

BACTERIAL DIVERSITY IN THE SEDIMENTS OF THREE LAGOONS IN CENTRAL VIETNAM

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ABSTRACT

The prokaryotic community in sediment plays an important role in the nutrient cycles of lagoon ecosystems. However, the diversity of microbiota in sediments of Vietnamese lagoons has not been discovered. In this study, sediment samples from three lagoons in Central Vietnam were collected. The microbial community structure in the sediments was determined using Illumina sequencing of 16S rRNA at V3V4 regions. Mother software was used to analyze the data and identify the Operational Taxonomic Units (OTUs). *Proteobacteria*, *Firmicutes*, *Campilobacterota*, and *Bacteroidetes* were the major phyla in three lagoons, while *Fusobacteria*, *Verrucomicrobia*, *Acidobacteria*, *Actinobacteria*, and *Chloroflexi* were the minor. These groups of bacteria have been reported to be involved in organic metabolism cycles in sediments. Tam Giang lagoon showed more diversity in species composition when compared with Nai and Thi Nai lagoons. Class *Clostridia* were predominant in Tam Giang sediment (46%), which may indicate the presence of organic sewage in the environment. Thi Nai and Nai lagoons witnessed the significant presence of *Lactobacilales* and *Vibrionales*. While *Vibrionales* is an indicator of urban pollution, *Lactobacilales* and other groups of phylum *Actinobacteria* were potential materials for the screening of natural antibiotics.

Keywords: 16S rRNA, Bacterial diversity, Lagoon, Nai, Tam Giang, Thi Nai, V3V4 regions.

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INTRODUCTION

The lagoon ecosystem is an important environment for the biodiversity of coastal countries. With different types of habitats such as estuaries, swamps, grasslands, mangroves, tidal flats, etc., the lagoon shows a diverse species composition of animals and plants. In the lagoon, the sediment acts as a storage pool for organic matter, inorganic nutrients, and toxic metals larowem, nayak. The nutrients in the lagoon ecosystem are accumulated in the lagoon sediment and then gradually diffused into the environment (Forsberg, 1989). Due to the presence of high amounts of nutrients in the sediments, resident microbial communities are very dense (Ajao & Fagade, 1990; Borin et al., 2009; Chaouni et al., 2022). These microorganisms are involved in the maintenance and control of the biochemical cycle of the basic elements of life (H, C, N, O, S, and P), therefore, play an important role in the re-mineralization of nutrients (Chapelle, 1995; Herbert, 1999). The composition and diversity of microorganisms in the sediment of the lagoon have a strong influence on the conservation and sustainable development of lagoon ecosystems.

Many studies have targeted lagoon systems to explore biodiversity and develop biotechnology applications (De Vuyst & Leroy, 2007; de Wit, 2011; Garrido et al., 2011). Biodiversity in the lagoons of central Vietnam has been reported (Beleneva et al., 2007; Ha et al., 2021; Nguyen & Truong-Si, 2006), but only for the water layer. The diversity of the prokaryotic community in the sediment remains unexplored. In this study, three typical coastal lagoon systems of Central Vietnam including Tam Giang, Thi Nai, and Nai lagoon were selected to analyze the biodiversity of prokaryotic communities in the sediment of these lagoons using 16S amplicon sequencing due to their importance to the coastal ecosystem.

MATERIALS AND METHODS

Study sites and sediment sampling Tam Giang - Cau Hai system is the largest brackish water lagoon in Southeast Asia. This area

stretches for 68 km with a total water surface area of 216 km², accounting for 48.2% of the water surface area of lagoons in Vietnam. Thi Nai lagoon has an area of 50 km², 15.6 km long and 3.9 km wide, and Nai lagoon has an area of 8 km², 3.5 km long and 3 km wide. Tam Giang (16°62'02"N - 107°49'73"E), Thi Nai (13°49'44"N - 109°14'06"E) and Nai lagoons (11°37'17"N - 109°1'41"E) are distributed along the latitude, located in the north, in the middle in the south of Central Vietnam, respectively, corresponding to the provinces of Thua Thien Hue, Binh Dinh and Ninh Thuan provinces. They are all located in the tropical monsoon region, with the typical tropical coastal ecological system.

