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Abundance and diversity of carbon-fixing bacterial communities in karst wetland soil ecosystems

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ABSTRACT

Autotrophic carbon-fixing bacteria are the main drivers of carbon sequestration and elemental cycle in wetland ecosystems. Their relationship with environmental factors in karst soils such as soil organic carbon (SOC) fractions, which are affected by natural degradation and human disturbance, is key to understanding the biological mechanisms of karst wetland ecosystem deterioration and restoration. In this study, the abundances of the Calvin cycle functional genes *cbbL* and *cbbM* as well as the characteristics of carbon-fixing bacterial communities were compared in soil samples from a native wetland, naturally degraded wetland, and reclaimed farmland wetland in the Huixian karst wetland, Guilin, China. The abundances of the *cbbL* gene in the degraded wetland and reclaimed wetland soils varied significantly among the seasons ($P < 0.05$, summer > winter). The influence of the season on the abundances of the *cbbL* and *cbbM* genes was more significant than that of the wetland state. The structures of carbon-fixing bacterial communities were similar in the three states of karst wetland soils, mainly consisting of *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* in the phylum *Proteobacteria*. However, the relative abundances of some dominant bacterial genera were significantly different. *Thiomonas* and *Bradyrhizobium* had a relatively high abundance in the native wetland soil and the degraded wetland soil, respectively, while *Ferriphaselus* and *Sulfuricaulis* were more abundant in the reclaimed farmland wetland soil. The main soil factors affecting the structure of the carbon-fixing bacterial communities were the SOC and its fractions dissolved organic carbon (DOC), microbial biomass carbon (MBC) and readily oxidizable organic carbon (ROC), as well as the soil temperature. Anthropogenic activities such as wetland transition to farmland have caused significant changes in karst soils and the characteristics of carbon-fixing bacterial community in this karst wetland soil ecosystem.

1. Introduction

Wetland soil carbon reserves account for 1/3 of the total terrestrial soil carbon pool, and maintaining high carbon reserves in wetland ecosystems plays an important role in mitigating climate warming caused by rising CO_2 concentration (Bridgham et al., 2006; Song, 2003). In karst areas the wetland soil environment is relatively fragile due to the congenital deficiency of carbonate soil formation materials and lack of soil resources (Yuan, 2001; Zhang et al., 2013). Excessive, unreasonable utilization of soil resources by local people leads to the degradation of some karst wetland soils and reduction of soil organic carbon (SOC) content (Tian et al., 2004; Cheng et al., 2015). Due to the influence of karstification, the CO_2 concentration in karst soils is 10 ~ 300 times higher than in non-karst areas (Yuan, 1988). Autotrophic carbon-fixing microbes can assimilate CO_2 and eventually it becomes part of the SOC pool (Berg et al., 2010; Yuan et al., 2011). Thus, the role of the autotrophic carbon-fixing bacterial communities is important for the carbon sequestration potential and protecting and restoring soil functions in karst wetland soils.

There are six known CO_2-fixation pathways in autotrophic microorganisms: the Calvin-Benson cycle, reductive tricarboxylic acid cycle, reductive acetyl-CoA pathway, 3-hydroxypropionate bicycle, 3-hydroxypropionate/4-hydroxybutyrate cycle, and dicarboxylate/4-hydroxybutyrate cycle (Hügler and Sievert, 2011; Jae-Hun et al.,

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2014; Thauer, 2007; Berg, 2011).The Calvin cycle is the most important CO_2-fixation pathway for microorganisms. The key enzyme involved in the cycle is ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO), which catalyzes the carboxylation of ribulose 1,5-bisphosphate to yield two molecules of 3-phosphoglycerate (Hügler and Sievert, 2011). RubisCO form I and form II are the two major and most studied forms of RubisCO enzymes. The *cbbL* and *cbbM* genes encode the large subunits of RubisCO form I and II, respectively. These two functional genes are highly conserved, have appropriate gene length, and can be used as specific probes for molecular analysis of the autotrophic carbon-fixing bacterial communities in the environment (Ji et al., 2016).

Studies on soil carbon-fixing bacterial communities have mainly focused on the effects of vegetation type, land use, litter input, fertilization and rhizosphere environment on the community structure, as well as the carbon fixation potential in agricultural farmland soil (Bai et al., 2018; Su et al., 2020; Yuan et al., 2012a; Yousuf et al., 2014). Natural degradation and human interventions may impact these bacterial communities and soil carbon cycles in karst wetland ecosystems. Research in biological mechanisms of karst wetland ecosystem degradation and restoration has been minimal in the literature. Determining seasonal effects on the abundances of *cbbL* and *cbbM* genes as well as diversity of carbon-fixing bacterial communities in karst wetland soils may provide insight into biological mechanisms in the degradation and amelioration of karst wetland ecosystems.

The Huixian wetland (Guilin, China) is the most representative and largest natural karst wetland in China and even in the global tropical and subtropical middle and low altitude karst areas (Cai et al., 2012). In recent decades, soil degradation in the Huixian wetland has been aggravated due to commercial development and resource utilization, causing decrease in SOC content (Yao and Tong, 2009). In this study, three states of the Huixian karst wetland soils were selected that represented the native wetland, naturally degraded wetland, and reclaimed farmland wetland soils. The main purpose of this study was to elucidate seasonal change in the abundances of the Calvin cycle functional genes *cbbL* and *cbbM* as well as the characteristics of carbon-fixing bacterial communities in the three states of karst wetland soils.

2. Materials and methods

2.1. Study area

The study area (110°09′50″~110°14′30″E, 25°05′20″~25°06′46″N) is in the Huixian karst wetland, Fengjia Village, Huixian County, Guilin, China. The Huixian karst wetland belongs to subtropical monsoon climate with an annual average temperature of 16.5 ~ 20.5 °C. The temperature difference between summer and winter is large, while the temperature difference between spring and autumn is small, and the temperature in autumn is slightly higher than that in spring. The average annual rainfall is about 1890.4 mm. The rainy season is mainly concentrated from April to July, and October to March of the following year is the dry season, in which January is the driest and coldest period (Cai et al., 2012). There is a large farming area in the Fengjia Village, and agricultural activities have led to the degradation of some surrounding wetland soil. Three states of wetland areas were selected according to their water surface and vegetation types. (1) Native wetland (designated Y), a well-preserved wetland area covered with *Phragmites australis* as main vegetation. (2) Naturally degraded wetland (degraded wetland for short, designated T) that can only partially support growth of large hydrophilic vegetation. The covering vegetation was mainly *Ranunculus japonicu* and *Zoysia japonica*. (3) Reclaimed farmland wetland (reclaimed wetland for short, designated K), which has been reclaimed as a paddy field. The schematic map of study area and sampling sites in the Huixian karst wetland are shown in Fig. 1.

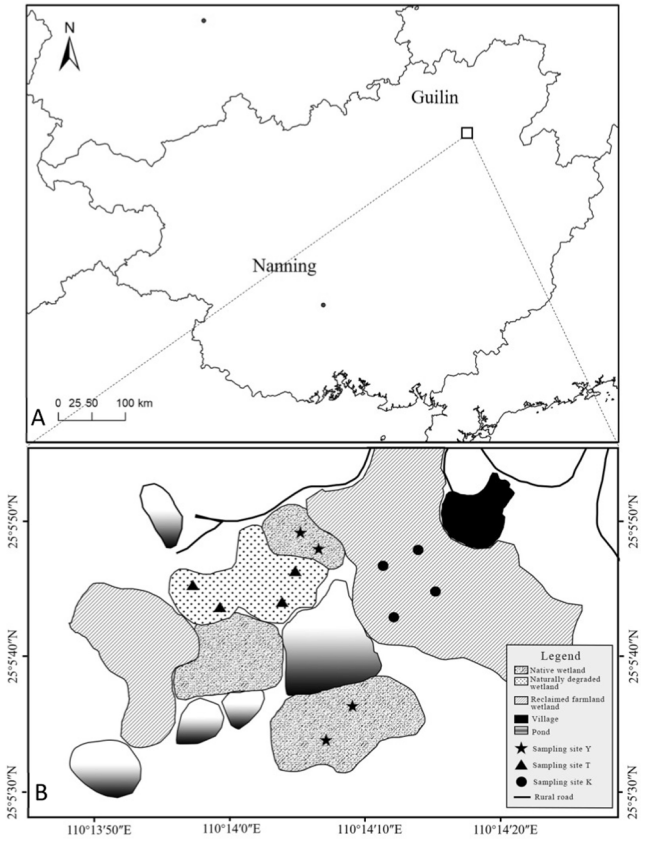

Fig. 1. Schematic map of study area and sampling sites in the Huixian karst wetland. A: Location of the Huixian karst wetland; B: Location of sampling sites in the Huixian karst wetland.

