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Distribution and correlation of iron oxidizers and carbon-fixing microbial communities in natural wetlands

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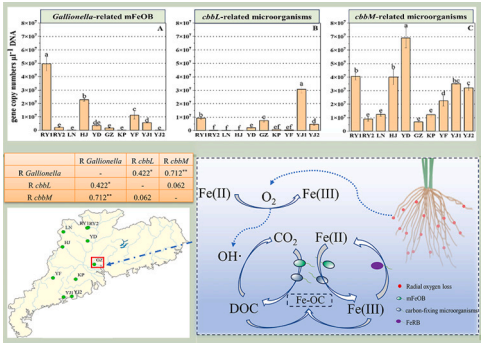
HIGHLIGHTS

- Soil Fe(II)-oxidizing and carbon-fixing microbial communities in wetlands were characterized.
- The carbon-fixing microorganisms were mainly composed of Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria.
- *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2 were dominant mFeOB in different wetland soils.
- A significant positive correlation was found between the abundances of mFeOB and carbon-fixing gene.
- Acetate-extracted Fe(II) and redox potential affected carbon-fixing microbial community structure.

GRAPHICAL ABSTRACT



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ABSTRACT

Most microaerophilic Fe(II)-oxidizing bacteria (mFeOB) belonging to the family *Gallionellaceae* are autotrophic microorganisms that can use inorganic carbon to drive carbon sequestration in wetlands. However, the relationship between microorganisms involved in Fe and C cycling is not well understood. Here, soil samples were collected from different wetlands to explore the distribution and correlation of *Gallionella*-related mFeOB and carbon-fixing microorganisms containing *cbbL* and *cbbM* genes. A significant positive correlation was found between the abundances of mFeOB and the *cbbL* gene, as well as a highly significant positive correlation between the abundances of mFeOB and the *cbbM* gene, indicating the distribution of mFeOB in co-occurrence with carbon-fixing microorganisms in wetlands. The mFeOB were mainly dominated by *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2 in all wetland soils. The structures of the carbon-fixing microbial communities were similar in these wetlands, mainly consisting of Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. The extractable Fe(II) concentrations affected the community composition of mFeOB, resulting in a significant difference in the relative abundances of the dominant FeOB. The main factors affecting *cbbL*-related microbial communities were dissolved inorganic carbon and oxygen, soil redox potential, and

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sodium acetate-extracted Fe(II). The composition of *cbbM*-related microbial communities was mainly affected by acetate-extracted Fe(II) and soil redox potential. In addition, the positive correlation between these functional microorganisms suggests that they play a synergistic role in Fe(II) oxidation and carbon fixation in wetland soil ecosystems. Our results suggest a cryptic relationship between mFeOB and carbon-fixing microorganisms in wetlands and that the microbial community structure can be effectively altered by regulating their physicochemical properties, thus affecting the capacity of carbon sequestration.

1. Introduction

Wetlands are dynamic areas formed by the interplay between soil and water systems that play crucial roles in maintaining biodiversity, regulating climate, degrading pollutants, purifying water quality, and sequestering carbon (Junk et al., 2013). Due to their periodic flooding and drought, wetlands exhibit intense biogeochemical activities to facilitate the cycling of elements, such as carbon (C), iron (Fe), and sulfur (S). As a vast carbon reservoir in natural ecosystems, the rate of carbon fixation in wetlands is affected by both wetland plants and microorganisms. The Calvin–Benson–Bassham (CBB) pathway is an important mechanism of microbial carbon dioxide (CO_2) fixation, wherein ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) serves as a key enzyme (Hügler and Sievert, 2011). The *cbbL* and *cbbM* genes, encoding RubisCO form I and form II, respectively, are the most widely studied genes involved in CO_2 fixation in various environments. These genes possess highly conserved features and appropriate gene lengths which are frequently employed as specific probes to directly target groups of microorganisms involved in CO_2 fixation (Ji et al., 2016).

As one of the most sensitive elements in biogeochemical redox processes (Emerson et al., 2010; Kappler et al., 2021), Fe cycling interacts with other elements, thereby affecting the growth and activities of microorganisms, such as methane-oxidizing and CO_2-fixing microorganisms (Kappler et al., 2021; Wang et al., 2022a, 2022b). Under anoxic conditions, Fe(III)-reducing bacteria (FeRB), which are widespread in wetlands, utilize small molecular organic carbons to reduce Fe(III) to Fe (II) (Wang et al., 2011). This Fe(II) can serve as an electron donor for Fe (II)-oxidizing bacteria (FeOB) to complete their growth metabolism. A unique microaerobic zone can be formed in wetlands due to the infiltration of oxygen (O_2) and radial oxygen loss (Khan et al., 2016). In these microaerobic zones, O_2 concentrations are lower than saturated levels, resulting in lower chemical Fe(II) oxidation which potentially allows microaerophilic FeOB (mFeOB) to compete with O_2 to perform effective biological Fe(II) oxidation (Maisch et al., 2019). Cultivation experiments have shown that microbial Fe(II) oxidation accounts for 50 %–60 % of the total Fe(II) under microaerobic conditions (Neubauer et al., 2002), confirming the importance of mFeOB for Fe(II) oxidation in wetlands. Wang et al. (2011) demonstrated that *Gallionellaceae* (*Gallionella*-related mFeOB) were the typical mFeOB in wetland soils and sediments at near-neutral pH. However, little is known about the distribution of these mFeOB and the effects of environmental factors on the diversity and composition of mFeOB in wetlands.

The reduced form of Fe is an inorganic electron donor that can fuel the chemolithoautotrophic fixation of inorganic carbon to organic carbon. Most mFeOB are chemolithoautotrophic microorganisms that can use inorganic carbon as a carbon source to oxidize Fe(II) (Li et al., 2019). Previous studies have shown that the CBB cycle is an important carbon fixation pathway for mFeOB under circumneutral conditions (Badger and Bek, 2008; Kato et al., 2015). RubisCO form I and II enzymes have different preferences for O_2 and CO_2 in the environment. The *cbbL*-encoded RubisCO I predominates in microorganisms that favor high O_2-niches, whereas the *cbbM*-encoded RusbiCO II prevails in microorganisms that have adapted to low-O_2 or high-CO_2 habitats (Thomas et al., 2019; Wang et al., 2021). The obligatory aerobic metabolism of most carbon-fixing microorganisms suggests that their environmental distribution and demands are similar to those of mFeOB. Among these mFeOB, genomic analysis showed that *Ferriphaselus amnicola* OYT1 and *Ferriphaselus strain* R-1 contained *cbbM* genes, while *Ferriphaselus strain* R-1 carried *cbbL* genes. Considering this, the various environments for mFeOB growth may be extrapolated, suggesting the possibility of co-occurring carbon-fixing microorganisms in wetlands. However, current research on the relationship between mFeOB-mediated Fe(II) oxidation and CO_2 fixation is mostly concentrated on paddy soils, mainly through simulated cultivation (Chen et al., 2022; Tong et al., 2021). The distribution and characteristics of microbial communities involved in both Fe(II) oxidation and carbon sequestration in natural wetlands have yet to be investigated in detail.

