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Soil bacterial community structure in Chinese wetlands

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ABSTRACT

Soil microbial communities are crucial in maintaining the functions of wetland ecosystems. Understanding the microbial community structure and the key factors driving the assemblages of wetland soil microbiota are important to reveal the connections between microorganisms and functions of wetland ecosystems. In this study, soil bacterial community compositions and the factors shaping them were investigated in three groups of wetlands across China, including Tibet plateau wetlands (TW), inland wetlands (IW) and coastal wetlands (CW). Overall, Bacterial community structure and turnover showed distinct patterns in different groups. Bacterial phyla were mainly composed of *Proteobacteria* followed by *Chloroflexi*, *Acidobacteria*, *Actinobacteria* and *Bacteroidetes* in all groups of wetland samples. At genus level, random forest model showed that *Coprothermobacter* and *Acetobacter* were two most important genera explaining the differences among groups. The abundances of these genera were very low in IW relative to the other two groups. The alpha diversity of IW was significantly higher than those of TW and CW. The relative contribution of environmental factors was larger in the assemblages of bacterial communities in TW and CW than that in IW. The pH and conductivity were recognized as the most important measured environmental factors influencing bacterial community structure. Our results suggested that the bacterial communities of wetlands in different regions were shaped with different mechanisms. The communities in CW and TW regions owned lower alpha diversity and were more influenced by deterministic processes than those in IW. In conclusion, the spatial pattern of soil bacterial community assembly in Chinese wetland was scale-dependent.

1. Introduction

Wetlands are unique ecosystems frequently or continually inundated with water, including swamps, marshes, bogs and fens (Mitsch and Gosselink, 2000). Wetland soils constitute big reservoirs of terrestrial carbon including decayed herbaceous and woody organic matter (Gorham, 1991). Microorganisms play key roles in biogeochemical processes in the wetlands such as greenhouse gas emission and nutrient cycling. Therefore, the structure of microbial communities and their responses to environmental changes may greatly influence the ecological functions of wetlands. Many studies have investigated microbial community assembly of wetlands at local scale (Dedysh et al., 2006; Foulquier et al., 2013; Hartman et al., 2008; Peralta et al., 2010; Serkebaeva et al., 2013). However, few studies provide comprehensive understandings on the bacterial community assemblages and driving factors at regional scale (Wu et al., 2013).

Although ecologists have made great progresses in understanding the mechanisms shaping biogeographical patterns of macroorganism community, the community assembly of microorganisms is still not fully understood. The high-throughput sequencing technique makes it available to survey the microbial biogeographic patterns at large spatial scales without culturing, and to test the applications of ecological theories on microbial communities (Barberán et al., 2014; Kirchman et al., 2010; Marsh, 1999; Muyzer et al., 2004; Muyzer et al., 1993; Prosser et al., 2007; Riesenfeld et al., 2004; Schmidt et al., 1991; Tringe et al., 2005).

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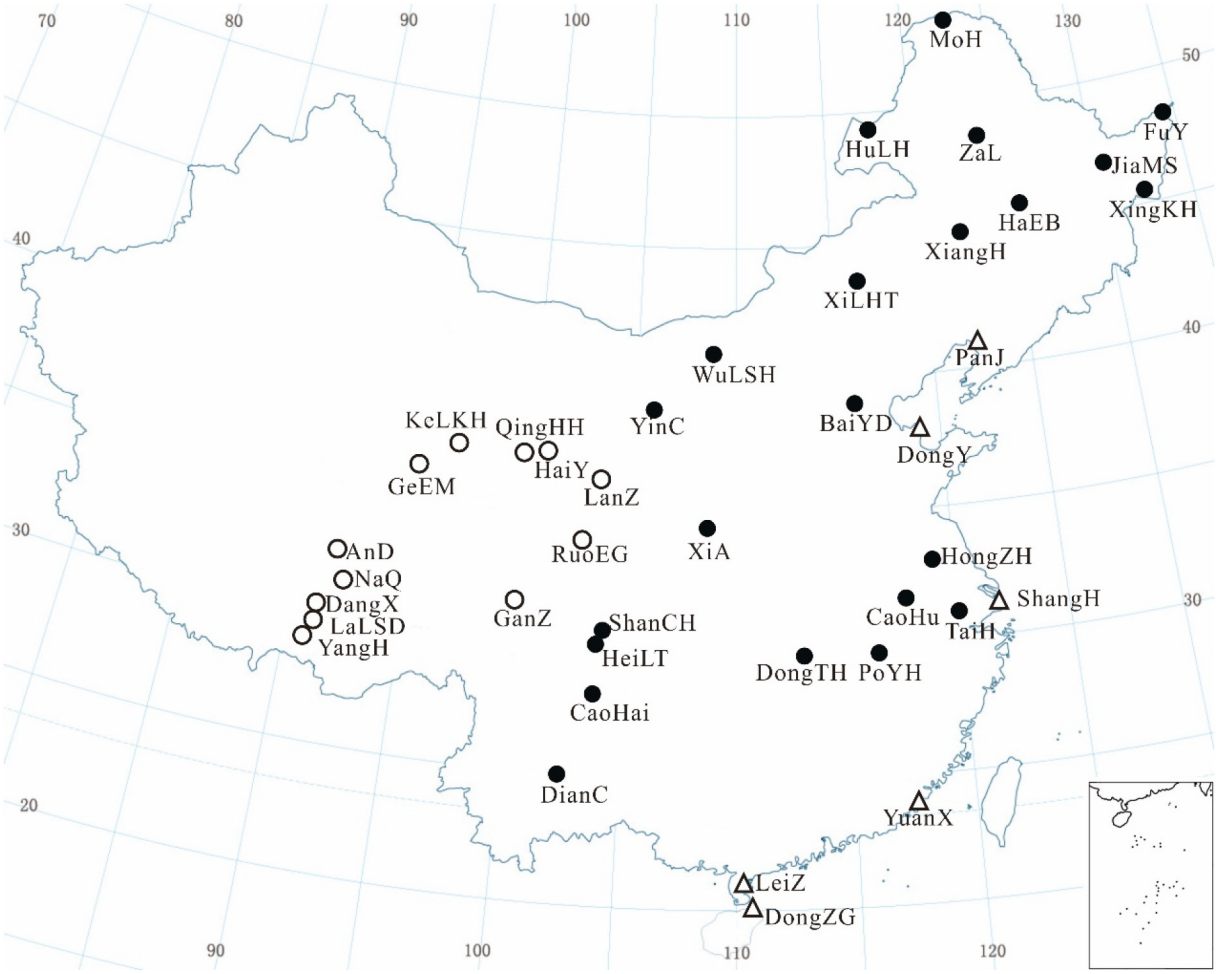

Fig. 1. Sampling sites of the wetland soils in China. Hollow circle: samples from Tibet plateau wetland (TP), solid circle: samples from inland wetland (IW), hollow triangle: samples from costal wetland (CW).

