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Effects of water tables and nitrogen application on soil bacterial community diversity, network structure, and function in an alpine wetland, China

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Abstract: Nitrogen deposition and water tables are important factors to control soil microbial community structure. However, the specific effects and mechanisms of nitrogen deposition and water tables coupling on bacterial diversity, abundance, and community structure in arid alpine wetlands remain unclear. The nitrogen deposition (0, 10, and 20 kg N/(hm^2•a)) experiments were conducted in the Bayinbulak alpine wetland with different water tables (perennial flooding, seasonal waterlogging, and perennial drying). The 16S rRNA (ribosomal ribonucleic acid) gene sequencing technology was employed to analyze the changes in bacterial community diversity, network structure, and function in the soil. Results indicated that bacterial diversity was the highest under seasonal waterlogging condition. However, nitrogen deposition only affected the bacterial Chao1 and beta diversity indices under seasonal waterlogging condition. The abundance of bacterial communities under different water tables showed significant differences at the phylum and genus levels. The dominant phylum, Proteobacteria, was sensitive to soil moisture and its abundance decreased with decreasing water tables. Although nitrogen deposition led to changes in bacterial abundance, such changes were small compared with the effects of water tables. Nitrogen deposition with 10 kg N/(hm^2•a) decreased bacterial edge number, average path length, and robustness. However, perennial flooding and drying conditions could simply resist environmental changes caused by 20 kg N/(hm^2•a) nitrogen deposition and their network structure remain unchanged. The sulfur cycle function was dominant under perennial flooding condition, and carbon and nitrogen cycle functions were dominant under seasonal waterlogging and perennial drying conditions. Nitrogen application increased the potential function of part of nitrogen cycle and decreased the potential function of sulfur cycle in bacterial community. In summary, composition of bacterial community in the arid alpine wetland was determined by water tables, and diversity of bacterial community was inhibited by a lower water table. Effect of nitrogen deposition on bacterial community structure and function depended on water tables.

Keywords: nitrogen application; alpine wetland; bacterial community; bacterial network; water tables

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1 Introduction

Spatial changes in regional moisture due to climate change and increased nitrogen deposition from anthropogenic activities will profoundly impact the structure, functioning, and material flows (Liu et al., 2011; Li et al., 2021a). Bacterial communities are the most abundant members of soil microbial community and play an important role as producers and key decomposers in the ecosystem (Sun et al., 2023). Earlier investigations had found that bacterial communities were more responsive than fungal communities to environmental change (Ackerman et al., 2019; Zhang et al., 2023). Bacteria typically had higher growth rates and shorter metabolic cycles. Bacteria were often involved in a wider range of nutrient transformations and soil organic matter degradation processes (Isobe et al., 2019).

Bacterial community structure was sensitive to factors such as nitrogen deposition in the external environment. In response to elevated nitrogen deposition, bacteria altered their metabolic pathways and growth strategies to enhance the utilization of additional nitrogen sources (Dai et al., 2018). Extensive research across various ecosystems, including forest lands (Zhu et al., 2015), farmlands (Sun et al., 2020), grasslands (Stevens et al., 2022), and wetlands (Deng et al., 2015), has demonstrated the profound impact of nitrogen application on bacterial communities. Nitrogen deposition not only affected the diversity of soil bacterial communities, but also altered soil bacterial structure (Ramirez et al., 2010; Wang et al., 2019). Nitrogen deposition decreased the phylogenetic alpha diversity of bacterial communities and changed their structure, primarily through species loss processes (Li et al., 2019; Li et al., 2021a). Nitrogen deposition increased the relative abundance of certain bacterial phyla, such as Actinobacteria and Chloroflexi, and decreased other bacterial phyla, such as Acidobacteria and Verrucomicrobia in the coastal wetland of the Yellow River Delta, China (Lu et al., 2021). Furthermore, the alterations in bacterial community structure due to nitrogen deposition have cascading effects on ecosystem processes (Lu et al., 2021). Nitrogen and sulfur cycles were impaired after nitrogen application although bacterial functional diversity remained relatively stable (Yan et al., 2020a; Hu et al., 2021). In addition, the response of bacterial community to nitrogen was influenced by the timing and concentration of nitrogen input (Treseder, 2008; Yin et al., 2022). The effects were not uniform across the globe, due to factors such as climate and altitude (Liu et al., 2013).

Changes in the water tables also had an important effect on the soil bacterial community (Wang et al., 2022a). Water tables controlled the activity of aerobic and anaerobic bacteria by changing the position of oxidation-anaerobic layer (Zhang et al., 2022b). Denitrifiers and methanogens were affected by changes in water tables (Kim et al., 2008). Growth process of bacteria was highly dependent on moisture and nutrient conditions in the soil (Fan et al., 2022; Zhang et al., 2022c). Methanogen populations demonstrated a linear increase as soil moisture content surpassed 60.00% (Zhang et al., 2022a). Impact of wetting and drying on the soil microbe of wetland was investigated, revealing significant alterations in both bacterial communities and metabolites (RoyChowdhury et al., 2022). Soil drying decreased bacterial colony-forming units and substrate utilization, but rewetting could lead to physiological recovery (Griffiths et al., 2003). Extreme water tables, such as prolonged drought or excessive saturation, led to significant shifts in microbial community structure and activity (Siebielec et al., 2020). Although similar bacteria and dominant taxa existed at different water tables, the composition and structure of bacterial communities at different water tables inevitably changed with nitrogen deposition (Fan et al., 2022). However, it has been shown that lower water tables mitigate the effects of nitrogen deposition on microbial community diversity (Li et al., 2021b). However, there were fewer studies on the effects of both water tables and nitrogen deposition on changes in bacterial diversity, network structure, and community function. Therefore, it was important to reveal the response mechanism and degree of bacterial community structure to nitrogen deposition and water tables to explore effective mitigation and adaptation strategies for wetlands in arid areas to cope with climate change and human disturbance.

Wetlands are sensitive to environmental change (Liu et al., 2011). Water tables of wetlands were affected to some extent under climate change, and with the increase in nitrogen deposition, it inevitably affected the carbon and nitrogen cycles of wetland ecosystem. Changes in the abundance of key species of bacterial communities had a profound impact on the function of material cycling and the stability of wetland ecosystems. However, the response and mechanism of bacteria communities to water tables and nitrogen deposition in alpine wetlands located in arid areas are not clear.

The Bayinbulak alpine wetland is situated in the middle Tianshan Mountains of Xinjiang Uygur Autonomous Region, northwestern China. It plays a critical role in maintaining the regional water balance, acting as an ecological carbon sink and providing various ecosystem services (Liusui et al., 2019; Hu et al., 2021). Previous studies have found changes in soil physical and chemical properties and carbon cycle under different water tables in this area (Liusui et al., 2019). In recent years, due to the development of industry and agriculture in this area, nitrogen deposition has been significantly intensified by human activities (Li et al., 2021a). Nitrogen deposition with the rate of 8 kg N/(hm^2·a) had been reported in this area (Li et al., 2015). Previous research on this wetland found that adding nitrogen changed the soil microbial structure (Hu et al., 2021). However, the specific effects and mechanisms of nitrogen deposition and water tables coupling on bacterial diversity, abundance, and community structure in this area remain unclear. The aim of this study was therefore to investigate the effects of (1) water tables and nitrogen deposition on the diversity of bacterial communities in the soil of alpine wetland; (2) the response of bacterial community network structure to water table changes and nitrogen deposition; and (3) the effects of nitrogen deposition and water tables on soil bacterial community functions.

2 Methods

2.1 Study area

The research was conducted in the Bayinbulak alpine wetland in Hejing County, Xinjiang, northwestern China (42°40′–43°00′N, 83°40′–84°35′E). The wetland covers approximately 770 km^2 and belongs to a typical temperate continental arid climate. Summer is short and cool, while winter is long and harsh (Bobbink et al., 2010), and the main surface water sources in the area come from precipitation and snowmelt (Liusui et al., 2019).

2.2 Experimental design and sample collection

We chose three distinct water tables along a long-term monitoring transect, according to water tables, groundwater depth, and dominant plants (Table 1). The distance between water tables was more than 150 m. The experiment was a randomized block design consisting of three water tables (perennial flooding, seasonal waterlogging, and perennial drying), three nitrogen deposition levels (0, 10, and 20 kg N/(hm^2·a), abbreviated as CK, N1, and N2, respectively), and five plots (2 m×2 m). Thus, the experiment comprised 45 plots (3 nitrogen levels×3 water tables×5 plots). To prevent water, nitrogen, and other substances from spreading and mixing between experimental areas, we set the space between plots at 10 m. Nitrogen (a 3:7 mixture of $CO(NH_2)_2$ and NH_4NO_3) was applied twice in May and July 2020 (Liu et al., 2013). Samples were collected in July 2021. To ensure the accuracy and reliability of the experiment, we collected 5 sub-samples of 0–20 cm soil depth from each plot and then mixed together to create a more representative composite sample. A total of 45 samples were collected and divided into two parts after removal of stones, roots, and visible plant debris. One part of the soil samples for analyzing of soil physical-chemical properties was air-dried and sieved through a sieve. The other part of the soil samples was stored in liquid nitrogen at –80°C in refrigerated tubes for the determination of soil microorganisms.

