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Biogeography and the driving factors affecting forest soil bacteria in an arid area

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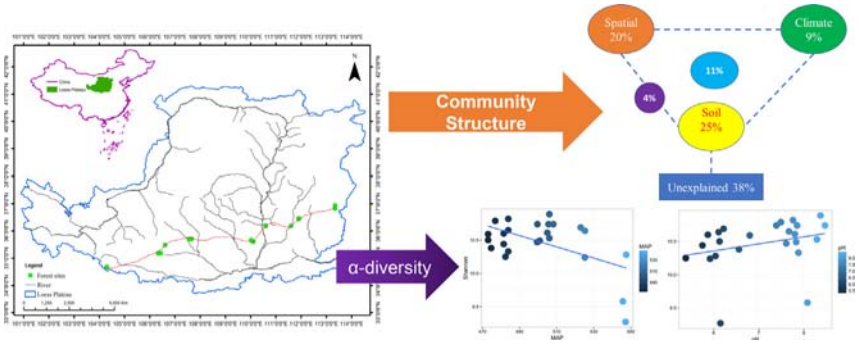
HIGHLIGHTS

- The biogeography and its driving factors of forest soil bacteria in the arid area were studied.
- Distinct biogeography for soil bacteria in the Loess Plateau.
- Climate significantly affected soil bacterial α-diversity.
- Soil properties contributed more to bacterial community variation than combined historical contingencies.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacteria are one of the most abundant and diverse groups and mediate many critical terrestrial ecosystem processes. Despite the crucial ecological role of bacteria, our understanding of their large-scale biogeography patterns across longitude (east-west transect), and the processes that determine these patterns lags significantly behind that of macro-organisms. Here, we used 16S rRNA gene sequencing to evaluate the geographic distributions of bacterial diversity and their driving factors across different longitude sites along an 800-km east-west transect in the Loess Plateau. Twenty-four phyla were detected across all soil samples and the most sequence-abundant bacterial phyla were Acidobacteria, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Chloroflexi and Gemmatimonadetes (average relative abundance >5%). Soil bacterial α-diversity, expressed by the richness of soil bacterial communities and Shannon diversity, differed among climates (MAP) but showed strong correlations with MAP ($r = -0.537$ and $r = -0.42$, respectively; $p < 0.05$ in both bacterial diversity indices). Variation partition analysis demonstrated that the bacterial community structure was closely correlated with environmental variables and geographic distance, which together explained 62% of the community variation. Soil properties contributed more to bacterial community variation than the combined geographic distance (historical contingencies) and climate factors. Among all environmental factors, soil pH exhibited a dominant role in structuring bacterial communities in this arid area. Our findings provide new evidence of bacterial biogeography patterns in an arid area (MAP ranged from 473 mm to 547 mm). Additionally, the results indicated a close linkage among soil bacterial community, climate and edaphic variables, which is critical for predicting promoting sustainable ecosystem services in the Loess Plateau.

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1. Introduction

Soil microbes play predominant roles in regulating ecosystem functioning and maintaining ecosystem stability (Fuhrman, 2009; Maestre et al., 2015). Bacteria influence the essential soil processes and the recycling of elements. Although the geographic patterns of microbes have been largely studied (Chu et al., 2010; Fierer and Jackson, 2006; Liu et al., 2014; Tian et al., 2018), there are still some important knowledge gaps in our understanding of the biogeography of microbes (Bardgett and Wh, 2014; Lauber et al., 2009; Martiny et al., 2006), especially in arid areas (mean annual precipitation <600 mm). Precipitation is an important factor driving the distribution and the diversity of soil bacteria (Bahram et al., 2018). Therefore, studying the effects of precipitation on soil bacterial communities will enhance our understanding of these soil processes and explore their contributions to natural climate change.

Previous studies have reported that biotic and abiotic factors have an impact on patterns of soil microorganisms, such as geographic distance (Jesus et al., 2009; Martiny et al., 2006; Martiny et al., 2011), soil pH (Chu et al., 2010; Fierer and Jackson, 2006), human activities (land use change) (Rodrigues et al., 2013), aridity (Wang et al., 2015), and soil organic carbon availability (Liu et al., 2014; Tian et al., 2018). A number of studies have examined bacterial biogeography patterns in terrestrial ecosystems across different spatial scales and have obtained different results (Caporaso et al., 2011; Lauber et al., 2009; Liu et al., 2014; Tian et al., 2018; Xiong et al., 2012). Such inconsistent conclusions may be caused by the study scales and the types of ecosystems. However, to date, limited information is available concerning the geographic patterns of soil bacterial diversity and the underlying drivers of that diversity (Fierer and Jackson, 2006; Lozupone and Knight, 2007; Nemergut et al., 2011), especially in arid areas. Within an arid environment, precipitation may cause changes in soil moisture, nutrient availability and plant compositions, and these changes subsequently shape the geographic patterns of soil bacterial diversity (Angel et al., 2009; Clark et al., 2009; Wang et al., 2015). In addition, whether soil nutrients and climate drivers can explain variations in soil bacterial biogeography patterns in different latitudes in arid areas (MAP <600 mm) similar to that seen in other areas, and their relative importance remains poorly unknown.

