

RESEARCH ARTICLE

Plant diversity is coupled with soil fungal diversity in a natural temperate steppe of northeastern China

Dan Liu¹, Guohua Liu^{2,3,*}, Li Chen⁴, Wangya Han⁵, Dongbo Wang⁶

¹ Institute of Qinghai-Tibetan Plateau, Southwest Minzu University, Chengdu 610041, China

² State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

³ University of Chinese Academy of Sciences, Beijing 100049, China

⁴ Torch High Technology Industry Development Center, Ministry of Science & Technology, Beijing 100049, China

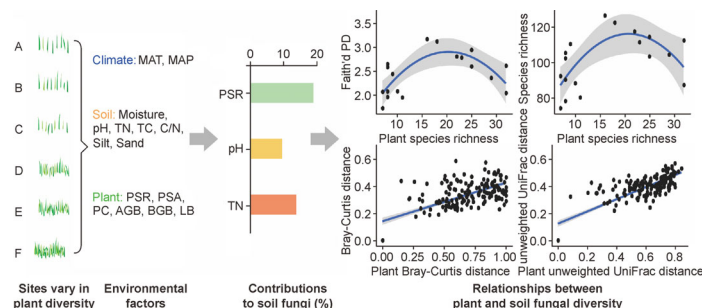
⁵ Institute of Ecology, School of Applied Meteorology, Nanjing University of Information Science & Technology, Nanjing 210044, China

⁶ Monitoring Station for Eco-environment of Hulunbeir, Hulunbeir 021000, China

HIGHLIGHTS

- Soil fungal community composition varied significantly between study sites.
- Plant species richness (PSR) contributed most to the variation in soil fungi community.
- Both α and β diversity of soil fungi coupled well with that of plant.
- Plant diversity can predict soil fungal diversity in the temperate steppe of northeastern China.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil fungi and aboveground plant play vital functions in terrestrial ecosystems, while the relationship between aboveground plant diversity and the unseen soil fungal diversity remains unclear. We established 6 sites from the west to the east of the temperate steppe that vary in plant diversity (plant species richness: 7–32) to explore the relationship between soil fungal diversity and aboveground plant diversity. Soil fungal community was characterized by applying 18S rRNA gene sequencing using MiSeq PE300 and aligned with Silva 132 database. As a result, soil fungal community was predominately composed of species within the Ascomycota (84.36%), Basidiomycota (7.22%) and Mucoromycota (6.44%). Plant species richness occupied the largest explanatory power in structuring soil fungal community (19.05%–19.78%). The alpha (α) diversity of the whole soil fungi and Ascomycota showed a hump-backed pattern with increasing plant species richness, and the beta (β) diversity of the whole soil fungi and Ascomycota increased with increasing plant β diversity. Those results indicated that soil fungi and external resources were well balanced at the 20-species level of plant and the sites were more distinct in the composition of their plant communities also harbored more distinct soil fungal communities. Thus, plant diversity could predict both soil fungal α and β diversity in the temperate steppe of northeastern China.

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* Corresponding author

E-mail address: ghliu@rcees.ac.cn (G.H. Liu)

1 Introduction

Soil fungi are widely dispersed in terrestrial ecosystems and contribute a lot to ecosystem processes, including litter decomposition, nutrient cycling and soil development (Wardle et al., 2004; Delgado-Baquerizo et al., 2016). Moreover, soil fungal diversity could have profound impacts on the ecosystem stability (Pennekamp et al., 2018). Nevertheless, soil fungi represent the unseen creature in soil and it is hard to visually estimate soil fungal diversity. There is evidence that aboveground plants could directly affect soil fungal diversity by mediating the food resource inputs to soil (Broeckling et al., 2008; Gao et al., 2015; Moll et al., 2015; Carrara et al., 2018) and indirectly affect soil fungal diversity by their effects on the microclimate and soil physicochemical properties (Hodge et al., 2001; Kuzyakov and Xu, 2013; Tedersoo et al., 2014). Thus, understanding the relationship between aboveground plant diversity and soil fungal diversity could help for prediction and conservation of the unseen soil fungal diversity (Waldrop et al., 2006; Prober et al., 2015; Yang et al., 2017).

Studies concerning the relationship between soil fungal diversity and plant diversity primarily investigated community α diversity and β diversity, the latter of which can imply the variation in community structure and function. Waldrop et al. (2006) suggested that plant diversity could not predict either α diversity or β diversity of soil fungal community in the CCNHA biodiversity experiment, while some studies suggest that both α and β diversity of soil fungi are effectively predicted by plant diversity (Peay et al., 2013; Chen et al., 2017; Yang et al., 2017). Additionally, α and β diversity of soil fungal community could also respond inconsistently with increasing plant diversity. Porazinska et al. (2018) found a positive relationship of plant diversity with soil fungal α diversity, and lack of relation with fungal β diversity in an early successional alpine ecosystem. However, a positive correlation between plant diversity and fungal β diversity was reported in a semiarid grassland (Chen et al., 2018). Another study investigated the grassland worldwide and demonstrated that fungal β diversity but not α diversity was coupled with plant diversity (Prober et al., 2015). The inconsistent relationships between plant diversity and soil fungal diversity described above made it difficult to predict and protect the diversity of soil fungi.

The response of soil fungal diversity to plant diversity could be affected by various factors, such as sampling design and soil physicochemical parameters. For example, the Jena Experiment manipulated a gradient in plant species richness from 1 to 60, and they found that plant diversity negatively affected fungal pathogen diversity (Lange et al., 2014; Rottstock et al., 2014). However, the CCNHA biodiversity experiment manipulated a gradient in savanna species richness from 1 to 16 and found that plant diversity was unrelated to fungal diversity (Waldrop et al., 2006). Those different sampling designs could result in the different plant-fungal diversity links. Another study suggested that the positive correlation between fungal β diversity and plant

diversity was controlled by the N-induced soil physicochemical variables, including soil available phosphorous, pH and inorganic nitrogen (Chen et al., 2018). Since environmental factors chronically interact with plant community in natural ecosystems, the underlying mechanism driving soil fungal community remains unclear. More studies concerning the relationship between plant diversity and soil fungal diversity are urgently needed to predict the unseen soil fungal diversity.

Temperate steppes are one of the largest terrestrial ecosystems worldwide, and they play an important role in regional climate change and global carbon cycling (Guo et al., 2018). The temperate steppe in Inner Mongolia comprises approximately 22% of the total grassland area of China (Tang et al., 2020). The plant community in Inner Mongolia varies sharply from west to east, which makes Inner Mongolia a natural laboratory for research on the relationship between plant diversity and soil fungi diversity (Liu et al., 2008; Ma et al., 2015). Recent studies in temperate steppes primarily focused on the aboveground plants whereas very few studies unraveled the linkages between plants and soil microbes (Ma et al., 2015; Tian et al., 2016; Tu et al., 2017). Liu et al. (2008) found tight relationships between the aboveground plant and microbial biomass as well as microbial activity in a temperate steppe. However, the response of soil fungal diversity to plant diversity remains unclear.

In this study, we sampled sites that represent a wide range of plant diversity in the temperate steppe of Inner Mongolia, and high-throughput Illumina sequencing was performed to analyze the relationship between plant diversity and soil fungal diversity. We hypothesized that: 1) Soil fungal communities vary significantly between sampling sites that vary in plant diversity; and 2) Plant diversity could partially predict soil fungal diversity in the temperate steppe.

