The differentiation of soil bacterial communities along a precipitation and temperature gradient in the eastern Inner Mongolia steppe

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A B S T R A C T

Understanding the differentiation of soil bacterial communities under precipitation and temperature gradients is crucial for assessing the impacts of environmental filters on ecosystem structure and function. Here we investigated spatial variations of bacterial communities along a precipitation and temperature transect in the eastern Inner Mongolia steppe, China. The aims are to understand the biogeographic pattern and key drivers shaping soil bacterial communities along the transect. Our results showed that the soil bacterial community along the 451-km transect exhibited the distance-decay relationship, decreasing in community similarity with geographic distance. Obvious changes in the bacterial community structure occurred at an annual precipitation from 215 to 261 mm, which was close to the boundary between arid and semi-arid systems. The relative abundances of Proteobacteria, Bacteroidetes, and Acidobacteria increased with precipitation, while those of Actinobacteria, Chloroflexi, and Gemmatimonadetes decreased. Environmental filtering was observed to be the key in shaping bacterial biogeographic patterns, and climate conditions exert indirect effects on soil bacterial communities mainly through direct effects on soil properties. Soil pH and plant coverage were important to soil bacterial communities in both arid and semi-arid steppes. Our findings provided insights for understanding the linkages among geographic distance, environmental filters, and soil bacterial communities in Eurasian steppe ecosystems.

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1. Introduction

Climate has a large impact on plant community, soil properties, biodiversity, and functions of terrestrial ecosystems (Kardol et al., 2010; Kelly and Goulden, 2008). It is well documented that aboveground net primary production increases with mean annual precipitation across various ecosystem types (Huxman et al., 2004). Similarly, changes in precipitation regimes may alter the soil microbial community structure and function, ultimately inducing the shifts of belowground ecosystem processes such as decomposition and nutrient cycling (Schimel et al., 2007; Schimel and Schaeffer, 2012). Some studies have investigated biogeographic patterns of the soil microbial community along the precipitation and temperature gradients, and most studies show that microbial biogeography is primarily controlled by soil variables (Angel et al., 2010; Bachar et al., 2010; Fierer and Jackson, 2006). However, previous studies using microbial biomass measurements (Serna-Chavez et al., 2013) or low-resolution microbial profiling methods (Chen et al., 2015; Hu et al., 2014) were not able to discern whether the microbial community structure or particular taxonomic groups were more or less sensitive to precipitation and temperature gradients.

A greater amount of evidence indicates that microbial assembly displays non-random environmental distributions (Angel et al., 2010; Fierer, 2008; Hanson et al., 2012; Lee et al., 2012; Prober et al., 2015). It is expected that an assortment of microbial communities (biogeographic patterns) will be shown along an environmental gradient based on certain mechanisms if environmental factors play key roles. Spatial scale is important in determining the variation patterns of microbial community structures, because environmental conditions are often scale-dependent (Bardgett and van der Putten, 2014). Under
such conditions, a distance-decay relationship often emerges in which taxonomy dissimilarity increases with geographic distance. The distance-decay relationship, as one example of biogeographical patterns for microbes, has a strong context-dependency (Martiny et al., 2006; Wang et al., 2013). It may vary depending on microbial groups, sampling seasons, and ecosystem types (Pasternak et al., 2013) or the interactive effects of multiple factors.

Environmental factors influencing the microbial community and diversity mainly include climate, soil physical and chemical properties, and plant properties (Angel et al., 2010; Fierer and Jackson, 2006; Prober et al., 2015). Abiotic factors, such as soil moisture, pH, and soil available carbon, may result in predictable shifts in the soil bacterial compositions (Fierer et al., 2007; Fierer and Jackson, 2006; Goldfarb et al., 2011). Several studies show that abiotic factors could potentially be the primary driving forces changing microbial diversity and community in desert soil (Cary et al., 2010; Fierer et al., 2012; Lee et al., 2012). These abiotic factors include extreme moisture and temperature conditions, high soil pH and salinity, or negligible plant-carbon inputs. However, the responses of the microbial biomass, diversity, and community structure to environmental factors may be different. It shows that the bacterial biomass increases with precipitation; however, bacterial diversity is often independent of precipitation depending on survey scales. Moreover, the compositions of bacterial communities are distinct to each site along the transect from the Mediterranean to semi-arid areas and arid sites in Israel (Bachar et al., 2010). It supports the notion that the microbial community is unique to each ecosystem (Bachar et al., 2010; Pasternak et al., 2013). Significant differences in soil microbial communities and functional attributes are also observed between desert and non-desert systems based on metagenomic analysis (Fierer et al., 2012). This implicates the occurrences of different mechanisms shaping bacterial communities along a specific transect.