Many evidences support that microbial assemblages show obvious biogeographic patterns which are determined by multiple biotic and abiotic factors, such as pH (Fierer and Jackson, 2006; Lauber et al., 2009), temperature (Barcenas-Moreno et al., 2009; Hoj et al., 2007), phosphorus (Allison et al., 2007; Andersson et al., 2010), carbon availability (Drenovsky et al., 2004; Hu et al., 2014), salinity (Lozupone and Knight, 2007; Wang et al., 2011) and vegetation (Saetre, 1999). However, the driving factors that determine biogeographical patterns within a single habitat may be different from that in a region or a biome (Fierer, 2008; Ganderton and Coker, 2005).

From the view point of ecological processes, both deterministic and stochastic processes are recognized to contribute to microbial community assembly (Dumbrell et al., 2009; Langenheder and Székely, 2011; Ofiţeru et al., 2010; Stegen et al., 2012b; Wang et al., 2013). At large temporal or spatial scale, historic factors are also important for the community assembly pattern (Hanson et al., 2012; Liu et al., 2017; Vellend, 2010). The niche-based deterministic processes (including abiotic and biotic selections) imply that microbial community assemblage patterns reflect the effects of contemporary environmental conditions, and similar environments may harbor similar compositions. Stochastic processes (dispersal and ecological drift) also strongly shape microbial community patterns, as indicated for animals and plants (Caruso et al., 2011; Stegen et al., 2012a; Van Der Gast et al., 2008). Many studies indicate that the variations in communities are primarily influenced by dispersal limitation (Caruso et al., 2011; Dumbrell et al., 2009; Lekberg et al., 2012; Martiny et al., 2011; Martiny et al., 2006). In most cases, microbial community assembly is simultaneously influenced by deterministic and stochastic processes (Chase and Myers, 2011; Fierer, 2008; Green et al., 2008; Liu et al., 2017). Deciphering the effects of deterministic and stochastic factors often depend on the complex calculating approaches, such as variance partitioning and null models (Vellend et al., 2014). Fierer et al. indicate that the most robust approach for quantifying the effects of dispersal limitation on microbial biogeography is to compare microbial communities across identical habitats at various geographic scales (Fierer, 2008).

Local environmental conditions and spatial factors have been extensively considered to be the major competitive elements driving microbial biogeography (Bokulich et al., 2014; Langenheder and Ragnarsson, 2007; Lindström and Langenheder, 2012). Many studies demonstrate that the relative contributions of environmental and spatial factors in microbial distribution patterns are likely scale-dependent (Martiny et al., 2011; Martiny et al., 2006). Environmental variables have stronger effects on the variations in microbial communities at small spatial scales, while geographic distance can better explain the variations at larger spatial scales (Hollister et al., 2010; Horner-Devine et al., 2004; Kou et al., 2017; Yao et al., 2017).

China has various types of wetlands. In Tibet plateau, wetlands are mainly associated to lakes with high salinity water. In the inland areas except Tibet plateau, wetlands mainly distribute along with lakes and rivers. Large areas of wetlands also distribute among the Chinese coastline. The commons and differences of microbial communities in these wetlands at large spatial scale are not revealed. Previous studies have shown that the Tibet Plateau has distinctive soil microbial community structure (Yang et al., 2014; Yun et al., 2014). Coastal wetlands

are affected by tides and seawater with significant differences in environmental factors compared to other wetlands. In this study, we divided Chinese wetlands into three main groups, including Tibet plateau wetlands (TW), inland wetlands (IW) and coastal wetlands (CW). We examined the diversity and compositions of bacterial communities in 200 soil samples collected from 42 wetlands across China. The compositions of bacterial communities in these wetland soils and the driving forces shaping community assembly were evaluated. The aims were to investigate (i) the distribution of bacterial community compositions and diversity in Chinese wetlands; (ii) important environmental factors shaping bacterial community structure; and (iii) the relative contributions of environmental factors and spatial distance to bacterial community assemblage at different wetlands.

2. Materials and methods

2.1. Sampling site description and soil sampling method

From June to October of 2013, we collected 200 soil samples from 42 wetland sites covering various types of wetlands in China. The IW group contained 120 samples, and TW and CW contained 47 and 33 samples, respectively (Fig. 1). At each sampling site, 3 to 14 samples were collected from flooded soil at 0 to 10 cm depth (soil immersed by free water but not completely covered by water). Soil was put into a sterilized 50 ml centrifuge tube, transported with ice bags to the laboratory, frozen-dried and stored at −20 °C.

For each sampling site, the geographic information (latitude, longitude and altitude) was recorded with a GPS device. The annual mean temperature and annual precipitation were inferred from the program of DIVA-GIS V7.5 (http://www.diva-gis.org/) with 2.5 arc-minutes resolution climate data (WorldClim, http://www.worldclim.org) based on the GPS information. The distance between any two sample sites was calculated using the “Haversine formula” (The Haversine Formula, n.d.) with latitude and longitude data.

2.2. Soil property measurements and genomic DNA extraction

Soil conductivity and pH were measured in the soil-water slurry (1: 5, soil: water, w: w) with a conductivity meter (FE38, METTLER TOLEDO) and a pH meter (PB-10, Sartorius), respectively. Total organic carbon (TOC) was determined with the dichromate digestion method (Walkley and Black, 1934), and total nitrogen (TN) with the Kjeldahl method (Bremner, 1965). Soil was extracted using 2 M KCl solution at soil: solution ratio of 1: 4 with shake for 1 h at 200 rpm. The $NH_4^+ - N$ and $NO_3^- - N$ in the supernatant were quantified using a phenol-hypochlorite reaction method (Weatherburn, 1967) and an improved Griess reagent method (Doane and Horwáth, 2003). Soil genomic DNA was extracted from 0.5 g freeze-dried soil with Ezup Column Soil DNA Purification Kit (Sangon Biotech, Shanghai, China) according to the manufacturer's instructions. All the geographic, climate and soil chemical properties were summarized in the Table S1.

2.3. Miseq sequencing of 16S rRNA gene amplicons

For investigating bacterial community, the V4 region of 16S rRNA gene was amplified using primer set 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) with a 12-mer barcode at 5′-end of primer 806R. The PCR reaction (25 μl) consisted of 5 ng of total DNA, 1 Unit of EX Taq (TaKaRa, Dalian, China), 1 × Ex Taq buffer, 0.2 mM of each dNTP and 0.4 μM of each primer. Amplification condition consisted of an initial denaturation step of 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 50 s and a final extension at 72 °C for 5 min. Replicate PCR reactions were carried out for each sample, and their PCR products were pooled and subject to 1% agarose gel electrophoresis. The band with a correct size was excised and purified using SanPrep DNA Gel Extraction Kit (Sangon Biotech, Shanghai, China). All PCR products were quantified with Nanodrop and pooled together with equal molar amount from each sample. The sequencing sample was prepared using TruSeq DNA kit according to manufacturer's instruction. The purified library was diluted, denatured, re-diluted, mixed with PhiX (equal to 30% of final DNA amount) as described in the Illumina library preparation protocols, and then applied to an Illumina Miseq system for sequencing with the Reagent Kit v2 2 × 250 bp at the Environmental Genome Platform of Chengdu Institute of Biology, CAS.