For each lagoon, 5 sites were selected for sampling, including the lagoon mouth, the middle of the lagoon, the shallow area 100 m from the shore, the estuaries, and the mangrove site. Sampling sites were described in Figure 1. At each sampling site, 4 sediment samples were taken to 30 cm depth by collecting pipes. These samples were then stored in sterile tubes, and transported into the lab with ice packs for DNA extraction. Microbial DNA in the sediment samples was extracted by PowerMax® Soil DNA Isolation Kit, following the manufacturer's instructions (MO BIO Laboratories, Carlsbad, CA, USA). The extracted DNA from each site was pooled in equal amounts to make a bulk DNA sample representing for each lagoon.

DNA extraction and 16S V3V4 amplification

The V3V4 region of 16S rRNA was then amplified by PCR reaction with primers 341F (5'-CTACGGGNGGCWGCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') (NovogeneAIT, Singapore). Each 10 µL reaction included 0.5 µL of forward primer (10 µM), 0.5 µL of reverse primer (10 µM), 0.2 µL of DMSO, 4.5 µL of Phusion Master Mix (2x), and 4.3 µL of template DNA. The PCR program for 16s rRNA gene was as follows: pre-denaturation at 98 °C for 2 min, then 30 cycles of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C and finally 5 min at 72 °C. The PCR products were then

checked by electrophoresis on 2% agarose gel. The target band was extracted from gels

using the Qiagen Gel Extraction Kit (Qiagen, Germany).

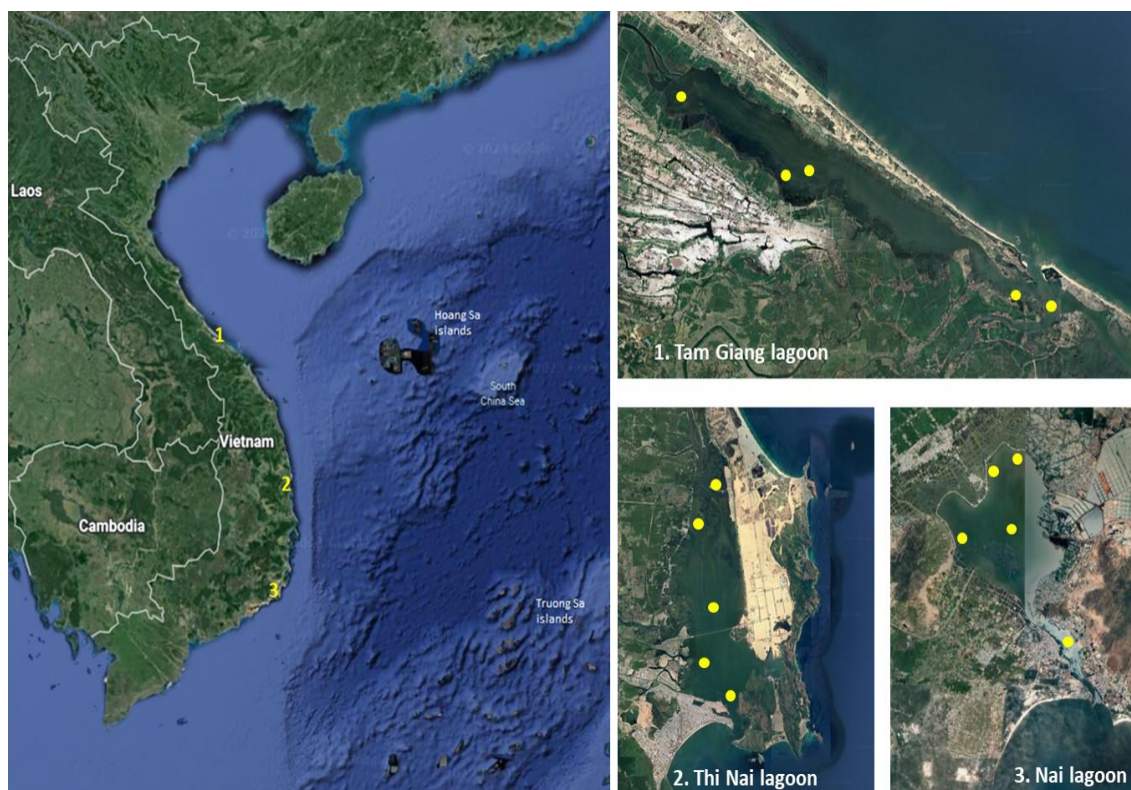


Figure 1. Sampling sites in overview (a) with three lagoons of Tam Giang (1), Thi Nai (2) and Nai (3) and positions of collected samples in each lagoon, respectively (b, c, d)

[Source: Google Earth, 2022]

Sequencing and data analysis

The sequencing library was constructed using the NEBNext Ultra DNA Library Pre® Kit for Illumina (New England Biolabs, UK) following the manufacturer's instructions and index codes were added. The quality of the library was assessed by Qubit @ 2.0 Fluorometer spectrometer (Thermo Scientific, USA) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina Novaseq 6000 platform with 250 bp paired-end reads.

