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ORIGINAL ARTICLE

Community structure and functional diversity of epiphytic bacteria and planktonic bacteria on submerged macrophytes in Caohai Lake, southwest of China

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Abstract

Purpose Epiphytic bacteria on the surfaces of submerged macrophytes play an important role in lake biodiversity and ecological processes. However, compared with planktonic bacteria, there is poor understanding of the community structure and function of epiphytic bacteria.

Methods Here, we used 16S rRNA gene high-throughput sequencing and functional prediction analysis to explore the structural and functional diversity of epiphytic bacteria and planktonic bacteria of a typical submerged macrophyte (*Potamogeton lucens*) in Caohai Lake.

Results The results showed that the species composition of epiphytic and planktonic bacteria was highly similar as 88.89% phyla, 77.21% genera and 65.78% OTUs were shared by the two kinds of samples. *Proteobacteria* and *Bacteroidetes* were dominant phyla shared by the two kinds of communities. However, there are also some special taxa. Furthermore, the epiphytic bacterial communities exhibited significantly different structures from those in water, and the abundant OTUs had opposite constituents. The explained proportion of the planktonic bacterial community by aquatic environmental parameters is significantly higher than that of epiphytic bacteria, implying that the habitat microenvironment of epiphytic biofilms may be a strong driving force of the epiphytic bacterial community. Functional predictive analysis (Functional Annotation of Prokaryotic Taxa, FAPROTAX) found that epiphytic bacteria and planktonic bacteria are dominated by heterotrophic functions, but epiphytic bacteria have more prominent fermentation and denitrification functions (nitrate reduction, nitrate respiration, and nitrite respiration) than planktonic bacteria.

Conclusion This study has increased our understanding of the communities and functions of epiphytic bacteria on submerged macrophyte leaves, and their role in lake denitrification cannot be ignored.

Keywords Epiphytic bacteria · Planktonic bacteria · Diversity pattern · Functional traits

Introduction

Epiphytic biofilms are widely distributed on solid surfaces such as rocks, sediments, and submerged plants in ponds, rivers, lakes, and marine environments, which harbor a combination of algae, protists, fungi, and bacteria (Palmer Jr. and White 1997; Writer et al. 2011; Lu et al. 2016; Zhao et al. 2018). Biofilms play a major role in regulating the nutrient cycle and energy flow in water bodies, and there is growing research interest in utilizing natural periphytic biofilms in wastewater treatment, nonpoint source pollution control, and remediation of polluted waters (Furey et al. 2016; Singh et al. 2017; Su et al. 2017; Wu et al. 2018). The ecological functions of biofilms are closely linked to the chelation, recycling, or metabolic degradation activities of microorganisms on

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nutrients and pollutants (Wu et al. 2012; Zhao et al. 2018). Therefore, the community structure and ecological functional characteristics of epiphytic biofilm microorganisms are the hotspots of current water ecology research (Wu et al. 2012; Wu et al. 2018). Submerged macrophytes are widely distributed in shallow water ecosystems, and their leaves provide epiphytic areas for the growth of microorganisms and have special niches. However, compared with that of planktonic bacteria, the understanding of the structure and function of epiphytic bacteria on submerged macrophyte leaves is still very limited.

Some previous studies have shown that distinct and shared microorganisms exist between epiphytic and planktonic bacterial communities (Burke et al. 2011; He et al. 2014). Planktonic bacteria have been regarded as a major seed bank for epiphytic bacteria, which has an important influence on the assemblage of epiphytic bacteria (Dolan 2005; Garulera et al. 2016). Certainly, host-specific communities can be selected by complex physical or biochemical characteristics on different plant leaves. Plants and their secretions at different growth stages can also shape the composition of epiphytic bacterial communities (Herrmann et al. 2008; Lachnit et al. 2011; He et al. 2012). Environmental factors also have an important impact on epiphytic bacterial communities, such as pH, redox potential, water flow, light, temperature, and nutrient availability(Bouletreau et al. 2012; Kuehn et al. 2014; Hao et al. 2017). For example, the abundances of *Actinobacteria*, *Nitrospirae*, and *Verrucomicrobia* in biofilms vary with the conductivity of river water, while the presence of *Acidobacteria*, *Gemmatimonadetes*, and *Proteobacteria* in biofilms is associated with pH changes (Wilhelm et al. 2013; Battin et al. 2016). Similarly, epiphytic bacteria may spread into planktonic bacterial communities (Underwood et al. 2007; Kurian et al. 2012). Clearly, there is a complex interrelationship between epiphytic and planktonic bacteria. However, current reports lack the understanding of the similarities and differences between the structure and function of epiphytic and planktonic bacteria.

Species richness or diversity may not be sufficient to understand how community composition and composition affect ecosystem function (Loreau et al. 2001; Cardinale et al. 2006). This shows that in addition to revealing which microorganisms are in the environment, it is particularly important to determine the functional profile of microbial communities. To this end, researchers have developed a variety of methods based on 16S rRNA high-throughput sequencing to predict bacterial community functions, including the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al. 2013), Tax4Fun (Aßhauer et al. 2015), and the Functional Annotation of Prokaryotic Taxa (FAPROTAX) (Louca et al. 2016). Among them, FAPROTAX is the most commonly used method for exploring the biogeochemical cycle functions of microorganisms. It depends on the prokaryotic functional data of culturable bacteria and species classification (OTU table) to obtain community function information, which is applicable to a variety of environmental samples and has the advantages of reliable results and economical and practical benefits (Louca et al. 2016; Galand et al. 2018). This provides an effective solution for understanding the differences in the structure and function of epiphytic and planktonic bacterial communities.

This study focuses on the typical macrophytic lake (Caohai) in southwest China and compares the structure and function of epiphytic and planktonic bacteria by 16S rRNA gene high-throughput sequencing and functional predictive analysis. The study aims to address the following three topics: (i) characteristics of epiphytic and planktonic bacterial community species composition, (ii) community structure of epiphytic and planktonic bacteria and their relationship with the environment, and (iii) epiphytic bacterial community function and its environmental significance.

