Pyrosequencing investigation into the bacterial communities in the Qinghai-Tibet Plateau soils associated with soil characteristic factors

Liang Cheng[1, 3, 4], Xin Wang[2] & Qingyun Guo[1, 3, 4*]

[1]*Plant Protection Institute, Qinghai Academy of Agriculture and Forestry Sciences, Qinghai University, Xining 810016, China*

[2]*Soil and Fertilizer institute, Qinghai Academy of Agriculture and Forestry Sciences, Qinghai University, Xining 810016, China;*

[3]*Key Laboratory of Agricultural Integrated Pest Management in Qinghai Province, Xining 810016, China*

[4]*Scientifc Observing and Experimental Station of Crop Pests in Xining, Ministry of Agriculture and Rural Affairs, Xining 810016, China*

E-mail: liangcheng1979@163.com; wangx710@163.com;guoqingyunqh@163.com*

*corresponding author

ABSTRACT

The Qinghai Tibet Plateau (QTP) is one of the most important regions of the earth's ecosystem that is vulnerable to climate and human activities due to its complex climate and terrain. However, knowledge about soil bacterial communities and their effect on the ecosystem within the QTP environments is still scarce. Metagenomic approaches on the structure and diversity of bacterial communities and their relationship with the environment from eighteen selected sites of the five major QTP ecosystems (gray-cinnamon soils, chernozems, castanozems, mountain meadow soils, gray desert soils) are presented in this paper. The dominant bacterial phyla in five type soils were *Proteobacteria* and *Actinobacteria*, whereas *Actinobacteria* and *Chloroflexi* predominated in gray desert soils. The bacteria diversity in castanozeras and mountain meadow soils was significantly higher than that of the other three soil types ($P < 0.05$). Phylogenetic diversity in gray desert soil was significantly lower than that of other four soil types ($P < 0.05$). Phylotype richness was the lowest in gray-cinnamon soils. There were significant correlations between the phylotype richness and soil moisture ($r = -0.578$) and potassium ($r = -0.529$). Phylogenetic diversity (PD) was significantly correlated with total organic carbon ($r = -0.548$). The redundancy analysis (RDA) showed that the diversity and composition in the bacterial communities differed greatly among the five soil types and that they were closely correlated with the soil moisture, soil organic carbon and potassium. These results indicated that the bacterial community structures of QTP soils were obviously influenced by soil characteristics and soil environmental characteristics and provided a theoretical basis for the optimal management and sustainable utilization of the QTP soil ecosystem, which is of great significance.

Keywords: bacterial community; environmental variables; high-throughput sequencing; Qinghai-Tibet Plateau; redundancy analysis

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Tengsl bakteríusamfélaga við jarðvegseiginleika á Qinghai-Tibet hásléttunni rannsökuð með hitaraðgreiningu. Qinghai Tibet hásléttan (QTP) sem er fjölbreytt að jarðvegsgerð og loftslagi, sætir miklum áhrifum vegna landnýtingar og loftslagsbreytinga. Þekking á jarðvegsbakteríum og vistfræðilegri þýðingu þeirra á hásléttunni er mjög takmörkuð. Með greiningu erfðamengja í jarðvegi var reynt að varpa ljósi á samsetningu

bakterísamfélaga á 18 völdum stöðum QTP í 5 algengustu jarðvegsgerðunum (gray-cinnamon, chernozems, castanozems, mountain meadow og gray desert soils). Ríkjandi fylkingar baktería reyndust vera *Proteobacteria*, *Actinobacteria, Actinobacteria* og *Chloroflexi*. Fjölbreytileiki baktería í „castanozeras" og „mountain meadow" soils var marktækt meiri en í öðrum jarðvegsgerðum (P < 0.05). Fjölbreytileiki baktería í „gray desert" jarðvegi var marktækt minni en í hinum fjórum jarðvegsgerðunum (P < 0.05). Skyldleikagerðir voru fæstar í „gray-cinnamon" jarðvegi. Það var marktæk fylgni fjölda skyldleikagerða baktería við jarðvegsraka (r = -0.578) og kalímagns jarðvegs (r = -0.529). Skyldleikafjölbreytileiki (PD) fylgdi marktækt heildar lífrænu kolefni jarðvegsins (r = -0.548). Tölfræðileg greining (redundancy analysis, RDA) sýndi að fjölbreytileiki og samsetning bakteríusamfélaga var mjög mismunandi eftir jarðvegsgerðum þar sem miklvægustu fylgnibreytur reyndust vera jarðvegsraki, ásamt heildamagni lífræns kolefnis og kalímagn jarðvegsins. Niðurstöðurnar benda því til að bakteríuvist jarðvegs QTP sé háð jarðvegsgerð og umhverfisþáttum, og að fræðileg þekking á þessum þáttum geti verið mikilvæg til að stuðla að sjálbærri nýtingu jarðvegsins.

INTRODUCTION

Soil microorganisms play a leading role in a series of key ecosystem functions and processes, such as soil organic matter regulation and nutrient cycling, and then affect plant species diversity and soil structure (Giller et al. 2004). The soils of the Qinghai-Tibet Plateau have distinguishing microbial diversity, and these microorganisms have been well adapted to the extreme environment (Duan et al. 2020). The effects of biodiversity have been caused by climate change and human activities, which means that research on the microbial diversity plays an increasingly important role in environmental monitoring (Winding et al. 2005), energy and material fluxes (Poli et al. 2017) to develop genetic studies in species conservation (Liu et al. 2004, Li & Zheng 2003, Arias et al. 2005) and other relevant factors. The unique environment in this region, abundant wildlife and their profound impact on the climate and environment of the surrounding areas have always attracted the attention of the scientific community. In the future, this region will remain a research hotspot and key area for the sustainable development of biodiversity and human-environment interaction in the world.

