

RESEARCH PAPER

Post-glacial determinants of regional species pools in alpine grasslands

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Abstract

Aim: Alpine habitats support unique biodiversity confined to high-elevation areas in the current interglacial. Plant diversity in these habitats may respond to area, environment, connectivity and isolation, yet these factors have been rarely evaluated in

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concert. Here we investigate major determinants of regional species pools in alpine grasslands, and the responses of their constituent species groups.

Location: European mountains below 50° N.

Time period: Between 1928 and 2019.

Major taxa studied: Vascular plants.

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

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

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

KEYWORDS

alpine grasslands, area effects, Europe, glaciations, island biogeography, neutral theory, species pools, species richness, species–area relationships

1 | INTRODUCTION

Alpine habitats occur globally in mountain areas between the climatic tree line and the snowline (if present), yet they are mainly distributed in extratropical regions (Körner et al., 2011; Testolin et al., 2020). Climatic history has had a lasting imprint on the biodiversity of these habitats (Kadereit et al., 2004; Schmitt et al., 2010) due to the cycles of glaciation and deglaciation that occurred during the Pleistocene (2.6 Ma – 12 ka BP). These cycles alternated long glacial ages with short and warm interglacial periods that resulted in the expansion and contraction of alpine-like habitats, respectively (Birks & Willis, 2008; Flantua et al., 2019). In temperate mountains, glacial cold periods are associated with the extinction of cold-sensitive species, while post-glacial warming produced local extinctions (extirpations or exterminations, Birks, 2019) of cold-adapted species due to physiological constraints and the upward expansion of forests and

competitive species (Birks, 2008). After the Last Glacial Maximum (LGM, 21 ka BP), climate warming triggered upslope shifts of cold-adapted species for the last time, and alpine habitats reached their current extent at the beginning of the Holocene, c. 11 ka BP (Tinner & Theurillat, 2003). The biodiversity we observe today in alpine habitats is thus the outcome of the post-glacial reorganization of cold-adapted species during the present interglacial (Hewitt, 1999).

The geographical extent of current alpine habitats has been recognized as a major determinant of alpine diversity (Hadley, 1987; Riebesell, 1982), following the ecological principle of species–area relationships applied to island-like habitats, that is, island species–area relationships (ISARs; Matthews et al., 2016). Since alpine habitats are generally located around mountaintops and surrounded by a matrix of non-alpine habitats (Itescu, 2019; Schmitt, 2009), they look like habitat islands (also known as sky islands) aggregated along mountain ranges in

networks or archipelagos (Flantua et al., 2020). This spatial configuration has prompted the investigation of alpine habitats under the theory of island biogeography, with a focus on the influence of area and isolation on species richness under an equilibrium between immigration and extinction (MacArthur & Wilson, 1967). However, the foundational theory developed for oceanic islands has evolved into a complex geodynamic system driven by evolutionary and ecological drivers (Whittaker et al., 2017). Using mountaintops of North America as a study system, Brown (1971) originally suggested a non-equilibrium scenario with few extinctions and low colonization rates, making high-elevation relict faunas relatively stable over the last 8,000 years. Intriguingly, the analysis of regional alpine floras has shown that area and isolation explain species richness and composition better in tropical (Gehrke & Linder, 2014; Sklenář et al., 2014) than in temperate regions (Hadley, 1987; Virtanen et al., 2002). It has been further suggested that other determinants (e.g. geology, habitat heterogeneity, or climate) should be taken into account when analysing alpine plant diversity, assuming a common biogeographical history among the compared units (Nagy & Grabherr, 2009).

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

Altogether, the aforementioned factors may explain the size and composition of regional pools that have persisted in alpine habitats during the current interglacial, and will likely play a role in their response to ongoing climate change (Malanson et al., 2019). For a better understanding of the main determinants of regional pools, we should consider that species inhabiting alpine habitats are heterogeneous in their ecological requirements (Jiménez-Alfaro, Marcenó et al., 2014; Klanderud, 2008). Many cold-adapted species are

specialists with physiological adaptations to cold conditions typical of low-temperature alpine habitats, or with low competitive abilities (Körner, 2003; Ross et al., 2010). Other cold-adapted species occurring in alpine habitats frequently have wide elevation ranges and a distribution centre below the tree line (Jiménez-Alfaro, Gavilán et al., 2014; Nagy & Grabherr, 2009). Such ecological differences may have shaped the diversity of regional species pools in response to their post-glacial spatial reorganization, leading to either elevation shifts (Dullinger et al., 2012) or extirpations (Jiménez-Alfaro et al., 2016). It is, therefore, expected that the determinants of species pools will differ between alpine species (those whose populations are mainly distributed above the tree line) and non-alpines (those that mainly occur at lower elevation but also can thrive in alpine habitats).