Table 1 Information of the study area

Water table	Surface water and groundwater depth	Soil moisture (%)	Dominant plant species
Perennial flooding	Surface waterlogging depth of 20–50 cm	92–115	*Carex rhynchophysa* C. A. Mey., *Utricularia vulgaris* L.
Seasonal waterlogging	There is sometimes 0–10 cm of water on the surface (usually in the spring snowmelt period and the rainy season), and the groundwater depth is 80–100 cm below the surface	60–81	*Carex melanantha* C. A. Mey., *Carex stenocarpa* Turcz ex V. I. Krecz, and *Triglochin maritima* L.
Perennial drying	Dry surface and no water logging. Groundwater depth of 200 cm below the surface.	36–52	*C. melanantha*, *Glaux maritima* L.

2.3 Soil physical and chemical properties

Soil moisture was measured by drying method (Wang et al., 2022b). Soil pH was measured at a water:soil ratio of 5:1 using a pH meter (Mettler Toledo FE28-Standard, Zurich, Switzerland) (Chen et al., 2020). Total carbon (TC) and total nitrogen (TN) contents were determined by an elemental analyzer (EA3000, Euro Vector, Milanuo, Italy) (Hu et al., 2021). Available nitrogen (AN) content was determined by alkali diffusion method (Abulaizi et al., 2023). Available phosphorus (AP) was extracted by using $NaHCO_3$ and then determined by spectrophotometry (UV-1780, Shimadzu Corporation, Tokyo, Japan) (Hu et al., 2021). Available potassium (AK) content was extracted by using CH_3COONH_4 and determined with a flame photometer (Shanghai Jinke 6400A, Shanghai, China). Soil organic carbon (SOC) content was determined by a potassium dichromate oxidation method (Yuan et al., 2023).

2.4 Deoxyribonucleic acid (DNA) extraction and sequencing

Soil DNA was extracted with the MoBio Power Soil DNA Separation Kit (Mo Bio Laboratories, Inc., Carlsbad, USA). DNA concentration and quality were determined using NanoDropTM One UV-Vis spectrophotometer (Thermo Fisher, Lenexa, USA). V3-V4 region of the bacterial 16S ribosomal ribonucleic acid (rRNA) gene was amplified with primer pairs 338F (5′-ACTCCTAGGGAGCA-3′) and 806R (5′-GGACTCHVGGGTWTTAT-3′) (Quast et al., 2013). Polymerase chain reaction (PCR) was used to homogenize, quantify, and purify the results. The purified amplicons were sequenced on the Illumina NovaSeq 6000 platform by Beijing Biomarker Biotechnology Co., Ltd., Being, China (Chen et al., 2021). To reduce the repetition rate in high-throughput sequencing data, we employed various approaches in this study. Firstly, Trimmomatic v.0.33 software was used to filter raw sequencing data. Subsequently, Cutadapt v.1.9.1 software was employed to detect and eliminate primer sequences in clean reads, further enhancing the quality of reads. Next, the clean reads from each sample were combined using USEARCH v.10.0 software. Finally, Usearch v.8.1 software was used to obtain operational taxonomic units (OTUs) with 97% similarity, which provided a reliable basis for bacterial composition analysis (Hu et al., 2021; Abulaizi et al., 2023). Bacterial gene sequence information was derived from functional annotation of prokaryotic taxa (FAPROTAX) and used to predict bacterial community function. All raw sequences had been deposited into a National Center for Biotechnology Information (NCBI) sequence read archive (the BioProject accession: PRJNA1029374).

2.5 Data analysis

Impact of different nitrogen applications on soil physical and chemical characteristics was investigated using SPSS v.26.0 software (IBM SPSS Statistics, Chicago, USA) through multivariate analysis of variance (MANOVA). Post-hoc analysis was conducted using Duncan's multiple range test to identify any significant differences in soil parameters between nitrogen applications.

Bacteria data were analyzed and visualized using R v.4.3.0 software with "ggplot2" package for generating the box plot for bacterial diversity index. Principal coordinate analysis (PCoA) and redundancy analysis (RDA) based on Bray-Curtis distance were performed using Vegan v.2.6.4

software to assess bacterial community diversity. Wilcoxon rank sum test was used to compare pairwise differences in bacterial communities. An overlaid histogram of the relative abundance of bacterial communities was shown using package in R software. The dominant phylum and genus were identified by their relative abundance at the top 10 phylum level and top 20 genus level, respectively. To calculate the relative abundance of species, we used linear discriminant analysis (LDA) and effect size (LEfSe) analyses. LEfSe analysis was employed to compare differences between water tables from phylum to genus level and determine factors contributing to these differences (Zhou et al., 2019). The cutoff for the LDA score was 4.0 for different water tables and 3.0 for different nitrogen levels.

The "ggClusterNet" package in R software was used for modular grouping in network visualization analysis. Spearman's correlation coefficient was applied to calculate the correlation between OTUs of various microorganisms and create network of bacterial co-occurrences (Wen et al., 2022). Using this approach, the number of edges, average degree, clustering coefficient, and average path length within bacterial network were determined. These metrics were carefully considered to assess the network's natural connectivity and robustness, which served as indicators for evaluating its stability (Hu et al., 2023). Total natural connectivity of the network is calculated from the first 500 OTUs. We used the method of removing critical module hubs. Robustness was calculated by randomly removing 50.00% of the nodes from each network. Furthermore, the combination of every two communities was compared to assess the stability of community composition (Yuan et al., 2021). The online ChiPlot tool (https://www.chiplot.online/) was used to analyze the differences in functional genes between different treatments and bubble plots. Additionally, heatmap plots were utilized to illustrate the link between functional genes and soil physical and chemical parameters.

3 Results

3.1 Soils physical and chemical properties

Treatments of different water tables and nitrogen application had significant interaction on the pH, TC, SOC, AP, and AK ($P<0.050$; Table 2). Compared with CK treatment, soil pH significantly decreased under N1 and N2 treatments under the same water tables ($P<0.050$; Table 3). Soil TC, SOC, and AP contents significantly increased in nitrogen application under perennial flooding and seasonal waterlogging conditions ($P<0.050$). Soil TN and AN content increased with increasing nitrogen application.

With increasing nitrogen application, SOC contents increased by 13.61%–19.43% under perennial flooding condition and 15.15%–16.40% under seasonal waterlogging condition. Compared with different water tables, soil pH under perennial drying condition was the highest. Soil TC under perennial flooding condition was significantly higher than that of the other water tables ($P<0.050$). SOC content was the highest under seasonal waterlogging condition. Soil AP

Table 2 Variance analysis of soil properties under nitrogen application and water tables

Treatment	Significance test	pH	TC	TN	AN	AP	AK	SOC	Soil moisture
Water tables	F	2454.70	782.01	195.49	46.25	240.64	18659.38	444.06	256.96
	P	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
Nitrogen application	F	40.79	30.61	365.54	51.63	25.91	44.38	40.50	0.26
	P	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	0.31
Water tables× Nitrogen application	F	3.60	3.96	1.62	1.48	3.45	17.77	12.50	0.15
	P	0.025*	0.018*	0.213	0.249	0.003*	<0.001***	<0.001***	0.96

Note: TC, total carbon; TN, total nitrogen; AN, available nitrogen; AP, available phosphorus; AK, available potassium; SOC, soil organic carbon. The abbreviations are the same in the following tables and figures. *, $P<0.050$ level; ***, $P<0.001$ level.