It is becoming increasingly evident that mFeOB are widespread in wetland soils, where they play an important role in Fe and carbon cycles (Wang et al., 2011). Determining the distribution of *Gallionella*-related mFeOB and carbon-fixing microorganisms and their interplay in wetlands may provide insight into biological mechanisms in soil carbon sequestration. In particular, the characterization of mFeOB which are key players in Fe mineral-organic carbons (Fe-OC) aggregate formation and their interplay with carbon-fixing microorganisms are crucial to understand the links between the microbial Fe and carbon cycles in wetlands. Guangdong province in southern China has high rainfall and is therefore rich in water resources. The environment and the water resources present exceptional advantages and the area of wetlands is vast (21 % of the total area) and encompasses a number of habitat types, which represent most wetland types in the tropics and subtropics in southern China. In the present study, the soil and water samples collected from various wetlands in Guangdong province were used to analyze their physicochemical properties and mFeOB and carbon-fixing bacterial communities. Real-time quantitative polymerase chain reaction (qPCR), high-throughput sequencing, and clone libraries with *Gallionella*, *cbbL*, and *cbbM* gene-specific primers were applied to quantify these functional microorganisms and genes, and characterize *Gallionella*-related mFeOB and carbon-fixing microorganisms in wetlands. In addition, the environmental factors of wetlands affecting the diversity and distribution of these microbial communities were determined.

2. Materials and methods

2.1. Site and sampling

Samples from different wetlands were collected at ten sites from eight locations in Guangdong Province, China, comprising lake wetlands, river wetlands, and mangrove wetlands (Fig. S1). These eight wetland locations are as follows: Ruyuan (RY1 and RY2), Yingde (YD), Liannan (LN), Huaiji (HJ), Guangzhou (GZ), Yunfu (YF), Yangjiang (YJ1 and YJ2), and Kaiping (KP). Three replicate samples were collected at each sampling site, including water and soil. Water samples for Fe speciation analysis were collected using a 50-mL syringe and filtered through a 0.22-μm filter into a centrifuge tube containing 0.5 M hydrochloric acid to avoid Fe(II) oxidation. Samples for dissolved inorganic carbon (DIC) measurement were filtered using a syringe into a vacuum tube to store 2 mL of each sample. Soil samples for Fe speciation analysis were collected using a wooden spatula, stored in 50-mL serum bottles, and then flushed with nitrogen gas and sealed with butyl rubber stoppers and aluminum crimp seals to prevent Fe(II) oxidation by oxygen. Soil samples for microbial analysis were stored in 50-mL sterilized

centrifuge tubes with a vehicle-mounted refrigerator at 4 °C and then stored at −80 °C immediately upon arrival at the laboratory. Soil samples for the analysis of physicochemical properties were stored in self-sealing bags, air-dried in the laboratory, then ground and crushed in an agate mortar, sieved through a 100-mesh sieve and stored.

2.2. Sample analysis

The pH and redox potential of water, the temperature (T), the dissolved oxygen (DO) , and the soil redox potential were measured in situ during sample collection. Water pH, T, and DO were detected with a portable water quality meter (AZ-86031, Hengxin, China). Water redox potential and soil redox potential were measured with an Eh meter (Seven2Go, Mettler Toledo, Greifensee, Switzerland). The water samples were collected for analysis of dissolved Fe(II), SO_4^{2-}, and NO_3^-, and DIC. The concentration of dissolved Fe(II) was determined using the 1,10-phenanthroline method (Tamura et al., 1974). To determine the water DIC content, 100 % phosphoric acid was added to a vacuum tube containing water samples (Salata et al., 2000), and the CO_2 concentration in the headspace was determined using gas chromatography (GC, Agilent 7890B, CA, USA). The dissolved SO_4^{2-} and NO_3^- were determined using ion chromatography (IC, ICS-600, Thermo Scientific, CA, USA).

Two methods were used to extract the bioavailable Fe(II) from soil: 1) extraction with 1 M sodium acetate (pH = 2.8), and 2) extraction with 0.5 M HCl (Wang et al., 2011). The free iron oxide, DCB-Fe, was extracted using a mixture of sodium bisulfate-sodium citrate-sodium bicarbonate (Wang et al., 2011). The extractable Fe(II) concentrations were determined using the 1,10-phenanthroline method (Tamura et al., 1974). The total Fe concentrations were determined by reducing Fe(III) with hydroxylamine hydrochloride, and the concentrations of Fe(III) were calculated based on the difference between the total Fe concentrations and the Fe(II) concentrations. The total organic carbon (TOC) in soil was determined by a TOC analyzer (TOC-SSM-5000A, Shimadzu, Kyoto, Japan), and the soil organic matter (OM) was analyzed using the combustion method (Wang et al., 2022a, 2022b). The total nitrogen (TN), total phosphorus (TP), and total iron (TFe) contents were determined according to soil and agricultural chemistry analysis (Bao et al., 2000).

2.3. DNA extraction, high-throughput sequencing, and bioinformatics analysis

Genomic DNA was extracted from triplicate soil samples using the DNeasy PowerSoil Pro Kit (Qiagen, USA), and quantified by Qubit 2.0 Fluorometer DNA (Invitrogen, USA). PCR amplification of 16S rRNA and the carbon-fixing microbial *cbbL* and *cbbM* genes was performed using the relevant primers in Table S1. After purification and normalization, the PCR products were subjected to high-throughput sequencing by Magigene Biotechnology (Guangzhou, China). The bioinformatics analysis was performed using QIIME 2 (Caporaso et al., 2010). Low-quality and chimeric sequences were identified and removed. Individual sequences were assigned to each sample using a 12-bp barcode. First, operational taxonomic units (OTUs) were identified using UCLUST at the 97 % sequence similarity level. Then, PyNAST was used to select representative sequences for each OTU. The taxonomic classification of each representative sequence was determined using the Ribosomal Database Project (RDP) at the 80 % threshold. The details of bioinformatics analysis are described in previous studies (DeSantis et al., 2006; Edgar, 2010). The raw sequences were deposited in the SRA database at the National Center for Biotechnology Information (NCBI, Accession Nos. PRJNA1043402 and PRJNA1043390).

2.4. Clone library for mFeOB

Seven clone libraries were constructed in this study to investigate the diversity of *Gallionella*-related mFeOB. PCR amplification was performed with primers M122F and Beta3R under the following conditions: 95 °C for 10 s; 45 cycles of 40 s at 95 °C, 30 s at 55 °C, 60 s at 72 °C; and then 15 s at 95 °C, 30 s at 65 °C, 15 s at 95 °C. The PCR products were purified using the OMEGA PCR purification kit (OMEGA Biotek, USA). The purified PCR products were ligated into the pGEM-T Easy vector (Promega, Madison, USA) and transformed into *Escherichia coli* JM109 competent cells (Takara, Shiga, Japan). Randomly selected positive clones from each clone library underwent sequence analysis. Quality checks and sequence clustering were performed via Mothur software (Schloss et al., 2009). Taxonomic classification was performed with the BLAST database (https://blast.ncbi.nlm.nih.gov/) and RDP sequence match tool (http://rdp.cme.msu.edu/).

2.5. The qPCR for 16S rRNA genes, Gallionella-related mFeOB, cbbL and cbbM genes

The abundances of 16S rRNA genes, *Gallionella*-related mFeOB, and the carbon-fixing genes *cbbL* and *cbbM* in different wetlands were determined using qPCR on a MyiQ™ 2 Optics Module (BIO-RAD, USA). The primer information and reaction conditions of the *cbbL* gene, the *cbbM* gene, mFeOB, and the 16S rRNA gene are presented in Table S1. Serial dilutions (ranging from 1×10^2 to 1×10^9 copies μL^{-1}) of plasmids containing the cloned target sequences were used to generate the qPCR calibration curves. Qubit 2.0 Fluorometer (Invitrogen, NY, USA) was employed to quantify the plasmid DNA concentration, and the relative gene copy number was determined based on the plasmid size, insert lengths and Avogadro number (Whelan et al., 2003).

2.6. Statistical analysis

Microsoft Excel 2016 and SPSS 26.0 were used to process the data. The data presented are the means of three replicates. Statistical analysis was conducted using analysis of variance (ANOVA) and Pearson correlation tests in SPSS 26.0, with a significance level of $p < 0.05$. The "devtools" and "gastonstat/plspm" packages in R were used to conduct partial least squares path modeling (PLS-PM). High-throughput sequencing analysis on the Magichand platform (http://www.magichand.online) was used to investigate the α-diversity (rarefaction curves) of the *cbbL* and *cbbM* genes related to carbon-fixing microorganisms. Canoco 5 was used to perform β-diversity Principal component analysis (PCA) of mFeOB and a redundancy analysis (RDA) of the wetland environmental factors and three microbial communities.

