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**Title: A multi-scale approach reveals random phylogenetic patterns at the edge of vascular plant life.**

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**ABSTRACT**

Mountain plant diversity results from a myriad of factors, including evolutionary history, species pools, abiotic and biotic constraints. For instance, increasing stress (e.g., elevation) often selects communities with species originating from fewer, and more closely-related clades. We assessed phylogenetic diversity and turnover of plant communities by considering multiple drivers simultaneously: 1) the species pools; 2) the regional context, i.e., two regions of the arid Trans-Himalaya (the Karakoram Range and Little Tibet) with distinct history, climate and species richness (regional scale); 3) the abiotic constraint with communities spread out along an elevation gradient in each region (community scale); 4) the biotic constraint, i.e., species co-existing inside a competitive dominant species (cushion plant)vs. its adjacent open area in each community (neighbourhood scale); and 5) two phylogenetic scales (overall vs. recent events in the assembled phylogeny). We found random phylogenetic patterns for all spatial and phylogenetic scales, irrespective of the regional context, and the abiotic and biotic conditions under consideration. Contrary to expectations, we observed a tendency for phylogenetic evenness in Little Tibet and in the Karakoram Range with increasing elevation. The observed phylogenetic patterns were mostly explained by region, elevation and interactions among the multiple drivers under considerations. Phylogenetic scale, species pools and cushion plants explained patterns to a lower but significant extent. The studied phylogenetic patterns emerge from the complex interplay between multiple drivers, and challenge the common view that increasing spatial and phylogenetic scales, as well as increasing biotic and abiotic constraints, select communities with species originating from fewer, and more closely-related clades.

**Keywords:** alpine communities; elevation gradient; extreme environments; phylogenetic scale; phylogenetic diversity; scaling approach.

**Introduction**

Understanding how evolutionary and ecological processes interplay to generate patterns of species diversity and distribution remains a key challenge in ecology (Lavergne et al., 2010; Ricklefs, 1987). Phylogenetic information can shed light on the evolutionary history of a region, past species niche evolution and mechanisms of contemporary species coexistence (Cavender-Bares et al*.*, 2009; Webb et al., 2002). However, inferring the multiple drivers of species coexistence from phylogenetic patterns requires cautious interpretation (Gerhold et al., 2015; Losos, 2008; Mayfield and Levine, 2010; Pausas and Verdú, 2010; Soliveres et al., 2012).

Phylogenetic diversity varies over a wide range of spatial scales (e.g., Cavender-Bares et al., 2009; Graham and Fine, 2008; Münkemüller et al., 2014). Past species diversification, migration events and past and current landscape dynamics primarily shape phylogenetic diversity at biogeographical scales (see Graham and Fine, 2008 for review). Abiotic factors and biotic interactions are more likely to shape diversity at regional and community scales (Webb et al., 2002). The interplay of these processes can lead to patterns shifting from low (phylogenetic evenness) to high relatedness among co-occurring species (phylogenetic clustering) with increasing spatial scale (e.g., Cavender-Bares et al., 2006; Swenson et al., 2007).

The observed patterns of phylogenetic diversity also vary with the depth of the phylogenetic tree under consideration (phylogenetic scale hereafter, sensu Graham et al., 2016). These variations in patterns of phylogenetic diversity may emerge from different underlying mechanisms operating at different depths (Benton and Emerson, 2007). For instance, deep phylogenetic analyses have suggested that environmental filtering shape communities, while analyses focusing on more recent events often highlight additional mechanisms involved (e.g. biotic interactions). As a result, patterns of phylogenetic diversity are often observed to shift from phylogenetic evenness to clustering with increasing phylogenetic scale (Cavender-Bares et al., 2006; Swenson et al., 2006, 2007).

Multi-scale approaches (spatial and phylogenetic) allow to better understand the interplay between multiple drivers shaping phylogenetic diversity (Emerson and Gillespie, 2008; Graham and Fine 2008; Graham et al., 2016). These approaches are highly needed to develop conservation efforts prioritizing targeted species with respect to their evolutionary uniqueness or distinctiveness (Mouquet et al., 2012). Multi-scale approaches have been successfully used in tropical (e.g., Kembel and Hubbell, 2006) and temperate systems (e.g., Cavender-Bares et al., 2006), but received far less investigations in alpine environments (but see Münkemüller et al., 2014).

Mountain systems exhibit strong environmental changes at various scales, and host many endemic species (Graves, 1985; Körner, 2000). Many recent studies have focused on elevation gradients as a primary driver of phylogenetic diversity. However, elevation gradients encompass a range of underlying factors, such as evolutionary history, climate, or local environmental heterogeneity (see Graham et al., 2014 for a review). Increasing elevation has been associated with phylogenetic clustering for microbes (Bryant et al., 2008), insects (Machac et al., 2011; Smith et al*.*, 2014), birds (Graham et al*.*, 2009), and plants (Jin et al., 2015; but see Bryant et al., 2008). Phylogenetic clustering is often viewed as a signature of habitat filtering, where species from only few clades can withstand environmental conditions of higher elevations. In particular, this should be the case if fitness related traits under selection at high elevations are more similar for closely related species than for species from different clades (Webb et al, 2002).

Mountain phylogenetic diversity has been poorly investigated at neighbourhood scale, i.e., within microhabitats (e.g., Butterfield et al., 2013; Piston et al., 2016). Mountain communities are shaped by local environmental heterogeneity and by foundation species such as cushion plants (Al Hayek et al. 2014; Cavieres et al., 2014; Choler et al., 2001). Cushion plants can profoundly modify the nature and intensity of local abiotic filters, ultimately determining community assembly (Cavieres and Badano, 2009; Reid et al., 2010). Cushion plants can increase plant phylogenetic diversity (e.g., Piston et al., 2016; Soliveres et al., 2012) by creating favourable microhabitats under stressful conditions, in which less tolerant lineages are facilitated and diverge from those of microhabitats without cushion (Butterfield et al., 2013). Cushion plants with compact forms can also have strong competitive effects (Al Hayek et al., 2014), and act as a biotic filter influencing phylogenetic diversity (Piston et al., 2016). Whether and how competitive plant cushions modulate the effect of elevation on phylogenetic diversity remains poorly understood.

In our study, plant diversity was assessed in the arid Trans-Himalaya, one of the highest elevation limits for vascular plants worldwide (Dvorsky et al., 2015). We investigated phylogenetic diversity, and used a complementary approach that explicitly quantifies changes in phylogenetic patterns (phylogenetic turnover hereafter: Graham and Fine, 2008) across multiple spatial scales ranging from the neighbourhood (~ 1m²) up to supra-regional levels (Fig. 1). Our study focused on two geographically distinct regions, i.e., the Karakoram Range and Little Tibet, which differ in terms of history, climate and species richness (Dvorsky et al*.*, 2015). We investigated phylogenetic diversity for each region, as well as between-region and between-site phylogenetic turnovers along elevation gradients within each study region. Observed patterns were all compared with the supra-regional and their respective regional reference pools (Karakoram Range and Little Tibet), to shed light on coarse-scale processes such as biogeographical history (Swenson et al., 2006). In each site, we used the dominant species *Thylacospermum caespitosum* (Caryophyllaceae) — a strong competitive cushion plant that excludes subsets of the local species richness (de Bello et al., 2011; Dvorsky et al*.*, 2013) — and adjacent open areas (i.e., microsites hereafter) to investigate phylogenetic diversity within each microsite, as well as between-microsite turnovers. Phylogenetic patterns were compared to the local reference pool (i.e., the local species pool of a study site) to shed light on processes such as species interactions and fine scale environmental filtering (Swenson et al., 2006). For all spatial scales considered, phylogenetic patterns were finally assessed at two phylogenetic scales (Graham et al., 2016), by comparing phylogenetic diversity observed for the most recent speciation events (tip level) to phylogenetic diversity across the whole tree (e.g., Jin et al., 2015).

