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Alpine soil microbial community structure and diversity are largely influenced by moisture content in the Zoige wetland

S. Fan[1] · J. Qin[2] · H. Sun[1] · Z. Jia[3] · Y. Chen[1]

Abstract

The Zoige alpine wetland is one of the most sensitive ecosystems to global climate change. It is the largest alpine wetland in the world and currently suffers from serious drought and degradation. In this study, soil microbial communities of five soils with different moisture content were investigated by Illumina MiSeq high-throughput sequencing of 16S rRNA. The results showed that soil acidity and the content of soil nutrients decreased with the decrease in soil moisture. The microbial richness indices (Chao1 and ACE) and diversity index (Shannon) were highest in the flooded wetland (FW) and lowest in the unflooded mound near the flooded wetland (UW). The relative abundance showed four dominated phyla among all the soil microbes in five soils: *Proteobacteria* (36.5%), *Acidobacteria* (26.1%), *Actinobacteria* (9.4%) and *Bacteroidetes* (5.8%). Moreover, *Proteobacteria* (51.4%) of UW was significantly ($P < 0.05$) higher than that of other soils, while *Actinobacteria* (1.6%), *Gemmatimonadetes* (0.9%) and *Nitrospirae* (0.03%) of UW were significantly ($P < 0.05$) lower than those of other soils. Principal component analysis (PCA) and redundancy analysis (RDA) revealed that soil samples of UW differed most from samples of other soils. *Proteobacteria* was positively correlated with water-soluble phosphorus, while *Actinobacteria* and *Bacteroidetes* were negatively correlated with total organic carbon and total nitrogen. Our findings revealed that soils in transitional unstable state from flooded to aridification had the lowest microbial diversity, and drought led to long-term changes in the microbial community in the Zoige wetland, which may cause further degradation of alpine wetland ecosystem functions.

Keywords Zoige alpine wetland · Microbial community · High-throughput sequencing · 16S rRNA · Soil moisture

Introduction

The Zoige alpine wetland is the most typical high-altitude and low-latitude wetland in the world, and it is sensitive to climate change (Cui et al. 2015a; Huo et al. 2013). The soil organic carbon density in the Zoige alpine wetland is three times greater than the wetland average in China and six times greater than the national average (Ma et al. 2016). It has the biggest peat deposition in China (Iqbal et al. 2019) and is an important global carbon storage reservoir for water conservation and biodiversity maintenance (Xiang et al. 2009). In recent years, due to the influence of natural and human activities, the trend of fragmentation and aridification of the Zoige alpine wetland has become increasingly serious (Pang et al. 2010). Wetland degradation promotes soil organic matter mineralization and carbon dioxide emission (Huo et al. 2013), accelerating carbon transfer from the soil to atmosphere and surface water. At present, research on the Zoige Wetland focuses on climate change (Bai et al. 2013; Li et al. 2014; Liang et al. 2015; Zhang et al. 2016), wetland degradation and restoration (Gao et al. 2014b; Jiang et al. 2017; Tang et al. 2012), carbon mineralization and carbon quality (Gao et al. 2011b; Gavazov et al. 2018; Luan et al. 2014), and specific microbial communities (Cui et al. 2015a;

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Fu et al. 2015; Tian et al. 2015; Wu et al. 2015; Zhang et al. 2008).

Soil microbes are the most active components in the soil. They play an important role in soil nutrient cycling, the maintenance of soil fertility and ecosystem function stability (Fierer 2017), and are important in exploring natural life mechanisms and the response of ecosystems to natural and anthropogenic disturbances such as global warming (Andersen et al. 2013). Microbial abundance and diversity in soil are mainly dependent on the physiochemical properties of the soil (e.g., pH, texture and nutrient status), rather than many macro-biogeographic factors which predict plant and animal diversity (Dequiedt et al. 2011; Fierer and Jackson 2006; Lauber et al. 2008). Research showed that moisture availability is the primary driver of microbial biomass carbon (it accounts for 34% of the global variance) (Serna-Chavez et al. 2013). Recently developed molecular biology techniques have the advantages of high data output and have been used to study the response of soil microbial communities to wetland degradation. Zhong et al. (2017) studied the depth-dependent response of microbial communities to water table drawdown. Other research focused on the different degradation stages of the Zoige wetland and analyzed the response of nitrogen-cycling microbial communities (Gu et al. 2019; Wu et al. 2016) and the association between microbial community changes and soil properties (Gu et al. 2018; Tang et al. 2012).