2.2. Sampling

The karst wetland soils of three states were sampled in October 2018 and January, April and July 2019. Four sampling sites were selected for each of the three states of wetland soils. The distance between the sites was about 80–100 m. Three sampling points were randomly selected in each site. Surface soil samples of 0 ~ 20 cm were collected from the three sampling points. The samples were pooled and non-soil materials such as plant debris and stones were removed. The samples were placed in 50 mL sterile centrifuge tubes and sterile self-sealing bags, which were stored in an ice box for transportation to the laboratory. The samples in centrifuge tubes were stored at −80 °C for extraction of soil microbial DNA. Part of the soil in the self-sealed bags was used for the determination of moisture content, while part of the soil was air-dried, ground, sifted and kept in a cool dry place for determination of soil physicochemical properties.

2.3. Determination of soil physicochemical properties

The soil temperature (T) was measured on site using a JRS-LCD105 digital thermometer (Ruilong, China). Soil water content (SWC), conductivity, pH, Ca^{2+} content, soil organic carbon (SOC), soil inorganic carbon (SIC), dissolved organic carbon (DOC), microbial biomass carbon (MBC) and readily oxidizable organic carbon (ROC) were measured in the laboratory. The SWC, pH, conductivity and Ca^{2+} content were determined as detailed in the Analytic Methods of Soil Agricultural Chemistry (Lu, 2000).

The SOC and SIC were measured as previously described (Dean,1974) with slight modification. 10 g of air-dried soil samples were weighed, ground, and then passed through a 200-mesh stainless steel sieve. An excess of 0.5 mol/L HCl was used to remove the carbonate, and

the samples were heated at 105 °C to constant weight in an oven. After the samples were re-ground and passed through a 200-mesh stainless steel sieve, a Vario Micro cube elemental analyzer (Elementar, Germany) was used to determine the SOC of the soil sample. At the same time, 10 g soil samples without HCl treatment were dried and ground, and then the soil carbon (SC) content of the soil sample was determined by an elemental analyzer. The total soil inorganic carbon (SIC) content can be calculated by the formula SIC = SC-SOC.

DOC was measured as follows. 5 g of air-dried soil was weighed and added in a 150 mL flask followed by addition of 50 mL of distilled water and shaking at 180 rpm for 1 h. The solution was subsequently passed through a 0.45 μm membrane filter and the DOC in the filtrate was determined using a Multi N/C 2100 total organic carbon analyzer (Analytik Jena, Germany).

MBC was determined by the fumigation-extraction method (Wu et al., 1990). ROC was determined by $KMnO_4$ oxidation method (Zhang et al. 2016).

2.4. Analysis of soil carbonic anhydrase activity

The soil carbonic anhydrase (CA) activity was determined based on the method of Li et al. (2005). The basic physicochemical properties and CA activity in the three states of the Huixian karst wetland soils are shown in Table 1.

2.5. Extraction of total DNA of soil microorganisms

A PowerSoil DNA Isolation Kit (MOBIO, Qiagen, Germany) was used to extract the total DNA of soil microorganisms according to the manufacturer's instructions. DNA quality was determined by 1% agarose gel electrophoresis, and DNA concentration and purity were determined by NanoDrop ND-1000 spectrophotometer. DNA samples were stored at −80 °C for subsequent analysis.

2.6. Detection of carbon fixation gene abundance

The abundances of 16S rRNA gene and carbon fixation functional genes *cbbL* and *cbbM* of bacteria in the Huixian karst wetland soils were analyzed by quantitative PCR (qPCR). The amplification primers are shown in Table S1. The reaction mixture (10 μL) contained 5 μL SYBR PremixEX TaqTM, 0.5 μL (10 mM) forward primer, 0.5 μL (10 mM) reverse primer, 1 μL template DNA, and 3 μL ddH_2O. The reaction conditions were: pre-denaturation at 95 °C for 60 s; followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 62 °C (*cbbL*) or 57 °C (*cbbM*) for 10 s, and extension at 72 °C for 30 s. The melting stage was 95 °C for 15 s, 60 °C for 60 s, and 95 °C for 15 s. The amplification efficiency was 92 ~ 103% with R^2 values more than 0.994. Melting curve analysis was performed at the end of qPCR to check the specificity of amplification.

Table 1
Basic physical and chemical properties and CA activity in the three states of the Huixian karst wetland soils.

Site	Y	T	K
T (°C)	20.94 ± 7.14	22.04 ± 7.67	20.88 ± 6.64
pH	6.99 ± 0.73	6.96 ± 0.62	6.63 ± 0.70
SWC (%)	34.06 ± 7.53	35.19 ± 10.08	32.81 ± 6.02
Ca^{2+} (mmol/kg)	243.36 ± 103.89	249.27 ± 122.80	205.42 ± 65.89
Conductivity (mS/cm)	0.12 ± 0.07	0.13 ± 0.07	0.11 ± 0.04
SOC (g/kg)	30.75 ± 11.54	33.23 ± 15.16	28.29 ± 5.08
MBC (mg/kg)	461.54 ± 109.90	448.41 ± 155.69	427.13 ± 244.80
ROC (mg/g)	7.65 ± 4.07	7.76 ± 3.47	6.51 ± 1.11
DOC (mg/kg)	390.67 ± 144.56	380.33 ± 135.75	355.76 ± 158.29
SIC (g/kg)	4.48 ± 3.47	3.49 ± 1.83	3.59 ± 1.61
CA (U/g)	3.81 ± 0.76	3.72 ± 0.61	3.04 ± 0.35

The data are the mean ± standard deviation of the measured values of soil samples in four seasons.

2.7. High-throughput sequencing

PCR amplification of the carbon-fixing bacterial *cbbL* and *cbbM* genes was performed using the corresponding primers in Table S1 with a 30 μL reaction mixture containing 3 μL template DNA, 25 μL 2 × Premix Taq, 1 μL (10 mM) forward primer, and 1 μL (10 mM) of reverse primer. The reaction conditions were: pre-denaturation at 94 °C for 5 min; followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C (*cbbL*) or 57 °C (*cbbM*) for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min. PCR amplification products were detected by 2% agarose gel electrophoresis, and target fragments were cut and recovered with gel recovery kit (Omega Bio-tek, Norcross, GA). The recovered target gene fragments were sequenced on an Illumina MiSeq 300 platform by Shanghai Personalbio Technology Co., Ltd.

2.8. Sequence analysis

The Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) pipeline was employed to remove low-quality bases and adaptor contamination sequences to complete the data filtering (Caporaso et al., 2010). Briefly, the low-quality sequences were filtered through following criteria (Gill et al., 2006; Chen and Jiang 2014): sequences that had a length of < 150 bp, sequences that had average Phred scores of < 20, sequences that contained ambiguous bases, and sequences that contained mononucleotide repeats of greater than 8 bp were filtered out. Paired-end reads were assembled using FLASH (Magoc and Salzberg 2011). After chimera detection, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity by UCLUST (Edgar, 2010). OTU-level alpha diversity indices, such as Chao 1, ACE, Shannon, and Simpson indices were calculated using MOTHUR software, and then OTU taxonomic classification was conducted by BLAST searching the representative sequences set against the NCBI Database.

The raw sequences of carbon-fixing bacteria were deposited in the NCBI Sequence Read Archive under the accession number PRJNA608833.

2.9. Statistical analysis

Differences in the abundances of functional genes among different seasons as well as the soils of the three states of wetland were analyzed by one-way ANOVA at $P < 0.05$ using SPSS 21.0. Two-way ANOVA at $P < 0.05$ was performed (using SPSS 21.0) to compare the influence of season and wetland state on the functional gene abundance. Pearson correlation coefficients between abundances of the *cbbL* and *cbbM* genes as well as community diversity indices of carbon-fixing bacteria and soil environmental parameters were calculated using SPSS 18.0. The linear discriminant analysis effect size (LEfSe) analysis was performed to identify significant differences in the soil carbon-fixing bacterial taxa among the three states of karst wetland soils using the platform (http://huttenhower.sph.harvard.edu/galaxy/). Histograms of linear discriminate analysis (LDA) and cladograms of phylogenetic distribution were prepared to present the differentially abundant carbon-fixing bacterial taxa in the karst wetland soils. The redundancy analysis (RDA) was conducted to explore the relationship between the carbon-fixing bacterial community composition and soil environmental factors using the R software package.

3. Results

3.1. Carbon fixation gene abundance in the karst wetland soils

In the karst wetland soils, the abundance of the *cbbL* gene was significantly higher than that of *cbbM* ($P < 0.05$). The abundance of the

cbbL gene in all samples ranged from 4.42×10^9 to 9.57×10^{10} copies/g dry soil, while the abundance of the *cbbM* gene ranged from $3.71 \times 10^4 \sim 9.80 \times 10^8$ copies/g dry soil (Fig. 2, Table S2).

