae*) and xeric grasslands (*Daphno-Festucetea*). Tundra-like vegetation from Scandinavia and
174 Scotland was not included because these regions have a different post-glacial history mostly driven by long-
175 distance colonization (Birks & Willis, 2008).

176 **Data collection**

177 We compiled a database of community-level surveys to investigate the size and composition of the species
178 pool in the study system. We queried the European Vegetation Archive (EVA; Chytrý et al., 2016) as the main
179 repository of vegetation data in Europe. Using the literature about the vegetation of the study regions, we
180 identified data gaps in the Balkan Peninsula, the Maritime Alps and the Apennines. To cover these gaps, we
181 digitalized data referring to 6,500 vegetation plots sampled in high-mountain grasslands and integrated them
182 into EVA databases (<http://euroveg.org/eva-database>). The initial criterion for including vegetation plots in our
183 data was based on the elevation of the climatic tree line for each region (Supporting Information Table S1.1),
184 assuming alpine grasslands mainly occur above these elevations (Körner, 2003). For each region, we defined
185 the lowest average elevation of the tree line minus 100 m as the threshold for selecting plots, thus allowing a
186 certain amount of variation in the lowest elevations of the tree line.

187 To restrict our data to alpine grasslands only, we removed plots containing tree species > 2 m in height (e.g.
188 transitional vegetation at the tree line), and plots with > 50% cover of dwarf-shrubs (shrublands dominated by
189 genera *Rhododendron*, *Juniperus*, *Calluna*, *Bruckenthalia*, *Vaccinium*, *Erica*, etc.). Using the assignment of
190 plots to vegetation types (available in > 70% of cases) and an inspection of species composition, we also
191 removed plots recorded in wetlands (mires) and rocky outcrops. Taxonomical names were standardized at the
192 species level using The Plant List with the taxonstand R package (Cayuela et al., 2012), and then revised by
193 experts. Species from the genera *Alchemilla*, *Hieracium*, *Pilosella* and *Taraxacum* (with apomictic
194 reproduction and difficult identification), as well as nonvascular plants and lichens, were removed. The final
195 dataset consisted of 16,804 plots with 2,838 vascular plant species (from 3,334 taxa originally recorded), which
196 represent a habitat-specific species pool (Cornell & Harrison, 2014; Zobel, 2016) of alpine grasslands in the
197 study regions. To evaluate the consistency of the regional pool size under different sampling strategies, we

198 used interpolation and extrapolation techniques implemented in iNEXT (Hsieh et al., 2016). The data showed
199 good sampling coverage (Supporting Information Table S2.2, Figure S2.1) while two estimates based on equal
200 sample size and equal sampled areas provided similar values (Supporting Information Table S2.3) and the
201 same results in our models. We, therefore, assumed a nearly complete sampling and kept regional observed
202 richness as the response variable.

203 **Species data**

204 To distinguish between alpine species and non-alpines, we evaluated the elevation preferences of all species
205 using the approach of Gottfried et al. (2012). This approach uses expert assessment and regional literature to
206 define the lower, central and upper elevational parts of species ranges in five belts: colline (co), montane (mo),
207 tree line ecotone or subalpine (tl), alpine (al) and subnival/nival (nl). As in Gottfried et al. (2012), the
208 classification of vegetation belts from Mediterranean mountains was assimilated into this scheme. In most
209 cases, species evaluated by experts were classified similarly in different regions. In case of divergence (i.e.
210 species assigned to different elevational ranges in two or more regions), we kept the largest possible elevation
211 range. As a result, we defined 791 species (28% of the species pool) as alpine species, which are mainly
212 distributed (or restricted to) the alpine belt. The other 2,047 species (72%) were considered non-alpines
213 because the centre of their elevational distribution lies below the tree line (see Data Accessibility Statement
214 for the species data).

215 **Predictors**

216 We selected 12 predictors hypothesized to influence the size and composition of regional species pools and
217 divided them into four conceptual groups (Table 1). To estimate the current alpine area for each region, we
218 calculated the area above each regional tree line using an elevation model in ARCGIS 10.6 (ESRI, Redlands,
219 CA). We also estimated the extent of grasslands above the tree line from the maximum normalized difference
220 vegetation index (NDVI) value recorded between 2013 and 2020 for each pixel using Landsat 8 images with
221 Google Earth Engine, as explained in Testolin et al. (2020). For all regions, we defined alpine grasslands as
222 areas with maximum NDVI values between .25 and .75 (assuming values < .25 and > .75 corresponded to
223 rocky areas and shrubs, respectively), based on Landsat interpretation from the Cantabrian mountains (authors'
224 unpublished data) and the Alps (Choler, 2015; Fontana et al., 2008). To describe topography, we used an

225 estimate of surface roughness, that is, the mean topographic ruggedness for each region, based on Riley's index
226 (Evans et al., 2014).

