RESEARCH ARTICLE

Differential responses of dominant and rare epiphytic bacteria from a submerged macrophyte to elevated CO_2

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Abstract

Epiphytic bacteria develop complex interactions with their host macrophytes and play an important role in the ecological processes in freshwater habitats. However, how dominant and rare taxa respond to elevated atmospheric CO_2 remains unclear. A manipulated experiment was carried out to explore the effects of elevated CO_2 on the diversity or functional characteristics of leaf epiphytic dominant and rare bacteria from a submerged macrophyte. Three levels (high, medium, normal) of dissolved inorganic carbon (DIC) were applied to the overlying water. The physicochemical properties of the overlying water were measured. Elevated atmospheric CO_2 significantly decreased the pH and dissolved oxygen (DO) of overlying water. Proteobacteria, Cyanobacteria, Bacteroidetes, Planctomycetes, and Actinobacteria are the dominant phyla of leaf epiphytic bacteria from *Myriophyllum spicatum*, occupying over 90% of the accumulated relative abundances. The aquatic DIC level and further pH significantly drove the epiphytic community composition differences among the three DIC levels. For dominant epiphytic bacteria, the functional potential of nutrient processes and mutualistic relationships were strongly affected by a high DIC level, while responses of rare epiphytic bacteria were more related to trace element processes, pathogens, and defense strategies under a high DIC level. Our results showed the responses of epiphytic bacteria to elevated CO_2 varied across dominant and rare taxa.

Keywords *Myriophyllum spicatum* · Dissolved inorganic carbon · Epiphytic bacteria · PICRUSt · Multivariate cutoff level analysis

Introduction

As the major components of aquatic ecosystems, submerged macrophytes serve as primary producers and provide food and shelter for fish and zooplankton (Hempel et al. 2009; Rejmankova 2011). The vast surfaces offered by densely rooted macrophytes become excellent microhabitats for attached bacteria, and such surfaces have a positive influence on bacterial activity due to exuded organic compounds (Eriksson 2001). The attached bacteria always form biofilms on the surfaces of submerged macrophytes, which are either beneficial or detrimental to submerged macrophytes. For example, epiphytic bacteria can provide carbon dioxide through respiration and enhance nutrient recycling (Wetzel 1993; Wijewardene et al. 2022). Negative influences on macrophytes included light attenuation by the biofilms and the assembly of pathogen bacteria (Hempel et al. 2008; Gere et al. 2017; Wijewardene et al. 2022). Although the important role of leaf epiphytes has been verified, how dominant

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and rare taxa respond to elevated atmospheric CO_2 remains unclear.

Elevated atmospheric CO_2 will possibly influence the dissolved inorganic carbon (DIC) concentrations in overlying water and further lead to decreased pH or weak acidification in some freshwater ecosystems (Yin et al. 2017; Hasler et al. 2018). Free CO_2 (or H_2CO_3), HCO_3^-, and carbonate (CO_3^{2-}) comprised the total inorganic carbon in freshwater systems, and the relative proportions of these compounds are dependent on the pH of the overlying water (Maberly and Spence 1983). Recent studies indicated that the contents of dissolved organic carbon (DOC) in the surface water were also significantly increased by elevated CO_2 (Guo et al. 2011; Song et al. 2014). The labile fraction of the DOC in freshwater supports high rates of bacterial metabolism (Nelson et al. 2011; Pollard 2013). Therefore, enhanced performance of submerged macrophytes due to the fertilization effect under elevated CO_2 can interact with aquatic chemistry changes (Hussner et al. 2019), further regulating the assembly of the epiphytic bacteria on the surfaces of macrophytes in the aquatic ecosystem.

Microbial communities comprise a large number of species, in which a small proportion of these species are highly dominant, while a large proportion is present at a lower abundance and with an extremely high diversity (Pedrós-Alió 2012). Dominant species with high growth rates account for the majority of bacterial biomass and regulate biogeochemical cycling in ecosystems (Cottrell and Kirchman 2003; Shu et al. 2018). Rare microorganisms can act as a diverse source pool that responds to disturbance events and thereby help promote ecological stability (Hanson et al. 2015; Lynch and Neufeld 2015). In a study of coastal marine sediment, changes in bacterial community composition were associated with a global increase in pCO_2, but most of these bacterial taxa were resilient (Kerfahi et al. 2014). Another study of soil microbial community structure under elevated CO_2 showed that PLFA (phospholipid fatty acid) profiles were not affected by CO_2 enrichment (Ebersberger et al. 2004). This is possibly attributed to the negligence of different responses by dominant and rare taxa. Therefore, distinguishing the responses of dominant and rare taxa will shed sufficient light on the changes of epiphytic bacterial communities to elevated atmospheric CO_2 and relatively few empirical studies have examined such responses in freshwater submerged macrophytes.

As is known, *Myriophyllum spicatum* L. is a submerged aquatic species with branching leafy shoots and finely dissected whorls of leaves, whose canopy characteristics provide sufficient space for the epiphytic bacteria (Strand and Weisner 2001). Due to the fact that *M. spicatum* has a low requirement for nutrients and can grow on various sediment types, it is widely distributed in freshwater lakes of the Yangtze River Basin (Zhang et al. 2012; Fan et al. 2021),

including the typical macrophyte-dominated mesotrophic Liangzi Lake (Xie et al. 2013; Ma et al. 2021). As other macrophytes, *M. spicatum* is also limited to CO_2 depletion and evolves a strategy of HCO_3^- utilization (Hussner and Jahns 2015). Taking these into consideration, *M. spicatum* was selected as the host plant species. To illustrate the epiphytic microbial response under elevated CO_2, three levels of overlying water DIC content were set up in a pot experiment. Using high-throughput sequencing of 16S rRNA genes, we aim to reveal the shifts of epiphytic microbial compositions and their potential changes in functional profiles. Our research questions were (1) whether the epiphytic community compositions changed in response to elevated CO_2; (2) whether there were differences in the responses between dominant and rare taxa to elevated CO_2. Since elevated CO_2 would change the pH and dissolved oxygen in the overlying water, we hypothesized that elevated CO_2 would significantly alter the epiphytic community compositions. Furthermore, because of the different adaptive availabilities of dominant and rare bacterial taxa to environmental changes, we hypothesized that the sensitive responses of dominant taxa would be related to nutrient process and mutualistic relationships, while sensitive responses of rare taxa were more related to trace element processes, pathogens, and defense strategies under elevated CO_2.

