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Microbial ecological and biogeochemical processes in the soil-vadose zone-groundwater habitats

Edited by
Zifang Chi, Huai Li and Jiuling Li

Coordinator by
Yi-Hao Luo

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Microbial ecological and biogeochemical processes in the soil-vadose zone-groundwater habitats

Topic editors

Zifang Chi — Jilin University, China

Huai Li — Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences (CAS), China

Jiuling Li — The University of Queensland, Australia

Topic Coordinator

Yi-Hao Luo — Arizona State University, United States

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Table of contents

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Bradley M. Tebo,
Oregon Health and Science University,
United States

*CORRESPONDENCE
Zifang Chi
chizifang@jlu.edu.cn

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Editorial: Microbial ecological and biogeochemical processes in the soil-vadose zone-groundwater habitats

Huai Li[1], Zifang Chi[2]*, Jiuling Li[3] and Yihao Luo[4]

[1]Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, China, [2]Key Laboratory of Groundwater Resources and Environment, Ministry of Education, Jilin University, Changchun, China, [3]Australian Centre for Water and Environmental Biotechnology, The University of Queensland, St. Lucia, QLD, Australia, [4]Swette Center for Environmental Biotechnology, Biodesign Institute at Arizona State University, Tempe, AZ, United States

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Editorial on the Research Topic
Microbial ecological and biogeochemical processes in the soil-vadose zone-groundwater habitats

Microorganisms regulate biogeochemical cycles and serve various functions within soil, vadose zone, and groundwater habitats (e.g., Chi et al., 2018, 2022; Zhang et al., 2021; Li et al., 2022). The composition and function of these microorganisms can be influenced by both biotic and abiotic factors, which in turn affect biochemical processes and ecosystem functions (e.g., Li et al., 2019; Chi et al., 2021). Thus, there is significant interest in studying these habitats and their connection to multiple microbial pathways, particularly those involved in material cycling, pollution control, and carbon neutrality. Therefore, to develop a healthy-stable-sustainable ecosystem, this Research Topic concentrates on microbial ecological/biogeochemical processes in the soil-vadose zone-groundwater habitat. The objectives of this Research Topic are: (1) to compile new research on microbial ecological processes in these habitats; and (2) to highlight the possibilities for achieving sustainable processes. The articles included in this Research Topic have undergone careful review, and the following eleven articles have been accepted.

Zheng et al. conducted a comparative analysis of bacterial communities under different chemical oxygen demand to nitrogen (COD/N) and nitrogen to phosphorus (N/P) ratios in restored wetlands. They discovered that variations of nitrogen source controlled the bacterial composition, while imbalance in organics and nutrients resulted in bacterial community differentiation.

Yan et al. investigated the impact of various remediation measures on the removal of SO_4^{2-}, Pb, Zn, and Mn in rare earth mine (REM) soils. The results indicated that chemicals ($Ca(OH)_2$, 3.0 g/kg) plus sulfate-reducing bacteria (SRB) (CM-M) exhibited greater efficiency in removing of SO_4^{2-}, Pb, Zn, and Mn. The inoculation of SRB was beneficial for increasing sulfur and nitrogen cycling. This study provided a good method for REM contaminant removal.

Kuang et al. identified Proteobacteria and Actinobacteria as the dominant phyla in Baiyangdian sediment under eutrophication. These phyla actively participated in C, N, P, and S cycles. The genes associated with these cycles were correlated with the presence of the reductive-citric acid cycle pathway. Carbon-metabolism was dominant for the bacterial community. The abundances of genes related to nitrogen cycle were consisted with high total nitrogen (TN) level.

Wang M. et al. investigated the response of soil property, enzyme activity, and bacterial community to different hydrological practices in the Changbai Mountains. Their findings revealed that a high water level promoted the recovery of soil nutrients and bacterial activities and communities. These results provide valuable guidance for implementing effective strategies to restore peatlands.

Zhao et al. explored the effect of soil depth and altitude gradients on microbial abundance in the Changbai Mountain. They determined that soil depth, rather than altitude, served as the primary controlling factor. Consequently, changes in soil depth may lead to greater disturbances for microorganisms in the face of future climate change.

Song et al. examined the influence of rising temperature and adding nitrogen on CO_2 and N_2O emissions and microbial abundances in permafrost-peatlands. They observed that increased temperature, nitrogen availability and their combined effect remarkably increased CO_2 and N_2O emissions. These findings demonstrated that soil microorganisms and available nitrogen were favorable for controlling carbon emissions.

Chen et al. presented a study on the benefits and drawbacks of two methods for removal of antibiotic resistance genes (ARGs) from soil. They highlighted how constructed wetlands could regulate ARG removal by utilizing different plants, substrates, wetlands, and hydraulic conditions. Photocatalysis, facilitated by catalysts and radiation intensities, effectively deactivated ARGs by producing reactive oxygen species. Combining constructed wetland with photocatalysis technology was feasible for ARG removal.

Wang Y. et al. explored antibiotic removal and biological response of different constructed wetlands. Their findings demonstrated that a combination of gravel substrate and algal/bacteria communities could effectively enhance the antibiotic wastewater removal, and maintain harmonious biological communities.

Zhang, Bai, Zhang et al. investigated the response of bacterial communities in rhizosphere and non-rhizosphere sediments of reed in Baiyangdian Lake under antibiotics stress. They found that total antibiotics and ciprofloxacin played a dominant role in regulating bacterial diversity. Antibiotics dramatically affected bacterial community composition and had a potential risk for the dissemination of ARGs in shallow lakes.

The same authors (Zhang, Bai, Zhai et al.) further explored the characteristics of bacterial communities in wild and cultivated reed zones. Their results indicated that antibiotic pollution resulting from planting activities had significant impacts on the bacterial community. These findings provide useful references for managing antibiotics in lake systems.

Liang et al. conducted a comprehensive summary of microbial communities in coastal wetlands, highlighting their controlling factors. They also discussed patterns of functional genes, revealed environmental functions, and proposed future directions for research in this field. These findings offer valuable insights into the potential application of microbes in material circulation and pollution remediation.

We think that all accepted articles in this Research Topic will provide new knowledge on microbial processes in soil-vadose zone-groundwater habitats.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Huai Li,
Northeast Institute of Geography and Agroecology (CAS), China

REVIEWED BY
Yuanchun Zou,
Northeast Institute of Geography and Agroecology (CAS), China
Yanyu Song,
Northeast Institute of Geography and Agroecology (CAS), China

*CORRESPONDENCE
Shangqi Xu
shangqixu@ahnu.edu.cn

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Responses of soil enzyme activities and bacterial community structure to different hydrological regimes during peatland restoration in the Changbai Mountain, northeast China

Ming Wang[1], Shangqi Xu[2]*, Shengzhong Wang[1,3], Cong Chen[1], Yuting Wang[1] and Lei Liu[4]

[1]Key Laboratory of Geographical Processes and Ecological Security in Changbai Mountains, Ministry of Education, School of Geographical Sciences, Northeast Normal University, Changchun, China, [2]School of Ecology and Environment, Anhui Normal University, Wuhu, China, [3]State Environmental Protection Key Laboratory of Wetland Ecology and Vegetation Restoration, Institute for Peat and Mire Research, Northeast Normal University, Changchun, China, [4]Institute of Scientific and Technical Information of Jilin, Changchun, China

Appropriate hydrological management is critical for peatland restoration. An important prerequisite for peatland restoration is a recovery of soil biological processes. However, little is known about the effects of different hydrological management practices on soil biological processes during peatland restoration. In this study, the variations in soil properties, enzyme activities, and bacterial communities across different peatlands, namely natural peatland (NP), peatland restored under high water level (HR), peatland restored under alternating high-low water level (HLR), peatland restored under low water level (LR), and degraded peatland (DP), in the Changbai Mountains were investigated. Results showed that soil organic carbon, soil water content, and total nitrogen in NP were significantly higher than those in restored and degraded peatlands, and these soil properties in restored peatlands increased with the water level. The activities of soil hydrolases including β-1, 4-glucosidase, β-1, 4-n-acetylglucosidase, and acid phosphatase in NP were higher than in restored and degraded peatlands, while the activity of polyphenol oxidase in NP was the lowest. In restored peatlands, all measured enzyme activities decreased with the decline in water level. Both bacterial diversity and richness in NP were the lowest, while the highest diversity and richness were observed in HR. Redundancy analysis indicated that soil organic carbon, water level, soil water content, total nitrogen, and pH were the most important factors that affected the soil enzyme activities and bacterial community. Our findings give insight into the effects of different hydrological regimes on soil biological processes during peatland restoration. Maintaining a

high water level early in the restoration process is more beneficial to restoring the ecological functions of peatlands than other hydrological regimes.

KEYWORDS

Changbai Mountain, peatland restoration, soil enzyme activity, soil bacteria, soil properties, water regime

Introduction

Boreal peatlands are important ecosystems because they store approximately one-third of the planet's terrestrial carbon (Yu, 2012; Turetsky et al., 2015). However, human activities pose a major threat to peatland stability and cause various degrees of damage to natural peatlands (Dohong et al., 2017). Due to human disturbance, approximately 12.5% of the world's peatlands have been lost or degraded (Frolking et al., 2011). The primary threat to peatlands is agricultural cultivation, which can destroy the native vegetation and hydrological regimes (Hallema et al., 2015). With the removal of native vegetation and the decline of the water level, organic matter decomposed quickly under aerobic conditions and soil carbon storage decreased (Berglund and Berglund, 2010; Heller and Zeitz, 2012; Hallema et al., 2015). These changes have shifted the world's peatlands from a sink to a source of carbon (Kløve et al., 2010; Leifeld et al., 2019), which may have profound effects on global climate change. Therefore, it is urgent to develop suitable and sustainable restoration methods to restore degraded peatlands.

Peatland restoration measures mainly include plant reintroduction, hydrological mediation, ditch blocking, and alteration of microtopography (Peacock et al., 2015; Guo et al., 2016). The main purpose of peatland restoration is to restore ecological functions close to or to their undisturbed state by restoring hydrological conditions and plant communities (Lazcano et al., 2018; Ahmad et al., 2020). Planting has been considered to be an effective way to restore the dominant peatland species in degraded peatland, but vegetation alone cannot ensure the persistence of the restoration (Guo et al., 2016). In addition to vegetation, the primary challenge associated with restoration is hydrological restoration (Ahmad et al., 2020). Hydrological regimes strongly control the form and function of peatlands, because the water flow, dissolved minerals, and nutrients regulate the diversity and characteristics of the plant community (Belyea and Baird, 2006; Mitsch and Gosselink, 2007), as well as the production and decomposition dynamics that lead to the accumulation of peat (Moore et al., 2002). Therefore, a key consideration in peatland restoration is the management of the hydrological regime, which aims not only at the reestablishment of the original peatland vegetation but also at the rapid recovery of ecological functions.

Soil microorganisms play a key role in the biogeochemical functions of soils, such as soil organic matter formation, decomposition, and nutrient cycling, which further affect the carbon balance (Hill et al., 2014; Soares and Rousk, 2019; Qin et al., 2021). Soil biochemical properties, including microbial community structure and enzymatic activities, reacted quickly to alterations in soil physicochemical properties and water regimes (Sardans et al., 2008; Lagomarsino et al., 2009; Ma et al., 2020). Therefore, these soil biochemical properties were used as sensitive indicators of soil functions (Veres et al., 2015; Qin et al., 2021). Some studies have demonstrated that soil microbial communities and extracellular enzyme activities responded sensitively and drastically to peatland drainage, reclamation, grazing, and mining (Freeman et al., 1996; Ward et al., 2007; Burns et al., 2013). Peatland restoration, through the reestablishment of the original plant community or recovery of the hydrological regime, is always accompanied by increasing soil carbon, nitrogen, and soil water content (Lucchese et al., 2010; Putkinen et al., 2018), all these changes are likely to affect biological processes that drive soil functions (Purre et al., 2019; Ahmad et al., 2020). However, few studies have investigated the effects of peatland recovery under different hydrological management practices on soil biological processes.

The Changbai Mountain is the largest peatlands region in northeast China. The area of peatlands in this region is approximately 463.31 km^2 (Ma et al., 2013). Since the 1950s, large areas of peatlands in this region have been cultivated into paddy fields after soil amendment. The area of peatlands was greatly reduced with the original hydrological patterns being destroyed (Li, 2013; Wang et al., 2020; Xu et al., 2021). Recently, the Chinese government issued the National Wetland Protection Law, which call for the restoration of the peatlands in China according to their types and degradation status. The Jilin Provincial Government developed and implemented plans for peatland restoration and intends to restore >6,000 hm^2 of cultivated peatland in the Changbai Mountain. In this study, different hydrological management practices were implemented in the restored peatlands in the Changbai Mountain. The purposes of this study were (1) to reveal how soil microbial community structure and enzyme activities respond to different hydrological management practices, and (2) to explore the optimal hydrological management measure to restore the ecological functions of degraded peatlands.

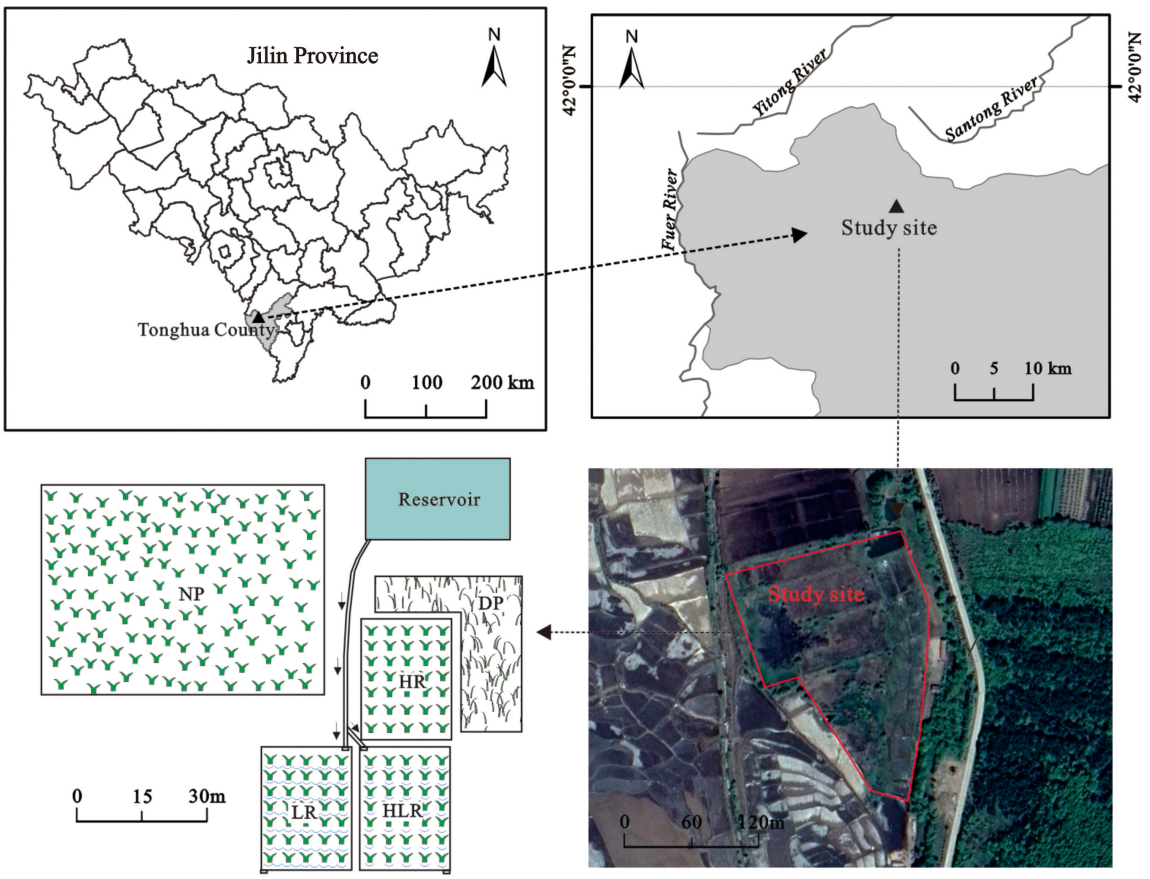

FIGURE 1
Study site locations in the Jilin province, northeastern China. NP, natural peatland; HR, peatland restored under high water level; HLR, peatland restored under an alternating high-low water level; LR, peatland restored under low water level; DP, degraded peatland.

TABLE 1 Environmental variables of the natural peatland and abandoned paddy fields before experiments.

	Soil organic carbon (g/kg)	Total nitrogen (g/kg)	Total phosphorus (g/kg)	pH	Soil water content (%)	Water level (cm)
Natural peatland	318.63 ± 12.53	21.77 ± 1.23	1.35 ± 0.14	5.46 ± 0.47	445.00 ± 23.14	1.37 ± 0.22
Abandoned paddy field	54.85 ± 4.37	4.39 ± 0.46	1.47 ± 0.11	6.12 ± 0.33	55.87 ± 5.67	−10.24 ± 1.06

The data in each column are shown as mean ± SE ($n = 4$).

Materials and methods

Study site

The study site is located at the west foot of the Changbai Mountain, approximately 1 km to the north of Sipeng Town, Tonghua City (41.858°N, 125.580°E), with an altitude of 512 m. The study area has a temperate continental monsoon climate. The mean annual precipitation and mean annual temperature are 790 mm and 5°C, respectively. The weather is usually cold and damp with about 110–150 frost-free days in a year (Song et al., 2005).

Before the 1980s, the study site was a typical peatland with a peat thickness of 0.8–1.2 m. The peatland area was approximately 40 hm^2, and the dominant species was *Carex schmidtii*. In the early 2000s, about 90% of these peatlands were reclaimed as paddy fields, dry cropland, or fishponds. Then, in 2010, some paddy fields were abandoned under the call of the local government to protect wetlands. However, due to long-term agricultural cultivation, the native vegetation, biodiversity, topsoil peat layer, and hydrological regimes of the original peatland have been destroyed, and ecological services have declined dramatically (Planas-Clarke et al., 2020; Wang et al., 2020). In the abandoned paddy fields, although the peat layer

TABLE 2 Environmental variables in natural, restored, and degraded peatlands.

Environment variables	NP	HR	HLR	LR	DP
Soil organic carbon (g/kg)	325.00 ± 7.51a	73.23 ± 4.48b	60.65 ± 2.86bc	57.86 ± 3.10c	61.16 ± 2.40bc
Total nitrogen (g/kg)	24.90 ± 0.97a	5.57 ± 0.26b	5.47 ± 0.47b	4.86 ± 0.29b	5.62 ± 0.15b
Total phosphorus (g/kg)	1.41 ± 0.08d	1.88 ± 0.05a	1.75 ± 0.02ab	1.61 ± 0.04bc	1.58 ± 0.07c
Soil water content (%)	432.65 ± 70.37a	122.65 ± 40.35b	110.68 ± 6.35b	98.19 ± 4.74b	87.67 ± 3.47b
pH	5.56 ± 0.06bc	5.72 ± 0.15b	5.43 ± 0.56c	6.10 ± 0.04a	6.14 ± 0.02a
C/N	13.08 ± 0.29a	13.31 ± 1.36a	11.26 ± 0.78ab	11.92 ± 0.08ab	10.87 ± 0.15b
Water level (cm)	2.28 ± 0.44b	7.56 ± 0.17a	1.45 ± 0.10b	−6.89 ± 0.49c	−7.85 ± 0.26c

The data in each column are shown as mean ± SE ($n = 4$). The different letters behind the data indicate significant differences among different treatments ($p < 0.05$, LSD test). C/N, the ratio of SOC to TN; NP, natural peatland; HR, peatland restored under high water level; HLR, peatland restored under an alternating high-low water level; LR, peatland restored under low water level; DP, degraded peatland.

still existed 30 cm below the surface soil layer, the vegetation were dominated by *Juncus bufonius*, *Echinochloa crusgali*, and *Bidens pilosa*, which are not typical peatland plants. The water level was significantly lower than that of the original peatland.

Experiments and sample collection

In April 2019, to simulate different restoration measures, experiments were conducted on abandoned paddy fields that had been converted from natural peatland around 2,000 and were abandoned in 2010. The area of the paddy fields is approximately 0.5 hm^2. There was a reservoir and a natural peatland near the paddy fields. The water of the reservoir came from rainfall and some nearby underground springs. The reservoir provides the water needed by the natural peatland, as well as water to conduct hydrological management in different experimental treatments (Figure 1). A natural peatland (NP), with an area of approximately 1.8 hm^2, was taken as a control to evaluate the effectiveness of different restoration measures. The natural peatland has never been cultivated. The main vegetation community in the natural peatland is *C. schmidtii* and the peat thickness is 0.8–1.2 m. Before experiments, the abandoned paddy fields were under the same original hydrology and management. Environmental variables of the natural peatland and abandoned paddy fields are shown in Table 1.

To study the restoration process of peatland under different water regimes, the paddy fields were divided into four plots, with each plot (>700 m^2) represent one treatment. Then, different restoration measures were implemented in the four plots, namely: (1) peatland restored under high water level (HR), the plot was permanently flooded through water supplement, and the water level was kept at a relatively high level: between 5 and 10 cm on average, (2) peatland restored under low water level (LR), the plot was under a relatively drained hydrological regime with a relatively low water level: among −10–0 cm on average, (3) peatland restored under an alternating high-low water level (HLR), the plot was under alternating flooded-drained hydrological regime with the water level alternating between high and low semimonthly, and (4) degraded peatland (DP), the plot maintained the status of abandoned paddy field with no restoration measures being conducted (Figure 1). At the beginning of the experiment, the three restored treatments (HR, LR, and HLR) were harrowed and then transplanted with *C. schmidtii*, with vegetation coverage of approximately 30–50%. After plant colonization, the restored peatlands were implemented with different hydrological management practices, hydrological management was carried out from May to October each year. The mean water levels of the restored peatlands during the growing season are shown in Table 2.

In October 2020, four 1 m^2 sample plots were randomly selected from each treatment for soil sampling. At each sample plot, soil samples were collected from 4 points and mixed into a composite sample. The soil at the depth of 0–20 cm was collected using a soil borer with a diameter of 5 cm. Each soil sample was divided into two parts in the lab: one part was kept at −80°C for DNA extraction; the other part was kept at 4°C for soil enzyme activity and physicochemical analysis.

Analysis of soil properties

The water level in different treatments was recorded using an Odyssey Logger (Dataflow Systems, Christchurch, New Zealand) installed in a PVC pipe. Soil pH was measured using a glass electrode (PHS-3E meter with E-201-C electrode, Leici, China). Soil water content was determined gravimetrically by drying at 105°C to a constant weight and then calculating the mass ratio of the water to the dried soil. Soil organic carbon (SOC) was determined after wet digestion with $K_2Cr_2O_7$-H_2SO_4 and titration with $FeSO_4$. The levels of total phosphorus and total nitrogen were determined by the Molybdenum blue method and the Kjeldahl method, respectively (Lu, 1999).

Analysis of soil enzyme activities

The activities of three soil hydrolases and one oxidase: β-1, 4-glucosidase (βG), β-1, 4-n-acetylglucosidase (NAG),

acid phosphatase (AP), and polyphenol oxidase (PPO), were determined by microplate fluorescence method (Saiya-Cork et al., 2002) using a multi-plate reader (Synergy H4 Hybrid Reader, Synergy H4BioTek, United States). The substrates used for the βG, NAG, AP, and PPO were 4-methyl umbelliferyl-BD-glucopyranoside, 4methyl umbelliferyl-BD-glucopyra-noate, 4-methyl parumone phosphate, and 4-dihydroxyphenylalanine, respectively. In brief, soil suspension was prepared by mixing about 0.5 g fresh soil sample and 125 mL sodium acetate buffer (pH = 5, 50 mmoL/L). To determine the activities of three soil hydrolases, 200 μL soil suspensions and 50 μL substrates were incubated in a 96-well microplate. The microplates were placed in the dark at 20°C for 4 h, and 10 μL of 1 mol/L NaOH solution was added and measured by the fluorescence detection method. To determine the activity of PPO, 600 μL soil suspension and 150 μL substrates were added to a transparent plate with a shallow mouth and incubated in the dark at 20°C for 5 h. Then the incubated solution was centrifuged under 3,000 r/min for 5 min and 250 μL supernate was sampled and measured using a microplate reader.

Soil DNA extraction and high-throughput sequencing

Soil DNA was extracted from 1 g of soil from each sample using a Fast DNA^{TM} SPIN Kit (MP Biomedicals, CA, United States). The concentration and purity of DNA extractives were examined and sent to Shanghai Majorbio Technology Co., Ltd., China, for high-throughput sequencing. The primer pairs used to amplify the V3-V4 hypervariable regions of the bacterial 16S rRNA gene were 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). An Illumina MiSeq platform was used for paired-end sequencing. The sequencing length of the target fragment was 250–500 bp.

The raw sequences were primarily processed with the standard Illumina pipeline. In brief, the raw sequences that passed through the mass screening were merged using Flash software (v1.2.11)[1] to obtain raw tags (Magoč and Salzberg, 2011). Then, the raw tags were filtered using QIIME (v1.9.1)[2] to identify the query sequences, and USEARCH (v7.0)[3] was invoked *via* QIIME to examine and eliminate chimeric sequences, obtaining effective tags (Caporaso et al., 2010; Edgar et al., 2011). The effective tags were clustered into operational taxonomic units (OTUs) based on a sequence similarity threshold of 97% using the UPARSE pipeline (v7.0.1090)[4] (Edgar, 2013), and each OTU was represented by the most abundant sequence in the OTU. An OTU table was created with the number of sequences of each OTU in each sample. Taxonomy was assigned for each OTU using the RDP Classifier (v2.11)[5] based on SILVA (v138)[6] (Quast et al., 2013). The low-abundance OTUs with a combined abundance of less than 10 sequences across all samples were eliminated.

Statistical analysis

The Shannon and ACE indices were calculated for each sample using Mothur (v 1.30.2)[7] (Schloss et al., 2009). One-way ANOVA followed by multiple comparisons using the LSD test was conducted to study the difference in microbial richness and diversity, soil properties, and enzyme activities among treatments. The significance level of $p < 0.05$ was considered asstatistically significant.

To study the effects of peatland restoration on soil enzyme activities and bacterial community composition, principal coordinate analyses (PCoA) were used on the Bray-Curtis dissimilarity matrices of the corresponding data. An ANOSIM test was used to verify the significance of the effects of hydrological regimes on bacterial community structure. Redundancy analysis (RDA) was used to explore the relationship between environmental variables and soil enzyme activities as well as microbial communities. Environmental variables were log-transformed and centered to equalize the weight of variables with ranges of different orders of magnitude. All data analyses were performed with R (v4.1.3)[8] in R Studio (v 1.0.153)[9] (Racine, 2012), the online platform of Majorbio Cloud Platform[10] (Ren et al., 2022), and the SPSS 26.0 as needed.

Results

Environmental variables among different peatlands

Environmental properties differed significantly among different peatlands (**Table 2**). The highest SOC, total nitrogen, and soil water content were observed in NP, which had the lowest total phosphorus ($p < 0.05$). DP was characterized by the lowest water level, soil water content, and the ratio of soil carbon to nitrogen (C/N), and the highest pH ($p < 0.05$).

For restored peatlands, SOC and total phosphorus in HR were higher than that in LR, while pH in HR was lower than in LR ($p < 0.05$). Total nitrogen, total phosphorus, SOC, and

1 https://ccb.jhu.edu/software/FLASH/
2 http://qiime.org/
3 http://www.drive5.com/usearch/
4 http://www.drive5.com/uparse/
5 https://sourceforge.net/projects/rdp-classifier/
6 https://www.arb-silva.de/
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8 https://www.r-project.org/
9 https://www.rstudio.com/
10 http://www.majorbio.com

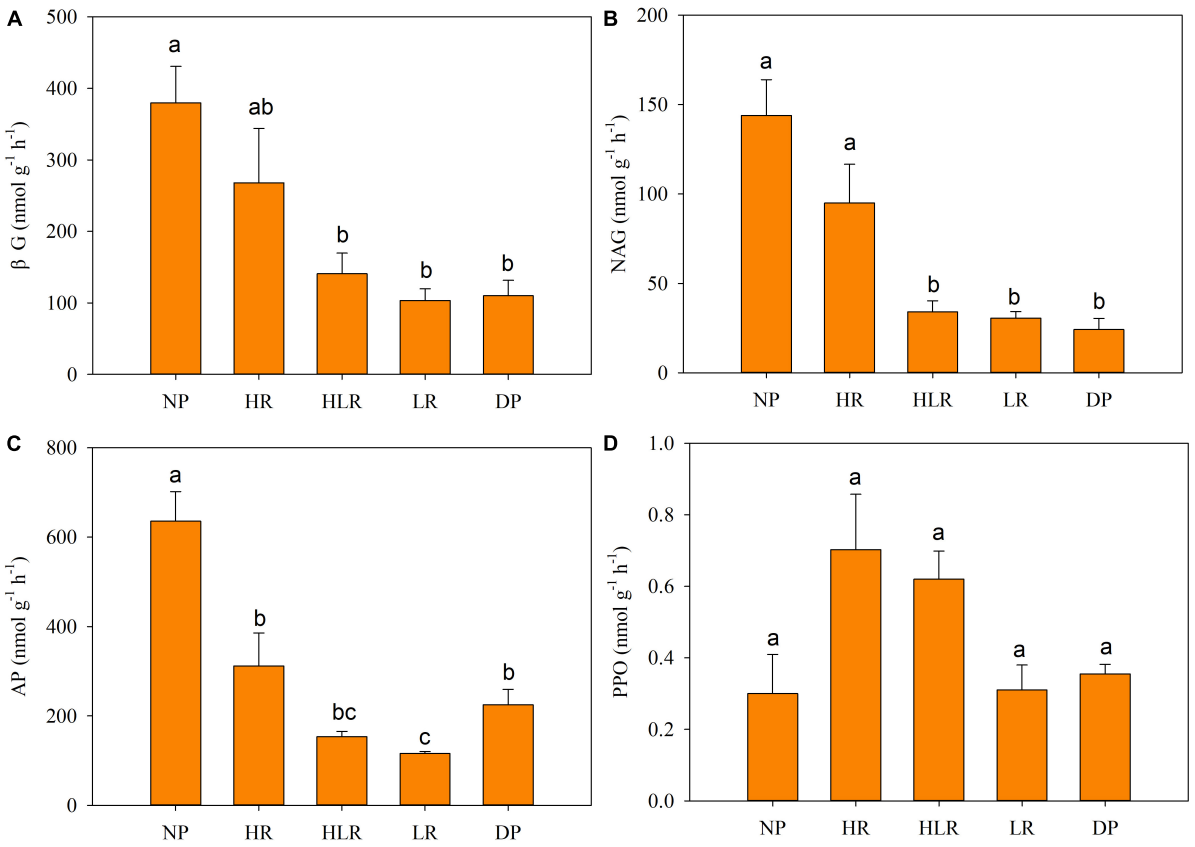

FIGURE 2
Soil enzyme activities in different peatlands. Bars represent average with standard error (n = 4). Different letters above the column indicate significant differences among different treatments, based on one-way ANOVA and LSD test ($p < 0.05$). βG, β-1, 4-glucosidase **(A)**; NAG, β-1, 4-n-acetylglucosidase **(B)**; AP, acid phosphatase **(C)**; PPO, polyphenol oxidase **(D)**; NP, natural peatland; HR, peatland restored under high water level; HLR, peatland restored under an alternating high-low water level; LR, peatland restored under low water level; DP, degraded peatland.

soil water content decreased along with water level from HR to HLR to LR. No significant difference was found in total nitrogen, soil water content, and C/N among the three restored peatlands ($p > 0.05$). Furthermore, no significant difference was found in all the detected variables between LR and DP.

Soil enzyme activities among different peatlands

The NP was characterized by higher soil hydrolase activities and lower oxidase activities (Figure 2). The activities of βG, NAG, and AP in NP were significantly higher than those in other treatments ($p < 0.05$). The lowest activities of the four detected enzymes were in the LR or DP. The AP activity of LR was significantly lower than that of DP, no significant differences were found between DP and LR for the other three enzymes. In restored peatlands, the activities of all detected soil enzymes decreased along with decreasing water level from HR to HLR to LR.

The PCoA showed that soil enzyme activities were significantly different across different peatlands. The first and second axes explained 82.96 and 6.68% of the total variation, respectively (Figure 3). All the points of NP and HR were present in the left quadrant, while all the points representing HLR, LR, and DP treatments were present in the right quadrant (Figure 3). In addition, the HR points were close to NP points, while most HLR and LR points were close to DP points.

Soil bacterial community structure among different peatlands

In total, 5,540 bacterial OTUs with 7,74,127 sequences in all samples were obtained. These 5,540 OTUs were classified into 854 genera within 52 phyla. The largest phylum was Proteobacteria, followed by Chloroflexi, and Acidobacteria. The relative abundance of different bacterial groups changed markedly in different peatlands (Figure 4). NP was characterized by the highest relative

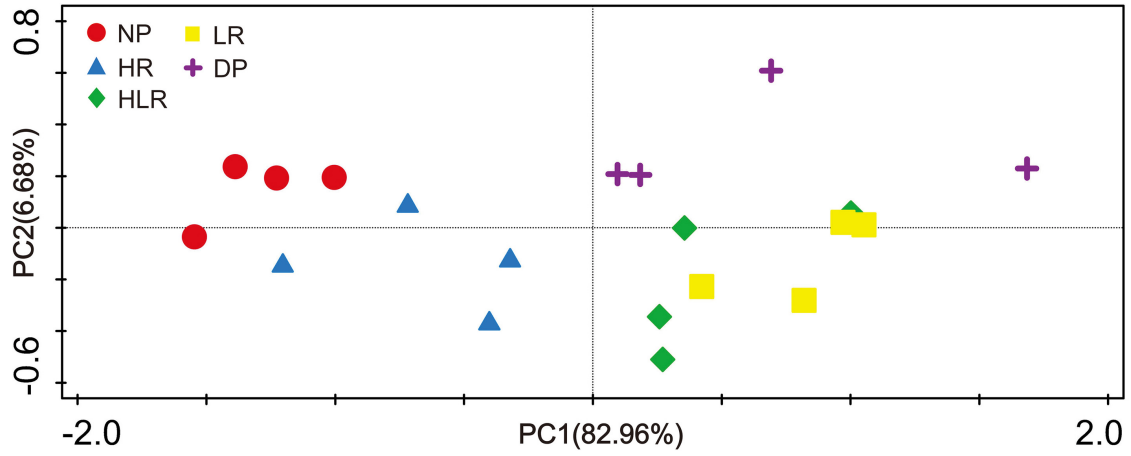

FIGURE 3
Principal coordinate analysis of soil enzyme activities across different peatlands. NP, natural peatland; HR, peatland restored under high water level; HLR, peatland restored under an alternating high-low water level; LR, peatland restored under low water level; DP, degraded peatland.

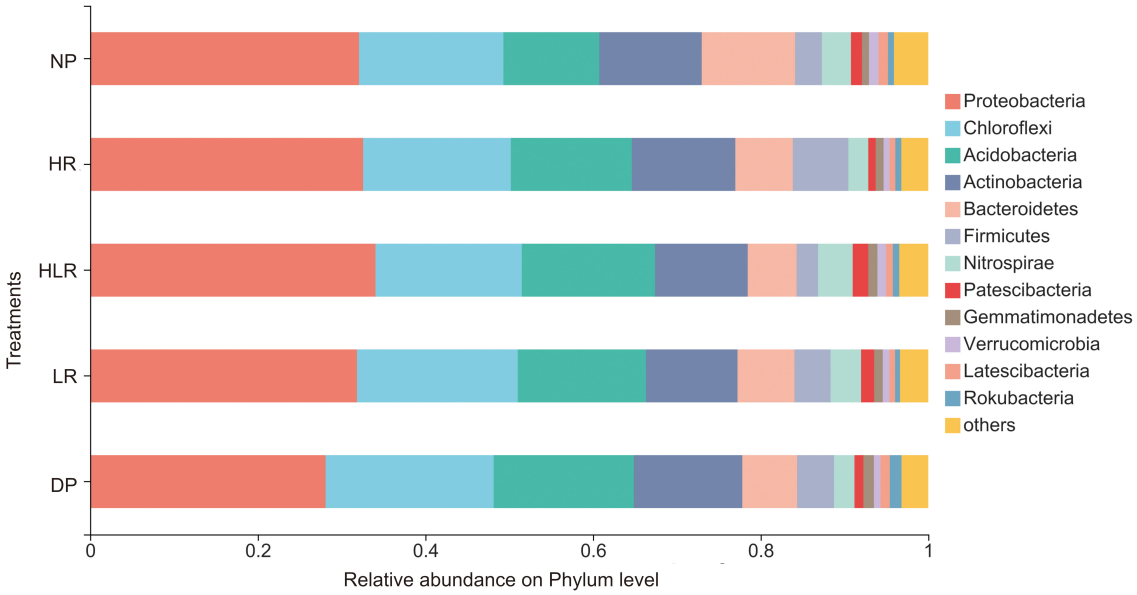

FIGURE 4
Relative abundance of different bacterial phyla under different peatlands. Only the top 12 phyla were displayed. NP, natural peatland; HR, peatland restored under high water level; HLR, peatland restored under an alternating high-low water level; LR, peatland restored under low water level; DP, degraded peatland.

abundance of Bacteroidetes and the lowest relative abundance of Acidobacteria. HR was characterized by a higher relative abundance of Firmicutes, while HLR had a higher relative abundance of Nitrospiraeand and Patescibacteria.

The results of microbial alpha-diversity analysis indicated that HR is beneficial to the increase of bacterial diversity and richness (Figure 5). The Shannon index of HR was higher than NP and HLR ($p < 0.05$), and the ACE index of HR was higher than NP and LR ($p < 0.05$). The NP had the lowest Shannon and ACE indices among all treatments, with its ACE index lower than all other treatments, including DP ($p < 0.05$).

The PCoA showed that bacterial community structure was significantly different across different peatlands. The first and second axes explained 31.85 and 15.74% of the total variation, respectively (Figure 6). Points representing bacterial communities of NP showed a clear separation from the points of the restored and degraded peatlands, indicating different community structures between natural peatland and the other treatments. Furthermore, the bacterial communities of restored

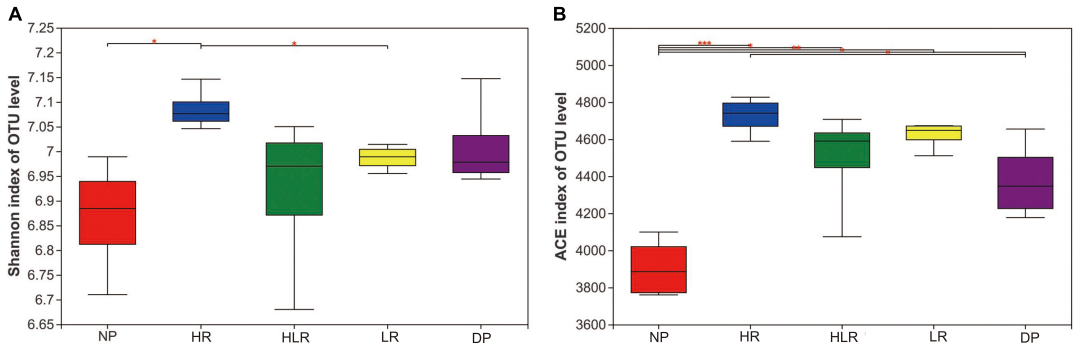

FIGURE 5
Shannon **(A)** and ACE **(B)** indices of soil bacteria from different peatlands. *, **, and *** mean $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, based on one-way ANOVA and LSD test. NP, natural peatland; HR, peatland restored under high water level; HLR, peatland restored under an alternating high-low water level; LR, peatland restored under low water level; DP, degraded peatland.

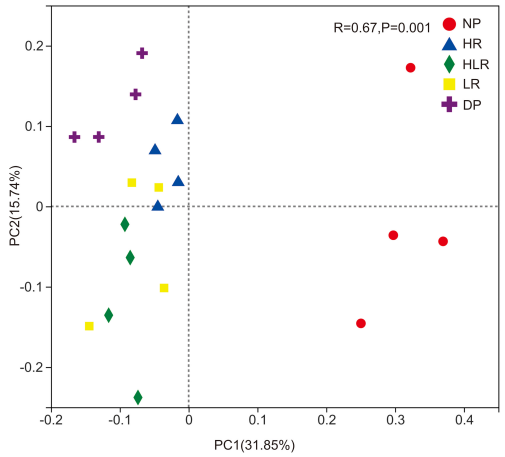

FIGURE 6
Principal coordinate analysis of the Bray-Curtis dissimilarity matrices of bacterial communities across different peatlands. The R and *p* were the statistical significance in bacterial community structure among different peatlands assessing by the ANOSIM test. NP, natural peatland; HR, peatland restored under high water level; HLR, peatland restored under an alternating high-low water level; LR, peatland restored under low water level; DP, degraded peatland.

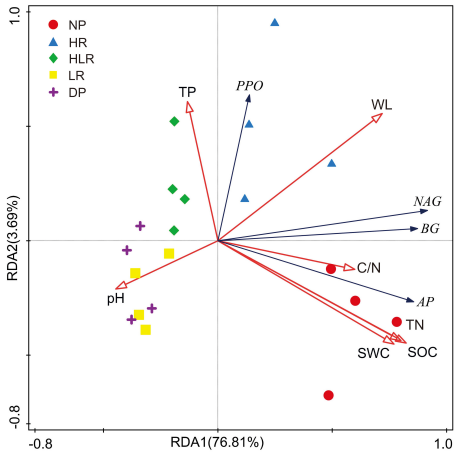

FIGURE 7
Redundancy analysis of soil enzyme activities across different peatlands using environmental variables as explanatory variables. βG, β-1, 4-glucosidase; NAG, β-1, 4-n-acetylglucosidase; AP, acid phosphatase; PPO, polyphenol oxidase; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; WL, water level; SWC, soil water content; C/N, the ratio of SOC to TN; NP, natural peatland; HR, peatland restored under high water level; HLR, peatland restored under an alternating high-low water level; LR, peatland restored under low water level; DP, degraded peatland.

peatlands were different from those of degraded peatlands, with their points showing a clear separation. For the restored peatlands, bacterial community structures showed similarities among the different treatments, shown as points clustered with each other in the plot.

Effects of soil properties on enzyme activities and bacterial community

The RDA indicated that soil enzyme activities were significantly influenced by environmental variables, with the first two axes explaining 80.5% of the total variations among different peatlands (Figure 7). Among these environmental variables, SOC explained the highest proportion of the variations in soil enzyme activities (51.0%, $p = 0.002$), followed by water level (21.2%, $p = 0.002$) and pH (4.3%, $p = 0.044$). The other measured soil properties, including soil water content, total phosphorus, total nitrogen, and C/N, explained only a small portion of the variations in soil enzyme activities, less than 2% in total.

The RDA showed that 50.53% of the total variations in the bacterial community were explained by environmental variables

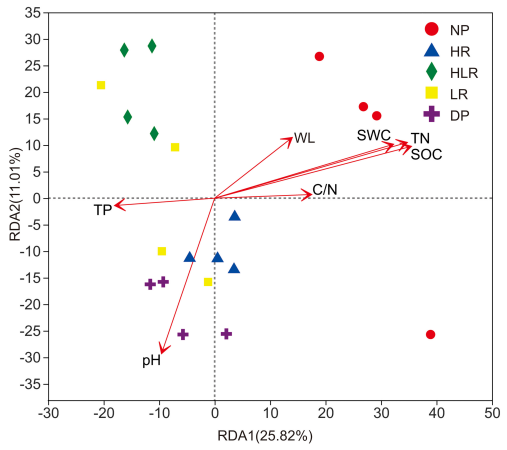

FIGURE 8
Redundancy analysis of the Bray-Curtis dissimilarity matrices of soil bacterial communities using environmental variables as explanatory variables. SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; WL, water level; SWC, soil water content; C/N, the ratio of SOC to TN; NP, natural peatland; HR, peatland restored under high water level; HLR, peatland restored under an alternating high-low water level; LR, peatland restored under low water level; DP, degraded peatland.

(**Figure 8**). Total nitrogen, SOC, soil water content, and pH were the most important factors affecting bacterial community. The bacterial community of DP was mainly affected by its higher pH, while the bacterial community of NP was mainly regulated by the higher SOC, total nitrogen, and soil water content.

Discussion

Responses of soil enzyme activities to different hydrological management practices

Soil enzyme activities play a key role in organic matter decomposition (Veres et al., 2015; Soares and Rousk, 2019). The detected soil enzyme activities of natural, restored, and degraded peatlands differed significantly (**Figure 2**), indicating that they were significantly affected by hydrological management practices during restoration. The hydrolysis enzyme activities (βG, NAG, and AP) in degraded peatland were significantly lower than those in natural peatland, this may be due to the sharp decrease in soil organic matter after peat reclamation. Previous studies also reported lower soil enzyme activities in agricultural or farm abandoned peatland than those in natural peatland (Wang et al., 2021). Peat cultivation can lead to rapid organic matter decomposition, and the nutrients released change to mobile forms that can be easily leached (Glina et al., 2016). These changes in degraded peatland result in a reduced carbon source, thus limiting soil enzyme activity (Hallema et al., 2015). However, the activity of polyphenol oxidase in NP was lower than other sites. This may be due to the high soil water content and poor soil air permeability in the natural peatland (**Table 2**), which is unfavorable to the survival and reproduction of aerobic microorganisms. As proposed by enzyme latch theory, under water logging conditions, a lack of oxygen can limit the activity of polyphenol oxidase (Freeman et al., 2001, 2004, 2012).

The soil enzyme activities of restored peatlands with high water level were higher than those of degraded peatland. Moreover, the soil enzyme activities in restored peatlands increased with water level, with enzyme activities being highest in HR and lowest in LR (**Figure 2**). Interestingly, the variations in enzyme activities in restored peatlands, unlike the condition in natural peatland, did not support the enzyme latch theory. A possible explanation is that flooding can hinder the decomposition of polyphenols in anoxic peat but not other organic matter (McGivern et al., 2021). For restored peatlands, after long-term rice cultivation and farm abandonment, the surface peat layer was completely destroyed, thoroughly changing the vegetation of restored peatlands. Therefore, most soil nutrients in restored peatlands were obtained from plants that were not typical peatland plants before peatland restoration, these nutrients were not hindered by the flooding environments. Instead, the higher soil enzyme activities in HR may benefit the accretion of organic matter, which benefited from the increasing plant biomass. The peatland was restored with *C. schmidtii* plantation, and *C. schmidtii* can easily adapt to the flooding environment with dense growth and multiply roots (Qi et al., 2021). In particular, the water depth of 11.2 cm, similar to the water level of HR in this study, promotes the rapid propagation of *C. schmidtii* and increases productivity to the maximum extent (Zhang et al., 2020). In addition, waterlogging in HR may have reduced the concentration of iron and decreased the protective effect on soil organic carbon (Wen et al., 2019). All these changes in plants and soil provided more available substrates for microorganisms and enhanced soil enzyme activities. The variations in soil properties also support this explanation.

Responses of soil microbial communities to different hydrological management practices

The soil microbial community structure is a good indicator of changes in soil quality (Balser and Firestone, 2005). In this study, the bacterial community structure was significantly affected by different hydrological regimes during peatland restoration. Specifically, the bacterial community structure of HR was similar to that of NP, while LR was similar to DP. This was consistent with previous studies, which demonstrated that rewetting is an effective measure to restore peatland

(He et al., 2015; Emsens et al., 2020). Moreover, our study further illustrates that rewetting with a higher water level was more effective than with a relatively lower water level.

Soil microbial diversity and richness can serve as indicators of ecosystem stability (Chaer et al., 2009). Previous studies indicated that degradation of peatlands leads to a decrease in diversity and richness of soil microorganisms, restored wetlands also showed lower microbial diversity and richness than natural wetlands (Xu et al., 2017; Kitson and Bell, 2020). However, in this study, the lowest bacterial diversity and richness were observed in NP among all peatlands, while the highest was found in HR, these results were unlike the variations in bacterial community structure. The same phenomenon was also observed in other studies (Andersen et al., 2013). This may be because the special environments of natural peatland, specifically, higher cellulose fraction and anaerobic environments, may restrict some microbes such as Acidobacteria. In this study, the relative abundance of Acidobacteria in natural peatland was lower than in restored and degraded peatlands, which was in line with the previous study (Emsens et al., 2020). A reasonable explanation for this is that some bacterial groups were restricted in natural peatland because they could not decompose recalcitrant organic matter such as cellulose and lignin, only being able to obtain nutrient substrates from other biology such as fungi that can decompose these recalcitrant organic matter (Boer et al., 2005; Jiao et al., 2022). The higher hydrolase activities but lower oxidase activities in the natural peatland also support this explanation. This further reinforces that the enzyme latch theory is effective only in natural peatlands, not restored peatlands, giving insight into the accumulative process of organic matter.

On the other hand, the higher diversity and richness of bacteria in peatland restored under high water levels may benefit from the combination of fresh organic matter derived from *C. schmidtii*, vascular plants, and some shrubs in abandoned rice paddies, which are thought to create favorable conditions for the development of active microbial biomass (Andersen et al., 2013). The RDA results indicated that SOC and total nitrogen were the most influential factors affecting the bacterial community, further supporting that bacteria were restricted by nutrient availability in natural peatland. Taken together, the environmental variables in natural peatland derived a different bacterial community that promotes organic matter accumulation but does not necessarily have a higher diversity and richness.

Implication for peatland restoration

Against the background of growing agricultural needs, peatland drainage has become a common stress globally (Cris et al., 2014). Restoration is necessary to recover degraded peatlands (Kimmel and Mander, 2010; Lazcano et al., 2018). Generally, planting was conducted as the optimal strategy (Guo et al., 2016; Qi et al., 2021). However, we found no significant difference in soil enzyme activities and soil bacterial community structure between LR and DP. This result indicates that vegetation restoration alone, without recovery of the water regime, has difficulty restoring peatland ecosystem functions. In this study, among the three different hydrological management practices in the restored peatlands, the HR has the highest soil nutrients, soil enzyme activities, and soil microbial diversity and richness. The results of the current study along with previous studies indicate that rewetting with a relatively high water level can be an appropriate hydrological management measure to achieve carbon accumulation and biological activities during peatland restoration (Kimmel and Mander, 2010; Schulte et al., 2019; Ahmad et al., 2020). Our findings highlight hydrological management as an effective way to improve the soil nutrient cycling processes, promote the restoration of soil ecological function, and accelerate the restoration process of peatland. Our results also indicate that soil microbial properties are important biological indices that are sensitive to environmental changes and can be used as indicators to assess wetland restoration.

Conclusion

In this study, we evaluated the effects of short-term peatland restoration in both hydrological management and original vegetation plantations on soil biological processes to support ongoing restoration efforts for the Changbai Mountain and other degraded peatland regions. Our results show that peatland restoration with a high water level (between 5 and 10 cm) can better promote the recovery of soil nutrients, enzyme activities, and bacterial communities compared to other hydrological management practices. This study gives insight into the mechanism underlying biological process changes affected by the water regime during peatland restoration and provides management strategies for northern peatland restoration.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA861699.

Author contributions

MW and SW conceived and designed the experiments. CC and YW were responsible for the management of water level

in the field. MW, SX, and LL analyzed the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Jiuling Li,
The University of Queensland, Australia

REVIEWED BY
Vineet Kumar,
National Environmental Engineering Research Institute (CSIR), India
Zhiyuan Yao,
Zhejiang University, China

*CORRESPONDENCE
Xin Leng
lengx@nju.edu.cn
Shuqing An
anshq@nju.edu.cn
Lu Xia
lulu8668@yeah.net

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Comparison and interpretation of freshwater bacterial structure and interactions with organic to nutrient imbalances in restored wetlands

Fuchao Zheng[1,2], Tiange Zhang[1,2], Shenglai Yin[3], Ge Qin[1], Jun Chen[1], Jinghua Zhang[1], Dehua Zhao[1], Xin Leng[1]*, Shuqing An[1,2]* and Lu Xia[1]*

[1]School of Life Sciences, Institute of Wetland Ecology, Nanjing University, Nanjing, Jiangsu, China, [2]Nanjing University Ecology Research Institute of Changshu, Changshu, Jiangsu, China, [3]College of Life Sciences, Nanjing Normal University, Nanjing, Jiangsu, China

Chemical oxygen demand to nitrogen (COD/N) and nitrogen to phosphorus (N/P) ratios have distinct effects on bacterial community structure and interactions. However, how organic to nutrient imbalances affect the structure of freshwater bacterial assemblages in restored wetlands remains poorly understood. Here, the composition and dominant taxa of bacterial assemblages in four wetlands [low COD/N and high N/P (LH), low COD/N and low N/P (LL), high COD/N and high N/P (HH), and high COD/N and low N/P (HL)] were investigated. A total of 7,709 operational taxonomic units were identified by high throughput sequencing, and Actinobacteria, Proteobacteria, and Cyanobacteria were the most abundant phyla in the restored wetlands. High COD/N significantly increased bacterial diversity and was negatively correlated with N/P (R^2 = 0.128; p = 0.039), and the observed richness (Sobs) indices ranged from 860.77 to 1314.66. The corresponding Chao1 and phylogenetic diversity (PD) values ranged from 1533.42 to 2524.56 and 127.95 to 184.63. Bacterial beta diversity was negatively related to COD/N (R^2 = 0.258; $p < 0.001$). The distribution of bacterial assemblages was mostly driven by variations in ammonia nitrogen (NH_4^+-N, $p < 0.01$) and electrical conductivity (EC, $p < 0.01$), which collectively explained more than 80% of the variation in bacterial assemblages. However, the dominant taxa Proteobacteria, Firmicutes, Cyanobacteria, Bacteroidetes, Verrucomicrobia, Planctomycetes, Chloroflexi, and Deinococcus-Thermus were obviously affected by variation in COD/N and N/P ($p < 0.05$). The highest node and edge numbers and average degree were observed in the LH group. The co-occurrence networkindicated that LH promoted bacterial network compactness and

bacterial interaction consolidation. The relationships between organic to nutrient imbalances and bacterial assemblages may provide a theoretical basis for the empirical management of wetland ecosystems.

KEYWORDS

bacterial assemblages, co-occurrence network, nutrient imbalance, organic matter, restored wetlands

Introduction

Great efforts have long been made to conserve, restore, and construct wetlands because of the important functions and services provided by these ecosystems, including water purification, environmental improvement, and biodiversity maintenance (Rebelo et al., 2018; Sampaio et al., 2018; Chen et al., 2019). Previous studies mainly focused on meeting water index standards based on wetland restoration technology and decreasing the water nutritional index, which is closely related to the interactions between microbes in the aquatic environment (Cardinale, 2011; Brisson et al., 2020; Garibay et al., 2021). The anaerobic bacteria in vertical-flow constructed wetlands usually contribute to organic matter degradation, in turn efficiently decreasing the chemical oxygen demand (COD) (Chang et al., 2015). Proteobacteria play a dominant role in artificial–natural coupled wetlands and significantly affect nitrogen removal due to their metabolic versatility (Zhang et al., 2021). Phosphorus (P)-accumulating organisms take up orthophosphate (PO_4^{3-}) from wastewater under aerobic conditions and then hydrolyze the stored poly-P for survival under anaerobic conditions, which can effectively reduce P pollution (Salehi et al., 2019). However, less attention has been given to the relationship between organic to nutrient imbalances and bacterial assemblages in freshwater environments since water quality standards are met.

In aquatic environments, microbial community patterns are dominantly influenced by several environmental factors and nutritional conditions (Baxter et al., 2012; Wu et al., 2016; Hou et al., 2017) that may themselves be influenced by deterministic processes involving non-random and niche-based mechanisms (Vellend, 2010). For example, environmental filtering by factors such as pH, dissolved oxygen (DO) and salinity greatly impacts the structure of bacterial assemblages (Hou et al., 2017; Shang et al., 2022). Interspecific interaction, *e.g.*, mutualism, competition, and predation, may also shape the patterns of bacterial communities in freshwater lakes (Sadeghi et al., 2021). However, a past study indicated that microbial diversity can be promoted by an increase in nutrition levels under moderate eutrophication (Duarte et al., 2009). Generally, the factors that affect the dynamic variations of microbial communities are the nutritional preferences and metabolic differences of the microorganisms, which are affected by organic matter, nitrogen (N), P and N/P ratios in the environment (Lyu et al., 2017; Aanderud et al., 2018; Chen et al., 2021). Notably, nutrient supply ratios play a vital role in microbial growth and cultivation because specific nutrient balances are needed for microbial growth, and limitation of organic matter, N or P may restrict microbial growth and metabolism (Elser et al., 2007; Jarvie et al., 2018). Lai et al. (2020) found that a high COD/N ratio increased microbial diversity by providing a richer carbon source, and the dominant taxa varied depending on the COD/N ratio. For instance, COD/N ratios of 6 and 12 were beneficial to Actinobacteria, Firmicutes, and Chloroflexi, which mainly participate in the process of denitrification (Lai et al., 2020). Lipizer et al. (2011) revealed that seasonal variations in N/P ratios dramatically affected microbial activities and phytoplankton blooms in the Gulf of Trieste.

Moreover, the interactions of microorganisms, which are greatly affected by the ratios between nutrients, are gradually being highlighted in the literature (Aanderud et al., 2018; Jabir et al., 2020). In some instances, low N/P efficiently decreased the negative interactions among bacterial taxa under P addition, and simultaneously improving C, N, and P fertilizer application decreased bacterial connections, which was attributed to the alleviation of bacterial competition for nitrients (Wei et al., 2020). The bacterial taxa in a lower-C/P environment were more diffuse and less connected due to affluent P (Aanderud et al., 2018). A COD/N ratio greater than 2 was conducive to the cultivation of heterotrophic aerobic bacteria and nitrite-oxidizing bacteria and enhanced the relationship between anammox bacteria and heterotrophic bacteria (Wang et al., 2018). However, little is known about the effects of COD/N and N/P on freshwater bacterial diversity, structure and interactions, especially in freshwater environments with low concentrations of COD, total N (TN), and total P (TP).

In this study, we compared the responses of variation in freshwater bacterial assemblages to different COD/N and N/P ratios in restored wetlands, with the following objectives: (1) to evaluate the effects of COD/N and N/P on bacterial diversity and community differentiation, (2) to analyze the effects of COD/N and N/P on bacterial community composition and dominant taxa, and (3) to depict the effects of COD/N and N/P on bacterial interactions.

Materials and methods

Sampling locations

We selected four wetlands that have been restored for more than a decade, and are barely affected by human activities. All the wetlands are located in Lake Taihu Basin, Eastern China. A total of 34 sampling sites were established in the four wetlands in August 2019. Nine sites were sampled each in the Shanghu Wetland (120.6853° E, 31.6537° N), Shajiabang Wetland (120.8021° E, 31.5548° N), and Nanhu Wetland (120.6307° E, 31.5927° N), and seven sites were sampled in the Taihu Wetland (120.3596° E, 31.3238° N) (**Figure 1A**). Three replicate samples were mixed at each site and a total of 34 freshwater samples (0–10 cm depth) were collected with plastic bottles [polyethylene (PE), volume 2 L, height 22 cm]. We analyzed the COD/N and N/P ratios of freshwater and designated the four wetlands as having low COD/N and high N/P (LH), low COD/N and low N/P (LL), high COD/N and highN/P (HH), or High COD/N and low N/P (HL), with LH, LL, HH, and HL corresponding to the Shanghu, Shajiabang, Taihu and Nanhu wetlands, respectively (**Figures 1B,C**).