We aimed at considering a large panel of potential drivers of phylogenetic patterns and their interactions to test the common assumption that increasing spatial and phylogenetic scales, or increasing abiotic and biotic constraints select communities with species originating from fewer, and more closely-related clades (so called phylogenetic clustering). Specifically, we hypothesized an overall increase in phylogenetic clustering i) from neighbourhood to supra-regional scales, ii) at higher elevation within each region (e.g., Jin et al., 2015; Machac et al., 2011); and iii) at micro-scale due to the competitive effect of the cushion plant considered (Butterfield et al., 2013).

**Materials and Methods**

*Study regions*

The study supra-region is located in Ladakh, in the Jammu & Kashmir State, NW India. The area is part of the Trans-Himalaya region, delimited by the Eastern Karakoram Range in the north and by the Great Himalaya Range in the south. Ladakh is an arid mountain region that receives little precipitation due to its position in the rain-shadow of the Himalaya Range (Dvorsky et al*.*, 2013; Wang, 1988). Our study focused on two regions of Ladakh that uplifted around 55 Ma ago, i.e., the Karakoram Range and Little Tibet. Their recent history differs substantially since the Karakoram Range was deglaciated more recently than Little Tibet. Climates of the two regions also differ, with Little Tibet experiencing harsher environmental conditions (Dvorsky et al., 2015). Finally, species diversity is higher in the Karakoram Range, while Little Tibet exhibits a high rate of plant endemism (Dvorsky et al*.*, 2015).

*Elevation gradients*

Plant phylogenetic diversity and between-site turnovers were assessed using eight sites, i.e., four sites in the Karakoram Range (34°45’N, 77°35’E) and four sites in Little Tibet (32°59’N, 78°24’E). The eight sites were distributed along two elevation gradients ranging from 4850 m to 5250 and from 5350 to 5850 m a.s.l. in the Karakoram Range and in Little Tibet, respectively. In each region, the four sites covered the entire elevation range occupied by *T. caespitosum*. It is also important to note that the two elevation gradients were ecologically comparable. Both gradients covered the complete sequence of mountain vegetation types: steppes at the lowest site of each gradient, alpine communities at the two intermediate sites and subnival communities at the highest site of each gradient (Dvorsky et al*.*, 2013).

*Impact of the cushion plant*

The phylogenetic diversity within microsites and between-microsite turnovers were assessed using the presence of the cushion plant *T. caespitosum* and the adjacent open areas. *T. caespitosum* is one of the most dominant high-alpine cushion plants in the Himalayas, and one of the few dominant species in the studied area (Klimesova et al*.*, 2011). It is a perennial plant with a woody taproot, forming very dense and solid cushions (Klimesova et al*.*, 2011). The largest cushions can be more than 150 cm in diameter (Dvorsky et al., 2013), suggesting that they may live for decades or even centuries (Le Roux and McGeoch, 2004). *T. caespitosum* occurs along an elevation range of 4600–5900 m, although this range can vary with the geographical locations (Klimesova et al*.*, 2011). *T. caespitosum* is a strong competitor that influences the structure and diversity of plant communities by locally excluding a subset of the site pools (de Bello et al*.*, 2011; Dvorsky et al*.*, 2013)**.**

*Vegetation survey*

The vegetation survey was carried out during the peak of the growing season (August) in 2011. The eight sites were selected at 4850 m, 5000 m, 5100 m and 5250 m a.s.l. in the Karakoram Range, and at 5350 m, 5600 m, 5750 m and 5850 m a.s.l. in Little Tibet. In each site, cushions were systematically surveyed in an area of ca. 1 ha (n = 66, 61, 69, 77, 70, 70, 70, 73 cushions in each site). The studied cushions represented all size classes with a diameter up to 132 cm. The most common size class was 40–60 cm in the Karakoram Range (n= 79) and 20–40 cm in Little Tibet (n= 102). Two microsites were considered for each cushion: a first microsite covering the cushion itself; a second microsite with the same size and shape randomly placed in the open adjacent area at a distance equal to the cushion diameter. In total, 1112 microsites were surveyed (556 pairs). All vascular plant species rooting in the microsites were recorded and their percentage cover was estimated visually. Because phylogenetic diversity can only be calculated for communities with more than one species, all microsites with fewer than two species were excluded from the data before further analyses, leaving 420 microsites (210 pairs).

*Species phylogeny*

All sequences used for building the tree were extracted from genBank ([www.ncbi.nlm.nih.gov/nuccore/](http://www.ncbi.nlm.nih.gov/nuccore/)). A combined multigene attitude was applied. Maximum data density was achieved with four loci: internal transcribed spacer (ITS), trnT-trnL intergenic spacer, matK + trnK region, and the gene for rubisco large subunit (rbcL). Missing sequences were filled with Ns (unknown data) or patched by corresponding nucleotides from closely related taxa (*Artemisia minor* was fixed with *A. glacialis*, *Desideria pumila* with *D. linearis*, *Elymus schrenkianus* with *E. dahuricus*, *Potentilla saundersiana* with *P. nivea* and *Sibbaldia tetrandra* with *S. cuneata*).

The L-INS-i algorithm implemented in the online version of MAFFT 6 (<http://mafft.cbrc.jp/alignment/server>, Katoh and Toh, 2008) was employed to align the sequence datasets. Partial alignments were concatenated, manually adjusted in BioEdit (Hall, 1999) and cleaned with the *automated1* algorithm in trimAll software (Capella-Gutierrez et al., 2009) to exclude highly divergent and gap-rich regions. Prior to the phylogenetic analysis, the best-fit model was selected by Kakusan4 (Tanabe, 2011). Baseml software (Adachi and Hagesawa, 1996) served as the computational core. Both non-partitioned and partitioned models were evaluated. Based on the Bayesian information criterion (Schwarz, 1978), we decided to use the GTR model with rate variation across locations simulated by discrete gamma distribution (Γ8), autocorrelated by the AdGamma rates prior and unlinked for particular gene partitions. To reflect the increased probability of transitions over transversions in non-coding loci, the substitution rates prior (revMatPr) was set for the ITS, trnT-L and petB-D partitions to the Dirichlet function with values of 1 and 3.