Table 3 Effect of nitrogen application and water tables on soil chemical properties

Water table	Treatment	pH	TC (g/kg)	TN (g/kg)	SOC (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)	Soil moisture (%)
Perennial flooding	CK	7.52 ± 0.02^{Ca}	169.02 ± 1.53^{Ab}	8.43 ± 0.39^{Aa}	105.42 ± 3.53^{Bb}	127.24 ± 3.44^{Ab}	16.67 ± 8.59^{Bb}	102.67 ± 4.01^{Ca}	101.50 ± 2.26^{Aa}
	N1	7.48 ± 0.01^{Cb}	184.38 ± 1.20^{Aa}	8.59 ± 0.12^{Aa}	125.91 ± 3.44^{Aa}	136.47 ± 5.66^{Aa}	26.90 ± 1.14^{Cab}	100.93 ± 2.05^{Ca}	107.56 ± 11.62^{Aa}
	N2	7.47 ± 0.01^{Cb}	184.94 ± 4.73^{Aa}	8.64 ± 0.17^{Aa}	119.77 ± 0.84^{Ba}	144.52 ± 1.79^{Aa}	27.74 ± 2.69^{Ca}	102.93 ± 1.80^{Ca}	104.82 ± 2.07^{Aa}
Seasonal water-logging	CK	7.83 ± 0.02^{Ba}	145.06 ± 1.60^{Bb}	7.73 ± 0.25^{Bb}	114.24 ± 2.35^{Ab}	99.45 ± 1.61^{Bc}	51.04 ± 3.47^{Ab}	413.47 ± 2.44^{Aa}	73.87 ± 6.17^{Ba}
	N1	7.72 ± 0.04^{Bb}	164.04 ± 5.70^{Ba}	8.23 ± 0.73^{Ab}	131.55 ± 2.29^{Aa}	105.57 ± 2.75^{Bb}	70.22 ± 1.97^{Aa}	417.87 ± 2.66^{Aa}	76.94 ± 1.63^{Ba}
	N2	7.75 ± 0.02^{Bb}	168.42 ± 2.57^{Ba}	8.68 ± 0.12^{Aa}	132.98 ± 2.28^{Aa}	113.08 ± 2.07^{Ba}	70.33 ± 4.54^{Aa}	426.67 ± 2.41^{Ab}	76.89 ± 4.66^{Ba}
Perennial drying	CK	8.07 ± 0.03^{Aa}	97.71 ± 5.63^{Ca}	5.40 ± 0.16^{Cc}	83.96 ± 3.90^{Ca}	96.13 ± 1.97^{Bb}	46.61 ± 3.86^{Aa}	345.73 ± 3.40^{Ba}	49.52 ± 3.36^{Ca}
	N1	8.03 ± 0.01^{Ab}	103.62 ± 2.41^{Ca}	5.89 ± 0.12^{Bb}	82.13 ± 1.25^{Ba}	102.24 ± 2.42^{Ba}	50.72 ± 1.85^{Ba}	363.97 ± 5.97^{Bb}	51.62 ± 2.20^{Ca}
	N2	8.04 ± 0.02^{Ab}	101.71 ± 8.15^{Ca}	6.44 ± 0.12^{Ba}	85.06 ± 1.77^{Ca}	105.46 ± 2.77^{Ca}	51.99 ± 1.43^{Ba}	381.63 ± 5.91^{Bc}	50.62 ± 1.61^{Ca}

Note: CK, N1, and N2 are the nitrogen application levels of 0, 10, and 20 kg N/(hm^2·a), respectively. Different lowercase letters within the same water table indicate significant differences among different nitrogen levels at $P<0.050$ level; different uppercase letters within the same nitrogen application indicate significant differences among different water tables at $P<0.050$ level.

and AK contents under seasonal waterlogging condition were significantly higher than those of the other water tables ($P<0.050$).

3.2 Soil bacterial community diversity

Sequencing of all samples revealed that the number of bacterial OTUs ranged from 1541 to 1720 (Fig. 1). All OTUs were utilized for subsequent statistical analysis. PCoA analysis, based on the Bray-Curtis distance matrix (Fig. 1c), demonstrated significant changes in bacterial community structures under different water tables ($P<0.050$). These two main coordinates, PCoA1 (66.67%) and PCoA2 (15.60%) explained 82.27% of the variation in bacterial community structure. The Chao1 indices under perennial flooding and seasonal waterlogging conditions were significantly higher than that of perennial drying condition in the same nitrogen application (Fig. 1a). Under seasonal waterlogging condition, bacterial Chao1 index significantly decreased under N1 treatment ($P<0.050$). The Shannon index under seasonal waterlogging condition was significantly higher than those of the other water tables ($P<0.050$; Fig. 1c). The beta diversity of bacterial community was significantly higher under seasonal waterlogging condition than those of the other water tables under CK and N2 treatments ($P<0.050$; Fig. 1d). Furthermore, bacterial community beta diversity under N1 treatment was significantly lower than those of CK and N2 treatment ($P<0.050$) under seasonal waterlogging condition.

3.3 Soil bacterial community structure

There were 33 bacterial phyla and 402 bacterial species (Fig. 2). In Figure 2a, we evaluated the changes in the composition of soil bacterial community based on relative abundance of the top 10 dominating phyla. Average relative abundances of Proteobacteria under CK, N1, and N2 treatments were compared: they were 39.48%, 40.81%, and 39.35%, respectively under perennial flooding condition; 37.80%, 28.19%, and 37.78%, respectively under seasonal waterlogging condition; and 25.95%, 27.53%, and 28.00%, respectively under perennial drying condition. Proteobacteria was the dominant phylum in all soils.

Relative abundance of Chloroflexi was higher under perennial flooding condition than the other water tables, and average relative abundances of Chloroflexi under perennial flooding, seasonal water-logging, and perennial drying were 21.83%, 14.88%, and 16.28%, respectively, followed by Bacteroidetes (18.33%, 6.35%, and 9.73%, respectively) and Nitrospirae (3.99%, 1.14%, and 1.36%, respectively) under CK treatment (Fig. 2a). Relative abundance of bacteria was lower under perennial flooding condition than under seasonal waterlogging and perennial drying conditions. Under perennial flooding condition, compared with CK, Acidobacteria and Gemmatimonadetes under N1 and N2 treatments increased significantly by 21.36%–22.26% and 21.68%–33.40%, respectively. *Hydrogenophilaceae* and *Bacteroidetes_ vadinHA*17 were the dominant soil bacterial genera in the soil under perennial flooding condition, and *Geminicoccaceae*

