Abstract. The Napahai plateau wetland, located in northwestern Yunnan province, China, is a unique seasonal plateau wetland of low-latitude and high-altitude, yet the microbial community is still unknown. To address this shortcoming, MiSeq high-throughput sequencing was used to analyze the composition and diversity of bacteria and archaea. The bacterial community comprised of 64 phyla, 164 classes and 484 genera, in which Proteobacteria was the most abundant, followed by Actinobacteria, Chloroflexi and Acidobacteria. Archaea comprised of 3 phyla, 5 classes, and 7 genera, in which Thaumarchaeota dominated. The results indicated that the composition and diversity of the bacterial community were more influenced by seasonal changes than by soil types, whereas the archaeal community was mostly resistant to these factors. Additionally, canonical correlation analysis (CCA) showed that the diversity of the bacterial community was closely correlated with nitrogen (N), total nitrogen (TN) and soil organic matter (SOM) in the dry season. However, no significant correlation of any of the factors was observed in the archaeal community. In conclusion, these results indicated that microbial communities in the soil of Napahai plateau wetland have unique diversity and composition, and Napahai plateau wetland as a microbial resource requires protection and restoration.

Keywords: wetland ecosystem, high throughput sequencing, bacterial community diversity, archaeal community diversity, environmental factors

Introduction

Wetlands are important links between terrestrial and aquatic systems. They are large reservoirs of biodiversity and ecologically powerful natural ecosystems. Ecological services of wetlands have high social and economic value. In wetland ecosystems, microbial community diversity is much better than vegetation species diversity to reflect environmental changes. The microbial community diversity and composition of wetland soils are more sensitive and comprehensive to reflect the wetland ecological conditions. Also they have a great significance for maintaining the balance of wetland ecosystems, repairing damaged wetlands and carrying out comprehensive environmental management.

Recently, the rapid development of high-throughput DNA sequencing has allowed the detailed study of the diversity of microbial communities in wetland soils, with fruitful results. For instance, soil samples were collected from six wetlands on the Qinghai-Tibetan plateau, and high-throughput 16S rRNA gene sequencing was used to assess the composition and localization of enriched microbial communities. Overall, microbial communities from the Qinghai-Tibetan plateau wetlands showed significant potential in converting cellulose and chitin to methane at low temperatures (Dai et al., 2016). In a subarctic wetland in Russia, the major bacterial groups identified in peat by high-
throughput sequencing of the 16S rRNA genes were *Acidobacteria* (35.4-41.2%), *Alphaproteobacteria* (19.1-24.2%), and *Gammaproteobacteria* (7.9-11.1%). The distinctive feature of this community was a high proportion of two subdivisions of *Acidobacteria*, which are not characteristic for boreal sphagnum peat bogs (Danilova et al., 2016). In coastal mangrove wetlands, MiSeq high throughput sequencing was used to understand the microbial composition and diversity pattern. The five most abundant phyla within the bacterial and archaeal communities remained stable between two distinctive seasons, suggesting that the microbial community in the Mai Po wetland exhibits a mild seasonal dynamic (Zhou et al., 2017).

In a wetland ecosystem, geographic distance and environmental factors are two important drivers of the distribution of microorganisms (Widder et al., 2014; Savio et al., 2015). However, in the whole space, environmental factors are more important. Previous studies have demonstrated that soil microbial communities can be significantly influenced by mineral nutrients (Su et al., 2015). In Qinghai-Tibet plateau, soil organic matter and pH were the main factors affecting the soil macrofauna communities of composition and diversity. In addition to environmental factors, the sampling site can influence the microbial community. For instance, Cao studied soil samples across a regional scale in China (including Jiangxi, Hubei, and Henan provinces). Henan has the largest amount of bacterial and fungal phospholipid fatty acids (PLFAs), while Jiangxi shows the lowest amount of PLFAs (Cao et al., 2016). Moreover, the dominant microbial communities generally differ in different sites. For instance, the dominant bacterial phyla are *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* in Beijing (Tian et al., 2014), but they are *Proteobacteria*, *Acidobacteria*, and *Chloroflexi* in Jiangsu (Zhao et al., 2014).

