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Phylogeny and ecological processes influence grass coexistence at different spatial scales within the steppe biome

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Abstract

Phylogenetic analyses are essential for disentangling how environmental filtering and competition determine species coexistence across spatial scales. Inner Mongolia steppe has strong environmental gradients, but how the phylogenetic relatedness of co-occurring species and phylogenetic signals of functional traits change across spatial scales remain unclear. We investigated the phylogenetic structure of grass assemblages along environmental gradients from regional to local scales, and measured functional traits within assemblages. We compared phylogenetic signals of plant traits between the same numbers of species randomly selected from the regional pool and species observed at the local scale, did phylogenetic principal component analysis to infer the main factors driving species coexistence, and examined the key plant trait-environment relationships across the phylogeny to reveal ecological adaptation mechanisms. Regionally, grass species were phylogenetically clustered with contrasting climate preferences. With decreasing spatial scales, species richness declined, changing from phylogenetically clustered to overdispersed, and phylogenetic signals of plant traits became weaker. At the local scale, grass assemblages were structured by soil water content and neighbor density, and the trait-environment relationships were less clear than those at the regional scale. This study demonstrated that at smaller scales, co-occurring grass species in the steppe tended to be more phylogenetically overdispersed, and that phylogenetic signals of plant functional traits became weaker with increasing abiotic and biotic interactions. Our findings contributed evidence for understanding species coexistence and maintenance at scales spanning regional to local communities in the East Asia steppe biome.

Keywords Inner Mongolian steppe, phylogenetic niche conservatism, plant functional trait, Poaceae, scale dependence.

Introduction

The phylogenetic structure of species coexistence in different spatial units (Kraft and Ackerly 2010; Swenson et al. 2006; Trisos et al. 2014) or in different ecosystems (Kunstler et al. 2012; Swenson 2013) is basic information that is essential for understanding species assemblage patterns and the underlying processes (Weber et al. 2017). It is likely to change across spatial scales because the dominant processes that structure communities are different when spatial scale shifts (Cavender-Bares et al. 2018; Mouquet et al. 2012). When the spatial scale is sufficiently large, species are more likely to be phylogenetically clustered (co-occurred species are more closely related than the random expectation), due to larger species pools, geographic separation and climatic restrictions (Swenson et al. 2007; Webb et al. 2002). Also the importance of species interactions in determining the phylogenetic structure is relaxed at the large spatial scale compared to small scale because plant individuals tend to interact only with their nearest neighbors (Uriarte et al. 2010; Wiegand et al. 2017). At smaller spatial scales, phylogenetic overdispersion (co-occurring species tend to be more distantly related than the random expectation) or a random species assemblage might occur, and in some cases phylogenetic clustering might also be observed such as the clustering of C_4 Andropogoneae in burned grasslands (Forrestel et al. 2014). Therefore, continuous species sampling across the geographical gradients could help to elaborate phylogenetic patterns in terms of different ecological processes and to determine the scale at which the phylogenetic patterns shift.

However, the linkage between phylogenetic patterns and ecological processes is complex, and strongly associates with species strategies in the trait-based context (Cadotte 2017; Gerhold et al. 2015; Mayfield and Levine 2010). For example, if closely related species have similar fitness with advantageous functional traits, they can coexist through competition and lead to phylogenetic clustering, which is often attributed to environmental filtering (HilleRisLambers et al. 2012; Kunstler et al. 2012; Mayfield and Levine 2010). Conversely, if critical functional traits that adapt to the environment and influence distributions are convergent across co-occurring species, this environmental filtering can also cause phylogenetic overdispersion, which often indicates competition exclusion (Cavender Bares et al. 2004; Kraft et al. 2007). Furthermore, the relative contributions of environmental filtering and competition are expected to be strongly scale-dependent (Wiegand et al. 2017). Thus, it is fundamental to consider both phylogenetic structures and functional traits in studying species assemblages across spatial scales.

Phylogeny reveals the past dispersal and evolutionary patterns (Donoghue 2008; Swenson 2013; Wiens 2018), and studies that only consider ecological processes will lose the historical information. Recent plant community studies on functional traits also suggest that different phylogenetic structures might depend on whether the functional traits of coexisting species are convergent or divergent (Wilcox et al. 2018; Zhang et al. 2018). On one hand, the determinate functional traits of coexisting species may be phylogenetically conserved (closely related species are more similar, i.e. phylogenetic niche conservatism, PNC) (Wiens and Graham 2005). On the other hand, these traits could also arise through convergent evolution in unrelated groups (Webb et al. 2002). Therefore, phylogenetic tests on the key functional traits in species assemblages (Pavoine et al. 2011) can predict how different lineages respond differently to global changes, and thus how new distribution patterns are formed (Edwards and Smith 2010). It can also reveal more clearly the underlying species assemblage rules (Weber et al. 2017), and/or even macroevolution patterns such as the role of diversification (Cavender-Bares et al. 2018; Gerhold et al. 2015) and dispersal (Wiens 2018).

To gain insights into the interplay of phylogenetic patterns, ecological processes and functional traits on species coexistence, especially to integrate results across spatial scales, we chose the Inner Mongolian steppe as our study object. It covers a large area, and is the main component of Mid-Asian temperate grasslands, with gradually increasing altitude and decreasing temperature and precipitation from east to west. The low (120 - 460 mm) mean annual precipitation (MAP) in the steppe makes it important to explore species distribution patterns in relation to restricted water availability. At the regional scale, studies have reported productivity (Bai et al. 2008), biodiversity (Zhang 1998) and species distribution (Pyankov et al. 2000) along climatic gradients, but no literature has yet carried out phylogenetic analyses of plant ecophysiological traits and species coexistence mechanisms in this steppe biome. However, such studies could not only test the theories about how phylogeny and ecological processes structure assemblages, but also are extremely important to understand this fragile biome, where species composition might change under future climatic changes such as greater drought incidence.

We comprehensively sampled the grass species pool of the Inner Mongolian steppe, investigating how species were filtered across different spatial scales. Here we refer to the regional, intermediate and local scales by reference to the whole area of Inner Mongolia, the administrative county and the field site scales, respectively (Fig. 1). We then selected local habitats with contrasting soil water gradients, and directly measured ecophysiological traits of all coexisting grasses, including plant functional traits related with water and nutrient use, soil water and nitrogen content, and neighbor density. To infer the main factors driving species coexistence in Poaceae assemblages, we used phylogenetic principal component analysis (PPCA) and analyzed how the functional traits associated with environmental gradients against the phylogenetic background. Our hypotheses are: (H1) Phylogenetic structure of co-occurring species will be more clustered at regional and intermediate spatial scales than that at local scale. (H2) Phylogenetic signals in functional traits and environmental variables become weaker at the local scale. (H3) Based on phylogenetic principal component analysis, both environmental filtering and competition will affect species coexistence at the local scale. (H4) Based on phylogenetic general linear models, the relationships between functional traits and environmental variables persist across scales.

Materials and methods

Study area

The Inner Mongolian Autonomous Region (IMAR) in China covers an area of 1.2 million km^2, of which 66% is natural grasslands. The Inner Mongolian steppe biome has a strong east-west altitude (230~1400 m) and climatic gradients (Fig. 1). The mean annual temperature (MAT) ranges from -1.7 to 8.6 °C, and the mean annual precipitation (MAP) from 120 to 460 mm, ~70-80% of which coincides with peak temperature during the growing season (May to September).