Methods and materials

Host species and free-air CO_2 enrichment experiment

The free-air CO_2 enrichment (FACE) experiment was carried out at the greenhouse of the College of Life Sciences in Wuhan University, Hubei Province, China. The details of the pot experiment were described in the previous work (Li et al. 2019). *M. spicatum* is widely distributed in the submerged areas and lakeshores of Liangzi Lake (Figure S1). Thus, plant materials (stem fragments of *M. spicatum*) were collected from Liangzi Lake (30°05′-30°18′ N, 114°21′–114°39′ E) in April 2018. As a reference, we also collected the overlying water DIC data in Liangzi Lake in the same time, and the average DIC content was 13.71 ± 2.47 mg/L based on the 30 plots from the field investigation. And the total nitrogen of the overlying water where *M. spicatum* occurred was 0.57 ± 0.091 mg/L and the total phosphate was 0.014 ± 0.007 mg/L. Three fragments of *M. spicatum* (3.60 g ± 0.684 g, fresh weight) were cultivated in plastic pots (diameter of 30 cm, the height of 25 cm; filled with substrates of 7 cm depth), which were placed in the experiment tanks (length of 80 cm, width of 40 cm, height of 50 cm). Three levels of DIC availability of overlying water were applied by continuous bubbling with different

Table 1 General description of all, abundant, and rare OTU data sets at 97% sequence similarity level

	OTU number	Sequence number	ACE	Chao	Coverage
All OTUs	926	321,759	777.49 ± 52.76	780.16 ± 55.82	0.997 ± 0.001
Abundant OTUs	42 (4.5%)	221,825 (68.94%)			
Rare OTUs	708 (78%)	25,947 (8.37%)			

concentrations of CO_2: "normal DIC level (N)" without bubbling any gas (DIC, 14.75 ± 0.43 mg/L), "medium DIC level (M)" with bubbling ambient atmospheric air (DIC, 16.16 ± 0.17 mg/L), "high DIC level (H)" with bubbling atmospheric air enriched with pure CO_2 from a pressurized gas cylinder (DIC, 20.99 ± 3.14 mg/L). For each treatment, three replicates were selected for the following analyses of high-throughput sequencing. The duration of the experiment was approximately 4 weeks.

Physicochemical properties of overlying water

The overlying water was sampled every week to determine dissolved inorganic carbon (DIC) and total carbon (TC) contents by a TOC analyzer (TOC-L analyzer, Shimadzu, Japan). Overlying water dissolved oxygen (DO), conductivity (C), total dissolved solids (TDS), salinity (SAL), and pH were determined weekly by the YSI Professional Plus handheld multiparameter meter (YSI Inc., USA). The total nitrogen (TN) of overlying water was analyzed by a flow injection analyzer (QC8500, LACHAT, USA).

Analysis of epiphytic microbial communities

At the end of the FACE experiment, healthy and intact fresh leaves of *M. spicatum* (about 15 cm in length from the apex)

were selected to characterize the epiphytic microbial communities associated with the surfaces. Leaf samples were enclosed individually inside sterilized press-seal bags and immediately brought to the lab. The surfaces were rinsed with sterilized ultrapure water and then wiped gently with sterile cotton swabs (Steinberg et al. 2015). The swab samples were then stored at −80 °C in the lab until processing. Microbial DNA was extracted from swab samples using the PowerSoil DNA kit (MoBio Laboratories, Solana Beach, CA) following the manufacturer's protocols. The purified DNA was stored at −20 °C for downstream analyses.

The forward primer 338F (5′-barcode-ACTCCTACGGGAGGCAGCAG-3′) and the reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the V3–V4 regions of the bacterial 16S rRNA gene (Dennis et al. 2013). PCR protocols were as follows: 95 °C for 3 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. PCR reactions were performed in a 20 μL mixture containing 4 μL of 5×FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, 0.2 μL BSA, and 10 ng of template DNA. The purified amplicons were then pooled in equimolar concentrations, and the amplicon library was quantified using the QuantiFluorŋ™-ST Fluorometer (Promega, USA). The amplicon library combined with the PhiX Control library was loaded on an Illumina MiSeq platform (Majorbio, Shanghai, China), and

Table 2 The contribution (contrib) of abundant OTUs to average Bray–Curtis dissimilarity between epiphytic communities of *M. spicatum* from high DIC and normal DIC level

OTU ID	Contrib	Taxon	High DIC	Normal DIC
OTU361	0.0472	Proteobacteria; Acetobacteraceae	2472.33 ± 1295.2	90.33 ± 42.34
OTU299	0.0081	Proteobacteria; Acetobacteraceae	442 ± 254.14	34.67 ± 10.6
OTU334	0.0291	Proteobacteria; Bradyrhizobiaceae	2401.67 ± 256.86	939.67 ± 516.06
OTU337	0.0055	Proteobacteria; Caulobacteraceae	362 ± 191.49	86 ± 19.7
OTU919	0.0104	Proteobacteria; Rhizobiaceae	547.67 ± 192.38	23 ± 17.78
OTU636	0.0087	Proteobacteria; Rhizobiaceae	546 ± 148.52	106.67 ± 46.92
OTU340	0.0068	Proteobacteria; Sphingomonadaceae	380 ± 214.57	39 ± 20.66
OTU485	0.0056	Proteobacteria; Methylophilaceae	330.67 ± 25.01	46.33 ± 19.66
OTU461	0.0380	Proteobacteria; Methylophilaceae	1973 ± 798.2	48 ± 36.51
OTU75	0.0237	Cyanobacteria	1380 ± 343.04	185.67 ± 241.94
OTU643	0.0155	Proteobacteria; Acetobacteraceae	114 ± 17.69	898.33 ± 379.52
OTU674	0.0054	Proteobacteria; Acetobacteraceae	99.67 ± 15.37	374.67 ± 118.95
OTU815	0.0125	Proteobacteria; Erythrobacteraceae	755 ± 406.59	1366.33 ± 182.53
OTU822	0.0176	Proteobacteria; Sphingomonadaceae	1578 ± 303.23	2437 ± 873.97
OTU74	0.0044	Cyanobacteria	186 ± 109	408.67 ± 18.72
OTU638	0.0158	Planctomycetes; Phycisphaeraceae	414 ± 277.57	1174.67 ± 551.44