Environmental parameter measurements

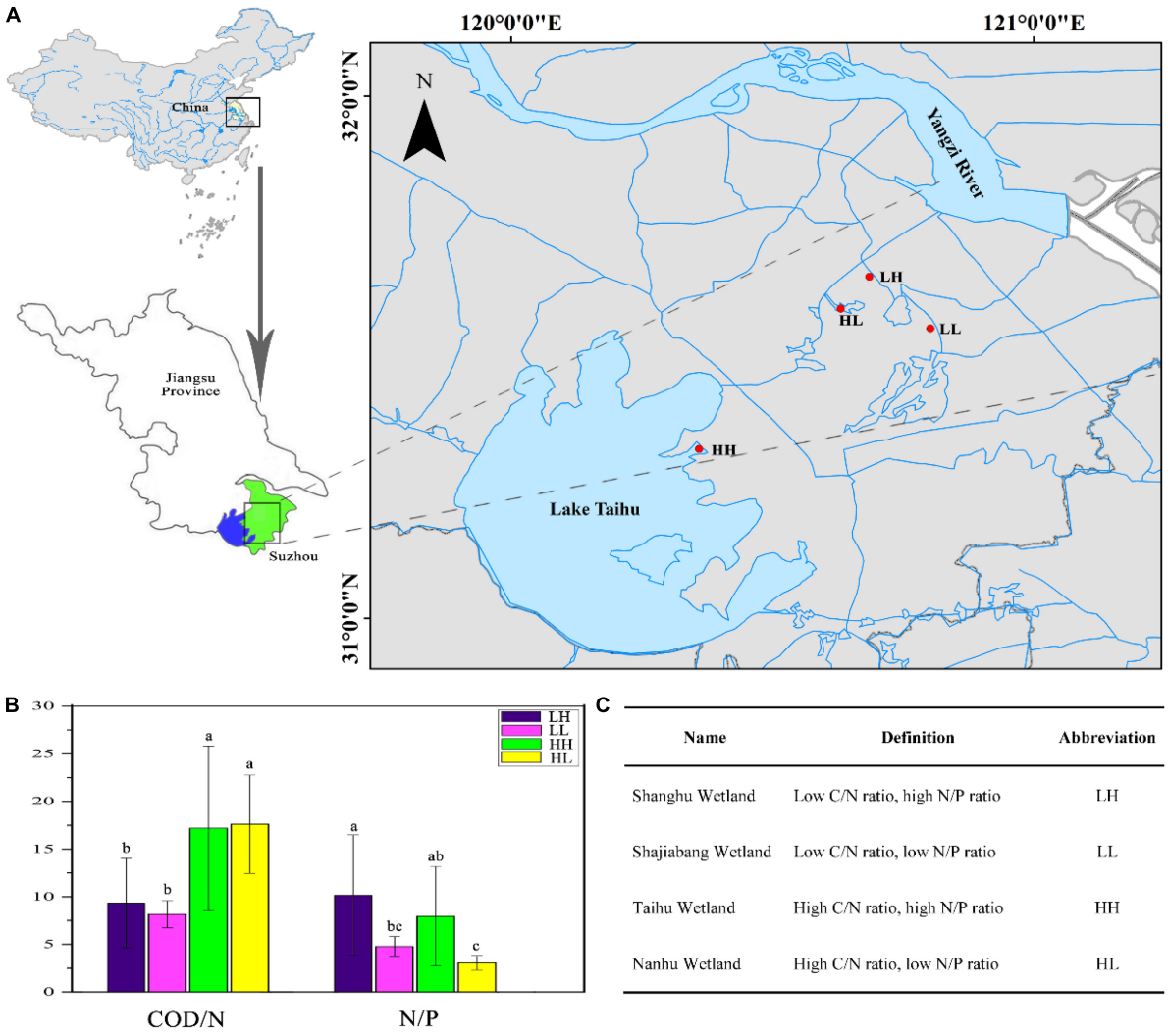
The physical parameters of the surface freshwater were measured *in situ* using a multiprobe instrument (HQd Portable Meter, Edition 6, HACH, USA) before sample collection. The measured parameters included pH, electrical conductivity (EC), and DO. Moreover, TN, TP, COD, nitrate nitrogen (NO_3^--N) and ammonia nitrogen (NH_4^+-N) were measured by a water quality analysis system (DRB200 and DR3900, HACH, USA). The biochemical oxygen demand (BOD_5) and chlorophyll-a (Chla) concentration were determined with the HJ505-2009 and HJ897-2017 methods (Ministry of Ecology and Environment of the People's Republic of China, 2009, 2017), respectively. The freshwater samples were passed through microporous membranes with a pore size of 0.22°μm and a diameter of 50 mm (Millipore, USA), and microbiological analysis was then conducted on microorganisms attached to the membranes. The microbial filter membrane samples were stored at −80°C until DNA extraction.

Microorganism measurement and analysis

Microbial community genomic DNA was extracted from freshwater samples using the FastDNA® SPIN Kit (Omega Biotek, Norcross, GA, USA) following the manufacturer's instructions. The DNA extract was checked on a 1.0% agarose gel, and DNA concentration and purity were determined with a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The hypervariable V3–V4 region of the bacterial 16S rDNA gene was amplified with the primer pair 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). PCR amplification of the 16S rDNA gene was performed as follows: initial denaturation at 95°C for 3 min; followed by 27 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 45 s; a single extension at 72°C for 10 min; and a final extension at 4°C. The PCR mixtures contained 5 × TransStart FastPfu buffer 4°μL, 2.5 mM dNTPs 2°μL, forward primer (5°μM) 0.8°μL, reverse primer (5°μM) 0.8°μL, TransStart FastPfu DNA Polymerase 0.4°μL, template DNA 10°ng, and enough ddH_2O to reach a total volume of 20°μL. PCRs were performed in triplicate. PCR products were extracted from a 2.0% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using a Quantus Fluorometer (Promega, USA). The library was constructed using the NEXTFLEX Rapid DNA-Seq Kit, the Illumina MiSeq PE300 platform was used for sequencing, and bacterial DNA fragments of freshwater samples were obtained. The raw 16S rDNA gene sequencing reads were demultiplexed, quality-filtered using Trimmomatic and merged with FLASH 1.2.11 (Center for Computational Biology, Baltimore, MD, USA) (Magoc and Salzberg, 2011). Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE v.7.1 (Edgar, 2013), and chimeric sequences were identified and removed. The taxonomy of each representative OTU sequence was assigned by RDP Classifier v.7.1 (Wang et al., 2007) against the 16S rRNA database v.132 by using a confidence threshold of 0.7 (Quast et al., 2013).

Data analysis

One-way analysis of variance (ANOVA) was used to test for differences in the environmental parameters between groups. We used the observed richness (Sobs), Shannon, Chao1, and whole-tree phylogenetic diversity (PD) indices to analyze bacterial diversity, which were calculated with QIIME (Version 1.7.0, Mothur v.1.30.2) (Schloss et al., 2009) and tested for differences with ANOVA. Linear regression analysis was performed to test for relationships of alpha diversity with COD/N and N/P. Pearson correlation analysis was performed between the environmental parameters and diversity indices. The Bray–Curtis distance matrices of bacterial genera were visualized using unconstrained principal coordinate analysis (PCoA), and the correlations of environmental parameters were analyzed with redundancy analysis (RDA) in CANOCO 5.0 (Wageningen University and Research, Wagenin–gen, Netherlands). Rank regression analysis based on PCoA was used



Name	Definition	Abbreviation
Shanghu Wetland	Low C/N ratio, high N/P ratio	LH
Shajiabang Wetland	Low C/N ratio, low N/P ratio	LL
Taihu Wetland	High C/N ratio, high N/P ratio	HH
Nanhu Wetland	High C/N ratio, low N/P ratio	HL

FIGURE 1
(A) Sampling locations in the four wetlands. LH: Shanghu Wetland; LL: Shajiabang Wetland; HH: Taihu Wetland; and HL: Nanhu Wetland. **(B,C)** One-way analysis of variance (ANOVA) of Chemical oxygen demand to nitrogen (COD/N) and nitrogen to phosphorus (N/P) in the four wetlands. Different superscripted lowercase letters indicate $p < 0.05$.

to test for correlations between environmental factors and beta diversity (Ren et al., 2022). Permutational multivariate ANOVA (Adonis test) based on Bray–Curtis distances was performed between the groups (Hartman et al., 2018). Community composition analysis was carried out with the "*vegan*" package (R Studio Inc., Massachusetts, USA). Random forest (RF) analysis was used to identify the important bacterial taxa that responded to organic and nutrient ratios (Ren et al., 2022). We identified the 10 most abundant phyla and classes in the bacterial assemblages which were analyzed by ANOVA. Duncan's test was performed to determine the statistical significance of differences ($p < 0.05$) in bacterial abundance among the dominant in the four groups. Pearson correlation analysis was used to test for correlations between environmental parameters and the 10 most abundant phyla and classes. All statistical tests were performed with the statistical program SPSS 25.0 (SPSS Inc., Chicago, IL, USA).

To analyze the effects of freshwater COD/N and N/P on the bacterial interactions, we calculated the spearman correlation coefficients between bacterial genera using the corr.test function and "*psych*" (Brisson et al., 2019). All bacterial sequencing data analyses were performed in R v3.6.1. Spearman correlations with a magnitude $r > 0.6$ or $r < -0.6$ and statistically significant at $p < 0.05$ were included. To analyze the relationships of highly abundant taxa, we adjusted the filter threshold to identify abundant taxa with an overall frequency greater than 0.5% among all the samples and used them to construct a bacterial co-occurrence network (Chen and Wen, 2021). A set of network topological properties (e.g., nodes, edges, degree, diameter, density modularity coefficient, and average

cluster coefficient) were calculated. Bacterial genera with the highest standardized scores for high degree, high closeness centrality, high transitivity, and low betweenness centrality were statistically identified as the keystone taxa (Berry and Widder, 2014). Networks were visualized using the interactive platform Gephi (Bastian et al., 2009).

Results

Alpha diversity

The results showed that 90% of the physicochemical parameters significantly differed among the groups ($p < 0.05$, Table 1). For example, the COD concentrations ranged from 8.33 to 22.14 mg/L, and the highest value appeared in the HH group. The NH_4^+-N and NO_3^--N concentrations ranged from 0.015 to 0.187 mg/L and 0.385 to 0.600 mg/L, respectively, and both were highest in the LL group. The values of TP ranged from 0.138 to 0.348 mg/L, and the highest value appeared in the HL group (Table 1).

A total of 1,719,396 16S rDNA sequences were selected for classification from freshwater, and 7,709 OTUs were obtained. The most common sequence length was approximately 414 bp. The alpha diversity indices showed significant differences among groups ($p < 0.05$, Table 2). Specifically, the Shannon indices ranged from 4.16 to 4.55, and that of the LL group was the lowest. The Sobs, Chao1, and PD values ranged from 860.77 to 1314.66, 1533.42 to 2524.56, and 127.95 to 184.63, respectively, and were markedly higher in the HL group than in the LL group ($p < 0.01$, Table 2).

The alpha diversity was closely correlated with COD/N ($R^2 = 0.097$; $p = 0.072$) and N/P ($R^2 = 0.128$; $p = 0.039$) (Figure 2). Pearson's correlation analysis performed on the alpha diversity indices and several physicochemical parameters revealed that TP was significantly correlated with Sobs ($r = 0.352$; $p < 0.05$), Chao1 ($r = 0.501$; $p < 0.01$), and PD ($r = 0.384$; $p < 0.05$), and the N/P ratio was significantly correlated with the Sobs ($r = -0.342$; $p < 0.05$), Chao1 ($r = -0.358$; $p < 0.05$), and PD indices ($r = -0.342$; $p < 0.05$) (Appendix Supplementary Table 1).

TABLE 2 Observed bacterial community richness and diversity indices (mean ± SE, $n = 34$) for the freshwater of four wetlands.

	Sobs	Shannon	Chao1	PD
LH	1207.44 ± 171.13^{ab}	4.39 ± 0.18^{a}	2077.60 ± 301.22^{ab}	169.05 ± 20.00^{ab}
LL	860.77 ± 45.76^{b}	4.16 ± 0.11^{a}	1533.42 ± 126.75^{b}	127.95 ± 5.99^{b}
HH	1099.71 ± 63.23^{ab}	4.55 ± 0.21^{a}	2016.19 ± 137.15^{ab}	167.45 ± 8.32^{ab}
HL	1314.66 ± 111.60^{a}	4.50 ± 0.11^{a}	2524.56 ± 243.52^{a}	184.63 ± 13.13^{a}

Different lowercase letters indicate significant differences ($p < 0.05$) among the different sampling areas.

Beta diversity and environmental effects

In the PCoA, PC 1, and PC 2 explained 27. 52 and 21.33% of the variation, respectively, and the two axes together explained 48.85%. The two axes divided the 34 sample locations into four clusters. The LH and LL groups were separated from the HH and HL groups, and the three locations of the LH group overlapped with those of the LL group (Figure 3A). Thus, the bacterial assemblages in the LH and LL groups differed from those in the HH and HL groups. The Adonis test showed that the bacterial assemblages were significantly different between all pairs of groups (Supplementary Table 2; $p < 0.01$). The RDA1 and RDA2 axes explained 69.31 and 15.82%, respectively, of the variation in the physicochemical parameters, and in the RDA of dominant bacterial phyla and classes, they explained 60.67 and 20.41%, respectively, collectively explaining more than 81.08%

TABLE 1 Physicochemical properties (mean ± SE, $n = 34$) of freshwater in the four wetlands in Jiangsu, China.

	pH	EC (μ s·cm^{-1})	DO (mg·L^{-1})	COD (mg·L^{-1})	NH_4^+-N (mg·L^{-1})
LH	9.274 ± 0.169^{a}	349.888 ± 9.943^{b}	10.027 ± 0.635^{a}	8.333 ± 1.054^{c}	0.042 ± 0.008^{b}
LL	8.384 ± 0.084^{c}	415.555 ± 5.527^{a}	4.464 ± 0.529^{b}	9.444 ± 1.094^{c}	0.187 ± 0.045^{a}
HH	9.071 ± 0.142^{ab}	325.571 ± 3.637^{c}	9.620 ± 0.422^{a}	22.142 ± 3.269^{a}	0.160 ± 0.024^{a}
HL	8.738 ± 0.022^{b}	359.000 ± 3.184^{b}	5.777 ± 0.481^{b}	16.444 ± 1.434^{b}	0.015 ± 0.006^{b}
	NO_3^--N (mg·L^{-1})	TN (mg·L^{-1})	TP (mg·L^{-1})	BOD_5 (mg·L^{-1})	Chla (μg·L^{-1})
LH	0.422 ± 0.027^{b}	1.066 ± 0.166^{a}	0.138 ± 0.025^{b}	5.222 ± 0.296^{c}	4.519 ± 0.758^{c}
LL	0.600 ± 0.076^{a}	1.166 ± 0.132^{a}	0.243 ± 0.017^{ab}	7.488 ± 0.312^{b}	5.919 ± 0.445^{c}
HH	0.385 ± 0.014^{b}	1.585 ± 0.478^{a}	0.235 ± 0.054^{ab}	9.242 ± 0.470^{a}	14.493 ± 2.997^{b}
HL	0.477 ± 0.052^{ab}	0.966 ± 0.072^{a}	0.348 ± 0.054^{a}	4.355 ± 0.232^{c}	20.559 ± 1.739^{a}

Different lowercase letters indicate significant differences ($p < 0.05$) among the different sampling areas; LH: Shanghu Wetland; LL: ShajiabangWetland; HH: Taihu Wetland; and HL: Nanhu Wetland. The same applies below.

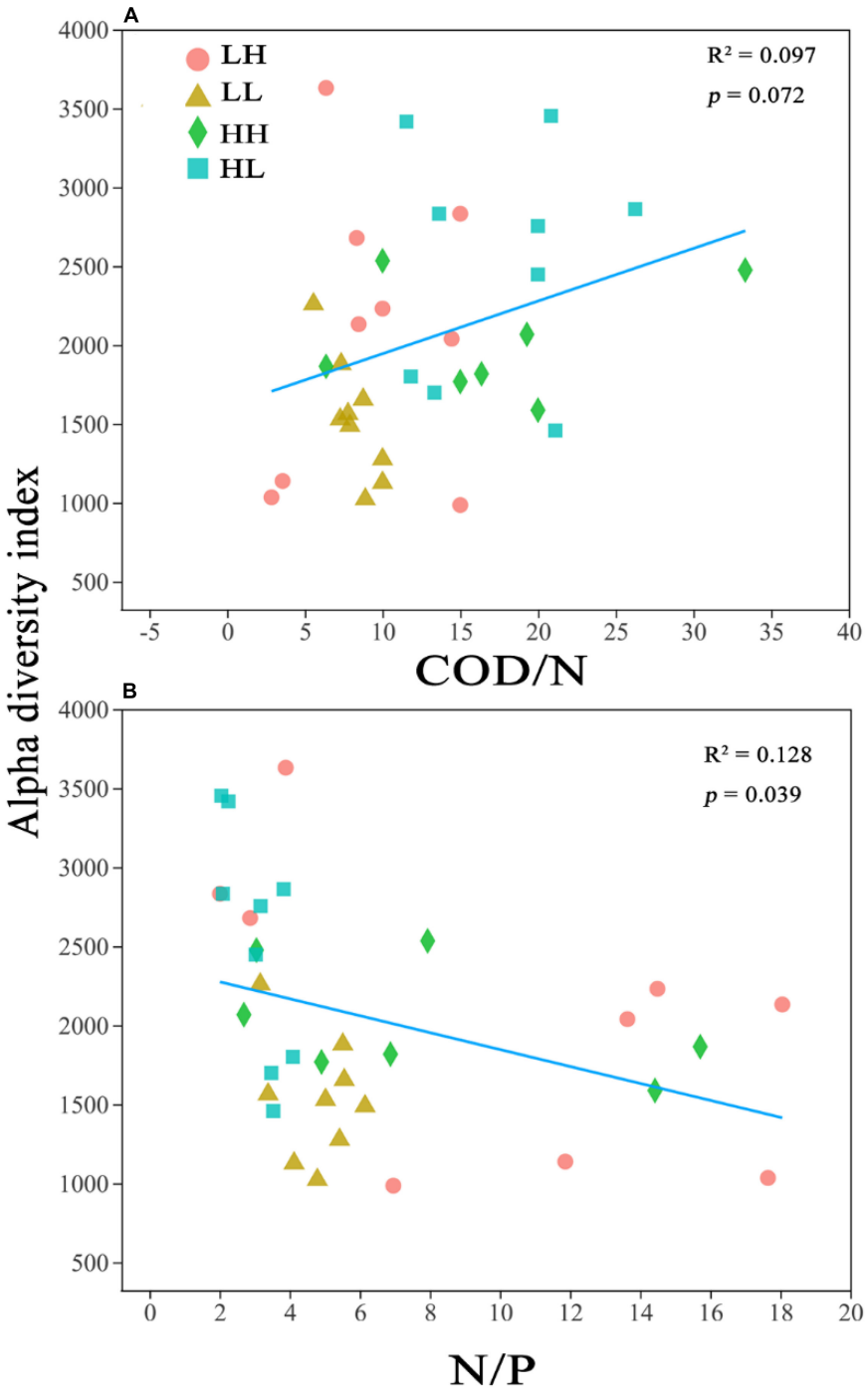


FIGURE 2
Relationship between bacterial alpha diversity (Chao1) and **(A)** Chemical oxygen demand to nitrogen (COD/N) and **(B)** nitrogen to phosphorus (N/P). R^2 represents the correlation coefficient, $p < 0.05$ indicates significance and $p > 0.05$ indicates non-significance.

of the variation in bacterial assemblages. In addition, NH_4^+-N and EC had a significant influence on the distribution of bacterial phyla and classes, especially some typical bacterial taxa (Figures 3B,C). In addition, we analyzed the relationships between environmental factors and beta diversity, and the results indicated that COD and COD/N were negatively and significantly related to beta diversity (R^2 = 0.435 and 0.332, respectively; $p < 0.001$), while NH_4^+-N was positively connected with beta diversity (R^2 = 0.149 and p = 0.02) (Figures 4A,C,D). There was not a significant correlation

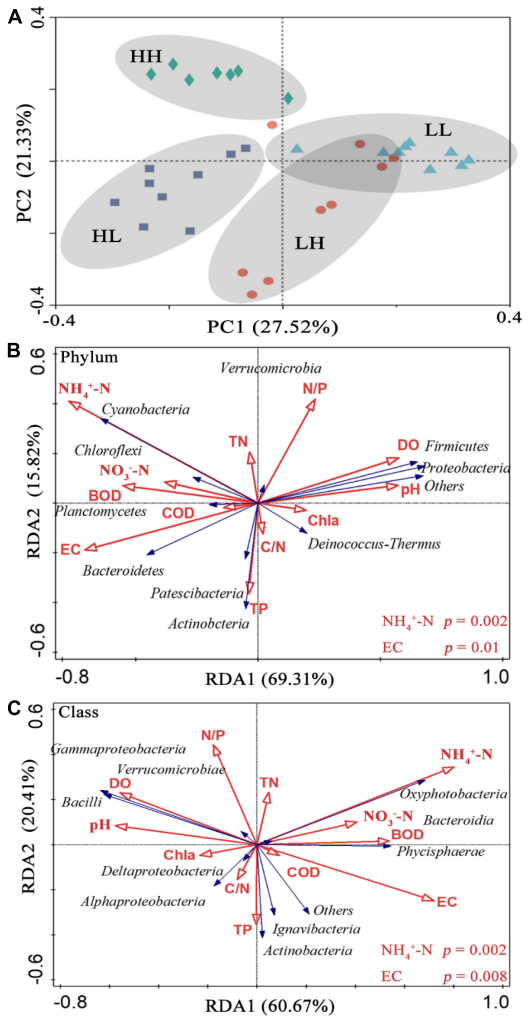


FIGURE 3
(A) Principal coordinate analysis (PCoA) of bacteria based on Bray–Curtis dissimilarity in freshwater of the four wetlands; redundancy analysis (RDA) diagram illustrating the relationships between the compositions of freshwater bacteria at the **(B)** phylum, and **(C)** class levels from different sampling sites with variable environments. Blue arrows show bacterial community composition; red arrows show physicochemical properties in the freshwater.

between N/P and beta diversity (R^2 = 0.016; p = 0.467), and TP presented a negative and significant relationship with beta diversity (R^2 = 0.136 and p = 0.02) (Figures 4B,E). Moreover, a positive and significant relationship was observed between EC and beta diversity (R^2 = 0.491; $p < 0.001$) (Figure 4F).

Taxonomy of and variation in bacterial assemblages

According to the taxonomic identification results, Actinobacteria, Proteobacteria, and Cyanobacteria were the most abundant phyla, and Oxyphotobacteria, Gammaproteobacteria, and Bacteroidia were the most abundant classes, as shown in Supplementary Figure 1. RF analysis indicated that Planctomycetes, Cyanobacteria, Actinobacteria, Bacteroidetes, and Proteobacteria were the most important phyla and that Planctomycetacia, Oxyphotobacteria, Actinobacteria, Bacteroidia, Gammaproteobacteria, and Alphaproteobacteria were the key bacterial classes that responded to the variation in COD/N and N/P (Supplementary Table 3). The proportions of both the dominant bacterial phyla and classes in freshwater varied among the groups. At the phylum level, the LH and HL groups showed significantly increased abundances of Proteobacteria and Firmicutes ($p < 0.01$), and the LL group showed large increases in the abundance of Cyanobacteria and Bacteroidetes ($p < 0.01$). The abundance of Verrucomicrobia, Planctomycetes, and Chloroflexi significantly increased in the HH group ($p < 0.01$), and the abundance of Deinococcus-Thermus significantly increased in the HL group ($p < 0.01$) (Figure 5A). At the class level, a markedly increased abundance of Oxyphotobacteria was observed in the LL group ($p < 0.01$), significantly improved richness of Gammaproteobacteria and Bacilli was also found in both the LH and HL groups ($p < 0.01$), and the HH group had significantly increased abundances of Verrucomicrobiae and Phycisphaerae ($p < 0.01$). However, the richness of Bacteroidia was decreased in the HL group ($p < 0.01$) (Figure 5B). According to the correlation analysis of the main physicochemical parameters and the 10 most abundant phyla and classes, Verrucomicrobia (r = 0.636; $p < 0.01$), Planctomycetes (r = 0.469; $p < 0.01$), Chloroflexi (r = 0.487; $p < 0.01$), Phycisphaerae (r = 0.556; $p < 0.01$), and Bacteroidetes (r = −0.345; $p < 0.05$) were significantly correlated with COD. In addition, the NH_4^+-N concentration was significantly associated with Cyanobacteria (r = 0.619; $p < 0.01$), Oxyphotobacteria (r = 0.623; $p < 0.01$), Bacteroidia (r = 0.479; $p < 0.01$) and Proteobacteria (r = −0.461; $p < 0.01$), and the NO_3^--N concentration was significantly related to Cyanobacteria (r = 0.384; $p < 0.05$) and Oxyphotobacteria (r = 0.386; $p < 0.05$). Furthermore, TN and COD/N were significantly correlated with Verrucomicrobiae (r = 0.359 and 0.415; $p < 0.05$), while COD/N was also related to Chloroflexi (r = 0.343; $p < 0.05$), Deinococcus-Thermus (r = 0.342; $p < 0.05$), and Phycisphaerae (r = 0.381; $p < 0.05$) (Figure 6).

Variation in the bacterial co-occurrence network

We measured the topological properties of the obtained networks to examine differences in bacterial taxon correlations among the groups (Figure 7). The largest numbers of nodes and edges were observed in the LH group, whereas the lowest numbers appeared in the HL group. The average

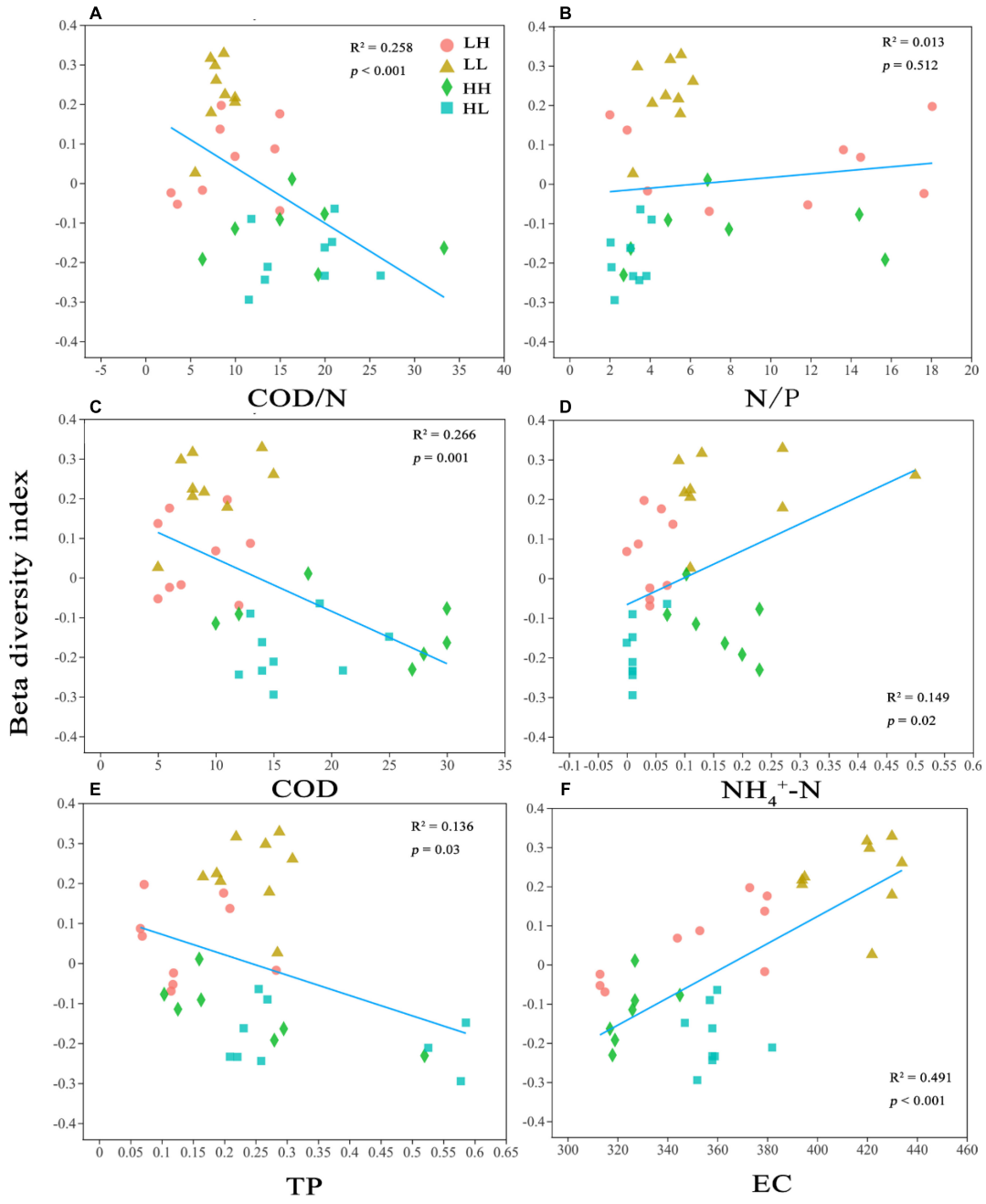


FIGURE 4
Relationships between bacterial beta diversity and **(A)** Chemical oxygen demand to nitrogen (COD/N), **(B)** nitrogen to phosphorus (N/P), **(C)** Chemical oxygen demand (COD), **(D)** ammonia nitrogen (NH_4^+-N), **(E)** total P (TP), and **(F)** electrical conductivity (EC). R^2 represents the correlation coefficient; $p < 0.05$ indicates significance and $p > 0.05$ indicates non-significance.

degree was 7.816, 4.061, 2.151, and 1.259 in the LH, LL, HH, and HL groups, respectively. Similarly, for the diameter of the networks, the highest value also appeared in the LH group, and the lowest value was observed in the HL group. Additionally, the peak network density was detected in the LH group, whereas the lowest value appeared in the HH group, and the highest modularity coefficient and average cluster coefficient were observed in the HH group, while the lowest values appeared in the LL and HL groups (Table 3). However, the keystone taxa obviously differed among these groups. For example, the predominant taxa of the LH group were *Paludibaculum*, WWE3, *Gemmatimonas*, Actinobacteria, Gemmataceae, *Silvanigrella*, Sporichthyaceae, and *Chryseomicrobium*; the major taxa of the LL group were

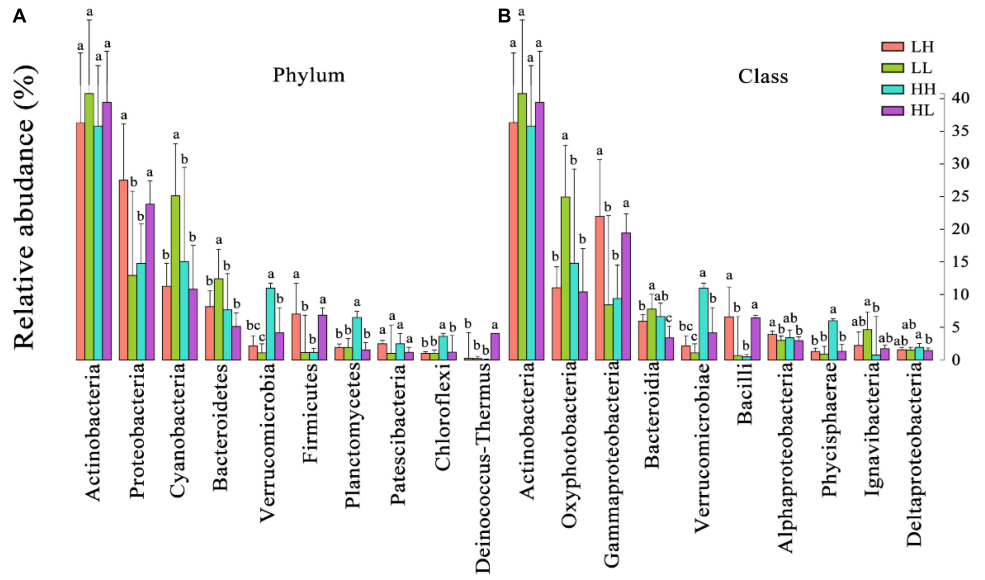


FIGURE 5
The relative abundances of the 10 most abundant bacteria at the **(A)** phylum and **(B)** class levels in freshwater of the four wetlands. Different superscripted lowercase letters indicate $p < 0.05$.

Mycobacterium, *Clostridium_sensu_stricto_1*, Pedosphaeraceae, *Roseomonas*, *Aurantimicrobium*, *29_marine_group* and Mitochondria; Rhodocyclaceae, PeM15, *Noviherbaspirillum*, Chloroplast, and *Terrimonas* were mainly present in the HH group; and Sphingomonadaceae, *Chryseobacterium*, Gammaproteobacteria, Subgroup_6, *Pseudorhodobacter*, *Rhodobacter*, Limnobacter, Brevundimonas, and OPB56 were dominant in the HL group, as shown in the **Supplementary Tables**.

Discussion

Bacterial assemblages are enriched when nutrients or organic matter is abundant, and COD/N is a crucial determinant of the bacterial diversity in freshwater (Han et al., 2016; Lai et al., 2020). With increasing nutrient levels, bacterial diversity can increase under moderate eutrophication and decrease under hypereutrophication (Duarte et al., 2009; Zhang et al., 2022). In this study, we found that a high COD/N (HH group) significantly increased bacterial diversity (**Table 2**). A negative and significant relationship was observed between N/P and alpha diversity (**Figure 2B**), and TP presented a positive and significant correlation with Sobs, Chao1 and PD indices (**Supplementary Table 1**), indicating that a higher N/P ratio of freshwater may have a negative effect on bacterial diversity. A higher N/P ratio may result in P limitation, which is not conducive to the growth of bacterial communities (Cui et al., 2018; Jarvie et al., 2018). Among the N sources, NO_3^--N showed the lowest concentration in the HH group, whereas the concentrations of both NH_4^+-N and TN were lowest in the HL group (**Table 1**). Consistent with our study, several previous studies also revealed that autotrophic nitrifying bacteria, Betaproteobacteria, and Gammaproteobacteria, contributed to N depletion, which promoted a high COD/N (Begum and Batista, 2013; Wang et al., 2019). In terms of nutritional balance, bacterial diversity usually increases when the COD/N increases under conditions of both sufficient N and hypereutrophication (Duarte et al., 2009; Jarvie et al., 2018). However, there were no significant relationships between COD or TN and bacterial diversity (**Supplementary Table 1**). Thus, the results confirmed that nutritional balance is more important than a single nutrient for the growth of bacterial assemblages, and a higher COD/N increases the diversity of bacterial assemblages in freshwater environments.

Our results revealed that variation in COD/N and N/P obviously impacted the structure of bacterial assemblages in the freshwater environments (**Figure 3A**), which was consistent with the findings of a previous report (Jabir et al., 2020). Although organic matter is usually regarded as an energy substance, N and P sources are frequently deficient in freshwater environments, which further restrict microbial growth (Elser et al., 2007; Jarvie et al., 2018). Thus, fluctuations in N and P are considered the major factors separating bacterial assemblages (**Figures 4D,E**). However, NH_4^+-N may be one of the most important factors regulating the distribution of bacterial assemblages (**Figures 3B,C**), which is consistent with the results of several previous studies (Baxter et al., 2012; Wu et al., 2016). First, the bacterial taxa prefer to use NH_4^+-N as the

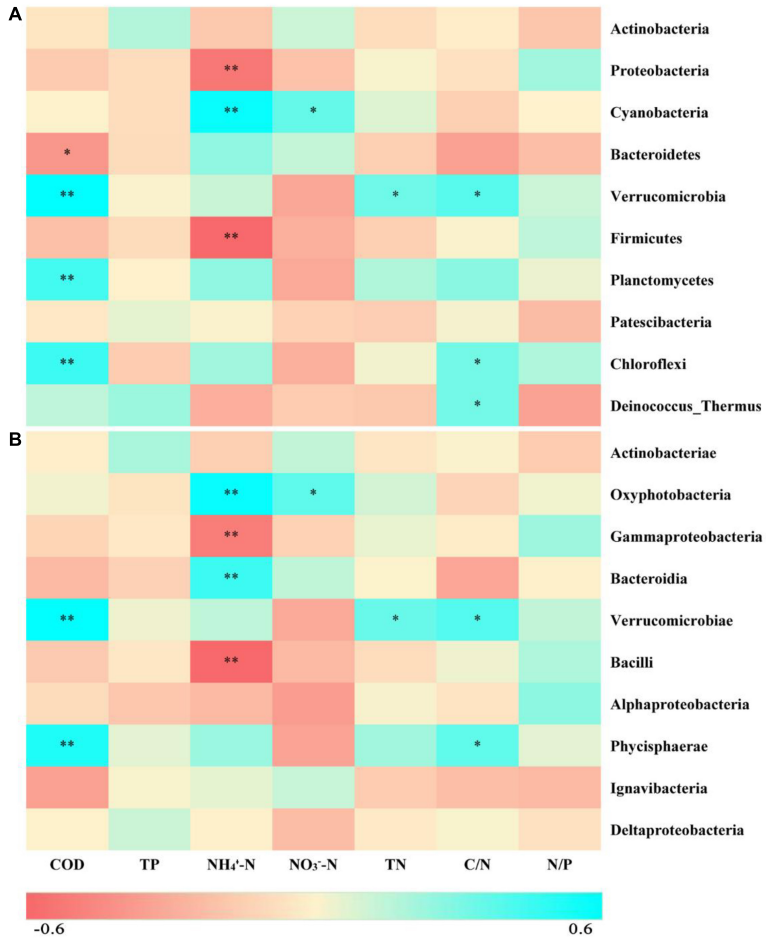


FIGURE 6
Heatmap depicting correlations between physicochemical properties of the freshwater and the 10 most abundant bacteria at the **(A)** phylum and **(B)** class levels. Red indicates negative correlations, while cyan indicates positive correlations. ** represents $p < 0.01$, and * represents $p < 0.05$.

electron donor for energy metabolism, which can significantly affect bacterial assemblage structure (Li et al., 2020). Second, NH_4^+-N can serve as the N source for ammonia-oxidizing bacteria or archaea through its oxidation to hydroxylamine and conversion to nitrite or nitrate due to diverse pathways derived from exogenous inputs or litter decomposition (Gusewell and Gessner, 2009; Kuypers et al., 2018; Yang et al., 2020). The results indicated that COD/N was negatively related to beta diversity (**Figure 4A**) and suggested that a higher COD/N ratio was favorable to bacterial differentiation mainly due to COD effects. The main reasons may be that higher organic matter availability facilitates bacterial reproduction, and bacteria with rapid propagation may lead to community differentiation (Blaz˘ina et al., 2009).

In addition, our results revealed that EC was a key factor explaining variation in the bacterial assemblages (**Figures 3B,C**), and a significant linear relationship was observed between EC and beta diversity (**Figure 2F**). These results indicated that EC played a major role in dividing bacterial communities. Korber et al. (1996) reported that high EC negatively influenced bacterial diversity, indicating the bactericidal effect of salinity. EC is related to salinity, which is signified by more extensive anions and cations in the freshwater environment and can limit ion uptake by microorganisms due to osmotic potential and ion competition (Mavi and Marschner, 2013; Ma et al., 2016). A past study showed that EC can potentially impact nutrient bioavailability by mediating alterations in bacterial assemblages (Hessini et al., 2019). Thus, our results showed that beta diversity increased with increasing EC (**Figure 4F**), indicating that the bacterial community is more stable in freshwater and that EC is a mechanism of positive selection on bacteria. In summary, the differences in bacterial assemblages were driven by organic to nutrient imbalances, COD/N, especially COD, presented negative selection on bacteria, and NH_4^+-N and EC also synergistically influenced the distributions of bacterial assemblages.

The structure and relative abundances of bacterial phyla and classes identified in the freshwater samples of different groups

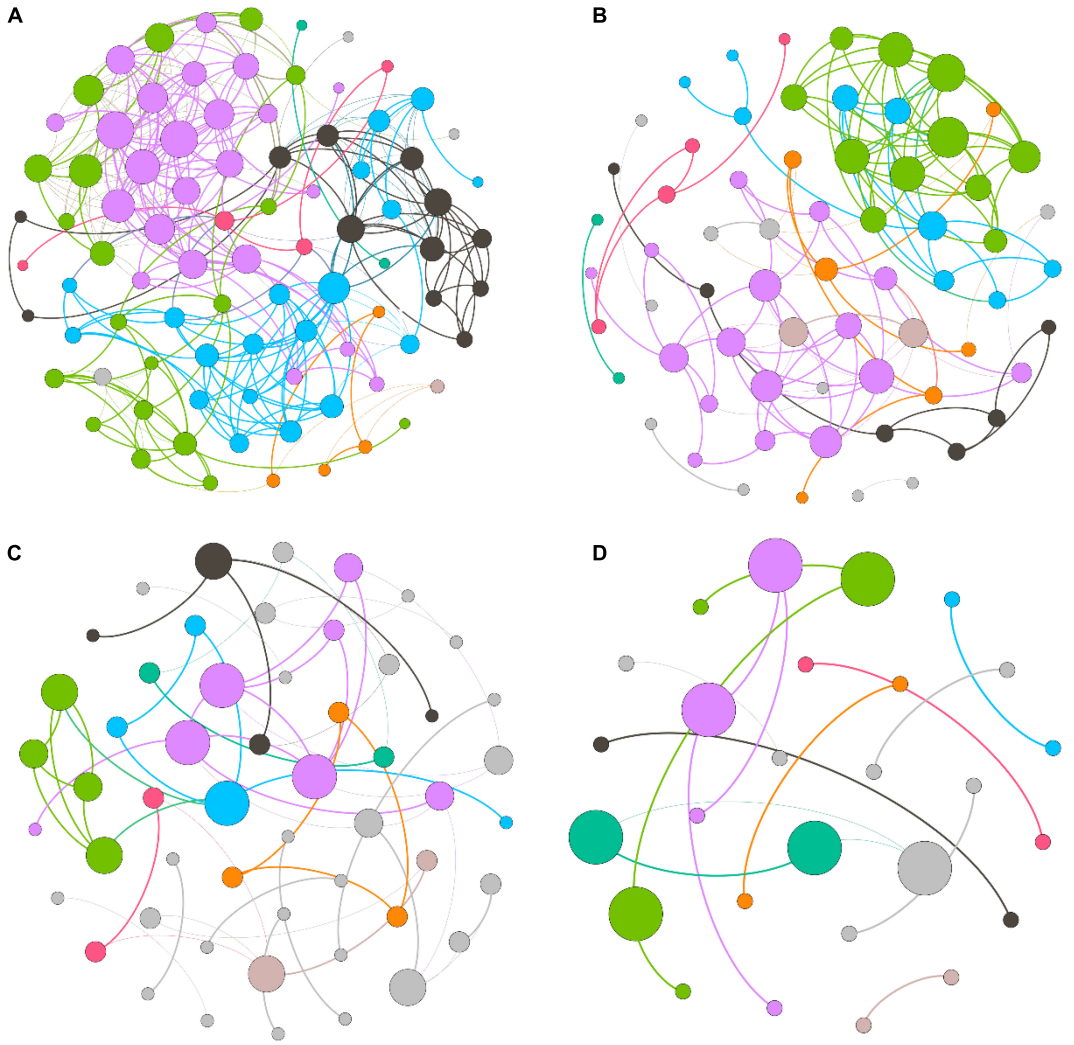


FIGURE 7
Co-occurrence network of freshwater bacterial assemblages in different wetlands. **(A)** high N/P (LH), **(B)** low N/P (LL), **(C)** high N/P (HH), and **(D)** low N/P (HL). Each node represents a bacterial genus, the size of the node represents the degree, and nodes of the same color represent a network module. The connections between nodes represent significant relationships between genera ($r > 0.6$ or $r < -0.6$, $p < 0.05$), and the thickness of the lines connecting nodes represent the size of the correlation coefficient.

TABLE 3 Network topological properties of freshwater bacterial communities in the four wetlands.

	Nodes[a]	Edges[b]	Average degree[c]	Diameter[d]	Density[e]	Modularity coefficient[f]	Average clustering coefficient[g]
LH	87	340	7.816	10	0.091	1.226	0.662
LL	66	134	4.061	9	0.062	0.795	0.548
HH	53	57	2.151	5	0.041	1.584	0.785
HL	17	27	1.259	3	0.048	1.051	0.429
Total	234	2804	23.966	8	0.103	1.637	0.505

[a]Number of species with at least one correlation > 0.6 or < -0.6, and statistically significant at $p < 0.05$.
[b]Number of strong and significant correlations between nodes.
[c]Node connectivity depicts how many connections (on average) each node has to another unique node in the network.
[d]The longest distance between the nodes in the network.
[e]The number of edges divided by the number of edges of a complete graph with the same number of vertices.
[f]A value > 0.4 indicates that the partition produced by the modularity algorithm can be used to detect distinct communities within the network. This indicates that there are nodes in the network that are more densely connected to each other than with the rest of the network and that their density is noticeably higher than the graph's average density.
[g]How nodes are embedded in their neighborhood and thus the degree to which they tend to cluster together.

were revealed in this study (**Figure 5** and **Supplementary Figure 1**). In general, Actinobacteria are dominant in soil and aquatic surroundings (Newton et al., 2011; Han et al., 2016). Similarly, this study also showed that Actinobacteria were the most abundant bacterial group (**Figure 5** and **Supplementary Figure 1**). Meanwhile, similar abundances of Actinobacteria among the groups were found in this study, which might be attributable to their extensive nutritional adaptability under eutrophy or oligotrophy (Yan et al., 2021). In addition, our results indicated that variations in COD/N and N/P significantly influenced the 10 most abundant bacterial taxa at both the phylum and class levels (**Figure 5**). For example, Gammaproteobacteria (Proteobacteria), which make up a large number of heterotrophic and mixotrophic species (Gong et al., 2016), and Bacilli (Firmicutes), which are chemoorganotrophs and mainly related to carbon sources (Gaugué et al., 2013), were both closely related to NH_4^+-N and affected by the imbalance of nutrients (*e.g.*, in the LH and HL groups) (**Figures 5**, **6**). Although Bacteroidetes and Oxyphotobacteria were also significantly correlated with NH_4^+-N, they increased in the LL group because of the low organic matter (**Figures 5**, **6**). Bacteroidetes are heterotrophic taxa and play a key role in polysaccharide degradation (Unfried et al., 2018). Oxyphotobacteria (Cyanobacteria) are photoautotrophs and are sensitive to fluctuations in nitrogen sources (Lindell et al., 2002; Chen and Bibby, 2005), and it has been shown that the reproductive rate of Cyanobacteria is affected by alterations in the concentrations of N and P (Gobler et al., 2016; Paerl, 2017). Moreover, Verrucomicrobia, Chloroflexi, and Phycisphaerae were significantly related to COD and were improved by high organic matter and N concentrations (*e.g.*, in the HH group, **Figures 5**, **6**). As previously reported, Verrucomicrobia is heterotrophic and able to degrade organic matter (Wang et al., 2019), and Chloroflexi can scavenge organic compounds derived from an anammox reactor (Kindaichi et al., 2012). Phycisphaerae-related (Planctomycetes) microbial ecological processes include anammox and mineral encrustation (Shu et al., 2011). Notably, Deinococcus-Thermus can utilize organic matter produced by autotrophs (Kadnikov et al., 2021) and are promoted by rich organic matter and P sources (*e.g.*, in the HL group, shown in **Figure 5A**). Nonetheless, significant decreases in the abundance of Bacteroidia (Bacteroidetes) were found in the HL group (**Figure 5B**), possibly due to Bacteroidia being chemo-organoheterotrophs with growth on carbohydrates or peptide mixtures and proteins (Podosokorskaya et al., 2020). Therefore, the dominant taxa presented different ecotypes, nutritional preferences, and responses to nutrient fluctuations.

Previous studies have shown that bacterial assemblages have the ability to exploit resources efficiently, which manifests as a more complex co-occurrence network, when the relationships between bacterial taxa are stronger (Aanderud et al., 2018; Santia et al., 2019). In this study, the co-occurrence network revealed that the bacterial assemblages in the low-COD/N groups presented many more nodes and connections than those in the high-COD/N groups, and the high-N/P groups showed improved network complexity under the same COD/N conditions (**Figure 7**). Our findings were consistent with those of several previous studies (Aanderud et al., 2018; Jabir et al., 2020). Bacterial taxa form several microbiotas (modules) based on their connections and interactions, which may be due to similar nutritional preferences or resource complementarity (Zheng et al., 2021). Furthermore, more nodes and connections appeared in the low-COD/N and high-N/P groups (**Figures 7A,B**). One possible explanation for this result is that the bacterial taxa needed a nutrient source with greater reuse efficiency, and the higher N concentrations helped increase the connections between interacting bacteria (Jarvie et al., 2018). While higher bacterial diversity appeared under high COD/N, fewer taxa and connections were observed (**Figures 7C,D**). One possible explanation is that sufficient organic matter may lead to less nutrient exchange as a result of less nutritional competition among bacterial taxa (Gralka et al., 2020). However, the keystone taxa sensitive to nutritional imbalances were identified in the co-occurrence networks, which greatly differed among the groups (**Supplementary Tables**). Previous studies have indicated that keystone taxa play a vital role in pollutant degradation, resource transformation, and ecosystem stability maintenance (Banerjee et al., 2018; Shi et al., 2022; Zhang et al., 2022). For instance, the keystone taxa of Sporichthyaceae (Actinobacteria) under high N (*e.g.*, in the LH group) can use various carbohydrates and nitrite (Tamura, 2014), which explains their importance in maintaining the efficient utilization of carbon sources by the microbiota. *Mycobacterium* (Actinobacteria) has strong acid resistance and the ability to grow on simple substrates (*e.g.*, in the LL group) (Nakai et al., 2015), and this taxon may contribute to bacterial assemblage stability under acid perturbation. Members of Rhodocyclaceae (Proteobacteria) prefer a high-organic matter and high-N source environment (*e.g.*, in the HH group) and can play a crucial role in water purification processes, such as carbon source provision, ammonium degradation and nitrogen fixation (Oren, 2014; Aanderud et al., 2018). Sphingomonadaceae (Proteobacteria) dominate in high-organic matter environments (*e.g.*, in the HL group) and perform many functions related to metabolizing and degrading aromatic and heterocyclic compounds, as well as producing extracellular biological polymers, which have shown strong adaptability to surrounding humic surface water and oxidative stress (Glaeser and Kämpfer, 2014). Thus, the fluctuation of nutrient imbalances, especially in relation to N sources, was the determining factor of bacterial network complexity and impacted the diverse functions of keystone taxa.

Currently, a number of water bodies are polluted by excessive organic matter, N and P, such as Tai Lake, where eutrophication has led to algal blooms that have adversely

affected industrial and agricultural production since the 1990s, and the regional direct economic loss was estimated at approximately 30 million US dollars (Duan et al., 2009). Some previous studies have indicated that the imbalanced nutrients caused by eutrophication always have marked influences on bacterial communities and functions (Jarvie et al., 2018; Wang et al., 2018), which was also confirmed by the results of our study. Therefore, this study reveals a response mechanism of aquatic bacterial assemblages to imbalanced nutrients (*e.g.*, COD/N and N/P), as well as a basic methodology for wetland management (*e.g.*, constructed wetlands) involving organic and nutritional balance and bacterial manipulation (Zeng et al., 2021). This approach is also conducive to converting pollutants into resources and could support improved provisioning of ecological services by the freshwater of wetlands.

Conclusion

The responses of bacterial assemblage structure to different COD/N and N/P ratios were revealed in this study. These results suggested that fluctuation of N sources was a predominant factor controlling the structure of bacterial assemblages in the freshwater environment. A high COD/N significantly increased bacterial alpha diversity, while this measure of diversity was negatively impacted by N/P. COD/N had a negative effect on beta diversity, and NH_4^+-N and EC greatly affected the distribution of bacterial assemblages. Organic to nutrient imbalance led to the differentiation of bacterial communities. However, LH increased the complexity of bacterial co-occurrence networks, and the more abundant connections of bacterial assemblages might be attributed to the balance of COD/N and N/P in the environment with increases in N sources. However, in aquatic environments, organic matter, N, and P are customarily regarded as pollutants. Constructed wetlands are used to improve the removal rate of pollutants by regulating organic to nutrient ratios. Our results shed light on the effects of organic to nutrient imbalance on bacterial diversity and interactions and represent considerable progress in advancing the functional management of bacterial communities and improving water quality, thereby enabling future research efforts to forecast the effects of freshwater bacterial communities on ecosystem function.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI, PRJNA865028.

Author contributions

FZ: methodology, data curation, investigation, formal analysis, writing—original draft, and resources. TZ, GQ, JC, and JZ: investigation. SY: visualization. DZ, XL and LX: writing—review and editing and validation. SA: project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.946537/full#supplementary-material

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EDITED BY
Jiuling Li,
The University of Queensland, Australia

REVIEWED BY
Xi Min,
Qingdao University,
China
Li Zongxing,
Northwest Institute of Eco-Environment and Resources (CAS), China

*CORRESPONDENCE
Xiaofei Yu
yuxf888@nenu.edu.cn

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Algae-bacteria symbiotic constructed wetlands for antibiotic wastewater purification and biological response

Yiqi Wang[1], Pingping Chen[1], Xiaofei Yu[1,2]* and Jingyao Zhang[1]

[1]State Environmental Protection Key Laboratory for Wetland Conservation and Vegetation Restoration, Jilin Provincial Key Laboratory of Ecological Restoration and Ecosystem Management, Key Laboratory of Geographical Processes and Ecological Security of Changbai Mountains, Ministry of Education, School of Geographical Sciences, Key Laboratory of Vegetation Ecology of Ministry of Education, School of Environment, Northeast Normal University, Changchun, China, [2]Key Laboratory of Wetland Ecology and Environment, Jilin Provincial Joint Key Laboratory of Changbai Mountain Wetland and Ecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, China

In this work, the removal efficiency and mechanism of various constructed wetlands microcosm systems on antibiotic wastewater, as well as the biological community response of microalgae and microorganisms were explored. Overall, the algal-bacteria symbiosis in conjunction with the gravel matrix had the most comprehensive treatment efficiency for antibiotic wastewater. However, pollutants such as high-concentration antibiotics impaired the biological community and functions. In the systems fed with microorganisms, both abundance and diversity of them were significantly reduced comparing with the initial value. According to the correlation analysis revealed that the pollutants removal rate increased with the addition of the relative abundance of some bacterial genera, while decreased with the addition of relative abundance of other bacterial genera. The presence of gravel matrix could lessen the stressful effect of antibiotics and other pollutants on the growth of microalgae and microorganisms, as well as improved treatment efficiency of antibiotic wastewater. Based on the findings of the study, the combination of gravel matrix and algal-bacteria symbiosis can considerably increase the capacity of constructed wetlands to treat antibiotic wastewater and protect biological community, which is an environmentally friendly way.

KEYWORDS

constructed wetlands, algal-bacterial symbiosis, gravel matrix, antibiotics, biological community

Introduction

In recent years, antibiotics have been widely used as bacteriostatic or bactericidal drugs in many fields including human disease treatment, livestock, poultry, and aquaculture (Cheng et al., 2014). They are secondary metabolites that interfere with the development of other active cells. However, 30–90% of antibiotics are ingested directly by microorganisms and discharged into surface water in the forms of prototypes or metabolites rather than metabolized (Hu et al., 2010; Baran et al., 2011). Antibiotics in the water environment will not only cause organic pollution, but also induce environmental microorganisms to produce resistance genes, which spread among microbial community through gene level, threatening public health and safety (Xu and Lv, 2019). Hence, it is critical to develop effective methods for treating antibiotic wastewater. At present, many biological, physical and chemical methods for removing antibiotics from the aquatic environment have been studied at home and abroad (Li et al., 2021), and treatment methods such as adsorption, chlorination, activated carbon filtration, advanced oxidation process (AOP), photocatalysis, nanomaterials and ferrate have been introduced (Homem and Santos, 2011; Wang X. et al., 2019; Du et al., 2021). However, these methods have some obvious limitations. For example, the adsorption method treating antibiotics relies on high-cost adsorbents (Chen et al., 2020). AOP and photocatalysis, although have high efficiency, require expensive chemical reagents or catalysts (Cuerda-Correa et al., 2019), and secondary pollutants such as metal sludge may be produced (Leng et al., 2020). The mass production and use of nanomaterials makes it easy to diffuse in the environment, and it difficult to recycle which results in adverse effects on the ecological environment.

Constructed wetlands (CWs), as an ecological treatment technique, achieve ecological restoration of water by utilizing the synergistic efficiency of sediment adsorption, plant absorption, and microbial metabolism. Also, CWs have the advantages of low cost, easy maintenance, excellent treatment efficiency and good environmental benefits, among others (Tao et al., 2021). The gravel matrix in CWs can not only improve the removal efficiency of pollutants, its developed pore structure and large specific surface area also provide attachment sites and carbon source for microorganisms (Yuan et al., 2020). CWs have enormous potential for removing antibiotics (Liu et al., 2019), and CWs do well in removing various antibiotics such as tetracyclines, sulfonamides and quinolones, reaching 59–99.9%. The presence of microorganisms is the key factor for the transformation and mineralization of CWs to degrade organic pollutants (Pei et al., 2009) and the changes in its community structure lead to the modification of CWs biodegrading antibiotics (Aydin et al., 2016). Antibiotics will affect the purification performance of CWs, and may cause two effects on bacterial community: one is the selection of antibiotic-resistant bacteria, and the other is the impairment of microbial physiological functions (Novo et al., 2013). Meanwhile, the removal performance of pollutants like antibiotics is decreased by CWs' drawbacks, which include easy clogging, large areas, and susceptible to environmental conditions (Huang et al., 2013).

Biodegradation is an economical and effective way to remove pollutants, and has the advantages of low cost, less energy consumption and environmental protection. To make up for the defects of CWs in treating antibiotic wastewater, the incorporation of microalgae is a viable solution. Golueke et al. proposed the algal-bacteria symbiosis for the first time in the 1960s (Golueke et al., 1967), and the method of co-treatment of wastewater by bacteria and algae drew widespread attention. It treats wastewater using the synergistic effect of bacteria and algae, which is a new energy-saving and environmental protection technology (Song et al., 2015). Algal-bacteria symbiosis can efficiently treat a wide range of wastewaters and have a high potential for producing microalgal biomass (Diao et al., 2018). As a result, establishing algal-bacterial symbiosis between microorganisms and microalgae in CWs significantly improve the efficiency on treating antibiotic wastewater. The abundance of microorganisms foster the development of algal-bacterial symbiosis to remove organic pollutants such as antibiotics, and the strong adsorption ability of gravel matrix in CWs is also beneficial to wastewater treatment. This study built CWs microcosms with different types of biological dosing, and reveal which approach can encourage biological growth and increase the treatment efficiency. In this study, the microcosms treated antibiotic water containing Cephradine Velosef (CED), a representative β-lactams antibiotic which is currently widely used broad-spectrum in order to realize the wider application and higher efficiency of CWs.

Materials and methods

Material collection and preparation

The sediment was collected in Jinchuan quagmire swamp wetlands, passed through a 100-mesh sieve, and air-dried for later use. Chlorella from Heiers Bio-Enterprise was used as the representative organism of microalgae and nurtured in BG11 medium, and microalgae was cultivated according to the light and dark times (12 h: 12 h), with the temperature keeping at 25 ± 1°C. CED was provided by Huijin Baili Biological Company.

Wastewater characterization

This experiment focused on the treatment antibiotic wastewater, using CED as the model antibiotic. The wastewater was tested using the sewage treatment plants' secondary effluent quality standards for COD (232 mg L^{-1}), total phosphorus (TP; 4.76 mg L^{-1}), nitrate-N (NO_3^--N; 23.26 mg L^{-1}), ammonium-N (NH_4^+-N; 23.26 mg L^{-1}) and CED (35 mg L^{-1}).

Every 24 h effluent water was collected at the bottom of the devices, and the test samples were obtained after filtration through 0.45 μm filter membranes for analysis. COD was measured by

potassium dichromate method. The ascorbic acid colorimetric method was used to measure TP. Total nitrogen (TN) was determined by alkaline potassium persulfate digestion-ultraviolet spectrophotometry. Ultraviolet spectrophotometry was used to measure the concentration of NO_3^--N and nessler reagent photometry determined NH_4^+-N. Ultra-high performance liquid chromatography (UPLC) was used for CED detection. Chlorella growth over time was assessed using a spectrophotometer analysis by absorbance at 680 nm. Furthermore, all the target samples were detected in 3 replicates.

Experimental design and operation

A total of 6 groups of microcosms were set up in the experiment, namely: sediment microcosm (S), sediment-gravel matrix microcosm (SG), algae microcosm (A), algae-gravel matrix microcosm (AG), algal-sediment microcosm (AS) and algal-sediment-gravel matrix microcosm (ASG). Each microcosm was assigned three parallel samples. The devices were made of PVC board (20 cm inner length, 15 cm inner width, 16 cm inner height), and the water outlets were located on the lower right (Figure 1). The same amount of wastewater was pumped into each microcosm device, and different types of biological material combination were added respectively: S was made up of with 100 g of sediment. Based on S, SG was mixed with 100 g of gravel matrix. Chlorella was added to A, and ensured its concentration reaching $OD_{680} = 0.1$ (Kao et al., 2014). A combined with 100 g of gravel matrix to constitute AG. Meanwhile, 100 g of sediment based on A was added to create AS, and added 100 g of gravel matrix into AS to construct ASG. The devices filled with Chlorella were exposed to simulated sunlight, and the experiment ran for 7 days.

Biological growth analysis

The initial collected sediment was stored at-80°C prior to the operation of the systems. Following the operation, the microorganisms of sediment in the systems were sent to Biomac Co., Ltd. The marker gene was sequenced using the single-molecule real-time sequencing (SMRT Cell) method, and the Circular consensus sequencing (CCS) sequence was filtered, clustered or denoised to divide the features, and the species classification was obtained basing on the sequence composition of the features in order to perform species annotation and relative abundance analysis for revealing species composition. Alpha Diversity was analyzed by indices such as Chaol, Shannon and Simpson, as well as Beta Diversity and significant species differences were analyzed by weighting method.

Because Chlorella is abounded of chlorophyll, and it has better light absorption at the wavelength of 680 nm, OD_{680} was chosen to reflect its growth in the experiment. 3 ml of algal cell suspension was taken to measure the absorbance of algal cells at 680 nm using a UV spectrophotometer. The biomass of algal cells can be calculated using the wet weight method (Hillebrand et al., 1999; Eq. 1), and converted the value to the biomass of Chlorella (mg L^{-1}) using the linear relationship between OD_{680} and biomass:

$$\text{biomass} = 4021.9 \times OD_{680} - 8.6817 \quad \left(r^2 = 0.9995\right) \tag{1}$$

Comprehensive evaluation and analysis of the efficiency of microcosms on antibiotic wastewater treatment

The KOM value calculated by the factor analysis applicability test method was 0.631 (> 0.6), indicating that there was a certain correlation between the indicators. The Sig value is 0.000, showing that correlation co-efficient was not a unit matrix, and each indicator was related. Both tests indicated that the data was suitable for factor analysis.