The Inner Mongolia steppe lies in the eastern part of the Eurasian steppe. Some studies have assessed the effects of environmental factors on microbial communities at arid and semi-arid Inner Mongolian steppe ecosystems. It is reported that soil organic carbon is the main positive predictor of microbial biomass (Hu et al., 2014). Apart from the dominant effect of precipitation, plant and soil properties could also regulate soil microbial communities characterized with the phospholipid fatty acid method in the Inner Mongolia steppe (Chen et al., 2015; Yao and Zhu, 2015). However, biogeographic patterns and key driving factors shaping bacterial community structure remain further elusive at a fine taxon detail in this cold and dry steppe ecosystem. It is necessary to test the relative importance of climate, soil, and plant community factors simultaneously on bacterial communities at the regional scale.

In this study, we surveyed soil bacterial communities using the pyrosequencing technique in nine sites covering arid and semi-arid steppes in the east Inner Mongolia, China, which spans 451 km along a precipitation and temperature gradient. We focused on two questions in this study. First, we studied the variation patterns of soil bacterial communities associated with the climate gradient in the Inner Mongolia steppe. Second, we simultaneously tested the relative importance of climate, soil, and plant community factors on bacterial communities and analyzed the different environmental factors shaping bacterial community assemblage between arid and semi-arid systems.