Considerable numbers and biodiversity of bacteria have been found in QTP soils. These studies focused on soil (Lin et al. 2012), glaciers (Chu et al. 2010, Wu XK et al. 2012), permafrost area (Zhang et al. 2013), alpine meadow (Bai et al. 2006, Djukic et al. 2010), lakes (Xiong et al. 2012), and the diversity of microorganisms and their correlation with the environment. Geographical distance and pH value have been found to be the main drivers of bacterial diversity in sediments (Xiong et al. 2012). Bacterial diversity in alpine grassland was positively correlated with plant root biomass, soil organic carbon, soil nitrogen content and aboveground biomass nitrogen and phosphorus content based on the study of 60 sample belts in the northeastern and central Qinghai-Tibet Plateau (Jing et al. 2015). Sample scale studies have shown that different vegetation types have had important effects on the bacterial diversity of alpine grassland, in which the bacterial diversity of alpine meadow was higher than that of alpine grassland (Zhang et al. 2016, Zhao et al. 2017). This is because the nutrient and water content of alpine meadow soil is higher than that of alpine grassland, and wet and fertile soil is conducive to shaping bacterial community composition (Drenovsky et al. 2004). So far, little is known about bacterial communities in the different soil ecosystems in the QTP. Conventionally, the biodiversity of QTP soil microbes can be rapidly profiled by the DNA fingerprint methods, including PCR-denaturing gradient gel electrophoresis and the clone library. However, these methods require expensive facilities and reagents and are relatively time-consuming. Currently, pyrosequencing was prevalently used in the analysis of microbial community composition in both broad and fine scales (Acosta-Martínez

et al. 2008). This method can provide a much more detailed description of the microbial communities, especially the low-abundant species, than the above traditional methods. Therefore, this method has been shown to be a very effective technique in microbial ecology research (Roesch et al. 2007).

Faced with the current anthropogenic pressure on soil ecosystems, such as the pressure caused by the intensification of agricultural and animal husbandry and climate change, it is necessary to better understand the effects of these factors in order to predict the impacts of such changes. However, the soil microorganisms in this unique soil remain relatively unexplored except for a few culturable diversity studies (Bai et al. 2006, Zhang et al. 2007).We therefore collected soil samples from five types across the QTP, and used pyrosequencing to evaluate the bacterial communities with respect to three broad and related aims: (i) to explore the taxonomic diversity of the bacteria on QTP soils, (ii) to determine key factors in shaping the bacterial communities distribution, soil environmental characteristics, and soil characteristics, and (iii) to quantify their relative importance to bacterial community variation.

MATERIALS AND METHODS
Soil Sampling and Physicochemical Analysis
This study was conducted in the northeastern part of the Qinghai-Tibet Plateau (36°03′-37°35′ N, 97°37′-102°48′ E; 2146-3815 m above sea level), located in Qinghai Province, and northwestern China. The local climate is highland continental, characterized as cold and long in winter, but warm and short in summer. The annual mean air temperature and precipitation are approximately 1.7 °C and 560 mm, respectively (Zhao et al. 2006). Soil samples of different sites were collected in August, 2015, including Gahai (GH, at Gahai), Halihatu (HLHT, at Ulan), Delhi (DLH, at Haixi), Daotanghe (DTH, at Hainan), Lajishan (LJS, at Guide), Qunjia (QJ, at Huangzhong), Datong beichuan (DT at Datong), Haiyan (HY, at Haiyan), Xihai (XH, at Haibei), Ledu putai (LD, at Ledu), Heimahe (HMH, at Hainan), Xiangpihan (XPS, at

Hainan), Huangzhong (HZ, at Huangzhong), Huangyuan shenzhong (HYSZ, at Huangyuan), Datong shuobei (DTSB, at Datong), Guide hexi (GDHX, at Guide), Chaka (CHK, at Ulan), Keke (KK, at Ulan)(Figure S1). Eighteen soil samples which represent five different types of soil in the QTP (five castanozeras [CA], five mountain meadow soils [MMS], two gray cinnamon soils [GCS], three chernozems [CH] ,and three gray desert soils [GDS]) were sampled. Eighteen 10×10 cm sampling plots with similar environmental characteristics were randomly selected within the sampled areas. Each sampling plots was separated by 10 m. At each of the eighteen sampling plots, three subsamples (approximately 1000 g each) were collected from the organic layer (15 cm deep) and mixed after the removal of visible roots and fresh litter, resulting in one composite sample per plot, and immediately placed into an incubator with ice packages and transported to the laboratory within 24 h. The fresh soils were sieved through a 2-mm mesh and divided into two subsamples. One was stored at 4 °C to determine physical and chemical properties, and the other was stored -80 °C for extracting DNA. The protocols used for determining soil classification were copied from the methods described in previous studies (Zhang et al. 2014, Shi et al. 2010, ISS-AS and ISRIC, 1994). Soil pH was determined in a soil/water (1: 5 w/v) suspension with a pH meter (Delta-320, Mettler-Toledo Instruments [Shanghai] Co., Ltd). Total organic carbon (TOC) was measured with the $K_2Cr_2O_7$ oxidation method (Islam et al. 1998). Total nitrogen (TN), phosphorus (P) and potassium (K) were analyzed using a Eurovector Elemental Analyzer (Isoprime-EuroEA 3000, Milan, Italy). Soil salt content was measured with the soil solution electrical conductivity method. Soil moisture (SM) was measured gravimetrically. The soil microbial biomass carbon and microbial biomass nitrogen were estimated using the chloroform-fumigation-extraction methods (Brookes et al. 1985, Vance et al. 1987). The selected soil properties are shown in Table S1.

