Seasonality and vertical structure of microbial communities in the alpine wetland

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Research Article

Keywords: Soil microbes, Depth, Seasonal variation, Alpine wetlands

Posted Date: October 16th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3435035/v1

Additional Declarations: No competing interests reported.

Abstract

The soil microbial community plays an important role in wetland ecosystem material transformation and energy flow. The temporal dynamics and spatial distribution of soil microbial communities have always been central questions in ecology. Numerous studies on wetland microbial community structure have focused on low altitudes, while patterns of microbial diversity across seasons and depths and their environmental determinants remain poorly studied. Here, we collected soil samples across four seasons at 0–5, 5–10, and 10–30 cm in the NamCo wetland on the Tibetan Plateau to study the seasonality and vertical patterns of soil microbial communities and their main drivers. We found clear seasonal variation in bacterial community composition, most pronounced in winter, but nonsignificant seasonal variation in archaea. In particular, *Proteobacteria* decreased by 11.5% in winter compared with other seasons ($P < 0.05$). The alpha diversity, indicated by the Chao1 index, showed hump-shaped seasonal patterns with a lower diversity in winter for bacteria but nonsignificant patterns in archaea across depths. PERMANOVA showed that the bacterial community structure had significant differences between winter and the other three seasons ($P < 0.05$). In addition, bacterial and archaeal community structures differed between surface (0–5 cm) and deeper (5–30 cm) soils ($P < 0.01$). Redundancy analysis demonstrated that soil total nitrogen, soil total phosphorus, and total soil organic carbon had significant effects on bacteria and archaea ($P < 0.05$). Furthermore, the bacterial community structure was strongly affected by soil moisture content and temperature ($P < 0.001$). Our findings highlighted the seasonal variation in the microbial community and the profound influence of soil moisture and temperature on microbial structure in alpine wetlands on the Tibetan Plateau.

1 Introduction

Soil microorganisms act as decomposers of ecosystems, regulating ecological circulation and energy flow[1], which are extremely sensitive to environmental changes. For instance, microbial community composition and diversity are influenced by soil physical and chemical properties, seasonal precipitation, and soil depth[2]. A series of studies have focused on microbial temporal variation or vertical distribution patterns and their main controlling factors in wetland ecosystems[3]. However, the seasonal dynamics of soil microorganisms across depths and their main drivers are less studied.

The seasonal changes or spatial distribution of wetland microbial communities and their relationship with environmental changes are fundamental research topics in ecology. Some studies have shown that the soil microbial community exhibited significant seasonal variation, with the highest diversity in spring and the lowest diversity in winter in the Zhalong wetland[4]. Furthermore, some research found that the seasonal variation in the microbial community was mainly affected by regional temperature and available substrates[5]. With increasing soil depth, the diversity of the bacterial community decreased, while the diversity of the archaeal community increased. In addition, the community structure of soil bacteria and archaea presented a regular layered distribution in wetlands in the Yellow River Delta wetland[6]. Likewise, an obvious vertical distribution of archaea was also found in the Huixian karst wetland[7]. In research on the variation characteristics of CH_4 flux and its relationship with methanogenic community composition in peat wetlands, it was found that season and depth had direct effects on the methanogenic diversity index and community composition[8, 9]. To date, most studies have focused on the microbial community structure in low-altitude wetlands, and there are few studies on seasonal changes in the microbial community at different depths in alpine wetlands.

The Tibetan Plateau has the largest wetland area in China[10]. Due to the low temperature and thin oxygen in high-altitude areas[11], alpine wetlands are vulnerable and sensitive to climate change and human activities[12]. A recent study showed that large areas of alpine wetland are experiencing rapid mineralization of soil organic carbon and rapid loss of soil nutrients[13]. In this study, soil samples from four seasons at 0–5 cm, 5–10 cm, and 10–30 cm were collected in the NamCo wetland, and we aimed to explore 1) the seasonality and vertical patterns of soil microbial communities and 2) the underlying drivers for the observed patterns. This study will provide comprehensive insights into the spatiotemporal distribution of soil microbial communities under global climate change.

2 Materials and methods

2.1 General situation of the research area

The NamCo wetland (29°57'-30°33' N, 89°49'-90°17' E) is located between Damxung County of Lhasa City and Bango County of Nagqu Prefecture, with an area of 6,300 hm^2 and an elevation of 4,720 m. The sampling site is covered with alpine swampy meadow of *Kobresia tibetica, Kobresia pygmaca and Kobresia humilis* with an average height of 15–20 cm and a total coverage of 60% ~ 80%[14]. The region has a typical plateau continental climate. The annual mean temperature is 0°C, with the coldest monthly temperature in January (mean temperature − 18.2°C) and the monthly warmest temperature in July (mean temperature 14.7°C). The annual precipitation is primarily concentrated in May-October, with distinct rainy and dry seasons[15]. The temperature in 2017 used in this study was recorded at the NamCo Multilayer Comprehensive Observation and Research Station.

2.2 Sample collection

Soil samples were collected from the Namco wetland on June 23, August 25, October 6, and January 23 in 2017. Three soil columns were obtained using a soil driller with an inner diameter of 4 cm. Soil samples were collected from 3 layers (0–5 cm, 5–10 cm, 10–30 cm) and homogenized with a sterilized spoon. After discarding the visible stones and the plant root, 500 g of soil was sieved through a 2-mm mesh and then stored at 4°C for physiochemical analyses or at − 80°C for microbial community analyses.

