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Particle size shapes the prokaryotic microbial communities in mangrove sediments: A case study of Sanya, China

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Abstract

The prokaryotic microbial communities in the sediments play crucial roles in the ecological functions of mangrove ecosystems. Therefore, the environmental factors that affect the structures of these prokaryotic microbial communities could indirectly participate in the regulation of mangrove functions, which is of great value for mangrove studies. The particle size (PS) of soils is recently demonstrated as a key environmental factor for shaping the microbial communities; however, this hypothesis has rarely been tested for mangrove environments. A case study of three tropical mangroves from Sanya, China was performed in this work to assess the influence of PS on the prokaryotic microbial community structures of bacteria, archaea, diazotrophs, and denitrifiers in the sediments. Results showed the variability in the spatial scale and the stability in the temporal scale for the prokaryotic communities, indicating that the tropical mangrove sediments could be a versatile but stable environment. Among the collected environmental factors, PS, salinity, and humidity had the greatest impacts, and PS mostly affected the structures of these prokaryotic communities based on its highest R^2 values of canonical correspondence analysis, Mental test, and linear fitting ($p \leqslant 0.05$). Furthermore, PS was positively correlated with the diversity and abundance of diazotrophic communities and negatively correlated with the abundances of methanogenic communities including Methanobacteriaceae, Methanospirillaceae, Methanoregulaceae, and Methanosaetaceae. Former studies show the increasing trend of PS caused by the rise of sea level and the intensification of human activities. Therefore, our findings indicate that PS could be a potential intermediate that links climate change and human activities with the possible ecological function migration of mangroves; meanwhile, the increase of PS could in turn release the stress of these environmental changes by increasing the abundance and diversity of the diazotrophic community and decreasing the abundances of methanogens.

Key words: mangrove sediments, particle size, prokaryotic community, environmental factors, high throughput sequencing

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1 Introduction

Mangroves are productive wetland ecosystems in the world and occur in the intertidal zones on tropical and subtropical coasts (Luo and Gu, 2018; Tue et al., 2012). This special ecosystem has attracted many research interests because of the various ecological functions of mangroves, including runoff and flood prevention, storage and recycling of nutrients and wastes, cultivation, energy conversion, and importance on science and education (Ruitenbeek, 1995). Mangroves act as the interface of land, ocean, and atmosphere and are among the centers for the flow of energy and matter among these ecosystems. As typical blue carbon ecosystems, mangroves store considerable amounts of carbon into sediments, thus making them the important regulators for climate change (Alongi et al., 2016).

The prokaryotic microorganisms in mangrove sediments including bacteria and archaea have been proven with crucial ecological roles for driving the material cycling and providing the nutrients (Li et al., 2021; Lin et al., 2019). Besides, the diazotrophic and denitrifying communities that contribute to the fixation (Flores-Mireles et al., 2007) and loss of nitrogen (Alongi et al., 1992; Hinsley, 2011) in the mangrove sediments are also important communities for the ecological balance of mangrove ecosystems. These prokaryotic microbial communities in nature can be affected and shaped by several environmental factors (Curtis and Sloan, 2004), and among them, a key factor that mostly driven the assembly of microbial communities could be found (Zhalnina et al., 2015; Liu et al., 2015). Addressing the key environmental factors for the assembly of microbial communities can facilitate us to forecast and manage the ecosystems. However, the key environmental factor that shaped the prokaryotic microbial

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communities including bacteria, archaea, diazotrophs, and denitrifiers in mangrove sediments remains poorly understanding.

The particle size (PS) is an important environmental factor for shaping the microbial communities in soils by controlling the nutrient contents (Sessitsch et al., 2001; Zhang et al., 2007), enzyme activities (Kandeler et al., 1999, 2000; Stemmer et al., 1998a, b), and air permeability (Gangi, 1985), and protective habitat space (Postma and van Veen, 1990; Oades, 1984). Based on these former studies, PS is a comprehensive influence factor for the assembly of microbial communities and has been demonstrated as a crucial environmental factor for shaping the microbial communities in terrestrial soils. Although the environmental parameters including humility (HUM), total carbon (TC), cordgrass, pH, salinity (SAL), and depth are shown to be the abiotic factors that shaped the microbial community structures in mangrove sediments (Zhou et al., 2017; Liu et al., 2017; Vanegas et al., 2019; Basak et al., 2015), we reasonably assume that PS could be another environmental factor which significantly affects the microbial communities in mangrove sediments. Unfortunately, this hypothesis has not yet been tested.

Sanya has typical tropical climate characteristics and has three mangroves located in the Yalongqingmei Bay (YLW), Sanya River (SYH), and Tielu Bay (TLG). The TLG mangroves possess almost all the mangrove trees with oldest ages, indicating that this area is the most ancient mangrove forest in China; the SYH mangrove forest is a very rare one growing along the riverbank of a brackish-water river in the city; the mangroves in YLW are the largest and best-protected ones in Sanya. Therefore, the diversity and primitiveness of environments prompt the three mangroves in Sanya to be the hot and representative areas for the studies of microbial ecology and others (Jin et al., 2019; Zhang et al., 2017; Qiu et al., 2019; Guo et al., 2018). In this study, we take the three mangroves in Sanya as a case study, aim to determine the impact degree of PS on the prokaryotic microbial communities including bacteria, archaea, diazotrophs, and denitrifiers in the sediments, and try to reveal the key environmental factors for shaping the microbial communities from multiple spatiotemporal scales.