The phylogenetic analysis was represented by the Bayesian inference (BI), conducted in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The phylogenetic analysis was provided using two independent runs with four Metropolis-coupled MCMC chains of 1×107 generations sampled after every 1000th generation. In each run, one Markov chain was cold and three were incrementally heated by a parameter of 0.3. The first 25% of entries were discarded as burn-in and the rest was used to compute the majority-rule consensus to eliminate trees sampled before reaching apparent stationarity. The resulting tree was edited to be compatible in future ecological studies, since it contained more terminals (unknown multispecies genera were substituted with five terminals). The internal phylogeny of these genera was collapsed to polytomy, branch lengths were averaged and excessive branches were cut out. The nomenclature was unified with the Ladakh plant list (Klimes and Dickore, 2006).

*Phylogenetic diversity and turnover*

Phylogenetic patterns were investigated by the Mean Pairwise Distance (MPD) and the Mean Nearest Taxon Distance (MNTD) indices. These two indices allowed assessing the whole-tree species relatedness in each community, and tip-level species relatedness between closely-related species in each community, respectively (Fine and Kembel, 2011; Jin et al., 2015). Comparing MPD and MNTD can therefore inform at which phylogenetic scale patterns of phylogenetic diversity are most evident (Jin et al., 2015). The Mean Nearest Taxon Distance index indicates patterns occurring for the most recent speciation events. The Mean Pairwise Distance indicates patterns occurring across the entire phylogeny (Cadotte and Davies, 2016). While MPD and MNTD allow to investigate the diversity in the studied samples, phylogenetic turnovers assess how much species in a sample are related to all other species in a second sample, either on average, or only for the nearest taxa. Hence, whereas diversities are calculated per plot, phylogenetic turnovers are calculated on a pairwise plot basis.

We investigated phylogenetic diversity for both MPD and MNTD using the functions *mpd* and *mntd* in the *picante* package (Kembel et al. 2010). We computed the phylogenetic turnovers with the functions *comdist* and *comdistnt* that arethe analogues of *mpd* and *mntd*. Abundance data were log-transformed after adding 1 to avoid negative values. We used standardized effect sizes (SES) of MPD and MNTD indices. Standardized effect sizes were calculated as *SESMPD = (MPDobs – mean(MPDrand))/ sd(MPDrand)*, where *MPDobs* is the observed phylogenetic diversity index, *mean(MPDrand)* is the mean MPD of random communities generated from a null model, and *sd(MPDrand)* is the standard deviation of MPD of those random communities.

For phylogenetic diversity, positive values of *SESPD* indicate greater distances among species than expected by chance (phylogenetic evenness). Negative values of *SESPD* indicate lower phylogenetic distances among species than expected by chance (phylogenetic clustering). *SESPD* values ranging between -1.96 and 1.96 indicate a pattern that is not significantly different from random, under the assumption that indices are normally distributed. For phylogenetic turnover, negative values of *SESPD* indicate that the species in two plots show less phylogenetic turnover (i.e., are more related to each other) than expected by chance, while positive values indicate higher showing phylogenetic turnover (i.e. species less related to each other) than expected by chance.

To compute SES scores for each community plot, random communities were generated by randomly drawing species from a species pool with the same species richness as in the observed plots, meaning that abundances where shuffled within plots across species, as executed by the “richness” null model of the *randomizeMatrix* function in the *picante* package (Kembel et al. 2010). The used species pools differed in size according to each tested hypothesis (see Fig. 1 and next paragraph): (1) all species sampled in both regions (supra-regional reference pool), (2) all species occurring in a single region (regional reference pool), (3) all species present in a given study site (local reference pool).

The combinations of different diversity indices and reference pools helps testing the strength of different species filters at different scales and according to ecological factors (elevation, cushion plants). SES of alpha diversities and spatial turnovers were achieved by using the supra-regional and regional species pools to test for increased filtering effects with spatial scale, stronger filtering in Little Tibet, and increased filtering with elevation (hypotheses i – ii). Comparing microsite alpha diversities and between-microsites turnovers against randomisations from the local species pool allowed testing the occurrence of biotic filters due to the presence of cushion plants (hypothesis iii).

*Statistical analyses*

First, we analysed the effects of region, elevation, the presence of cushions, and their interactions on diversities and turnovers with mixed effect ANOVA models. All predictors were categorical. Pairs of sampled microsites (cushion and adjacent open area) were used as a random factor, and nested within site. We also performed a linear regression model with the between-site turnovers within single region used as the response variable, and with the elevation difference between sites, the region, and their interaction as predictors. We used post-hoc Tukey tests to evaluate if between-site turnover differed between the two regions.

Second, we used a mixed effect ANOVA model to test whether alpha phylogenetic diversity of different sites differed between regions, species pools (supra-regional vs. regional reference pools), elevation, phylogenetic scale (MPD vs. MNTD) and their interactions (Appendix S1). We repeated this analysis at the microsite scale with region, elevation, cushions, phylogenetic scale (MPD vs. MNTD) and their interactions as predictors. At each scale (microsite and site), we evaluated the relative effect of the predictors on phylogenetic diversity by calculating their proportional contribution to the overall explained variance of the model from their sums of square. The obtained relative effects of predictors are grouped into the following identifiable variance fractions: i) region, ii) elevation, iii) phylogenetic scale and iv) interactions among drivers. Note that the following variance fractions v) pool and vi) cushion were also considered at the site and the microsite scales, respectively.

All statistical analyses were performed using R 2.15.1 (R Core Team, 2012).

**Results**

The observed *SES* values of phylogenetic diversity mostly ranged between -1.96 and 1.96, a signature of random patterns (Figs. 2 and 4). Yet, our subsequent analyses revealed significant effects of our drivers and their interactions on phylogenetic diversity. Region and elevation were the most important drivers explaining 45% of the variation in phylogenetic diversity at the site scale and 63% at the microsite scale (Appendix S1). Phylogenetic scale and species pool had lower, but significant effects at the site level (7% and 6%, respectively). Cushion explained 3% of variation at the microsite scale. Finally, interactions among drivers explained 28% of variation in phylogenetic patterns at the site scale and 11% at the microsite scale.

Our results were consistent when comparing the observed phylogenetic patterns to both supra-regional (Fig. 2: upper panels) and regional reference species pools (lower panels). Nonetheless, *SESMPD* values were generally higher in Little Tibet when considering the regional reference pool rather than the supra-regional pool (Figs. 2a and c).

We found significantly higher - and more often positive - *SES* values in Little Tibet than in the Karakoram Range (Table 1) for both whole-tree (*SESMPD*: Fig. 2c) and tip level diversity metrics (*SESMNTD*: Figs. 2b and d). SES values computed at the tip level also increased with elevation in the Karakoram Range (Table 1) (*SESMNTD*: Fig. 2b and d). These results suggest a tendency for phylogenetic evenness in Little Tibet, and in the Karakoram Range at high elevation.

