COMMUNITY ECOLOGY – ORIGINAL RESEARCH

Plant–plant interactions influence phylogenetic diversity at multiple spatial scales in a semi-arid mountain rangeland

Maral Pashirzad[1] · Hamid Ejtehadi[1] · Jamil Vaezi[1] · Richard P. Shefferson[2]

Abstract

Molecular phylogenies are increasingly used to understand how biotic interactions and environment shape phylogenetic community structure (PCS). However, we do not understand the effects of plant–plant interactions and environment on PCS and phylogenetic diversity across spatial scales, particularly in rangelands. Here, we ask: (1) do plant–plant interactions and environment affect PCS and phylogenetic diversity differently across the three spatial scales of the patch, the community, and the habitat? (2) What are the impacts of dominant cushion-nurse plants on the phylogenetic structure of plant communities? We assessed the PCS of semi-arid plant communities along an elevation gradient at the patch, community and habitat scales. Then, we assessed co-occurrence patterns along two sample slopes. Our results indicated important roles for biotic interactions and environmental filtering in determining phylogenetic diversity, with biotic interactions, in particular, having a stronger tendency to increase phylogenetic diversity. This is most likely due to the asymmetrical effects of nurse plants across the three spatial scales on our two different slopes. The impact of biotic interactions caused non-random phylogenetic patterns in more severe environments. In conclusion, biotic interactions influence phylogenetic diversity by altering PCS across aspects and along elevation gradients.

✉ Hamid Ejtehadi
hejtehadi@um.ac.ir; hejtehadi@yahoo.com

Maral Pashirzad
Maral.Pashirzad@stu.um.ac.ir

Jamil Vaezi
vaezi@um.ac.ir

Richard P. Shefferson
dormancy@gmail.com

[1] Quantitative Plant Ecology and Biodiversity Research Lab., Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

[2] Organization for Programs on Environmental Sciences, Faculty of Arts and Sciences, University of Tokyo, Tokyo, Japan

Introduction

Understanding the mechanisms shaping plant communities is a fundamental goal of ecology (Mo et al. 2013; Webb et al. 2002). The study of phylogenetic community structure (PCS) through the comparison of relatedness among species across space, particularly at different spatial scales, offers insights into these mechanisms (Cavender-Bares et al. 2006; Swenson et al. 2006; Fine and Kembel 2011; Machac et al. 2011; Chun and Lee 2017; Fournier et al. 2017; Qian et al. 2017; Head et al. 2018; Pashirzad et al. 2018). Recent studies suggest that biotic interactions drive differences among communities in ways unaccounted for by existing models (Anderson et al. 2011; Cavender-Bares et al. 2009). Assessments of the impacts of biotic interactions and environmental conditions are needed but have rarely been performed (Lortie et al. 2004; Soliveres et al. 2012), especially in relation to one another at different spatial scales (Verdú et al. 2009).

Species interactions among plants influence the structure and diversity of plant communities in terrestrial ecosystems

(Brooker et al. 2008). Realized species niches can expand over evolutionary time through the impacts of plant–plant interactions (Valiente-Banuet and Verdú 2013). The abiotic environment and evolutionary relationships between interacting species strongly influence species interactions (Maestre et al. 2009; Valiente-Banuet and Verdú 2013), and these impacts may translate into altered community structure. Several empirical and theoretical models have been created to explain these impacts (Butterfield et al. 2013). One such model, the 'compensation hypothesis', states dominant plant species acting in a nurse role (i.e. positive and negative effects of dominant plants on habitat suitability for different species) contract environmental filters in benign conditions (competition), but expand filters in severe conditions (facilitation) (Callaway et al. 2002). Another model, the 'severity divergence hypothesis', states that such plants simultaneously expand and contract environmental filters in more severe environments. The Mean Pairwise Distance Index (Webb 2000) as a measure of PCS can illuminate these different patterns in PCS at microsites influenced by the presence or absence of nurse species (Butterfield et al. 2013).

Most studies investigating the impacts of biotic factors on phylogenetic diversity have considered only interactions between a few species (Maestre et al. 2009), and few have focused on the community scale (Maltez-Mouro et al. 2010). The latter is particularly problematic, because most inference of community effects is based on studies at the patch scale, rather than at the higher community scale (Brooker et al. 2008; Soliveres et al. 2012). Moreover, patterns of PCS are highly scale-dependent and different conclusions about the relative importance of biotic and abiotic factors can result from PCS analyses at different spatial and environmental scales (Cavender-Bares et al. 2006; Swenson et al. 2006). Recent studies reinforce this by showing that habitat specialization drives different patterns in phylogenetic structure of communities both among and within habitats (Kembel and Hubbel 2006; Fine and Kembel 2011; Yang et al. 2015; Pashirzad et al. 2018). Therefore, quantifying the phylogenetic structure of communities in different habitat types (i.e. quantifying the PCS at habitat scale) while simultaneously studying other spatial scales should profoundly increase our understanding of community assembly processes (Fine and Kembel 2011; Yang et al. 2015; Pashirzad et al. 2018).

Studies on the impacts of biotic and abiotic factors on PCS have been carried out often in alpine regions (Soliveres et al. 2012). However, we are unaware of any empirical evidence from mountainous arid and semi-arid rangelands, where much of PCS theory was developed (Flores and Jurado 2003). Several important factors, such as environmental severity, heterogeneous topography, and the presence of cushion plants, make mountainous rangelands particularly suitable ecosystems for the investigation of the ecology of biotic interactions and topography over different spatial

scales of a landscape (Alejandro et al. 2009; Farzam and Ejtehadi 2016). In mountainous rangelands, better climatic conditions of mesic slopes (northern aspect in the Northern Hemisphere) support the presence of fragile plant species (i.e. more sensitive to disturbance), whereas harsher conditions along drier slopes (southern aspect in the Northern Hemisphere) lead to the establishment of more spiny shrubs (stress-tolerant species) (Farzam and Ejtehadi 2016). Therefore, shrubs may have stronger effects on PCS on xeric rather than mesic exposures through the evolutionary divergence of taxa in microhabitats in harsh environments (Alejandro et al. 2009; Butterfield et al. 2013). They may also exhibit such patterns across elevation, assuming a gradient of environmental severity across elevation (Piston et al. 2016).

Our goal in this study was to understand the factors shaping phylogenetic structure in mountainous rangeland plant communities. We analyzed the effects of biotic and abiotic factors on PCS and phylogenetic diversity across three spatial scales, along an environmental gradient spanning the North and South aspects of a semi-arid mountain ecosystem, linking such effects to microhabitat conditions under the canopy of shrub species. To evaluate the relative effects of factors at the patch scale and at the community scale, we also analyzed the contribution of species co-occurrence patterns to community phylogenetic diversity. We tested the hypothesis that the factors determining phylogenetic structure at the patch scale also defined phylogenetic structure at the community scale, and asked whether biotic interactions were as important as environmental filtering in determining phylogenetic structure and patterns. Finally, we tested whether patterns observed at one spatial scale can reflect patterns and processes at others. To this effect, we determined whether a coarser scale categorical perspective can provide results comparable to those generated from fine scale continuous representations of biotic and abiotic factors.