To our knowledge, no previous study has examined how entire soil bacterial communities are structured across large spatial scales for the forest soils in the whole Loess Plateau, which is an important area influencing climate change and ecosystem function in western China. The west-east transect of the Loess Plateau was selected as the subject which has distinct gradients in climate, edaphic and geographic distance factors. More importantly, most of this area has <600 mm of precipitation, providing an ideal study area to explore the responses of bacterial diversity and community structure to different environmental gradients in arid areas. We hypothesize that soil bacterial communities do exhibit biogeographical patterns and that these patterns are predictable. Therefore, the specific objectives of this study are listed as follows: (1) to determine and compare the biogeographical patterns of soil diversity and community structure; (2) to quantitatively assess the relative importance of multiple environmental variables, such as climate, geographic distance and edaphic factors, in shaping bacterial diversity and community structure; and (3) to determine whether soil organic carbon controls the biogeographical pattern of soil bacteria. It thus may have underestimated changes in diversity that occurred over larger geographic scales in the Loess Plateau (western China) and provides a more comprehensive understanding of the factors controlling the Earth's biodiversity and biogeochemistry.

2. Materials and methods

2.1. Site description and field sampling

The Loess Plateau is one of the most important serious erosion areas, which has different vegetation ecosystems, including forest, grassland, shrub land and cropland. As a limitation of MAP, most forests are distributed in areas with a higher MAP (>~500 mm). In this study, we chose forests that were distributed at similar latitudes on the Loess Plateau as research areas (Fig. S1). Soil samples were collected from 24 sites from an east-west transect, spanning ~800 km from east to west (Fig. S1). The mean annual temperature (MAT) at these sites ranges from 7.0 to 12.4 °C, while the mean annual precipitation (MAP) ranges from 473 to 547 mm (Table S1). The soil had similar types (loessial soils) (Zeng et al., 2016), and Entisols (USA. taxonomy).

Soil samples were collected during July 2016. We selected well-protected nature areas as sampling sites to reduce the influence of anthropogenic disturbances. At each sampling site, we randomly established 3 plots (20 m × 20 m). At each plot, we collected 10–20 surface soil scores (0–10 cm soil layers). The roots, stones and plant litters were removed from the fresh soils, and then they were homogenized and sieved with a 2-mm mesh. All of the soil samples were divided into two parts. One part was used to analyze the soil bacterial community, and it was stored at −80 °C until soil DNA extraction. The remaining part was used to analyze the soil properties, including soil organic carbon (SOC), total nitrogen (TN), soil total phosphor (TP), pH, electric conductivity (EC), available phosphor (AVP), soil nitrate nitrogen (NO3N) and soil ammonia nitrogen (NH4N).

2.2. Climate and soil variables analysis

The climate variables (MAP and MAT) were collected from the Chinese meteorological database (http://data.cma.cn/). Soil properties were determined by the methods described by Zeng et al. (2017). Soil pH was determined using a pH meter (1:2.5 w/v). Soil EC was measured using a conductivity meter (1:5 w/v). SOC was determined using a modified Mebius method after digestion with H_2SO_4 at ~180 °C for 5 min (Ren et al., 2015). Soil TN was measured by the Kjeldahl acid-digestion method (Thomas et al., 1967). Soil available nitrogen (NO3N and NH4N) were extracted from 1 mol/L KCl and the content was determined by a Seal AutoAnalyzer3 continuous-flow (Zeng et al., 2016). Soil available phosphorus was extracted with 0.5 mol/L $NaHCO_3$ and then determined by the molybdenum blue method (Olsen, 1954). The average values of soil properties in each sample are presented in Table S1.

2.3. Soil DNA extraction

Soil DNA was extracted from 0.5 g soil samples using the PowerSoil kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The quality of the purified DNA was evaluated with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) based on the absorbance ratios at 260/280 nm and 260/230 nm. The extracted DNA was stored at −80 °C until use.

2.4. 16S rRNA gene amplification and sequencing

An aliquot of the purified DNA from each sample was used as a template for amplification. The V3-V4 regions of the bacterial 16S rRNA genes were amplified using the universal primers 515F (5′-GTGCCAGCAGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011). PCRs were performed in triplicate with a 20 μl mixture containing 4 μl of 5× FastPfu Buffer, 2 μl of 2.5 mM dNTPs, 0.8 μl of each primer (5 μM), 0.4 μl of FastPfu Polymerase, and 10 ng of template DNA. PCR amplification was conducted with the following thermal program: 95 °C for 3 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 10 min. PCR amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), according to the manufacturer's instructions and quantified

using QuantiFluor™-ST (Promega, USA). The purified amplicons from all samples were pooled in equimolar concentrations. Sequencing was conducted on an Illumina MiSeq platform at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

2.5. Processing of sequencing data

Sequencing data were processed using QIIME 1.17. Raw sequences >150 bp with an average quality score >20 and without ambiguous base calls were quality processed. The high-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity using uparse (Edgar et al., 2011) (version 7.1 http://drive5.com/uparse/). After quality filtering, all samples were normalized to 11,020 sequences per sample which were the smallest among all the samples, to conduct downstream analyses for all samples at the same sequencing depth. OTU abundance information was normalized using a standard sequence number, corresponding to the sample with the least sequences (11020). The taxonomic assignment was performed using the Ribosomal Database Project (RDP) classifier (http://rdp.cme.msu.edu/). Soil bacterial α-diversity indices (Shannon index and Simpson index) and β-diversity metrics were constructed based on the OTU table in QIIME.