We also found negative values of SESMPD for between-region and between-site turnovers, although they all ranged between -1.96 and 1.96 (Fig. 3a). Our results highlight a tendency of phylogenetic similarity between regions for whole-tree diversity indices (*SESMPD*), i.e., a lower phylogenetic turnover across sites than expected from the supra-regional reference pool. SESMPD were also significantly lower within regions than between regions, indicating an even lower phylogenetic turnover across sites within regions than between regions (Fig. 3a). Positive *SESMNTD*were observed between regions, although they were lower than 1.96 (Fig. 3b). This indicated a tendency for phylogenetic turnover at the tip level between regions.

Elevation tended to increase the degree of phylogenetic evenness when considering either the site (Fig. 2, Table 1) or the microsite scales (Figs. 4a and b, Table 2). We also observed an effect of the elevation distance on the between-site turnover along the elevation gradients for both whole-tree (*SESMPD*) and tip level analyses (*SESMNTD*, Appendix S2). Closer sites exhibited a phylogenetic turnover of closely related clades (negative *SES* values) while no trend occurred for spatially distant communities.

Finally, the presence of the cushion plant slightly increased the degree of phylogenetic evenness (Table 2) for whole-tree diversity metrics (Fig 4a) and at low elevation for tip level diversity metrics (marginally significant elevation x cushion in Table 2, Fig. 4b, significant elevation x cushion in AppendixS1). However, cushion plant did not seem to drive a significant turnover of plant phylogenetic diversity between sampled microsites (Figs. 4c and d).

**Discussion**

Our multi-scale approach revealed random phylogenetic patterns, irrespectively of the species pool, the regional context (history, climate and species richness), the abiotic and the biotic conditions. Detecting consistent random patterns does not support the common view that phylogenetic patterns depend on spatial scale (e.g., Münkemüller et al., 2014; Swenson et al., 2006), shifting from phylogenetic evenness (Swenson et al., 2007) to clustering with increasing spatial grain and extent (Cavender-Bares et al., 2006; Swenson et al., 2006, 2007). A consistent pattern across multiple spatial scales has also been observed in bee communities (i.e., a consistent phylogenetic clustering in Harmon-Threatt and Ackerly, 2013), and therefore spotlights a possible idiosyncrasy in scaling phylogenetic patterns (Cadotte and Davies, 2016). This discrepancy between our results and other similar studies does not seem to arise from the geographic scale under consideration. Indeed, the range of spatial scales considered in our study (from ~ 1 m² to ~20000 km²) fully encompassed the scales of previous studies (e.g., Kembel and Hubbell, 2006 and Swenson et al., 2007: from < 100m² to 1ha).

The observed random patterns of plant community phylogenetic diversity indicate that particular sets of morphological/anatomical adaptations allowing species to cross the environmental filters and to persist into extreme environments can be found within and across different lineages (Butterfield et al., 2013; Dolezal et al., 2016; Webb et al., 2002). For instance, species growing at the highest elevation of Little Tibet recruit mostly from Brassicaceae, Asteraceae, Caryophyllaceae and Poaceae (Appendix S3). Despite their general high phylogenetic dispersion, these species are generally small, exhibit a similar growth form (cushion) with a relatively high water content and water-use efficiency, and contain more nutrients and soluble carbohydrates than species from lower elevation (Dvorsky et al., 2015, 2016). Further studies simultaneously combining phylogenetic and functional (multi-trait) approaches would certainly allow better understanding the processes governing community assembly in these extreme environments (Swenson, 2013), and addressing the issue of plant adaptation in relation to evolutionary history (Schweingruber et al*.*, 2014).

Elevation was the major driver of community assembly at microsite-scale, explaining 63% of the variation in phylogenetic diversity. The tendency for phylogenetic evenness with increasing elevation in the Karakoram Range is opposite to previous findings generally showing an increase in phylogenetic clustering (Bryant et al., 2008; Graham et al., 2009; Jin et al., 2015; Machac et al*.*, 2011; Smith et al., 2014). Our study system illustrates that communities from extreme environments are not necessarily composed of species originating from fewer, and more closely-related clades (see also Le Bagousse-Pinguet et al., 2017 for similar findings on functional diversity in global drylands).

The regional context was the main driver of our studied communities at the site scale, explaining 45% of the variation in phylogenetic patterns. This suggests that floristic regional context is a major drivers of phylogenetic patterns of plant communities. The difference between the two study regions may stem from the difference in their deglaciation history (Dvorsky et al*.*, 2015). The tendency for phylogenetic evenness in Little Tibet may arise from a longer time for distantly related species to converge in their habitat-use along the elevation gradient. In contrast, the shorter time from deglaciation in the Karakoram Range may have led the species to converge in their habitat-use at the highest elevation only, and more time might be required for this pattern to emerge at low elevation.

Several other underlying processes may explain the observed phylogenetic evenness at high elevation in the Karakoram Range. Elevation gradients are often complex, comprising multiple underlying gradients and notably acting on dispersal limitation (Körner, 2000; Michalet et al., 2014). Phylogenetic turnover of more closely related species between communities of closer elevation (Appendix S2), associated with the absence of phylogenetic clustering at any scale, supports the idea that dispersal limitation could be one of the underlying drivers shaping phylogenetic patterns along the studied elevation gradients (Dvorsky et al. 2016).

The local biotic context, represented by the presence or absence of cushion plants at the neighbourhood scale was also a significant driver of phylogenetic patterns in the studied sites, although it was weaker than the other factors considered (3% of the variations in phylogenetic diversity). The presence of the cushion tended to be associated with phylogenetic evenness when considering the whole tree, although no plant phylogenetic turnover was observed between microsites. These patterns indicate that subsets of species from open areas (similar to the site reference pool) were filtered by the cushion plants, with distantly-related species converging in their use of micro-habitats (Webb et al*.*, 2002). Interestingly, we also found that the net effect of the cushions on phylogenetic diversity changed along the elevation gradient. The competitive effect of the cushion *sensu stricto* remains similar along the entire gradient (Dvorsky et al., 2013). The more pronounced difference between cushions and adjacent open areas observed at low elevation may therefore result from the higher occurrence of competitive species exhibiting the set of morphological/anatomical adaptations to invade the cushions (see also Liancourt et al., 2017 for the role of the species pool on the outcome of biotic interactions). Altogether, our results therefore support the view that considering neighbourhood scale processes is relevant when assessing phylogenetic patterns of plant communities (Valiente-Banuet and Verdu, 2007; Verdu et al*.*, 2009), and that investigating the role of plant-plant interactions may bring interesting insights into the mechanisms driving vegetation structure in extreme habitats (Butterfield et al., 2013; Piston et al., 2016; Soliveres et al., 2012).

The phylogenetic scale explained 7% of the variations in phylogenetic patterns, demonstrating the importance of accounting for different phylogenetic scales to better understand the evolutionary history of regional floras (Fine and Kembel, 2011; Graham et al., 2016; Swenson et al., 2007). The observed changes in patterns were clearer when considering recent speciation events rather than the whole tree, reinforcing the view that the phylogenetic scale under consideration can largely influence our ability to detect non-random patterns in plant communities (e.g., Jin et al., 2015; Swenson et al., 2007). Phylogenetic evenness observed at the tip levels may result from the effect of local abiotic factors and biotic interactions (Cavender-Bares et al., 2006), or alternatively from allopatric speciation (Pigot and Etienne, 2015). This pattern is also likely to become more common when communities are more finely defined taxonomically (Cavender-Bares et al., 2006; Swenson et al., 2006, 2007). We therefore acknowledge that the stronger signals of phylogenetic evenness observed at the tip levels may simply result from a decrease in phylogenetic signal with decreasing the phylogenetic scale of the analysis.