The results of one-way ANOVA showed that the abundances of the *cbbL* gene in degraded wetland (T state) and reclaimed wetland (K state) soils were significantly different among the seasons ($P < 0.05$), both expressed as summer > winter. The *cbbL* gene abundance in native wetland (Y state) soil was not significantly different among the seasons. The abundance of the *cbbM* gene in the degraded wetland soil was significantly higher in the summer than in winter ($P < 0.05$). However, the abundance of the *cbbM* gene in the native wetland and reclaimed wetland soils did not vary significantly with the season. In the winter, the *cbbL* gene abundance was significantly higher in native wetland soil than in degraded wetland soil ($P < 0.05$). In the spring, the *cbbL* gene abundance was significantly higher in reclaimed wetland soil than in native wetland soil ($P < 0.05$). There were no significant differences in the *cbbM* gene abundances among the three states of wetland soils.

Based on the 16S rRNA and the *cbbL* and *cbbM* gene abundances, the proportion of potential carbon-fixing bacteria using RubisCO I and RubisCO II via the Calvin cycle in the bacterial community was estimated. In all samples, the proportion of the *cbbL* gene was significantly higher than that of *cbbM* ($P < 0.05$). The ratio of *cbbL*/16S rRNA ranged from 0.04 to 0.67, while the ratio of *cbbM*/16S rRNA ranged from 1.00×10^{-7} to 9.13×10^{-3} (Fig. 3).

The results of one-way ANOVA showed that the *cbbL*/16S rRNA ratio in the degraded wetland and reclaimed wetland soils was significantly different among the seasons ($P < 0.05$), both expressed as summer > winter. The *cbbM*/16S rRNA ratio in the degraded wetland soil was significantly higher in the summer than in winter ($P < 0.05$). In the winter, the *cbbL*/16S rRNA ratio was significantly higher in the native wetland soil than in the reclaimed wetland and degraded wetland soils ($P < 0.05$). In other seasons, there were no significant differences in the *cbbL*/16S rRNA ratio among the wetland soils. The *cbbM*/16S rRNA ratio was not significantly different among the wetland soils.

All data of gene abundances and environmental parameters were log transformed before analysis. The results showed that the abundance of the *cbbL* gene was significantly positively correlated with soil T, SWC, ROC, and SIC (Table 2).

3.2. Diversity of carbon-fixing bacterial community in the karst wetland soils

The soil samples collected in the summer representing the rainy season and those in the winter representing the dry season were selected for high-throughput sequencing analysis of carbon fixation functional genes.

A total of 24 sequencing samples were obtained from the karst wetland soils for the summer and winter seasons. 1,336,596 sequences and 143,106 OTUs were obtained from the *cbbL* gene sequencing, and 1,455,046 sequences and 103,187 OTUs were obtained from the *cbbM* gene sequencing after quality control.

The rarefaction curves of the *cbbM* gene reached the saturation plateau (Fig. S1), indicating that the sequencing depth covered all species in the samples. The number of species of the carbon-fixing bacterial community containing the *cbbM* gene in all samples was within 1600. Although a few rarefaction curves of the *cbbL* gene did not reach the plateau stage (Fig. S1), these rarefaction curves of the *cbbL* gene tend to approach the saturation plateau. In addition, according to the calculation results of the Good's coverage index (0.9043 ~ 0.9889), the higher the index of the sample the less the proportion of undetected species. Therefore, the sequencing results can reflect the diversity of the carbon-fixing bacterial communities containing the *cbbL* gene.

The diversity indices for the carbon-fixing bacterial community in the karst wetland soils are shown in Table S3 and Table S4. The richness index (ACE, Chao 1) and diversity (Simpson, Shannon) indices of the carbon-fixing bacterial community containing the *cbbL* gene in the karst wetland soil samples were higher than those of the *cbbM* gene with exception of the reclaimed wetland soil samples in the summer.

The richness indices (ACE and Chao 1) of the carbon-fixing bacterial community containing *cbbL* gene in the native wetland soil was significantly higher in the summer as compared to winter ($P < 0.05$). There was no significant difference in the richness and diversity indices for the carbon-fixing bacterial community containing the *cbbL* gene in the degraded and reclaimed wetland soils between the winter and the summer. For the carbon-fixing bacterial community containing the *cbbM* gene, the Shannon index in the degraded wetland soil was significantly higher in the summer than in the winter ($P < 0.05$). The richness (ACE,

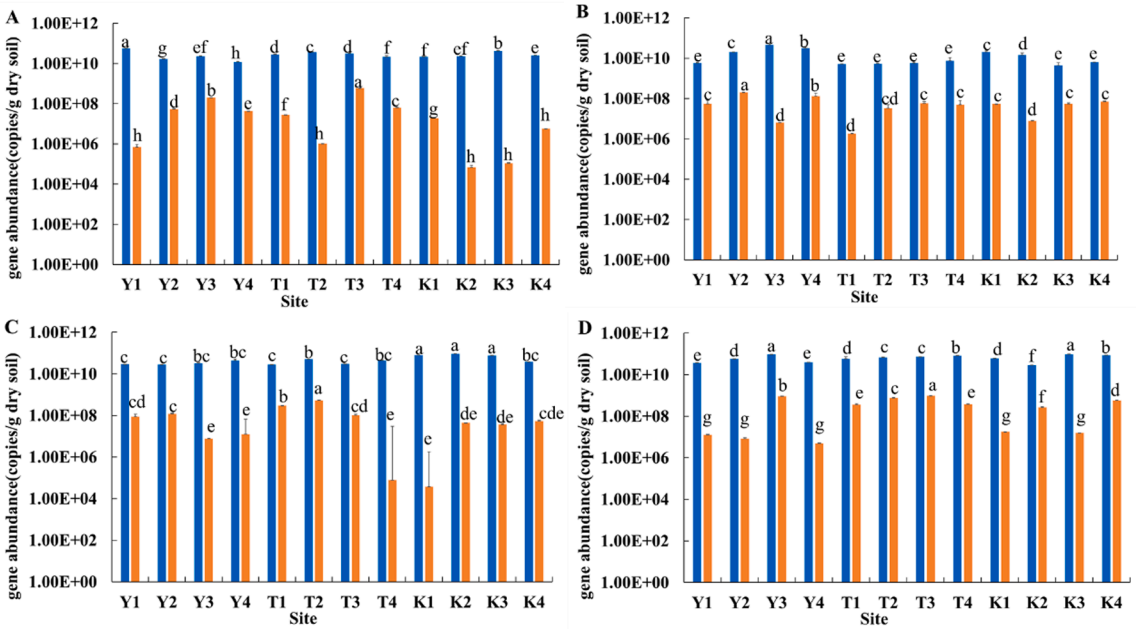

Fig. 2. Abundances of the *cbbL* (left, blue) and *cbbM* (right, orange) genes in the Huixian karst wetland soils. A: Autumn (201810); B: Winter (201901); C: Spring (201904); D: Summer (201907). Significant differences among sites ($P < 0.05$) were denoted with different lower case letters. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

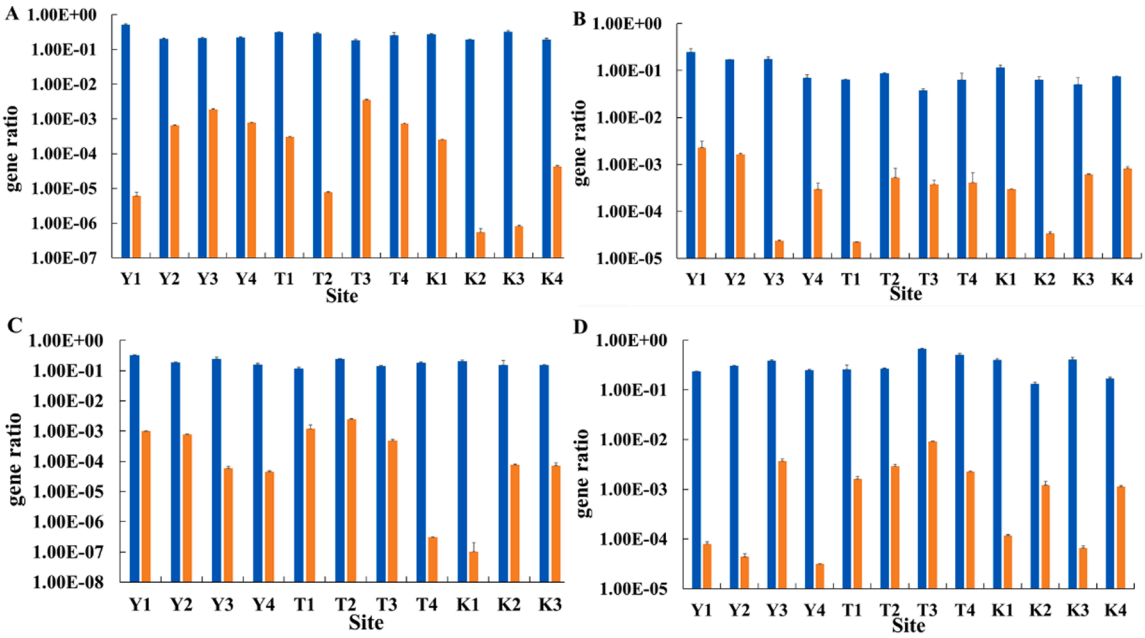

Fig. 3. Gene copy number ratios of the *cbbL* (left, blue) or *cbbM* (right, orange) gene to 16S rRNA gene in the Huixian karst wetland soils. A: Autumn (201810); B: Winter (201901); C: Spring (201904); D: Summer (201907). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Results of correlation analysis between carbon fixation gene abundances and environmental factors in the Huixian karst wetland soils.

	cbbL	*cbbM*
T	0.766**	0.117
SWC	0.443**	0.067
Conductivity	0.090	−0.082
pH	−0.028	−0.216
Ca^{2+}	−0.036	−0.146
SOC	0.004	−0.178
SIC	0.636**	−0.046
MBC	−0.049	−0.164
ROC	0.285*	−0.112
DOC	−0.153	0.149
CA	0.155	−0.003

Note: *: $P < 0.05$; **: $P < 0.01$.