3. Results and discussion

3.1. Water and soil characteristics

The physicochemical properties of the water and soil samples are shown in Tables S2 and S3. The pH values ranged from 5.3 to 6.4, which were conducive to the growth of mFeOB (Emerson et al., 2010; Gulay et al., 2018). The Eh values of the soil indicated reducing conditions, ranging from −251.5 to −23.3 mV. The total Fe content of the wetland soil samples ranged from 11.39 to 47.47 g kg^{-1}. The 0.5 M HCl-extracted Fe(II) content ranged from 0.44 to 25.12 g kg^{-1}, which was higher than the Fe content in other wetland soils, such as in typical wetland soils of the Sanjiang Plain (about 0.02–5.00 g kg^{-1}) (Chi et al., 2016). The water samples had DIC contents of 66.74–296.19 μM. The high abundance of Fe(II) and the presence of DIC in the wetland systems provided sufficient nutrients for the growth of mFeOB under microaerobic conditions (Emerson and Moyer, 1997). The concentrations of NO_3^- and SO_4^{2-} in water were 0.02–3.50 and 0.04–57.57 mg L^{-1}, respectively, except for the YJ2 sample, which had higher NO_3^- and SO_4^{2-} concentrations of 1382.63 mg L^{-1} and 1653.66 mg L^{-1}, respectively. This disparity could be attributed to the different wetland types. YJ2 is a coastal mangrove wetland located in the intertidal zone that is influenced by tides as well as biological activity, resulting in high concentrations of NO_3^- and SO_4^{2-}

(Rahman et al., 2013).

3.2. Relative abundance of mFeOB and carbon-fixing genes in soils

3.2.1. Results of qPCR

The copy numbers of *Gallionella*-related mFeOB and carbon-fixing genes (*cbbL* and *cbbM*) were detected using qPCR in all of the soil samples (Fig. 1). The results demonstrated that *Gallionella*-related mFeOB and carbon-fixing microorganisms related with *cbbL* and *cbbM* were prevalent in various types of wetlands. The copy numbers of the *cbbM* gene were higher than the copy numbers of the *cbbL* gene and mFeOB. This may be due to the microaerobic or anaerobic conditions of the collected soil samples, accompanied by a high concentration of inorganic carbon, which provides favorable conditions for the growth of microbes containing RubisCO II, encoded by the *cbbM* gene (Badger and Bek, 2008). Moreover, the *cbbM* gene accounted for 0.24 %–5.21 % of the 16S rRNA gene copy numbers in wetlands, which was significantly higher than the proportion contributed by the *cbbL* gene (0.003 %–0.526 %) and mFeOB (0.003 %–2.798 %) (Fig. S2). The difference in gene abundances suggests that the flooded wetland soil may serve as an ecological niche for functional microbes related to *cbbM*.

3.2.2. Possible mechanism of iron oxidation coupled to CO_2 fixation

Autotrophic microorganisms can fix inorganic carbon through six distinct pathways: the CBB cycle, reductive tricarboxylic acid cycle, reductive acetyl-CoA pathway, 3-hydroxypropionate cycle, 3-hydroxypropionate/4-hydroxybutyrate cycle, and dicarboxylate/4-hydroxybutyrate cycle (Hügler and Sievert, 2011; Jae-Hun et al., 2014; Thauer, 2007; Berg, 2011). Among these pathways, the CBB cycle, including RubisCO genes, is the most significant carbon fixation pathway for CO_2 fixation. The *cbbL* and *cbbM* genes, encoding RubisCO are widely present in mFeOB that could obtain energy through Fe(II) oxidation coupled with CO_2 fixation (Kato et al., 2015; Li et al., 2019) In the present study, the relationships among mFeOB, *cbbL*, and *cbbM* were investigated and the results showed a significant correlation between the abundances of mFeOB and the *cbbL* gene, as well as a highly significant correlation between the abundances of mFeOB and the *cbbM* gene (Table 1). Previous reports have shown that the *cbbM* gene encoding RubisCO II is found in the most isolated mFeOB genomes (Kato et al., 2013; Kato et al., 2015), which is consistent with the relationship between FeOB and the *cbbM* gene. The results of genomic analysis of two typical mFeOB, *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2, revealed that both contained the *cbbM* gene (Kato et al., 2013), while *Sideroxydans lithotrophicus* ES-1 only contained the *cbbL* gene (Emerson et al., 2013). Interestingly, no significant correlation was found between the abundances of the *cbbL* and *cbbM* genes, which may be due to the different environmental preferences of the enzymes encoded by the *cbbL* and *cbbM* genes (Badger and Bek, 2008). RubisCO I, encoded by the *cbbL* gene, is primarily present in aerobic autotrophs and photosynthetic microorganisms (Watson and Tabita, 1997), while RubisCO II is commonly found in environments with low O_2 and high CO_2 concentrations (Thomas et al., 2019). The copy number of *cbbM* gene was much higher than the copy number of *cbbL* gene, which is consistent with the microaerophilic lifestyle with low O_2 concentration. Co-existence of *cbbL* and *cbbM* genes in mFeOB may produce a synergistic effect in CO_2 fixation when the redox conditions change in wetlands, which allows mFeOB to adapt to a wider range of O_2 and CO_2 environments.

Table 1
Correlation between functional microorganisms (genes).

	R *Gallionella*	R *cbbL*	R *cbbM*
R *Gallionella*	–	0.422*	0.712**
R *cbbL*	0.422*	–	0.062
R *cbbM*	0.712**	0.062	–

Note: *$p < 0.05$, significant correlation; **$p < 0.01$, highly significant correlation.

3.3. Community structure of Gallionella-related mFeOB

The clone library was applied to analyze the community structure of *Gallionellaceae* (*Gallionella*-related mFeOB). In total, 939 sequences were obtained from all of the samples. The results revealed that mFeOB comprised predominantly of *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2, and the physicochemical properties of wetlands resulted in differences in the mFeOB community structures (Fig. 2). Based on the differences in the total abundances of *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2, the wetland soils could be clustered into three distinct groups. The first group comprised RY1, RY2, HJ, YF, GZ, and YJ1; the second group included KP and LN; and the third group comprised YD and YJ2. Cluster I had the highest total abundance of *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2, accounting for 94 %–100 % of the community (Fig. 2B). In the RY1, HJ, and YJ1 samples, all of the sequences matched these two mFeOB. Cluster II had a total abundance of 59 % (LN) and 87 % (KP) of these two bacteria, while cluster III had the lowest abundance, accounting for 44 % (YD) and 42 % (YJ2). *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2 are representative mFeOB that are closely associated with carbon fixation in the environment. As outlined in Section 3.2, *Sideroxydans lithotrophicus* ES-1 carries the *cbbL* and *cbbM* genes, while *Gallionella capsiferriformans* ES-2