2.4. Sequencing data processing

Low quality reads, primers and barcode sequences were removed when split libraries according to unique barcodes using Qiime (v1.7) with default parameters (Caporaso et al., 2010). Chimeric sequences were detected and removed using UCHIME (Edgar et al., 2011). Operational taxonomic units (OTUs) clustering was performed based on 97% sequence similarity. The representative sequence was annotated using the Greengenes reference library (v13_8). Sequences affiliated to Archaea and singletons were removed. All sequencing data were deposited in GenBank short-read archive with access number of SRP115116.

2.5. Data analysis

All the downstream data analysis were performed in R (Team, R.C., 2016). In case of the influences of sequencing depth on community diversity, the OTU table was rarified to make all samples holding 10,000 reads. Chao1, Simpson and Shannon indices were used to evaluate the species richness and the alpha diversity. Kruskal-Wallis rank sum test was performed to show the significance of the difference on alpha diversity and taxon abundance among different groups. Random Forests analysis was applied to obtain the important indicator taxa using randomForest package (Liaw and Wiener, 2002) with 1000 trees and all default settings. To avoid the effects of rare taxa, we removed less abundant genera (average abundance < 0.01% in all samples) in the Random Forest analysis. The spearman correlation method was used to calculate the correlations between the relative abundances of important genera and environmental factors. Bray-Curtis dissimilarity was calculated using Vegan (Wagner, 2013) to show the distances between each pair communities. Non-metric Multi-Dimensional Scaling (NMDS) was performed to show the differences among communities using package vegan. To identify if there were significant differences among different groups, permutational multivariate analysis of variance (PERMANOVA) was performed based on the Bray-Curtis dissimilarity matrix. We used distance-based redundancy analysis (dbRDA) to partition variation in beta-diversity into fractions explained by environmental variables. The functions “capscale”, “anova.cca”, and “vif.cca” in R package Vegan were used to perform the model construction, variables and axes significance permutation test, and variance inflation factor analysis.

Principal coordinates of neighbor matrices (PCNM) eigenfunctions were computed across all the points of each sampling region. PCNM eigenfunctions represent a spectral decomposition of the spatial relationships among the points; they describe all spatial scales that can be accommodated in the sampling design. They are obtained by principal coordinate analysis (PCoA) of the truncated geographic distance matrix. Variation partitioning for dbRDA enable us to determine the various unique and combined fractions of variation of the community explained by the environmental data, the broad scale spatial pattern (trend surface analysis) and the finer scale spatial patterns (PCNMs). We independently forward selected the X-Y coordinates, the environmental variables and the PCNM variables before variation partitioning using vegan function ordiR2step. Variation partitioning was performed using function varpart.

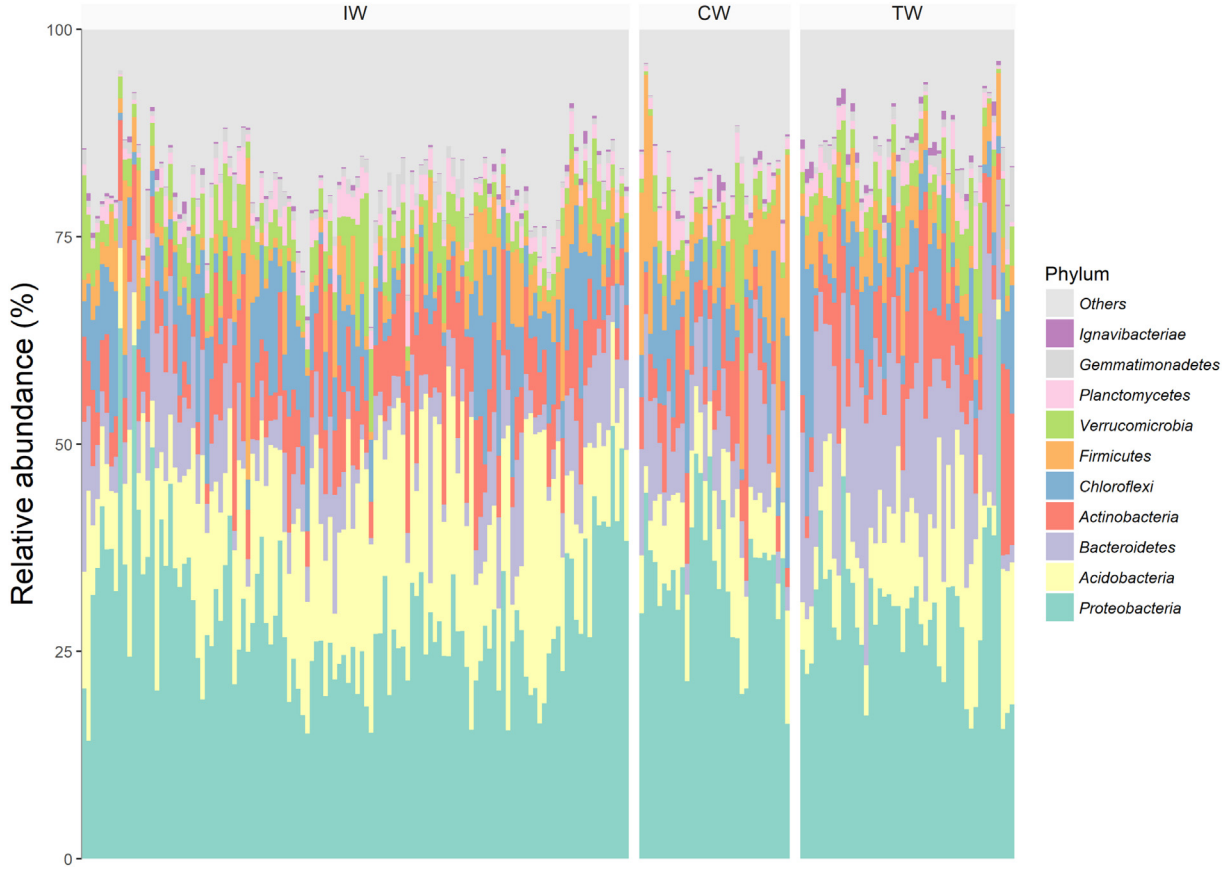

Fig. 2. The relative abundances of bacterial communities at phylum level in wetland samples. The relative abundances of top 10 abundant phyla were shown, while other less abundant phyla and unclassified reads were integrated into others.