The Napahai plateau wetland, an important water source for the upper reaches of the Yangtze River and located in northwestern of Yunnan Province, China, is a unique, seasonal plateau wetland in low latitude and at high altitude. The Napahai plateau wetland has been listed as an internationally important wetland, and has the significance of being a representative plateau wetland (Xiang et al., 2018). However, the microbial community composition and diversity of this region have not become the subjects of research. Soil microbes have ecological functions of central importance, playing crucial roles in nutrient cycling and soil fertility. Their diversity is a sensitive indicator of soil quality that can determine its ecological function (Anderson et al., 2009). Thus, research on microbial communities in the Napahai plateau wetland has become a fundamental need. To provide new insight into the Napahai plateau wetland microbial resources, six sets of samples from three different soil types were studied, with high-throughput 16S rRNA gene sequencing used to investigate the bacterial and archaeal community diversity in the soil. The major aims of this research were to address the following two key questions. (i) What are the characteristics of the bacterial and archaeal communities, with respect to diversity in the soil of Napahai plateau wetland? (ii) What are the influential factors and their contributions to the compositions of the two communities within the Napahai wetland environments?

**Materials and methods**

**Soil sample sites and collection**

Soil samples were collected from the Napahai plateau wetland, where the whole year split between the dry season (October to April) and the rainy season (May to September). According to the region’s geomorphology, samples were collected from the areas of three
different soil types, including bog soil (YN), swamp meadow soil (SD) and peat soil (NT).

Soil samples were collected in November 2014 (dry season) and June 2015 (rainy season) from three sampling areas (YN, SD and NT). For each sampling area, two samples were collected and for each sample, and eight mixed sample plot soils were selected (“S” distribution) at 10 cm soil layer. Table 1 shows the general conditions of each sample, and Figure 1 shows the sampling sites. YN samples were collected from the vicinity of Lalang from areas less disturbed by human activities. The work regarded as the environmental background area. NT soil sample area was overgrazed by livestock, belonging to a tourism hotspot. Stellera chamaejasme Linn., a plant that is a sign of wetland degradation, was also found. Therefore, this area was regarded to have significant disturbance by human activities. The soil from YN, SD and NT sampling areas represented the transition from native swamp soil and swamp meadow soil to meadow soil formed due to the degradation of the Napahai plateau wetland. The twelve soil samples were obtained and stored at 4 °C, and then transported to the lab for DNA extraction and property analysis.

Analysis of physical and chemical properties

Soil samples for property analysis were crushed and sifted using sieves with apertures of 1 mm. Physical and chemical factors, including soil organic matter (SOM), total nitrogen (TN), total phosphorus (TP), total potassium (TK) and nitrogen (N), were measured using ion chromatography (Dionex™ ICS-6000, Thermo Scientific, USA) at the Ministry of Agriculture Agricultural Products Quality Supervision and Testing Center (Kunming, China).

DNA extraction and PCR amplification

Total soil DNA was extracted from 0.1 g of each soil sample, which had roots removed and was ground using the Power Soil DNA kit (MoBio Laboratories Inc., CA, USA) according to the manufacturer’s instructions (Zhou et al., 2017). The DNA was confirmed using 1.0% agarose gel electrophoresis and a NanoDrop (ND2000, Thermo Fisher, USA), then stored at -80 °C.

For bacterial and archaeal communities, the primer pairs bac-515F 5’GTGCCAGCMCGCGGTAA3’ and bac-806R 5’GGACTACHVGGGTWTCTAAT3’; arc-U515F 5’CAGYMGCCRCGGKAAHACC3’ and arc-U806R 5’GGACTACNSGGGTMTCTAAT3’ were used for amplifying the V4 region of the 16S rRNA genes of bacterial and archaeal, respectively, including barcodes and adapters (Peiffer et al., 2013; Shehab et al., 2013). The PCR mixtures (50 μl) contained 25 μl 2×MightyAmp Buffer Ver.2, 1 μl of each primer (10 μmol/L), 1.0 μl MightyAmp DNA Polymerase, and 2.0 μl template DNA (10-200 ng), with nuclease-free water added to 50 μl. The PCR amplifications used an initial denaturation step at 94 °C for 4 min, followed by 30 cycles of 30 s at 94 °C, 45 s at 56 °C and 35 s at 72 °C and were held at 72 °C for 10 min. The 16S rRNA gene PCR products were sequenced using the Illumina platform (Peiffer et al., 2013).