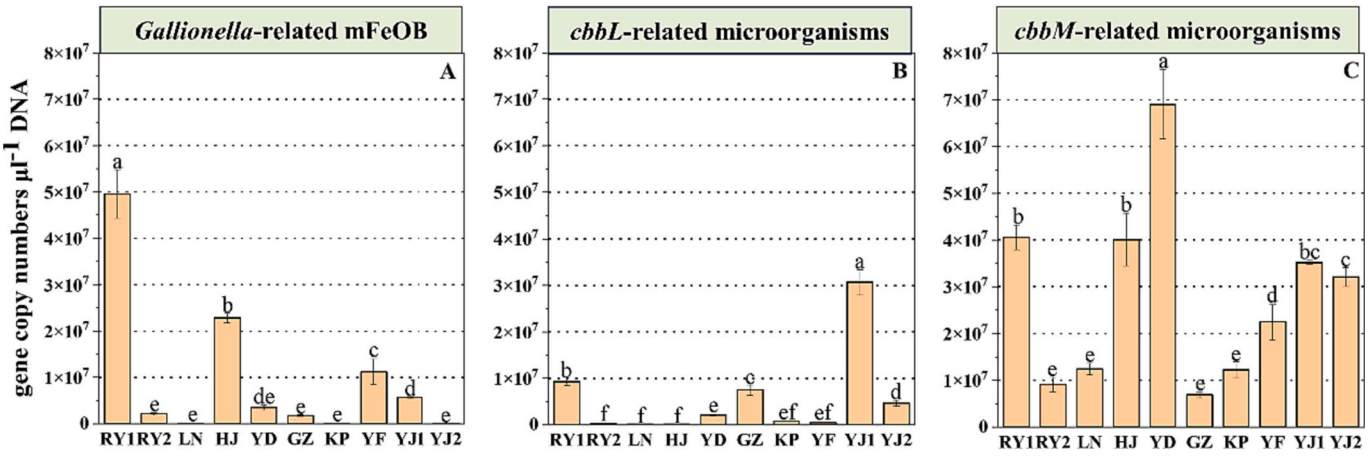

Fig. 1. The gene copy numbers of (A) *Gallionella*-related mFeOB, and (B) *cbbL* gene and (C) *cbbM* gene. Different letters in each graph show significant difference ($p < 0.05$).

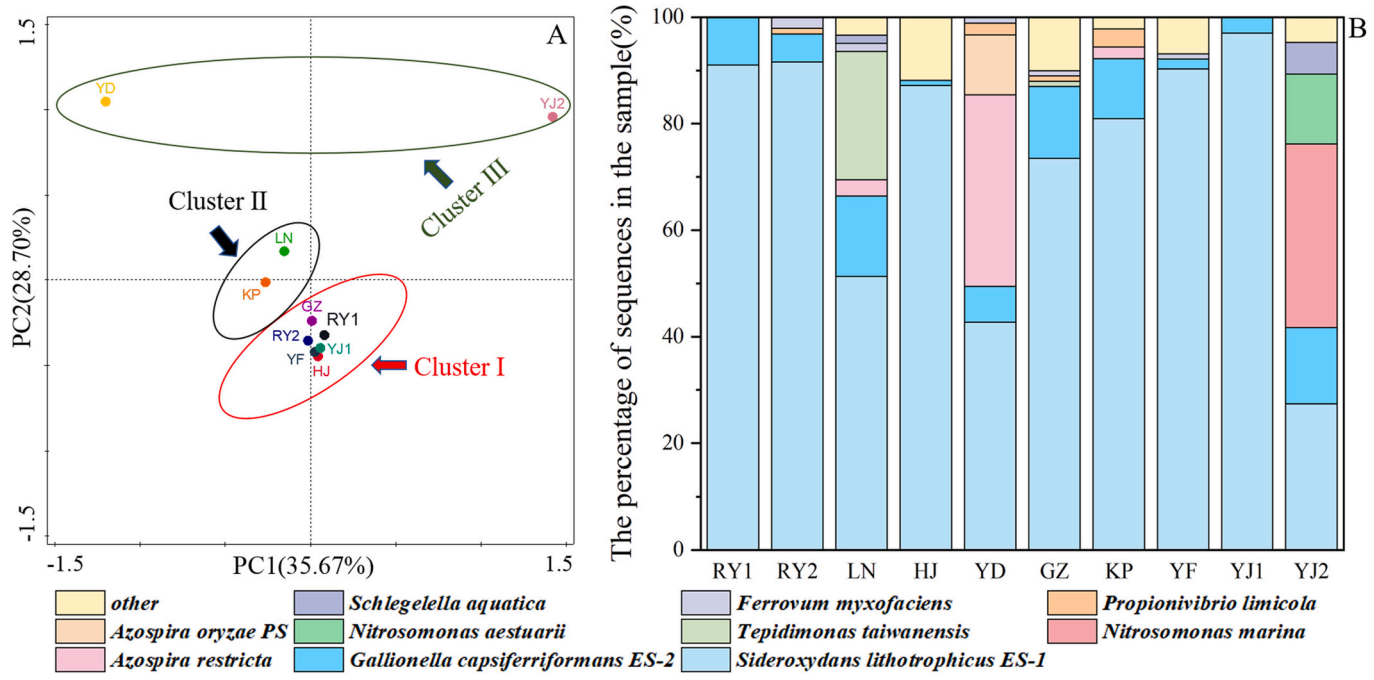

Fig. 2. PCA analysis (A) and microbial composition (the abundance ranks in the top 10, B) of *Gallionella*-related mFeOB in different samples. The relative abundance and PC are presented as the average of the three replicates.

carries only the *cbbM* gene, both of which can fix inorganic carbon through the CBB cycle (Emerson et al., 2013; Kato et al., 2013). Generally, the similarity of the microbial community structure decreases as the distance increases between different samples (An et al., 2019). However, samples YJ1 and YJ2, which were collected from Yangjiang city, exhibited a significant difference in microbial community composition (Fig. 2A). In the YJ1 sample, all of the sequences matched *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2, while these two bacteria accounted for only 42 % of the total abundance in YJ2. The difference may be due to the variation of chlorion concentrations in these wetlands. The result of RDA analysis showed that chlorion (pseudo-F = 7.7, P = 0.03) was a significant factor affecting the community variation of mFeOB with a contribution of 56.6 % to the community variation (Fig. S3). The previous studies showed that salinity is a critical factor that shapes the microbial community composition to adapt to specific salinity levels at the regional scale (Lin et al., 2013; Wang et al., 2011). In YJ2 sample, *Nitrosomonas marina* and *Nitrosomonas aestuarii* were also the dominant FeOB, accounting for 34.52 % and 18.03 % of the total abundance, respectively (Fig. 2B). These two microorganisms are halophiles and are mainly found in