Material and methods

Study site and sampling

The sampling sites were located in the karst area of southwest China, Caohai National Nature Reserve (104°1 2′–104° 18′ E, 26° 49′–26° 53′N) with a subtropical semi-humid monsoon climate. With a water area of 25 km^2 and an average temperature of 10.5 °C, it is one of the three highest plateau freshwater lakes in China (2171 m above sea level). It is a typical macrophytic lake ecosystem with abundant aquatic vegetation and a water depth of approximately 3 m. Samples were gathered from 9 sites on the lake in November 2017 (Fig. 1). Weining County is at the northeast region of the lake, where a large amount of domestic sewage enters the protected area (S1, S2, S3, and S4, named HP), and has relatively heavy pollution. The southwestern part of the lake has less pollution (S5, S6, S7, S8, and S9, named LP). Submerged macrophytes (*Potamogeton lucens*) were collected with a hook that was cleared with in situ water. Approximately 10 g of fresh-weight leaf samples was cut away from three to five plant replicates and transferred into a sterile 500-mL polyethylene bottle containing 400 mL of 50 mM phosphate-buffered saline (PBS, pH = 7.4) solution for epiphytic bacterial community analysis (Zhang et al. 2016). In addition to plant sampling, 1.5-L water samples from the area surrounding the sampling area for physicochemical and planktonic bacterial community analysis were collected. All leaf samples were collected in three replicates, mixed, kept with ice bags, and quickly returned to the laboratory. Dissolved oxygen and pH were determined using a portable instrument (HQ30d, HACH, USA). The physicochemical properties of the water column

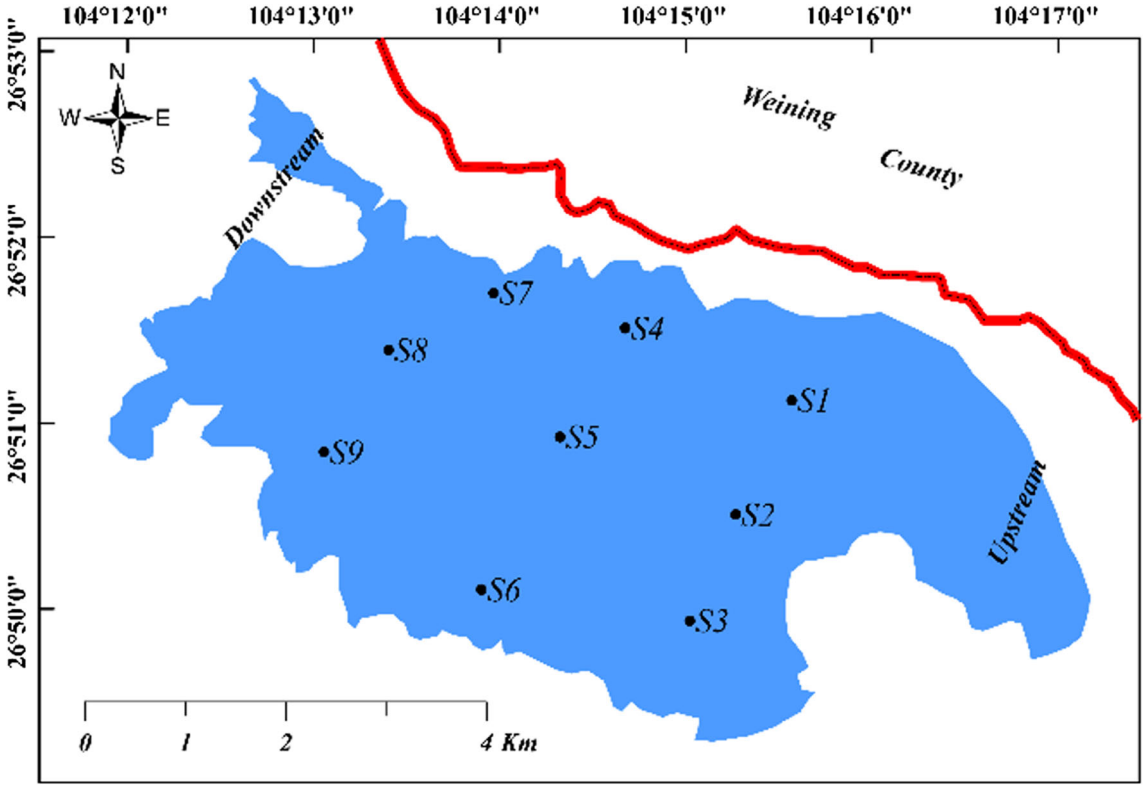

Fig. 1 Map of Caohai lake, Guizhou, showing the location of the sampling sites. Meanwhile, the main submerged plant is *Potamogeton lucens* during the season of our sampling period

were determined according to standard methods for the surface water of China (GB3838-2002). Total nitrogen was assayed using alkaline persulfate digestion and UV spectrophotometry (UV mini-1240, Shimadzu, Japan). Total phosphorus was determined with acidified molybdate to form reduced phosphor-molybdenum blue, which was measured spectrophotometrically. Ammonia nitrogen was measured using a spectrophotometric method with Nessler's reagent, chemical oxygen demand was measured using the potassium permanganate index method, and the chlorophyll a concentration was estimated spectrophotometrically after extraction in 90% ethanol.