Paired-end reads are assembled using the make.contig command in Schloss et al. (2009). Assembled sequence reads containing ambiguous bases and contigs longer than 500 bps were removed. Cleaned reads were then

aligned with the Silva databases version 138. The aligned reads were classified using the classify.seqs, cluster.split and classify.otus commands. The 16S Silva 138 99% OTU full-length sequences database and the RPD version 18 training dataset (July, 2020) were used for assigning sequences to Operational Taxonomic Units (OTUs) with 97% similarity. Chimera and non-bacterial sequences (Chloroplast, Mitochondria, Eukaryota, unknown) were filtered out of the data.

RESULTS AND DISCUSSION

16S rRNA amplification and sequencing characteristics

The V3V4 sequence region of the 16S rRNA gene was amplified using primers 341F

and 806R. PCR product was examined by 2% agarose gel electrophoresis, showing a single and clear band, about 450 bp in size. After sequencing, about 16,000 sequence reads were obtained for each sample. These sequences were classified by Mothur and OTUs were identified at 3% cutoff. After filtering out low-quality sequences, chimaeras and non-bacterial sequences as described earlier, about 110,000 clean sequence reads per sample, representing 70% of the original data, were assigned to OTUs. The rarefaction curves of three samples with the number of sequences reads and OTUs were displayed in Figure 2. Tam Giang lagoon showed the highest richness at the species level with 15,978 OTUs from 113,445 16S rRNA sequence reads. Meanwhile, Thi Nai and Nai lagoons only produced 8,414 and 7,115 OTUs with 122,026 and 128,944 sequence reads, respectively.

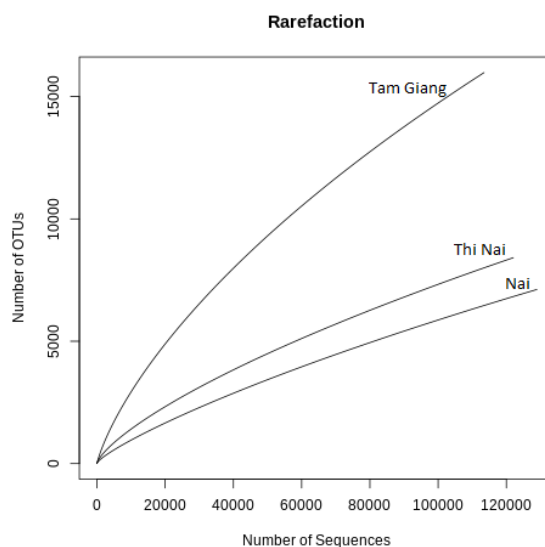


Figure 2. The number of OTUs and the corresponding number of reads in the samples of three lagoons Tam Giang, Thi Nai, and Nai were exhibited by rarefaction curves

Table 1. Characteristics of 16S rRNA sequencing

Sample ID	Number of raw reads	Number of cleaned reads	Number of OTUs
Tam Giang	160,947	113,445	15,978
Thi Nai	166,837	122,026	8,414
Nai	165,205	128,944	7,115

Composition of bacterial communities

The relative abundance of major phyla was shown in Figure 3. *Proteobacteria* were predominant in Thi Nai and Nai lagoons, followed by *Firmicutes*. Meanwhile, in Tam Giang lagoon, *Firmicutes* accounted for the most significant percentage. *Proteobacteria* accounted for 55.60%, 43.54%, and 25.35% in Nai, Thi Nai, and Tam Giang lagoons, respectively, while *Firmicutes* were 30.75%, 32.68%, and 48.13%. *Campilobacterota* was the third largest phylum in the Nai and Thi Nai lagoon samples with more than 10% per each but was found in a tiny proportion in the Tam Giang lagoon (0.09%). The phylogenetic composition at the phylum level in the Thi Nai and Nai lagoon samples was highly similar, however, there was an exception for the phylum *Bacteroidetes*. *Bacteroidetes* accounted for 7.78% in the Thi Nai lagoon,

while were less presented in the Nai lagoon (1.26%).