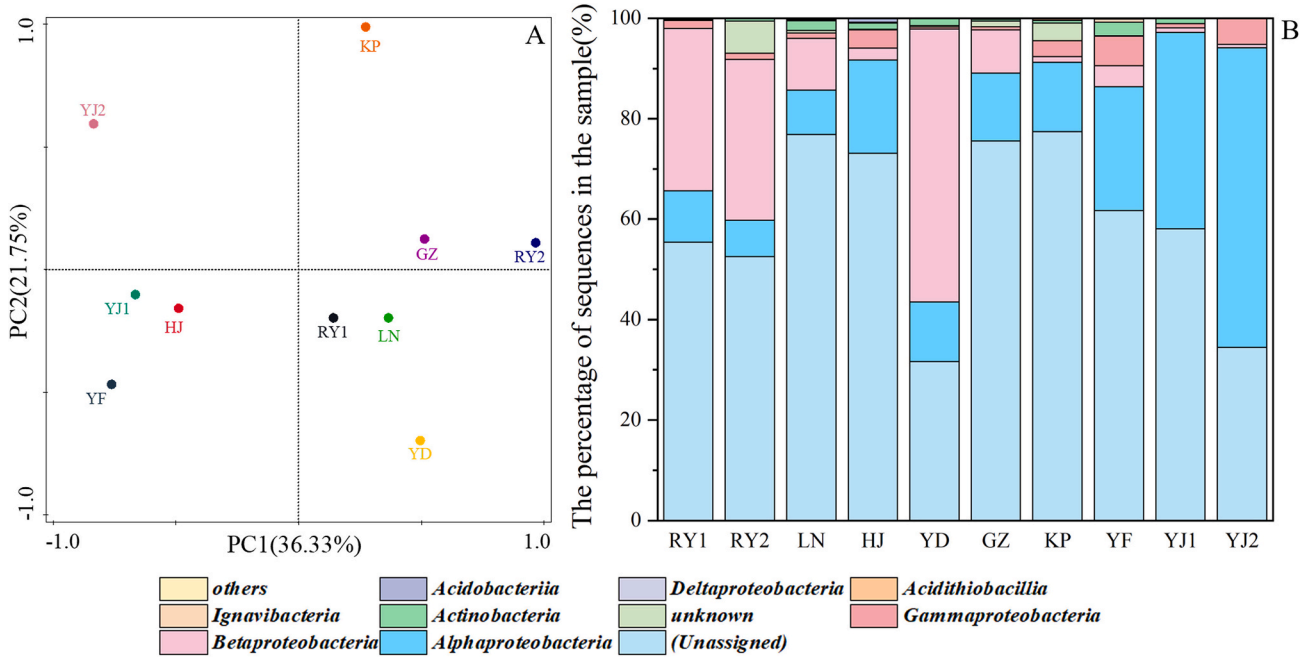

Fig. 3. PCA analysis (A) and microbial composition (the abundance ranks in the top ten, classified by class, B) of *cbbL* gene-related microorganisms in different samples. The relative abundance and PC are presented as the average of the three replicates.

marine sediments with a salinity of 300–400 mM (Ling et al., 2018; Zhang et al., 2015). In addition, the type of vegetation in coastal wetland could secrete different root exudates, thereby affecting the structure of the mFeOB microbial community (Meng et al., 2022). Although the mFeOB community structure in coastal wetland (YJ2) was different from that in inland wetlands, *Gallionellaceae* was still the most important FeOB in wetlands (Fig. S4).

3.4. Community structure of the carbon-fixing microorganisms in wetland soil

This study obtained 100,428 sequences and 4806 OTUs by sequencing the *cbbL* gene from different soil samples. The rarefaction curves of the *cbbL* gene reached the saturation plateau (Fig. S5A), indicating that the sequencing depth covered all of the species in the sample. The carbon-fixing microbial communities related to the *cbbL* gene in different wetland samples are shown in Fig. 3. The microbial community carrying the *cbbL* genes exhibited significant variation among the wetland samples (Fig. 3A), and the microbial community related to the *cbbL* gene was mainly composed of Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria (Fig. 3B). The microbial community composition based on class level showed that Alphaproteobacteria was the most abundant, ranging from 7.26 % to 59.67 %, followed by Betaproteobacteria and Gammaproteobacteria, with abundances of 0.68 %–54.34 % and 0.41–5.89 %, respectively (Fig. 3B). Among the top 10 classes in abundance, Acidobacteria was only detected in the HJ sample, with an abundance of 0.76 %. This may be due to the lower pH of the HJ sample (5.3) compared to the other samples (5.5–6.4). A significant negative correlation between the abundance of Acidobacteria and the soil pH value was reported by Jones et al. (2009), with a decrease in Acidobacteria abundance as the pH increased. The *cbbL* gene can be classified into two categories: *cbbL* G (Green-like) and *cbbL* R (Red-like), the former of which commonly exists in plants, green algae, blue-green algae, and some fungi, while the latter is typically found in eukaryotic non-green (red) algae and "purple" bacteria (Watson and Tabita, 1997). The diversity of *cbbL* gene leads to wide-ranging differences in *cbbL* gene-related functional microbial communities across various wetland samples.

A total of 79,114 sequences and 2567 OTUs were obtained from *cbbM* gene sequencing. The rarefaction curve of the *cbbM* gene likewise reached a saturation plateau (Fig. S5B), and the sequencing depth covered all of the species in the different samples. The *cbbM*-related carbon-fixing microbial communities in different soil samples are shown in Fig. 4. Microbial community analysis at the class level revealed that the composition of the *cbbM*-related carbon-fixing microbial communities was relatively similar (Fig. 4B). Alphaproteobacteria were the most prevalent, with an abundance ranging from 1.37 % to 45.46 %, followed by Gammaproteobacteria and Betaproteobacteria, with abundances of 0–4.84 % and 0–0.70 %, respectively. PCA indicated that the community composition of the YJ2 and KP samples differed from that of the other samples (Fig. 4A). As discussed in Section 3.3, YJ2 is a coastal mangrove wetland, where salinity plays a crucial role in shaping microbial communities at the local scale. Therefore, the YJ2 samples exhibited a distinctive community composition compared to other samples (Fig. 4A). In contrast, the KP samples were collected from Kongque Lake wetland, which was under construction during the sampling period and heavily disturbed by human activities. This human interference might have caused differences in the *cbbM*-related microbial communities between KP and other samples.

According to a previous report, members of the phylum Proteobacteria are the most abundant soil bacteria, constituting an average of 39 % (range: 10 %–77 %) of libraries derived from soil bacterial communities (Janssen, 2006). Proteobacteria exhibit a diverse range of physiology and metabolism, which play important roles in global Fe, C, N, and S cycling (Kersters et al., 2006). In the present study, the high-throughput sequencing of carbon-fixing genes and the clone library of mFeOB revealed that Proteobacteria were the dominant phylum, with an abundance ranging from 28.04 % to 57.02 % in all of the samples. Within the Betaproteobacteria class of Proteobacteria, the dominant *Gallionella*-related mFeOB of *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2 exhibited the highest abundance. These results indicate that the *Gallionella*-related mFeOB might be closely related to CO_2 fixation.

3.5. Effect of wetland physicochemical properties on mFeOB and carbon-fixing microbial community structure

The effect of wetland environmental factors on the functional

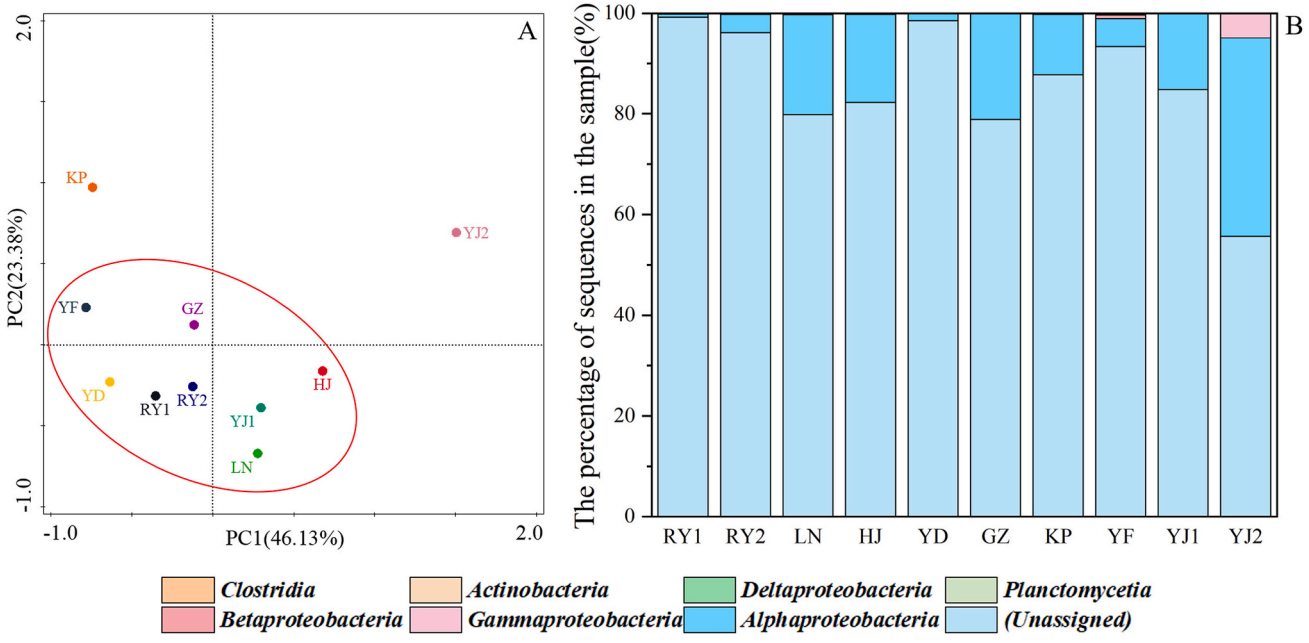

Fig. 4. PCA analysis (A) and microbial composition (classified by class, B) of *cbbM* gene-related microorganisms in different samples. The relative abundance and PC are presented as the average of the three replicates.