Materials and methods

Study area

Our study region is a 2500-ha mountainous rangeland in the southern range of the Hezar-Masjed Mountains, located in the eastern Khorassan-Kopet Dagh floristic province in the Irano-Turanian region in northeastern Iran. This floristic province is an important center of plant endemism in the Irano-Turanian region (Memariani et al. 2016). It is between 36°40′ and 36°55′N, 59°17′ and 59°31′E, and elevation ranges from 1300 to 2000 m, generally increasing from south to north (Fig. 1). Mean monthly precipitation ranges from 0 to 45 mm, and mean monthly temperature is from 0 to 25 °C. Significant rainfall occurs in Fall and Winter, whereas Spring and Summer are dry and hot, respectively
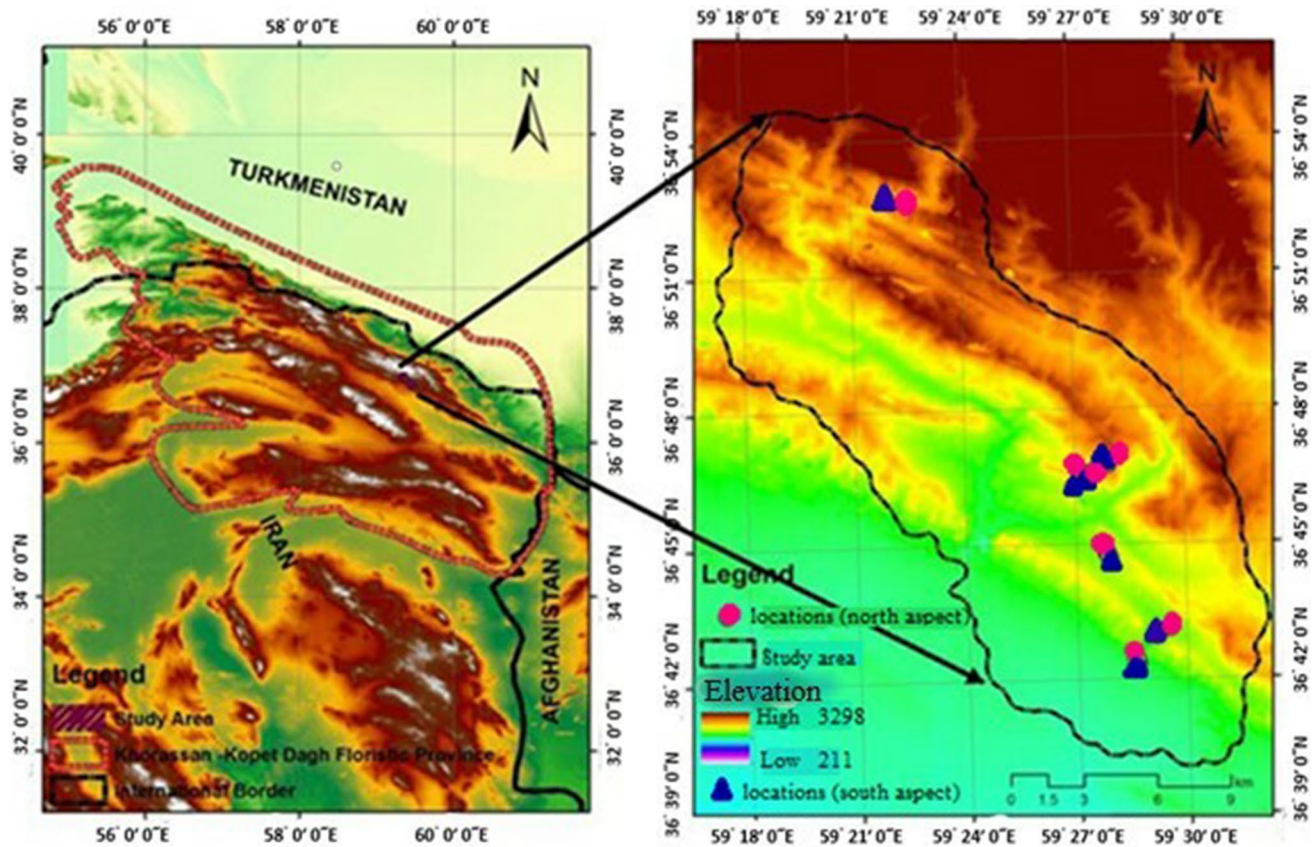
Fig. 1 Map showing the study region and the locations of the seven study sites in the eastern part of Khorassan-Kopet Dagh Floristic province, located in northeastern Iran

(Appendix S1). Plant communities are typically dominated by herbaceous plants and shrubs.

The dominant shrub species are typically *Acantholimon pterostegium* Bunge. (Plumbaginaceae) and *Acanthophyllum diezianum* Hand.-Mazz. (Caryophyllaceae). Other shrub species such as *Artemisia kopetdaghensis* Krasch., Popov & Lincz. *ex* Poljakov (Asteraceae), *Juniperus polycarpos* var. *turcomanica* (B.Fedtsch.) R.P.Adams (Cupressaceae) and *Berberis integerrima* Bunge (Berberidaceae) are only found in certain habitats. In the herbaceous layer, *Holosteum glutinosum* (M.Bieb.) Fisch. & C.A.Mey., (Caryophyllaceae), *Androsace maxima* L. (Primulaceae), *Taeniatherum caput-medusae* (L.) Nevski (Poaceae), *Minuartia meyeri* (Boiss.) Bornm., (Caryophyllaceae), *Carex stenophylla* Wahlenb., (Cyperaceae), *Eremurus stenophyllus* (Boiss. & Buhse) Baker (Asphodelaceae), *Gladiolus atroviolaceus* Boiss. (Iridaceae) and *Allium giganteum* Regel.(Amaryllidaceae) are indicator species for different habitat types (Pashirzad et al. 2018). Our analyses focused on the two most dominant shrubs at these locations, *A. pterostegium* Bunge. and *A. diezianum* Hand.-Mazz., both of which occurred at all of the sites sampled. Both species form dense cushion-like canopy structures. *A. diezianum* is a cushion forming plant covered by long hairs, occurring exclusively in the Irano-Turanian region, especially in Iran and Afghanistan (Pirani and Rabeler 2017). *A. pterostegium* is an Iranian endemic species restricted mostly to NE Iran (Memariani et al. 2016). Jankju et al. (2008) found higher soil fertility under the canopy of *Acantholimon* and *Acanthophyllum* as compared with that of open areas, and they suggested that this difference was due to higher litter accumulation under the canopy of cushion-like nurse species.

Data collection

We selected seven sites on each slope (i.e. the northern slopes and the southern slopes) of the Hezar-Masjed Mountains at 1350 m to 1950 m elevation. To examine the relationship between elevation gradient and climate, we extracted annual precipitation (at millimeters) and annual mean temperature (in degrees Celsius) for each plot at each location (community) from the WorldClim v1.4 database (http://www.worldclim.org); (Hijmans et al. 2005). Mean annual temperature and annual precipitation for each location were calculated as mean values of annual mean temperature and annual precipitation across the seven plots

in each location (community). Annual mean temperature decreases across elevation about 3.3 °C on the north slopes and 2.8 °C on the south slopes, while annual precipitation increases around 37 mm and 31 mm across the north and south slopes, respectively (see Online supplementary material Appendix S2).