2. Materials and methods

2.1. Study site descriptions

Nine sites were selected along the precipitation and temperature transect from the west to east in eastern Inner Mongolia, northern China (Fig. 1). These sites were distributed from latitude 43°37.926′ to 44°00.411′ N and from longitude 112°10.276′ to 117°45.856′ E. The ecosystem types along the precipitation and temperature transect change from arid steppe (T1 to T4) to semi-arid steppe (T5 to T9) from west to east. The topography consists of gently rolling hills and tablelands, with an elevation ranging from 933 m in the west to 1251 m in the east. Based on the long-term meteorological data (1901–2009), the mean annual temperature ranges from 0.91 to 3.48 °C, and the mean annual precipitation ranges from 167.5 to 333.3 mm, 70–80% of which occurs in the growing season (May–August). The main plant species in arid and semi-arid steppes were shown in Table 1. In this study, we employed an aridity index (AI, the ratio of average annual precipitation to potential evapotranspiration) based on United Nations Environment Programme (Middleton and Thomas, 1992) to quantify the drought occurrence at each study site. The AI can reflect the interactive effects of...
| Site | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 |
|------|----|----|----|----|----|----|----|----|----|
| Latitude (N) | 43°37.926′ | 43°43.971′ | 43°45.136′ | 43°50.502′ | 43°52.660′ | 43°59.726′ | 44°01.310′ | 44°03.759′ | 44°04.411′ |
| Longitude (E) | 112°10.276′ | 112°37.850′ | 113°06.321′ | 113°34.748′ | 114°14.179′ | 115°04.279′ | 116°12.403′ | 117°15.977′ | 117°45.856′ |
| Altitude (m) | 954 | 933 | 1035 | 1037 | 1027 | 1160 | 1059 | 1055 | 1251 |
| Temperature (°C) | 3.19 | 3.08 | 3.48 | 2.75 | 2.4 | 1.96 | 1.35 | 1.01 | 0.91 |
| Potential evapotranspiration (mm/yr) | 1096 | 1109 | 1142 | 1137 | 1144 | 1162 | 1143 | 1137 | 1141 |
| Precipitation (mm/yr) | | | | | | | | | |
| Aridity index | 0.153 | 0.163 | 0.170 | 0.189 | 0.200 | 0.225 | 0.243 | 0.277 | 0.292 |
| Plant coverage (%) | 40 | 40 | 50 | 40 | 50 | 80 | 90 | 95 | 95 |
| Plant richness | 22 | 17 | 20 | 24 | 26 | 25 | 20 | 30 | 30 |
| Main plant species | Stipa krylovii Roshev, Cleistogenes squarrosa, Allium anisopodium, et al. | Stipa krylovii Roshev, Salsoia collina, Bassia dasyphylla, et al. | Stipa krylovii Roshev, Salsoia collina, Convovulus ammamnii, et al. | Stipa krylovii Roshev, Salsoia collina, Allium anisopodium, et al. | Stipa krylovii Roshev, Allium polyrhizum, Leymus chinensis, et al. | Stipa krylovii Roshev, Leymus chinensis, Artemisia frigida, et al. | Stipa krylovii Roshev, Leymus chinensis, Allium anisopodium, et al. | Stipa krylovii Roshev, Leymus chinensis, Allium chinensis, et al. | Stipa krylovii Roshev, Cleistogenes squarrosa, Leymus chinensis, et al. | Stipa krylovii Roshev, Leymus chinensis, Allium anisopodium, et al. |
| pH | 8.49 ± 0.09b | 8.91 ± 0.09a | 9.10 ± 0.01a | 8.56 ± 0.10b | 9.10 ± 0.01a | 6.73 ± 0.10b | 7.14 ± 0.03c | 7.21 ± 0.14c | 6.75 ± 0.04d |
| Conductivity (μS/cm) | 41.12 ± 1.39c | 57.60 ± 4.54b | 85.70 ± 2.14a | 43.32 ± 0.94c | 80.06 ± 0.03d | 50.04 ± 2.34b | 58.06 ± 2.14a | 53.10 ± 2.10b | 61.02 ± 4.65b |
| TN (%) | 0.03 ± 0.001f | 0.04 ± 0.005ef | 0.06 ± 0.003de | 0.06 ± 0.002de | 0.07 ± 0.001c | 0.08 ± 0.002c | 0.09 ± 0.002c | 1.61 ± 0.18b | 0.27 ± 0.02a |
| NH₄-N (mg/kg dry soil) | 2.14 ± 0.29b | 2.51 ± 0.04e | 3.17 ± 0.01d | 1.46 ± 0.17b | 2.48 ± 0.12b | 1.72 ± 0.19b | 2.21 ± 0.03b | 4.93 ± 3.44a | 7.67 ± 0.04d |
| Cmic (mg/kg dry soil) | 185.65 ± 10.43d | 242.80 ± 24.23d | 275.41 ± 11.68d | 289.74 ± 12.34d | 436.21 ± 12.34d | 471.55 ± 12.34d | 449.10 ± 12.34d | 1150.30 ± 5.54a | 6.67 ± 0.02d |
| Nmic (mg/kg dry soil) | 20.57 ± 0.81d | 22.83 ± 2.37d | 34.88 ± 1.50cd | 32.96 ± 1.69cd | 54.66 ± 3.36b | 56.74 ± 3.73b | 51.98 ± 3.06b | 112.30 ± 14.93a | 79.22 ± 2.93a |
| R (mg/kg dry soil/d) | 7.71 ± 0.43ef | 11.57 ± 0.77def | 22.90 ± 4.01bced | 25.30 ± 13.8abc | 46.30 ± 4.01bced | 48.80 ± 13.8abc | 72.50 ± 18.90a | 3.09 ± 0.10a | 2.8 ± 0.106a |

Soil samples were collected at the depth of 0–10 cm. Values with different letters in a row means significant difference at p = 0.05. Values are means of five replicates ± SE. Cmic: soil microbial biomass C; Nmic: soil microbial biomass N; R: soil microbial respiration rate; Aridity index (AI) is calculated by the average annual precipitation divided by potential evapotranspiration (Middleton and Thomas, 1992).
mean annual precipitation and temperature and classify the climate as arid (0.05 < AI < 0.2) or semi-arid (0.2 < AI < 0.5) (Middleton and Thomas, 1997).

2.2. Soil collection and property measurements

Soil samples were collected at nine sites along the transect in August 2012. At each site, five replicate soil samples of the 0–10 cm depth were collected. Firstly, we selected a 50 m × 50 m plot for soil samplings in each site, then, we established five sampling lines with 10 m apart in this plot. We collected 5 individual soil cores 10 m apart along a sampling line and pooled as a replicate sample. Soil samples were transported to the lab on ice and stored at −20 °C.