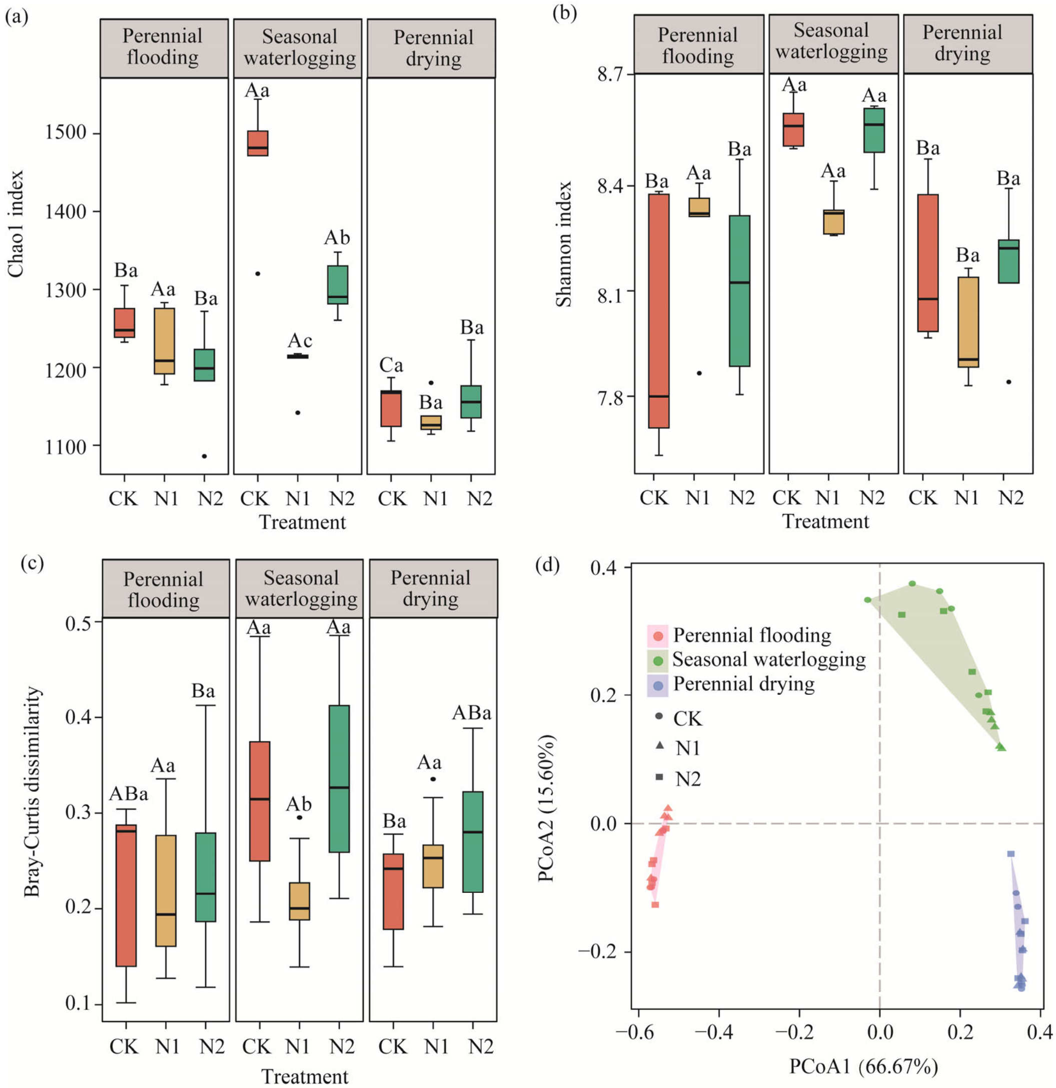

Fig. 1 Effects of nitrogen application on alpha and beta diversity of soil bacterial community under different water tables. Different uppercase letters within the same nitrogen application indicate significant differences under different water tables at $P<0.050$ level, and different lowercase letters within the same water tables indicate significant differences under different nitrogen application levels at $P<0.050$ level. (a), Chao1 index; (b), Shannon index; (c), Bray-Curtis dissimilarity; (d), principal coordinate analysis (PCoA) of bacterial community based on Bray-Curtis dissimilarity. CK, N1, and N2 are the nitrogen application levels of 0, 10, and 20 kg N/(hm^2•a), respectively. Boxes in Figure 1a, b, and c indicate the IQR (interquartile range, 75th to 25th of the data). The median value is shown as a line within the box. Outlier is shown as black circle. Whiskers extend to the most extreme value within 1.5×IQR.

and *c_subgroup*_6 were the dominant bacterial genera in the soil under seasonal waterlogging and perennial drying conditions (Fig. 2b). Effects of water tables and nitrogen deposition on bacterial phyla and genera, as well as their interaction, were evident (Table 4). Water tables had a highly significant effect on both bacterial phylum and genus ($P<0.001$), while nitrogen deposition had a significant effect on 7 bacterial phylum and 8 bacterial genera ($P<0.050$).

We identified significant changes in the bacterial community of 83 bacterial groups among different water tables, according to the LEfSe analysis results (Fig. 3a). However, under different nitrogen levels, significant changes were found in the microbes of only 3 bacterial groups (Fig.

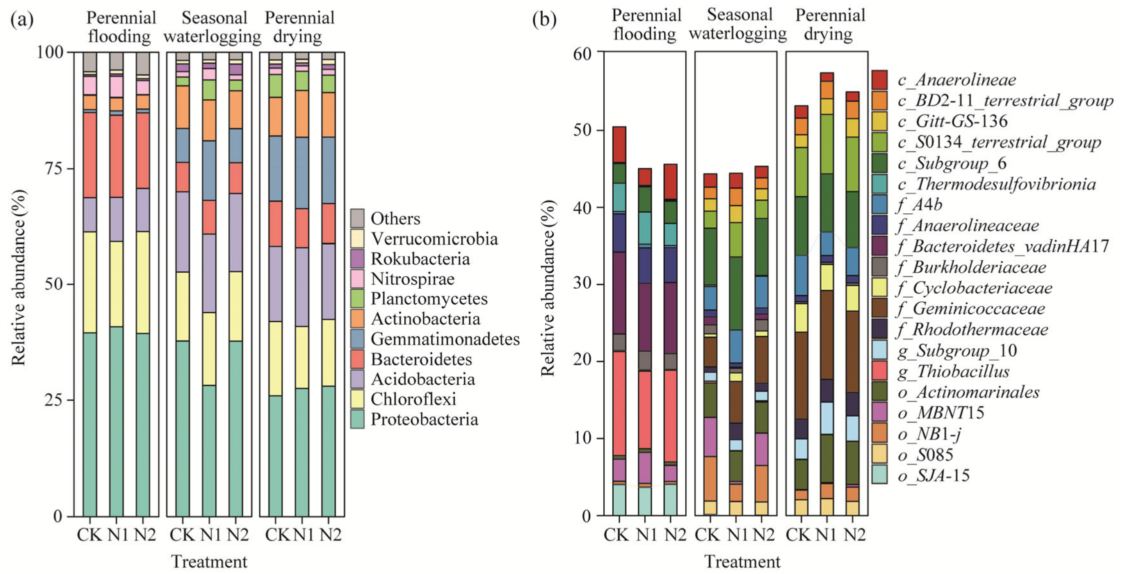

Fig. 2 Effects of nitrogen application on soil bacterial community structure under different water tables. (a), top 10 bacteria at phylum level; (b), top 20 bacteria at genus level.

3b). There were 29 biomarkers under perennial flooding condition, with the dominant phyla being Planctomycetes, Bacteroidetes, Chloroflexi, and Nitrospirae. Under seasonal waterlogging condition, there were 15 biomarkers, with dominant phylum being Acidobacteria. Under perennial drying condition, there were 39 biomarkers, with Gemmatimonadetes, Actinobacteria, and Planctomycetes being the dominant phylum.

3.4 Relationships between soil bacterial communities and soil properties

RDA1 axis explained 5.14% of the variation in the bacterial community, while RDA2 axis explained 81.02% of the variation. Combined, both axes explained a total of 86.16% of the variation (Fig. 4a). Through RDA, we found that differences between samples from each treatment were caused by water tables. However, bacterial community structure showed similarities among treatments with nitrogen application under the same water table. Soil properties under perennial drying and perennial flooding conditions had less effect on bacterial community diversity. OTUs and Chao1 indices were significantly correlated with soil pH, TC, and SOC under seasonally waterlogged condition (Fig. 4b).

In addition, an inter group correlation analysis was conducted between soil chemical properties and the top 10 bacterial phyla (Fig. 4c). The results showed that the relative abundance of Acidobacteria and Verrucomicrobia were significantly and positively correlated with SOC and TC under perennial flooding condition. Soil pH showed significant correlations with relative abundance of Planctomycete and Nitrospirae under seasonal waterlogging condition. In addition, Verrucomicrobia was significantly correlated with soil AN and AK contents. Relative abundance of Proteobacteria, Planctomycete, and Verrucomicrobia were significantly correlated with soil TN, AN, and AK contents under perennial drying condition.

3.5 Soil bacterial community network structure and stability

We constructed 9 bacterial interaction networks based on Spearman's correlation method to analyze the interactions among bacterial communities and the changes in species under different nitrogen application and water tables (Fig. 5). The number of network nodes and edges were both reduced under N1 and all water tables. CK had the highest number of network nodes and paths under seasonal waterlogged condition.

Negative edges and average degree of the network were reduced under nitrogen application, perennial flooding, and seasonal waterlogging conditions. Conversely, an increase in these

Table 4 Variance analysis of bacterial communities at phylum and genus levels under different nitrogen application and water tables

Phylum/genus	Water tables		Nitrogen application		Water tables×Nitrogen application	
	F	*P*	*F*	*P*	*F*	*P*
Phylum						
Proteobacteria	85.143	<0.001***	4.716	0.015*	8.942	<0.001***
Chloroflexi	46.730	<0.001***	3.346	0.046*	2.384	0.070
Acidobacteria	151.436	<0.001***	1.320	0.280	1.277	0.300
Bacteroidetes	232.009	<0.001***	1.766	0.185	1.430	0.240
Gemmatimonadetes	282.100	<0.001***	10.802	<0.001***	5.381	0.002**
Actinobacteria	147.073	<0.001***	0.366	0.696	2.038	0.110
Planctomycetes	294.773	<0.001***	11.530	<0.001***	17.168	<0.001***
Nitrospirae	113.675	<0.001***	12.159	<0.001***	5.131	0.002**
Rokubacteria	35.207	<0.001***	4.575	0.017*	2.459	0.060
Verrucomicrobia	5.511	<0.001***	8.970	0.001**	2.711	0.045*
Others	321.968	<0.001***	4.364	0.020*	4.671	0.004**
Genus						
*c_Subgroup_*6	127.480	<0.001***	4.871	0.013*	2.070	0.105
Geminicoccaceae	424.470	<0.001***	1.442	0.250	2.788	0.041*
Hydrogenophilaceae	155.358	<0.001***	1.287	0.288	1.058	0.392
*Bacteroidetes_vadinHA*17	576.490	<0.001***	4.533	0.018*	1.777	0.155
o_Actinomarinales	113.628	<0.001***	1.793	0.181	3.684	0.013*
*c_S*0134*_terrestrial_group*	314.537	<0.001***	9.834	<0.001***	3.468	0.017*
Anaerolineaceae	101.599	<0.001***	0.615	0.546	6.984	<0.001***
*A*4*b*	39.338	<0.001***	5.085	0.011*	5.112	0.002**
c_Anaerolineae	61.026	<0.001***	7.275	0.002**	21.784	<0.001***
*o_MBNT*15	615.364	<0.001***	1.074	0.352	1.045	0.398
*o_NB*1*-j*	42.549	<0.001***	2.791	0.075	5.105	0.002**
*Solibacteraceae_Subgroup_*3	133.354	<0.001***	4.781	0.014*	2.339	0.074
Gemmatimonadaceae	359.449	<0.001***	0.572	0.570	0.300	0.876
Thermoanaerobaculaceae	222.265	<0.001***	11.317	<0.001***	6.044	0.001**
o_SJA-15	138.860	<0.001***	3.136	0.056	2.758	0.042*
Ilumatobacteraceae	199.072	<0.001***	4.971	0.012*	5.321	0.002**
Cyclobacteriaceae	164.618	<0.001***	0.342	0.713	0.980	0.431
Rhodothermaceae	327.543	<0.001***	3.861	0.030*	4.312	0.006**
*c_BD*2-11*_terrestrial_group*	167.426	<0.001***	1.048	0.361	0.399	0.808
Burkholderiaceae	31.589	<0.001***	0.314	0.733	1.194	0.330
Others	42.020	<0.001***	0.148	0.863	3.055	0.029*