We considered three spatial scales in this study: the habitat scale (largest), the community scale and the vegetation patch scale (smallest). We systematically selected our seven locations, each approximately 100 m higher than the previous, from 1350 to 1950 m elevation on each slope (north vs. south) (Fig. 1). Within each location, seven 25 m² plots, with approximate distance of 100–150 m, were randomly established. In each plot, species abundance was determined by recording the number of individuals of all present plants at the quadrat. Plant specimens were collected for identification, and 129 plant taxa were identified in total. Vegetation data were collected in spring and summer 2017.

The community level included vegetation composition at each location (the sum of vegetation composition of seven plots located in each location). To evaluate interactions at the patch level, we randomly selected eight individuals of each shrub species (i.e. *A. diezianum* and *A. pterostegium*) at each location (community) and on each aspect. We sampled the area under their canopy using 0.5 m × 0.5 m quadrats. Eight paired open areas using 0.5 m × 0.5 m quadrats were randomly selected adjacent to these shrubs (but at least 1 m away the shrub canopy). The abundance (number of individuals) of all plant species was recorded within each quadrat.

At the habitat scale, we identified habitats using topographic variables including elevation and slope aspect. We used multivariate regression trees (MRT) via the classifications and regression tree analysis (CART) extension to handle our response variables (De'ath 2002; Borcard et al. 2012). MRT was employed to predict habitats as MRT clusters from communities (Krishnadas et al. 2016). The clusters and their dependence on the environmental and topographic variables were represented graphically by a tree. Our selected tree was based on minimum CVRE (i.e. cross-validated relative error, where lower CVRE indicates a better fit) (De'ath 2002). Finally, five habitat types (H1, H2, H3, H4 and H5) were identified via multivariate regression tree analysis. The best fit tree in this analysis had a CVRE of 0.75 ($R^2 = 0.25$). Each 25 m² plot was assigned to one of the five habitat types (H1, H2, H3, H4 and H5) in the study region (the spatial distribution of the five habitat types is given in Appendix S3). The first split based on elevation (≥ or < 1450, asp = N,S) explained 25% of the variation in community composition across all plots. High elevation plots were further segregated based on elevation (> or ≤ 1750, asp = N,S; See Supporting Information Appendix S5). This analysis was performed via the package *mvpart* ver.1.3-1 (De'ath 2010) in *R* version 3.2.5 (R Core Team 2017).

Data analysis

Phylogenetic analysis

A species pool was constructed from all of the plant species within the communities for phylogenetic community analysis. The final list included 129 species of angiosperms and gymnosperms. For each plant species, we used two sequences: (1) plastid DNA gene (rbcL) and (2) nuclear DNA gene (ITS, containing ITS1, 5.8 s and ITS2). Some sequences were obtained from GenBank and for other species silica-dried samples and herbarium leaves were used for DNA extraction using a modified CTAB protocol (Joly et al. 2006). We PCR amplified samples using the standard methodology for Takara ExTaq and sequenced the plant ITS and rbcL regions with primers ITS1 and ITS4 (White et al. 1990) and rbcLa-F and rbcLa-R (Kores et al. 1997), respectively. Detailed sources for the DNA sequences of each species have been reported elsewhere (Pashirzad et al. 2018). Finally, plant sequences were aligned using MAFFT software (Katoh and Standley 2013).

Phylogenetic reconstruction

In the field of community ecology, most community studies lack DNA sequence data, and therefore, phylogenies have been organized with programs reflecting taxonomic structure (Kress et al. 2009). Such phylogenetic trees are usually only resolved at the order and family levels. Therefore, we conducted additional analyses on DNA sequences for a more thorough exploration of tree topology and branch lengths. We generated a maximum likelihood phylogeny using our sequence data in PHYML, using a BIONJ starting tree (Guindon and Gascuel 2003) (see Online supplementary material, Appendix S4). The branch lengths of this tree were estimated using penalized maximum likelihood to estimate divergence times (Sanderson 2002). According to previous findings, the species pool at different spatial scales overestimates the impacts of environmental filtering and biotic interactions on phylogenetic patterns (Cavender-Bares et al. 2006; Kraft and Ackerly 2010). Hence, we constrained our species pool to those species occurring in the studied communities (our locations), and not to species present in ecosystems throughout the study region as a whole (Soliveres et al. 2012). Therefore, the community spatial scale has an appropriate size to include both of these processes (Rajaniemi et al. 2006).

Evaluating plant–plant interactions and environment on phylogenetic diversity and PCS in three spatial scales

Patch scale

Phylogenetic structure within open and cushion microsites were calculated with mean phylogenetic distance (MPD)

as an indicator of phylogenetic pattern (Webb et al. 2002). Phylogenetic structure between cushion and open microsites was calculated based on the mean phylogenetic distance between taxa across the open and cushion microsites (bMPD). We compared these indices with null models to test whether phylogenetic structures differed from random communities generated by null models. Independent-swap randomization was used to represent the expected distribution of MPD under the null model. This algorithm maintains species richness and species frequency at each site (Gotelli 2000). Standardized effect sizes (SES) for MPD were calculated and these analyses were performed in *R* using package *picante* (Kembel et al. 2010). Positive SES values (SES values > 1.96) indicate significant phylogenetic over-dispersion in the community, while negative SES values (SES values < − 1.96) represent phylogenetic clustering (Lopez-Angulo et al. 2018). Phylogenetic structure between open and cushion microsites (bSES.mpd) was estimated using the independent swap algorithm, and random communities were generated to estimate observed abundance distributions and occupancy rates of species across communities (Bryant et al. 2008). Positive values of bSES.mpd indicate that two microsites consist of more phylogenetically distantly related taxa than random, and negative values suggest the presence of more phylogenetically closely related taxa than random. To minimize the effects of particularly abundant species (such as ruderal species with high population turnover), we measured the phylogenetic structure of different microsites based on log10-transformed abundance.

Evaluation of both species interactions and environmental factors on phylogenetic structure is important because species interactions are highly dependent on environmental variables. Therefore, we analyzed variation in the phylogenetic structure of communities relative to the presence and absence of nurse plants (species interaction effect) along elevation gradients (abiotic effect) at the patch level via regression models. We modeled SES.mpdCushion, SES.mpdopen and bSES.mpd as response variables, and elevation and slope as independent factors using a general linear model framework.