Although similar microbial biomass and dominant taxa may exist in various wetlands, different environment conditions and correlations between microbial communities and environmental factors can help us further understand the complex biochemical processes in alpine ecosystems. In this paper, 16S rRNA gene sequencing combined with other data processing and statistical analysis was used to study microbial communities of five soils with different moisture contents in the Zoige alpine wetland of China. Sampling was carried out in June 2016. Our purpose was to (1) identify the specific taxa and microbial community diversity in different soils, (2) compare our results with previous studies, and (3) analyze the association and potential mechanism between soil microbes and environmental factors. This study aims to comprehensively explain the impact of current degradation on the structure and diversity of microbial communities in the Zoige alpine wetland and provide scientific evidence for the protection and restoration of alpine wetland ecosystems.

Materials and methods

Study area and soil sampling

The Zoige alpine wetland (32° 20′–34° 20′ N, 102° 10′–103° 50′ E; 3400–3600 m.a.s.l.), located on the eastern margin of the Qinghai-Tibet Plateau, known as the third pole (Qiu 2008), covers about 31.5% of the whole Zoige plateau (Hao et al. 2011). It is dry in winter and wet in summer, with mean annual precipitation of 650 mm and mean annual temperature of 1.1 °C (Hao et al. 2011). With the current degradation of the Zoige alpine wetland, this area is now continuously distributed with plateau hills, rivers, terraces, lakes, peatlands and meadows. In June 2016, we collected five soils with decreasing moisture levels in the adjacent habitats of Zoige alpine wetland, namely flooded wetland (FW), unflooded mound near the flooded wetland (UW), arid wetland (AW), low meadow (LM) and high meadow (HM) (Fig. 1). There were three replicate samples for each soil, and each replicate was mixed by three points. The sampling depth was 0–10 cm. After removing litter and fine debris, it was ground and passed through a 100 mesh sieve and stored at 4 °C. One part of the soil was used to determine the physiochemical properties of the soil; the other part was used for high-throughput sequencing of 16S rRNA.

Physicochemical properties determination

Soil pH was determined by a pHS-25CW acidity meter; total organic carbon (TOC) and total nitrogen (TN) were determined by Elementar vario MACRO cube elemental analyzer; dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were extracted and determined by Elementar vario TOC cube total organic carbon analyzer (Jones and Willett 2006); water-soluble phosphorus (WSP) was determined by ICP-AES (US, Thermo Jarrell Ash).

DNA extraction and PCR amplification

Soil genomic DNA was extracted using MP Biomedicals' FastDNA® Spin Kit for Soil kit, performed according to the instructions. The resulting DNA solution was stored at − 20 °C until use. The DNA was thawed and diluted and then subjected to PCR amplification as a template. The dilution factor was determined by the DNA concentration (50 times in this study). The soil microbial 16S rRNA gene (v4–v5 region) was amplified using universal primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). The 50 μL PCR amplification system contained 1 μL of forward and reverse primers, 2 μL of DNA sample template, 25 μL of TaKaRa Taq version 2.0 plus dye (TaKaRa, Dalian, China) and 21 μL of sterile water. The PCR reaction conditions were: 94 °C, 5 min; 32x (94 °C, 45 s; 55 °C, 45 s; 72 °C, 1 min); 72 °C, 10 min.

Illumina MiSeq high-throughput sequencing

The PCR product was purified by Takara Agarose MiniBEST DNA Fragment Purification Kit Ver.2.0 kit and dissolved in

Fig. 1 Soil sampling site in Zoige alpine wetland. Soil moisture gradually decreases in five soils, namely flooded wetland (FW), unflooded mound near the flooded wetland (UW), arid wetland (AW), low meadow (LM) and high meadow (HM) (the same below)
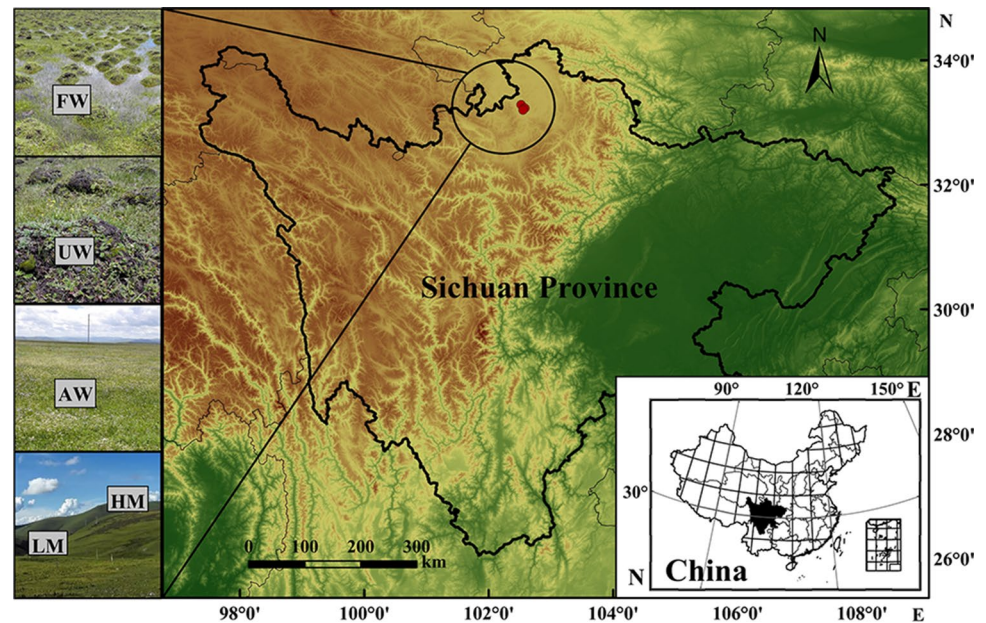
30 μL of DNase-free H_2O, and then, the concentration was measured with a Nano Drop® ND-1000 UV spectrophotometer using TruSeq Nano DNA LT Sample Prep Kit. After the kit was built, the sample concentration was calculated, and the soil samples were mixed in equimolar numbers, and high-throughput sequencing was performed using the Illumina MiSeq sequencer. The sequence was submitted to the National Center for Biotechnology Information (NCBI) Genbank repository under BioProject number PRJNA603635.