2.6. Statistical analysis

The phylotype richness was represented by the number of OTUs. The correlation between bacterial diversity (Shannon index) and phylotype richness and environmental factors (climate and soil properties) were tested with Pearson correlation analyses using SPSS 21.0. To assess the potential for variables to explain variations in soil bacterial diversity, multiple regression was performed using R 3.5. We used Akaike Information Criterion (AIC) scores to evaluate multiple regression models using the step function in the MASS package in R (Ripley et al., 2013). In order to meet the assumption of homoscedasticity, all of the response variables (climate and edaphic properties) were log transformed. Based on the multiple regression model, the relative importance (RI) of each variable can be calculated using the lmg function in the relaimpo package which can be used to evaluate the relative influence of each parameter (Grömping, 2007; Reinhart et al., 2016).

Similarity matrices for soil properties (Euclidean distances), climate factors (Euclidean distances) and bacterial communities (Bray-Curtis and Unifrac) were constructed with the vegan package in R 3.5 (Team, 2014). A Mantel test was used to assess the correlations between the bacterial community structure and the environmental variables or geographic distance. Non-metric multidimensional scaling (NMDS), principal coordinate analyses (Pcoa) and canonical correlation Analysis (CCA) were used to assess variations in the bacterial community structure using the vegan package in R 3.5. CCA was also used to determine the impacts of the environmental variables on the soil bacterial community structure. A Monte Carlo permutation test was used to assess the significance of CCA correlations. A canonical correspondence analysis-based variation partitioning analysis (VPA) was used to assess the relative importance of geographic distance, climate and edaphic factors in shaping bacterial community structure. Distance-based multivariate (DistLM) analysis was used to assess the importance of each environmental factor observed in this study on bacterial community structure with Primer 7 (Freedman and Zak, 2015). Principle coordinates of neighbor matrices (PCNM) performed from grid coordinates was used as geographic distance explanatory variable (Abarenkov et al., 2010) using the PCNM package in R 3.5. PCNM decomposes the total spatial variation into a finite set of spatial variables (Ramette and Tiedje, 2007). Pearson correlations between the relative abundances of bacterial taxa with pH and between scores for the first principal coordinate from NMDS and pH were performed in R 3.5.

3. Results

3.1. Distribution of taxa and phylotypes

In our study, 845,072 valid reads were obtained in total across all soil samples with an average of 35,211 reads per sample. At the 97% similarity level, these valid reads were grouped into 256,752 OTUs (10,698 OTUs per sample). We used the Shannon index and phylotype richness (OTU numbers) to express the bacterial α-diversity. Soil bacterial Shannon diversity ranged from 9.27 to 10.89, with the higher values at lower MAP areas. Soil bacterial Shannon diversity ($r = -0.537$, $p < 0.01$) and phylotype richness ($r = -0.42$, $p < 0.05$) were significantly related to MAP (Fig. 1). Other environmental variables (MAP, SOC, TN, TP, pH and available P) had no significant effects on soil bacterial α-diversity. The multiple regression showed that soil pH emerged as the most important factor influencing the soil bacterial diversity with a relative importance of 50% (Table 1).

Across all of the soil samples, we detected 24 phyla, including 5 dominant phyla: α-proteobacteria (with an average abundance of 18.2%), β-proteobacteria (with an average abundance of 5.2%), Actinobacteria (with an average abundance of 31%), Acidobacteria (with an average abundance of 14%), Chloroflexi (with an average abundance of 10%) and Gemmatimonadetes (with an average abundance of 5%), accounting for 90% of the total obtained reads (Fig. 2). In addition, we also obtained some phyla in all soils at low relative abundances (<1%), including Planctomycetes, Verrucomicrobia, Nitrospirae, Bacteroidetes, Firmicutes, Latescibacteria, Deinococcus-Thermus, Tectomicrobia, Cyanobacteria, Parcubacteria and Armatimonadetes.

Among these phyla, we found that their relative abundances were significantly related to soil chemical properties (soil pH, SOC, TN and NH4N). For example, the relative abundance of soil Alphaproteobacteria, Actinobacteria, Thermoleophilia, Acidimicrobiia, KD4-96, Nitrospira and Solibacteres were significantly related with soil pH (Table S2). These results indicated that soil pH shaped the bacterial community structure. Significant relationships were observed between soil TN and SOC and the relative abundances of Nitrospira, Actinobacteria, Spartobacteria and Subgroup_17. In addition, soil NH4N content had strong effects on soil relative abundances of Nitrospira, Actinobacteria, Spartobacteria, Subgroup_17, Alphaproteobacteria and Solibacteres (Table S2). Such strong correlations demonstrated that soil C, N and pH were important factors affecting soil bacterial community compositions. The multiple regressions showed that soil pH was the best predictor of soil community, followed by SOC (Table 2), with a relative importance of 0.58 and 0.30, respectively.