Community scale

We estimated species interactions at the community level (studied locations) based on patterns of species co-occurrence (Gotelli and Graves 1996). Seven matrices (seven sites located in each location) were analyzed using the checkerboard score (C-score) on each aspect to evaluate species co-occurrence patterns in each community. This index measures how often different species pairs appear at the same sites (Gotelli 2000). Fourteen observed C_scores (seven observed C_scores for each studied aspects) were compared to C_score indices derived from 5000 null matrices (Gotelli

2000). Similar algorithms at the patch scale were selected to create null matrices for the community level. Lastly, we calculated standardized effect sizes (SES) of C_score for each community (Bowker et al. 2010). Less co-occurrence (indicator of competition) and more co-occurrence (indicator of facilitation) than expected by chance would yield positive and negative C score SES values, respectively (Lopez et al. 2013). Moreover, at the community scale, we measured standard effect sizes of MPD (SES.MPD) as indicators of phylogenetic pattern (Webb et al. 2002) for each community based on the above algorithm (Webb et al. 2008).

At the community scale, first, we analyzed variation in species interactions along elevation on both aspects using regression models. We developed first, second and third order polynomial regressions to evaluate our two community-level phylogenetic hypotheses. We compared fitted models using second-order Akaike information criteria (AICc). Next, we conducted multiple regression analyses with MPD as the response variable and independent variables including elevation, C_score, and their interaction (elevation and C_score vs. MPD) on both aspects. We used Akaike's information criterion (AIC) to determine the best regression model and the most effective variable (or variables) on PCS.

Habitat scale

We assessed whether factors influencing PCS at local scales (i.e. plant–plant interactions and elevation) impact habitat specialization and habitat segregation of species into different habitats. To quantify this, we calculated SES.mpd for each community within the same habitats, and then used box plots to compare the SES.mpd of communities within habitats.

Results

At the patch scale, measures of PCS correlated with one another and with environmental variation along both north and south facing slopes when compared with random communities drawn from the community species pool (Fig. 2). However, we observed different phylogenetic patterns along the two slopes as elevation increased. On north-facing slopes, SES.mpdOpen (Fig. 2b) indicated greater phylogenetic clustering ($r = − 0.30$), while SES.mpdCushion (Fig. 2a) indicated phylogenetic overdispersion in cold environments ($r = 0.41$). Thus, with increasing environmental severity on north-facing slopes, clades present in open microsites became more restricted than clades in cushion microsites (Fig. 3a). The phylogenetic composition of cushion microsites converged in more severe environments from that found in open microsites (Fig. 3a),
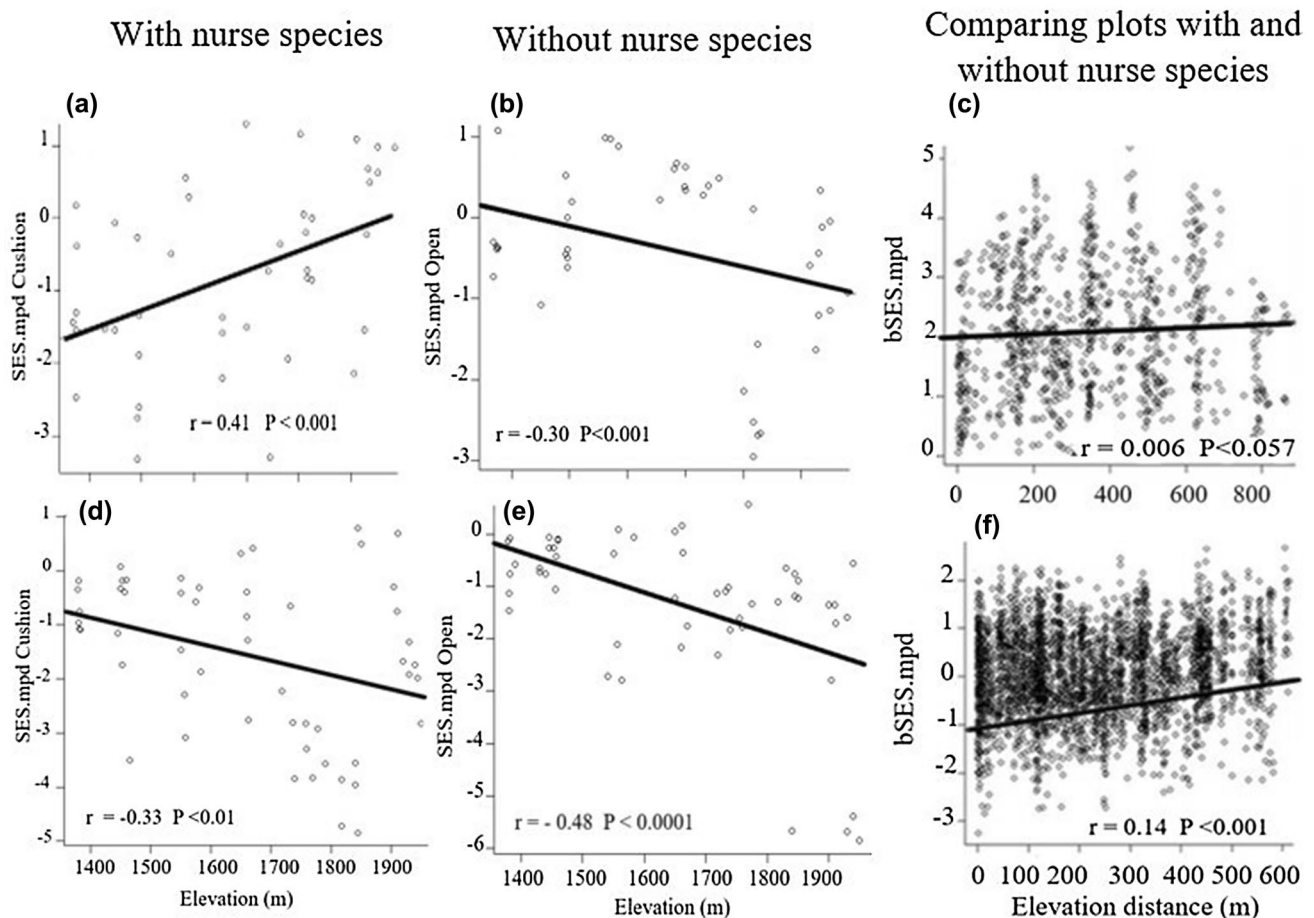
With nurse species

Without nurse species

Comparing plots with and without nurse species

Fig. 2 Relationships between the dimensions of phylogenetic community structure relative to elevation measured as standard effect sizes of mean phylogenetic distance on **a–c** north-and **d–f** south-fac-

ing slopes. Left panels (**a**, **d**) sites with dominant nurse species present; middle panels (**b**, **e**) excluding sites with the nurse species; right panels (**c**, **f**) comparing sites with and without nurse species

resulting in no relationship in turnover of between total community PCS (Fig. 2c). In contrast, on south-facing slopes, SES.mpdOpen (Fig. 2e) and SES.mpdCushion (Fig. 2d) indicated greater phylogenetic clustering in cold environments (stressful environments) and greater phylogenetic dispersion in less severe environments, while phylogenetic relatedness between open and cushion microsites within communities (bSES.mpd) increased with environmental severity ($r = 0.14$) (Fig. 2f). At least on south-facing slopes, open and cushion microsite filters tended to be restrictive, but open microsites became more restrictive in severe environments. This suggests that the clades present in open vs. cushion microsites should diverge from one another (Fig. 3b).