Note: *, $P<0.050$ level; **, $P<0.010$ level; ***, $P<0.001$ level.

parameters was observed under perennial drying condition. Under the same water table, the average path length of bacterial network decreased under N1 treatment and increased under N2 treatment (Fig. 6a). Stability of the network was compromised when key modular hubs were removed, and nitrogen affected key modular hubs in bacterial network (Fig. 6b). Robustness of bacterial network was increased under nitrogen application and perennial flooding condition, however, robustness decreased under nitrogen deposition, seasonal waterlogging, and perennial drying conditions (Fig. 6c). Nitrogen application enhanced the community stability under perennial flooding condition, decreased under seasonal waterlogging condition, and had little effect under perennial drying condition (Fig. 6d). An investigation of correlations revealed a connection between the parameters of bacterial network and soil properties, and soil AK, SOC, and TC contents were significantly correlated with parameters of bacterial network (Fig. 6e).

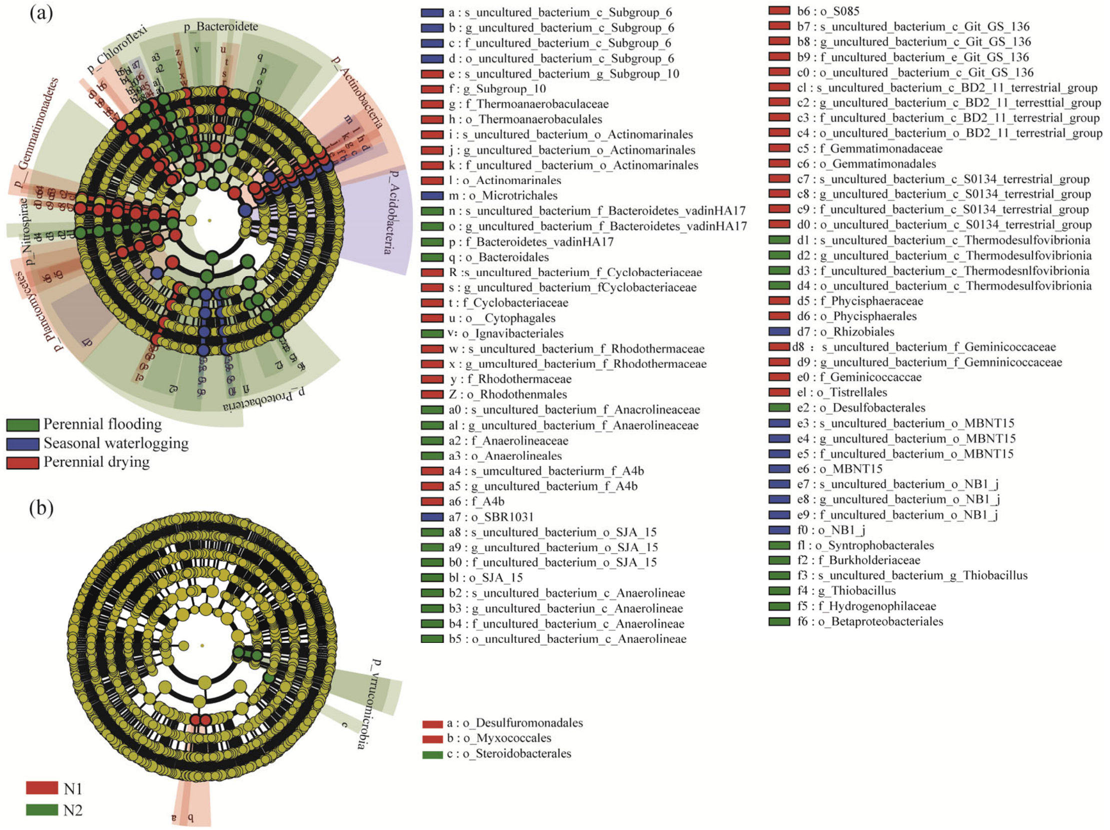

Fig. 3 Analysis of soil microbial community structure using linear discriminant analysis (LDA) and effect size (LEfSe) under different water tables. (a), phylogenetic trees of bacterial biomarkers under perennial flooding, seasonal waterlogging, and perennial drying conditions. The LDA threshold was 4.0; (b), phylogenetic trees of bacterial biomarkers under N1 and N2 treatments. The LDA threshold was 3.0.

3.6 Soil bacterial community function

Our analysis revealed 48 functional categories (Fig. 7). Water tables significantly altered bacterial community function (P<0.050). Under perennial flooding condition, the main functions of soil bacterial community were dark oxidation of sulfur compounds (19.78%–25.35%), dark sulfide oxidation (19.76%–25.25%), respiration of sulfur compounds (10.26%–11.29%), and sulfate respiration (10.13%–11.00%). The main functions of soil bacterial community were nitrification (10.41%–15.55%) and chemoheterotrophs (11.99%–14.41%) under seasonal waterlogging condition. The main functions were nitrification (13.54%–15.47%), chemoheterotrophy (13.99%–15.17%), and aerobic nitrite oxidation (10.90%–13.12%) under perennial drying condition. The number of significant functional differences between different nitrogen levels was 17, 27, and 10 under perennial flooding, seasonal waterlogging, and perennial drying conditions, respectively. Compared with CK, nitrate reduction and denitrification functions under N1 treatment were significantly increased under perennial flooding condition, while functions of aerobic chemoheterotrophy, aromatic compound degradation, aromatic hydrocarbon degradation, and fermentation under N2 treatment were significantly increased (P<0.050). Under seasonal waterlogging condition, compared with CK, bacterial community under N1 treatment