227 Climatic variation across regions was reduced to the first two axes of a principal component analysis (PCA)
228 computed with 19 bioclimatic variables extracted for cells of c. 1-km resolution within our alpine areas using
229 the Climatologies at high resolution for the earth's land surface areas (CHELSA, Karger et al., 2017). The first
230 axis (PC1, 35% of explained variation) was correlated with variables reflecting temperature gradients, whereas
231 the second axis (PC2, 24%) was positively correlated with precipitation (Supporting Information Figure S3.2,
232 Table S3.4). We used the centroid of each region along PC1 and PC2 as a descriptor of its climate. The third
233 axis (15%) was not considered since a preliminary test showed no effects on the variation of species pools. We
234 also calculated the percentage of calcareous bedrock for each region (Ca bedrock) by overlapping alpine
235 patches with the lithological units referred to calcareous substrates in the International Hydrogeological Map
236 of Europe, scale 1:500,000 (BGR & UNESCO, 2019). As an estimate of the pH variability within each region,
237 we calculated the standard deviation of soil pH (averaged values from 5 and 15 cm depths) across all alpine
238 patches using the soil pH layer from SoilGrids at 250-m resolution (Hengl et al., 2017). We measured the
239 current connectivity of alpine islands using three non-correlated metrics that showed significant effects on
240 species pool sizes in a previous analysis: clumpiness index (CLUMPY), which ranges from -1 (patches fully
241 disaggregated) to 1 (maximum clumped); proximity mean (PROXmn) as the sum of patch area (m²) divided
242 by the nearest edge-to-edge distance squared (m²) among patches using a distance of 100 m; and connectance
243 index (CONNECT) as the proportion of functional joining among patches based on a distance of 100 m. Our
244 threshold of 100 m assumes that dispersal events mostly occur below this distance in alpine environments, but
245 relative differences among regions in terms of these two indices were similar when using other distances
246 between 10 and 1,000 m. These metrics were calculated in FRAGSTATS (McGarigal et al., 2012).

247 As an estimate of regional isolation, we first calculated the minimum distance between a focal region and all
248 other regions in the LGM with the Cost distance function in ARCGIS, prioritizing corridors at medium
249 elevation and penalizing coastal areas and the highest summits (Supporting Information Figure S4.3). All
250 distances were calculated over the shape of inland areas during the LGM, thus including terrestrial routes to
251 Corsica and Sicily. The resulting predictor (Distance) is a measure of "snapshot isolation" (Flantua et al., 2020)
252 as the degree of isolation in the LGM. We also calculated cost distances for the present time, but these showed

253 very similar values (Pearson's $r > .93$; $p < .001$; without Corsica and Sicily), indicating that relative distances
254 among regions are comparable in both periods. To analyse temporal variability of isolation, we calculated: (a)
255 the extent of non-glaciated alpine areas in the LGM (Area.LGM), predicting that larger alpine areas were more
256 likely to receive immigrants from other regions; and (b) the difference between LGM and current alpine areas
257 (Area.change) to reflect spatial changes driven by temperature fluctuations (Flantua et al., 2020). Area.LGM
258 was based on the assumption that the tree line was 1,000 m below current regional elevation, following
259 palaeobotanical evidence from tree line shifts (Burga, 2000; Tinner, 2013).

260 **Data analysis**

261 Statistical analyses were performed in R version 3.6.3 (R Core Team, 2020). We first computed island species–
262 area relationships (ISARs) for all regions using the Arrhenius power function (Arrhenius, 1921) with the sars
263 package (Matthews et al., 2019). Since the regions of the Alps are much larger than others, and not fully
264 independent from each other, we re-computed ISARs for the subset of regions ($n = 18$) without the Alps.

265 We evaluated the relative effects of area and other predictors on regional richness using generalized linear
266 mixed models (GLMMs) and a Poisson error distribution with the lme4 package (Bates et al., 2015). Given
267 our relatively small sample size, we first identified the variable within each group (as in Table 1) that explained
268 the highest amount of deviance in univariate models. The four best-fitting variables were combined in a multi-
269 factor GLMM after scaling the predictors with the 'scale' R function to ensure model convergence and to make
270 the effect sizes comparable. Model performance was evaluated with the Akaike information criterion corrected
271 for small sample sizes (AICc) and the marginal (mR²) and conditional (cR²) R² values, using the 'dredge'
272 function in the MuMin package (Bartoń, 2020) to select the simplest model with $\Delta\text{AICc} < 2$. We then computed
273 a second GLMM to compare the effect of area with the second-best predictors identified in the univariate
274 models, to test the relative effect of other determinants and their potential effects on species subsets. Different
275 GLMMs were fitted to all combinations of regional subsets (all regions and without the Alps) and species
276 subsets (all, alpine species and non-alpines) for a total of six model sets. The regions were grouped into macro-
277 regions (Alps, Carpathians, Iberian Peninsula, Italian Peninsula and the Balkans) as a random factor level to
278 consider geographical constraints.

279 We calculated floristic dissimilarities between regions based on the Simpson index (β_{sim}), a metric
280 independent of species richness, using the 'beta.pair' function in the R package betapart (Baselga & Orme,
281 2012). We further investigated the relative importance of predictors on β_{sim} distances using generalized
282 dissimilarity modelling (GDM) with the 'gdm' function in the R package gdm (Fitzpatrick et al., 2020). The
283 matrix of cost distances among regions (Distance) was used as the geographical component, while the two
284 climatic PCA axes were used as environmental predictors. The other predictors were tested in an exploratory
285 analysis but did not contribute significantly and were excluded. All GDMs were computed using a stepwise
286 procedure with 100 permutations to keep only significant predictors ($p < .05$). The relative importance of
287 selected variables was estimated with the 'varImp' function in the gdm package. To estimate past dispersal
288 routes among regions, we used the 'bgdispersal' function (Legendre & Legendre, 2012) in vegan (Oksanen et
289 al., 2016). This function calculates a coefficient of dispersal direction (DD) between each pair of regions that
290 increases when (a) the number of shared species between two regions is large; and (b) the number of unique
291 (non-shared) species in the first region is substantially larger than the number of unique species in the second
292 region. The coefficient is not influenced by richness and uses a corrected version of the McNemar test for
293 calculating the significance of asymmetry between pairs of regions (Legendre & Legendre, 2012). We used
294 the DD2 version to give double weight to the number of shared species, as in the Sørensen coefficient. Using
295 DD1 (with single weights as in Jaccard coefficient) provided lower absolute values but the same results. DD2
296 coefficients were calculated only for regions less than 1,000 km apart to focus on dispersal routes within
297 southern peninsulas, or between the Alps and the Carpathians and nearby regions. The significant coefficients
298 for each focal region were summed to estimate the net effect of positive (outbound) and negative (inbound)
299 connections. To evaluate geographical trends, we calculated Pearson's r correlations between DD2 absolute
300 values and the latitudinal and longitudinal differences of pairwise regional centroids.