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

2 | METHODS

2.1 | Study system

We defined 23 geographical regions (Figure 1) as distinct biogeographical features (see Supporting Information Appendix S1). These regions cover the mid-latitude mountains of Europe (between 37° and 49° N), which have a comparable post-glacial history and related floras (Kadereit et al., 2004; Ozenda, 1985; Schmitt, 2009). We focused on alpine grasslands as the dominant habitats that occur above the climatic tree line (Testolin et al., 2020). These habitats are dominated by graminoids, typically grasses (Poaceae), sedges (Cyperaceae), rushes (Juncaceae), and several chamaephytes and forbs. They occupy relatively large areas in the Alps but small patches in the Iberian, Italian and Balkan peninsulas (Figure 1). According to the European vegetation classification (Mucina et al., 2016), the study system includes zonal vegetation related to acidophilous alpine grasslands (*Juncetea trifidi*), alpine or subalpine calcicolous swards (*Elyno-Seslerietea*), Mediterranean silicicolous fescue grasslands (*Festucetea indigestae*), dry calcicolous grasslands (*Festuco-Poetalia ligulatae*) and xeric grasslands (*Daphno-Festucetea*). Tundra-like vegetation from Scandinavia and Scotland was not included because

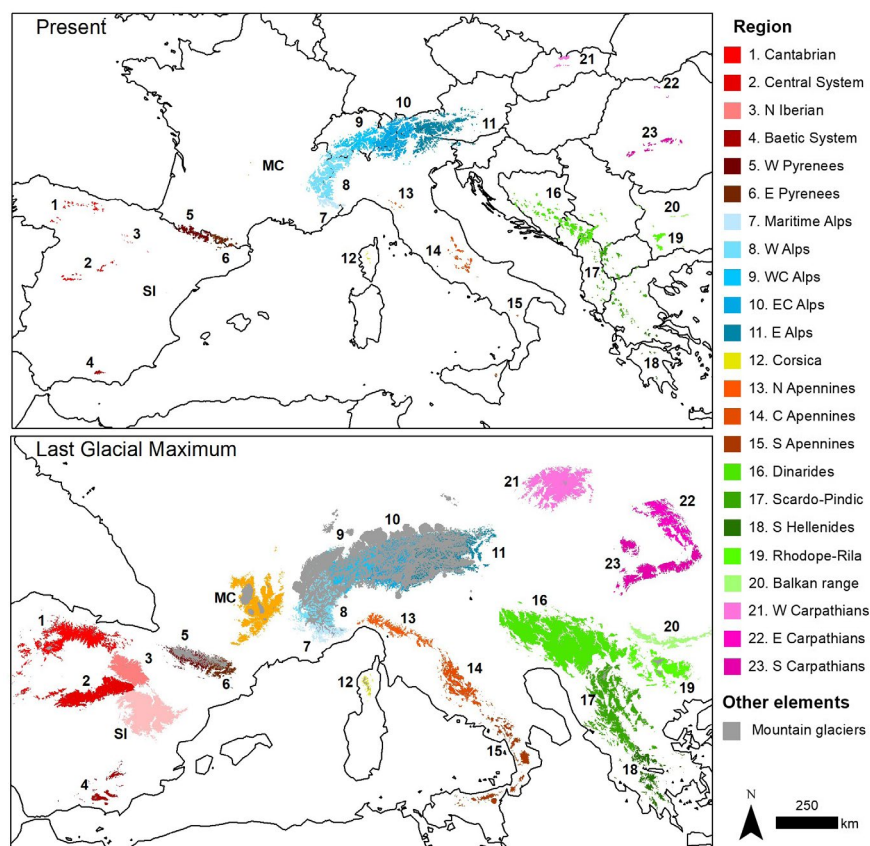


FIGURE 1 Geographical distribution of 23 regions with alpine grasslands in Europe. The extent of alpine grasslands was estimated for the current interglacial (Present) using elevation thresholds of regional tree lines and a filter based on normalized difference vegetation index (NDVI) values to exclude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

these regions have a different post-glacial history mostly driven by long-distance colonization (Birks & Willis, 2008).

2.2 | Data collection

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

To restrict our data to alpine grasslands only, we removed plots containing tree species > 2 m in height (e.g. transitional vegetation at the tree line), and plots with > 50% cover of dwarf-shrubs (shrublands dominated by genera *Rhododendron*, *Juniperus*, *Calluna*, *Bruckenthalia*, *Vaccinium*, *Erica*, etc.). Using the assignment of plots

to vegetation types (available in > 70% of cases) and an inspection of species composition, we also removed plots recorded in wetlands (mires) and rocky outcrops. Taxonomical names were standardized at the species level using The Plant List with the *taxonstand* R package (Cayuela et al., 2012), and then revised by experts. Species from the genera *Alchemilla*, *Hieracium*, *Pilosella* and *Taraxacum* (with apomictic reproduction and difficult identification), as well as non-vascular plants and lichens, were removed. The final dataset consisted of 16,804 plots with 2,838 vascular plant species (from 3,334 taxa originally recorded), which represent a habitat-specific species pool (Cornell & Harrison, 2014; Zobel, 2016) of alpine grasslands in the study regions. To evaluate the consistency of the regional pool size under different sampling strategies, we used interpolation and extrapolation techniques implemented in iNEXT (Hsieh et al., 2016). The data showed good sampling coverage (Supporting Information Table S2.2, Figure S2.1) while two estimates based on equal sample size and equal sampled areas provided similar values (Supporting Information Table S2.3) and the same results in our models. We, therefore, assumed a nearly complete sampling and kept regional observed richness as the response variable.