There are 88 counties in total in the IMAR, each with a fully documented species pool, which allowed us to investigate species filtering into smaller areas. In the middle of the steppe, we chose the Hunshandake sandland within the Zhenglan county for the local scale study (Fig. 1). Our local field site is located in the Hunshandake sandland, where around 27 km^2 land area has been fenced since 2001 to exclude livestock for vegetation recovery and environmental protection. Data for 1960~1999 from the local meteorological bureau showed a temperate arid and semi-arid climate, with MAT, average January and average July temperatures of 1.8°C, -17.9°C and 18.7°C, respectively. The MAP is 378 mm, fluctuating from 150 to 450 mm, 64% of which concentrated in the growing season.

The four main habitat types at the local field site are moving dune, fixed dune, lowland meadow and wetland. There are strong increasing gradients of soil water content, soil nitrogen contents and species density in each habitat along the gradient from moving dune to wetland (Appendix S1).

Data collection at the regional scale

We assembled three datasets for the Inner Mongolian grasses:

(1) Records of 265 Poaceae species from the Flora of Inner Mongolia (Editorial Committee on Flora Intramongolica 1998), including culm height, leaf length and width, flowering date, leaf type (flat and/or folded/rolled, use "folded" hereafter), and county-scaled occurrences for each species (Appendix S2 Table S1). Folded leaf is an important adaptation strategy of grass species to avoid drought and its occurrence differs among grass subfamilies (Liu and Osborne 2014). The taxonomy was based on *Grass Genera of the World* (Watson 1992 onwards). There are six subfamilies of Poaceae in Inner Mongolia: the largest, Pooideae, accounted for nearly 80% of the total species, the second and third largest subfamilies, Panicoideae and Chloridoideae, had 35 and 21 species, respectively (Appendix S3).

(2) Geographical and climatic information for 88 counties of the IMAR for 1971~2000, including temperature, precipitation, area and population for each county (China Meteorological Data Sharing Service System 2005, Standard climate dataset for international exchange on Chinese surface weather stations 1971-2000. http://data.cma.cn/site/index.html; Appendix S2 Table S2). Based on the species distribution table (Appendix S2 Table S3), we calculated species richness and proportions of each subfamily/tribe for each county, and arithmetic means of the climatic values associated with the county-scaled distribution for each species, including mean growing

season (May to September) temperature (MGT) and precipitation (MGP). We tested phylogenetic structures of co-occurring species in each county (Appendix S2 Table S4).

(3) For each species in Inner Mongolia, we calculated species water requirement (Editorial Committee on Flora Intramongolica 1998) (Appendix S2 Table S1). We assigned a numerical score to each plant ecological tolerance describing water availability, giving equal weighting to the extremes (Hydrophyte = 5, Helophyte = 4, Mesophyte = 3, Xerophyte = 1), resulting in a continuous sequence of values for each species. These four values were summarized as a range "water range" and a mean "water score" for each species (Osborne and Freckleton 2009). This "water score" was positively related with MGP (regional scale) and soil water content (local scale), supporting the consistency of species water requirements across spatial scales (Appendix S1 Figure S2).

Sampling and measurements at the local scale

To investigate community structure in the Hunshandake sandland, we carried out field surveys and measurements in July and August, 2010. First, three sites (each ~0.3 km^2) were chosen from each of the four habitats (moving dune, fixed dune, lowland meadow and wetland), with 12 sites in total. At each site, we collected all distinguishable grass species for identification. Second, we randomly sampled three individuals for each species from each habitat, apart from two rare and one early season species, where only two individuals were available. We sampled 147 individuals of 32 Poaceae species in total.

For each individual, we dug the soil from the surface down to 30 cm depth (30 cm covers the main root distribution layer due to the shallow roots of most grass species), measuring the water content of three layers (0~10, 10~20 and 20~30 cm) using a soil moisture probe (ThetaKit type TK3; Delta-T Devices Ltd, Cambridge, UK), then took soil samples separately. We then ground dried soil samples with a ball mill (Mixer Mill MM

200; Retsch, Haan, Germany), and measured total soil nitrogen through Kjeldahl determination on an Auto Distillation Unit (KejeltecTM 2200; FOSS, Denmark). To quantify the biotic environment, we recorded relative species abundance (assessed by Drude's scale), together with neighbor number (within a 30cm radius), distance to the nearest neighbor and the height of the tallest neighbor.

Functional traits can explain and predict the dramatic variations in species abundances and compositions (Adler et al. 2014), therefore we selected traits to reflect life history strategies of each species, including plant growth (e.g. culm and leaf sizes, root depth), resource acquisition (e.g. leaf nutrient concentration, stomatal pore area index) and abiotic stress adaptation (e.g. folded leaf and midday water potential). We directly measured culm height, leaf height, leaf length and width, and the number of living leaves per tiller, and referenced root depths for each species from a previous study (Chen 1986), with five missing species measured by completely excavating their roots in the field. During soil moisture measurements, we took roots connected to the aboveground culms to ensure they were from the same species. After carefully washing and sieving, we scanned a part of the fine roots (diameter < 1mm) on a flatbed scanner (PhantomF60; Microtek, Shanghai, China) to calculate specific root length (SRL).

From 12:00 to 14:00 on sunny days, we cut one or two young mature leaves at the ligule for each individual to measure midday water potential using a psychrometer (PSYPRO; Wescor, Utah, USA) with C-30 Sample Chambers. We calibrated the psychrometer beforehand in an air-conditioned laboratory (25°C) with a series of standard NaCl solutions. To keep the samples at a stable temperature (~25°C) under strong sunlight, we used an insulated ice box with a thick towel separating ice packs and sample chambers. We scanned leaf samples after measurements and imported both leaf and root images into ImageJ (Abramoff *et al.* 2004) to obtain leaf area and root length (Kimura *et al.* 1999).

We calculated specific leaf area (SLA) and specific root length (SRL) after weighing the oven dried (65°C, 24h) samples. We ground dried leaf samples using a ball mill (Tissuelyser, Retsch, Qiagen GmbH, Germany), and determined leaf carbon and nitrogen concentrations and stable isotope rations using a stable isotope ratio mass spectrometer (PDZ Europa 20-20; PDZ Europa Ltd, Cheshire, UK).

According to the leaf economic spectrum, photosynthetic capacity is one of the most important axes in plant life history strategies, and is closely related with stomatal conductance (Wright et al. 2004). For dried leaf samples, to estimate stomatal conductance and photosynthetic potential, stomatal pore area index (SPI) was calculated based on stomatal morphological traits, on the assumption that stomata would open completely at their maximum photosynthetic rate (Franks and Beerling 2009). We made dental putty (President Plus-light body, Coltène/Whaledent Ltd., Burgess Hill, West Sussex, UK) impressions from the rehydrated leaf samples, and prepared microscope slides of both adaxial and abaxial surfaces using nail polish imprints of the impressions. We observed slides under an inverted microscope equipped with a digital camera (Leica Laborlux S, Wetzlar, Germany) and an image analysis system (Leica Quantimet 500 Q win software). From each peel, we randomly chose three images as replicates and measured: guard cell length (GL) and width of the guard cell pair (SW), the stomatal density (SD) and SPI (a dimensionless index of stomata pore area per lamina area), where $SPI = SD \cdot GL^2$ (Sack et al. 2003).