Table 3 The contribution (contrib) of rare OTUs at genus level to average Bray–Curtis dissimilarity between epiphytic communities of *M. spicatum* from high DIC and normal DIC level

Genus	Contrib	Taxon	High DIC	Normal DIC
norank	0.0248	Gemmatimonadetes	13 ± 6.08	275.67 ± 176.32
OM27_clade	0.0109	Proteobacteria; Bdellovibrionaceae	25 ± 35.59	137.33 ± 43.25
Hyphomonas	0.0147	Proteobacteria; Hyphomonadaceae	68 ± 11.36	225 ± 115.57
Unclassified	0.0195	Proteobacteria; Rhodospirillaceae	4.67 ± 0.58	198 ± 162.73
Silanimonas	0.0093	Proteobacteria; Xanthomonadaceae	10.33 ± 3.06	108.33 ± 37.63
norank	0.0195	Proteobacteria	13.67 ± 11.59	222.33 ± 136.05
Geitlerinema	0.0104	Cyanobacteria	25.33 ± 13.32	131.67 ± 21.73
Ideonella	0.0174	Proteobacteria; Comamonadaceae	192.33 ± 167.85	11 ± 5.2
Blastomonas	0.0082	Proteobacteria; Sphingomonadaceae	104.67 ± 38.03	21 ± 8.19

Note: because of the great high diversity of the rare bacteria, the analyses were performed at the genus level

sequencing was performed using a 2×300 paired-end (PE) configuration (Wu et al. 2015; You et al. 2016).

The paired-end reads were extracted from each sample according to the barcode sequence, and all reads with a considerable proportion of either poor quality bases (low-quality score, ambiguous bases, or homopolymer) or mismatches in primers or barcodes must be removed (Schloss et al. 2011; Li et al. 2014). After quality trimming, paired-end reads of sufficient length (a minimum of 10 base overlap between forward and reverse reads) were merged into full-length sequences by FLASH v1.2.5 (Magoč and Salzberg 2011). Potential chimera sequences were identified and removed
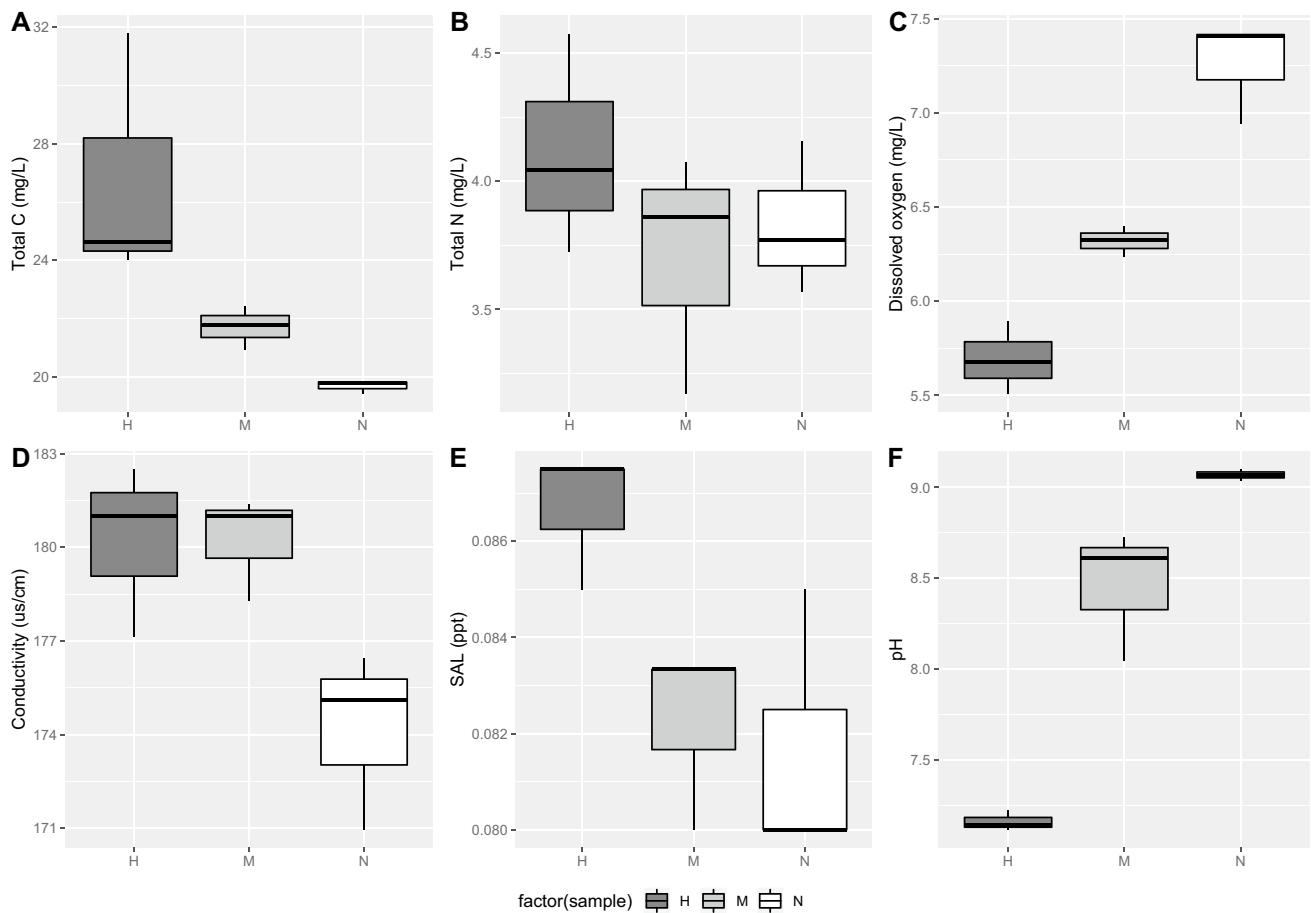