2 Materials and methods

2.1 *Sampling and physicochemical analysis*

Sediments were sampled from two depths (surface: 0–2 cm, subsurface: 12–14 cm), three locations in Sanya, China including the YLW, SYH, and TLG (Fig. 1), and four seasons (March for spring, June for summer, September for autumn, and December for winter) by using a core sampler. However, two samples from the YLW in December were missing because the sampling path was blocked by the municipal engineering unit. Therefore, a total of 22 sediment samples was collected in this study. The sample name was formatted as "location_month_depth". For example, the label "YLW_6_surf" indicated that the sample was collected from the surface sediments of YLW mangroves in June, whereas the label "TLG_9_sub" indicated that the sample was collected from the subsurface sediments of TLG mangroves in September.

Approximate 500 g of bulk sediments were collected in the core area of the forest. The sediments were taken by using a sterilized medicine spoon and were placed into several sterilized centrifuge tubes at each site. The samples were stored in dry ice. After returning to the lab, the samples were repackaged in a super clean bench for further analysis. Thirteen environmental abiotic factors, namely, SAL, sediment temperature (TEMP), pH, total nitrogen (TN), ammonia nitrogen (AN), nitrate-nitrogen

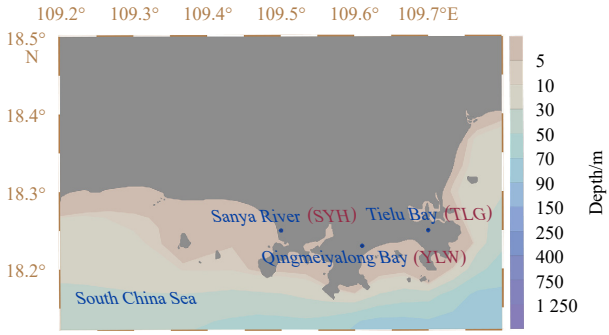

Fig. 1. The sampling sites of this study in Sanya, China. The surface and subsurface sediments were collected from the Yalongqingmei Bay (YLW), Sanya River (SYH), and Tielu Bay (TLG) mangroves in four seasons.

(NN), total phosphorus (TP), TC, organic carbon (OC), HUM, PS, monthly mean temperature (MMT), and monthly mean precipitation (MMP), were collected. The SAL of sediments was determined using a handheld SAL meter (ATAGO, Japan). The HUM was defined as the percentage of water content in the wet sediment samples. The MMT and MMP were queried at https://www.wunderground.com/history/. The Nicomp 380 laser particle size analyzer (PSS, USA) was used to determine the sediment PS. The pH and TN, TP, TC, AN, NN, and OC contents were measured by Qingdao Hengli Testing Co., Ltd. (China) following the national standards of China. The TEMP was measured using an alcohol thermometer *in situ*. All measured values were derived from three biological replicates, and the mean values were calculated and used for further analyses.

2.2 *DNA extraction, high-throughput sequencing, and data processing*

DNA extraction was performed using the PowerSoil DNA isolation kit (MO BIO, USA). For each sample, 0.5 g sediment was used for DNA extraction, and the procedures were strictly operated in three replicates in accordance with the kit instructions. The repeated DNA from the same sample was pooled to avoid extraction bias. The DNA quality was determined using 1% agarose gel electrophoresis, and the DNA concentrations extracted from the samples were also measured using the Nanodrop 2000 (Thermo, USA).

Subsequently, 515F and 806R primers (Fan et al., 2014) with barcodes were used to amplify the V4 regions of 16S rRNA genes from bacteria and archaea, cd3aF and R3cd primers (Rösch et al., 2002) were used to amplify the nirS gene, and nifH-F and nifH-R primers (Dong et al., 2014) were used to amplify nifH genes. Amplicon sequences were amplified using the TransStart FastPfu DNA polymerase (TransGen, China) with the following components: 4 μL FastPfu buffer, 2 μL dNTPs (2.5 mmol/L), 0.8 μL forward primer (5 μmol/L), 0.8 μL reverse primer (5 μmol/L), 0.4 μL FastPfu polymerase, 0.2 μL albumin from bovine serum, 10 ng DNA, and double distilled water to obtain a total volume of 20 μL. The amplification reaction was performed using the ABI GeneAmp® 9700 PCR device (ABI, USA), and the PCR program was as follows: 1 cycle of preheating at 95°C for 3 min; 27 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s; 1 cycle of final extension at 72°C for 10 min; and storage at 10°C until halted by the user. Purified amplicons were pooled in equimolar and paired-end sequenced (2×300) on the Illumina MiSeq platform (Illumina, San Diego, USA) in accordance with the standard protocols by Majorbio

Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Raw fastq files were quality-filtered using the Trimmomatic and merged using the FLASH on the basis of the following criteria: (1) the reads were truncated at any site that received an average quality score <20 over a 50 bp sliding window; (2) sequences with overlap longer than 10 bp were merged in accordance with their overlap with mismatch no more than 2 bp; (3) the sequences of each sample were separated in accordance with the barcodes (exact matching) and Primers (allowing two nucleotide mismatching), and reads containing ambiguous bases were removed. Raw sequencing data in this study were deposited in the NCBI SRA database under the BioProject Numbers of PRJNA484097 (bacterial and archaeal communities), PRJNA484108 (diazotrophic community), and PRJNA484114 (denitrifying community).

Operational taxonomic units (OTUs) with 97% (bacteria and archaea) and 95% (diazotrophs and denitrifiers) similarity cutoffs were clustered using the UPARSE (version 7.1, http://drive5.com/uparse/) with the novel "greedy" algorithm that performed chimera filtering and OTU clustering simultaneously. The taxonomic information of the amplicon sequences was analyzed using the Ribosomal Database Project classifier algorithm (http://rdp.cme.msu.edu/) against the Silva 16S rRNA (bacteria and archaea) and FunGene (diazotrophs and denitrifiers) databases with a confidence threshold of 70%.

2.3 *Statistical analysis*

The alpha diversity indices were calculated using the picante package of R and visualized using the ggpubr package of R. The bar graphs of bacterial, archaeal, and fungal communities were generated in the R software in accordance with the result of the Qiime processing. Canonical correlation analysis (CCA) was used to evaluate the influences of environmental factors on the microbial diversity patterns. The "cca()" function in the ade4 package of R was used to execute this analysis. The "vif.cca()" function in R was used to reveal the variance inflation factor (VIF) values of abiotic environmental factors, and the factors with VIF $\geqslant$ 10 were removed until all the VIF values of abiotic factors were less than 10. The principal component analysis (PCA) was conducted using the "principal()" function in the psych package of R, and the PC1 values were obtained from the PCA results. The linear equation fitting was used to determine the most important shaping factors for microbial communities by using the "lm()" function with the "singular.ok=FALSE" parameter to void a singular fit. The factors with VIFs less than 10 were fitted separately with the PC1 values from PCA results. The correlation coefficient and significance were analyzed and visualized using the "corrgram()" function in the corrgram package of R with the Pearson method to display the relationship among the environmental factors. Analysis of Similarities (ANOSIM), Adonis, and Mantel tests were performed using the "anosim()", "adonis()", and "mantel.rtest()" functions, respectively, in R with the Bray–Curtis distance and 999 permutation tests, and "metaMDS()" function in vegan package of R was used to perform the nonmetric multidimensional scaling (NMDS) analysis.

3 Results

3.1 *The correlation among the environmental factors*

A total of 13 environmental factors, including SAL, TEMP, pH, TN, AN, NN, TP, TC, OC, HUM, PS, MMT and MMP, were measured in this study. SAL, PS, and MMP violently fluctuated among the seasonal samples. PS was significantly (Pearson, $p \leqslant 0.05$) and negatively correlated with nitrogen (TN, AN, and NN) and phosphorus (TP) contents and HUM (Fig. S1).

3.2 *Compositions of the prokaryotic microbial communities*

Proteobacteria was the dominant phylum for the bacterial community in all samples, and the bacterial phyla Bacteroidetes, Chloroflexi, Cyanobacteria, Planctomycetes, Acidobacteria, Firmicutes, Verrucomicrobia, Nitrospirae, Latescibacteria, Spirochaetae, Actinobacteria, and Gemmatimonadetes were detected in all sediment samples (Fig. S2). For the archaeal community, Euryarchaeota, Woesearchaeota_DHVEG-6, Bathyarchaeota, and Thaumarchaeota were the dominant phyla in the samples (Fig. S2). Proteobacteria and Firmicutes were the dominant diazotrophic phyla (Fig. S3) and Proteobacteria was the dominant denitrifying phylum in the samples (Fig. S4).

3.3 *Alpha-diversity indices of the prokaryotic microbial communities*

The indices of Ace and Chao and the index of Shannon were selected to describe the abundance and diversity of the prokaryotic microbial communities in the mangrove sediments, respectively. The depths significantly affected the abundance and diversity of bacterial community (ANOVA, $p \leqslant 0.05$; Fig. S5a). The abundance and diversity of archaeal community were significantly affected by locations (ANOVA, $p \leqslant 0.05$; Fig. S5b). The locations affected the abundance and diversity of diazotrophic community; meanwhile, the depths can only affect the abundance instead of the diversity of diazotrophic community (ANOVA, $p \leqslant 0.05$; Fig. S5c). The depth affected the abundance and diversity of denitrifying community in the samples (ANOVA, $p \leqslant 0.05$; Fig. S5d). Consistently, the seasons of sampling had no significant influence on the abundances and diversities of bacterial, archaeal, diazotrophic, and denitrifying communities (ANOVA; Figs S5a–d).

