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The local environment regulates biogeographic patterns of soil fungal communities on the Loess Plateau

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ABSTRACT

Soil fungi play critical ecological roles in terrestrial ecosystem function, soil formation and element cycles. However, the biogeography of soil fungi in the forest ecosystem is poorly understand, especially in arid or semiarid areas. Here, a regional scale study in an arid area was conducted on the Loess Plateau to illustrate the geographic distributions of soil fungi and their driving forces. The results showed that Ascomycota, Basidiomycota and Zygomycota were the most dominant phyla in 24 sample sites. Fungal Shannon diversity and OTUs richness were significantly correlated with mean annual precipitation (MAP), total soil nitrogen (TN), ammonium nitrogen (NH4N) and soil organic carbon (SOC) levels. The decay-curve analysis demonstrated that soil fungal Bray-Cutis dissimilarities were significantly regressed with geographic distance, revealing that the soil fungal community structure was affected by historical factors. Variation partitioning analysis revealed that the soil properties (15%) contributed more to the fungal community variations than geographic distance (9%). Additionally, there were many predictors of soil fungi geography that were not detected. These results suggest that the fungal geography on the Loess Plateau is mainly regulated by soil properties or other unmeasured variables. Such findings advance our understanding of fungal diversity patterns on the Loess Plateau, which will help us better understand the functions and services in underground ecosystems in arid areas.

1. Introduction

Healthy soil is fundamental for a wide range of global ecosystem activities. The research into the biogeography of soil fungi and its driving factors has raised fundamental questions about the roles of soil fungi in regulating soil health and the services of soil ecosystems (Ding et al., 2016; Fujita et al., 2001; Oksanen et al., 2013). Plants and animals have clear distributions with varied latitudes or longitudes throughout the world, and their interactions with environmental factors are well understood (Capinha et al., 2017; Xiong et al., 2012). Soil microbes, in terms of their complex diversities and compositions, are not well understand. Recently, an increasing number of researchers began to study soil microbes at different scales to explore the link between their biogeography and their specific functions (Gumiere et al., 2016; Queloz et al., 2011; Rousk et al., 2010; Tedersoo et al., 2014). A deeper understanding of the distribution of soil microorganisms will help us to understand the mechanisms of nutrient cycling and ecological processes mediated by microbes. However, the limited methods for studying soil microbes make these research studies difficult; therefore, their compositions and roles in biogeochemistry are not well understood. Although substantial progress has been made in pyrosequencing, deeper studies in the biogeography of soil fungi are still limited; therefor, further study on different ecosystems or at the regional scale will help us understand the specific roles of soil microbes and their regulation of global biogeochemistry.

Soil fungi are the most important drivers of the biogeochemical cycles, and they do so via the mineralization of soil organic matter, which significantly influences global climate change (Voříšková and Baldrian, 2013). Soil fungi are sensitive to climate change, and their biogeographic distributions are affected by temperature and precipitation (Tedersoo et al., 2014). In warmer and wetter conditions, soil fungi can decompose more soil organic matter leading to more CO_2 flux into the atmosphere. This increase in decomposition rate also provides more substrates and promotes more abundant and diverse soil fungi. These interactions suggest that soil fungi are greatly influenced by the climate. Some researchers have reported that soil fungi varied greatly depending on the climate, plant community and edaphic factors (Tedersoo et al., 2014). For example, a global scale study using 454

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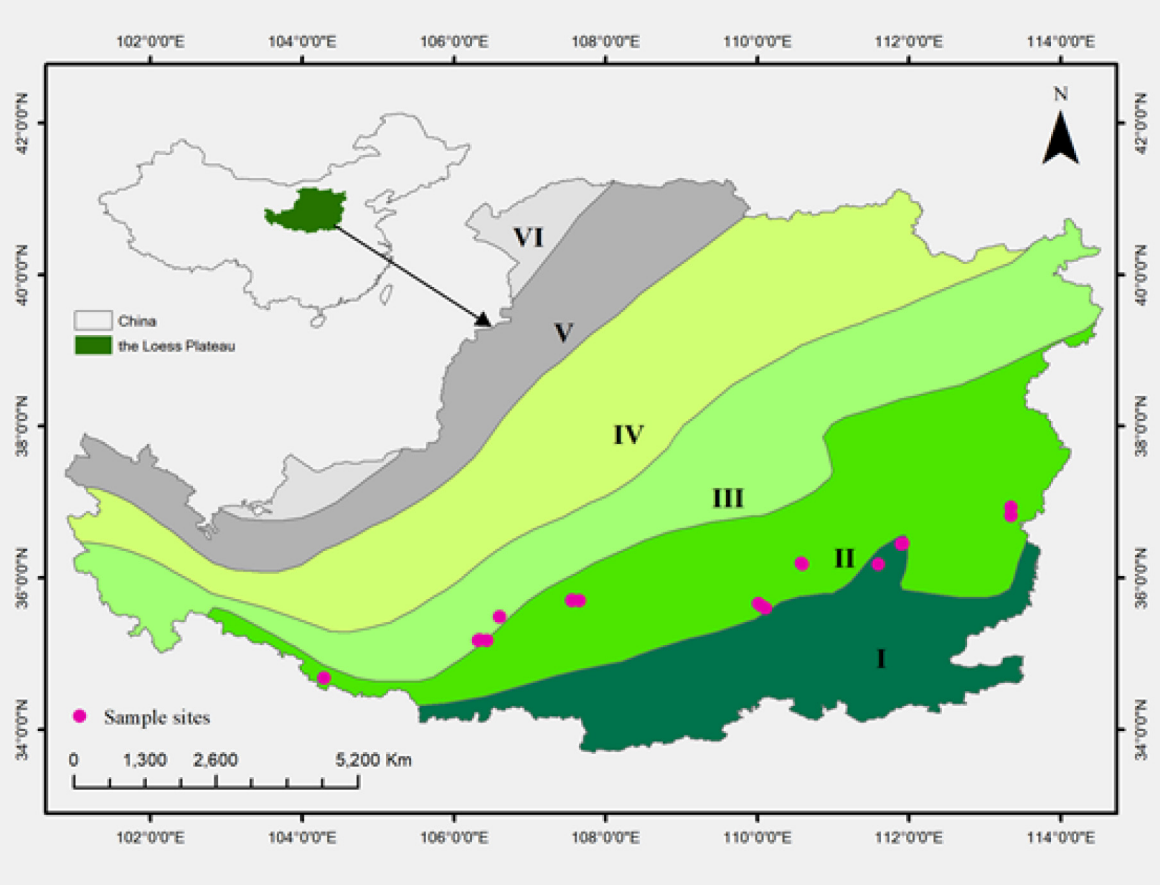