The soil bacterial community structure was clearly separated from each other from the NMDS (stress = 0.08) and Pcoa plots. There was a strong influence of soil pH on the composition of the bacterial communities across the gradient. This influence is evident from the NMDS plot ordination of the pairwise UniFrac distances that illustrate how bacterial communities of different pH align along the first dimension of the plot (Fig. 3a). A regression between the scores on the first NMDS axis (Fig. 3c; $p < 0.0001$; $r^2 = 0.79$) and the first principal coordinate axis (Pcoa1) (Fig. 3b; $p < 0.0001$; $r^2 = 0.743$) and soil pH demonstrated this finding, indicating soil pH that shaped the bacterial community structure.

3.2. Environmental factors shaping the bacterial community structure

Environmental variables had significant influences on soil the bacterial community structure. The results from the CCA indicated MAP ($r^2 = 0.4671$, $p = 0.001$), MAT ($r^2 = 0.2502$, $p = 0.047$) and Elevation ($r^2 = 0.4510$, $p = 0.003$) had significant influence on soil bacterial community structure among the climate drivers (Table S3). Among soil properties, SOC ($r^2 = 0.4173$, $p = 0.004$), TN ($r^2 = 0.3811$, $p = 0.006$), pH ($r^2 = 0.8069$, $p = 0.001$), EC ($r^2 = 0.4274$, $p = 0.032$) and NH4N ($r^2 = 0.4216$, $p = 0.006$) were important factors, especially soil

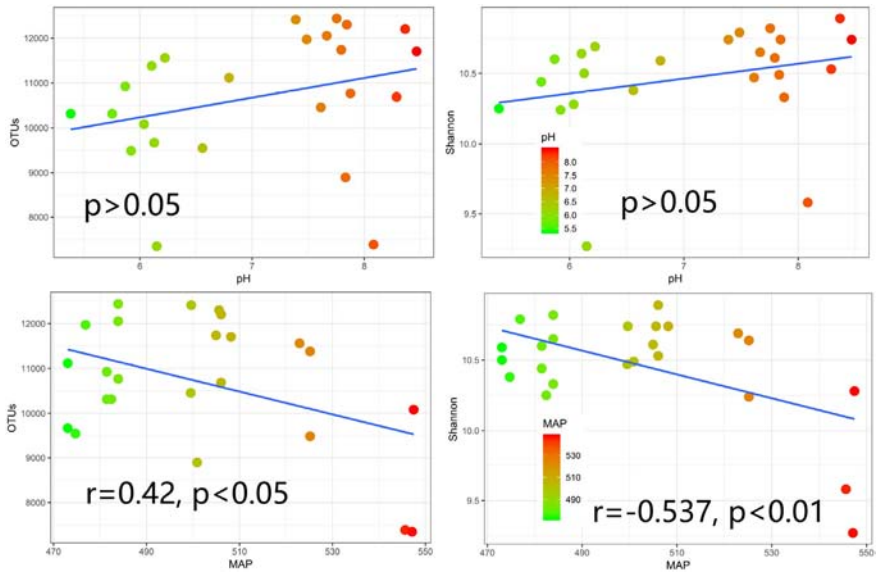

Fig. 1. The correlations between soil bacterial diversity (Shannon and OTUs) and soil pH and MAP.

pH, which had the strongest r^2 and lowest p value (Table S3). Compared with climate drivers, we found that soil properties had more important influences on soil community structure based on r^2 and p values. The correlations between first components from NMDS analysis and soil environmental variables (Table 3) confirmed that soil properties had strong effects on the soil bacterial structure.

We estimated the relationship between bacterial community similarity and geographic distance, chemical distance (soil properties distance based on Euclidean distance) and climate distance (climate factors distance based on Euclidean distance) using the distance-decay approach. Distance-decay curves revealed that soil bacterial community similarity was significantly correlated with geographic distance (historical factor) (Fig. 4), which revealed that spatial isolation affected community structure, presumably via dispersal limitations. Strong relationships were also observed between the microbial community structure and environmental distance (including soil Euclidean distance and climate Euclidean distance) similarity. These regression results indicated that soil properties were similarly based on Euclidean distance ($r = 0.265$ for Unweighted Unifrac distance, $r = 0.241$ for Weighted Unifrac distance and $r = 0.310$ for Bray distance) and had a stronger effect on soil bacterial structure than geographic distance ($r = 0.297$ for Unweighted Unifrac distance, $r = 0.185$ for Weighted Unifrac distance and $r = 0.287$ for Bray distance) and climate distance ($r = 0.230$ for Unweighted Unifrac distance, $r = 0.188$ for Weighted Unifrac distance and $r = 0.224$ for Bray distance). These results revealed that soil properties had more important influences on soil bacterial community structure.

