Drivers of Soil Bacterial Diversity in Sandy Grasslands in China

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Abstract

Bacteria constitute great abundances and groups on Earth and control many important processes in terrestrial ecosystems. However, our understanding of the interactions between soil bacteria and environmental factors remains limited, especially in sensitive and fragile ecosystems. In this study, geographic patterns of bacterial diversity across the four sandy grasslands along a 1600 km north-south transect in northern China were characterized by high-throughput 16S rRNA gene sequencing. Then, we analyzed the driving factors behind the patterns in bacterial diversity. The results showed that of the 21 phyla detected, the most abundant were Proteobacteria, Actinobacteria, Acidobacteria and Firmicutes (average relative abundance > 5%). Soil bacterial α diversity, calculated as the bacterial phylotype richness and Faith's phylogenetic diversity, was highest in the Otingdag Sandy Land and lowest in the Mu Us Sandy Land. Soil EC was the most influential factor driving bacterial α diversity. The bacterial communities differed significantly among the four sandy grasslands, and the bacterial community structure was significantly affected by environmental factors and geographic distance. Of the environmental variables examined, climatic factors (MAT and MAP) and edaphic properties (pH and EC) explained the highest proportion of the variation in bacterial community structure. Biotic factors such as plant species richness and aboveground biomass exhibited weak but significant associations with bacterial α diversity. Our findings revealed the important role of climate and salinity factors in controlling bacterial diversity; understanding these roles is critical for predicting the impacts of climate change and promoting sustainable management strategies for ecosystem services in these sandy lands.

Introduction

Soil microbes have great abundances and groups on Earth, and control many important processes in terrestrial ecosystems[1]. Microbe spatial patterns and their driving forces have received much attention in recent years and are important subjects in community ecology. Numerous studies have reported patterns in the diversity and community structure of soil microbes at different scales in different ecosystems[2–5]. Several studies have demonstrated large-scale patterns of soil microbes in China, for instance forest[5], alpine grassland[6] and agricultural ecosystems[7]. However, little is known about the geographic patterns of soil microbial diversity and factors controlling that diversity in sandy land ecosystems. There are four sandy lands in China, i.e., Hulun Buir, Horqin, Otindag and Mu Us, and they account for approximately 1.7% of the land area in China. Investigating the driving factors of soil microbial diversity in these sensitive and fragile ecosystems is essential due to their importance to nutrient cycling, climate change resistance and human activities.

The patterns of microbial diversity are influenced by a wide range of biotic and abiotic factors. At small scales, the patterns of the microbial community are influenced by the nutrient status[8], texture[9], moisture 4, and pH of soil[2,10]. Microbial communities have also been found to be controlled by climatic features, geographic distance, and vegetation and soil properties at large spatial scales[5,11,12]. Generally, these driving factors vary with the scales and ecosystems studied. However, no study has investigated how soil

microbial communities are organized in the soils of the four sandy grasslands in China until now. In summary, soil microbial communities are closely correlated with abiotic variables, such as climate and edaphic properties[6,13], and biotic variables, namely plant diversity[14]. Studying microbial diversity and community structure and their relationships with environmental factors would expand our knowledge about the factors that regulate Earth's biodiversity and biogeochemistry.

Additionally, most studies in sandy lands have been performed at the site scale and in limited areas[15,16]. A comprehensive analysis of the effects of environmental variation on microbial diversity and community structure across the four sandy grasslands is still lacking. In the present work, bacterial diversity and community structure were estimated based on high-throughput sequencing data, and their relationships with environmental variables were analyzed. We aimed to (i) explore and compare the bacterial diversity and community structure patterns among the four sandy grasslands, and (ii) identify the main environmental factors controlling the patterns in the bacterial community.

Results

Patterns of taxa and phylotypes

A total of 4,307,217 high-quality sequences were obtained from the 56 soil samples. The sequences were clustered into 3,097 OTUs at the 97% similarity level. Across all samples, 21 bacterial phyla were identified in all samples at an equivalent sequencing depth (26,600 sequences per sample). The dominant bacterial phyla across the four sandy grasslands were Proteobacteria (39.42%), Actinobacteria (27.12%), Acidobacteria (9.81%) and Firmicutes (7.47%) (relative abundance > 5%) (Fig. 1). Bacteroidetes, Chloroflexi, Gemmatimonadetes, and Cyanobacteria were identified at relatively low abundances, accounting for 11.38% of the obtained bacterial sequences, and the other 12 phyla (Verrucomicrobia, Nitrospirae, Armatimonadetes, Rokubacteria, Fibrobacteres, Melainabacteria, Deinococcus-Thermus, Latescibacteria, Planctomycetes, Fusobacteria, Parcubacteria, unidentified_Bacteria) detected were even rarer.

Relationships between bacterial α diversity and climate, vegetation and edaphic factors

The bacterial α diversity (number of observed OTUs and Faith’s phylogenetic diversity) changed significantly across the four sandy grasslands. The observed bacterial phylotype richness and Faith’s phylogenetic diversity were the highest in the Otindag Sandy Land, followed by the Horqin Sandy Land and the Hulun Buir Sandy Land, and were the lowest in the Mu Us Sandy Land (Fig. 2).