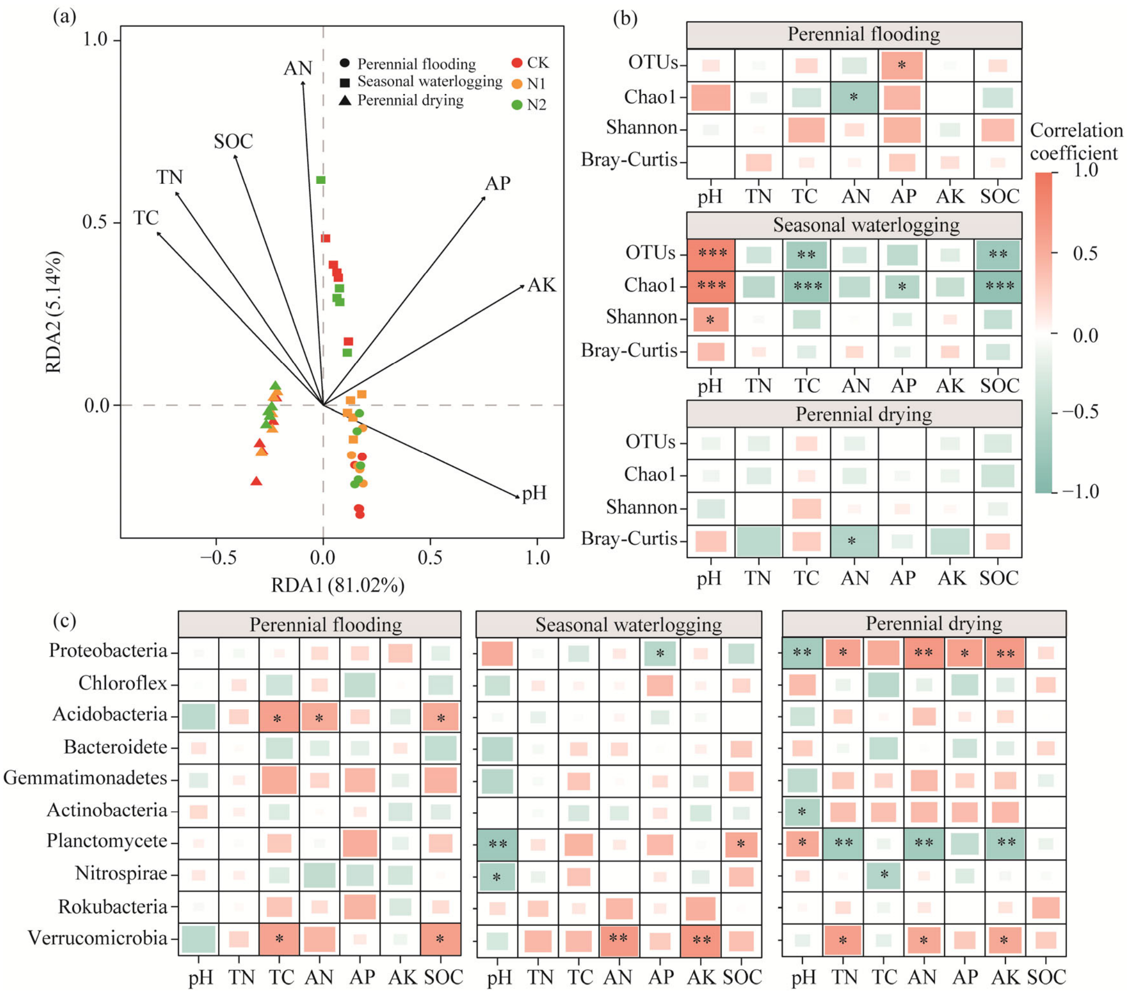

Fig. 4 Correlation analysis of nitrogen application on bacterial community structure and soil properties under different water tables. (a), redundancy analysis (RDA) between soil bacterial populations and soil properties; (b), Pearson's correlation between diversity index and soil properties; (c), Pearson's correlation between dominating phyla and soil properties. OTUs, operational taxonomic units; *, $P<0.050$ level; **, $P<0.010$ level; ***, $P<0.001$ level.

showed a significant increase in aerobic nitrite oxidation, nitrate reduction, photoheterotrophy, oxygenic photoautotrophy, and sulfur respiration functions, but a significant decrease in methanotrophy and sulfate respiration functions ($P<0.050$) was found. Compared with CK, the aerobic chemoheterotrophy, aromatic compound degradation, fermentation, and nitrification under N2 treatment were significantly reduced. Under perennial drying condition, compared with CK, the chlorate reducers, respiration of sulfur compounds, and sulfate respiration of bacteria were significantly increased ($P<0.050$). Soil TC was significantly associated with 21 bacterial community functions under perennial flooding condition ($P<0.050$; Fig. 8). Most bacteria were significantly correlated with soil properties under seasonal waterlogging condition.

4 Discussion

4.1 Effects of water tables and nitrogen application on soil bacterial community structure

Microbial Chao1 index, Shannon index, and beta diversity under seasonal waterlogging condition were significantly higher than those under perennial flooding and perennial drying conditions.

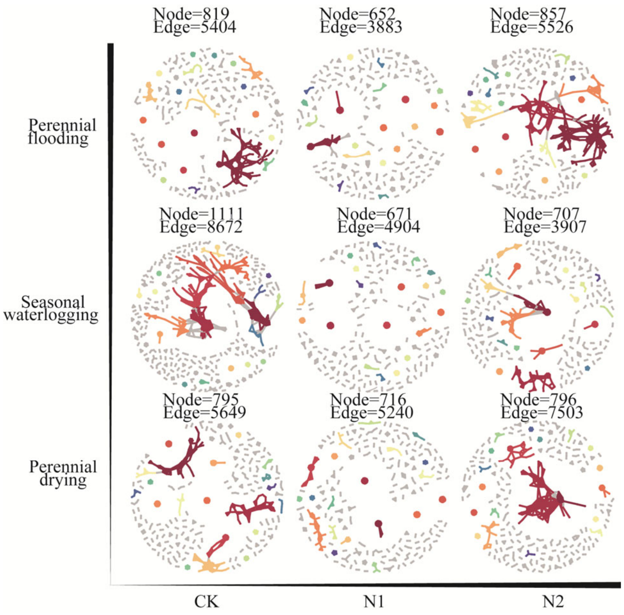

Fig. 5 Effects of nitrogen application on bacterial networks under different water tables. Nodes with the same color had the same size. Larger nodes were shown in different colors, and smaller nodes were shown in gray.

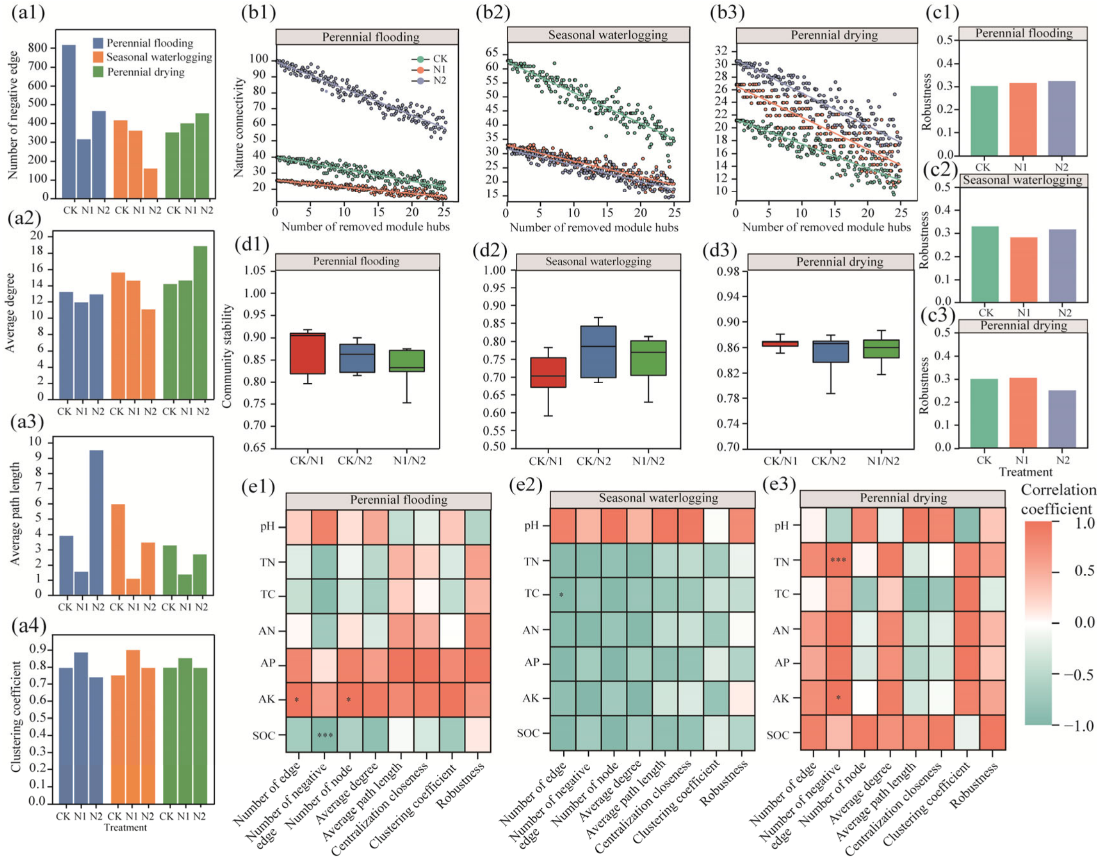

Fig. 6 Network topology properties of bacteria. (a1–a4), parameters of bacterial network under different nitrogen application and water tables; (b1–b3), nature connectivity of bacterial network under numbers of removed module hubs; (c1–c3), robustness; (d1–d3), community stability; (e1–e3), Spearman's correlation between parameters of bacterial network and soil properties. *, $P<0.050$ level; ***, $P<0.001$ level.