Patterns of whole-tree phylogenetic turnover were low between and within regions, and relatively high at the tip level between regions (Fig. 3). Therefore, our results indicate that patterns of diversity are phylogenetically representative of the supra-regional reference pool and similar between the Karakoram Range and Little Tibet. In both regions, most of the families occurring in the supra-regional reference pool are represented, e.g., Poaceae, Cyperaceae, Asteraceae, Fabaceae, Brassicaceae and Rosaceae. Rather, phylogenetic turnover mostly occurs at the tip level, indicating a recent diversification within families and genera, such as greater diversifications in *Oxytropis,* *Potentilla* or *Carex* in the Karakorum Range or Caryophyllaceae in Little Tibet, and therefore reflecting the unique phytogeographical history of each region.

Our multi-scale approach, from neighbourhood to supra-regional scales and across phylogenetic scales, revealed random phylogenetic patterns at the edge of vascular plant life. This finding challenges the common view that increasing spatial and phylogenetic scales, or increasing abiotic and biotic constraints select communities with species originating from fewer, and more closely-related clades. Using multiple drivers simultaneously, we also quantified and ranked the importance of commonly investigated drivers of phylogenetic patterns. We showed that phylogenetic patterns could clearly emerge from the complex effects of pools, regional context (history), phylogenetic scale, abiotic and biotic constraints and their interactions (reaching up to 28% of the explained variance). On one hand, our approach illustrates the difficulty to directly infer ecological processes underlying species coexistence and community assembly from phylogenetic patterns (Gerhold et al., 2015; Losos, 2008; Mayfield and Levine, 2010; Pausas and Verdú, 2010; Soliveres et al., 2012). On the other hand, our study clearly illustrates that phylogenetic patterns contain a great deal of information and therefore supports the recent claim that these patterns *“are not only proxies of community assembly processes, they are far better”* (Gerhold et al., 2015).

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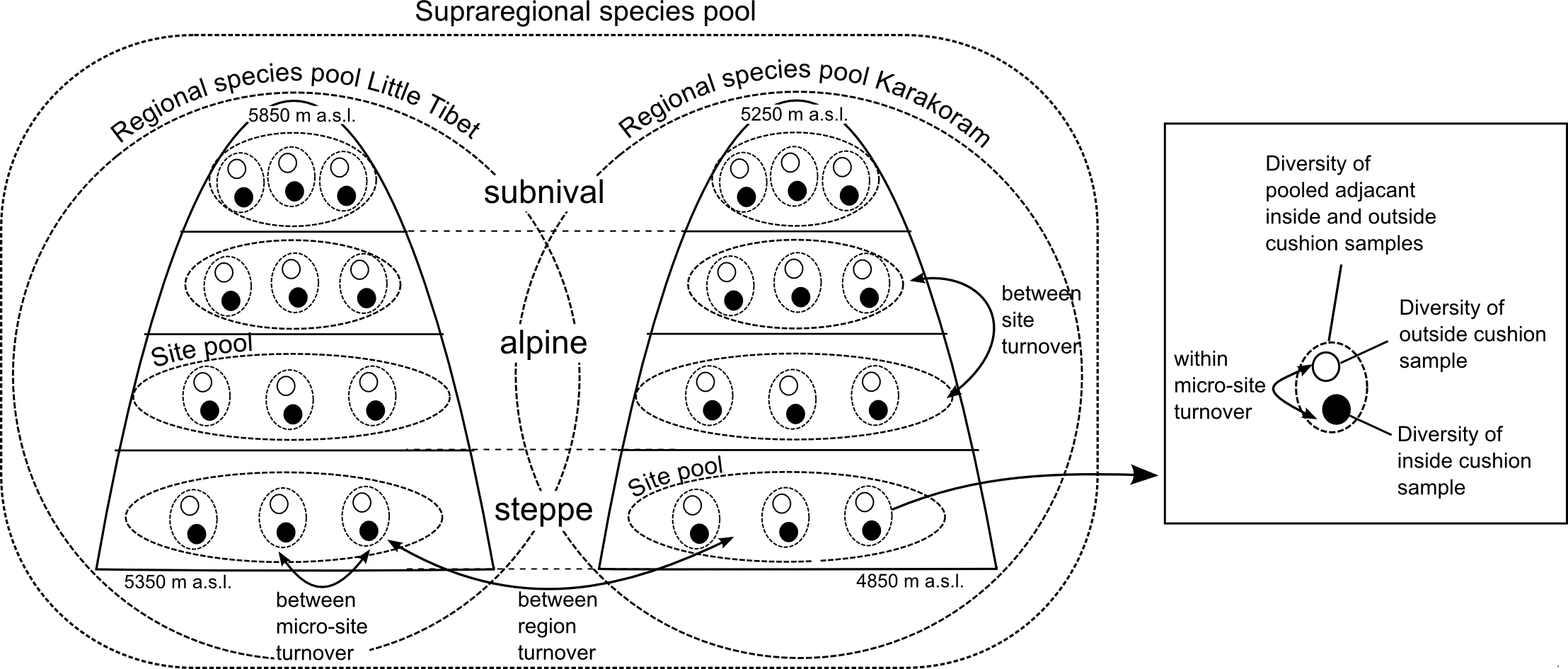
**Figure legend**

**Figure 1**. Graphical representation of the analytical framework used in this study. We represent the different spatial scales, as well as the reference pools used. Note that we applied this framework across the whole-tree and for most recent speciation events (tip level).