2 Materials and methods

2.1 Site description

The study sites are located in Hulunbeir (115°31' E–126°04' E, 47°05' N–53°20' N), Inner Mongolia, China (Fig. 1). This area has a continental monsoon climate, with large temperature and precipitation gradients. Mean annual temperature (MAT), and mean annual precipitation (MAP) at this spatial scale range from approximately 0.9 to 2.1°C and 138 to 197 mm, respectively. Similarly, aboveground plants vary significantly from west to east, including typical steppe and meadow steppe. The main soil types are chernozem and chestnut, and all the pH values vary from 6.6 to 7.4. Six sites (A, B, C, D, E and F) were selected across a longitudinal gradient, representing a wide range of plant diversity in this part of the temperate steppe. A 100 m × 100 m plot was selected for plant investigation in each site, and a 100 m line transect was set in the middle position, and three 1 m × 1 m quadrats were randomly set on the transect line at least 10 m distant from

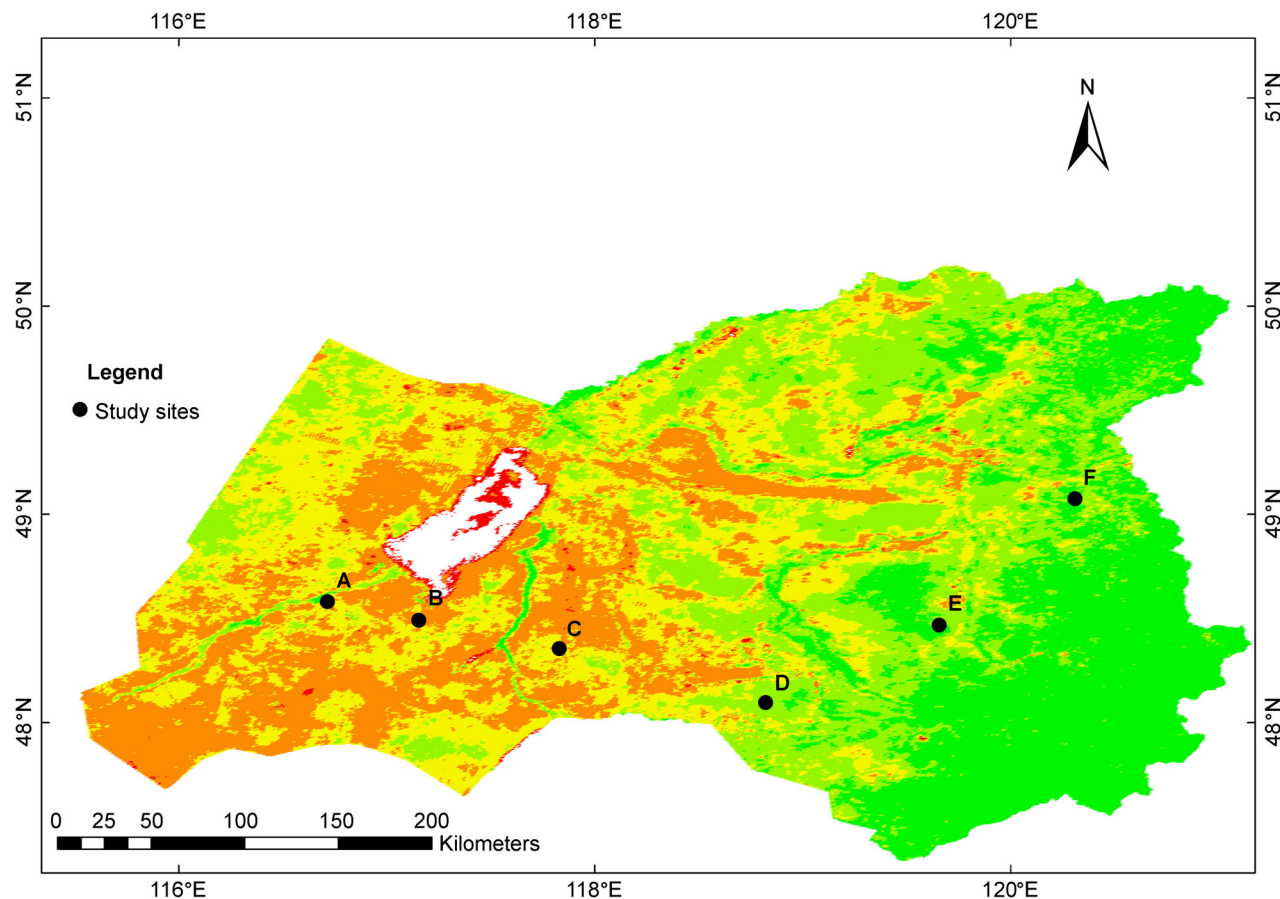


Fig. 1 Map of the study sites across Hulunbeir steppe, Inner Mongolia, China. The map in this figure was generated by ArcGIS 10.2 based on the monthly Normalized Difference Vegetation Index (NDVI) of August 2015 (ESRI, Redlands, CA, USA, <http://resources.arcgis.com/en/home/>).

one another, producing 18 quadrats in total. All the 18 quadrats selected from regions without grazing and farming activities to avoid anthropic disturbance. A detailed description of geographic locations, climate parameters, soil and vegetation properties of the six sites can be found in Table 1.

2.2 Vegetation survey and soil sampling

Vegetation survey was conducted in August 2015, at the peak of aboveground biomass and plant species richness. All the plants in the 18 quadrats were recorded. We obtained the following plant traits: PSR, plant species abundance (PSA), plant coverage (PC), plant aboveground biomass (AGB), plant litter biomass (LB), and plant belowground biomass (BGB). The total number of plant species in a given quadrat was calculated as PSR, and PSA represented the total number of plant individuals. The percentage cover of herbaceous vegetation in each quadrat was estimated as PC. The aboveground plants, plant litter and roots in each quadrat were collected and dried at 65°C to a constant weight to determine the AGB, LB and BGB, respectively. Taxonomic information of plants was obtained based on the Angiosperm Phylogeny Group (APG) III system (Chase and Reveal,

2009). Phylogenetic information of plants was calculated using the online phylogenetic query tool Phylomatic (<http://phylodiversity.net/phyloomatic/>) (Webb and Donoghue, 2005; Zanne et al., 2014).

In each quadrat, five randomly located soil cores at 0–20 cm depth were collected using an auger (diameter = 7 cm). Each soil sample was sieved (<2 mm) before dividing into two subsamples. One subsample was stored at 4°C for soil physicochemical measurements, and the other subsample was stored at –80°C for soil DNA extraction. Soil moisture was determined by weighing method. Soil texture was examined using a Mastersizer 2000 Laser Diffraction Particle Analyzer (Malvern Instruments Ltd, Malvern, UK) according to the universal criteria of soil particle size. Soil pH was measured after shaking a soil water (1:5 mass/volume) suspension for 30 minutes. Soil total carbon (TC), TN and carbon/nitrogen ratio (C/N) were determined using a Vario EL III analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Mean annual temperature (MAT) and mean annual precipitation (MAP) values of sampling points were computed using ordinary Kriging interpolation based on the 2015 climate data from the nearest meteorological stations in Hulunbeir, China.

Table 1 Summary of ecological characteristics of the study sites in Hulunbeir, China.

Environmental variables	A	B	C	D	E	F
MAT (°C)	2.09	1.54	1.78	1.47	1.19	1.08
MAP (mm)	138.32	158.86	197.13	195.72	189.55	188.00
Soil type	kastanozem	kastanozem	kastanozem	chernozem	chernozem	chernozem
Moisture (%)	6.32±0.28b	1.91±0.20a	4.54±0.95ab	5.16±0.38ab	19.08±2.22c	18.51±4.00c
Soil pH	7.04±0.02b	6.99±0.25b	7.02±0.14b	6.87±0.01ab	6.70±0.07a	7.30±0.12c
TN (mg · g ⁻¹)	1.78±0.07b	1.06±0.13a	1.85±0.16b	1.88±0.02b	2.88±0.05d	2.40±0.07c
TC (mg · g ⁻¹)	18.80±0.49b	9.66±1.54a	18.69±1.84b	21.52±0.04c	34.64±0.88e	32.38±1.56d
C/N	10.55±0.14b	9.07±0.54a	10.12±0.12b	11.42±0.12c	12.01±0.21d	13.48±0.35e
Silt (%)	4.60±2.23b	2.89±0.21ab	2.82±0.35ab	4.23±0.20ab	2.59±0.07a	2.75±0.31a
Sand (%)	95.33±2.35a	97.11±0.21ab	97.18±0.35ab	95.77±0.20ab	97.41±0.07b	97.25±0.31b
Dominant plant	<i>Cleistogenes squarrosa</i>	<i>C. squarrosa</i>	<i>C. squarrosa</i>	<i>C. squarrosa</i>	<i>Serratula centauroides</i>	<i>C. pediformis</i>
	<i>Carex korshinskii</i>	<i>Stipa grandis</i>	<i>C. korshinskii</i>	<i>C. korshinskii</i>	<i>Carex pediformis</i>	<i>Thalictrum petaloideum</i>
	<i>Stipa krylovii</i>	<i>Agropyron cristatum</i>	<i>S. grandis</i>	<i>Artemisia scoparia</i>	<i>Pulsatilla turczaninowii</i>	<i>Potentilla verticillaris</i>
PSR (number of plant species)	8±1a	9±1a	9±2a	19±3b	24±1c	31±2d
PSA (count of plant individual)	1151±264bc	381±165a	602±147ab	1312±64c	1300±315c	1483±393c
PC (%)	56.67±11.55b	23.33±2.89a	53.33±5.77b	78.33±10.41c	71.67±5.77c	80.00±10.00c
AGB (g)	409.47±35.38a	455.33±65.41ab	717.87±104.26bc	537.07±56.78ab	887.47±140.76c	1303.73±286.91d
BGB (g)	1289.61±731.43abc	560.93±233.45a	812.56±565.37ab	1420.67±300.19bcd	1729.97±175.13cd	2191.29±380.17d
LB (g)	224.00±103.66a	253.87±120.34a	288.67±29.61a	176.67±94.43a	232.13±103.38a	307.20±217.75a