3.4 *The influences of spatiotemporal scales on the structures of prokaryotic microbial communities*

The results of NMDS showed the clear clusters for the samples from the same location or depth, indicating that the locations and depths could affect the structures of bacterial, archaeal, diazotrophic, and denitrifying communities in mangrove sediments (Fig. 2). Furthermore, ANOSIM also demonstrated that the locations could significantly affect the structures of bacterial, archaeal, diazotrophic, and denitrifying communities ($p \leqslant 0.01$; Table 1). The depths could significantly affect the community structures of bacteria and diazotrophs ($p \leqslant 0.05$; Table 1) but did not influence the archaeal and denitrifying communities. Similarly, the seasons could not significantly impact the prokaryotic microbial communities in mangrove sediments.

3.5 *Assessment of the influences of environmental factors on the alpha-diversities and structures of prokaryotic microbial communities*

The environmental factors including TN, NN, TC and MMT with the VIF values larger than 10 were removed, and CCA, Mantel test, and linear fitting were performed to assess the influence degree of the remaining environmental factors on the structures of prokaryotic microbial communities in mangrove sediments. Consistently, PS possessed the highest R^2 values in these models for the prokaryotic microbial communities including bacteria, archaea, diazotrophs, and denitrifiers ($p \leqslant 0.05$; Fig. 3). In addition to PS, SAL and HUM were the environmental factors who also had higher R^2 values in the results from CCA, Mantel test, and

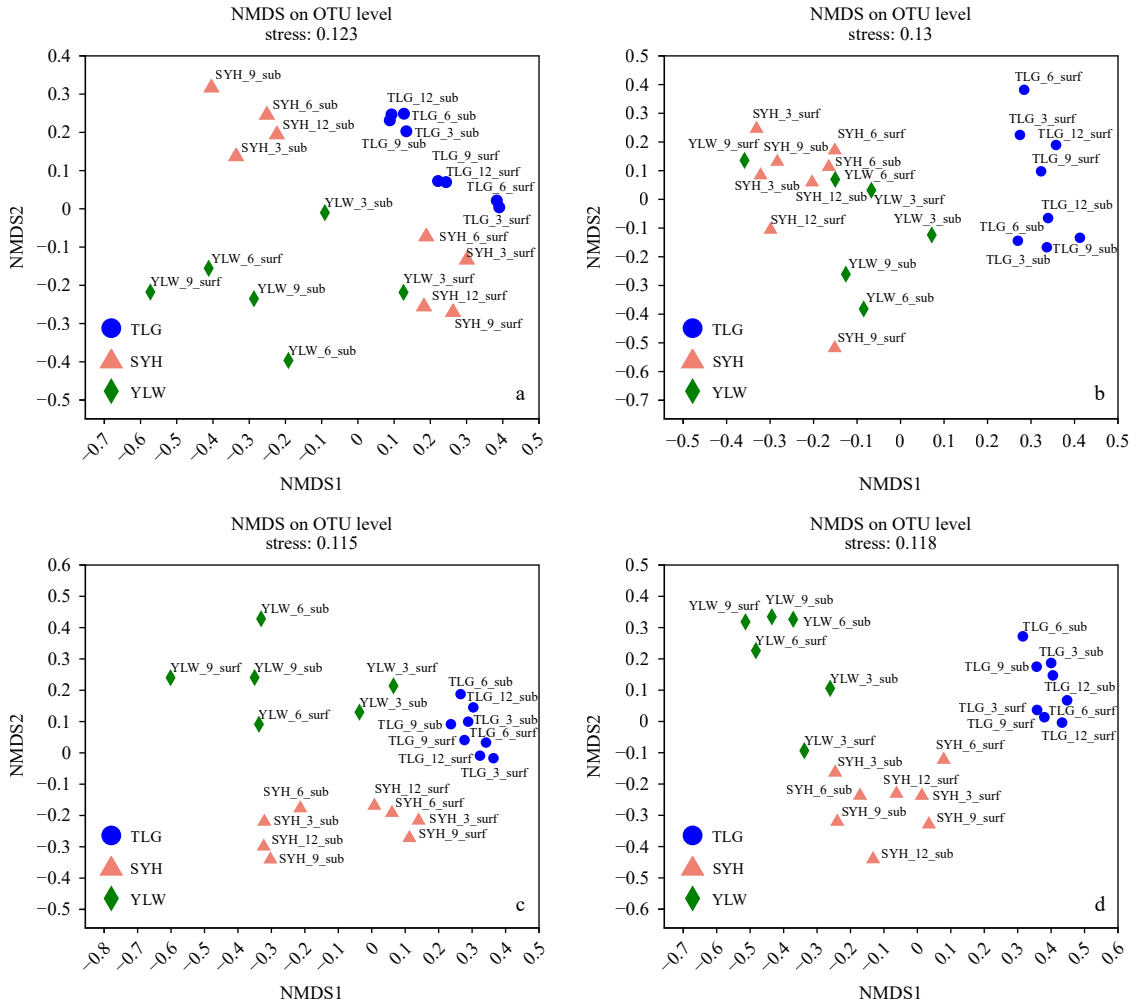

Fig. 2. Results of nonmetric multidimensional scaling (NMDS) analysis of the community structures of bacteria (a), archaea (b), diazotrophs (c), and denitrifiers (d) in sediment samples. TLG is the abbreviation of the Tielu Bay mangrove; YLW, Yalongqingmei Bay mangrove; SYH, Sanya River mangrove.