2.3 | Species data

To distinguish between alpine species and non-alpines, we evaluated the elevation preferences of all species using the approach of Gottfried et al. (2012). This approach uses expert assessment and regional

TABLE 1 Main predictions and measurements for 12 variables selected as potential determinants of regional pool sizes (S) and composition as a function of taxonomical dissimilarity (D) in European alpine grasslands. The variables are aggregated into four major groups to compare the effects of area and topography, environment, connectivity of alpine patches and regional isolation

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.

Abbreviations: LGM, Last Glacial Maximum; NDVI, normalized difference vegetation index; PC, principal component.

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

2.4 | Predictors

We selected 12 predictors hypothesized to influence the size and composition of regional species pools and divided them into four conceptual groups (Table 1). To estimate the current alpine area for each region, we calculated the area above each regional tree line using an elevation model in ArcGIS 10.6 (ESRI, Redlands, CA). We also estimated the extent of grasslands above the tree line from the maximum normalized difference vegetation index (NDVI) value recorded between 2013 and 2020 for each pixel using Landsat 8 images with Google Earth Engine, as explained in Testolin et al. (2020). For all regions, we defined alpine grasslands as areas with maximum NDVI values between .25 and .75

(assuming values $< .25$ and $> .75$ corresponded to rocky areas and shrubs, respectively), based on Landsat interpretation from the Cantabrian mountains (authors' unpublished data) and the Alps (Choler, 2015; Fontana et al., 2008). To describe topography, we used an estimate of surface roughness, that is, the mean topographic ruggedness for each region, based on Riley's index (Evans et al., 2014).

Climatic variation across regions was reduced to the first two axes of a principal component analysis (PCA) computed with 19 bioclimatic variables extracted for cells of c. 1-km resolution within our alpine areas using the Climatologies at high resolution for the earth's land surface areas (CHELSA, Karger et al., 2017). The first axis (PC1, 35% of explained variation) was correlated with variables reflecting temperature gradients, whereas the second axis (PC2, 24%) was positively correlated with precipitation (Supporting Information Figure S3.2, Table S3.4). We used the centroid of each region along PC1 and PC2 as a descriptor of its climate. The third axis (15%) was not considered since a preliminary test showed no effects on the variation of species pools. We also calculated the percentage of calcareous bedrock for each region (Ca bedrock) by overlapping alpine patches with the lithological units referred to calcareous substrates in the International Hydrogeological Map of Europe, scale 1:500,000 (BGR & UNESCO, 2019). As an estimate of the pH variability within each region, we calculated the standard deviation of soil pH (averaged values from 5 and 15 cm depths) across all alpine patches using the soil pH layer from SoilGrids at 250-m resolution (Hengl et al., 2017).

We measured the current connectivity of alpine islands using three non-correlated metrics that showed significant effects on species pool sizes in a previous analysis: clumpiness index (CLUMPY), which ranges from -1 (patches fully disaggregated) to 1 (maximum clumped); proximity mean (PROXmn) as the sum of patch area (m^2) divided by the nearest edge-to-edge distance squared (m^2) among patches using a distance of 100 m; and connectance index (CONNECT) as the proportion of functional joining among patches based on a distance of 100 m. Our threshold of 100 m assumes that dispersal events mostly occur below this distance in alpine environments, but relative differences among regions in terms of these two indices were similar when using other distances between 10 and 1,000 m. These metrics were calculated in FRAGSTATS (McGarigal et al., 2012).

As an estimate of regional isolation, we first calculated the minimum distance between a focal region and all other regions in the LGM with the Cost distance function in ArcGIS, prioritizing corridors at medium elevation and penalizing coastal areas and the highest summits (Supporting Information Figure S4.3). All distances were calculated over the shape of inland areas during the LGM, thus including terrestrial routes to Corsica and Sicily. The resulting predictor (Distance) is a measure of "snapshot isolation" (Flantua et al., 2020) as the degree of isolation in the LGM. We also calculated cost distances for the present time, but these showed very similar values (Pearson's $r > .93$; $p < .001$; without Corsica and Sicily), indicating that relative distances among regions are comparable in both

periods. To analyse temporal variability of isolation, we calculated: (a) the extent of non-glaciated alpine areas in the LGM (Area.LGM), predicting that larger alpine areas were more likely to receive immigrants from other regions; and (b) the difference between LGM and current alpine areas (Area.change) to reflect spatial changes driven by temperature fluctuations (Flantua et al., 2020). Area.LGM was based on the assumption that the tree line was 1,000 m below current regional elevation, following palaeobotanical evidence from tree line shifts (Burga, 2000; Tinner, 2013).