Soil DNA extraction and bacterial 16 S rRNA amplification

Soil DNA was extracted from a 0.5 g wet soil sample using an Omega Soil DNA Kit (QIAGEN Inc.; Valencia, CA, USA). The concentration of DNA was measured by using a NanoDrop 2000 spectrophotometer (Thermo Scientific; Wilmington, DE, USA). The extracted soil DNA was dissolved with 60 µL TE buffer and stored at -20℃ until use.

An equal amount (0.5 µg) of purified DNA from each sample was used as an amplification template. The V3-V4 hypervariable regions of bacterial 16S rRNA were amplified using Bac 319F/Uni 806R primers containing a barcode sequence, pad bases, and linker bases. PCR reactions were conducted with 2.0 µL of template DNA (10 ng), 1.0 µL of each primer (30 µmol L^{-1}), 2.0 µL dNTPs, 0.125 µL ExTaq DNA polymerase, 2.5 µL 10 × ExTaq buffer, and water to achieve a volume of 25 µL. PCR was performed with an initial 5 minute denaturation at 94 °C. followed by 30 cycles of denaturation at 96 °C for 1 second, annealing at 55 °C for 55 seconds, and extension at 72 °C for 1 minute (plus 2-second autoextensions per cycle), with a final extension of 10 minutes at 72 °C. The same amount of PCR products from each sample was mixed into a single microcentrifuge tube to be run on an Illumina Hiseq 250PE instrument of Macrogen Inc. (Seoul, Korea).

Sequence and data analysis

The quality of the raw data was first processed using QIIME (Caporaso et al. 2010). Bacterial sequences were grouped by sample based on having the same barcode. Barcode and primer sequences were deleted, and only the first 350 bp after primer-F319 were included for further analysis. Bacterial phylotypes were identified using cd-hit and assigned to operational taxonomic units (OTUs, 97 % similarity). The taxonomic identity of each phylotype was determined using the ribosomal database project classifier (Cole et al. 2009, Altschul et al.1990, Wang et al. 2007). At the OTUs level, the bacterial community comparison between samples was analyzed by Principal Coordinate Analysis (PCoA) using UNIFRAC (Lozupone& Knight 2005). Phylogenetic diversity (PD) was estimated using Faith's index (Faith 1992, Faith et al. 2009), which provides an integrated index of phylogenetic breadth across taxonomic levels. Correlation between soil factors and soil bacterial diversity index were examined by linear regression analysis ($P < 0.05$). Statistical analysis was carried out by using SPSS 20.0 software (IBM Corp., Armon, NY Inc, USA). Data were analyzed by one-way ANOVA, using soil type as factor, followed by Fisher's least significant difference (LSD) with significance at $P < 0.05$. Alpha diversity was calculated by using the taxonomic and functional metrics. Redundancy analysis (RDA) was used to analyze the relationship between environmental factors and sampling sites with CANOCO 5.0 software. All Illumine sequences data in this study were deposited to the SRA of the NCBI database under BioProject PRJNA658924.

RESULTS

Soil physicochemical characteristics

Based on the similarity of soil-forming processes and morphological feature 18 soil samples

Table 1. Comparison of SM, pH, TOC, TN, P and K in five different soil types.

Soil type	SM^a (%)	pH	TOC(g kg^{-1})	TN(g kg^{-1})	P(g kg^{-1})	K(g kg^{-1})
CA	29.42±2.07b	8.14±0.04ab	10.24±0.48a	1.4±0.01a	0.16±0.01b	2.43±0.03a
MMS	38.68±0.59a	8.20±0.02ab	8.03±0.39b	0.9±0.01ab	0.18±0.01b	2.54±0.04a
CH	34.04±0.84ab	8.31±0.01a	8.61±0.75ab	1.0±0.01ab	0.17±0.01b	2.52±0.01a
GDS	12.71±1.16c	8.04±0.09ab	4.79±0.77c	0.6±0.01b	0.19±0.02b	2.19±0.01b
GCS	39.01±1.62a	7.99±0.09b	4.65±0.65c	1.2±0.01a	0.39±0.04a	2.38±0.03a

Data are presented as the mean ± s.e.m. (n = 4). Different letters indicate significantly different values ($P < 0.05$).

^{a} SM TOC, TN, P, K indicate soil moisture, total organic carbon, total nitrogen, phosphorus and potassium.
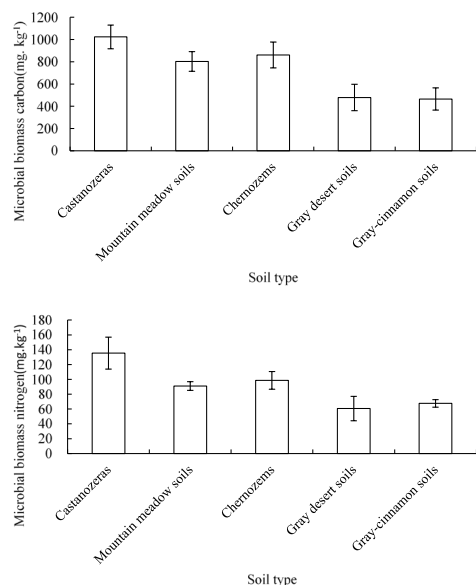
Table 2. Pearson correlation (r) among soil characteristic**s.**