Notes: MAT: mean annual temperature; MAP: mean annual precipitation; TN: total nitrogen; TC: total carbon; C/N: carbon/nitrogen ratio; PSR: the total number of plant species; PSA: the total number of plant individuals; PC: plant coverage; AGB: plant aboveground biomass; BGB: plant belowground biomass; LB: plant litter biomass.

2.3 Soil fungal community analyses

Soil DNA was extracted from the 0.25 g soil using the MOBIO Powersoil DNA extraction kit (Carlsbad, USA) as directed by the manufacturer. DNA concentrations and purity were determined using a NanoVue™ Spectrophotometer (GE Healthcare, USA) and stored at -80°C before sequencing. The V4 + V5 variable regions of fungal 18S rRNA genes were amplified with the primers pair 817F (5'-TTAGCATGGAA-TAATRAATAGGA-3') and 1196R (5'-TCTGGACCTGGT-GAGTTTCC-3'), which could make our findings most generalizable at the family level or above (Rousk et al., 2010). PCR amplification was carried out in 20 μL volume, containing 10 ng template DNA, 4 μL FastPfu Buffer (TransGen Biotech, Beijing, China, 0.8 μL 5 μM primers.), 2 μL dNTPs, and 0.4 μL TransStart FastPfu DNA Polymerase (TransGen Biotech, Beijing, China). The PCR amplification program consisted of denaturation at 95°C for 3 min; 32 cycles at 95°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 45 s; final elongation at 72°C for 10 min, and cooling at 10°C . The PCR products were detected on QuantiFluor™ -ST Blue fluorescence quantitative system (Promega, USA), and the amplicons were mixed and sequenced on an Illumina Miseq sequencer (Illumina Inc., San Diego, USA).

The raw sequences were quality-filtered and demultiplexed using Quantitative Insights into Microbial Ecology (QIIME) pipeline version 1.9.1 (Caporaso et al., 2010) with the following criteria: 1) the raw sequences were truncated where the average quality was less than 20 over a 50 bp sliding window and sequences shorter than 50 bp were truncated; 2) paired-end sequences were merged with overlap length threshold of 10 bp (mismatch ratio < 0.2); 3) the joint sequences were assigned to samples with specific barcodes and primers (barcode mismatch = 0, primer mismatch = 2). Then dereplication and discard of singletons were performed by USEARCH (Edgar, 2013). The residual sequences were then clustered into operational taxonomic unit (OTU) at 97% similarity using Usearch version 7.1 (<http://drive5.com/uparse/>), and chimeras were detected and removed. Taxonomic classification was performed using RDP classifier (<http://rdp.cme.msu.edu/>) and Silva 132 database with 0.7 confidence (Quast et al., 2013). A subsampling procedure (single_rarefaction.py) was conducted at a depth of 29 150 sequences per sample for fungal diversity calculation. filter_taxa_from_out_table.py was performed to retain the sequences of specify fungal groups.

2.4 Statistical analyses

The species richness and Faith's PD for communities were calculated as α diversity. Variations of the dominant fungal phyla, orders, families and α diversity indices between study sites were compared using one-way ANOVA, and the post hoc test was performed using TukeyHSD method. Principal Coordinate Analysis (PCoA) of the fungal Bray–Curtis distance and unweighted UniFrac distance was adopted to

reduce dimensionality and to summarize the variations of fungal structure between the different sampling sites. We assessed whether fungal community structure differed according to the sampling sites by using Permutation Multivariate Analysis of Variance (PERMANOVA) with 9999 permutations (Anderson et al., 2006). In order to reflect true differentiation in fungal community structure rather than differences in variation between sites, Permutation Analyses of Multivariate Dispersion (PERMDISP) was performed to test the dispersion of the fungal communities using betadisper function (Oksanen et al., 2007).

Bray–Curtis distance and unweighted UniFrac distance of soil fungi were used in the distance-based redundancy analysis (dbRDA) to estimate the effect of environmental factors on soil fungal community. The vif.cca function was performed to diagnose the collinearity among different environmental factors. Soil C/N, sand and TC had strong collinearity with other soil parameters (vif value > 10), and those three factors were removed in the subsequent analysis. Variance partitioning analysis (VPA) was performed to test the relative contributions of soil, climate and plant to changes of soil fungal community structure by the varpart function, and the significance of the each fraction was tested using the anova function. Explanatory variables describing samples were grouped in the following partitions: 1) soil (pH, TN, silt and moisture); 2) plant (PSA, PC, AGB, BGB and PSR) and 3) climate (MAT and MAP). The dbRDA was further conducted to examine the contribution of each environmental variable to soil fungal community. The best fitting statistical model was selected using ordistep function in vegan (Blanchet et al., 2008). The best fitting statistical model contained environmental variables that had the lowest AIC value, and p -values were obtained under 9999 permutations. Furthermore, linear and quadratic regression analyses were performed to see whether soil fungal diversity indices were coupled with aboveground plant diversity. Mantel test was performed to examine the correlation between plant composition and soil fungal composition, and partial Mantel test was performed to tease apart the impacts of soil properties and climate from the impact of plant composition in driving fungal composition across sites. All these analyses were performed in R 3.4.4 (R Core Team, 2013).

3 Results

3.1 Climate, soil and vegetation properties

The climate varied widely from west to east in the temperate steppe (Table 1). The MAT ranged from 2.25°C to 0.56°C , and it declined from site A to F, while the MAP values increased from west to east. The soil moisture content varied widely in the following order: $\text{E} > \text{F} > \text{A} > \text{B} > \text{C} > \text{D}$. The soil pH varied significantly in the topsoil ($P < 0.01$), while the soil texture varied insignificantly between different sites (silt: $P > 0.05$; sand: $P > 0.05$). Soil TN varied from 0.91 mg g^{-1} to

2.91 mg g⁻¹, and it increased significantly from western to eastern sampling sites ($P < 0.01$). Similarly, soil TC and C/N increased with increasing longitude ($P < 0.01$) in the temperate steppe. A total of 76 plant species were recorded across the study sites, and they were derived from 25 families. The top three plant families with the highest relative abundance were Poaceae (33.77%), Cyperaceae (27.57%) and Asteraceae (13.58%). PSR, PSA, PC, AGB and BGB of site F were significantly higher than that of other sites ($P < 0.05$ in all cases), while LB varied slightly between sampling sites ($P > 0.05$).