microorganisms and the correlations among these functional microorganisms were assessed using RDA and correlation analysis (Fig. 5). The results showed that pH was weakly correlated with the abundance of mFeOB, which was inconsistent with previous research (Kersters et al., 2006). This discrepancy may be due to the fact that all of the samples in the present study were near-neutral pH, which is optimal for the growth of mFeOB. Furthermore, the abundances of mFeOB were influenced by other environmental factors such as OM, which may mask or weaken the effect of pH. The results showed a significant negative correlation between the abundance of mFeOB and the soil OM contents (Fig. 5). Previous reports have shown that many microorganisms that collaborate in Fe(II) oxidation are heterotrophic and require OM to promote their growth, thereby accelerating Fe(II) oxidation and promoting the growth of mFeOB (Emerson et al., 2010; Kato et al., 2013). Moreover, OM can combine with Fe(II) in soil, which reduces its availability and affects the abundance of mFeOB (Daugherty et al., 2017). A highly significant correlation between soil redox potential values and mFeOB abundance was detected. Eh can be used to evaluate redox conditions and reflects the redox characteristics of the environment (Husson, 2013). The Eh ranged from −251.5 to −23.3 mV in the present study (Table S3), suggesting a reducing environment in the soil. An increase in Eh implied a shift toward an oxidized environment, which favored mFeOB growth and Fe(II) oxidation. The wetland pH was not significantly correlated with *cbbL* gene abundance. It has been shown that pH can affect CO_2 ionization and solubility, which may influence the carbon available to functional microorganisms (Schick et al., 2023; Xiao et al., 2014). The *cbbL* gene encoding RubisCO I can be subdivided into *cbbL* G and *cbbL* R, which show differential sensitivity to pH. A significant correlation between the *cbbL* gene abundance and pH has been demonstrated by previous study (Zhu et al., 2021), whereas others have reported no significant correlation (Long et al., 2015), indicating the different response of various groups to pH. The highly significant negative correlation observed between the soil OM and the *cbbM* gene abundance likely arose from the predominance of mFeOB, particularly *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2. Notably, both *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2 were found to carry the *cbbM* genes. Furthermore, a highly significant negative correlation was found between OM and mFeOB, thereby establishing a consequential link between the *cbbM* gene abundance and OM, ultimately resulting in a highly significant negative correlation between OM and *cbbM* gene abundance. Hence, the abundances of these functional microorganisms and genes in wetlands are significantly affected by various environmental factors.

Water is a crucial component of wetland ecosystems (Keddy and Fraser, 2000), and understanding its influence on the community structure of mFeOB and carbon-fixing microorganisms is essential. The results showed that the physicochemical properties of wetland waters had a significant effect on the composition of mFeOB and *cbbL* and *cbbM* gene-related carbon-fixing microbial communities (Figs. S4 and 6). DIC (pseudo-$F = 2.1$, $P = 0.02$) and DO (pseudo-$F = 2.1$, $P = 0.03$) are significant environmental factors (water) that affect the composition of carbon-fixing microbial communities related to *cbbL* (Fig. 6B). RubisCO I is closely associated with the CO_2 concentration mechanism (Iniguez et al., 2020). When the CO_2 concentration is low, microorganisms rely on HCO_3^- absorption to fix CO_2 (Trimborn et al., 2009). The efficiency of carbon sequestration by RubisCO I may be related to the DO concentration because its active sites are contested by O_2 and CO_2 (Badger and Bek, 2008; Thomas et al., 2019). Sulfur is an important variable valence element in the environment and an essential nutrient element for life, playing a critical role in the composition of the microbial community (Cao et al., 2014). The results confirmed that dissolved SO_4^{2-} (pseudo-$F = 2.5$, $P = 0.02$) was a significant environmental factor affecting the carbon-fixing microorganisms related to the *cbbM* gene (Fig. 6C and Table S4). Microbial analyses of limestone aquifers have revealed that most microorganisms rely on the oxidation of reduced sulfur compounds for carbon assimilation, including *Sulfuricella*, *Sideroxydans* and *Acidithiobacillus* (Herrmann et al., 2015). Cao et al. (2014) showed that sulfur-oxidizing bacteria are mainly distributed in Alphaproteobacteria, Gammaproteobacteria and Deltaproteobacteria, which was consistent with our results that *cbbM*-related functional microorganisms were mainly distributed in the classes of Alphaproteobacteria, Gammaproteobacteria, and Deltaproteobacteria (Fig. 3).

Fig. 7 depicts the results of the RDA of *Gallionella*-related mFeOB and carbon-fixing microbial communities with the soil physicochemical properties. The primary factors affecting the structure of mFeOB communities were 0.5 M HCl-extracted Fe(II) (pseudo-$F = 1.8$, $P = 0.07$) and DCB-Fe (pseudo-$F = 1.9$, $P = 0.06$), which explained 18.3 % and 17.3 % of the community variation, respectively (Fig. 7A). Under microaerobic conditions, Fe(II) serves as an electron donor for the growth metabolism of mFeOB (Neubauer et al., 2002), which depends on the availability of Fe(II) in the environment. For example, the typical mFeOB of *Leptothrix ochracea* thrive only in environments with a high concentration of Fe(II), whereas *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2 require lower Fe(II) concentrations for growth in various wetlands (Emerson et al., 2010). Conversely, the Fe(III) hydroxyl oxides generated by FeOB as part of DCB-Fe can be reduced by FeRB under anaerobic conditions, thus providing more Fe(II) for FeOB consumption (Emerson and De Vet, 2015). Therefore, the community structure of mFeOB in the present study largely depended on the concentrations of extractable Fe (II), including 0.5 M HCl-extracted Fe(II) and DCB-Fe.

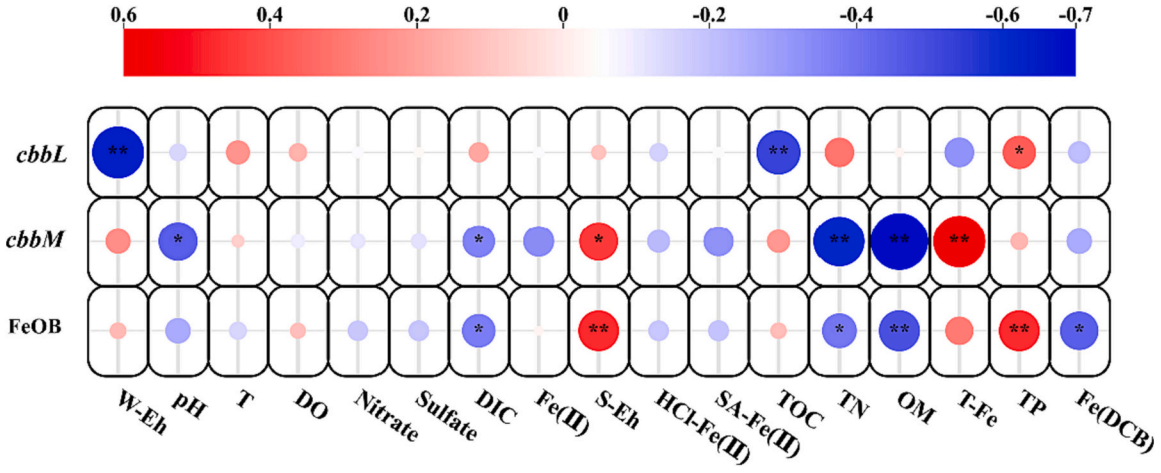

Fig. 5. Correlations between abundance of functional microorganisms (genes) and environmental factors.* - $p < 0.05$, the correlation is significant; ** - $p < 0.01$, the correlation is highly significant.