The bacterial phylotype richness and Faith’s phylogenetic diversity in the four sandy grassland soils were both significantly correlated with soil EC and PR (Table S1). The bacterial phylotype richness was also significantly correlated with soil pH and AB, while Faith’s phylogenetic diversity was significantly

correlated with MAP. Among all 10 environmental variables examined, EC was the most influential factor influencing the bacterial phylotype richness and Faith's phylogenetic diversity. The results of the stepwise regression further confirmed the best prediction of EC for both phylotype richness and Faith's phylogenetic diversity. The variation in EC explained 45.6% and 48.4% of the variation in phylotype richness and Faith's phylogenetic diversity, respectively (Table 1).

Correlations of bacterial β diversity with geographical distance and environmental factors

NMDS showed that the soil samples from the four sandy grasslands were clearly separated from each other, revealing that the structure of bacterial communities differed significantly across the four sandy grasslands (Fig. 3). This finding was further confirmed by the results of ANOSIM, MRPP and PERMANOVA (Table 2). The structure of soil bacterial communities was significantly related tospatial factors (geographic distance) and climate factors (MAT, MAP) (Fig. 4), suggesting that spatial isolation and climate characteristics affected the degree of similarity between bacterial communities. Significant correlations were also observed between the structure of bacterial communities and salinity factors (pH and EC) (Table S2). The results of DistLM showed that climate factor (MAT, 6.37%, $p < 0.001$) explained the largest proportion of the variation in the bacterial community structure, followed by SOM (5.50%, $p < 0.001$) and salinity factors (EC, 4.17%, $p < 0.001$; pH, 3.01%, $p < 0.001$). Climate factor, namely MAP ($p < 0.01$; Table 3), also played an important role in explaining the variation in the bacterial community structure.

Discussion

The results of this study demonstrate that a group of biotic and abiotic variables can explain the variation in soil bacterial diversity across the four sandy grasslands in China. Specifically, our results show that the soil bacterial communities differed significantly across the sandy grasslands and were mainly controlled by a suite of variables, including edaphic variables, for instance soil EC and pH; climate variables, such as MAT and MAP; and spatial variables, namely geographic distance. Surprisingly, vegetation-related variables played a minor role in explaining the variation in bacterial community diversity.

Soil properties played an important role in explaining the variation in the diversity of bacterial communities. Soil EC was the most important factor regulating diversity patterns across a large scale. This is surprising because saline soils are often controlled by EC. In this study, both bacterial phylotype richness and phylogenetic diversity decreased with increasing EC (Figure S2); however, EC was the third most important variable for β diversity. These results are in agreement with other results obtained in saline environments[27–29]. The role of soil EC in determining microbial diversity can be explained by examining the variations in the relative abundances of specific taxonomic groups, especially the dominant groups. For example, in this study, the relative abundance of Actinobacteria decreased with increased EC, but the relative abundance of Proteobacteria increased (Table S3). Proteobacteria and

Actinobacteria accounted for 66.56% of the obtained bacterial sequences. This indicates that salinity is highly influential not only in saline soils but also in nonsaline soils.

Soil EC is not the only stressor for soil biota in desert environments and is often accompanied by high pH[30]. Our results indicate that soil pH was another important factor influencing the structure of soil bacterial communities at a large scale. The contribution of soil pH in regulating the structure of soil bacterial communities has also been widely estimated by previous studies[5,31–33]. The strong relationship between soil pH and the structure of microbial communities can be attributed to the interaction between pH and other environmental variables. The intracellular pH of most microorganisms is usually within 1 pH unit of neutral[34]. Any significant variation in environmental pH imposes stress on single-celled organisms, which has significant effects on microbial community composition in a variety of environments[35–37].

In addition, our findings demonstrate that soil nutrient contents, such as C,N and P, were not strong predictors of soil bacterial α and β diversity, which is consistent with the findings of a study conducted in the Loess Plateau by Zeng et al.[11]. However, this is inconsistent with the global soil bacterial diversity patterns identified in a meta-analysis of 600 soil samples[13]. This inconsistency arose because in the current study, there were no obvious differences in soil C, N and P levels across the four sandy grasslands. Thus, no significant correlations were observed between soil bacterial α and β diversity and soil C, N and P contents. However, multicollinearities were excluded from the step wise regression, and only variables that were closely related, such as soil EC, were selected, as they contributed to most of the variance.

Vegetation properties (PR and AB) exhibited weak but significant correlations with bacterial phylotype richness and phylogenetic diversity but not with bacterial community structure (Table 3; Table S1). Many studies have examined the relationships between vegetation characteristics and soil bacterial community properties; however, the results have been inconsistent. Some studies in grassland ecosystems have reported the significant effect of plant diversity on the diversity of soil microbial communities[38,39]. Others have shown that vegetation properties do not significantly affect the soil microbial community[40]. Plant diversity and productivity control the contents of limiting resources and thereby control the soil biota[41,42]. The results of this study confirm the importance of resource availability in regulating the soil microbial community in these sandy grasslands and indicate that the influences of vegetation properties on soil biodiversity in sandy grassland ecosystems should also be considered in future studies.

Climate also played an important role in explaining the variation in the structure of soil bacterial communities. Our findings reveal that MAT and MAP significantly affected the structure of soil bacterial communities. This finding is consistent with studies demonstrating the importance of MAT and MAP in influential the structure of bacterial communities at different scales[2,43], especially in arid and semiarid regions such as temperate grasslands in northern China[44]. Any change in MAT or MAP directly affects the processes of microbial metabolism[45]. Furthermore, MAT and MAP are the main factors affecting

plant community composition and AB, which determine the quantity and quality of resources available to soil bacterial communities[5,46,47]. In this sense, MAT and MAP control the structure of soil bacterial communities through their impacts on vegetation properties and soil nutrient levels.