3.2 Fungal composition and diversity

All the rarefaction curves were asymptotic lines, suggesting the sequencing effort is sufficient to represent the soil fungal community (Fig. S1). A total of 613 897 eukaryotic sequences were obtained from the examined soil samples, in which 606 480 (98.79%) fungal sequences were kept in the sequential analyses. On average, 33 693 fungal sequences were found in each sample. There were 186 fungal phylotypes in the complete data set, with an average of 102 fungal phylotypes per sample. At the phylum level, fungal communities were predominately composed of species within the Ascomycota (84.36%), Basidiomycota (7.22%) and Mucoromycota (6.44%) phyla (Fig. 2A). One-way ANOVA tests suggested

that the relative abundance of Zoopagomycota varied significantly between the study sites ($F = 5.30$, $P < 0.01$, Table S1), and the TukeyHSD test suggested that the relative abundance of Zoopagomycota in E was significantly higher than that in C, A and F (Table S2). About 20.38% of the total sequences were identified to the order level (Fig. 2B), and the top 10 orders were Hypocreales (6.26%), Mortierellales (4.35%), Agaricales (3.14%), Archaeorhizomycetales (1.94%), Chaetothyriales (1.50%), Pleosporales (1.32%), Pezizales (1.32%), Capnodiales (0.27%), Tremellales (0.11%) and Glomerellales (0.11%). One-way ANOVA test suggested that the relative abundance of Archaeorhizomycetales ($F = 3.32$, $P < 0.05$, Table S1) and Pezizales ($F = 4.27$, $P < 0.05$, Table S1) varied significantly between study sites. The TukeyHSD test indicated that the relative abundance of Archaeorhizomycetales in site F was significantly higher than that in A and B (Table S2). Only 8.81% of the total sequences were identified to the family level, and the relative abundance of Archaeorhizomycetaceae in site F was significantly higher than that in A and B ($P < 0.05$, Fig. 2C, Table S2).

One-way ANOVA tests showed that soil fungal α diversity varied significantly between study sites (species richness: $F = 5.52$, $P < 0.01$; Faith's PD: $F = 6.33$, $P < 0.01$, Table S3). The TukeyHSD test suggested that the values of fungal species richness (Fig. 3A) and Faith's PD (Fig. 3B) of site D were

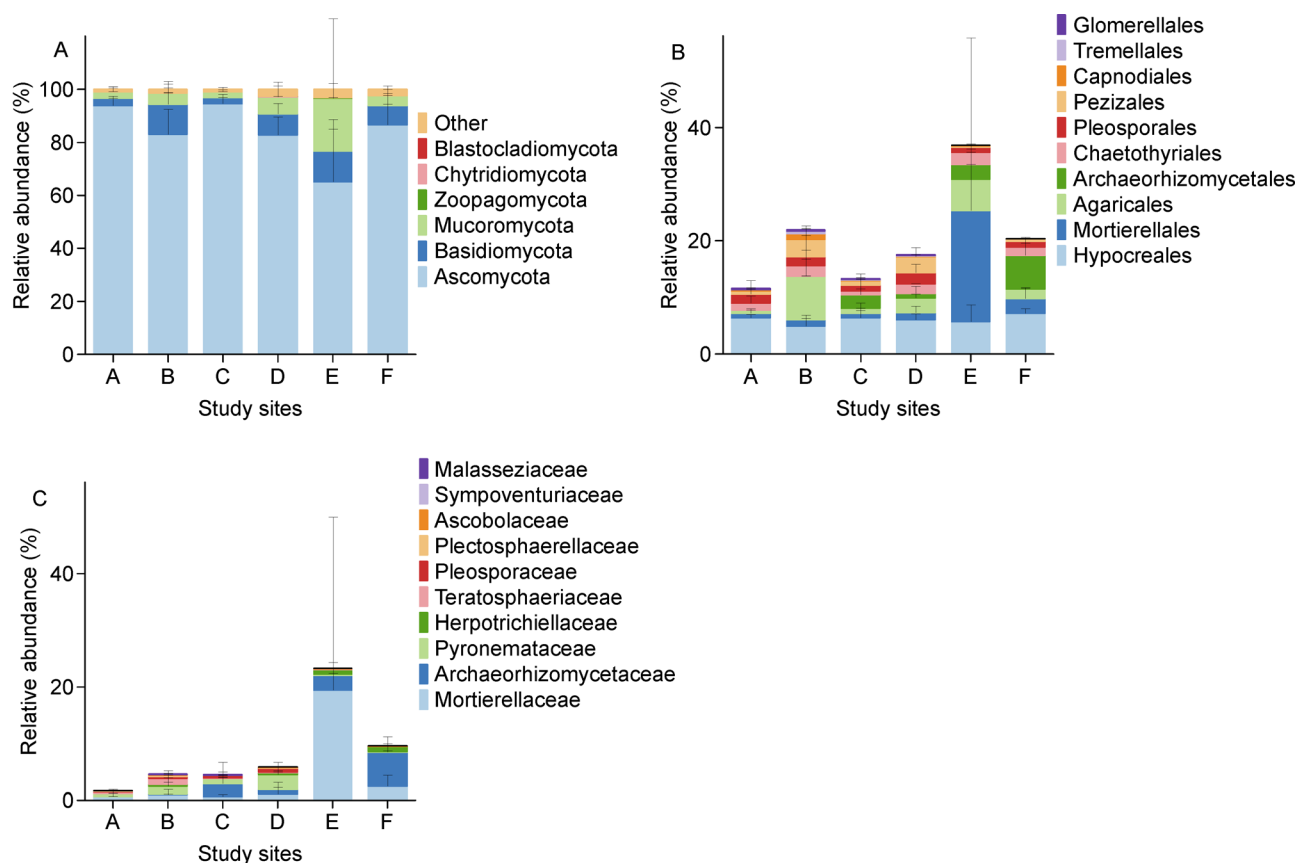


Fig. 2 Soil fungal composition across the sampling sites. Relative abundance of dominant phyla (A), orders (B) and families (C) of fungal community. The relative abundance values were presented as mean \pm SD calculated from biological triplicates.

significantly higher than that of A and C ($P < 0.05$ in all cases). At the phylum level, species richness and Faith's PD values of Ascomycota and Basidiomycota were the highest in site D (Table 2). However, species richness and Faith's PD of Mucoromycota varied insignificantly between sampling sites (Table 2).

β diversity estimation indicated fungal communities were distinct across the sampling sites (PERMANOVA: $P < 0.05$ in all cases, Fig. 3C, D; Table 3). Furthermore, there was no difference in community heterogeneity across the study sites (PERMDISP: $P > 0.1$ in all cases). Things were similar at the phylum level that the dispersion of Ascomycota, Basidiomycota and Mucoromycota varied slightly between sites (Table 3).

3.3 The relationship between plant diversity and soil fungal diversity

The VPA analyses showed that the independent effects of soil (12.96%, $P < 0.01$) and plant (8.7%, $P < 0.05$) accounted for

21.66% of the total variation in fungal Bray–Curtis distance, and 17.60% of the total variation was explained by the shared effect of plant and climate (Fig. 4A). The independent effects of plant (11.53%, $P < 0.05$) contributed to most of the variation explained in soil fungal unweighted UniFrac distance, and the shared effect of plant and climate accounted for 11.44% of the total variance (Fig. 4B). The dbRDA analyses based on the Bray–Curtis distance and unweighted UniFrac distance were further performed to measure the relative contribution of each environmental factor impacting fungal composition. After removing the collinear environmental factors, environmental factors contributed 56.2% of the total variance in fungal Bray–Curtis distance, while 29.76% of the total variance in fungal unweighted UniFrac distance. PSR (19.05%), soil pH (9.83%) and TN (14.07%) were the most significant parameters influencing the variation of the fungal composition based on Bray–Curtis distance (Fig. 4C). PSR (19.78%) hold the largest explanatory power of the variation of fungal unweighted UniFrac distance, followed by soil pH (9.97%, Fig. 4D).