In addition, we used variance partition analysis to dissect the relative contributions of geographic distance, edaphic factors and climate drivers of soil bacterial community structure (Fig. 3d). A total of 62% of the community variations could be explained by all measured variables. Geographic distance, climate and soil variables independently explained 20%, 9% and 25% of the community variation, respectively. There was no interaction detected between edaphic factors and climate drivers. Once the soil environmental variables were considered, geographic distance and climate drivers had lower explanations, suggesting that soils with similar environmental characteristics have similar bacterial communities regardless of geographic distance. We also used the DistLM to test relative explanations of each environmental factor on the soil bacterial community structure. The results showed that soil pH (23.6%, $p < 0.001$) had the greatest explanation for the bacterial

Table 1
Multiple regression model to explain variation in soil bacterial alpha diversity (Shannon).

Variables	Estimate	t value	p	R^2	RI
(Intercept)	7.692012	8.958	<0.0001	–	
MAP	0.002309	2.307	0.0258	–	0.50
pH	0.140574	2.100	0.0415	–	0.25
SOC	0.008976	1.982	0.0538	–	0.25
Total		F = 2.86	0.04759	0.16	

RI, relative importance based on the multiple regression models.

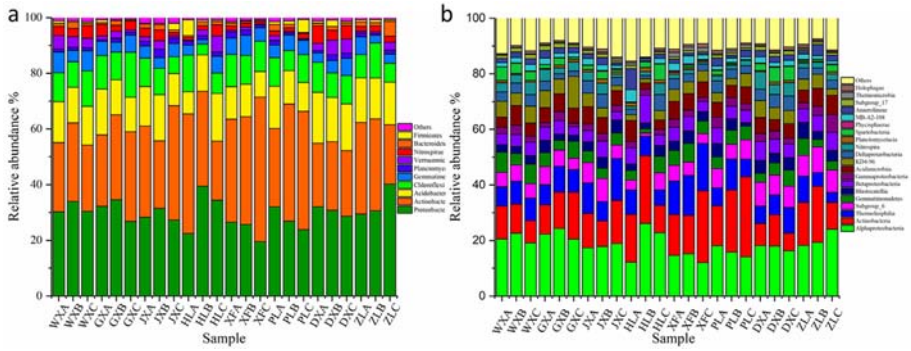

Fig. 2. The distributions of soil bacterial dominant taxa at the different sampling sites (a represents the phylum level; b represents the class level).

Table 2
Multiple regression model to explain variation in soil bacterial beta diversity using the NMDS1.

Variables	Estimate	t value	*p*	R^2	RI
(Intercept)	2.8644958	3.038	0.00404	–	
MAP	0.0023847	1.944	0.05851	–	0.05
pH	−0.5293209	−7.701	<0.0001	–	0.58
SOC	0.0112960	2.451	0.01838	–	0.30
EC	−0.0007948	−2.881	0.00616	–	0.07
Total		F = 56.81	<0.0001	0.84	

Table 3
The correlations between environmental variables and the first component from NMDS.

Variables	Weighted NMDS1	Unweighted NMDS1
MAP	0.066	0.017
MAT	0.105	−0.029
Elevation	−0.548**	−0.410*
SOC	−0.570**	−0.516**
TN	−0.549**	−0.482*
TP	−0.05	−0.128
pH	0.696**	0.889**
EC	0.587**	0.528**
NO3N	−0.29	−0.321
NH4N	−0.612**	−0.649**
AVP	−0.38	−0.318

* indicate significant correlations at 0.05 level; **indicates significant correlations at 0.01 level.

community variation, followed by climate factors (elevation, 8.6%, $p < 0.001$; MAT, 4.6%, $p < 0.05$; MAP, 4.5%, $p = 0.023$) (Table 4).

4. Discussion

4.1. Effects of soil properties in shaping bacterial community structure

The predominant role of soil pH in shaping bacterial structure has recently been demonstrated by many researchers at different scales and in different ecosystems (Chu et al., 2010; Lauber et al., 2009; Shen et al., 2013; Tian et al., 2018). The soil pH was found to be the most important factor shaping soil bacterial community structure with in a range of 3.7 pH units. The different bacterial communities across the pH gradient were clearly related to shifts in the relative abundances of bacterial taxa at the phylum and class level.