At the community scale, communities located in lower elevations on north-facing slopes exhibited less co-occurrence than expected by chance, as indicated by positive C-score SES values (Fig. 4a). This is evidence of competition among species observed in lower elevation communities. Strong association between species (negative SES

values) indicating facilitation was observed only in one community of higher elevation (community 6). Therefore, with increasing environmental severity (increasing elevation) on north-facing slopes, interactions among species across communities changed from primarily competitive to facilitative (Fig. 4a). Additionally, elevation was nonlinearly related to the degree of co-occurrence among species (significant second order polynomial model with AICc = 17.90, Table 1), revealing a tendency toward facilitation in the mid-range of stress gradients (Fig. 4a). A significant linear component (elevation coefficient = − 5.48) in our second-order polynomial model suggests increasing co-occurrence (Fig. 4b, Table 1).

On south-facing slopes, communities located at the lowest elevation exhibited negative SES (− 1.89), suggesting a greater association than expected by chance, but this value was not significant (Fig. 4b). At intermediate elevation severity, communities tended to have either positive or negative SES values. Co-occurrence patterns along elevation were more complex on south-facing slopes. Finally,

Fig. 3 Schematic diagrams showing two phylogenetic hypotheses tested in this study. **a** Compensation hypothesis: variation in composition of communities from low to high elevation on north-facing slopes. **b** Severity divergence hypothesis: variation in species composition of communities along elevation gradients on south-facing slopes. Blue and purple squares indicate taxa present in the community in the absence and presence of nurse species, respectively
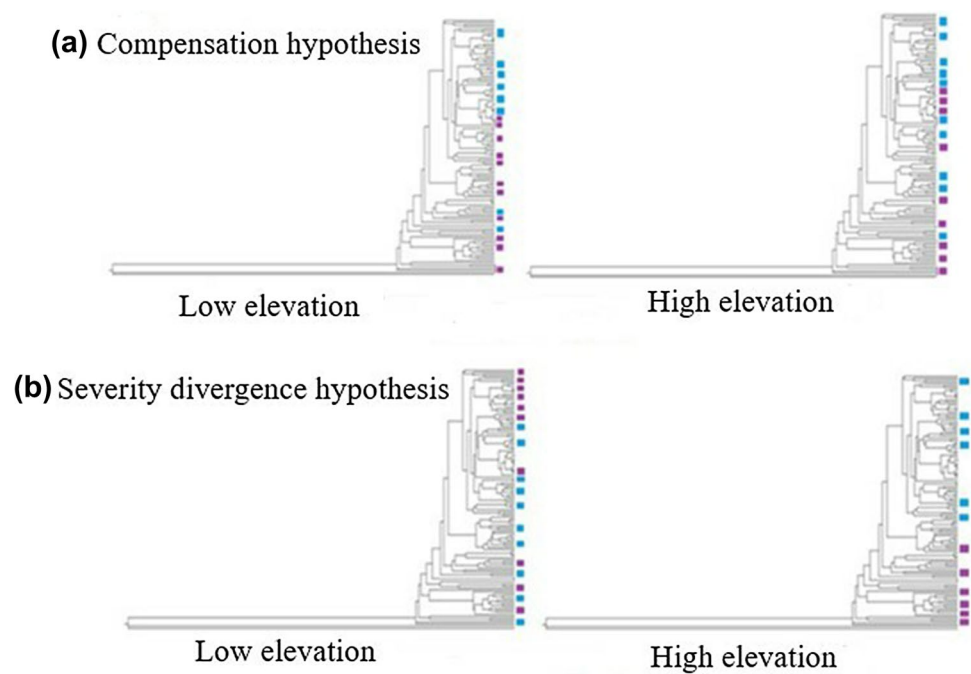
(a) Compensation hypothesis

Low elevation   High elevation

(b) Severity divergence hypothesis

Low elevation   High elevation

Fig. 4 Significant non-linear relationships between elevation and species co-occurrence (C-score index) across seven communities on **a** north-facing (second order polynomial) and **b** south-facing slopes (third order polynomial)
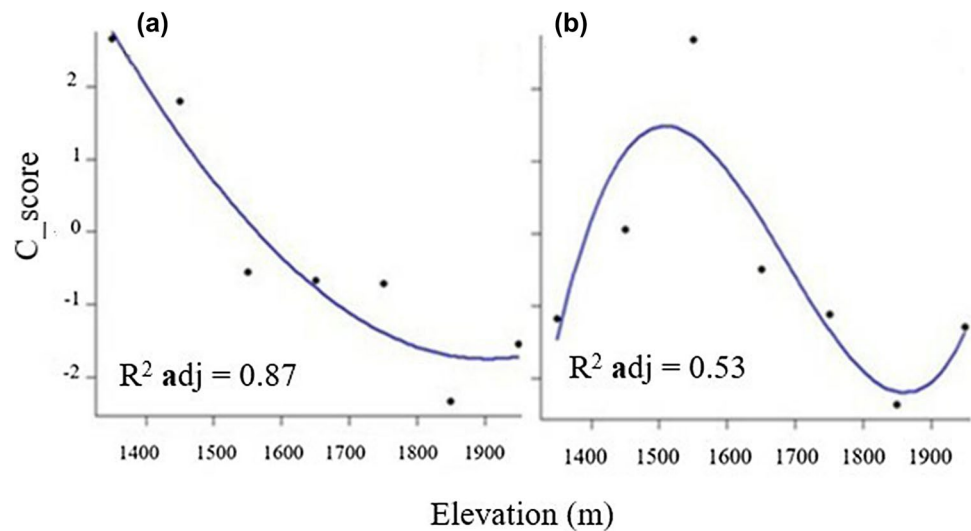

Table 1 Effects of elevation on standard effect size of C-scores (SES.C_scores) on north and south slope-facing to test two studied phylogenetic hypotheses in community level

Slope	Model	Elevation	Elevation^2	Elevation^3	R^2 adj	AICc
North slope	1st order	−0.007			0.79	20.68
	2nd order	−5.48	1.43		0.87	17.59
	3rd order	−3.90	4.70	1.94	0.83	19.59
South slope	1st order	−0.003			0.02	29.89
	2nd order	5.56	−1.87		0.04	30.20
	3rd order	1.42	−8.56	1.69	0.53	25.15

Elevation, Elevation^2 and Elevation^3 are slope parameters of linear (1st order), quadratic (2nd order) and cubic (3rd order) polynomial models, respectively

the best-fit model was our third-order polynomial regression model (AICc = 25.15), and all regression coefficients in that model were significant (Table 1). At the lowest elevations, communities with neutral and more co-occurrence than random were dominant. There was a linear component suggesting a trend from low (at intermediate of elevation) to high (at higher elevation) co-occurrence. Finally, at the highest elevations, strong co-occurrence emerged again (Fig. 4b).

Lastly, biotic interactions (co-occurrence index) exerted stronger impacts on PCS at the community level on north-facing slopes than the abiotic environment did, as indicated by our regression models (R^2 adj = 0.54, AIC = 21.73, Table 2). In contrast, the interaction between biotic interactions and abiotic environment was strong on south-facing slopes (R^2 adj = 0.92, AIC = 9.49, Table 2).