Linear and quadratic regressions showed that PSR coupled with fungal α diversity significantly (Fig. 5). For

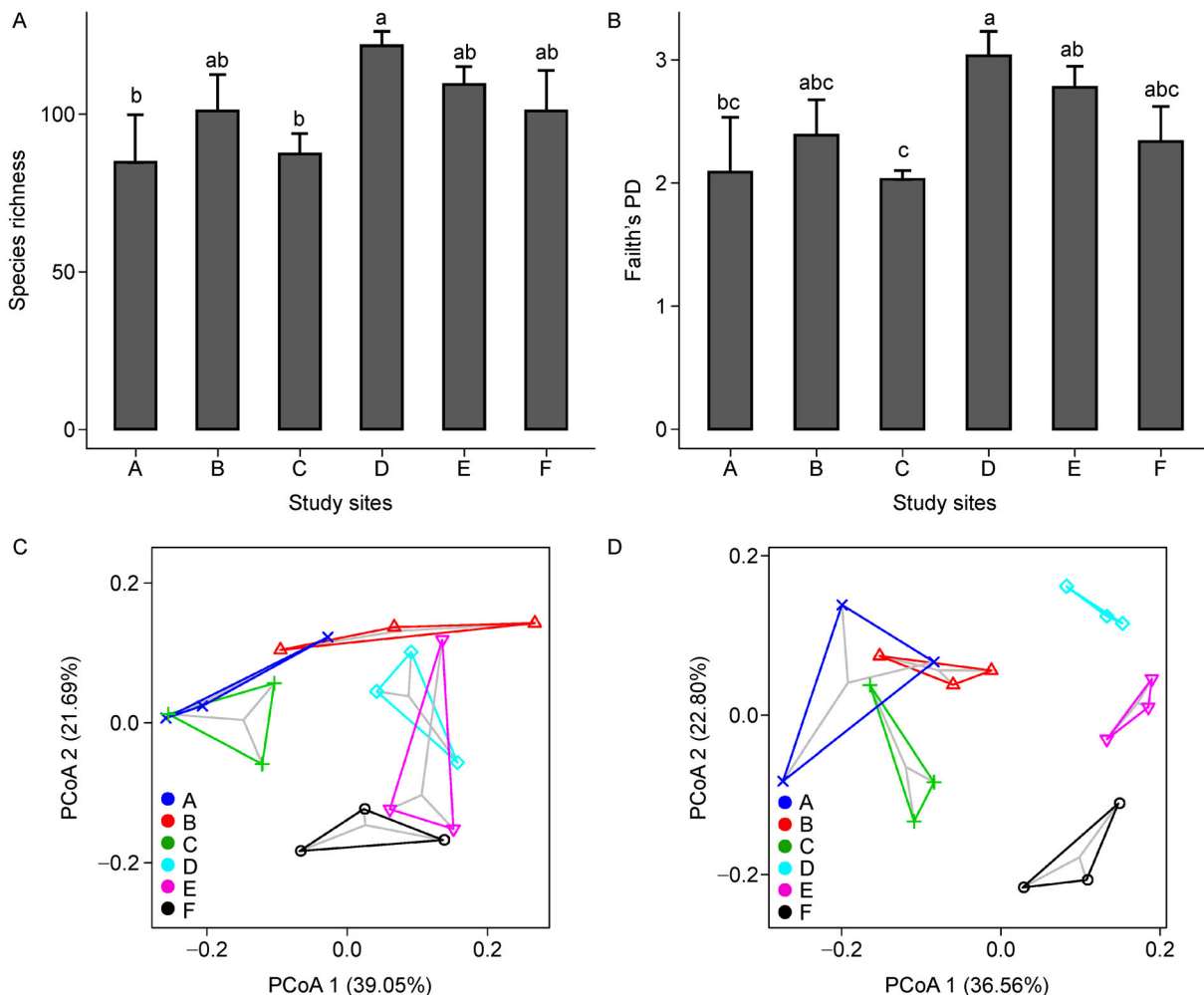


Fig. 3 The variances of soil fungal α diversity and β diversity in the temperate steppe. Species richness (A) and Faith's PD (B) were calculated as α diversity. We used Principal Coordinate Analysis (PCoA) of the fungal Bray–Curtis distance (C) and unweighted UniFrac distance (D) to reduce dimensionality and to summarize the β diversity.

Table 2 Species richness and Faith's PD of the dominant phyla in the temperate steppe.

Phylum	Index	A	B	C	D	E	F
Ascomycota	Faith's PD	1.07±0.12ab	1.16±0.14abc	1.04±0.08a	1.36±0.10c	1.31±0.06bc	1.20±0.09abc
	Species richness	48.67±6.43a	55.33±6.66ab	48.33±2.89a	64.00±4.00b	58.33±2.08ab	56.00±5.00ab
Basidiomycota	Faith's PD	0.54±0.13ab	0.62±0.06ab	0.50±0.06a	0.81±0.04b	0.68±0.12ab	0.49±0.15a
	Species richness	17.00±2.65a	22.00±2.00ab	19.67±2.52ab	27.00±1.73b	22.67±3.21ab	20.33±4.16ab
Mucoromycota	Faith's PD	0.16±0.12a	0.25±0.12a	0.23±0.10a	0.30±0.13a	0.31±0.03a	0.33±0.04a
	Species richness	3.33±1.53a	5.00±0.00a	4.00±1.00a	6.67±1.53a	4.67±0.58a	6.33±2.52a

Notes: The diversity values (mean±SD) with different letters were significantly different from one another ($P < 0.05$).

Table 3 Summary of PERMANOVA and PERMDISP analyses for soil fungal community.

	PERMANOVA				PERMDISP			
	unweighted UniFrac distance		Bray–Curtis distance		unweighted UniFrac distance		Bray–Curtis distance	
	Pseudo-F	<i>P</i>	Pseudo-F	<i>P</i>	Pseudo-F	<i>P</i>	Pseudo-F	<i>P</i>
The whole fungi	2.39	0.00**	2.55	0.00**	1.29	0.33	0.27	0.92
Ascomycota	1.96	0.00**	2.73	0.00**	0.29	0.91	0.18	0.97
Basidiomycota	3.12	0.00**	2.37	0.00**	0.44	0.81	0.45	0.81
Mucoromycota	3.75	0.14*	1.62	0.02*	0.73	0.61	0.42	0.83

Note: **: $P < 0.01$, *: $P < 0.05$.

example, the fungal Faith's PD showed a hump-backed pattern with increasing PSR ($R^2 = 0.47$, $P < 0.01$; Fig. 5A) and the species richness of fungal community also showed a hump-backed pattern with increasing PSR ($R^2 = 0.55$, $P < 0.01$; Fig. 5B). At the phylum level, both of the Faith's PD and species richness of Ascomycota (Fig. 5C, D) exhibited a hump-backed pattern with PSR. The Faith's PD of Basidiomycota increased and then decreased with increasing PSR (Fig. 5E), while the species richness of Basidiomycota showed no significant relationship with PSR (Fig. 5F). Both of the Faith's PD and species richness of Mucoromycota showed no significant pattern with PSR (Fig. 5G, H).

Mantel test indicated that plant β diversity (compositional distance between sites) was substantially correlated with fungal β diversity. For example, plant Bray–Curtis distance was significantly related with fungal Bray–Curtis distance ($r = 0.35$, $P < 0.01$, Table 4), and plant unweighted UniFrac distance was significantly correlated with fungal unweighted UniFrac distance ($r = 0.55$, $P < 0.01$, Table 4). At the phylum level, Ascomycota, Basidiomycota and Mucoromycota β diversity were also significantly related with plant β diversity ($P < 0.05$ in all cases, Table 4). Partial Mantel test was performed to tease apart the impacts of soil properties and climate from the impact of plant β diversity in driving fungal β diversity across sites (Table 4). Partial Mantel test indicated that plant β diversity was significantly correlated with the whole fungal β diversity when the effect of soil and climate

were held constant ($P < 0.05$ in all cases). The significant correlation between plant β diversity and soil fungal β diversity did extend to Basidiomycota when the effect of soil and climate were controlled respectively ($P < 0.05$). Consistently, plant β diversity was also significantly correlated with Ascomycota β diversity when the climate was controlled ($P < 0.05$). When the effect of soil was held constant, plant unweighted UniFrac distance showed no significant correlation with the Ascomycota unweighted UniFrac distance ($P > 0.05$), while plant Bray–Curtis distance was significantly correlated with Ascomycota Bray–Curtis distance ($P < 0.05$). Only the plant unweighted UniFrac distance was significantly correlated with the Mucoromycota unweighted UniFrac distance ($P < 0.05$) when the effect of soil was held constant, and only the plant Bray–Curtis distance was significantly correlated with Mucoromycota Bray–Curtis distance when the climate was held constant.