3. Results

3.1. Overall bacterial community structure and diversity

After removing the chimeras and resampling, 20,365 OTUs were obtained with 842 to 2194 (mean: 1594 ± 253) OTUs in each sample. For all wetland soil samples, bacterial profiles were dominated by *Proteobacteria* (31.04%), *Acidobacteria* (12.36%), *Bacteroidetes* (11.21%), *Actinobacteria* (8.09%), *Chloroflexi* (7.12%), *Firmicutes* (5.77%), *Verrucomicrobia* (3.85%), *Planctomycetes* (2.34%), *Gemmatimonadetes* (0.99%), *Ignavibacteriae* (0.45%), and *Armatimonadetes* (0.26%) (Fig. 2).

In three group wetlands, the average relative abundance of *Proteobacteria* was highest, ranging from 34.12% in CW to 29.17% in TW and 29.38% in IW (Fig. S1). In IW group, *Acidobacteria* (16.95%) was second abundant after *Proteobacteria* followed by *Actinobacteria* (8.53%), *Chloroflex* (7.05%) and *Bacteroidetes* (6.94%). However, in Tibet Plateau, *Bacteroidetes* (16.47%) was more abundant followed by *Acidobacteria* (11.76%), *Actinobacteria* (8.66%) and *Chloroflex* (6.67%). In CW, *Proteobacteria* was overpresented, followed by *Bacteroidetes* (9.47%), *Acidobacteria* (8.99%) and *Firmicutes* (8.62%).

The α-diversity (Observed number of OTUs, Chao1 index, Simpson index, Shannon index) of IW was higher than those of TP and CW (Fig. 3). The Venn diagram showed that 6874 OTUs were shared by all three groups (Fig. S2), and these shared OTUs represented 87.21% of total reads. This implied that the similarity of bacterial compositions in all wetland soils was high, and the wetland soil bacterial communities had relatively stable compositions to ensure their ecological functions. The numbers of OTUs co-occurred in TW-IW, IW-CW, CW-TW were 10,156, 9130 and 7621, respectively. The results were possibly due to the differences in spatial distances between any two groups. The distances between IW and TW/CW were relatively smaller than that between CW and TW, which was separated by large spatial distance.

The richness of bacterial taxa between groups and group sets (contained two or three groups) were also analyzed at phylum and genus levels. The co-occurred phyla between CW, IW and TW groups had high proportions of *Proteobacteria*, although it had relatively lower proportion in some groups (Fig. S2). However, the co-occurred genera were very different in each group and group sets.

The plot of NMDS ordination based on Bray-Curtis distance combined with geographical location of sample sites indicated that the bacterial communities were separated by geographical locations (Fig. 4a). The PERMANOVA test indicated that bacterial community structures in these three groups were significantly different from each other at phylum level ($p < 0.001$) (Table S2).

3.2. Environmental factors associated with beta-diversity of bacterial community

Measured environmental factors, including temperature, precipitation, TOC, NH_4^+, NO_3^-, pH, TN, conductivity and altitude were used to analyze their relationships with bacterial communities (Fig. S3, Table S1). RDA with forward selection showed that all factors, except NO_3^-, were significantly correlated with the variations in bacterial community structure, and the pH ($F = 11.67$, $p < 0.001$) and conductivity ($F = 11.28$, $p < 0.001$) were the most influential variables affecting bacterial community structure (Table 1) (Fig. 4b).

We also investigated the most important genera for classifying samples into different groups using random forest approach. The results showed that *Coprothermonbacter*, *Acetobacter*, *Polaromonas*, *Anaerobaculum* and *Staphylococcus* were the most important genera (Fig. 5), and the abundances of these genera were more similar between

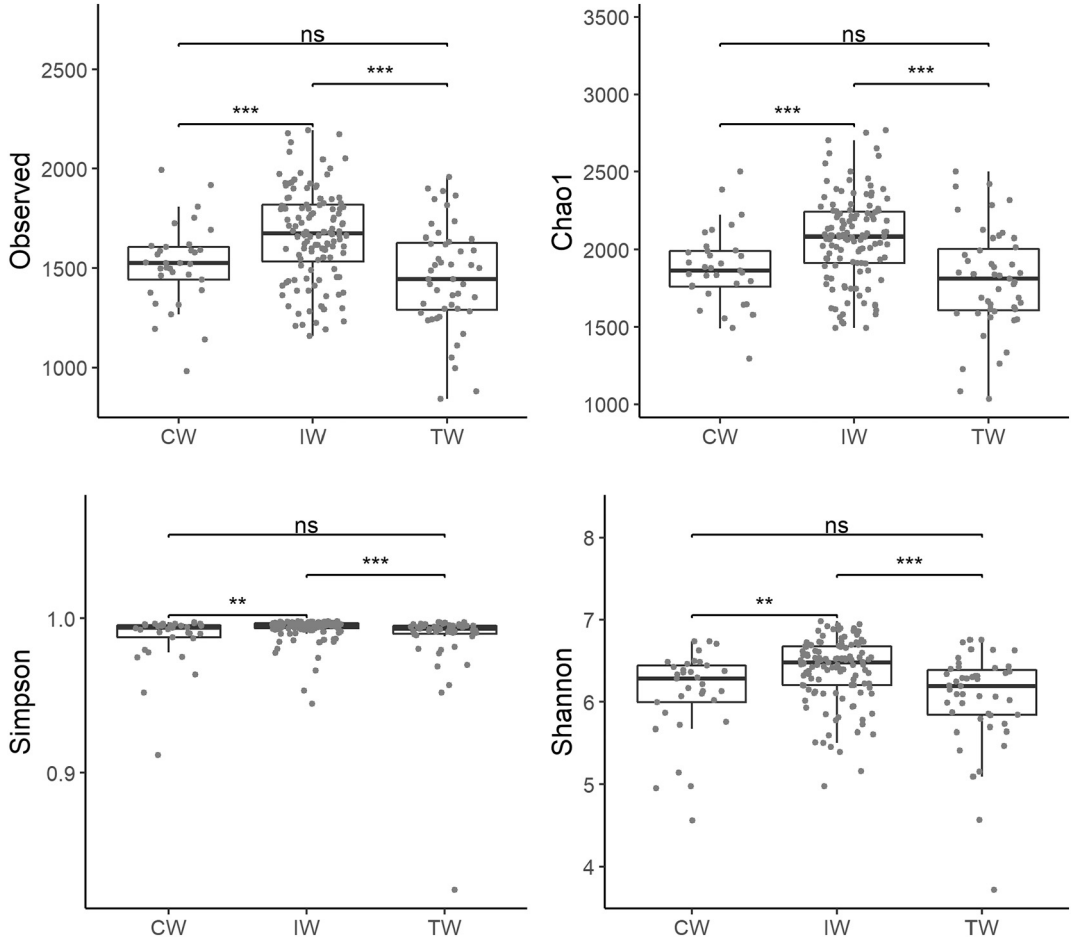

Fig. 3. The α-diversity indices in different wetland groups. The differences between any two groups were tested by Wilcox test. ns, not significant; **, $p < 0.01$; ***, $p < 0.001$.