Sample pretreatment and sequencing

Epiphytic bacteria were detached after 3 min of ultrasonication (KQ5200DE, Kunshan, China), 30 min of shaking (225 r/min) (SHZ-82A, Changzhou, China), and subsequent ultrasonication for 3 min. After complete detachment, 100 mL of mixed liquor was filtered through 0.22-μm membrane filters (Millipore Ireland Ltd., Ireland) to collect epiphytic bacteria. Planktonic bacteria were collected by filtering 500-mL water samples through 0.22-μm membrane filters. Then, all filters were stored at − 20 °C before bacterial DNA extraction (He et al. 2012). Extraction of bacterial DNA was performed according to the instructions of the FastDNA® Spin Kit for Soil (MP, USA), DNA concentration and purity were detected using a NanoDrop 2000 (Thermo Fisher Scientific, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3–V4 hypervariable regions of the bacteria 16S rRNA gene were amplified using the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Xu et al. 2016). The PCRs were performed in a 20-μL reaction 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, 10 ng of template DNA, and ddH_2O up to 20 μL. The amplification program included an initial denaturation step at 95 °C for 3 min, followed by 25 cycles at 95 °C for 30 s (denaturation), 55 °C for 30 s (annealing), and 72 °C for 30 s (extension), with a final extension at 72 °C for 5 min (Mori et al. 2013), using 2% agarose gel to recover PCR products; purification using AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) with Tris–HCl elution was performed. Quantitative detection using QuantiFluor™-ST (Promega, Madison, WI, USA) and sequencing using Illumina's MiSeq PE300 platform (Illumina, San Diego, USA) were performed. The raw sequences were quality-filtered by Trimmomatic and merged by FLASH (http://ccb.jhu.edu/software/FLASH/). Operational taxonomic units (OTUs) were clustered using 97% similarity cut-off with UPARSE version 7.1 (http://drive5.com/uparse/) with a novel "greedy" algorithm that

performs chimera filtering and OTU clustering simultaneously. The taxonomy of each 16S rRNA gene sequence was analyzed by the RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU128) 16S rRNA database using a confidence threshold of 70% (Cole et al. 2014). Then, sequences were subsampled at 27353 bp (sample minimum sequence length). In addition, alpha diversity matrix was calculated by Mothur (version 1.30.1) based on the OTU level.

Data analysis and functional predication

The sampling diagram was drawn using ArcGIS (version 10.5). Information was analyzed with different approaches. The distinction of alpha diversity between the two types of sample compared by analysis of variance (ANOVA). Data visualization was performed using R (version 3.4.3, https://www.r-project.org/). Boxplots were drawn by "ggplot2" for visualization of the physical–chemical properties of water and the bacterial alpha diversity index. The "pheatmap" package was applied to display the relative abundances of OTUs across different sample sites. Nonmetric multidimensional scaling analysis (NMDS) and the ANOSIM test based on Bray–Curtis distance were used to calculate the beta diversity matrix by the "vegan" package. Redundancy discriminant analysis (RDA) was performed using the vegan package. Linear discriminant analysis (LDA) effect size (LEfSe) was generated from Python (version 2.7) to estimate which microbiome attributes differ significantly between the two types of communities. Differences were evaluated via Kruskal–Wallis and Wilcoxon rank-sum testing, with an alpha value of 0.05 for the factorial Kruskal–Wallis test among classes and pairwise Wilcoxon rank-sum test between subclasses and a threshold for the logarithmic linear discriminant analysis score for discriminate features of 4.0.

To further analyze the biogeochemical cycle functions of microorganisms, we also applied FAPROTAX (version 1.1). FAPROTAX is a manually constructed database that maps prokaryotic taxa (e.g., genera or species) to metabolic or other ecologically relevant functions (e.g., nitrification, denitrification, or fermentation) based on the literature of cultured representatives. Functions represented in FAPROTAX focus on marine and lake biogeochemistry, and the program includes a Python script for converting OTU tables into putative functional tables based on the taxa identified in a sample and their functional annotations in the FAPROTAX database. One weakness of applying this approach to our data is the implicit assumption of FAPROTAX that if all cultured members of a taxon (cultured and noncultured) can perform that function, then all members of the taxon (cultured and noncultured) can perform that function (Louca et al. 2016; Kumar et al. 2018). Even considering this limitation, we believe that predicting putative functional groups using this approach is superior to genomic prediction approaches based on sequence homology (Louca et al. 2016; Kumar et al. 2018). Differences in functional profiles between the two groups were compared via the Wilcoxon rank-sum test using the Statistical Analysis of Metagenomic Profiles (STAMP v2.1.3, http://kiwi.cs.dal.ca/Software/STAMP) software (Parks et al. 2014).

Result

Physical–chemical characteristics of the water column

The physical–chemical properties of the water column displayed clear distinctions between HP and LP (Fig. S1). The pH of the water column ranges from 8.32 to 9.47 and generally presented as weakly alkaline, with the highest at S6 of 9.47 and the lowest at S2 of 8.32. The ammonia nitrogen concentrations in the HP and LP areas were 0.356–0.716 mg L^{-1} and 0.338–0.393 mg L^{-1}, respectively (ANOVA, $p < 0.05$). The total nitrogen values were 0.676–0.952 mg L^{-1} in the HP areas and 0.418–0.587 mg L^{-1} in the LP areas ($p < 0.05$). The chemical oxygen demand values were 7.35–8.05 mg L^{-1} (HP) and 5.54–7.20 mg L^{-1} (LP) ($p < 0.05$). The water quality of the sampling point was evaluated by the Carlson comprehensive nutrition index method, and the weights were calculated by four parameters: chemical oxygen demand, levels of chlorophyll a, total nitrogen, and total phosphorus. The results showed that the comprehensive eutrophication index (TLI) of Caohai Lake ranged from 26.61 to 38.75; the maximum appeared in the S1 sample, the average in the HP region was 36.17, and the average in the LP region was 31.75. The difference in the eutrophication index between the two regions was significant (ANOVA: $p = 0.044$).