Sequence analysis

For each sample, three amplicons were pooled together and purified according to the Gene JET kit (Thermo Scientific, USA) instructions. Sequencing of the purified
amplicons was performed on an Illumina MiSeq platform (MiSeq PE300, Illumina, USA) at the Computer Center (Beijing, China). Sequence analysis was performed with Fast Length Adjustment of Short reads (FLASH), discarding the low-quality sequences. Using UPARSE v7.1, sequences were clustered into operational taxonomic units (OTUs) with a 97% similarity cutoff, and Usearch7.1 was used to filtered chimaeras (Edgar, 2013). The remaining sequences, including 530,418 bacterial and 561,217 archaeal, were assigned to OTUs at the 97% similarity level using Mothur v1.34.4 (Schloss et al., 2009). Each representative OUT sequence was assigned to a taxonomic level using the Quantitative Insights into Microbial Ecology (QIIME) pipeline and the RDP classification method (Caporaso et al., 2010).

**Table 1. The overview of Napahai plateau wetland sampling areas**

| Soil samples | Sampling points | Longitude       | Latitude       | Average altitude (m) |
|--------------|-----------------|-----------------|----------------|----------------------|
| YN1          | NPH-YN1-YN8     | E99°37’43.00”-41.26” | N27°54’26.00”-24.38” | 3290                |
| YN2          | NPH-YN1’-YN8’   | E99°37’22.88”-20.75” | N27°53’41.21”-36.81” | 3282                |
| SD1          | NPH-S1-SD8      | E99°38’6.46”-0.67”  | N27°51’36.33”-40.22” | 3277                |
| SD2          | NPH-S1’-SD8’    | E99°38’7.73”-15.63” | N27°50’44.27”-36.02” | 3275                |
| NT1          | NPH-NT1-NT8     | E99°38’0.00”-3.63”  | N27°50’1.00”-49.58”  | 3273                |
| NT2          | NPH-NT1’-NT8’   | E99°38’0.96”-1.97”  | N27°49’35.63”-34.87” | 3272                |

**Figure 1. Distribution of Napahai plateau wetland sampling areas. Each sampling area is composed of eight sampling points and eight sampling points distributed as “S”**
Statistical analysis

A number of alpha diversity indices were assessed at the 97% similarity level using mothur 1.34.4 including the Shannon-Wiener and Simpson index, the abundance based coverage estimator (ACE), terminal richness estimation (Chao1), and Good’s coverage estimator (Schloss et al., 2009). Beta diversity was measured using Bray-Curtis distances among samples, and community differences were assessed using complete-linkage clustering analysis. Based on Weighted_Unifrac, the Unweighted Pair-group Method with Arithmetic Means (UPGMA) was applied. The heatmap and Venn diagrams were generated using the Vegan Package for R v3.0.2 (Oksanen et al., 2013). Canonical correspondence analysis (CCA) was performed using Canoco v4.5.1 to reveal the relationships between microbial community diversity and soil environmental factors (Legendre et al., 2001).

Results

Physical and chemical properties of soil samples

Table 2 shows the physical and chemical properties of the soil. It can be concluded that soil nutrient contents (SOM, TN, TP, TK and N) show no significant differences in different seasons \((p > 0.05)\), but are significantly different in different soil types \((p < 0.05)\).

| Sample   | SOM, g/kg | TN, g/kg | N, mg/kg | TP, g/kg | TK, g/kg |
|----------|-----------|----------|----------|----------|----------|
| DS.YN1   | 105.8     | 0.568    | 393      | 0.032    | 0.416    |
| DS.YN2   | 117.4     | 0.624    | 368      | 0.062    | 0.414    |
| DS.SD1   | 31.2      | 0.26     | 121      | 0.102    | 0.664    |
| DS.SD2   | 26.8      | 0.166    | 97.9     | 0.106    | 0.675    |
| DS.NT1   | 43.7      | 0.445    | 246.3    | 0.279    | 0.795    |
| DS.NT2   | 38.7      | 0.392    | 233      | 0.369    | 0.806    |
| RS.YN1   | 145.8     | 0.659    | 381      | 0.088    | 0.647    |
| RS.YN2   | 160.9     | 0.823    | 346      | 0.081    | 0.643    |
| RS.SD1   | 22.3      | 0.18     | 106      | 0.09     | 0.732    |
| RS.SD2   | 17.4      | 0.156    | 91.3     | 0.127    | 0.764    |
| RS.NT1   | 59.7      | 0.435    | 259      | 0.12     | 0.950    |
| RS.NT2   | 51.2      | 0.358    | 227      | 0.142    | 0.908    |