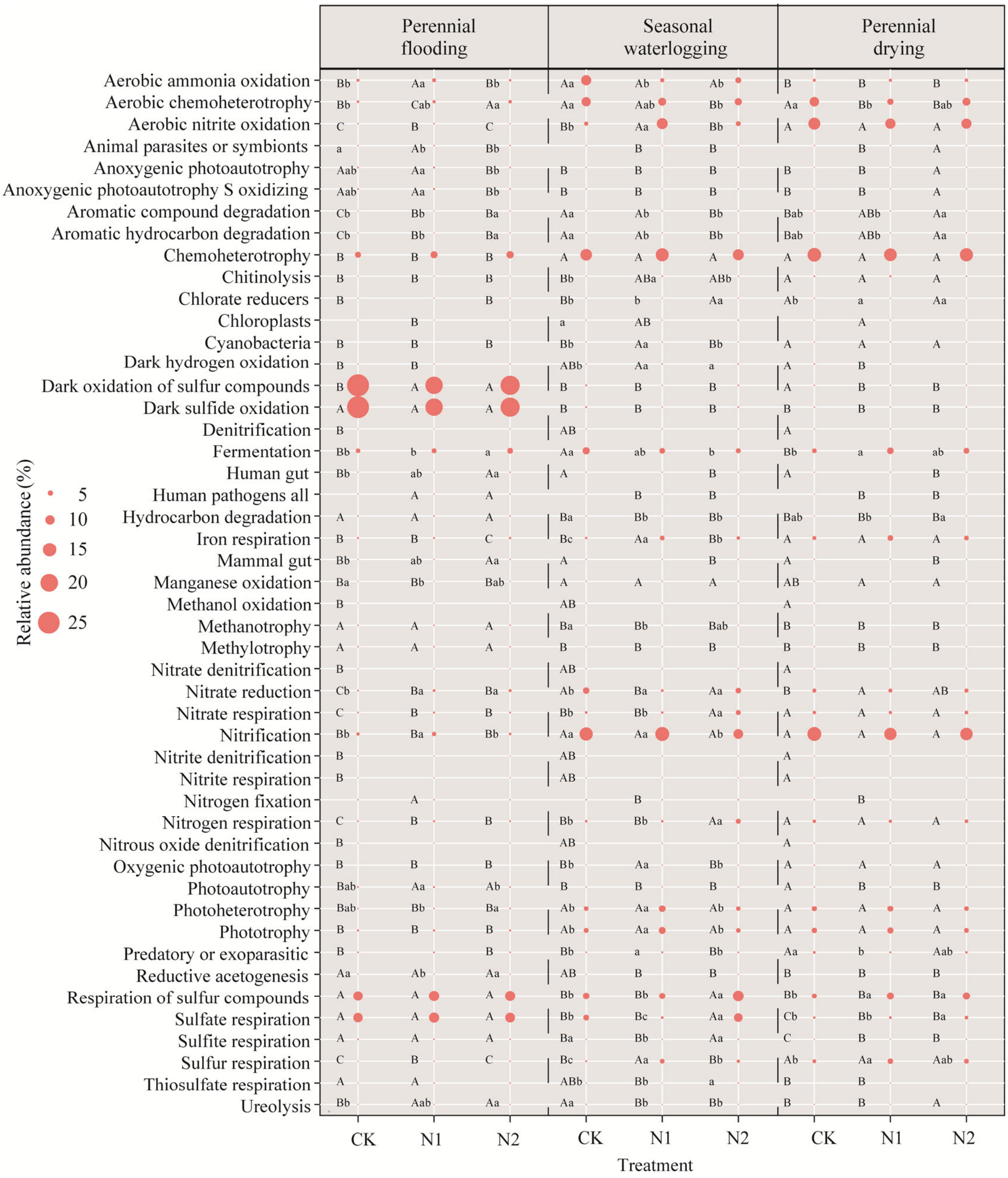

Fig. 7 Soil bacterial function under different nitrogen application and water tables. Different lowercase letters within the same water tables indicate significant differences among different nitrogen application levels at *P*<0.050 level. Different uppercase letters within the same nitrogen application indicate significant differences among different water tables at *P*<0.050 level. The red circle represents the relative abundance of bacterial function.

The distinctive feature of seasonal waterlogging condition was the cyclical variation in their hydrological condition, which led to increased environmental heterogeneity. Seasonal waterlogging condition altered not only physical but also influenced chemicals such as nutrient availability and redox state (Yin et al., 2022). This environmental heterogeneity provided suitable ecological niches for a wide range of bacterial taxa, thus contributing to an increase in bacterial diversity. A study with 8 a nitrogen deposition showed a significant increase in the diversity index of bacteria. The duration of nitrogen application might explain the inconsistency with this study (Ma et al.,

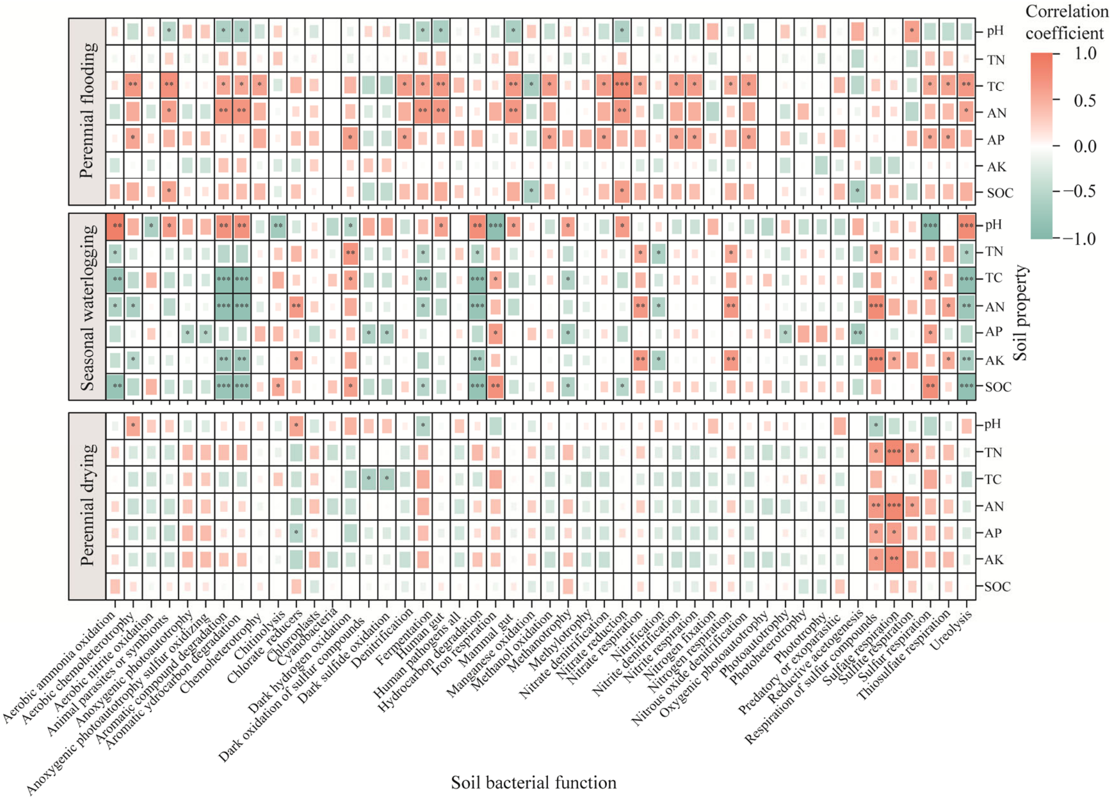

Fig. 8 Correlation analysis between soil bacterial function and soil properties under different water tables. *, *P*<0.050 level; **, *P*<0.010 level; ***, *P*<0.001 level.

2021; Wang et al., 2023). However, a meta-analysis on a global scale showed that nitrogen application significantly reduced alpha diversity of soil bacteria (Wang et al., 2023). Recent research has demonstrated that variations in soil moisture and soil properties significantly influence the diversity of soil microbial community (Sokol et al., 2022). This result implied that the impact of nitrogen application on bacterial diversity was dependent on soil background (Yang et al., 2022).

The microbial community composition depended on the cumulative effect of soil moisture (Williams and Rice, 2007). The main reason behind this was that soil moisture had a significant impact on the transcription and activity of microbes, leading to changes in soil microbial communities (Li et al., 2017a). Different soil moisture altered the physiological state and metabolic activity of microbes, thereby influencing their growth, reproduction, and function (Yu and Ehrenfeld, 2009). Compared with the perennial flooding condition with high soil moisture, the abundance of Ascomycetes, Chloroflexi, and Bacteroidetes bacterial significantly decreased under perennial drying condition, while Acidobacteria, Actinobacteria, and Gemmatimonadetes increased significantly (Fig. 2a). Most Acidophilus phylum was considered aerobic bacteria, which could make it favorable in the arid environment (Urbanová and Bárta, 2016). Actinomycetes live in neutral or slightly alkaline soils with low soil moisture (Bao et al., 2021), so this might be the reason for the low distribution under perennial flooding condition. As soil moisture decreased, methanogenic bacteria were inhibited (Jassey et al., 2018). In this study, members of the phyla Proteobacteria and Gemmatimonadetes were found to be more sensitive to drought under different water tables, which was consistent with the findings of Potter et al. (2017).