The strong relationship between the degree of dissimilarity among bacterial communities and geographical distance indicates that the probability of dispersion is the main constrainton the spatial patterns of bacterial communities across the four sandy grasslands. The impacts of dispersal limitation in soil bacterial communities have also been extensively described in previous studies[31,48,49]. However, the results of this study are inconsistent with the results of Fierer and Jackson[2], who quantitatively compared the diversity and structure of bacterial communities across North and South America, and found that there was no significant relationship between the degree of dissimilarity among soil bacterial communities and geographic distance. This discrepancy may be attributed to the different scales of that study and our study[50,51]. Our results are in agreement with those of Martiny et al.[52], who suggest that the effects of dispersal limitation on the biogeography of soil bacterial communities are most likely to be observed at intermediate scales (10–3,000 km).

In summary, this work is the first attempt to comprehensively detect the geographic patterns of soil bacteria across the four sandy grasslands in northern China. A combination of biotic and abiotic factors controlling soil bacterial α and β diversity were identified. Our study provides evidence for the influence of soil EC on the diversity of bacterial communities, which is often neglected in nonsaline soils. Soil pH was another important predictor of the structure of soil microbial communities. Geographic distance and climate factors (MAT and MAP) explained the largest proportion of the variance in the structure of soil microbial communities. To improve our knowledge about the dynamics of these sensitive and fragile ecosystems, future efforts should be put into exploring the general patterns that microbes respond to climate change and human activities. The results of this study strengthen our knowledge about the processes that generate and maintain microbial biogeographic patterns.

Materials And Methods

Study area and field sampling

This study was performed at 19 sites in the four sandy grasslands in China (107.5°E to 120.7°E, 37.2°N to 49.2°N; Figure S1). There were 6 sites in the Hulun Buir Sandy Land, 5 sites in the Horqin Sandy Land, 5 sites in the Otindag Sandy Land and 3 sites in the Mu Us Sandy Land. The mean annual temperature (MAT) ranged from − 0.85 to 8.25 °C, and the mean annual precipitation (MAP) ranged from 290 to 439 mm.

Field sampling was conducted in August 2018 during the period with the highest vegetation productivity. Fifty-six soil samples (0–10 cm; 3 samples per site except at one ofthe HulunBuir Sandy Land) were collected. At each site, three plots (1×1 m^2) were established at least 100 m apart. Within each plot, all plant species were identified to the species level. The aboveground biomass of each species was

determined by clipping the plants at ground level, drying them at 65 °C for 72 h, and then weighing them[17]. In addition, 5 soil corsamples (3.5 cm in diameter) were collected within each plot. The samples were combined and then divided into two portions in the field. One portion was stored in polyethylene bags, placed in a portable refrigerator and then refrigerated at − 20 °C in the laboratory until processed for molecular analysis; the other was air-dried for the determination of its edaphic physicochemical properties.

Climate, Vegetation And Edaphic Properties

Climate factors, such as MAT and MAP, were collected from the WorldClim database[18] (www.worldclim.org).

Soil organic matter (SOM) was determined by the $K_2Cr_2O_7$-H_2SO_4 oxidation method. Total nitrogen (TN) was measured using an elemental analyzer (VarioMacro Cube elementar, Germany). Total phosphorus (TP) was examined using the Mo-Sb colorimetric method after H_2SO_4-H_2O_2 digestion. Available phosphorus (AP) was measured by the Mo-Sb colorimetric method after extraction with 0.5 M $NaHCO_3$. Electrical conductivity (EC) and pH were measured in 1: 5 and 1: 2.5 soil-water extracts, respectively.

Plant species richness (PR) was expressed as the total number of species[18]. The total aboveground biomass (AB) was estimated as the sum of each species' biomass[18].

Soil Dna Extraction, Pcr And 16s Rrna Sequencing

DNA from each sample was extracted from 0.5 g fresh soil using a magnetic bead DNA extraction kit (Tiangen Biochemical Technology Co. Ltd., Beijing, China). The primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) were used to amplify the V3–V4 hypervariable regions ofthe 16S rRNA genes in bacteria. PCRs were conducted in a 30 μl mixture containing 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 1 μl of each primer (10 μM), and ~ 10 ng of template DNA (gDNA). Thermal cycling was performed at 95 °C for 5 min, followed by 34 cycles at 91 °C for 1 min, 57 °C for 45 s, 72 °C for 1 min and finally at 72 °C for 10 min. Sequencing was carried out on an Ion S5 XL platform (Thermo Fisher Scientific) with single-end reads at Novogene Bioinformatics Technology Co. Ltd., Beijing, China.

The raw sequences were first trimmed using Cutadapt to remove short and low-quality sequences (version 1.9.1, http://cutadapt.readthedocs.io/en/stable/). Chimera removal was conducted with the UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html). The high-quality sequences were clustered into operational taxonomic units (OTUs) at the 97% similarity level using UPARSE (version 7.0.1001, http://drive5.com/uparse/). OTUs containing fewer than 100 reads were removed; the representative sequences for the remaining OTUs were assigned by comparison with the

SILVA database (http://www.arb-silva.de/) using Mothur[19]. Sequences were rarefied at 26,600 sequences per sample.