Microbial community alpha-diversity analysis

To estimate the bacterial and archaeal community diversity and richness, the work applied the Shannon-Wiener index and Simpson’s index, ACE, Chao1 and Good’s coverage estimation (Table 3). In general, high OTU richness in both the bacterial and archaeal communities was found. Good’s coverage estimator showed high coverage and reasonable sequencing depth. Regarding the seasons, the diversity (Shannon-Wiener and Simpson’s) and richness (ACE, Chao1) indices for the bacterial community showed higher diversity in the rainy season than in the dry season, whereas there was no obvious change of index in the archaeal community. The soil type also influenced the
diversity of the bacterial and archaeal communities. In the NT sampling area, both the bacterial and archaeal communities had higher diversity than in the other sampling areas.

### Table 3. The diversity analysis of bacterial and archaeal community at the 97% similarity level

| Season | Sample  | OTUs | Goods coverage | Shannon | Simpson | ACE | Chao1 |
|--------|---------|------|----------------|---------|---------|-----|-------|
| **Bacteria** | | | | | | | |
| Dry season | DS.YN1 | 2505 | 0.84 | 8.38 | 0.96 | 5457.84 | 4955.89 |
| | DS.YN2 | 2351 | 0.83 | 7.98 | 0.95 | 5447.82 | 4938.42 |
| | DS.SD1 | 2137 | 0.86 | 7.69 | 0.95 | 5197.22 | 4757.14 |
| | DS.SD2 | 2715 | 0.84 | 8.88 | 0.97 | 5286.83 | 4700.81 |
| | DS.NT1 | 3454 | 0.77 | 10.28 | 0.99 | 8248.06 | 7169.99 |
| | DS.NT2 | 3238 | 0.78 | 10.02 | 0.99 | 7951.63 | 7305.19 |
| Rain season | RS.YN1 | 5324 | 0.93 | 10.14 | 0.99 | 8614.29 | 8058.17 |
| | RS.YN2 | 6020 | 0.92 | 10.78 | 0.99 | 9550.27 | 9129.80 |
| | RS.SD1 | 4606 | 0.95 | 10.11 | 0.99 | 6413.36 | 6067.05 |
| | RS.SD2 | 6332 | 0.92 | 11.12 | 0.99 | 9824.72 | 9371.37 |
| | RS.NT1 | 5845 | 0.92 | 10.57 | 0.99 | 9708.51 | 9282.13 |
| | RS.NT2 | 5908 | 0.93 | 10.87 | 0.99 | 8813.01 | 8408.68 |
| **Archaeal** | | | | | | | |
| Dry season | DS.YN1 | 1411 | 0.98 | 7.16 | 0.98 | 1402.50 | 1369.08 |
| | DS.YN2 | 1553 | 0.99 | 6.52 | 0.93 | 1592.97 | 1567.33 |
| | DS.SD1 | 1344 | 0.99 | 7.43 | 0.98 | 1306.80 | 1275.64 |
| | DS.SD2 | 1221 | 0.99 | 7.06 | 0.97 | 1206.14 | 1180.20 |
| | DS.NT1 | 1743 | 0.98 | 7.17 | 0.98 | 1913.99 | 1915.81 |
| | DS.NT2 | 1276 | 0.99 | 6.68 | 0.97 | 1362.58 | 1256.25 |
| Rain season | RS.YN1 | 1771 | 0.98 | 7.71 | 0.98 | 1966.28 | 1922.17 |
| | RS.YN2 | 1448 | 0.98 | 6.61 | 0.96 | 1668.68 | 1627.47 |
| | RS.SD1 | 1270 | 0.99 | 6.58 | 0.96 | 1255.94 | 1215.25 |
| | RS.SD2 | 1631 | 0.99 | 7.77 | 0.99 | 1646.68 | 1562.00 |
| | RS.NT1 | 1620 | 0.99 | 7.63 | 0.99 | 1627.14 | 1553.94 |
| | RS.NT2 | 1480 | 0.99 | 7.25 | 0.98 | 1508.16 | 1476.85 |

**Taxonomic composition of the microbial community**

Analysis of the bacterial community composition in the Napahai plateau revealed 64 phyla, 164 classes, 271 orders, 330 families and 484 genera. In addition, 2 archaeal phyla were detected to comprise 5 classes, 8 orders, 7 families and 7 genera. Figure 2 shows the relative abundances of bacteria and archaea at the phylum and genus levels.