301

302 **Results**

303 Observed richness (Supporting Information Table S2.3) ranged from 75 to 801 species (mean = 459; median
304 = 514; $n = 23$), including 29 to 301 (mean = 148; median = 156) alpine species and 41 to 622 (mean = 311;
305 median = 343) non-alpines. The Dinarides region had the highest richness for all species and non-alpines,

306 followed by the Western Alps and the Scardo-Pindic regions. The highest number of alpine species was found
307 in the Western Alps, followed by the Dinarides and the Eastern Alps.

308 According to the ISARs, current alpine area had a significant effect on regional pool sizes (Figure 2), with the
309 best fit for alpine species ($R^2 = .69$; $p < .01$ for c and z estimates), followed by all species ($R^2 = .59$; $p < .001$
310 for c and z) and non-alpines ($R^2 = .39$; $p < .01$ for c and z). For the 18 regions without the Alps, the differences
311 between subsets were less pronounced, with better fit for all species ($R^2 = .63$; $p < .01$ for c , $p < .001$ for z)
312 than for non-alpines ($R^2 = .56$; $p < .05$ for c , $p < .001$ for z), and alpine species ($R^2 = .53$; $p < .05$ for c , $p <$
313 $.001$ for z).

314 In the univariate GLMMs fitted to the whole dataset (Supporting Information Table S5.5), alpine area was the
315 best predictor as measured by lowest AICc and highest mR^2 . Within the other groups of predictors, the best
316 performing variables across the three datasets were the total alpine areas during the LGM, connectance, and
317 the proportion of calcareous bedrocks. These predictors showed significant effects in the GLMMs computed
318 in combination with area, reaching mR^2 values from .41 to .46 (Table 2). For the whole dataset, calcium
319 bedrock and connectance had the strongest effects, while for the subset without the Alps the most important
320 predictor was the alpine area (Figure 3a). There were relevant differences among the two subsets of species,
321 with stronger effects of area and Area. LGM on alpine species, and stronger effects of connectance for non-
322 alpines.

323 The second set of GLMMs was computed with alpine area together with roughness, distance and PC2 climate,
324 as they were the three second-best predictors in the univariate models (Supporting Information Table S5.5).
325 New models (Table 3) showed higher AICc and thus lower performance than the previous models (Table 2).
326 Besides a general positive effect of alpine area, alpine species were most affected by roughness and by the
327 distance to the nearest mountain regions, while non-alpines were influenced by PC2 climate, that is,
328 precipitation gradients (Figure 3b), a variable that was not even included in the models for alpine species.
329 These results were consistent for all regions and the subset of regions without the Alps.

330 The GDMs showed higher relative importance of distance between alpine regions than climatic gradients (PC1
331 and PC2), with remarkable differences among the three datasets (Table 4). Total explained deviance reached
332 almost 70% for all species and alpine species, but 41% for non-alpines. For alpine species, 21% of regional

333 dissimilarity was explained by the precipitation gradient (PC2), whereas for non-alpine species the only
334 predictor with significant effects was Distance. The GDMs for all species also showed much stronger effects
335 of Distance than precipitation (PC2).

336 The number of significant connections (Figure 4) for dispersal distance (DD₂) was slightly higher for the
337 subset of all species (78) than non-alpines (72) and alpine species (68). However, the average weight of
338 connections was higher for alpine species (.14) than for the other subsets (.10 for all species and for non-
339 alpines). For alpine species, the strongest connections were found between the Pyrenees and the rest of the
340 Iberian Peninsula, and between the Alps and the closest regions. The Alps and the Pyrenees were also the most
341 important sources of alpine species, while the Dinarides had the highest number of net outbound connections
342 for all species and non-alpines. The values of DD₂ were correlated with latitudinal differences between
343 regions for all species (Pearson's $r = -.31; p = .005$), alpine species ($r = -.31; p = .008$) and non-alpines ($r = -.27;$
344 $p = .021$); but not with longitudinal differences ($p > .05$ in the three datasets).

345

346 **Discussion**

347 **Determinants of species pool sizes**

348 This study analyses the drivers of species pool sizes in mountain regions with comparable vegetation and a
349 common biogeographical and evolutionary history (Schmitt, 2009; Vargas, 2003). Therefore, a basic
350 expectation across the study regions is that the extent of alpine areas has a dominant effect on regional pool
351 sizes, that is, a pure area effect (Matthews et al., 2016). Our results provide partial support to this expectation
352 as they show that ISARs are well fitted by a power function computed for habitat-specific regional pools,
353 indicating that area matters to explain plant species richness in the study regions, with a stronger effect on
354 alpine species. Nevertheless, our results were influenced by the disproportionately larger areas of the five
355 regions defined in the Alps, equalling area effects on alpine species and non-alpines in the ISARs computed
356 without the Alps. This supports the view of southern European mountains as current interglacial refugia for
357 cold-adapted plants, with the Alps representing the largest reservoir of alpine species (Birks & Willis, 2008;
358 Frate et al., 2018) as a function of the current larger extent of alpine areas. Together with area effects, our
359 models support that the proportion of calcareous bedrock, the connectivity of alpine habitat within regions and

360 the extent of alpine areas in the LGM are major determinants of species pool sizes, with consistent effects
361 when analysing all regions and the subset without the Alps. The effect of calcareous bedrock was especially
362 relevant in the whole dataset, in agreement with the highest species richness known to occur in base-rich
363 substrates (Ewald, 2003) and the relatively larger number of vegetation types found in calcareous-rich habitats
364 (Virtanen et al., 2002). In contrast with our initial prediction, we found that connectivity of alpine patches has
365 a negative effect on species richness, with a stronger effect on non-alpines. The regions with the highest
366 connectivity (Baetic System, South Apennines, Balkans) have relatively smooth relief and a lower number of
367 summits connected along continuous ranges. This spatial configuration might decrease habitat heterogeneity
368 and the number of niches available for cold-adapted species, favouring a larger proportion of non-alpines from
369 lower elevations through a mass-effect metacommunity process (Leibold et al., 2004). In fact, the richness of
370 non-alpines positively increased with distance among regions, indicating that the main sources of dispersal
371 come from the surrounding mountain flora. In contrast, the pools of alpine species decreased at larger distances
372 from other mountain regions, supporting dispersal limitation and the effect of regional isolation predicted to
373 occur in alpine habitats (i.e. snapshot isolation, Flantua et al., 2020).