Moreover, regression analyses suggested that soil fungal β diversity increased linearly with increasing plant β diversity. For example, soil fungal Bray–Curtis distance increased with increasing plant Bray–Curtis distance (Fig. 6A), and soil fungal unweighted UniFrac distance increased with increasing plant unweighted UniFrac distance (Fig. 6B). This increase pattern did extend to the phylum level that unweighted UniFrac distance and Bray–Curtis distance of Ascomycota, Basidiomycota and Mucoromycota (Fig. 6C–H) increased with that of plant community.

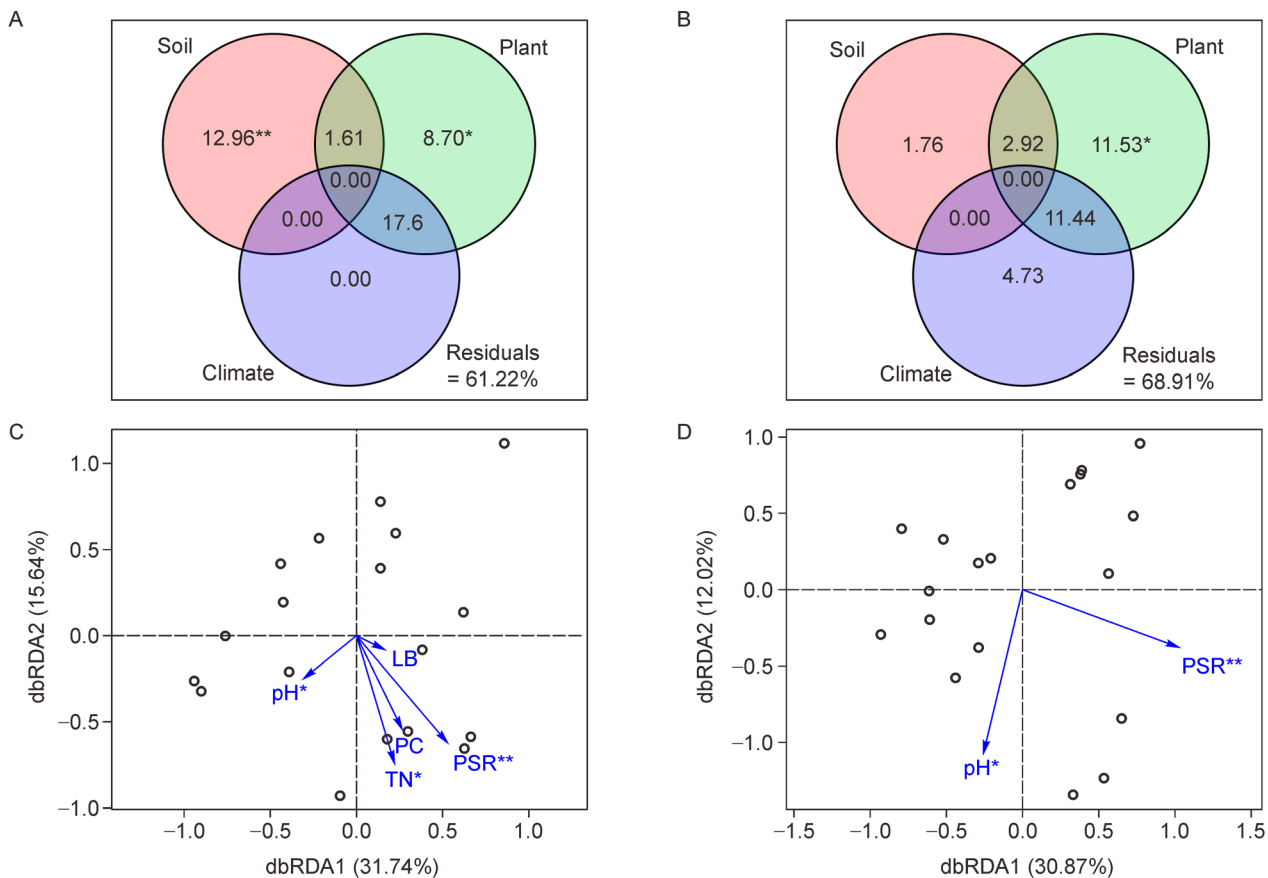


Fig. 4 Effects of environmental factors on soil fungal community structure. (A) Bray–Curtis distance based variance partitioning analysis; (B) unweighted UniFrac distance based variance partitioning analysis; (C) Bray–Curtis distance based redundancy analysis; (D) unweighted UniFrac distance based redundancy analysis. Note: Values in variance partitioning plots indicate the proportion of explained variation. PSR: plant species richness; PC: plant coverage; LB: plant litter biomass; TN: total nitrogen. *: $P < 0.05$; **: $P < 0.01$.

4 Discussion

Our study demonstrated that although the relative abundance of dominant fungal phyla across the study sites was similar, their composition and diversity differed substantially. Variations in fungal diversity between sites were strongly related to the variations in plant diversity, and plant species richness had a strong association with fungal diversity. This indicated that aboveground plant diversity could predict soil fungal diversity in the temperate steppe.

4.1 Soil fungal composition and diversity across the temperate steppe

Sequences derived from Ascomycota, Basidiomycota and Mucoromycota occupied the largest proportions (Fig. 2). Ascomycota and Basidiomycota were found in grassland soils from other regions, such as in the Xilingol region of Inner Mongolia, China (Li et al., 2017), the US (Ochoa-Hueso et al., 2018), the Alps (Pellissier et al., 2014), the Tibetan Plateau (Zhang et al., 2013), even across grasslands worldwide

(Prober et al., 2015). Furthermore, Ascomycota and Basidiomycota were also found to dominate the fungal community in forest soil (He et al., 2005; Curlevski et al., 2010). These results indicate that these groups of fungi can survive in diverse ecosystems. Additionally, the low relative abundance of Zoopagomycota, Chytridiomycota and Blastocladiomycota could be owing to the bias related to the fungi-specific primers, given that 817F/1196R primer pair was designed to amplify the rDNA from the major fungal phyla (Borneman and Hartin, 2000). At the order level, results from one-way ANOVA analysis showed that the relative abundance of Pezizales varied significantly between study sites (Fig. 2). The higher relative abundance of Pezizales in site B could be related to the soil moisture content, since the Pezizales are known as prevalent mutualistic symbionts in various ecosystems, particularly in habitats subjected to drought (Smith et al., 2007). The soil moisture content was likewise the lowest in site B (Table 1). Another study reported that water addition reduced the relative abundance of drought-tolerant Pezizales in a 2-year field experiment (She et al., 2018). In addition, both the relative abundance of order Archaeorhizomycetales and family Archaeorhizomycetaceae were higher in site F than