2.3 Determination methods

2.3.1 Determination of soil physical and chemical properties

Five grams of soil was added to distilled water (1:2.5, soil—water ratio), after which the mixture was stirred for 1 min and allowed to stand for 30 min, and the pH was determined with a pH meter (Sartorius PB-10, Germany). Five grams of natural air-dried soil was sieved through a 1-mm sieve and baked in a 105°C oven to constant weight. The weight loss was measured, and the water content was calculated. Then, total organic carbon (TOC) and total nitrogen (TN) were measured with a CHNS/O Analyzer (PerkinElmer, Waltham, MA, USA) after 1 N-HCl acidification and 50°C oven drying. Total phosphorus (TP) was measured by the molybdenum-antimony resistance method [16]. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured by dissolving water-soluble substances in water through mixing soil in water with a weight ratio of 1:5[16]. After soil—water mixing and acidification with a drop of phosphoric acid, the soil was shaken for 120 r/min at room temperature for 1 h[17]. The contents of DOC and TDN in the supernatant were determined by a Multi N/C 2100S (Jena, Germany).

2.3.2 High-throughput sequencing of the 16S rRNA gene in the soil microbial community

The soil metagenomic DNA was extracted by the FastDNA® SPIN kit for Soil. One microliter of DNA was amplified by PCR using a 96-well Veriti Thermal Cycler (Veriti 96-well) with the forward primer 515F (5’-GTGCCAGCMGCCGCGGTAA-3’) and the reverse primer 806R (5’-GGACTACHVGGGTWTAAT-3’) to amplify the 16S rRNA gene V4 region[18]. The PCR products were loaded into a 1% agarose gel and run for 30 min at 120 V. The amplified fragments were recovered and purified by using SV Gel and PCR Clean-Up System. The purified PCR products were dissolved in 20 μl of purified water and quantified by Picogreen reagent (Quant-iT tm Picogreen DNA Reagent and Kits, Invitrogen, Carlsbad, California).

The purified DNA was amplified by emulsion PCR using an Ion PGM™ Template OT2 400 Kit (Catalog No. 4479878, Life Technologies, USA), and then the amplified product was enriched by an Ion OneTouch ES System. The enriched DNA template-positive ISPs were retained in a 0.2 ml PCR centrifuge tube. The preenriched ISPs were subjected to thermal cycling with sequencing primers, depolymerized at 95°C for 2 min, annealed at 30°C for 2 min, and then added to 3 μL of ION PGM™ Sequencing 400 Polymerase. The sample (30 μL) was gently added to the sequencing Chip (318 tm Chip V2, cat. No. 4484354) and placed at the designated position of the IonTorrent sequencer for high-throughput sequencing.

2.4 Data analysis

Using Uparse software[19], the valid data of samples were clustered by operational taxonomic unit (OTU) sequences under 97% similarity. The MOTHUR method (threshold value 0.8-1.0) and SSU rRNA database[20] of SILVA[21] were used for species annotation analysis of OTU representative sequences. The histogram of species composition was drawn by OriginPro 2018. One-way analysis of variance (ANOVA) was used to test the significance among soil physical and chemical properties with SPSS 18.0. The Chao1 index was used to represent alpha diversity. Community dissimilarity and multivariate variance analysis (PERMANOVA) based on the Bray—Curtis distance matrix was used to test the seasonal variation and vertical structure of the microbial community. Redundancy analysis (RDA) was used to determine the impact of soil physiochemical properties and climate variables on the microbial community structure. Most statistical analyses and plots were conducted in R statistical software V3.6.3 by using the packages vegan and ggplot2.

3 Results

3.1 Soil physicochemical properties in alpine wetlands

Soil physicochemical properties varied with depth and different seasons. For example, the average contents of TDN, TOC and SMC in 0–5 cm depth soil in spring, summer, and autumn were 4.78 mg/kg, 0.13 mg/kg and 0.018 mg/kg, respectively, which were higher than the respective contents of 4.1 mg/kg, 0.09 mg/kg and 0.013 mg/kg in winter. In contrast, the pH was the highest in the winter soil depth. At different depths in the same season, soil physicochemical properties showed a general decreasing trend (Table 1), especially at depths of 10–30 cm. For example, in June, the average contents of TN, TP, and DOC at 0–10 cm were 10.21 mg/kg, 1.73 mg/kg, and 26.99 mg/kg, respectively, while the contents of these properties at 10–30 cm were only 8.03 mg/kg, 1.34 mg/kg and 18.01 mg/kg, respectively.

Table 1
Soil physical and chemical properties across different seasons and depths in alpine wetlands