Soil microbial biomass C (Cmic) and N (Nmic) were determined by the chloroform fumigation-incubation method. Soil microbial respiration rate (R, representing microbial activity) was calculated based on the basal respired CO2 from control incubation without fumigation. Bacterial abundance was measured with quantitative real-time polymerase chain reaction (qRT-PCR). Details procedures for above measurements and other soil properties (pH, conductivity, TOC, TN, NH4+ -N) were described by Yao et al. (2014).

2.3. DNA extraction and 454 pyrosequencing

Soil genomic DNA was extracted using a method described by Lueders et al. (2003). To amplify the V4-V5 hypervariable regions of 16S rRNA genes, universal primers 515F (5′-GTGCRYCAGCMCCGGGTA-3′) and 909R (5′-CCCGYCAATTCCMTTTRAGT-3′) were used, and the experimental procedures were described in detail by Li et al. (2014). The barcoded amplicons were pooled in an equal molar concentration for 454 pyrosequencing using a Roche 454 GS FLX Titanium system.

2.4. Pyrosequencing data and statistical analysis

The QIIME Pipeline (http://qiime.org/tutorials/tutorial.html) was used to process the sequencing data. Sequences with high quality (length > 300 bp, with no ambiguous bases “N”, and average base quality score > 30) were used for downstream analysis. The aligned sequences were checked for chimeras using the Uchime algorithm (Edgar et al., 2011). Operational taxonomic units (OTUs) were picked at the threshold of 97% identity using cd-hit in the QIIME pipeline. Singleton sequences were filtered out. Each sample was rarefied to a soil sample exhibiting the lowest number of reads (2735 sequences) for further analyses. The Shannon index, Simpson index, Chao1 estimator, and sample exhibiting the lowest number of reads (2735 sequences) for further analyses. The Shannon index, Simpson index, Chao1 estimator, and other soil properties (pH, conductivity, TOC, TN, NH4+ -N) were described by Yao et al. (2014).

2.5. Analysis of bacterial communities with geographic distance

One approach to test the spatial distribution pattern is the “distance-decay relationship”, which shows that the dissimilarities in bacterial communities increase with geographic distance. This dissimilarity is the phylogenetic distance of the bacterial community between samples. We used unweighted UniFrac distance (Lozupone and Knight, 2005) and the beta mean nearest taxon distance (bacterMNTD) (Stegen et al., 2012) to quantify the phylogenetic distance in bacterial communities along the transect. BetaMNTD is a “terminal” metric of phylogenetic beta-diversity that quantifies phylogenetic turnover based on the phylogenetic distance among the closest relatives (Fine and Kembel, 2011).

2.6. Path model

Partial Least Squares Path Modeling (PLS-PM) was used to explore the relationships among soil bacterial communities (including main phyla with the relative abundance higher than 1%), climate (precipitation, temperature), soil properties (pH, TOC, TN, and NH4+ -N), and plant factors (plant coverage and plant richness). PLS-PM is a data analysis approach for studying observed variables that can be summarized by the use of a latent variable and the fact that linear relationships exist between latent variables (Sanchez, 2013). Since PLS-PM does not rely on any distributional assumptions (Tenenhaus et al., 2005), we run the path model using 999 bootstraps to validate the estimates of path coefficients (representing the direction and strength of the linear relationships between variables) and explained variability (R²).

2.7. Network analysis of bacterial communities

The phylogenetic molecular ecological networks (pMENs) (Deng et al., 2012) were constructed following the molecular ecological network analyses pipeline (MENAP) (http://ieg2.ou.edu/MENA/main.cgi) with default parameters based on an OTU table calculated from 16S rRNA gene-sequencing data. This network construction method aims to produce an understanding of the interactions among different species within a community and their responses to environmental changes. Only the OTUs detected in more than half of all samples were kept for network construction. Modules were detected by fast greedy modularity optimization. Each node in a module signifies an OTU. Modules with >6 nodes were used for module network analysis. Cytoscape 3.0.2 software was used to visualize the network graphs (Saito et al., 2012).