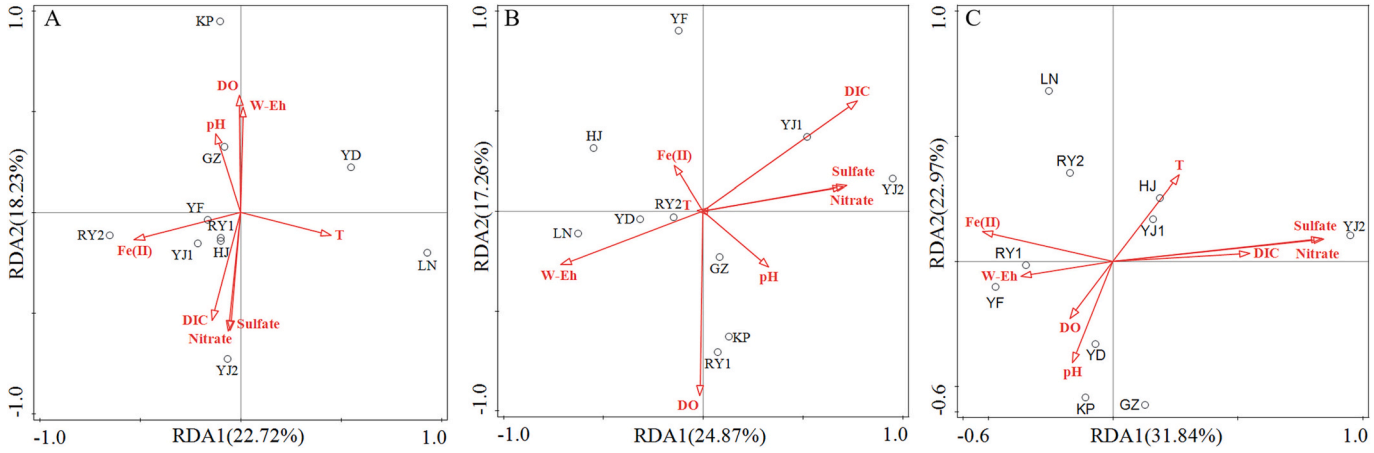

Fig. 6. Results of RDA analysis of *Gallionella*-related mFeOB (A) and carbon-fixing microbial community structure related to *cbbL* gene (B) and *cbbM* gene (C) with water physicochemical properties.

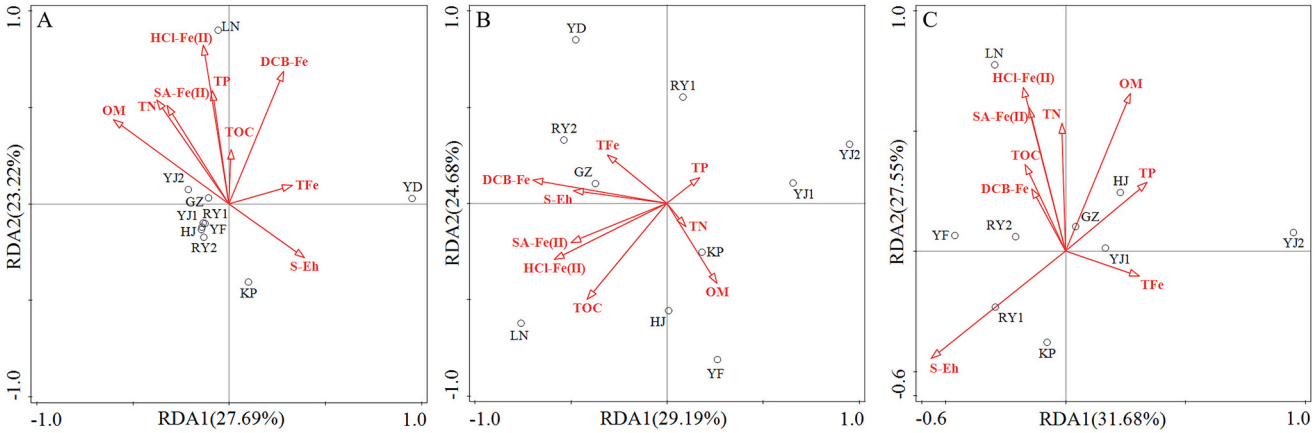

Fig. 7. Results of RDA analysis of *Gallionella*-related mFeOB (A) and CO_2-fixing microbial community structure related to *cbbL* gene (B) and *cbbM* gene (C) with soil physicochemical properties.

Fig. 7B and C illustrate the impact of soil physicochemical properties on the carbon-fixing microbial communities related to the *cbbL* and *cbbM* genes. The OM (pseudo-$F = 2.0$, $P = 0.04$) was the most significant factor influencing the composition of the *cbbL*-related carbon-fixing microbial community. Additionally, DCB-Fe (pseudo-$F = 1.8$, $P = 0.08$) and TP (pseudo-$F = 1.8$, $P = 0.08$) were important contributors to the variation in the microbial community, explaining 18.0 % and 14.9 % of the variation, respectively (Fig. 7B). Huang et al. (2018) reported an increase in the abundance of *cbbL*-related carbon-fixing microorganisms following the addition of straw to increase the soil OM content. Under anoxic conditions, microbial communities can mediate the production of hydroxyl radicals through the redox of Fe (Wan et al., 2022), which can split the chemical bonds of small-molecule OM, thus enhancing its bioavailability and affecting the carbon cycle in soil (Du et al., 2020). As described in Section 3.3, the most abundant FeOB isolated from wetlands contain *cbbL* genes. Therefore, the relationship between DCB-Fe and *cbbL*-related microorganisms may be due to the fact that bioavailable Fe(II) promotes the growth of mFeOB containing *cbbL* genes. Moreover, TP has an important effect on the community composition of *cbbL*-related carbon-fixing microorganisms. Phosphorus, as an essential nutrient for life, is closely related to various microbial community compositions. Studies have shown that increased P concentrations enhance the activity and abundance of soil microorganisms associated with denitrification, nitrogen fixation, and methanogenesis (Wang et al., 2022a, 2022b). The sodium acetate-extracted Fe(II) (pseudo-$F = 2.0$, $P = 0.06$) and soil redox potential (pseudo-$F = 2.0$, $P = 0.09$) were the main factors influencing the *cbbM*-related microbial community, explaining 20.3 % and 17.9 % of the microbial community variation, respectively (Fig. 7C). As described in Section 3.3, *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2 as the dominant mFeOB, both contained *cbbM* genes and were capable of using CO_2 as a carbon source for Fe(II) oxidation. Eh, as a measure of the redox conditions, reflects the redox properties of the environment and is closely related to the biogeochemical cycle of Fe in soil (Husson, 2013). Therefore, sodium acetate-extracted Fe(II) and soil redox potential in soil may indirectly affect the community structure of *cbbM*-related carbon-fixing microorganisms through influencing the growth of mFeOB.