The dominant bacterial phyla across all samples were **Proteobacteria**, **Actinobacteria**, **Chloroflexi** and **Acidobacteria**, accounting for 72.4 to 81.8% of the bacterial sequences. It depended on the season and soil type (Fig. 3). The abundance of the phylum **Proteobacteria** was much higher (p < 0.1), and that of **Chloroflexi** and **Acidobacteria** was much lower (p < 0.1) in the dry season, while **Proteobacteria**, **Chloroflexi** and **Acidobacteria** showed the differences (p < 0.1) in soil type. The archaeal sequence information included approximately 40% unknown; the dominant archaeal phylum, **Thaumarchaeota** (*Cenarchaeum*), accounted for 50% without exhibiting differences in seasons and soil types.
Figure 2. Relative abundance of bacterial phyla (A) and archaeal phyla (B) detected in soil samples. At different taxonomic levels, the classification of microbial community structure in soil samples to the currently known taxonomic unit is defined as “unknown”; the definition of “others” in which the proportion of microbial community structure is less than 1%; although the microbial community information is aligned to sequences in the database, there is no definitive annotation for the data defined as “unclassified”

Microbial community analysis of OTU distributions

Based on the statistics of the total OTUs, Venn diagrams were created to display the unique and OTUs were shared among samples (Fig. 4). Under a similarity threshold of 0.97, the numbers of overlapping bacterial and archaeal OTUs in the rainy season (2,634 and 828, respectively) were more than in the dry season (1,187 and 741, respectively) in the three soil types. For archaea, the YN sampling area contained more OTUs (1329) than the NT (1020), and SD (935) areas. Each sample contained specific OTUs. For bacteria, the NT sampling area contained more OTUs (2,547) than the SD (1,391) and YN (1,368) areas in the dry season, while the YN sampling area contained more OTUs (4,127) than the SD (3,344) and NT (2,919) areas in the rainy season.

The authors also performed heatmap cluster analysis to discern the community compositional differences (Fig. 5). The overall taxonomic diversity of the bacterial community was significantly higher than that of the archaeal community at the genus level. Bacterial and archaeal taxonomic diversity was considerably lower in the dry season compared to the rainy season. The heatmap of bacterial communities showed that Proteobacteria, Actinobacteria and Bacteroidetes were most differentiated between seasons. Additionally, bacterial communities differed slightly in the soil samples from the SD and YN sampling areas in the dry season, but were more irregular in the rainy season. In contrast, Thaumarchaeota (Cenarchaeum) was the predominant phylum in the archaeal communities. The abundance of archaeal was independent of seasons and soil types.

Microbial community beta-diversity analysis

Based on Weighted_Unifrac distances, the work assessed the similarities and differences in the composition of the microbial communities with respect to seasonal and spatial variations (Fig. 6). The UPGMA analysis showed that the diversity and composition of the bacterial community were more impacted by seasonal changes than
by soil types, the opposite of the low-abundance archaeal community. The archaeal community composition did not differ in soil samples from the SD and YN sampling areas, but dissimilar to the NT sampling area.

![Figure 3](image-url)

**Figure 3.** Comparisons of the bacteria four mainly phyla taxonomy abundance of soil samples (YN, SD and NT) in dry and rainy seasons (DS and RS). The bacteria four mainly phyla taxonomy are Proteobacteria, Actinobacteria, Chloroflexi and Acidobacteria. **A** The soil samples are collected in dry season (DS). **B** The soil samples are collected in rainy season (RS)

**Effects of environmental factors on microbial community diversity**

CCA was performed to identify the relationships between major environmental factors and microbial community diversity (Fig. 7). In the bacterial communities, the first two axes of the CCA plot explained 21.7 and 2.7% (in the dry season) and 77 and
26.92% (in the rainy season), respectively, of the total variation in the data. These results indicate that the bacterial community is closely correlated with N, TN and SOM in the dry season. In the archaeal community, the first two axes of the CCA plot explained 34.38 and 27.05% (in the dry season) and 35.12 and 23.32% (in the rainy season), respectively, of the total variation in the data. However, no significant correlation of any of the factors was observed for the archaeal community.