Statistical analysis

The bacterial phylotype richness was calculated as the number of OTUs, while phylogenetic diversity was determined with Faith's index[20]. Pearson correlation was performed to assess the associations between bacterial phylotype richness and phylogenetic diversity and the measured environmental variables using DPS software[21]. To avoid collinearities among the environmental variables, the most discriminating variables for bacterial phylotype richness and diversity were selected by stepwise multiple regression analysis. A Mantel test was also performed with PCORD 5.0to relate the structure of bacterial communities based on Bray-Curtis distance to the environmental variables. The importance of each environmental variable to bacterial community structure was estimated with a distance-based multivariate linear model (DistLM) with the 'forward3' procedure in Primer 7[22].

Nonmetric multidimensional scaling (NMDS) was applied to test the variation in the bacterial community using the VEGAN package in R[23]. Analysis of similarity (ANOSIM)[24], multiresponse permutation procedure (MRPP)[25], and permutational multivariate analysis of variance (PERMANOVA)[26] were also performed to confirm the significance of differences among the bacterial communities.

Declarations

Availability of data and materials

All raw sequences have been deposited at the NCBI Sequence Read Archive (SRA) the accession number PRJNA667483.

Declaration of competing interest

The authors declare that they have no competing interests.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the author.

References

1. Maestre, F. T. *et al.* Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proceeding of the National Academy of Sciences of the United States of America. 112, 15684–15689(2015).
2. Fierer, N. & Jackson, R. B. The diversity and biogeography of soil bacterial communities. Proceeding of the National Academy of Sciences of the United States of America. 103, 626–631(2006).
3. Lozupone, C. A. & Knight, R. Global patterns in bacterial diversity. Proceeding of the National Academy of Sciences of the United States of America. 104, 11436–11440(2007).
4. Bastida, F. *et al.* Differential sensitivity of total and active soil microbial communities to drought and forest management. *Globle Change Biology.* **23**, 4185–4203 (2017).
5. Tian, J. *et al.* Soil organic matter availability and climate drive latitudinal patterns in bacterial diversity from tropical to cold temperate forests. *Funct. Ecol.* **32**, 61–70 (2018).
6. Jing, X. *et al.* The links between ecosystem multifunctionality and above- and belowground biodiversity are mediated by climate. *Nature Communication.* **6**, 8159 (2015).
7. Jiao, S. & Lu, Y. Soil pH and temperature regulate assembly processes of abundant and rare bacterial communities in agricultural ecosystems. *Environ. Microbiol.* **22**, 1052–1065 (2020).
8. Koorem, K. *et al.* Soil nutrient content influences the abundance of soil microbes but not plant biomass at the small-scale. *Plos One.* **9**, e91998 (2014).
9. Xia, Q., Rufty, T. & Shi, W. Soil microbial diversity and composition: Links to soil texture and associated properties. *Soil Biology Biochemistry.* **149**, 107953 (2020).
10. Nacke, H. *et al.* Fine spatial scale variation of soil microbial communities under European beech and Norway spruce. *Frontior Microbiology.* **7**, 2067 (2016).
11. Zeng, Q., An, S., Liu, Y., Wang, H. & Wang, Y. Biogeography and the driving factors affecting forest soil bacteria in an arid area. *Science of the Total Environment.* **680**, 124–131 (2019).
12. Wang, X. B. *et al.* Habitat-specific patterns and drivers of bacterial β-diversity in China's drylands. *ISME Journal.* **11**, 1345–1358 (2017).
13. Delgado-Baquerizo, M. *et al.* Carbon content and climate variability drive global soil bacterial diversity patterns. *Ecol. Monogr.* **86**, 373–390 (2016).
14. Chen, Y. L. *et al.* Plant diversity represents the prevalent determinant of soil fungal community structure across temperate grasslands in northern China. *Soil Biology Biochemistry.* **110**, 12–21 (2017).
15. Wang, S. *et al.* Dominant plant species shape soil bacterial community in semiarid sandy land of northern China. *Ecology and Evolution.* **8**, 1693–1704 (2018).
16. Yan, R. & Feng, W. Effect of vegetation on soil bacteria and their potential functions for ecological restoration in the Hulun Buir Sandy Land, China. *Journal of Arid Land.* **12**, 473–494 (2020).
17. Feng, P. C. C., Liu, Q., Li, J. L., Li, Y. L. & Yu, X. Y. Community structure of grassland ground-dwelling arthropods along increasing soil salinities. *Environmental Science and Pollution Research.* **25**, 7479–7486 (2018).

18. Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology.* **25**, 1965–1978 (2005).
19. Schloss, P. D. *et al.* Introducing mothur: open source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology.* **75**, 7537–7541 (2009).
20. Faith, D. P. Conservation evaluation and phylogenetic diversity. *Biol. Conserv.* **61**, 1–10 (1992).
21. Tang, Q. Y. & Zhang, C. X. Data processing system (DPS) software with experimental design, statistical analysis and data mining developed for use inentomological research. *Insect Sci.* **20**, 254–260 (2013).
22. Freedman, Z. & Zak, D. R. Soil bacterial communities are shaped by temporal and environmental filtering: evidence from a long-term chronosequence. *Environ. Microbiol.* **17**, 3208–3218 (2015).
23. Team, R. C. R. A language and environment for statistical computing. (2014).
24. Clarke, K. R. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology.* **18**, 117–143 (1993).
25. Sickle, J. V. Using mean similarity dendrograms to evaluate classifications. *Journal of Agricultural Biological and Environmental Statistics.* **23**, 70–88 (1997).
26. Zapala, M. A. & Schork, N. J. Multivariate regression analysis of distance matrices for testing associations between gene expression patterns and related variables. Proceeding of the National Academy of Sciences of the United States of America. 103, 19430–19435(2006).
27. Zhang, K. *et al.* Salinity is a key determinant for soil microbial communities in a desert ecosystem, Msystems. 4, e00225-18(2019).
28. Herlemann, D. P. *et al.* Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME Journal.* **5**, 1571–1579 (2011).
29. Campbell, B. J. & &Kirchman, D. L. Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. *ISME Journal.* **7**, 210–220 (2013).
30. Rath, K. M. & Rousk, J. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: a review. *Soil Biol. Biochem.* **81**, 108–123 (2015).
31. Chu, H. *et al.* Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. *Environ. Microbiol.* **12**, 2998–3006 (2010).
32. Shen, C. C. *et al.* Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. *Soil Biol. Biochem.* **57**, 204–211 (2013).
33. Xiong, J. B. *et al.* Geographic distance and pH drive bacterial distribution in alkaline lake sediments across Tibetan Plateau. *Environ. Microbiol.* **14**, 2457–2466 (2012).
34. Madigan, M., Martinko, J. & Parker, J. *Brock Biology of Microorganisms* (Prentice Hall, Upper Saddle River, NJ, 1997).
35. Hornstrom, E. Phytoplankton in 63 limed lakes in comparison with the distribution in 500 untreated lakes with varying pH. *Hydrobiologia.* **470**, 115–126 (2002).

36. Bååth, E. & Anderson, T. H. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biol. Biochem.* **35**, 955–963 (2003).
37. Schnittler, M. & Stephenson, S. L. Myxomycete biodiversity in four different forest types in Costa Rica. *Mycologia.* **92**, 626–637 (2000).
38. Stephan, A., Meyer, A. H. & Schmid, B. Plant diversity affects culturable soil bacteria in experimental grassland communities. *Journal of Ecology.* **88**, 988–998 (2000).
39. Loranger-Merciris, G., Barthes, L., Gastine, A. & Leadley, P. Rapid effects of plant species diversity and identity on soil microbial communities in experimental grassland ecosystems. *Soil Biology & Biochemestry.* **38**, 2336–2343 (2006).
40. Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D. & Tilman, D. Plant diversity, microbial communities, and ecosystem function: are there any links?. *Ecology.* **84**, 2042–2050 (2003).
41. Kaspari, M., Alonso, L. & O'Donnel, S. Three energy variables predictant abundance at a geographical scale. Proceedings of the Royal Society B. 267, 485–489(2000).
42. Hooper, D. U. *et al.* Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. *Bioscience.* **50**, 1049–1061 (2000).
43. Griffiths, R. I. *et al.* The bacterial biogeography of British soils. *Environ. Microbiol.* **13**, 1642–1654 (2011).
44. Yang, H. J. *et al.* Plant community responses to nitrogen addition and increased precipitation: the importance of water availability and species traits. *Globle Change Biology.* **17**, 2936–2944 (2012).
45. Zhou, J. *et al.* Temperature mediates continental-scale diversity of microbes in forest soils. *Nature Communication.* **7**, 12083 (2016).
46. Bai, Y. F. *et al.* Primary production and rain use efficiency across a precipitation gradient on the Mongolia plateau. *Ecology.* **89**, 2140–2153 (2008).
47. Cross, W. F., Hood, J. M., Benstead, J. P., Huryn, A. D. & Nelson, D. Interactions between temperature and nutrients across levels of ecological organization. *Globle Change Biology.* **21**, 1025–1040 (2015).
48. Talbot, J. M. *et al.* Endemism and functional convergence across the North American soil mycobiome. Proceeding of the National Academy of Sciences of the United States of America. 111, 6341–6346(2014).
49. Wang, X. *et al.* Scale-dependent effects of climate and geographic distance on bacterial diversity patterns across northern China's grasslands. *FEMS Microbiology Ecology.* **91**, fiv133 (2015).
50. Ge, Y. *et al.* Differences in soil bacterial diversity: Driven by contemporary disturbances or historical contingencies?. *ISME Journal.* **2**, 254–264 (2008).
51. Hendershot, J. N., Read, Q. D., Henning, J. A., Sanders, N. J. & Classen, A. T. Consistently inconsistent drivers of patterns of microbial diversity and abundance at macroecological scales. *Ecology.* **98**, 1757–1763 (2017).