Sample depth	**Index parameter**	**Spr**	**Sum**	**Fal**	**Win**
0–5 cm	TN (mg/kg)	10.29 ± 1.91ab	11.29 ± 1.10a	8.35 ± 0.43c	7.63 ± 1.30d
	TP (mg/kg)	1.80 ± 0.22a	1.87 ± 0.20a	1.46 ± 0.19a	1.65 ± 0.21a
	DOC (mg/kg)	26.88 ± 12.08bc	18.34 ± 1.77c	37.15 ± 1.98bc	41.38 ± 12.79a
	TDN (mg/kg)	5.81 ± 3.05a	3.06 ± 0.31ab	5.48 ± 1.16a	4.15 ± 0.46a
	TOC (mg/kg)	0.15 ± 0.025a	0.14 ± 0.007ab	0.10 ± 0.006b	0.09 ± 0.026b
	pH	7.43 ± 0.01a	7.22 ± 0.16a	7.32 ± 0.07a	7.50 ± 0.25a
	SMC (%)	91.47 ± 3.86a	121.77 ± 7.53ab	139.71 ± 17.47ab	88.39 ± 0.1.98b
5–10 cm	TN (mg/kg)	10.12 ± 0.85ab	8.35 ± 2.80ab	8.91 ± 0.31ab	7.53 ± 2.66bc
	TP (mg/kg)	1.66 ± 0.28a	1.44 ± 0.08ab	1.45 ± 0.23ab	1.69 ± 0.45ab
	DOC (mg/kg)	27.11 ± 6.71ab	25.96 ± 4.78b	26.78 ± 10.23b	27.83 ± 12.09ab
	TDN (mg/kg)	4.51 ± 0.58ab	5.40 ± 1.41a	3.76 ± 1.45c	4.54 ± 2.60ab
	TOC (mg/kg)	0.12 ± 0.009a	0.09 ± 0.046a	0.09 ± 0.023a	0.72 ± 0.024a
	pH	7.35 ± 0.09bc	7.38 ± 0.13abc	7.28 ± 0.05bc	7.60 ± 0.09a
	SMC (%)	121 ± 22.72a	133.07 ± 7.37a	124.68 ± 22.97a	56.28 ± 20.47b
10–30 cm	TN (mg/kg)	8.03 ± 2.22b	8.74 ± 0.76ab	8.96 ± 1.31ab	4.93 ± 0.92c
	TP (mg/kg)	1.34 ± 0.11bc	1.46 ± 0.26abc	1.57 ± 0.24abc	1.31 ± 0.09c
	DOC (mg/kg)	18.01 ± 1.51c	20.84 ± 4.98c	21.83 ± 2.51b	28.66 ± 6.64ab
	TDN (mg/kg)	3.37 ± 0.43ab	3.45 ± 1.09ab	3.22 ± 0.98ab	1.02 ± 0.20c

Note: Spr, Sum, Fal and Win represent spring, summer, fall and winter, respectively. TN: Total nitrogen; TP: Total phosphorus; TOC: Total organic carbon of soil; DOC: Dissolved organic carbon; TDN: Total dissolved nitrogen; pH: Soil pH; SMC: Soil moisture content; T: atmospheric temperature. The data are the mean ± standard, and different letters in the same column indicate significant differences (n = 3, $P < 0.05$).

Sample depth	Index parameter	Spr	Sum	Fal	Win
	TOC (mg/kg)	0.08 ± 0.041ab	0.08 ± 0.009ab	0.09 ± 0.037a	0.05 ± 0.019d
	pH	7.42 ± 0.21abc	7.29 ± 0.02bc	7.19 ± 0.25c	7.35 ± 0.09bc
	SMC (%)	111.12 ± 12.28b	136.41 ± 19.16a	111.45 ± 3.94b	34.15 ± 0.83c
T (℃)		2.94 ± 0.91b	11.16 ± 0.66a	-1 ± 2.68b	-9.88 ± 1.63c

Note: Spr, Sum, Fal and Win represent spring, summer, fall and winter, respectively. TN: Total nitrogen; TP: Total phosphorus; TOC: Total organic carbon of soil; DOC: Dissolved organic carbon; TDN: Total dissolved nitrogen; pH: Soil pH; SMC: Soil moisture content; T: atmospheric temperature. The data are the mean ± standard, and different letters in the same column indicate significant differences (n = 3, $P < 0.05$).

3.2 Soil microbial community composition in alpine wetlands under different soil depths and seasons

After filtering low-quality sequences, a total of 20021 OTUs were obtained, and a total of 25 phyla, 50 orders, 6 orders, 128 families, 214 genera of bacteria, 2 phyla, 4 orders, 7 orders, 9 families, and 8 genera of archaea were detected. Overall, bacteria accounted for 97.4%, and archaea accounted for 1.5%. Within bacteria, the dominant phyla included Proteobacteria (relative abundance 50.2%), Acidobacteria (12.6%), Actinomycetes (10.4%), Verrucomicrobia (5.8%), Bacteroidetes (3.5%), Firmicutes (3.0%), and Planctomycetes (8.5%) (Fig. 1a). Proteobacteria was mostly composed of Alphaproteobacteria (20.8%), Betaproteobacteria (17.8%), Gammaproteobacteria (3.0%), and Deltaproteobacteria (4.5%). From the perspective of seasonal variation, the relative abundances of the main phyla in spring, summer, and autumn did not change much, but the relative abundances of the dominant bacteria differed in winter; in particular, the dominant Proteobacteria phylum decreased significantly ($P < 0.05$), from 41.5–11.5%, among which Alphaproteobacteria decreased by 5.1% in winter compared with the other three seasons. The three dominant classes of archaea were Thermoprotei (92.21%), Methanomicrobia (4.85%), and Methanobacteria (2.94%) (Fig. 1b). The relative abundance of Thermoprotei in winter was 77.7%, with increases of 5.7%, 10.0%, and 8.1% in comparison with spring, summer, and autumn, respectively. In terms of depth, there was no significant change in bacterial composition at 0–5 cm, 5–10 cm and 10–30 cm, and the relative abundance of Thermoprotei at 0–5 cm depth was 0.7% and 0.2% higher than that at 5–10 cm and 10–30 cm, respectively, while Methanomicrobia and Methanobacteria decreased by approximately 0.33%. Overally, the relative abundance of the soil microbial composition changed more significantly among the seasons than the soil depths, while the relative abundance of bacteria changed more significantly with the seasons than that of archaea.