At the habitat spatial scale, standard effect sizes of mean pairwise distance (SES.mpd) as a predictor of phylogenetic community structure were higher than expected by chance within some of habitat types, as indicated by predominantly negative and positive SES.mpd values (Fig. 5). Variation in phylogenetic community structure indicated a trend from lower than expected by chance in habitats located in less harsh environments to higher than expected by chance in harsh habitats (i.e. significant phylogenetic clustering and phylogenetic overdispersion; Fig. 5).

Discussion

The dominant cushion-like plants, i.e. *A. diezianum* and *A. pterostegium*, increased phylogenetic diversity at multiple spatial scales in our studied rangeland. The responsible mechanisms differed on north vs. south facing slopes, with the compensation hypothesis supported along north-facing slopes and the severity divergence hypothesis supported along south-facing slopes (Fig. 3). On north-facing slopes,

Fig. 5 Box plots illustrating phylogenetic patterns of plant communities (Standard effect sizes of mpd) within five habitat types (H1, H2, H3, H4 and H5). Different phylogenetic patterns indicating phylogenetic overdispersion or phylogenetic clustering (based on lack of overlap with SES.mpd ± 1.96 SE) in plant communities located in H5 (more severe environment habitat) indicated habitat specialization in more severe environments

patch microsites with cushion-nurse plants *A. diezianum* and *A. pterostegium* created increasingly phylogenetically unique communities with increasing elevation relative to communities on open ground, consistent with the compensation hypothesis. We observed higher phylogenetic diversity in more abiotically stressful environments as distantly related taxa were observed in higher, severe communities, creating more phylogenetic overdispersion (Pashirzad et al. 2018). However, in the absence of cushion plants, abiotic factors increasingly restricted phylogenetic diversity and PCS changed from dispersed to clustered. On south-facing slopes, cushion and open microsites exhibited strongly divergent PCS, and phylogenetic restriction in these microsites supported more strongly divergent clades. More strongly divergent clades on south facing slopes compared to north facing slopes suggests that cushion-nurse plants create unique microenvironments in higher stress environments on south facing slopes by enhancing suitability for some clades while decreasing suitability for others (Butterfield et al. 2013). For example, south facing slopes have lower temperature, have lower variation in precipitation across elevation, and are relatively dry in comparison to north facing slopes (Piston et al. 2016). A trade-off related to ecological restriction can explain the development of such unique microenvironments through increasing divergence of clades in cushion vs. open microsites with increasing elevation. In this trade-off, stress-tolerator clades are excluded from cushion microhabitats (with low ecological restriction) by clades

Table 2 Effects of biotic interaction (i.e. C_score index), elevation and their interaction on phylogenetic patterns [standardized effect size of the mean phylogenetic distance (SES.mpd)] of the studied communities on north and south facing slopes using regression models

Slope	Introduced variables	R^2 adj	AIC	P value
North slope	C_score	0.54	21.73	0.03
	Elevation	0.06	28.28	0.45
	C_score × elevation	0.43	23.73	0.09
South slope	C_score	0.90	11.50	0.003
	Elevation	0.40	23.65	0.07
	C_score × elevation	0.92	9.49	0.0003

Akaike's information criterion (AIC) was used for determining the best regression model and the most effective variable (or variables) on PCS
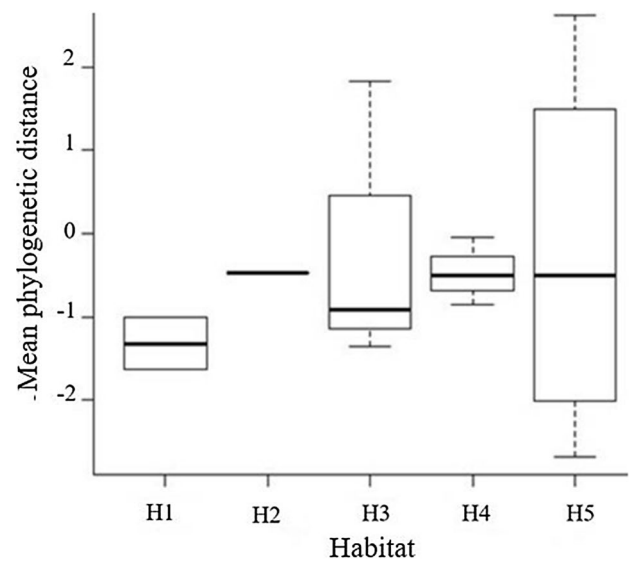
of stronger competitors, while high stress environments in open microsites exclude competitors through their low stress tolerance (Butterfield et al. 2013). Such a trade-off would not be realized in environments with less restricting ecological conditions, such as mesic conditions on north-facing slopes (Piston et al. 2016).

At the community scale, we found two different co-occurrence patterns among plant communities on north and south slopes. Hump-shaped co-occurrence patterns on north-facing slopes support the compensation hypothesis (Lopez et al. 2013). Co-occurrence patterns of species in plant communities along an elevation gradient on south slopes were more complex, with more extreme temperature and precipitation (i.e. generally lower temperature and precipitation; Appendix S2) possibly masking the influence of biotic interactions. Additionally, the presence of more phylogenetically distantly related species and higher phylogenetic diversity on north-facing slopes in sites with more severe environments is consistent with predictions of greater phylogenetic overdispersion in extreme environments through interactions between facilitators and competitors (Valiente-Banuet and Verdú 2007). For example, species interactions explained the association of phylogenetically distant species in communities across North and South America, Europe and New Zealand (Butterfield et al. 2013; Iyengar et al. 2017), and the desert and Mediterranean communities of Central America (Valiente-Banuet and Verdú 2007). More specifically, the facilitative and competitive roles of species have been shown to organize community structure and diversity in rangelands (Pashirzad et al. 2018).

In sharp contrast with north-facing slopes, phylogenetic diversity increased at higher elevation on south-facing slopes through the phylogenetic clustering of closed related taxa in more severe environments. Therefore, community-scale patterns related to phylogenetic diversity on the two different aspects were similar to phylogenetic patterns at the patch scale. We believe that our statistical power allows us to be confident about the important effects of biotic interactions on PCS, especially the positive impacts of the cushion species *A. diezianum* and *A. pterostegium* on other plant species at the community level. However, complex patterns in co-occurrence, and the results of our multiple regression analysis, indicate effects of both environment and biotic interactions on south-facing slopes (Soliveres et al. 2012). Therefore, biotic interactions at both the community and patch scales were stronger predictors of phylogenetic community structure on both aspects than elevation.

Several factors may explain the relatively weak effect of environment on PCS, especially on north-facing slopes. First, environmental conditions may not have been sufficiently harsh at higher elevation (i.e. cold and low relative humidity environments) to act as environmental filters in our north-facing slope communities (Soliveres et al. 2012; Piston et al. 2016). This suggests that negative and positive relationships between environment and MPD on north- and south-facing slopes, respectively, could be mediated in part by the effects of environmental conditions on species co-occurrence (Lopez et al. 2013). Moreover, it is clear that environmental conditions increased phylogenetic clustering on south-facing slopes, and these results indicate that high elevation areas were sufficiently harsh (Cavender-Bares et al. 2009). However, our results on north-facing slopes also suggest an indirect effect of environmental conditions on community assemblage through a reduction in the importance of positive species interactions at the highest elevations (Lortie et al. 2004).