In accordance with previous study, there was a significant negative relationship between soil pH and the relative abundance of Acidobacteria, whereas a positive relationship was observed between soil pH and the relative abundance of Actinobacteria (Shen et al., 2013). In this study, Nitrospirae were more abundant in lower pH (5–6) soils. However, some studies reported that soil pH (4–7) had positive effects on the relative abundance of Nitrospirae (Chu et al., 2010; Tian et al., 2018; Zhalnina et al., 2015; Zhou et al., 2015), which was not in accordance with the findings of this study. In addition, Zhou et al. (2015) found that Proteobacteria was more abundant in the lower pH (4–5) soils with a strong correlation ($r = -0.97$, $p < 0.001$) which was in accordance with this study's findings

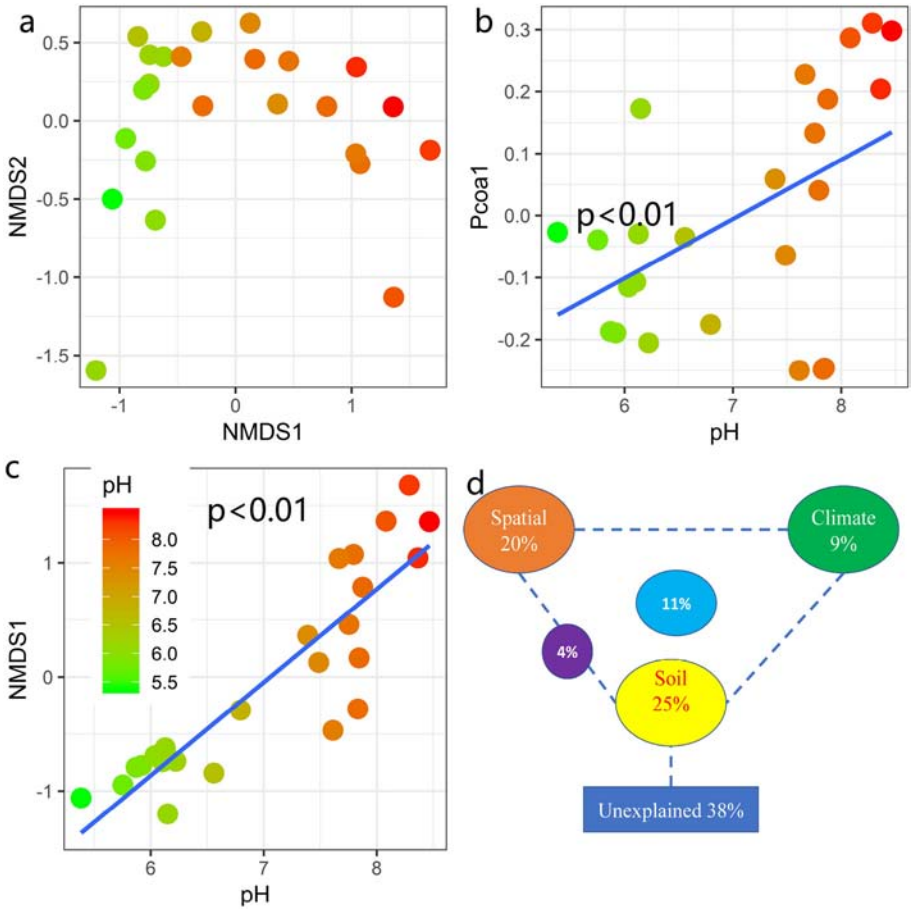

Fig. 3. Non-metric multidimensional scaling (NMDS) plot based on the Unweighted Unifrac distance matrix with symbols coded by pH gradient (a). The first component from a principal coordinate analysis of the Bray distances (b) and Unweighted Unifrac distance (c) regressed against soil pH using a linear function. Variation partitioning analysis of the bacterial community explained by climate and spatial factors and soil factors and their interactions (d).

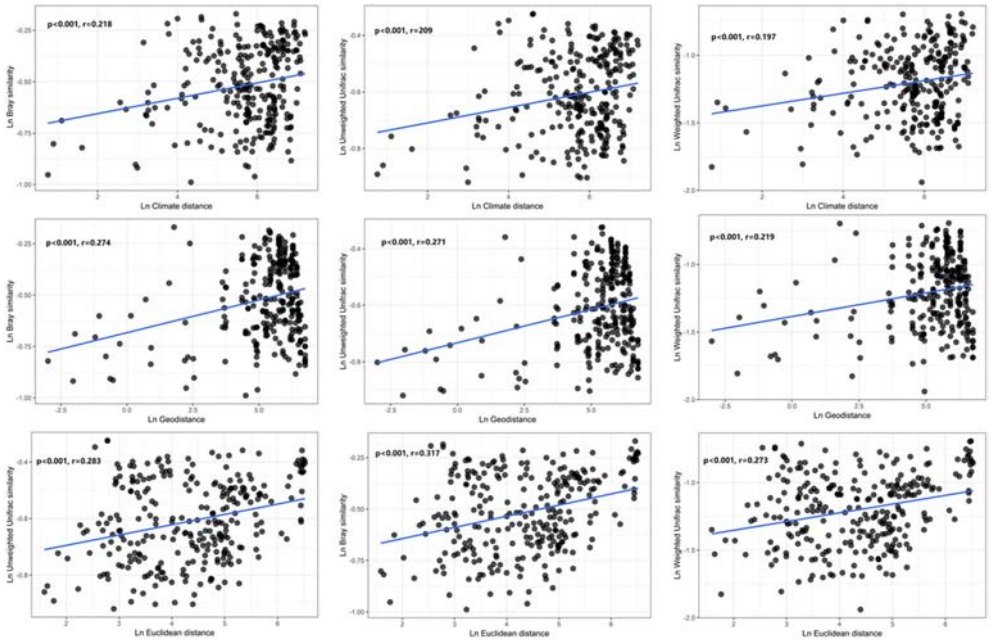

Fig. 4. Distance decay curves of the bacterial community similarity and geographic distances and environment similarity based on Euclidean distances.