Description of overall sequences

Subsamples normalized to the smallest sample sequence length (27,353 bp) are from 1,089,908 total high-quality sequences (with 443.89 ± 46.27 bp of average length, mean ± SD), which were binned into 976 OTUs based on 97% similarity. Phylogenetic classification results showed a total of 27 microbial phyla in all samples, including 59 classes, 115 orders, 204 families, 373 genera, 560 species, and many unclassified species (67.80%). Among the identified taxonomic groups of all samples, 88.89% phyla, 81.36% classes, 79.13% orders, 78.43% families, and 77.21% genera were shared by epiphytic bacteria and planktonic bacteria (Table S1). At the phylum level, *Proteobacteria* and *Bacteroidetes* were the most shared groups for epiphytic and planktonic bacteria, *Nitrospirae* and *SR1_Acondconditabacteria* were distinct species in epiphytic bacterial communities (EBC), and *Peregrinibacteria* is a unique component of the planktonic bacterial community (PBC). The relative number of *Proteobacteria* in the EBC

was as high as 65.83 ± 9.13% (mean ± SD, $N = 9$), but only 41.98 ± 6.82% in the PBC. *Bacteroidetes* in both communities were maintained at approximately 20%. The *Actinobacteria* in the EBC accounted for only 0.43 ± 0.27%, while those in the PBC were up to 20.11 ± 9.36%. Compared with EBC, the proportion of *Verrucomicrobia* and *Cyanobacteria* in the PBC increased (0.28 ± 0.22% vs 6.83 ± 3.81% (EBC vs PBC), 2.14 ± 1.72% vs 5.46 ± 5.67% respectively). Likewise, the largest decrease in the PBC was that of the *Firmicutes*, which decreased from 11.32 ± 7.62% in the EBC to 0.63 ± 1.05% in the PBC (Fig. 2a). This indicates that although the EBC and PBC share many different classification levels, the relative quantity distribution of the different groups varies greatly.

Significant differences in alpha and beta diversity between epiphytic and planktonic communities

The alpha diversities of epiphytic and planktonic bacterial communities were compared according to OTU (at 3% cut-off) levels. The Shannon indices ranged from 2.51 to 3.50 (EBC) and 3.36 to 4.57 (PBC) (ANOVA: $p < 0.001$). Moreover, the OTU richness Chao1 indices ranged from 410 to 577 (EBC) and 465 to 595 (PBC) ($p < 0.05$). The Shannon evenness indices ranged from 0.427 to 0.569 (EBC) and 0.570 to 0.720 (PBC) ($p < 0.001$). The phylogenetic diversity (PD) ranged from 25.64 to 46.67 (EBC) and 37.16 to 49.77 (PBC) ($p < 0.01$). We found that the alpha diversity of the EBC was significantly lower than that of the PBC (Fig. 2b). The OTU numbers of the EBC and PBC were 334.6 ± 73.83 vs 432.66 ± 45.71; the PBC's richer OTU composition confirms its high alpha diversity. Likewise, at the genus level, the relative abundance less than 1% of the phytoplanktonic bacterial communities increased to 23% vs 8.7% of the epiphytic bacteria, further confirming the high alpha diversity of the planktonic bacterial community.

Although the OTUs shared by EBC and PBC reached 65.78%, the dominant OTUs were markedly different in the two sample types (Fig. S2). Relatively abundant species (except *Flavobacterium*) of each other showed a significant inverse relationship, and the abundant species in one group were at a lower abundance level in the other group. The dominant genera (> 5% of total reads) in the EBC were *Pseudomonas* (mean value 43%, $N = 9$), *Flavobacterium* (11%), *Chryseobacterium* (8.9%), and *Exiguobacterium* (7.4%). In contrast, *Flavobacterium* (13%), *hgcI_clade* (9.1%), *Acinetobacter* (7%), and *Limnohabitans* (7%) were abundant in the PBC. To further reveal the difference between EBC and PBC species composition, an NMDS analysis based on Bray–Curtis distances was performed according to the OTU distribution (Fig. 3). The epiphytic bacterial samples were clearly separated from planktonic bacterial samples (stress = 0.056), and an ANOSIM test based on Bray–Curtis similarity distances further confirmed the separation between epiphytic and planktonic bacterial communities ($R = 0.9979$, $p = 0.001$). To further define differences, LEfse analysis at the phylum to genus level uncovered the great difference in taxonomic units between EBC and PBC (Fig. 4). There are 8 differential indicator species at the genus level for epiphytic bacteria: *Pseudomonas*, *Aeromonas*, *Janthinobacterium*, *Rahnella*, *unclassified_f_Enterobacteriaceae* (*Proteobacteria*),