Table 1. The R^2 values of ANOSIM analysis for the prokaryotic microbial communities in the samples from different spatiotemporal scale

Spatiotemporal scales	Bacteria	Archaea	Diazotrophs	Denitrifiers
Location	0.508 2**	0.574 6**	0.811 4**	0.948 3**
Depth	0.094 3**	0.100 1	0.155 1*	−0.034 9
Season	−0.100 4	−0.105 1	−0.074 3	0.084 4

Note: * represents the p values $\leqslant$0.05 and $\geqslant$0.01; ** represents the p value <0.01.

linear fitting than other factors ($p\leqslant0.05$; Fig. 3). Except for PS, SAL and HUM, AN and TP possessed the higher R^2 values among the remaining environmental factors ($p\leqslant0.05$; Fig. 3). Similarly, PS also had a positive impact on the alpha-diversities of bacterial, archaeal, and diazotrophic communities. However, no apparent influence of PS was found on the indices of Ace, Chao, and Shannon of denitrifying community (Fig. 4).

3.6 *The correlation between the environmental factors and the abundances of prokaryotic microbial communities*

The environmental factors showed a high correlation with the abundances of microbial communities on the level of family, especially the variables of PS, HUM, and SAL. PS was significantly correlated with the abundances of 15 bacterial, 10 archaeal, 12 diazotrophic, and 6 denitrifying families. SAL was significantly correlated with the abundances of 15 bacterial, 4 archaeal, 10 diazotrophic, and 4 denitrifying families. HUM was significantly correlated with the abundances of 15 bacterial, 10 archaeal, 8 diazotrophic, and 3 denitrifying families (Pearson, $p\leqslant0.05$; Figs S6–S9). For instance, PS and SAL were positively and HUM was negatively correlated with the abundances of Rhodocyclaceae and Comamonadaceae from bacterial families (Pearson, $p\leqslant0.05$; Fig. S6). PS was significantly and negatively correlated with the abundances of Methanobacteriaceae, Methanospirillaceae, Methanoregulaceae (Fig. S7), and Methanosaetaceae (Pearson, $p\leqslant0.05$; Figs S7 and S8). Besides, a negative correlation was observed in most denitrifying families that were significantly correlated with PS (Pearson, $p\leqslant0.05$; Fig. S9).