3. Results

3.1. The changes of basic soil and microbial parameters along the transect

The AI was positively correlated with mean annual precipitation (r = 0.999, p < 0.01) and negatively correlated with mean temperature (r = −0.975, p < 0.01) along the transect. Soil microbial biomass (Cmic, Nmic), microbial activity (R), and soil TOC, TN, and NH4+ -N increased, while soil pH decreased with precipitation. The increase of soil microbial biomass with precipitation was corroborated by qRT-PCR. Meanwhile, the aboveground plant coverage and plant richness also increased with precipitation (Table 1).
3.2. Changes of bacterial diversity and community structure

Observed species and bacterial alpha-diversity indices based on the Chao1 richness, Shannon’s diversity, and Simpson indices were not significantly different with the increased precipitation (Table S1). The general difference in the bacterial community structure was visualized by the PCoA tool with an unweighted UniFrac distance matrix. When annual precipitation reached around 215 to 261 mm, the bacterial communities shifted strongly in a different direction (Fig. 2). The variations of bacterial communities were larger among those at the high precipitation sites (>300 mm) compared to those at the low precipitation sites (<200 mm). AI was supposed to be the main factor in the first principle coordinate axis (PCo1), which contributed 29.81% of total variation. The significant difference of the overall soil bacterial community structure was further tested by PerMANOVA based on unweighted UniFrac distance measures, showing significant differences of bacterial community structures from site to site (p < 0.05) (Table S2), except for the sites with precipitation between 168 mm and 215 mm, 229 mm and 261 mm, 229 mm and 278 mm, and 278 mm and 315 mm. Our results further showed the increase of bacterial community dissimilarity with geographic distance, which satisfied the distance-decay relationship along this transect using both unweighted UniFrac and betaMNTD (Fig. 3).

3.3. Distribution of bacterial community compositions along the transect

The relative abundances of Proteobacteria, Bacteroidetes, and Acidobacteria increased with precipitation, while those of Actinobacteria, Chloroflexi, and Gemmatimonadetes decreased with precipitation. Especially when the precipitation was between 215 and 261 mm/yr, the relative abundances of some bacterial groups (such as Proteobacteria and Bacteroidetes) increased significantly with precipitation (Fig. 4). The increase of Proteobacteria with precipitation was mainly driven by the increase of class Alphaproteobacteria, including order Rhodospirillales, Sphingomonadales, Rhizobiales (mainly family Hyphomicrobiaceae and genus Rhodoplanes, Bradyrhizobium (OTU3)), Betaproteobacteria, including order Burkholderiales (mainly family Comamonadaceae and Oxalobacteraceae), and Gamma-proteobacteria, including order Xanthomonadales (mainly family Xanthomonadaceae) (Table S3). These Proteobacteria are largely either plant symbionts or recognized copiotrophic microbes. Changes in the relative abundance of phylum Acidobacteria were mainly driven by Chloracidobacteria (OTU5, OTU13, OTU17). However, some OTUs, e.g., OTU23 (order Bacillales of class Bacilli) in Firmicutes, responded negatively to precipitation. The decrease of the relative abundance of phylum Actinobacteria with precipitation was mainly driven by the change of family Micrococcaceae, and the decrease of phylum Chloroflexi was mainly driven by classes Chloroflexi and Thermomicrobia.

3.4. Relationships of bacterial communities with environmental properties

In order to explore the key drivers shaping soil bacterial communities along the transect, we supplied comprehensive proof by using a variety of statistical methods. The partial Mantel test showed no significant effects of geographic distances on the bacterial community structure (p > 0.05) when the effects of environmental factors (including climate and soil and plant properties) were controlled (Table 2). In
contrast, when the effects of geographical distances were controlled, there was a significant ($r = 0.170, p = 0.004$) correlation between the bacterial communities and environmental factors. For environmental factors, the climate (e.g., precipitation and temperature) was significantly ($r = 0.462, p = 0.001$) correlated with bacterial communities when soil and plant properties were controlled. The soil properties were correlated to bacterial communities significantly ($r = 0.177, p = 0.010$) when climate and plant factors were controlled. However, the correlations of plant properties and bacterial communities were not significant ($p > 0.05$) when climate and soil properties were controlled.