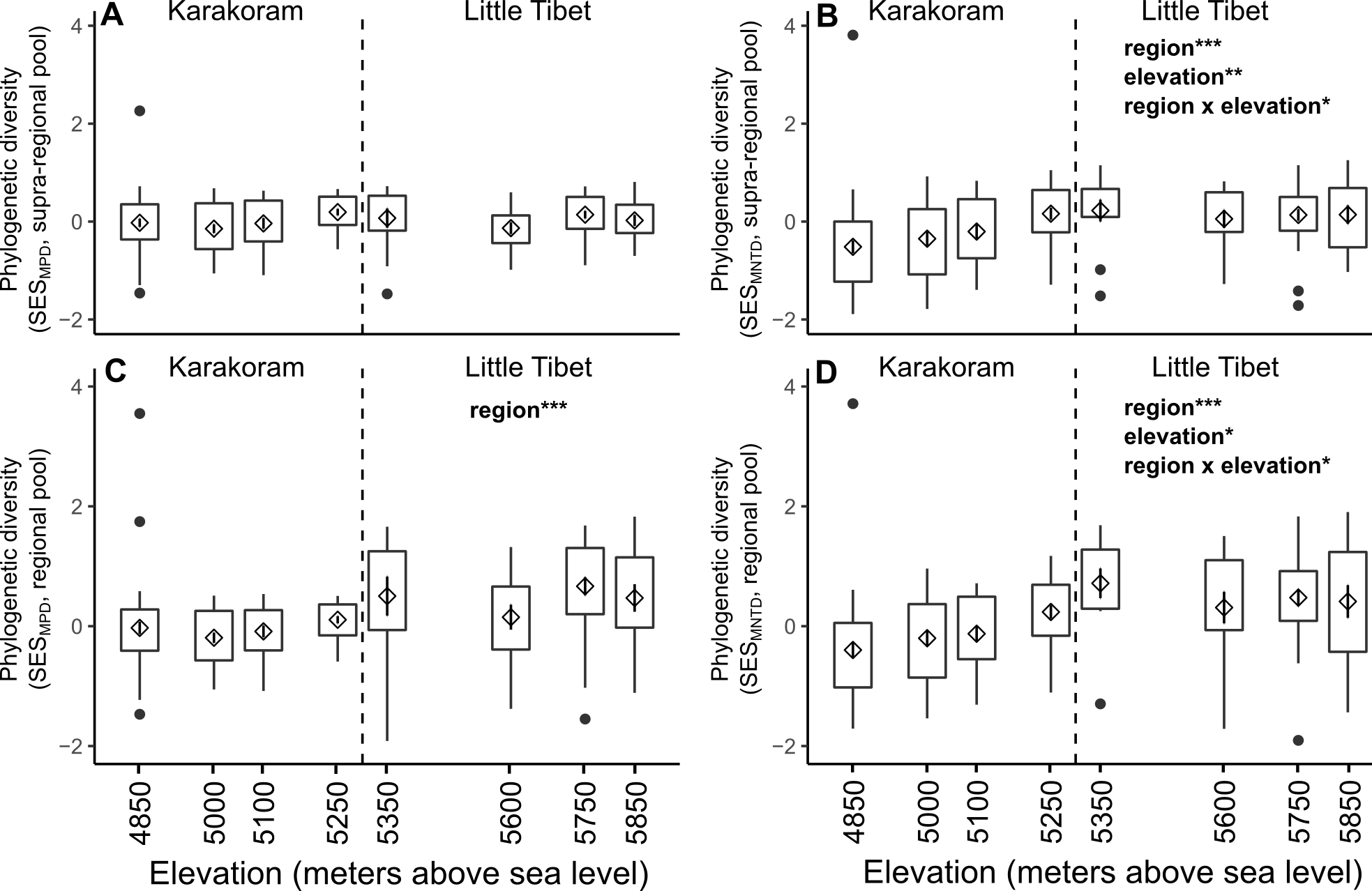
**Figure 2**. Boxplot of standardized effect sizes (SES), their mean (diamond) and error around the mean (bar through the diamond) of phylogenetic diversity at the community level (pairs of microsites inside and outside cushions) along the elevation gradient. A and B show SES of mean pairwise phylogenetic distance (MPD) and mean phylogenetic distance to the nearest neighbor (MNTD), respectively, compared to communities randomly drawn from the supra-regional reference pool with the same species richness. C and D show SES of mean pairwise phylogenetic distance (MPD) and mean phylogenetic distance to the nearest neighbor (MNTD), respectively, compared to communities randomly drawn from the regional reference pools with the same species richness. Bold terms describe the significant terms in a linear model with region, elevation, and their interaction as explanatory variables (see Table 1). NS: not significant; \* significant with P < 0.05; \*\* significant with P < 0.01; \*\*\* significant with P < 0.001

**Figure 3**. Phylogenetic turnover based on MPD (A) and MNTD (B), respectively, for all pairwise comparisons between communities (i.e., between pairs of microsites across communities) from the two different regions, and between communities within each of the regions. Boxplots depict the distribution of SES values; the diamond and bar their mean and error around the mean, respectively. Significance above the lines are results of a post-hoc Tukey HSD test to compare the means of the three groups. NS: not significant; \* significant with P < 0.05; \*\* significant with P < 0.01; \*\*\* significant with P < 0.001

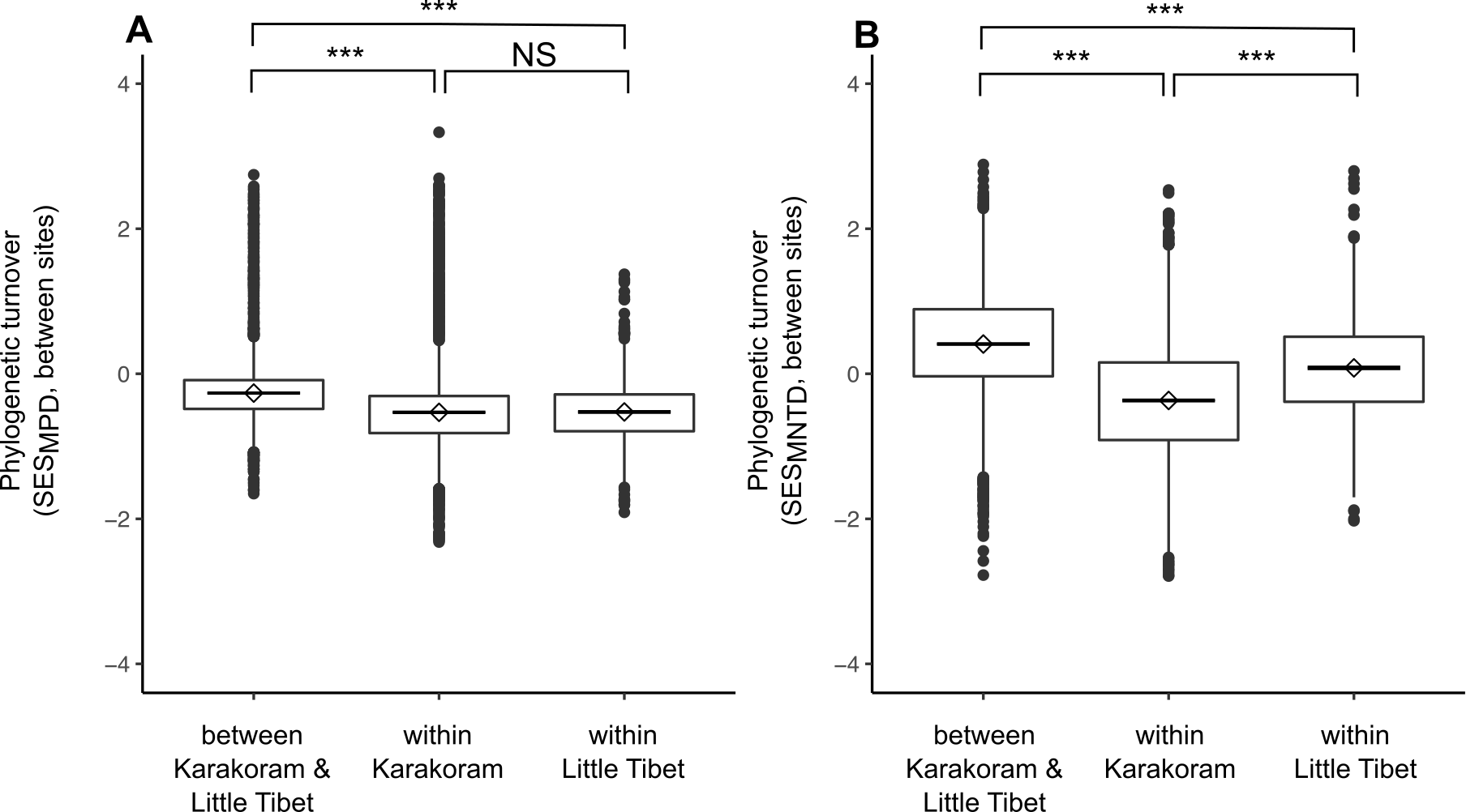
**Figure 4**. Phylogenetic diversity and turnover with the local reference pool for randomization (i.e., all species found in the investigated site at a given elevation range). Boxplots depict the distribution of SES values; the diamond and bar their mean and error around the mean, respectively. A and B show the diversity at the community level separately for microsites inside and outside of the cushion plant *Thylacospermum caespitosum* across the elevation gradient as mean pairwise phylogenetic distance (MPD, subfigure A) or mean phylogenetic distance to the nearest neighbor (MNTD, subfigure B), compared to communities randomly drawn from the local reference pool with the same species richness. Bold terms describe the significant terms in a linear mixed effect model with region, elevation, position inside or outside the cushion, and all possible interactions as fixed factor, and pairs of microsites (cushion and adjacent open area) nested within a site as random factor. C and D show phylogenetic turnovers based on MPD and MNTD, respectively, for pairs of adjacent inside and outside cushion microsites along the elevation gradient, compared to communities randomized by shuffling species abundances within a site (see methods for details). Bold terms describe the significant terms in linear models with region, elevation, position outside or inside the cushion and their interactions (for phylogenetic diversity), and with region, elevation, and their interaction (for turnover), as explanatory variables (see Table 2). NS: not significant; \* significant with P < 0.05; \*\* significant with P < 0.01; \*\*\* significant with P < 0.001.