3.3 Soil microbial alpha diversity in alpine wetlands under different soil depths and seasons

The Chao1 index is an indicator for estimating the total number of species in soil samples, and the higher the value is, the higher the diversity of species in the sample. Overall, the alpha diversity of bacteria was higher than that of archaea, and the diversity of bacteria and archaea at different depths did not change significantly among the seasons. However, there were significant differences in bacterial diversity at depths of 0–5 cm and 5–10 cm in winter compared with the other three seasons ($P < 0.01$), while the diversity at 10–30 cm showed significant differences between spring and the other three seasons ($P < 0.05$) (Fig. 2a). There were no significant differences in archaea in different seasons ($P < 0.05$) (Fig. 2b). Therefore, the soil microbial diversity also exhibited higher variability among depths.

3.4 Soil microbial beta diversity in alpine wetlands under different soil depths and seasons

PERMANOVA was used to reveal the differences in bacterial and archaeal community structure with season and soil depth. The bacterial community exhibited significant temporal decay rates (Fig. 3a), whereas archaea had no obvious seasonal patterns (Fig. 3b). Furthermore, the dissimilarity of the bacterial and archaeal communities exhibited an increasing pattern with increasing depth in spring ($P < 0.001$, Fig. 3c, d). The microbial community structure (including bacteria and archaea) between the surface soil (0–5 cm) and deep soil (5–30 cm) differed significantly ($P < 0.01$). For the variation in the seasons, the bacterial community structure in the winter differed significantly from that in the other seasons ($P < 0.01$), while the archaeal community structure did not change significantly with season (Table 2).

Table 2

Soil microbial community structure across different seasons and depths in alpine wetlands by using PerManova analysis

Sample name	Index parameter		Mean squares	F.Model	Variation	Pr(> F)
Bacteria	Depth (cm)	0–5/5–10	0.272	2.418	0.099	0.001**
		0–5/10–30	0.348	2.898	0.116	0.001**
		5–10/10–30	0.127	1.207	0.052	0.169
	Season	Spr/Sum	0.106	1.019	0.060	0.359
		Spr/Fal	0.155	1.480	0.085	0.067
		Spr/Win	0.295	2.438	0.132	0.002**
		Sum/Fal	0.106	1.035	0.061	0.383
		Sum/Win	0.306	2.567	0.138	0.003**
		Fal/Win	0.301	2.516	0.136	0.003**
Archaea	Depth (cm)	0–5/5–10	0.254	2.860	0.115	0.007**
		0–5/10–30	0.387	3.626	0.141	0.003**
		5–10/10–30	0.172	2.054	0.085	0.061
	Season	Spr/Sum	0.064	0.598	0.036	0.755
		Spr/Fal	0.078	0.693	0.042	0.653
		Spr/Win	0.168	1.481	0.085	0.212
		Sum/Fal	0.051	0.541	0.033	0.882
		Sum/Win	0.154	1.635	0.093	0.114
		Fal/Win	0.102	1.030	0.060	0.361

Note: Spr, Sum, Fal and Win represent spring, summer, fall and winter, respectively. *, ** indicate significance at $P < 0.05$ and $P < 0.01$, respectively.

3.5 Main environmental factors influencing soil microbial communities in alpine wetlands

Redundancy analysis (RDA) was performed to determine the strength of the association between the soil microbial community and climate, soil physical, and chemical properties (Fig. 4). The first two canonical axes explained 21% (29% by the RDA1 axis and 22% by the RDA2 axis) and 58% (36% by the RDA1 axis and 22% by the RDA2 axis) of the variance for the bacterial and archaeal communities, respectively. The RDA indicated that TN, TP, TOC, SMC and temperature had a significant influence on the bacterial community ($P < 0.05$); TN, TP and TOC had significant effects on the archaeal community ($P < 0.05$) (Table 3).

Table 3
The influence of environmental factors on soil microbial community structure in alpine wetlands by using redundancy analysis

Environment variables	**Bacteria**			**Archaea**		
	Variance	F	Pr(> F)	Variance	F	Pr(> F)
TN	0.014	1.840	0.005**	0.014	2.273	0.015*
TP	0.016	2.219	0.005**	0.014	2.297	0.013*
DOC	0.009	1.243	0.126	0.006	1.048	0.335
TDN	0.010	1.407	0.063	0.009	1.445	0.117
pH	0.009	1.222	0.15	0.005	0.815	0.609
TOC	0.010	1.415	0.046*	0.015	2.298	0.011*
SMC	0.018	2.247	0.001***	0.005	0.870	0.527
T	0.018	2.208	0.001***	0.006	1.043	0.346
	Inertia	Proportion		Inertia	Proportion	
Constrained	0.104	0.302		0.074	0.296	
Unconstrained	0.206	0.697		0.175	0.703	

Note: TN: Total nitrogen; TP: Total phosphorus; TOC: Total organic carbon of soil; DOC: Dissolved organic carbon; TDN: Total dissolved nitrogen; pH: Soil pH; SMC: Soil moisture content; T: atmospheric temperature. * indicates significance when $P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