Using the removal rate of each pollutant (COD, TP, TN, NO_3^--N, NH_4^+-N and CED) as indicators variable, the total

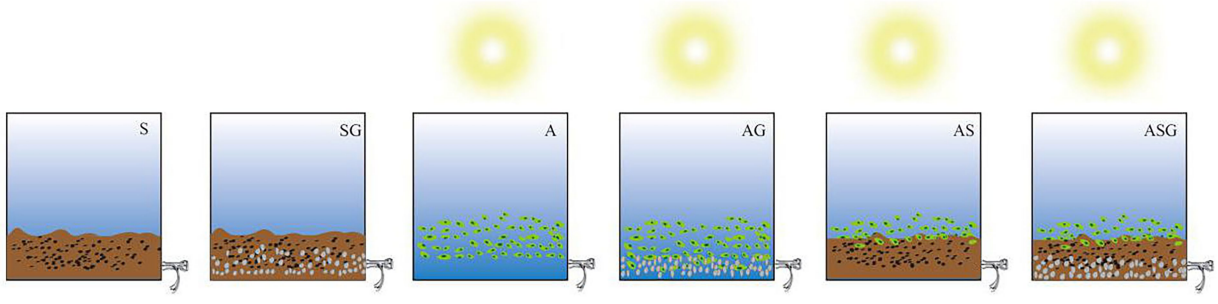

FIGURE 1
Six microcosm devices diagram.

explained variance was obtained by factor analysis (Table 1). According to the initial eigenvalue and variance percentage, two main evaluation factors were determined, and the cumulative variance percentage of these two main factors reached to 86.673%, which summarized majority of the information on the comprehensive treatment efficiency of microcosm systems on antibiotic wastewater. Other variables were considered to have little effect on the variance, and divided them into these two main factors through factor analysis.

In addition, due to the different eigenvalue and variance percentage, the weight of the two main evaluation factors in the information reflecting the comprehensive treatment efficiency of antibiotic wastewater were different. The weight of factor 1 was 0.569, and the weight of factor 2 was 0.43 (Eq. 2), both of which could be confident in evaluating the comprehensive ability of the microcosm systems to treat antibiotic wastewater.

$$T_i = \frac{\lambda_i\%}{\sum_{i=1}^{k}\lambda_i\%} \quad (2)$$

Where T_i and λ_i represent the weight of the component in reflecting the overall information and the contribution rate of the component, respectively; $\sum_{i=1}^{k}\lambda_i\%$ reflects the cumulative contribution rate of each component.

The factor loading of each index variable in the two main factors screened was shown in Table 2. The indexes of COD removal rate, NO_3^--N removal rate and CED removal rate had a higher loading in factor 1, indicating that they had a strong correlation with factor 1 and it was mainly composed of the information of these three indicators. Additionally, the indicators included in factor 2 were TP, TN and NH_4^+-N removal rate.

Since each factor was a collection of indicators, and the indicator was the smallest functional unit of weight, the final weight of each indicator could be calculated basing on the weight of the indicators in each factor (Eq. 3).

$$\text{weight of indicator} = 0.569 \times factor_1 + 0.43 \times factor_2 \quad (3)$$

Finally, the weight of each indicator was calculated (Table 3). Used the different weight of each index and Eq. 4 to reflect the comprehensive treatment efficiency of different microcosm systems on antibiotic wastewater.

$$E = \sum_{i=1}^{n} Ai\% \times Bi \quad (4)$$

Where n, A_i% and B_i represent the number, removal rate value and final weight of indicators, respectively.

Statistical analysis

The mean and standard deviation of the data were calculated by SPSS 23 software. For the treatment efficiency of each pollutant, repeated measures ANOVA and one-way ANOVA were used. Principal component analysis and factor analysis were used to reduce the dimension of the original data. For the application of factor analysis to evaluate the synthesis of antibiotic wastewater by different systems, the KOM test and the Bartlett sphere test were used. Origin 2020 software was applied to draw graphs. R was used to analyze the correlation heat map. The clade diagram was analyzed by LEfSe. Gephi was used to make a network analysis map of microcosms.

Results

Dynamic change of pollutants in microcosms

The dynamic changes and final efficiency of pollutants treatment in microcosm systems were showed in Figures 2, 3. The addition of the gravel matrix significantly improved the COD treatment efficiency in microcosm systems (SG, AS and ASG) comparing with microorganisms and microalgae alone (S and A; $p < 0.05$), Compared with S and A, the efficiency of AS was also obviously increased ($p < 0.05$). The repeated measures ANOVA results showed that, with the change of

TABLE 1 Total variance interpretation table.

Component	Initial eigenvalue			Extract the sum of load squares		
	Total	Variance percentage	Perception	Total	Variance percentage	Perception
1	3.865	64.409	64.409	3.865	64.409	64.409
2	1.336	22.264	86.673	1.336	22.264	86.673
3	0.408	6.793	93.466			
4	0.231	3.854	97.320			
5	0.135	2.249	99.568			
6	0.026	0.432	100.000			

TABLE 2 Table of the factor load matrix after the orthogonal rotation.

Metric	Component 1	Component 2
COD removal rate	0.88	0.35
TP removal rate	0.309	0.795
TN removal rate	0.591	0.744
NO_3^--N removal rate	0.948	0.126
NH_4^+-N removal rate	0.017	0.949
CED removal rate	0.919	0.125

TABLE 3 Final weight of each indicator.

Metric	Weight
COD removal rate	0.1856
TP removal rate	0.1588
TN removal rate	0.1955
NO_3^--N removal rate	0.1649
NH_4^+-N removal rate	0.1349
CED removal rate	0.1603

time, the treatment efficiency of each system on pollutants improved significantly ($p < 0.001$), but from d5 onwards, the improvement of the COD treatment efficiency slowed down obviously ($p < 0.05$). The final removal efficiency of the six microcosm systems ranked as following: ASG > AG > AS > SG > S > A. It can be seen that the algal-bacterial symbiosis combined with the gravel matrix had the highest removal efficiency of COD.

The microcosm systems containing microorganisms (S, SG, AS and ASG) had higher treatment efficiency for TP, and the final removal rate reached 63.36, 63.84, 64.14 and 65.95%, respectively. Compared with other systems, microalgae alone had a lower removal efficiency of TP at the beginning of the experiment, but with time, the utilization efficiency of A on TP was significantly enhanced ($p < 0.001$). Simultaneously, the presence of gravel matrix considerably improved the efficiency of biological treatment of phosphorus ($p < 0.05$). After the experiment period completed, the final treatment efficiency of ASG on TP reached highest level, which was 65.95%.

At the start of the experiment, the removal efficiency of NO_3^--N in each microcosm system was low, as was the removal efficiency of TN with NO_3^--N as the main form, and the removal efficiency of NO_3^--N and TN of each system increased significantly with time ($p < 0.001$). Compared with other microcosms, the removal efficiency of A on NH_4^+-N was poor, and the final removal rate was only 36.8%. The figure demonstrated that the algal-bacterial symbiosis (AS) had a remarkably better removal effect of nitrogen than the two treatments alone ($p < 0.05$), and the presence of gravel matrix also significantly improved the removal of nitrogen ($p < 0.05$). Following the experiment, the order of the efficiency of each microcosm system on the nitrogen treatment was: ASG > AS > SG > AG > S > A. In addition, the removal rate was as following: 90.79, 88.94, 88.80, 80.53, 77.63 and 71.21%.

At the end of the experiment (d7), the CED treatment efficiency of S and SG containing microorganisms reached 75.69 and 79.55%, which were slightly lower than other systems. The removal rate of CED by A and AG added with Chlorella increased with operation time, and the removal efficiency of the two improved significantly ($p < 0.001$).The final removal rate of CED by the two systems reached 98.76, and 98.84%, respectively, which were remarkably better than other systems ($p < 0.05$). The final removal rate of CED by AS and ASG were 90.75 and 92.40%, which were lower than the treatment efficiency of A and AG. At the same time, the addition of gravel matrix improved the efficiency to remove CED and enhanced the capacity of biodegrading antibiotics.

Comprehensive efficiency of microcosms on the treatment of antibiotic wastewater

In reflecting the treatment efficiency of antibiotic wastewater, the weight of each pollution index was different. The comprehensive treatment scores of antibiotic wastewater in the six microcosm systems over time were calculated (Supplementary Table S1). The outcome demonstrated that at the end of the experiment period (d7), all microcosms had the highest comprehensive treatment efficiency. The final composite scores were 0.7641, 0.8264, 0.7465, 0.8224, 0.8494 and 0.8729, respectively. Meanwhile, ASG, the algal-bacteria symbiosis combined with the gravel matrix, had the best comprehensive treatment capacity for antibiotic wastewater.

Community response of microorganisms under the operation of the microcosms

In order to reveal the changes in the microbial community species diversity and relative abundance of microorganisms in different microcosm systems after the treatment of antibiotic wastewater, the samples obtained by sequencing were processed and analyzed to get the difference of the α-diversity index between each system (Table 4). In addition, different lowercase letters indicated significant differences ($p < 0.05$).

Compared with the initial community structure of microorganisms, the species diversity and relative abundance of each system were significantly reduced ($p < 0.05$). The relative abundance of microorganisms increased clearly after the addition of the gravel matrix to S and AS (SG and ASG), but the diversity of SG decreased significantly ($p < 0.05$), indicating that some stress-resistant microorganisms could adapt to the new environment with antibiotics and other pollutants and grew on the gravel matrix. The relative abundance of microorganisms increased, while the diversity of

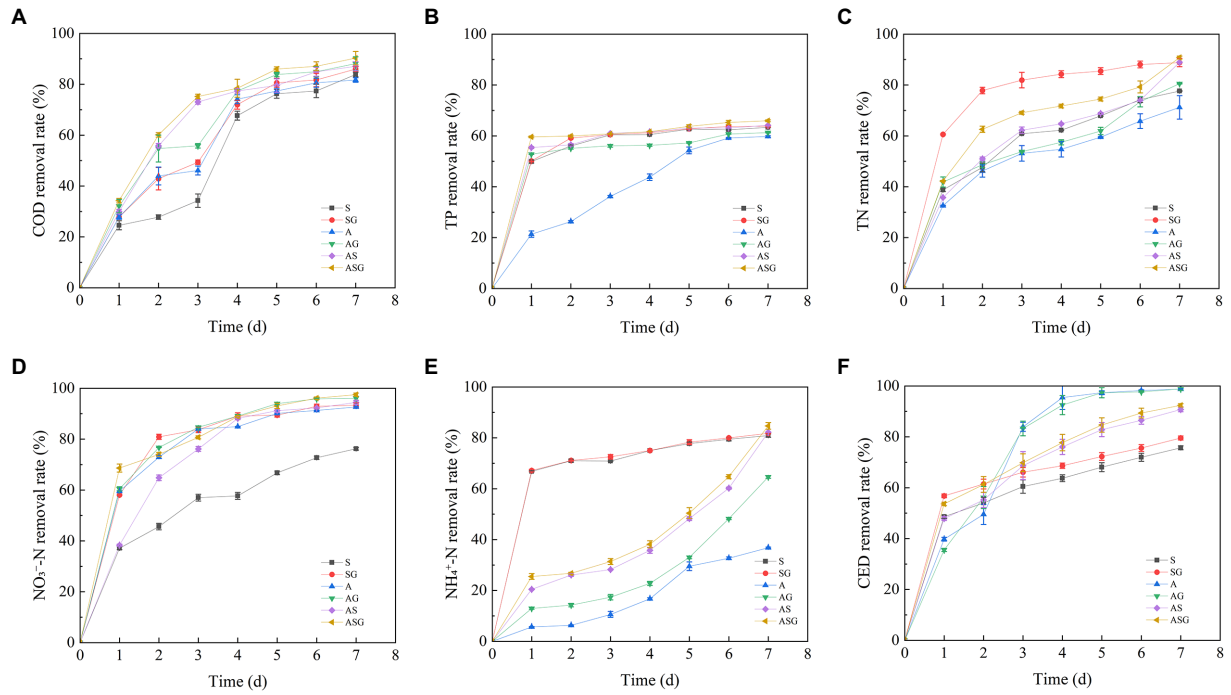

FIGURE 2
Dynamic changes in pollutants treatment efficiency of different microcosmic systems. **(A)** COD removal rate; **(B)** TP removal rate; **(C)** TN removal rate; **(D)** NO_3^--N removal rate; **(E)** NH_4^+-N removal rate; **(F)** CED removal rate.

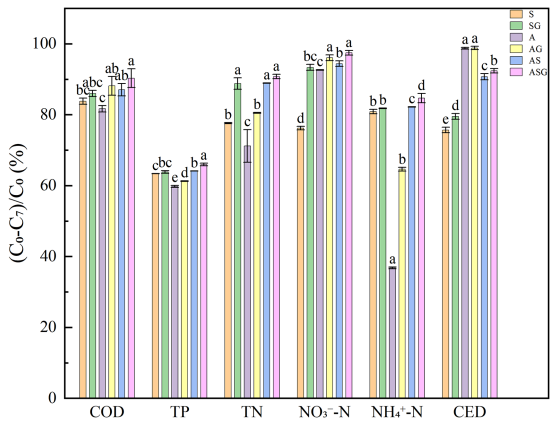

FIGURE 3
Final removal rate of pollutants in different microcosmic systems. Different letters indicate p <0.05, with significant difference.

other sensitive microbial community decreased. Different from SG, Chlorella in ASG could provide nutrient such as oxygen to microorganisms, which remarkably increased the relative abundance and diversity of them ($p < 0.05$).

The results revealed that the relative abundance and diversity of various microbial systems changed significantly after the antibiotic wastewater treatment ($p < 0.05$). The addition of antibiotics and other pollutants had a screened

TABLE 4 Diversity index of microbial community in different treatment systems.

	OTU	Chao1	Shannon	Simpson	Coverage(%)
Initial value	916	1018.42^a	8.27^a	0.9896^a	97
S	643	863.69^b	6.24^b	0.9574^b	96
SG	598	957.6^a	5.24^c	0.8910^c	97
AS	322	530.96^d	3.11^d	0.6446^d	98
ASG	504	797.91^c	4.95^c	0.8909^c	97

Different lowcase letters indicated significant difference among treatments ($p < 0.05$).

function on microbial community, promoted the growth of stress-resistant bacteria, inhibited or killed sensitive microorganisms, and reduced the relative abundance and diversity of microbial community in the systems. However, gravel matrix provided growth conditions for microorganisms in an adverse environment.

Using β-diversity analysis, obtained unweighted pair-group method with arithmetic mean (UPGMA) dendrogram of the 10 species with the highest relative abundance at the microbial phylum-level in the initial community (blank control) and the microcosms containing microorganisms (S, SG, AS, and ASG; Figure 4A). The relative abundance of microorganisms in different microcosm systems changed comparing with the initial microbial community and Proteobacteria was the dominant phylum. In addition, the relative abundance of Bacteroidetes, Firmicutes,

Acidobacteria, Patescibacteria, Verrucomicrobia and Chloroflexi were also higher.

Following the treatment of antibiotic wastewater, the relative abundance of Firmicutes was significantly increased by 19.64 and 11.66% in S and SG ($p < 0.05$), respectively, compared to the blank control. The relative abundance of Proteobacteria and Bacteroidetes increased the most obviously in AS and ASG. Acidobacteria, Patescibacteria, Verrucomicrobia and Chloroflexi relative abundance were significantly reduced in each system ($p < 0.05$). During the treatment of antibiotic wastewater, the relative abundance of microbial community in different microcosm systems reacted and changed to varying degrees, with SG retaining a more primitive microbial community structure.

The genera with high relative abundance (> 0.1%) in the blank control and each microcosm system containing microorganisms was subjected to dendrogram analysis (Figure 4B), and it can be seen that different treatment modes also had obvious effects on the structure at the microbial genus-level. Except for other unclassified species, the dominant genera in the microcosm systems were *Enterobacter*, *Paludibacter*, *Janthinobacterium*, and *Pseudomonas*, and the relative abundance of *Clostridium*, *Kluyvera*, *Paludibacterium*, and *Chryseobacterium* were also higher.

After the antibiotic wastewater treatment, the relative abundance of *Enterobacter* and *Paludibacter* were significantly increased ($p < 0.05$), as well as the relative abundance of *Janthinobacterium* and *Clostridium* also raised. Except for ASG, the relative abundance of *Pseudomonas* in the other three systems decreased significantly ($p < 0.05$), indicating that the growth of dominant genera was significantly affected by pollutants and living environment.

LEfSe differences between the initial microbial community (T0) and the four microcosm systems: S (T1), SG (T2), AS (T5), and ASG (T6) were investigated (Figure 5). *Clostridium* and *Paludibacterium* were significantly enriched in S (T1). The representative genera of SG (T2) were *Kluyvera*, *Enterobacter*, and *Chryseobacterium* mainly existed in AS (T5). *Paludibacter*, *Pseudomonas*, and *Janthinobacterium* were mainly present in ASG (T6).

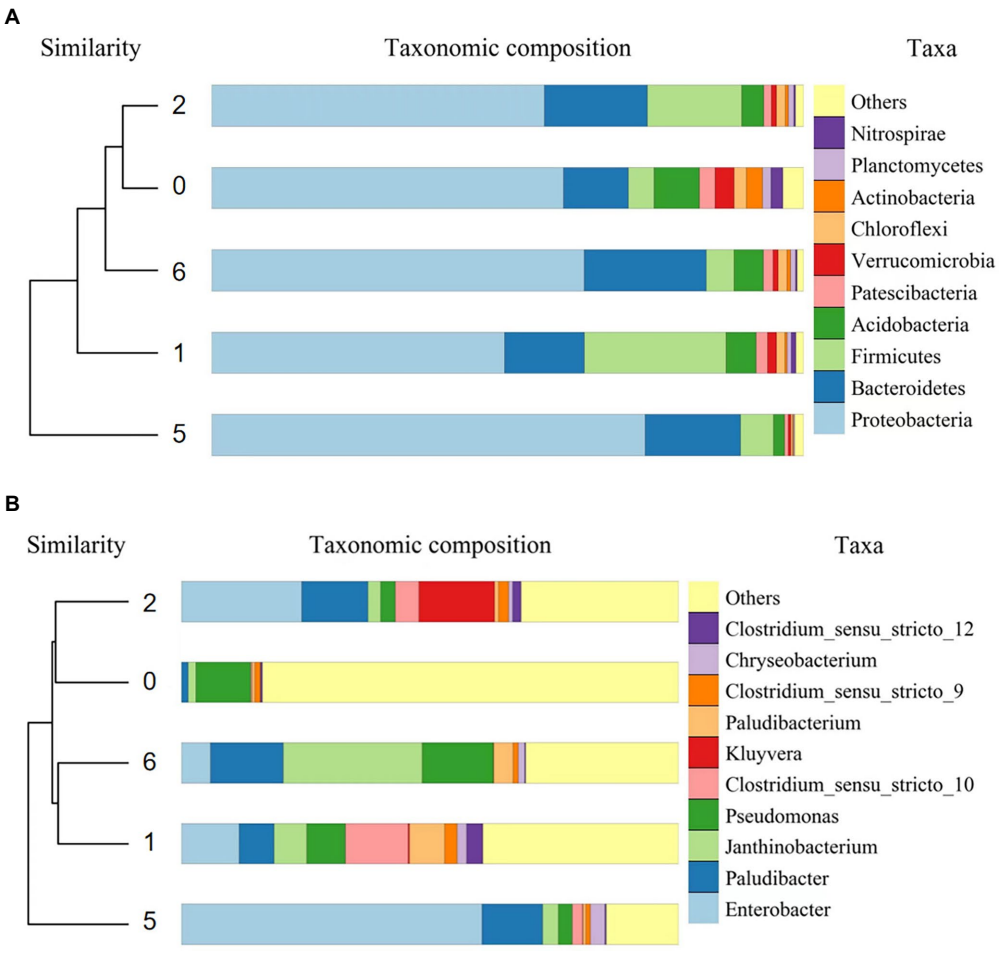

FIGURE 4
Dendrograms of the bacterial phylum-level UPGMA **(A)** and genus-level UPGMA **(B)** in the microcosmic systems.

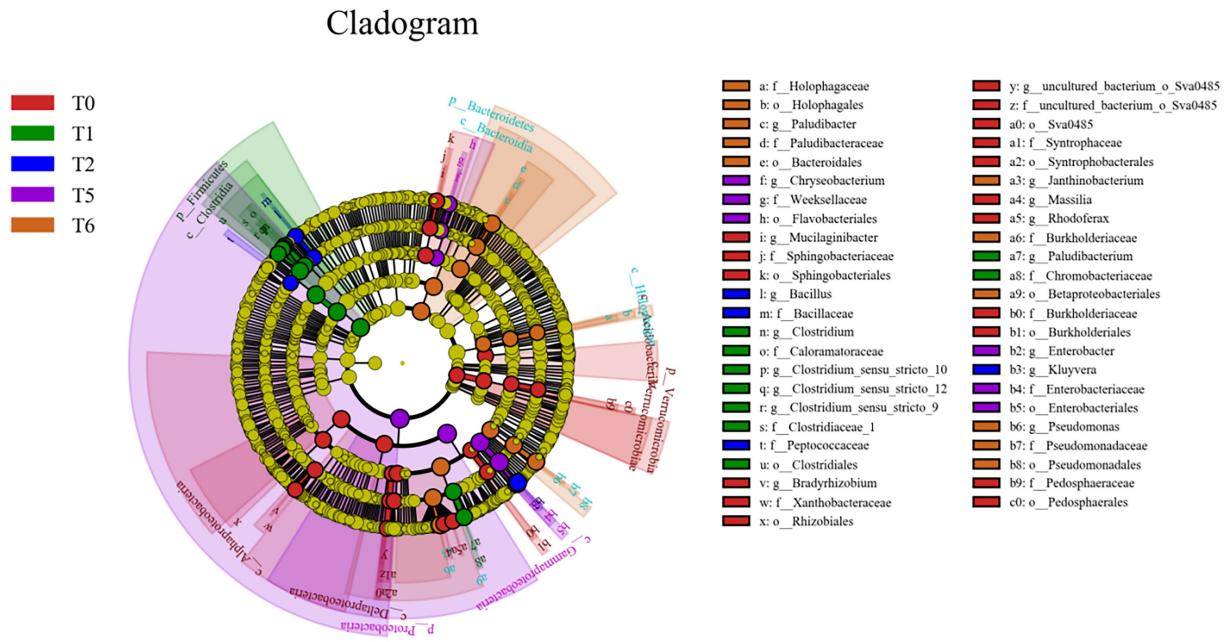

FIGURE 5
The LEfSe results of the differences between the initial system (T0) and S (T1), SG (T2), AS (T5) and ASG (T6) microbial community.

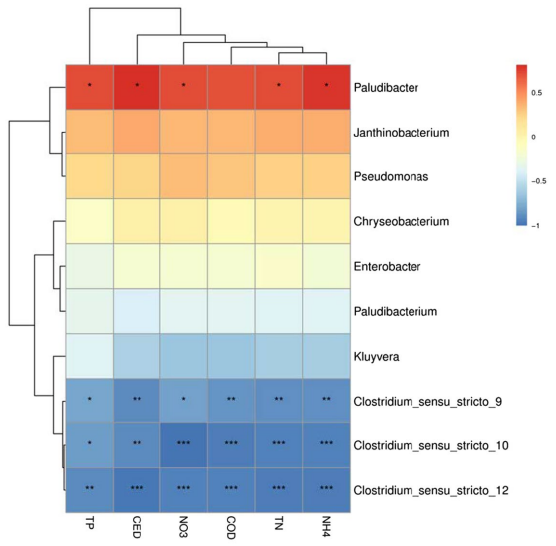

FIGURE 6
Heatmap analysis of correlation between microbial dominant genera and pollutants removal rate.

The relationship between microorganisms and pollutants removal rate

To understand the potential relationship between the microbial community and the removal rate of pollutants, the relative abundance of dominant genera was used as the species data, and the removal rate of each pollutant was used as the material variable for correlation heatmap analysis (Figure 6). The removal rate of pollutants increased as the relative abundance of *Paludibacter*, *Janthinobacterium*, *Pseudomonas*, and *Chryseobacterium* raised, particularly *Paludibacter*, which was significantly positively correlated with the removal rate of each pollutant ($p < 0.05$). In addition to having no obvious relationship with the removal rate of TP, *Enterobacter* was positively correlated with the removal rate of other pollutants. The removal rate of each pollutant was negatively correlated with *Clostridium*, *Kluyvera*, and *Paludibacterium*, especially *Clostridium*, with the increase of removal rate of each pollutant, its relative abundance decreased significantly ($p < 0.05$). Correlation analysis revealed that the relative abundance of different genera interacted and influenced the removal rate of pollutants like antibiotics.

The network analysis plot based on relative abundance of microbial dominant genera and pollutants concentration show that the different colors nodes represent different pollutants and dominant genera, and their size represent relative abundance or concentration (Figure 7). Furthermore, the edges show the relationship between microorganisms and pollutants (colors represent positive and negative, and thickness represents correlation strength). The relative abundance of *Paludibacter*, *Janthinobacterium*, and *Pseudomonas*, which removed pollutants such as antibiotics, was negatively correlated with pollutants concentration, indicating that with the decrease of pollutants concentration, these genera were less disturbed and grew more vigorously. The relative abundance of *Chryseobacterium* was inversely correlated with the concentration of pollutants except CED, while the relative abundance of *Chryseobacterium* and

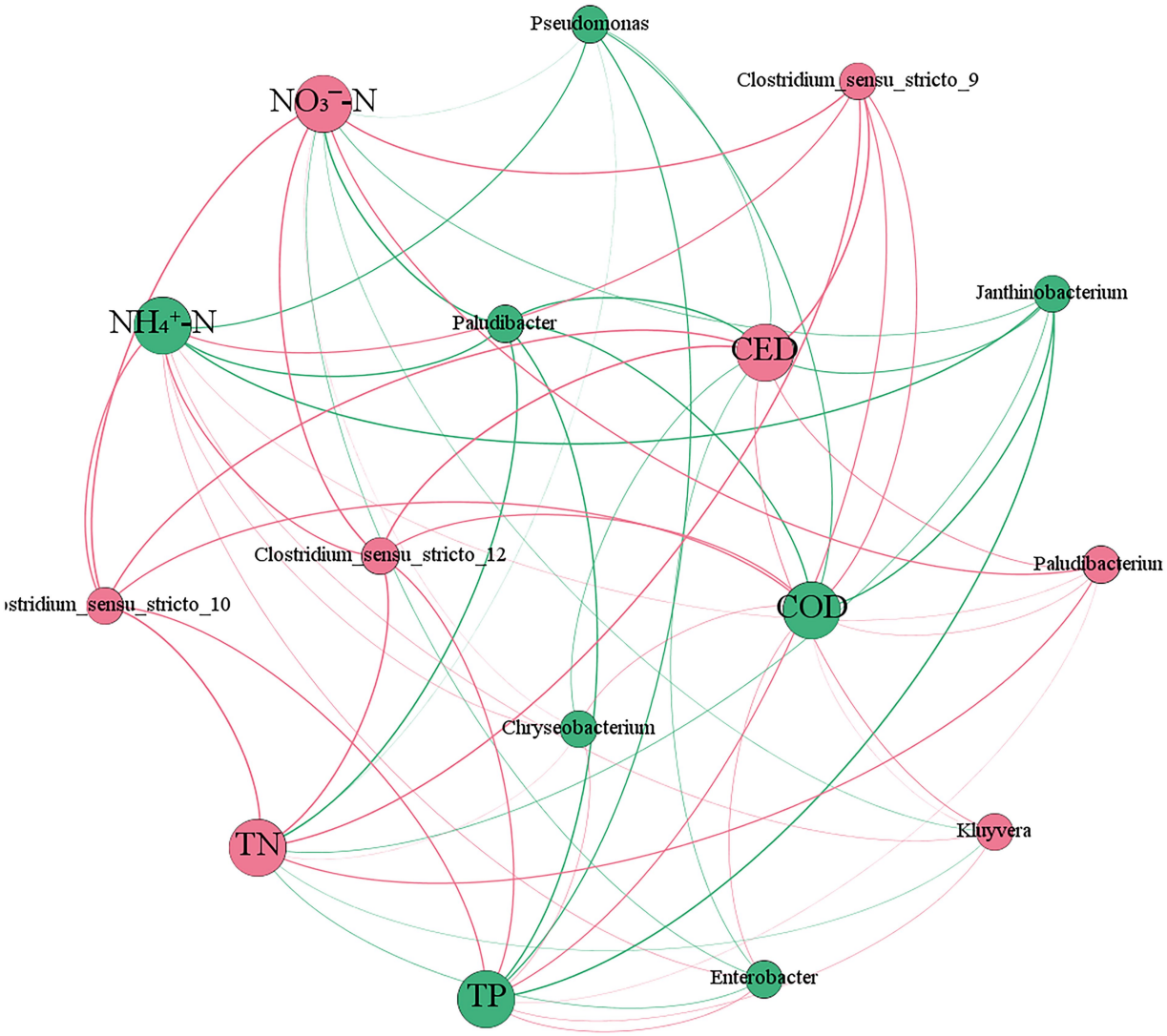

FIGURE 7
Network analysis plot of microbial dominant genus relative abundance and pollutants concentration.

Clostridium was positively correlated with pollutants concentration, that is, the lower the concentration of pollutants were, the fewer nutrient was provided to these microbial community, resulting in the relative abundance of them decreasing.

Effects of different microcosm system operation on the growth of microalgae

The wet weight method was used to measure and calculate the pigment value (OD_{680}) of Chlorella in A, AG, AS and ASG, and the dynamic analysis of the biomass change was performed (Figure 8). In the operation of removing pollutants, the growth of algal cells was not significantly inhibited, but when they were combined with microorganisms and gravel matrix, their growth was significantly increased ($p < 0.05$).

Discussion

The purification mechanism of microcosms for antibiotic wastewater

After being put into a new environment, organisms need a period of time to adapt to achieve community stability, and high concentration of antibiotics and other pollutants have stressful effect on the their growth.

Under the induction of environmental pressure by pollutants such as antibiotics, the resistance genes of some microorganisms were gradually generated, antibiotic-resistant bacteria appeared, and the inhibitory effect of antibiotics on them become smaller. After the microbial community stabilized over time, the removal efficiency of each microcosm system was gradually increased. The reason for this was that pollutants such as organic matter in

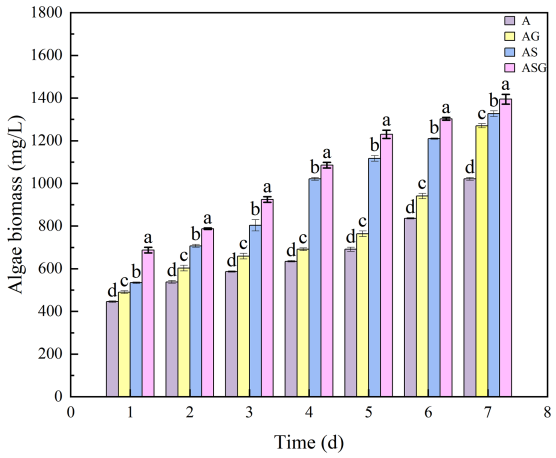

FIGURE 8
Changes of microalgae biomass in different microcosm systems. Different letters indicate p <0.05, with significant difference.

wastewater was the material basis for microorganisms growth and metabolism, and there was a high relative abundance of phosphate solubilizing bacteria (such as Proteobacteria and Firmicutes; Niu et al., 2016) and nitrifying bacteria, which can effectively remove COD, TP and TN. Under certain conditions, microorganisms also produced enzymes and other substances through metabolism, directly or indirectly modified the structure of antibiotics to render them inactive. CED is β-lactam antibiotic, and Pseudomonas is the bacteria that can degrade it (Lin et al., 2015). Although it was present in the added sediment microorganisms, the number was limited and its growth cycle was short. At the same time, because microorganisms were sensitive to high concentration of antibiotics, the community system was easily affected, limiting their capacity to deal with CED.

Phosphorus and nitrogen are involved in energy transfer and nucleic acid synthesis in Chlorella, as well as metabolic processes involving lipids, proteins and carbohydrates. As a result, the utilization rate of COD, TP and TN by Chlorella in each system was gradually increased. Because the high concentration of antibiotics would inhibit the growth of Chlorella, A and AG adding Chlorella had a low removal rate of CED in the initial treatment process, and its hydrolyzed products also had a higher toxic effect on Chlorella. Hence, when Chlorella was added to the antibiotic wastewater in the beginning, its removal rate of CED was low, but by the end of the experimental operation, the removal efficiency was significantly increased, and even better than other systems. This is because Chlorella adsorbed and degraded antibiotics through a variety of biological ways. After gradually adapting to the new environment, Chlorella adsorbed antibiotics to its own cell wall or secretion (Saavedra et al., 2018; Sutherland and Ralph, 2019). Also, Chlorella removed antibiotics *via* intracellular and extracellular biodegradation or bioaccumulated in the body (Zhong et al., 2021).

The algal-bacterial symbiosis did not outperform the pure algae system in terms of CED removal because Chlorella and microorganisms competed for nutrient in the nutrient-limited environment, which led to the limited growth of both (Wang H. et al., 2019). Meanwhile, in the process of bacteria and algae systems processing organic matter such as antibiotics, microorganisms primarily removed antibiotics through biological co-metabolism. However, when the concentration of organic matter like CED was high, it would affect the metabolic activity of microorganisms (Li, 2005). Microalgae tolerated to high concentration of refractory substances well, and CED was absorbed, enriched and biodegraded by it to be removed. In summary, the pure algae system removed refractory substances such as antibiotics more effectively.

The presence of the gravel matrix significantly improved the performance of the microcosm systems to remove pollutants, according to the experimental results, because phosphate substances reacted with the metal ions in the gravel matrix to form precipitation, which could be removed (Sakadevan and Bavor, 1998; Vymazal, 1998). While serving as a growth medium for microorganisms and microalgae, the gravel matrix directly removed pollutants *via* precipitation, filtration and adsorption which significantly enhanced the ability of the systems to remove pollutants.

The comprehensive treatment efficiency scores showed that the algal-bacterial symbiosis combining with gravel matrix (ASG) demonstrated the best efficiency in the treatment of antibiotic wastewater. The reason for this was that in the algal-bacterial symbiosis, Chlorella produced oxygen to supply microorganisms through photosynthesis, and microorganisms would generate carbon source to provide Chlorella photosynthesis (Liang and Zhang, 2019). Aerobic bacteria used O_2 produced by Chlorella to degrade carbon organic matter to produce CO_2 and ammonia nitrogen organic matter, followed by nitrification to generate ammonia nitrogen, nitrite and nitrate, at the same time, phosphorus organic matter was degraded into orthophosphate. These substances were supplied to the growth of Chlorella (Xing et al., 2009). The two provided favorable conditions for the growth and metabolism of each other. Simultaneously, the gravel matrix adsorbed and treated pollutants like antibiotics, and had ecological effects such as providing living carriers and nutrient for organisms, which was more conducive to their growth. The system (ASG) played greater potential in the process of antibiotic wastewater treatment.

Interaction between microbial community structure and pollutants removal rate

As the important component of wetland ecosystem, microorganisms play the pivotal role in the process of geochemical material cycling and energy transformation (Huang, 2014; Moche et al., 2015). The introduction of pollutants into the environment, such as antibiotics, caused changes in pH, nutrient concentration and community structure of microorganisms. At the same time, the relative abundance and diversity of species which were

sensitive to environmental changes decreased (such as Acidobacteria and Verrucomicrobia). The species with high stress resistance (such as Firmicutes) can produce spores to resist external harmful factors and survive to form new species community (Dai et al., 2019), with the number increasing and accumulation to form dominant species. The relative abundance of Firmicutes in each system was significantly increased under the stress of antibiotics and other pollutants, while the other sensitive phyla decreased, according to an analysis of microbial community diversity with a blank control. This demonstrated that high concentration of antibiotics screened microbial population, and they could be retained and gradually expanded. On the contrary, it had a toxic affect on sensitive microorganisms and caused them to die.

Paludibacter, *Janthinobacterium*, and *Pseudomonas* absorbed carbon, phosphorus and nitrogen elements in order to synthesize lipopolysaccharide, cellulase and xylanase and other constituent substances. Meanwhile, *Pseudomonas* produced antibacterial active enzymes such as β-lactamase and cephalosporinase (Huang et al., 2010), which aided in CED removal. With the high relative abundance of the three genera in each microcosm system, the removal rate of pollutants in antibiotic wastewater increased significantly, and then the environmental stress weakened which resulted in an increase relative abundance of them. *Paludibacter*, *Janthinobacterium*, and *Pseudomonas* were mainly enriched in the ASG, which was one of the reasons why ASG performed more efficiently on antibiotic wastewater treatment. *Chryseobacterium* is a fermentative bacteria that can utilize organic matter and produce β-lactamase, which is highly resistant to cephalosporins and other drugs (Lin et al., 2003), its relative abundance was positively correlated with the concentration of each pollutant in this study. In contrast to these three genera, the concentration of each pollutant decreased, so did the relative abundance of *Chryseobacterium*, indicating that the concentration of nutrient required for their growth was higher. *Paludibacterium*, and *Clostridium* are anaerobic bacteria (Kang et al., 2016), lacking a complete metabolic enzyme system, and their removal efficiency of pollutants such as antibiotics was low, however, these microorganisms were antibiotics resistant, and their growth affected slightly by external interference. Thus, the response of different genera to the stress of exogenous high-concentration antibiotics and other pollutants were related to the physiological and metabolic characteristics.

The growth of microalgae and the efficiency of treatment of antibiotic wastewater

The cephalosporin antibiotic wastewater contains bioinhibitory substances such as antibiotics and naturally occurring nutrient including ammonia nitrogen, nitrate and phosphate (Liu, 2017). Antibiotics had effects on the growth of microalgae in the process of antibiotic wastewater treatment. Chlorella is a single-celled microalgae in the Chlorophyta Oocystaceae family that grow both heterotrophically and autotrophically. Meanwhile, it can purify water and recover bio-energy such as oil and fat (Arita et al., 2015). Chlorella synthesized phosphorus into phospholipids, ATP, nucleic acids and other substances in photosynthesis and signal transduction (De-Bashan et al., 2002). It also absorbed NO_3^--N and converted it into NH_4^+-N for absorption and utilization (Hellebust and Ahmad, 1989; Crofcheck et al., 2012). Chlorella made use of the nutrient source substances in the wastewater, and reduced the concentration of COD, TP and TN. Furthermore, Chlorella removed antibiotics *via* biological processes such as biosorption, bioaccumulation and biodegradation, which effectively reduced antibiotics concentration.

Antibiotics primarily inhibited the biological growth and metabolic process. Microalgae and bacteria were very different in cell structure and physiological metabolism, and Chlorella demonstrated good antibiotics resistance and tolerance. Besides inhibiting the growth and reproduction of microorganisms, the presence of higher concentration of antibiotics in water also decreased the activity of Chlorella and the synthesis of chlorophyll to a certain extent (Halling-Sorensen, 2000). However, recent researches have revealed that microalgae have toxic stimulatory effects at specific concentration, further activate proteases, regulate synthesis and induce gene expression to promote their own growth, demonstrating the performance of "low promotion and high inhibition"(Ma et al., 2012). This was also one of the reasons why the degradation efficiency of CED was higher in the pure algae microcosm system. The existence of the gravel matrix provided attachment sites for Chlorella, and adsorbed pollutants that were harm to its growth. Meanwhile, microorganisms provided nutrient for Chlorella during the metabolic process. Thus, in the systems where gravel matrix and microorganisms existed, Chlorella was more adaptable to the environment of antibiotic wastewater, and its biomass also increased significantly over time.

Conclusion

The results of this study and the comprehensive calculation scores demonstrated that ASG had the best comprehensive capacity in treating antibiotic wastewater. Antibiotics screened microbial population and affected microbial diversity and community structure. It promoted the growth and reproduction of antibiotic-resistant microbial population, such as Firmicutes. In contrast, it had a stressful effect on the growth of sensitive microorganisms like Acidobacteria. The relative abundance of microorganisms interacted with the removal rate of pollutants. *Paludibacter*, *Janthinobacterium*, *Pseudomonas*, and *Chryseobacterium* were all positively correlated with the removal rate of each pollutant, while *Clostridium* was the opposite. The relative abundance of microorganisms was also related to their growth and metabolism characteristics. For

example, *Chryseobacterium*, although its relative abundance increased with the rise of the removal rate of each pollutant, the required nutrient concentration was higher, so it was positively correlated with the pollutants concentration. The growth of Chlorella in different microcosm systems treating antibiotic wastewater was also significantly different, and the presence of gravel matrix and microorganisms provided a more suitable living environment for Chlorella, effectively reduced the damage caused by antibiotics and other pollutants, which significantly improved the efficiency of microcosm systems to treat antibiotic wastewater. In summary, algal-bacterial symbiosis is an environmentally friendly method to purify the wastewater that will be widely used in the future.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

YW: data curation and analysis and writing—original draft. PC: data curation. XY: worked on the technical details, supervised the findings of the work, and helped in the development of manuscript. JZ: writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1044009/full#supplementary-material

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EDITED BY
Zifang Chi,
Jilin University,
China

REVIEWED BY
Feng Li,
Institute of Subtropical Agriculture (CAS),
China
Xi Min,
Qingdao University,
China

*CORRESPONDENCE
Guodong Wang
wanggd@iga.ac.cn

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Soil microbial abundance was more affected by soil depth than the altitude in peatlands

Meiling Zhao[1,2], Ming Wang[3], Yantong Zhao[1,2], Nanlin Hu[1,2], Lei Qin[1,3], Zhibin Ren[1,3], Guodong Wang[1,3]* and Ming Jiang[1]

[1]Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, China, [2]College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, China, [3]State Environmental Protection Key Laboratory of Wetland Ecology and Vegetation Restoration, Institute for Peat and Mire Research, Northeast Normal University, Changchun, China

Soil microbial abundance is a key factor to predict soil organic carbon dynamics in peatlands. However, little is known about the effects of altitude and soil depth and their interaction on soil microbial abundance in peatlands. In this study, we measured the microbial abundance and soil physicochemical properties at different soil depths (0–30cm) in peatlands along an altitudinal gradient (from 200 to 1,500m) on Changbai Mountain, China. The effect of soil depth on soil microbial abundance was stronger than the altitude. The total microbial abundance and different microbial groups showed the same trend along the soil depth and altitudinal gradients, respectively. Microbial abundance in soil layer of 5–10cm was the highest and then decreased with soil depth; microbial abundance at the altitude of 500–800m was the highest. Abiotic and biotic factors together drove the change in microbial abundance. Physical variables (soil water content and pH) and microbial co-occurrence network had negative effects on microbial abundance, and nutrient variables (total nitrogen and total phosphorus) had positive effects on microbial abundance. Our results demonstrated that soil depth had more effects on peatland microbial abundance than altitude. Soil environmental change with peat depth may lead to the microorganisms receiving more disturbances in future climate change.

KEYWORDS

microbial abundance, altitudinal gradient, depth gradient, peatland carbon dynamics, co-occurrence network

Introduction

Soil microorganisms are a vital part of peatland ecosystems and play a critical role as a "carbon pump" in the process of organic matter decomposition (Liang et al., 2020; Li J. et al., 2022). Ecologists have tried to comprehend the patterns of soil microbial communities along the environmental gradient. With the increasing awareness of the significance of microbial participation in the carbon cycle process and the development of biomarker technology, the

pattern of soil microorganisms along the altitudinal and depth gradients has attracted scholars' attention (Wang et al., 2019; Looby and Martin, 2020). However, existing research so far have not reached the same conclusion. Some studies indicated that the altitude had a stronger impact on the community composition of microbes than soil depth (Li J. et al., 2022), while other studies suggested that soil microbial community activity and composition was more depended on soil depth (Dove et al., 2021; Lamit et al., 2021; Zhao H. et al., 2021). Moreover, several studies demonstrated the complex interaction between altitude and depth had significantly influenced soil microbial abundance and their ratios (Xu et al., 2022). These examples suggest the ongoing debate on the pattern of microbial abundance and communities along the altitudinal and depth gradients. The reason is that these studies focus on different ecosystems or vegetation zones, little is known about the pattern within one ecosystem.

Environmental conditions could affect the distribution of microorganisms in peatlands. For example, soil pH, soil water condition, and dissolved organic carbon (DOC) were found to be major factors influencing the biogeographic patterns of microorganisms (Rousk et al., 2010; Wagner et al., 2015; Wang et al., 2021), and soil nutrients including total nitrogen and total phosphorus significantly affected soil microbial abundance (Fenner and Freeman, 2011). However, the importance of the internal interaction of microorganisms in regulating the microbial abundance has been ignored. In fact, biotic variables (e.g., microbial co-occurrence network) are also supposed to be an important factor influencing the spatial pattern of soil microorganisms (Fan et al., 2017). The symbiosis, predation, and competition relationships between soil microorganisms formed a complex microbial ecological interaction network (Faust and Raes, 2012). The co-occurrence network has been widely used in forest, farmland and other ecosystems to evaluate microbial interactions (Fan et al., 2017; Tu et al., 2020; Xie et al., 2020). However, there are limited data about the living microbial lipid co-occurrence network along the altitudinal and depth gradients in peatlands.

The Changbai Mountain in northeastern China are exceedingly vulnerable to climate change, and the peatlands are widely distributed in this region (Wang et al., 2018; Zhao M. L. et al., 2021), which provides an ideal place to study the pattern in microbial abundance along the altitudinal and depth gradients. In the present study, we set four altitudinal gradients and six depth gradients in peatlands in the Changbai Mountain to understand the change in microbial abundance and their responses to abiotic and biotic factors along the altitudinal and depth gradients.

Materials and methods

Study area

The Changbai Mountain is located in Jilin Province, northeastern China (Zhao et al., 2022). The peatlands in the Changbai Mountain are dominated by sedges (Bao et al., 2010; Wang et al., 2018). The climate in this region is a typical continental monsoon climate; the mean annual temperature (MAT) in the study area ranges from −0.2°C to 3.9°C, and the mean annual precipitation (MAP) ranges from 580 to 770 mm in the study area (Zhao et al., 2022).

Sample collection

Samples were collected in July 2020. Soil cores at 0–30 cm depth with an interval of 5 cm were collected in four altitude gradients (200–500, 500–800, 800–1,200 and 1,200–1,500 m; Figure 1). Two peat samples were collected in each altitudinal gradient. Peat samples were kept at −20°C immediately after collection. Each sample was separated into two subsamples. One was used for the measurement of soil water content (SWC), and the other was freeze-dried and sieved for the measurement of extracted microbial lipids and soil physicochemical properties.

Phospholipid fatty acids analysis

The analysis of microbial abundance was used by phospholipid fatty acids (PLFAs) technology. The extraction and separation of PLFAs were performed followed the Bligh Dyer method (Bligh and Dyer, 1959). The sample test method was described by Zhao M. L. et al. (2021). The detected compounds were identified in the MIDI library (MIDI, Inc., Newark, United States) (Zhang et al., 2019). The PLFAs were used as the microbial biomarkers according to Joergensen (2021). The gram-positive bacteria (G+) were the sum of the abundances of Firmicutes and Actinobacteria, the G+ and gram-negative bacteria (G-) belong to bacteria (B). The total microbial abundance was the sum of the abundances of bacteria, fungi (F) and unspecific microbial biomarkers.

Soil physicochemical property measurement

Soil water content (SWC) was measured by the gravimetric method. Soil pH was measured by a potentiometric test with a soil to water ratio of 1:10. The total organic carbon (TOC) and dissolved organic carbon (DOC) were detected on a TOC analyzer (Shimazu, Japan). The total phosphorus (TP) and total nitrogen (TN) were determined by an automated analyzer (Smartchem140, AMS-Alliance, and French; Lu, 2000).

Microbial co-occurrence network analysis

Co-occurrence network was used to show microbial biomarker interactions, the analysis was visualized using Gephi

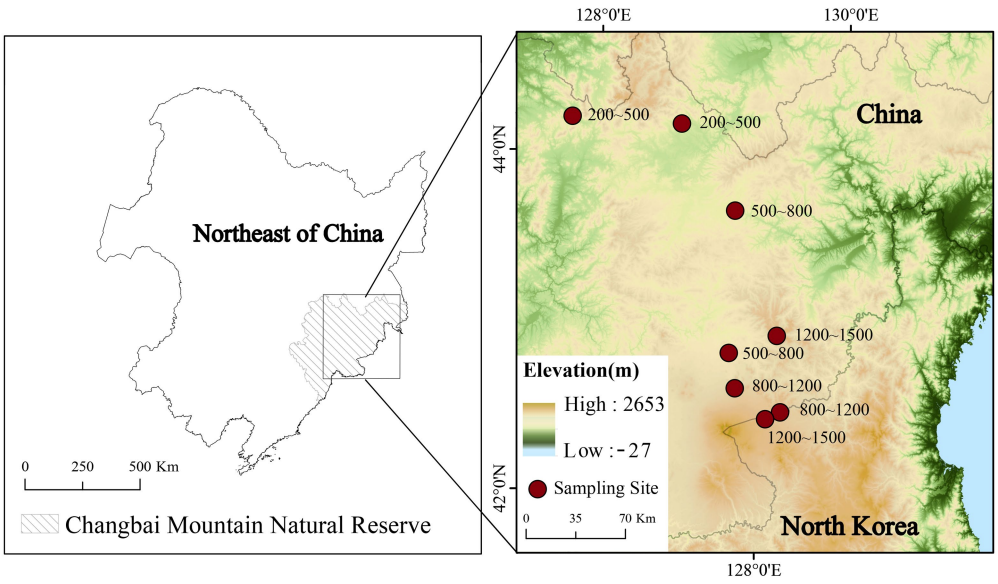

FIGURE 1
Site locations of the sedge peatlands along an altitudinal gradient on Changbai Mountain, China.

v.0.9.1. Each node means one microbial lipid and each edge means a strong relationship between two nodes. The topology of the co-occurrence networks was assessed referred to previous studies (Tu et al., 2020; Li J. et al., 2022). Briefly, average degree indicates the complexity of the co-occurrence network. Average path length indicates the distance between any two members of the co-occurrence network. A higher average degree indicates a higher complexity of the network, a shorter average path length suggests a stronger correlation between members.

Data analysis

Two-way ANOVAs were used to examine the main effect of altitude and soil depth and their interaction on peat physicochemical properties and soil microbial abundance. Redundancy analysis (RDA) was conducted to analyze the relationship of microbial abundance to peat physicochemical properties. Variation decomposition analysis (VDA) was used to analyze the effects of peat physicochemical properties and microbial co-occurrence networks on soil total microbial abundance. The physical (pH and SWC) and nutrient (DOC, TN, TOC, and TP) variables were used as abiotic factors and the microbial ecological interaction network was used as the biotic factor in the prediction model. Based on previous studies, the microbial co-occurrence networks are represented by the first two axes' scores of the principal component analysis for microbial community composition (Purahong et al., 2016). A positive coefficient of VDA indicates a positive effect on the prediction of the total microbial abundance, and a negative coefficient suggests the opposite (Gross et al., 2017). The data were $\log_{10}$-transformed to conform to normality and homogeneity of variance. The analyses were performed using SPSS 21.0, Canoco 5.0, Origin 29.0, and R 4.1.1 with the packages vegan (Oksanen et al., 2020), MuMIn (Bartoń, 2022), performance (Lüdecke et al., 2021), ggplot2 (Wickham, 2016), and ggh4x (van den Brand, 2021).

Results

Soil microbial abundance changes with depth and altitude

Soil depth and altitude significantly affected the total microbial abundance (Table 1). The effect of soil depth on the abundance of the microbial group was stronger than the altitude (Table 1). The soil layer of 5–10 cm had the highest total microbial concentration, which then decreased with soil depth (Figure 2A). The concentration of total microbial PLFAs was higher at 500–800 m than at other altitudes (Figure 2B). The concentrations of G+, G−, and F showed a similar trend with the total microbial concentration (Figure 2).

Soil properties and co-occurrence network change with depth and altitude

Soil depth significantly affected DOC, TN, and pH (Table 1). As soil depth increased, DOC decreased and TN generally increased. No significant difference was found in SWC, TOC, and TP between different depths. Altitude significantly affected SWC (Table 1). SWC generally increased with the altitude. No

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TABLE 1 A summary of analysis of variance (ANOVA) on the effects of altitude and soil depth for soil physicochemical properties and microbial groups.

Variable	Altitude		Depth		Altitude × depth	
	f value	Value of *p*	*f* value	Value of *p*	*f* value	Value of *p*
SWC	4.04	0.019*	1.702	0.173	0.776	0.690
TOC	2.03	0.136	1.813	0.148	0.622	0.828
DOC	2.759	0.064	40.149	<0.001**	1.181	0.348
TN	1.22	0.324	68.85	<0.001**	1.376	0.236
TP	1.043	0.391	0.676	0.646	0.824	0.644
pH	0.829	0.491	5.84	0.001**	1.039	0.454
Firmicutes	5.731	0.004**	17.948	<0.001**	1.739	0.110
Actinobacteria	5.428	0.005**	12.255	<0.001**	0.871	0.601
G+ bacteria	6.273	0.003**	18.868	<0.001**	1.418	0.216
G− bacteria	11.777	<0.001**	10.928	<0.001**	0.663	0.793
Fungi	4.29	0.015*	5.696	0.001**	0.700	0.761
Bacteria	11.648	<0.001**	19.048	<0.001**	1.024	0.466
Unspecific	2.427	0.09	10.681	<0.001**	1.011	0.477
Total	9.442	<0.001**	19.526	<0.001**	0.967	0.514

f value is the value of *F*-test, *Difference was significant at $p < 0.05$, **Difference was significant at $p < 0.01$. SWC, soil water content; TOC, soil total organic carbon; DOC, dissolved organic carbon; TN, total nitrogen; TP, total phosphorus.

significant difference was found in soil pH, TN, TP, and TOC along the altitudinal gradient.

Microbial co-occurrence network did not show significant differences along the altitudinal gradient (Figure 3A), but it had significant differences between soil depths (Figure 3B). As soil depth increased, the negative interaction ratio and average path length of co-occurrence networks decreased, and the positive interaction ratio, average degree, average clustering coefficients, and graph density of co-occurrence networks gradually increased (Table 2; Figure 3B).

Relationship between the microbial abundance and biotic and abiotic factors

The RDA analysis found that the first and second axes together explained over 60.0% of the total variation in microbial groups (Figure 4). Soil pH, TN, DOC, and depth were the main factors that significantly affected the microbial abundance. Samples from the top soil layers were located in the lower quadrant of the RDA graph while samples from deeper soil layers were located in the upper quadrant (Figure 4).

The VDA analysis showed that the total microbial abundance was significantly affected by both abiotic and biotic factors. Soil nutrients including DOC and TN had a positive correlation with the total microbial abundance. Soil physical properties (pH and SWC) and the microbial interaction network had a negative relationship with the total microbial abundance (Figure 5).

Discussion

Soil physicochemical properties differed between depths and significantly affected microbial abundance in peatlands

Soil depth has a great impact on soil microbes because of the unequal distribution of plant roots and soil nutrients across soil profiles (Rousk et al., 2010). Soil nutrients and physical environments were the main factors affecting the total microbial abundance in peatlands. Soil nutrients provided energy for the growth, metabolism, and reproduction of microorganisms (Zhang et al., 2022). In our study, soil depth significantly affected soil physicochemical properties and microbial abundance in peatlands. Soil nutrients had a positive influence on the soil microorganisms and soil physical factors had a negative impact on the abundance of soil microbes (Figure 5).

Soil nutrient is a key factor affecting microbial abundance in peatlands. DOC can be used as a carbon substrate for soil microbes, and TN can alleviate carbon limitation on soil microorganisms (Guo et al., 2011; Zhou et al., 2017). In our study, DOC and TN differed significantly between soil depths, and they significantly affected the total microbial abundance (Table 1; Figure 5). This result was consistent with former research (Bradley et al., 2006; Jia et al., 2020; Zhao M. L. et al., 2021). DOC is the most active intermediate in carbon cycle process because of its high mobility and bioavailability (Marschner and Kalbitz, 2003). DOC is utilized as a substrate, leading to microbial mineralization and CO_2 emissions in peatlands (Battin et al., 2008; Zhang et al.,

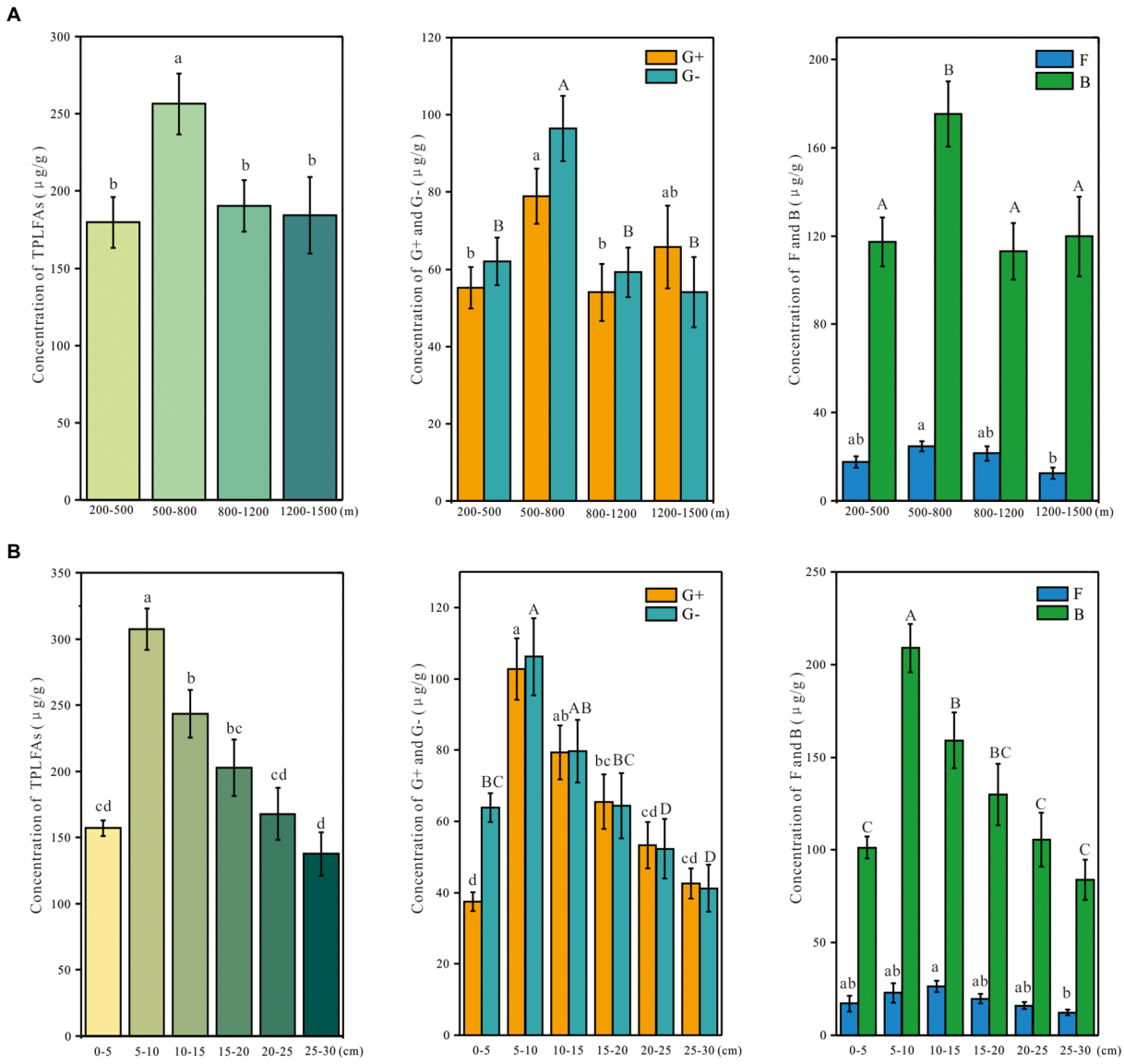

FIGURE 2
Concentration of various microbial groups based on the phospholipid fatty acids along the depth **(A)** and altitudinal **(B)** gradient on Changbai Mountain, China. Values were shown as Means±SE. Different letters suggest significant differences among study sites based on one-way ANOVA and Tukey's test (p<0.05). TPLFAs: total phospholipid fatty acids abundance; G+: gram-positive bacteria; G−: gram-negative bacteria; F: fungi; B: bacteria.

2022). Nitrogen accumulation increases microbial abundance because it increases the utilization rate of nitrogen resources, which can alleviate carbon limitation on soil microorganisms or inhibit the limitation of carbon caused by soil acidification (Guo et al., 2011; Zhou et al., 2017). This finding indicated that nitrogen accumulation had a positive effect on microbial abundance. The terrestrial surface temperature is projected to exceed 2°C by the end of this century, and the atmospheric nitrogen deposition and the intensity of extreme precipitation events will increase (IPCC, 2022). These changes may lead to more DOC exports from the peatlands (Cole et al., 2002; Clark et al., 2010) and an increasing nitrogen accumulation in peatlands (Zhang et al., 2018, 2022). Our findings suggest that DOC and TN positively affect soil microbial abundance and microbial activities, which may lead to higher CO_2 emissions in peatlands.