Phylogenetic trees

We constructed phylogenetic trees for the 265 species (76 genera) of Poaceae in the Flora of Inner Mongolia by integrating published phylogenies. First, we used a large phylogenetic tree of over 3000 Poaceae species as a framework (Edwards et al. 2010), then extracted 110 matched species from it after accounting for synonymies (Clayton 2002 onwards). Second, we comprehensively searched published phylogenies for different genera and identified 60 more species (10 genera) (Appendix S3 Table S5). We assembled all the 60 species (10 small trees) into the 110-species tree, using function *bind.tree* in the R (https://www.r-project.org) package *ape* (Paradis et al. 2004), such that a 170-species tree (species for which DNA sequence data were measured) was used in the following phylogenetic tests (Appendix S3 Figure S4 tree A). Finally, we added 58 more species by replacing their congeners' positions based on the phylogeny from Edwards *et al.* (2010) using species name replacement (Appendix S3). Due to the potential uncertainty of this 238-species tree, we only used it as a phylogenetic background (all species in the region) in the sampling test (Table 1), and used the 170-species tree for all the phylogenetic models. We also compared our phylogenetic tree with the latest phylogenetic work on Poaceae (Soreng et al. 2017), finding that the two phylogenetic trees were consistent with each other.

There were 88 species recorded in the Zhenglan county, and 32 of them were found in the four habitats within the field sites. Corresponding phylogenetic trees of each of the 88 counties and the 32-species tree were extracted from the 170-species tree for the phylogenetic analyses in each case (Appendix S3 Figure S4 tree BC).

Statistical analysis

Based on the strong climatic gradients in the steppe, we firstly analyzed the relationship between species richness of different phylogenetic lineages and climatic conditions at the regional scale, to see if different lineages have different climatic niches. For example, if a dominant lineage occurred more in dry areas, it generally will lead to a phylogenetically clustered pattern in dry areas due to niche conservatism (Wiens and Graham 2005). In this case, it is simple and direct to link biogeographical with phylogenetic perspectives.

For hypothesis (H1), to analyze phylogenetic structure across spatial scales, net relatedness index (NRI) was used as a standardized index based on the mean phylogenetic distance (MPD) of species co-occurring in an assemblage. NRI is defined as (rndMPD-MPD)/sdrndMPD, where MPD was calculated from the phylogenetic tree of the regional species pool, rndMPD and sdrndMPD were the average and standard deviation of MPDs obtained from a null model by reshuffling the species tips 9999 times across the same phylogenetic tree (Faith 1992). NRI indicates whether co-occurring species in one assemblage are phylogenetically clustered (NRI > 0) or overdispersed (NRI < 0) (Webb et al. 2002). Many studies have also calculated the nearest taxon index (NTI), but NTI focuses on specific differences and is more sensitive to tree topology than NRI (Letcher 2010). Here we tried to compare among different trees and communities, thus only NRI was calculated using the R package *picante*.

Phylogenetic signal measures the statistical dependence among species' trait values based on their phylogenetic relationships. For binary variables, we used Fritz and Purvis's D to calculate phylogenetic signals (Fritz and Purvis 2010). D measures the sum of changes in estimated nodal values of a binary trait along edges in a phylogeny. To calibrate for phylogenetic size, observed D is compared with simulated D values using the same phylogeny under two models: Brownian threshold model and phylogenetic randomness, in order to set points of 0 and 1, respectively. $D < 0$ indicates a highly phylogenetically conserved trait, while $D > 1$ suggests a phylogenetically overdispersed trait. Although species occurrence in each county (a binary variable, 1 and 0) is not a trait, we interpreted its phylogenetic signal as a niche similarity measurement of local species filtered from a larger species pool. If local species are more closely related than expected by chance, it represents the influences of biogeographic or climatic heterogeneity on species distributions and local diversification (Levin 1993).

For hypothesis (H2), to test ecophysiological traits, Pagel's λ was used based on a Brownian model of quantitative trait evolution (Pagel 1999). The extent to which traits evolve by random drift from a common ancestor gives a λ value between 0 and 1, where $\lambda = 1$ indicates strong phylogenetic dependence, while $\lambda = 0$ implies no phylogenetic dependence (Freckleton et al. 2002). Our interpretation of conservatism here is that high λ values indicate that traits of closely related species are more similar than expected by chance (Cooper et al. 2010). Both D and λ were calculated using the R package *caper*.

Furthermore, to test whether the λ values for quantitative traits differed significantly between the 170 (regional) and 32 (local) species samples, we used a randomization method by drawing 1000 random samples of 32 species from the 170-species phylogeny and calculated the average λ value. The results were compared with those obtained from the real assemblage. If the observed λ were lower than the average λ based on random samples, we interpreted this as a scale-dependent decline in PNC at the local scale (32 species) compared with the regional scale (170 species).

For hypothesis (H3), to distinguish the driving factors in determining species distributions within local assemblages, phylogenetic principal component analysis (PPCA) was used (Felsenstein 1985). Data were log-transformed to meet the requirement of a normal distribution. PPCA was carried out using the *phyl.pca* function in the R package *phytools*. The variables for each of the 32 species in the Hunshandak sand were divided into two groups, 10 environmental variables and 17 plant traits. Since the 32 species scattered among the four habitats with a contrasting water gradient (Appendix S1), we expected that both the driving environmental variables and plant intrinsic traits should be related with water availability, absorption and consumption.

For hypothesis (H4), the key traits that distinguished species at the local scales detected by PPCA were used to analyze plant-environment relationships. Phylogenetic general linear models (*PGLM*) were used, based on the same functions in estimating λ values in the R package *cape*r. Currently *PGLM* is the best suitable method to test correlations between trait pairs, other methods such as phylogenetic independent contrast (PIC) is an extreme case of *PGLM* when phylogenetic signal λ is set to one.

Results

H1: Phylogenetic structure of grass assemblages across spatial scales

The three largest monophyletic lineages (subfamilies) of Poaceae in the Inner Mongolian steppe showed distinct patterns along the regional climatic gradients from east to west (Fig. 2). With increasing MGT, species richness of Pooideae dropped significantly, while that of Chloridoideae and Panicoideae slightly increased (Fig. 2a). However, at higher MGP, species richness of Chloridoideae decreased while that of Panicoideae increased, and species richness of Pooideae varied widely (Fig. 2bc). Among the monophyletic lineages in Pooideae, species richness of Aveneae and Stipeae had positive and negative relationships with MGP, respectively (Fig. 2d), but all the others showed no associations. General linear models with county area as an additive factor confirmed that sampling area had no significant effects on these relationships.

Across spatial scales, the sampling distribution of the 170-species across the 238-species showed a phylogenetically clustered trend (NRI > 0 and D = 0.44; Table 1). This was mainly due to the lack of DNA sequence data for native Chinese species in several large genera like *Festuca*, *Poa*, *Roegneria* and *Stipa* (Appendix S3 Figure S4 tree A). The 88-species pool of the Zhenglan county showed phylogenetic overdispersion against the regional species pool represented by the 238-species tree (NRI < 0, D = 0.84; P < 0.05 for

$D = 1$), but phylogenetic clustering against the 170-species tree (NRI > 0, $D > 1$, Table 1; Appendix S3 Figure S4 tree B). However, 32 out of 88 counties in the IMAR were phylogenetically clustered, most of these formed a contiguous cropland-grassland border area, dominated by species from Paniceae, Eragrostideae, Triticeae, Bromeae and Aveneae (Appendix S2 Table S4). The 32-species local assemblage in the Hunshandake sandland also showed phylogenetically overdispersed patterns within either the 238- or 170-species tree (NRI < 0 ns, D values ns from 1, Table 1; Appendix S3 Figure S4 tree C). For the four habitats, species assemblages showed no patterns from NRI values, but were phylogenetically overdispersed based on D values (all D ns from 1; Table 1).