Chao 1) and diversity (Simpson, Shannon) indices in the reclaimed wetland soil were significantly higher in the summer than in the winter ($P < 0.05$). However, there were no significant differences in the richness and diversity indices for the carbon-fixing bacterial community containing the *cbbM* gene in the native wetland soil between the winter and the summer.

Differences in the diversity of carbon-fixing bacterial communities containing the *cbbL* or *cbbM* genes in the three states of the karst wetland soils were compared by one-way ANOVA. In the winter, the richness indices (ACE and Chao 1) of the carbon-fixing bacterial communities containing the *cbbL* and *cbbM* genes were significantly different among the soils from the three wetland states ($P < 0.05$). The richness index of the carbon-fixing bacterial communities containing the *cbbL* gene was the highest in the reclaimed wetland soil, followed by degraded wetland and native wetland soils. The richness of the carbon-fixing bacterial community containing the *cbbM* gene was the highest in the reclaimed wetland soil, followed by native wetland and degraded wetland soils. In the summer, there were significant differences in the richness (ACE, Chao 1) and Shannon indices for the *cbbM*-containing carbon-fixing bacterial community among the soils from the three wetland states ($P < 0.05$), both expressed as reclaimed wetland > degraded wetland > native wetland. There was no significant difference in the richness (ACE, Chao 1) and diversity (Simpson, Shannon) indices of the *cbbL*-containing bacterial communities in the wetland soils.

3.3. Community structure of the carbon-fixing bacteria in the karst wetland soils

In the *cbbL* sequencing results, all OTUs belonged to 13 phyla, 30 classes, 64 orders, 107 families and 193 genera. At the level of phylum, *Proteobacteria* and *Actinobacteria* were the dominant phyla in the karst wetland soils. The average relative abundance of *Proteobacteria* was 81.3%, with the maximum of 96.4%. The average relative abundance of *Actinobacteria* was 9%, with the maximum of 73.5%. At the class level (Fig. 4A), *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Actinobacteria* were dominant with the average relative abundance of 30.9%, 14.4%, 19.2% and 8.9%, respectively. A large number of OTUs belonging to *Proteobacteria*, accounting for 41.4%, could not be identified to the genus level. The top 20 genera with relative abundance were selected to construct the distribution chart of species relative abundance (Fig. 4B). *Bradyrhizobium*, *Dokdonella*, *Hydrogenophaga*, *Mesorhizobium*, *Methylibium*, *Sulfuricaulis*, and *Thermomonospora* were most abundant. Their average relative abundances were 5.35%, 3.09%, 3.20%, 7.60%, 3.63%, 8.91%, and 2.82%, respectively.

In the *cbbM* sequencing results, all OTUs belonged to 2 phyla, 5 classes, 10 orders, 17 families, and 25 genera. At the level of phylum, almost all species belonged to *Proteobacteria*, with an average relative abundance of 99.9%. At the class level (Fig. 5A), *Betaproteobacteria* was the most dominant with an average relative abundance of 51.0%, followed by *Gammaproteobacteria* and *Alphaproteobacteria*, with an average relative abundance of 27.3% and 21.4%, respectively. At the genus level, the top 20 genera with relative abundance were selected to construct the distribution chart of species relative abundance (Fig. 5B). *Ferriphaselus*, *Halothiobacillus*, *Rhodopseudomonas*, *Sinorhizobium*, *Sulfuritalea*, *Thiobacillus*, and *Thiohalorhabdus* were among the more abundant genera, with average relative abundances of 14.35%, 11.78%, 3.11%, 11.43%, 25.41%, 12.54%, and 4.74%, respectively.

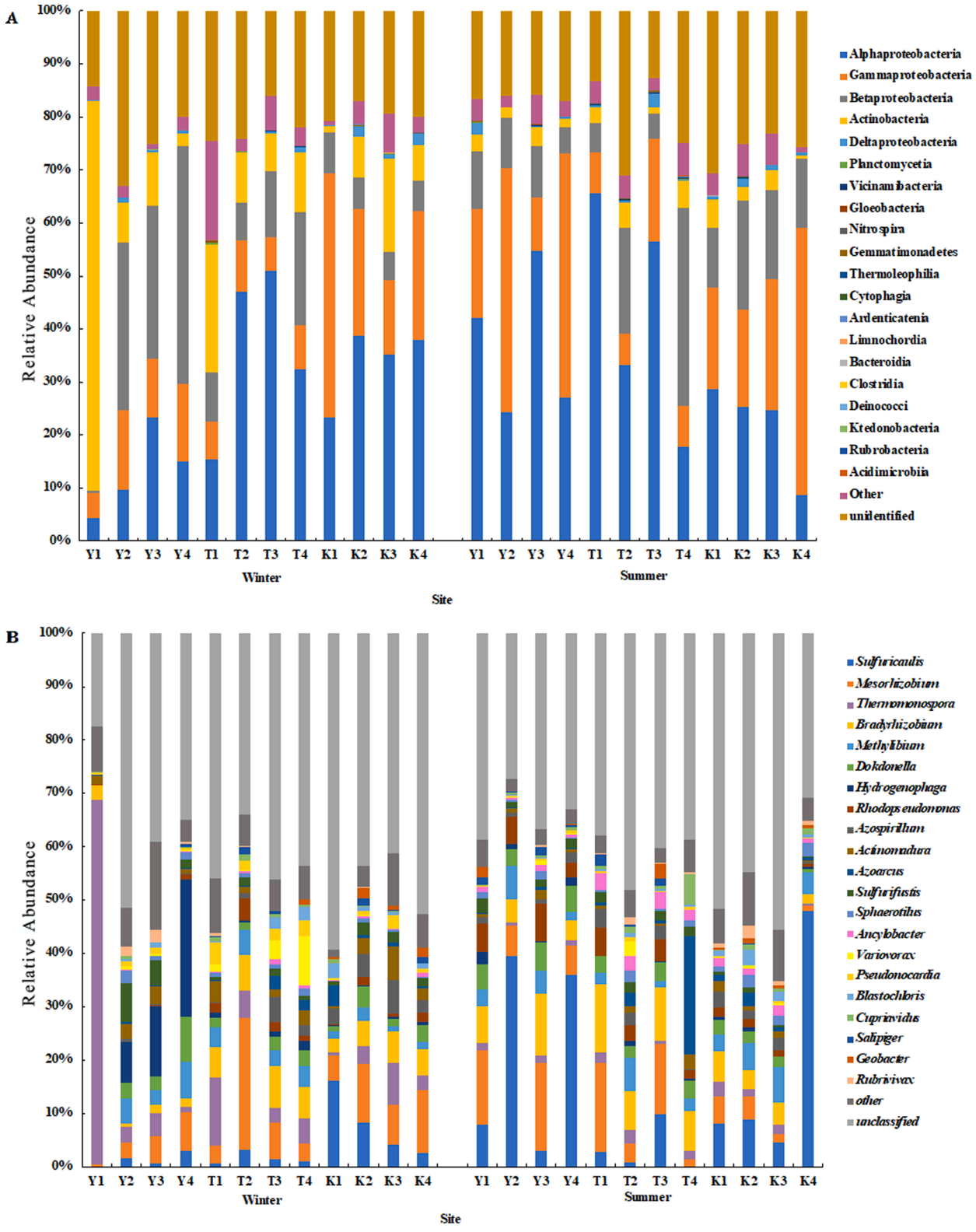

Fig. 4. Relative abundance and composition of the carbon-fixing bacterial community containing the *cbbL* gene at the class level (A) and genus level (B) in the three states of the Huixian karst wetland soils.