4 Discussion

Our results showed that the composition of bacterial communities was strongly affected by seasonal changes. In particular, the abundance of Proteobacteria at the surface depth (0–5 cm) decreased by 5.1%, 3.0% and 1.3% in winter compared with the other three seasons. Previous studies have shown that Proteobacteria prefer a soil environment with a high nutrient content, which is significantly related to the content of soil organic matter and total nitrogen[22]. The physical and chemical parameters of this study showed that the nutrient content in the 0–5 cm depth soil was the highest, and Proteobacteria had the most significant seasonal changes in the 0–5 cm surface among the main bacterial lineages. In addition to the influence of soil nutrients in each soil layer, the actual temperature and precipitation data were measured by Wei et al[23]. At Namco station, the soil temperature is 2.94 (°C), 11.16 (°C), -1.01 (°C), and − 9.89 (°C) in spring, summer, autumn and winter, respectively, precipitation is mainly concentrated in July August (242 mm), and the soil temperature and precipitation are unimodal. Some studies have shown that the abundance of microbial communities is closely related to seasonal changes in temperature and precipitation[24–26]. In this study, the relative abundance of Firmicutes was 6.7% in summer > 3.4% in spring > 3.3% in autumn > 2.9% in winter, and the relative abundance of Methanomicrobia was 4.2% in summer > 3.2% in spring > 2.7% in autumn > 2.2% in winter. Because Firmicutes are facultative anaerobes and Methanomicrobia are anaerobes, they can maintain their growth under anoxic conditions compared with other microorganisms, and their relative abundance is higher in summer. In summer, 63% of the annual precipitation is concentrated in Namco[27], and the dissolved oxygen in soil is low[28], which may have inhibited the growth of other aerobic bacteria.

Higher bacterial diversity than archaeal diversity is similar to other wetlands, such as the Huixian wetland and the Nanniwan wetland in China[29, 30]. However, some major microbial lineages may differ due to their different locations. In addition, the bacterial Chao1 index showed significant differences between winter and the other three seasons, and the Chao1 index of archaea did not show significant differences with season. At the same time, the soil moisture content ($P = 0.001$, < 0.05) and temperature ($P = 0.025$, < 0.05) also decreased significantly in winter. A study on the Ruoergai Wetland has proven that soil moisture has a direct impact on the microbial community and diversity[31]. Based on the results of studies on the effects of biome time series[32, 33], the temperature will affect the biological enzyme activity, and the longer frost period and freezing period will also affect the nutritional status of the soil and increase the environmental pressure on microorganisms. Therefore, the long-term low temperature in winter might inhibit biological enzyme activity, thus affecting microbial activity and reducing the diversity of the microbial community. The changes in biological enzyme activity and soil microbial community structure in Tanggula Mountain show that biological enzyme activity is directly related to microbial community structure and diversity[34]. In this study, the relative abundance of Thermoproteus bacteria increased by 7.0% in winter. It is speculated that some cryophilic microorganisms were more durable in winter in the NamCo wetland.

PerManova analysis showed that there were significant differences between the microbial community in the surface soils and those in the deep soils, which was in accordance with the microbial community structure with a layered distribution in the wetland soil of the Yellow River Delta [6]. In the surface soil, the bacterial community structure is more complex. In subsurface soil, the bacterial community structure is relatively simple because there are fewer nutrients, the survival conditions of microorganisms are harsh, and archaea become the main component of the microbial community. In addition, the archaeal community structure in the subsurface soil is simpler than that in the surface soil, which is mainly composed of methanogenic bacteria, sulfur-reducing bacteria and archaea. This is because the oxygen content in deeper soils is lower, and archaea are usually anaerobic organisms that can survive and thrive in such conditions. Moreover, there are fewer nutrients in deep soil, and archaea are usually able to metabolize using simple organic and inorganic materials, so they are better able to survive and reproduce in this environment. These results showed that the layered distribution of aerobic or anaerobic bacteria and archaea is very obvious at different soil depths, indicating that with increasing soil depth, the difference in oxygen supply creates an aerobic or anaerobic environment, resulting in the regular layered distribution of the microbial community structure. Additionally, plant roots may also be one of the influencing factors responsible for the differences in microbial community structure at different depths[35].

RDA revealed that the soil microbial community structure is closely related to soil physical and chemical properties, and the impact of soil physical and chemical properties on the bacterial community is significantly higher than that on archaea. Specifically, bacteria were significantly affected by soil moisture content (SMC) and temperature (T) in the NamCo wetland. Many studies have proven that soil TN, TP, TOC and soil water content are significant factors affecting the structure of the microbial community[36, 37]. The seasonal and soil depth changes in the soil microbial community in this study area are mainly concentrated in the 0–5 cm surface soil, except that the nutrient content in the surface soil of the NamCo wetland is high and the water content is sufficient. It may also have an important impact on vegetation type [38], the input of regulated falling objects [39] and trace elements other than C, N and P [40]. However, the negative correlation between pH and bacterial and fungal abundance is inconsistent with previous studies, which reported that decreased pH inhibits microbial growth[41], which may be due to special habitats such as low temperature and low nutrients in the alpine zone of the Namco wetland, causing the changes in special functional populations in this area to be different from those in other soil ecosystems. For example, Proteobacteria and Acidobacteria dominate at the bacterial genus level and prefer acidic environments. Therefore, the drop in pH may promote the proliferation of microbes in the area[42].

5 Conclusion

In summary, based on the high-throughput sequencing of the 16S rRNA gene, this study depicted the seasonality and vertical patterns of soil microbial communities and their main drivers in the NamCo wetland on the Tibetan Plateau. We found that the soil microbial community in alpine wetlands exhibited clear seasonal variation, especially the diversity and composition of the bacterial community in winter, which were significantly different from those in the other three seasons, which may be caused by the soil nutrients, temperature, and moisture. More interestingly, the differences in soil microorganisms under different soil layers are mainly reflected in spring, when the community composition of bacteria and archaea in surface soil (0–5 cm) is significantly different from that in subface soils (5–30 cm). This may be mainly due to the lowest water level in spring, and the soil water content of the surface soil layer has not yet reached saturation. Overall, these findings highlighted the importance of seasonality variation and the vertical pattern of the microbial community in the alpine wetlands on the Tibetan Plateau.