Fig. 1 Physicochemical properties of overlying water under three DIC levels (H, high DIC level; M, medium DIC level; N, normal DIC level). **A** Total carbon (total C); **B** total nitrogen (total N); **C** dissolved oxygen (O); **D** conductivity (C); **E** salinity (SAL); **F**, pH
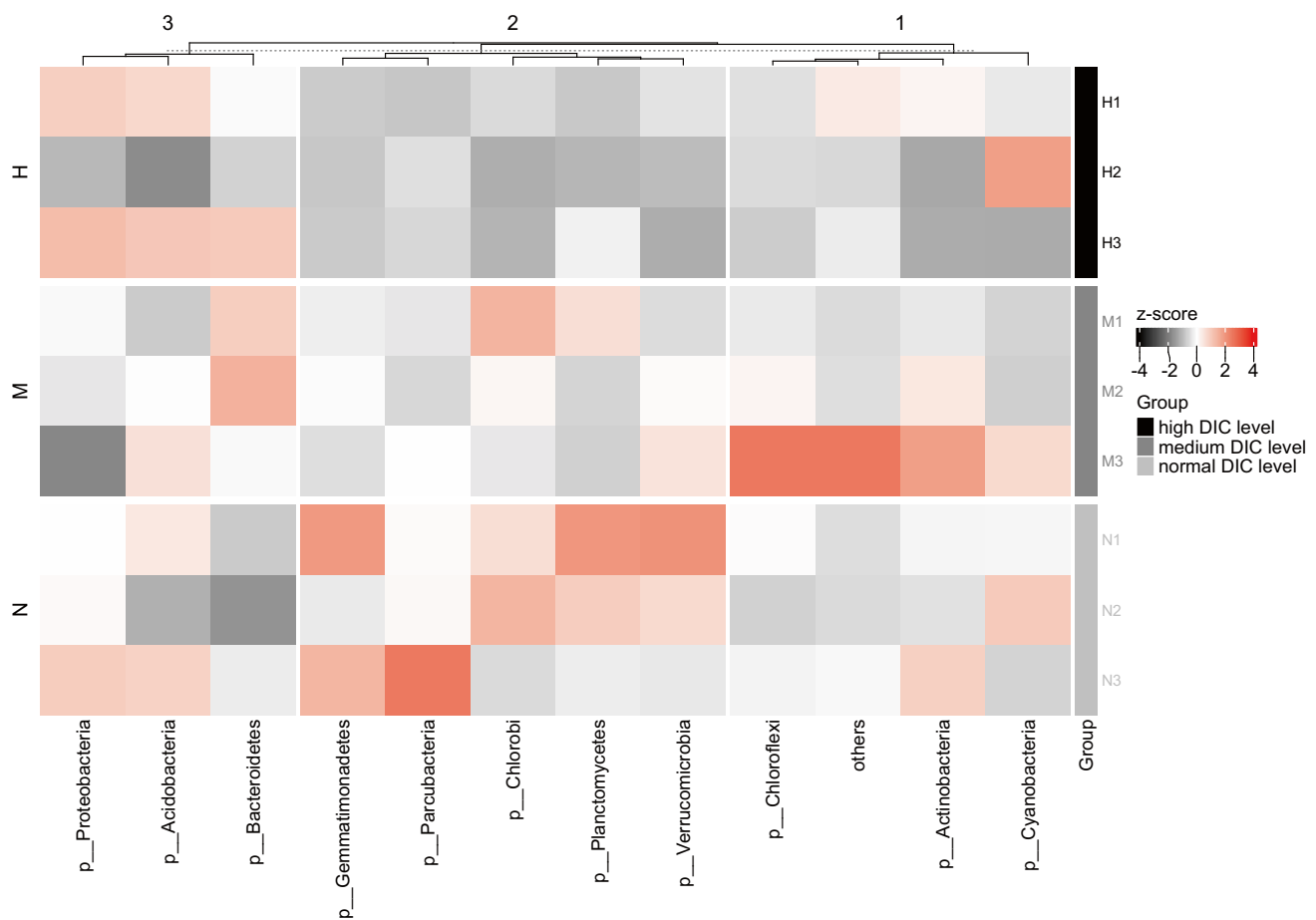
Fig. 2 Hierarchical heatmap of epiphytic bacterial communities associated with *M. spicatum* leaves under three DIC levels (H, high DIC level; M, medium DIC level; N, normal DIC level)

based on a prediction by UCHIME (USEARCH v9.2) using the reference database mode. The clean sequences were clustered into operational taxonomic units (OTUs) using UCLUST (USEARCH v9.2) at a 97% similarity level. The taxonomy assignment of OTUs was performed using RDP Release 11 for bacteria16S rRNA genes as a reference database source. The chloroplast and mitochondria from the host plant were checked and excluded by filtering the sequences from the OTU table.

Definition of dominant and rare taxa

Multivariate cutoff level analysis was used for the definition of dominant and rare OTUs (Gobet et al. 2010; Liu et al. 2015). The data set was sorted according to the decreasing total sum of OTU sequences. A series of cutoff levels are assigned to the original data set according to several percentages of the total number of sequences in the data set (0, 1, 5–95, and 99%). Pairwise distance matrices were calculated using the Bray–Curtis dissimilarity index and then the resulting dissimilarity matrices were compared with one

another using the nonparametric Spearman rho correlation coefficient. Variations in the main axes of extracted variation in community structure were explored via nonmetric multidimensional scaling (NMDS), and then the Procrustes method was then used to compare the NMDS ordination results. The OTUs that had a mean relative abundance of > 0.15% were defined as regionally dominant OTUs (4.5% cutoff, Figure S2), whereas the OTUs with a mean relative abundance of < 0.0195% were defined as regionally rare OTUs (78% cutoff, Figure S3).