374 In contrast with our expectation, we found negative effects of the extent of LGM alpine areas on species pool
375 sizes, probably due to the LGM presence of large ice shields in the richest mountains, especially the Alps and
376 Pyrenees. Although the LGM was a relatively short glacial period, and for most of the glacial era the ice shield
377 was smaller, the ice blocks were unoccupied by plants, with the exception of in-situ glacial refugia
378 (Holderegger & Thiel-Egenter, 2009). Since the effect of LGM area was negative for all the datasets, but
379 univariate models showed slightly positive effects on regional richness, our results are not conclusive enough
380 to interpret this determinant thus new research will need to consider LGM areas at a finer scale (e.g. species
381 richness from single mountaintops). We also found a positive effect of topographic roughness on species pool
382 sizes, supporting that heterogeneity of alpine landscapes favours multiple niches and microrefugia (Graae et
383 al., 2018). This effect was especially relevant for alpine species, suggesting that regions with more topographic
384 heterogeneity facilitate local refugia during glacial periods, as predicted by genetic data (Christe et al., 2014).
385 Interestingly, we did not find any effect of temperature gradients across regions, likely because our study
386 system is already filtered on low-temperature areas. Although regional pool sizes responded to regional
387 precipitation regimes, this effect was only significant for non-alpines as an effect of general floristic variation

388 across regions. This supports the hypothesis that topographical refugia determine the persistence of alpine
389 species in spite of macroclimatic conditions (Dullinger et al., 2012; Frate et al., 2018).

390 **Determinants of species pool composition**

391 Among our set of predictors, geographical distance and a climatic gradient of precipitation were the only
392 drivers of species pool dissimilarities. The influence of distance supports the idea that dispersal limitation
393 shaped the configuration of species pools across the study regions. Distance was also the main determinant for
394 non-alpines, reflecting the dominant role of geography in determining floristic dissimilarities across mountain
395 floras (Ozenda, 1985). Although the composition of non-alpine species is also expected to respond to climatic
396 gradients at the continental scale (Jiménez- Alfaro, Suárez-Seoane et al., 2018), we note that our set of non-
397 alpines is limited to the species that are able to thrive in alpine habitats (i.e. a subset of the mountain floras).
398 In contrast, alpine species, which are mainly distributed in the alpine zone, were further filtered by current
399 precipitation gradients. Indeed, the study system is characterized by a north–south gradient from temperate
400 climates with wet summers to Mediterranean mountains with a dry season (Nagy, 2006; Pauli et al., 2012). In
401 consequence, the postglacial reorganization of alpine pool composition was regulated by precipitation, an
402 effect that may also reflect the length of the snow free season (Choler, 2015). This agrees with the view of
403 alpine habitats as cold systems that are mostly differentiated by humidity gradients at global and continental
404 scales (Billings, 1974; Testolin et al., 2020).

405 Assuming that alpine species are unlikely to disperse among mountain regions in the current interglacial, the
406 influence of regional isolation supports past dispersal events as a major determinant of regional pool
407 composition (Gehrke & Linder, 2014; Hadley, 1987). Our estimates of dispersal direction further suggest that
408 the more plausible dispersal routes between regions had a north–south direction, with stronger connections for
409 alpine species than for non-alpines. This can be explained by the spatial configuration of the southern
410 peninsulas, where alpine habitats represent current climatic refugia for alpine grasslands (Birks & Willis, 2008;
411 Feliner, 2011). The results for the three species datasets also indicate stronger connections originating from the
412 Pyrenees and the Alps, validating their role as major centres of alpine plant diversity (Schmitt, 2009). We also
413 found a key role of the Dinarides as a donor of mountain flora in the Balkans and nearby regions (including
414 the Italian Peninsula), as has been widely recognized (Ozenda, 1985; Redžić, 2011). Although our results are

415 in line with the known dispersal routes of European alpine plants, past connections occurred much before the
416 LGM, and the interplay of dispersal, isolation and vicariance is still unresolved given the lack of phylogenetic
417 data (Kropf et al., 2006; Schmitt, 2009).

418 Our results on dispersal directions also suggest a prominent role of stepping-stones for long-distance dispersal
419 (Saura et al., 2014), by which medium-size regions may have acted as hubs in glacial expansions and as
420 reservoirs of alpine flora during interglacial contractions. This might be the case for regions with high net
421 connections with respect to their size, like the Cantabrian mountains (Spain), the Rila-Rhodope massif
422 (Bulgaria) and the Southern Carpathians (Romania). Indeed, phylogeographical studies have traced continuous
423 gene flow between the Cantabrian range and the Pyrenees (Kropf et al., 2003) and a key role of the Carpathian
424 mountains in long-term migrations of high-mountain plants within Eurasia (Ronikier, 2011). We note, however,
425 that our approach is based on the number of shared species among regions and a-priori assumptions about their
426 implication on past dispersal, thus we lack proper evolutionary links among species lineages (Schmitt, 2009;
427 Vargas, 2003), or information about macrofossil remains (Birks, 2019). Despite this, our results reinforce the
428 general view that alpine habitats from southern Europe are interglacial refugia for cold-adapted plants (Felinier,
429 2011) formerly connected by dispersal but currently isolated from each other.