Declarations

Data Availability Statement

The 16S rRNA gene sequences and geochemistry data for this paper are available at SRA under a BioProject No. PRJNA1026561.

Acknowledgments

This research was supported by grants from the Second Tibetan Plateau Scientific Expedition and Research Program (STEP) (2019QZKK0503) and the National Natural Science Foundation of China (41871066 and 41471055). We also thank the Nagqu Alpine Grassland Ecosystem National Field Scientific Observation and Research Station for field sampling.

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Figures

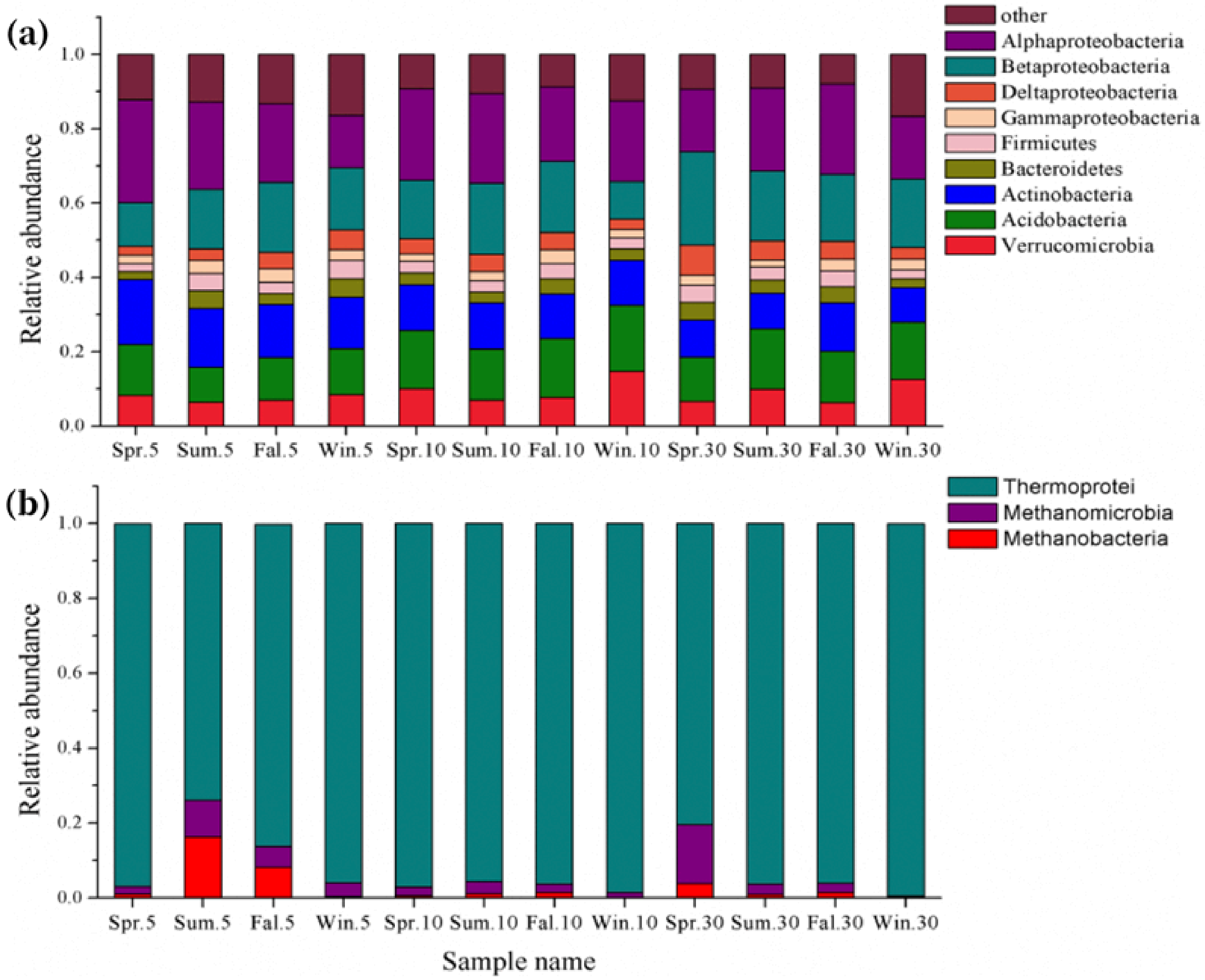

Figure 1

The relative abundance of the dominant phyla of the bacterial community (a) and the dominant classes of the archaeal community (b). Spr: Spring; Sum: Summer; Fal: Fall Win: Winter. 5:5 cm, 10:10 cm, 30:30 cm.