2.5 | Data analysis

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

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

We calculated floristic dissimilarities between regions based on the Simpson index (β_{sim}), a metric independent of species richness, using the 'beta.pair' function in the R package *betapart* (Baselga & Orme, 2012). We further investigated the relative importance of predictors on β_{sim} distances using generalized dissimilarity modelling (GDM) with the 'gdm' function in the R package *gdm* (Fitzpatrick et al., 2020). The matrix of cost distances among regions (Distance) was used as the geographical component, while the two climatic PCA axes were used as environmental predictors. The other predictors were tested in an exploratory analysis but did not contribute

significantly and were excluded. All GDMs were computed using a stepwise procedure with 100 permutations to keep only significant predictors ($p < .05$). The relative importance of selected variables was estimated with the 'varImp' function in the *gdm* package.

To estimate past dispersal routes among regions, we used the 'bg-dispersal' function (Legendre & Legendre, 2012) in *vegan* (Oksanen et al., 2016). This function calculates a coefficient of dispersal direction (DD) between each pair of regions that increases when (a) the number of shared species between two regions is large; and (b) the number of unique (non-shared) species in the first region is substantially larger than the number of unique species in the second region. The coefficient is not influenced by richness and uses a corrected version of the McNemar test for calculating the significance of asymmetry between pairs of regions (Legendre & Legendre, 2012). We used the DD₂ version to give double weight to the number of shared species, as in the Sørensen coefficient. Using DD₁ (with single weights as in Jaccard coefficient) provided lower absolute values but the same results. DD₂ coefficients were calculated only for regions less than 1,000 km apart to focus on dispersal routes within southern peninsulas, or between the Alps and the Carpathians and nearby regions. The significant coefficients for each focal region were summed to estimate the net effect of positive (outbound) and negative (inbound) connections. To evaluate geographical trends, we calculated Pearson's r correlations between DD₂ absolute values and the latitudinal and longitudinal differences of pairwise regional centroids.

3 | RESULTS

Observed richness (Supporting Information Table S2.3) ranged from 75 to 801 species (mean = 459; median = 514; $n = 23$), including 29 to 301 (mean = 148; median = 156) alpine species and 41 to 622 (mean = 311; median = 343) non-alpines. The Dinarides region had the highest richness for all species and non-alpines, followed by the Western Alps and the Scardo-Pindic regions. The highest number of alpine species was found in the Western Alps, followed by the Dinarides and the Eastern Alps.

According to the ISARs, current alpine area had a significant effect on regional pool sizes (Figure 2), with the best fit for alpine species ($R^2 = .69$; $p < .01$ for c and z estimates), followed by all species ($R^2 = .59$; $p < .001$ 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 between subsets were less pronounced, with better fit for all species ($R^2 = .63$; $p < .01$ for c , $p < .001$ for z) 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 , $p < .001$ for z).

In the univariate GLMMs fitted to the whole dataset (Supporting Information Table S5.5), alpine area was the best predictor as measured by lowest AICc and highest mR^2 . Within the other groups of predictors, the best performing variables across the three datasets were the total alpine areas during the LGM, connectance, and the proportion of calcareous bedrocks. These predictors showed significant effects in the GLMMs computed in combination with area,