Second, while we used elevation as our only measure of environmental severity, other environmental filters could be important in shaping our plant assemblages (Cavender-Bares et al. 2009; Lopez et al. 2013). Some factors may interact in ways that generate seemingly random patterns at coarser scales. Such scale effects have been noted previously with interactions between environmental filtering and competitive exclusion (Algar et al. 2011). Our chosen biotic and abiotic factors seemed to explain the mechanisms structuring our plant communities well. Moreover, several previous studies have indicated effects of environment and biotic interactions on the shaping of semi-arid plants (Soliveres et al. 2012; Piston et al. 2016).

In conclusion, our results demonstrate that nurse species increase phylogenetic diversity within landscapes (Butterfield et al. 2013). Cushion species affect habitat specialization in plant communities through the expansion and contraction of various local environmental filters (Valiente-Banuet and Verdú 2013), and habitat specialization yields non-random patterns within habitat types (Fine and Kembel 2011). Our observation of non-random community patterns within habitat types suggests a stronger role of species interactions than of elevation at relatively fine scales. We believe particularly that nurse species and microhabitat variation may counteract trends in community assembly driven by altitudinal severity gradients, or similar broad-scale factors (Keppel et al. 2012). Indeed, nurse plants may be recognized as micro-refugia that create biotically stable microenvironments supporting unique plant lineages (Dobrowski 2011).

Author contributions statement MP performed the project, wrote the MS and analyzed all of data as a Ph.D. student. HE defined the project as the main supervisor. JV collaborated as the co-supervisor of the project. RS edited the MS, also allocated his laboratory in Tokyo university to carry out molecular experiments.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Alejandro GFB, Chinchilla FA, Magrach A, Romero V, Reyos M, Velilla M, Serrano JM, Amador-Vargas S (2009) Slope orientation enhances the nurse effect of a paramo shrub, *Hypericum irazuense* (Hypericaceae) in Costa Rica. J Trop Ecol 25:331–335. https://www.jstor.org/stable/25562622

Algar AC, Kerr JT, Currie DJ (2011) Quantifying the importance of regional and local filters for community trait structure in tropical and temperate zones. Ecology 92:903–914. https://doi.org/10.1890/10-0606.1

Anderson TM, Shaw J, Olff H (2011) Ecology's cruel dilemma, phylogenetic trait evolution and the assembly of Serengeti plant communities. J Ecol 99:797–806. https://doi.org/10.1111/j.1365-2745.2011.01795.x

Borcard D, Gillet F, Legendre P (2012) Numerical ecology with R. Springer, New York, pp 1–306

Bowker MA, Soliveres S, Maestre F (2010) Competition increases with abiotic stress and regulates the diversity of biological soil crusts. J Ecol 98:551–560. https://doi.org/10.1111/j.1365-2745.2010.01647.x

Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA et al (2008) Facilitation in plant communities: the past, the present, and the future. J Ecol 96:18–34. https://doi.org/10.1111/j.13652745.2007.01295.x

Bryant JA, Lamanna C, Morlon H, Kerkhoff AJ, Enquist BJ, Green JL (2008) Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. Proc Natl Acad Sci USA 105:11505–11511. https://doi.org/10.1073/pnas.0801920105

Butterfield BJ, Cavieres LA, Callaway RM, Cook BJ, Kikvidze Z, Lortie CJ et al (2013) Alpine cushion plants inhibit loss of phylogenetic diversity in severe environments. Ecol Lett 16:478–486. https://doi.org/10.1111/ele.12070

Callaway RM, Brooker RW, Choler P, Kikvidze Z, Lortie CJ, Michalet R et al (2002) Positive interactions among alpine plants increase with stress. Nature 417:844–848. https://doi.org/10.1038/nature00812

Cavender-Bares J, Keen A, Miles B (2006) Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. Ecology 87:109–122. https://doi.org/10.1890/00129658(2006)87%5b109:PSOFPC%5d2.0.CO;2

Cavender-Bares J, Kozak KH, Fine PVA, Kembel SW (2009) The merging of community ecology and phylogenetic biology. Ecol Lett 12(7):693–715

Chun JH, Lee CB (2017) Disentangling the local-scale drivers of taxonomic, phylogenetic and functional diversity in woody plant assemblages along elevational gradients in South Korea. PLoS ONE 12(10):e0185763. https://doi.org/10.1371/journal.pone.0185763

De'ath G (2002) Multivariate regression trees: a new technique for modeling species–environment relationships. Ecology 83:1105–1117. https://doi.org/10.1890/00129658(2002)083%5b1105:MRTANT%5d2.0.CO;2

De'ath G (2010) mvpart: Multivariate partitioning. R package version 1.3-1

Dobrowski SZ (2011) A climatic basis for microrefugia: the influence of terrain on climate. Glob Change Biol 17:1022–1035. https://doi.org/10.1111/j.1365-2486.2010.02263.x

Farzam M, Ejtehadi H (2016) Effects of drought and canopy facilitation on plant diversity and abundance in a semiarid mountainous rangeland. J Plant Ecol. https://doi.org/10.1093/jpe/rtw070

Fine PVA, Kembel SW (2011) Phylogenetic community structure and phylogenetic turnover across space and edaphic gradients in western Amazonian tree communities. Ecography 34:552–565. https://doi.org/10.1111/j.1600-0587.2010.06548.x

Flores J, Jurado E (2003) Are nurse-protege' interactions more common among plants from arid environments? J Veg Sci 14:911–916. https://doi.org/10.1111/j.1654-1103.2003.tb02225.x

Fournier B, Mouly A, Moretti M, Gillet F (2017) Contrasting processes drive alpha and beta taxonomic, functional and phylogenetic diversity of orthopteran communities in grasslands. Agr Ecosyst Environ 242:43–52. https://doi.org/10.1016/j.agee.2017.03.021

Gotelli NJ (2000) Null model analysis of species co-occurrence patterns. Ecology 81:2606–2621

Gotelli NJ, Graves GR (1996) Null models in ecology. Smithsonian Institution Press, Washington DC

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704. https://doi.org/10.1080/10635150390235520

Head CEI, Koldewey H, Pavoine S et al (2018) Trait and phylogenetic diversity provide insights into community assembly of reef-associated shrimps (Palaemonidae) at different spatial scales across the Chagos Archipelago. Ecol Evol 8:4098–4107. https://doi.org/10.1002/ece3.3969

Hijmans RJ, Cameron SE, Parra JL et al (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–1978. https://doi.org/10.1002/joc.1276

Iyengar SB, Bagchi S, Barua D, Mishra CH, Sancaran M (2017) A dominant dwarf shrub increases diversity of herbaceous plant communities in a Trans-Himalayan rangeland. Plant Ecol 1:1. https://doi.org/10.1007/s11258-017-0734-x