Soil physical properties also affect soil microbial abundance. Soil pH changed cell membrane charge and thus influenced the nutrient absorption by soil microorganisms and the enzyme activity in metabolic processes (Rousk et al., 2010). The acidic environment in peatlands is conducive to the growth of microorganism, and the microbial abundance generally decreased as soil pH increased in our study, which is consistent with former research (Anderson et al., 2010). Water regime could also affect soil microbial abundance. The effects of water drainage on soil

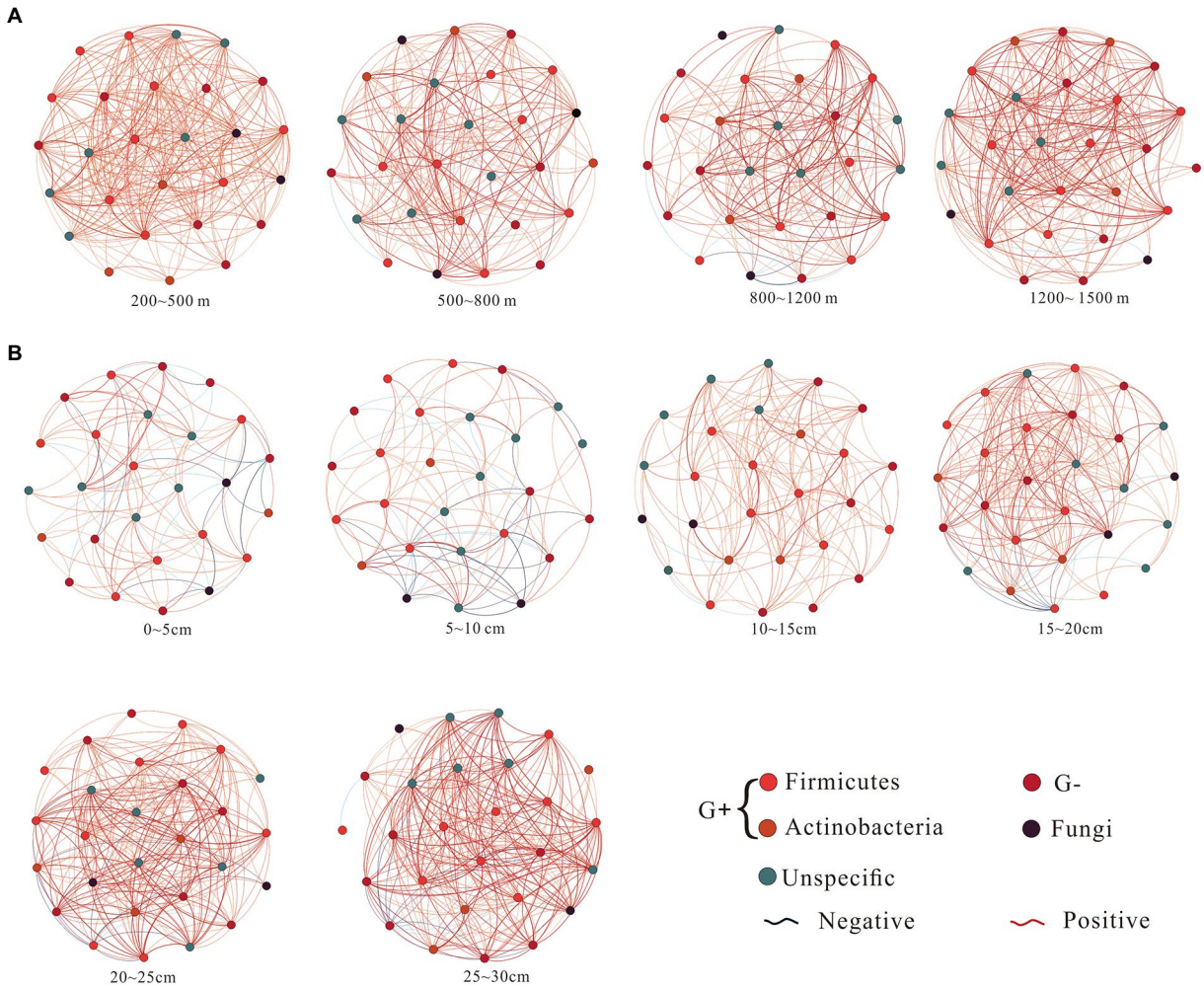

FIGURE 3
Microbial co-occurrence networks along the altitudinal **(A)** and depth **(B)** gradient on Changbai Mountain, China. The networks of co-occurring microbial biomarkers were determined based on Pearson correlation analysis. The node suggests the individual microbial biomarker based on the phospholipid fatty acid. The co-occurrence network nodes are colored by microbial groups. Blue edges indicate negative relationships between two individual nodes, while red edges suggest positive relationships. A connection stands for a strong correlation coefficient (r) >0.5. Each depth network was constructed from eight samples. Each altitudinal network was constructed from 12 samples.

TABLE 2 Topological parameters of network analysis in different altitudes and soil depths.

Network attributes	Altitude (m)				Soil depth (cm)					
	200–500	500–800	800–1,200	1,200–1,500	0–5	5–10	10–15	15–20	20–25	25–30
Nodes	27	27	27	27	26	26	27	27	27	27
Edges	267	204	200	240	124	116	168	212	250	252
Interaction positives	100%	96.08%	95.5%	98.75%	79.03%	73.27%	98.21%	94.34%	95.2%	93.65%
Interaction negatives	0%	3.92%	4.5%	1.25%	20.97%	26.73%	1.79%	5.66%	4.8%	6.35%
Average degree	9.889	7.556	7.407	8.889	4.769	4.462	6.222	7.852	9.259	9.333
Modularity	0.073	0.104	0.164	0.111	0.293	0.264	0.186	0.088	0.072	0.046
Graph density	0.38	0.291	0.285	0.342	0.191	0.178	0.239	0.302	0.356	0.359
Average clustering coefficient	0.442	0.368	0.352	0.411	0.296	0.250	0.338	0.389	0.418	0.437
Average path length	1.207	1.336	1.305	1.328	1.542	1.652	1.48	1.394	1.213	1.204

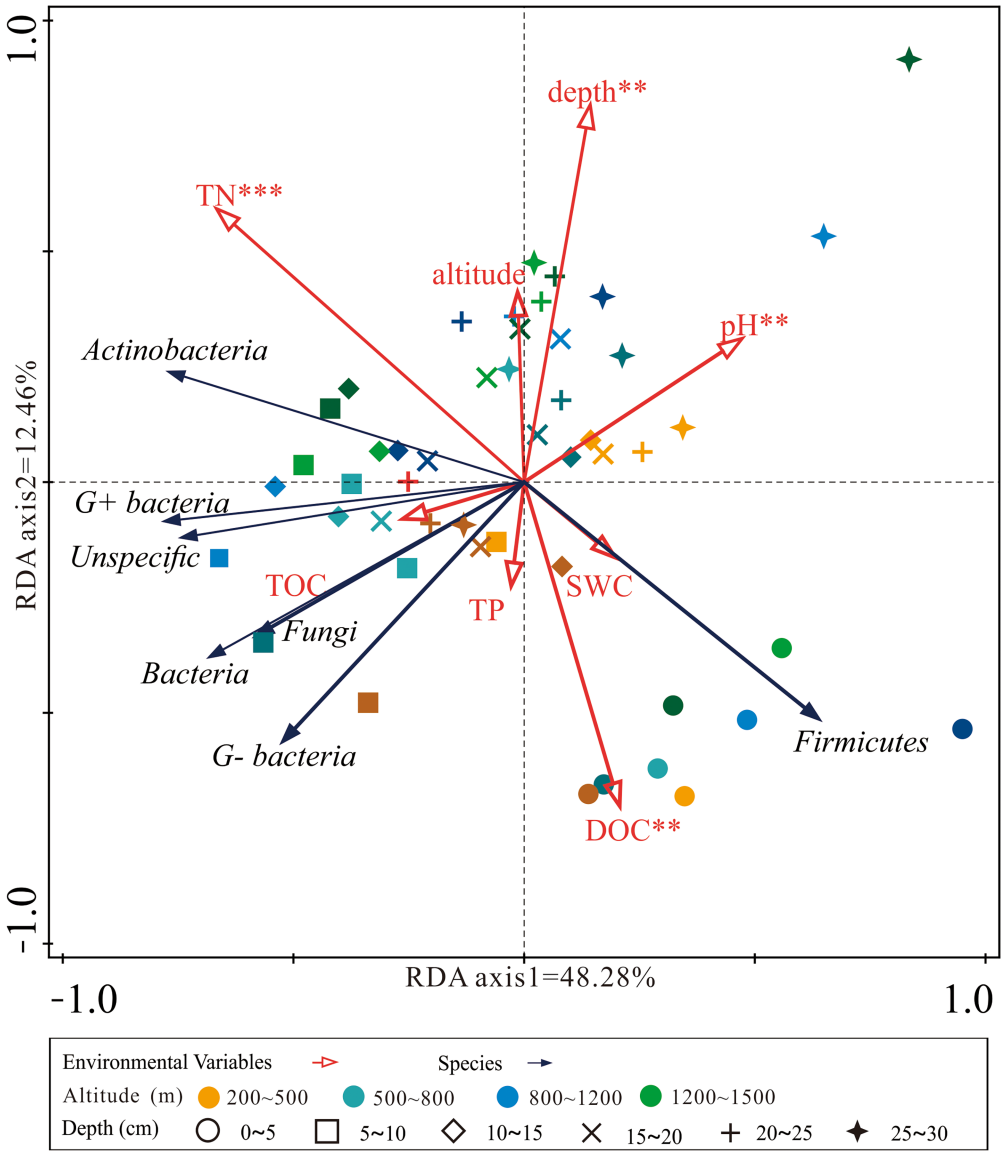

FIGURE 4
Redundancy analysis (RDA) ordination plot of the concentration of microbial groups constrained by the altitude, soil depth, and physicochemical properties. DOC, dissolved organic carbon; TOC, soil total organic carbon; TP, total phosphorus; TN, total nitrogen; SWC, soil water content.

microbial community and enzyme activity were dependent on soil depth in peatlands (Xu et al., 2021). When SWC was high, soil microbial abundance decreased because microbial heterotrophic respiration of microorganisms was inhibited (Wagner et al., 2015).

The complexity of the co-occurrence network increased with soil depths and affected microbial abundance in peatlands

The complexity of the co-occurrence network indicated microbial interactions along the environmental gradient (Ma et al., 2020). Environmental variables are essential for microbial niche differentiation, which enables distinct microbial groups to obtain adequate substrate and survive under various environmental conditions (Wiens et al., 2010; Li et al., 2020). The wide range of edaphic environments has driven the assembly process of soil microorganism (Dini-Andreote et al., 2015; Tripathi et al., 2018). In our study, the complexity of microbial co-occurrence networks increased and the microbial abundance decreased with soil depth (Table 2), and the VDA analysis indicated that microbial community assembly was one major factor affecting microbial abundance (Figure 5). The availability of carbon, energy and oxygen decreased with soil depth in peatlands, the competition of microorganisms for the resource increased, and their interactions increased. Resource limitation leads to the reduction of microbial abundance (Lu et al., 2020). Our results are consistent with a recent

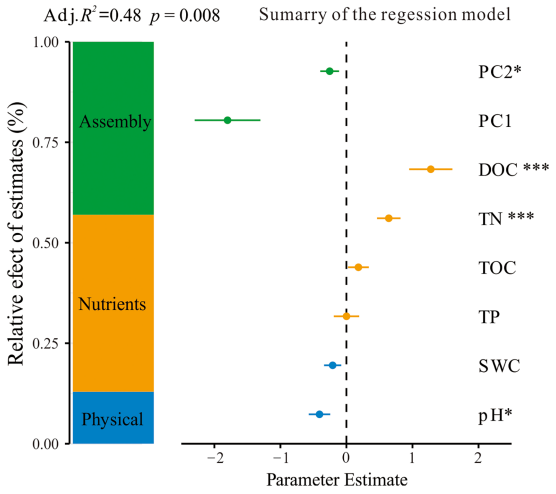

FIGURE 5
Effect of abiotic and biotic factors on total microbial abundance. Average parameter estimates (standardized regression coefficients) of model predictors, associated 95% confidence intervals, and relative importance of each factor, expressed as the percentage of explained variance. The adjusted (adj.) R^2 of the averaged model and the p-value of each predictor were given as: *p<0.05; **p<0.01; ***p<0.001. TOC, soil total organic carbon; DOC, dissolved organic carbon; TN, total nitrogen; TP, total phosphorus; SWC, soil water content. PC1 and PC2, the first and two axes' scores of the principal component analysis for microbial community composition.

study which found the depth effect on bacterial community assembly processes in paddy soils (Li W. T. et al., 2022).

Conclusion

We explored the change in microbial abundance in peatlands along the depth and altitudinal gradients on Changbai Mountain, China. Soil microbial abundance was more affected by soil depth than the altitude. The microbial abundance at 5–10 cm was higher than that at depth of 0–5 cm and 10–30 cm. The microbial abundance at 500–800 m was higher than that at altitude of 200–500 m and 800–1,500 m. The change in total microbial abundance was driven by both soil physiochemical properties and microbial co-occurrence network. Our study provides a new insight into the significance of microbial participation in peatland carbon cycling along the environmental gradient. It is important to consider the depth effects on soil microbial abundance when assess the peatland carbon dynamic under climate change.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MZ, MW, and GW designed the study. MZ, MW, YZ, NH, LQ, ZR, and MJ performed the field investigation and collected the data. MZ and GW conducted the statistical analysis and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer FL declared a shared affiliation with the authors MZ, YZ, NH, LQ, ZR, GW, and MJ to the handling editor at the time of review.

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EDITED BY
Huai Li,
Northeast Institute of Geography and Agroecology (CAS), China

REVIEWED BY
Xiaofei Yu,
Northeast Normal University, China
Weihua Guo,
Shandong University, China

*CORRESPONDENCE
Junhong Bai
junhongbai@163.com

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Characterizing bacterial communities in *Phragmites australis* rhizosphere and non-rhizosphere sediments under pressure of antibiotics in a shallow lake

Ling Zhang[1,2], Junhong Bai[1]*, Kegang Zhang[3], Zhuoqun Wei[1], Yaqi Wang[1], Haizhu Liu[1], Rong Xiao[4] and Milko A. Jorquera[5]

[1]School of Environment, Beijing Normal University, Beijing, China, [2]School of Chemistry and Chemical Engineering, Qinghai Normal University, Xining, China, [3]Department of Environmental Engineering and Science, North China Electric Power University, Baoding, China, [4]College of Environment and Safety Engineering, FuZhou University, Fuzhou, China, [5]Laboratorio de Ecología Microbiana Aplicada (EMALAB), Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco, Chile

Introduction: Antibiotics are ubiquitous pollutants and widely found in aquatic ecosystems, which of rhizosphere sediment and rhizosphere bacterial communities had certain correlation. However, the response of bacterial communities in *Phragmites australis* rhizosphere and non-rhizosphere sediments to antibiotics stress is still poorly understood.

Methods: To address this knowledge gap, the samples of rhizosphere (R) and non-rhizosphere (NR) sediments of *P. australis* were collected to investigate the differences of bacterial communities under the influence of antibiotics and key bacterial species and dominate environmental factors in Baiyangdian (BYD) Lake.

Results: The results showed that the contents of norfloxacin (NOR), ciprofloxacin (CIP) and total antibiotics in rhizosphere sediments were significantly higher than that in non-rhizosphere sediments, meanwhile, bacterial communities in non-rhizosphere sediments had significantly higher diversity (Sobs, Shannon, Simpsoneven and PD) than those in rhizosphere sediments. Furthermore, total antibiotics and CIP were found to be the most important factors in bacterial diversity. The majority of the phyla in rhizosphere sediments were *Firmicutes*, *Proteobacteria* and *Campilobacterota*, while *Proteobacteria*, *Chloroflexi* was the most abundant phyla followed by *Bacteroidota*, *Actinobacteriota* in non-rhizosphere sediments. The dominate factors of shaping the bacterial communities in rhizosphere were total antibiotics, pH, sediment organic matter (SOM), and NH_4-N, while dissolved organic carbon (DOC), NO_3-N, pH, and water contents (WC) in non-rhizosphere sediments.

Discussion: It is suggested that antibiotics may have a substantial effect on bacterial communities in *P. australis* rhizosphere sediment, which showed potential risk for ARGs selection pressure and dissemination in shallow lake ecosystems.

KEYWORDS
antibiotics, rhizosphere, bacterial community, sediments, shallow lake

Introduction

Antibiotics are widely used in medical treatment, agriculture, breeding, etc., and their superior performance has brought huge benefits. Most antibiotics ingested in humans or animals are not fully absorbed and metabolized, but enter the environment through excrement (Githinji et al., 2011). Some scholars found that higher concentrations of antibiotics were detected in the water and sediments of lakes, due to the discharge of waste water from pharmaceutical factories, sewage treatment plants, hospitals and farm in upstream or surrounding lakes (Xu et al., 2016; Wang et al., 2022).

Pollutants circulate slowly in the lake and are vulnerable to pollutants such as antibiotics due to slower pollutant circulation in the lakes than in other water environments (Nnadozie and Odume, 2019). Aquatic plants, as an important part of the lake ecosystem, also play an important role in the fate of pollutants in lakes (Zhang et al., 2022). Rhizosphere sediment regulates rhizosphere interactions, processes, antibiotics migration and transformation and thus play a vital role in maintaining plant health and ecosystem stability (Li et al., 2022). However, rhizosphere bacterial community also received much attention due to their associations with plant growth and pollution in lake ecosystems (Marschner et al., 2004). The bacterial community in rhizosphere soil can carry out material transformation. In turn, plants transfer their metabolites to the bacterial community in the form of root exudates, thereby affecting the changes of bacterial community (Huang et al., 2020). Pantigoso et al. (2020) presented that aquatic plants were sensitive to external pollution stimulation. However, rhizosphere bacterial communities with maximum microbial activities around plant roots could improve the plant's response to environmental stress, such as environmental pollution (Otto, 2021). There is multitude of interaction between the numerous microbes and plants in their habitats, which can greatly affect the migration and transformation of pollutants (Thacker and Quideau, 2021). Exogenous pollutants are frequently identified as the most important influencing factors in shaping the bacteria community (Nino Garcia et al., 2016). Therefore, the contaminated environment can result in the difference in microbial community structure and diversity between rhizosphere and non-rhizosphere soils (Thacker and Quideau, 2021; Fortin Faubert et al., 2022).

It has been well documented that plant roots shape and benefit from associated rhizosphere bacterial communities (Edwards et al., 2015; Perez-Jaramillo et al., 2018). What's more, rhizosphere bacterial communities play a role in supporting pest tolerance (Tronson et al., 2022) and increasing yield (Su et al., 2022). Therefore, the study of rhizosphere bacterial communities is important for the transformation of pollutants, plant health and agricultural production in lake ecosystems. Previous studies have shown that antibiotics can change the structure and function of rhizosphere soil bacterial communities in wetland systems (Tong et al., 2020). Shen et al. (2021) reported that alpha microbial diversity values were decreased for the lettuce non-rhizosphere soil rather than rhizosphere soil with antibiotic exposure in agricultural soil habitat.

However, few studies will involve the response of rhizosphere microorganisms to antibiotics in lake ecosystems, especially, simultaneous responses of bacterial communities to antibiotic stress in rhizosphere and non-rhizosphere sediments are poorly understood. Therefore, our research proposed the following primary objectives were: (1) To identify the differences in bacterial community structure and function under antibiotic exposure in *Phragmites australis* rhizosphere and non-rhizosphere sediments; (2) to explore key bacterial species in *P. australis* rhizosphere and non-rhizosphere sediments; and (3) to identify which environmental factors act as the main force for structuring bacterial communities in *P. australis* rhizosphere and non-rhizosphere sediments.

Materials and methods

Site description

Baiyangdian (BYD) Lake (38°43′ ~ 39°02'N, 115°38′ ~ 116°07′E, Figure 1) is the largest freshwater lake wetland in North China, known as the Kidney of North China, located in the Xiong'an New District of Hebei Province, with a total area of about 366 km^2. It has a typical temperate semi-arid continental monsoon climate, with the average annual rainfall of 552.7 mm and the evaporation of 1,637 mm, and its water level remains within the range of 1 ~ 3 m throughout the year. BYD Lake is covered by various species of aquatic macrophytes, of which *P. australis* is one of the dominant emergent macrophytes. The complex aquatic ecological conditions of BYD Lake provides various habitats for aquatic organisms. At present, as an important water body in the Xiong'an New Area, BYD has been given more and more concerns.

Sample collection and analysis

A total of 9 sampling sites were set up at the vegetation areas covered by *P. australis* of BYD Lake. We paired collected 9 samples of rhizosphere sediments (R) and non-rhizosphere soil (NR)

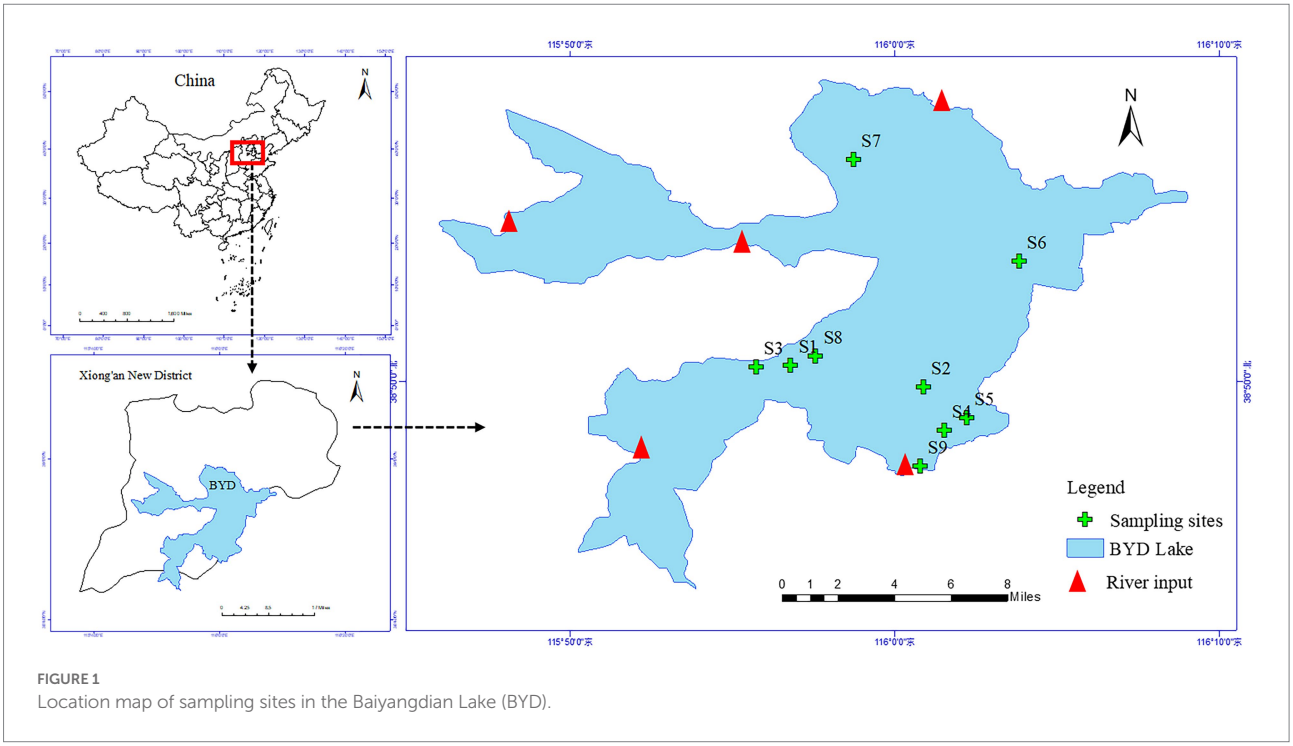

FIGURE 1
Location map of sampling sites in the Baiyangdian Lake (BYD).

surrounding *P. australis* in October 2020, respectively (Figure 1). Rhizosphere sediments were obtained from the root zone of *P. australis* following the protocol of Huang et al. (2020) by shaking off sediment that was loosely adhering to the roots. For each sampling site, non-rhizosphere sediments without any *P. australis* roots were collected by stainless steel static gravity dredger at least 50 cm away from vegetated areas. The collected sediments are stored in three parts to determine antibiotics, physicochemical properties and extract DNA for bioinformatics analysis. All samples were stored in the freezer and send to the laboratory as soon as possible. In the laboratory, one part of samples were freeze-dried in a vacuum freeze dryer, ground in an agate mortar, passed through a 100-mesh sieve, sealed in a plastic bag to physicochemical properties analyses. Another part of samples was stored at −20°C in the dark before antibiotic extraction and extracted and determined as soon as possible. The remaining samples were stored at −80°C to bacterial community analyses.

Zhang et al. (2022) the pH and EC values of the sediments were measured with a pH meter (model ST3100-F, OHAUS Co., Parsippany, NJ, United States, soil to water ratio is 1: 5) and a conductivity meter (HQ40d, Hach Co., Loveland, CO, United States). The water contents (WC) of the sediments were obtained by drying the soils at 105°C for 24 h in an oven. Sediment organic matter (SOM) was measured by dichromate oxidation-colorimetric method (Nelson and Sommers, 1982). The dissolved organic carbon (DOC) and total phosphorus (TP) in the sediments were measured using a Shimadzu TOC meter (TOC-LCPN, Japan) and an inductively coupled plasma atomic emission spectrometer (ICP-MS, Thermo Fisher Scientific, United States), respectively. The NO_3-N and NH_4-N in the sediments were measured on an element flow analyzer (AACE, Germany).

Quality assurance and quality control

The concentrations of nine antibiotics [norfloxacin (NOR), ofloxacin (OFL) and ciprofloxacin (CIP), oxytetracycline (OTC), tetracycline (TC), sulfapyridine (SPD) and sulfadiazine (SDZ), erythromycin (ERM) and roxithromycin (ROM)] was extracted and determinated according to our previous study (Zhang et al., 2022; Supplementary Table S1). Briefly, the antibiotics in the extract were determined by liquid chromatography-mass spectrometry (LC-ESI-MS/MS) in multiple reactive ion detection mode (MRM). An API 4500 QTrap liquid chromatography-mass spectrometer and Waters column (2.1 mm × 100 mm, particle size 1.7 μM) were used for the determination. The best separation conditions were a gradient elution of eluent A (0.1% formic acid ultrapure water) and eluent B (acetonitrile). Tandem mass spectrometry analysis was performed on a Micromass Quattro triple quadrupole mass spectrometer.

The optimized MS/MS parameters for the target antibiotics and antibiotic recoveries in sediment samples were tested, and the detailed results are shown in Supplementary Tables S2, S3. The antibiotic recovery rates ranged from 77.4 to 105.3% in rhizosphere sediments and from 76.9 to 108.6% in non-rhizosphere sediments. The limit of quantification (LOQ) calculated with a signal/noise ratio of 10 was 0.14 ng/g to 1.4 ng/g for rhizosphere sediments, and 0.4 ng/g to 2.6 ng/g for non-rhizosphere sediments.

DNA extraction and illumina sequencing

The FastDNA® SPIN kit for soil (MP Biomedicals, Solon, OH, United States) was used to extract DNA from 0.5 g homogenized soil. The concentration and purity of the DNA were determined with NanoDrop 2000 UV–vis spectrophotometer (Thermo Fisher Scientific, Wilmington, United States). Polymerase chain reaction (PCR) amplification was performed using the universal primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) designed against the V3-V4 region of bacterial 16S rRNA gene in an ABI GeneAmp® 9,700 PCR thermocycler (ABI, CA, United States). Amplicon sequencing was performed using the Illumina MiSeq platform at the Shanghai Majorbio Bio-pharm Technology Co., Ltd., in China.

The PCR amplification of 16S rRNA gene was performed as follows: initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, and single extension at 72°C for 10 min. The PCR was carried out in triplicates in a 20 μL reaction mixture containing 5× FastPfu Buffer, 2.5 mM dNTPs, 5 μM of each forward and reverse primer, FastPfu DNA Polymerase, BSA and 10 ng template DNA. PCR products were purified from a 2% agarose gel using an AxyPrep DNA Gel Extraction Kit (Axygen, United States), quantified using a QuantiFluor™-ST Blue Fluorescence Quantitative System (Promega Company), and pooled together for library preparation using a TruSeqTM DNA Sample Prep Kit (Illumina, San Diego, CA, United States) following the manufacturer's recommendations. Sequencing was performed using Illumina's Miseq PE300 platform. After splitting the PE reads obtained by MiSeq sequencing, the double-end reads were first quality-controlled and filtered according to the sequencing quality, and spliced according to the overlap relationship between the double-end reads to obtain optimized data after quality control splicing. Then, the sequence noise reduction method (DADA2/Deblur, etc.) is used to process the optimized data to obtain amplicon sequence variants (ASVs) representative sequence and abundance information. Based on ASV representative sequence and abundance information, bioinformatics analysis can be performed. Alpha diversities were used in our study to evaluate bacterial diversities (i.e., Shannon), abundance (i.e., Sobs), bacterial evenness (i.e., Simpsoneven), and phylogenetic diversities (PD). The sequence data were submitted to NCBI Sequence Read Archive with the accession number PRJNA899316.

Statistical analysis

SPSS software was used for descriptive statistical analysis of antibiotic contents in different environmental media. One-way ANOVA analysis was performed to test the differences in the concentrations of nine antibiotics among different sediments. The Random Forests model was used to identify the importance of environmental factors and the significance of the models and cross-validated R^2 values were assessed with 1,000 permutations of the response variable using the A3 package R. The significance level was described as $p < 0.05$, $p < 0.01$, or $p < 0.001$. These statistical analysis were performed using SPSS 24.0 for Windows and R (v4.1.1).[1]

Alpha diversity was calculated at the ASV levels. ANOVA with Duncan's test was performed to determine the differences in bacterial alpha diversities. Beta diversity expressed as principal coordinate analysis (PCoA) based on the Bray-Curtis distance matrix. The Spearman's correlation will be considered to be significant if the value of p is <0.05. Linear discriminant analysis effect size (LEfSe) analysis was conducted to identify the significantly changed bacterial and the relationships between sediments bacterial communities and the environmental variables were determined using canonical correlation analysis (CCA). These analyses were conducted using the vegan package R in free online platform of Majorbio Cloud Platform[2] (Yu et al., 2022).

Results

Antibiotics characteristics of *Phragmites australis* rhizosphere and non-rhizosphere sediments

The changes in antibiotic and physicochemical properties of *P. australis* rhizosphere and non-rhizosphere sediments properties are shown in Figure 2 and Supplementary Table S4. The total antibiotic concentrations in rhizosphere sediments ranged from 92.62 ng/g to 275.57 ng/g, with the average concentration of 153.45 ng/L, while that varied from 70.4845 ng/g to 285.72 ng/g, with the average concentration of 126.80 ng/g in non-rhizosphere sediments. Overall, rhizosphere sediments significantly enriched the antibiotics ($p < 0.05$, Figure 2A). Similarly, the concentrations of NOR (17.55 ± 0.98 ng/g) and CIP (53.97 ± 30.59 ng/g) were significantly higher in rhizosphere sediments than that in non-rhizosphere sediments (NOR: 14.62 ± 1.55 ng/g; CIP: 6.18 ± 4.84 ng/g) ($p < 0.05$, Figure 1D), while other antibiotics such as SPD, SDZ, OTC, TC, ERM, and ROM showed no significant difference between rhizosphere and non-rhizosphere sediments ($p > 0.05$, Figures 1B,C,G–J). However, significantly higher NH_4-N, NO_3-N, WC, and TP were observed in rhizosphere sediments than that in non-rhizosphere sediments ($p < 0.05$, Supplementary Table S4), while DOC and SOM were significantly higher in non-rhizosphere sediments ($p < 0.05$, Supplementary Table S4). The pH varied from 7.35 to 7.84 in non-rhizosphere sediments and from 7.45 to 7.54 in rhizosphere sediments ($p > 0.05$, Supplementary Table S4).

1 https://www.r-project.org/

2 https://cloud.majorbio.com/

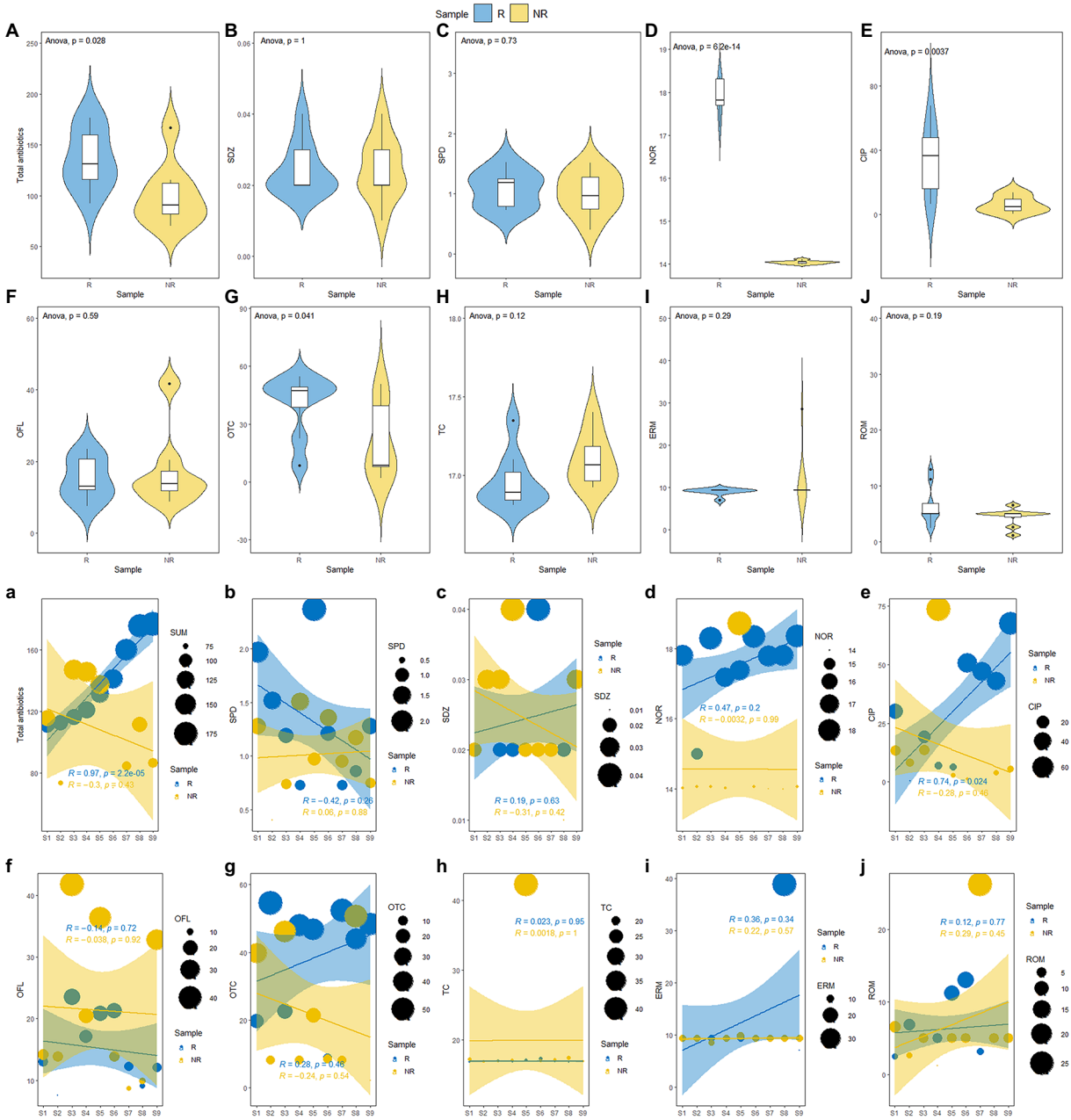

FIGURE 2
(A–J) Changes of antibiotic concentration *P. australis* rhizosphere and non-rhizosphere sediments and **(a-j)** along a gradient of increasing antibiotic concentration.

Our results showed that the total antibiotics were accumulated in *P. australis* rhizosphere sediments. Accordingly, we ranked the concentrations of total antibiotics in rhizosphere sediments from low to high (from S1 to S9, Figure 1A). The results showed that the total antibiotics showed various trends in the rhizosphere and non-rhizosphere sediments. The total antibiotics in *P. australis* rhizosphere sediments showed a significant linear increase from S1 to S9 ($R = 0.97$, $p < 0.001$, Figure 2A) and no significant ($p > 0.05$, Figure 2A) relationships in *P. australis* non-rhizosphere sediments with inflection point at S3 (147.12 ng/g). Similarly, there was a significant linear increase in the CIP concentration in rhizosphere sediments from S1 to S9 ($R = 0.74$, $p < 0.05$, Figure 2E) Besides, the concentrations of NH_4-N, NO_3-N, DOC, WC, and TP showed higher levels in non-rhizosphere sediments than those in rhizosphere sediments from S1 to S9, except for SOM. What's more, TP concentration showed a linear increase from S1 to S9 in non-rhizosphere sediments ($R = 0.65$, $p = 0.06$, Supplementary Figure S1). A different changing trend with a decrease before increasing was observed for NH_4-N ($R = -0.39$,

Supplementary Figure S1) and WC in non-rhizosphere sediments ($R = -0.20$, Supplementary Figure S1).

Effects of antibiotics on bacterial diversity

A total of 468,192 and 543,524 bacterial sequences were detected, 1,232 and 934 ASVs were clustered in rhizosphere and non-rhizosphere sediments, respectively. As shown in Figure 3. We evaluated the community richness (Sobs indices), community diversity (Shannon indices), community evenness (Simpsoneven indices) and phylogenetic diversity (PD) to further compare the bacterial community structure in rhizosphere and non-rhizosphere sediments. The alpha diversity of the bacterial communities in different sediment samples also exhibited certain differences (Figure 3). Results showed that non-rhizosphere sediments had significantly higher diversity (Sobs: 1271.22 ± 192.56, Shannon: 6.44 ± 0.18, Simpsoneven: 0.17 ± 0.08 and PD: 160.57 ± 22.37) compared with rhizosphere sediment (Sobs: 943.33 ± 137.77, Shannon: 5.48 ± 0.84, Simpsoneven: 0.071 ± 0.052 and PD: 131.16 ± 11.33) ($p < 0.05$, Figure 3). Correlation analysis results showed that bacterial Sobs indices ($r = -0.67$, $p < 0.05$, Supplementary Figure S2) and PD indices ($r = -0.80$, $p < 0.01$, Supplementary Figure S2) were significantly negatively correlated with NO_3-N concentrations in rhizosphere sediments, while positively correlated with NH_4-N ($r = 0.72$, $p < 0.05$) and CIP ($r = 0.65$, $p < 0.05$) in non-rhizosphere sediments (Supplementary Figure S2). In contrast, Shannon indices were positively correlated with OFL ($r = 0.72$, $p < 0.05$, Supplementary Figure S2), and simpsoneven indices were negatively correlated with SOM ($r = -0.75$, $p < 0.05$, Supplementary Figure S2) and positively correlated with NH_4-N, ROM and OFL ($r > 0.71$, $p < 0.05$, Supplementary Figure S2).To disentangle the potential main factor for bacterial diversity under antibiotics stress, we explored the relative importance of environmental factors to influence the bacterial diversity by the random forest analysis (Figure 4A). Total antibiotics (SUM) and CIP were found to be the most important factors in bacterial diversity. Meanwhile, the bacterial diversity (PD) exhibited strong linear correlation with total antibiotics ($R = 0.73$, $p < 0.05$, Figure 4C) and CIP ($R = 0.70$, $p < 0.05$, Figure 4C) in non-rhizosphere sediment. However, no significant differences ($p > 0.05$) in Sobs, Shannon and Simpsoneven indices were

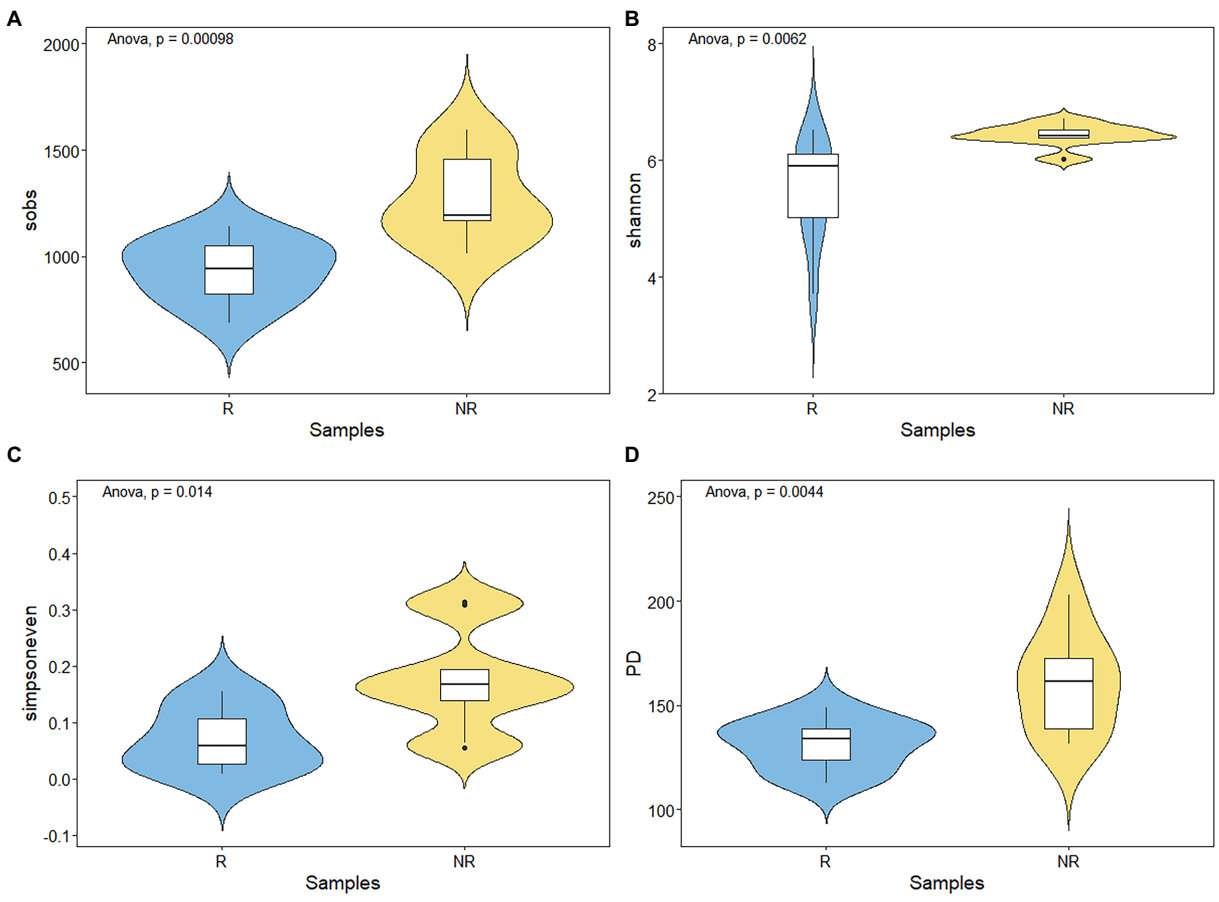

FIGURE 3
(A–D) Variations of bacterial alpha diversity in rhizosphere and non-rhizosphere sediments.

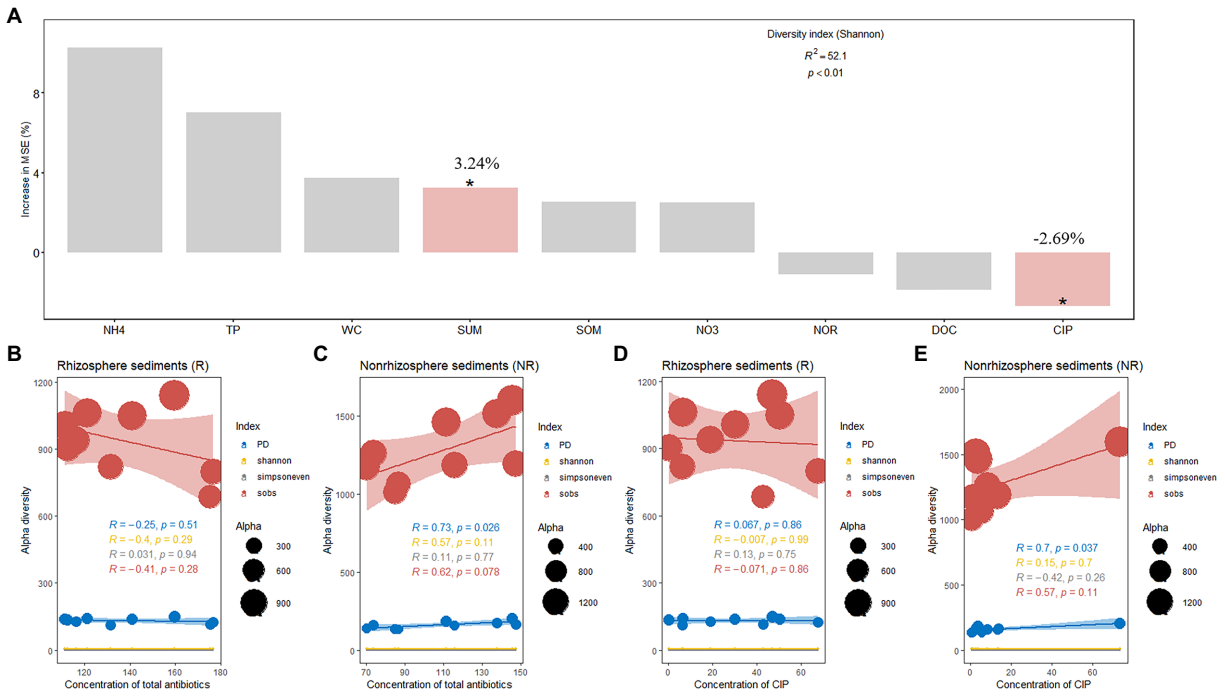

FIGURE 4
(A) Random forest indicating the potential factors of variation in bacterial alpha diversity and **(B–E)**, Effect of antibiotic concentration on bacterial alpha diversity.

observed both in rhizosphere and non-rhizosphere sediments as the antibiotic concentration increased (Figures 4B,C).

The variations in bacterial communities between rhizosphere and non-rhizosphere sediments were assessed by principal coordinate analysis (PCoA) based on Bray-Curtis distance measure (Supplementary Figure S3). Rhizosphere sediments showed significant differences in bacterial beta diversity compared with non-rhizosphere sediments ($p < 0.05$, Supplementary Figure S3), and The PC1 and PC2 axes explained 31.16% of the variation in the bacterial community. Along the PC2 axis, the bacterial community in rhizosphere sediments was separated from that in non-rhizosphere sediments.

Bacterial community changes in rhizosphere and non-rhizosphere sediments

As shown in Figure 5A, the ASVs were assigned to 53 phyla and 1,593 species for rhizosphere bacterial communities, and 62 phyla and 1714 species for non-rhizosphere bacterial communities. The majority of the bacterial ASVs were classified as *Firmicutes* (21.80% of the total relative abundance), *Proteobacteria* (19.94%), *Campilobacterota* (17.20%), *Chloroflexi* (13.56%), *Bacteroidota* (7.19%), *Actinobacteriota* (5.85%) at the phylum level in rhizosphere sediments, while *Proteobacteria* (24.75%), *Chloroflexi* (18.60%), *Firmicutes* (9.34%), *Acidobacteriota* (7.91%), *Actinobacteriota* (7.88%), *Desulfobacterota* (7.36%) dominated the sample bacterial community in non-rhizosphere sediments.

Variations in bacterial community composition with increasing antibiotic concentrations (from S1 to S9) is shown in Figure 5B. *Proteobacteria*, *Chloroflexi* and *Actinobacteriota* were the main phyla in sediment samples, with an increasing tendency, while *Firmicutes* and *Campilobacterota* demonstrated a slight decreasing tendency, in the rhizosphere sediments from S1 to S9 sampling sites. However, different trend has been observed from bacterial community composition in non-rhizosphere sediment samples. *Proteobacteria*, *Chloroflexi* and *Actinobacteriota* showed a decreasing tendency. Whereas, *Firmicutes* and *Campilobacterota* posed an increasing tendency from S1 to S9 sampling sites.

The LEfSe was performed to identify high-dimensional biomarker taxa with significantly different bacterial abundances in rhizosphere and non-rhizosphere sediments (Figure 6). In total, 14 and 28 bacterial abundant taxa were identified with an LDA threshold of 3.5 in rhizosphere and non-rhizosphere sediments, respectively (Supplementary Figure S5). The LEfSe results revealed that the specific bacterial biomarkers in rhizosphere sediments were phylum *Firmicutes* (including the class *Clostridia* and genera *unclassified_f__Lachnospiraceae*), and *Campilobacterota* (from phylum to genus *Pseudarcobacter* and class *Campylobacteria*). For the non-rhizosphere sediments, specific bacterial biomarkers were

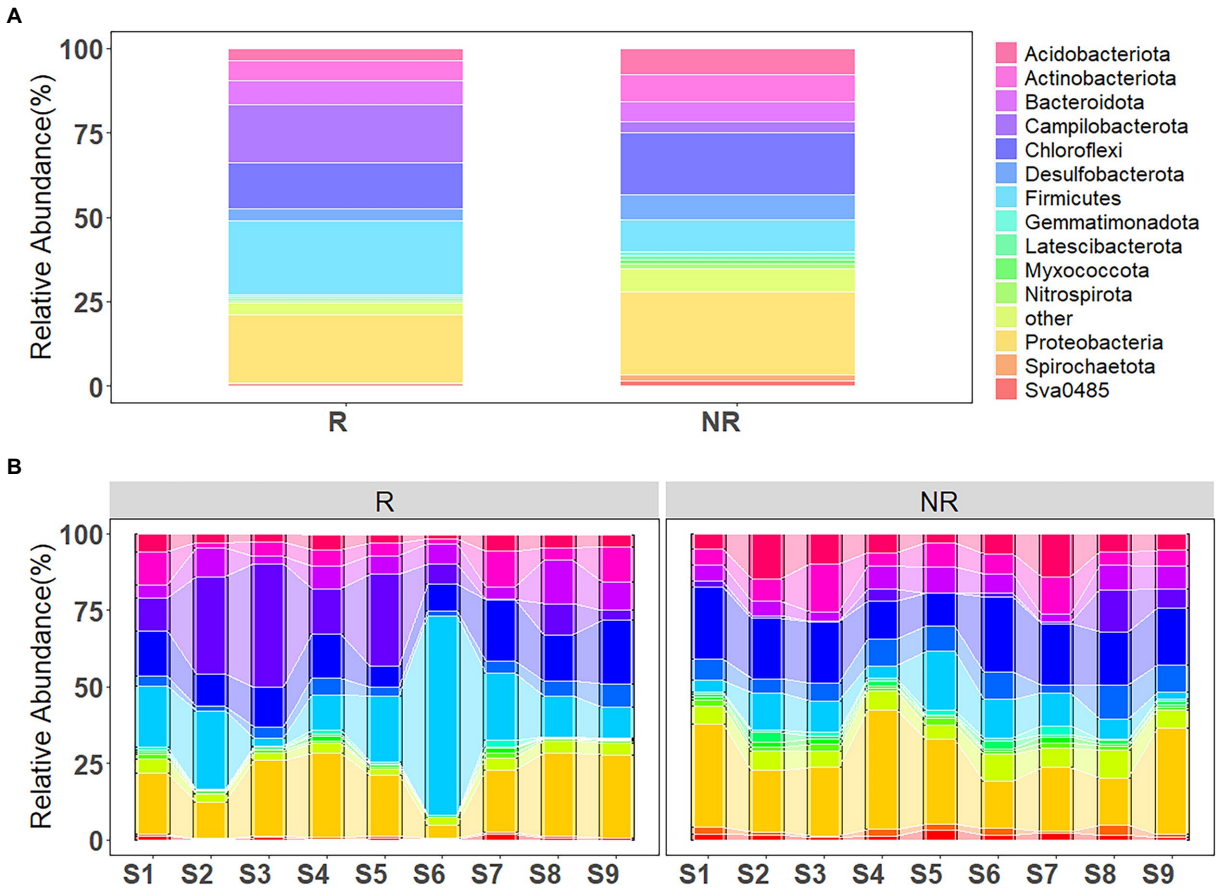

FIGURE 5
(A) Bacterial community composition in rhizosphere and non-rhizosphere sediments and **(B)** along the concentration gradient of antibiotics.

Acidobacteriota (from phylum to its genus *norank_f__norank_o__Vicinamibacterales*), *Desulfobacterota* (from phylum to its genus *norank_f__norank_o__Syntrophales and Desulfatiglans*), and *Vicinamibacteria* class, *Desulfobacteria* class.

Relationships between the soil environment and the bacterial communities

The relationships between the bacterial community and rhizosphere or non-rhizosphere sediments environmental factors were analyzed by CCA (Figures 7A,B). As for rhizosphere sediments (Figure 7A), the first CCA axis was mainly positively correlated with total antibiotics and negatively correlated with pH, NH_4-N and DOC, while the second CCA axis was mainly positively correlated with pH and total antibiotics and negatively correlated with TP and SOM. However, the first CCA axis was mainly positively correlated with NH_4-N, pH and DOC and negatively correlated with SOM, TP, NO3 and WC in non-rhizosphere sediments (Figure 7B).

Compared with non-rhizosphere bacterial communities, the rhizosphere bacterial communities had a more regular correlation with environmental factors in the correlation heatmap analysis (Figure 8). For rhizosphere bacterial communities, the relative abundance of the two most dominant phyla *Firmicutes* ($r = -0.68$) and *Proteobacteria* ($r = 0.68$) significantly correlated with sediment pH ($p < 0.05$). Nevertheless, the relative abundance of *Campilobacterota* had significant correlations with DOC ($r = 0.67$, $p < 0.05$), CIP ($r = -0.7$, $p < 0.05$) and SPD ($r = 0.76$, $p < 0.05$). Specifically, the relative abundance of *Bacteroidota* had significant negative correlations with TC ($r = -0.82$, $p < 0.01$), NO_3-N ($r = -0.72$, $p < 0.05$) and SOM ($r = -0.71$, $p < 0.05$). However, OFL and TC showed significant correlations with *Nitrospirota* ($r > 0.91$, $p < 0.001$), *Sva0485* ($r > 0.76$, $p < 0.05$), *Methylomirabilota* ($r > 0.85$, $p < 0.01$), *Latescibacterota* and TP was significantly correlated with $r = -0.86$ in rhizosphere sediment, specifically. *Patescibacteria* ($r = -0.85$, $p < 0.01$), *Sva0485* ($r = 0.71$, $p < 0.05$) was significantly correlated to SOM.

In non-rhizosphere, NOR and SOM were significantly negatively correlated with the relative abundances of *Cyanobacteria* ($r > -0.72$, $p < 0.05$) and *Campilobacterota* ($r > -0.68$, $p < 0.05$),

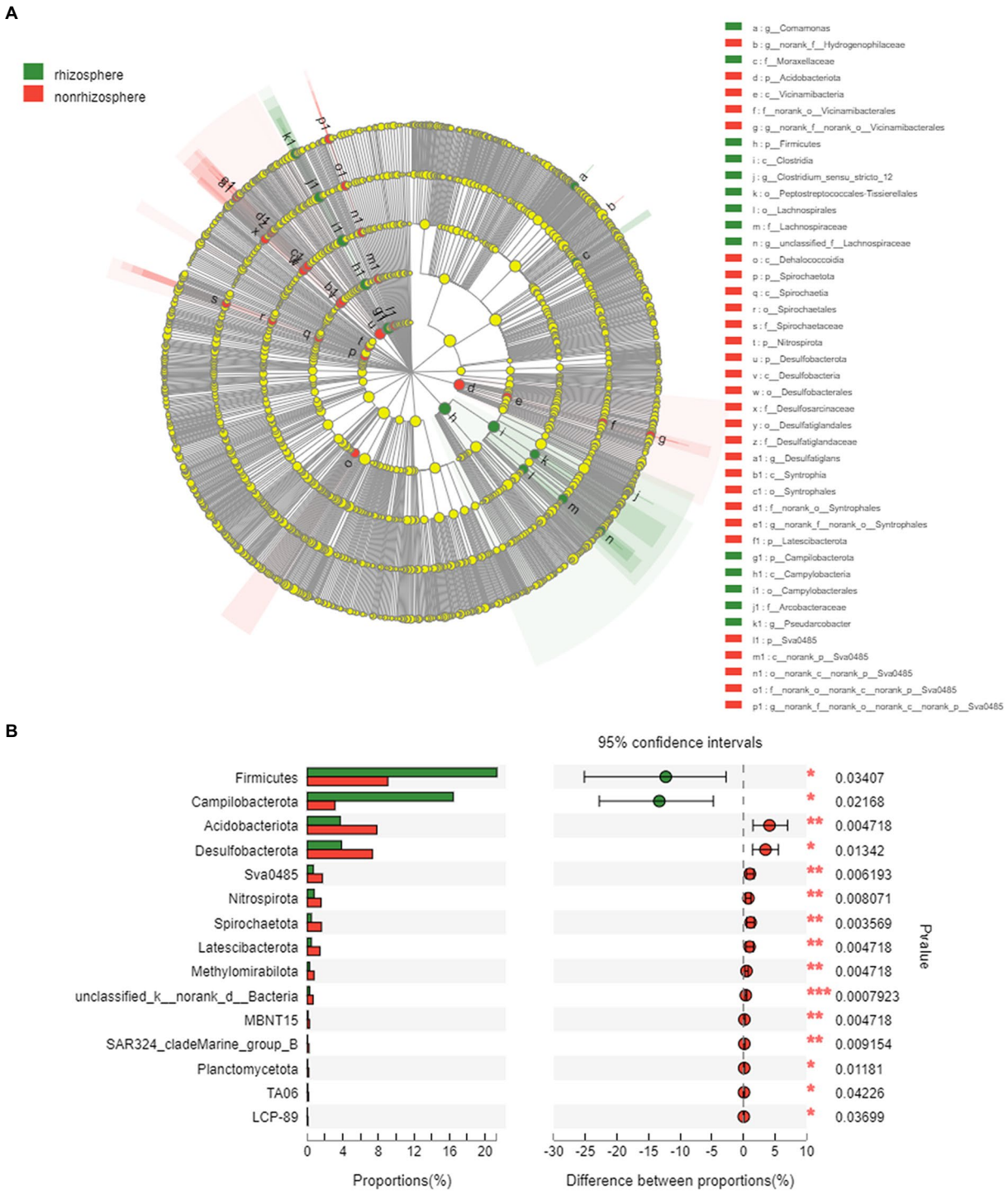

FIGURE 6
(A) LEfSe analysis of bacteria on rhizosphere and non-rhizosphere sediments (LDA>3.0) and **(B)** Key differential species in rhizosphere and non-rhizosphere sediments.

while significantly positively correlated with the relative abundances of *Nitrospirota* ($r > 0.83$, $p < 0.01$). Moreover, *Chloroflexi* was significantly positively correlated with OTC ($r = 0.7$, $p < 0.05$). Besides, the relative abundances of *Nitrospirota* were significantly positively correlated with OFL ($r = 0.78$, $p < 0.05$) and WC ($r = 0.68$, $p < 0.05$). Similarly, the relative abundances of *Actinobacteria* and *Desulfobacterota* were significantly positively correlated with SOM ($r = 0.76$, $p < 0.05$) and pH ($r = 0.67$ $p < 0.05$), respectively.

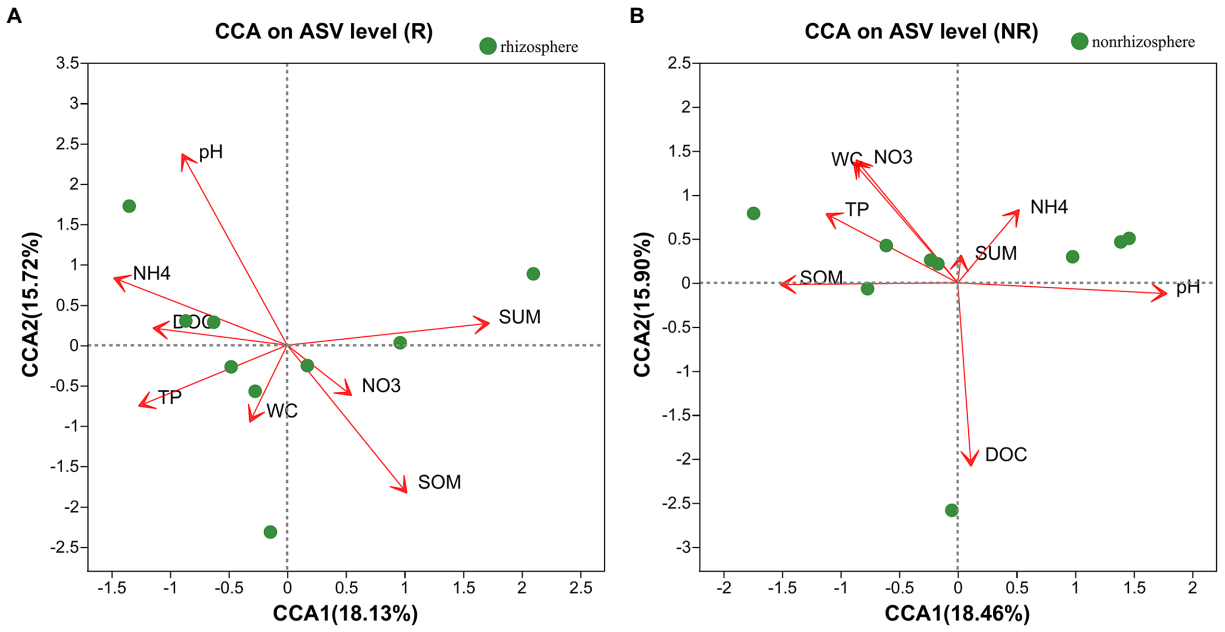

FIGURE 7
(A,B) CCA analysis between bacterial communities and environmental factors.