Fig. 1. The sampling site distribution in the Loess Plateau of China. I, broadleaved forest zone; II, forest steppe zone; III, steppe zone; IV, desert-steppe zone; V, steppe-desert zone; VI, desert zone.

pyrosequencing datasets indicated that distance from the equator and mean annual precipitation had the strongest effects on fungi richness, including most fungal taxonomic and functional groups (Tedersoo et al., 2014). Liu et al. (2015) found that the soil organic carbon (SOC) content was significantly related with fungal diversity and several abundant fungal groups, and soil properties had more important effects on soil fungal biogeography in northeast China (Liu et al., 2015). Shi et al., (2014) also found that temperature, latitude and plant diversity mainly determined soil fungal community compositions in the forest ecosystem . These studies concluded that the climate had strong effects on the geographic patterns of soil fungi (Barberán et al., 2015; Tedersoo et al., 2014). However, soil fungi in the temperate and boreal forests had no biogeographic patterns; instead, tree species had significant effects on them (Queloz et al., 2011). These inconsistent results may be explained by the study scales, climate conditions, plant types and other unpredicted factors (Schauer et al., 2010).

Soil microbes are under the influence of various environmental factors, such as the contemporary environment and historical contingencies (i.e., geographic distances), but the specific influence degree depends on the study scales (Ramette and Tiedje, 2007). For instance, large latitudinal gradient related studies reported stronger effects from historical contingencies compared with contemporary environment conditions (Ge et al., 2008; Liu et al., 2019; Schauer et al., 2010). Some reginal scale studies indicated that contemporary factors played more important roles in the biogeography of soil microbes than historical contingencies (Liu et al., 2014; Liu et al., 2015). However, most of the studies that set out to discover possible mechanisms of soil microbial biogeography were focused on soil bacteria rather than soil fungi. A widely accepted concept is that soil pH dominantly shapes the biogeographical distribution of soil bacterial communities (Chu et al., 2010; Fierer and Jackson, 2006; Liu et al., 2014; Nicol et al., 2008; Shi et al., 2014). In contrast, there is no widely accepted conclusion related to soil fungal biogeographic distributions. Although there were some studies that set out to determine the biogeography of soil fungi, these limited studies were conducted at various ecosystems, and few related studies were conducted in arid or semiarid areas.

The loess Plateau represents an area of severe erosion, which has caused many threats to local societies and human survival. The clearance of vegetation disturbed the stability and diversity of the ecosystem. In the 1990s, the Chinese government implemented some projects to recover the local ecosystem and migrate the serious erosion (Chen et al., 2007). Many plant types were planted in non-vegetation areas and on sloping lands (Zhao et al., 2013). After approximately 30 years of revegetation, the plant cover and diversity have improved greatly. The soil quality also has been improved, according to many researchers (Dray et al., 2006; Goslee and Urban, 2007; Jiao et al., 2011; Jin et al., 2014; Li and Shao, 2006). Until now, most studies have focused on soil quality and the above ground ecosystem. However, changes in soil fungi were ignored, especially at the regional scale. The Loess Plateau is approximately 640,000 km^2, spanning 750 km from north to west and 1000 km from east to west. The climate and soil properties vary greatly, which may significantly affect soil microbes, as suggested by previous studies in other areas (Gumiere et al., 2016; Rousk et al., 2010; Tedersoo et al., 2014). The difference is that the whole plateau is located in arid or semiarid areas with an annual mean precipitation of 150–750 mm. As it contains varied climates and a diverse vegetation ecosystem, this is an ideal studying area to explore the biogeography of soil fungi. Therefore, this study was conducted to investigate the biogeography of forest soil fungi and their driving factors on the Loess Plateau. We hypothesized that (1) soil fungal diversity and

communities are mainly influenced by climate factors, such as similar ecosystems (forest soils); and (2) soil pH is the most important effecting factor among soil properties, as suggested by previous studies (Chen et al., 2015; Rousk et al., 2010).

2. Materials and methods

2.1. Sampling sites

The Loess Plateau is an important ecozone in China, and it includes all kinds of vegetation types. In this study, we chose forests as research subjects, which are mainly distributed south of the Loess Plateau with similar latitudes and mean annual precipitation (~500 mm) (Zeng et al., 2019). We choose 24 well-protected forest sites along an east-west transect (~800 km) on the Loess Plateau to determine the influence of anthropogenic disturbances (Fig. 1). The latitudes, longitudes and elevations at each site were recorded through a GPS device. At each site, three plots (10 m × 10 m) were established as replicates, and 20 soil cores at 10 cm soil depth were collected randomly in each plot. All these soil cores were mixed and then roots, stones and animals were removed by hand as one composite sample. After sieving through a 2-mm mesh, each soil sample was divided into 2 parts for different analyses. One part was stored at −80 °C for DNA extraction, while the other part was air-dried for soil property analysis. The soil property values are listed in Table 1.

To obtain the specific mean annual temperature (MAT) and mean annual precipitation (MAP) of each site, we used the Cokring method as described by Ding et al. (2016) (Ding et al., 2016). First, we collected climate data from 154 weather stations on the Loess Plateau from the China Meteorological Data Sharing Service System (http://cdc.nmic.cn/home.do) from 1982 to 2015. Second, we interpolated climate data (MAT and MAP) with different altitudes as a covariant to account for the topographic effects, and then retrieved the interpolated climate data for each sampling site at a spatial resolution of 10 × 10 km^2, using ArcMap 10.0 (Environmental Systems Research Institute, Inc., Redlands, CA, USA).