Data processing and statistical analysis

Pairs of reads were merged by Flash (Magoc and Salzberg 2011). Quality filtering was performed using QIIME as described by Bokulich et al. (2013). Then, the DNA sequences were clustered to the operation taxonomic unit (OTU) under 97% similarity using UPARSE algorithm (Edgar 2013), with 24,386 to 44,381 reads sampled (36,331 on average). The RDP classifier was used to annotate the OTU representative sequence from domain to genus level (Wang et al. 2007). All samples were rarefied at 10,000 sequences to standardize sampling efforts. The relative abundance was plotted using Origin 9.0. VennDiagram and vegan packages in R 3.5.2 were used to draw Venn diagrams and calculate community diversity indices. Sample clustering of UPGMA (unweighted pair group method with arithmetic mean) was performed using Past 4.01, based on the Bray–Curtis distances. STAMP was used to perform principal component analysis (PCA) and pairwise comparisons of OTUs (Parks and Beiko 2010). Redundancy analysis (RDA) was performed using Canoco for Windows 4.5, and statistical testing was performed using SPSS 21.0.

Results and discussion

Results

Soil physicochemical properties and microbial diversity

The physicochemical properties of the Zoige wetland samples are shown in Table 1. All five soils were acidic with a pH of 5.41–6.49. When the soil moisture decreased, meadow soil acidity (LM and HM) was significantly ($P < 0.05$) lower than that of wetland with water (FW and UW) (Table 1 and Table S1). TOC was also significantly ($P < 0.05$) different, which was highest in FW and UW, middle in AW, and lowest in LM and HM. TN of the five soils presented approximately the same order and significance of TOC. DOC of HM and WSP of LM were significantly lower than that of other soils ($P < 0.05$). In addition, DON of FW was 3–5 times higher ($P < 0.05$) than other soils, with an average of 0.66 g kg^{-1}.

Changes in the diversity of soil microbial community are illustrated in Table 2. The results showed that OTUs and total reads of UW were the lowest in the five soils. Chao1, ACE, and Shannon of FW were the highest, and Pielou of FW was in the middle. All diversity indices (chao1, ACE, Shannon, Simpson and Pielou) of UW were the lowest. Most of the diversity indices (except for Simpson) of HM were higher than those of LM. However, one-way analysis of variance (ANOVA) results showed that most diversity indices' differences did not reach the significant level ($P > 0.05$), except for chao1 of FW that was significantly higher than that of UW.

Shared and unique OTUs at the genus level of five soils are represented in Fig. 2. UW contains the least OTUs of

Table 1 Soil physicochemical properties of the Zoige Alpine Wetlands

Soils	pH*	TOC (g kg⁻¹)	TN (g kg⁻¹)	DOC (g kg⁻¹)	DON (g kg⁻¹)	WSP (mg kg⁻¹)
FW	5.41 ± 0.15c	155.62 ± 18.80a	14.46 ± 1.66a	0.73 ± 0.04a	0.66 ± 0.08a	9.53 ± 0.64abc
UW	5.43 ± 0.07c	167.26 ± 8.43a	14.42 ± 0.70a	0.66 ± 0.03a	0.24 ± 0.02bc	9.73 ± 0.65a
AW	5.80 ± 0.08bc	119.50 ± 25.77b	10.43 ± 2.58b	0.73 ± 0.12a	0.27 ± 0.14b	8.47 ± 0.78bc
LM	6.19 ± 0.28a	86.54 ± 19.77c	7.76 ± 1.56bc	0.67 ± 0.04a	0.18 ± 0.02bc	7.27 ± 0.42d
HM	5.94 ± 0.35ab	69.47 ± 3.84c	6.15 ± 0.21c	0.52 ± 0.05b	0.12 ± 0.02c	8.50 ± 0.36bc