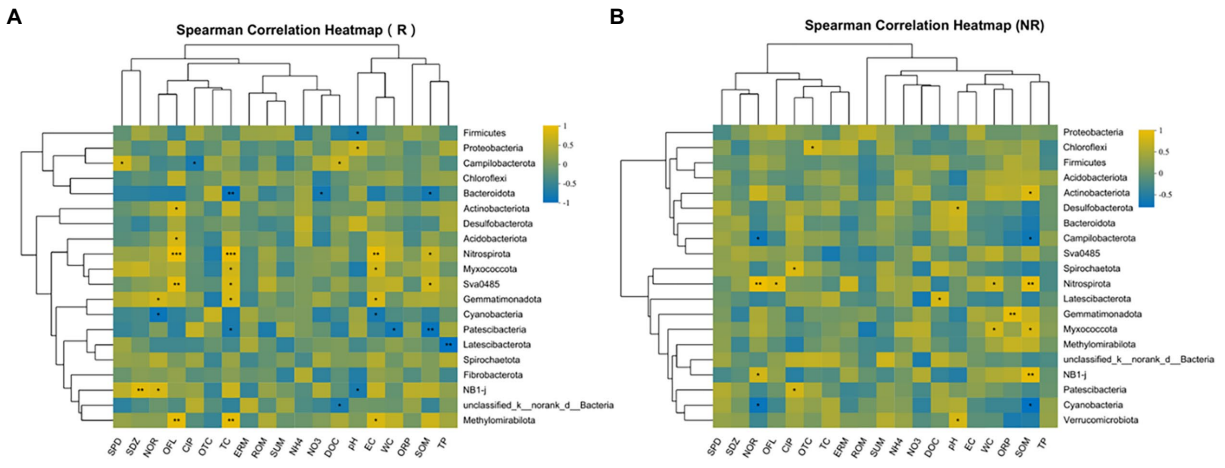

FIGURE 8
(A,B) Correlation between environmental factors and microorganisms at phylum level.

Discussion

Changes in bacterial communities under antibiotics stress

The rhizosphere is the critical zone where roots access water and nutrients, and interact intimately with the physical, chemical, and biological components of the soil or sediments (Wang et al., 2020). Rhizosphere processes have an important role in the fate of nutrient pollutants in the rhizosphere environment (Lynch, 2019). Rhizosphere bacterial communities also are important for plant growth by influencing SOM breakdown and improving pollutant accumulation by plant roots in the rhizosphere (Marschner et al., 2004; Reinhold-Hurek et al., 2015). Our results showed that the CIP and NOR and total antibiotics in rhizosphere sediments were higher than that in non-rhizosphere sediments, which is consistent with previous result by Chen et al. (2018). This indicated that the

accumulation of antibiotics by plant roots in the rhizosphere was stronger due to rhizosphere bacteria may stimulate some transporter protein (Souza et al., 1999). However, CIP and NOR are more easily deposited in sediments due to the adsorption of suspended matter in water and organic matter in sediments (Gong et al., 2012; Zhang et al., 2022). Therefore, the accumulation of CIP and NOR in aquatic plants mainly occurs in roots (Hoang et al., 2013). However, the degradation of microorganisms in rhizosphere was not strong enough to cause a significant difference between rhizosphere and non-rhizosphere sediments (Song et al., 2020).

Our results showed that the richness, diversity, evenness and phylogenetic diversity of bacterial community in rhizosphere and non-rhizosphere sediments were significantly affected by antibiotics (such as NOR, OFL TC, OTC, etc., Figure 8). Previous studies have also confirmed that the alterations in composition and diversity of bacteria were caused by pollutants from the effluent discharge (Pascual-Benito et al., 2020). Moreover, alpha diversity of bacterial communities observed in non-rhizosphere sediments was significantly higher than that in rhizosphere sediments (Figure 3), which indicates that different root zones have certain effects on microbial diversity. Zhou et al. (2022) also reported that habitat (rhizosphere or bulk) accounted for the greatest amount of variation in bacterial community composition, overriding the importance of environmental interference (such as seasonal and flooding conditions) on these bacterial communities. In our study, the main factors causing the differences of microbial diversity in rhizosphere and non-rhizosphere sediments may be the antibiotics mentioned above and the physicochemical properties of sediments, which could change bacterial community diversity (Zhang et al., 2022).

Rhizosphere and non-rhizosphere sediments have very distinct bacterial communities and specific bacterial biomarkers (Figure 6), which are the integrated result of many different selection factors (Carelli et al., 2000). These factors include the physical and chemical properties of the sediments (e.g., DOC, SOM and pH, etc., Figure 7) and environmental factors such as pollutants. In rhizosphere sediments, the concentrations of total antibiotics, NH_4-N, NO_3-N, WC and TP were significantly higher than those in non-rhizosphere sediments, while DOC and SOM were significantly higher in non-rhizosphere sediments (Supplementary Table S4). Previous studies reported that rhizosphere microorganisms are affected by the sediment properties, such as pH (Han et al., 2020). Therefore, the significant change in sediment properties could cause the variations in the bacterial community composition in current study. Marschner et al. (2004) also presented similar results that rhizosphere soil bacterial communities can be influenced by soil chemical and physical properties. As a result, *Firmicutes*, *Proteobacteria* were the most abundant phyla followed by *Bacteroidota*, *Actinobacteriota* in rhizosphere sediments, while *Proteobacteria*, *Chloroflexi*, *Firmicutes*, *Acidobacteriota* were dominated bacterial phyla in non-rhizosphere sediments (Figure 5).

Links between sediment bacterial communities and environmental factors

The rhizosphere sidiment is important zone where sediments interact with bacterial communities, and environmental factors are responsible for the shaping of bacterial communities (Wang et al., 2020). It has been previously reported that root exudates from plant rhizosphere sedimentscan alter the chemical composition of rhizosphere sediments and affect the behavior of contaminants (Chen et al., 2018). Our result showed that major environmental factor affecting bacterial diversity in rhizosphere were SOM, pH, and total antibiotics (Figure 7A) and in non-rhizosphere sediment were DOC, pH, WC and NO_3-N (Figure 7B). What' more, total antibiotics was accumulated in rhizosphere sediment. This is consistent with Katoh et al. (2016) study that the rhizosphere sediments increase the availability of pollution by reducing the rhizosphere pH.

The nutrients provided by root exudates can enrich the bacterial community in rhizosphere sediments and may promote the biodegradation of pollutants (2016). However, in our study this phenomenon is not obvious, as we mentioned earlier, may be the effect of antibiotic bioaccumulation greater than biodegradation. It has been reported that environmental factors were the main driver of bacterial communities (Song et al., 2020). NOR, OFL, and CIP showed strong significant correlations with the dominated bacterial phyla ($p < 0.05$; Figure 8). This could be explained by the fact that these antibiotics (i.e., NOR, OFL, and CIP) are more likely to be retained in sediments (Gong et al., 2012) and exert selective pressure on microorganisms (Gong et al., 2012; Karkman et al., 2019). Additionally, We also found that WC and SOM were significantly related to the dominated bacterial phyla ($p < 0.05$, Figures 7, 8), which is also identified by previous result that moisture and organic matter as significant factors controlled the variation in bacterial communities in the aquatic environment (Chen et al., 2019; Song et al., 2020). Although Song et al. (2020) reported that pH and EC played the most significant role in shaping bacterial communities. However, in current study, pH and EC presented no significant correlation with all phyla in non-rhizosphere sediments and significant correlation with several phyla in rhizosphere sediments, which might explain the positive association of oxygen regulation by bacterial communities (Huang et al., 2020) and root exudates (Lv et al., 2020).

Conclusion

This study investigated the differences in bacterial community and key bacterial species as well as their main influencing factors in rhizosphere and non-rhizosphere sediments. Our study highlighted the antibiotics remarkably altered the diversity and composition of bacterial communities between *P. australis* rhizosphere and non-rhizosphere sediments. More antibiotics were accumulated in *P. australis* rhizosphere sediments, while non-rhizosphere sediments had higher bacterial diversity, which

may be mainly influenced by total antibiotics and ciprofloxacin (CIP). The main environmental factors affecting bacterial community structure in rhizosphere and non-rhizosphere sediments are different. Total antibiotics, pH and SOM played essential roles in shaping the bacterial communities in *P. australis* rhizosphere sediments, while DOC, NH_4-N, pH and WC could be responsible for the variations bacterial communities in non-rhizosphere sediments. Meanwhile, rhizosphere sediments showed higher potential risks for ARGs selection pressure and dissemination. Overall, these findings suggest that antibiotics significantly changed diversity and structure of bacterial communities, which indicates the utmost importance of developing corresponding control, monitoring, management strategies for antibiotics pollution. Further studies should be carried out to explore selective pressure of antibiotics on bacterial communities from sediments to waters.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

LZ: writing—investigation and original draft. JB: funding acquisition, and writing—review and editing. KZ, RX, and MJ: review and editing. ZW, YW, and HL: writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1092854/full#supplementary-material

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EDITED BY
Zifang Chi,
Jilin University,
China

REVIEWED BY
Bin Chen,
Beijing Normal University,
China
Meng Wang,
Northeast Normal University,
China

*CORRESPONDENCE
Changchun Song
songcc@iga.ac.cn

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Soil CO_2 and N_2O emissions and microbial abundances altered by temperature rise and nitrogen addition in active-layer soils of permafrost peatland

Yanyu Song[1], Xiaofeng Cheng[1], Changchun Song[1,2]*, Mengting Li[1,3], Siqi Gao[1,4], Zhendi Liu[1,4], Jinli Gao[1] and Xianwei Wang[1]

[1]Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, China, [2]School of Hydraulic Engineering, Dalian University of Technology, Dalian, China, [3]College of Tourism and Geographical Science, Jilin Normal University, Siping, China, [4]College of Resource and Environment, University of Chinese Academy of Sciences, Beijing, China

Changes in soil CO_2 and N_2O emissions due to climate change and nitrogen input will result in increased levels of atmospheric CO_2 and N_2O, thereby feeding back into Earth's climate. Understanding the responses of soil carbon and nitrogen emissions mediated by microbe from permafrost peatland to temperature rising is important for modeling the regional carbon and nitrogen balance. This study conducted a laboratory incubation experiment at 15 and 20°C to observe the impact of increasing temperature on soil CO_2 and N_2O emissions and soil microbial abundances in permafrost peatland. An NH_4NO_3 solution was added to soil at a concentration of 50mgNkg^{-1} to investigate the effect of nitrogen addition. The results indicated that elevated temperature, available nitrogen, and their combined effects significantly increased CO_2 and N_2O emissions in permafrost peatland. However, the temperature sensitivities of soil CO_2 and N_2O emissions were not affected by nitrogen addition. Warming significantly increased the abundances of methanogens, methanotrophs, and *nir*K-type denitrifiers, and the contents of soil dissolved organic carbon (DOC) and ammonia nitrogen, whereas *nir*S-type denitrifiers, β-1,4-glucosidase (βG), cellobiohydrolase (CBH), and acid phosphatase (AP) activities significantly decreased. Nitrogen addition significantly increased soil *nir*S-type denitrifiers abundances, β-1,4-N-acetylglucosaminidase (NAG) activities, and ammonia nitrogen and nitrate nitrogen contents, but significantly reduced bacterial, methanogen abundances, CBH, and AP activities. A rising temperature and nitrogen addition had synergistic effects on soil fungal and methanotroph abundances, NAG activities, and DOC and DON contents. Soil CO_2 emissions showed a significantly positive correlation with soil fungal abundances, NAG activities, and ammonia nitrogen and nitrate nitrogen contents. Soil N_2O emissions showed positive correlations with soil fungal, methanotroph, and *nir*K-type denitrifiers abundances, and DOC, ammonia nitrogen, and nitrate contents. These results demonstrate the importance of soil microbes, labile carbon, and nitrogen for regulating soil carbon and nitrogen emissions. The results

of this study can assist simulating the effects of global climate change on carbon and nitrogen cycling in permafrost peatlands.

KEYWORDS

climate warming, nitrogen availability, soil microbial abundance, enzyme activity, boreal peatland

Introduction

Soil carbon dioxide (CO_2) emissions represent the second largest carbon (C) flux in terrestrial ecosystems, accounting for 70–90% of total ecosystem respiration (Schlesinger and Andrews, 2000; Cascio et al., 2017). Losses of soil C to the atmosphere through soil heterotrophic respiration play an important role in regulating atmospheric CO_2. These losses are predicted to increase due to climate change, resulting in a positive C-climate feedback loop (Yuan et al., 2019; Dacal et al., 2022). The availability of nitrogen (N) changes the source-sink dynamics of ecosystem C by changing the soil CO_2 flux (Cascio et al., 2017). Soils also act as an important source-sink for nitrous oxide (N_2O; Wu et al., 2013, 2015). Climate warming and the input of N could change mineralization of soil N and N_2O emissions (Ma et al., 2011). The increases in N_2O emissions can cause changes in global warming potential, thus affecting the C sinks and CO_2 emissions (Muhammad et al., 2022). However, little is known about how increases in temperature and N inputs interact to regulate soil emissions of CO_2 and N_2O and their temperature sensitivity. An increased comprehension of the microbial mechanisms under warming and N addition impact emissions of CO_2 and N_2O is vital for accurately simulating the consequences of a changing global climate on the C and N balance.

Low temperatures and nutrient concentration limited soil microbial activities and soil organic matter (SOM) decomposition (Koyama et al., 2014). An increase in temperature results in enhanced microbial growth and in the activation of the functional genes involved in C and N cycling (Xue et al., 2016; Wang et al., 2019). These result in increased soil C decomposition and respiration (Han et al., 2013). However, a previous study noted a reduction in N_2O production with increasing temperature, especially due to denitrification (Duan et al., 2019), whereas the abundances of *amo*A, *nif*H, and *nir*K increased (Jung et al., 2011; Han et al., 2013). Warming could increase N limitation of microorganisms, which, in turn, could limit the impact of increased temperature on SOM mineralization. Previous studies found that N addition increased the abundances of C decomposition and N cycling genes (Jung et al., 2011; Wang et al., 2019), leading to a stronger positive correlation between soil available N and microbial properties exposed to elevated temperature (Huang et al., 2022). Greater insight into the impacts of warming and the addition of N on soil microorganisms can assist in improving understanding of the reactions of soil C and N emissions to a global changing climate.

Soil enzymes catalyze breakdown of high molecular weight compounds, and play important functions in SOM degradation (Yao et al., 2015), measuring their activities can provide useful indicators of soil emissions of CO_2 and N_2O (Chen et al., 2017). Soil enzyme activities can be used to investigate microbial nutrient cycling due to their connections with active microbial biomass, including microbial responses to environmental changes, transformation rates, and the location of the most active biomass (Wang et al., 2015). Warming can result in changes in enzyme activities, leading to functional changes in soil ecosystem processes (Xu et al., 2015). An improved understanding of decomposition and mechanisms of microbial enzyme production can assist in constraining long-term responses to warming (Sihi et al., 2016). Moreover, enzyme activities were applied as indicators of the impacts of N input within many recent experiments since they reflect the metabolic needs of soil microbial communities relative to available nutrients (Ochoa-Hueso et al., 2013). Nitrogen addition significantly stimulated activities of N- and phosphorus-acquiring hydrolytic enzymes and depressed the activities of oxidative enzymes (Tu et al., 2014). Maslov and Maslova (2021) investigated the effect of increased N availability on changes in soil enzyme activities to better understand the internal mechanisms of soil C and N cycling processes. Improved comprehension of soil enzymes and their regulatory mechanisms is needed to enhance comprehension of the impacts of temperature and N availability on soil CO_2 and N_2O emissions.

Peatlands represent an important C pool on Earth, storing 1,055 Gt of soil C, even though they only cover 3% of the land surface of the Earth (Nichols and Peteet, 2019). In particular, permafrost peatlands experience increased storage and emissions of C, and can act as key contributors to global warming. Permafrost thaw in northern peatlands results in alterations to ground thermal conditions, moisture, and chemistry, which, in turn, regulate microbial activities responsible for generating greenhouse gases (GHGs) from decomposing organic matter (Kirkwood et al., 2021). Newly thawed permafrost in Western Canada is predicted to release 0.2 to 25% of stored C by 2,100 (Jin and Ma, 2021). An increase in annual temperature by 1°C was predicted to increase respiration by up to 60% in an experiment conducted in Arctic blanket peatland (Dorrepaal et al., 2009). Moreover, increases in N input affected N_2O emissions in northern peatlands due to increased N availability and/or changing vegetation composition

(Le et al., 2020). Nitrogen addition could mitigate the positive effect of warming on methane fluxes in a coastal bog (Gong et al., 2021). However, the synergistic environmental parameters regulating GHGs emissions in northern permafrost peatlands remain largely unknown (AminiTabrizi et al., 2020). Clarifying the synergistic effects of both climate warming and a rising nitrogen availability on permafrost emissions of CO_2 and N_2O can provide a reference for future studies on potential responses of C and N sequestration of high latitude peatlands to climate change.

Northeastern China contains the second largest expanse of permafrost in China, primarily known as Xing'an-Baikal permafrost. This permafrost area lies on the southeastern edges of the Eurasian cryolithozone and is thermally unstable and sensitive to external changes (Wei et al., 2011). By the 2010s, the area of Xing'an-Baikal permafrost in Northeast China had declined by 40.6% compared with that in the 1960s (Li et al., 2021). The present study aimed to understand the synergistic effects of both climate warming and rising N availability on soil emissions of CO_2 and N_2O and its regulation mechanism in permafrost peatlands. An incubation experiment with temperature increase of 5°C and nitrogen addition of 50 mg N kg^{-1} was conducted in the Great Xing'an mountain peatland, Northeast China. The objectives of this research were to explore the response of CO_2 and N_2O emissions from permafrost peatland soil to warming and nitrogen addition, and clarify their driving mechanisms, which can help improve future predictions of responses of soil C and N cycling to climate warming.

Materials and methods

Site description and soil sampling

The study site of the present study is a typical permafrost peatland nearby the Tuqiang Forestry Bureau, Great Xing'an Mountain (52°44′N, 122°39′E), Heilongjiang Province, China. Average yearly temperature and average yearly precipitation are −3.9°C and 452 mm, respectively. The dominant species of plants are *Vaccinium uliginosum* L., Moench, Sphagnum spp., *Ledum palustre* L., *Eriophorum vaginatum* L., and *Chamaedaphne calyculata* L. The soil type of the study area according to the United States Department of Agriculture (USDA) classification system is Glacic Histoturbels (Soil Survey Staff, 2010). A soil sample of the active layer (0–20 cm) was obtained using a hand auger soil core sampler, which was filtered through a 2-mm sieve. The total C (TC) and total N (TN) of the soil sample before incubation experiments were 408.74 and 15.34 g kg^{-1}, respectively, whereas soil moisture and pH were 77.18% and 5.49, respectively.

Laboratory incubation

Fresh soil samples (15 g according to completely dry soil) were placed in 500-ml glass flasks and preincubated at 15°C for 7 days. NH_4NO_3 solution (2 ml) was uniformly added to soil at a concentration of 50 mg N kg^{-1}, with four replicates prepared. Deionized water (2 ml) was added to the control treatment. The flask lids were sealed with rubber septa to allow the analysis of rates of emissions of CO_2 and N_2O at 15 and 20°C (maximum monthly mean temperature in July of 18.4°C). These soils were incubated continuously for 18 days. Trapped air in the jars was removed for CO_2 and N_2O determination at intervals of 2 h, 1, 2, 3, 5, 7, 9, 12, 15, and 18 days. Headspace gas in the jars was extracted using a 50-ml syringe with a three-way valve. The concentrations of CO_2 and N_2O were measured utilizing a gas chromatograph (Agilent 7890B, United States). Deionized water corresponding to the reduction in weight after each collection of gas was added. Soil samples were collected to determine soil microbial abundances, enzyme activities, and labile C and N contents at the end of incubation.

Soil microbial abundances analysis

Soil DNA was extracted from a 300-mg subsample using a FastDNA spin Kit (MPbio, Santa Ana, CA, United States) in accordance with the manufacturer's instructions. Bacterial 16S rRNA, fungal IST, and functional genes encoding *mcr*A, *pmo*A, *nir*S, and *nir*K were quantitatively evaluated *via* qPCR using an ABI StepOne instrument (Applied Biosystems, San Francisco, CA, United States). Supplementary Table S1 lists the primers and amplification details used in the present study. The PCR mixture contained 10 ng soil DNA, 0.4 µl primers (10 µM), and 12.5-µl of SYBR Buffer (TaKaRa, Beijing, China) in a final volume of 25 µl. qPCR standard curves were created by purifying amplicon products of functional and phylogenetic markers using a cyclic purification kit (Omega Bio-Tek, United States), ligated to the pMD18-T (TaKaRa) vector, and transforming into *Escherichia coli*. A plasmid mini kit (Omega Bio-Tek, United States) was utilized to remove the plasmids, with a standard local alignment searching tool used to identify specificity of plasmids. Standard curves were produced by plasmid serial dilution (Song et al., 2021).

Soil enzyme activities measurement

The potential activities of acid phosphatase (AP), β-1,4-glucosidase (βG), cellobiohydrolase (CBH), and NAG were measured for absorbance using a microplate spectrophotometer. Aliquots (200 µl) of slurry (1 g fresh soil sample homogenized in 125-ml 50-mM acetate buffer, pH 8) and 50-µl of substrate solution (200 µM) were placed into 96-well microplates. Every microplate had eight replicate wells per assay, as well as negative and positive controls for quench correction. The microplates were incubated in darkness at 20°C for 4 h. Excitation and emission fluorescence were identified at 365 and 450 nm, respectively using Cell Imaging Multi-Mode Reader (BioTek Cytation 5, United States).

Soil carbon and nitrogen content measurement

Soil ammonia nitrogen (NH_4^+-N), nitrate (NO_3^--N), and dissolved organic N (DON) were extracted through the addition of 2 M KCl at a 1:15 ratio, followed by 1 h of shaking at 150 rpm at a temperature of 20°C. DON concentrations of soil were calculated as the difference between total dissolved N and inorganic N. Soil dissolved organic C (DOC) contents were analyzed using a Multi N/C 2100 analyzer (Analytik Jena AG, Germany) after extracting fresh soil with a 2 M KCl solution. Soil TN contents were analyzed after digestion with sulfuric acid (H_2SO_4) and potassium sulfate (K_2SO_4), with cupric sulfate ($CuSO_4$) used as a catalyst. The products of digestion were subsequently analyzed using an AA3 continuous flow chemical analyzer (Seal Analytical, Germany). Quantification of soil moisture was by oven drying of fresh soil at 105°C to a constant weight. The pH of soil was measured using a 1:10 soil-deionized water slurry.

Data analyses

Statistical analyses were performed in the SPSS 24.0 package. Results are shown as the average ± standard error. A two-way analysis of variance (ANOVA) was performed to evaluate the interactions between increasing temperature and addition of N on soil emissions of CO_2 and N_2O, microbial abundances, enzyme activities, and contents of soil C and N. Linear regression analysis was conducted to explore relationships between the soil CO_2 and N_2O emissions and soil microbial abundances, enzyme activities, and soil C and N contents.

The temperature sensitivities (Q_{10}) of soil CO_2 and N_2O emission rates per 10°C were calculated as follows:

$$Q_{10} = \left(\frac{K_2}{K_1}\right)^{\frac{10}{T_2 - T_1}}$$

where T_1 and T_2 is the incubation temperatures for 15 and 20°C, respectively. K_1 and K_2 is the CO_2 (mg CO_2-C kg^{-1} d^{-1}) and N_2O (μg N_2O-N kg^{-1} d^{-1}) emission rates at 15 and 20°C, respectively.

Results

Emissions of soil CO_2 and N_2O and their sensitivity to temperature

An increase in temperature significantly stimulated soil emissions of CO_2 and N_2O in the permafrost peatlands (Figures 1A,B). Soil CO_2 and N_2O emissions in the control increased by 53.57 and 45.50% at 20°C compared to that at 15°C, respectively. The addition of N resulted in increases in CO_2 and N_2O emissions by 52.34 and 54.53% at 20°C compared to that at 15°C, respectively. The cumulative CO_2 and N_2O emissions were significantly higher under N addition than that in the control at 15°C and 20°C (Figures 2A,B). The increase in cumulative N_2O emissions after the addition of N was significantly higher than the increase in CO_2. There were significant interactions between rising temperature and addition of N on both CO_2 and N_2O emissions ($p < 0.05$; Table 1). The sensitivities of soil CO_2 and N_2O emissions to temperature in the control were 2.37 and 2.36, respectively. The addition of N did not impact the Q_{10} values of CO_2 and N_2O emissions of 2.50 and 2.44, respectively (Figure 2C).

Soil microbial abundances

Among the microbial community, bacteria were the most abundant ($6.08–14.52 \times 10^{12}$ copies g^{-1} dry soil). At 20°C, bacterial abundances in the control and N addition treatment decreased to 36.89 and 50.54% of that at 15°C (Figure 3A), respectively, indicating the preference of bacteria for lower temperature. At 20°C, fungal abundances increased significantly by 60.73% in the N addition treatment (Figure 3B). N addition appeared to reduce the abundances of bacteria under both temperatures, whereas fungal abundances were significantly stimulated at 20°C. Increased temperature resulted in the proliferation of methanogen (*mcr*A) by 28.04 and 31.46% in the control and N addition treatments, respectively (Figure 3C). However, N addition reduced methanogen abundances by 19.30 and 17.14% at 15 and 20°C, respectively. The abundances of methanotrophs (*pmo*A) significantly increased by 28.49-, 14.31-, and 18.16-fold under a rising temperature, N addition, and both increased temperature and N addition, respectively (Figure 3D). Adding N at 15°C significantly increased the abundances of *nir*K-type denitrifiers by 21.89% (Figure 3E). An increase in temperature resulted in decreases in the abundances of the *nir*S-type denitrifiers by 25.59 and 22.75% in the control and N addition treatments, respectively (Figure 3F). The addition of N resulted in increases in the abundances of *nir*S-type denitrifiers by 19.48 and 24.04% at 15 and 20°C, respectively. The increase in temperature and N addition had an interactive impact on the abundances of fungi and methanotrophs; however, there was no synergistic effect on bacterial, methanogen, and denitrifier abundances ($p < 0.01$; Table 2). There were significant relationships between the abundances of fungi and the contents of NH_4^+-N, NO_3^-N, as well as emissions of CO_2. This result indicated that fungi contributed to CO_2 emissions and were affected by N concentrations. The significant correlations between N_2O emissions and the abundances of fungi, methanotrophs, and *nir*K-type denitrifiers indicated the significant contribution of the microbial community to N_2O emissions ($p < 0.05$; Figure 4).

Soil enzymes activities

The activities of the four soil enzymes responded significantly to a rising temperature and the addition of N (Figure 5). The

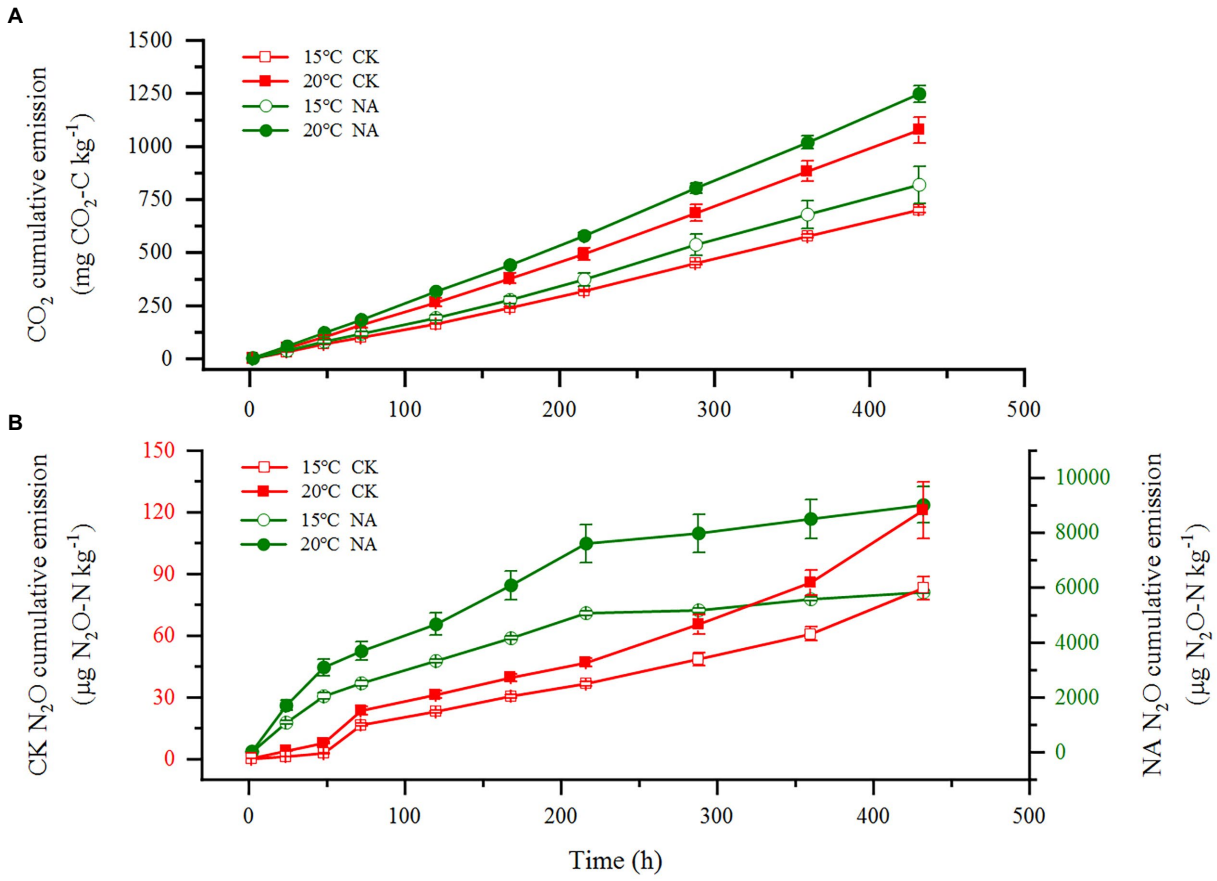

FIGURE 1
Effects of temperature rising and nitrogen addition on soil CO_2 **(A)** and N_2O **(B)** emissions in permafrost peatland. CK, control; NA, add 50mgNkg^{-1} soil.

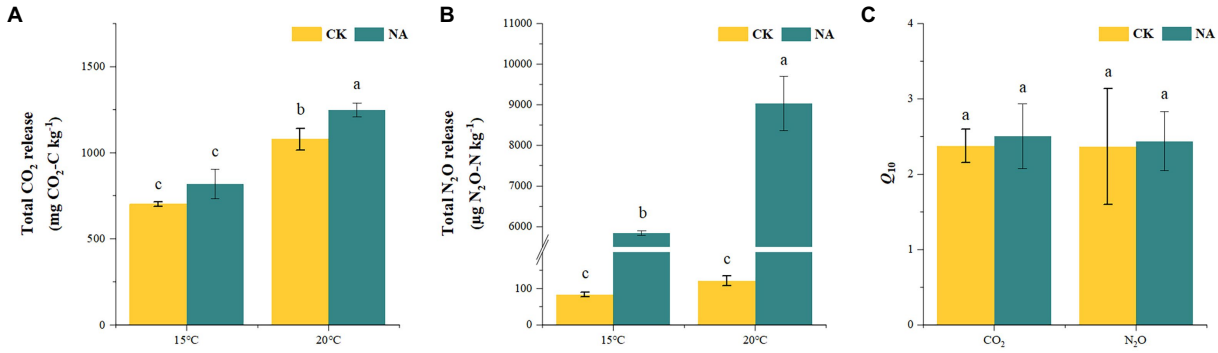

FIGURE 2
Effects of temperature rising and nitrogen addition on soil total CO_2 **(A)** and N_2O **(B)** release and their temperature sensitivity (Q_{10}) **(C)** in permafrost peatland. CK, control; NA, add 50mgNkg^{-1} soil. Different lowercase letters in the figure indicate significant differences in the means between different treatments.

C-cycling-related activities of βG and CBH decreased by 22.63 and 22.46% with a rising in temperature in the control, whereas they decreased by 12.40 and 46.03% in the N addition treatment, respectively (Figures 5A,B). The rise in temperature resulted in an increase in soil NAG activities by 11.83 and 48.57% in the control and N addition treatments, respectively (Figure 5C). Significant interactive effects were observed between the rising temperature and addition of N on soil NAG activities ($p < 0.01$; Table 2). NAG

TABLE 1 Two-way ANOVA of effects of temperature rising and nitrogen addition on soil CO_2, N_2O release, and soil carbon and nitrogen contents.

	CO_2 emission rate	N_2O emission rate	DOC	DON	NH_4^+-N	NO_3^--N
Temperature rising	49.824**	23.030**	8.890*	0.190	65.101**	6.160*
Nitrogen addition	6.427*	476.718**	0.003	13.075**	324.349**	11.814**
Temperature rising × Nitrogen addition	0.217*	21.961**	4.935*	31.132**	2.469	1.985

DOC, dissolved organic carbon; DON, dissolved organic nitrogen. *$P < 0.05$; **$P < 0.01$.

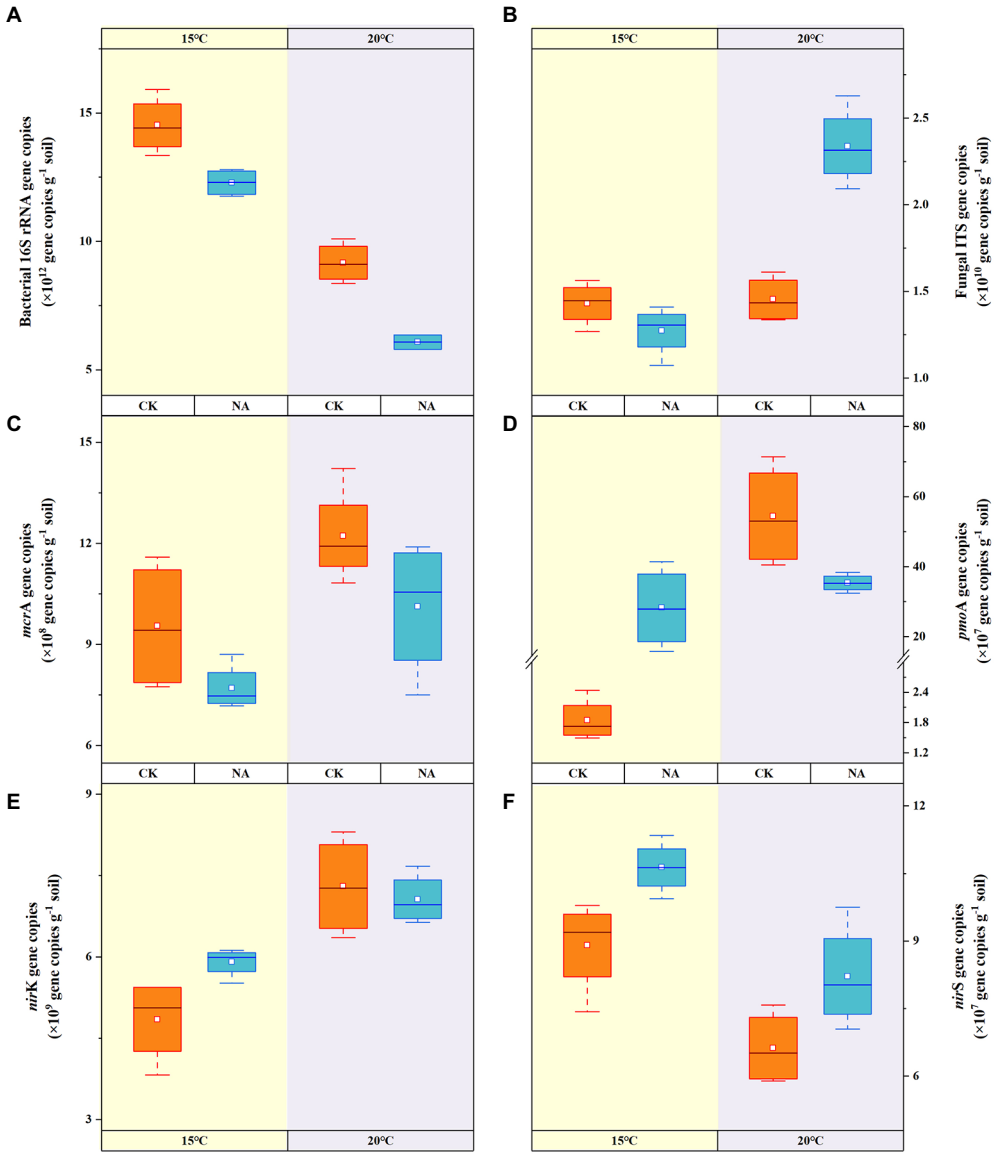

FIGURE 3
Effects of temperature rising and nitrogen addition on soil bacterial **(A)**, fungal **(B)**, *mcr*A **(C)**, *pmo*A **(D)**, *nir*K **(E)**, and *nir*S **(F)** abundances in permafrost peatland. CK, control; NA, add 50mgNkg^{-1} soil.

activities showed significant positive correlations with soil emissions of CO_2 and contents of NO_3^--N and NH_4^+-N ($p < 0.05$; Figure 4). Soil AP activities decreased with a rising temperature and the addition of N, with the highest and lowest activities of 2,089.23 and 1,730.22 nmol g^{-1} h^{-1} obtained at 15°C without N addition and 20°C with N addition, respectively (Figure 5D). There were no synergistic effects of the rising temperature and addition of N on soil βG, CBH, and AP activities ($p > 0.05$; Table 2).

TABLE 2 Two-way ANOVA of the effects of nitrogen addition and temperature rising on soil microbial abundances and enzyme activities.

	Bacteria	Fungi	*mcr*A	*pmo*A	*nir*K	*nir*S	βG	CBH	NAG	AP
Temperature rising	241.018**	45.530**	9.914**	39.356**	30.183**	25.749**	8.811*	23.166**	1.936	20.320**
Nitrogen addition	51.150**	20.357**	5.907*	0.597	1.564	12.909**	3.625	42.317**	67.208**	13.070**
Temperature rising × Nitrogen addition	1.308	41.716**	0.024	22.850**	3.897	0.024	1.226	0.630	19.659**	0.027

βG, β-1,4-glucosidase; CBH, cellobiohydrolase; NAG, β-1,4-N-acetylglucosaminidase; AP, acid phosphatase. *$P < 0.05$; **$P < 0.01$.

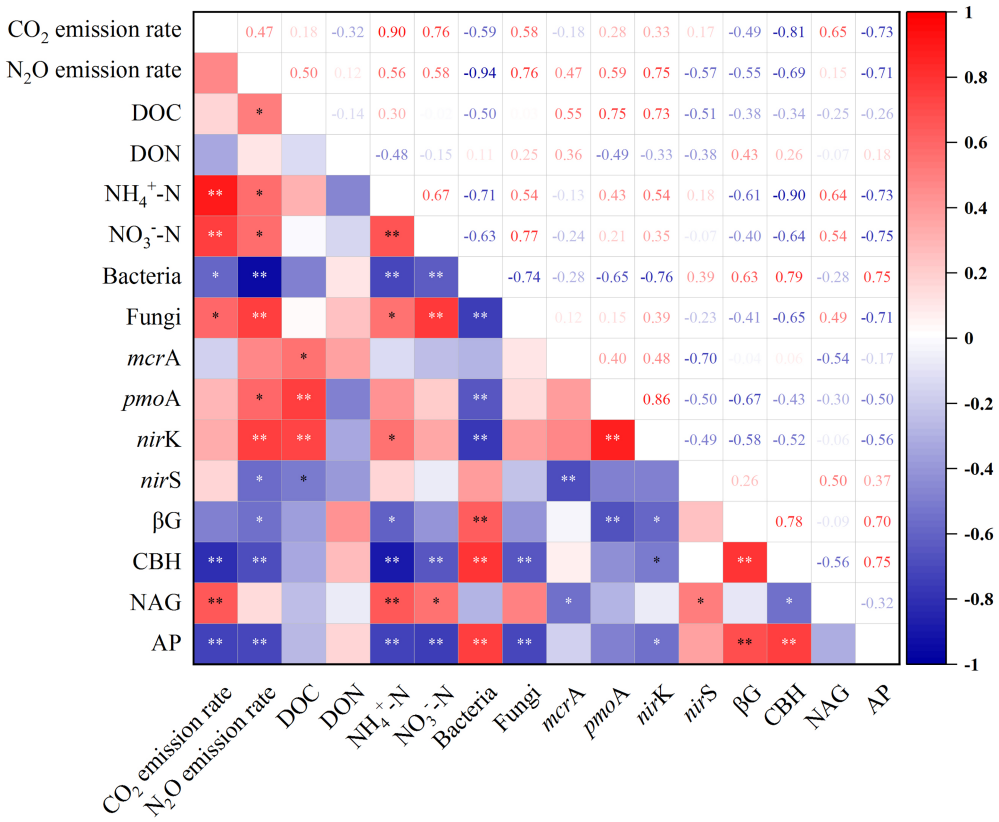

FIGURE 4
Pearson's correlation analysis of soil CO_2 and N_2O emissions, carbon and nitrogen contents, microbial abundances, and enzyme activities. βG, β-1,4-glucosidase; CBH, cellobiohydrolase; NAG, β-1,4-N-acetylglucosaminidase; AP, acid phosphatase; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; NH_4^+-N, ammonium nitrogen; NO_3^-N, nitrate nitrogen. * indicates significant $p<0.05$; ** indicates significant $p<0.01$.

Soil labile carbon and nitrogen contents

An increase in temperature increased DOC contents in the permafrost peatlands from 531.05 to 628.25 mg kg^{-1} in the control treatment (Figure 6A). However, the increase in temperature did not result in a significant change in soil DOC contents under the N addition treatment. N addition had a significantly negative impact on DON contents at 15°C, with DON decreasing from 169.80 to 116.80 mg kg^{-1}, whereas soil DON was not significantly affected at 20°C (Figure 6B). NH_4^+-N in soil ranged from 35.70 to 62.25 mg kg^{-1}. Both a rise in temperature and the addition of N resulted in increased contents of soil NH_4^+-N (Figure 6C). The contents of soil NO_3^--N under N addition (19.73 mg kg^{-1}) were significantly higher than that in the control (14.96 mg kg^{-1}) at 20°C (Figure 6D). The increase in temperature and N addition had significant interactive impacts on soil DOC and DON contents ($p < 0.05$; Table 1), whereas the effects on NO_3^--N and NH_4^+-N were not significant. The soil emissions of CO_2 and N_2O showed significant positive correlations with contents of soil NH_4^+-N and NO_3^--N, whereas N_2O emissions were positively correlated with DOC contents ($p < 0.05$; Figure 4).

Discussion

Effect of soil microbial abundances on emissions of soil CO_2 and N_2O

The rise in temperature and addition of N stimulated emissions of soil CO_2 and N_2O. Moreover, the rise in temperature

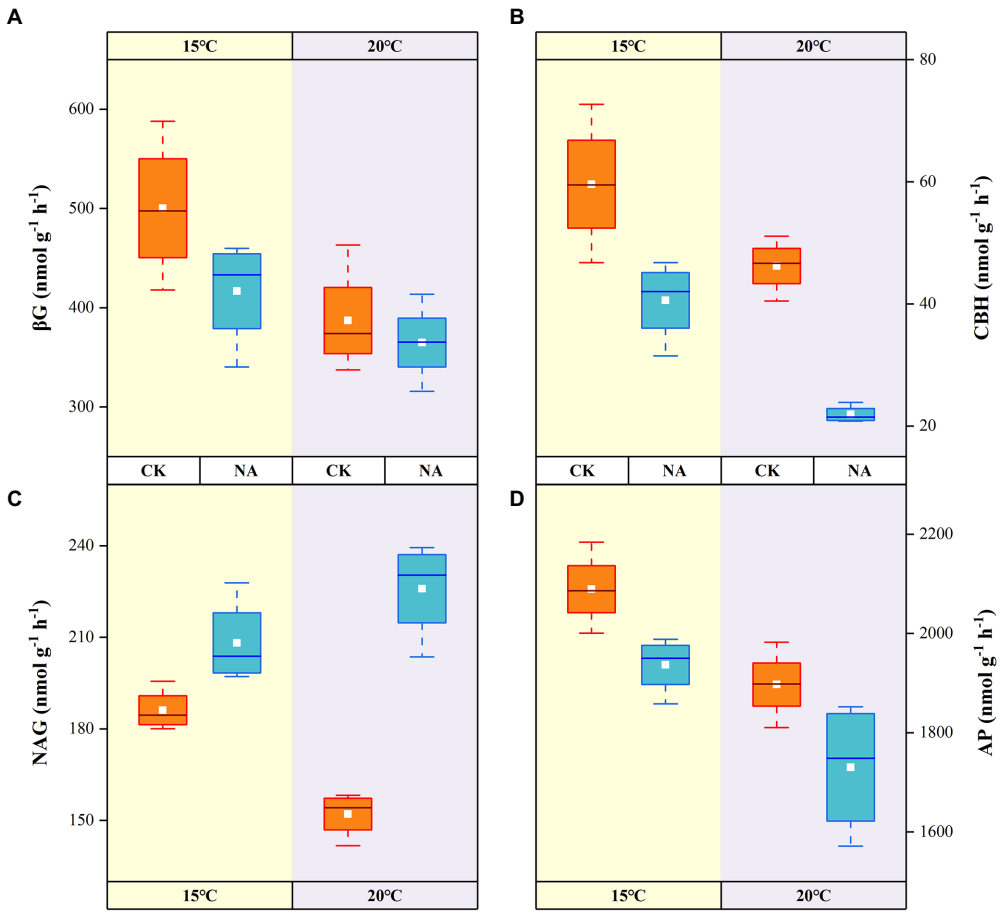

FIGURE 5
Effects of temperature rising and nitrogen addition on soil enzyme activities in permafrost peatland. CK, control; NA, add 50mgNkg^{-1} soil. βG, β-1,4-glucosidase **(A)**; CBH, cellobiohydrolase **(B)**; NAG, β-1,4-N-acetylglucosaminidase **(C)**; AP, acid phosphatase **(D)**.

and addition of N interacted within their effect on soil emissions of CO_2 and N_2O. However, the results of the current study demonstrated a strong negative effect of rise in temperature and the addition of N on the abundances of bacteria. This result indicated that bacteria in permafrost peatlands were adapted to a low temperature and N-limited environment. In line with our results, warming reduced 37% of bacterial abundance and microbial metabolic capacity in the deep organic layer of an Alaska tundra (Wu et al., 2022). Our results showed that the combined effects of temperature rising and N addition significantly increased fungal abundances and there were significantly positively correlations between fungal abundances and the emissions of CO_2 and N_2O, suggesting that there were differences in sensitivity of different microbial communities to environmental changes and fungi communities played a vital part in the variations of CO_2 and N_2O emissions at higher temperature and under the addition of N. Consistent with the outcomes of the current study, Xu et al. (2017) determined that fungal tolerance to high temperatures played a significant part in N_2O emissions.

The results of the present study showed that methanotrophs were more sensitive to a changing temperature and the addition of N compared to other microbial communities. The higher abundances of *nir*K-type denitrifiers at 20°C compared to at 15°C observed in the present study were consistent with results of previous studies in which the abundances of *nir*K genes were promoted by higher temperatures (Jung et al., 2011; Cui et al., 2016). Declines the abundances of *nir*S-type denitrifiers were observed at 20°C compared to those at 15°C. This result demonstrated that *nir*S-type denitrifiers were better adapted to low temperature conditions. The significant positive correlations between the abundances of *nir*K-type denitrifiers and NH_4^+-N contents and N_2O emissions observed in the present study indicated that the increase in emissions of N_2O could be primarily attributed to the denitrification pathway mediated by *nir*K denitrifiers. Jung et al. (2011) similarly observed an increase in *nir*K genes abundances under both warming and the addition of N. The *nir*K denitrifiers mentioned above are bacterial *nir*K, fungal *nir*K also have clear relevance for N_2O-producing, future understanding the abundance and distribution of denitrifying fungi may provide new insight into soil N_2O emissions under various environmental settings (Chen et al., 2016).

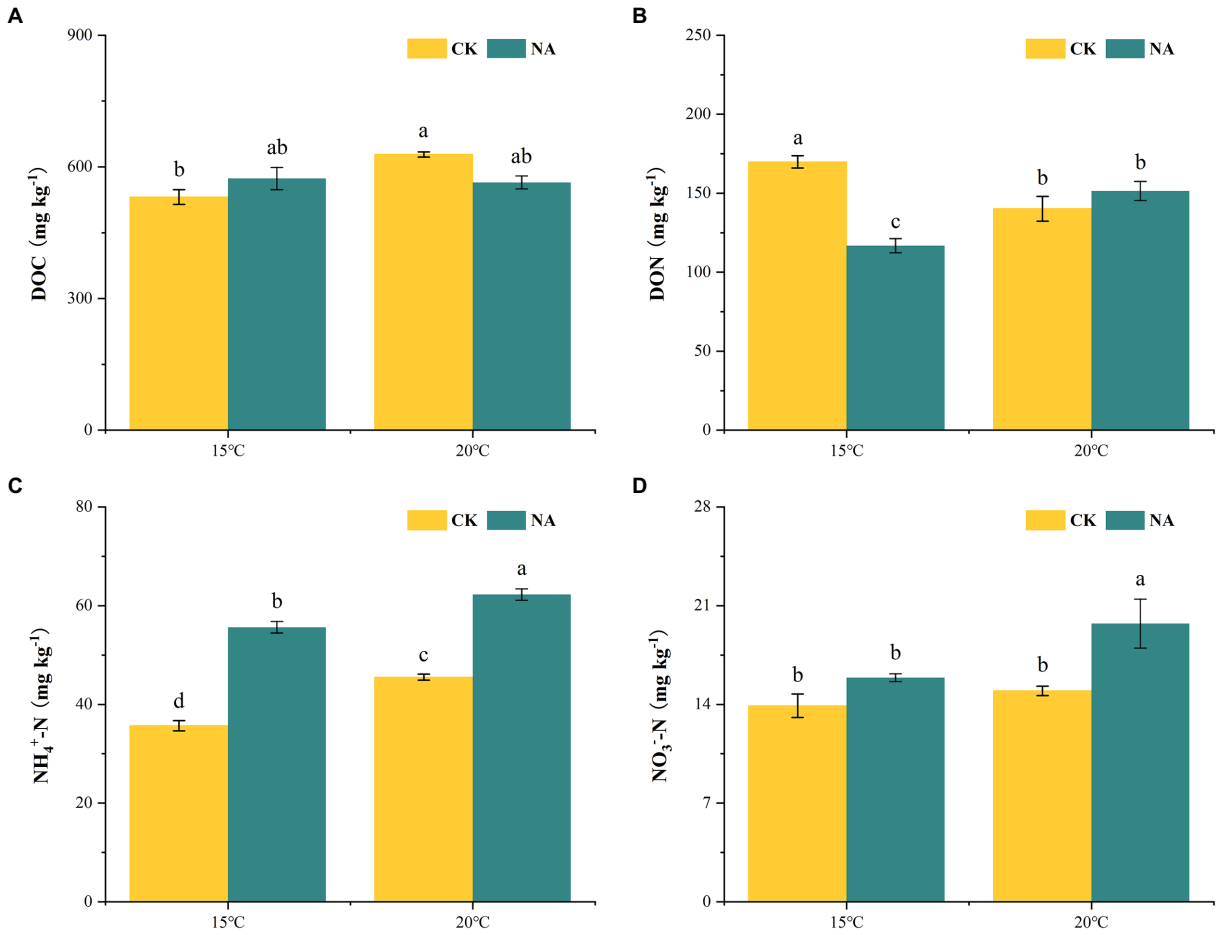

FIGURE 6
Effects of temperature rising and nitrogen addition on soil dissolved organic carbon **(A)**, dissolved organic nitrogen **(B)**, NH_4^+-N **(C)**, and NO_3^--N **(D)** contents in peatland. CK, control; NA, add 50mgNkg^{-1} soil; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; NH_4^+-N, ammonium nitrogen; NO_3^-N, nitrate nitrogen. Different lowercase letters in the figure indicate significant differences in the means between different treatments.

Impacts of soil enzyme activities on emissions of soil CO_2 and N_2O

Soil enzymes play an important role in the mineralization of soil C and N. Therefore, an improved comprehension of the reaction of soil enzyme activities to increasing temperature and the availability of N is crucial for understanding the mechanisms under which emissions of soil CO_2 and N_2O occur. An increased temperature can alter the nutrient acquisition strategies of microbial communities. This is achieved by changing extracellular enzyme activities through the priming of decomposition of SOM, which leads to increased emissions of CO_2 from peatlands (AminiTabrizi et al., 2022). NAG participates in N conversion and plays a significant part in the decomposition of nitrogenous substances in soil as it facilitates the degradation of chitin (Liu et al., 2019). Chitin is a major source of soil organic N. The addition of N may affect the decomposition of chitin and peptidoglycan, which, in turn, accelerates the activities of NAG (Liu et al., 2019). Consistent with the outcomes of the present study, Chen et al. (2018) and Liu et al. (2019) determined that N addition significantly increased the activities of NAG by 5.5% and 56.40–204.78%, respectively. The increase in the activities of NAG can be attributed to soil acidification induced by the addition of N. A decrease in pH was shown to positively affect soil NAG activities (Chen et al., 2018). pH is a key driver for the turnover of organic matter in cold soil, regulatory role of pH needs consideration in the future studies (Leifeld et al., 2013). Although the rise in temperature decreased NAG activities in the control treatment, the increase in NAG activities in the N addition treatment indicated that within the combined effect of an elevated temperature and addition of N, the latter had the dominant effect on soil enzyme activities.

The rise in temperature inhibited the activities of soil βG, CBH, and AP. This result could be attributed to the decrease in enzyme activities possibly being related to a decrease in substrate (e.g., microbial biomass) availability at elevated temperatures.

Wang J. Y. et al. (2020) determined that enzyme activities reduced with increasing incubation time, suggesting that the responses of enzymes reflected changes in the availability of substrate due to warming. The rate of enzyme production has been shown to decrease as substrate is exhausted. The outcomes of the current study illustrated that the warming stimulation of soil respiration readily depleted hydrolysable substrates during incubation without inputs of C sources. Therefore, decreases in the active pool due to warming can result in microbial C starvation (Metcalfe, 2017; Wang J. Y. et al., 2020). In addition, bacterial conversion of NH_4^+-N to NO_2^--N in the first step of nitrification can further acidify soils through the release of H^+ into soil solution. Accelerated acidification, in turn, is an important factor inhibiting soil microbial enzyme activities to acute nutrient amendment (Fatemi et al., 2016). Previous studies have also suggested that a decline in soil enzyme activities was attributable to their more rapid inactivation due to warming can help explain attenuation of the warming impact on mineralization of soil C (Alvarez et al., 2018). Changes in redox conditions driven by temperature can result in abiotic destabilization of Fe-organic matter (phenol) complexes. This is a peatland decomposition pathway that was previously underestimated and can result in increased production of CO_2 and the accumulation of polyphenol-like compounds that could further inhibit the activities of extracellular enzymes (AminiTabrizi et al., 2022).

Effect of substrate availability on emissions of soil CO_2 and N_2O

The emissions of soil CO_2 and N_2O were related to the concentrations of NO_3^--N and NH_4^+-N. Also, soil emissions of N_2O were related to the concentrations of DOC. Similarly, correlations between the soil CO_2 release and NO_3^--N and NH_4^+-N concentrations were revealed by Zhang et al. (2018) in mountain forest and meadow ecosystems. These results indicated that higher substrate availability enhanced the activities of soil microbes, which, in turn, resulted in increased emissions of CO_2 and N_2O. Soil DOC is composed of low molecular weight organic compounds and drives the growth and activity of microbes by acting as an energy source and a substrate (Wang C. M. et al., 2020). The results of the present study showed an increase in DOC with increasing incubation temperature in the control. An elevated temperature accelerated microbial processes and increased C availability in the control, resulting in higher heterotrophic respiration rates and increased release of CO_2. However, soil DOC tended to decrease with the addition of N at a higher incubation temperature, indicating that N addition may limit available C. Warming significantly increased inorganic N (NH_4^+-N and NO_3^-N; Table 1) due to higher mineralization and nitrification of TN. The above results are consistent with the earlier study of Yuan et al. (2018), and suggest that warming increases soil N mineralization. Increase in N mineralization resulted in an increase in soil available N contents with increasing incubation temperature. Higher temperatures have been shown to accelerate the denitrification and nitrification processes (Inclan et al., 2012; Zhang et al., 2016). These processes are major pathways of soil emissions or production of N_2O (Zhang et al., 2018; Li et al., 2019). N_2O emissions due to nitrification accounted for 60–80% of total emissions (Zhang et al., 2020). Therefore, the increased availability of C and N in the soil substrate stimulated N_2O emissions by accelerating N transformation under warming.

In addition to soil temperature, the addition of N had profound influences on the emissions of CO_2 and N_2O. N addition significantly elevated NH_4^+-N and NO_3^--N, alleviated microbial N limitation, and promoted soil CO_2 and N_2O emissions, thereby accelerating soil C and N cycling. Menyailoa et al. (2014) similarly found an increase in heterotrophic activity by 20–30% after the addition of N. Increase in the availability of N often accelerates soil denitrification and nitrification processes and results in increased emissions of N-oxide (Davidson et al., 2000; Benanti et al., 2014). Especially, when C are available for microbial activity, N availability will have pronounced impacts on nitrification and denitrification (Lu et al., 2015). Consistent with the result of Guo et al. (2020), the results of the present study showed a positive correlation between DOC and N_2O emissions. This result indicated that both labile C and available N concentrations were the dominant factors influencing the emissions of N_2O. DOC is an important factor regulating denitrification and autotrophic and heterotrophic nitrification (Ferrarini et al., 2017). DOC concentrations influence the emissions of greenhouse gasses by regulating microbial metabolism, whereas soil ammonium and nitrate do not have the same regulatory function (Chen et al., 2020). Increased C availability enhances microbial activity, and, in turn, O_2 consumption, which may lead to sub-aerobic microsites facilitating N_2O emissions by denitrification and nitrifier denitrification (Ma et al., 2022). Consistent with the outcomes of the current study, Zhu et al. (2016) concluded that the sensitivity of soil respiration to temperature was not influenced by the addition of N, indicating that the availability of C substrate may be more important than that of N substrate.

Conclusion

This study showed that a rise in temperature and the addition of N promoted soil CO_2 and N_2O emissions. This result implies that future increases in temperature and availability of N will stimulate C and N cycling in the permafrost peatlands. The abundances of fungi were positively correlated with emissions of soil CO_2 and N_2O, suggesting that fungal communities may play a significant part in driving the exchange of C and N at the soil-atmosphere interface in permafrost peatlands. The abundances of the *nir*K-type

denitrifiers were positively correlated with DOC and NH_4^+-N contents, and emissions of N_2O, suggesting that the denitrification process mediated by *nir*K-type denitrifiers and available substrate may play a significant part in emissions of N_2O. The activities of soil NAG increased with the addition of N and a rise in temperature, and were positively correlated with soil CO_2 emissions. This result indicated that the activities of soil NAG are more important than those of other enzymes for regulating CO_2 emissions. The results of the current study improve understanding of how temperature and N availability regulate soil emissions of greenhouse gasses in permafrost peatlands. However, a laboratory study cannot completely reflect the actual response of greenhouse gasses to global warming, and future research should focus on how plants and their interactions with soil microbes regulate greenhouse gas emissions under field conditions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

YS: conceptualization, writing – review and editing, and funding acquisition. XC: methodology, data curation, and writing – review and editing. CS: supervision and funding acquisition. ML, ZL, JG, and XW: writing – review and editing. SG: methodology. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1093487/full#supplementary-material

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EDITED BY
Huai Li,
Northeast Institute of Geography and Agroecology (CAS), China

REVIEWED BY
Feng Li,
Institute of Subtropical Agriculture (CAS), China
Weiqi Wang,
Fujian Normal University, China

*CORRESPONDENCE
Xiaofei Yu
✉ yuxf888@nenu.edu.cn

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New and traditional methods for antibiotic resistance genes removal: Constructed wetland technology and photocatalysis technology

Pingping Chen, Xiaofei Yu*, Jingyao Zhang and Yiqi Wang

State Environmental Protection Key Laboratory for Wetland Conservation and Vegetation Restoration & Jilin Provincial Key Laboratory of Ecological Restoration and Ecosystem Management & Key Laboratory of Vegetation Ecology of Ministry of Education, School of Environment, Northeast Normal University, Changchun, China

Antibiotic resistance genes (ARGs) are a new environmental contaminant that poses a major hazard to humans and the environment. This research discusses the methods and drawbacks of two ARG removal approaches, constructed wetlands (CWs) and photocatalysis. CWs primarily rely on the synergistic effects of substrate adsorption, plant uptake, and microbial processes to remove ARGs. The removal of ARGs can be influenced by wetland plants, substrate type, wetland type, and hydraulic conditions. The absolute abundance of ARGs in effluent decreased, but their relative abundance increased. Photocatalysis deactivates ARGs predominantly through reactive oxygen species, with removal effectiveness determined by catalyst type, radiation type, and radiation intensity. The drawback is that it exposes intracellular resistance genes, perhaps increasing the risk of ARG spread. To address the current shortcomings, this paper proposes the feasibility of combining a constructed wetland with photocatalysis technology, which provides a novel strategy for ARG removal.