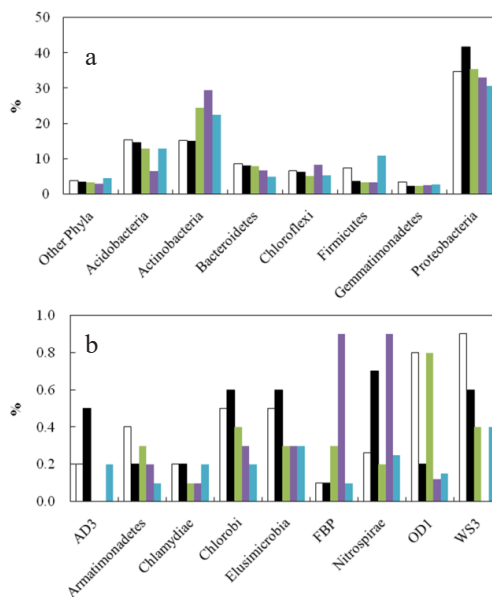| r	Elevation	pH	TOC	TN	P	K	Salt	SM	T[b]
Elevation	0								
pH	-0.088	0							
TOC	-0.424	0.127	0						
TN	-0.394	-0.281	**0.566**[*]	0					
P	0.291	**-0.667**	-0.306	0.256	0				
K	0.134	0.297	**0.543**	0.245	-0.200	0			
Salt	0.202	**-0.802**	-0.458	-0.085	**0.578**	-0.358	0		
SM	0.346	0.369	0.252	-0.057	0.004	**0.582**	-0.276	0	
T	**-0.562**	0.346	0.298	0.142	-0.313	-0.083	**-0.536**	-0.059	0

[*] Values in bold type indicate factors that had significant correlations ($P < 0.05$).

[b] T indicate mean annual temperature.

were grouped into five different types, namely Castanozeras (CA), Mountain meadow soil (MMS), Gray cinnamon soil (GCS), Chernozem (CH), and Gray desert soil (GDS). As can be seen from Table S1, each soil sample has its own corresponding nomenclature in the Genetic Soil Classification of China (GSCC). For example, GH was collected under the *Achnatherum splendens* in the Gahai, which is located in Haixi city. In GSCC, this soil was named GDS. According to the standard of the IUSS Working

Group WRB (2006), we found that there is great variability in the maximum referencibility between soil great groups of GSCC and the World Reference Base for soil resources (WRB)

Figure 1. Comparison of soil microbial biomass carbon and nitrogen in five different soil types.

Figure 2. Frequency of bacterial phyla in different QTP soils according to the pyrosequencing analysis using Ribosomal Database Project classifiers. (a) Dominant phyla with relative frequencies >1 %. (b) Rare phyla with relative frequencies <1 %. (CA: Castanozeras □; MMS: Mountain meadow soils ■; CH: Chernozems ▨; GDS: Gray desert soils ▨ ; GCS: Gray-cinnamon soils ▨.)

soil groups, which ranged from 30.7% to 78.1%. For example, GCS cannot be sorted into Cambisols in WRB by reference, with maximum referencibility only being 30.7%. the main reasons for this were the large differences in climatic zones, richness of soil types, and levels of economic and technological development on different continents and in different countries. The authors therefore decided that the GSCC system should be used below.

Analysis of physicochemical properties of the QTP soils showed that the soils were alkaline, ranging from pH 7.99 to 8.31. The SM, pH, TN and TOC differed significantly among the CA, MMS, CH, GDS and GCS. The highest average soil moisture and lowest average pH were found in GCS soils, followed by MMS and CH soils, but no significant differences were observed between the different soil types (Table 1). There was no correlation between the soil pH (r = 0.088, P = 0.727), TOC (r = 0.424, P = 0.079), TN (r = 0.394, P = 0.106) and the altitude of the sampling sites (Table 2). On the contrary, the TOC was significantly positively correlated with the TN (r = 0.566, P < 0.05) and K content (r = 0.543, P < 0.05). Soil pH were significantly negatively correlated with K content (r = -0.667, P < 0.01) and salt content (r = -0.802, P < 0.01). The SM was positively correlated with the K content (r = 0.582, P < 0.05).

The results showed that soil microbial biomass varied on the order of CA, CH, MMS, GDS and GCS (Fig.1). The average microbial biomass carbon of CA was 1023.68 mg·kg⁻¹, which was significantly higher than that of GDS and GCS (P < 0.05). There was no significant difference in average microbial biomass carbon content between GDS and GCS (P > 0.05). Similarly, the same order of soil microbial biomass nitrogen content was found, and CA content was significantly (P < 0.05) higher than GDS and GCS.

Distribution of taxa and phylotypes
Across all soil samples, we obtained 1,463,157 quality sequences in total, and 58,395- 98,766 sequences per sample (mean = 81,286). The read lengths ranged from 362 to 550 bp, with an average of 446 bp. A total of 93.8%

could be classified in these sequences. When grouped at the 97% similarity level, there were 44,265 different phylotypes in all of the soils, with an average of 3,992 phylotypes per sample.
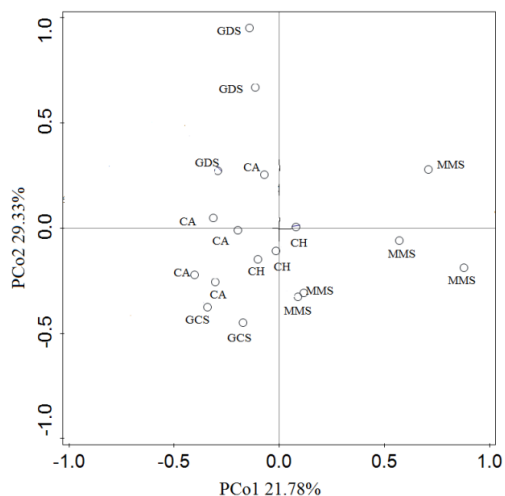