2.2. Soil physical and chemical analyses

Soil properties were analyzed by the universal methods conducted by other researchers. Specifically, soil organic carbon was measured by the modified Mebius method (Ren et al., 2015). Soil total nitrogen (TN) was digested with concentrated H_2SO_4 and then extracted with 0.02 mol/L sulfuric acid (Thomas et al., 1967). The available nitrogen in the soil, including nitrate nitrogen (NO3N) and the ammonia nitrogen (NH4N) in the soil, were extracted with 1 mol/L KCl, and then they were measured by a Seal AutoAnalyzer3 (Zeng et al., 2017). Soil pH and electric conductivity (EC) were measured with a glass electrode. The total phosphorus (TP) in the soil was digested by H_2SO_4-$HClO_4$, and the digestive solution was determined with the molybdenum blue method (Olsen, 1954). The amount of available P (AVP) in the soil was determined following extraction with 0.5 mol/L $NaHCO_3$, and the P content in the extraction was determined with the molybdenum blue method (Olsen, 1954).

2.3. DNA extraction, amplification and MiSeq sequencing

Soil microbial DNA was extracted from 0.5 g soil, which was stored at −80 °C. The FastDNA® SPIN Kit for Soil (Q-BIOgene, Carlsbad, CA, USA) was used and all the extraction details are listed in the manufacturer's instructions. The extracted DNA was examined on a 1% agarose gel and quantified using a NanoDrop® ND-2000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). For fungi, the ITS1 variable region gene was amplified using primer sets for ITS3 (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4 (5′-TCCTCCGC TTATTGATATGC-3′) (Fujita et al., 2001). Each sample was amplified in triplicate and pooled prior to purification with the QIAEX Gel Extraction Kit (Qiagen, Valencia, CA, USA) and quantification with a Quant-iT dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA). The final PCR pools were created by combining equimolar ratios of amplicons from individual samples. The MiSeq V2 kit was used for 2 × 250-bp paired-end sequencing on a MiSeq machine (Illumina, San Diego, CA, USA) at Shanghai Personal Biotechnology Co., Ltd.

Table 1
Environmental variables (climate and edaphic factors) and fungal diversities at different forest sampling sites on the Loess Plateau (Zeng et al. 2019).

Site	MAP	MAT	Elevation	SOC	TN	TP	pH	EC	NO3N	NH4N	AVP	Shannon	OTUs	PD
WXA	525	9.9	1851	56.82	4.47	0.63	5.92	111.67	10.49	18.08	2.72	6.31	2613	258
WXB	523	9.8	1800	62.89	5.07	0.82	6.22	150.33	23.49	18.72	4.83	7.81	3009	345
WXC	525	9.9	1781	27.81	2.31	0.52	6.11	76.33	8.58	14.14	3.72	7.78	2849	305
GXA	481	11.6	1429	25.05	1.91	0.47	5.75	45	5.78	14.57	4.51	5.34	1963	173
GXB	481	11.6	1424	35.87	2.62	0.53	5.87	75.67	8.97	25.65	3.88	6.32	2581	224
GXC	482	11.5	1263	25.17	1.74	0.64	5.38	52.67	1.63	13.04	1.79	4.89	1846	164
JXA	506	11.4	1419	35.06	3.01	0.53	7.85	232.67	11.16	13.96	6.27	7.12	2592	249
JXB	477	12.4	1490	20.38	1.71	0.32	7.49	214	3.19	11.95	4.29	5.85	2861	280
JXC	505	11.4	1269	29.27	2.25	0.54	7.8	289.33	2.52	15.56	4.74	5.75	1986	190
HLA	546	12	1027	8.92	0.9	0.48	8.08	723	10.62	9.04	1.95	5.71	1790	207
HLB	547	11.9	1264	10.37	1	0.39	6.15	82.17	6.53	15.84	4.88	3.31	841	66
HLC	547	11.8	1745	24.14	2.14	0.49	6.04	81.33	9.44	13.62	3	5.83	1964	219
XFA	506	9.6	1173	13.55	1.44	0.62	8.37	96.17	7.25	9.38	2.25	6.79	1886	198
XFB	508	9.6	1173	12.67	1	0.64	8.47	95.33	1.59	9.47	1.68	5.35	1440	125
XFC	506	9.6	1300	11.01	1.03	0.64	8.29	92.83	2.15	10.96	5.57	4.05	1063	101
PLA	484	9	1724	18.51	1.4	0.21	7.76	198.33	1.65	8.97	1.64	6.06	1554	171
PLB	484	9	1721	20.77	1.39	0.26	7.67	242.67	3.55	7.9	2.04	5.97	3491	318
PLC	484	9	1695	13.67	1.28	0.56	7.88	365.33	5.05	7.13	2.85	5.72	2806	273
DXA	475	7	2040	72.77	5.63	0.74	6.56	131.17	10.21	22.73	11.18	7.87	4895	452
DXB	473	7	2341	35.59	2.73	0.47	6.13	88.17	4.68	16.79	6.02	7.01	3878	360
DXC	473	7	2361	46.59	3.99	0.56	6.8	101.5	6.35	20.45	9.68	7.45	2355	255
ZLA	500	9	2023	29.15	2.59	0.49	7.39	84.67	3.01	10.19	4.03	6.39	2040	203
ZLB	501	9.2	2038	20.58	1.38	0.52	7.83	64.67	1.41	11.49	5.38	6.35	2352	202
ZLC	500	8.9	2036	22.48	1.59	0.51	7.61	69	2.02	12.43	4.95	7.01	3309	318

MAP, mean annual precipitation; MAT, mean annual temperature; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphor; EC, electric conductivity; NO3N, nitrate nitrogen; NH4N, ammonium nitrogen; The same as below.