430

431 **Conclusions**

432 Besides demonstrating area effects on species pool sizes, we found a key role of edaphic, topographic and
433 spatio-temporal factors linked with the post-glacial retreat of alpine grasslands. These factors may even
434 outcompete area effects, supporting the need to use multiple determinants to understand alpine habitats as
435 isolated systems (Flantua et al., 2020; Itescu, 2019). We also found that the size and composition of regional
436 pools are better understood when subsets of species with different ecological profiles are distinguished. Thus,
437 the regional pools of alpine species seem to be primarily driven by alpine areas, topographic heterogeneity and
438 regional isolation, but less so by temperature gradients across regions. Although climate-driven extirpations of
439 relict populations may still occur (Jiménez-Alfaro et al., 2016), our results are in line with the idea that full
440 species extinctions of alpine species are rare in interglacial periods (Birks, 2008), suggesting that the pools of
441 alpine species may have been relatively unaffected by extinction or immigration since the climate stabilized

442 around 10,000 yr BP. In contrast, the regional number of non-alpines might be more sensitive to climate, likely
443 explaining recent elevation shifts (Jiménez-Alfaro, Gavilán et al., 2014; Steinbauer et al., 2018), even though
444 their composition is determined by mountain regional floras. The central question of the theory of island
445 biogeography about the role of immigration (reaching equilibrium) versus the predominance of extinction
446 (non-equilibrium) might, therefore, be biased by the assumption that all species from the species pool respond
447 similarly to climatic oscillations (as it is essentially a neutral model). To develop a consistent framework for
448 understanding the diversity of alpine habitats, and to anticipate the effects of ongoing climate change, we
449 conclude that empirical research should contextualize the role of post-glacial determinants on regional species
450 pools and the different responses of species ecological groups.

451

452 **Data availability statement**

453 All the raw data and the R scripts necessary to replicate the analyses are accessible at
454 <https://doi.org/10.5061/dryad.0cfxpnw1h>. These datasets include plot species data for estimating regional
455 completeness, the full list of species and their elevation ranges, and regional predictors. The geographical
456 distribution of different estimates of alpine areas can be visualized at [https://torpignattaro.users.earth
457 engine.app/view/european-alpine-areas](https://torpignattaro.users.earthengine.app/view/european-alpine-areas)

458

459 **Tables**

460 **Table 1.** Main predictions and measurements for 12 variables selected as potential determinants of regional
 461 pool sizes (S) and composition as a function of taxonomical dissimilarity (D) in European alpine grasslands.
 462 The variables are aggregated into four major groups to compare the effects of area and topography,
 463 environment, connectivity of alpine patches and regional isolation. Abbreviations: LGM, Last Glacial
 464 Maximum; NDVI, normalized difference vegetation index; PC, principal component.

Variable	Predictions	Measurement
<i>Area and topography</i>		
Alpine area	Larger areas have higher habitat heterogeneity and lower extinction rates, increasing S.	Current extent of alpine grasslands estimated above the regional tree line based on NDVI at 30 m × 30 m.
Roughness	Higher roughness favours micro-niche variation and topographical refugia, increasing S.	Topographic roughness estimated for alpine areas using a digital elevation model at 250 m × 250 m.
<i>Environment</i>		
PC1 climate	Higher temperature provides energy and increases S. Variation drives D between warm and cold regions.	First climatic gradient representing mean and minimum temperature across regions.
PC2 climate	Water availability and the lack of drought season increases S. Variation drives D between wet and dry regions.	Second climatic gradient representing annual and summer precipitation across regions.
Ca bedrock	Calcium bedrocks favour the availability of calcium and magnesium, increasing S. Regional variation drives D.	Percentage of calcareous bedrocks in regional alpine patches estimated from lithological maps.
pH variation	High pH variation implies regional heterogeneity of soil composition, increasing S.	Standard deviation of pH values in a region extracted from soil models at 250 m × 250 m.
<i>Connectivity of alpine patches</i>		
Clumpiness	High clumpiness indicates clustering of alpine islands, reducing isolation, increasing persistence and S.	Disaggregation (-1) or contagion (1) of alpine patches in relation to a random distribution (0).
Proximity	High proximity index among patches favour dispersal events within a region and increases S.	A measure of isolation for all alpine patches in a region using a reference distance of 100 m (mean and area-weighted values).
Connectance	High connectance reduces the number of isolated alpine patches, increasing S.	Percentage of the maximum possible connections among alpine patches at distance of 100 m.
<i>Regional isolation</i>		
Distance	Larger distances to other mountain regions reduce dispersal and increase local extinctions, reducing S and increasing D.	Pairwise minimum distances among regions based on cost distances to optimize mountain routes.
Area.LGM	Larger alpine area in the LGM reduces temporal isolation and benefits from past area effects, increasing S and decreasing D.	Extent of total alpine areas in the LGM estimated by decreasing tree line elevation, removing glaciated areas.
Area change	Large post-glacial changes in alpine areas increase temporal variability in isolation, decreasing S.	Change between Area.LGM and current total alpine areas above the regional tree line.

465

466

467 **Table 2.** Results of generalized linear mixed models (GLMMs) testing the effect of alpine area and the best
 468 performing variables for isolation (Area.LGM), patch connectivity (Connectance) and soil composition
 469 (Ca.Bedrock) to explain regional pool sizes in European alpine grasslands. The analyses were computed for
 470 the whole dataset and a subset of regions without the Alps. Numbers for variables show the z statistic of model
 471 significance. mR^2 and cR^2 indicate the fit of the models without (marginal) and with (conditional) random
 472 effects, respectively. Abbreviations: AICc, Akaike information criterion corrected for small sample sizes;
 473 LGM, Last Glacial Maximum. *** $p < .001$. ** $p < .01$.