Figure 3. Bacterial community principal coordinates analysis (PCoA) using a weighted UniFrac distance matrix. The percentage variation explained by each principal coordinate is shown on the axes.
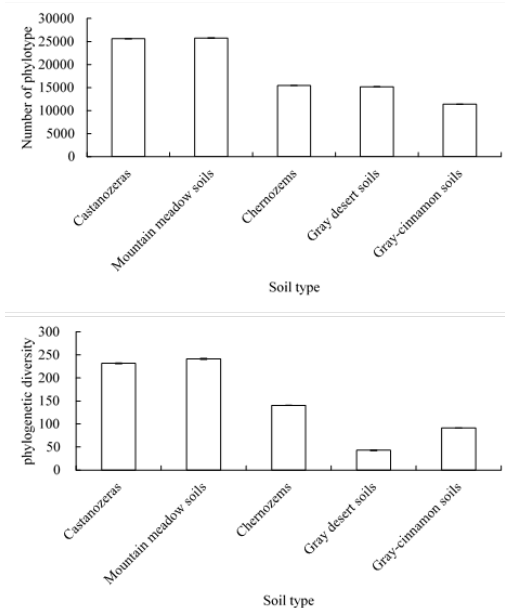

Figure 4. Soil bacterial phylotype richness and phylogenetic diversity of five morphological feature types.
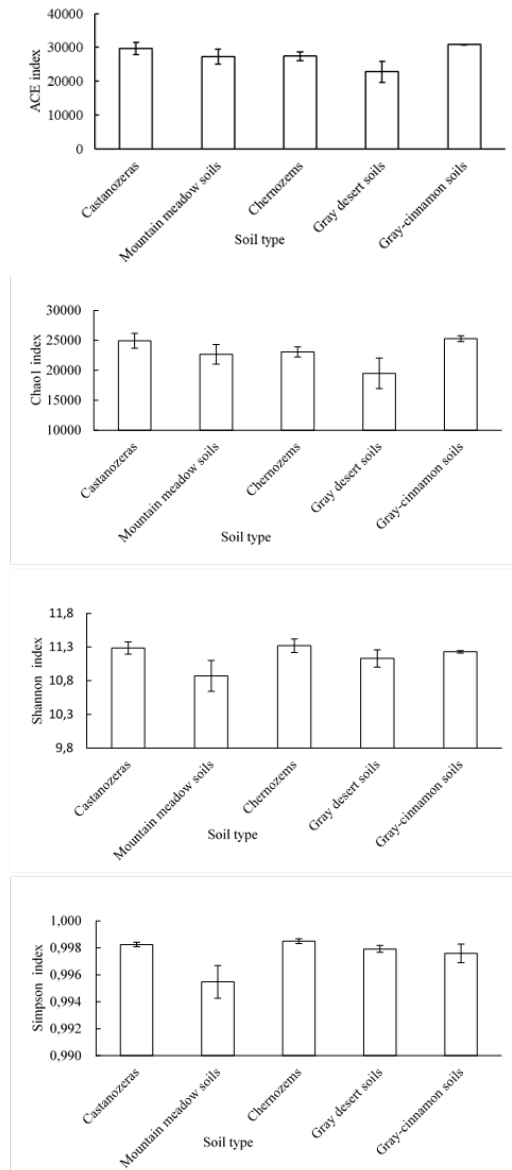
Table 3. The correlation (r) and significance (*P*) values of linear regressions between bacterial diversity (phylogenetic diversity and phylotype richness) and soil characteristics.

r/*P*		Elevation	pH	TOC	P	Salt	SM	TN	P
phylogenetic diversity (PD)	r	-0.120	0.241	**-0.548***	**-0.558***	-0.122	**-0.475***	**-0.529***	-0.391
	P	0.635	0.336	0.038	0.031	0.630	0.046	0.046	0.108
phylotype richness (OTUs)	r	-0.308	-0.327	**0.547***	**-0.529***	0.155	**-0.578***	**0.499***	0.084
	P	0.214	0.185	0.044	0.024	0.540	0.012	0.048	0.741

* Values in bold type indicate factors that had significant correlations (*P* < 0.05).

Eight phyla (*Proteobacteria, Gemmatimonadetes, Firmicutes, Chloroflexi, Bacteroidetes, Actinobacteria* and *Acidobacteria*) were considered abundant with sequence frequencies of >1% (Fig.2a), whereas 9 phyla (AD3, *Armatimonadetes, Chlamydiae, Chlorobi, Elusimicrobia*, FBP, *Nitrospirae*, OD1 and WS3) were considered low abundance with sequence frequencies of <1% (Fig.2b). The relative abundances of the dominant bacterial group varied among the CA, GCS, CH, MMS and GDS. The prevalent taxon in five type soils was *Proteobacteria*, which was followed in relative abundance by *Actinobacteria* and *Acidobacteria*. In GDS, the abundance of *Proteobacteria* and *Acidobacteria* decreased, whereas that of *Bacteriodetes* increased significantly compared with MMS and CA. Nevertheless, the GCS were dominated by *Proteobacteria* and *Actinobateria*, followed in abundance by *Acidobacteria* and *Firmicutes*. Generally, *Proteobacteria* abundance decreased gradually from MMS through CH to GDS, that is, from 41.71 to 35.40% and 33.12%, respectively. In contrast, the abundance of *Actinobacteria* increased from 15.04% in MMS to 24.44% in CH and 29.36% in GDS. Compared with GCS, the abundance of *Proteobacteria* in MMS and CA increased significantly (*P* < 0.05), while the abundance of *Actinobacteria* in MMS and CA decreased significantly (*P* < 0.05).