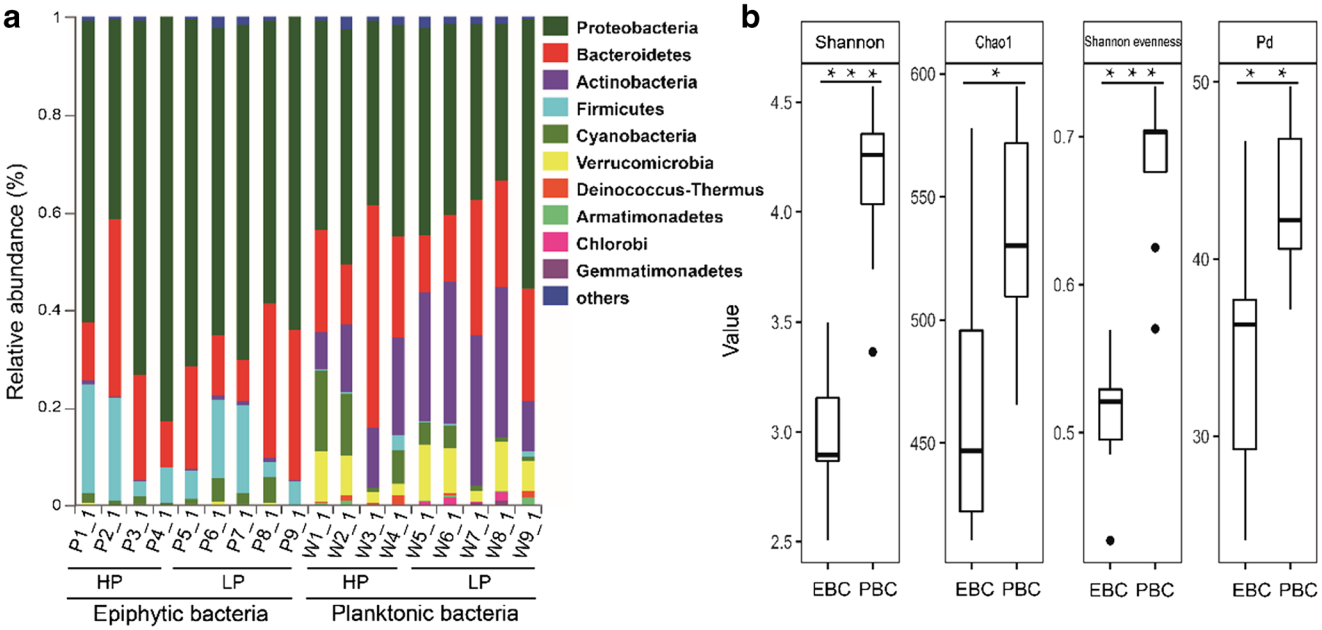

Fig. 2 **a** The percent of community abundance on phylum level in the two kinds of samples. EBC: epiphytic bacterial community, PBC: planktonic bacterial community. HP (heavy pollution): S1, S2, S3, and S4. LP (light pollution): S5, S6, S7, S8, and S9. Only groups' percentage over 0.01 of all samples were displayed; the lower were pooled together and referred to as "other". **b** The alpha diversity on the OTU level of bacterial communities. DP: phylogenetic diversity. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

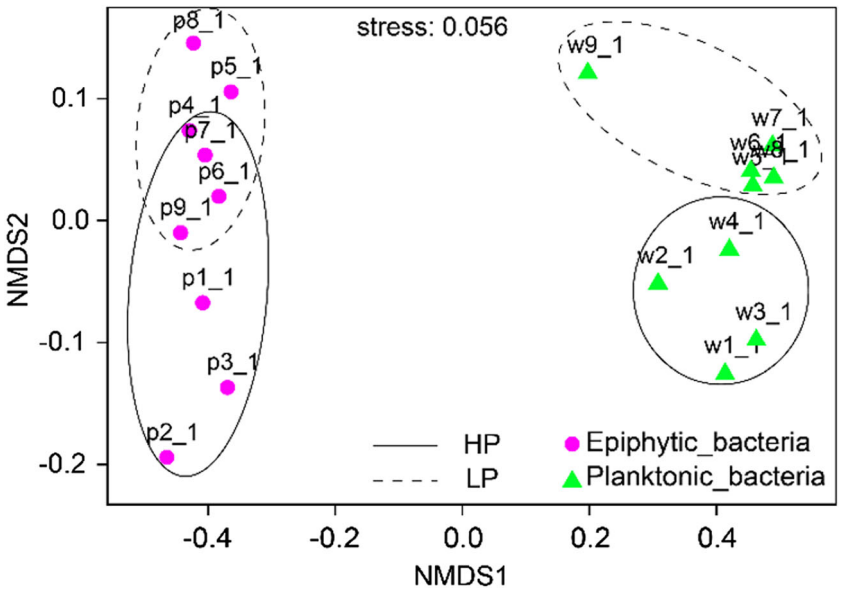

Fig. 3 NMDS similarities between all samples. The planktonic samples were clustered far apart from the epiphytic samples. ANOSIM test indicated great distinction between the two kinds of samples ($R = 0.9979$, $p = 0.001$). HP (heavy pollution): S1, S2, S3, and S4. LP (light pollution): S5, S6, S7, S8, and S9

Trichococcus, *Exiguobacterium* (*Firmicutes*), and *Chryseobacterium* (*Bacteroidetes*). The 10 differential indicator species at the genus level for planktonic bacteria were *Methylocystis*, *Limnohabitans*, *Brevundimonas*, *Acinetobacter*, *Polynucleobacter*, *norank_f_Sphingomonadaceae* (*Proteobacteria*), *hgcI_clade*, *Mycobacterium*, *CL500-29_marine_group* (*Actinobacteria*), and *Synechococcus* (*Cyanobacteria*).

Relationship between bacterial communities and environmental factors

Using RDA to link bacterial community variations with environmental parameters could also explain bacterial beta diversity. According to variance inflation factors (VIF), less than 10 environmental parameters were selected by the forward selection principle, eliminating chlorophyll a. In our results (Fig. 5), there