($r = -0.51$, $p < 0.05$). The most likely mechanism of soil pH affecting soil microbial community compositions is via regulating the availability of soil nutrient (.i.e., N, Ca, Mg and Al) (Ma et al., 2018; Pan et al., 2014). The strong relationships between soil pH and SOC, TN and ammonium N confirmed that soil pH influenced the soil bacterial community via soil C and N availability. Another mechanism of the soil pH driving the variations in soil bacterial communities is that most bacteria exhibited a relative narrow growth tolerance (Rousk et al., 2010). From a culture study, individual bacterial species had a narrow pH range, and the variation was usually 3–4 pH units (Rosso et al., 1995). This narrow pH optima may explain the different correlations between bacterial community compositions and soil pH. Such results have confirmed that soil pH is a critical driver of soil bacterial community structure at different scales and in different ecosystems (Chu et al., 2010; Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010; Zhalnina et al., 2015; Zhou et al., 2015).

This shift in the relative abundances of specific taxonomic groups across this SOS gradient are similar to the findings of other studies. In this study, SOC strongly affected soil bacterial structure, which was consistent with the studies reported by Tian et al. (2018) and Liu et al. (2014). We observed that the relative abundances of Actinobacteria, Nitrospirae, Verrucomicrobia, Acidobacteria and Armatimonadetes had significantly positive or negative relationships with SOC in forest soils (Table S2). Similarly, some studies reported that SOC shaped the variations in the soil bacterial community in tropical agricultural soils (Sul et al., 2013) and balk soils (Liu et al., 2014). The difference lies in the positive or negative relationship between Actinobacteria and organic carbon. Liu et al. (2014) reported that Actinobacteria was more abundant in the higher C content soils; however, in our study we found that Actinobacteria are more favorable in lower C content soils which is consistent with the study conducted by Sul et al. (2013). The opposite patterns observed in response to soil organic carbon are mainly because of the ranges of soil organic carbon content in these studies (Liu et al., 2014).

In addition, soil N content (TN and ammonium N) was significantly correlated with some phyla. This finding supported the hypothesis that soil nutrients (e.g., SOM and N contents) are the most important soil variables influencing the bacterial community composition (Delgado-Baquerizo et al., 2016; Zeng et al., 2017; Zhang et al., 2016a). Our results showed that the relative abundance of Actinobacteria significantly decreased with SOC, TN and ammonium N content, which differed from a study of long term fertilization of black soil in northeastern China (Zhou et al., 2015).

Acidobacteria are considered to be oligotrophic bacteria and are more abundant in lower nutrient soils (Fierer et al., 2007) which is not consistent with the findings of this study. Proteobacteria are copiotrophic bacteria and are usually found in nutrient-rich conditions (Fierer et al., 2007; Newton and Mcmahon, 2011; Wang et al., 2017). The life strategy of soil bacterial groups may also explain these differences. In our study, Acidobacteria preferred to live in the higher SOC and TN forest soils and Proteobacteria were more abundant in lower pH soils. Soils in our study had a much higher SOC (mean = 28.3 g/kg) and TN (mean = 2.27 g/kg), which may explain the correlations between the relative abundances of Proteobacteria with SOC and TN. When soil has sufficient C and N availability, the copiotrophic bacteria (Proteobacteria) may be affected by other environmental variables (pH). Soil pH is the most important factor affecting soil bacterial compositions. In addition, copiotrophic bacteria and oligotrophic bacteria were classified at the coarse taxonomic level, and their relationships with nutrients depend on the levels and availability of nutrients in soils, which may cause inconsistent results. Therefore, other soil nutrients and

Table 4
DistLM analysis identified the relative importance of environmental variables on soil bacterial variations when all the variables combined in sequence.

Variable	Adj R^2	P	Prop.	Cumul.
pH	0.20141	0.001	23.6%	23.6%
Elevation	0.25968	0.001	8.8%	32.4%
EC	0.27791	0.03	4.8%	37.2%
MAT	0.29568	0.026	4.6%	41.8%
MAP	0.31423	0.023	4.5%	46.3%
NO3N	0.33206	0.035	4.3%	50.6%
AVP	0.33737	0.279	3.3%	53.9%
TN	0.34287	0.291	3.2%	57.1%
SOC	0.35802	0.103	3.8%	60.9%
TP	0.36296	0.325	3.1%	64.0%

Prop: percentage variance explained by specific variable; Cum.: cumulative percentage of variance explained.

variables should be taken into account when analyzing the environmental contributions to the bacterial community structure in a future study. Thus, the pattern of soil bacteria in response to SOC is not uniform, and it depends on the specific range of SOC.