3.4. LEfSe analysis of the carbon-fixing bacterial communities in the karst wetland soils

The results of LEfSe analysis showed that there were significant differences in the distribution of some carbon-fixing bacterial taxa among the soils from the three wetland states ($P < 0.05$). In the carbon-fixing bacterial communities containing the *cbbL* gene, *Acidihalobacter* and *Confluentimicrobium* were relatively abundant in the native wetland soil. The relative abundances of *Bradyrhizobiaceae* and *Bradyrhizobium* in the degraded wetland soil were relatively high. *Gammaproteobacteria*

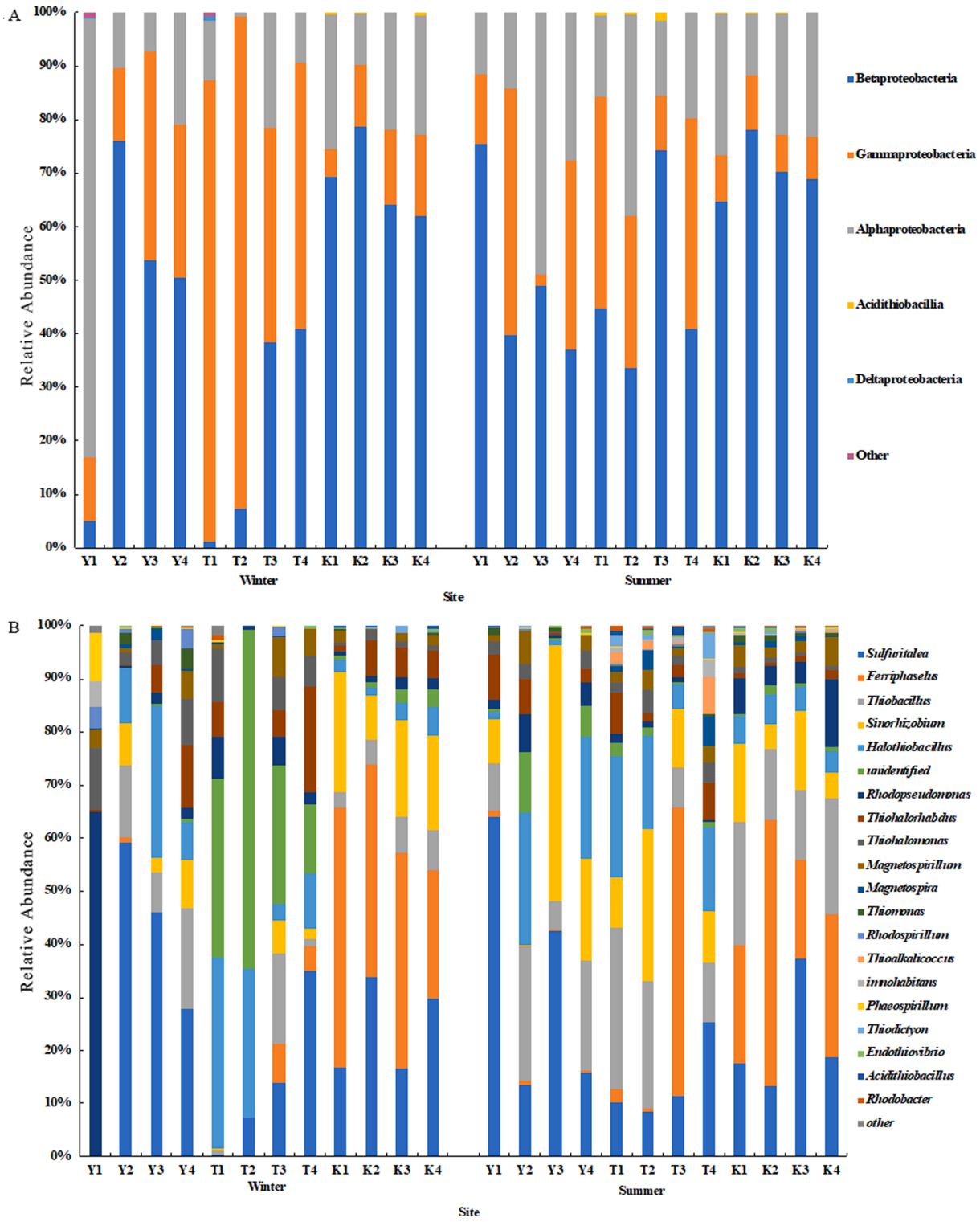

Fig. 5. Relative abundance and composition of the carbon-fixing bacterial community containing the *cbbM* gene at the class level (A) and genus level (B) in the three states of the Huixian karst wetland soils.

and *Sulfuricaulis* had relatively high abundances in the reclaimed wetland soil. In the *cbbM* gene containing carbon-fixing bacterial communities, Ectothiorhodospiraceae (family), *Thiohalomonas*, *Thiohalomonas*, and *Thiomonas* were relatively abundant in the native wetland soil. *Gammaproteobacteria* (class), *Chromatiales* (order) and *Acidithiobacillus* had relatively high abundances in the degraded wetland soils. *Nitrosomonadales* (order), *Gallionellaceae* (family) and *Ferriphaselus* had higher relative abundances in the reclaimed wetland soils. Details in other differentiated microbial communities are shown in Fig. 6.

3.5. Effects of soil environmental factors on community structure and diversity of carbon-fixing bacteria

In order to explore the influence of soil environmental factors on the diversity of carbon-fixing bacterial community, Pearson correlation analysis was conducted between the richness indices (ACE and Chao1)

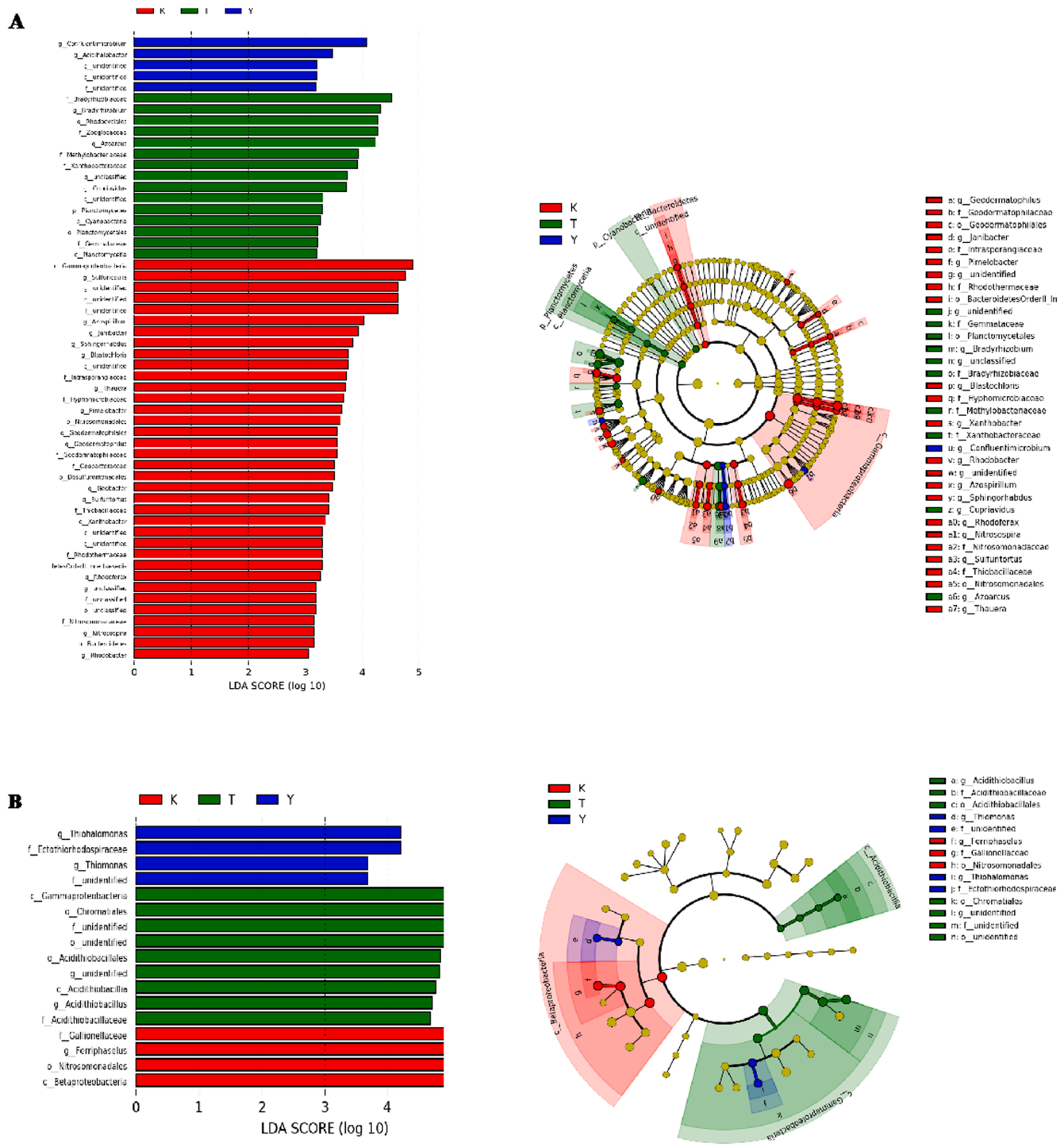

Fig. 6. Differentially abundant carbon-fixing bacterial taxa as assessed using histogram of linear discriminate analysis (LDA) and cladogram of phylogenetic distribution with effect size measurements (LEfSe) in the three states of the Huixian karst wetland soils. A: *cbbL* gene; B: *cbbM* gene.