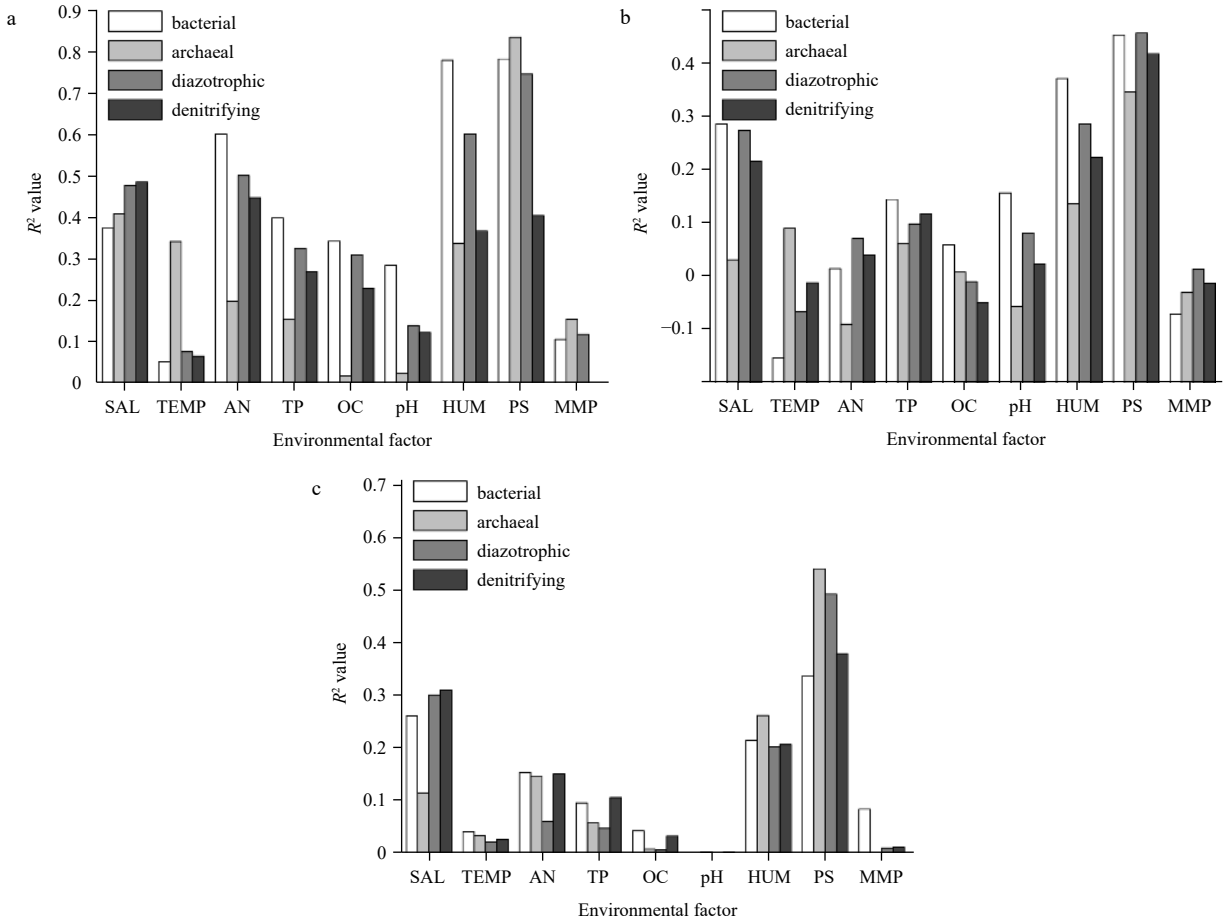

Fig. 3. The values of R^2 in the results of Canonical correlation analysis (CCA) (a), Mantel test (b), and linear fitting (c) for the impact assessment of environmental factors on the bacterial, archaeal, diazotrophic, and denitrifying communities in sediment samples. SAL is the abbreviation of salinity; TEMP, sediment temperature; AN, ammonia nitrogen; TP, total phosphorus; OC, organic carbon; HUM, humility; PS, particle size; MMT, monthly mean temperature.

4 Discussion

4.1 *The structures of prokaryotic microbial communities in tropical mangrove sediments are stable against time-varying*

Comprehensive information for the biotic and abiotic processes across space and time needs to be established to fully understand ocean-related ecosystems (Sunagawa et al., 2020). In this work, we selected the communities of bacteria, archaea, diazotrophs, and denitrifiers in mangrove sediments to investigate the influences of spatiotemporal variation on these typical prokaryotic communities. The results demonstrated that the locations significantly affected the alpha-diversities of archaeal and diazotrophic communities (Pearson, $p \leqslant 0.05$; Figs S5b, c), and also influenced the structures of all these communities (Fig. 2, Table 1); meanwhile, depths served as another scale to affect the communities of bacteria, denitrifiers (Figs S5a, d), and diazotrophs (Table 1) in mangrove sediments, therefore indicating that the distribution of prokaryotic microbial communities in mangrove sediments could be highly stratified and territorialized even in a fairly close sampling range.

We failed to detect significant differences among the seasonal samples, indicating the weak seasonal influence on the prokaryotic microbial communities. The influence degree of seasonality on the mangrove microbial community structures remains controversial. The previous study (Zhou et al., 2017) demonstrates the weak influence of seasonality on bacterial and archaeal community structures in mangrove sediments due to the stable environment of tropical area; however, it is inconsistent with other studies (Behera et al., 2019; Basak et al., 2015). The current study has drawn the same conclusion and further proofed that the seasonal temporal scale has no significant effect on bacterial, archaeal, diazotrophic, and denitrifying community structures in tropical mangrove sediments ($p \leqslant 0.05$), supporting the previous result (Zhou et al., 2017). Overall, the current work indicates the variability in spatial scale and the stability in the temporal scale of the microbial communities in tropical mangroves in Sanya. This characteristic could ensure the diversity of microbial functions in different areas of mangroves and guarantee the stability of the microbial functions against time-varying, suggesting a versatile but stable environment in tropical mangrove sediments.

4.2 *PS serves as a crucial environmental factor for shaping the prokaryotic microbial communities in tropical mangrove sediments*

The non-nutritive factors including PS, SAL, and HUM were more influential on the assembly of prokaryotic microbial communities than the nutritive ones including AN, NN, TN, TP, OC and TC (Fig. 3). SAL and HUM were closely related with the osmotic pressure (Wang et al., 2018) and O_2 concentration (Flores-