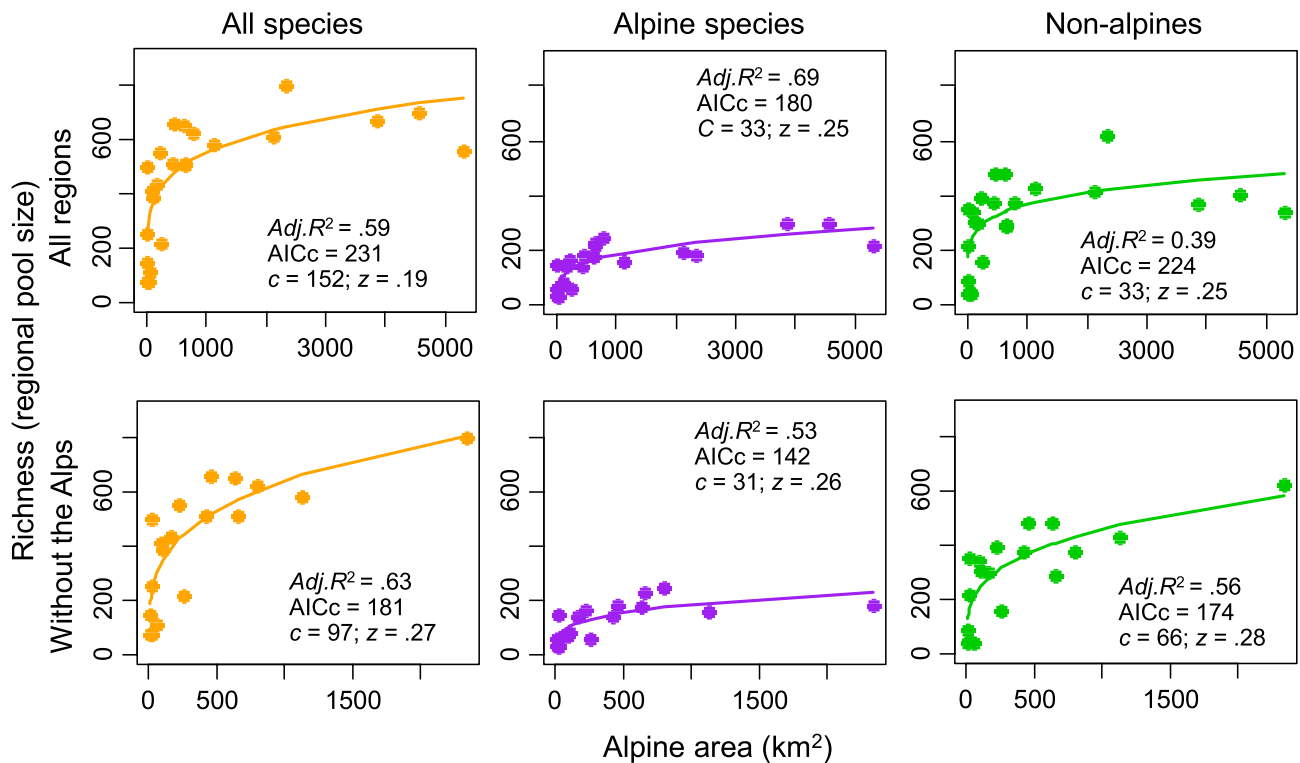


FIGURE 2 Island species–area relationships (ISARs) computed for all study regions ($n = 23$) and for a subset without the Alps ($n = 18$). ISARs were calculated with a power function ($S = cA^z$) where S is the number of species, A is the area, and c and z are the fitted parameters. The goodness of fit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

	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

Abbreviations: AICc, Akaike information criterion corrected for small sample sizes; LGM, Last Glacial Maximum.

*** $p < .001$. ** $p < .01$.

reaching mR^2 values from .41 to .46 (Table 2). For the whole dataset, calcium bedrock and connectance had the strongest effects, while for the subset without the Alps the most important predictor was the alpine area (Figure 3a). There were relevant differences among the two subsets of species, with stronger effects of area and Area. LGM on alpine species, and stronger effects of connectance for non-alpines.

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

The GDMs showed higher relative importance of distance between alpine regions than climatic gradients (PC1 and PC2), with remarkable differences among the three datasets (Table 4). Total explained deviance reached almost 70% for all species and alpine species, but 41% for non-alpines. For alpine species, 21% of regional dissimilarity was explained by the precipitation gradient (PC2), whereas for non-alpine species the only predictor with significant effects was Distance. The GDMs for all species also showed much stronger effects of Distance than precipitation (PC2).

The number of significant connections (Figure 4) for dispersal distance (DD_2) was slightly higher for the subset of all species (78) than non-alpines (72) and alpine species (68). However, the average weight of connections was higher for alpine species (.14) than for the other subsets (.10 for all species and for non-alpines). For alpine species, the strongest connections were found between the Pyrenees and the rest of the Iberian Peninsula, and between the Alps and the closest regions. The Alps and the Pyrenees were also the most important sources of alpine species, while the Dinarides

TABLE 2 Results of generalized linear mixed models (GLMMs) testing the effect of alpine area and the best performing variables for isolation (Area.LGM), patch connectivity (Connectance) and soil composition (Ca.Bedrock) to explain regional pool sizes in European alpine grasslands. The analyses were computed for the whole dataset and a subset of regions without the Alps. Numbers for variables show the z statistic of model significance. mR^2 and cR^2 indicate the fit of the models without (marginal) and with (conditional) random effects, respectively

had the highest number of net outbound connections for all species and non-alpines. The values of DD_2 were correlated with latitudinal differences between regions for all species (Pearson's $r = -.31$; $p = .005$), alpine species ($r = -.31$; $p = .008$) and non-alpines ($r = -.27$; $p = .021$); but not with longitudinal differences ($p > .05$ in the three datasets).

4 | DISCUSSION

4.1 | Determinants of species pool sizes

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

Together with area effects, our models support that the proportion of calcareous bedrock, the connectivity of alpine habitat within regions and the extent of alpine areas in the LGM are major determinants of species pool sizes, with consistent effects when analysing all regions and the subset without the Alps. The effect of calcareous bedrock was especially relevant in the whole dataset, in agreement with the highest species richness known to occur in base-rich