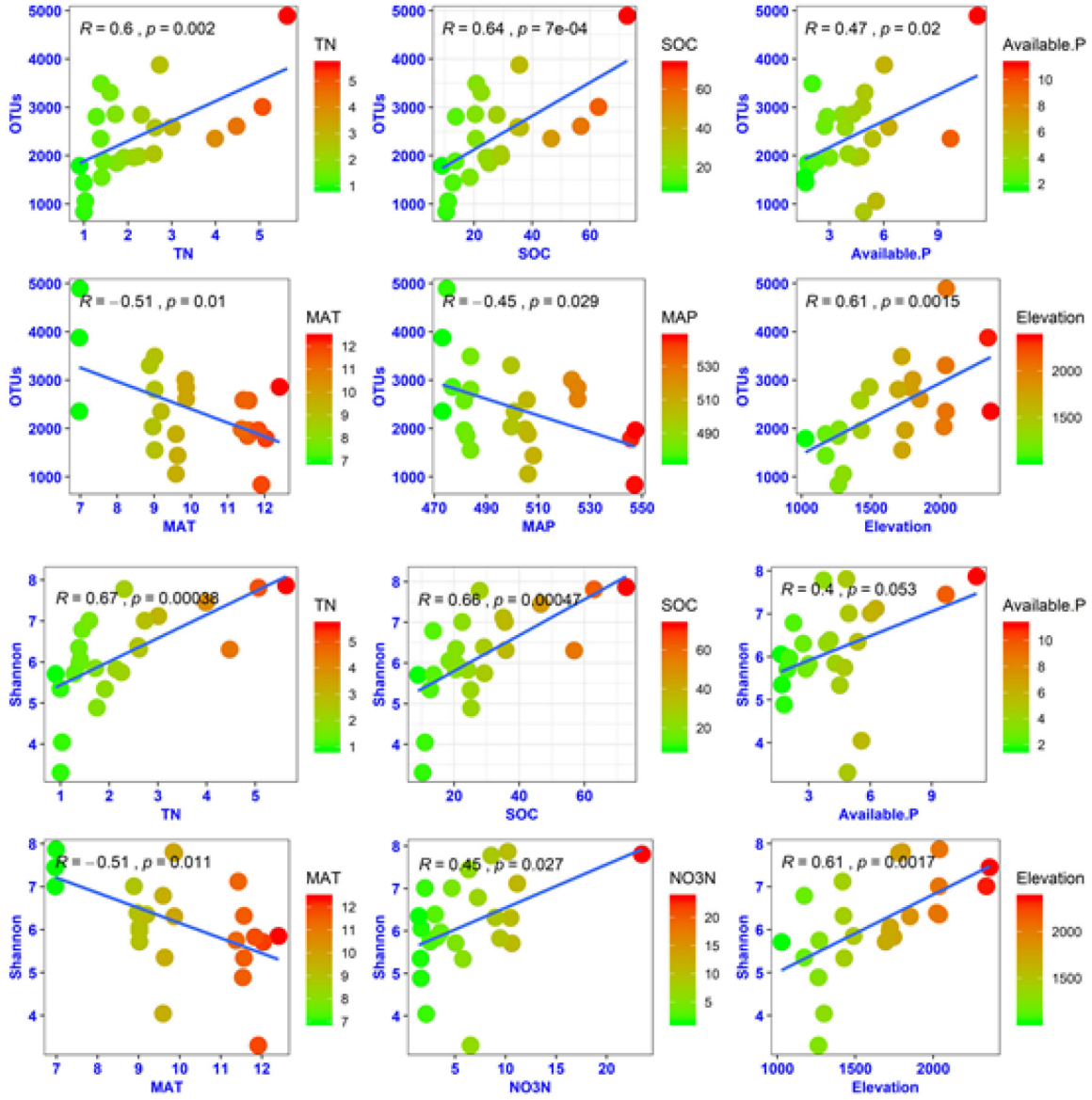

Fig. 2. The correlations between soil fungal diversity and environmental factors.

2.4. Bioinformatics analysis

Raw reads without adapters from the MiSeq sequencer were assigned to different samples based on barcodes. Paired-end reads with at least a 50-bp overlap, and < 5% mismatches were combined using flash (version 1.0.0) (Magoč and Salzberg, 2011). A threshold of average quality scores > 30 over a 5-bp window size was used to trim the unqualified sequences using btrim (version 1.0.0). Any joined sequences with ambiguous bases and lengths < 200 bp were discarded. High-quality sequences were clustered into operational taxonomic units (OTUs) with 97% similarity using uparse (version usearch v7.0.1001_i86), while the chimeras and singletons were discarded (Edgar, 2013). We determined taxonomic annotations using the Ribosomal Database Project Classifier (version 1.0.0) with a 50% confidence score (Wang et al., 2007). ITS sequences were assigned using the unite database (Abarenkov et al., 2010). To correct for sampling effects, we normalized all samples to an even number of sequences (27,000) per sample for downstream community analysis.

2.5. Statistical analyses

Alpha diversity metrics including the Shannon-Wiener index and phylogenetic diversity of the whole tree (PD, whole tree) were calculated (Oksanen et al., 2013). Pearson correlations were used to assess the associations between the fungal alpha diversity and environmental factors. A value of $p < 0.05$ was considered statistically significant. These statistical analyses were performed using SPSS 20.0 (IBM Corporation, Armonk, NY, USA). To assess the potential for variables to explain variations in soil fungal diversity and community structure, multiple regressions were performed using R 3.5. We used Akaike Information Criterion (AIC) scores to evaluate the multiple regression models using the step function with the MASS package in R 3.5 (Ripley et al., 2013). Based on the multiple regression model, the relative importance (RI) of each variable can be calculated using the lmg function with the relaimpo package, which can be used to evaluate the relative influence of each parameter (Grömping, 2007; Reinhart et al., 2016). Canonical correspondence analysis (CCA) was used to further test the effects of environmental factors on soil fungal community structure with the vegan package in R 3.5.

The fungal community structure was visualized by nonmetric

multidimensional scaling (NMDS) ordinations that were based on the Bray–Curtis dissimilarity matrices using the vegan package (Oksanen et al., 2013) in R 3.5. The Mantel test was performed to assess correlations between microbial beta diversity and environmental variables and geographic distance with the ecodist package (Goslee and Urban, 2007) in R 3.5. During this analysis, the Bray-Curtis dissimilarity matrix was used for fungal community datasets, and the distances among samples were calculated based on Euclidean dissimilarity for the environmental factors. The principal coordinates of neighbor matrices (PCNM) were calculated to reflect the spatial distance (Dray et al., 2006), and the most significant PCNM variables were selected by conducting forward selection procedures with 999 permutations using the pcnm function in the vegan package in the R software. Variation partitioning analysis was then performed to determine the relative importance of the environmental variables and spatial distances in explaining the microbial community compositions by a redundancy analysis using the varpart function in the vegan package in R 3.5.