	Area	Area. LGM	Connectance	Ca. Bedrock	mR^2	cR^2	AICc
All data ($n = 23$)							
All species	6.4***	-12.6***	-14.9***	19.0***	.45	.72	796
Alpine species	6.1***	-7.9***	-7.4***	10.4***	.45	.67	388
Non-alpines	3.2**	-9.7***	-13.3***	15.7***	.46	.73	632
Without the Alps ($n = 18$)							
All species	14.7***	-17.9***	-12.5***	14.8***	.41	.76	571
Alpine species	12.4***	-12.4***	-5.5***	5.9***	.41	.80	296
Non-alpines	8.6***	-11.6***	11.5***	13.5***	.43	.73	515

474

475

476 **Table 3.** Results of generalized linear mixed models (GLMMs) testing the effect of alpine area and a selection
 477 of second- best predictors related to topography (Roughness), isolation (Distance) and environment [principal
 478 component 2 (PC2) climate, reflecting precipitation gradients] to explain regional pool sizes in European alpine
 479 grasslands. The analyses were computed for the whole dataset and a subset of regions without the Alps.
 480 Numbers for variables show the z statistic of model significance. mR2 and cR2 indicate the fit of the models
 481 without (marginal) and with (conditional) random effects, respectively. Abbreviation: AICc, Akaike
 482 information criterion corrected for small sample sizes. ***p < .001. **p < .01.

	Area	Roughness	Distance	PC2 climate	mR ²	cR ²	AICc
All data (n = 23)							
All species	9.7***	4.0**		-15.4***	0.36	0.46	1,277
Alpine species	4.0***	7.2***	-7.7***		0.48	0.48	559
Non-alpines	7.5***		3.9***	-14.9***	0.32	0.56	1,003
Without the Alps (n = 18)							
All species	11.8***	7.4***		-5.3***	0.39	0.43	1,079
Alpine species	9.1***	8.8***	-4.5***		0.41	0.54	391
Non-alpines	9.7***	3.1**	4.1***	-6.6***	0.34	0.47	903

483

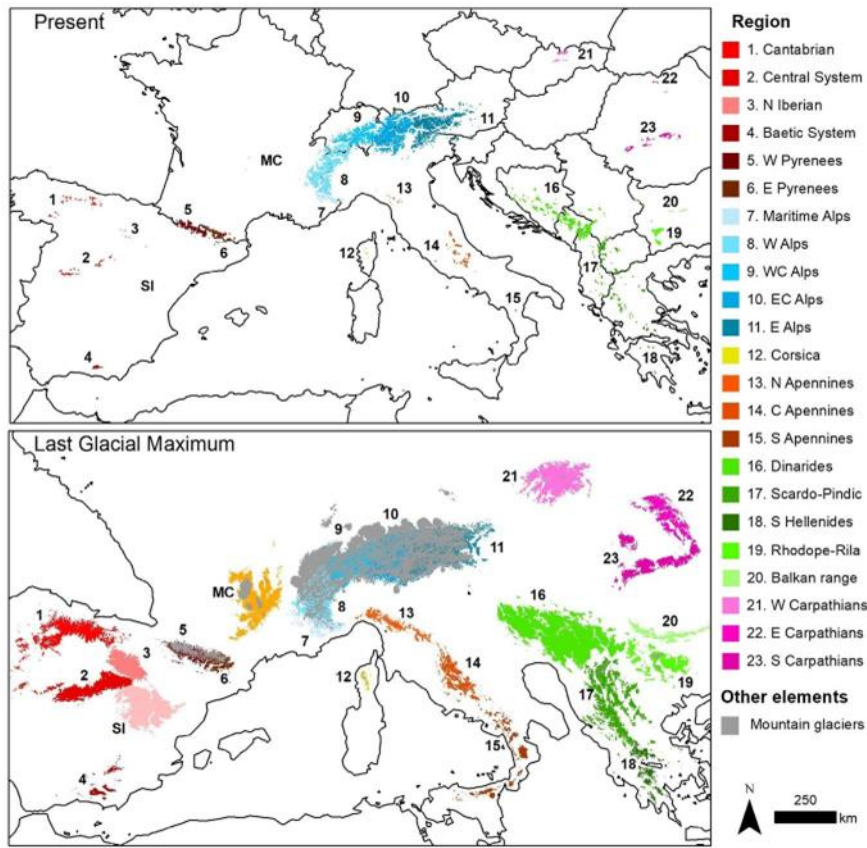
484

485 Table 4. Results of generalized dissimilarity models (GDMs) testing compositional differences of regional
486 pool composition across European alpine grasslands. Numbers indicate variable importance of predictors.
487 Principal component axes 1 (PC1) and 2 (PC2) reflect temperature and precipitation gradients, respectively.
488 ***p < .001. nsp > .05.