*One-way analysis of variance (ANOVA) shows that the differences of all parameters reach the significant level ($P < 0.05$) ($n = 3$),

The results of multiple comparisons are indicated by values (mean $\pm$ SD) with different letters

Table 2 Soil microbial community diversity of the Zoige alpine wetland

Soil	OTUs	Reads	Richness		Diversity		Evenness
			chao1*	ACE	Shannon	Simpson	Pielou
FW	3578 ± 110	$38,414 \pm 3654$	5227a	5244	6.61	0.995	0.808
UW	2853 ± 211	$34,082 \pm 6060$	4325b	4385	6.12	0.991	0.769
AW	3289 ± 199	$36,650 \pm 4961$	4677ab	4681	6.56	0.995	0.810
LM	3287 ± 407	$37,538 \pm 3900$	4774ab	4795	6.50	0.995	0.802
HM	3340 ± 347	$34,971 \pm 10,049$	4999ab	4962	6.60	0.995	0.814
Significance based on one-way ANOVA							
F**		0.252	6.219	5.635	2.877	3.354	2.564
P		0.902	0.043	0.052	0.080	0.055	0.104

*One-way AVOVA shows that only Chao1′s difference reaches the significant level ($P < 0.05$) ($n = 3$)

**F values of chao1 and ACE are represented by *Welch* statistic because homogeneity of variance is not satisfied
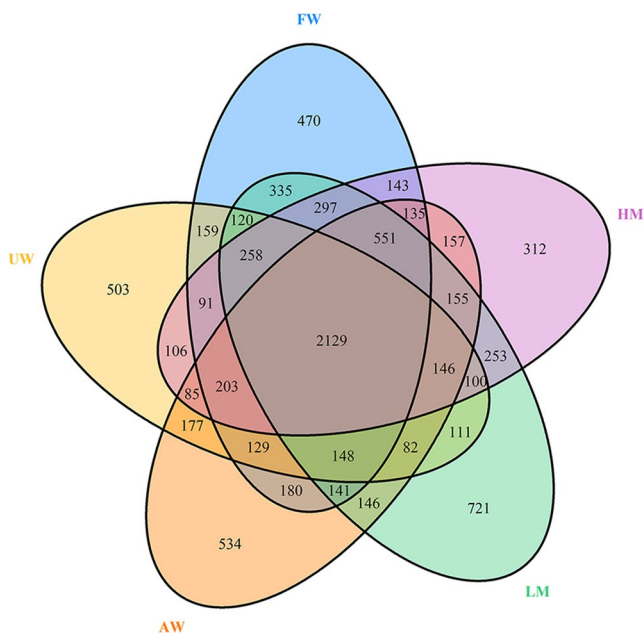
Fig. 2 Shared and unique OTUs at the genus level of five soils in the Zoige alpine wetland

4547 (total of three replicates), followed by AW (5089), HM (5121), FW (5489) and LM (5693). OTUs shared by five soils (2129) accounted for 37.4–46.8% of each soil, with that of UW being the largest (46.8%). The specific OTUs in LM accounted for the largest proportion of its total (12.7%), followed by UW (11.1%), AW (10.4%), FW (8.6%) and HM (6.1%). When comparing the proportions of two-soil-shared OTUs to the total number of a certain soil (Table S2), the proportions of OTUs shared by UW and other soils (FW, AW, LM and HM) were always the smallest (54.3–60.8%), while the proportions of OTUs shared by FW and other soils (UW, AW, LM and HM) were relatively large (69.9–74.3%).