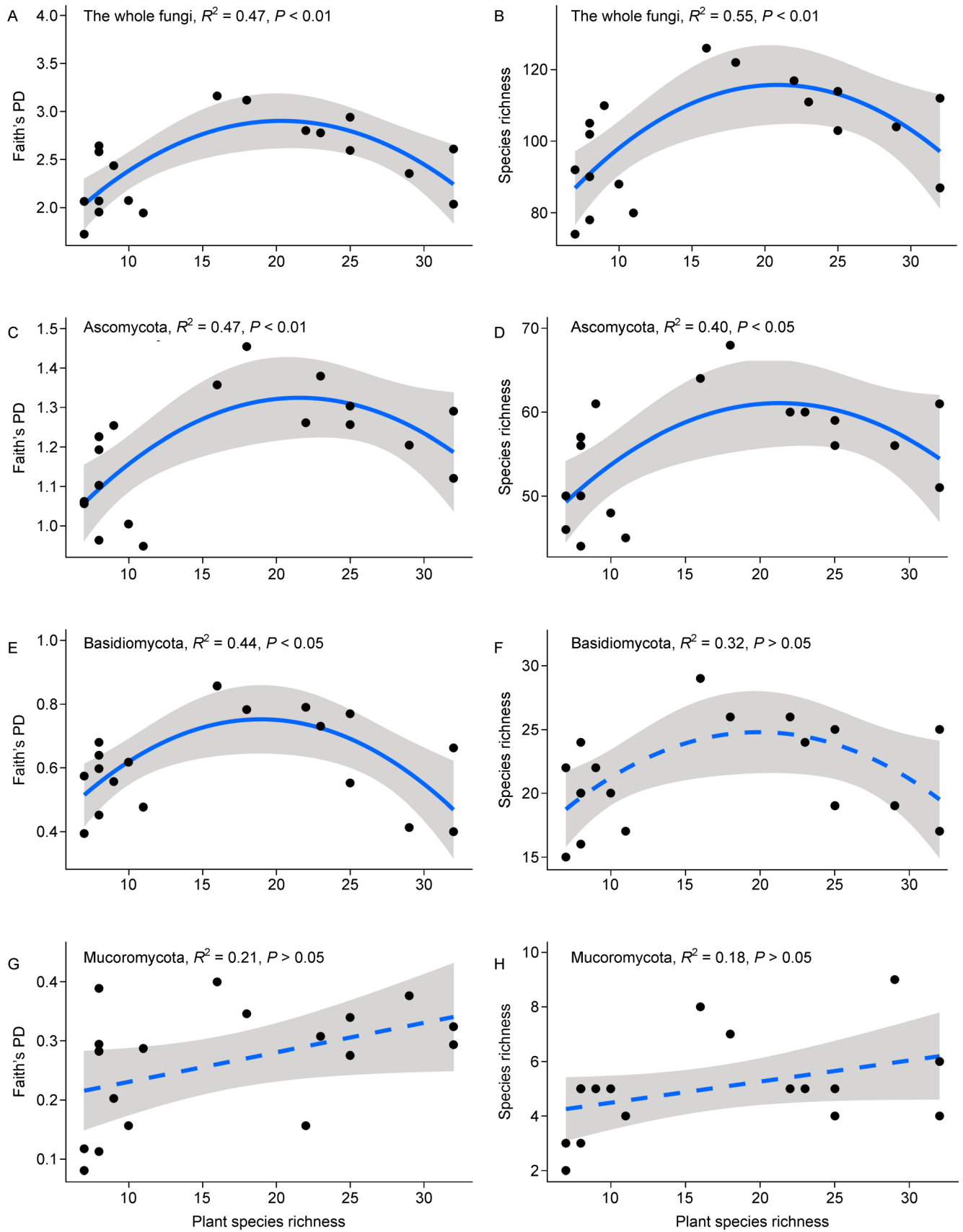


Fig. 5 Coupling of plant species richness and fungal α diversity indices across the Hulunbeir, China. (A) Faith's PD of the whole fungi, (B) species richness of the whole fungi, (C) Faith's PD of Ascomycota, (D) species richness of Ascomycota, (E) Faith's PD of Basidiomycota, (F) species richness of Basidiomycota, (G) Faith's PD of Mucoromycota, (H) species richness of Mucoromycota.

Table 4 Mantel test and partial Mantel test analyses for the association between plant composition and fungal composition.

	Control for	Anweighted UniFrac distance	Bray–Curtis distance
Mantel test			
The whole fungi		0.55**	0.35**
Ascomycota		0.36**	0.27**
Basidiomycota		0.37**	0.41**
Mucoromycota		0.23*	0.24*
Partial Mantel test			
The whole fungi	Soil	0.47**	0.20*
	Climate	0.47**	0.33**
Ascomycota	Soil	0.12	0.22*
	Climate	0.25**	0.24**
Basidiomycota	Soil	0.22*	0.35**
	Climate	0.30**	0.37**
Mucoromycota	Soil	0.18*	–0.16
	Climate	0.09	0.27*

Note: **: $P < 0.01$, *: $P < 0.05$.

that in A and B (Fig. 2). Archaeorhizomycetales was introduced to play as slow-growing ectomycorrhizal fungi associated with plant roots (Pec et al., 2017). The highest relative abundance of Archaeorhizomycetales in site F was consistent with the highest plant diversity and belowground biomass in this study (Table 1).

We observed that both fungal α and β diversity varied significantly between the study sites (Fig. 3; Tables 2, 3). Previous studies found that soil fungal diversity varied significantly at the regional scale. For example, Bahram et al. (2012) reported that ectomycorrhizal fungal diversity varied sharply along an altitudinal gradient in Iran. Another study sampled 213 sites across the Western Swiss Alps, and it also found that soil fungal diversity varied significantly along elevation gradients (Pellissier et al., 2014). These studies in concert indicate that soil fungi were not randomly distributed, and environmental filtering may be the plausible mechanism structuring soil fungal community. Previous studies have reported that the shifts in soil fungi could be associated with soil physicochemical properties (Waldrop et al., 2006; Pellissier et al., 2014), vegetation (Bahram et al., 2012) and climate (Tedersoo et al., 2012). Our sampling sites in the temperate steppe could represent an environmental gradient to some extent, since the temperature, soil properties and plant traits changed sharply from west to east (Table 1). In addition, we found that environmental factors, such as MAT, MAP, soil TC, TN, moisture, PSR and PC exhibited noticeable variations between study sites (Table 1). Therefore, we assumed that the diversity pattern of fungal community in the temperate steppe was also related to environmental factors.

4.2 Relationships between soil fungal diversity and plant diversity

In this study, plant contributed to the variation of soil fungal community significantly according to the VPA analysis, suggesting a close relationship between aboveground plant and soil fungi. Moreover, we found that PSR occupied the largest explanatory power for fungal community composition in the temperate steppe. A comparable study in temperate grasslands also found that PSR was the best predictor of the whole microbial biomass and activity (Liu et al., 2008). The close relationship between plant diversity and soil fungal community has been reported in central European mesophilic grassland (Eisenhauer et al., 2010), Dutch grassland (Bezeemer et al., 2006), Japanese grassland (Hossain and Sugiyama, 2011) and American tallgrass prairie preserve (Eom et al., 2000). The productivity-diversity hypothesis suggests that the significant impact of plant diversity on soil microbes could be owing to the changes in plant detritus production (Spehn et al., 2000; Zak et al., 2003). However, our results do not support the productivity-diversity hypothesis, because there was no significant correlation between plant productivity (AGB, BGB and LB) and fungal diversity. The redundancy hypothesis of biodiversity suggests only the functional groups are needed to maintain ecosystem functioning (Scherer-Lorenzen, 2008). Grasses, sedges, legumes and forbs groups are regarded as key plant functional groups for soil microbial communities. The four functional groups are present in all the six sites, which indicates that the significant variation in fungal diversity in Hulunbeir is not due to the presence of particular plant functional groups. The singular