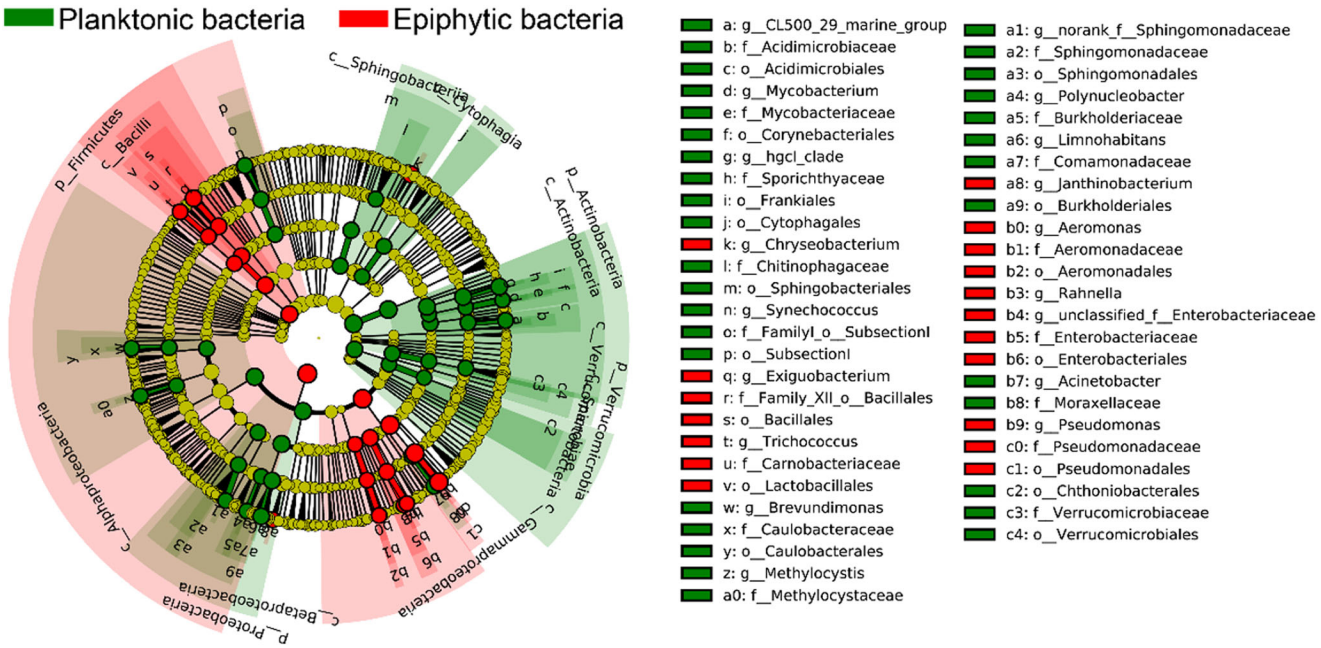

Fig. 4 Taxonomic cladogram comparing all samples categorized in the two groups by least discriminant analysis (LDA) effect size (LDA > 4, $p < 0.05$) and applied all-against-all (more strict) comparative strategy. The innermost circle represents the phylum taxonomy level, and the outer circle in turn represents the taxonomy level of class, order, family, and genus. The size of the node represents the abundance, significantly discriminant taxon nodes were colored, and the branch areas are shaded according to the highest ranked group for that taxon. When the taxon was not significantly differentially represented among the sample groups, the corresponding node was colored yellow. Highly abundant and selected taxa are indicated

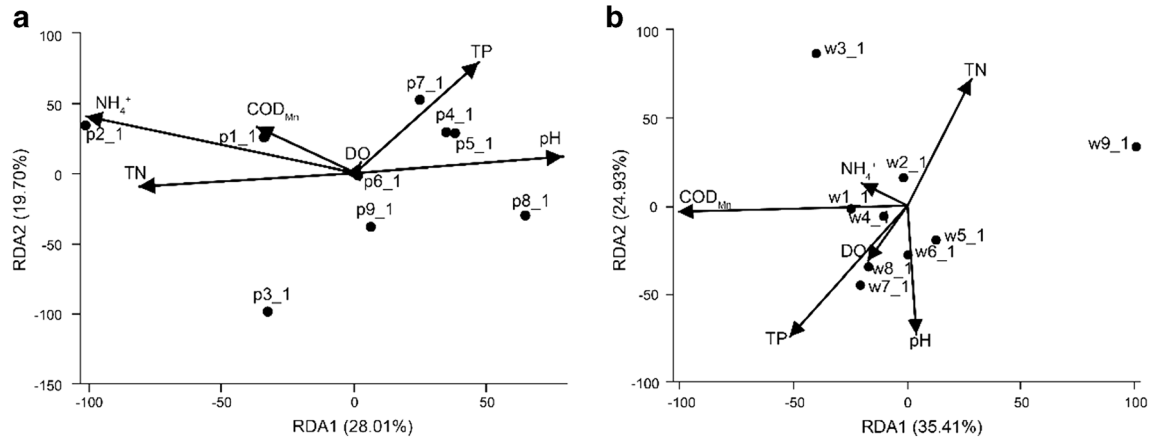

Fig. 5 RDA analysis ordination diagram of environment factors related to bacterial phylogenetic groups at the OTU level. The arrow length and direction correspond to the variance that can be explained by the environmental and response variables. The direction of an arrow indicates the extent to which the given factor is influenced by each RDA variable. The perpendicular distance between the sample sites and environmental variable axes in the plot reflects their correlations. The smaller the distance, the stronger the correlation. **a** RDA analysis of epiphytic bacteria. **b** RDA analysis of planktonic bacteria. DO: dissolved oxygen, TN: total nitrogen, TP: total phosphorus, NH_4^+: ammonia nitrogen, COD_{Mn}: chemical oxygen demand

is a close interaction between the bacterial community and the water quality, but the two communities respond differently to water quality. Chemical oxygen demand ($R^2 = 0.808$, $p = 0.037$) and total phosphorus ($R^2 = 0.604$, $p = 0.015$) were significantly associated with planktonic bacteria variations, while changes in epiphytic bacteria can be well explained by ammonia nitrogen ($R^2 = 0.817$, $p = 0.011$), total phosphorus ($R^2 = 0.604$, $p = 0.073$), pH, and total nitrogen. All selected parameters on the first two axes explained 47.71% (EBC) and 60.34% (PBC) of the bacterial community changes, suggesting that the complex microenvironment in the biofilms of submerged macrophytes drives the differences between the epiphytic and planktonic community structures.

Functional traits of epiphytic and planktonic bacterial communities

A number of microorganisms are involved in crucial biogeochemical processes and interspecies interactions. The putative functions of FAPROTAX are mainly used to further analyze the functions of biogeochemical cycles of microorganisms, especially the circulatory functions of sulfur, carbon, hydrogen, and nitrogen. Among the putative functions, a total of 48 putative biogeochemical cycle functions were identified from the epiphytic community and planktonic community. Although the most common functions of epiphytic and planktonic communities were chemoheterotrophy and aerobic chemoheterotrophy, there was a significant gene abundance difference between the two community types ($p < 0.01$) (Fig. 6). Chemoheterotrophy and aerobic chemoheterotrophy were mainly contributed by the abundant bacteria such as *Acidobacteria*, *Flavobacteria*, *Proteobacteria*, and *Verrucomicrobia*. The special biogeochemical functions in EBC were the fermentation and nitrogen cycle (especially denitrification), which included nitrate reduction, nitrate respiration, nitrogen respiration, nitrite respiration, nitrate ammonification, and nitrite ammonification. PBC is rich in autotrophic functions (phototrophy, photoautotrophy, oxygenic photoautotrophy) in animal parasites or symbionts, human pathogens all, aromatic compound degradation, methylotrophy, and hydrocarbon degradation.