H2: Phylogenetic signals in plant traits and environmental variables across spatial scales

At the regional scale, significant phylogenetic signals were detected for most of the traits (Table 2). Morphological traits, flowering start and end date showed λ values from 0.29 to 0.95 ($P < 0.05$ for both $\lambda = 0$ and 1), although leaf shape had λ around 0. Measures of environmental niches (water score, MGT and MGP) showed phylogenetic signals (λ from 0.14 to 0.92; $P < 0.05$ for both $\lambda = 0$ and 1), with the exception of water range.

For the traits measured at both regional and local scales, random selection models of 32 species from the 170-species dataset showed λ values lower than those for the full regional sample of species (except water range), but higher than those for the full local species sample (except culm height) (Table 2). Of the traits measured only at the local scale, specific root length (SRL), stomatal density, guard cell length, stomatal width and leaf δ^{13}C showed phylogenetic signals. Meanwhile, no phylogenetic signals were detected in either biotic or abiotic factors except the tallest neighbour ($\lambda = 0.48$, $P < 0.01$ for both $\lambda = 0$ and 1), water score and SWC ($\lambda = 0.21$ and 0.39, but both P ns for $\lambda = 0$) (Table 2).

H3: PPCA results at the local scale

PPCA on environmental variables showed that local species distributions were separated first by abiotic factors, and then biotic factors (Table 3a). Three principal components (PC) together explained 77% of the variance. The first PC indicated soil nitrogen content (SNC) of three soil layers and soil water content (SWC) of the 20-30cm soil layer (positive loadings). The second PC showed neighbor density, relative abundance and tallest neighbor (negative loadings). The third PC was tallest neighbor, SWC of the first layer (positive loadings), and SNC of the first and second layers (negative loadings).

For the whole set of morphological traits, three PCs explained 68% of the variance, indicating that leaf morphology, stomatal and rooting traits were three axes that distinguished species (Table 3b). The first PC included leaf length and width, single leaf area and mass (negative loadings). The second PC was mainly from stomatal width, SLA (negative loadings) and root depth (positive loadings). The third PC corresponded to guard cell length and width (negative loadings), and stomatal density (positive loadings).

Water-related traits were plotted across the phylogenetic tree (Appendix S4). It was hard to discern patterns for culm and leaf size, except two extreme values in Stipeae. However, larger but less dense stomata were seen in Pooideae compared with other subfamilies (Appendix S4, column 1 to 4). Meanwhile, there was no phylogenetic signal in leaf water potential, which was inversely associated with culm height (Appendix S4, column 5). Both neighbor density and SWC showed high values in Poeae and Aveneae, without overall phylogenetic dependences (Appendix S4, column 6 and 7).

H4: Phylogeny-based trait-environment relationships

Phylogenetic models of trait-environment relationships did not show consistent patterns at the regional and local scales (Table 4).

Regionally, culm height unexpectedly decreased with increasing MGT, although the variance explained was low. Morphological traits had significant positive relationships with both MGP and water score, and water score (habitat-scale measurements) was better in explaining variance (Table 4a). Since all the regressions had low explanatory power, and all λ values were greater than zero (0.16 to 0.80, $P < 0.05$ for both $\lambda = 0$ and 1), the three morphological traits were not explained well by environmental variables, but depended on phylogeny.

At the local scale, most models showed no patterns, only SWC explained variance in culm height and SRL, and neighbor density explained variance in culm height and root depth. The λ values of models were similar to those for the univariate analysis, and were only higher for culm height and stomata related traits (Table 4b). Results were also insensitive to whether SWC was averaged over the whole profile or taken from the surface layer only. No trends were found for leaf traits with increasing SWC or neighbor density, but there were significant positive relationships between culm height, SRL and SWC (Fig. 3ab), and between culm height, root depth and neighbor density (Fig. 3cd).

Discussion

H1: Scale dependence within the steppe: from phylogenetic clustering to overdispersion

At the regional scale, Poaceae species in the steppe biome indicated phylogenetically clustered patterns, which was consistent with the global picture (Edwards and Smith 2010; Hartley 1950; Liu et al. 2012; Visser et al. 2014), and patterns in other regions, such as Australia (Hattersley 1992), North America (Taub 2000), Mongolia (Pyankov et al. 2000) and South Africa (Visser et al. 2012). Pooideae had more species in low MGT areas with an early flowering date, indicating its cold tolerant strategy (Vigeland et al. 2013). Chloridoideae occurred more in drier habitats than Panicoideae, which was supported by our previous findings that shorter height and narrower leaves, denser and smaller stomata, and smaller seeds, better adapt Chloridoideae to low water availability (Liu et al. 2012). Different climatic niches of the main grass lineages indicated phylogenetic niche conservatism (Wiens and Graham 2005), emphasizing the importance of phylogeny in understanding species geographical distributions. Specifically, the contrasting responses to climatic gradients of different Poaceae lineages in our study might indicate their different distribution patterns under future global changes (Edwards and Smith 2010).

With decreasing spatial scales, co-occurring species in the steppe ecosystem shifted from phylogenetically clustered to overdispersed patterns (Table 1). Our results agreed with other community-scale studies showing phylogenetic overdispersion, as in oak communities across the US (Cavender-Bares et al. 2018) and English meadows (Silvertown et al. 2006), but contrasted with the phylogenetic clustering observed for other plant communities at small scales (Forrestel et al. 2014; Prinzing et al. 2008). One possible explanation for the phylogenetic overdispersion of grass assemblages at smaller scales in our results was interspecific interactions, such as competition, because at large spatial scales, biotic interactions are less important in structuring communities, causing overdispersion to diminish as reported in previous work on oaks across spatial scales (Cavender-Bares et al. 2018). It was also notable that at intermediate scale of Zhenglan and other counties, grass assemblages could be either phylogenetic overdispersed or clustered depending on different species pools. This indicated the important role of species pool in determining phylogenetic structures, and suggested that county could be the spatial scale at which the phylogenetic pattern of grass assemblages started to shift.

H2: Weaker phylogenetic signals in functional traits and environmental variables

As expected, phylogenetic signals in the same traits tested at regional scale became weaker at intermediate and local scales. The simulated random draw from the regional dataset also produced higher λ values of these traits than the observed dataset, verifying a scale-dependent decline in PNC at the local scale compared with the regional scale (Table 2). PNC is generally caused by stabilizing selection within the same climate zone or at the regional scale (Donoghue 2008), but smaller spatial scales restricted not only the species pool but also the trait variation. This tends to weaken the phylogenetic signals of traits, either through the narrower phylogenetic range caused by environmental filtering, or the tendency towards convergence caused by competitive exclusion, leading to the phylogenetic overdispersion of co-occurring species (Webb et al. 2002). For example, grass species have a wide global range of MAT (-20~40 °C) and MAP (10~810 mm), but the narrow range of both MAT (-2~9 °C) and MAP (100~500 mm) in the Inner Mongolia steppe constrained both co-occurring species and their trait variability.