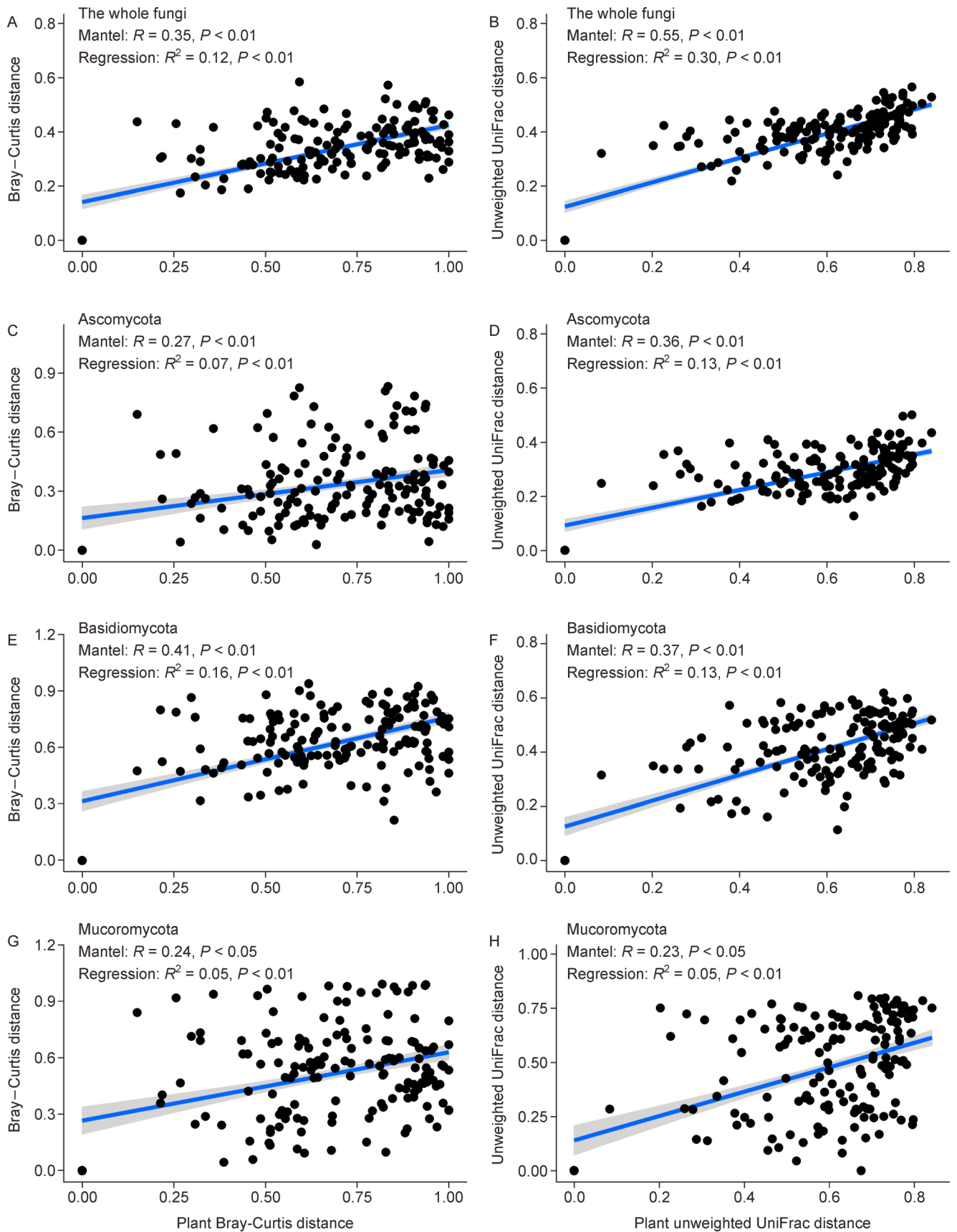


Fig. 6 Coupling of plant β diversity and fungal β diversity in the temperate steppe. (A) regression between Bray–Curtis distance of plant and the whole fungi, (B) regression between unweighted UniFrac distance of plant and the whole fungi, (C) regression between Bray–Curtis distance of plant and Ascomycota, (D) regression between unweighted UniFrac distance of plant and Ascomycota, (E) regression between Bray–Curtis distance of plant and Basidiomycota, (F) regression between unweighted UniFrac distance of plant and Basidiomycota, (G) regression between Bray–Curtis distance of plant and Mucoromycota, (H) regression between unweighted UniFrac distance of plant and Mucoromycota.

hypothesis of biodiversity suggests that each species contributes to ecosystem functioning (Hooper et al., 2000; Eisenhauer et al., 2010). The partial Mantel tests support the singular hypothesis of biodiversity, because the significant effect of plant composition on soil fungal composition when the effects of soil and climate are held constant. In addition, our findings show a great shift in the dominant plant species (Table 1), with *Cleistogenes squarrosa* dominating in western sites (A, B, C and D), while some forbs in eastern sites (E and F).

Regression analyses showed that α and β diversity of soil fungi were closely related to that of plant. The hump-backed pattern in soil fungal α diversity with increasing PSR, which was different from the results in other studies. Tedersoo et al. (2016) reported an increase in fungal species richness along with greater PSR in a clear-cut boreal forest. Another N addition experiment in a semiarid grassland found that soil fungal species richness varied insignificantly with increasing PSR (Chen et al., 2018). Lange et al. (2014) manipulated a gradient in sown plant species next to a river in Germany, and they reported that there was no significant trend between soil fungal biomass and PSR. Those increased patterns and a lack of a significant pattern differed with the hump-backed pattern between fungal α diversity and PSR in our study. It is worth noting that we sampled across a natural temperate steppe, while the studies described above resulted from human-impacted ecosystems. The effect of plants on soil fungi may be masked by mankind disturbance in the human-impacted ecosystems (e.g. plow, irrigation and fertilization). Soil fungal species richness in Hulunbeir decreased after the 20-species level of plants, while another study sampled from Tibetan Plateau suggested that soil fungal species richness still increased in parallel with PSR at the 20-species level of plants (Yang et al., 2017). In general, the peak of species number shifts toward a higher disturbance level as the resource increases (Kondoh, 2001). Thus, we deduced that those distinct trends were probably owing to the difference in soil nutrients. Accordingly, the maximum soil TN in the Tibetan Plateau (14.1 mg g^{-1}) was far higher than that in Hulunbeir (2.91 mg g^{-1}), and the dbRDA analysis demonstrated the significant effect of soil TN on soil fungal composition in the temperate steppe. Moreover, the middle site D lies in the transition area from the typical steppe to meadow steppe and the soil microbial distribution pattern might shift in the transition area (Li et al., 2016). The close relationship between plant β diversity and fungal β diversity was consistent with many previous studies from different grassland ecosystems (Prober et al., 2015; Yang et al., 2017; Chen et al., 2017). The increased pattern of fungal β diversity with increasing plant β diversity indicated that the sites were more distinct in the composition of their plant communities also harbored more distinct soil fungal communities. According to the Mantel analysis of the pairwise Bray–Curtis distances for soil fungal and plant communities, the correlations between plant β diversity and fungal β diversity in the Tibetan Plateau ($R = 0.46, P < 0.001$) (Yang et al., 2017) and North America ($R =$

$0.45, P < 0.001$) (Prober et al., 2015) were stronger than that in Hulunbeir ($R = 0.35, P < 0.01$). The reason for the difference might be that the climate, dominant species and soil properties in the Tibetan Plateau and North America are obviously different from that in Hulunbeir. Another comparable research in the whole northern China suggested a weaker correlation between plant β diversity and fungal β diversity (Mantel: $R = 0.29, P < 0.001$) (Chen et al., 2017). The sampling scale in the whole northern China (approximately 4000 km) was larger than that in this study (approximately 360 km), and many previous studies suggested that environmental variables have strong effects at small spatial scales (Kou et al., 2017; Yao et al., 2017).

Soil pH has been found to play a key role in constructing fungal community in various studies (Pellissier et al., 2014; Liu et al., 2018; Ren et al., 2018), and it significantly contributed to the variation of soil fungal community in the temperate steppe. Although climate parameters were removed due to the high collinearity they shared with other environmental factors, the effects of MAT and MAP on soil fungal community and plant community should not be ignored. Previous studies have shown the importance of temperature and precipitation influencing fungal metabolism (Hawkes et al., 2011; Frey et al., 2013; Chen et al., 2017). The regression analysis suggested that PSR and soil TN content decreased sharply with MAT ($P < 0.01$ in both cases), whereas they increased significantly with MAP ($P < 0.05$ in both cases). Those strong correlations indicate that climatic factors could play a potential role in soil fungal community construction.

5 Conclusions

The results that soil fungal α and β diversity varied sharply between sampling sites supported the first hypothesis, and it suggests that soil fungi are distributed determinately in the temperate steppe of Hulunbeir, northeastern China. Moreover, the large contribution of PSR to soil fungal community and the close relationships between plant diversity and soil fungal diversity demonstrated that plant diversity could partially predict the unseen soil fungal diversity in the temperate steppe. The hump-backed trend of fungal α diversity with increasing plant diversity indicates the turnover of fungal community composition at the botanically diverse sites. The stronger correlation between plant β diversity and fungal β diversity in Hulunbeir than that in the whole northern China indicates that the relationship between plant and soil fungi is scale-dependent. Future challenges are to explore the internal linkages between plant and soil fungal communities and to unravel the functions of specific fungi in different vegetation conditions.

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Disclosure statement

The authors declare no conflict of interest.

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