The PLS path model was constructed to integrate the complex inter-relationships among environmental factors and bacterial communities (Fig. 5). The PLS model was best represented here with the GoF 0.73. According to the PLS-PM, climate conditions exerted significant direct effects on soil properties ($r = 0.909, p < 0.001$) and significant direct effects on plant properties ($r = 0.914, p < 0.001$). Interestingly, there was no significant direct effect of climate on bacterial community composition ($p > 0.05$). Instead, there was a significant direct effect of soil properties on bacterial community composition ($r = 0.551, p = 0.003$), but not the plant properties ($p > 0.05$). Together, this suggested that climate

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**Table 2**

The influences of geographic distance and environmental factors on bacterial communities by partial Mantel test.

| Effect of Environmental factors | Geographic distance | Climate$^b$ | Soil$^c$ | Plant$^d$ |
|---------------------------------|---------------------|-------------|-----------|-----------|
| Controlling for Geographic distance | Environmental factors | Soil and plant | Plant and climate | Climate and soil |
| Bacterial communities | $r$ | $p$ | $r$ | $p$ | $r$ | $p$ | $r$ | $p$ | $r$ | $p$ |
|---------------------------|---------------------|-------------|-----------|-----------|
| $0.170$ | $0.004$ | $0.042$ | $0.495$ | $0.462$ | $0.001$ | $0.177$ | $0.010$ | $-0.024$ | $0.641$ |

Bold means significant correlation ($p < 0.05$).

$^a$ Environmental factors include climate (precipitation and temperature), soil (pH, conductivity, TOC, TN, and NH$_4^+$-N), and plant (plant coverage and richness).

$^b$ Climate (precipitation and temperature).

$^c$ Soil (pH, conductivity, TOC, TN, and NH$_4^+$-N).

$^d$ Plant (plant coverage and richness).
conditions exerted indirect effects on bacterial communities mainly through direct effects on soil properties.

To further analyze how individual environmental factors influenced the relative abundances of particular bacterial phyla, redundancy analysis (RDA) was used. The results showed that 46% and 9% of the variations at the phylum level can be explained by X and Y-axes, respectively. There were positive correlations between pH and temperature, between precipitation and plant coverage, plant richness, soil TN, NH₄⁺-N, and TOC (Fig. 6). The relative abundances of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacteroidetes, and Acidobacteria showed positive correlations to precipitation, plant coverage and richness, TN, NH₄⁺-N, and TOC. Plantomycetes showed positive correlations to conductivity. Actinobacteria, Chloroflexi, and Gemmatimonadetes showed positive correlations to temperature and pH. These results indicated varied responses of main phyla (the relative abundance was >1%) to different factors.

To reveal the interactions among different species within a community and their responses to environmental factors, we conducted pMEN network analysis. The pMEN using all the transect samples showed 8 main modules generated by environmental properties (Fig. 7A). Each module is a group of OTUs that may have similar ecological niches of co-occurring species and are well connected among themselves, but are less linked with OTUs belonging to other modules. Modules M1, M5, M6, and M7 showed positive correlations \( (p < 0.01) \) with precipitation, plant coverage and diversity, soil TN, and TOC, but negative correlations with temperature and soil pH. These modules were mainly composed of relatively more abundant copiotrophic phyla, e.g., Proteobacteria and Bacteroidetes. The modules M2, M3, and M4 were mainly composed of relatively more abundant oligotrophic phyla, e.g., Actinobacteria and Chloroflexi, which showed positive correlations \( (p < 0.01) \) with temperature and pH, but negative correlations with precipitation, plant coverage and diversity, soil TN, and TOC (Fig. 7B).

We further confirmed the effects of environmental factors on bacterial communities in arid and semi-arid steppes by using the partial Mantel test. The results showed that plant coverage, plant richness, and soil pH provided the highest correlations \( (p < 0.05) \) with bacterial communities in arid steppes (sites T1–T4, 0.15 < AI < 0.2). However, in the semi-arid steppes (sites T5–T9, 0.2 < AI < 0.3), soil pH, plant coverage, and soil NH₄⁺-N provided the highest correlations \( (p < 0.05) \) with bacterial communities (Table 3).
4. Discussion

This study demonstrated significant changes of bacterial community structure and compositions along a precipitation and temperature gradient in the Inner Mongolia steppe, and the responses of various bacterial taxa to environmental gradient depended on their sensitivities to different environmental factors.