Of the traits only measured at the local scale, we used a number of ecophysiological traits that have not been commonly tested in previous studies on species coexistence. However, the phylogenetic signals of these traits were very weak within habitats (Table 2). The loss of phylogenetic signals is potentially due to: (1) lowered species richness at smaller scales, where the phylogenetically overdispersed pattern will weaken the statistical power to detect phylogenetic signals; (2) Other functional traits fluctuated over time like leaf water potential, so that they might also be unrepresentative of long-term species values for the phylogenetic tests; (3) The sensitivity of phylogenetic tests among species could be reduced by using average values across the four habitats. Phenotypic plasticity and the ability to extract limiting resources from the environment determine the niche breadth of each species, and crucially affect interactions among and within species (Genung et al. 2012). In our data, some species were exclusively in one habitat, indicating strong, specific environmental adaptations, while others had wide niche breadths. Therefore, phylogenetic niche conservatism in traits alone was insufficient to explain species distribution, and environmental factors had to be added at the small spatial scales (Wiegand et al. 2017).

However, for traits that still retained phylogenetic signals at smaller scales, the divergences among major lineages might have contributed to species coexistence, since our previous work found that separate phylogenetic tests on the two largest C_4 subfamilies would weaken the strong phylogenetic signals for the whole dataset (Liu et al. 2012). In this study, leaf $\delta^{13}C$ had strong phylogenetic signals due to the large difference in CO_2 discrimination between C_3 and C_4 species (Farquhar et al. 1989), and the clustering of C_4 photosynthesis into particular lineages (Grass Phylogeny Working Group II 2012; Sage and Monson 1999).

H3: Environmental drivers for species assemblage at local scales

The SWC and SNC in deeper soil layers were the best predictors of species distribution across the four habitats, suggesting that water and nitrogen at depth are most important for sorting species in semiarid steppes (Sala et al. 1992), in agreement with previous studies in the same area of Inner Mongolia based on soil water and nutrient measurements for 102 communities (Song and Guo 2007). Environmental filtering could sort species into different niches, increase species coexistence through the complementary use of water and nutrient resources (Cardinale 2011). The complementarity is also related to neighbor-related indices and leaf morphology in our data, emphasizing the importance of competition intensity. Phylogenetic overdispersion of grass species at the local scale was confirmed by niche complementarity of species in the same genus using different strategies to cope with neighbors (Gubsch et al. 2011). For example, in the wetland, *Puccinellia macranthera* has a very dense population and tall culms in or around water; but its congener, *Puccinellia hauptiana*, is tiny and dispersed near water margins. Unlike plant trait hierarchy-driven interactions in tree communities (Kunstler et al. 2012), we argue that strong correlations between biotic and abiotic factors, as well as trait variation are all involved in differentiating niche gradients in this semi-arid region (Table 3).

H4: Weaker functional trait-environment relationships

Regionally, trait-environment relationships were influenced more by phylogenetic background than the same tests both at the global (Liu et al. 2012) and the intermediate scales (Table 4). This scale dependence supported the idea that phylogeny and environment both play roles in species distribution (Webb et al. 2002). Within the region, morphological traits correlated better with water availability than MGT/MGP, suggesting that species growth was associated more closely with habitat wetness than regional precipitation (Appendix S1). Meanwhile, these positive trait-environment relationships became weaker at smaller spatial scales, implying that more environmental factors must be invoked to explain variance in plant traits (Table 4). Specifically, positive relationships between culm height and SWC suggested more aboveground growth with more soil water, while SRL increased with SWC indicated that with more water availability, species tended to use a resource acquisitive strategy with a high root surface area per unit investment (Eissenstat 1991). Culm height and root depth increased with neighbor density showing the importance of above- and below-ground competition in understanding species coexistence (Kraft et al. 2015).

Conclusions

This study has demonstrated that co-occurring grass species in the steppe biome tend to be more phylogenetically overdispersed, and phylogenetic signals of plant ecophysiological traits become weaker at smaller spatial scales. At the local scale, abiotic and biotic interactions are tested to be more important than the regional scale, at which scale other processes such as dispersal limitation might be more determinant. Our findings contribute to the understanding of species coexistence and maintenance at scales spanning regional to local communities in the East Asia steppe biome.

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Electronic supplementary material The online version of this article contains supplementary material, which is available to authorized users.

Appendix S1 Environmental gradients and the consistency of water availability across spatial scales

Appendix S2 Poaceae species list with morphological traits, habitat, biogeographic data and species occurrence in the 88 counties in Inner Mongolia. County-level phylogenetic signals and phylogenetic structure were also reported

Appendix S3 A phylogenetic tree of 238 Poaceae species in Inner Mongolia, showing species filtered across spatial scales

Appendix S4 A phylogenetic tree of 32 Poaceae species at the local scale (Hunshandake sandland), with species abundance in four habitats and main variables mapped across the tree based on PPCA results

Tables

Table 1 Phylogenetic analyses for grass species occurrences filtered from the regional (238 and 170 species pools) to the intermediate (88 species) and then local (32 species) scales in the Inner Mongolian steppe. The 238-species phylogenetic tree is an overall species pool; it merged the 170-species tree that was strictly constructed based on DNA data and another 58 congeners. Sample size (*n*), *D* and *P* values for $D = 0$ and 1 are reported. Level of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant. SR, species richness; NRI, net relatedness index. C, phylogenetically clustered; O, phylogenetically overdispersed

	SR	NRI	*P*	Pattern	*n*	*D*	$P_{(D=0)}$	$P_{(D=1)}$	Pattern
Filtered from 238 spp. tree									
170 spp. in the region	170	0.63	ns	C	238	0.44	***	***	C
88 spp. in the county	88	-1.93	ns	O	238	0.84	***	*	O
32 spp. in the local field	32	-4.82	ns	O	238	1.03	***	ns	O
Filtered from 170 spp. tree									
88 spp. in the county	88	0.10	ns	C	170	1.12	***	ns	O
32 spp. in the local field	32	-2.03	ns	O	170	0.70	*	ns	O
Filtered from 32 spp. tree									
5 spp. in the moving dune	5	-1.07	ns	O	32	1.20	***	ns	O
10 spp. in the fixed dune	10	0.04	ns	C	32	1.28	***	ns	O
21 spp. in the meadow	21	-1.26	ns	O	32	0.88	*	ns	O
12 spp. in the wetland	12	2.14	ns	C	32	0.77	*	ns	O

Table 2 Phylogenetic signals in plant functional traits, abiotic and biotic environmental variables for grasses in the Inner Mongolian steppe at both regional and local scales. Random selection models give the mean ± SD of λ values based on 1000 random selections of 32 species from the regional dataset. Data are natural logged in tests except those with †. Sample size (n), λ and P values for both $\lambda = 0$ and 1 are reported. Level of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant. SLA, specific leaf area; SRL, specific root length; DNN, distance to the nearest neighbor; MGT, mean growing season (May to September) temperature; MGP, mean growing season precipitation