as well as the diversity indices (Simpson and Shannon) of the carbon-fixing bacterial community and various environmental factors. The results are summarized in Tables 3 and 4. For the carbon-fixing bacterial communities containing the *cbbM* gene, soil T and SWC were significantly positively correlated with richness indices (ACE, Chao1) and Shannon index ($P < 0.05$). SIC was significantly positively correlated with Chao1 index as well as Shannon index ($P < 0.05$). SOC was significantly negatively correlated with richness indices (ACE, Chao1) ($P < 0.01$) and diversity indices (Simpson, Shannon) ($P < 0.05$). There was a significant negative correlation between MBC and the richness indices (ACE and Chao1) ($P < 0.05$). For the carbon-fixing bacterial communities containing the *cbbL* gene, no significant correlation was found between richness indices, diversity indices and soil environmental factors.

The results of RDA analysis indicated that the main environmental factors affecting the community structure of carbon-fixing bacteria containing the *cbbL* gene in the winter were conductivity ($r^2 = 0.6294$, $P = 0.0211$), pH ($r^2 = 0.589$, $P = 0.0098$), Ca^{2+}($r^2 = 0.6301$, $P = 0.0470$), SOC ($r^2 = 0.5360$, $P = 0.0297$) and DOC ($r^2 = 0.7019$, $P = 0.0089$), while in the summer they were SOC ($r^2 = 0.5179$, $P = 0.0413$), MBC (r^2

Table 3
Results of correlation analysis of *cbbL* gene containing carbon-fixing bacterial community diversity with environmental factors in the Huixian karst wetland soils.

Environmental factors	ACE	Chao1	Simpson	Shannon
T	0.290	0.295	−0.016	0.090
SWC	0.082	0.133	−0.020	0.196
Conductivity	−0.146	−0.138	0.251	0.188
pH	−0.109	−0.112	0.246	0.161
Ca^{2+}	−0.317	−0.309	0.116	−0.003
SOC	−0.185	−0.208	−0.073	−0.181
SIC	0.013	0.030	−0.004	0.027
DOC	−0.316	−0.307	0.051	−0.078
MBC	0.014	−0.022	0.051	−0.024
ROC	0.084	0.079	−0.052	−0.059
CA	−0.035	−0.013	0.288	0.076

Note: *: $P < 0.05$; **: $P < 0.01$.

Table 4
Results of correlation analysis of *cbbM* gene containing carbon-fixing bacterial community diversity with environmental factors in the Huixian karst wetland soils.

Environmental factors	ACE	Chao1	Simpson	Shannon
T	0.430*	0.436*	0.360	0.570**
SWC	0.537**	0.548**	0.399	0.643**
Conductivity	−0.104	−0.104	0.246	0.128
pH	−0.226	−0.228	0.197	−0.021
Ca^{2+}	−0.224	−0.223	0.118	−0.08
SOC	−0.613**	−0.616**	−0.476*	−0.550**
SIC	0.402	0.415*	0.395	0.496*
DOC	−0.390	−0.396	−0.538**	−0.594**
MBC	−0.480*	−0.480*	−0.319	−0.359
ROC	−0.255	−0.251	−0.212	−0.116
CA	−0.295	−0.284	0.070	0.038

Note: *: $P < 0.05$; **: $P < 0.01$.

$= 0.5426$, $P = 0.0304$), and ROC ($r^2 = 0.5195$, $P = 0.0379$) (Fig. 7). The main environmental factors affecting the community structure of carbon-fixing bacteria containing the *cbbM* gene in the winter were the soil T ($r^2 = 0.4623$, $P = 0.0424$) and DOC ($r^2 = 0.6518$, $P = 0.0214$), while in the summer it was the CA ($r^2 = 0.5336$, $P = 0.0311$) (Fig. 8) .

Spearman correlation heatmap analysis was conducted between the relative abundance of carbon-fixing bacterial community at the genus level and environmental factors (Fig. 9). The results showed that the relative abundance of some bacterial genera in the carbon-fixing bacterial community containing the *cbbL* gene was significantly correlated with SOC and its components in wetland soils. For example, the relative abundances of *Ancylobacter* and *Sulfuricaulis* were significantly negatively correlated with SOC and DOC, but were significantly positively correlated with SIC ($P < 0.05$). The relative abundances of *Thermomonospora* and *Actinomadura* were significantly positively correlated with DOC content, but were significantly negatively correlated with SIC ($P < 0.05$). The relative abundances of some bacterial genera in the carbon-fixing bacterial community containing the *cbbM* gene also significantly correlated with SOC and its components in the wetland soils (Fig. 9). In particular, *Ferriphaselus* was significantly negatively correlated with ROC, MBC and SOC ($P < 0.01$). *Thioalkalicoccus* and *Thiobacillus* were significantly positively correlated with SIC, but significantly negatively correlated with DOC ($P < 0.01$). In addition, the relative abundances of some carbon-fixing bacterial genera were affected by soil T and SWC. *Bradyrhizobium*, *Rhodopseudomonas, Ancylobacter* and *Cupriavidus* in the carbon-fixing bacterial genera containing the *cbbL* gene were significantly positively correlated with soil T, while *Actinomadura* was significantly negatively correlated with T ($P < 0.05$). *Ancylobacter* and *Sphaerotilus* were significantly positively correlated with SWC ($P < 0.05$). In carbon-fixing bacterial genera containing *cbbM* gene, *Sulfuricella*, *Limnohabitans*, *Thiobacillus*, *Thioalkalicoccus*, and *Magnetospira* were significantly positively correlated with soil T and SWC ($P < 0.05$).

4. Discussion

4.1. Community abundance and diversity of soil carbon-fixing bacteria in the karst wetland soils

The *cbbL* and *cbbM* genes were detected abundantly ($10^4 \sim 10^{10}$ copies/g dry soil) in the karst wetland soils, which indicated that there are indeed a considerable number of autotrophic carbon-fixing bacterial communities in the karst wetland soils. In general, the abundance of these genes in this study was significantly higher than reported for typical paddy soils ($10^5 \sim 10^8$ copies/g dry soil) in non-karst areas in south China (Liu et al., 2017). The highest abundance of these genes was almost comparable to 10^9 gene copies/g dry soil reported for non-wetland soils in karst area (Wang et al., 2018). Because of karstification, the concentration of soil inorganic carbon in karst areas is

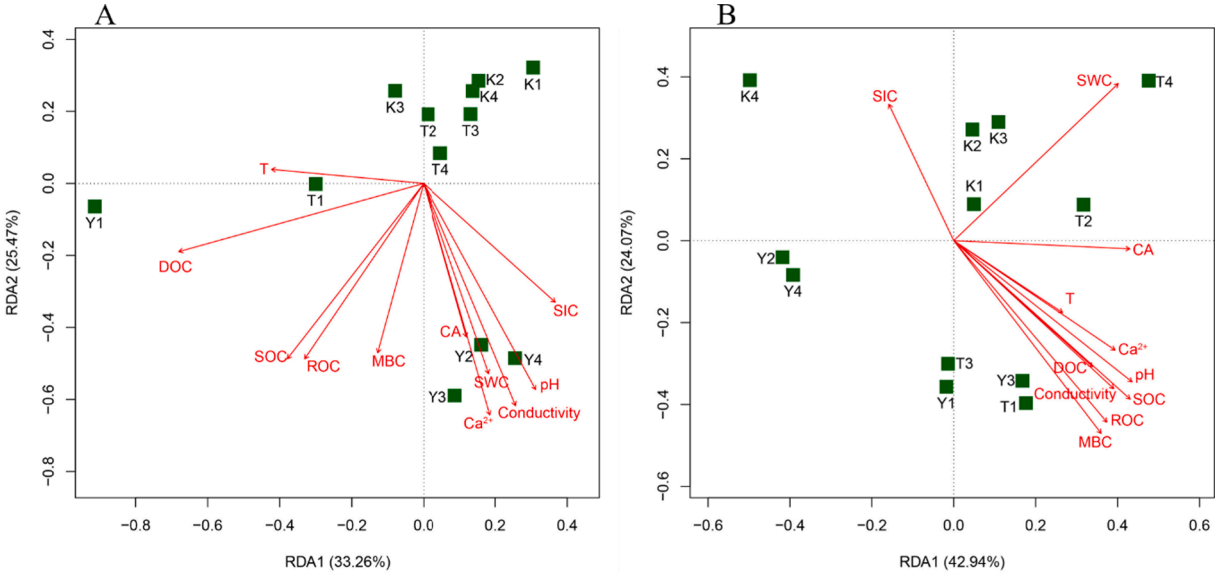

Fig. 7. Results of RDA analysis of the carbon-fixing bacterial community structure containing the *cbbL* gene with environmental factors in the Huixian karst wetland soils. A: Winter (201901); B: Summer (201907).