KEYWORDS

antibiotic resistance genes, constructed wetlands, photocatalysis, removal mechanism, combination

1. Introduction

Antibiotics have accumulated massively in the environment as a result of their widespread use in recent years. Antibiotics not only cause chemical pollution, but they may also induce the production of resistance genes (ARGs) and resistant bacteria (ARB) in the environment, hastening resistance spread and diffusion. As a result, the evolution and variation of bacterial resistance, as well as the spread of ARGs, have received increased attention in the field of environmental research. ARGs have been found in abundance in a variety of environmental media, including surface water, groundwater (Jiang et al., 2013),

sediment, soil (Wang et al., 2019), and air detection. Antibiotic resistance has emerged as a serious global environmental health issue (Ying et al., 2017; Li et al., 2020). This antibiotic resistance can be spread between microorganisms *via* a horizontal gene transfer (HGT) mechanism (Berendonk et al., 2015). ARGs are classified as extracellular ARGs (eARGs) and intracellular ARGs (iARGs), both of which are transmitted *via* HGT.

ARGs enter the environment *via* a variety of routes, including municipal water, sewer runoff, livestock wastewater, landfill leachate, and hospital wastewater (Ezeuko et al., 2021; Koch et al., 2021). Antibiotics leave significant levels of ARGs in humans and animals, which are eventually discharged into wastewater treatment plants *via* fecal wastewater runoff. The wastewater discharged from wastewater treatment plants, as well as the ARGs present in biosolids, enter the soil and aquatic environments and are absorbed in a cycle by plants, animals, and so on. ARGs have been found in a range of water environments, and some investigations have revealed that they are present in tap water (Bergeron et al., 2015). ARG removal solutions that are cost-effective are urgently needed, and ARGs pollution must be addressed.

Some of the current tactics for removing ARGs from wastewater include disinfection procedures, membrane treatment technologies, advanced oxidation technologies, and constructed wetlands (CWs). Disinfection techniques, such as chlorine disinfection, which increases antibiotic resistance and the genera of bacteria that can carry antibiotic resistance, are ineffective in eliminating ARGs and also encourage the transmission and spread of ARGs (Cheng et al., 2021). ARGs are physically removed by membrane treatment technologies; nevertheless, when membrane filtration is used, ARGs accumulate in membrane fouling and sewage sludge, which can re-enter the environment. Advanced oxidation technologies and CWs, to the contrary hand, have relatively good ARG removal. ARGs can be effectively eliminated by photocatalytic advanced oxidation based on hydroxyl radicals, and a TiO_2/UV treatment can reduce 5.8 log of *mecA* and 4.7 log of *ampC* (Guo et al., 2017). Furthermore, CWs is an efficient and sustainable wastewater treatment technology that efficiently removes organic matter, bacteria, antibiotics, pharmaceuticals and personal care products (PPCPs) from wastewater (Hartl et al., 2021), and has great potential in ARGs removal (Chen et al., 2016a; Huang et al., 2017). Thus, this study summarizes recent research and uses of CWs and photocatalysis in the removal of ARGs. The basic mechanisms, affecting factors, and limitations of CWs and photocatalysis for ARG removal are summarized. The feasibility of using CWs in conjunction with photocatalysis to remove ARGs is considered. Some novel approaches to removing ARGs from aquatic environments are proposed.

2. CWs for the removal of ARGs

CWs are an ecosystem made up of water, microbial communities, plants, and substrate (Chen et al., 2019). It uses a synergistic process of physical, chemical, and biological processes to remove contaminants. Its advantages over conventional wastewater treatment technologies include low cost, simplicity of use, and good maintenance (Faulwetter et al., 2009). CWs are currently being utilized to treat domestic wastewater (Adrados et al., 2014), agricultural wastewater (Liu J. et al., 2013), and landfill leachate (Sundberg et al., 2007). CWs are effective in removing both new pollutants as well as conventional contaminants like nitrogen and phosphorus. CWs remove ARGs mainly by substrate sorption, plant uptake, and microbial removal (Figure. 1). The type of wetlands, the plants used, the type of substrate, and other factors all have an impact on how successfully CWs remove ARGs. ARGs can currently be removed from aquatic habitats using CWs, albeit the bulk of these techniques are still in the experimental stage and the precise process is unknown, needing additional research.

2.1. Removal efficiency of ARGs for different CWs types

Surface flow constructed wetlands (SFCWs) and subsurface flow CWs are the two types of CWs. Subsurface flow CWs are classified as either horizontal subsurface flow constructed wetlands (HFCWs) or vertical subsurface flow constructed wetlands (VSFWs) based on the direction of the water flow (VFCWs). The removal effectiveness of ARGs varied dramatically amongst CW types (Table 1).

CWs have the ability to remove most common ARGs, with removal rates ranging from 14.5% to 100%. Furthermore, the ARG removal efficiency of several CWs was much higher than that of conventional wastewater treatment plants (Xu et al., 2015). Various types of CWs have their own targeted ARG species that

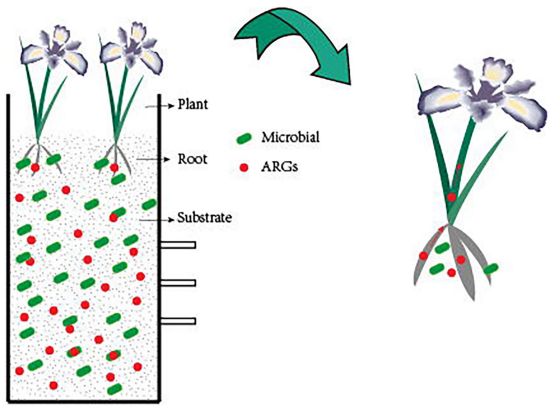

FIGURE 1
Mechanisms for the removal of ARGs in CWs. The substrate can absorb a high amount of ARGs, while the plant can also absorb ARGs. As the plant grows, ARGs migrate from the roots to the stems and leaves, and the plant root system's biofilm structure removes ARGs by mechanisms of filtration, adsorption, absorption, and transformation of ARGs, and bacteria within the CWs can also remove ARGs.

TABLE 1 ARGs removal effects in different CWs.

CW types	Target ARGs	Removal efficiency (%)	References
SFCWs	*sul1*, *sul2*, *tetG*, *floR*	47.2–82.8	Chen et al. (2016b)
SFCWs	*sul1*, *sul2*, *tetG tetM*, *qnrB*, *qnrS*	59.5–77.8	Fang et al. (2017)
HFCWs	*sul1*, *sul2*, *tetG*, *floR*	59.3–90.5	Chen et al. (2016b)
HFCWs	*sul1*, *tetW*, *tetG*, *dfrA1*, *aphA*, *tetX*, *ermC*, *tetO*	14.5–94.1	Du et al. (2021)
VFCWs	*sul1*, *sul2*, *tetG*, *floR*	79.1–94.6	Chen et al. (2016b)
VFCWs	*tetM*, *tetO*, *tetW*	90	Liu L. et al. (2013)
VFCWs	*tetO*, *tetM*, *tetW*, *tetA*, *tetX*, *intI1*	45.9–99.9	Huang et al. (2014)
VFCWs	*tetW*, *tetA*, *tetX*, *intI1*	33.2–99.1	Huang et al. (2017)

are efficiently removed. For example, VFCWs significantly reduced the concentration of tetracycline resistance genes in livestock effluent (Liu L. et al., 2013), with the absolute abundance of *tetM*, *tetW*, and *tetO* being reduced by 90%. HFCWs was also effective in removing ARGs, especially sulfonamide ARGs. The abundance of *sul1*, *sul2*, *tetM*, *tetO*, *tetQ*, *tetW*, and *intI1* was reduced by 1–3 orders of magnitude by HFCWs (Chen and Zhang, 2013a). HFCWs was more effective in eliminating sul1-carrying bacteria than some typical wastewater treatment facilities (Czekalski et al., 2012), indicating that it can be used as a supplement to standard wastewater treatment plants, particularly to reduce the amount of sulfonamide ARGs. HFCWs and VFCW are more effective at removing contaminants than SFCWs, with VFCWs having the highest removal efficacy, ranging from 33.2 to 99.9%. The differences in removal effectiveness may be connected to the adsorptive filtration, biochemical processes, and redox conditions found in each wetland. Furthermore, the direction of water flow in VFCWs induced disparities in removal results, with upflow VFCWs having a higher relative abundance of tetracycline ARGs and *intI1* than downflow VFCWs (Chen et al., 2019).

2.2. ARGs uptake by plants in CWs and biofilm degradation in the root system

ARGs are primarily removed by plants in CWs *via* two pathways: absorption and root biofilm breakdown. Bacteria can reproduce in plant tissues and expand their populations during plant growth *via* hydraulic transport and active plant absorption. This allows plants to effectively reduce the abundance of microorganisms in the feed water, thus facilitating the removal of ARGs (Vacca et al., 2005). There were significant differences in the distribution of ARGs among different tissues of the plant, with ARG abundance higher in plant leaves than stems (Guo et al., 2021). The total abundance of ARGs observed in plants, however, was much lower than that found in the substrate.

The huge root systems of wetland plants can form a special biofilm structure together with the filler surface to remove ARGs through the processes of filtration, adsorption, absorption, and transformation of ARGs. Tetracycline ARGs, especially *tetW*, can rapidly migrate to the biofilm surface and are therefore more easily removed by CWs (Cheng et al., 2013). Furthermore, it has been demonstrated that plants can indirectly participate in the removal of ARGs, primarily by filtering solid particles and delivering oxygen to the microbial community, enhancing the role of inter-rhizosphere bacteria, or providing a medium for biofilm development, which improves the removal capacity of microorganisms and reduces the accumulation of ARGs, resulting in a reduction in ARGs abundance (Anderson et al., 2013; Fang et al., 2017). The type and amount of inter-root secretions vary among plants, thus affecting the inter-root microorganisms, resulting in different removal efficiencies of ARGs by different plants, with *Thalia dealbata* Fraser being more effective than *Iris tectorum* Maxim in removing ARGs (Chen et al., 2016b). Reed is a major aquatic plant for reducing ARGs contamination, with a removal effectiveness of more than 90% for ARGs such as *sul1*, *sul2*, *ermB*, *qnrS*, and bla_{TEM-1} (Avila et al., 2021). However, biological processes in CWs not only degrade ARGs as described above, but also lead to the transfer and increase of ARGs (Ghosh and LaPara, 2007; Diehl and LaPara, 2010; Guo et al., 2014; Yang et al., 2014), thus the role in ARGs removal is complex and needs to be studied in more depth.

2.3. Substrate effect on ARGs removal

In CWs, the matrix serves as an essential vehicle for physicochemical processes (Chen et al., 2019). The particle size distribution, surface charge, porosity, and pH of the matrix all have an impact on ARGs removal (He et al., 2021). Substrate adsorption and microbial degradation on the substrate surface are two important pathways for ARG removal. The inadequate elimination of macrolide resistance genes by CWs may be explained by substrate adsorption (Chen et al., 2019). Bacteria, particularly gut microorganisms, are easily absorbed by substrates (Huang et al., 2017). Furthermore, because ARGs are generally carried by gut bacteria, the high removal effectiveness of ARGs by CWs is most likely due to the substrate's high adsorption efficiency on intestinal microorganisms (Huang et al., 2014). ARGs from *tenericutes*, *cyanobacteria*, and *acidobacteria* were more likely to be lost (Su et al., 2019). In addition, small pore size substrates enable bacterial filtration and precipitation and have a great ability

to remove microorganisms from water. Gravel, zeolite, oyster shell, medicinal stone, ceramic, and tuff are the most typical substrates used in CWs for ARGs removal (Table 2). Zeolites have a microporous structure and silica hydroxyl groups, which provide surface area for chemisorption and microbial attachment, while silica hydroxyl groups are catalytically active for various chemical reactions, and their average pore size (4.32 nm) is smaller than that of volcanic rocks (10.78 nm; Liu L. et al., 2013), making zeolites more efficient for the removal of ARGs (Gorra et al., 2007). Ceramics have a porous morphology with a greater specific surface area, but they have a macroporous structure (Chen et al., 2016a), hence they are less effective at removing ARGs than zeolites. Tuff has a more porous structure and a bigger surface area, allowing for more bacterial adsorption and stronger biofilm development (Abou-Kandil et al., 2021), and hence has a better ability to remove ARGs. Both oyster shell and medical stone have an ordered lamellar structure, but oyster shell has considerable agglomeration that medical stone does not, and hence oyster shell is more efficient at removing ARGs than medical stone.

2.4. CWs remove the shortcomings of ARGs

The mechanism of ARG removal by CWs is complex, and the mechanism of migration and removal of ARGs in CWs is not well understood. While CWs are effective in removing ARGs, they also have the risk of enriching them. Despite a decrease in absolute abundance, the relative abundance of resistance genes in CWs effluent increased (Yi et al., 2017). ARGs are generally transmitted *via* vertical and horizontal gene transfer, i.e., genetic transfer between parents and transmission between microbes. Once ARGs produce resistance in pathogenic microbes or spread across pathogenic microorganisms, CWs can evolve into a large reservoir of ARGs, with potentially disastrous ecological and health effects. According to research, reducing the overall number of microorganisms in wastewater can successfully limit ARGs transmission (Song et al., 2018). Therefore, research on the removal of ARGs by CWs still faces a great challenge.

3. Photocatalytic removal of ARGs

Photocatalytic oxidation is a method of removing pollutants from water or the atmosphere that involves a sequence of reactions between a catalyst and oxygen in solution that produce powerful oxidizing ·OH under the action of solar radiation. The photocatalyst is central to photocatalytic technology, and there are many different types of photocatalysts, including TiO_2, ZnO, and WO_3, which are now the most researched photocatalysts. Because of its non-toxicity, low cost, and great photocatalytic efficiency, TiO_2 is the most often used photocatalyst.

In comparison to the activated sludge method, membrane separation method, and chemical oxidation method, the photocatalytic oxidation method has the advantages of low energy consumption, rapid reaction, simple operation, and no secondary pollution, and it has become a popular research direction in recent years. Advanced photocatalytic oxidation processes have the potential to remove microbial contaminants, such as photocatalytic titanium dioxide, which can generate reactive oxygen species (ROS, e.g., OH), which kill microorganisms by oxidative damage to cell membranes, RNA, DNA, proteins, and lipids (Umar et al., 2019). TiO_2 multiphase photocatalytic oxidation offers various advantages as a "green" water disinfection technology, including better efficiency in eliminating ARB from wastewater than standard disinfection processes and the absence of disinfection by-products.

3.1. Mechanism of photocatalytic removal of ARGs

Photocatalytic creation of holes and electrons under UVA irradiation causes redox processes in which oxidants first target microbial cell walls, membranes, and enzymes, and later interior components such as RNA and DNA (Koivunen and Heinonen-Tanski, 2005). Although bacteria have self-defense systems to defend themselves from ROS damage, excessive ROS can cause oxidative stress and assault membrane lipids, eventually leading to DNA damage (Li et al., 2011). ARG abundance is decreasing owing to photocatalyst light exposure, which causes DNA damage in bacterial cells (Figure 2). Furthermore, photocatalytic treatment breaks down long DNA strands into shorter nucleotides, allowing the deoxyribose phosphate backbone to be broken (Hirakawa et al., 2009). Free ARGs-containing deoxyribonucleic acids are due to a lack of protective bacterial cell walls, which can also be rapidly removed by photocatalysis. TiO_2 can produce hydroxyl radicals, which are thought to be the most active oxidants for destroying ARGs (Pham and Lee, 2014). Therefore, the majority of current research employs TiO_2 or modified TiO_2 as a photocatalyst.

TABLE 2 Comparison of adsorption performance of CWs substrate.

CWs	CWs substrates	Adsorption performance	References
VFCWs	Tuff, gravel	Tuff > gravel	Abou-Kandil et al. (2021)
VFCWs	Oyster shell, zeolite, medical stone, ceramic	Zeolite > oyster shell > medical stone > ceramic	Chen et al. (2016a)
VFCWs	Zeolite, volcanic rocks	Zeolite > volcanic rocks	Liu J. et al. (2013)

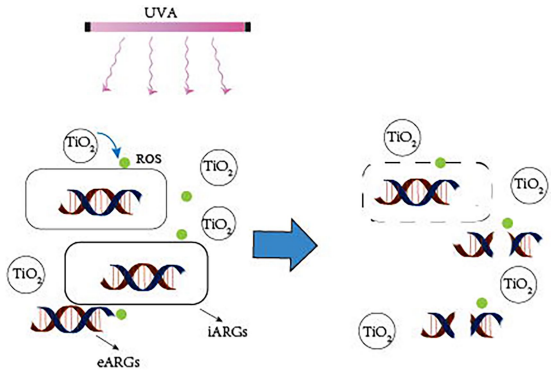

FIGURE 2
Mechanism of photocatalytic removal of ARGs. UVA irradiation causes the catalyst to create ROS, which attack the bacterial cell wall, breaking it down and converting iARGs to eARGs. Simultaneously, ROS attack eARGs, breaking the DNA strand, and inactivating ARGs.

3.2. Factors influencing photocatalytic removal of ARGs

Among the large number of photocatalytic materials, TiO_2 and graphitic carbon nitride (g-C_3N_4) are two materials that have been studied more. The effects of different catalyst types, radiation types, and radiation intensities on the removal of ARGs varied significantly (Table 3). Radiation intensity is significant in photocatalysis efficiency (Yu et al., 2020), and the higher the radiation intensity, the more effective the removal of ARGs. UV removal of ARGs is more effective than solar radiation, and the decrease of ARGs by TiO_2 under UV irradiation is 4–5 log, whereas it is only 0.5 log under sunlight irradiation, which may be related to TiO_2's low activation effectiveness under sunshine. To address TiO_2's low activation efficiency in solar radiation, the synthesis and application of semiconductor-reinforced TiO_2 composites have emerged as a hot research area. TiO_2-rGO composites are found to be much more effective than TiO_2 at removing ARGs. The removal effect of different ARGs under the same treatment conditions is different, most likely because their sensitivity to oxidation radicals varies depending on their composition. Because reactive oxygen species react more quickly with guanine bases in DNA, ARGs with a high GC% content, such as sulfonamide ARGs, are degraded to a greater extent (Ren et al., 2018). In addition, the amount of photocatalyst used also affects the removal of ARGs, and the size of the composite also influences the treatment efficacy, as smaller composites are more likely to cross cell membranes and promote rupture (Guo and Tian, 2019). Hence, the development of non-toxic nanocomposites should be explored in the development of new photocatalysts for the removal of ARGs. Inorganic ions and organic debris in wastewater can both limit photocatalytic activity by adsorbing on the TiO_2 surface and blocking active sites, and some anions can function as cavity scavengers, slowing the rate of chemical breakdown or disinfection on the TiO_2 surface. In addition, organic matter in wastewater can also reduce the disinfection rate through a variety of mechanisms, including ROS scavenging, competition for active sites on the TiO_2 surface, and direct UV absorption.

3.3. Effect of nanomaterials on the diffusion of ARGs

The effect of nanomaterials on the HGT of ARGs in the environment has aroused the interest of many researchers. Related research has revealed that gene level transfer is a significant factor in the spread of ARGs (Cheng et al., 2021). ARGs can move from one bacterium to another when combined with mobile genetic elements like plasmids, integrons, transposons, and so on, allowing bacteria to acquire ARGs. Most nanomaterials can contribute to the diffusion of ARGs in pure bacterial systems. Nano-TiO_2 significantly improved the splice transfer between RP4 plasmids in *E. coli* (Qiu et al., 2015), and with slight inhibition of bacterial growth, it could increase the splice transfer efficiency by 56-fold. ARB activity was reduced by graphene oxide (GO). However, at the level of ARGs, all GO greatly boosted transfer efficiency (Guo and Zhang, 2017). These findings suggest that the excellent adsorption characteristics of insoluble nanoparticles boost the binding of ARGs and bacteria, which can considerably contribute to the transfer efficiency of ARGs. The environmental conditions in actual water and wastewater treatment are complex, as are the technologies used. The environmental conditions in actual water and wastewater treatment are complicated, with a generally mixed flora of microorganisms. The impact of nanomaterials on the spread of ARGs in the actual world is more complicated. The effluent from a secondary wastewater treatment plant was treated using polyvinylidene fluoride ultrafiltration membranes enhanced with nano-TiO_2 (Ren et al., 2018). The results showed that nano-TiO_2 on this membrane was successful in eliminating 98% of ARGs when exposed to UV light, thus effectively controlling the HGT of ARGs. To remove resistant bacteria and genes from municipal wastewater, TiO_2-rGO material was utilized (Karaolia et al., 2018). These results can be attributed to the ability of the reactive oxygen species generated by photoexcitation of nanomaterials to oxidatively damage DNA, thus enabling the control of the proliferation of ARGs in the real environment.

3.4. Photocatalysis' shortcomings in ARG removal

Photocatalysis is an excellent method for removing ARGs by directly destroying cellular deoxyribonucleic acid; however, the continuous use of high-intensity UV light during wastewater treatment is difficult (Chen and Zhang, 2013b; Lee et al., 2017; Mauter et al., 2018). Moreover, after UV damage to the cell wall, iARGs flow out and are converted into eARGs, which can survive in the environment and bind to other

TABLE 3 Effect of different catalysts, radiation type and radiation intensity on the removal effectivness of ARGs.

Catalyst	Radiation type	Radiation intensity	ARGs	ARGs removal (log units)	References
TiO_2	UVA	120 mJ/cm^2	*ampC*, *mecA*	4.7log (*ampC*), 5.8log (*mecA*)	Guo et al. (2017)
TiO_2	UVA	8 W/m^2	Bla_{NDM-1}	0.7–1.5log	Chen et al. (2022)
TiO_2	Solar simulator	500 W/m^2	*sul1*, *sul2*, bla_{TEM}, *int11*, *uidA*, *efec*	98.9% (*sul1*), 74.6% (*sul2*), 93.26% (bla_{TEM}), 93.45% (*int11*),99.96% (*uidA*),71.96% (*efec*)	Felis et al. (2022)
TiO_2, TiO_2-rGO	Solar simulator	63 W/m^2	*ampC*, *sul1*, *ermb*, *mecA*	2log *ampC* (TiO_2-rGO); 0.5 log *ermB* (TiO_2)	Karaolia et al. (2018)
Ag/AgBr/g-C_3N_4	UVA	9. 6 W/m^2	*tetA*, *tetM*, *tetQ*, *intI1*	49% (*tetA*), 86% (*tetM*), 69% (*tetQ*), 86% (*intI1*)	Yu et al. (2020)
g-C_3N_4	UVA	3 W/m^2	*tetA*, *tetB*,	41.77% (*tetA*), 37.59% (*tetB*)	Hu et al. (2022)
TiO_2, GO-TiO_2	Solar radiation	40 W/m^2	*int11*, *qnrS*, bla_{CTK-M}, *sul1*	3.5 log bla_{CTK-M} (GO-TiO_2)	Moreira et al. (2018)

bacteria *via* transformation, transduction, and other mechanisms, resulting in the spread of ARGs in the environment. In addition, genes fragmented by oxidation can integrate with other pathogens in the wastewater (De Vries and Wackernagel, 2002; Nielsen et al., 2007), thus requiring additional treatment. Bacteria with ruptured cell membranes can cause cell lysis and an increase in eARGs levels, resulting in secondary water contamination. The removal of ARGs released by ARBs is critical. Furthermore, injured bacteria treated with photocatalysis will recover, and in addition to bacterial regrowth, ARGs transfer may rise if pathogens are not completely inactivated. The release and transfer of ARGs may contribute to the subsequent development of resistant bacteria in the aquatic environment unless the duration of treatment is managed (Dunlop et al., 2015).

4. Prospect

CWs can efficiently remove ARGs, and the removal efficiency of VFCWs and HFCWs is greater than that of SFCWs. VFCWs effectively remove tetracycline ARGs, whereas HFCWs effectively remove sulfonamide ARGs. Furthermore， photocatalysis successfully removes ARGs, and the higher the intensity of the irradiation, the better the efficacy of ARGs removal. For the elimination of ARGs, nanocomposites outperformed TiO_2. Combining the advantages of CWs and photocatalysis in the removal of ARGs, a combination of CWs and photocatalysis could be proposed to remove ARGs. With remarkable success, the combination of photocatalysis and CWs has been studied for the treatment of high-salt chromium-containing wastewater (Li et al., 2020) and municipal wastewater. More importantly, in the combination system of CWs and photocatalysis, CWs could further eliminate the eARGs. Under UVA irradiation, the catalyst generates ROS, and the ROS break the cell wall and allow intracellular DNA to flow out, converting the difficult-to-remove iARGs into eARGs. While the ROS also attacks the eARGs, breaking the DNA strands and inactivating the ARGs. eARGs and iARGs are removed further in the artificial wetland by substrate adsorption, plant uptake, and microbial action. Furthermore, the combination would be increased the removal efficiency of COD, BOD_5, and Cr (VI) with no negative effect on plant production indicators (Li et al., 2019). In addition, photocatalysis can extend the life of the wetland because it eliminates clogging with refractory substances, reduces total phosphorus concentrations and reduces wetland loading.

Therefore, CWs paired with photocatalytic wastewater treatment technology provide the advantages of steady water quality, minimal investment, and low running costs. When CWs are combined with photocatalytic technology, they may remove both iARGs and eARGs, and the treatment is complete, lowering the risk of ARGs spreading. Moverover, CWs in combination with photocatalysis could produce a flexible and operable wastewater treatment system. This method combines ecological treatment technology with photocatalysis technology to produce a new technology that is superior to traditional wastewater treatment methods to remove resistance genes.

Author contributions

PC: data curation and analysis, writing—original draft. XY: worked on the technical details, supervised the findings of the work and helped in the development of manuscript. JZ: aided in interpreting the results and worked on the manuscript. YW: writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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EDITED BY
Huai Li,
Northeast Institute of Geography and Agroecology (CAS), China

REVIEWED BY
Lu Zheng,
Institute of Soil Science (CAS), China
Xiaofei Yu,
Northeast Normal University, China

*CORRESPONDENCE
Rong Xiao
✉ xiaorong@fzu.edu.cn

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Metagenomics reveals biogeochemical processes carried out by sediment microbial communities in a shallow eutrophic freshwater lake

Bo Kuang[1], Rong Xiao[1]*, Yanping Hu[1], Yaping Wang[1], Ling Zhang[2], Zhuoqun Wei[2], Junhong Bai[2], Kegang Zhang[3], Jacquelinne J. Acuña[4], Milko A. Jorquera[4] and Wenbin Pan[1]

[1]College of Environment and Safety Engineering, Fuzhou University, Fuzhou, China, [2]State Key Laboratory of Water Environment Simulation, School of Environment, Beijing Normal University, Beijing, China, [3]Department of Environmental Science and Engineering, North China Electric Power University, Baoding, China, [4]Department of Chemical Sciences and Natural Resources, University of La Frontera, Temuco, Chile

Introduction: As the largest shallow freshwater lake in the North China Plain, Baiyangdian lake is essential for maintaining ecosystem functioning in this highly populated region. Sediments are considered to record the impacts of human activities.

Methods: The abundance, diversity and metabolic pathways of microbial communities in sediments were studied by metagenomic approach to reveal patterns and mechanism of C, N, P and S cycling under the threat of lake eutrophication.

Results: Many genera, with plural genes encoding key enzymes involved in genes, belonging to Proteobacteria and Actinobacteria which were the most main phylum in bacterial community of Baiyangdian sediment were involved in C, N, S, P cycling processes, such as *Nocardioides* (Actinobacteria), *Thiobacillus*, *Nitrosomonas*, *Rhodoplanes* and *Sulfuricaulis* (Proteobacteria). For instance, the abundance of *Nocardioides* were positively correlated to TN, EC, SOC and N/P ratio in pathways of phytase, regulation of phosphate starvation, dissimilatory sulfate reduction and oxidation, assimilatory sulfate reduction, assimilatory nitrate reduction and reductive tricarboxylic acid (rTCA) cycle. Many key genes in C, N, P, S cycling were closely related to the reductive citrate cycle. A complete while weaker sulfur cycle between SO_4^{2-} and HS^- might occur in Baiyangdian lake sediments compared to C fixation and N cycling. In addition, dissimilatory nitrate reduction to ammonia was determined to co-occur with denitrification. Methanogenesis was the main pathway of methane metabolism and the reductive citrate cycle was accounted for the highest proportion of C fixation processes. The abundance of pathways of assimilatory nitrate reduction, denitrification and dissimilatory nitrate reduction of nitrogen cycling in sediments with higher TN content was

higher than those with lower TN content. Besides, *Nocardioides* with plural genes encoding key enzymes involved in *nasAB* and *nirBD* gene were involved in these pathways.

Discussion: *Nocardioides* involved in the processes of assimilatory nitrate reduction, denitrification and dissimilatory nitrate reduction of nitrogen cycling may have important effects on nitrogen transformation.

KEYWORDS

metagenomics, sediment, microbial community, biogeochemical processes, Baiyangdian

1. Introduction

As an important component of global aquatic ecosystems, lakes only account for a small fraction (2.8%) of the land surface (Downing et al., 2006), however play a unique and essential role in biogeochemical cycles due to their possession of diverse microbes, and capability to hasten the nutrients cycling (Hakulinen et al., 2005; Liu et al., 2009; Song et al., 2012; Small et al., 2014; Verpoorter et al., 2014; Li et al., 2015; Huang and Jiang, 2016; Frade et al., 2020). Microorganisms are monitors to variation in the external environment in the lake and may be one of the most sensitive indicators (Kuang et al., 2022). Metabolism of organic and inorganic elements can be changed by microbial communities which impact biogeochemical environments of lake sediments (Song et al., 2012). Moreover, it is reported that the carbon cycle is dominated by the balance between carbon-fixing and carbon-consuming occurred in microorganisms; microbial nitrogen-transforming networks both attenuate and exacerbate human-induced global change; microbial P mineralization is a side-effect of microbial C acquisition and can be driven by it; the remineralization of the organic matter of seafloor is facilitated by sulfate-reducing microorganisms (Spohn and Kuzyakov, 2013; Bowles et al., 2014; Gougoulias et al., 2014; Kuypers et al., 2018). Such natural diverse conditions harbor hotspots of microbes.

Increasing attention have been paid to the environmental regulatory factors leading to eutrophication and changing the constitute of sediment bacterial community, such as pH, N, P, N:P ratio (N/P) and soil organic carbon (SOC; Howarth et al., 2011; Wen et al., 2012; Yi et al., 2021; Kuang et al., 2022). And overdosing or excessive enrichment of nutrients lead to eutrophication. In the past half century, human activities have greatly accelerated the flow of nutrients to lake ecosystem, resulting in extensive eutrophication (Rabalais, 2002). In many industrialized countries, although the input of P drops sharply due to the improvement of wastewater treatment plants, the N pollution is still high (Howarth et al., 2011). In sediments, the decay of algal biomass generates organic carbon and consumes oxygen, which is conducive to N loss through denitrification. Under natural conditions and control experiments, when algae gather, N loss will be reduced (Zhu et al., 2020). Eutrophication may produce positive feedback to increase phosphorus supply. For example, P storage in many sediments decreases and P flux into water increases (Bol et al., 2018). In addition, in sediment, with the progress of eutrophication, the increase of C input and the decrease of oxygen input, sulfide will accumulate faster (Corredor et al., 1999). Even when the organic carbon (OC) content is low, sulfate reduction also accounts for a lot. When the OC concentration is high, the sulfate reduction contribution is higher (Howarth, 1984). Relationship between N, P and OC are also reported that the loss of N and the increase of P leads to lower N/P but higher trophic level index (calculated by Chl-a, Secchi disk transparency and TP). Specifically, in eutrophic lakes, the enrichment of total organic carbon (TOC) and all forms of P in sediment can promote potential denitrification rate and eventually leads to N loss by regulating community composition and increasing the abundance of *nirS* denitrifiers (Zhang et al., 2018). The eutrophication of surface water is an endemic global problem, and the nutrient load from agriculture is an underlying and persistent driving factor (Withers et al., 2014). Pesticides containing organophosphorus (OPPs) which were widely used in agriculture for their insecticidal properties are one of the most commonly used chemical pesticides in China (Wu et al., 2009). It is common for OPPs to leave residues in ecosystems due to their indiscriminate application (Montuori et al., 2016; Liu et al., 2019). To the best of our knowledge, there are various levels of toxicity associated with the acute and chronic exposure of humans, animals, plants, and insects to OPPs (Sidhu et al., 2019). It is demonstrated that pesticide pollution exists in agricultural ecosystems. For example, in sediments of Sundays and Swartkops Estuary, the highest concentration of OPPs is, respectively, up to 8.07 μg/kg and 13.6 μg/kg (Olisah et al., 2021a,b). The concentration of parathion and paraoxon-methyl (the degradation product of parathion-methyl) which are the most common OPP pollutants in Tai Lake varies from 5.88 to 506 ng/l (Wei et al., 2022).

In shallow lakes, eutrophication is widespread which leads to phytoplankton and algal blooms and then hypoxic or anoxic episodes when the organisms submerge and decompose, and is generally caused by increased P, N and organic matter (Gao et al., 2021; Li et al., 2016). As the largest shallow freshwater lake in the North China Plain, Baiyangdian lake is essential for maintaining ecosystem functioning in this highly populated

region (Han et al., 2020). However, increased anthropogenic activities in recent decades have gradually increased the amounts of nutrients discharged into the lake in domestic sewage and industrial wastewater from the Baoding City in the upper reaches via the Fu River. These discharges and nutrients transported from farmland and residential areas have caused nutrient enrichment, resulting in eutrophication of Baiyangdian lake (Zhu et al., 2019). And then, the activities of C, N, P and S cycling in Baiyangdian lake have changed. A vast and uncultured diversity of microorganisms is exposed by molecular genetic techniques, so that there is a huge breakthrough of environmental microbiology (Handelsman, 2004). This is the main advantage of metagenomics, because cultivating new microorganisms is a difficult technology. Massive sequence data generated by metagenome technology allows linking microbial communities with their biogeochemical functions and provides insights about abiotic and biological interactions of microbial communities (Grossart et al., 2020). However, there are a few studies on the influence of environmental factors on the biogeochemistry cycle of shallow lakes under the condition of eutrophication. In this study, we (1) characterize the microbial community structure in the sediments of Baiyangdian, and (2) reveal the effect of environmental factors in controlling microbiome composition of sediment, and (3) show the relationship between relative abundance of important taxa and functional genes for regulating C, N, P and S cycling, as well as their metabolism pathway for further quantifying bacterial functional potential in biogeochemical cycling processes in inland freshwater lake floors under eutrophic threats.

2. Materials and methods

2.1. Study area and sample collection

In northern China, Baiyangdian Lake is located in the center of Hebei province at 38°43′ to 39°02′ N, 115°38′ to 116°07′ E (Figure 1) with a watershed area of 781 km^2. Baiyangdian is the ecological hinterland of Xiong'an New Area. As a carrier of ecological resources and a sink of environmental load, water environment of Baiyangdian is tightly related to the ecological security of Xiong'an New Area. In our study, we want to basically reflect the whole physicochemical property of Baiyangdian Lake, so total 10 typical sediment samples (0–10 cm depth) which were intensively mixed of 4–6 buckets with grab bucket were collected during November 25 ~ 26, 2021. Specific sampling sites were marked as S1 to S10 in Figure 1. S1, S2, S3 and S8 are typical human gathering areas locating near the village. S6 and S7 are located near the inner river. S9 is located in open water and S10 nears to entrance of lake. S4 and S5 are near to the Anxin County. The villages of Beihezhuang (BHZ), Nanliuzhuang (NLZ), Duancun (DC), Quantou (QT) and Caiputai (CPT) were also marked on Figure 1. The samples were transported to the lab on ice and were kept at 4°C and −20°C, respectively, for further analysis.

2.2. Determination of physicochemical property and organophosphorus pesticides

Sediment samples were air-dried, crushed, and passed through meshes of 0.25 mm or 2 mm during laboratory analysis. Electrical conductivity (EC) and pH of sediment were measured at a soil to water mass ratio of 1:5 using a surveying instrument (P16 pH/EC/DO determinator, Yoke instrument, shanghai, China) equipped with a calibrated combined glass electrode (Kuang et al., 2022). SOC was determined using wet oxidation with $K_2Cr_2O_7$; total nitrogen (TN) concentration was measured using the classical Kjeldahl digestion method and the total phosphorus (TP) concentration was determined by alkaline digestion followed by molybdate colorimetry (Liu et al., 2013).

Eight OPPs (diazinon, parathion-methyl, fenitrothion, malathion, chlorpyrifos, fenthion, quinalphos, ethion) were measured. Briefly, 10 g of sediment after being centrifuged was extracted by 10 ml acetonitrile. After shaking by hand for 30 s, 7.5 g anhydrous $MgSO_4$ and 1 g NaCl were added. Subsequently, the mixture was centrifuged at 5000 rpm for 5 min after shaking for 30 s. The extract (1.8 ml) was mixed with 100 mg primary secondary amine sorbent, 150 mg anhydrous $MgSO_4$ and 30 mg graphitized carbon black. They were shaken vigorously for 30 s and centrifuged at 9000 rpm for 5 min (Olisah et al., 2021b). The final extracts were used to analyze the OPPs by Gas Chromatography–Mass Spectrometry (GCMS-QP2020 NX, Shimadzu, Japan) fitted with SH-Rxi-5Sil MS column (30 m × 250 μm × 250 μm), with 2 μl volume being injected automatically. The split less mode was applied for injection and the injector inlet temperature was 250°C. The column temperature was programmed as follows: from 90°C to 180°C for 1 min at 25°C/min, from 180°C to 270°C for 1 min at 3°C/min, from 270°C to 310°C at 20°C/min for 3 min, given a total run time of about 41 min. Helium (99.9%) was used as the carrier gas at a constant flow rate of 1.0 ml/min, and nitrogen was the make-up gas. Full-scan analysis (50–450 m/z) was used to determine cleanup effects, and selected ion monitoring mode was used for measure.

2.3. DNA extraction and metagenomic sequencing

Total genomic DNA of sediment were extracted using ZNA® Bacterial DNA Kit for Soil (Omega Biotech, United States) according to the manufacture's instruction. The fresh sediment sample was used directly for DNA extraction. Extracted DNA was checked using 2% agarose gels. After quality assessment, DNA was stored at −80°C until further analyses. Metagenomic libraries of ten

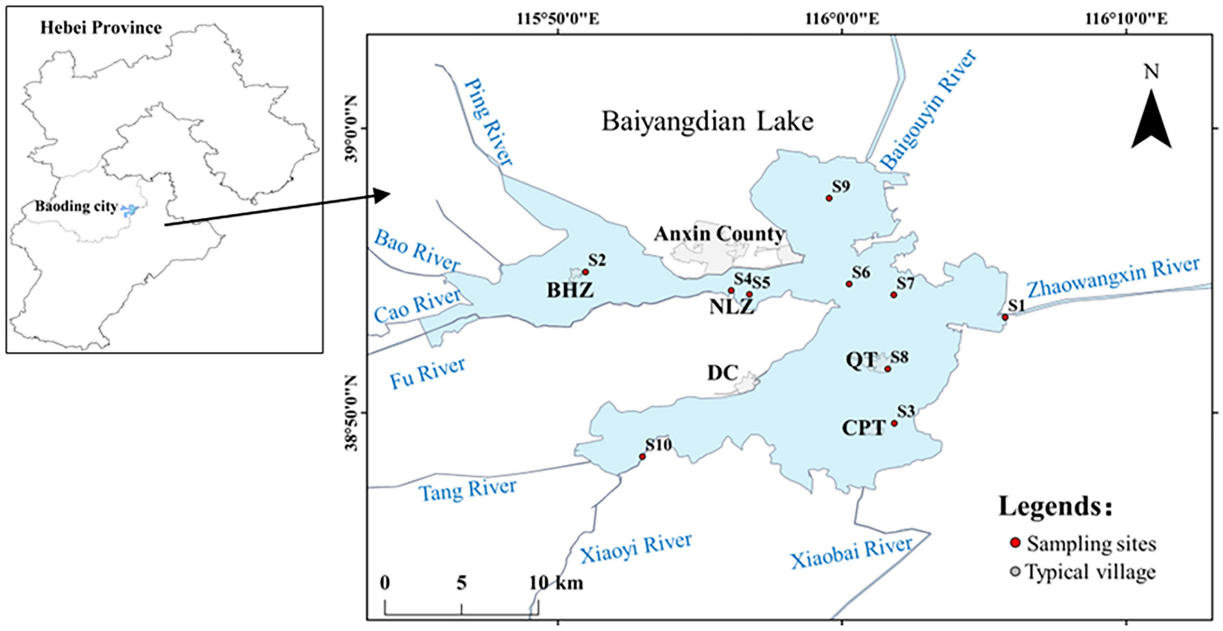

FIGURE 1
Sampling sites of Baiyangdian Lake.

sediment samples were performed by Illumina HiSeq 2,500 platform. Raw sequence data (40.37 Gb) were generated. Sequences were cleaned and assembled using Seqprep, Sickle, BWA, and SOAPdenovo (Version 1.06), and the length of contigs >500 bp were retained for further bioinformatics analyses. CD-HIT was used to build a non-redundant gene catalog, and MetaGene software was used for ORF prediction. Taxonomic assignment was carried by using BLASTP alignment against the integrated non-redundant (NR) database of the National Center for Biotechnology Information (NCBI; Tatusov et al., 2003). In addition, the resulting genes were annotated and classified in species and function. Raw reads were uploaded to the NCBI database under BioProject PRJNA908081.

2.4. Bioinformatics analysis

On one hand, to carry out composition annotation, Unigenes were blasted to sequences of bacteria, eukaryota, archaea and viruses extracted from the NR database (Version 20,200,604) of NCBI using DIAMOND software (Version 0.8.35). On the other hand, for function analysis, DIAMOND software was adopted to blast Unigenes to KEGG (Kyoto Encyclopedia of Genes and Genomes) database (Version 94.2; Kanehisa et al., 2014; Buchfink et al., 2015). Then, genes encoding key enzymes involved in C fixation, N, S and P cycling were identified according to KEGG. Moreover, correlation analysis, calculated by IBM SPSS Statistics (Version 26), was used to investigate the relationships between environmental parameters and different functional profiles such as C, N, P, S cycling. Additionally, the dominant metabolic type of different functions was also determined by DIAMOND.

3. Results

3.1. Physicochemical property and organophosphorus pesticides

As shown in Table 1, the pH of sediment varied from 7.78 to 8.35 reflecting the slightly alkaline in the Baiyangdian Lake; the average value of EC in sediment was 410 μS/cm. TN varied significantly across sample sites with average value around 2,400 (±809) mg/kg and TP varied with average value around 515 (±92) mg/kg. No significant correlation was found between TP, OPPs and pH, EC, TN, SOC, N/P in the sediments from Baiyangdian Lake (Supplementary Table S1). Among the eight OPPs, the detection rates of diazinon and fenitrothion in sediment reached 50 and 60%, respectively, and were higher than the rest of six pesticides. The detection rate of parathion-methyl and malathion in sediment were lower than those of diazinon and fenitrothion, while the concentrations were higher than those of diazinon and fenitrothion. The highest level of total OPPs was measured in site S10 reaching around 97 mg/kg (average value) and the lowest concentration of total OPPs was determined in the sits S6 as low as 3.2 mg/kg (average value). TN had significant negative correlation with pH ($p < 0.05$) and positive correlation with EC, SOC, N/P ($p < 0.01$). In addition, EC had significant negative correlation with pH ($p < 0.05$) and positive correlation with N/P ($p < 0.01$; Supplementary Table S1).

TABLE 1 Physicochemical properties of sediment samples from Baiyangdian Lake.

Sites	pH	TN (mg/kg)	TP (mg/kg)	EC (μS/cm)	SOC (%)	N/P	OPPs (μg/kg)
S1	8.35	1446.84 (152.96)^{d}	436.67 (102.15)^{ab}	230	1.33 (0.47)^{bc}	3.31	4.38 (±6.19)^{b}
S2	7.78	3320.52 (193.71)^{ab}	572.22 (70.83)^{ab}	419	1.38 (0.23)^{bc}	5.8	15.47 (±1.11)^{b}
S3	7.86	3634.56 (356.97)^{a}	402.22 (24.66)^{b}	559	3.34 (1.28)^{a}	9.04	16.97 (±1.05)^{b}
S4	7.83	2724.03 (262.19)^{bc}	689.26 (145.01)^{a}	547	2.9 (0.25)^{ab}	3.95	79.94 (±40.88)^{a}
S5	7.99	1613.51 (136.73)^{d}	595.93 (68.24)^{ab}	247	1.65 (0.44)^{abc}	2.71	34.48 (±11.46)^{b}
S6	8.16	2497.72 (60.22)^{c}	513.7 (167.87)^{ab}	270	2.7 (0.25)^{abc}	4.86	3.2 (±1.54)^{b}
S7	8.31	1631.05 (151.87)^{d}	554.45 (126.35)^{ab}	262	1.36 (0.3)^{bc}	2.94	17.28 (±2.78)^{b}
S8	8.06	1588.95 (235.26)^{d}	355.93 (44.97)^{b}	405	1.02 (0.29)^{c}	4.46	14.6 (±7.45)^{b}
S9	8.10	2064.39 (191.91)^{cd}	524.07 (54.03)^{ab}	383	1.31 (0.45)^{bc}	3.94	9.29 (±6.54)^{b}
S10	7.98	3481.93 (651.02)^{a}	511.85 (125.7)^{ab}	777	2.74 (1.62)^{abc}	6.8	97.47 (±15)^{a}

Values were presented by means (with standard deviation). Lower case letters-superscripts denote significant differences among the different groups within a column ($p < 0.05$).

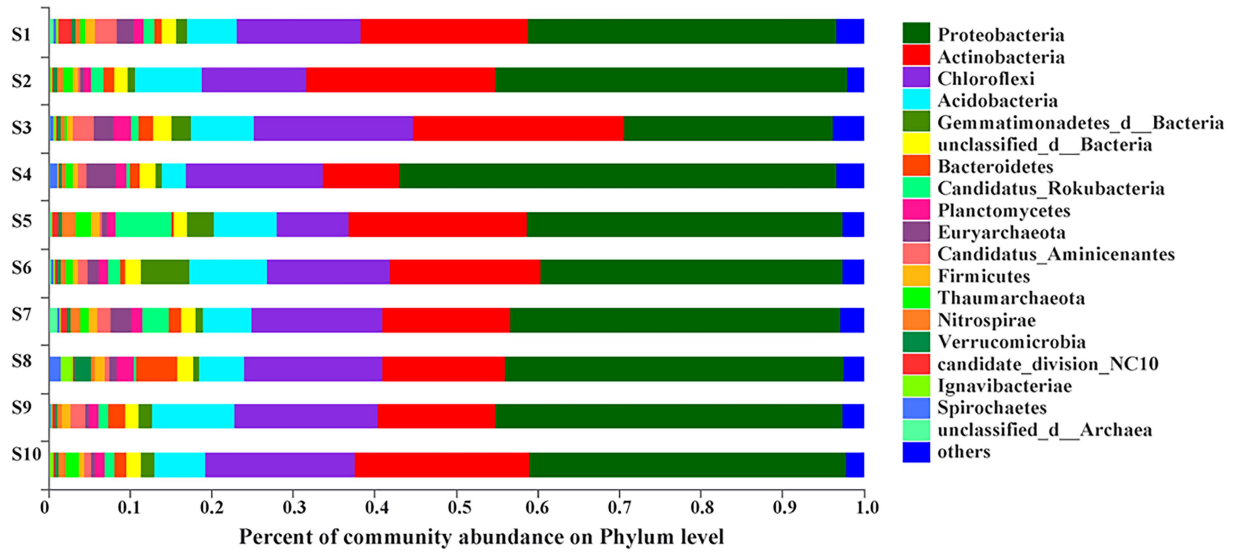

FIGURE 2
Bacterial communities' composition in sediments at phylum level of Baiyangdian lake.

3.2. Taxonomic profiles of bacterial community in sediment

At the phylum level, more than 1% of total microbial community composition in each sediment sample was picked out to analyze species and abundance of bacterial community. The top eight phyla followed the order of Proteobacteria (25.64% ~ 53.63%), Actinobacteria (9.39% ~ 25.96%), Chloroflexi (8.83% ~ 19.45%), Acidobacteria (2.99% ~ 10.01%), Gemmatimonadetes (0.67% ~ 5.97%), unclassified_d__Bacteria (1.55% ~ 2.16%), Bacteroidetes (0.33% ~ 5.09%), Candidatus_Rokubacteria (0.33% ~ 6.93%; Figure 2). At site S3, Proteobacteria had the lowest relative abundance among all samples while Actinobacia and Chloroflexi had the highest. In addition, the sediment sample from site S4 had the highest relative abundance of Proteobacteria but the lowest relative abundance of Actinobacteria, Acidobacteria and Gemmatimonadetes. Candidatus_Rokubacteria had the highest relative abundance in S5 but lowest in S8. Bacteroidetes (opposite to Candidatus_Rokubacteria) had the highest relative abundance in S8 but lowest in S5. Chloroflexi was the most abundant in site S5. The relative abundance of Gemmatimonadetes and Acidobacteria were the highest in S6 and S9, respectively. As shown in Table 2, many genera were involved in C, N, S, P cycling processes, such as, *Thiobacillus*, *Nitrosomonas*, *Aromatoleum*, *Rhodoplanes*, *Sulfuricaulis*, *Pseudomonas*, *Desulfatitalea* and *Desulfobulbus* of Proteobacteria; *Nocardioides* of Actinobacteria; *Methanothrix* of Euryarchaeota. A few key genes involved in each cycle were showed in Supplementary Table S2. For example, *napAB, narGHI, nasAB, nifDHK, nirBD, nirKS, norBC* and *nrfAH* were involved in nitrogen cycling; *aprAB* was involved in phosphorus cycling. Many genera with plural genes encoding key enzymes involved in these genes. Such as *Thiobacillus*,

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TABLE 2 Major taxa involved in C, N, P, S cycling in Baiyangdian lake sediments based on KEGG annotation results of metagenomes.

Cycling processes	Metabolic pathways	Major taxa
Carbon fixation	*1: 3-HP/4-HB*	*Rhodoplanes, Syntrophus, Methylibium*
	2: 3-HP	*Nocardioides, Aestuariivirga, Sulfurisoma*
	3: WL	*Methanothrix, Desulfobacca, Desulfobulbus*
	4: rTCA	*Nocardioides, Thiobacillus, Anaeromyxobacter*
	5: CBB	*Thiobacillus, Sedimenticola, Sulfuricaulis*
Nitrogen cycling	*6: Nitrification*	*Nitrosomonas, Sulfurifustis, Streptomyces*
	7: N fixation	*Thiobacillus, Desulfobulbus, Geobacter*
	8: Denitrification	*Thiobacillus, Dechloromonas, Anaeromyxobacter*
	9: Assimilatory nitrate reduction	*Nocardioides, Thiobacillus, Dechloromonas*
	10: DNRA	*Thiobacillus, Nocardioides, Gaiella*
Sulfur cycling	*11: Sox system*	*Thiobacillus, Aromatoleum, Sulfuricaulis*
	12: Dissimilatory sulfate reduction and oxidation	*Thiobacillus, Sulfuricaulis, Nocardioides*
	13: Assimilatory sulfate reduction	*Nocardioides, Nitrososphaera, Thiobacillus*
Phosphorus cycling	*14: Regulation of phosphate starvation*	*Thiobacillus, Steroidobacter, Nocardioides*
	15: Phosphorus	*Thiobacillus, Nocardioides, Candidatus_Methanoperedens*
	16: Inorganic phosphate solubilizing	*Pseudomonas, Nocardioides, Thiobacillus*
	17: Phosphonate degradation	*Desulfatitalea, Thiobacillus, Candidatus_Methanoperedens*
	18: Phytase	*Solirubrobacter, Nocardioides, Kribbella*
	19: Phosphoesterase	*Nocardioides, Cryobacterium, Aromatoleum*

3-HP/4-HB, 3-hydroxypropionate/4-hydroxybutylate cycle; 3-HP, 3-hydroxypropionate bicycle; WL, Wood-Ljungdahl pathway; rTCA, reductive tricarboxylic acid cycle; CBB, Calvin–Benson–Bassham cycle; DNRA, dissimilatory nitrate reduction to ammonium.

Nocardioides, *Gaiella*, *Actinomadura*, *Thioalkalivibrio*, *Ramlibacter*. Then, at the phylum level, for the bacterial community in the sediments of Baiyangdian lake, the relative abundance of Chloroflexi had significant positive correlation with EC while the relative abundance of Candidatus_Rokubacteria had negative correlation with it.

3.3. Carbon fixation, nitrogen, sulfur, phosphorus cycling metabolism through metagenomic analysis

As shown in Supplementary Figure S1, at KEGG pathway level 2, global and overview maps were the dominant metabolic subsystems in the sediment system. Metabolism was also the indispensable function in the system of sediment, such as, metabolism of carbohydrate, amino acid, energy, cofactors and vitamins, lipid, nucleotide, xenobiotics, terpenoids and polyketides. In addition, carbohydrate metabolism, amino acid metabolism and energy metabolism had relative abundances of 10.47, 7.64, and 5.69%, respectively. Carbon fixation pathway, N, S cycling modules and genes encoding key enzymes involved in P cycling were analyzed based on the KEGG database. Major taxa involved in C, N, P, S cycling based on KEGG annotation results of metagenomes were shown in Table 2. Most of them belonged to Proteobacteria phylum. In addition, among the major taxa, the genus *Nocardioides* had played an important role in many metabolic pathways of C, N, P, S cycling and was affected by many environmental factors. As shown in Figure 3, for example, the abundance of *Kribbella* was negatively correlated with TP in pathway of phytase. The relative abundance of *Thiobacillus* was positively correlated with the concentration of OPPs in pathways of regulation of phosphate starvation, inorganic phosphate solubilizing, assimilatory nitrate reduction and CBB (Table 2). The abundance of *Nocardioides* were positively correlated to TN, EC, SOC and N/P in pathways of phytase, regulation of phosphate starvation, dissimilatory sulfate reduction and oxidation, assimilatory sulfate reduction, assimilatory nitrate reduction and rTCA. The abundance of *Candidatus_Methanoperedens* was positively related to pH (negatively related to EC) in pathway of phosphonate degradation and was negatively related N/P in pathway of phosphorus. Sulfur metabolism pathway was mainly composed of 4 reaction modules (Figure 4A): assimilatory sulfate reduction, dissimilatory sulfate reduction and oxidation, thiosulfate oxidation by SOX complex and cysteine biosynthesis; C fixation pathway was mainly composed of 7 reaction modules (Figure 4B): incomplete reductive citrate cycle, phosphate acetyltransferase-acetate kinase pathway, reductive acetyl-CoA pathway, 3-Hydroxypropionate, hydroxypropionate-hydroxybutylate, dicarboxylate-hydroxybutyrate and reductive citrate cycle; N metabolism was mainly composed of 6 reaction modules (Figure 4C): complete nitrification, assimilatory nitrate

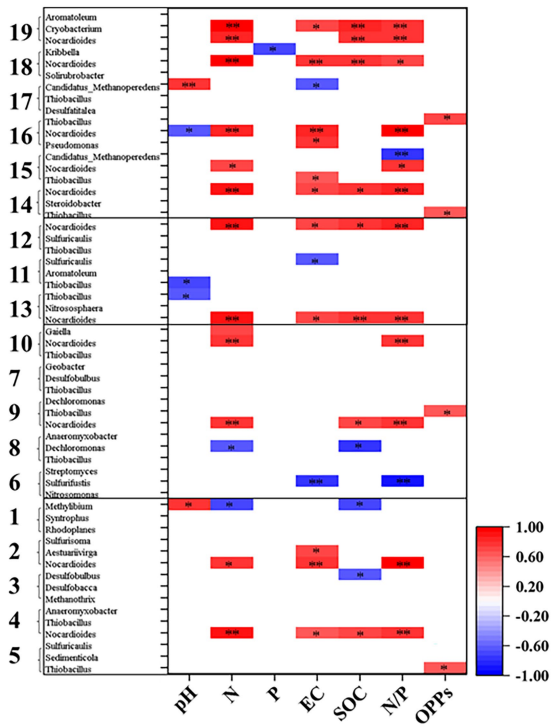

FIGURE 3
Spearman's correlation coefficients between major taxa involved in C, N, P, S cycling and sediment properties. Colored squares indicate significant correlations at $p < 0.05$ (*) or $p < 0.01$ (**) level and blank space indicates no significant correlation. The digits of y-axis represent different pathways showed in Table 2.

reduction, dissimilatory nitrate reduction, denitrification, nitrification and nitrogen fixation; and key genes of P cycling were divided into 6 types (Figure 4D). P-cycling genetic signatures in sediment metagenome involved in phosphoesterase, phytase, phosphonate degradation, inorganic phosphate solubilizing, phosphorus and regulation of phosphate starvation. For example, high abundance genes *phoURB* involved in regulating of phosphate starvation; *pstABC* involved in P transport; *glpQ* and *phoD* involved in phosphoesterase. Moreover, the highest proportion M00176 was the main pathway of S metabolism and the highest proportion M00377 was the main pathway of C fixation. In the N metabolism module M00377 occupying the highest proportion, nitrates were converted to nitrogen by denitrification. As shown in Figure 5, many key genes in C, N, P, S cycling were correlated with module M00357 and M00173. In these two modules (Figure 6), these genes were mainly involved in the module reductive citrate cycle of C fixation. In addition, the abundance of genes *corBD* were proved to be negatively correlated with M00173. The module methanogenesis in methane metabolism was positively correlated with abundance of genes *pmoB-amoB* and *narB* and negatively correlated with abundance of *phoN* and *phnO*. Moreover, many genes in P cycling (e.g., *phoABR*, *phnAEKLMNI*, *ugpABCQ*, *pstABC*) were positively correlated with the module M00173.

4. Discussion

Environmental factors are important to determine the composition of microbial communities. Many studies had shown that TN and TP played important roles in composition of bacterial communities (Song et al., 2012; Bergkemper et al., 2016; Wei et al., 2018). In our study, TN and TP greatly affected the bacterial community composition. TN likely showed a relatively greater impact on bacterial communities than TP. We divided the ten points into group 1 (S2, S3, S10) and group 2 (the rest of the samples sites; Figure 7). The values of TN, EC and N/P of group 1 was significantly higher than group 2 ($p < 0.05$). And, there is no obvious difference in total phosphorus content. Compared with the highly contaminated situation (value of TN and TP contents were approximately 4,000 and 800 mg/kg) of Baiyangdian in 2018 reported in January this year (Wang et al., 2022), TN and TP have dropped to around 2,400 and 515 mg/kg, respectively now because of somewhat significant results that the government has achieved in pollution control in recent years. But it is still under eutrophication threat. In addition, in site S3, in the condition of the highest N/P up to 9.04, Proteobacteria had the lowest relative abundance while Chloroflexi had the highest. Also, the function of assimilatory nitrate reduction of N fixation was also the strongest but the functions of C fixation (e.g., reductive citrate cycle, dicarboxylate-hydroxybutyrate cycle, reductive acetyl-CoA pathway) were at an average level. The high N/P might be caused by strong capacity of N removal of Proteobacteria (Nielsen et al., 1999; Ren et al., 2018) and the effects of Chloroflexi in N cycling (Rao et al., 2022), because of the lowest abundance of Proteobacteria and the highest abundance of Chloroflexi. TN and TP were important macronutrients for all biota on earth and were two essential components of the energy metabolism and the genetic backup and stable cell structures. For example, recent researches had provided evidence for that TN was the dominant environmental factor to drive the formation of bacterial community structure and that TP concentration was significantly correlated with the distribution of bacterial communities (Song et al., 2012; Fan et al., 2019). In addition, previous researches showed that microbial community composition could varied with addition of N (Wang et al., 2018). A study showed that the effect of TN on the community structure was mainly caused by pushing the variation of the dominated phyla and influencing the functional microbes of C and N metabolism (Liao et al., 2021). For TN, many studies had shown that the relative abundance of Actinobacteria might be restrained by TN (Pascault et al., 2014; Mao et al., 2019; Ouyang et al., 2020). It was consistent with the community distribution of sample S4, but not the communities of other samples. In addition, at site S4, 3-Hydroxypropionate cycling of C fixation was the strongest while the weakest function of denitrification and complete nitrification of N cycling (might be caused by low abundance of Actinobacteria) which might lead to the high level of TN. TN fixation of sediment might be weaker in areas with high water velocity (Duan et al., 2016). Sample S6, S7, S9 were basically sampled from open water; Sample S1 was sampled from Zhaowangxin river estuary. The

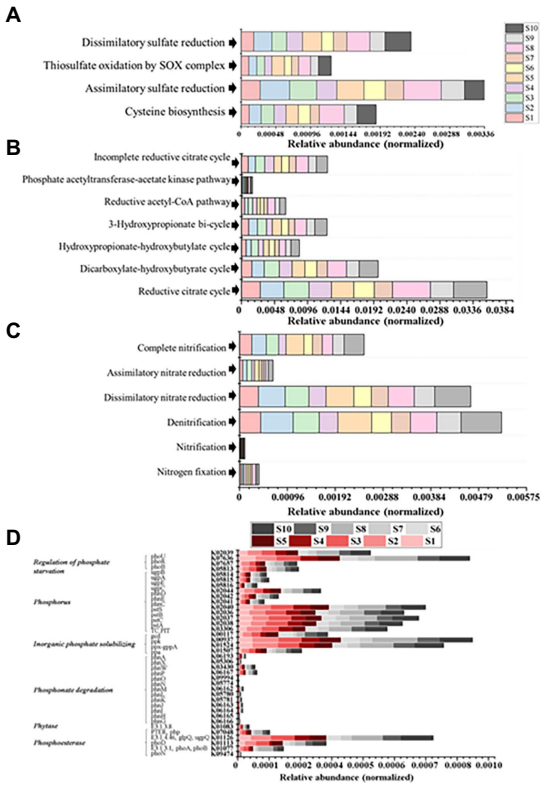

FIGURE 4
(A) Relative abundance of S metabolism modules. **(B)** Relative abundance of C fixation modules. **(C)** Relative abundance of N metabolism modules. **(D)** Relative abundances of genes encoding key enzymes involved in P cycling.