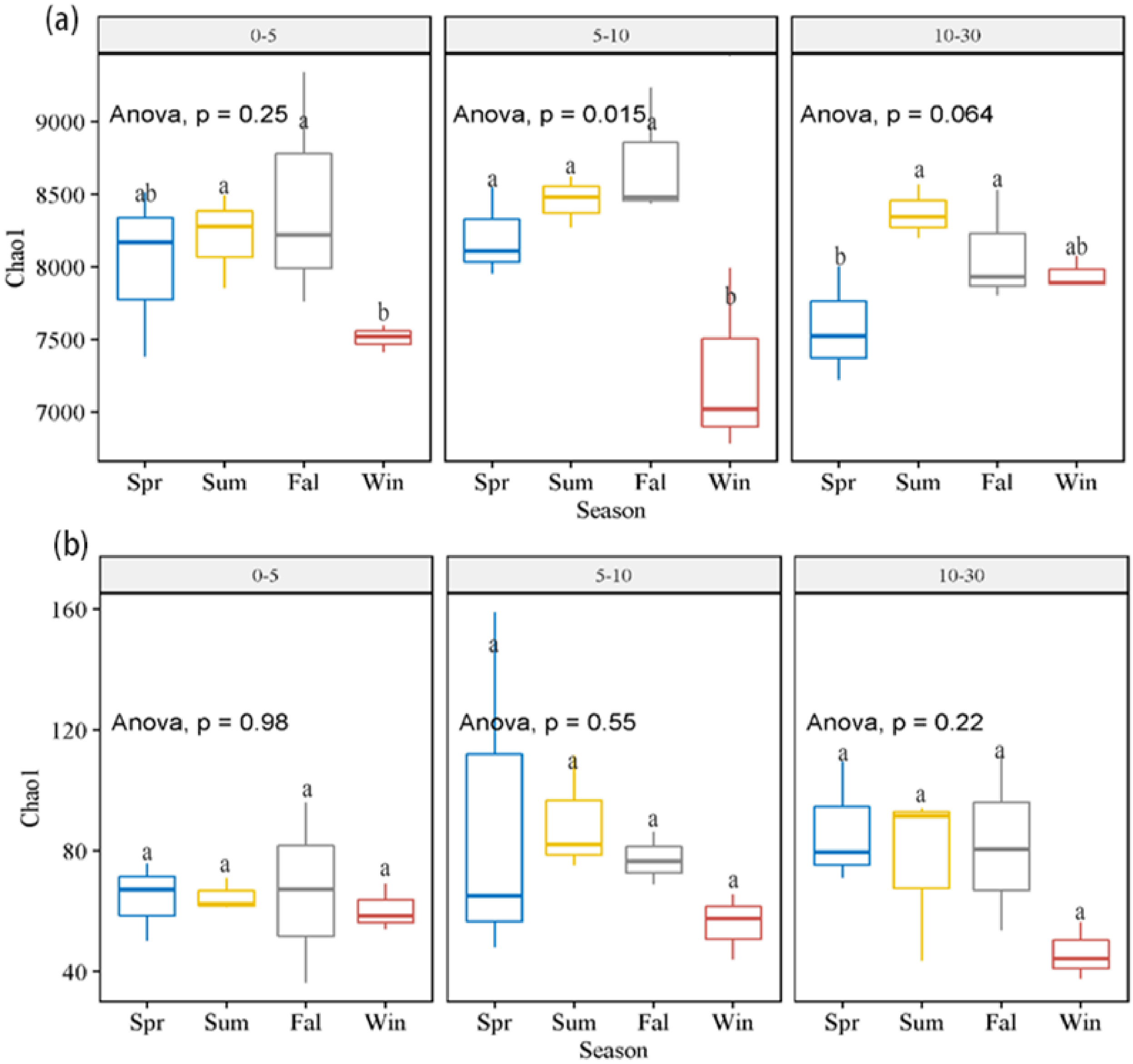

Figure 2

Microbial Chao1 index of different seasons at the same depth in the alpine wetland. (a) Bacterial and (b) archaeal Chao1 index across different seasons and depths. Different capital letters indicate significant differences ($P < 0.05$) between seasons at the same soil depth. Spr, Sum, Fal and Win represent spring, summer, fall and winter, respectively.