Composition of soil microbial communities

Sample clustering and relative abundance of soil microbes at the phylum level are shown in Fig. 3. There were eight dominant phyla (mean relative abundance > 1.0%) of soil microbes in the Zoige alpine wetland, and the sum of their mean relative abundance was 85.3% (Table S3). The mean relative abundance of *Proteobacteria* (36.5%), *Acidobacteria* (26.1%), *Actinobacteria* (9.4%) and *Bacteroidetes* (5.8%) was more than 5%, and mean relative abundance of *Planctomycetes* (2.6%), *Gemmatimonadetes* (2.0%), *Chloroflexi* (1.7%) and *Firmicutes* (1.1%) was 1–5%. One-way
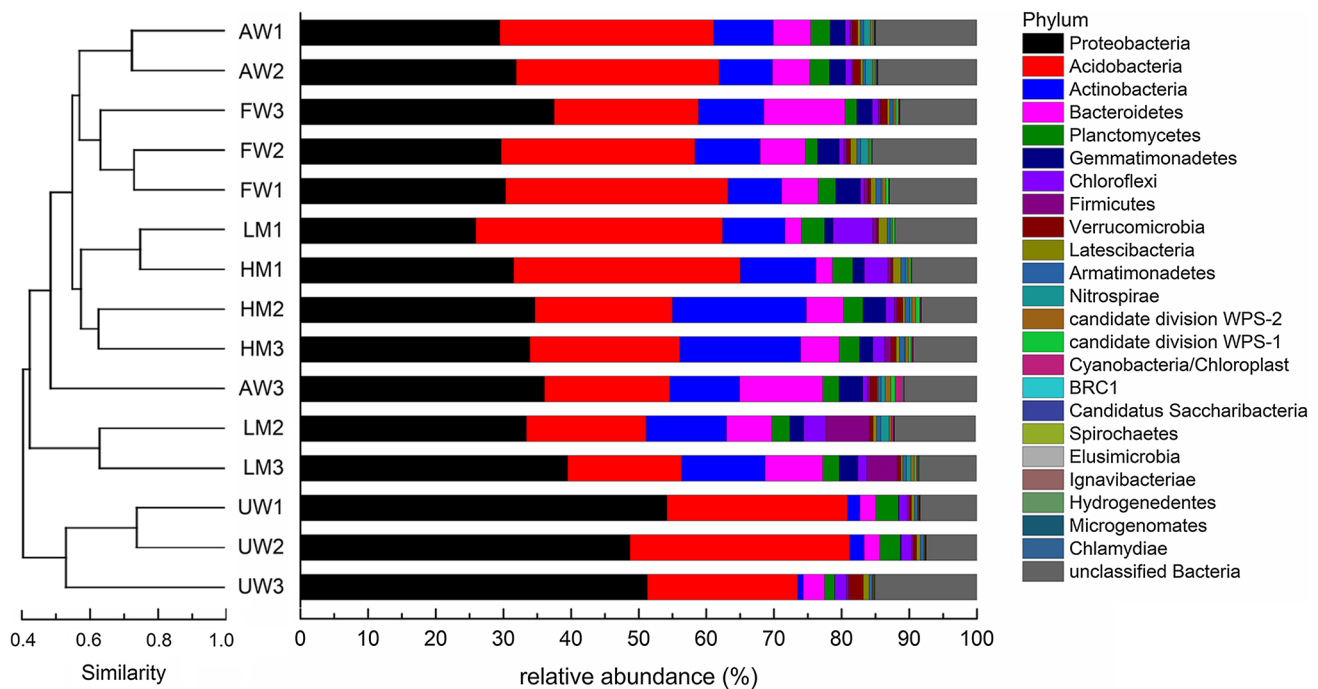
Fig. 3 Sample dissimilarity and relative abundance (%) of microbes at the phylum level in the Zoige alpine wetland soils

ANOVA showed that relative abundance of *Proteobacteria* ($P = 0.001$), *Actinobacteria* ($P = 0.001$), *Gemmatimonadetes* ($P = 0.003$) and *Nitrospirae* ($P = 0.026$) was significantly different (Table S4). *Proteobacteria* of UW (51.4%) was significantly higher than that of the other four soils, and UW's relative abundance of *Actinobacteria* (1.6%), *Gemmatimonadetes* (0.9%) and *Nitrospirae* (0.03%) was significantly lower than those for other soils. Although one-way ANOVA showed no significant differences in the relative abundance of *Firmicutes* ($P = 0.289$), the value of LM (3.8%) was nearly 10 times the average of other soils (0.4%).

The numbers of OTUs with significant difference ($P < 0.05$) at the genus level between different soils were counted according to the phylum to they belonged (Table S5). There were more OTUs with significant differences between FW, UW and AW (433 with 67.4% were FW > UW, 334 with 54.5% were FW > AW, and 396 with 39.9% were UW > AW), less between AW and LM (224 with 79.8% were AM > LM), and the least between LM and HM (108 with 15.7% were LM > HM) (Tables S6–S10). In general, dominant phyla (*Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Planctomycetes*, *Gemmatimonadetes*, *Chloroflexi* and *Firmicutes*) had more significant differences, except for *Bacteroidetes* between LM and HM (only one). Among other phyla with low relative abundance (< 1.0%), there were also significant differences in *Armatimonadetes*, *candidate division WPS-1* and *candidate division WPS-2* between FW and UW (with number of 5–7),

Nitrospirae between UW and AW (with number of 6), and *Armatimonadetes* between LM and HM (with number of 7).