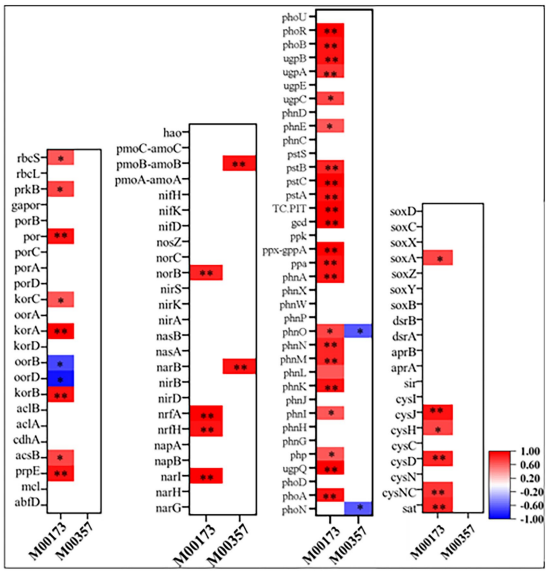

FIGURE 5
Spearman's correlation coefficients between key genes in C, N, P, S cycling and modules of methane metabolism and carbon fixation. Colored squares indicated significant correlation at p <0.05(*) or p <0.01(**) level while blank space indicated unsignificant correlation between key genes abundance and the corresponding modules abundance showing below in the x-axis.

relatively low TN content and N/P might be caused by high mobility of water. In addition, sample S10 was sampled from entrance of lake and S2, S3 were, respectively, sampled from the area where was more likely facing pressure of extensive water pollution and eutrophication near to Beihezhuang and Caiputai villages, all of them were relatively severely affected by human activities. The N enrichment in these samples was likely due to the geographic location of the sampling sites. Sample S5 was located near the village but the TN content was relatively low, which suggested that the situation was complicated under field conditions, and there might also be an area with relatively light pollution with less human activities. Sediment pH greatly impacted the TN contents by influencing microbial activities. Microorganisms were most active in neutral conditions while were inhibited in the alkali condition (Bai et al., 2005). Besides, from a global perspective, soil salinity was one of the major environmental factors limiting plant growth and productivity (Huang L. et al., 2017). We found that sediment TN was significantly negatively correlated with pH and positively correlated with EC. It was consistent with the findings of Mei et al. (2014). In lakes, reduction of the external P loading was a prerequisite for water quality improvement. However, lakes showed hardly any signs of recovery after reducing of the external P and it was mainly caused by recycling of P from the P-rich sediments (Lürling and van Oosterhout, 2013). The P cycle genes in Baiyangdian area were relatively rich (Figure 4) indicating that the P cycle in Baiyangdian sediments was relatively strong. This might be explained by the statement that the predominance of organic matter remineralization as the predominant pathway of P cycling in temporally varying bottom water redox conditions (Joshi et al., 2015).

Bacterial community played a key role in ecological functions of sediments. Proteobacteria was reported to contribute to N fixation, photosynthesis, S metabolism, methane metabolism and could degrade proteins, polysaccharides and other organic matter and removed N and P (Liao et al., 2019; Ouyang et al., 2020; Rathour et al., 2020; Duyar et al., 2021). As the dominant colonies in Baiyangdian sediment, the relative abundance of Proteobacteria in bacterial community was significant negatively correlated with Actinobacteria indicating their opposite distribution pattern. The dominance of Proteobacteria in bacterial community and opposite distribution pattern between Proteobacteria and Actinobacteria were also found in other plain lakes with different trophic status (Huang W. et al., 2017), suggesting that a universal mechanism was existed to shape the sediment bacterial community. Following Proteobacteria, Actinobacteria, Chloroflexi and Acidobacteria (Figure 2) were the large phyla within the bacterial community. Actinobacteria produced 70% of the world's natural antibiotics and had the ability to degrade complex organic compounds (Barka et al., 2016). Recent studies on antibiotics in Baiyangdian showed that there was a real risk of antibiotic contamination in the Baiyangdian area (Zhang et al., 2022). Actinobacteria widely

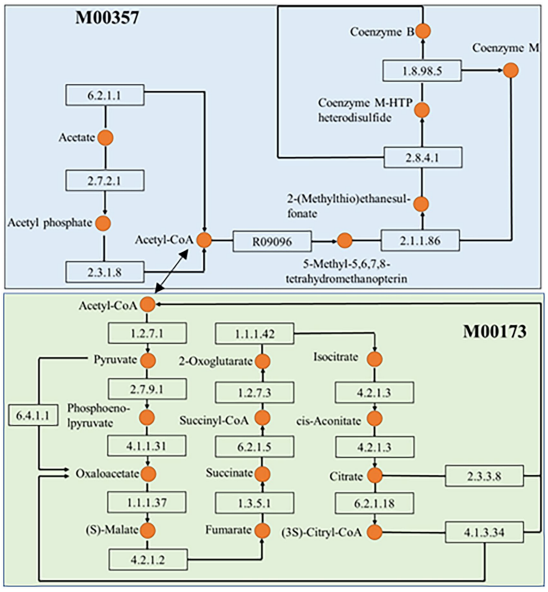

FIGURE 6
Interrelationship between the methanogenesis, acetate=>methane (M00357) in methane metabolism and the module reductive citrate cycle (Arnon-Buchanan cycle; M00173) in carbon fixation. The number in the rectangle represents the enzyme and the orange circle represents the substance noted on the left.

existed in ocean, soil and freshwater ecosystem, and many new species had been found (Wu et al., 2021). Actinobacteria had been identified to be potentially important DDT degraders and had been proved to degrade HCH by producing dechlorinating enzymes (Fudala-Ksiazek et al., 2018; Xu et al., 2021). In Baiyangdian Lake, Hu et al. (2010) had proved that organochlorine pesticides residue existed and HCHs were the predominant contaminants. This could explain why Actinobacteria occupied the second most abundant phylum of bacterial community in Baiyangdian, a typical shallow lake, which was also consistent with other studies (Liao et al., 2019; Ouyang et al., 2020). Then, noticeably, the third most abundance phylum of bacterial community in sediment was Chloroflexi. It was involved in the metabolism of organic matter and the transformation of contaminants (Liao et al., 2021). And, researcher had proved that Chloroflexi were rich in the sediments from eutrophic region (Huang W. et al., 2017).

Except for C metabolism, the biogeochemical processes of N and S also affected the biodegradation in sediments. Microbial groups related to N metabolism, including N_2 fixation groups and denitrification groups, could improve N availability, because bioavailable N was a major limiting factor in nutrient poor environments (Li et al., 2022). In our study, we found that the relative abundance of each module of the C-N-S cycle was consistent in each sample site, but its absolute abundance was different. It might indicate that in Baiyangdian lake, due to environmental properties, absolute abundance of each module of each sample site was different, but it showed consistent relative strength in the whole area. For examples, the relative abundance of reductive citrate cycle (M00173), denitrification (M00529) and assimilatory sulfate reduction (M00176) were, respectively, highest in C, N, S in all sediment samples. However, assimilatory nitrate reduction, denitrification and dissimilatory nitrate reduction of nitrogen cycling of group 1 were significantly high than group 2 (Figure 7). This indicated that higher N might accelerate the processes of assimilatory nitrate reduction, denitrification and dissimilatory nitrate reduction of nitrogen cycling. In addition, there were also significant differences in genus of *Nocardioides* involved in these pathways. This was consistent with the previous reports that the important effect of *Nocardioides* on nitrogen transformation (Zhang et al., 2021). Microbial processes associated with the S cycle had been extensively studied. Studies had affirmed that the microbial S cycle was among the most active in lakes (Sorokin et al., 2011; Vavourakis et al., 2019). Our research also found that a complete while weaker S cycle between SO_4^{2-} and HS^- might occur in Baiyangdian lake sediments compared to N cycle and C fixation (Figure 4). For N cycle, DNRA had been determined to co-occur with denitrification in estuary sediments (Baker et al., 2015). Also in our study, DNRA co-occurred with denitrification in shallow lake sediment and both of them were relatively active. The most abundant mode of C fixation of Baiyangdian lake was the reductive citrate cycle. It was noted that the reductive acetyl-CoA pathway was found to be most common mode of C fixation and regarded as the response to energy limitation (Hügler and Sievert, 2011). However, in our study, this C fixation pathway was not very strong. As we known, C metabolism pathways were one of the core metabolisms in soil bacterial community (Cai et al., 2020). Carbohydrate metabolism degraded complex organic matter into readily biodegradable substances and provided the necessary energy and matter for growth of bacteria. As a consequence, C fixation and methane metabolism and in Baiyangdian sediment were analyzed to reveal the pathways of organic matter conversion. For C cycle, organic matter was degraded by methanogenic archaea which used a battery of specific enzymes and coenzymes to cut CO_2 or methyl compounds down and produced CH_4 by means of biochemical reactions (Cheng et al., 2021). Moreover, in an aerobic state, organic matter could be broken down to produce CO_2 by microorganisms. Methanogenesis (M00357) in methane metabolism and the reductive citrate cycle (M00173) module in carbon fixation were further analyzed (Figure 6). The highest proportion of M00357 module was the main pathway of methane metabolism. In the methane metabolism M00357 module, acetate was converted to acetyl phosphate via ATP: acetate phosphotransferase (EC 2.7.2.1) again into acetyl-CoA via acetyl-CoA: phosphate acetyltransferase, or to acetyl-CoA via acetate: CoA ligase (EC 6.2.1.1). In addition, acetyl-CoA, coenzyme M-HTP heterodisulfide, 2-(Methylthio) ethanesul-fonate and 5-Methyl-5,6,7,8-tetrahydromethanopterin were interconverted through a series of enzymes and coenzymes. The highest proportion of M00173 module was the main pathway of C fixation. The same was, acetyl-CoA, Citrate and (3S)-Citryl-CoA were interconverted through C fixation process. M00357 module

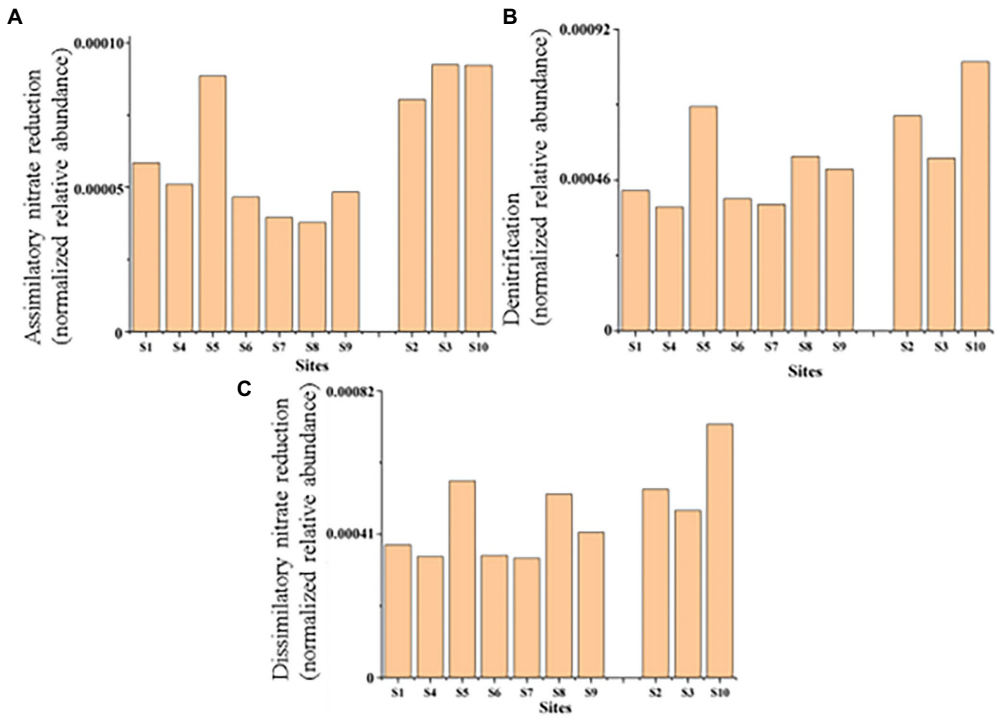

FIGURE 7
Parts of nitrogen metabolic pathways in sediments of ten typical sites from the Baiyangdian Lake. **(A)** Assimilatory nitrate reduction, **(B)** denitrification, **(C)** dissimilatory nitrate reduction.

and M00173 module were interconnected by acetyl-CoA. Moreover, many key genes in C, N, P, S cycling were closely related with C fixation. This showed that there was a complex interaction between the metabolic pathways of C fixation and key genes of C, N, P, S biogeochemical cycling. For the P cycle, genes involved in regulation of P starvation (such as *phoB*, *phoR*) were prevalent in soil ecosystem. K01078 (acid phosphatase) and K03788 (acid phosphatase (class B)) belonging to phosphoesterase were not detected in Baiyangdian sediment metagenome, possibly meaning the absence of phosphoesterase metabolism in microbiota. Gene *ppk* was reported to be a phylogenetic marker of phosphorus removal communities and was widely used to investigate P removal organisms. In addition, it was closely linked the patterns underlying endogenous P release from sediments (Lv et al., 2015). So, the dominance of S8 of K00937 (*ppk*) in Baiyangdian sediment metagenome might be associated with the release of underlying endogenous P. The same change trend in the ten sediment samples was also shown in some genes with relatively high abundance, such as, *pstABCS*, *phnCDE* and *ugpABCE*. It might suggest that the strength of P cycling in Baiyangdian lake. And, high microbial potential for efficient phosphate uptake systems (for example, *pstSCAB*) was also detected in P depleted soil (Bergkemper et al., 2016). These genes with high abundance also were screened in Baiyangdian sediments. Hence, the high abundance of these genes in lake sediments might be a common phenomenon. Phosphonoacetate hydrolase (*phnA*) might increase TP content by breaking C-P bonds. This interesting conclusion was also found in our previous studies (Campos et al., 2022). Acid phosphatase *phoN* was involved in the hydrolysis of P and occupied an important position in global P cycling (Rana et al., 2020). Therefore, it might increase the biologically available P by increasing the activity of P-related enzymes, resulting in the decrease of soil P content.

5. Conclusion

Baiyangdian lake sediment was under weak alkaline conditions. Compared with the highly contaminated situation of Baiyangdian in 2018, TN content have dropped almost 40% and TP content have dropped about 35%, however it was still under eutrophication threat. TN showed a relatively greater impact on bacterial communities than TP. The status of OPPs was at an acceptable level of risk and was not the main source of TP. Proteobacteria was the most main phylum in sediment, and was proved to indispensable contribute to C, N, P, S cycling. At site S4, 3-Hydroxypropionate cycling of C fixation was the strongest while the weakest function of denitrification and complete nitrification of N cycling. Many genera involved in C, N, S, P cycling processes, such as, *Thiobacillus*, *Nitrosomonas*, *Rhodoplanes* of Proteobacteria; *Nocardioides* of Actinobacteria. *Nocardioides* had played an important role in many metabolic pathways of C, N, P, S cycling. Many key genes in C, N, P, S cycling were closely related to the module reductive citrate cycle. The relative abundance of each module of C-N-P-S cycling was

consistent in each sample site, but its absolute abundance was different. However, the abundance of pathways of assimilatory nitrate reduction, denitrification and dissimilatory nitrate reduction of nitrogen cycling in sediments with higher TN content was higher than those with lower TN content. Besides, *Nocardioides* with plural genes encoding key enzymes involved in *nasAB* and *nirBD* gene were involved in these pathways. Carbon metabolism pathways was the core metabolisms in sediment bacterial community. In Baiyangdian lake, the methanogenesis process (involving genes such as *acs*, *hdrA2*…) was the main pathway of methane metabolism and the reductive citrate cycle module (involving genes such as *nifJ*, *oorA*…) accounted for the highest proportion of C fixation processes.

Data availability statement

The data presented in the study are deposited in the NCBI repository, accession number PRJNA908081.

Author contributions

BK was responsible for writing manuscript. RX was responsible for writing and correcting manuscript. YH, YW, LZ, and ZW were responsible for measurement of environmental factor. JB, KZ, JA, MJ, and WP were responsible for determining research direction. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1112669/full#supplementary-material

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EDITED BY
Huai Li,
Northeast Institute of Geography and Agroecology (CAS), China

REVIEWED BY
Xiaofei Yu,
Northeast Normal University, China
Weihua Guo,
Shandong University, China

*CORRESPONDENCE
Junhong Bai
junhongbai@163.com

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Antibiotics affected the bacterial community structure and diversity in pore water and sediments with cultivated *Phragmites australis* in a typical Chinese shallow lake

Ling Zhang[1,2], Junhong Bai[1]*, Yujia Zhai[1], Kegang Zhang[3], Zhuoqun Wei[1], Yaqi Wang[1], Haizhu Liu[1], Rong Xiao[4] and Milko A. Jorquera[5]

[1]School of Environment, Beijing Normal University, Beijing, China, [2]School of Chemistry and Chemical Engineering, Qinghai Normal University, Xining, China, [3]Department of Environmental Engineering and Science, North China Electric Power University, Baoding, China, [4]College of Environment and Safety Engineering, FuZhou University, Fuzhou, China, [5]Laboratorio de Ecología Microbiana Aplicada (EMALAB), Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco, Chile

The migration of antibiotics and bacterial communities between sediments and pore water occurring in the lake, which is affected by aquatic vegetation. However, the differences in bacterial community structure and biodiversity between pore water and sediments with plants in lakes under antibiotic stress are still poorly understood. We collected pore water and sediments in both wild and cultivated *Phragmites australis* regions in the Zaozhadian (ZZD) Lake to explore the characteristics of the bacterial community. Our results showed that the diversity of bacterial community in sediment samples were significantly higher than those in pore water samples in both *P. australis* regions. Due to higher antibiotic levels in sediments from the cultivated *P. australis* region, the composition of bacterial communities showed a difference, which reduced the relative abundance of dominant phyla in pore water and increased that in sediments. The higher bacterial variations in pore water could be explained by sediment in the cultivated *P. australis* region than that in wild *P. australis* region, therefore plant cultivation might change the source-sink pattern between sediments and pore water. The dominant factors shaping the bacterial communities in the wild *P. australis* region pore water or sediment were NH_4-N, NO_3-N, and particle size, while cultivated *P. australis* region pore water or sediment were oxytetracycline, tetracycline, etc. The findings of this work indicates that the antibiotic pollution caused by planting activities has a greater impact on the bacterial community, which will provide a reference for the use and management of antibiotics in lake ecosystems.

KEYWORDS

pore water, sediments, bacterial community, structure, biodiversity, shallow lake

1. Introduction

Lake sediment is one of the main media for the material cycle, including nutrient and pollution transformation and migration. Meanwhile, sediments had high microbial biomass and taxon richness, which plays an important role in driving the biogeochemical cycles of elements (Samuel et al., 2022). However, the aquatic plant growth (Bartelme et al., 2018), and

contaminants (such as antibiotics), sedimentation, and chemical gradient between water (or pore water) and sediments (Shade et al., 2012), could lead to the migration of bacterial communities (Zhu et al., 2022a). The toxicological effects (Välitalo et al., 2017) and the pressure selection (Zhu et al., 2019; Sun et al., 2021) of antibiotics on bacterial communities ultimately change the bacteria populations, which could affect the migration of bacterial communities and the balance of the ecosystem. However, the distribution of antibiotics in aquatic environments was different， such as in pore water and sediments (Xu et al., 2014; Zhang et al., 2022a,b). Keshri et al. (2018) found that pore water and sediment share 6.7–20.3% of operational taxonomic units (OTUs), which indicated a link between sediment bacterial communities and pore water bacterial communities. Nevertheless, the origin and differences between bacterial communities in sediments and pore water in lakes affected by antibiotic pollution are still unclear.

Aquatic plants were one of the important components of the lake ecosystem and play an important role in the fate of pollutants and bacterial communities in lakes (Perez-Jaramillo et al., 2018; Zhang et al., 2022a,b). Plants accumulated antibiotics (Zhang et al., 2022a,b) and transfer their metabolites to the bacterial community in the form of root exudates, thereby affecting the changes in the bacterial community (Huang et al., 2020). However, there is evidence that cultivated and wild plants respond differently to environmental stress. Pantigoso et al. (2020) reported that environmental stress has a significant effect on the rhizosphere soil microflora of cultivated and wild potato plants. Besides, the wild and cultivated tomatoes also showed microbial community structural differences caused by soil properties and environmental pressures (Tronson et al., 2022). Similarly, wild and cultivated *P. australis* also show different responses to antibiotics. Compared to wild Phragmites australis (*P. australis*), cultivated *P. australis* had a developed root system, high plant height, and large stem and leaf area, which lead to more antibiotics accumulation from sediments and pore water (Zhang et al., 2022a,b) and bacteria enrichment (Pantigoso et al., 2020). Similarity, the bacterial community has received much influence due to their associations with plant growth and environmental pollution in lake ecosystems (Marschner et al., 2004). While, few studies have involved in the difference in bacterial communities in pore water and sediments covered by wild and cultivated *P. australis*.

Baiyangdian Lake (38°43′ ~ 39°02′N, 115°38′ ~ 116°07′E) is the largest freshwater lake wetland in North China and is located in the Xiong'an New District. Zaozhadian Lake (ZZD, Supplementary Figure S1) is one of the seven large lakes belonging to the Baiyangdian Lake and has a relatively important ecological geographic location. However, ZZD was an important planting area, covering large areas of wild and cultivated *P. australis*. As well as there was occurred antibiotics pollution due to river runoff caused by Pu River inflow and heavy agricultural activities and rural domestic sewage discharge (Cai et al., 2021). Generally, compared with the wild *P. australis* region, total antibiotics in sediments of the cultivated *P. australis* region was higher, while no significant difference was observed in the pore water in our previous study. What is more, wild and cultivated *P. australis* had different antibiotic accumulation abilities (Zhang et al., 2022a,b). However, antibiotics will produce selection pressure on the bacterial community, and the bacterial community affects the growth and development of plants. Therefore, it is necessary to study the difference in bacterial communities in pore water and sediments covered by wild and cultivated *P. australis*, which will have important reference significance for the biological control and management of antibiotic pollution in the lake system.

2. Alpha and beta diversity of the bacterial community in the pore water and sediments

Using Illumina 16S rRNA gene sequencing, a total of 396,527 bacterial sequences were detected. 236,822 and 159,705 ASVs were clustered to further compare the bacterial community structure in pore water and sediments, respectively. We evaluated the community richness (Sobs indices), community diversity (Shannon indices), community evenness (Shannoneven indices), and phylogenetic diversity (PD) to further compare the bacterial community structure in pore water and sediment samples. As shown in Supplementary Figures S2A–D, no significant differences in Sobs and PD indices were observed in pore water and sediments in both *P. australis* regions ($p > 0.05$), while the community diversity in sediments (Shannoneven indices: about 0.93, Shannon indices: from 6.52 to 6.59) was significantly ($p < 0.05$) higher than those in pore water. However, there was no significant ($p > 0.05$, Supplementary Figure S3) difference in alpha diversity of the pore water bacterial community between the wild and cultivated *P. australis* regions.

Furthermore, the microbial structure was explored by PCoA and Adonis analysis based on the Bray–Curtis dissimilarity (Supplementary Figures S2E–G). It showed a clear separation in bacterial composition between the pore water and sediment samples in the wild *P. australis* region ($R^2 = 0.4277$, $p < 0.05$, Supplementary Figure S2F) and in cultivated *P. australis* region ($R^2 = 0.4158$, $p < 0.05$, Supplementary Figure S2G). However, no significant differences in bacterial community structure in the pore water and sediments from both regions ($p > 0.05$, Supplementary Figure S2E). In the wild *P. australis* region, the PC1 and PC2 axes explained 59.18% of the variation in the bacterial community (Supplementary Figure S2F) and 55.73% in the cultivated *P. australis* region (Supplementary Figure S2G). Along the PC1 axis, the bacterial community in the pore water and sediments was separated (Supplementary Figures S2F,G).

3. Composition of the bacterial community in the pore water and sediments

As shown in Figure 1A, in pore water samples from the wild *P. australis* region, the majority of sequences belonged to Proteobacteria and accounted for 54.97% of the total reads. Bacteroidota for 15.16%, Actinobacteriota accounted for 9.93%, and Patescibacteria for 7.23% of the total reads at the phylum level. However, Chloroflexi (22.74%), Proteobacteria (20.32%), and Actinobacteriota (16.18%), Acidobacteriota (9.00%) were predominant in sediment samples in the wild *P. australis* region. Generally, Proteobacteria (average relative abundance: 59.46%), Bacteroidota (15.01%), and Patescibacteria (5.94%), Firmicutes

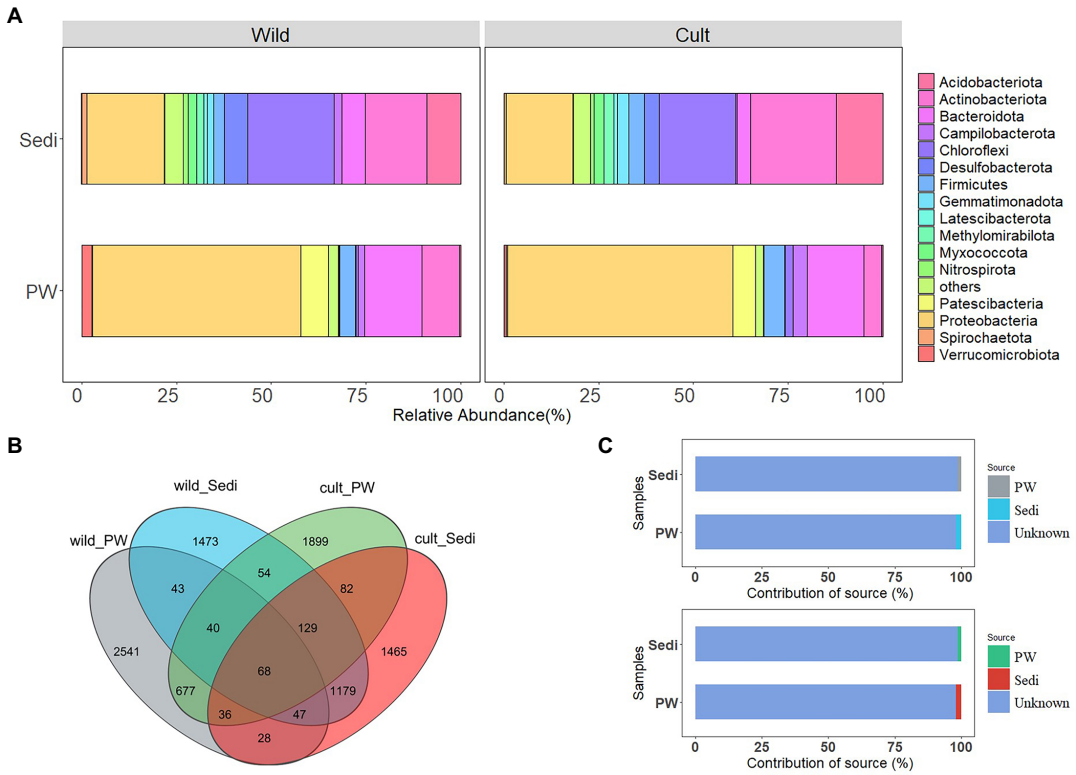

FIGURE 1
Variations in all bacterial alpha **(A)**, Venn diagram represents the number of ASVs detected **(B)** and contribution of the source to bacterial communities by the Source Tracker **(C)** in the pore water and sediment samples from the wild and cultivated *Phragmites australis* regions.

(5.47%), and Actinobacteriota (4.71%) were identified to be abundant in pore water from the cultivated *P. australis* region. In contrast, the dominant phyla were Actinobacteriota (22.64%), Chloroflexi (20.23%), and Proteobacteria (17.68%), Acidobacteriota (12.36%) in sediment samples in this region (Figure 1A).

Figure 1B illustrated that only 1.07% (43 ASVs) of ASVs were shared between the pore water and sediment samples in the wild *P. australis* region, while 2.44% (84 ASVs) of ASVs were shared in cultivated *P. australis* region. Comparatively, the less ASV number of bacterial communities was observed in sediment samples compared with pore water samples in both *P. australis* regions (Figure 1B). However, when we compare wild and cultivated *P. australis* region, 40.12% (1,179 ASVs) of ASVs were shared in sediment samples, while 15.25% (677 ASVs) of ASVs were shared in pore water.

The possible contributions of bacterial communities in the pore water and sediments to each other were determined using Source Tracker Analysis (Figure 1C). For sediment samples, approximately 1.28% of the variations in bacterial composition could be attributed to the contribution of the pore water in both *P. australis* region. As for pore water, only 1.01% of the bacterial variations in the pore water could be explained by sediment samples in the wild *P. australis* region, while 2.04% in the cultivated *P. australis* region.

One-way ANOVA results showed significant differences in the relative abundance of Proteobacteria ($p < 0.01$), Actinobacteriota ($p < 0.05$), Chloroflexi ($p < 0.001$), Bacteroidota ($p < 0.01$), and Acidobacteriota ($p < 0.001$) between pore water and sediment samples in wild and cultivated *P. australis* regions, while no significant differences in the relative abundance of Firmicutes were observed between four groups ($p > 0.05$, Figure 2A). However, Proteobacteria and Bacteroidota showed significantly ($p < 0.05$) higher relative abundance in pore water than sediments, while Chloroflexi and Acidobacteriota showed significantly ($p < 0.05$) lower relative abundance in pore water compared with sediments in both *P. australis* region (Supplementary Figure S4).

4. Influencing factors on bacterial community in the pore water and sediment

For bacterial communities in the pore water samples in the wild *P. australis* region, the relative abundance of the most dominant phyla Bacteroidota, and Verrucomicrobiota showed significant positive correlation with NH_4-N ($p < 0.001$), and Patescibacteria demonstrated significant positive correlation with NO_3-N and sulfapyridine(SPD; $p < 0.001$), respectively (Figure 2B). Similarity, the relative abundance of Proteobacteria and Actinobacteriota in sediment samples in the wild *P. australis* region showed significant positive correlation with NH_4-N and clay ($p < 0.001$), while significant negative correlation with NO_3-N and sand ($p < 0.001$).

In the cultivated *P. australis* region, significant correlations were observed between the relative abundance of the most dominant phyla Firmicutes and norfloxacin (NOR) and total antibiotics (SUM) in pore water samples ($p < 0.001$). What is more, the relative abundance of

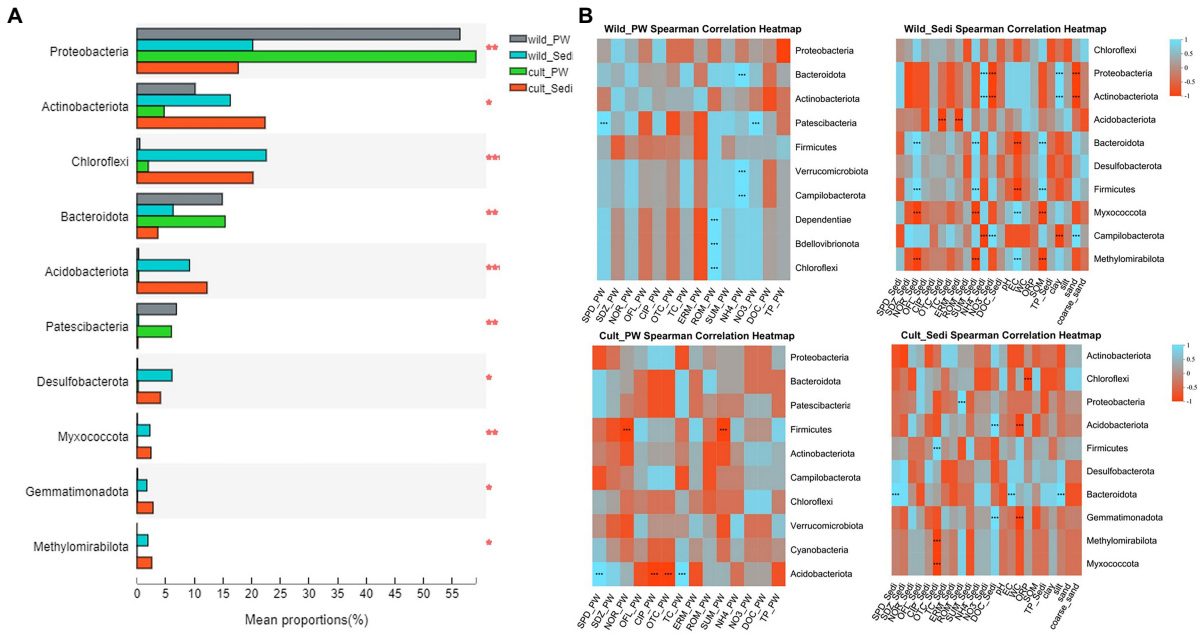

FIGURE 2
(A) One-way ANOVA analysis showed bacterial alpha difference between pore water and sediments in both *Phragmites australis* region and **(B)** spearman correlation between environmental factors and bacteria at the phylum level in the pore water and sediments from the wild and cultivated *P. australis* regions. PW, pore water; Sedi, sediments; SPD, sulfapyridine; SDZ, sulfapyridine; NOR, norfloxacin; OFL, ofloxacin; CIP, ciprofloxacin; OTC, oxytetracycline; TC, tetracycline; ERM, erythromycin; ROM, roxithromycin; and SUM, total antibiotic.

Acidobacteriota also has significant correlations with antibiotics, such as SPD, ciprofloxacin (CIP), oxytetracycline (OTC), and tetracycline (TC), in pore water samples from cultivated *P. australis* region ($p < 0.001$). As for sediments in the cultivated *P. australis* region, the relative abundance of Chloroflex, Acidobacteriota, and Firmicutes showed significant correlations with ORP, WC and DOC, and OTC ($p < 0.001$).

5. Discussion

Antibiotic distribution behavior exists between sediment and pore water (Cheng et al., 2014), moreover, the distribution coefficient of antibiotics from sediment to pore water was higher than that from sediment to overlying water (Xu et al., 2014). Therefore, the sediment, as a source of pollutants, is more likely to release antibiotics into pore water, which may lead to a significant difference in bacterial community diversity between pore water and sediment in both wild and cultivated *P. australis* regions. Previous studies have also shown that sediment-associated bacterial communities are more abundant and diverse than bacteria in pore water (Keshri et al., 2018). The aquatic plant root system can influence the connection between pore water and sediments, where the physical, chemical, and biological components interact closely (Li et al., 2019). Plants can rely on beneficial interactions between roots and bacterial communities to obtain nutrients, degrade contaminants, and promote growth (Edwards et al., 2015; Wang et al., 2022), so the interactions might be changed by rich roots in the cultivated *P. australis* region under antibiotic stress.

In the current study, the bacterial community diversity (i.e., Shannon and Shannoneven) in sediments was higher than that in pore water from both *P. australis* regions under the different antibiotics stress, and no significant difference in diversity in sediment was observed between wild and cultivated *P. australis* regions, which can be explained by the clear fact that wild and cultivated *P. australis* all promote the migration of bacterial communities between pore water and sediments. Moreover, there was no significant difference in the bacterial community structure in pore water between the wild and cultivated *P. australis* regions under the same antibiotics stress. The possible explanation was no significant difference in the total antibiotics in pore water between both regions (Zhang et al., 2022a,b), What is more, the similar bacterial community in sediments between both regions might be explained by the fact that the two *P. australis* regions were connected by inflow rivers, and the bacterial communities may coalesced between pore water and sediments (Ren et al., 2017; Gao et al., 2021). It is different from the previous research that the bacterial community in pore water in different types of wetlands can be distinguished (Wang et al., 2018).

Furthermore, the composition of bacterial communities changed under the influence of antibiotics, while Source Tracker analysis results showed that 1.28% variations in sediment bacterial composition could be attributed to the contribution of the pore water in both *P. australis* regions, which can be explained by the clear fact that pore water contains the same levels of antibiotics in both *P. australis* regions. Gao et al. (2021) report that the contribution of the bacterial community from the water to sediment is lower (<0.3%), similarly, little proportion or none from sediments to the water was observed. However, the contribution (2.04%) of the bacterial community from the sediment to pore water in the cultivated *P. australis* region is higher than that (1.01%) in the wild *P. australis* region. Therefore,

aquatic plant rich roots might alter the migration of bacterial communities between pore water and sediment. We speculated that plant cultivation might change the source-sink pattern between sediments and pore water. Similar dominant phyla (e.g., Actinobacteriota, Chloroflexi, and Proteobacteria) in sediments and different dominant phyla in pore water were observed in both *P. australis* regions. This might be associated with sediment textures and the hydraulic or zoobenthos disturbances (Zhu et al., 2022b) in both regions (Supplementary Figure S1). Additionally, the root exudates of wild and cultivated *P. australis* and water quality in both regions might be different, which will also affect the bacterial community in the pore water (Chen et al., 2019; Song et al., 2020). Previous studies have reported that the development of bacterial communities in the rhizosphere sediments is related to plant species (Marschner et al., 2004). Therefore, we hypothesized that the difference in bacterial communities in the pore water might be caused by different root systems of wild and cultivated *P. australis* in the current study.

In the current study, in the wild *P. australis* region, NH_4-N, NO_3-N, and sediment particle size were significantly associated with the relative abundance of major bacterial phyla in pore water or sediments, while antibiotics (e.g., TC, OTC, and NOR) were associated with the relative abundance of major bacterial phyla in the cultivated *P. australis* region pore water or sediments. TC and OTC are widely used in the treatmentof human and livestock diseases, livestock and poultry breeding (Van Boeckel et al., 2014; Xu et al., 2022), therefore these antibiotics were introduced to cultivated *P. australis* region and further affect the bacterial community (Karkman et al., 2019).

This study shows that under the influence of antibiotics, higher alpha diversity (e.g., Shannoneven and Shannon) of bacterial communities were observed in the sediments than those in the pore water in both *P. australis* regions. Different compositions of bacterial communities in the pore water and similar composition in sediments in both *P. australis* regions. The contribution of the bacterial community in pore water from cultivated *P. australis* region to sediment is higher (2.04%) than that in wild *P. australis* region (1.01%), therefore, plant cultivation might change the source-sink pattern between sediments and pore water. Generally, pore water and sediments bacterial communities in wild and cultivated *P. australis* regions showed significant differences. Therefore, further studies should be carried out to explore bacteria transfer and coalescence in different aquatic plants under antibiotic stress in a shallow lake in the future.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

LZ: writing—investigation and original draft. JB: funding acquisition and writing—review and editing. YZ, KZ, RX, and MJ: review and editing. ZW, YW, and HL: writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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EDITED BY
Zifang Chi,
Jilin University,
China

REVIEWED BY
Shangqi Xu,
Anhui Normal University,
China
Xiaomin Li,
South China Normal University,
China

*CORRESPONDENCE
Mingjiang Zhang
✉ zmj0630@163.com

†These authors have contributed equally to this work and share first authorship

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Insights into remediation effects and bacterial diversity of different remediation measures in rare earth mine soil with SO_4^{2-} and heavy metals

Xiao Yan[1,2†], Bowen Gao[1,2†], Jianlei Wang[1,2], Xuezhe Zhu[1,2,3,4] and Mingjiang Zhang[1,2*]

[1]GRINM Resources and Environment Tech. Co., Ltd., Beijing, China, [2]National Engineering Research Center for Environment-Friendly Metallurgy in Producing Premium Non-Ferrous Metals, GRINM Group Co., Ltd., Beijing, China, [3]School of Metallurgy, Northeastern University, Shenyang, China, [4]GRIMAT Engineering Institute Co., Ltd., Beijing, China

The increased demand for rare earth resources has led to an increase in the development of rare earth mines (REMs). However, the production of high-concentration leaching agents (SO_4^{2-}) and heavy metals as a result of rare earth mining has increased, necessitating the removal of contaminants. Here, a series of experiments with different remediation measures, including control (CK), sulfate-reducing bacteria (SRB) alone (M), chemicals ($Ca(OH)_2$, 1.5g/kg) plus SRB (CM-L), chemicals ($Ca(OH)_2$, 3.0g/kg) plus SRB (CM-M), and chemicals ($Ca(OH)_2$, 4.5g/kg) plus SRB (CM-H), were conducted to investigate the removal effect of SO_4^{2-}, Pb, Zn, and Mn from the REM soil. Then, a high-throughput sequencing technology was applied to explore the response of bacterial community diversity and functions with different remediation measures. The results indicated that CM-M treatment had a more efficient removal effect for SO_4^{2-}, Pb, Zn, and Mn than the others, up to 94.6, 88.3, 98.7, and 91%, respectively. Soil bacterial abundance and diversity were significantly affected by treatments with the inoculation of SRB in comparison with CK. The relative abundance of *Desulfobacterota* with the ability to transform SO_4^{2-} into S^{2-} increased significantly in all treatments, except for CK. There was a strong correlation between environmental factors (pH, Eh, SO_4^{2-}, Pb, and Zn) and bacterial community structure. Furthermore, functional prediction analysis revealed that the SRB inoculation treatments significantly increased the abundance of sulfate respiration, sulfite respiration, and nitrogen fixation, while decreasing the abundance of manganese oxidation, dark hydrogen oxidation, and denitrification. This provides good evidence for us to understand the difference in removal efficiency, bacterial community structure, and function by different remediation measures that help select a more efficient and sustainable method to remediate contaminants in the REM soil.

KEYWORDS

sulfate-reducing bacteria (SRB), SO_4^{2-}, heavy metals, rare earth mines soil, soil bacterial community, bacterial function

Highlights

- The SRB system has a positive effect on SO42- removal and heavy metals stabilization.
- The treatments with the inoculation of SRB clearly increased functional microbial community abundance and affected microbial community structure.
- Desulfobacterota was most sensitive to SO42- and heavy metals.
- Functional microbes (Desulfosporosinus, Desulfitobacterium, Desulfobulbus and Dethiosulfovibrio) promotes sulfur cycling and provides sustainable ecological remediation in the REEs mine soil.

Introduction

Rare earth elements are recognized as critical raw materials that are an important part of all high-tech devices, including electronics (TV, autocatalytic converters, and telephone), superconductors (high energy particle accelerator, maglev train, and energy storage devices), and fluorescent materials (indicator, glass additives, and clothes) (Lima and Ottosen, 2021). A series of environmental problems result from the development and utilization of rare earth mining (Amol et al., 2022). According to statistics, about 3,000 mg/l of SO_4^{2-} is produced in the rare earth industry every year (Zhou et al., 2022) in addition, some heavy metals (Pb, Zn, and Cu) from surrounding mines may be migrated to the REM soil (Du et al., 2022). The combined pollution of SO_4^{2-} and heavy metals not only destroys the soil quality and decreases the crop yield but also harms human health through the food chain (Sharma et al., 2021). More importantly, the contaminants could spread with rainfall or water flow, which results in the expansion of the polluted area (Tran et al., 2021; Wang F. et al., 2022). Considering potential risks, the remediation of these combined pollutants in rare earth soil has been widely concerned (Li L. et al., 2022). At present, the main remediation technologies including physical, chemical, and biological methods are applied to treat SO_4^{2-} and heavy metals of rare earth soil (Ali et al., 2022). Chemical agents, such as $Ca(OH)_2$, possess heavy metal absorption capability to remove dissolved heavy metals (Pb and Zn) effectively at high concentrations of up to 1,000 mg/L (Du et al., 2022). In addition, $Ca(OH)_2$ is often used as a reagent to regulate the pH of contaminated soil rapidly. However, excessive application of chemicals has the disadvantages of high operational cost, damaging soil quality, and production of secondary pollutants (Mokoena et al., 2022). In comparison with physical and chemical methods, the biological method was anticipated as one of the most promising technologies due to low-cost, environment-friendly, and sustainable remediation (Saxena et al., 2021). The combined pollution of SO_4^{2-} and heavy metals was transformed and immobilized by functional microbes through mechanisms of bioreduction, biosorption, and biomineralization (Zhang et al., 2020).

Sulfate-reducing bacteria (SRB) are a typical functional bacterial species that can transform SO_4^{2-} to S^{2-} by acting as the terminal electron acceptor in the process of dissimilatory sulfate reduction, which is an important reaction in global sulfur cycling (Santos et al., 2022). Previous studies indicated that the SRB belongs to different genera including *Desulfomicrobium*, *Desulfitobacterium*, *Desulfobulbus*, and *Dethiosulfovibrio* (Yang Z. et al., 2021; Tang et al., 2022). It has been reported that SRB are widely distributed in anaerobic environments, such as sewage sludge, polluted oil field, mine tailings, animal intestines, and even acidic mine drainage (Zhu et al., 2020; Gu et al., 2021). SRB can use SO_4^{2-} as electron acceptors to reduce SO_4^{2-} to S^{2-}. Then, S^{2-} can remove heavy metals in the water solution by synthesizing a variety of insoluble sulfides precipitation including PbS, ZnS, CuS, and MnS (Lv et al., 2022a,b). Previous studies concluded that SRB can effectively remove heavy metals (Pb, Zn, Cu, Cd, Cr, and U) in the water solution from acid mine drainage and sustainably improve the ecological environment (Gu et al., 2021; Wang et al., 2021; You et al., 2021; Yang et al., 2021a). For example, Yang X. et al. (2021) and Yang Z. et al. (2021) inoculated SRB into a biological filter disk carrier, which can decline SO_4^{2-} concentration from wastewater systems by over 90% in 60 days (Yang Z. et al., 2021). Lv et al. (2022a,b) reported that the 99% of U(VI) was solidified by SRB when the initial concentration of U(VI) is about 5 mg/L (Li J. et al., 2022). Therefore, the SRB was widely used to treat wastewater containing SO_4^{2-} and heavy metal contamination of soil (Li J. et al., 2022). However, for an extremely high concentration of SO_4^{2-} combined with heavy metal contamination soil, signal bacterial technology (SRB) is difficult to achieve the anticipated effects in the short term (Ali et al., 2022). More importantly, several contaminated sites containing SO_4^{2-} were considered too acidic for the colonization of SRB. Therefore, the combination methods of chemical and biological are considered the most efficient technology in harsh environments (Zhao et al., 2019).

The leaching reagents were widely used in the process of rare earth element leaching (Traore et al., 2022). So numerous studies focused on the screening and application of leaching reagents in the process of rare earth element leaching from the REM (Talan and Huang, 2022; Zhou et al., 2022), but a large amount of leaching reagents not only affected soil quality but also increased ecological risk for rare earth ores after closure (Mokoena et al., 2022). However, few studies focused on treating leaching reagents and variation of microbial community succession in the REM. Recent studies have found that combined pollution of SO_4^{2-} and heavy metals strongly affected soil quality and further regulated bacterial community structure and functions in process of treating acid mine drainage (Villegas-Plazas et al., 2019). Similarly, it was possible that bacterial community structure and ecological functions in the REM soil were significantly affected by combined pollution of SO_4^{2-} and heavy metals. It may be a close association and intense interaction between contamination removal efficiency and bacterial dominant flora. In order to find the truth from above conjecture, the main objectives of this study were as follows: (1) to compare the removal effects of SO_4^{2-} and heavy metals from the REM soil under different remediation measures; (2) to explore the differences of bacterial diversity, community structure, and analyze the effects between contaminants and bacterial community structure in the REM soil experiments under different remediation measures; and (3) to predict the abundance differences of metabolism or other ecological-related functions (such as nitrification and denitrification) under different treatments.

Materials and methods

Sampling and analysis

The contaminated soil used in this study was collected from the closure of the REM, located in Ganzhou, Jiangxi Province, China (25°42′N, 115°07′E). A total of 12 points were selected in the REM, and all samples were taken with a shovel at a spatial

interval of 20 m for each sample from the surface of approximately 0–20 cm. Then, the samples were stored in polyethylene seal pockets at 4°C and transported to the laboratory within 24 h for further measurement of the initial concentration of target pollutants. All samples were mixed over 20 times to form a sample for the remediation experimental study of columns. The chemical composition of the REM soil sample was described in our previous study, which primarily contains high concentrations of SO_4^{2-} and heavy metals such as Pb, Zn, and Mn (Zhou et al., 2022). The SO_4^{2-} concentration was determined by referring to the turbidimetric method (Tayar et al., 2022). According to the methods described by Singh, the concentration of heavy metals including Pb, Zn, and Mn from the leaching solution was determined by ICP-MS (Agilent Technologies 7700x, United States) (Singh et al., 2022). In addition, the pH and Eh were measured by a portable multi-parameter digital analyzer (HQ40d, HACH, United States).

Bacteria and rejuvenation

The SRB were isolated from activated sludge (Yan et al., 2019) and preserved in the National Engineering Laboratory of Biohydrometallurgy, China. First, 10 mL of the SRB bacterial fluid was added to 90 mL of LB medium (sterilized at 121°C for 30 min) to prepare an expand culture at 30°C for 48 h. Then, the supernatant liquid was transferred into the fresh LB medium with an inoculation volume of 10%. When the OD_{600} (the optical density value was measured when the wavelength is set at 600 nm) in the culture system was reaching to 0.4, the supernatant liquid was transferred again for rejuvenation. Finally, the activated SRB solution was added to remediate systems with 10% inoculation volume.

Experimental design

First, 10 cm quartz sand was placed at the bottom of the PVC experimental columns (0.7 cm diameter, 50 cm height). Then, a 1.4 kg soil sample after well mixing was added to each column. This experiment comprised five treatments, including no chemicals and SRB treatment (CK); inoculation with SRB alone (M); adding $Ca(OH)_2$ at a dosage of 1.5 g/kg for 7 days in the soil sample for treatment priority, and then, inoculated SRB for sustainable treatment for contaminants (CM-L); adding $Ca(OH)_2$ at a dosage of 3.0 g/kg for 7 days in the soil sample for treatment priority, and then, inoculated SRB for sustainable treatment for contaminants (CM-M); adding $Ca(OH)_2$ at a dosage of 4.5 g/kg for 7 days in the soil sample for treatment priority, and then, inoculated SRB for sustainable treatment for contaminants (CM-H). For each treatment (CK, M, CM-L, CM-M, and CM-H), three replicates were set to reduce experimental error. A total of 15 columns were used in this experiment. The treatment time was 42 days, and the leaching solution of each column was collected every 7 days for the detection of the variation of pH, Eh, SO_4^{2-} and heavy metal (Pb, Zn, and Mn) concentrations in different remediation measure systems. When the significant remediation effect was achieved (about 30 days), the bacterial community abundance, diversity, and functions of the REM soil under different treatments were measured by high-throughput sequencing technology.

DNA extraction

DNA was extracted from the REM soil under different treatments using a DNeasy PowerSoil Kit (QIAGEN, Germany) according to the manufacturer's instructions. The V3–V4 region of the 16S rRNA gene was amplified by polymerase chain reaction (PCR) with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTA CHVGGGTWTCTAAT-3′) (Lv et al., 2022a,b). Each sample was amplified with three technical replicates under the following conditions: 94°C for 5 min, and then 30 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 45 s, and a final extension at 72°C for 7 min. PCR products were purified using a QIAamp 96 PowerFecal QIAcube HT kit (QIAGEN, Germany). The purified products were mixed at equimolar concentrations and then applied to sequence on an Illumina HiSeq 2500 (PE250) platform at Shanghai Major Biomedical Technology Co., Ltd.

Bioinformatics analysis

The raw sequences were processed using the QIIME pipeline (Ma et al., 2022). We used the denoising software DADA2 to remove low-quality reads, putative chimera, and then, the result was parsed into amplicon sequence variants (ASVs) with default quality settings (Callahan et al., 2017). Based on the Silva V138 (99%) reference database, the ASVs were used as the more particular taxonomy unit than the species (Yang et al., 2022). The detailed taxonomic affiliation of ASVs was obtained from the National Center for Biotechnology Information (NCBI) website in order to conduct further analysis. ASVs present in only one sample was removed from the final dataset because they could be remaining sequencing errors not detected by DADA2, resulting in 1764 ASVs and 946,312 reads in the final dataset. The sequence counts per sample were rarefied to the smallest individual sample sequence.

Statistical analysis

Microsoft Excel and Origin 2017 software were used for the statistical analysis of the variation of physical and chemical properties and contaminations in the experiment system. The diversity metrics (species' richness and Shannon diversity) and bacterial community composition on the phylum/genus level (Bray–Curtis dissimilarity matrices) were analyzed on the online tool of Majorbio Cloud Platform.[1] The canonical correlation analysis (CCA) and Spearman correlational analyses were performed to examine the relationship between the contaminations and bacterial community structure. FAPROTAX was used to predict the biochemical cycle of different treatments. The prokaryotic taxa were mapped to metabolic or other ecologically relevant functions using FAPROTAX software based on the literature on cultured representatives (Ma et al., 2022).

1 https://cloud.majorbio.com/page/tools/

Results and discussion

Effect of different remediation measures on pH, Eh, SO_4^{2-}, and heavy metals in the REM soil

In order to understand the remediation effects of different measures on removing contaminants, the variation of pH, Eh, SO_4^{2-}, and heavy metals concentration was monitored in this study. The results showed that the initial values of soil pH and Eh were 4.20 and 537 mV, respectively, which means that this site was still in a strongly acidic and extremely oxidized state. Compared with CK, an increasing trend of pH is presented in the others. After 7 days, the pH reached 7.5 and continued throughout the treatment process (Figure 1A). With increasing pH, there was a decrease in Eh value in each treatment, but the decrease varied between treatments. The variation of Eh value in the CM-L, CM-M, and CM-H was rapid, decreasing to 0 mV at 7 days and continuing (Figure 1B), but a relatively slight decrease from 537 mV to 19 mV was presented in the M. The changes in Eh showed that the strong oxidation state was gradually transformed into the reduction state.

The phenomenon of increased pH with decreasing Eh may be influenced by two aspects: neutralization of chemical agents and transformation of microorganisms. The addition of $Ca(OH)_2$ directly neutralizes H^+ and rapidly changes pH and Eh values. This result was consistent with Florentino's conclusion that alkaline chemicals could quickly neutralize the acid from acid mine wastewater and affect redox conditions (Florentino et al., 2015). The SRB was a kind of common functional microbe that changed the system's extreme oxidization to a reduction state by converting SO_4^{2-} to S^{2-}, which was a process of acid consumption and alkali production (Villegas-Plazas et al., 2019).

In this study, we investigated the effects of different remediation measures on the SO_4^{2-} removal effects. As shown in Figure 1C, the SO_4^{2-} concentration in the CK was first decreased and then kept constant (about 2,700 mg/L). The increased SO_4^{2-} concentration in 7 days may be due to the dissolution of sulfate compounds in the soil under strongly acidic conditions. However, SO_4^{2-} concentration in treatments, except the CK, decreased over time. Among them, when the $Ca(OH)_2$ concentration was increased from 1.5 to 4.5 g/L, the variation of SO_4^{2-} concentration followed an order of the CM-M (from 2,610 to 138.5 mg/L) > the CM-L (from 2,610 to 278.9 mg/L) > the CM-H (from 2,610 to 339.6 mg/L). Such an anticipated removal effect of SO_4^{2-} by the addition of the middle $Ca(OH)_2$ concentration was reported in previous studies (Liu et al., 2022; Yang et al., 2022).

The SO_4^{2-} concentration in the CM-L was relatively higher than that of the CM-M, which may have a relationship with the relatively lower $Ca(OH)_2$ concentration. Meantime, it takes longer to convert SO_4^{2-} to H_2S by the SRB (Santos et al., 2022). In addition, $CaSO_4$ as a low solubility substance released SO_4^{2-} with time at relatively low pH conditions (Xing et al., 2022). In comparison with the CM-L, CM-M, and CM-H, the variation of SO_4^{2-} concentration in the M was relatively slow, from 2,610 mg/L to 460.5 mg/L during the remediation process, which was explained that the SRB needs to be satisfied for more time to adapt to a new environment first, and then, the population and activity of SRB gradually increase over time (Tang et al., 2022). This result was consistent with of the variation of Eh values. With the increase of SRB abundance, the systems gradually transformed from the oxidized state to the reduced state, and then, the SO_4^{2-} transformed into H_2S by RSB under the reduction conditions (Li W. et al., 2022). After remediation, SO_4^{2-} concentrations in the earlier treatment systems were all below the effluent discharge standard (800 mg/L).

The studied soil was collected from the REM under closed storage conditions in the south of Ganzhou city, Jiangxi Province, China. According to the previous investigation, many heavy metal mines such as lead–zinc gather around this studied site (Li J. et al., 2022). Due to the lack of appropriate management, the concentrations of Pb and Zn in the surrounding soil exceeded the standard to varying degrees (Ali et al., 2022). In this study, the Pb, Zn, and Mn concentrations were analyzed. The results showed that Pb, Zn, and Mn concentrations in the different remediation measures, except the CK, presented a declining trend over time (Figures 1D–F). In comparison with the CM-L, CM-M, and CM-H, the decline of Pb, Zn, and Mn concentrations in the M was relatively significant, ranging from 5.15, 8.56, 8.49 mg/L to 0.51, 0.22, and 1.05 mg/L, respectively. The removal rate reached 90.1, 97.4, and 87.6%. Such a predictable removal effect was mainly attributed to the large number and high activity of SRB (Yang et al., 2022). It was clear that abundant SRB in the system could increase the chance of contacting SO_4^{2-}, and the higher activity of SRB in the system was a key to transforming SO_4^{2-} rapidly (Wang C. et al., 2022). When the population and activity of SRB are met at the same time, SRB can quickly and efficiently convert

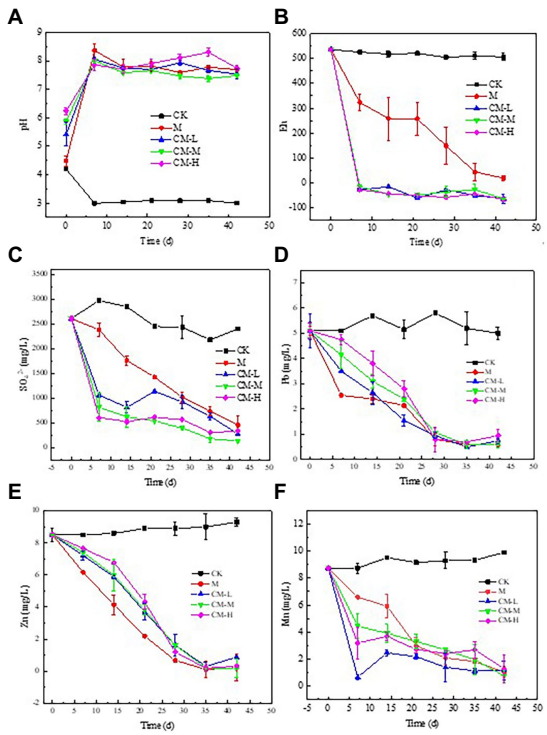

FIGURE 1
Variation of physicochemical properties [**(A)** pH, **(B)** Eh] and the contaminants [**(C)** SO_4^{2-}, **(D)** Pb, **(E)** Zn, and **(F)** Mn] in the experimental system of REM soil by different remediation measures: CK (no amendment), M [sulfate-reducing bacteria (SRB) alone], CM-L [chemicals ($Ca(OH)_2$, 1.5g/kg) plus SRB], CM-M [chemicals ($Ca(OH)_2$, 3.0g/kg) plus SRB], and CM-H [chemicals ($Ca(OH)_2$, 4.5g/kg) plus SRB].

from SO_4^{2-} to H_2S, and then, the H_2S combined with heavy metal ions (Pb and Zn) to form stable compound precipitation (PbS and ZnS), so as to achieve the purpose of solidification of heavy metals in the REM soil (Lv et al., 2022a,b). For the collaborative remediation group with the addition of $Ca(OH)_2$ and SRB, the remediation effects of Pb, Zn, and Mn were as follows: the CM-M (from 5.15, 8.56, and 8.49 to 0.60, 0.17, and 0.76) > the CM-L (from 5.15, 8.56, and 8.49 to 0.75, 0.87, and 1.19) > the CM-H (from 5.15, 8.56, and 8.49 to 0.95, 0.32, and 1.30). Such a CM-M treatment was anticipated as in the previous results. The addition of an appropriate amount of $Ca(OH)_2$ was beneficial to the removal of contaminants due to suitable soil pH. Meanwhile, the growth of the SRB would be inhibited in more acid or basic conditions. In comparison to CK and M, the rapid change in heavy metal concentration over 7 days may be attributed to the high efficiency of $Ca(OH)_2$. Subsequently, suitable pH and moderate heavy metal concentration provided a favorable condition for the removal of contaminants by SRB. This was a measure to realize the sustainable remediation of the REM soil under closed storage conditions. Previous study results showed that the SRB had strong tolerance against heavy metals and had a special capacity for solidified heavy metals, such as Pb, Zn Cu, U, Cr, and Mn (Lin et al., 2022). Solidification of heavy metals by SRB has been widely used in the bioremediation of acid mine wastewater and soil (Villegas-Plazas et al., 2019).