CW and TW groups than between IW and other two groups. The correlations between environmental factors and top 40 important genera were also analyzed, and the results showed that CW bacterial communities were more correlated to environmental variables than TW and IW (Fig. 5).

To understand the relationship of bacterial community diversity and spatial distance, linear regressions were performed based on Bray-Curtis dissimilarity and spatial distance. The results showed that the

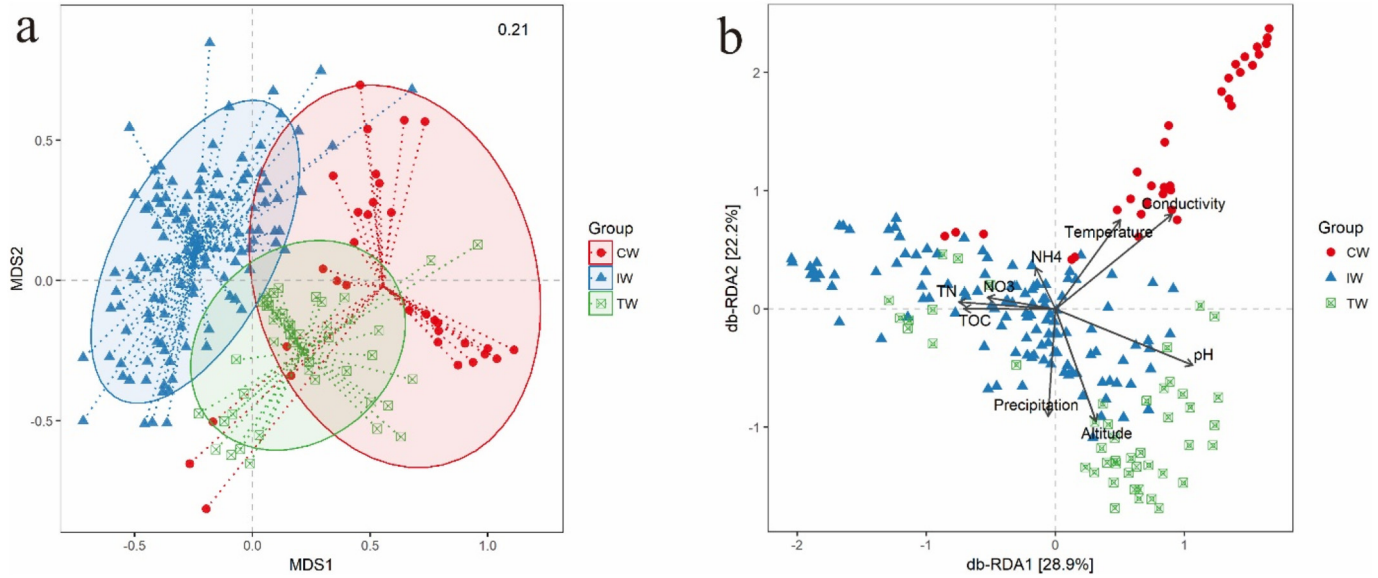

Fig. 4. (a) Non-metric multidimensional scaling ordination (NMDS) plot based on Bray-Curtis distances of bacterial communities in wetland soils. (b) Distance-based redundancy analysis (dbRDA) ordination plots of the community-environment relationships of bacterial communities.

Table 1
Pearson correlations between the Bray–Curtis dissimilarity score and the site characteristics using mantel test and partial-mantel test. The number represented the r values and the stars represented the p values (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

	ALL		CW		IW		TW	
	Mantel	Partial	Mantel	Partial	Mantel	Partial	Mantel	Partial
Altitude	0.1627 ***	0.1563 ***	0.3067 **	0.1605 *	0.0543	0.0761 *	0.1879 ***	0.1322 **
Temperature	0.2696 ***	0.2034 ***	0.5059 ***	0.4397 ***	0.3122 ***	0.249 ***	0.1362 *	0.0771
Precipitation	0.2208 ***	0.1865 ***	0.6361 ***	0.5763 ***	0.1771 ***	0.1422 ***	0.3688 ***	0.3256 ***
TOC	0.1013 **	−0.1591	0.0339	−0.3484	0.1889 **	−0.0848	0.0935	−0.0562
NH_4^+-N	−0.0256	−0.0591	−0.0177	−0.209	0.0386	0.0208	−0.0626	−0.0497
NO_3^--N	0.0559	−0.0118	0.1949 *	0.1983 *	0.117 ***	−0.0125	−0.087	−0.1109
pH	0.3237 ***	0.2689 ***	0.5317 ***	0.4768 ***	0.4984 ***	0.4117 ***	0.5575 ***	0.5254 ***
Conductivity	0.388 ***	0.3896 ***	0.1079	−0.1152	0.3109 ***	0.2991 ***	0.4537 ***	0.5009 ***
TN	0.091 **	−0.1521	0.0268	−0.3918	0.1874 **	−0.0967	0.0971	−0.0544

dissimilarity was positively correlated with the distance in each group and all samples ($p < 0.0001$) (Fig. 6). The highest correlation was found in CW group ($R^2 = 0.26$), and lower in IW ($R^2 = 0.11$), TW ($R^2 = 0.12$) and all samples ($R^2 = 0.12$).

To better assess the effects of spatial distances (as a proxy for dispersal barriers) and environmental parameters on the variations of the bacterial communities, we conducted a variance partitioning analysis and illustrated our results using a modified variation partitioning diagram (Fig. 7). We used principle coordinates of neighbor matrices (PCNM) to obtain the finer spatial scale pattern. Together, the whole set (the pure effects of environmental factors, the pure effect of spatial linear trend, spatial factors derived from PCNM) explained 44.2%, 31.9%, 30.5% and 31.3% of the variations in CW, IW, TW and all samples, respectively. When separately investigated within CW, IW and TW groups, the pure effects of environmental factors explained 17.0%, 5.2% and 13.4% of the variation. On the other hand, the pure effect of spatial linear trend contributed 1.7%, 1.0% and 2.5% of the variation, and PCNM explained 4%, 14.5% and 6.4% of the variation. The spatial variables (broad- and fine-scale) alone explained 8%, 14.6% and 9.4%, and the environmental variables explained 36.2%, 16.4% and 21.1% within three groups, respectively. It implicated that both environmental variables and geographic distance were important to shape bacterial community structure in IW, while in CW and TW, the environmental variables were more important. Overall, when assessed with all samples, PCNM contribution (13.9%) was more important than the effects of pure environment factors (5.7%) and spatial linear trend (0.6%),