In Nai and Thi Nai lagoons, more than 95% of sequence reads belonging to the four main phyla: *Proteobacteria*, *Firmicutes*, *Campilobacterota*, and *Bacteroidetes*, the other phyla only accounted for a small proportion (total less than 5%). Meanwhile, the Tam Giang lagoon showed high diversity at the phylum level, with a significant proportion of *Verrucomicrobia* (2.81%), *Acidobacteria* (2.93%), *Chloroflexi* (1.86%) and *Actinobacteria* (0.82%).

The composition of bacterial communities at lower taxonomic levels (phylum, class, and order) was visualized using the krona pie charts (Figs. 4, 5, 6). In phylum *Proteobacteria*, the Tam Giang lagoon showed the most diversity at class and order levels, with the relevant presence of all 3

classes *Beta*, *Delta*, and *Gammaproteobacteria* and a small proportion of *Alphaproteobacteria*. Meanwhile, Thi Nai and Nai samples were dominated by *Gammaproteobacteria*. Delta subgroup was observed with 4% in Thi Nai lagoon, but only 1% in Nai. *Proteobacteria* is a diverse phylum that has been reported to predominate in many lagoons (Borin et al., 2009; Chaouni et al., 2022). These include bacterial species involved in the biodegradation and synthesis of amino acids (Dang & Lovell, 2000; Gupta, 2000). *Alphaproteobacteria* is known for nitrate elimination (Vincent et al., 1994; Zhang et al., 2022), while *Deltaproteobacteria*, which includes the order *Desulfobacterales*

and *Desulfuromonadales*, is responsible for sulfate and sulfur reduction (Guyoneaud et al., 1997; Guyoneaud et al., 2002). *Chromatiales*, an order belonging to *Gammaproteobacteria*, is also involved in completing the nitrogen cycle in sediments (Guyoneaud et al., 1997). These bacterial groups were found in certain proportions in the Tam Giang lagoon but in a lower proportion in the Thi Nai lagoon and a very small component in the Nai lagoon. Nai lagoon was dominated by order *Vibrionales* (41%), the group of bacteria commonly found in polluted marine areas (Garay et al., 1985). This indicated that the Nai lagoon might be affected by urban pollution more than the other two lagoons.