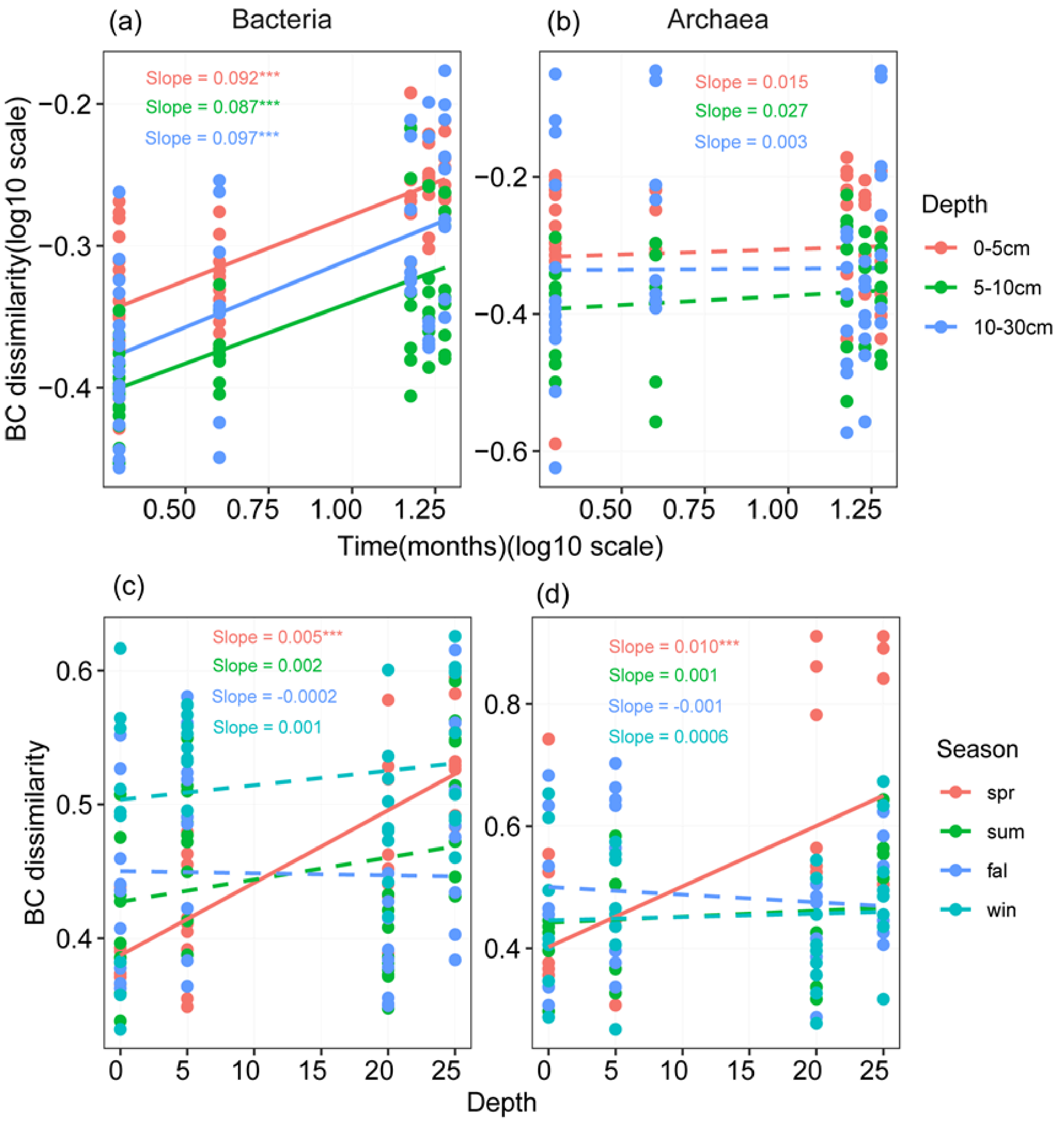

Figure 3

The dissimilarity of the bacterial and archaeal communities across months and depths. The community dissimilarity was calculated based on the Bray–Curtis distance matrix. The solid and dotted lines represent significant ($P < 0.05$) and nonsignificant ($P > 0.05$) results, respectively. The slope represents a linear model between community dissimilarity and temporal distance **(a, b)** or depth **(c, d)**. *, **, *** represent significant differences at the 0.05, 0.01 and 0.001 levels, respectively. Spr, Sum, Fal and Win represent spring, summer, fall and winter, respectively.

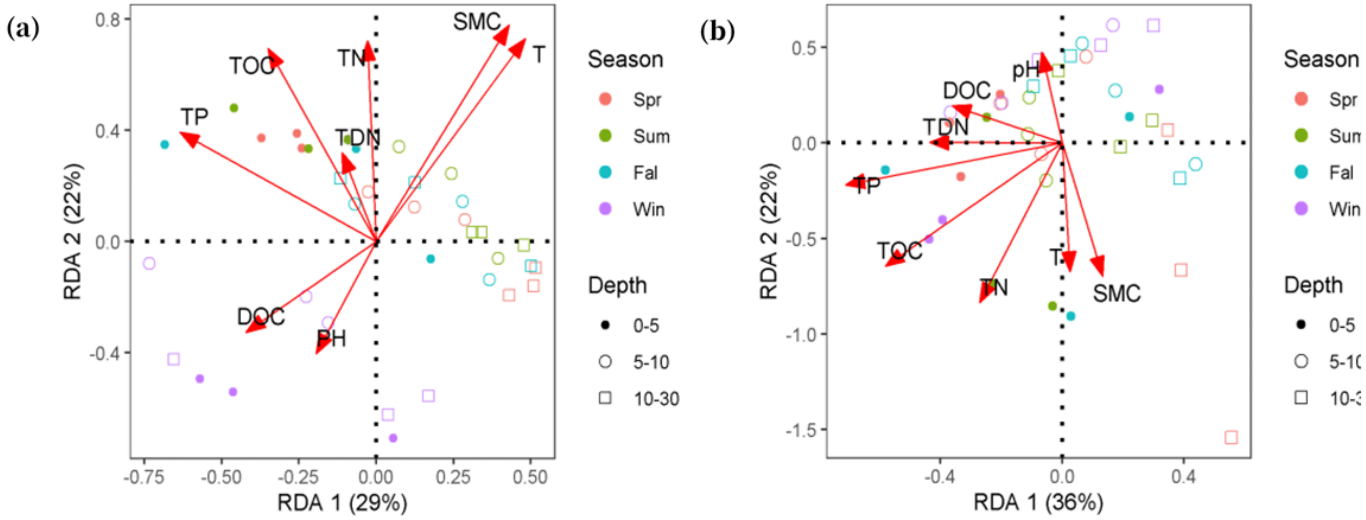

Figure 4

Redundancy analysis to explore the influence of environmental factors on soil bacterial (a) and archaeal (b) community structure in alpine wetlands. Spr, Sum, Fal and Win represent spring, summer, fall and winter, respectively. TN: Total dissolved nitrogen; TP: Total phosphorus; TOC: Total organic carbon of soil; DOC: Dissolved organic carbon; TDN: Total dissolved nitrogen; pH: Soil pH; SMC: Soil moisture content; T: atmospheric temperature.