	Distance	PC1 climate	PC2 climate	Explained deviance (%)
All species	51.4***	1.1 ^{ns}	14.0***	63.7
Alpine species	36.9***	2.5 ^{ns}	21.5***	72.0
Non-alpines	75.0***	2.0 ^{ns}	0.9 ^{ns}	40.9

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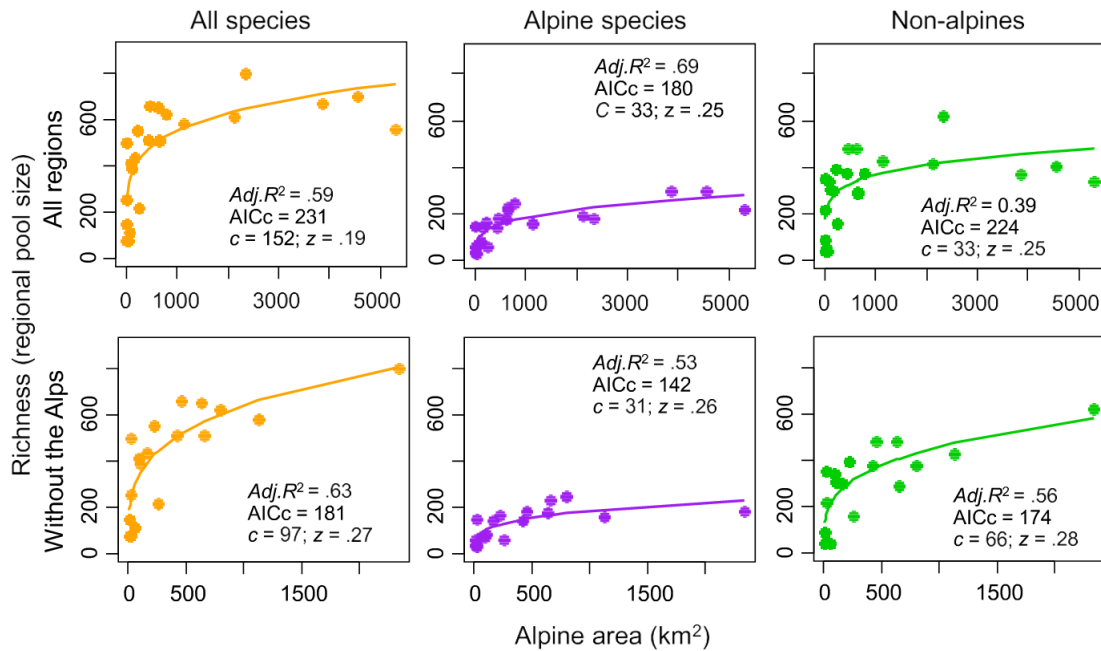
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492

493 **Figure 1.** Geographical distribution of 23 regions with alpine grasslands in Europe. The extent of alpine
 494 grasslands was estimated for the current interglacial (Present) using elevation thresholds of regional tree lines
 495 and a filter based on normalized difference vegetation index (NDVI) values to exclude woody vegetation and
 496 rocky outcrops. The extent of total alpine areas for the Last Glacial Maximum (LGM) was obtained by
 497 decreasing regional tree line elevation by 1,000 m. The Massif Central (MC) and Southern Iberian mountains
 498 (SI) are shown for completeness but not included in the study because they currently do not have a
 499 vegetation belt with alpine grasslands. Extent of inland areas in the LGM was obtained from Worldclim
 500 historical climatic data (www.worldclim.org). The extent of glaciers in the LGM are based on Ehlers et al.
 501 (2011).

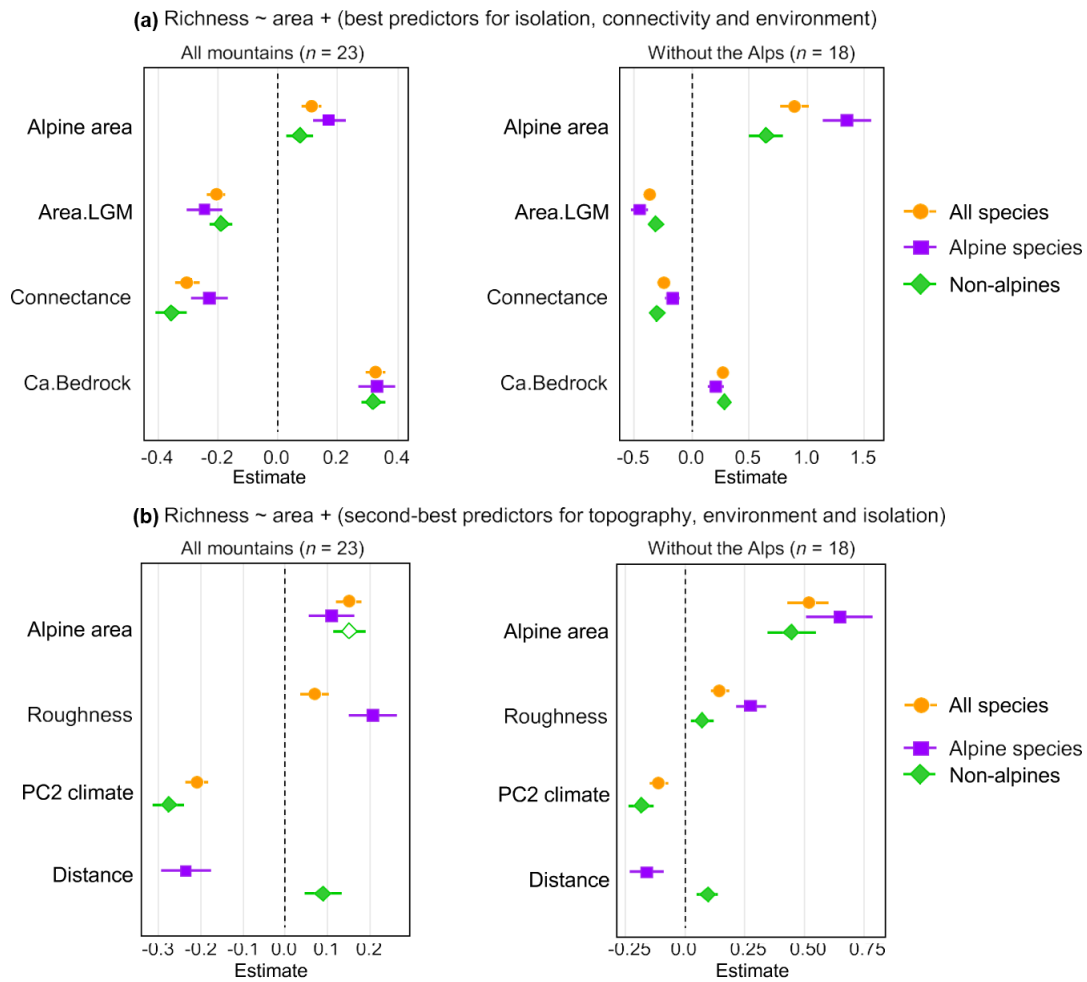
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503