52. Martiny, J. B. *et al.* Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiolgoy.* **4**, 102 (2006).

Tables

Table 1 Model summary for the stepwise multiple regression of soil bacterial phylotype richness and Faith's phylogenetic diversity on environmental variables

	$Adj.r^2$	Contribution of the individual predictor (%)		
	Full model	EC	Plant species richness	Aboveground biomass
Richness	0.728	45.6	5.8	16.7
Diversity	0.709	48.4	12.0	1.9

EC, electrical conductivity

Table 2 Difference tests for soil bacterial community structure between different sandy grasslands

		Hulun Buir	Otindag	Horqin
ANOSIM R(*p* value)	Otindag	0.6761(0.001)		
	Horqin	0.758(0.001)	0.5629(0.001)	
	Mu Us	0.9623(0.001)	0.8599(0.001)	0.7927(0.001)
ADONIS F(*p* value)	Otindag	10.094(0.001)		
	Horqin	10.752(0.001)	5.0173(0.001)	
	Mu Us	17.702(0.001)	11.728(0.001)	11.21(0.001)
MRPP δ (*p*value)	Otindag	0.4262(0.001)		
	Horqin	0.4616(0.001)	0.4386(0.001)	
	Mu Us	0.4815(0.001)	0.4545(0.001)	0.5014(0.001)

Table 3 Results of distance-based multivariate linear models (DistLM) for soil microbial community structure

Variable	pseudo-F	*p*	Ex. Var. (%)	Cum. (%)
MAT	12.26	0.001	18.50	18.50
pH	9.85	0.001	12.77	31.27
EC	6.13	0.001	7.24	38.51
SOM	5.15	0.001	5.64	44.15
MAP	3.10	0.001	3.26	47.41
PB	1.69	0.05	1.75	49.16
TN	1.22	0.22	1.27	50.42
AP	0.93	0.55	0.96	51.38
PR	0.84	0.66	0.87	52.25
TP	0.96	0.49	1.00	53.24

MAT, mean annual temperature; MAP,mean annual precipitation; SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; AP, available phosphorus; EC, electrical conductivity; PR, plant species richness; PB, plant aboveground biomass

Figures

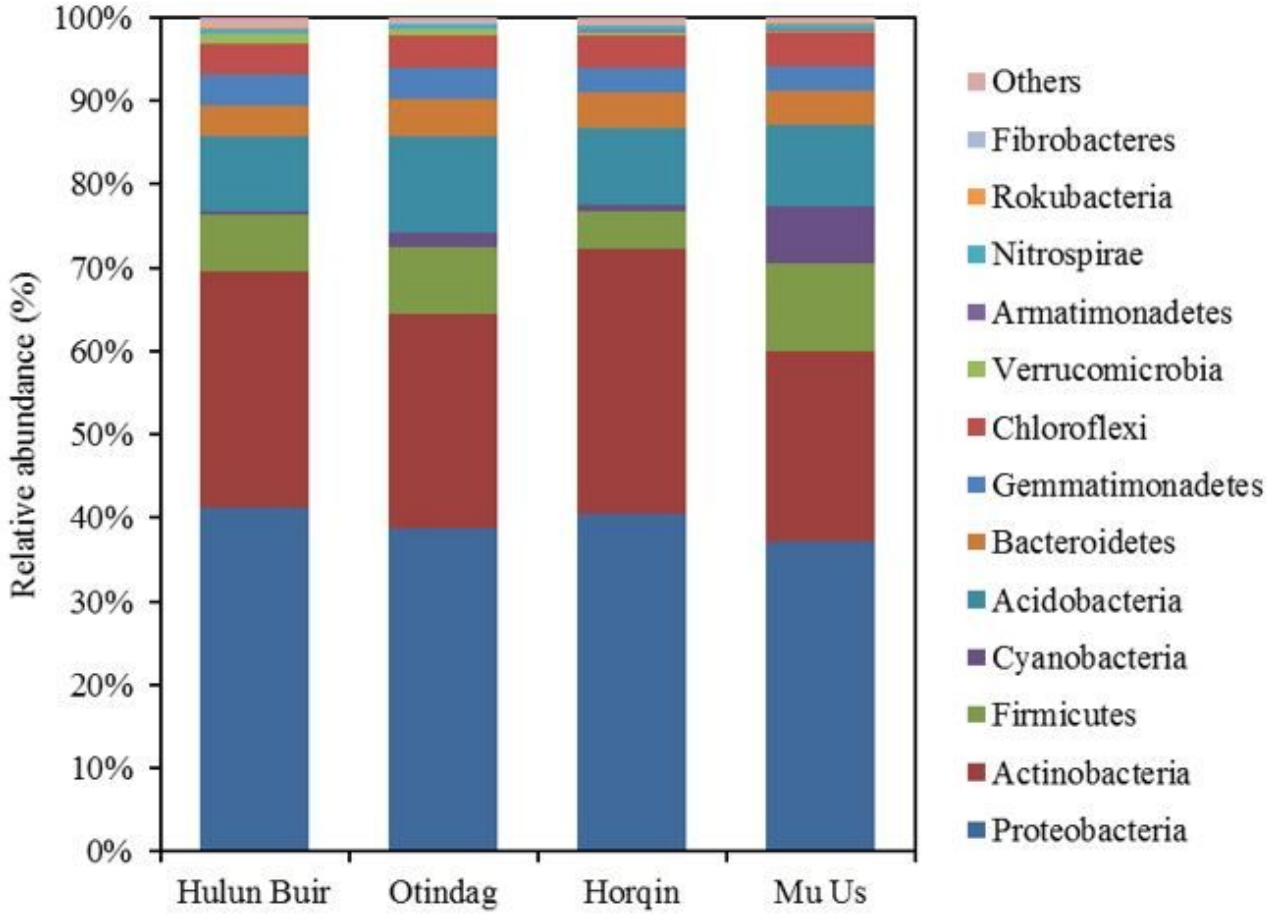

Figure 1

Relative abundances of the dominant soil bacteria groups in the four sandy grasslands in northern China