Nitrogen application provided additional nutrients to microorganisms, which altered the interactions between microbial species from symbiosis to competition (Hoek et al., 2016). It was possible that the bacterial response to nitrogen application was highly dependent on the environment or selectively conserved through evolution (Isobe et al., 2019), as demonstrated in this study. Eutrophic bacteria, specifically Actinomycetes (Zeng et al., 2016), may be impacted by nitrogen application in this study. Bacterial taxa exhibited ecological niche preferences, even within the same phylum (Fig. 2b). The observed differences in the response of soil microbial community to external nitrogen input attributed to the unique characteristics of alpine wetland ecosystem studied here, which differed from other ecosystems in terms of the sensitivity of soil bacteria to nitrogen input (Ramirez et al., 2010). In this study, although nitrogen application had a significant effect on the soil bacterial community, the bacterial community was more dependent on water tables.

The biodiversity of wetland ecosystems is very sensitive to natural changes in water tables. Microbial communities can be used as indicators of environmental change. Using these indicators, it is possible to monitor the quality of environment and to assess the impact of human activities on the ecosystem.

4.2 Effects of water tables and nitrogen application on structure and stability of soil bacterial network

There was a tendency for both network node and edge to initially decrease and then increase with rising nitrogen deposition and decreasing water tables (Fig. 5). A possible explanation for this occurrence was an increase in species diversity caused by nitrogen deposition (Li et al., 2017a). Nitrogen deposition provides greater opportunities for interactions between different bacteria (Sun et al., 2023). Our research showed that bacterial network structure under seasonal waterlogging condition was more complex than those of perennial flooding and perennial drying conditions. Changes in environmental pressures due to seasonal waterlogging, such as dewatering pressures during dry periods and hydrodynamic effects during wet periods, increased community adaptations and network complexity (Liu et al., 2020b). Soil moisture changes had a regulating effect on soil microbial network (Wang et al., 2022b). This study revealed that as soil moisture decreased, the negative interactions among microorganisms decreased (Fig. 6a). Bacterial network was highly sensitive to drought and had poor drought tolerance, with an increase in positive links and a decrease in negative links (Li et al., 2017b). This suggested that under perennial drying condition, cooperation between bacterial operational taxonomic units was enhanced and competition was reduced (de Vries et al., 2018).

The negative interactions among microorganisms increased under perennial drying condition (Fig. 6a). Positive and negative interactions among microorganisms represent cooperation and competition between corresponding microbial species (Che et al., 2019). Considering that the growth conditions without nitrogen deposition were not optimal, the degradation of organic matter in the soil was an important source of nitrogen for microorganisms (Nicolás et al., 2019). Microorganisms may experience an increased demand for nitrogen due to nitrogen deposition. Consequently, as the rate of nitrogen utilization rises, bacteria's nitrogen content and energy supply (electron acceptor) became limited (Ma et al., 2022). Under nitrogen deposition, the enhanced selection during community assembly also supported similar views, as the convergent effect increased the competition between bacteria (Sun et al., 2023). When microbes can utilize similar resources, they may compete strongly within a limited niche space (Götzenberger et al., 2012). Nitrogen deposition led to increased microbial network stability under perennial flooding condition and decreased stability under seasonal waterlogging and perennial drying conditions (Fig. 6c). This result may be attributed to alterations in the relative abundance and competitive interactions of different bacterial species in the soil, which in turn leads to changes in the structure of microbial network (Ma et al., 2022). The bacteria adapted to nitrogen deposition may gain a competitive advantage and gradually establish a more stable microbial network structure (Sun et al., 2023). Water tables had minimal impact on network stability, likely due to the adaptability of bacteria to environmental changes (Jiao et al., 2022), as evidenced by the analysis of soil properties and parameters of bacterial network (Fig. 6e).

The effects of nitrogen deposition and water table change on bacterial network are complex and multidimensional. It is thus imperative that future research should concentrate on the adaptation and resilience of microbial network to the changes in nitrogen deposition and water tables, and how microbial network serves as a buffer or respond to external disturbances through their diversity and complexity.

4.3 Effects of water tables and nitrogen application on soil bacterial community function

The alterations in soil microbial community structure and interactions could affect soil element cycles and potentially influence ecosystem stability under various water tables and nitrogen application (Chowdhury et al., 2019; Huang et al., 2020). In this research, variations in water tables and nitrogen deposition were found to alter carbon, nitrogen, and sulfur functions of bacterial communities (Fig. 7). Microbially driven sulfur cycle processes were critical to wetland nutrient cycle, and the sulfur cycle was coupled to carbon, nitrogen, and metal cycles (Yu et al., 2023). Flood and drought altered oxygen content and thus affected NO_3^- transformation rate. Microbial communities, bacterial symbiosis, and functional genes in aerobic-anoxic transitions were affected by dissolved oxygen (Liu et al., 2021). Low soil moisture inhibited denitrification, and the potential denitrification rate was highly sensitive to changes in soil moisture (Peralta et al., 2013). Water tables affect the denitrification function of bacteria, which was consistent with the research of Shi et al. (2018). According to the results of this study, we found that nitrate reduction, aerobic ammonia oxidation, and nitrification were all differentially affected by nitrogen application, which was also found in the study of Liu et al. (2020a). Under seasonal waterlogging condition, nitrogen affected the functional changes of nitrifying and denitrifying bacteria through synergistic effects (Li et al., 2022). Under nitrogen application, relative abundance of functional groups related to nitrate reduction was significantly reduced under low water table. This result may be due to the change of soil environment caused by nitrogen application, which in turn affects the growth and metabolic activity of nitrate-reducing bacteria (Liu et al., 2020a). Nitrogen application could promote the growth of ammonia-oxidizing bacteria, which was also found in the research of Reed et al. (2010). Other studies have suggested that nitrogen application may lead to soil acidification, which inhibits the growth of ammonia-oxidizing bacteria (Abirami et al., 2021).

The results showed that the function of bacterial communities was strongly influenced by environmental factors such as soil nutrient availability. Changes in the relative abundance of genes related to carbon, nitrogen, and sulfur cycles were closely related to soil nutrient content (Yan et al., 2020b). The correlation between bacterial community function and soil properties was the largest under seasonal waterlogging condition, but the least under perennial drying condition (Fig. 8). Correlation between bacterial community function and soil properties may be enhanced under seasonal waterlogging condition as bacterial community in the soil may experience rapid growth and metabolic activity due to cyclical changes in moisture, which may lead to rapid changes in soil properties (RoyChowdhury et al., 2022). In contrast, under perennial drying condition, the lack of moisture may limit bacterial growth and activity, leading to a decrease in the correlation between bacterial community function and soil properties (Uhlířová et al., 2008). The physical and chemical properties of the soil play a key role in regulating the functioning of bacterial communities, as demonstrated in previous studies (Tian et al., 2022).

Water tables affected functional gene expression by affecting oxygen and water status of the soil, altering the bacterial composition and thus affecting the functional genes. Nitrogen deposition affects bacterial metabolism by altering nitrogen availability in the soil. In this study, the functional potential of bacterial community was only inferred from amplicon sequencing data by FAPROTAX, and future studies could use macrogenomics to reveal the effects of nitrogen deposition and water tables on the expression of microbial functional genes and metabolic pathways.

5 Conclusions

Nitrogen application had an impact on bacterial abundance, but had minimal effect on bacterial community structure under the same water table. Nitrogen application mainly affected the relevant functions of nitrification and denitrification involved in the nitrogen cycle under different water tables. There were differences in the number of network node and edge at different water tables, but nitrogen deposition decreased the structure of bacterial network. Stability of microbial networks was altered by nitrogen deposition under different water tables. Nitrogen deposition and water table interaction affected soil pH, TC, SOC, AP, and AK in the alpine wetland. Effects of water tables on soil bacterial community structure and bacterial diversity were more significant than that of nitrogen application. The findings of this research revealed the response mechanism and degree of microbial community structure to nitrogen deposition and water tables, and explored effective mitigation and adaptation strategies for wetland in arid areas to cope with climate change and human disturbance.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

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