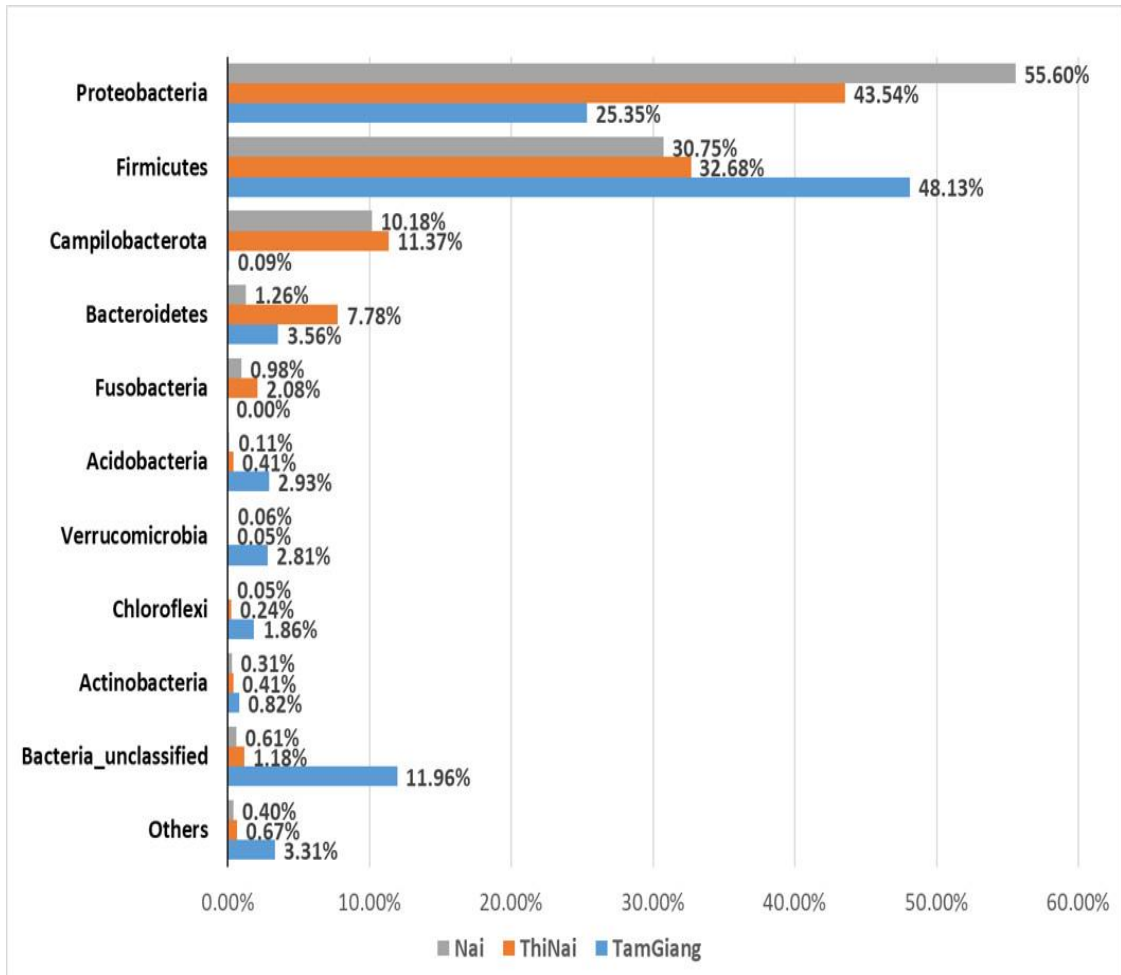


Figure 3. Relative abundance of main bacterial phyla isolated in bottom mud samples from three lagoons of Tam Giang, Thi Nai, and Nai

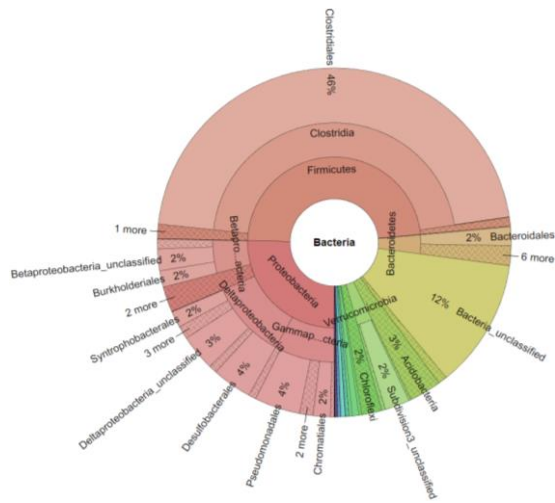


Figure 4. Taxonomic profile of the bacterial community in Tam Giang lagoon visualized using KRONA

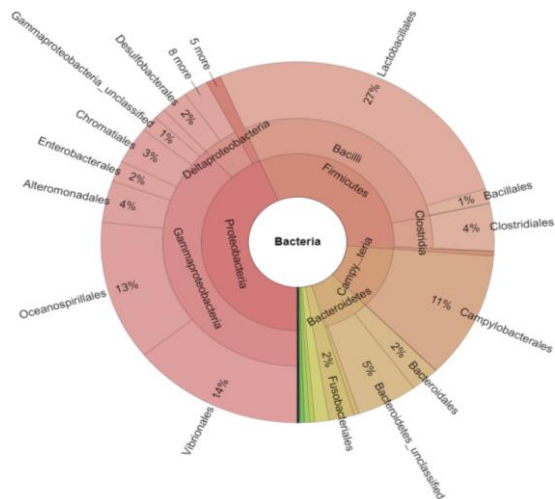


Figure 5. Taxonomic profile of the bacterial community in Thi Nai lagoon visualized using KRONA

The composition of bacterial communities at lower taxonomic levels (phylum, class and order) was visualized using the krona pie charts (Figs. 4, 5, 6). In phylum *Proteobacteria*, the Tam Giang lagoon showed the most diversity at class and order levels, with the relevant presence of all 3 classes *Beta*, *Delta* and *Gammaproteobacteria* and a small proportion of *Alphaproteobacteria*. Meanwhile, Thi Nai and

Nai samples were dominated by *Gammaproteobacteria*. Delta subgroup was observed with 4% in Thi Nai lagoon, but only 1% in Nai. *Proteobacteria* is a diverse phylum that has been reported to predominate in many lagoons (Borin et al., 2009; Chaoui et al., 2022). These include bacterial species involved in the biodegradation and synthesis of amino acids (Dang & Lovell, 2000; Gupta, 2000). *Alphaproteobacteria* is known for nitrate elimination (Vincent et al., 1994; Zhang et al., 2022), while *Deltaproteobacteria*, which includes the order *Desulfobacteriales* and *Desulfuromonadales*, is responsible for sulfate and sulfur reduction (Guyoneaud et al., 1997; Guyoneaud et al., 2002). *Chromatiales*, an order belonging to *Gammaproteobacteria*, is also involved in completing the nitrogen cycle in sediments (Guyoneaud et al., 1997). These bacterial groups were found in certain proportions in the Tam Giang lagoon, but in a lower proportion in the Thi Nai lagoon and a very small component in the Nai lagoon. Nai lagoon was dominated by order *Vibrionales* (41%), the group of bacteria commonly found in polluted marine areas (Garay et al., 1985). This indicated that the Nai lagoon might be affected by urban pollution more than the other two lagoons.