At the phylum level, the PCoA patterns indicated that there were distinct differences in bacterial community compositions among CA, MMS, CH, GDS and GCS (Fig. 3). All samples tended to cluster together according to their own soil characteristics. Bacteria communities

Figure 5. Diversity indices of five soil bacterial communities in QTP.

in CA, GCS and CH were clearly different from those in MMS, as well as those from GDS which were relatively similar and clustered together, explaining 21.78% of the total variation along the first axis, respectively. The MMS, GCS, CA, and CH bacterial communities were different from those in GDS along the second axis, which accounted for 29.33% of the variation, respectively.

Relationship between soil bacterial diversity and soil physicochemical properties

Soil bacterial OTUs and PD of five soil types varied from 11,402.33 to 25,751.33 and from 91.42 to 241.57, respectively (Fig. 4). The highest values of OTUs and PD were observed in CA and MMS, while the lowest values were found in GDS and GCS. The diversity of PD in GDS was significantly lower than that in the other four soil types ($P < 0.05$). In order to explore the relationship between soil bacterial alpha diversity and soil factors, the correlation between soil bacterial alpha diversity (OTUs and PD) and
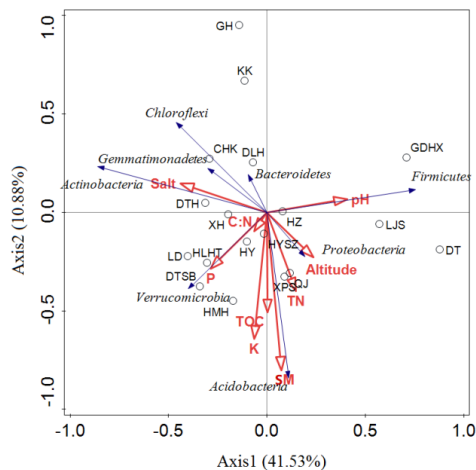

Figure 6. RDA showing associations between environmental factors and bacterial phyla.

(LD: Leduputai; HLHT: Halihatu; DTH: Datanghe;XH: Xihai; DLH: Delhi;DT: Datong Beichuan; LJS:Lajishan; GDHX: Guide hexi; XPS: Xiangpishan;QJ: Qunjia; HMH:Heimahe; DTSB: Datong shuobei; HZ: Huangzhong; HYSZ: Huangyuanshenzhong; HY: Haiyan; GH: Gahai;CK: Chaka; KK: Keke.)
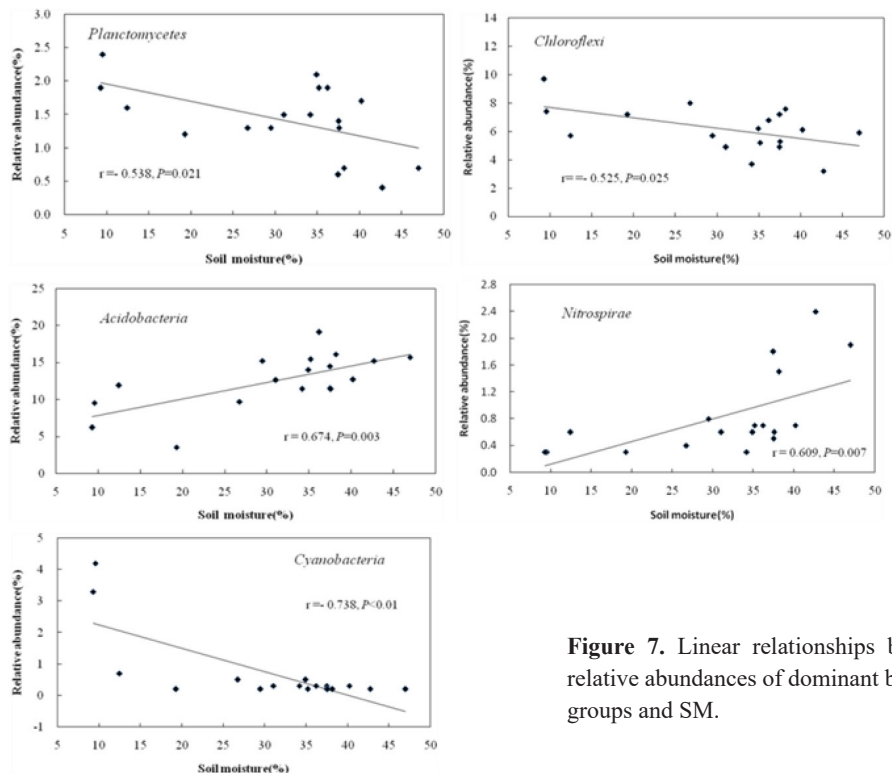