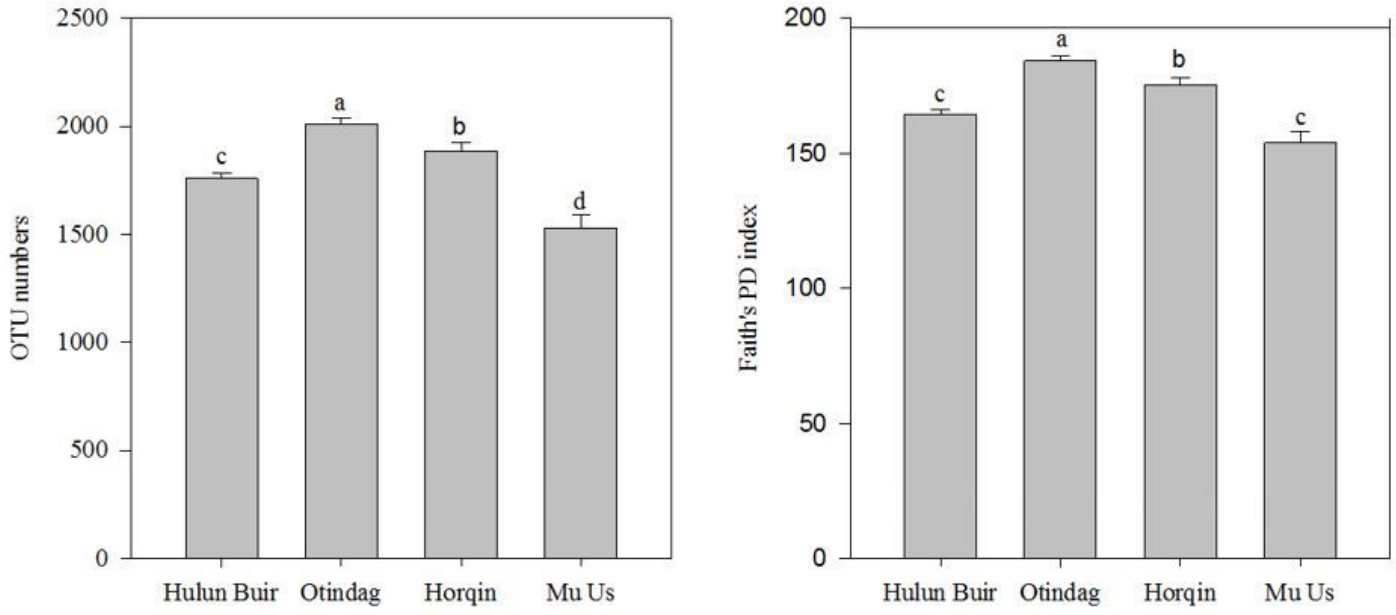

Figure 2

Soil bacterial phylotype richness and Faith's phylogenetic diversity across the four sandy lands in northern China. Means (±SE) with different letters indicate significant differences.