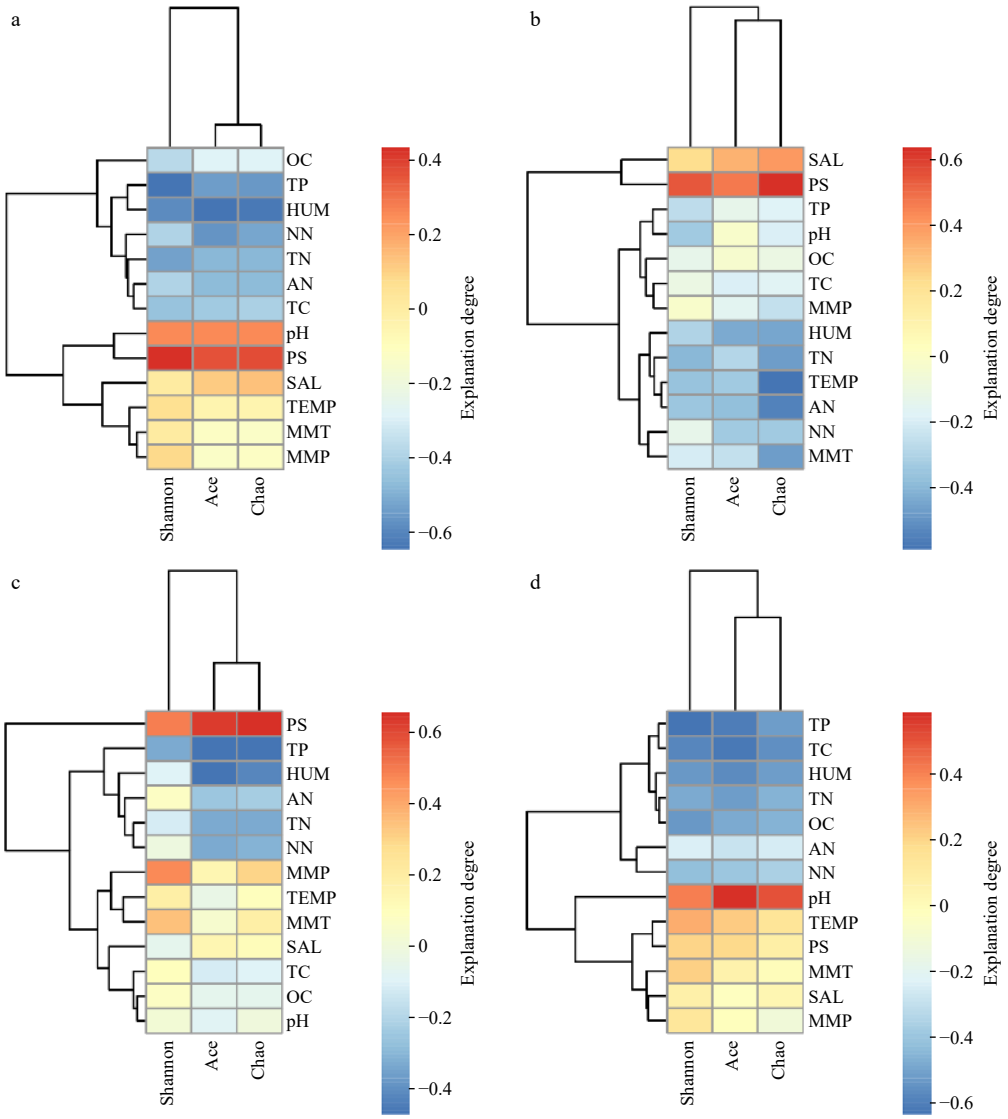

Fig. 4. Pearson correlations between the environmental factors and the alpha-indices of bacterial (a), archaeal (b), diazotrophic (c), and denitrifying (d) communities in sediment samples. SAL is the abbreviation of salinity; TEMP, sediment temperature; AN, ammonia nitrogen; NN, nitrate-nitrogen; TN, total nitrogen; TP, total phosphorus; OC, organic carbon; TC, total carbon; HUM, humility; PS, particle size; MMT, monthly mean temperature; MMP, monthly mean precipitation.

Mireles et al., 2007) in the sediments, respectively. Therefore, these two factors could affect the prokaryotic community in mangrove sediments. Besides, the limited nutrients in mangroves, AN and TP, possessed a higher impact on the prokaryotic communities among these nutritive factors (Fig. 3), which was consistent with the situation of nitrogen and phosphorus deficiency in mangroves (Boto and Wellington, 1983; Feller et al., 2003a, b).

Of the 13 measured environmental factors, PS was found to be the most crucial variable for shaping the communities of bacteria, archaea, diazotrophs, and denitrifiers in mangrove sediment samples based on the results of CCA, Mantel test, and linear fitting (Fig. 3). The importance of PS for shaping the prokaryotic microbial communities in mangrove sediments has been rarely reported until now, although PS has been widely studied in terrestrial soil-related ecosystems (Sessitsch et al., 2001; Zhang et al., 2007). Before our work, PS is only shown to be the factor that affected the diversity and the abundance of laccase-like bacteria with the order of sand>clay>silt in Mai Po mangrove sediments of China (Luo et al., 2015). This work enhances the value of PS for the assembly of not only laccase-like bacteria but also four typical microbial communities including bacteria, archaea, diazotrophs, and denitrifiers in mangrove sediments.