Taxonomic compositions of bacterial communities in different remediation measures

Sequencing of the V4 region of the 16S rDNA yielded a total of 835,812 high-quality reads for 15 samples of five different remediation measures. The richness indices (ACE and Chao) and diversity indices (Simpson and Shannon) were compared among different remediation measures (Figure 2). In comparison with the CK, ACE and Chao indices were significantly increased, which was attributed to the inoculation of SRB increase in the number of system bacterial species (Yin et al., 2022). Meanwhile, the collaborative remediation groups of CM-L, CM-M, and CM-H presented higher richness indices compared to the M. This was explained that the improvement of the harsh environment (strongly acidic soil) can provide a better living environment for various bacterial colonization (Zhu et al., 2022). In addition, sufficient nutrients can stimulate the growth and metabolism of rare indigenous microorganisms whose abundance was not counted previously (Xu et al., 2022; Yin et al., 2022). For the variation of diversity indices in different remediation measures, there was a significant decreasing trend for the Simpson and Shannon diversity indices with the inoculation of functional microbes, with the M having the least bacterial diversity indices, followed by the CM-H, CM-L, and CM-M. These results were consistent with the previous study (Koner et al., 2022). Artificial inoculation of functional microbes was a bioaugmentation process used to regulate indigenous bacterial community structure in the soil, resulting in several bacterial species with strong competitiveness occupying ecological niches while rare microbes with weak competitiveness did not survive (Chen et al., 2022). Moreover, improvements in soil quality may indicate that bacterial species with an ability to adapt to circumstances rapidly were enriched, whereas other bacterial taxa showed the opposite trend (Zong and Fu, 2021).

Barplots and Circos graphs showed bacterial community composition and abundance at the phylum and genus levels of bacteria (Figure 3). Dominant bacterial compositions of phylum (>1% relative abundance in at least one treatment) are shown in Figure 3A, and those with less than 1% relative abundance were classified as "others." Six major phyla were detected including Proteobacteria (29–54%), Firmicutes (5–45%), Bacteroidota (6–18%), Actinobacteriota (1–14%), *Desulfobacterota* (0.03–6%), and Campilobacterota (0.05–8%), which totally accounted for a high proportion above 90%. Chen

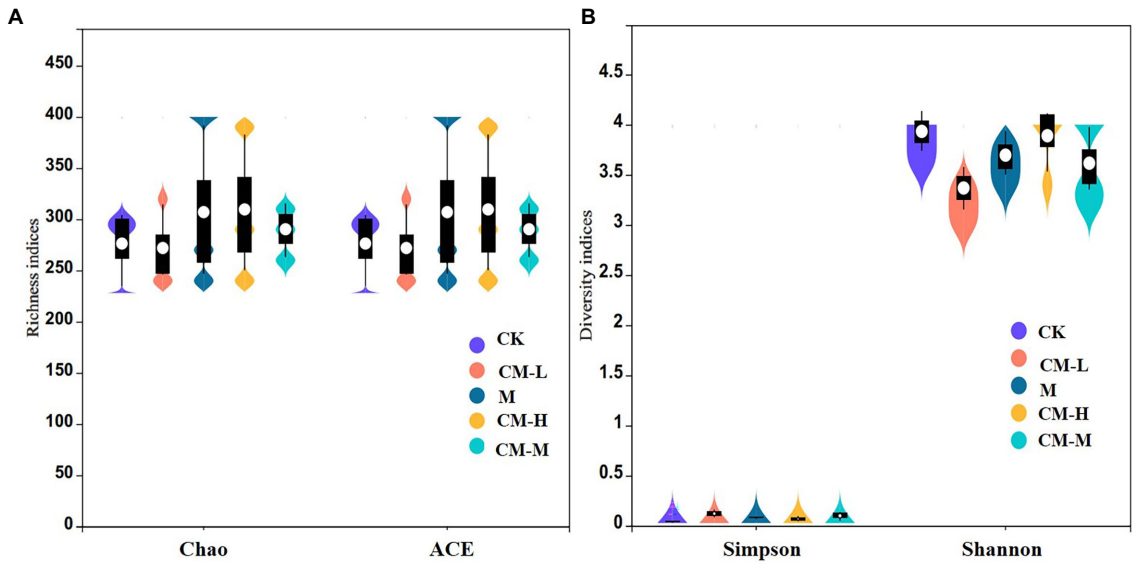

FIGURE 2
Alpha-diversity of samples collected from different remediation measures. **(A)** Richness indices (ACE and Chao) and **(B)** diversity indices (Simpson and Shannon).

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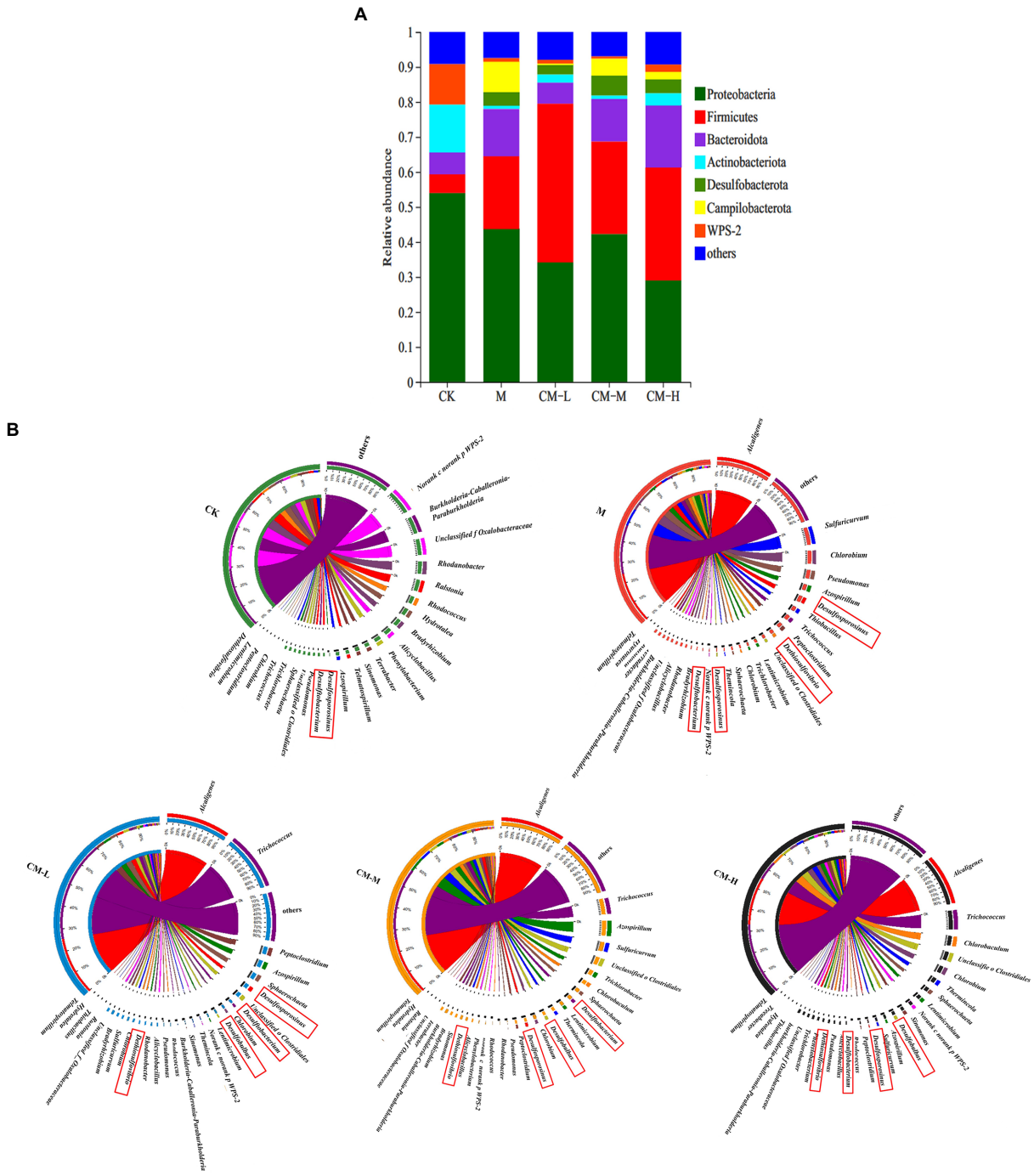

FIGURE 3
Relative abundances of bacterial community at the level of phylum **(A)** and genus **(B)** in the REM soil samples under different treatments.

et al. (2021) found that Proteobacteria, Firmicutes, Bacteroidota, and Actinobacteriota were the most common bacterial species found in contaminated soil (Chen et al., 2021). This was consistent with our study that Proteobacteria, Firmicutes, Bacteroidota, and Actinobacteriota were three major phyla in the CK. The difference was a high richness of Desulfobacterota and Campilobacterota presenting in the M, CM-L, CM-M, and CM-H. This may be related to Desulfobacterota and Campilobacterota belonging to the common phylum of SRB. Inoculation of SRB results in a significant increase in abundance. According to the variation in contaminant concentration, functional microbes play an important role in the remediation process of SO_4^{2-} and heavy metals. Scholars discovered that Desulfobacterota was the key phylum for removing SO_4^{2-} and solidified heavy metals in the acid mine drainage (Santos et al., 2022). The relative abundance of Desulfobacterota in the CM-L, CM-M, and CM-H was higher than the M, which explained that the physical and chemical properties of soil were improved rapidly by the addition of $Ca(OH)_2$, and then, inoculated functional microbes were easier to survive and produce a

marked effect. With the different concentrations of $Ca(OH)_2$, the abundance of Desulfobacterota was presented as CM-M > CM-L > CM-H, which was attributed to Desulfobacterota's inability to grow well in an acidic or alkaline environment. It was found by Li J. et al., (2022) that *Desulfobacterota* had a high abundance when the pH value of the system was 6–9 (Li W. et al., 2022).

To further characterize the responses of bacterial communities to different remediation measures, genus-level analysis was performed by Circos graphs to determine the differences in functional microbes (Figure 3B). In this study, the key genus including *Desulfosporosinus*, *Desulfitobacterium*, *Desulfobulbus*, and *Dethiosulfovibrio* belonged to Desulfobacterota. These microbes could convert SO_4^{2-} into H_2S and may be capable of absorbing heavy metals in their water-soluble state (Du et al., 2022). At the genus level, the relative abundance of *Desulfosporosinus*, *Desulfitobacterium*, *Desulfobulbus*, and *Dethiosulfovibrio* varied greatly in conjunction with different remediation measures. The bacterial community composition was similar to that of the bioremediation groups from acid mine drainage treatment but differed from the treatment of contaminated soil of heavy metals. In comparison with the CK (0.005%), the total abundance proportion of *Desulfosporosinus*, *Desulfitobacterium*, *Desulfobulbus*, and *Dethiosulfovibrio* was relatively higher and presented as the M (7.58%) > CM-M (6.13%) > CM-L (6.11%) > CM-H (3.91%). For the M, the relatively high abundance of *Desulfobacterota* may be attributed to the priority effects of bacterial colonization in the ecological theory. When the population of functional microbes was inoculated into the M, functional bacterial communities would prioritize colonization in the current environment by improving competitiveness and trophic resources. Overall, previous studies reported that predicting when and how the current community state impacts the success of newly arriving bacterial taxa was critical for the management of microbiomes to sustain ecological function (Li et al., 2021; Ma et al., 2022). The higher relative abundance of *Desulfobacterota in the* CM-M (6.13%) and CM-L (6.11%) were higher than it in the CM-H (3.91%) which due to adding of chemicals greatly changed the physicochemical properties of soil in the CM-H, and then, functional microbes need more time to resist and adapt to the environment, meanwhile, the competition of indigenous microbes was also a major obstacle affecting the colonization of *Desulfobacterota* (Geng et al., 2022). In addition, Santos et al. (2022) found that the addition of chemicals had a great influence on the abundance and diversity of bacterial communities (Santos et al., 2022). *Desulfobacterota* was the key player in the process of transforming SO_4^{2-} into H_2S, and then, achieving the solidification of heavy metals by H_2S combining with heavy metal in the water-solution state to form stable sulfide in the RM soil (Lima and Ottosen, 2021). In addition, many Firmicutes were related to the solidification of heavy metals. For example, *Trichococcus* and *Bacillus* (Firmicutes) were widely used for treating the contaminated soil of heavy metals (Pb, Zn, Cu, As, and Cd) (Jiang et al., 2021). The *Alcaligenes* had a relatively high abundance in the remediation group, which had the capacity of improving the acid soil environment (Wu et al., 2021). This result was consistent with Du et al. (2022), who found that the inoculation of *Alcaligenes* in acid wastewater can decline the concentration of organic acids and increase the pH value of the systems (Du et al., 2022).

Relationship between environmental factors and bacterial community structure

Canonical correlation analysis (CCA) by chi-square distance was used to reflect the relationship between different environmental factors and bacterial community (on the genus level). As shown in Figure 4, environmental factors (SO_4^{2-}, Pb, Zn, pH, and Eh) were chosen for CCA analysis. Five combinational variables accounted for 37.31% of observed changes in bacterial community composition, with axis 1 accounting for 29.14% and axis 2 accounting for 8.17%. The distributions of the bacterial community under the M, CM-L, CM-M, and CM-H were negatively correlated with Eh, SO_4^{2-}, Pb, and Zn (Figure 4A). It means that the relative abundance of the bacterial community increased with the decline of SO_4^{2-}, Pb, and Zn concentrations. Based on previous studies, Huang et al. (2022) concluded that the population and activity of microbes for bioremediation were the main factors in the process of removing SO_4^{2-} and heavy metals (Huang et al. 2022; Zhang et al., 2022). The distributions of the bacterial community in the CK were all positively correlated with all factors. This is consistent with previous results about the richness index and the concentration of SO_4^{2-}, Pb, and Zn in the CK. Due to the lack of management of the closed REM for a long time, many contaminants containing heavy metals and SO_4^{2-} continued to dissolve as a result of the action of acid rain. This was causing a decline in the richness and diversity of the bacterial community. In addition, the bacterial community structure in all treatments, except CK, was significantly and positively correlated with the pH value, which was explained as a process of consuming acid for transforming SO_4^{2-} by the SRB (Tang et al., 2022).

A Spearman's correlation analysis between the bacterial community structure and the main factors was calculated (Figure 4B). According to the descending order of bacterial abundance, the majority of the top 30 genera had a positive correlation with the pH value, indicating that these microbes can contribute to the improvement of soil pH. Due to potential resistance to the harsh environment and acid consumption in self-metabolism, many bacterial species can improve their pH value and colonize into the acid-contaminated system (Zhu et al., 2022). In particular, these key bacterial species involving *Desulfosporosinus*, *Desulfitobacterium*, and *Desulfobulbus* showed a negative correction with all factors, except for the pH above result indicated that these microbes can tolerate heavy metals and high sulfate conditions. A negative correction between functional microbes and Eh value, indicating that *Desulfosporosinus*, *Desulfitobacterium*, and *Desulfobulbus* can transform oxidation (Eh > 0 mV) into reduction condition (Eh < 0 mV) by reducing SO_4^{2-} into H_2S. Previous studies found that functional bacterial species through the reduction of SO_4^{2-} into H_2S had an influence on Eh value, which was transforming oxidation into reduction potential, reaching below 0 and maintaining reduction conditions. The earlier result will contribute to the sustainable prevention of heavy metal dissolution (Minari et al., 2020; Gu et al., 2021). A significantly negative correlation between functional bacterial species and Pb and Zn was attributed to these microbes producing enough H_2S to combine with heavy metal, causing the decline of Pb and Zn concentrations.

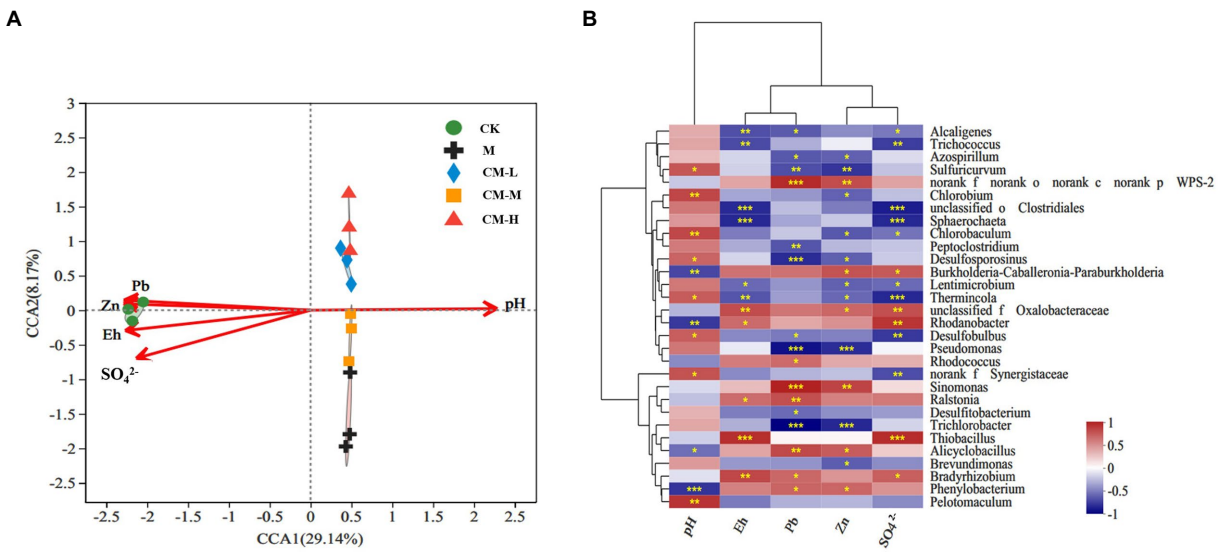

FIGURE 4
(A) Result from CCA to explore the relationship between soil bacterial community and soil physiochemical properties, and contaminants. **(B)** Heatmap of Spearman's rank correlation coefficients combined with a cluster analysis between soil physiochemical properties, contaminants and the relative abundances of the bacterial genus in the top 30. Horizontal row represents soil physiochemical properties and contaminants information, vertical row represents microbial community abundance information, red represents positive correlation, blue represents negative correlation, darker color indicates higher correlation, value of *p* is the correlation test result, * in the figure indicates $p<0.05$, and ** indicates $p<0.01$. *** in the figure indicates $p < 0.001$.

Sinharoy and Pakshirajan (2019) found continuous accumulation of stable sulfide (PbS and ZnS) with the production of H_2S. In addition, the negative correlation between functional bacterial species and SO_4^{2-} was due to these microbes' need for abundant SO_4^{2-} for growth, proliferation, and self-metabolism (Santos et al., 2022). With the consumption of SO_4^{2-}, the population and activity of functional microbes were continuously increasing. It was achieving the goal of removing SO_4^{2-} and solidification of heavy metals in the system.

The differences of bacterial metabolic function in different remediation measures

In order to compare the difference in functional characteristics in different remediation measures, the functional annotation of prokaryotic taxa (FAPROTAX) was used to predict prokaryotic clades to establish metabolic or micro-ecological relevant functions under different remediation measures. A total of 56 functional groups in the FAPROTAX database were identified, and the relative abundance of 34 out of 38 was differences in the dotted boxes among different remediation measures (Figure 5A). Therefore, these functional groups were defined as sensitive functional groups in different remediation measures. Of the 34 sensitive functional groups in the different remediation measures, six functional groups, including sulfate respiration, sulfite respiration, nitrogen fixation, manganese oxidation, dark hydrogen oxidation, and denitrification, were significantly different (Figure 5B). Among them, three functional groups of sulfate respiration, sulfite respiration, and nitrogen fixation were significantly increased after the inoculation of functional microbes in comparison with the CK. This result was related to the high SO_4^{2-} concentration of the system. SO_4^{2-} and SO_3^{2-} often acted as electron acceptors to participate in the respiration of functional microbes with the capacity of transforming SO_4^{2-} into H_2S. Previous studies concluded that the increase of sulfate respiration and sulfite respiration was related to the relatively high abundance of *Desulfosporosinus*, *Desulfitobacterium*, *Desulfobulbus*, and *Dethiosulfovibrio* in the high SO_4^{2-} system (Gao et al., 2022; Santos et al., 2022). The difference in nitrogen fixation in different remediation measures may be attributed to the stimulation of nutrients and nitrogen-fixing microorganisms (Azospirillum). After adjusting pH by $Ca(OH)_2$, the addition of nutrients stimulated the growth and activity of ingenious microbes with nitrogen fixation ability in a suitable pH of the system. This was the main reason why nitrogen fixation content presented as CM-M > CM-L > CM-H. In comparison with the CK, another three groups, such as manganese oxidation, dark hydrogen oxidation, and denitrification, had a declining trend with the inoculation of functional microbes. Oxidizing microbes associated with the manganese oxidation metabolism can promote the dissolution of heavy metal (Mn), which then significantly increases in the oxidized conditions with the abundance of these microbes. In this study, the initial oxidized conditions in the M, CM-L, CM-M, and CM-H transformed gradually into the reduction conditions by the inoculation of functional microbes and caused the decline of oxidizing bacterial abundance, and that was the reason for the decrease of manganese oxidation metabolism in all treatments, except for the CK. The difference in denitrification metabolism between the CK and the others was attributed to the high abundance of denitrification bacteria (Alcaligenes, Burkholderia-Caballeronia-Paraburkholderia, Pseudomonas, and Rhodococcus) in the CK. Previous studies reported that Pseudomonas, Alcaligenes, and Rhodococcus could promote the conversion of nitrate nitrogen to nitrogen.

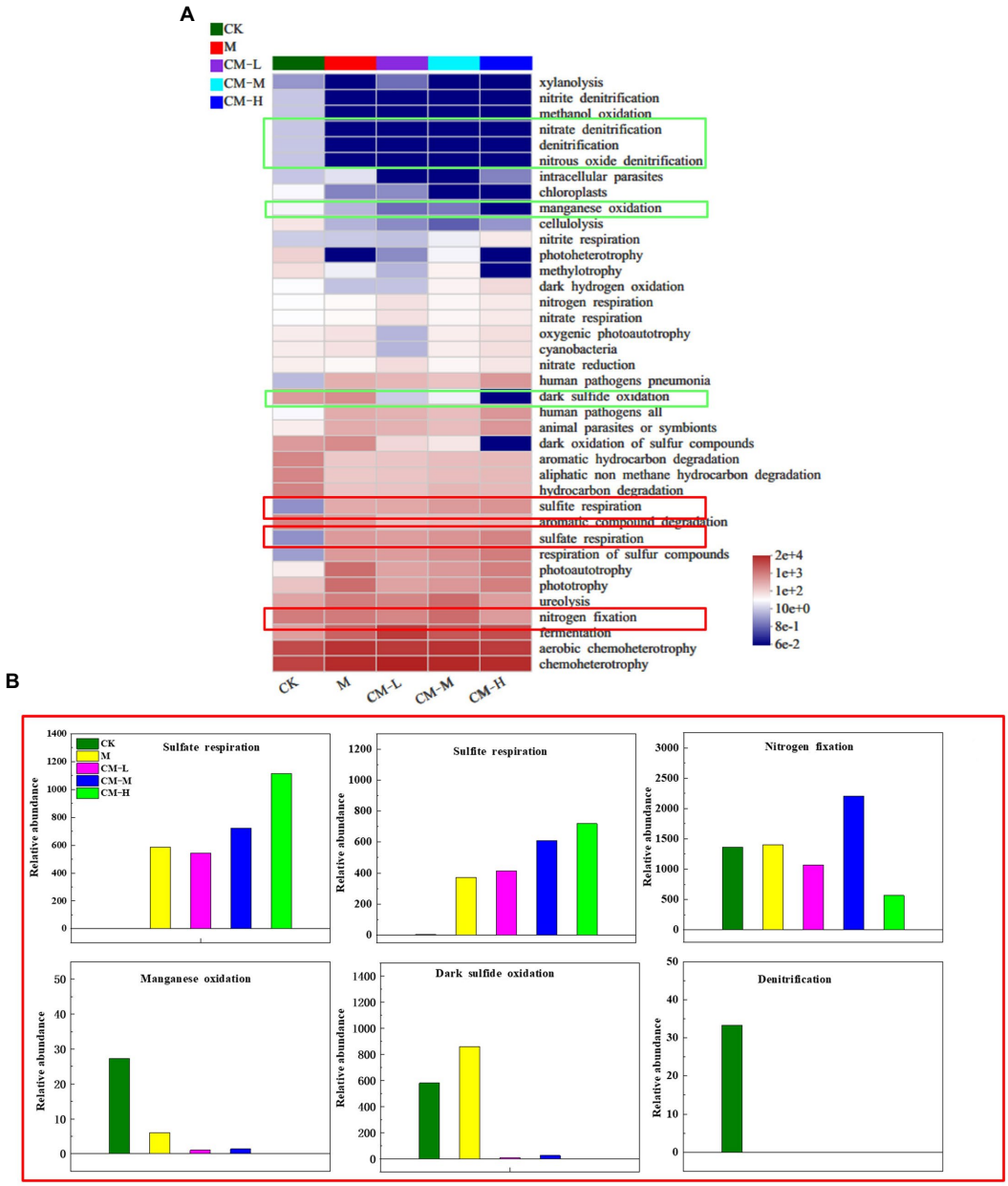

FIGURE 5
Microbial metabolism profiles in the REM soil with different treatments. **(A)** Heatmap analysis of the top 40 differential metabolites. **(B)** The relative abundance differences in different functional groups.

Conclusion

In this study, the concentration of contaminants from the REM soil was decreased in different remediation measures. The results indicated that the CM-M had a more efficient removal effect for SO_4^{2-}, Pb, Zn, and Mn than the others, up to 94.6, 88.3, 98.7, and 91%, respectively. The difference in bacterial community structure in different remediation measures was detected. Compared with the CK, *Desulfobacterota* with the ability to transform SO_4^{2-} into S^{2-} increased significantly. The correlation between environmental factors and bacterial community structure is presented as follows: SO_4^{2-} > Pb > pH > Zn > Eh. Among them, Eh, SO_4^{2-}, Pb, and Zn had a negative correlation with distributions of bacterial communities in the M, CM-L, CM-M, and CM-H. Functional prediction analysis showed significant differences in the five treatments. The functional groups' abundance including sulfate respiration, sulfite respiration, and nitrogen fixation significantly increased in all treatments, except for the CK, while the manganese oxidation, dark hydrogen oxidation, and denitrification decreased. The study provides an effective method for the removal of contaminants from the REM soil and establishes the theoretical foundation for harmlessness and reclamation of the REM.

Data availability statement

The original contributions presented in the study are included in the article. Because part of this batch of original data involves other research topics and paper publication, further inquiries can be directed to the corresponding author.

Author contributions

MZ provided the idea of this work. XY and BG performed the experiments, collected the samples, detected, analyzed the data, and involved in experimental design. XY prepared the figures and wrote the manuscript. XZ detected physiochemical properties. BG collected the samples. JW revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

XY, BG, JW, XZ, and MZ were employed by GRINM Resources and Environment Tech. Co., Ltd. and GRINM Group Co., Ltd. XZ was employed by GRIMAT Engineering Institute Co., Ltd.

Publisher's note

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EDITED BY
Ruiyong Zhang,
Institute of Oceanology, Chinese Academy of Sciences (CAS), China

REVIEWED BY
Jia Meng,
Harbin Institute of Technology, China
Rong Xiao,
Fuzhou University, China

*CORRESPONDENCE
Huai Li
✉ lihuai@iga.ac.cn

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Microorganisms in coastal wetland sediments: a review on microbial community structure, functional gene, and environmental potential

Shen Liang[1,2], Huai Li[1]*, Haitao Wu[1], Baixing Yan[1] and Aiwen Song[1,2]

[1]Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, China, [2]University of Chinese Academy of Sciences, Beijing, China

Coastal wetlands (CW) are the junction of the terrestrial and marine ecosystems and have special ecological compositions and functions, which are important for maintaining biogeochemical cycles. Microorganisms inhabiting in sediments play key roles in the material cycle of CW. Due to the variable environment of CW and the fact that most CW are affected by human activities and climate change, CW are severely degraded. In-depth understanding of the community structure, function, and environmental potential of microorganisms in CW sediments is essential for wetland restoration and function enhancement. Therefore, this paper summarizes microbial community structure and its influencing factors, discusses the change patterns of microbial functional genes, reveals the potential environmental functions of microorganisms, and further proposes future prospects about CW studies. These results provide some important references for promoting the application of microorganisms in material cycling and pollution remediation of CW.

KEYWORDS

coastal wetlands, microorganisms, community structure, functional gene, environmental potential

1. Introduction

CW are the transitional regions between the terrestrial and marine ecosystems, mainly including shallow seas, estuaries, mangroves, salt marshes, deltas, etc. CW have the vegetated zones (mangroves, salt marshes, and seagrass beds) and non-vegetated zones (mudflats and sandy beaches), which are critical areas connecting land, freshwater habitats, and the ocean (Levin et al., 2001). CW can provide many facilities for human activities such as fishing and breeding (Zhang and Shao, 2013), and also protect coastal zones in flooding (Narayan et al., 2017). Moreover, CW richen in biodiversity, material cycling, energy flow, and species migration and evolution, with high primary productivity (Cui et al., 2016). CW are the most vulnerable ecosystems due to ocean dynamics, river disturbance, and human activities (Wang et al., 2022), and its degradation (such as biodiversity decline, ecosystem function loss, and coastal vegetation reduction) may lead to biological invasions, water quality deterioration, and reduced coastal protection from flooding and storm events (Barbier et al., 2011).

Microorganisms are an important component of wetland ecosystems and play key roles in biogeochemical cycles (DeLong et al., 2006). Microbial community structure has significant differences depending on different soil properties and vegetation types. Soil properties and plant types are the main determinants of microbial community structure (Yu et al., 2014). The interdependence between plants and microorganisms has a critical role in regulating ecosystem services such as nutrient cycling, productivity, and pollutants degradation (Abdu et al., 2017). Microorganisms can decompose soil organic matter, promote sulfate reduction, sulfide/sulfur oxidation, iron reduction, nitrification, pollutant degradation, and help improve soil structure and enhance ecosystem stability (Behera et al., 2017). CW exhibit strong nutrient and salinity gradients due to freshwater and seawater interactions, affecting soil microbial composition. Furthermore, these changes in microorganisms can lead to the variations of the function and structure of CW (Webster et al., 2015).

Despite the importance of microorganisms in CW (Figure 1), few studies have reviewed and summarized them. Therefore, the purpose of this study is: (1) to provide an overview of microorganisms in CW sediments; and (2) to identify future research directions and possible difficulties. In this paper, we summarize the community structure characteristics, functional genetic variation and potential environmental functions of microorganisms in CW.

2. Microbial community structure in CW

The special soil characteristics and hydrological conditions of CW constitute a unique microbial community (Peralta et al., 2010). Linking microbial communities to physical, chemical, and biological factors can explore the drivers of microbial community formation (Fierer and Jackson, 2006), which is important for the restoration of environmental functions in wetland ecosystems.

2.1. Microbial community composition

Microbial species are abundant in CW, which are mainly divided into bacteria, archaea, and fungi (Table 1). Among them, bacterial communities have the highest richness, followed by archaeal and fungal communities (Cheung et al., 2018). Although the composition is the same at community level, there is some variation in microbial composition among different CW and different times of wetlands (Adame et al., 2012).

Proteobacteria is the most abundant bacterial phyla in CW sediments (Ling et al., 2015), and mainly includes *α-*, *β-*, *γ-*, *δ-, and ε-Proteobacteria* (Hu et al., 2014), and their composition varies somewhat in different wetland types. For example, *γ-Proteobacteria* dominates in coastal zone of Yellow River Delta, whereas *γ-* and *δ-Proteobacteria* dominate in brine-freshwater zone (Hu et al., 2016). *ε-Proteobacteria* is dominant Proteobacteria in Jiuduansha Wetlands of Yangtze Estuary, while *γ- and β-Proteobacteria* are abundant in Jiangyanan Shoal of the river (Fei Xi et al., 2014). On the contrary, Firmicutes is the dominant phylum near salt flats of the Yangtze River Delta (Zou et al., 2020). In addition to Proteobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Firmicutes, Acidobacteria, and Planctomycetes are also the main phyla in CW (Zhang et al., 2017; An et al., 2019). Although bacterial compositions are relatively similar, there are some differences in different wetlands types. Moreover, archaea are also an important component of microbial communities and play an important role in biogeochemical cycles of CW (Narrowe et al., 2017). The dominant phyla of archaea are mainly Euryarchaeota, Thaumarchaeota, Bathyarchaeota and Grenarchaeota (Zhao et al., 2020; Chi et al., 2021a,b,c,d). Fungi as an important component of microorganisms and its community are essential for maintaining soil versatility (Li H. et al., 2019). Ascomycota and Basidiomycota are the dominant taxa in CW (Mohapatra et al., 2021). Among them, *Dothidomycetes* and *Sordariomycetes* are the dominant classes, and the dominant orders include *Pleosporales*, *Agaricales*, and *Capnodiales* (Ye et al., 2022). Moreover, many fungi cannot be attributed to the known phyla (Cheung et al., 2018).

2.2. Factors shaping microbial community

2.2.1. Soil characterizations

Microorganisms in CW sediments are influenced by soil physicochemical properties, including salinity, pH, and nutrients (Jackson and Vallaire, 2009). These properties can affect microbial growth and metabolism as well as microbial activity (Figure 2).

Salinity can directly affect abiotic and biotic processes, and is considered as a major driver of ecosystem structure and function (Brucet et al., 2012). Previous study showed that salinity affected microbial communities and the associated biogeochemical cycles (Chambers et al., 2011). In general, salinity elevation usually has a negative impact on microorganisms, and low salinity environments are suited for microbial growth (Hu et al., 2016). High salinity can affect CW ecosystems through inhibiting plant growth and heterotrophic metabolism, and reducing soil quality and heterotrophic bacterial diversity (Abed et al., 2007). Microbial community structure varies along salinity gradients (Yang et al., 2018). It was found that halophilic bacteria such as *Fodinibius*, *Alkalilimnicola*, *Phycisphaera* and Gp21 were abundant in high-salinity zone of the Yangtze River Delta, and the dominant genera in the transition zone were *Rhodocyclus*, *Flavobacterium* and *Shin* (*Shinell*; Li J. et al., 2019).

pH has a significant effect on microbial community (Rousk et al., 2010). The bacterial composition and diversity in various ecosystems respond strongly to soil pH (Shen et al., 2013). Nitrospirae was lower in saline wetlands with high pH than in freshwater wetlands with low pH, and Nitrospirae was significantly negatively correlated with pH (Chi et al., 2021a,b,c,d).

Nutrients can also affect microbial growth. Total organic matter, total nitrogen and total phosphorus in the samples are usually tested as quantitative indicators of nutrient content when conducting experiments (Wang et al., 2012). The content of available nutrients affects microbial activity, and the addition of nutrients can effectively increase the abundance of bacterial strains (Meng et al., 2016). The unique structural composition of microbial communities in intertidal sediments of the Yellow River Delta is nutrient-related, and many saprophytic microorganisms are enriched (Zhang et al., 2017).

2.2.2. Vegetation types

Vegetation has important influences on microbial community. Plants can create a unique environment for rhizosphere microorganisms (Grayston et al., 1998). Studies have shown that

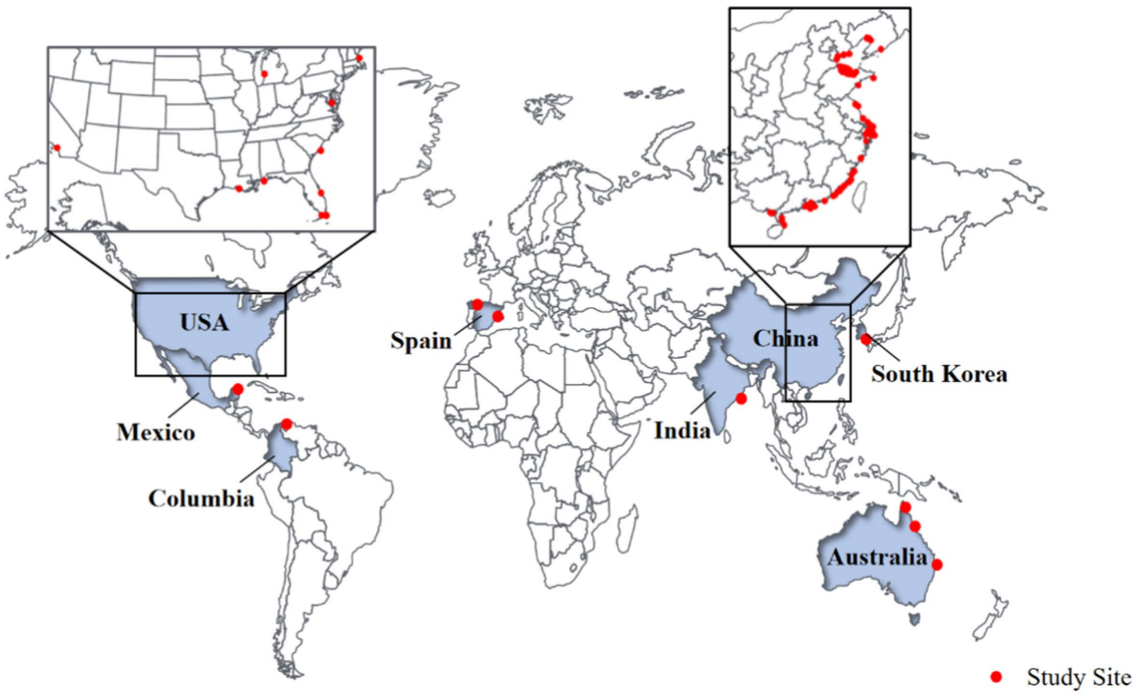

FIGURE 1
Location of CW studied by various groups for their microbial distribution.

nutrient acquisition strategies of plant can drive the structural and functional formation of soil surface microbes and that changes in vegetation lead to changes in soil microbial diversity and function (Bahram et al., 2020). Root-mediated changes in soil can provide oxygen or other substrates for soil microbes (Noll et al., 2005) and also alter microbial community (Lipson et al., 2015). Plants alter the physicochemical conditions of sub-canopy soils (Menon et al., 2013), such as leaf litter can improve soil fertility and plant roots can release a variety of compounds into the surrounding soils (Garbeva et al., 2004). Roots of woody plants, such as mangroves, have different chemical (Perry and Mendelssohn, 2009) and physiological properties (Skelton and Allaway, 1996), and can transport different root secretions (Bertin et al., 2003). Differences of microbial community composition in mangrove- and swamp-dominated soils in Florida may be due to differences in root secretions or oxygen availability between vegetation types (Barreto et al., 2018). The photosynthesis of plants lead to the adsorption of cyanobacteria on plant rhizomes with more than 50% abundance in Yellow River Delta (Li et al., 2021).

3. Microbial functional genes in CW

3.1. Nitrogen cycle-related genes

Microbially mediated nitrogen cycle is one of the important components of biogeochemical cycles in CW (see Figure 3A). Among them, denitrification and dissimilatory nitrate reduction to ammonia (DNRA) processes are particularly important, and the end-products of these pathways have different effects on ecosystem nitrogen effectiveness (Morina and Franklin, 2022).

Denitrification plays a key role in nitrogen removal (van Breemen et al., 2002), and there are some important metabolic enzymes, including nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos; Levy-Booth et al., 2014). Among these, Nir catalyzes the rate-limiting step in denitrification, encoding by the *nirK* and/or *nirS* genes. A previous study found that the enzyme genes associated with denitrification decreased with increasing distance from the river bank of Yellow River, and reached their highest levels at distances of 0–50 m (except 0 m; Li W. et al., 2019). Saline plants had no significant effect on the abundances of denitrification genes *nirK*, *nirS*, and *nosZ* in Suncheon Bay, South Korea (Chaudhary et al., 2018). Mesosaline soils affect negatively on *nirS* and *nirK* genes compared to freshwater soils in the east coast of the United States (Morina and Franklin, 2022). Moreover, the diversity of *nirS* genes in Chinese CW exhibited significant latitudinal heterogeneity, and it is speculated that temperature rather than salinity contributes significantly to the latitudinal distribution of *nirS*-based denitrifying bacteria (Gao et al., 2016).

DNRA can convert nitrate nitrogen to ammonia nitrogen, and is one of the potentially important nitrogen cycling processes in CW. The reduction of NO_2^- to NH_4^+ in DNRA is catalyzed by nitrite reductase, encoding by the *nrfA* gene. The abundance of *nirS* denitrifying bacteria is much greater than that of *nrfA*-DNRA microorganisms in the Chesapeake Bay watershed of United States, suggesting that denitrification is the primary nitrate reduction process (Franklin et al., 2017). The abundance of *nrfA* genes was low in tidal freshwater marshes in South Carolina of United States, suggesting weak DRNA process (Minick et al., 2019).

Nitrification and anammox are also important processes in the nitrogen cycle (Kuypers et al., 2018), but these two processes are weakly in CW ecosystems (Zhang X. et al., 2019). Functional genes

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TABLE 1 The dominant microbial phylum in CW.

Location	Bacteria	Archaea	Fungi	References
Asia				
Yellow River Delta, China	Proteobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, Gemmatimonadetes	Thaumarchaeota, Crenarchaeota, Euryarchaeota， Diapherotrites		Yu et al. (2012), Zhao et al. (2020), Lu et al. (2021) and Zhang et al. (2021)
Futian Mangrove Natural Reserve，China	Proteobacteria, Bacteroidetes, Firmicutes, Tenericutes, Chloroflexi			Tong et al. (2021)
Mai Po wetland, Hong Kong	Proteobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes, Acidobacteria	Aenigmarchaeota, Bathyarchaeota， Euryarchaeota, Thaumarchaeota	Ascomycota, Basidiomycota, Chytridiomycota	Cheung et al. (2018)
Hangu District of Tianjin Municipality, China	Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Chloroflexi			Li et al. (2016)
Senmao farm in Rudong county, China	Bacteroidetes, Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria,			Bai et al. (2019)
Jiulong River Estuary, China	Bacteroidetes, Chlorobi, Chloroflexi, Proteobacteria, Firmicutes			Su et al. (2016)
Nalabana Island, India	Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Bacteroidetes	Euryarchaeota, Candidatus Bathyarchaeota, Thaumarchaeota, Crenarchaeota, Candidatus	Ascomycota, Basidiomycota, Mucoromycota, Chytridiomycota	Mohapatra et al. (2021)
America				
Eastern Coast of Florida, United States	Proteobacteria, Chloroflexi, Acidobacteria Nitrospirota, Chlorobi			Barreto et al. (2018)
York River State Park，United States	Proteobacteria, Acidobacteriota, Desulfobacterota, Bacteroidota, Chloroflexi	Halobacterota, Thermoplasmatota, Nanoarchaeota		Morina and Franklin (2022)
Whitney Marine Laboratory, United States	Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Cyanobacteria			Ward et al. (2019)
Barataria Bay, United States	Proteobacteria, Bacteroidetes, Firmicutes, Chloroflexi, Acidobacteria,			Bae et al. (2018)
Mangrove in La Guajira, Colombia	Proteobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Firmicutes,			Torres et al. (2019)
Point Aux Pins peninsula, United States	Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Acidobacteria			Beazley et al. (2012)
Australia				
East Trinity, Cairns, Australia	Proteobacteria， Chloroflexi, Bacteroidetes, Firmicutes, Acidobacteria	Crenarchaeota, Euryarchaeota		Ling et al. (2015)

Column "bacteria" selected the five phyla with the highest relative abundance.

associated with nitrification are mainly genes encoding ammonia monooxygenase (*amoA/B/C*) and hydroxylamine dehydrogenase (*hao*; Mohapatra et al., 2021), while those associated with anammox are mainly genes encoding hydrazine synthase (*hszA*; Harhangi et al., 2012). The abundance of functional genes associated with nitrification in intertidal wetlands disturbed by crabs is increased compared to the

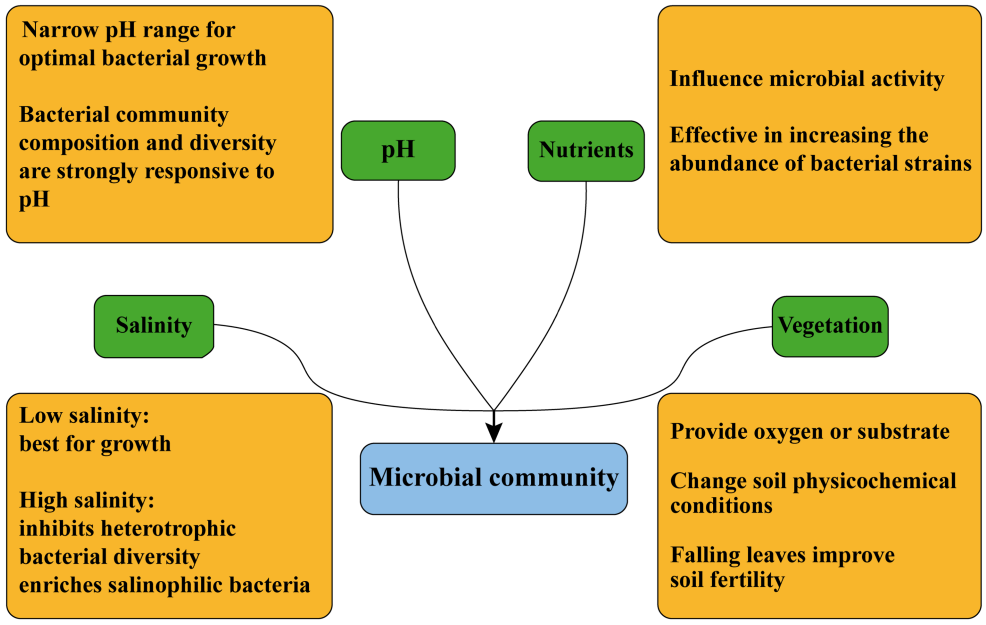

FIGURE 2
Factors influencing microbial community composition in CW.

surrounding undisturbed sediments (An et al., 2021). Moreover, the copy number of nitrification genes is significantly higher in oily marshes (Bae et al., 2018).

3.2. Methanogenesis-related genes

Methane production is a major process in anaerobic carbon-cycle of CW, and methanogenic bacteria are the main microorganisms involved in this process, encoding by *mcrA* gene (see Figure 3B; Oremland et al., 1982). A previous study showed that the abundance of methanogenic genes in wetlands affected by runoff and tidal seawater increased with distance from the river bank, while gene abundance in tidal wetlands increased first and then decreased in Yellow River Delta (Chi et al., 2021a,b,c,d). The abundance of *mcrA* was significantly lower in oiled marshes compared to non-oiled marshes along the United States coast (Bae et al., 2018).

3.3. Organics degradation-related genes

A large number of organic pollutants from human activities are released into wetlands with industrial development, adversely affecting the surrounding ecosystems (Qian et al., 2016). Petroleum hydrocarbons are the main pollutants that affect the material cycle and ecosystem function of wetlands (Yuan et al., 2014). Indigenous microorganisms in wetlands can degrade petroleum hydrocarbons, and lots of hydrocarbon-degrading bacteria isolated from petroleum-contaminated soils play key roles in petroleum hydrocarbons degradation (Tiralerdpanich et al., 2018). Therefore, the level of petroleum hydrocarbon contamination in different wetland soils can affect microbial community, which leads to changes in metabolic functions (see Figure 3C).

Both *alkB* and CYP 153A1 genes encoding alkane hydroxylases are enriched in tidal marshs from the Coacheco River in the United States under chronically contaminated petroleum hydrocarbons such as gasoline, n-hexane, and dodecane (Ní Chadhain et al., 2018). The gene *alkB* involving in aerobic alkanes degradation has high copy number in oil-bearing coastal salt marshes of the United States, whereas *bamA* related to anaerobic aromatics degradation has low copy number (Bae et al., 2018). Genes associated with the degradation of alkanes, cycloalkanes, aromatic carboxylic acids, chlorinated aromatics, polycyclic aromatic hydrocarbons, and other aromatic hydrocarbons are significantly reduced in salt marshes of Gulf Coast during oil concentration reduction (Beazley et al., 2012). The initial dioxygenase and open-loop dioxygenase associated with phenanthrene (PHE) degradation were expressed under PHE contamination in CW, indicating the presence of aerobic PHE degradation (Chi et al., 2021a,b,c,d).

4. Environmental potential of microorganisms in CW

Microorganisms contribute significantly to ecological functions (e.g., carbon and nitrogen cycle processes) in CW (Figure 4), which are critical in retaining chemical contaminants (e.g., organic pollutants) and excess nutrients (Horton et al., 2019).

4.1. Functional indicator

Previous studies have shown that the species composition and spatio-temporal dynamics of soil microbial communities are related to habitat characteristics, plant types, and human interferences (Eddie et al., 2010). Microorganisms are highly sensitive to environmental

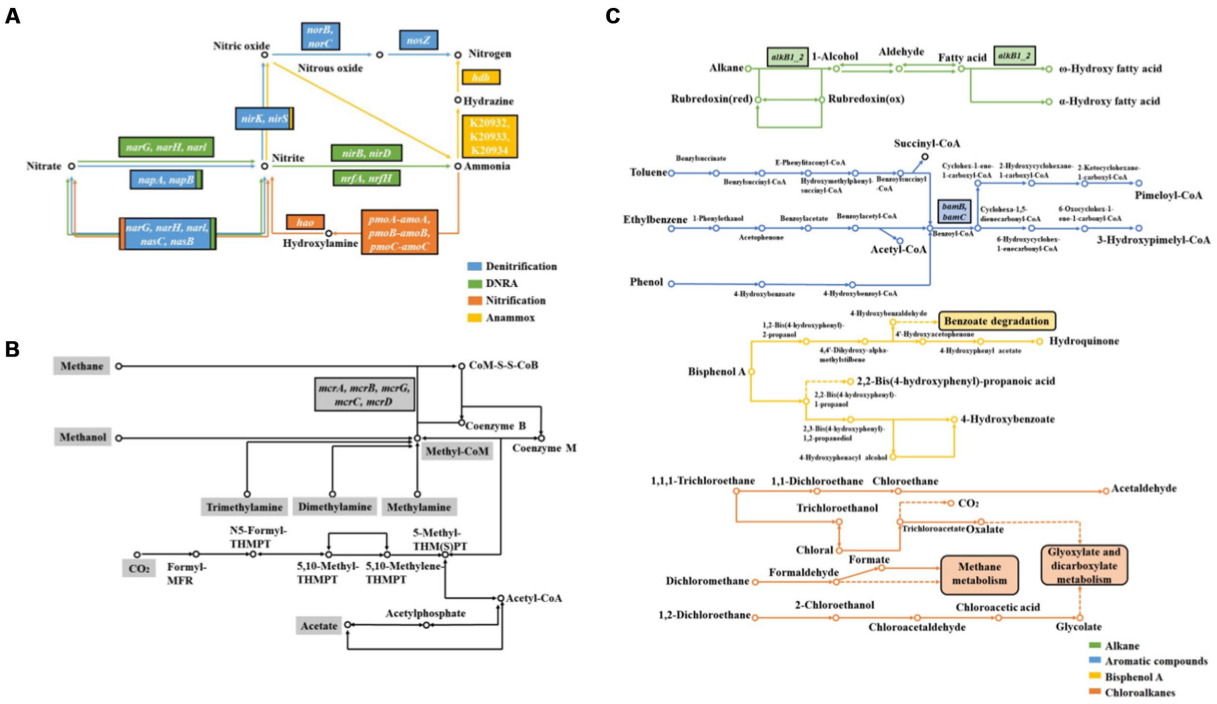

FIGURE 3
KEGG pathway of nitrogen cycle **(A)**, methanogenesis **(B)** and organics degradation **(C)** in CW.

changes and thus can be an ideal indicator for environment monitoring (Santos et al., 2010; Yang et al., 2012). The indicative effects of microbial communities are various (Table 2). Fungal community composition in different habitats varies and could be used as a bioindicator to assess the restoration process of mangrove ecosystems in Jiulongjiang estuary (Yu et al., 2014). Fungi as indicator species of *P. australis* soils is found in restoration area of the Yangtze River Delta (Ma et al., 2017). Differences in microbial community over a short period in Florida suggest that they can serve as early warning signals for sea-level rise (Chambers et al., 2016). The ratio of ammonia to nitrate nitrogen in CW of Pearl River Delta significantly affect bacterial community composition, and thus anaerobic ammonia-oxidizing bacteria is a bioindicator of terrestrial nitrogen input or pollution (Han and Gu, 2015).

4.2. Organic pollutant degradation

Microorganisms have become popularly alternatives for pollutant bioremediation because they are environmentally friendly and cost-effective (Macaulay, 2015). Hydrocarbon-degrading microorganisms are ubiquitous in many environments (Head et al., 2006). Most studies on microbial hydrocarbon degradation have focused on environments highly exposed to hydrocarbons, such as areas surrounding oil deposits and hydrocarbon spills. *Pseudomonas*-type alkane-degrading bacteria are enriched in marshes nearby oil contamination, suggesting that oil degradation is important at this zone (Ní Chadhain et al., 2018). Bacterial community in sediments of Mexican coastal zone can degrade toluene, naphthalene, chloroalkanes, and chlorinated alkanes, but has low removals of aromatics, fluorobenzoates, and xylenes (Reyes-Sosa et al., 2018). *Proteobacteria* is responsible for the degradation of some phenolic compounds including bisphenol A (BPA) in mangrove of Shenzhen, and shows significant variation with BPA biodegradation (Tong et al., 2021). The correlation between fungal abundance and phenol oxidase activities in the Mai Po wetlands of Hong Kong suggests that fungi can contribute to soluble phenols reduction (Luo et al., 2018). The relative abundance of hydrocarbon-degrading bacteria (*Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*) in hydrocarbon-contaminated sediments increases in salt marshes along the Gulf of Mexico (Beazley et al., 2012). PAHs-degrading bacteria (*Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Chloroflexi*) in mangrove increase under polycyclic aromatic hydrocarbons contamination in the Jiulongjiang estuary (Su et al., 2016).

4.3. Biogeochemical cycles

Functionally diverse microbial communities in CW contribute to the biogeochemical transformation of elements such as carbon (C) and nitrogen (N; Yuan et al., 2014; Yang et al., 2022), and these biological processes mainly include carbon formation and degradation, carbon fixation and nitrogen metabolism, methane metabolism, and exogenous biodegradation and metabolism (Mohapatra et al., 2021).

Halobacteria and *Thaumarchaeota* are found in Yellow River Delta which can fix CO_2 (Hu et al., 2016). Compared to mangrove, invasive *Spartina alterniflora* significantly can increase CH_4 emissions and decrease CO_2 emissions (Han and Gu, 2015). CH_4 production was high in soils with saline plants in Suncheon Bay, Korea (Chaudhary et al., 2018). Nutrient transformation is related to highly active and adaptive

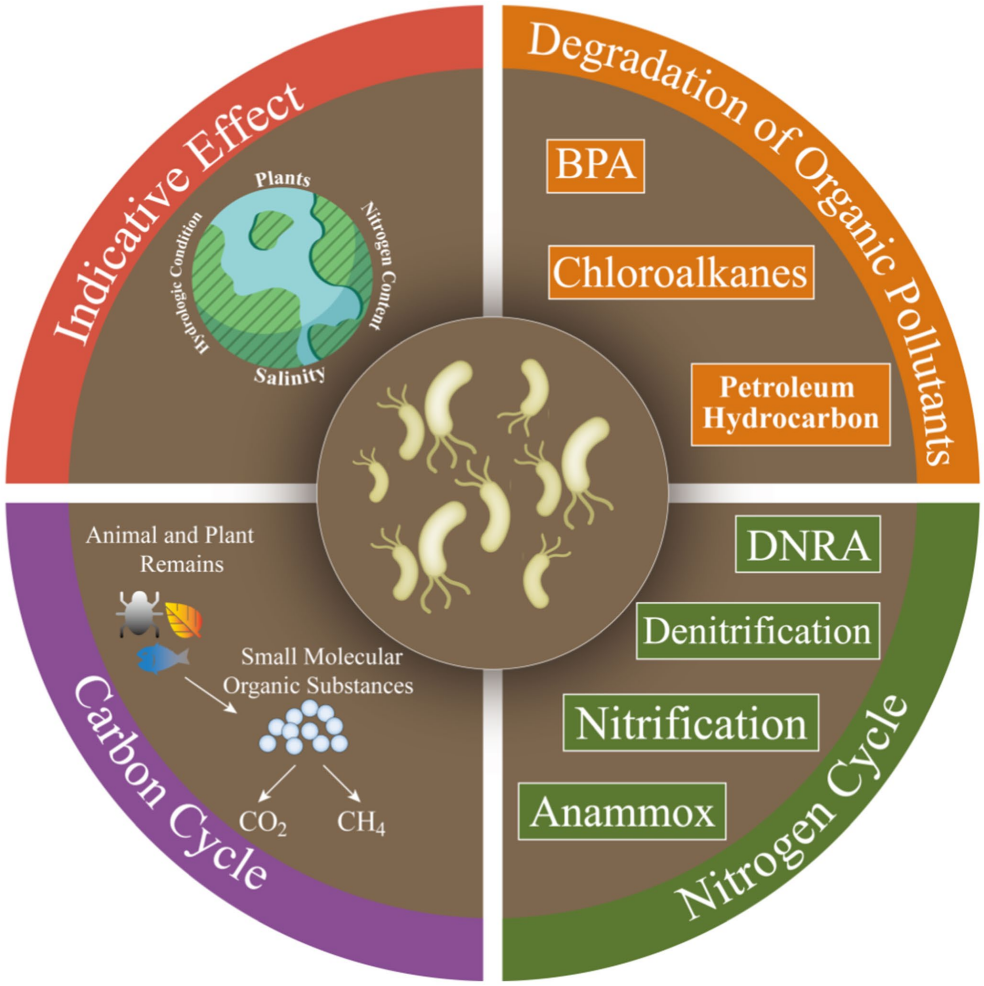

FIGURE 4
Potential environmental functions of microorganisms in CW.

TABLE 2 Summary of indicative effects of microbial communities in CW.

Location	CW	Indicative effect	References
Jiulong River Estuary, China	M	Microbial community structure could be bioindicator of the mangrove recovery	Yu et al. (2014)
Yellow River Delta, China	P	Fungi could be bioindicator for soils under *P. australis*	Ma et al. (2017)
Pearl River Delta, China	M	Anammox bacteria community structures could be bioindicator of the anthropogenic/terrestrial inputs	Han and Gu (2015)
Bohai Economic Rim, China	P	Functional genes could be bioindicator of denitrification potential	Zhang X. et al. (2019) and Zhang Y. et al. (2019)
Quangang District, China	M	The genera *Mangrovibacterium* and *Mangrovimonas* can both be potential bioindicators of wetland restoration	Lin et al. (2021)
Avicennia germinans, Columbia	M	Firmicutes, Chloroflexi, Cyanobacteria and Gemmatimonadetes may be bioindicators of anthropogenic pollution	Torres et al. (2019)

Column "CW" defines the vegetation type in coastal wetlands: *Mangrove* (M) and *Phragmites australis* (P).

bacterial metabolic channels in Chinese coastal zone (Zhang et al., 2022). When exposed to unstable substrates, microbial respiration is much higher and can produce more CO_2 in mangrove and marsh soils along the east coast of Florida, United States (Barreto et al., 2018). In Liaohe River estuary of China, there is a positive correlation between soil respiration rates and *Clostridia* abundance, suggesting that anaerobic carbon decomposition is important in brackish wetland soils (Yang et al., 2018).

Changes in biomass and community structure may enhance soil N sequestration due to the abilities of special heterotrophic metabolism and refractory organics degradation of soil microorganisms (Tang et al., 2011). Denitrification is the main mechanism for nitrogen removal in CW of Bohai Sea (Zhang Y. et al., 2019), and ammonia-oxidizing archaea and bacteria are also important in global nitrogen cycle (Bai et al., 2019). Nitrate reduction rates are associated with denitrifying bacterial community in the protected area in Spain, suggesting that microbial communities are closely associated with N_2O emissions (Bañeras and Ruiz-Rueda, 2012). Nitrogen and phosphorus additions can increase microbial denitrification in the absence of salinity (Chi et al., 2021a,b,c,d). Nitrification and denitrification rates are more higher in intertidal areas disturbed by crabs, and this process greatly contribute to N_2O emissions (An et al., 2021).

5. Future prospects

Microorganisms in CW sediments are highly biodiverse and spatial heterogeneous, and play a key role in maintaining biogeochemical cycles. With the rapid development of molecular biology technologies, our understanding of microbial community and their potential functions has grown substantially. However, several major challenges remain in CW studies.

(1) Accurate identification of key factors affecting microbial community structure. Microorganisms in coastal wetlands are important for maintaining the normal biogeochemical cycle, so it is important to explore the factors affecting the community structure. The most studies about influencing factors focused on single factor, ignoring a combination of multiple factors. Therefore, it is important to elucidate the dominant factors affecting microbial community, which will help to maintain the stability of coastal ecosystems, prevent CW destruction, and restore degraded CW.

(2) In-depth reveal the potential functions of microorganisms, and decipher the relationship between functional stability and microbial biodiversity. Microorganisms are important for environmental management due to the community and functional diversities. The stable microbial community not only ensure the normal biogeochemical cycle, but also decompose complex pollutants into harmless substances through metabolic activities. Furthermpre, clarifying the coupling relationship between microbial diversity and functional stability and parsing the function of biological elements in habitat function ascension will help to maintain and improve the stability of CW function.

6. Conclusion

Microorganisms in CW have become important players involving in biogeochemical cycles and potential solutions for the treatment of difficult-to-degrade pollutants. Microbial community structure usually rapidly changes in response to environmental changes. Therefore, they can be used as indicators to detect changes in CW. Our studies discuss the changes in microbial composition of CW, summarize the effects of different factors on microbial community structure and the important functional genes, and further reveal the potential environmental functions of coastal microbes. Microbial communities involving in organic pollutant degradation and material cycling of CW have been well developed, but their functions and the relationship between functional stability and microbial biodiversity need to be further explored.

Author contributions

SL: data curation and analysis and writing - original draft. HL: conceptualization, methodology, resources, supervision, writing - review and editing, project administration, and funding acquisition. HW, BY, and AS: conceptualization, methodology, data curation, and writing - review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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