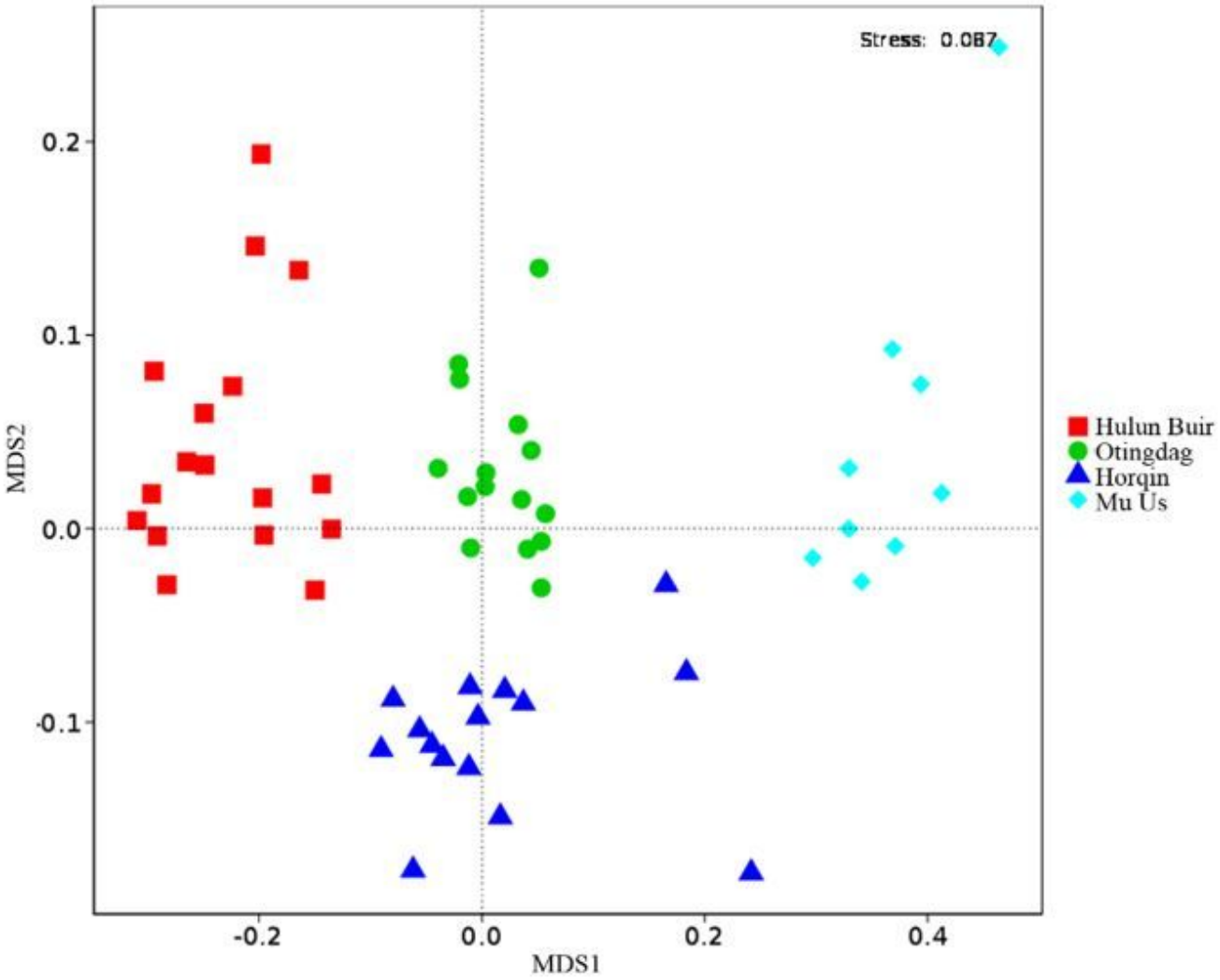

Figure 3

Nonmetric multidimensional scaling (NMDS) ordination of soil bacterial community structure based on Bray-Curtis distances in the four sandy grasslands in northern China

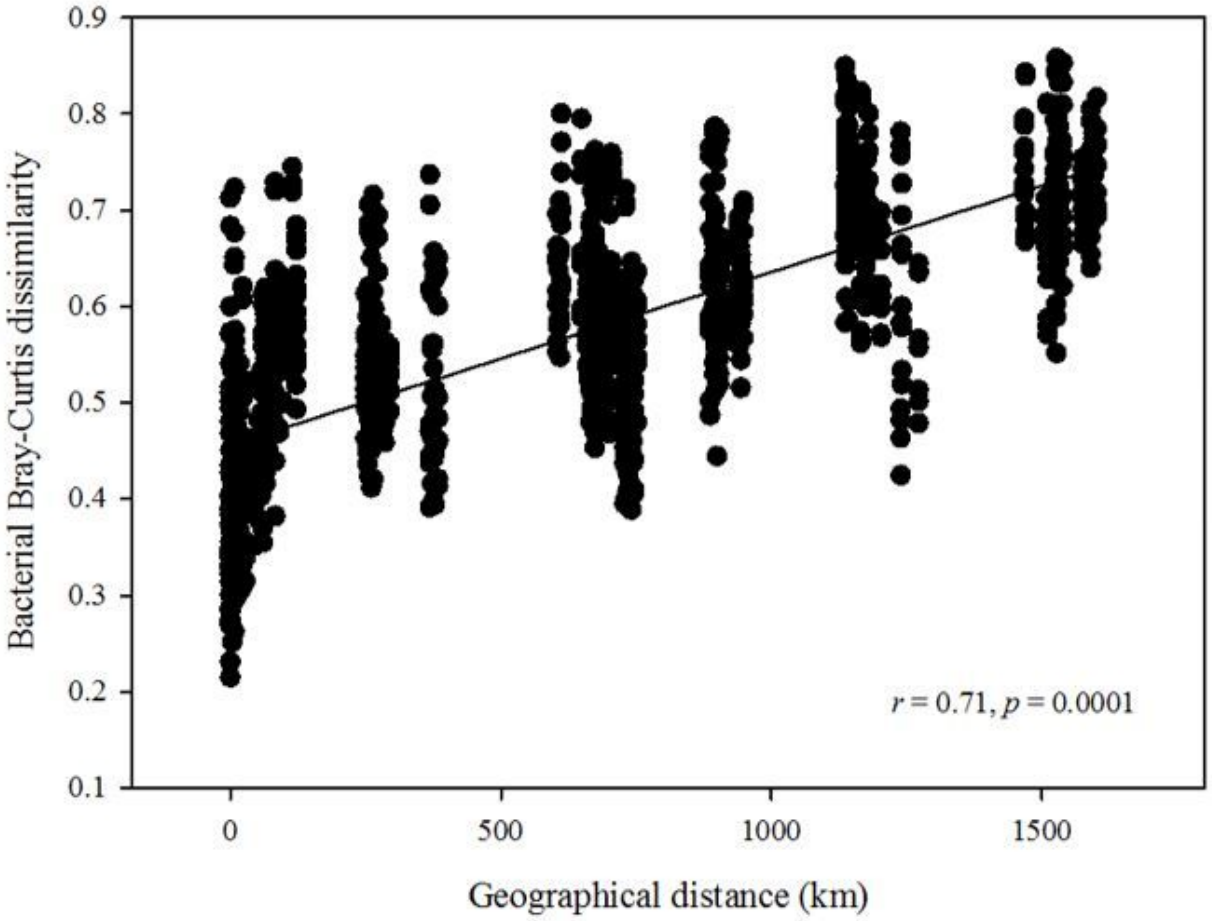

Figure 4

Correlations between soil bacterial Bray-Curtis dissimilarities and geographical distances