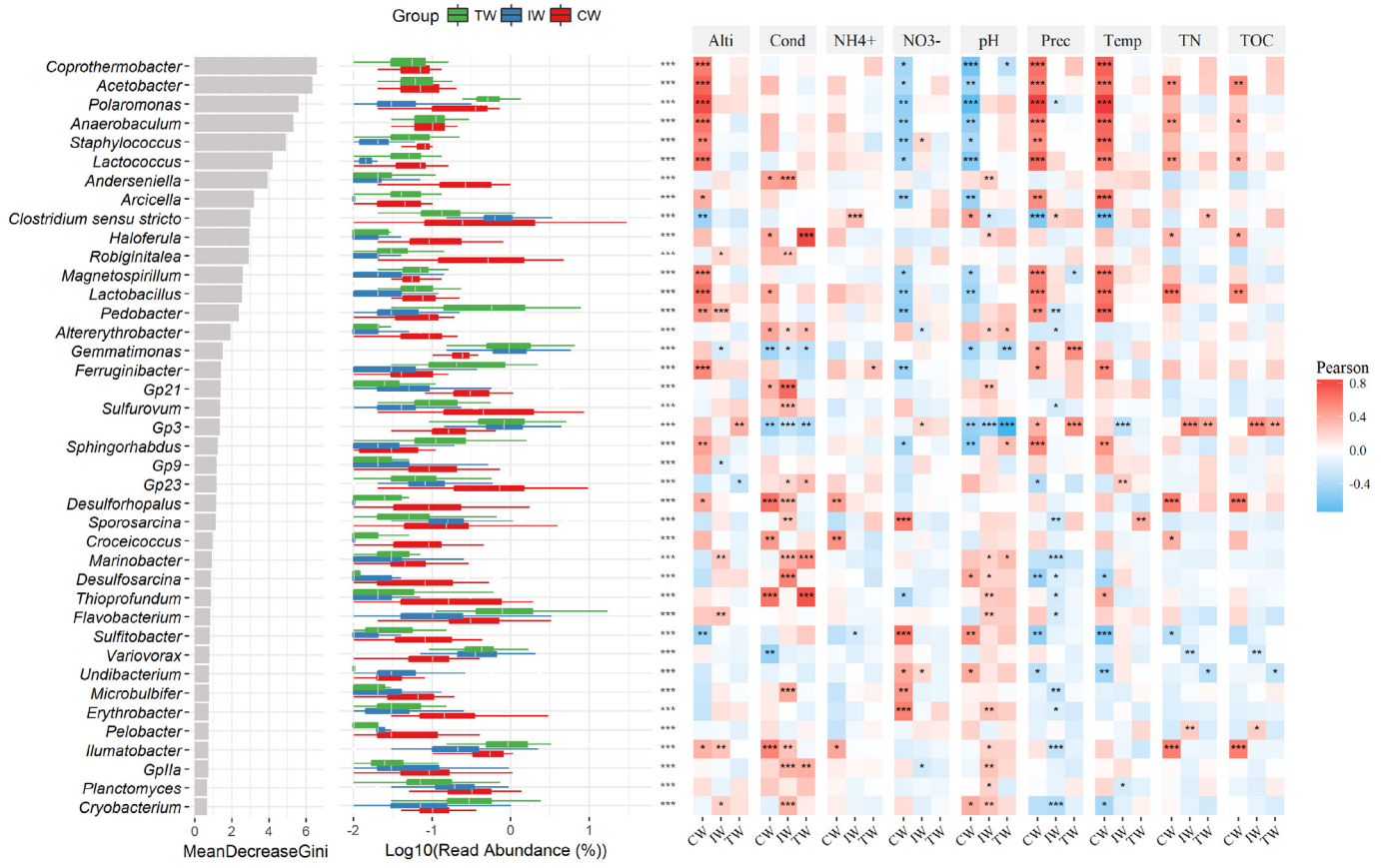

Fig. 5. Top 40 important genera for Random Forest Classification. Left, Top 40 taxa were assessed by Gini index, which represented the importance of each genus in distinguishing different groups; middle, read abundances of the top 40 genera; right, the Pearson correlations between the relative abundances of top 40 genera and the environmental factors. Alti, altitude; Temp, temperature; Prec, precipitation; TOC, total organic carbon; NH4+, NH_4^+ − N; NO3-, NO_3^- − N; Cond, conductivity; TN, total nitrogen; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