In order to find the most significant differences in the composition of microbial communities, OTUs with high (top 30) DPs (difference in mean proportions) in pairwise comparison were selected. Many significantly different-OTUs between FW and UW (Fig. S1), UW and AW (Fig. S2) belong to *Proteobacteria*, including *Rhizobiales*, *Burkholderiales*, *Myxococcales1* and *Xanthomonadales* at order level, and OTUs with three largest DPs all belong to *Rhizobiales* and are higher in UW. In the result between FW and AW (Fig. S3), there were many OTUs belongs to *Acidobacteria* (including *GP1*, *GP3*, *GP4*, *GP6*, *GP7* and *GP17* at class level) and *Proteobacteria* (including *Burkholderiales*, *Xanthomonadales*, *Rhodospirillales* and *Rhizobiales* at order level), with the number of 11 and 10, respectively. When comparing LM with AW (Fig. S4) and HM (Fig. S5), most significantly different-OTUs were lower in LM, and they belonged to *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, *Verrucomicrobia*, *Gemmatimonadetes*, *Bacteroidetes* and *Armatimonadetes* at the phylum level.

Soil microbial communities and environmental factors

The result of PCA is shown in Fig. 4. The PC1, PC2 and PC3 axes explained 30.9%, 25.3% and 11.7% of the changes, respectively. UW was in the positive direction of PC1 and PC2 axes, while FW, AW, LM, and HM were all distributed

Fig. 4 Principal component analysis (PCA) of soil microbial communities in the Zoige alpine wetland
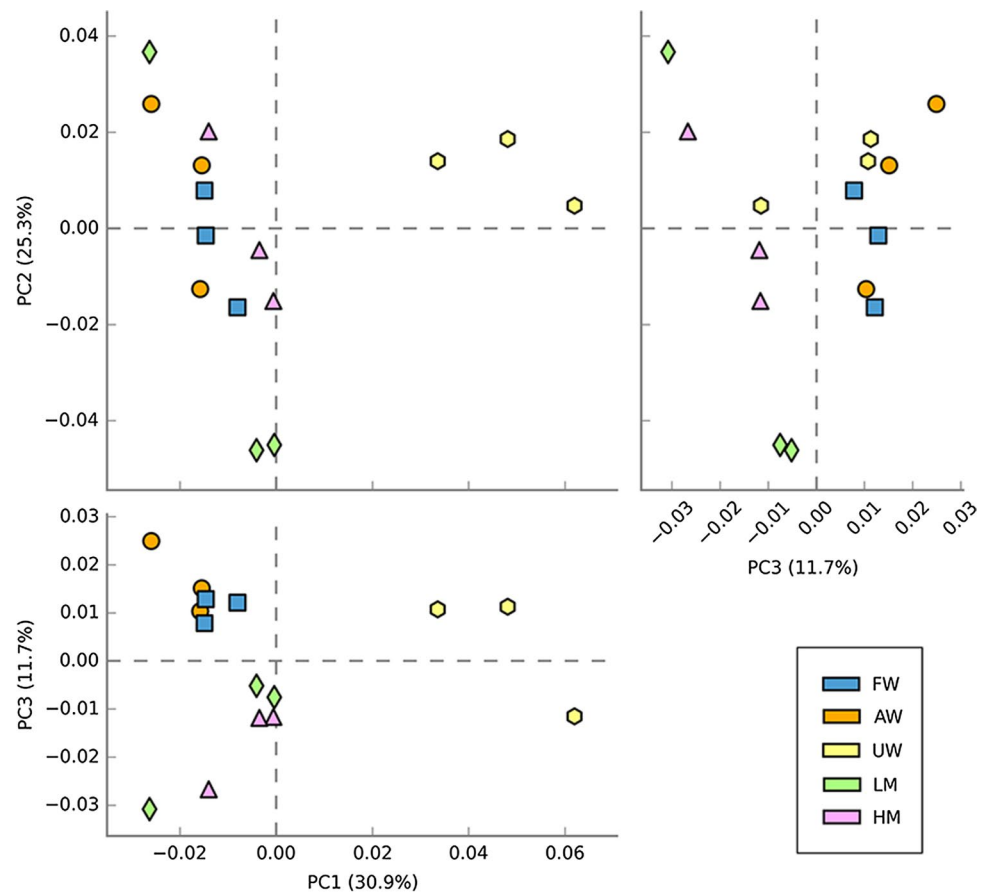
in the negative direction of PC1 axis, and both positive and negative directions of PC2 axis. FW and AW were both distributed in the negative direction of PC3 axis; LM and HM were both distributed in the positive direction of PC3 axis, while UW was distributed in both the positive and negative direction of PC3 axis. In general, the distribution of samples in UW was the most different from those in the other four soils, indicating that the structure of the soil microbial communities of UW was the most specific among the five soils in the Zoige wetland.

Constrained ranking can be used to study the impact of environmental factors on soil microbial species. Common methods include redundant analysis (RDA) (based on a linear model) and canonical correspondence analysis (CCA) (based on a single peak model). The length of the first axis in detrended correspondence analysis (DCA) was 0.65 (Table S11), so RDA was used in this study. The first two axes of RDA explain 48.7% and 7.1% of total variance (Fig. 5). The results showed that there were strong positive correlations between TOC and TN, DOC and DON; a strong negative correlation between pH and WSP in this study.