	Regional scale				Random selection models	Local scale ($n = 32$)		
	n	λ	$P_{(\lambda = 0)}$	$P_{(\lambda = 1)}$	λ	λ	$P_{(\lambda = 0)}$	$P_{(\lambda = 1)}$
Culm height (cm)	166	.83	***	***	.37±.01	.51	**	**
Leaf length (cm)	159	.95	***	***	.48±.01	.36	ns	***
Leaf width (mm)	154	.78	***	***	.56±.01	.00	ns	***
Leaf mass (g)						.07	ns	***
Leaf number per tiller						.00	ns	***
SLA (cm^2 g^{-1})						.19	ns	***
Root depth (cm)						.13	ns	***
SRL (cm g^{-1})						.41	*	***
Flat leaf (%) †	127	.39	ns	ns		.00	ns	***
Folded leaf (%) †	75	.43	ns	ns		.14	ns	***
Flowering start †	135	.29	***	***	.18±.01	.00	ns	*
Flowering end †	135	.50	***	***	.43±.01	.34	ns	*
Leaf nitrogen content (%)						.26	ns	***
Leaf δ^{15}N (‰)						.00	ns	***
Leaf carbon content (%)						.23	ns	***
Leaf δ^{13}C (‰)						.78	***	ns
Leaf water potential (MPa)						.12	ns	***
Stomatal density (mm^{-2})						.46	***	***
Guard cell length (μm)						.56	***	***
Stomatal width (μm)						.43	*	***

Stomatal pore index						.14	ns	***
Water range	166	.00	ns	***	.09±.01	.00	ns	***
Water score	166	.92	***	***	.31±.01	.21	ns	***
MGT (°C)	165	.24	**	***	.12±.01	.00	ns	***
MGP (mm y^{-1})	165	.14	*	***	.11±.01	.00	ns	***
Soil water content (%)						.39	ns	***
Soil nitrogen content (%)						.00	ns	***
Relative abundance						.00	ns	***
Neighbour density (m^{-2})						.09	ns	***
DNN (cm)						.15	ns	***
Tallest neighbour (cm)						.48	**	***

Table 3 Principal component (PC) loadings for phylogenetic PCA (PPCA) on (a) environmental variables and (b) plant functional traits of grasses at the local scale (Hunshandak sandland). SWC1-2-3, soil water content from 0-10, 10-20, 20-30 cm layers; SNC1-2-3, soil nitrogen content from 0-10, 10-20, 20-30 cm layers; ND, neighbour density; RA, relative abundance; TN, tallest neighbour; NN, nearest neighbour distance; LL, leaf length; LA, leaf area; LM, leaf mass; LW, leaf width; SW, stomatal width; RD, root depth; LC, leaf carbon content; GL, guard cell length; SD, stomatal density. For each PC, the first four variables with highest loadings are in bold

(a) PPCA on environmental variables				(b) PPCA on morphological traits			
Variable *n* = 10	PC1	PC2	PC3	Variable *n* = 17	PC1	PC2	PC3
SNC3	**0.886**	0.137	0.226	LL	**-0.957**	0.108	-0.064
SNC2	**0.81**	0.174	**0.418**	LA	**-0.947**	0.01	-0.095
SNC1	**0.803**	-0.122	**0.435**	LM	**-0.932**	0.193	-0.123
SWC3	**0.752**	0.165	-0.353	LW	**-0.891**	-0.3	0.105
ND	0.478	**-0.788**	0.05	Abxial SW	-0.185	**-0.8**	-0.396
RA	0.09	**-0.774**	-0.114	RD	-0.425	**0.751**	-0.142
TN	0.201	**-0.547**	**-0.619**	SLA	0.377	**-0.659**	0.199
NN	-0.581	**0.533**	0.006	LC	-0.435	**-0.581**	0.001
SWC1	0.553	0.468	**-0.546**	Abxial GL	-0.104	-0.149	**-0.876**
SWC2	0.722	0.442	-0.286	Adxial SD	-0.681	0.072	**0.595**
				Adxial GL	-0.516	-0.155	**-0.468**
				Adxial SW	-0.185	-0.8	**-0.396**
Total variance (%)	40.92	23.09	13.18		38.74	18.49	10.44
Cumulative variance (%)	40.92	64.01	77.19		38.74	57.23	67.67

Table 4 Phylogenetic general linear models (*PGLM*) of relationships between plant functional traits and environmental variables for grasses in (a) the whole Inner Mongolia region and (b) the local scale (Hunshandak sandland). Sample size (*n*), *F*, *P* and *λ* values, slopes and R^2 are listed. All data are natural-logged for standardization. Slopes and *F* values with significant fits are in bold. CH, culm height; LL, leaf length; LW, leaf width; water, water score; SWC, soil water content; ND, neighbor density; SNC, soil nitrogen content; LM, leaf mass; LA, leaf area; RD, root depth; GL, guard cell length; SW, stomatal width

(a) Regional scale	*n*	*λ*	$P_{(\lambda=0)}$	$P_{(\lambda=1)}$	F^P	*slope*	R^2
CH ~ MGT	161	.18	*	***	**7.9****	**-1.43**	.04
LL ~ MGT	155	.77	***	***	1.25^{ns}	-.66	.002
LW ~ MGT	149	.47	*	***	.59^{ns}	-.59	.003
CH ~ MGP	161	.16	*	***	**1.88****	**.50**	.058
LL ~ MGP	155	.73	***	***	**5.08***	**.41**	.026
LW ~ MGP	149	.42	**	***	**8.35****	**.65**	.047
CH ~ water	162	.43	*	***	**2.63*****	**.39**	.11
LL ~ water	155	.80	***	***	**22.66*****	**.50**	.12
LW ~ water	151	.48	*	***	**42.80*****	**.76**	.22

(b) Local scale	*n*	*λ*	*P*	*P*	F^P		
			(*λ* = 0)	(*λ* = 1)	SWC	ND	SNC
CH ~ SWC+ND+SNC	32	.63	**	**	**4.30** *	**6.22** **	1.64 ^{ns}
LL ~ SWC+ND+SNC	32	.35	ns	***	.01 ^{ns}	.52 ^{ns}	.04 ^{ns}
LW ~ SWC+ND+SNC	32	.00	ns	***	.81 ^{ns}	.05 ^{ns}	.15 ^{ns}
LM ~ SWC+ND+SNC	32	.00	ns	***	.01 ^{ns}	1.66 ^{ns}	.01 ^{ns}
LA ~ SWC+ND+SNC	32	.00	ns	***	.17 ^{ns}	.92 ^{ns}	.00 ^{ns}
SLA ~ SWC+ND+SNC	32	.00	ns	***	.56 ^{ns}	2.76 ^{ns}	.09 ^{ns}
RD ~ SWC+ND+SNC	32	.00	ns	***	1.64 ^{ns}	**4.20** *	.49 ^{ns}
SRL ~ SWC+ND+SNC	32	.37	ns	***	**7.78** **	.16 ^{ns}	2.06 ^{ns}
SD ~ SWC+ND+SNC	32	.45	**	***	.99 ^{ns}	.03 ^{ns}	.04 ^{ns}
GL ~ SWC+ND+SNC	32	.66	***	***	1.68 ^{ns}	.37 ^{ns}	3.19 ^{ns}
SW ~ SWC+ND+SNC	32	.58	**	***	.08 ^{ns}	3.95 ^{ns}	.17 ^{ns}