Discussion

Plant and animal diversity have been studied over the past decade in the Napahai plateau wetland. The research has been complemented with microbial diversity information through the measurement of the bacterial and archaeal community composition and diversity, allowing for a better assessment of the whole area’s ecosystem.

The composition and diversity of the microbial community in the Napahai plateau wetland were investigated using high-throughput sequencing of 16S rRNA V4 genes. *Proteobacteria*, *Actinobacteria*, *Chloroflexi* and *Acidobacteria* were the top four most abundant bacterial phyla (Fig. 2A). Similar results have been obtained in different land-
use types of soils in North and South America (Lauber et al., 2009), agricultural and forest soils in Salta and Jujuy (Montecchia et al., 2015) and soils from Antarctic and Arctic (Teixeira et al., 2010; Yergeau et al., 2010). However, compared with those derived from cucumber rhizosphere soils, Proteobacteria, Bacteroidetes and Actinobacteria in the work were the most abundant phyla, accounting for over 70% (Tian et al., 2013). Nevertheless, Proteobacteria is the most common phylum in soils worldwide, consistent with our results (Janssen, 2006; Spain et al., 2009). Actinobacteria is not only the dominant phylum in low temperature environments but also the dominant phylum in freshwater ecosystems (Zwart et al., 2002; Johnson et al., 2007). The Napahai plateau wetland has an average temperature of approximate 5°C with a freshwater lake, and its ecological environment is in line with Actinobacteria growth. Thus, Actinobacteria was also the most abundant phylum in the region. In contrast, the archaeal community compositions, especially the dominant phylum Thaumarchaeota, were clearly different from many other ecosystems, which tend to be dominated by Crenarchaeota and Euryarchaeota. The difference may be due to special geological features (Porat et al., 2010; Wang et al., 2010). Thaumarchaeota was the most abundant phylum accounting for almost 50%, which played an important role in the biochemical cycling of carbon, nitrogen and other elements (Cabello et al., 2004). In the Thaumarchaeota, most of the known sequences belong to the ammoxidation archaea, involved in the ammoniation of soil and affect the nitrogen cycle. Therefore, it is speculated that archaea play a more important role than bacteria in the cycling of nitrogen in this area. How archaea and bacteria coordinate with each other to accomplish biogeochemical cycling in the wetland needs further study.