504 **Figure 2.** Island species–area relationships (ISARs) computed for all study regions ($n = 23$) and for a subset
 505 without the Alps ($n = 18$). ISARs were calculated with a power function ($S = cAz$) where S is the number of
 506 species, A is the area, and c and z are the fitted parameters. The goodness of fit of the equations is presented
 507 for each ISAR curve with the adjusted R^2 and the Akaike information criterion corrected for small sample
 508 sizes (AICc).

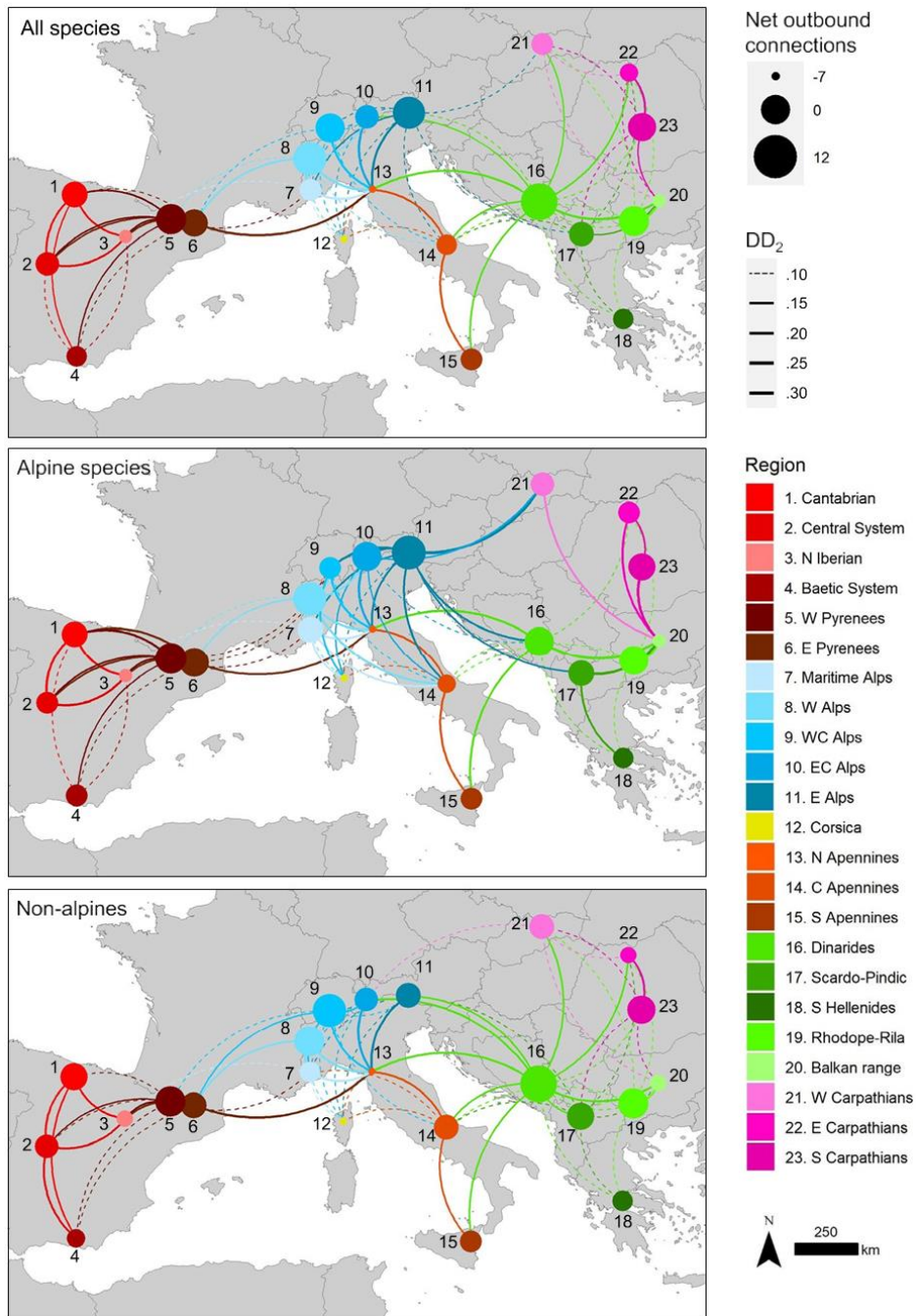
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510

511 **Figure 3.** Coefficient estimates (slopes) derived from generalized linear mixed models (GLMMs) fit to the
 512 species pool size (Richness) of European alpine grasslands, for all mountains and a subset without the Alps.
 513 Model (a) includes alpine area and the best predictors selected within variable groups reflecting regional
 514 isolation (Area.LGM), connectivity (Connectance) and environment (Ca.Bedrock). Model (b) includes alpine
 515 area, topography and the second-best predictors with a significant effect on species richness. LGM = Last
 516 Glacial Maximum; PC2 = second axis of principal component.

517



518

519 Figure 4. Coefficients of dispersal direction (DD₂) calculated for pairs of European regions using species
 520 pool composition from alpine grasslands. DD₂ reflects the strength of significant pairwise connections
 521 between regions. Outbound links have the same colour as the source (i.e. when dispersal direction is
 522 estimated from region A to region B, the link is coloured as A). Circle size is proportional to the number of
 523 net outbound connections (i.e. positive dispersal directions).

524

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