In total, 42 (4.5%) OTUs with 221,825 sequences were considered dominant taxa, while 708 (78%) OTUs with 25,947 sequences were classified as rare taxa (Table 1). The richness (OTU number) of rare taxa was six times greater than that of dominant taxa, but the abundance of rare OTUs was about one-twentieth of the dominant one. In addition, dominant taxa mainly came from Proteobacteria (25 OTUs) and Cyanobacteria (11 OTUs), while rare taxa distributed more evenly, mainly coming from Acidobacteria (22 OTUs), Actinobacteria (40 OTUs), Bacteroidetes (86 OTUs), Chloroflexi (58 OTUs), Cyanobacteria (65 OTUs), Proteobacteria
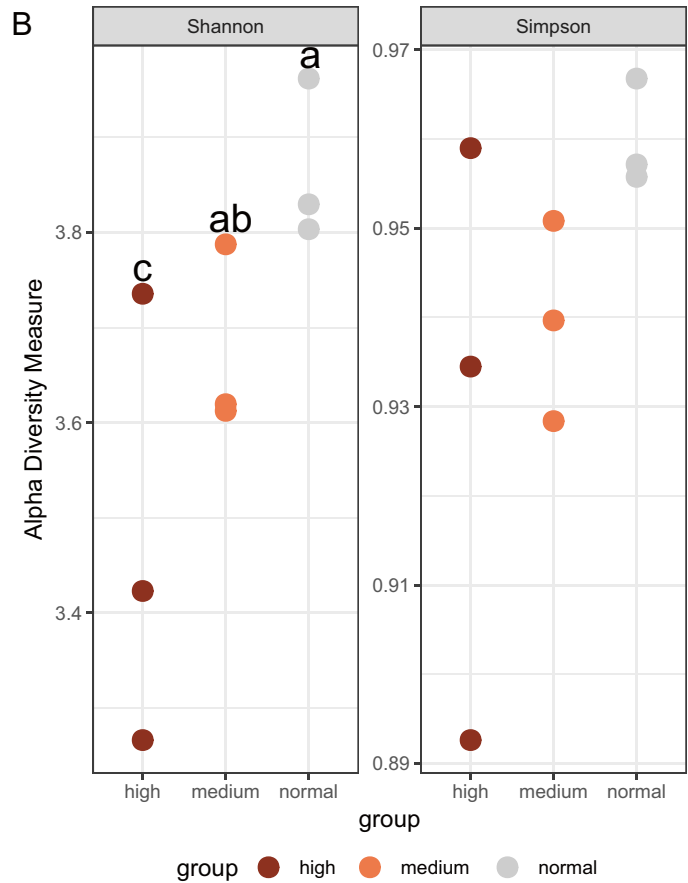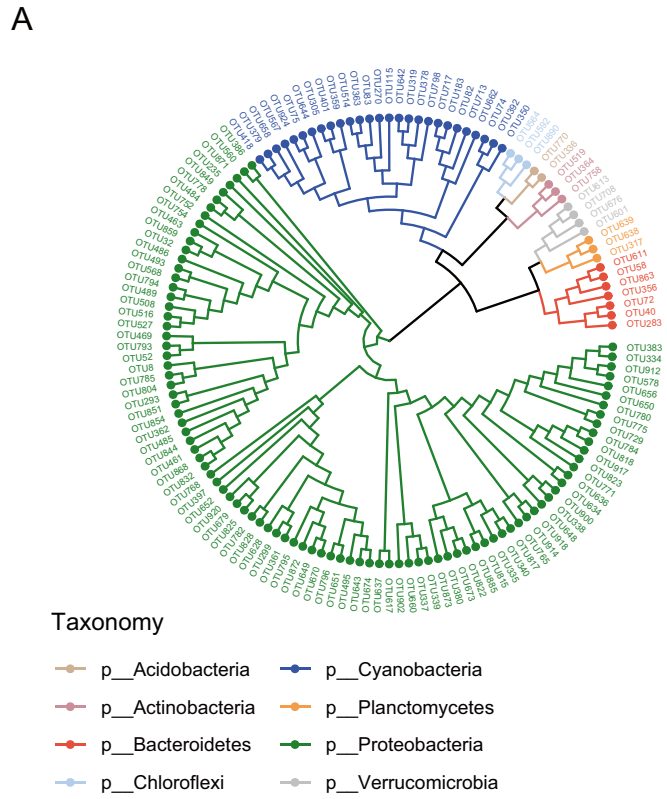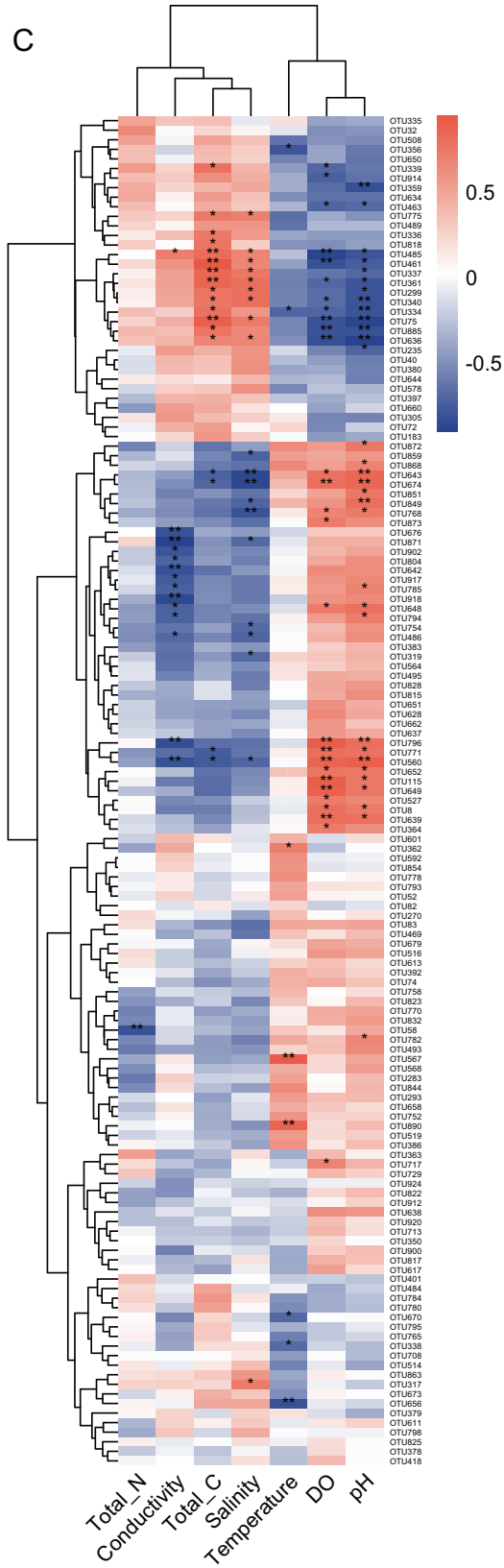
◄Fig. 3 Taxonomic information of dominant epiphytic bacterial taxa occurred in all samples (**A**), and the phylogenetic tree was constructed using the neighbor-joining method. The Shannon and Simpson diversity among the three DIC levels (**B**), and the different lowercase letters showed significant differences. The correlation between the dominant epiphytic bacterial taxa and overlying water properties (**C**). Total_C, total carbon content; Total_N, total nitrogen; DO, dissolved oxygen