Discussion

Species characteristics of epiphytic and planktonic bacterial communities

We found that the two kinds of environmental species have a higher rate of shared feature, which may be because planktonic bacteria are the main seed bank of epiphytic bacteria (Dolan 2005; Garulera et al. 2016), such as *Flavobacterium*, *Sphingomonadaceae*, *Caulobacteraceae*, and *Moraxellaceae*. They may be versatile in adapting to different kinds of habitats and represent the intercommunication of epiphytic bacteria and planktonic communities. However, our results indicated that both epiphytic and planktonic bacteria have specific microbial groups and that the community structures are significantly different.

There are obvious differences in structure between epiphytic and planktonic bacterial communities (Figs. 3 and 4 and S2). This result is consistent with studies of *Potamogeton crispus* and *Wolffia australiana* leaf epiphytic bacteria in freshwater (He et al. 2014; Xie et al. 2015). The most abundant OTUs of

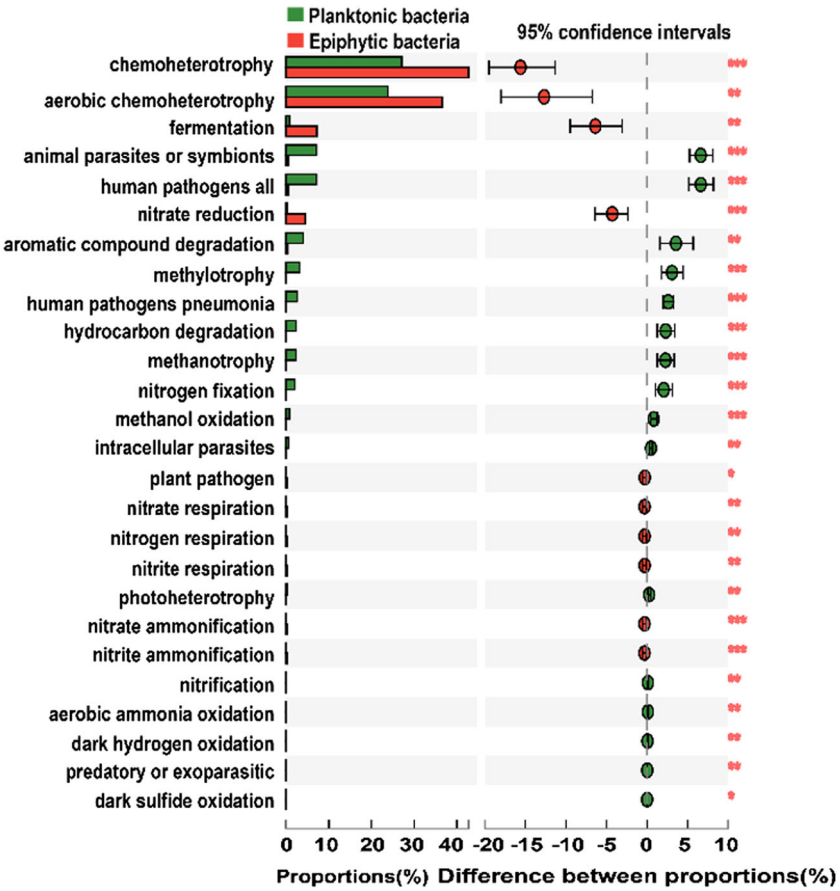

Fig. 6 Putative functions of samples only displayed the significant difference between epiphytic and planktonic communities in Wilcoxon rank-sum test. The left panel displayed the abundance ratio of different functional groups; the middle showed the percentage of functional group abundance within the 95% confidence interval; the right $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$

epiphytic bacteria were affiliated with *Pseudomonas* (Fig. S2), a genus that demonstrates substantial metabolic diversity and consequently is able to colonize a wide range of niches (Michael et al. 2005). *Pseudomonas* usually have one or more flagella to improve mobility and adhesion and can also produce a large number of extracellular polysaccharides related to biofilm formation (Hassett et al. 2002). Another abundant OTU in the epiphytic bacterial community was affiliated with *Chryseobacterium*; species in this genus can adapt to diverse habitats and have the ability to inhibit phytopathogenic fungi (Matu et al. 2019). *Exiguobacterium* and *Janthinobacterium* are important contributors to epiphytic community specificity (Fig. 4). They are able to withstand many environmental stresses (wide range of pH, cold, UV, etc.), can metabolize many types of substrates, and have strong viability (Ordoñez et al. 2013; Koo et al. 2016). Specific and abundant OTUs in the planktonic bacterial community were affiliated with *Hgcl-clade*, *Acinetobacter*, *Limnohabitans*, *Synechococcus*, and *Polynucleobacter*. These taxa are typical and dominant bacterioplankton taxa in freshwater columns, as some taxa need a strictly aerobic environment, sufficient light, etc. (Jezberová et al. 2010; Bitrian et al. 2013; Kasalický et al. 2013). They may be specialists for water columns and lack the capacity to live in certain interfaces, such as the leaf surfaces of submerged macrophytes.

Environmental regulation of epiphytic bacteria and planktonic bacteria communities

So far, there are only a few studies on the differences between epiphytic and planktonic bacterial communities. The alpha diversity of the epiphytic bacterial community on *Potamogeton crispus* leaves was significantly higher than that of the planktonic bacterial community (He et al. 2014). However, Bengtsson et al. (2012) and Xie et al. (2015) showed that the alpha diversity of epiphytic bacterial communities is significantly lower than that of planktonic bacterial communities. In this study, the alpha diversity of the epiphytic bacterial community on *Potamogeton lucens* leaves was significantly lower than that of the planktonic bacterial community ($p < 0.05$) (Fig. 2), and there were significant differences between epiphytic bacteria and planktonic bacteria communities (Fig. 3). Habitat type (microenvironmental) and aquatic environmental factors were the dominant factors driving the difference.