3. Results

3.1. The distributions of soil fungal α-diversity in forest soils

To compare the soil fungal community diversity among forest soils, 27,000 sequences was randomly selected from each sample. The results revealed that the phylotype richness, which is equivalent to the number of OTUs determined at the 97% similarity level, ranged from 841 to 4895, and the Shannon diversity ranged from 3.31 to 7.85 across the 24 forest soils (Table 1).

The regression analysis demonstrated that both the Shannon diversity ($p < 0.05$) and phylotype richness ($p < 0.05$) increased as the elevation increased (Fig. 2). When soil characteristics were considered, we found that phylotype richness was significantly positively correlated with SOC, TN, AVP, and significantly negatively correlated with MAP and MAT. The Shannon diversity values had similar correlations with the soil properties. SOC, TN and NO3N were positively significantly correlated with soil fungal Shannon diversity; however, MAP and MAT were negatively significantly correlated with soil fungal diversity. Other soil properties only exhibited weakly positive or negative relationships with phylotype richness and Shannon diversity. We used a multiple regression model to obtain the best predictor of soil fungal diversity and the results indicated that elevation was the predominant factor with a relative importance of 0.48, followed by NO3N with a relative importance of 0.38 (Table 2).

3.2. The distributions of soil fungal community compositions in forest soils

Across all soils, the dominant fungal phyla in all sampling sites were Ascomycota, Basidiomycota and Zygomycoata, with average relative abundances of 42.9%, 43.7%, 3.8%, respectively (Fig. 3). Other minor phyla (Chytridiomycota, Rozellomycota and Glomeromycota) were also determined at lower relative abundance (relative abundance < 1%). Taxonomical classification at the class level, Agaricomycetes, Sordariomycetes, Eurotiomycetes, Leotiomycetes, Tremellomycetes, Dothideomycetes, Pezizomycetes, Archaeorhizomycetes, Wallemiomycetes, Lecanoromycetes and Chytridiomycetes were more abundant than other groups (relative abundance > 1%), which accounted for 80% of the total fungal sequence. Other fungal classes were less abundant in the forest soils.

Taxonomical classifications at the order level indicated that > 25 orders were detected. Among them, Russulales, Agaricales, Thelephorales, Sebacinales and Hypocreales were the dominant orders, with mean relative abundances of 8.6%, 8.3%, 7.3%, 6.4% and 5.8%, respectively, which accounted for 36.5% of the fungal sequences. The Sordariales, Eurotiales, Pezizales, Boletales, Geminibasidiales, Helotiales, Mortierellales, Archaeorhizomycetales and Archaeorhizomycetales orders were also frequently observed, with relative abundances ranging from 2.4% to 4.9%. Taxonomical classification at the genus level indicated that Russula, Tomentella, Sebacina, Geminibasidium, Mortierella, Archaeorhizomyces, Humicola, Penicillium and Cortinarius were highly detected in all the soils and had mean relative abundances of 7.0%, 5.9%, 5.1%, 2.9%, 2.4%, 2.3%, 2.1% and 1.9%, respectively.

Table 2
The multiple regression model results that explain the effects of environmental factors on the fungal Shannon diversity. RI represents the relative importance of each variable.

Variable	F	*p*	RI
MAP	3.369	0.0821	0.113
pH	0.396	0.537	0.0405
NO3N	21.14	0.000196	0.383
Elevation	13.55	0.00159	0.464

3.3. Correlations between fungi community compositions and environmental variables

Regression analysis was used to explore the relationships between edaphic properties and the relative abundance of the most abundant fungal taxa at different levels (Fig. 4). The results revealed that the relative abundance of Zygomycota was positively significantly with SOC ($r = 0.459$, $p < 0.05$), TN ($r = 0.487$, $p < 0.05$) and NO3N ($r = 0.502$, $p < 0.05$) (Fig. 4); however, Basidiomycota and Ascomycota exhibited weak positive or negative correlations with climate and edaphic properties. At the order level, Mortierellales was positively correlated with MAP ($r = 0.491$, $p < 0.05$); Russulales was positively correlated with pH ($r = -0.636$, $p < 0.01$); soil TP was positively correlated with Sordariales ($r = 0.491$, $p < 0.05$) and negatively correlated with Archaeorhizomycetales ($r = -0.456$, $p < 0.05$); soil NO3N was positively correlated with Sordariales ($r = 0.476$, $p < 0.05$) and Mortierellales ($r = 0.586$, $p < 0.05$), and it was negatively correlated with Thelephorales ($r = -0.434$, $p < 0.05$). At the genus level, Archaeorhizomyces was negatively significantly related with TP ($r = -0.454$, $p < 0.05$); Russula was negatively significantly related to pH ($r = -0.710$, $p < 0.05$); Tomentella was negatively significantly related to NO3N ($r = -0.407$, $p < 0.05$) (Fig. 4).

3.4. Fungal community structure

The NMDS plots clearly showed that the fungal community structures of the 24 forest soils were significantly different with elevation and SOC gradient changes (Fig. 5). The regression analysis demonstrated that SOC ($r = -0.66$, $p < 0.01$), TN ($r = -0.68$, $p < 0.01$), pH ($r = 0.47$, $p < 0.05$), elevation ($r = -0.69$, $p < 0.01$), NO3N ($r = -0.43$, $p < 0.05$) and NH4N ($r = -0.44$, $p < 0.05$) were significantly correlated with scores of the first NMDS axis. This finding was also confirmed by the CCA analysis; the CCA clearly showed that MAP, elevation, SOC and EC significantly influenced soil fugal community structures (all $p < 0.05$; Table 3).

Multiple regression models showed that elevation and NO3N was the most important factors affecting soil fungal structure, with relative importance values of 0.43 and 0.22, respectively. Soil pH (0.12), MAP (0.08) and MAT (0.09) exhibited relatively low importance in explaining the soil fungal community structure (Table 4).

3.5. Fungal community links to soil properties and geographic distance

The regression analysis revealed that both environmental distance and geographic distance were positively correlated with fungal community dissimilarities (all $p < 0.0001$; Fig. 6), indicating a significant distance-decay relationship. Variation partitioning analysis was used to analyze the contributions of soil properties, climate and geographic

Fig. 3. The distributions of the main phyla at the different sampling sites in the forest soils.