(302 OTUs), and Verrucomicrobia (22 OTUs) (Table S1). SIMPER analysis was used to identify dominant and rare OTUs contributing to the average Bray–Curtis dissimilarity in community compositions among three DIC levels with the function "simper" in the "vegan" package. The dominant and rare OTUs with higher contribution values were used for sample discrimination (Lin et al. 2012). Further filtering processes were applied to get rid of OTUs that did not occur in all samples. At last, we selected dominant and rare OTUs that showed a significant difference in abundance between high and normal DIC levels to reveal the different responses of dominant and rare bacteria to elevated CO_2 (Table 2, 3).

Statistics

One-way ANOVA was used to determine the effect of DIC levels on TC, TN, DO, C, TDS, SAL, and pH of the overlying water. Variance homogeneity was tested using Levene's test, and data were log-transformed if necessary. LSD post hoc tests were used to identify significant differences between different DIC levels at the 5% significance level.

Abundance-based coverage estimator (ACE), Chao, and diversity indices (Shannon–Wiener diversity, Simpson diversity) based on the OTU table were estimated in the "vegan" package. One-way analysis of variance was performed to explore the effect of DIC levels on alpha diversity indices. The Pearson correlations between abundances of bacterial taxa and overlying water properties were evaluated with the function "corr.test" in the package "psych." The phylogenetic tree of dominant taxa (occurred in all samples) was constructed using the neighbor-joining method with the function "ggtree." A redundancy analysis (RDA) was performed to investigate the relationships between bacterial communities and environmental factors with the function "rda" in the "vegan" package. Permutational multivariate analysis of variance (MANOVA) was then performed to test the effects of overlying water DIC levels on the Hellinger-transformed bacterial community compositions with the function "adonis" in the "vegan" package. The above multivariate analyses were performed at three levels: all OTUs, dominant, and rare OTUs. The functional profiles of microbial communities were predicted using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) according to the online protocol

(Langille et al. 2013; Ortiz-Estrada et al. 2019). The predicted table of individual enzyme functions abundance was obtained. One-way ANOVA was performed to test the effect of different DIC levels on the predicted enzyme abundances in each sample. All statistical analyses and plots were performed in R (version 3.5.1).

Results and discussions

Physicochemical properties of overlying water

The physicochemical properties of the overlying water were significantly affected by the elevated atmospheric CO_2 concentration (Table S2). One major consequence of elevated atmospheric CO_2 concentrations is an increase in C availability in aquatic ecosystems (Olesen and Madsen 2000). As exhibited, there was a significant difference in the total C among three DIC levels (p value = 0.034), while the total N exhibited no such difference (Table S2, Fig. 1A, B). Significant differences in DO (p value < 0.001) and conductivity (p value = 0.038) were observed among three DIC levels (Table S2, Fig. 1C, D). Concentrations of DO possibly depend on gas exchange processes at a small scale (Hanson et al. 2006). The gas bubbling under high or medium DIC treatments possibly led to the decreased DO content in the overlying water. The salinity in overlying water differed among three DIC levels at the p value < 0.1 level (Table S2, Fig. 1E), which confirmed the fact that the salinity in overlying water was always correlated with conductivity (Roos and Pieterse 1995), The increased overlying water conductivity and salinity under increased DIC levels was possibly attributed to the decreased pH, which strongly influences nutrient availability and the solubility of metals (Rousk et al. 2009).