NMDS analysis showed that the compositions of bacterial communities were more similar within similar habitat samples (Fig. 3), strongly suggesting that a habitat microenvironment "stress filter" was involved in the compositional structuring on the leaves of submerged plants. For

example, the circadian rhythm of plants formed distinct day and night dissolved oxygen concentrations, unique products, and secretions (special carbon sources) on the surfaces of submerged plant leaves, and respiration of epiphytic algae led to an increase in the pH of the biofilm, the physical barrier of biofilm extracellular polymer (EPS) (dispersal limitation), and biological interactions (symbiosis and hostility) driving the two kinds of habitat-related bacteria distinction (Paul and Pohnert 2011; Song et al. 2015; Liu et al. 2016; Florez et al. 2017; Seymour et al. 2017). Therefore, habitat microenvironment-based species sorting is suggested as a key factor in determining bacterial community structures in aquatic environments (Filippini et al. 2009; Jones and McMahon 2009). In addition, there also exist differences in bacterial composition between HP and LP in each kind of sample, suggesting that factors other than habitat type also participate in the regulation of epiphytic and planktonic bacterial dynamics.

Physicochemical properties of the surrounding water column were critical drivers of variation observed in the epiphytic bacterial communities (Fig. 5). Epiphytic bacterial communities on *Potamogeton lucens* leaves were modulated significantly by concentrations of ammonia nitrogen and total nitrogen, implying that an increase in nitrogen nutrition can considerably influence the microbiology associated with submerged macrophytes (Yan et al. 2018). Increasing ammonia nitrogen levels can change the composition and content of submerged plant secretions, thus influencing the community structure of epiphytic bacteria (Cao et al. 2004). Total phosphorus is another important factor affecting the epiphytic community structure found on *Potamogeton lucens*. pH was a strong driving force of epiphytic bacterial communities on *Potamogeton lucens* leaves, as found in various environments (Hörnström 2002). Other factors, such as temperature, light intensity, and nutrient availability, are also important factors that influence epiphytic bacterial community structure (Bing et al. 2018; Zhao et al. 2018). This study only captured a small subset of physicochemical factors, which may lead to a lower explained proportion of environmental factors for epiphytic and planktonic bacterial community changes. Similarly, our results may be one-sided, because our sample size is low (although we collected three repetitions for each sample), and there are inherent errors in the core processes such as PCR preferences. Aquatic environmental factors cannot fully explain the variances in the epiphytic bacterial community (the explained proportion was only 47.71% by the first two axes), further demonstrating the important role of the habitat microenvironment (epiphytic biofilm) in the construction of epiphytic bacterial communities. This effect (the habitat microenvironment "stress filter") may be an important reason for the alpha diversity of epiphytic bacterial communities to be lower than that of planktonic bacteria.

The epiphytic bacterial community of submerged macrophytes has important denitrification functions

The most abundant biogeochemical cycle functions of epiphytic and planktonic bacterial communities were chemoheterotrophy and aerobic chemoheterotrophy (Fig. 6). Heterotrophic bacteria are often used as decomposers and are responsible for in situ pollution repair and degradation of organic matter in ecosystems (Wei et al. 2018). Interestingly, epiphytic bacteria have obvious fermentation and nitrogen cycle functions, especially denitrification. Similar studies have reported that biofilms attached to *Potamogeton crispus* and *Wolffia australiana* are rich in nitrogen cycle species and harbor-related functional genes (Xie et al. 2015; Yan et al. 2019). The special microenvironment and microbial composition of epiphytic biofilms drive the transfer of nitrogen. Some taxonomic groups are dominant, such as specific epiphytic bacteria that are known for biofilm formation and pollutant removal, which implied the possible divergence of functional traits between epiphytic bacteria and aquatic bacteria (Xie et al. 2015). Epiphytic biofilms can uptake a large amount of nitrogen nutrients from the water column (Levi et al. 2015). The increase in nitrogen concentration will stimulate the growth of biofilms and enhance the relative abundance of nitrifying and denitrifying genera (Levi et al. 2015; Yan et al. 2018). Due to the presence of day and night alternating oxygenic–anaerobic environments in epiphytic biofilms and the presence of many aerobic denitrifying microorganisms (Sandjensen et al. 1985; Ji et al. 2015), denitrification can be accomplished on plant leaves. For instance, the dominant genus of epiphytic bacterial communities was *Pseudomonas* and relevant studies indicated that *Pseudomonas* plays an important role in circulating nitrogen elements, mineralizing organic matter, and decomposing some organic compounds (Patel et al. 2014; Ma et al. 2015). Epiphytic bacteria of submerged plants have a high denitrification rate and compose the hot zone where denitrification occurs (Yan et al. 2018; Zhao et al. 2018). Hence, the epiphytic bacteria of submerged plants may play an important role in the denitrification of lakes, especially in macrophytic lakes, where submerged plants are widely distributed. The denitrification of epiphytic bacteria contributes to the process of water de-nitride and cannot be ignored.

Conclusions

Our research indicates the following. (i) Epiphytic bacteria and planktonic bacteria have a high ratio of shared species composition, but each has some specific microbial taxa. (ii) The community structures of epiphytic bacteria and planktonic bacteria are significantly different. Aquatic environment factors have a lower explained proportion of the changes in

epiphytic bacterial communities, indicating that the habitat microenvironment has an important influence on the construction of epiphytic bacterial communities. (iii) Epiphytic bacteria have a more prominent denitrification functions than planktonic bacteria, and the role of denitrification of epiphytic bacteria in the process of lake nitrogen removal cannot be ignored.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies performed by any of the authors with human participants.

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