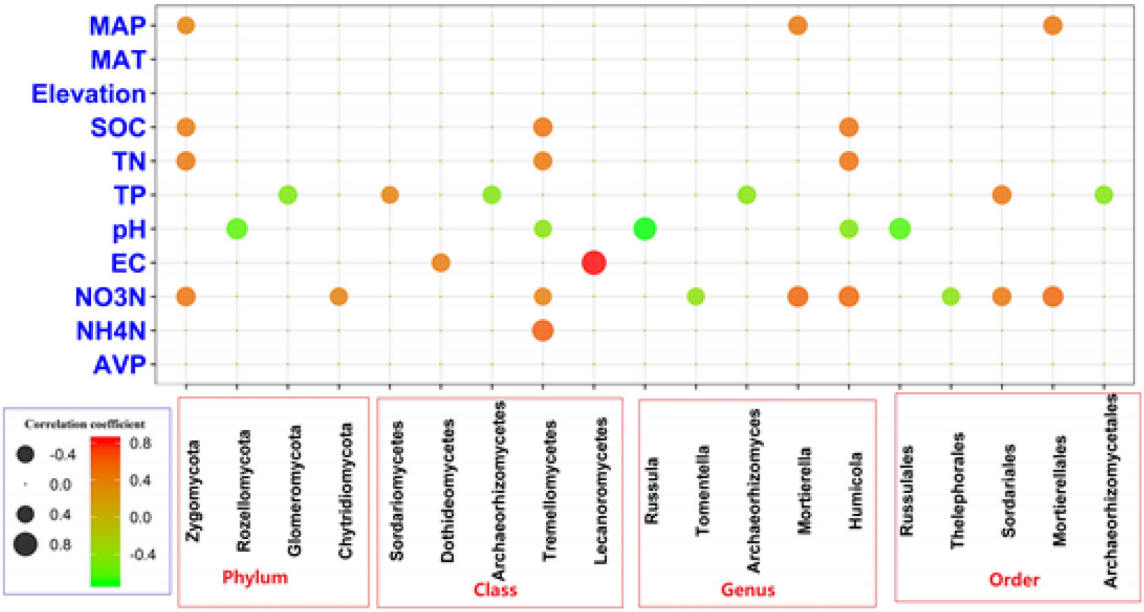

Fig. 4. The correlations between the soil fungal groups and the environmental factors.

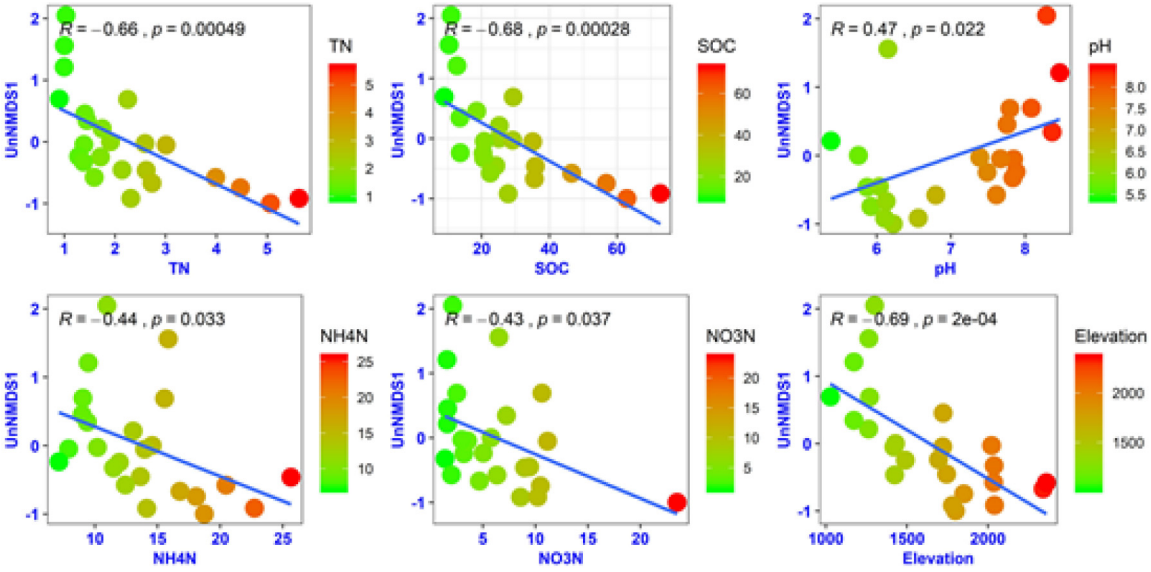

Fig. 5. The correlations between the soil fungal unweighted NMDS scores (first axis) and environmental factors (all $p < 0.05$).

Table 3
The results from CCA between environmental factors and soil fungal community structures on the Loess Plateau.

Variables	CCA_1	CCA_2	r^2	p
MAP	0.900	−0.435	0.331	0.026
MAT	0.980	−0.199	0.085	0.488
Elevation	−0.830	0.558	0.435	0.004
SOC	−0.805	0.594	0.333	0.033
TN	−0.813	0.583	0.277	0.070
TP	−0.805	−0.593	0.037	0.662
pH	0.923	−0.386	0.250	0.059
EC	0.862	0.508	0.939	0.001
NO3N	0.289	0.957	0.044	0.650
NH4N	−0.972	0.235	0.162	0.203
AVP	−1.000	−0.021	0.073	0.478

Table 4
The multiple regression model results that explain the effects of environmental factors on fungal community structures. RI represents the relative importance of each variable.

Variable	F	p	RI
MAP	5.49	0.032	0.083
MAT	4.46	0.049	0.088
pH	20.97	0.00027	0.13
NO3N	12.38	0.0026	0.22
Elevation	19.25	0.00040	0.43
AVP	3.27	0.088	0.043

distance to the soil fungal community structure and the results showed that these variables explained 30% of the fungal community variation, leaving 70% of the variation unexplained (Fig. 7). Soil properties (15%) and geographic distance (9%) better associated with the fungal community variations than climate factors (1%). These results suggest that the fungal geography on the Loess Plateau was regulated by soil properties and geographic distance in arid soils, while climate had a smaller influence, indicating that contemporary environment plays a more important role than geographic distance in shaping the compositions of fungal communities.