To explore the complex networks of the relationship among wetland physicochemical properties, *Gallionella*-related mFeOB, and *cbbL* and *cbbM*-related carbon-fixing microbial communities, PLS-PM was constructed (Fig. 8). The results showed that extractable Fe(II) was an important factor to regulate the distribution of three functional microorganisms, and it had a total positive effect on the abundances of mFeOB (0.0443), *cbbL* (0.1904), and *cbbM* (0.4613) (Fig. S6). The PLS-PM indicated that there was a significant positive effect of DIC on mFeOB (direct effect with 0.5987 and indirect effect with 0.6105), which was attributed to the ability of mFeOB to use inorganic carbon as a carbon source during Fe(II) oxidation (Li et al., 2019). Similar to the RDA results, anions had a significant positive direct effect on *cbbM*-related microorganisms (0.8785), while they had a significant negative direct effect on mFeOB (−0.7991) and *cbbL*-related microorganisms

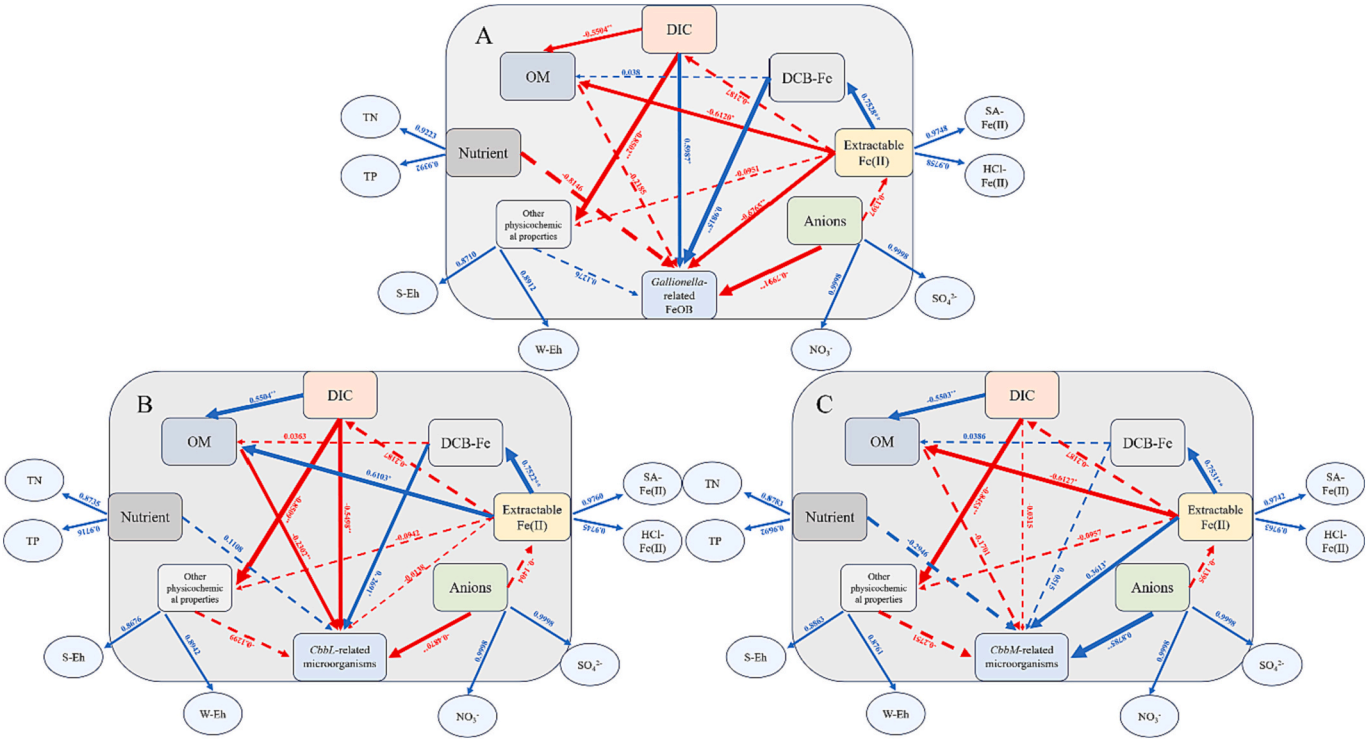

Fig. 8. Partial least squares path modeling (PLS-PM) disentangling the relationships between wetland physicochemical properties and the community composition of mFeOB (A), *cbbL*-related microorganisms (B) and *cbbM*-related microorganisms (C). The goodness of fit was 0.6129, 0.6448 and 0.6417, respectively. Numbers on arrows indicate significant standardized path coefficients. Path color represents the direction of the effect (blue and red indicate positive and negative effects, respectively). Path type indicates the level of significance (dashed lines indicate non-significant, solid lines indicate significant paths. $0.01 < p < 0.05$ indicates *, $p < 0.01$ indicates **).

(−0.5138). Hence, interpreting the similarity between the distribution of mFeOB and carbon-fixing microorganisms in different wetlands as being groups that have similar ecological characteristics would be rather speculative. The most likely explanations for the strong co-occurrence are the link between Fe(II) oxidation and carbon assimilation and the common dependence on the redox interfaces and microaerophilic conditions (Wang et al., 2012).

4. Conclusions and implications

Gallionella-related mFeOB and carbon-fixing microorganisms related to the *cbbL* and *cbbM* genes are widely distributed in wetland soils and play important roles in Fe and carbon cycles. Among these three groups of functional microorganisms, the *cbbM*-related carbon-fixing microorganisms have the highest copy number in wetland soils, followed by mFeOB and *cbbL*-related carbon-fixing microorganisms. Due to the high proportion of *cbbM*-related carbon-fixing microorganisms present in the total microorganisms and their sensitivity to low O_2 and high CO_2 environments, flooded wetland soils may provide a suitable ecological niche for their growth. Correlation analysis revealed a significant positive correlation between the abundance of *Gallionella*-related mFeOB and the abundance of the *cbbL* gene, and there was a highly significant positive correlation between *Gallionella*-related mFeOB and *cbbL* gene abundance. However, there was no significant correlation between the abundances of the *cbbL* gene and the *cbbM* gene. Community structure analysis revealed that all of the mFeOB in wetland soils belonged to Betaproteobacteria, mainly *Sideroxydans lithotrophicus* ES-1 and *Gallionella capsiferriformans* ES-2. These two microorganisms carry both *cbbL* and *cbbM* genes, indicating a close relationship between mFeOB and carbon-fixing microbial communities in wetland soils. The RDA of microbial communities and environmental factors showed that HCl-Fe(II) and DCB-Fe in soil had an important influence on the composition of mFeOB communities. The main soil physicochemical factors affecting the carbon-fixing microbial community structure in wetland soils were DIC, DO, and SO_4^{2-} in the water layer as well as the OM and Fe(II) concentrations in soils. The results indicate that these functional microorganisms play a synergistic role in Fe(II) oxidation and CO_2 fixation in wetland soil ecosystems. For this, it is crucial to further determine co-occurring Fe(II) oxidation rates and CO_2 fixation rates following Fe mineral formation to quantitatively assess the direct contribution of microbial Fe(II) oxidation to ecosystem fixation of CO_2 in wetlands. This includes incubation experiments in laboratory which evaluate the carbon fixation rates and accumulation of Fe mineral-organic carbon aggregate by FeOB or other microbial key players (enriched or isolated from wetlands). Additionally, the microbial community structure can be effectively altered by managing wetlands scientifically and regulating their physicochemical properties based on our findings, thus affecting the carbon sequestration capacity of wetlands.

CRediT authorship contribution statement

Leheng Dong: Investigation, Methodology, Writing – original draft, Writing – review & editing. **Xugang Wang:** Supervision, Writing – review & editing. **Hui Tong:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Yahui Lv:** Data curation, Investigation. **Manjia Chen:** Conceptualization, Supervision, Writing – review & editing. **Jiahui Li:** Data curation, Investigation. **Chengshuai Liu:** Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare no competing financial interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.168719.

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