Overlying water pH decrease is another major consequence of elevated atmospheric CO_2. Accumulated free CO_2 under elevated CO_2 possibly changes the equilibria between various inorganic carbon species (free CO_2, HCO_3^-, and carbonate) in the overlying water (Maberly and Spence 1983). The addition of CO_2 to overlying water possibly results in the production of carbonic acid and the accumulation of H^+, further reducing the water pH. Therefore, elevated CO_2 always leads to "freshwater acidification" in aquatic ecosystems, while the extent of the acidification is not comparable to acidification in oceans (Reitsema et al. 2018). While in systems with a great buffering capacity, such as a high density of macrophytes, the accumulated CO_2 would be consumed quickly by the primary producer and not lead to acidification of the water body (Hasler et al. 2016). So, the occurrence of a high density of macrophytes probably enhances the resistance of the freshwater ecosystem to elevated CO_2 in the future.
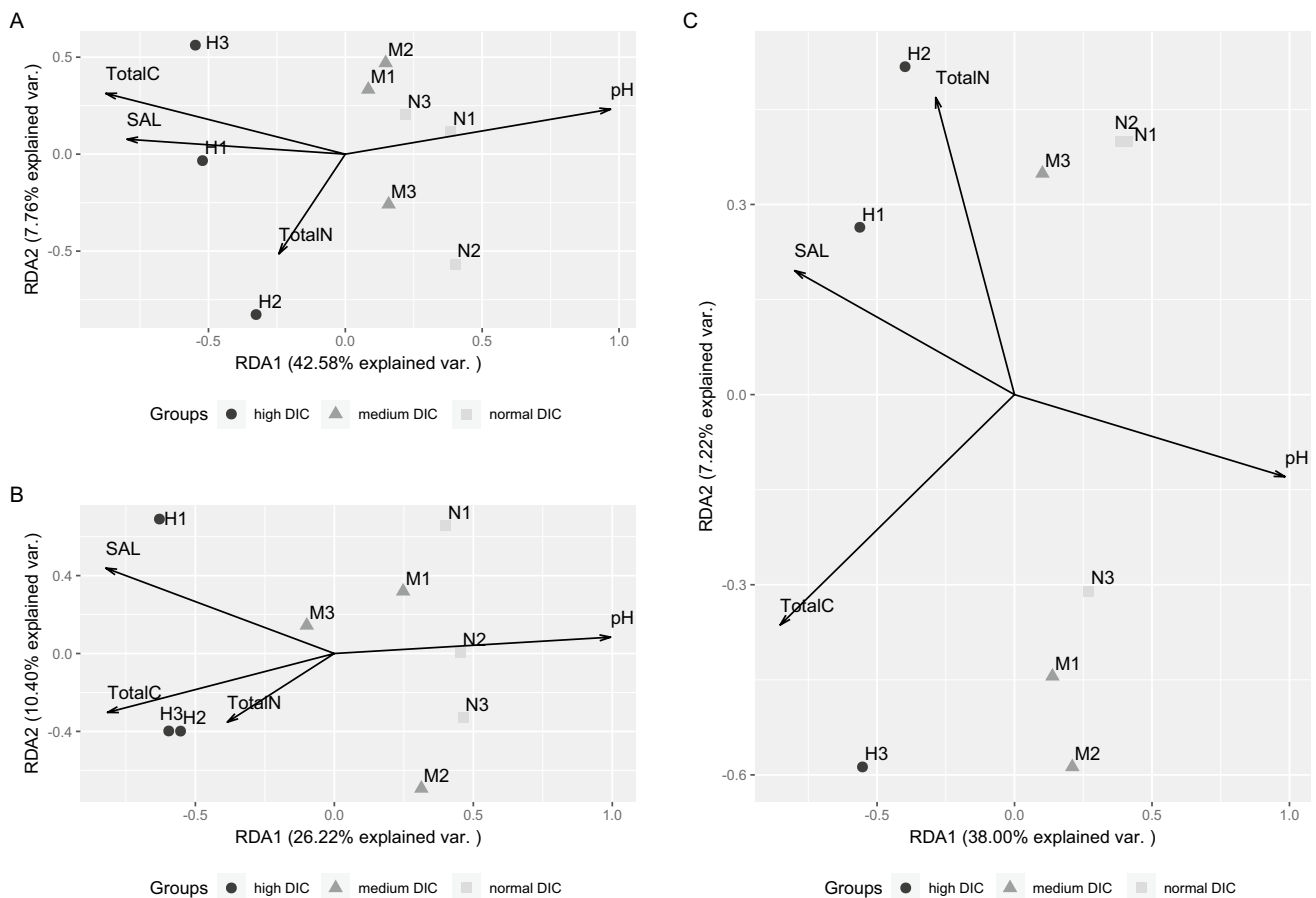
Fig. 4 Ordination diagram of redundancy analysis (the first two axes) based on the dominant subcommunities (**A**), rare subcommunities (**B**), or the whole epiphytic bacterial communities (**C**) with environmental factors

Epiphytic microbial community compositions under elevated CO_2

We recovered 321,759 high-quality sequences, which clustered into 926 OTUs at a 97% sequence similarity level (Table 1). The number of OTUs estimated by Chao1 (780.16 ± 55.82) was similar to the estimator of the ACE index (777.49 ± 52.76), and the mean of Good's coverage of all samples was 99.7% (Table 1). The phyla of Proteobacteria, Cyanobacteria, Bacteroidetes, Planctomycetes, and Actinobacteria occupied a large proportion of each sample, with an accumulative relative abundance ranging from 90.36 to 97.99% (Fig. 2, Table S3, Fig. 3A). The dominance of these bacterial phyla on the plant surfaces also occurred in previous studies (He et al. 2014; Hassenrück et al. 2015). The high DIC level in the overlying water caused by elevated CO_2 significantly reduced the Shannon diversity of epiphytic bacteria (Fig. 3B). We further examined the correlations of dominant bacterial taxa with overlying water properties. Several abundant bacterial taxa showed significant relationships with overlying water total carbon content, pH, DO,

conductivity, and salinity (Fig. 3C). This result possibly indicated that only a part of bacteria taxa had adapted to the lower pH level caused by elevated CO_2. This contrasted to a previous study that sediment microbes quickly adapted to the elevated CO_2 environment (Li et al. 2019).