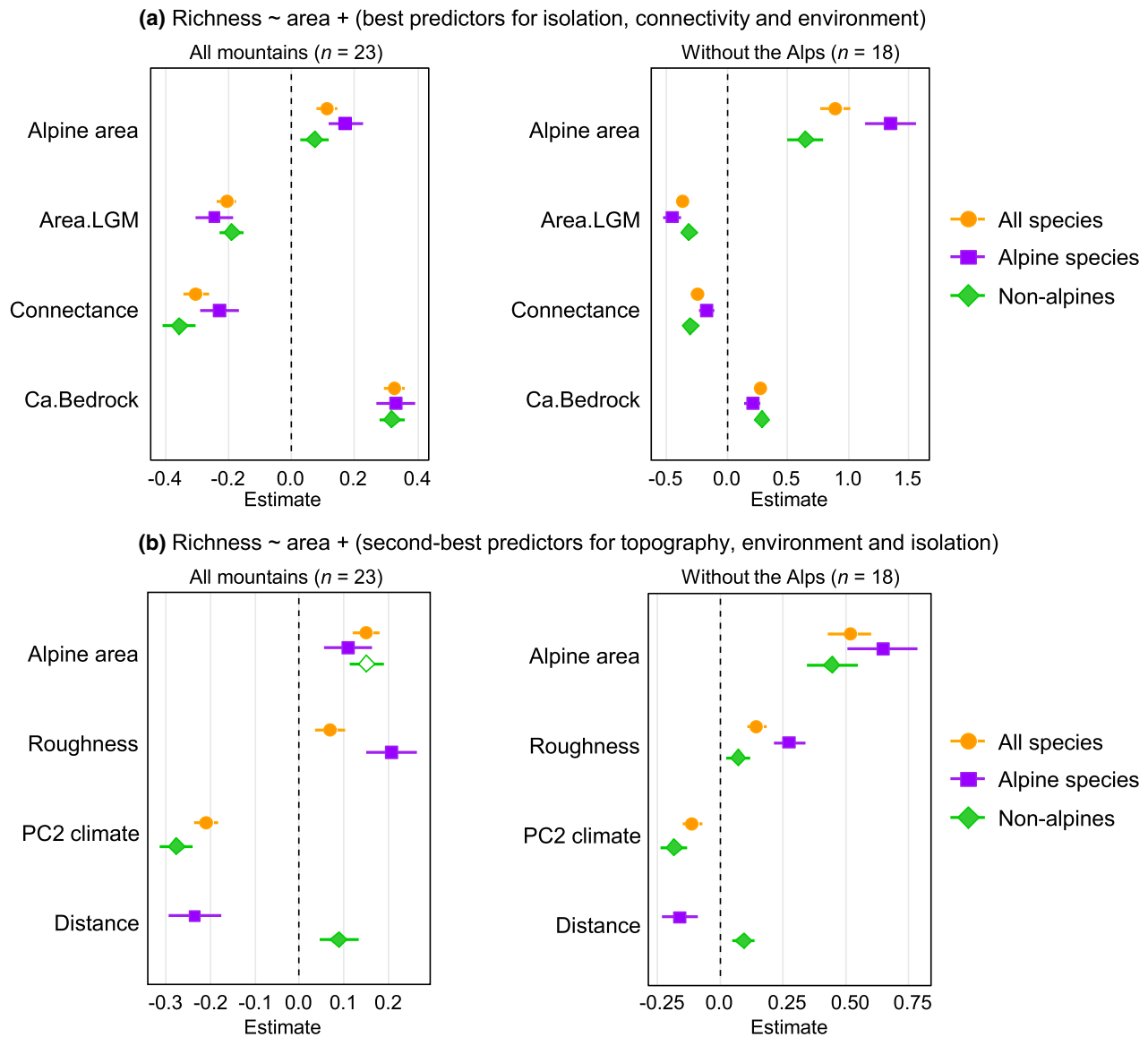


FIGURE 3 Coefficient estimates (slopes) derived from generalized linear mixed models (GLMMs) fit to the species pool size (Richness) of European alpine grasslands, for all mountains and a subset without the Alps. Model (a) includes alpine area and the best predictors selected within variable groups reflecting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

substrates (Ewald, 2003) and the relatively larger number of vegetation types found in calcareous-rich habitats (Virtanen et al., 2002). In contrast with our initial prediction, we found that connectivity of alpine patches has a negative effect on species richness, with a stronger effect on non-alpines. The regions with the highest connectivity (Baetic System, South Apennines, Balkans) have relatively smooth relief and a lower number of summits connected along continuous ranges. This spatial configuration might decrease habitat heterogeneity and the number of niches available for cold-adapted species, favouring a larger proportion of non-alpines from lower elevations through a mass-effect metacommunity process (Leibold et al., 2004). In fact, the richness of non-alpines positively increased with distance among regions, indicating that the main sources of dispersal come from the surrounding mountain flora. In contrast,

the pools of alpine species decreased at larger distances from other mountain regions, supporting dispersal limitation and the effect of regional isolation predicted to occur in alpine habitats (i.e. snapshot isolation, Flantua et al., 2020).

In contrast with our expectation, we found negative effects of the extent of LGM alpine areas on species pool sizes, probably due to the LGM presence of large ice shields in the richest mountains, especially the Alps and Pyrenees. Although the LGM was a relatively short glacial period, and for most of the glacial era the ice shield was smaller, the ice blocks were unoccupied by plants, with the exception of in-situ glacial refugia (Holderegger & Thiel-Egenter, 2009). Since the effect of LGM area was negative for all the datasets, but univariate models showed slightly positive effects on regional richness, our results are not conclusive enough to interpret this determinant thus

	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

Abbreviation: AICc, Akaike information criterion corrected for small sample sizes.