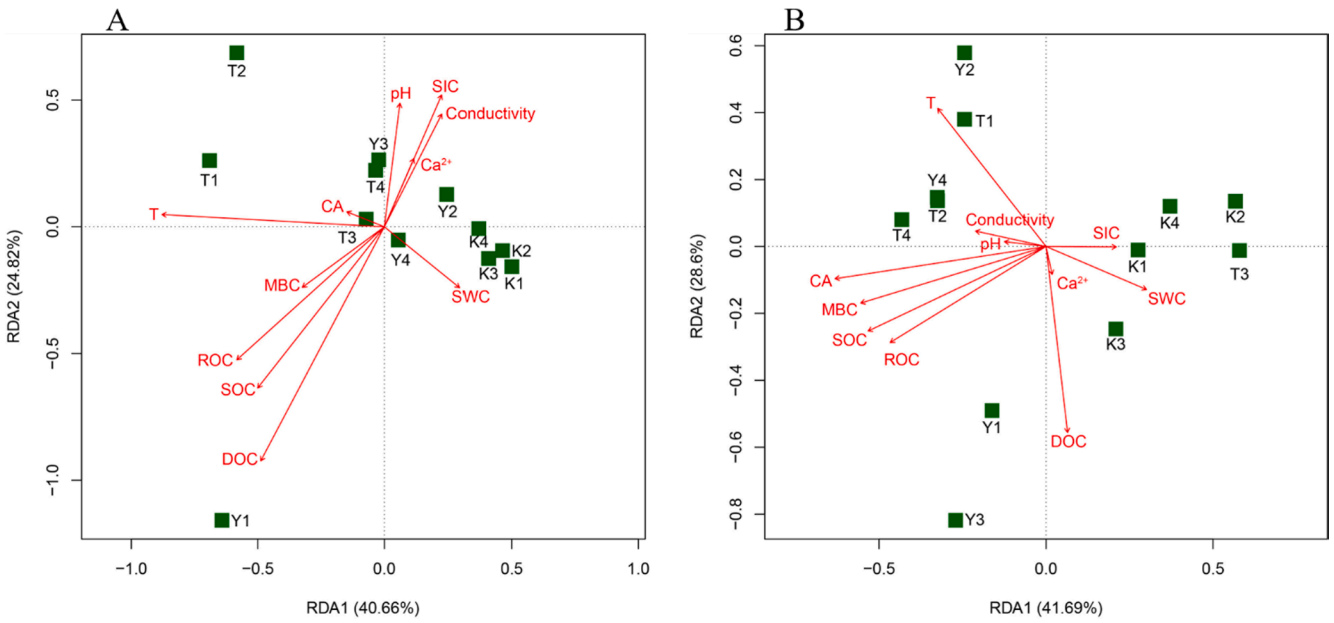

Fig. 8. Results of RDA analysis of the carbon-fixing bacterial community structure containing the *cbbM* gene with environmental factors in the Huixian karst wetland soils. A: Winter (201901); B: Summer (201907).

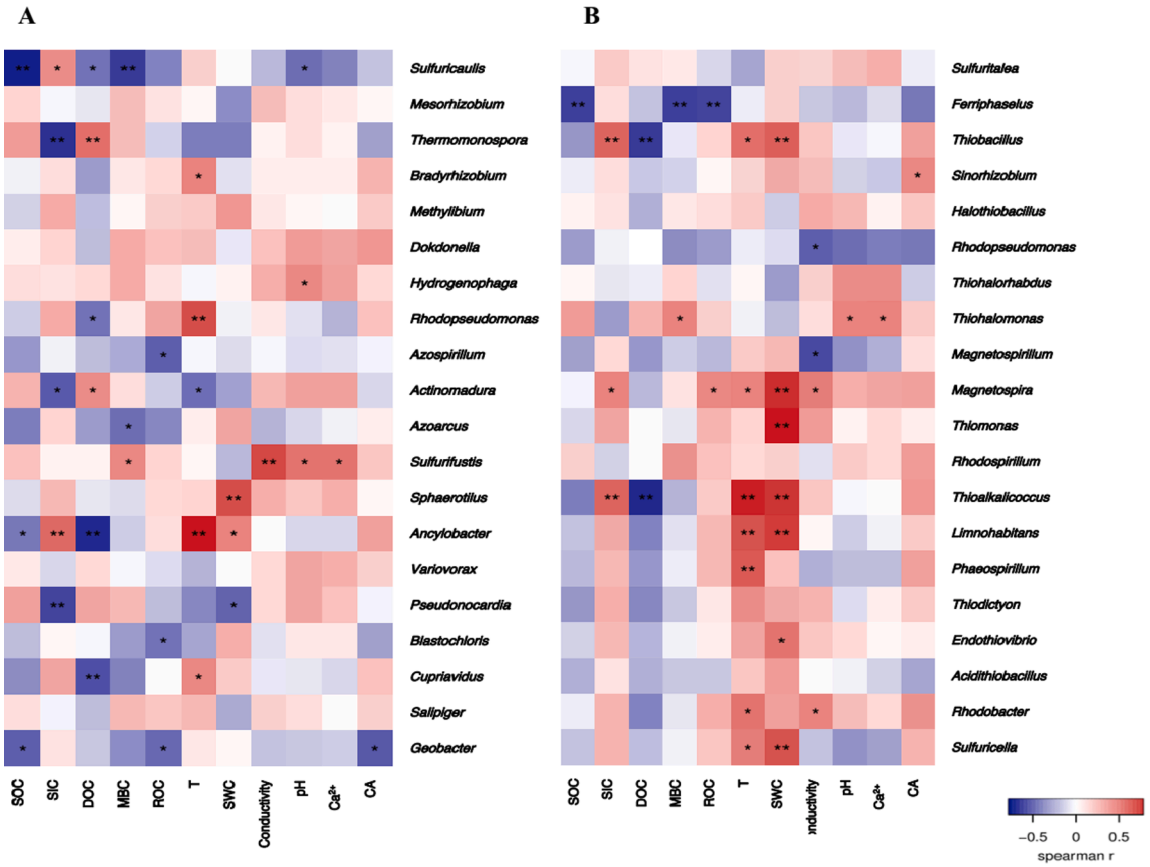

Fig. 9. Heatmaps of spearman correlation between the distribution of the carbon-fixing bacterial community containing the *cbbL* (A) or *cbbM* (B) genes and environmental factors in the Huixian karst wetland soils.

generally somewhat higher than in soils in non-karst areas (Yuan, 1988), and may thus provide more inorganic carbon sources for carbon-fixing bacteria. The abundance of the *cbbL* gene was significantly higher than that of *cbbM* gene ($P < 0.05$) in the present study. This difference may reflect the surface soil sampling whereby the higher O_2 concentration in soil may have inhibited the growth of carbon-fixing bacteria containing the *cbbM* gene. Moreover, two-way ANOVA analysis showed that the influence of the season on the abundances of *cbbL* and *cbbM*

genes was more significant than the wetland state. This result corresponded to the changes in soil physicochemical properties.

The richness and diversity indices are two important indicators that commonly describe the characteristics of microbial community. In this study, the diversity indices of the carbon-fixing bacterial community containing the *cbbL* gene was significantly higher than that containing the *cbbM* gene ($P < 0.05$). This indicated that the community structure of the autotrophic carbon-fixing bacteria containing RubisCO I was richer compared to those containing RubisCO II in the karst wetland soils. The richness indices of the carbon-fixing bacterial community containing *cbbL* gene in the winter and those containing the *cbbM* gene in the winter and summer in the reclaimed wetland soil were significantly higher than the indices in degraded wetland and native wetland soils ($P < 0.05$). The change in land use pattern can change the diversity of soil autotrophic carbon-fixing bacterial communities (Yuan et al., 2012b). According to field investigations in this study, local farmers apply NPK fertilizers as well as nitrogen fertilizers in the reclaimed wetland (paddy field) during the rice sowing and growing seasons. Zhou et al. (2019) reported that chemical fertilization significantly increased the diversity of soil carbon-fixing bacterial community containing the *cbbL* gene in newly formed soil compared to no fertilization. Therefore, fertilization may be one of the reasons for higher diversity of the carbon-fixing bacterial community in the reclaimed wetland soil.