4. Discussion

4.1. Distribution of fungal diversity and compositions

To our knowledge, this is the first study that provides a geography of patterns and drivers of forest soil fungal communities along an east-west transect on the Loess Plateau. Our results indicate that soil fungal α-diversity was mainly determined by soil properties (defined as SOC, TN and AVP) and climate factors (MAP, MAT and elevation), partly supporting our first hypothesis that fungal diversity is mainly predicted by contemporary environments.

MAT and MAP are considered as good indicators of plants and animals (Hawkins et al., 2003). Similarly, in this study, we found that soil fungal diversity was significantly correlated with climate differences, as confirmed by the Pearson correlation analyses between soil fungal diversity and MAP and MAT values. Altered precipitation regimens have been found to alter microbial community composition (Nielsen and Ball, 2015). Warmer and wetter environments have lower soil fungal diversities, as indicated by OTUs and Shannon indices. At the higher elevation conditions, the MAP and MAT values were much lower. Therefore, the effects of elevation on soil fungal diversity may be explained by the climate. Temperature and precipitation might indirectly influence fungal diversity by regulating soil nutrient availability (Oehl et al., 2004) and plant traits (Yang et al., 2017). Warmer environments enhance the decomposition of soil organic matter, leading to lower soil organic matter availability (Tian et al., 2018). For the selected soil properties, SOC, TN, NO3N or AVP were determined as the most important factors that affected soil fungal α-diversity. These results are consistent with previous studies in different study areas (Beauregard et al., 2010; Liu et al., 2015).

Consistent with a previous study, our soils did not exhibit significant correlations between soil pH and soil fungal α-diversity. In contrast, SOC significantly influenced it, which is similar with a previous study conducted in the black soil zone in China (Liu et al., 2015). Generally, soil pH has been considered to be the most important factor for the growth and distributions of soil microbes, especially for soil bacteria (Baker et al., 2009; Chu et al., 2010; Fierer and Jackson, 2006; Lauber et al., 2009; Liu et al., 2014). Although some studies also showed soil fungal diversity was influenced by soil pH (Rousk et al., 2010; Wang et al., 2015), soil pH did not influence soil fungal diversity in this study or a previous study (Liu et al., 2015). The incongruent findings for the influence of soil pH might be caused by the different responses in

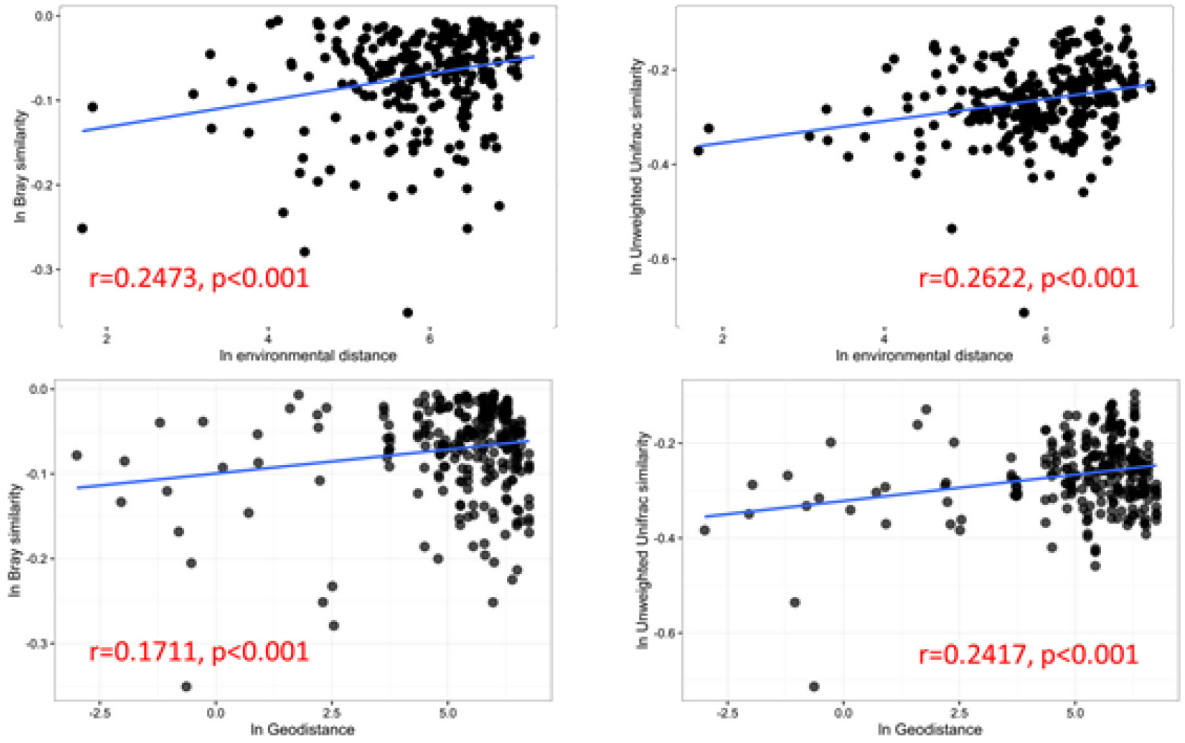

Fig. 6. The correlations between the soil fungal similarity distances (unweighted and Bray similarity distance) and the environmental distance (Euclidean distance and geographic distance).

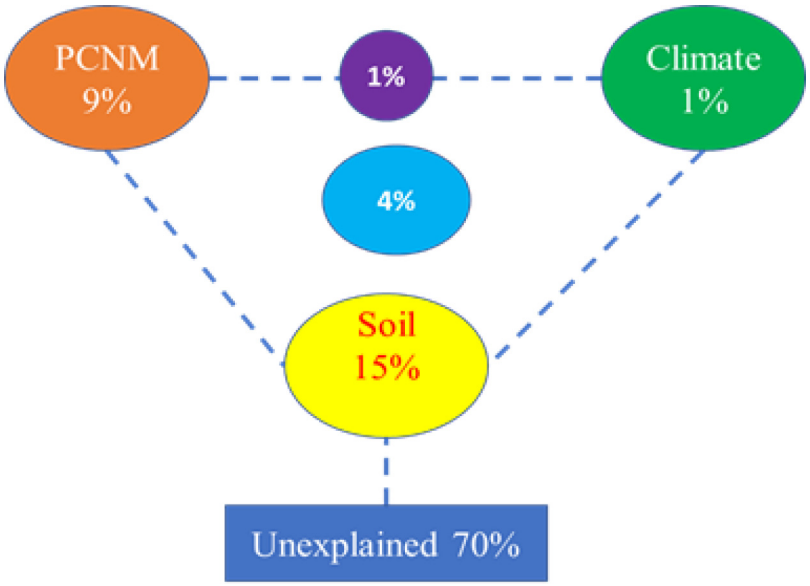

Fig. 7. Variation portioning analysis indicating the relative contribution of variance in fungal communities explained by the principal coordinates of neighbor matrices (PCNM), soil properties and climate in the forest soils.

bacteria and fungi growth characteristics to pH (Bååth et al., 2009). Generally, fungal species have a wider optimum growth pH than bacteria, which often ranges from 5 to 9 (Nevarez et al., 2009; Wheeler et al., 1991), suggesting a more subtle response of fungi to changes in environmental pH.