The impacts of environmental factors on microbes varied with microbial taxa. In this study (Fig. 5), *Proteobacteria* and *Verrucomicrobia* had positive correlations with WSP;
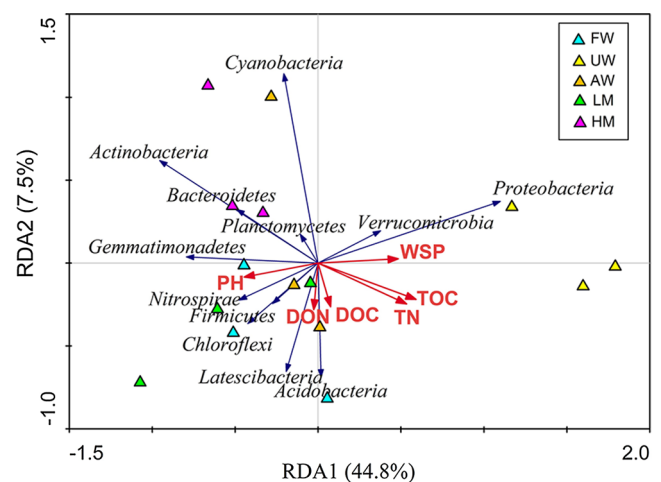

Fig. 5 Redundancy analysis (RDA) of soil microbial communities and Environmental factors of five soils in the Zoige alpine wetland

Acidobacteria had strong positive correlations with DON and DOC; *Actinobacteria* and *Bacteroidetes* had strong negative correlations with TN and TOC; *Gemmatimonadetes* was negatively correlated with WSP and positively correlated with pH. Moreover, UW was mainly affected by

WSP, TN and TOC; FW and LM were mainly affected by pH, DOC and DON; HM was affected by pH, while AW was moderately affected by all environmental factors. In addition, the distance between UW and other soils (FW, AW, LM and HM) was the largest, which was consistent with PCA.

Discussion

With current level of global warming, emission of stored carbon in wetlands may strengthen the greenhouse effects (Bridgham et al. 1995). Based on incubation experiments, Gao et al. (2011a) found that CO_2 and CH_4 emission rates in the Zoige alpine wetland increased with the increase in temperature, and increasing water table reduced CO_2 emissions and increased CH_4 emissions. The results also showed that the carbon mineralization process in the Zoige wetland was more sensitive at low temperature (Gao et al. 2011a). Our research found that with the decrease in soil moisture (from FW to HM), the acidity of soil was weakened, and the soil nutrients tended to decrease, which is consistent with previous studies (Gao et al. 2014a; Gu et al. 2018; Ma et al. 2016), possibly because the reduced soil moisture in the wetland increased soil permeability and thus promoted the decomposition of soil organic carbon and other nutrients. In addition, Fenner and Freeman (2011) found that aridification reduced the content of phenolic compounds in peatlands and promoted microbial growth, which in turn exacerbated soil carbon mineralization. Microbial activity also affects soil properties and nutrient conversion. Previous research showed that the mineralization rates of carbon, nitrogen and phosphorus are positively correlated with soil microbial biomass (McLatchey and Reddy 1998).

Soil organisms (especially microbes) are closely related to soil and water conservation, decomposition of organic matter, nutrient cycling, detoxification of poisons and inhibition of pathogenic bacteria in soil ecosystems (Doran and Zeiss 2000). Generally speaking, soil microbial diversity of natural wetland is higher than that of constructed wetland, and its microbial community difference is also smaller than that of constructed wetland (Ansola et al. 2014). In addition, previous studies found that drought has a long-term effect on microbial communities by affecting vegetation and soil moisture, with bacteria being more resilient but less resistant than fungi (Bapiri et al. 2010; Barnard et al. 2013; De Vries et al. 2018). In our study, there was no significant difference of richness and diversity indices of microbial communities between different soils, except for Chao1′s difference between FW and UW. Zhong et al. (2017) also found that statistical significance of diversity reduction only appeared in the middle depth of the soil, but not in the top soil or deep soil. In our study, samples of similar soil types were clustered together in PCA, with the distance between UW

and other soils being the largest, indicating that soil types largely shaped microbial communities in the Zoige alpine wetland (Tang et al. 2012). In addition, diversity indices of HM were higher than those of LM, probably due to the higher metabolic activity of the soil microorganisms in HM and their stronger ability to utilize substrates.