Figure legends

Fig. 1 Map of the Inner Mongolian Autonomous Region (IMAR), China, showing spatial and environmental gradients. (a) Main vegetation types of each county in IMAR, intermediate (Zhenglan county) and local field site (Hunshandak sandland, the red triangle) are labeled. (b) Altitude, (c) mean annual temperature (MAT), and (d) mean annual precipitation (MAP) and grass species richness of each county across IMAR. See Appendix S1 for detailed environmental data. This figure is available in color in the online version of the journal

Fig. 2 Species richness of different grass lineages along the mean annual growth (a) temperature and (b-c) precipitation gradients. Panel (b) is enlarged to show (c) Chloridoideae and Panicoideae, and (d) tribes in Pooideae. In (d), Aveneae and Stipeae are the only two of eight tribes in Pooideae that have significant regressions. In (a): Pooideae (triangles), $y = -7.7 \cdot x + 189.8$, $F_{1,86} = 48.9$, $R^2 = 0.36^{***}$. In (b, c): Chloridoideae (black dots), $y = -0.02 \cdot x + 14.0$, $F_{1,86} = 16.1$, $R^2 = 0.15^{***}$; Panicoideae (white dots), $y = 0.03 \cdot x + 9.2$, $F_{1,86} = 4.4$, $R^2 = 0.31^{***}$. In (d): Aveneae (white quares) $y = 0.03 \cdot x + 4.6$, $F_{1,86} = 24.9$, $R^2 = 0.22^{***}$; and Stipeae (black quares), $y = -0.03 \cdot x + 17.2$, $F_{1,86} = 58.3$, $R^2 = 0.40^{***}$

Fig. 3 Relationships between plant functional traits and environmental factors based on the models in Table 4. (a) culm height and (b) specific root length with soil water content; (c) culm height and (d) root depth with neighbour density. Data are mean ± SD. Subfamilies are Pooideae (black dot), Panicoideae (white dot), Chloridoideae (white square) and Arundinoideae (white triangle). Significant regressions based on *PGLM*s are (a): $y = 0.4 \cdot x + 2.6$, $F_{1,30} = 3.2$, $R^2 = 0.07^{ns}$; (b): $y = 0.4 \cdot x + 3.2$, $F_{1,30} = 7.9$, $R^2 = 0.18^{**}$; (c): $y = 0.3 \cdot x + 2.0$, $F_{1,30} = 6.9$, $R^2 = 0.16^{*}$; and (b): $y = 0.29 \cdot x + 1.6$, $F_{1,30} = 3.7$, $R^2 = 0.08^{*}$

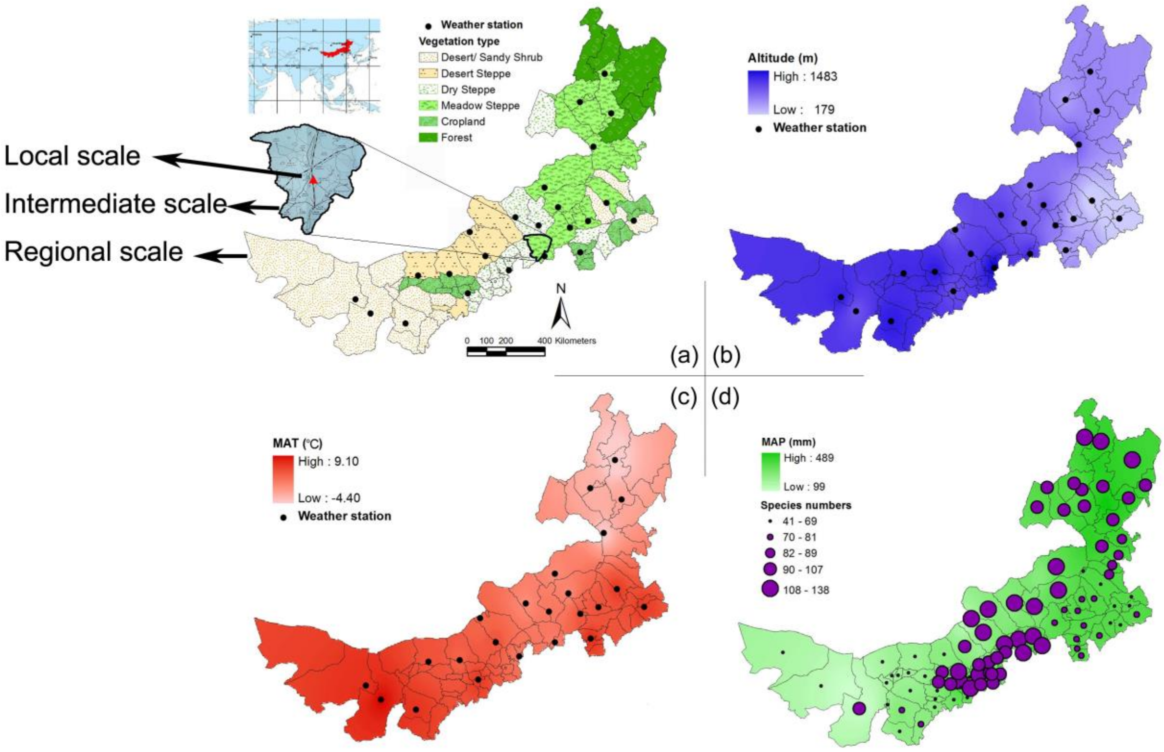
Weather station
Vegetation type
Desert/ Sandy Shrub
Desert Steppe
Dry Steppe
Meadow Steppe
Cropland
Forest
Local scale
Intermediate scale
Regional scale
N
0 100 200 400 Kilometers
Altitude (m)
High : 1483
Low : 179
Weather station
(a)
(b)
(c)
(d)
MAT (°C)
High : 9.10
Low : -4.40
Weather station
MAP (mm)
High : 489
Low : 99
Species numbers
41 - 69
70 - 81
82 - 89
90 - 107
108 - 138