In the forest soils, all the fungal communities were dominated by Ascomycota (42.9%) and Basidiomycota (43.7%), which is a lower percent for Basidiomycota and a higher percent for Ascomycota than the average relative abundance that was determined in a global scale study (Basidiomycota (55.7%), Ascomycota (31.3%)) (Tedersoo et al., 2014). When compared with a previous study on the Loess Plateau, the relative abundance of Basidiomycota was approximately 3 times higher than that reported by Liu et al. (2019); however, the relative abundance of Basidiomycota in this study was much lower than their observation (Liu et al., 2019). Our results are consistent with the study conducted on the Loess Plateau under different land use types (Yang et al., 2017). The differences for the two dominant fungal phyla may be explained by the local soil properties, land use types, climate and other unpredicted factors (Thomson et al., 2015). For example, large differences exhibited for soil fungal community compositions among different land use types were observed (Yang et al., 2017). In our study, we found that the SOC, TN and NO3N contents significantly influenced soil Zygomycota, which

was the third dominant phyla, with an average relative abundance of 3.8%. Soil properties and climate had no significant effects on Ascomycota and Basidiomycota at phylum level. However, further taxonomical classification at the order level revealed that the dominant classes (Russulales, Thelephorales, Sordariales, Mortierellales and Archaeorhizomycetales) had significant correlations with soil properties. More specifically, soil pH negatively affected soil Russulales; soil TP was positively correlated with Sordariales ($r = 0.491$, $p < 0.05$), and it negatively correlated with Archaeorhizomycetales ($r = -0.456$, $p < 0.05$); soil NO3N was positively correlated with Sordariales ($r = 0.476$, $p < 0.05$) and Mortierellales ($r = 0.586$, $p < 0.05$), and it was negatively correlated with Thelephorales ($r = -0.434$, $p < 0.05$). These correlations suggest that soil properties have important effects on soil fungi at finer levels of taxonomic resolution. In future studies, we should focus more attention on the specific taxa and their functions related to nutrient cycles.

Another important finding was that the soil fungal community compositions differed from other ecosystems. For example, in the Chinese black soils, Tremellomycetes and Dothideomycetes were the two dominant fungal classes, whereas Agaricomycetes was observed as the dominant class in the forest soils, which is in line with previous studies conducted with forest soils (Buée et al., 2009; Peay et al., 2013; Shi et al., 2014). Agaricomycetes can decompose wood and plant litter; therefore, they usually occur in forest soils. The Saprotrophic taxa occur in all orders of Agaricomycetes (Hibbett et al., 2014), and Agaricomycetes possesses multiple ligninolytic class II fungal peroxidases and other plant cell wall decaying enzymes, implying that it is capable of producing white rot (Floudas et al., 2012; Hibbett et al., 2014; Ruiz-Duenas et al., 2013). Wood decayers with multiple enzymes capable of attacking crystalline cellulose occur in diverse lineages of Agaricomycetes (Hibbett et al., 2014). This might be a reason why Agaricomycetes were dominant in the forest soils compared with the other soils.

4.2. Biogeographical distribution of fungal communities

Contemporary environment and historical contingencies (geographic distance) are two important process that influence the biogeographical distribution of soil microorganisms. Many studies have focused on the drivers of the biogeographical distributions of soil bacterial communities at different regional scales (Barberán et al., 2015; Ge et al., 2008; Liu et al., 2014). Limited studies have been conducted that evaluated the driving factors on the biogeography of soil fungal communities (Kivlin et al., 2014; Wu et al., 2013). In this study, we first explored the biogeographic distributions of forest soil fungal communities on the Loess Plateau, and we found that geographic distance had significant effects on soil fungal community structures with 9% of the variations coming from the VPA. Regression analysis also indicated that geographic and environmental distances (based on Euclidean distance with data set of soil properties and geographic distance) had significant influences on soil fungal community structure in this study. Similar studies were also reported by Liu et al. (2019) and Liu et al. (2015). Consistent with the first hypothesis, the forest soil fungal community compositions were strongly determined by spatial and environmental variables, which accumulatively explained 30% of the total variations; thus, 70% of the variations unexplained by unpredicted factors. As with a previous study, spatial and edaphic variables determined the compositions of soil fungal community (Tedersoo et al., 2016). Here, edaphic properties were the most important drivers for the fungal composition, which is consistent with previous studies. The geographic distance explained 9% of the variation in the soil fungal community, which was lower than the soil properties, suggesting that environmental factors mainly controlled the biogeography of the forest soil fungi on the Loess Plateau. This result is consistent with a previous study (Wu et al., 2013), whereas at the local scale (< 1000 km), the environmental factors explained major variations.

5. Conclusions

In summary, this study represents one of the largest attempts to comprehensively investigate the regulation of soil fungal diversity and community structure under multiple environmental gradients along the east-west transect in western China. Our study showed that climate factors (MAP and MAT) and edaphic properties were the most important factors influencing fungal α diversity (Shannon and OTU richness) on the Loess Plateau. The fungal community compositions in arid areas were sharply shaped by elevation. MAP and MAT had smaller influences on soil fungal community structure compared with soil properties and geographic distances. These results provide a better understanding of the link between soil fungal diversity and ecological ecosystem services. Future studies are necessary to investigate and compare the community turnover and interaction network of fungi in different vegetation ecosystems and climate zones.

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