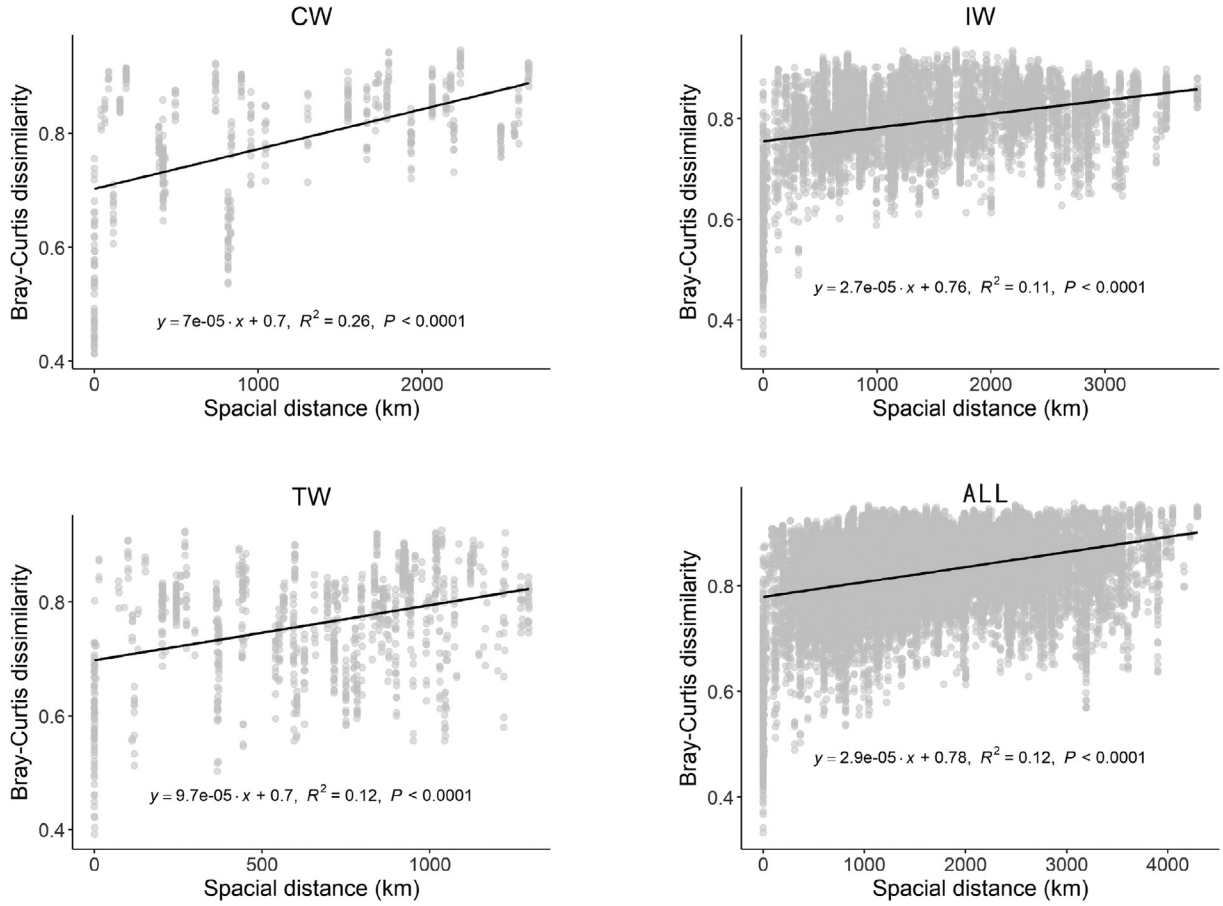

Fig. 6. Distance–decay relationships for bacterial communities in the wetlands. Each data point was based on the Bray–Curtis dissimilarity score between two samples and the geographic distance between them. The straight lines represented the linear relationships between the dissimilarity and distance matrices.

which implied there might be some unmeasured factors driving bacterial community assembly.

4. Discussion

In this study, bacterial community structures in wetland soils at large spatial scale across China were revealed using Miseq sequencing technique, and the effects of environmental factors and geographical distance were revealed on the bacterial communities and their assemblage mechanisms in wetland soils.

Although the geographical scale of these wetlands are very large and the environmental conditions are very different, some conditions are relatively similar. For example, wetland soil has high organic matter layer saturated by water, while temperature and oxygen contents are more stable than other types of soil. These may result in a high proportion of the shared OTUs (87.21% of total reads in this study) among various types of wetland soils, and this also implicate that wetland bacterial communities have relatively stable compositions to ensure its ecological function. However, there were still some taxa being significantly different in various groups, e.g. Acidobacteria had higher abundance in IW, Bacteroidetes was higher in TW, and higher abundance of Firmicutes was observed in CW (Fig. S1). Acidobacteria is reported to be more abundant in acidic soils and their abundances are negatively correlated with the pH level (Lauber et al., 2009; Yun et al., 2014). Wetland soils with acid pH in IW, are widely distributed, especially in Sanjiang plain of northeastern China (Table S1), which might result in the high abundances of Acidobacteria in IW.

The differences in bacterial community structures also reflected in alpha diversity indices. The alpha diversity of IW was quite different from those of CW and TW, but it was interesting that CW and TW did not show significant different, although there is a large spatial distance between the Tibetan Plateau and coastline, and the weather conditions are very different (such as temperature, precipitation and altitude). This may be due to the strong filtering effects of salinity on the bacterial communities in the Qinghai-Tibet wetlands and coastline wetlands. Salinity is reported as an important environment factor shaping bacterial communities at regional scale (Lozupone and Knight, 2007; Wang et al., 2011). It directly affects microbial community structure by selecting those microorganisms adapted to a particular salt concentration (Lin et al., 2013; Logares et al., 2009). Moreover, the alpha diversity of CW was mainly correlated with altitude and temperature, while the TW was mainly correlated with conductivity (Table S2). Random Forest analysis showed that the top 10 abundant taxa were less influenced by conductivity compared to altitude, temperature, precipitation, and pH (Fig. 5). This indicated that the low alpha diversities of the bacterial communities in CW and TW samples were caused not only by salinity but also by other environmental factors. Although we could not accurately explain how these environment factors affect the alpha diversity, these differences could be used as the intrinsic properties of wetlands in different regions to provide reference for future research on the microbial responses to global changes.

pH is recognized as an important environmental factor driving the variation of bacterial community assembly (Fierer and Jackson, 2006; Lauber et al., 2009; Siciliano et al., 2014). In this study, the pH effects were higher than other environmental factors in IW and TW samples, and this is consistent with other soil types in this region (Hu et al., 2014; Liu et al., 2014). On the other hand, conductivity and precipitation were major environmental factors influencing bacterial communities in CW groups. Wetland soils in this study were mostly neutral (average pH was 7.66, 7.45, 7.86 in IW, CW and TW,