Figure 7. Linear relationships between relative abundances of dominant bacterial groups and SM.
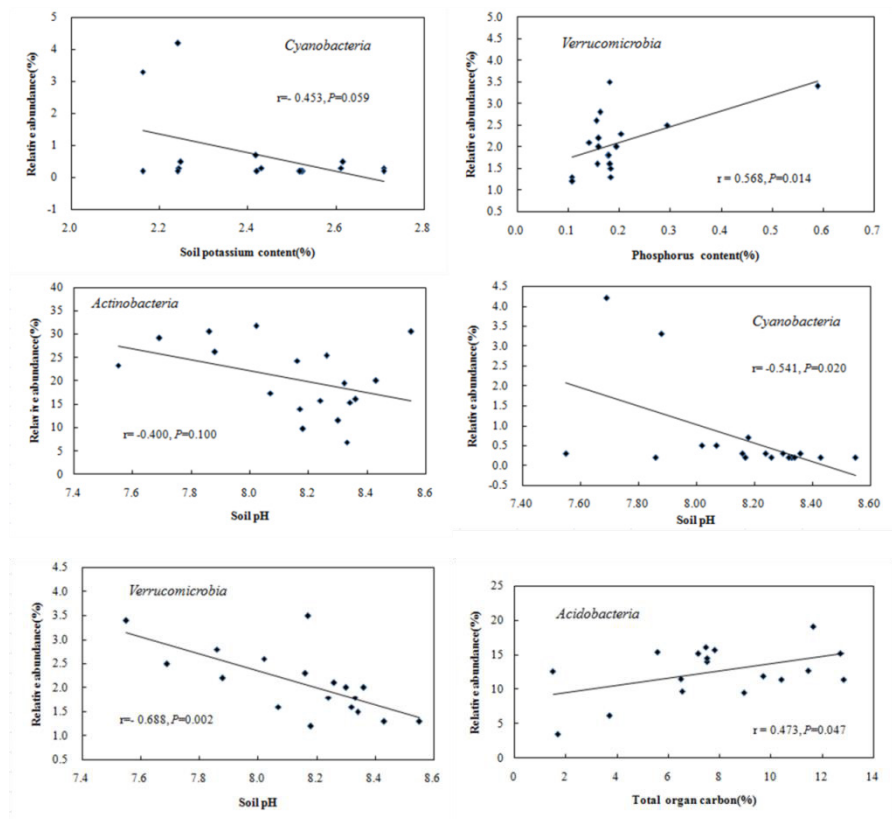
Figure 8. The relationships between relative abundances of dominant bacterial groups and soil factors, including altitude, K, P, pH, and TOC, respectively.

eight soil factors (such as soil pH, SM and TOC, etc.) was analyzed (Table 3). The results showed that SM, soil K, TOC and TN were significantly correlated with OTUs and PD ($P < 0.05$). OTUs and PD were negatively correlated with SM ($P = 0.012$; $P = 0.046$, respectively), and also negatively correlated with soil K ($P = 0.024$; $P = 0.031$, respectively). TOC and TN were positively correlated with OTUs ($P = 0.044$, $P = 0.048$), and negatively correlated with PD ($P = 0.038$, $P = 0.046$). Other soil factors and OTUs and PD did not reach significant levels ($P > 0.05$). The OTUs and PD of soil bacteria were not significantly correlated with altitude, pH or P content. The four microbial diversity indices of the five type soils were calculated respectively (Fig.5). The ACE and Chao1 indices showed that the diversity and richness of microbial communities in GDS were

the lowest, CA and GCS were the highest, and the Shannon and Simpson indices were very similar among the five soil types. Among the five soil types, the diversity of MMS was the lowest.

Effects of soil properties on the microbial community structure
The RDA revealed that the total percentage variance explained by the measured variables was 52.41% in the bacterial community model (Fig. 6). Across all samples, SM was the most important soil parameter for the variations in the bacterial community structures, followed by K, TOC and TN. SM had an obvious effect on the very rough classification resolution because the relative abundances of the dominant bacterial phyla (e.g. *Acidobacteria* and *Chloroflexi*), rarer phyla (e.g. *Planctomycetes*, *Nitrospirae*, and

Cyanobacteria), were significantly correlated across the SM gradient, although sometimes in opposite directions. For instance, the relative abundance of *Planctomycetes*, *Cyanobacteria* and *Chloroflexi* decreased as SM increased, but *Acidobacteria* and *Nitrospirae* increased (Fig. 7). Some soil elements related to SM, such as K and P, showed significant correlations with the relative abundance of *Acidobacteria*, *Cyanobacteria* and *Verrucomicrobia*.

The relative abundance of *Verrucomicrobia* (r = -0.688, *P* = 0.002), *Planctomycetes* (r = -0.486, *P* = 0.041) and *Cyanobacteria* (r = -0.541, *P* = 0.020) were negatively correlated with soil pH. The abundance of other bacterial groups had no relationship with the soil TOC, except *Acidobacteria*, which showed a not significant positive relationship with TOC (r = 0.473, *P* = 0.047). The soil C/N, TN, and temperature were found to have no significant relationship with the abundance of all the dominant bacterial groups. Elevation was significantly (*P* < 0.05 in all cases) correlated with the relative abundances of *Gemmatimonadetes*, *Verrucomicrobia* or *Nitrospira* (Fig. 8). In brief, the results strongly demonstrated that local SM , directly or indirectly, affected the bacterial community structure among sites across the QTP soils.