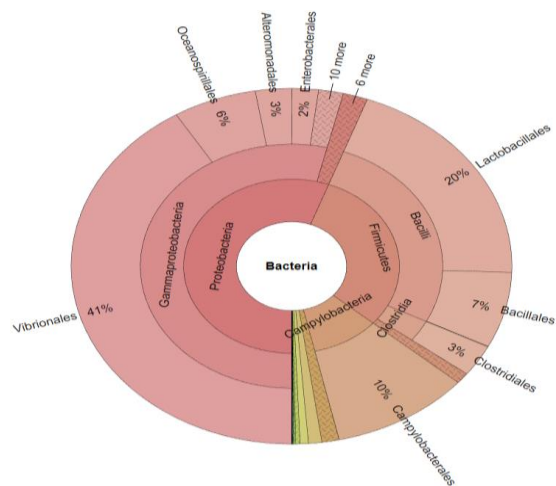


Figure 6. Taxonomic profile of the bacterial community in Nai lagoon visualized using KRONA

Phylum *Firmicutes* showed less diversity when compared with *Proteobacteria*. The composition in this phylum was significantly different between the Tam Giang lagoon and the other two lagoons in Tam Giang lagoon, phylum *Firmicutes* was presented by class *Clostridia* and order *Clostridiales* (46%), while in the Thi Nai and Nai samples, *Firmicutes* was dominated by class *Bacilli* and order *Lactobacilales* (27% and 20%). *Clostridiales* are involved in the anaerobic decomposition of organic matter (Mckew et al., 2013; Skanavis & Yanko, 2001), and the presence of this bacterial group in the sediments of Tam Giang lagoon was a potential indicator for the sewage solids from the surrounding animal and aquaculture farms (Skanavis & Yanko, 2001). In contrast, Thi Nai and Nai lagoons showed a high percentage of *Lactobacilales* (27% and 20%, respectively). This order belonged to Lactic acid bacteria (LAB), the group is known for their ability to synthesize antimicrobial substances (Alakomi et al., 2000; De Vuyst & Leroy, 2007). The isolation of *Lactobacillus* strains from sediments has received increasing attention in recent years, due to their potential applications as natural preservatives in the food industry (Issazadeh et al., 2012; Wu et al., 2021).

Most OTUs of *Campilobacterota* phylum were classified as order *Campylobacterales* and most members of *Bacteroidetes* belonged to the order *Bacteroidales*. *Campylobacterale* were responsible for sulfide-oxidizing in Nai and Thi Nai lagoons, while *Bacteroidales* might relate to the high level of organic matter (Mann et al., 2013) (fecal pellets, algae and invertebrates) in Tam Giang and Thi Nai lagoons. Other phyla like *Fusobacteria*, *Verrucomicrobia*, *Acidobacteria* and *Chloroflexi* have been reported to be involved in the degradation of biopolymers (Cardman et al., 2014; Pelikan et al., 2021; Zhao et al., 2004), methane (Greening et al., 2015) and halogenated solvents (Thiel et al., 2019). Some strains of *Actinobacteria* have been isolated from sediments for the discovery of

new natural antibiotics (Hussein et al., 2018; Ribeiro et al., 2020; Savitha et al., 2022).

CONCLUSION

This study, for the first time, investigated the diversity of microbial communities in the sediments of three important lagoons in Central Vietnam. *Proteobacteria* and *Firmicutes* were the two phyla that accounted for the largest proportion in all three lagoons, however, there were significant differences in the composition of each phylum. The microbial composition in the sediment of these lagoons was closely related to the carbon, nitrogen and sulfur cycles in the ecosystem, and reflected the presence of organic matter and urban pollutants in the environment. The microbial resources in the sediment of Tam Giang, Thi Nai and Nai lagoons also showed potential application for the identification and development of new bioactive natural products.

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