Fig. 1

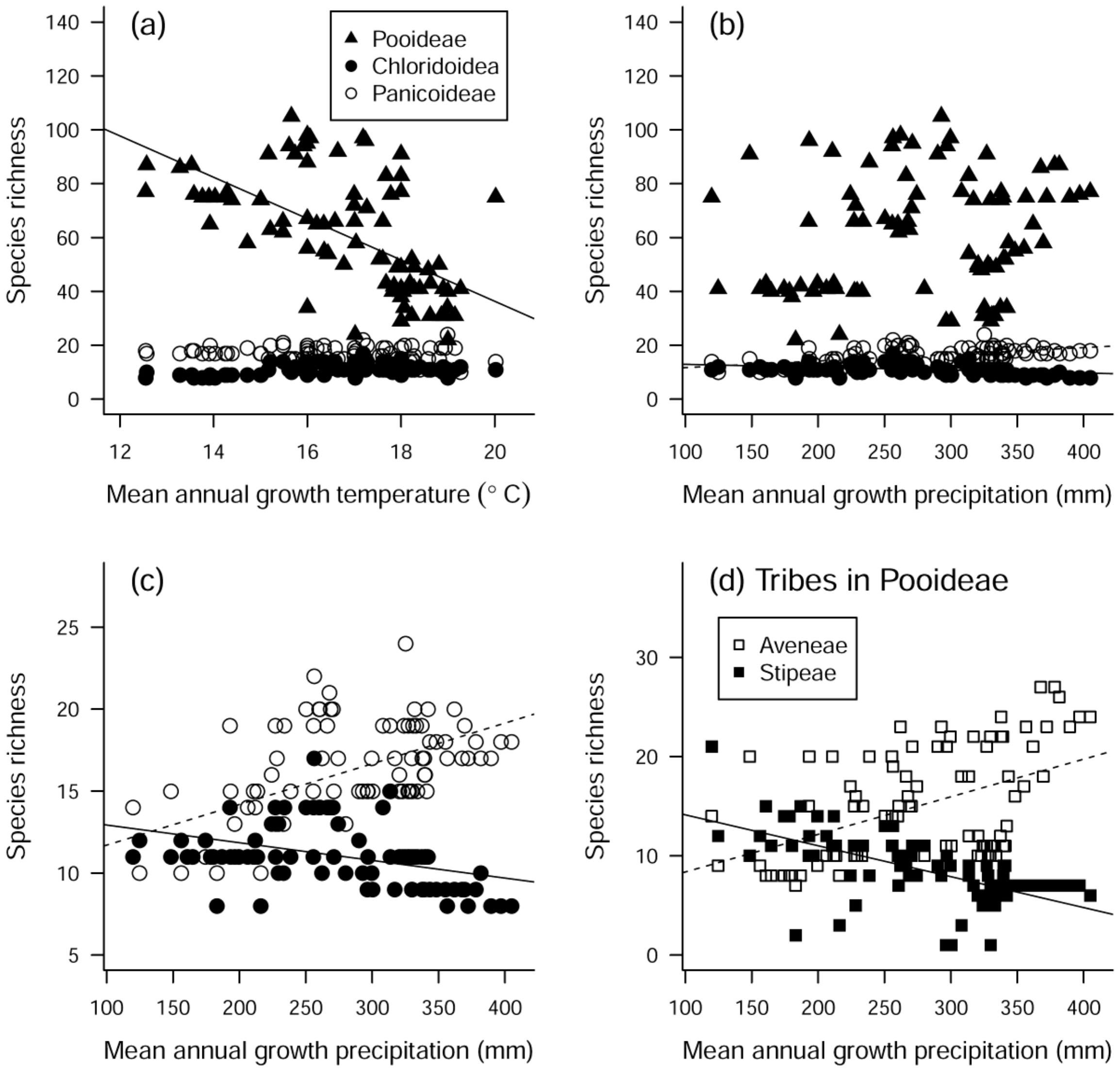
(a)
Pooideae
Chloridoidea
Panicoideae
Species richness
0
20
40
60
80
100
120
140
12
14
16
18
20
Mean annual growth temperature (° C)
(b)
Species richness
0
20
40
60
80
100
120
140
100
150
200
250
300
350
400
Mean annual growth precipitation (mm)
(c)
Species richness
5
10
15
20
25
100
150
200
250
300
350
400
Mean annual growth precipitation (mm)
(d) Tribes in Pooideae
Aveneae
Stipeae
Species richness
0
10
20
30
100
150
200
250
300
350
400
Mean annual growth precipitation (mm)

Fig. 2

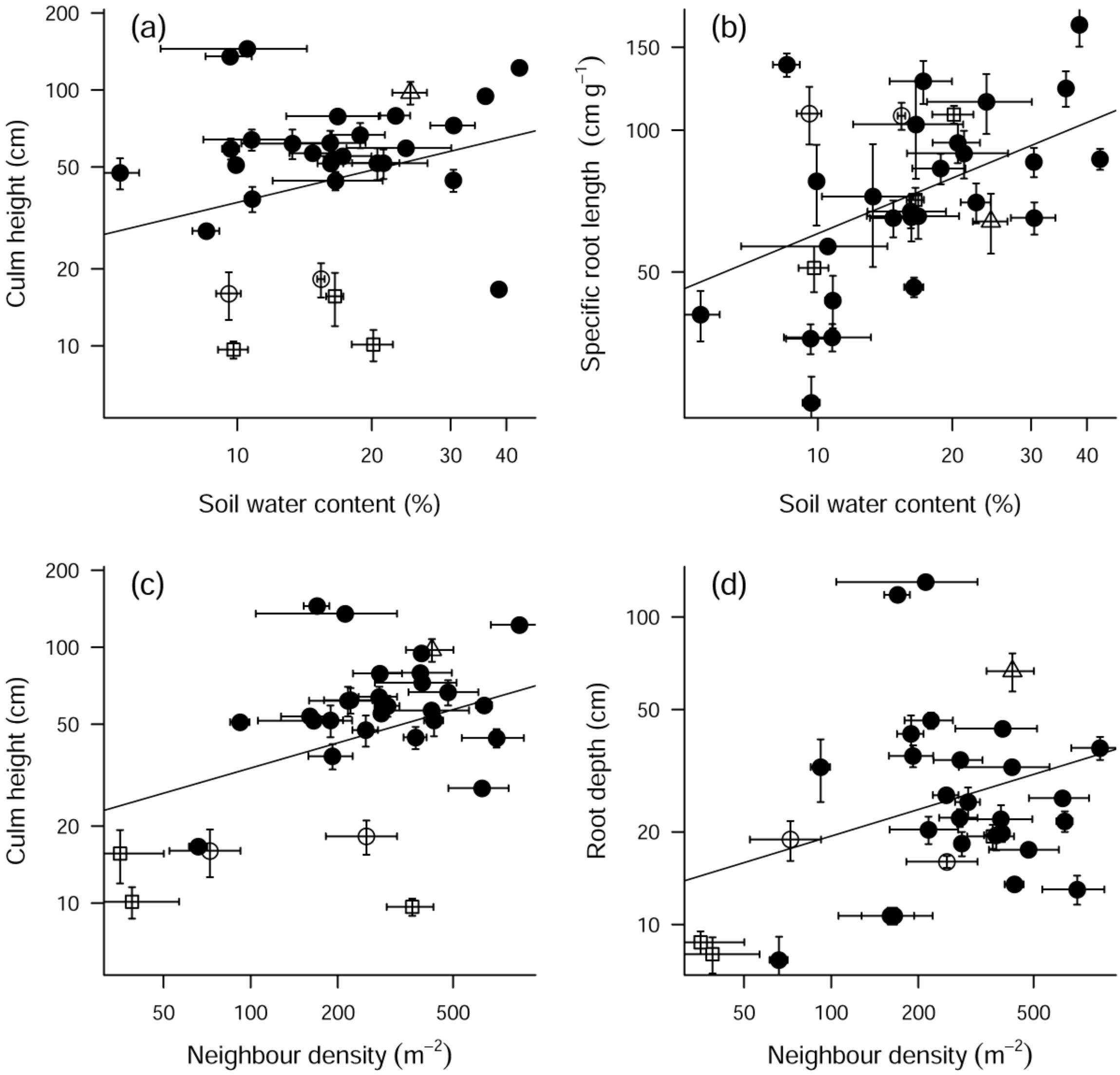
(a)
Culm height (cm)
200
100
50
20
10
10
20
30
40
Soil water content (%)
(b)
Specific root length (cm g^{-1})
150
100
50
10
20
30
40
Soil water content (%)
(c)
Culm height (cm)
200
100
50
20
10
50
100
200
500
Neighbour density (m^{-2})
(d)
Root depth (cm)
100
50
20
10
50
100
200
500
Neighbour density (m^{-2})

Fig. 3