****p* < .001. ***p* < .01.

TABLE 4 Results of generalized dissimilarity models (GDMs) testing compositional differences of regional pool composition across European alpine grasslands. Numbers indicate variable importance of predictors. Principal component axes 1 (PC1) and 2 (PC2) reflect temperature and precipitation gradients, respectively

	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

****p* < .001. ^{ns}*p* > .05.

new research will need to consider LGM areas at a finer scale (e.g. species richness from single mountaintops). We also found a positive effect of topographic roughness on species pool sizes, supporting that heterogeneity of alpine landscapes favours multiple niches and microrefugia (Graae et al., 2018). This effect was especially relevant for alpine species, suggesting that regions with more topographic heterogeneity facilitate local refugia during glacial periods, as predicted by genetic data (Christe et al., 2014). Interestingly, we did not find any effect of temperature gradients across regions, likely because our study system is already filtered on low-temperature areas. Although regional pool sizes responded to regional precipitation regimes, this effect was only significant for non-alpines as an effect of general floristic variation across regions. This supports the hypothesis that topographical refugia determine the persistence of alpine species in spite of macroclimatic conditions (Dullinger et al., 2012; Frate et al., 2018).

4.2 | Determinants of species pool composition

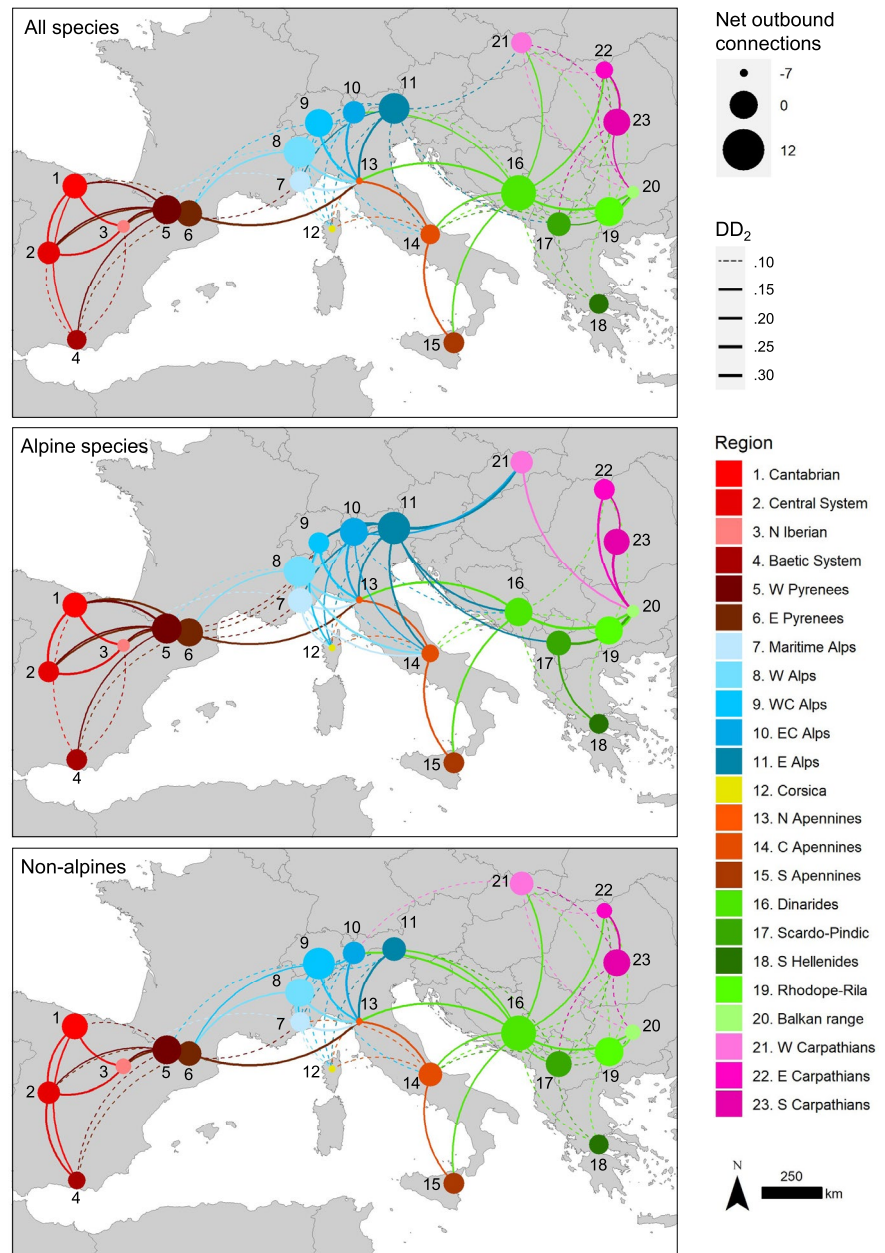
Among our set of predictors, geographical distance and a climatic gradient of precipitation were the only drivers of species pool dissimilarities. The influence of distance supports the idea that dispersal limitation shaped the configuration of species pools across