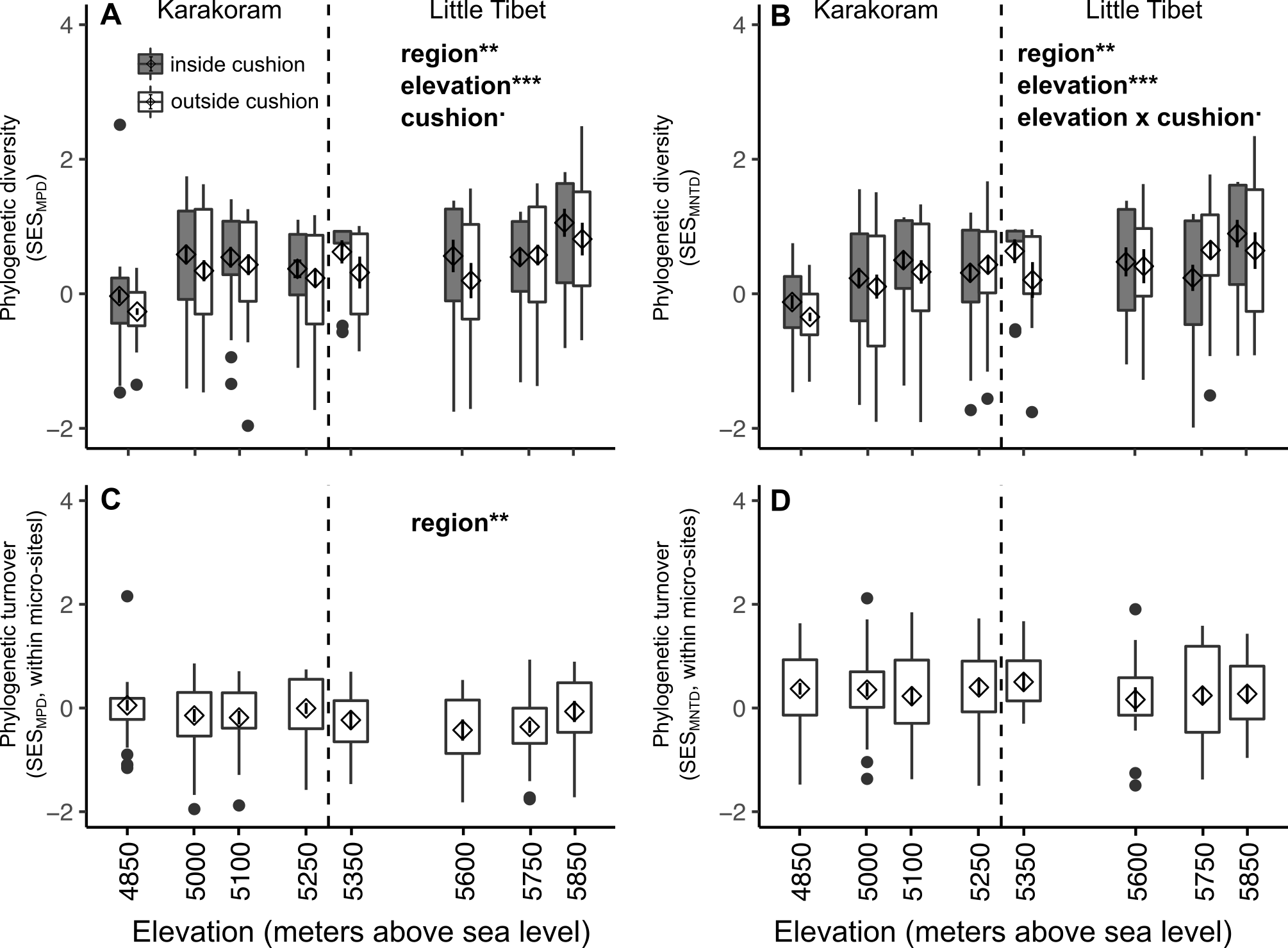
**Fig. 1.**



**Fig. 2**.



**Fig. 3**.



**Fig. 4**.

**Tables**

**Table 1.** Results of the mixed effect ANOVA models with phylogenetic diversity expressed as standardized effect size (SES) of MPD and MNTD as response variables. As reference pool for randomizations served either the supra-regional or the regional species pool. Region, elevation, and their interaction were used as fixed factors. Pairs of microhabitats (cushion and adjacent open area) were used as random factor and nested within communities. Significant factors are in bold.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | SESMPD | | | SESMNTD | | |
| Factor | df | F value | P | Df | F value | P |
|  | Supra-regional reference pool | | | | | |
| Region | 1 | 0.11 | 0.73 | 1 | 13.93 | **< 0.001** |
| Elevation | 1 | 0.70 | 0.40 | 1 | 8.89 | **< 0.01** |
| Region x Elevation | 1 | 0.21 | 0.65 | 1 | 5.86 | **< 0.05** |
|  |  |  |  |  |  |  |
|  | Regional reference pool | | | | | |
| Region | 1 | 30.71 | **< 0.001** | 1 | 21.72 | **< 0.001** |
| Elevation | 1 | 1.03 | 0.31 | 1 | 6.22 | **< 0.05** |
| Region x Elevation | 1 | 0.01 | 0.94 | 1 | 4.42 | **< 0.05** |