DISCUSSION
Soil bacterial community composition
QTP is the highest and largest plateau on earth and is considered to be one of the sensitive areas of biodiversity (Zheng et al. 2000). However, until now, only a very limited number of studies have explored the soil microbial communities in QTP using 16S rRNA gene clone library approaches (Liu et al. 2009, Liu et al. 2006). Recent studies found that QTP has a formed predominant group from different environmental samples with a similar group but different abundance. For example, the differences in soil bacterial community structure among cultivated farmlands, alpine meadow and salt lake ecosystem in QTP indicated that the dominant bacteria phyla were *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Acdiobacteria*, and *Verrucomicrobia* (Guan et al. 2013). Five dominant bacteria phyla *Actinobacteria*, α-*Proteobacteria*,

Acdiobacteria, *Chloroflexi* and *Gemmatimonadete* were observed across the northwest Tibetan Plateau surface soil (Chu et al. 2016). Yuan et al. (2014) and associates indicated that *Acdiobacteria*, *Proteobacteria* and *Gemmatimonadete* were confirmed in Nyainqentanglha Mountain alpine grassland soil samples. In this study, we found that *Proteobacteria*, *Actinomycete*, *Acidobacteria* and *Bacteroidetes* were the dominant phyla in QTP soils and high relative abundances of *Chloroflexi* and *Firmicutes* were observed, accounting for more than 87.1%. These observations were consistent with many past studies mentioned above. From the analysis of bacterial communities in different soil types, most bacterial groups were relatively stable among the GDS, GCS, CH, CA, and MMS, but several groups exhibited changes in their relative abundance. The alkalophilic *Actinobacteria* were consistently more abundant in GDS (Fig. 2a), which might be due to higher organic matter, low water and higher pH. On the contrary, *Proteobacteria* were abundant in MMS, indicating that the bacterial communities' structure was obviously different relative to those of GDS, which agreed with the PCoA pattern, the water content abundance in this sample. This finding was consistent with results in North America (Fierer et al. 2007) and QTP soils (Wang et al. 2008).

Bacterial community structure and environmental relevance
The QTP soil bacterial communities were different despite the fact that the soils from other environments such as arctic areas or farmland share some physicochemical characteristics. Although phyla have been detected in all collected soils (including QTP), the relative frequencies of bacterial phyla differ in different niches, which are usually considered to be controlled by local environmental factors including vegetation (Marschner et al. 2001, Kowalchuk et al. 2002, Weinert et al. 2011), soil characteristics (Hansel et al. 2008, Wu et al. 2008), soil texture (Schutter et al. 2001), land use (Kennedy et al. 2005, Yergeau et al. 2007), geographic distance (Fierer & Jackson 2006), and pH (Lauber et al. 2008), to name some relevant factors. When soil

physicochemical parameters were considered separately, SM was the most important factor to determine soil microbial diversity in the QTP soils (Fig.4). Previous studies have shown that the moisture content of soils in QTP is positively correlated with soil organic carbon storage and vegetation coverage (Wang et al. 2008, Wu XD et al. 2012, Chu et al. 2011). In addition, the soil nutrients and moisture in the alpine meadow were higher than in the alpine steppe, and there was an exponentially decreasing trend as the vegetative cover decreased (Chu et al. 2016). This may explain why the microbial communities in MMS with higher SM were different from those in GDS and GCS. In addition, the significant covariates between SM and the bacterial community composition may be related to *Acidobacteria*, *Nitrospirae*, *Chloroflexi*, *Planctomycetes*, and *Cyanobacteria*, while some dominant groups were obviously related to SM. This might further suggest that the SM was the controlling factor for the bacterial species structure in QTP. These results are consistent with surveys of Antarctic soils, the Canadian Low Arctic Tundra (Chong et al. 2010) and the QTP North Slope permafrost (Guan et al. 2013), where soil moisture has a great influence on the composition of soil bacterial communities.

Although SM is the main environmental factor affecting the composition of soil bacterial communities, other factors were also shown to affect the distribution of microbial communities in different environments. For example, the community of Estuarine bacterioplankton varies along the salinity gradient (Crump et al. 2004), and the bacterial structures in high salinity and sediment soils are significantly correlated with the content of TOC (Hollister et al. 2010). Phylotype richness was inversely correlated with soil TOC, which is in agreement with the results observed in the black soil zone in northeast China (Liu et al. 2014), suggesting that soil TOC is another important factor predicting bacterial communities. Therefore, considering that the significant positive correlation between TOC, TN and K contents in QTP soil, we deduce that K is also an important factor determining the

QTP soil bacterial community structures, which can be explained by a series of higher K levels with specific environmental conditions and geographical locations in QTP soil. Meanwhile, RDA analysis also showed that significant correlations between the K content and bacterial community composition were observed in QTP soils (Fig.6), indicating that the K content in soil was the main environmental factor affecting the bacterial community distribution.

The soil pH also has a strong impact on the structure and diversity of soil bacterial communities (Chu et al. 2010, Chong et al. 2010, Lauber et al. 2009). However, the soil pH was not the most important soil factor in the current study and the distribution pattern of bacterial community structure differed from previous studies. For instance, previous work found that the relative abundance of bacterial community has been shown to increase with higher pH (Chu et al. 2010, Rousk et al. 2010), but bacterial community structure and pH were not significantly associated in the current study. The narrow pH range of the samples from our eighteen locations may account for this difference. The pH ranged from 7.99 to 8.31 at eighteen locations, whereas the pH ranges in other investigations were 4.0-7.64 (Chu et al. 2010), 3.30-7.24 (Nacke et al. 2011), 3.30-7.37 (Zinger et al. 2011) and 3.50-8.50 (Fierer et al. 2006).

The elevational diversity gradient is one of the most basic models in animal and plant biogeography. Here we observed that there was no apparent elevation gradient of soil bacterial diversity on QTP, neither monotonous nor unimodal within microorganisms, which is in agreement with the findings of Zhang et al. (2013) and Fierer et al. (2011). These results suggest that bacterial distribution may not follow the patterns of plants and animals in conjunction with elevation.

CONCLUSION

This study showed that soil physicochemical factors, in particular SM, soil TOC, and K, were the main factors explaining the variation in bacterial communities in QTP soils, and a relatively high diversity of the bacterial

community was observed in CA and MMS. Further studies with more environmental variables on a larger scale will provide further insights into the factors that drive microbial communities in this unique environment.

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