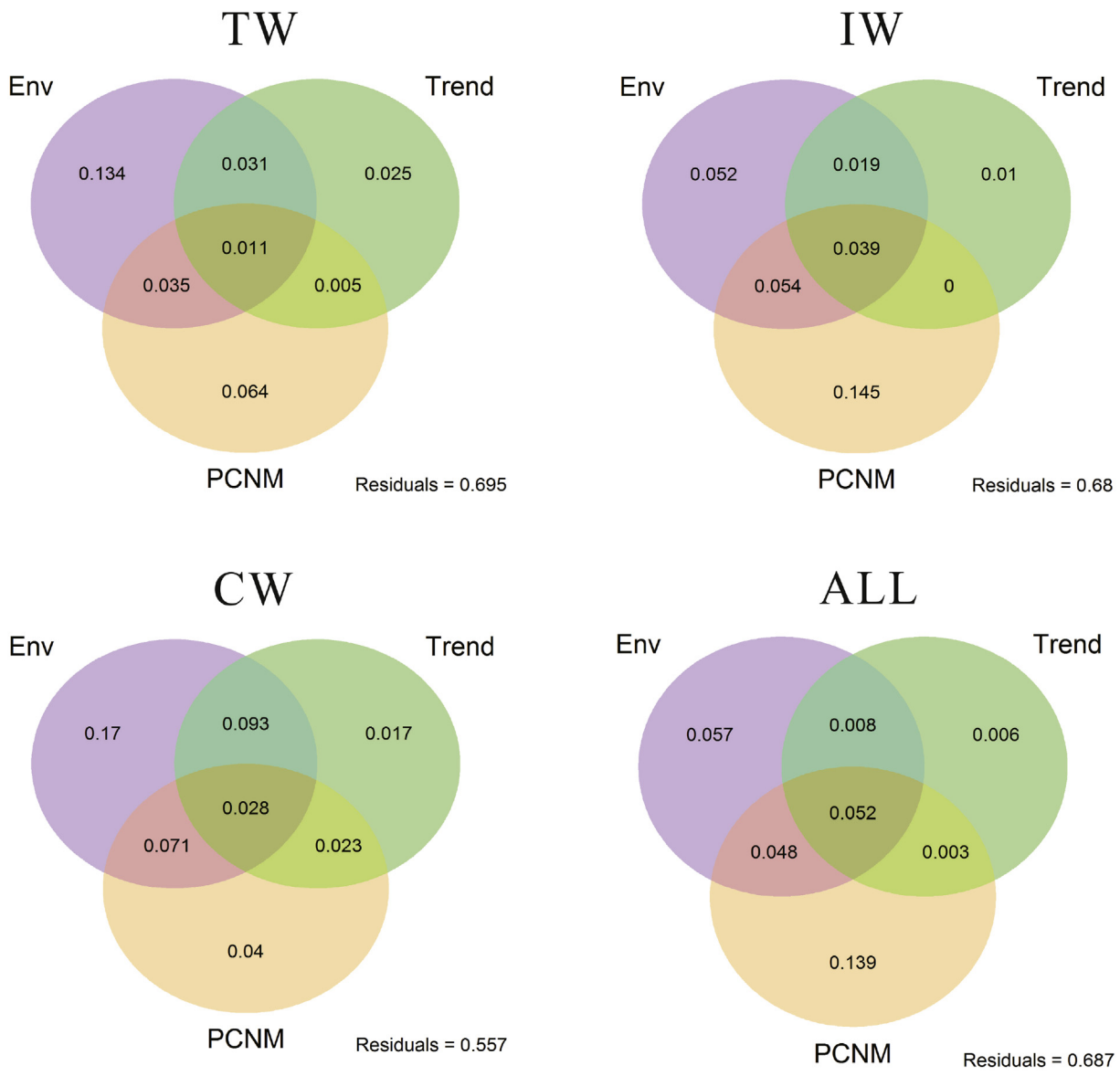

Fig. 7. Variation partitioning by principal coordinates of neighbor matrices (PCNM)-based analysis for the phylogenetic structure of bacterial communities into an environmental component (Env, top left), a linear trend (Trend, top right), and a finer spatial structure (PCNM, bottom). A forward selection procedure was used to select the best PCNM variables (no spatial correlation) important to the bacterial community structure.

respectively), and only a few samples exhibited acidic pH, these might cause less effects of pH on bacterial communities in our surveyed wetland soils in a large scale.

Nutrient-related environmental factors, such as TOC, $NH_4^+ - N$, $NO_3^- - N$ and TN, did not show high correlations to bacterial communities. Nutrients are possibly not limiting factors for microbial growth in the wetland soils due to the accumulation of high organic matter from decaying plants.

In many cases, microbial community similarity decays with increasing distance between samples, and distance-decay relationship is often used to assess the relative importance of environmental heterogeneity and dispersal history in controlling the spatial scaling of biodiversity (Green and Bohannan, 2006; Martiny et al., 2011). In this study, the bacterial community similarity decayed significantly with distance in all samples, but the relationships of beta-diversity and spatial distance were different in various wetland groups. The CW had a higher correlation than those in IW and TW. CW samples contained high salinity influenced by seawater, which might result in a low environmental heterogeneity among various CW wetlands. The VPA analysis also showed similar results that pure environment in CW contributed more to influence the community compositions than that in IW. This also implicated that bacterial community compositions of coastline wetlands would be more predictable than others using current measured variables.

It is well known that plants influence bacterial communities in soils, and plant diversity can be used to predict beta diversity of soil microbial community (Borga et al., 1994; Degens and Harris, 1997; Gömöryová et al., 2013; Kourtev et al., 2003; Prober et al., 2015; Ravit et al., 2006). The northern coastal wetlands are dominated by common reed (*Phragmites communis*), while mangroves are common in southern coastal wetlands. The differences in plant community compositions might contribute partly to the variations of bacterial communities in coastline wetlands.

Although TW and IW had similar distance attenuation relationships, however, the mechanisms that formed their relationships were possibly different. The community composition of TW was mainly shaped by pure environment, while the IW community was mostly influenced by PCNM. These results were likely related to the geographical

characteristics in the two regions. The Tibetan plateau is a high-altitude steppe with the average elevation above 4000 m above sea level, and it is interspersed with mountain ranges and many brackish lakes. The uplift of Tibet Plateau greatly influences the climate and biogeography of the fauna and flora (Shi, 1998), consequently, the Plateau has high species richness and endemism (Huang et al., 2008; Wang et al., 2009; Wu et al., 2013; Zhang et al., 2008). The environments of Tibet plateau are controlled by hypoxia, high solar UV radiation, and cold climate. The wetland samples in TW were collected from brackish lakes, fresh water lakes, and river banks. Many wetlands in this region are affected by brackish water, which are different from those in IW samples. The stronger influences of pH, conductivity and precipitation resulted in higher contribution of pure environment factors in TW samples than those in IW samples. We presume that the niche-based deterministic processes play more important roles on the bacterial community assembly, and the strong environmental selection in the Tibet plateau overwhelm the effects of dispersal.

The IW wetland samples were collected from the mainland of China across northern to southern regions. These wetlands are mainly freshwater marshes receiving the inputs of runoff from precipitation and/or surface water from surrounding lake and rivers. We collected more IW group wetland samples covering a larger region than CW and TW. We observed that the contributions of pure environmental factor and spatial distance to bacterial community assemblage were apparently weaker in IW than in other two groups. Moreover, the PCNM has more important contribution to community composition in IW. These wetlands are heavily affected by human activities which may introduce some human-caused factors in influencing bacterial community assembly, e.g., fertilizer application. In addition, the dispersal ability of wetland soil bacterial taxa is limited by the discontinuous distribution of IW wetlands, and dispersal limitation may contribute significantly to the variations of bacterial communities in IW wetlands.

When considering all the samples together, more than half of the total variation remained unexplained by both measured environmental factors and spatial distance. This might reflect the effects of non-measured environmental variables, biological interactions, or other historical events and factors that are not considered in this study.

Although it might not reflect the whole natural conditions by artificially clustering wetland communities into three groups, clustering approach provided a better resolution for understanding bacterial community assembly and key driving factors. The correlations between environmental variables and distinct bacterial community structures varied in various groups of wetlands, indicating scale-dependent key driving factors shaping bacterial communities.

In summary, this study revealed bacterial community structures in Chinese wetland soils, and the contributions from environmental variables and geographic distance in shaping bacteria community structure. These community structures and driving factors were significantly different in three wetland groups. It is likely that wetland bacterial community assembly in the same groups of wetlands tends to be similar, although different wetlands are far apart even within a group. The bacterial community is mainly shaped by environmental heterogeneity in natural conditions. On the other hand, the spatial distance exerts relatively weaker influence on bacterial communities in wetlands. However, it remains to elucidate the mechanisms of microbial community assembly at various scales.

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Conflict of interest

The authors declare no conflict of interest.

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