This study demonstrated PS was the most crucial variable for shaping the prokaryotic microbial communities among the 13 measured environmental factors, which is similar to former results from other studies on terrestrial soil-related ecosystems (Haines et al., 2019; Xia et al., 2019; Liu et al., 2019). The studies on terrestrial soil-related ecosystems show that the diversity of microbial communities decrease with the increase of PS due to the less enzyme activities (Kandeler et al., 1999; Stemmer et al., 1998a), nutrients (Zhang et al., 2007), and higher concentrations of heavy metal pollutions (Chen et al., 2014) in the sediments with larger PS. Our work also found that PS was significantly and negatively correlated with the contents of AN, NN, TN, and TP (Fig. S1), the limited nutrients in mangroves (Boto and Wellington, 1983; Feller et al., 2003a, b). However, the influence of the increasing PS on the diversities and abundances of microbial communities in mangrove sediments is different from the former

studies on terrestrial soil-related ecosystems. The abundances and diversities of bacterial, archaeal, and diazotrophic communities increased in mangrove sediments rather than decreased in terrestrial soils with the increase of PS (Fig. 4). We contribute this differential to the periodic inundation of mangrove eco-systems by seawater. The inundation makes oxygen become a key but limiting factor for the microbial communities in the sediments (Chambers et al., 2016), and the oxygen concentration is higher in the sediments with larger PS (Gangi, 1985), therefore increasing the diversities and abundances of bacterial, archaeal, and diazotrophic communities. Thus, the unique environment of mangroves brings special properties for the microorganisms in the sediments.

4.3 *PS could be a potential important intermediate variable for predicting the shifts of microbial communities and functions*

Global warming leads to the melting of glaciers and the thermal expansion of surface water, which eventually results in the rising of seawater level and the increase of sediment PS (Sanders et al., 2012). Another study based on an 800-year mangrove dynamic data also supports the conclusion (Punwong et al., 2018). In addition, due to the intensification of human activities such as surrounding dams, buildings, and aquaculture, a large number of engineering spoils flow into mangroves, which further increases the PS of surface sediments (Punwong et al., 2018). The PS increase has been observed in the investigation of sediments from Sanya coastal line due to these environmental changes (Mao et al., 2007).Therefore, the PS of mangrove sediments shows an increasing trend under the ecological background of climate warming and intensified human activities, which could change the structures and functions of microbial communities in mangrove sediment and further shift the ecological functions of mangrove ecosystems. Based on the result of this work, we speculate that PS could be a possible intermediate, linking climate change and human activities with the possible ecological function migration of mangroves.

In order to respond to climate change, mangroves could also slow down further global warming through PS variation. The increase of PS can promote the diversity and abundance of diazotrophic community but decrease those of the denitrifiers (Figs 4c, d, and Fig. S9), thereby improving the total AN in mangrove sediments. Mangrove ecosystems are typical nitrogen-deficient environments (Lovelock et al., 2006). Therefore, the increase of the diversity and abundance of nitrogen fixers can provide more bioavailable AN for the survival of other microorganisms and benthic flora in mangroves, which could further enhance the photosynthesis of photosynthetic bacteria and plants and fix more carbon dioxide. Besides, PS was negatively correlated with the abundances of methanogens (Figs S7 and S8) including Methanobacteriaceae (Oren, 2014), Methanospirillaceae (Yasin et al., 2015), Methanoregulaceae (Imachi and Sakai, 2015), and Methanosaetaceae (Karakashev et al., 2006), indicating the reduction of methane production from the mangroves with PS increasing. These results suggest that the increase of PS in mangroves due to climate change could, in turn, possibly release the stress of global warming through the increase input of AN and the decrease output of methane. Therefore, PS could be used as a potential important environmental variable in future studies to forecast where the mangrove functions are heading with the influences of climate change and anthropogenic activities.

5 Conclusions

This work verified the hypothesis that PS dramatically shaped the prokaryotic microbial communities including bacteria, archaea, diazotrophs, and denitrifiers in mangrove sediments through a case study of Sanya, China. However, the influence of PS on the abundances and diversities of prokaryotic microbial communities in mangrove sediments was different from other studies on terrestrial soil-related ecosystems. The increase of PS due to global warming and human activities could in turn release the stress of these environmental changes by increasing the abundance and diversity of diazotrophic community and decreasing the abundances of methanogens. In summary, PS could be a potential intermediate that linked global warming and human activities with the structures and functions of prokaryotic communities in mangrove sediments and predicted the future shift of ecological functions of mangrove ecosystems.

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Supplementary information:

Fig. S1. The correlation among 13 measured environmental factors.

Fig. S2. Bacterial and archaeal community components at phylum level.

Fig. S3. Diazotrophic community component at phylum level.

Fig. S4. Denitrifying community component at phylum level.

Fig. S5. The indices of Ace and Shannon of bacterial (A), archaeal (B), 43 diazotrophic (C), and denitrifying (D) communities in mangrove sediments.

Fig. S6. The correlation between environmental factors and the abundances of 49 bacterial community on the family level.

Fig. S7. The correlation between environmental factors and the abundances of 55 archaeal community on the family level.

Fig. S8. The correlation between environmental factors and the abundances of 61 diazotrophic community on the family level.

Fig. S9. The correlation between environmental factors and the abundances of 67 denitrifying community on the family level.

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