The compositions of leaf epiphytic bacterial communities were significantly altered by increased atmospheric CO_2. The results of MANOVA showed significant effects of elevated CO_2 on the epiphytic, dominant, and rare bacterial community compositions (Fig. 4). All the constrained axes in each of the RDA models explained great proportions of the total variations in the communities' dissimilarities (Fig. 4). This is possibly attributed to the niche-based mechanism. Greater environmental differences represent more available niche space to be colonized by specific microbes and thereby lead to stronger environmental filtering (He et al. 2014; Kerfahi et al. 2014; Cox et al. 2015). Elevated CO_2 significantly altered the overlying water properties (especially pH), led to a divergent species assemblage, and formed different bacterial communities on the leaf surfaces (Fig. 4). The composition changes under different DIC levels

were similar to a previous study that found bacterial epiphytes formed distinct communities at the CO_2-impacted site compared with the control site (Hassenrück et al. 2015), and a previous study that found epiphyte communities of a tropical seagrass meadow showed a decline in the abundance of coralline algae and increases in filamentous algae under elevated aquatic CO_2 (Campbell and Fourqurean 2014). Though we did not collect microbial data in the overlying water, there is evidence that epiphytic microbes on the plant surfaces are possibly recruited from the surrounding environment, and both the environment and the host plant have the capability to modulate the recruitment process (Burke et al. 2011; Mancuso et al. 2016; Roth-Schulze et al. 2018). This selective power of the host plant may be closely associated with the surface properties of the host plant and its exudates (Bringel and Couée 2015; Remus-Emsermann and Schlechter 2018).

Meanwhile, there existed a negative influence of elevated CO_2 on epiphytic bacteria. In our study, we observed a decrease in the Shannon diversity of an epiphytic bacterial community (high DIC level, 3.94 ± 0.26; normal DIC level, 4.62 ± 0.13). This is similar to a study of *Posidonia oceanica*, a significant decline in epiphytic cover was observed under ocean acidification (Cox et al. 2015). Further, the PICRUSt result showed that the abundance of predicted catalase was significantly increased by elevated CO_2 (Figure S4). As is known, the potential of enhanced catalase indicates a community response to exogenous stress (Chabot et al. 2020). Catalases are enzymes that use H_2O_2 as an electron acceptor and that manage oxidative stress caused by reactive oxygen species (Sinsabaugh 2010). For example, catalase activity was enhanced under the stress of saline-alkali soil (Zhang et al. 2016). In total, excessive CO_2 represents an unwanted waste product of metabolism and exerts negative influences (Fenner et al. 2007).

Responses of dominant and rare taxa to elevated CO_2

Generally, dominant and rare bacterial taxa respond to elevated CO_2 differently. The responses of dominant taxa to elevated CO_2 were mainly related to nutrient processes and mutualistic relationships. Responses of dominant taxa to elevated CO_2 varied across families and within one family, for example, the OTUs from Rhizobiaceae or Acetobacteraceae (Table 2). High DIC levels increased the abundance of OTU334 which is a species of *Bosea* (Bradyrhizobiaceae) with the capability of denitrification (de Souza et al. 2014). The species in Caulobacteraceae can exude alkaline phosphatase and better survive in oligotrophic aquatic habitats. In a previous study, the bacteria of *Caulobacter* is one of the primary microbial colonizers in the reformation process of a temporary river (Fazi et al. 2008). The abundances of

OTUs in Rhizobiaceae, characterized by their ability to develop biological nitrogen fixation (Carareto Alves et al. 2014), were upregulated under high DIC levels (Table 2). The effects of aquatic DIC levels on species in Sphingomonadaceae varied, with an increased abundance of OTU340 and a decreased abundance of OTU822 under high DIC levels (Table 2). Species in Sphingomonadaceae are important members of the freshwater planktonic bacterial community, most of which are chemoorganotrophic. And several genera are facultative photoheterotrophic owing to the bacteriochlorophyll a content, such as *Blastomonas*, *Sandaracinobacter*, and *Sandarakinorhabdus* (Glaeser and Kämpfer 2014). The abundances of two OTUs in Methylophilaceae were upregulated under high DIC levels (OTU485, OTU461, Table 2), possessing the ability to utilize the C1 compound. Methylobacteria in this family can consume methanol released by plants into the environment through leaf stomata, with a production of bioactive substances (Fedorov et al. 2011). Such changes implied that elevated atmospheric CO_2 strongly affected the nutrient potential of dominant epiphytic bacteria and their mutualistic relationship with their host.

Elevated atmospheric CO_2 downregulated the abundances of most rare species (genus level), except for the genera of *Ideonella* and *Blastomonas* (Table 3). This is possibly true because rare species are more vulnerable to being lost from ecosystems under climate change because of their low competitive ability (Pedrós-Alió 2012). Members of the family Comamonadaceae are free-living or plant associated, in which quite a number of species inhabit more oligotrophic freshwater habitats (Willems 2014). Members of *Blastomonas* contain bacteriochlorophyll a and are aerobic anoxygenic phototrophic bacteria (Glaeser and Kämpfer 2014). Members of Hydrogenophilaceae regulate the process of sulfide turnover in aquatic habitats (Willems 2014). The Bdellovibrionaceae are predatory bacteria that prey upon a variety of Gram-negative bacteria (Pineiro et al. 2004). Several genera of Xanthomonadaceae are categorized as generalist hydrocarbon-degraders based on their ability to also utilize various carbon substrates as a sole source of carbon and energy (Gutierrez 2019), and several genera are plant pathogens. In total, the response of rare epiphytic bacteria was more related to trace element processes, pathogens, and defense strategies under high DIC levels (Hol et al. 2010; Lynch and Neufeld 2015).

Conclusions

The presented study showed an obvious shift in the leaf epiphytic microbial community composition of *M. spicatum* under elevated CO_2. The responses of epiphytic bacteria to elevated CO_2 varied across dominant and rare taxa. For dominant epiphytic bacteria, the functional potential of nutrient

processes and mutual relationships were strongly affected by high DIC levels, while responses of rare epiphytic bacteria were more related to trace element processes, pathogens, and defense strategies under a high DIC level. The functional potential changes of leaf epiphytes under elevated CO_2 imply that submerged macrophytes can alternatively assimilate nitrogen and phosphate through the leaves from the water column and facilitatively enhance the fitness of their host plant. This exhibited a disparate pattern compared with their terrestrial counterparts, where terrestrial leaf epiphytes constantly need to cope with a combination of stresses, such as ultraviolet radiation exposure, low water and nutrient availability, and high-temperature fluctuations (Remus-Emsermann and Schlechter 2018).

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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