4.1. Biogeographic pattern of bacterial communities is context-dependency

The biogeographical pattern of bacterial communities across our sampled 451 km transect showed a distance-decay relationship based on both unweighted UniFrac and betaMNTD. This indicated that both geographic distance and environmental factors might play important roles in shaping the bacterial community structure. However, our study showed that a significant correlation was observed only between bacterial communities and combined environmental conditions, but not between bacterial communities and geographical distances, indicating that environmental filtering was more important than geographical distance for bacterial community assemblage along this transect. That is, the variation of bacterial communities is context-dependent along this precipitation and temperature transect in the eastern Inner Mongolia steppe. Fierer and Jackson (2006) reported that environmental factors, such as soil pH, were more important than geographic distance in influencing the continental scale spatial structure of microbial communities. Relationships between bacterial communities and context properties are likely scale-dependent (Wang et al., 2013). Surveys at smaller spatial scales usually found that environmental factors rather than geographic distance were primary determinants in microbial communities (Hollister et al., 2010; Horner-Devine et al., 2004), while the contribution of geographic distance (historical contingencies) might increase to shaping the spatial patterns of microbial communities across large spatial scales (Wang et al., 2015).

This study further suggested the context-dependent biogeographic patterns for many bacterial phyla in the Inner Mongolia steppe ecosystems. A previous study in North America showed that soil available carbon could explain the spatial variance of some bacterial phyla (Acidobacteria, Betaproteobacteria, Bacteroidetes) (Fierer et al., 2007).

**Table 3**
The correlations of soil and plant properties to bacterial communities based on weighted UniFrac distance using partial Mantel test ($p < 0.05$, permutation $= 999$).

| Arid steppe       | Correlation    | Plant coverage | Plant richness | pH        | Conductivity | TOC       | TN        | NH$_4$-N |
|-------------------|----------------|----------------|----------------|-----------|--------------|-----------|-----------|----------|
| Site T1–T4        | 0.287 ($p = 0.002$) | 0.227 ($p = 0.002$) | 0.147 ($p = 0.002$) | 0.127 ($p = 0.002$) | 0.066 ($p = 0.002$) | 0.021 ($p = 0.002$) | 0.005 ($p = 0.002$) |
| Semi-arid steppe  | 0.270 ($p = 0.001$) | 0.147 ($p = 0.001$) | 0.135 ($p = 0.001$) | 0.070 ($p = 0.001$) | 0.052 ($p = 0.001$) | 0.046 ($p = 0.001$) | 0.013 ($p = 0.001$) |

Bold means significant correlation ($p < 0.05$).
In this cold and dry transect of Inner Mongolia steppe, the relative abundances of Acidobacteria, Betaproteobacteria, and Bacteroidetes were also observed to be related to precipitation and soil nutrients. Our results confirmed the increase in predicted copiotrophic groups (Proteobacteria and Bacteroidetes) (Goldfarb et al., 2011; Ramirez et al., 2012) and microbial activity with precipitation, indicating that nutrient cycling activity might become higher with precipitation along the transect. The correlation between water availability and the high abundance of Actinobacteria was not observed along a low precipitation gradient in Israel (Bachar et al., 2010). In our surveyed transect, the variances of Alphaproteobacteria and Actinobacteria were related to precipitation. However, the variances of Nitrosiraea, Cyanobacteria, and Deltaproteobacteria did not significantly correlate to any measured soil properties. The relative abundances of phylum Actinobacteria, Acidobacteria, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Chloroflexi, and Bacteroidetes significantly correlated to multiple soil properties. All the above results collectively suggested that the distance-decay relationship and the variation of whole bacterial communities are strong context-dependent and are driven by environmental variables. However, various phyla may show different variation patterns. This confirms that different ecosystems may support unique microbial populations (Angel et al., 2010; Bachar et al., 2010; Fierer and Jackson, 2006; Nemerget et al., 2011; Zhou et al., 2002), and microbial assemblages can exhibit small- and large-scale biogeographic patterns.