4.2. Effects of climate variables and geographic distance in shaping bacterial α diversity and community structure

In addition to edaphic factors, we also noted significant correlations between bacterial diversity and other measured variables (climatic variables and geographic distance) as well as significant interactions between variables. These results suggest that bacterial diversity in the forest soils across a large spatial scale are a result of multiple complicated ecological processes. One of our hypotheses is that bacterial diversity differs across different climate factors and soil properties, but only MAP significantly shapes α-diversity patterns. Here, this study indicated that bacterial diversity and phylotype richness were significantly correlated with MAP across the east-west transect on the Loess Plateau. These results from the analysis using the multiple regression model confirmed that MAP was the main driver controlling soil bacterial Shannon diversity which is in line with a global study conducted by (Bahram et al., 2018). We also found that MAP controlled soil bacterial Shannon diversity using a multiple regression model. However, Shen et al. (2013) reported that MAP (ranging from 632 to 1154 mm) was not significantly correlated with bacterial α-diversity in the Changbai Mountain, which is inconsistent with this study's findings. These differences may be caused by the ranges of MAP. MAP can affect soil bacterial community compositions either directly or indirectly through affecting plant community composition and soil nutrient availability. For example, in a dynamic study assessing variations in soil bacterial communities during wet-up of dry soils, the results demonstrated that soil bacterial communities changed remarkably (Barnard et al., 2013).

In addition, we found soil C, N and P contents had no significant effects on soil bacterial α-diversity, which was not consistent with other related studies. For instance, Delgado-Baquerizo et al. (2016) reported that climate features, soil pH and SOC content drive the geographic patterns of soil bacterial α-diversity based on a meta-analysis of 600 soil samples at the global scale (Delgado-Baquerizo et al., 2016). However, soil C and N contents affected the relative abundance of bacterial groups. This observation may be explained by the life-strategy of bacteria.

Climate has been considered as an important driver of soil bacterial community structure across regional and large scale studies (Bahram et al., 2018; Fierer and Jackson, 2006; Tian et al., 2018; Zhang et al., 2016b). Similarly, our findings indicate MAT and MAP have significant influences on the soil bacterial community structure. Temperature and precipitation are two main factors directly or indirectly affecting the soil bacterial community. For example, MAT can directly affect microbial metabolic rates (Zhou et al., 2016). Moreover, higher precipitation may enhance the soil organic matter decomposition rate, leading to lower soil organic matter availability and subsequently affecting the soil bacterial community structure (Tian et al., 2018). Therefore, the main mechanism of MAP and MAT affecting the soil bacterial community structure is via changing the availability of soil nutrients and the plant traits (Tian et al., 2018).

We used a distance-decay method to test the influences of historical contingencies (dispersal limitation) and environmental heterogeneity on soil bacterial community structure, which are considered to be two important driving factors in shaping bacterial structure (Martiny et al., 2006; Ramette and Tiedje, 2007). A significant correlation between bacterial community dissimilarity and geographical distance (Fig. 4) suggests that historical contingencies are also an important driver of changes in bacterial communities across this 800-km transect line. Similar studies have been conducted and concluded that historical contingencies strongly affect the variations of soil bacterial communities (Chu et al., 2010; Liu et al., 2014; Martiny et al., 2006; Talbot et al., 2014; Wang et al., 2015). However, some studies showed that environmental factors were more important than geographic distance (Liu et al., 2018; Xia et al., 2016). For example, soil bacterial composition is strongly associated with soil pH and mean annual precipitation at the global scale (Bahram et al., 2018). These inconsistent results may be attributed to differences in the study scales (Hendershot et al., 2017; Tian et al., 2018). Our result was in accordance with previous studies that revealed that historical contingencies (edaphic properties) drive soil bacterial biogeography (Martiny et al., 2006; Tian et al., 2018).

Nevertheless, our results provide strong evidence that environmental factors, such as soil pH, are more important than other factors in influencing the continental-scale spatial structuring of microbial communities at higher taxonomic levels. This observation implies that pH is a greater driver of bacterial community composition than dispersal limitation and other environmental factors that vary between biomes.

5. Conclusions

In summary, this study is the first attempt to comprehensively investigate the geographic patterns of forest soil bacteria under multiple environmental gradients along the east-west transect in arid areas (northwestern China). Our study reveals that climate factor (MAP) is the most important factor influencing bacterial α-diversity (Shannon diversity and OTU richness) on the Loess Plateau. Other climate variables (MAT and elevation) and edaphic properties exhibited no significant correlations with bacterial α-diversity. The structure of the forest soil bacterial community in the arid areas was strongly shaped by soil pH. Climate variables had lower influences on soil bacterial community structure compared with soil properties and geographic distances. This finding corroborates the previously reported results that pH has emerged as the dominant factor driving global patterns of bacterial biogeography (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010). These results provide a better understanding of the link between soil bacterial diversity and ecological ecosystem services and provide a framework to predict microbial continental-scale responses under future climate change.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.04.184.

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