Jankju M, Delavari A, Ganjali A (2008) Interseeding Bromus kopetdaghensis, in shrublands. Rangeland J Iran Soc Range Manag 2:314–328

Joly S, Starr JR, Lewis WH, Bruneau A (2006) Polyploid and hybrid evolution in roses east of The Rocky Mountains. Am J Bot 93:412–425. https://doi.org/10.3732/ajb.93.3.412

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30(4):772–780. https://doi.org/10.1093/molbev/mst010

Kembel SW, Hubbel SP (2006) The phylogenetic structure of a neotropical forest tree community. Ecology 87:86–99. https://doi.org/10.1890/0012-9658(2006)87%5b86:TPSOAN%5d2.0.CO;2

Kembel SW, Cowan PD, Helmus MR et al (2010) Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26:1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Keppel G, Van Niel KP, Wardell-Johnson GW, Yates CJ, Byrne M, Mucina L, Schut AGT, Hopper SD, Franklin SE (2012) Refugia: identifying and understanding safe havens for biodiversity under climate change. Glob Ecol Biogeogr 21(4):393–404

Kores PJ, Cameron KM, Molvray M, Chase MW (1997) The phylogenetic relationships of orchidoideae and spiranthoideae (Orchidaceae) as inferred from *rbcL* plastid sequences. Lindleyana 12:1–11

Kraft NJB, Ackerly D (2010) Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. Ecol Monogr 80:401–422. https://doi.org/10.1890/09-1672.1

Kress WJ, Erickson DL, Jones FA, Swenson NG, Perez R, Sanjur O et al (2009) Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proc Natl Acad Sci USA 106:18621–18626. https://doi.org/10.1073/pnas.0909820106

Krishnadas M, Kumar A, Comita AS (2016) Environmental gradients structure tropical tree assemblages at the regional scale. J Veg Sci 27:1117–1128. https://doi.org/10.1111/jvs.12438

Lopez RP, Valdivia S, Rivera ML, Rios RS (2013) Co-occurrence patterns along a regional aridity gradient of the subtropical andes do not support stress gradient hypotheses. PLoS ONE 8:1–10. https://doi.org/10.1371/journal.pone.0058518

Lopez-Angulo J, Swenson NG, Cavieres LA (2018) Interactions between abiotic gradients determine functional and phylogenetic diversity patterns in Mediterranean type climate mountains in the Andes. J Veg Sci 29(2):245–254

Lortie CJ, Brooker RW, Choler P, Kikvidze Z, Michalet R, Pugnaire FI, Callaway RM (2004) Rethinking plant community theory. Oikos 107:433–438. https://doi.org/10.1111/j.0030-1299.2004.13250.x

Machac A, Janda M, Dunn RR, Sanders NJ (2011) Elevational gradients in phylogenetic structure of ant communities reveal the interplay of biotic and abiotic constraints on diversity. Ecography 34:364–371. https://doi.org/10.1111/j.1600-0587.2010.06629.x

Maestre FT, Callaway RM, Valladares F, Lortie CJ (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. J Ecol 97:199–205. https://doi.org/10.1111/j.1365-2745.2008.01476.x

Maltez-Mouro S, Maestre FT, Freitas H (2010) Co-occurrence patterns and abiotic stress in sand-dune communities: their relationship varies with spatial scale and the stress estimator. Acta Oecol 36:80–84. https://doi.org/10.1016/j.actao.2009.10.003

Memariani F, Akhani H, Joharchi MR (2016) Endemic plants of Khorassan-Kopet Dagh floristic province in Irano-Turanian region: diversity, distribution patterns and conservation status. Phytotaxa 249(1):031–117

Mo XX, Shi LL, Zhang YJ, Zhu H, Slik JWF (2013) Change in phylogenetic community structure during succession of traditionally managed tropical rainforest in Southwest China. PLoS ONE 8(7):e71464. https://doi.org/10.1371/journal.pone.0071464

Pashirzad M, Ejtehadi H, Vaezi J, Shefferson RP (2018) Spatial scale-dependent phylogenetic signal in species distributions along geographic and elevation gradients in a mountainous rangeland. Ecol Evol 00:1–11. https://doi.org/10.1002/ece3.4293

Pirani A, Rabeler RK (2017) Nomenclatural notes on *Acanthophyllum* (Caryophylleae, Caryophyllaceae). Phytotaxa 303(2):197–198. https://doi.org/10.11646/phytotaxa.303.2.11

Piston N, Schob C, Armas C, Prieto I, Pugnaire F (2016) Contribution of co-occurring shrub species to community richness and phylogenetic diversity along an environmental gradient. Perspect Plant Ecol 19:30–39. https://doi.org/10.1016/j.ppees.2016.02.002

Qian H, Chen SH, Zhang JL (2017) Disentangling environmental and spatial effects on phylogenetic structure of angiosperm tree communities in China. Sci Rep 7:5864. https://doi.org/10.1038/s41598-017-04679-5

Rajaniemi TK, Goldberg DE, Turkington R, Dyer AR (2006) Quantitative partitioning of regional and local processes shaping regional diversity patterns. Ecol Lett 9:121–128. https://doi.org/10.1111/j.1461-0248.2005.00855.x

Sanderson MJ (2002) Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol Biol Evol 19(1):101–109

Soliveres S, Torices R, Maestre F (2012) Environmental conditions and biotic interactions acting together promote phylogenetic randomness in semiarid plant communities: new methods help to avoid misleading conclusions. J Veg Sci 23:822–836. https://doi.org/10.1111/j.1654-1103.2012.01410.x

Swenson NG, Enquist BJ, Pither J, Thompson J, Zimmerman JK (2006) The problem and promise of scale dependency in community phylogenetics. Ecology 87:2418–2424

Valiente-Banuet A, Verdú M (2007) Facilitation can increase the phylogenetic diversity of plant communities. Ecol Lett 10:1029–1036. https://doi.org/10.1111/j.1461-0248.2007.01100.x

Valiente-Banuet A, Verdú M (2013) Plant facilitation and phylogenetics. Ann Rev Ecol Evol Syst 44(25):1–25

Verdú M, Rey PJ, Alcantara JM, Siles G, Valiente-Banuet A (2009) Phylogenetic signatures of facilitation and competition in successional communities. J Ecol 97:1171–1180. https://doi.org/10.1111/j.1365-2745.2009.01565.x

Webb CO (2000) Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. Am Nat 156:145–155

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and community ecology. Annu Rev Ecol Syst 33:475–505. https://doi.org/10.1146/annurev.ecolsys.33.010802.150448

Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics 24:2098. https://doi.org/10.1093/bioinformatics/btn358

White TJ, Burns T, Lee S, Taylor J (1990) Amplification and sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols. A guide to methods and applications. Academic Press Inc, San Diego, pp 315–322

Yang J, Swenson NJ, Zhang G, Ci X, Cao M, Sha L et al (2015) Local-scale partitioning of functional and phylogenetic beta diversity in a tropical tree assemblage. Sci Rep 5:12731. https://doi.org/10.1038/srep12731