Moreover, greater biodiversity in soil can lead to a more stable system, providing the enhanced combinations of vital microbial functions and processes (Wagg et al., 2014; Regar et al., 2019). In the work, the difference in microbial community composition in different soil types revealed the feedback mechanism of ecological environmental degradation and microbial composition. Acidobacteria distribution in acidic environments is contaminated by heavy metal, belonging to non-original colonies (Diamond et al., 2019). Among the YN, SD and NT sampling areas, no significant differences in Acidobacteria abundance were observed (Fig. 3B). This may indicate that the whole area is affected by heavy metal pollution. In contrast, Proteobacteria and Chloroflexi showed differences (p < 0.1) in different soil types (Fig. 3B). One of the predominant classes of Proteobacteria was Gammaproteobacteria, which can degrade organic matter and were most abundant in the NT sampling area (Picazo et al., 2019). The authors also observed that the abundance of Chloroflexi was significantly higher in the NT sampling area, and these bacteria are known to live in environment with high organic matter content (Denef et al., 2015). These similar results could probably be due to the NT sampling area having more nitrogen oxides caused by serious disturbance of human activities. In the archaeal community, Methanofollis only present in the NT sampling area in the rainy season (Fig. 5B). Methanofollis lived in anaerobic environments such as oceans, lake sediments and the digestive tract of ruminants (Pazinato et al., 2010). In the NT sampling area, there are a large number of animals such as cattle and sheep grazing in the rainy season; therefore, Methanogenus has had an ecological balance with host ruminants for a long time. All the results illustrated that from the microbial perspective, the NT sampling area can be regarded as consisting of meadow soil formed due to disruption by human activities. Soil microbial composition is important for soil quality and health.
Figure 5. Heatmap cluster of microbial community of soil samples (YN, SD and NT) in dry and rainy seasons (DS and RS). A show the distribution of community OTUs classified at the genus level. Colors correspond to the relative abundance of each OTU within the community and cluster distance based on Bray-Curtis distances. Only the most abundant 50 bacterial genera based on statistical analysis are shown and the numbers in the plate to stand for in supplement material. A Heatmap cluster of bacterial community of soil samples in dry season (DS). B Heatmap cluster of bacterial community of soil samples in rainy season (RS). C Heatmap cluster of archael community of soil samples in dry and rainy seasons (DS and RS).
In this work, the effects of seasons and soil types on soil microbial community characteristics were observed. Based on the analysis of OTU distributions (Venn diagrams and heatmap cluster) and beta-diversity analysis, the differences in two factors impact the microbial community (Figs. 4–6). Seasonal differences clearly impacted bacterial diversity, consistent with most of the literature reported (Bissett et al., 2007; Kara et al., 2013; Wilhelm et al., 2014). Unlike our result for bacterial diversity, archaeal diversity was affected by neither seasons nor soil types, especially in terms of abundance. Our finding is consistent with the numerous studies on the homogeneous spatial distributions of the archaeal community in various habitats, including field soil (Yuan et al., 2009) and sediment (Keuter et al., 2016); however, in ocean ecosystems, the diversity of archaea was influenced by spatial and temporal differences (Vik et al., 2017). This difference is probably due to unpredictable movements in ocean ecosystems. The soil ecosystem is relatively stable. However, one major drawback of our study was that the small number of sampling sites was in different soils samples, so the changes of microbial community diversity was relatively less convincing.

Our analysis of the relationship between major environmental factors and microbial community diversity showed that TN had the largest effect on bacterial community diversity, but the archaeal community was not significantly affected by environmental factors. This conclusion was also supported by our finding that archaeal diversity was not affected by seasons or soil types (Keuter and Rinkevich, 2016).

Finally, a large number of unclassified microorganisms were detected in the Napahai plateau wetland, with the abundance of unclassified bacteria and archaea at the phylum level being approximate 10 and 25%, respectively. The unclassified bacteria consisted of the novel candidate divisions WPS-2, WS3, GN04, MVP-21 and MVS-104, whereas the archaeal candidate divisions included GA55, VAL11 and FRD15. Their ecological and biological interactions are unknown. Therefore, the isolation of soil microbes combined with traditional methods under laboratory conditions is ongoing, which will help us to identify critical resources in the Napahai plateau wetland.
Figure 7. CCA plot of the relationship between the major environmental factors and microbial community composition of soil samples (YN, SD and NT) in dry and rain seasons (DS and RS). Samples are indicated by dots and environmental factors indicated by color arrows. Arrow vector length represents the strength of the correlation with the axes. A CCA plot of the relationship between the major environmental factors and bacterial composition of soil samples in dry season (DS). B CCA plot of the relationship between the major environmental factors and bacterial composition of soil samples in rainy season (RS). C CCA plot of the relationship between the major environmental factors and archaeal composition of soil samples in dry season (DS). D CCA plot of the relationship between the major environmental factors and archaeal composition of soil samples in rainy season (RS).

Conclusions

The work examined the composition and diversity of the bacterial and archaeal communities with respect to spatial and temporal distribution and explored the relationships between environmental variables in the Napahai plateau wetland. For the bacterial community, *Proteobacteria* prevailed was the most abundant, followed by *Actinobacteria*, *Chloroflexi* and *Acidobacteria*. Meanwhile, they were more influenced by seasonal changes than by soil types and was closely correlated with N, TN and SOM in the dry season. For the archaeal community, *Thaumarchaeota* dominated. Meanwhile, the archaeal community was neither associate with these factors nor significant correlation of physical and chemical factors. The microbial communities showed high and unique microbial diversity and composition, and influence with seasons and soil
types, which lays a foundation for understanding the microbial and environmental response mechanism of the Napahai plateau wetland.

In the future, we will combine transcriptomics and proteomics to conduct research on microbial communities, and understand how the main bacteria or archaea conduct ecological functions in the particular ecological environment, and also for the discovery of unknown microorganism.

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