The most dominant phyla in our study accounted for more than half of the total microbes, including *Proteobacteria* (36.5%), *Acidobacteria* (26.1%), *Actinobacteria* (9.4%), *Bacteroidetes* (5.8%), *Planctomycetes* (2.6%), *Gemmatimonadetes* (2.0%), *Chloroflexi* (1.7%) and *Firmicutes* (1.1%). Notwithstanding the heterogeneity in soil types and soil properties, microbial communities in the Zoige wetland shared similar dominant taxa. Based on DGGE, Tang et al. (2012) found bacterial phyla of *Proteobacteria* and *Bacteroidetes* in swamp soil, peat soil, meadow soil and sand soil. Gu et al. (2018) found that swamp soil, meadow soil and sandy soil were dominated by *Proteobacteria* (24.6–31.6%), *Acidobacteria* (12.3–19.7%) and *Chloroflexi* (10.8–19.7%). Cui et al. (2015b) observed similar pattern in different incubation groups, with the 10 top abundant phyla being *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Firmicutes*, *Acidobacteria*, *Planctomycetes*, *Verrucomicrobia*, *Chlorobi*, *Gemmatimonadetes* and OP8. Cui et al. (2015a) also found that methanogenic bacterial communities in wetlands constituted by different plant species remained relatively constant and were not significantly affected by the temperature increases. However, there were still specificities in the microbial communities of the Zoige wetland. We found that *Proteobacteria* (especially *Rhizobiales* at the order level) of UW was significantly higher than that of other soils, *Actinobacteria* and *Gemmatimonadetes* of UW were significantly lower than those of others, and *Firmicutes* of LM were higher than that of other soils. These differences might be caused by the physiological preferences (e.g., oxygen content, hydrothermal condition and nutrient content) of different phyla.

The microbial community in the Zoige wetland was complicatedly affected by environmental factors, and the complex mechanism of microbial metabolism can be explained by the relationship between specific microbial taxa and environmental factors. Our results suggested the strong negative correlations between TOC(TN) and *Actinobacteria* (*Bacteroidetes*), indicating that TOC and TN may be the key factors that inhibit the growth of *Actinobacteria* and *Bacteroidetes*. The strong positive correlations between *Acidobacteria* and dissolved nutrients (DOC and DON) also indicated the effect of nutrient conditions on *Acidobacteria*. Tang et al. (2012) found that soil organic carbon and TN were more strongly correlated with microbial biomass than soil water content. Zhong et al. (2017) found that soil pH, TOC and TN were the most important parameters influencing the structure of soil microbial communities, which confirmed our results of RDA. What is more, researchers also found that drought

indirectly increased the abundance of denitrification and ammonia-oxidizing genes to increase soil nitrogen availability (De Vries et al. 2018), and altitude change significantly affected ammonia-oxidizing microorganisms (Zhang et al. 2009). In addition, Gu et al. (2018) found that soil total potassium was the strongest driver that affected the shaping of microbial communities in the Zoige wetland.

In summary, changes in nutrient content and availability caused by aridification in the Zoige wetland would affect the metabolism of soil microorganisms and thus affect the function and stability of the entire wetland ecosystem. Some studies found that climate warming would promote the development of vascular plants in peat wetlands, while the rhizosphere initiation promotes the heterotrophic decomposition of peat microbes, thereby increasing the degree of humification of dissolved organic matter (Gavazov et al. 2018). In addition, seasonal freezing and thawing, extreme weather such as summer rainstorm in alpine regions, also cause soil moisture changes (Edwards et al. 2007), which will further affect soil physical properties, nutrient dynamics and microbial activity. Moreover, research showed that recovery of water may further accelerate carbon loss in peatlands (Fenner and Freeman 2011), which has an important reference value for the restoration of the Zoige alpine wetland, and also illustrates the need for further understanding the ecological role of microbial communities in the degradation of wetland ecosystem.

Conclusion

We studied the microbial community composition and the diversity of five soils with different moisture contents in the Zoige alpine wetland and found that (1) soil acidity and nutrients tended to decrease when the water content decreased; (2) the flooded wetland had the highest microbial diversity, while the unflooded mound near the flooded wetland had the lowest microbial diversity, and its microbial composition was significantly different from the other four soils; (3) dominant phyla in five soils were *Proteobacteria*, *Acidobacteria*, *Actinobacteria* and *Bacteroidetes*, and they responded differently to environmental factors, while the specific mechanism and quantitative relationship are still unclear. Alpine soil microbial composition and its variation with aridification caused by climate change in a natural environment are of great ecological significance. In the future, we can combine other advanced methods such as metagenomics and metabolomics to further study the spatial and temporal response of soil microbes to environmental changes such as warming, the mechanisms of carbon, nitrogen and phosphorus transformation, and the quantitative

relationships between certain functional bacteria and environmental processes in alpine wetlands.

Authors' contribution SF and HS conceived the idea and designed the study. SF, YC and ZJ collected samples and performed the experiment. SF and JQ interpreted the data and wrote this paper. All authors reviewed the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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

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