**Table 2**. Results of the mixed effect ANOVA models with phylogenetic diversity and turnover expressed as standardized effect size (SES) of MPD and MNTD as response variables. For diversity, region, elevation, location outside or inside the cushion, and interactions among these factors were used as fixed factors. Pairs of microsites (cushion and adjacent open area) were used as random factor and nested within site. For phylogenetic turnover between inside and outside cushions, region, elevation and their interaction were used as fixed factors. Significant factors are in bold.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | SESMPD | | | SESMNTD | | |
| Factor | df | F value | P | df | F value | P |
|  | **Phylogenetic diversity** inside and outside cushions, site as the reference pool | | | | | |
| Region | 1 | 9.45 | **< 0.01** | 1 | 10.20 | **< 0.01** |
| Elevation | 1 | 26.32 | **< 0.001** | 1 | 28.08 | **< 0.001** |
| Cushion | 1 | 3.43 | 0.06 | 1 | 0.20 | 0.65 |
| Region x Elevation | 1 | 0.20 | 0.65 | 1 | 0.56 | 0.45 |
| Region x Cushion | 1 | 0.11 | 0.74 | 1 | 0.97 | 0.33 |
| Cushion x Elevation | 1 | 2.37 | 0.12 | 1 | 3.54 | 0.06 |
| Region x Cushion x Elevation | 1 | 1.19 | 0.27 | 1 | 0.53 | 0.46 |
|  | **Phylogenetic turnover** between inside and outside cushions, site as the reference pool | | | | | |
| Region | 1 | 9.43 | **< 0.01** | 1 | 2.30 | 0.13 |
| Elevation | 1 | 0.001 | 0.97 | 1 | 0.03 | 0.85 |
| Region x Elevation | 1 | 1.00 | 0.32 | 1 | 0.17 | 0.68 |

**Appendices**

**Appendix S1**. Results of the mixed effect ANOVA models with phylogenetic diversity (standardized effect size: SES) as a response variable at a) the site and b) the microsite scale. Region, pools (supra-regional vs. regional reference pools), elevation, phylogenetic scale (MPD vs. MNTD) and their interactions were used as fixed factors at the site scale. Region, elevation, cushions, phylogenetic scale and their interactions were used as fixed factors at the microsite scale. Pairs of microhabitats (cushion and adjacent open area) were used as random factor and nested within communities. Significant factors are in bold. We also show the explained variance of each predictor.

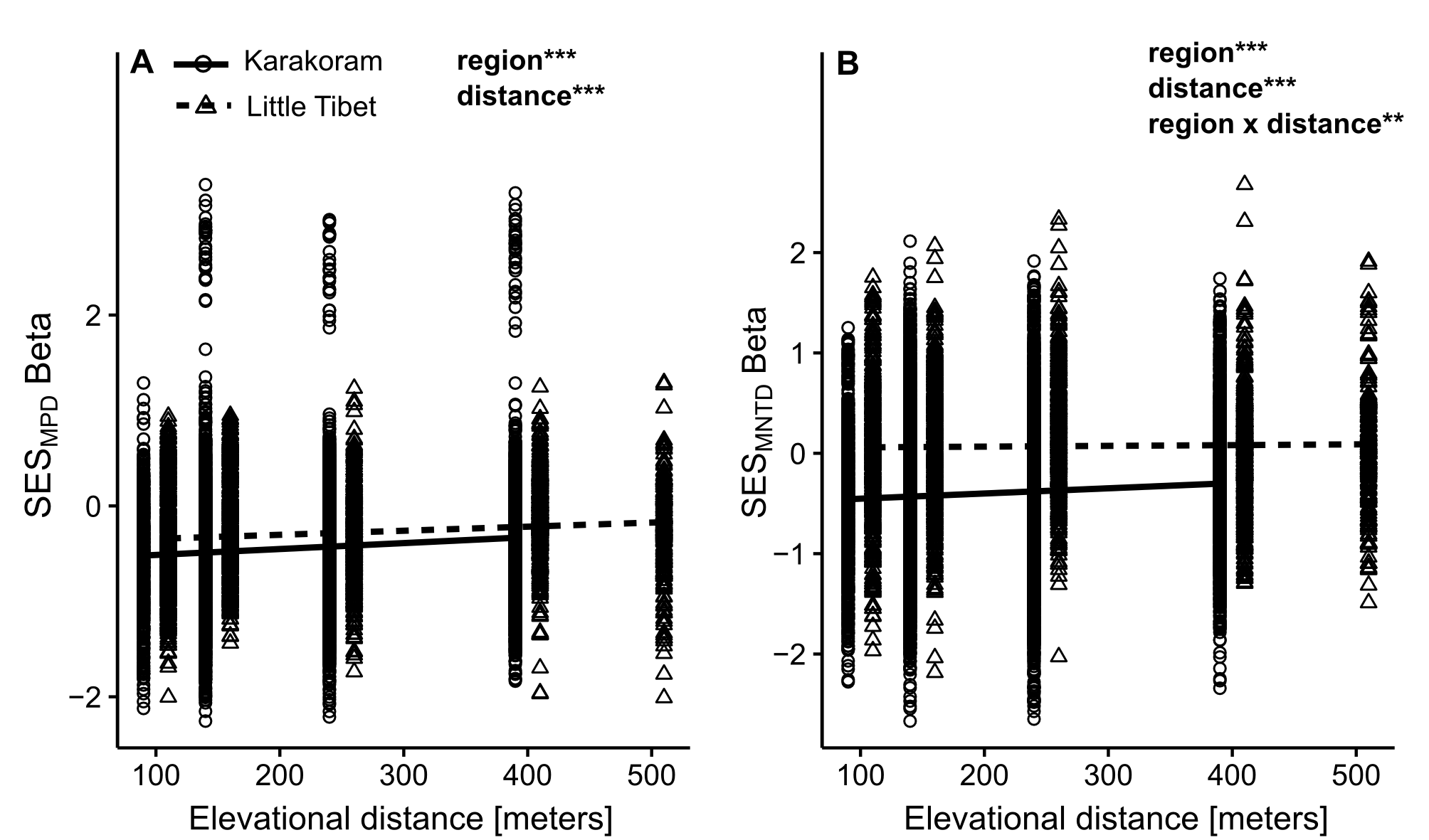
**a)**



**b)**



**Appendix S2**. Standardized effect sizes (SES) of phylogenetic turnover based on MPD (A) and MNTD (B), respectively, for all pairwise comparisons between communities within a region and their relationship with the elevational distance between the plot pairs. Bold terms describe the significant terms in a linear model with region, elevational distance, and their interaction as explanatory variables. NS: not significant; \* significant with P < 0.05; \*\* significant with P < 0.01; \*\*\* significant with P < 0.001.



**Appendix S3**. Phylogenetic trees of studied species. A: for the supra-regional reference pool containing all samples. B: for the regional species pool of Karakoram. C: for the regional species pool of Little Tibet.