4.2. Soil carbon-fixing bacterial community structure in Huixian karst wetlands of different status

In the three states of karst wetland soils, *Proteobacteria* was the predominant phylum of carbon-fixing bacterial communities, and *Alphaproteobacteria* and *Betaproteobacteria* were the predominant classes, which were similar to those found in alpine meadow soils on the northern Tibetan Plateau (Gao et al., 2018). *Bradyrhizobium*, *Hydrogenophaga*, *Mesorhizobium*, *Methylibium,* and *Sulfuricaulis* were the dominant bacterial genera in the carbon-fixing bacterial community containing the *cbbL* gene. *Ferriphaselus*, *Halothiobacillus*, *Rhodopseudomonas*, *Sinorhizobium*, *Sulfuritalea*, *Thiobacillus*, *Thiohalorhabdus*, and *Thiomonas* were the dominant bacterial genera in the carbon-fixing bacterial community containing the *cbbM* gene. Zhang et al. (2019) noted that *Bradyrhizobium* was dominant in the carbon-fixing bacterial community containing the *cbbL* gene in soils of a karst area, non-karst area and a mixing zone, and the relative abundance of *Methylibium* in the carbon-fixing bacterial community containing the *cbbL* gene was significantly higher in soils in karst areas as compared to non-karst areas. Yousuf et al. (2014) compared the carbon-fixing bacterial communities containing the *cbbM* gene in saline soil with those in farmland soil, and found that *Rhodopseudomonas* and *Thiobacillus* were the dominant bacterial genera in farmland soil. These results were similar to those of the present study of the three states of karst wetland soils.

In addition to fixing carbon through Calvin cycle by RubisCO I and RubisCO II, many bacterial genera in the carbon-fixing bacterial communities in the karst wetland soils were also related to the oxidation of inorganic nitrogen and sulfur compounds. For example, in the carbon-fixing bacterial communities containing the *cbbL* gene, *Sulfuricaulis* and *Methylibium* can participate in sulfur oxidation. *Bradyrhizobium* and *Mesorhizobium* are related to the nitrogen cycle. *Bradyrhizobium* can use NH_4^+, NO_2^-, S^{2-}, H_2S, H_2 and Fe^{2+} as energy sources for autotrophic growth (Tan, 2016; Kojima et al., 2016). *Sulfuritalea*, *Thiobacillus*, *Halothiobacillus*, and *Rhodopseudomonas* in the carbon-fixing bacterial community containing the *cbbM* gene are involved in the sulfur cycle (Liu et al., 2017; Xiao, 2017). *Rhodopseudomonas* can use thiosulfate as electron donor and can grow autotrophically or heterotrophically in aerobic or low oxygen conditions in the dark (Karr et al., 2003; Imhoff, 2001).

The composition of the dominant species in the carbon-fixing bacterial community was similar in the three states of karst wetland soils, but the relative abundance of some species was significantly different. The results of LEfSe analysis showed that *Thiomonas* was relatively abundant in the native wetland soil (Fig. 6). *Thiomonas* spp. are also the main dominant iron-reducing bacteria found in a coastal flooded reed wetland (Zhang et al., 2017), which had the same type of vegetation as the native wetland in the present study. In this study, the relative abundance of *Bradyrhizobium* was high in degraded wetland soil, which was similar to the result that *Bradyrhizobium* spp. were the dominant symbiotic N_2-fixing bacteria found in karst soil in northwestern Guangxi (Liu et al., 2015). Rhizobia can form symbiotic relationship with legumes, increase soil organic matter, and play an important role in restoring degraded soil fertility (Nie et al., 2014; Liu et al., 2015). Therefore, *Bradyrhizobium* may play a role in the restoration of degraded soil in karst wetlands by improving soil fertility through N_2-fixation. The abundances of *Ferriphaselus* and *Sulfuricaulis* were relatively high in the reclaimed wetland soil in this study. The results of Spearman analysis showed that the relative abundances of these two genera were significantly negatively correlated with SOC and MBC (Fig. 9), suggesting that these two genera may be beneficial to carbon fixation in barren, low carbon soils. *Sulfuricaulis* spp. are chemoautotrophs and oxidize inorganic sulfur compounds (Kojima et al., 2016). Some *Ferriphaselus* spp. fix CO_2 coupled with iron oxidation, reported also for an isolate from paddy soil (Khalifa et al., 2018). The iron redox directly or indirectly participates in the redox cycle in paddy soil and plays an important role in maintaining rice yield (Kyuma, 2004). Therefore, *Ferriphaselus* may play a role in the iron cycle of the reclaimed wetland soil in this study. To sum up, the soil physicochemical properties changed in the native wetland soil following natural degradation or reclamation, leading to shifts in the composition and/or abundance of the carbon-fixing bacterial communities in the karst wetland soil ecosystem.

4.3. Effects of soil organic carbon components and other environmental factors on the carbon-fixing bacterial community structure

The results of the RDA analysis showed that SOC and its fractions DOC, MBC, and ROC had significant effects on the carbon-fixing bacterial community structure in the karst wetland soils. It is well established that SOC affects the physicochemical properties and biological characteristics of soil and plays an important role in soil ecosystems (Gong et al., 2009; He et al., 2015). ROC, MBC, and DOC are active organic carbon in soil and usually respond quickly to soil changes (Chen et al., 2017). Changes in these soil carbon parameters can affect the carbon-fixing bacterial community structure (Selesi et al., 2005; Zhang et al., 2007; Xiao et al., 2014). Wu et al. (2015) reported that the SOC content was the main factor affecting the soil autotrophic carbon-fixing bacterial community structure in soils with different tillage patterns in the subtropical region of China. Their results are comparable to the present study of the karst wetland soils.

In the present study, both obligate and facultative autotrophic bacteria existed in the three states of karst wetland soils. For example, some *Thiobacillus* spp. are obligate autotrophs, while *Bradyrhizobium*, *Mesorhizobium*, *Rhodopseudomonas, Sinorhizobium, Methylibium*, and *Sulfuritalea* are facultative autotrophs (Sorokin et al., 2007; Wang et al., 2015; Zhou et al., 2017; Kojima and Fukui, 2011). Obligately autotrophic bacteria tend to grow in environments with low organic carbon content. Facultatively autotrophic bacteria can adapt to more environmental conditions. They not only can grow autotrophically through the Calvin cycle, but can also use organic carbon sources for energy and C assimilation (Selesi et al., 2005;Zhang et al., 2007). Therefore, differences in contents of SOC and its fractions may lead to differences in soil carbon-fixing bacterial community structure.

Soil temperature (T) is also the main factor affecting the carbon-fixing microbial community structure in the karst wetland soils. Similarly to the result of the present study, Gao et al. (2018) reported that soil temperature was the main factor driving the change in the carbon-fixing bacterial community structure in the meadow soil of northern Tibetan Plateau.

5. Conclusions

The abundance of the Calvin cycle functional genes *cbbL* and *cbbM* as well as the characteristics of the carbon-fixing bacterial community were compared in soil samples from the three states of Huixian karst wetland, Guilin, China. A considerable number of autotrophic carbon-fixing bacterial communities with two key enzymes, RubisCO form I and form II, were present in the karst wetland soils. The abundances of the genes encoding RubisCO form I and form II were $4.42 \times 10^9 \sim 9.57 \times 10^{10}$ copies/g dry soil and $3.71 \times 10^4 \sim 9.80 \times 10^8$ copies/g dry soil, respectively. The abundances of the *cbbL* gene in the degraded wetland and reclaimed wetland soils were significantly different among the seasons ($P < 0.05$), both expressed as summer > winter. The influence of the season on the abundances of the *cbbL* and *cbbM* genes was more significant than that of the states of wetland soils. The composition of dominant species in the carbon-fixing bacterial community was similar in the three states of karst wetland soils, mainly consisting of *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* in the phylum *Proteobacteria*. However, the relative abundances of some dominant bacterial genera were significantly different. *Thiomonas* had a relatively high abundance in the native wetland soil and *Bradyrhizobium* in the degraded wetland soil, while *Ferriphaselus* and *Sulfuricaulis* had relatively high abundances in the reclaimed farmland wetland soil. The main soil physicochemical factors influencing the carbon-fixing bacterial community structure in the karst wetland soils were SOC and its fractions DOC, MBC, and ROC as well as the soil temperature. Natural degradation or human activities such as wetland reclamation changed the physicochemical properties of the native wetland soil, leading to differences in distribution of soil carbon-fixing bacterial community in the karst wetland soil ecosystem. These results provide a scientific basis and reference for soil carbon sequestration and ecological protection in karst wetlands.

Declaration of Competing Interest

None.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2021.105418.

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