TABLE 3 Results of generalized linear mixed models (GLMMs) testing the effect of alpine area and a selection of second-best predictors related to topography (Roughness), isolation (Distance) and environment [principal component 2 (PC2) climate, reflecting precipitation gradients] to explain regional pool sizes in European alpine grasslands. The analyses were computed for the whole dataset and a subset of regions without the Alps. Numbers for variables show the *z* statistic of model significance. mR² and cR² indicate the fit of the models without (marginal) and with (conditional) random effects, respectively

the study regions. Distance was also the main determinant for non-alpines, reflecting the dominant role of geography in determining floristic dissimilarities across mountain floras (Ozenda, 1985). Although the composition of non-alpine species is also expected to respond to climatic gradients at the continental scale (Jiménez-Alfaro, Suárez-Seoane et al., 2018), we note that our set of non-alpines is limited to the species that are able to thrive in alpine habitats (i.e. a subset of the mountain floras). In contrast, alpine species, which are mainly distributed in the alpine zone, were further filtered by current precipitation gradients. Indeed, the study system is characterized by a north–south gradient from temperate climates with wet summers to Mediterranean mountains with a dry season (Nagy, 2006; Pauli et al., 2012). In consequence, the post-glacial reorganization of alpine pool composition was regulated by precipitation, an effect that may also reflect the length of the snow free season (Choler, 2015). This agrees with the view of alpine habitats as cold systems that are mostly differentiated by humidity gradients at global and continental scales (Billings, 1974; Testolin et al., 2020).

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

FIGURE 4 Coefficients of dispersal direction (DD_2) calculated for pairs of European regions using species pool composition from alpine grasslands. DD_2 reflects the strength of significant pairwise connections between regions. Outbound links have the same colour as the source (i.e. when dispersal direction is estimated from region A to region B, the link is coloured as A). Circle size is proportional to the number of net outbound connections (i.e. positive dispersal directions)



interplay of dispersal, isolation and vicariance is still unresolved given the lack of phylogenetic data (Kropf et al., 2006; Schmitt, 2009).

Our results on dispersal directions also suggest a prominent role of stepping-stones for long-distance dispersal (Saura et al., 2014), by which medium-size regions may have acted as hubs in glacial expansions and as reservoirs of alpine flora during interglacial contractions. This might be the case for regions with high net connections with respect to their size, like the Cantabrian mountains (Spain), the Rila-Rhodope massif (Bulgaria) and the Southern Carpathians (Romania). Indeed, phylogeographical studies have traced continuous gene flow between the Cantabrian range and the Pyrenees (Kropf et al., 2003) and a key role of the Carpathian mountains in long-term migrations of high-mountain plants within Eurasia (Ronikier, 2011). We note, however, that our approach is based on the number of shared

species among regions and a-priori assumptions about their implication on past dispersal, thus we lack proper evolutionary links among species lineages (Schmitt, 2009; Vargas, 2003), or information about macrofossil remains (Birks, 2019). Despite this, our results reinforce the general view that alpine habitats from southern Europe are interglacial refugia for cold-adapted plants (Feliner, 2011) formerly connected by dispersal but currently isolated from each other.

5 | CONCLUSIONS

Besides demonstrating area effects on species pool sizes, we found a key role of edaphic, topographic and spatio-temporal factors linked with the post-glacial retreat of alpine grasslands. These factors may

even outcompete area effects, supporting the need to use multiple determinants to understand alpine habitats as isolated systems (Flantua et al., 2020; Itescu, 2019). We also found that the size and composition of regional pools are better understood when subsets of species with different ecological profiles are distinguished. Thus, the regional pools of alpine species seem to be primarily driven by alpine areas, topographic heterogeneity and regional isolation, but less so by temperature gradients across regions. Although climate-driven extirpations of relict populations may still occur (Jiménez-Alfaro et al., 2016), our results are in line with the idea that full species extinctions of alpine species are rare in interglacial periods (Birks, 2008), suggesting that the pools of alpine species may have been relatively unaffected by extinction or immigration since the climate stabilized around 10,000 yr BP. In contrast, the regional number of non-alpines might be more sensitive to climate, likely explaining recent elevation shifts (Jiménez-Alfaro, Gavilán et al., 2014; Steinbauer et al., 2018), even though their composition is determined by mountain regional floras. The central question of the theory of island biogeography about the role of immigration (reaching equilibrium) versus the predominance of extinction (non-equilibrium) might, therefore, be biased by the assumption that all species from the species pool respond similarly to climatic oscillations (as it is essentially a neutral model). To develop a consistent framework for understanding the diversity of alpine habitats, and to anticipate the effects of ongoing climate change, we conclude that empirical research should contextualize the role of post-glacial determinants on regional species pools and the different responses of species ecological groups.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

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

DATA AVAILABILITY STATEMENT

All the raw data and the R scripts necessary to replicate the analyses are accessible at <https://doi.org/10.5061/dryad.0cfxpnw1h>. These

datasets include plot species data for estimating regional completeness, the full list of species and their elevation ranges, and regional predictors. The geographical distribution of different estimates of alpine areas can be visualized at <https://torpignattaro.users.earthengine.app/view/european-alpine-areas>

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BIOSKETCH

Borja Jiménez-Alfaro is interested in the patterns and processes regulating the distribution of vascular plants across scales and ecosystems. He coordinates the ALPVEG research group (<https://www.alpveg.com/>), a virtual research hub for understanding plant biodiversity in alpine vegetation.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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