4.2. Climate drives the differentiation of soil bacterial communities

This study and many previous studies showed that microbial biomass and activity (respiration) became higher with increasing precipitation in the Inner Mongolia steppe (Chen et al., 2015; Li and Chen, 2004) and other biomes (Manzoni et al., 2012). A broad array of indirect mechanisms under precipitation and temperature changes likely affects the microbial physiology and community composition in the Inner Mongolia steppe and these indirect effects likely co-occur with the direct effects of temperature and precipitation changes along the transect. This study highlighted the notion that environmental selection did not act independently on an individual environmental factor (Verberk et al., 2013). Instead of focusing on individual environmental factors relationships, our PLS-PM approach used latent variables of climate, soil and plant properties, and soil bacterial communities as combinations of related factors, and path models could well explain the cause and effect relationships among variables (Barberan et al., 2014; Tenenhaus et al., 2005). Our results revealed that climate conditions had important direct effects on soil properties and plants, and had indirect effects on soil bacterial communities mainly through the effects on soil properties, similar to the previous report that the climatic variables only indirectly affected microbial abundance according to the structural equation model analysis (Hu et al., 2014).

Different climate and soil environments may reflect the influence of spatial niche partitioning on microbial communities. The presence of various modules within a network serves as a prime example for the study of niche partitioning and synergistic relationships (Barberan et al., 2012; Chaffron et al., 2010). The pMEN analysis constructs some ecological relevant modules, which are expected to differentiate with ecological niches. The pMEN analysis in this study showed that some modules characterized by more abundant and well-connected copiotrophic groups positively correlated to precipitation and nutrient contents, which were high in semi-arid sites, while other modules characterized by more abundant and well-connected oligotrophic and dormancy groups positively correlated to temperature and pH, which were high in arid sites. This suggested that environmental variables observed along the transect were the main factors promoting multiple niches over a long time. Thus, spatial niche partitioning could be a good explanation for promoting phylotype coexistence in complex bacterial communities and their differentiation along the transect.

4.3. The contributions of different environmental factors shaping bacterial communities may vary in arid and semi-arid systems

Around an annual precipitation of 215 to 261 mm, which was close to the boundary between arid and semi-arid systems with an AI of 0.2 (Middleton and Thomas, 1997), bacterial community structures shifted strongly to a different direction, and the relative abundances of some dominant bacterial phyla, such as Proteobacteria and Bacteroidetes, increased significantly. These phenomena implicated the different assemblages of bacterial communities between arid and semi-arid regions. The contributions of different environmental factors shaping bacterial communities may vary in arid and semi-arid systems.

In the arid region, plant coverage and richness likely played important roles in shaping the bacterial community structure. It is likely that the changes in plant coverage and richness become especially important for nutrient and moisture maintenance in arid systems. Thus, arid climates drive the assemblage of a community to better adapt to desiccation stress, and to become less sensitive to the limited precipitation. Arid conditions would promote more oligotrophic bacterial occurrence; e.g., Actinobacteria, Chloroflexi, and other bacteria adapt to desiccation and thermal stress. Previous studies have shown that members of Rubrobacteria are tolerant to desiccation (Davinic et al., 2012; Singleton, 2003), and the phylum Chloroflexi is abundant at high temperature conditions (Acosta-Martínez et al., 2010). Our results also showed that more Rubrobacteria and Chloroflexi appeared in the arid steppe of Inner Mongolia. In the semi-arid region, besides soil pH, plant coverage and soil NH$_4$ play important roles in shaping the structure of the bacterial community. Precipitation effects on plants and nutrient cycling are likely important to drive the changes in bacterial communities in semi-arid steppe, and promotes soil bacterial communities to enrich those fast-growth and more active copiotrophic microbes, e.g., Proteobacteria and Bacteroidetes. This is supported by a metagenomic study that desert microbial communities have more abundant genes related to osmoregulation and dormancy, but grassland communities have more abundant genes related to nutrient cycling and the catabolism of plant-derived organic compounds (Fierer et al., 2012).

5. Conclusion

The current research revealed the differentiation of bacterial communities along the precipitation and temperature transect across 451 km in the Inner Mongolia steppe. The spatial distribution of the bacterial community is in accordance with the distance-decay relationship, implicating the eco-evolutionary trajectory of bacterial assemblages along the environmental gradient. Local environmental filtering is a dominant process shaping the bacterial community. Climate conditions have important direct effects on soil properties and plants, and indirect effects on soil bacterial communities mainly through their effects on soil properties. Dramatic changes in the bacterial community structure are approximately separated by the AI threshold between arid and semi-arid regimes. The contributions of different environmental factors driving bacterial community assemblage may vary in arid and semi-arid systems. This study established the linkages among geographic distance, environmental filters, and soil bacterial communities in the Inner Mongolia steppe and Eurasian grassland ecosystems.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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