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Seasonal variation of the bacterial diversity residing in sediments of Tam Giang lagoon, Central Viet Nam

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Abstract. Bacterial communities within sediment play a critical role in lagoon ecosystem nutrient cycling. Moreover, the sediments diversity of Vietnamese lagoons remains largely unexplored. Tam Giang Lagoon is one of the largest lagoons in the world, the largest lagoon in Southeast Asia. Therefore, the lagoon contains very high biodiversity potential. This study aimed to analyze variation between dry and wet seasons in bacterial diversity within the Tam Giang Lagoon, Central Vietnam. Sediment samples were collected for subsequent Illumina sequencing of the V3-V4 regions of the 16S rRNA gene to elucidate community composition. Mothur software was ultilized for the analysis and identification the Operational Taxonomic Units (OTUs). *Pseudomonadota* was the most dominant phylum in the lagoon in both seasons, followed by *Verrucomicrobiota*, *Acidobacteriota*, and *Chloroflexota*. Interestingly, there are bold variations of bacterial abundance between dry and wet season samples at all taxonomic levels. This study shows that different climatic conditions between the two seasons can change the physical and chemical environment in the lagoon, thereby greatly affecting the bacterial population structure. Thus, based on the results of this study, it is possible to identify beneficial bacterial populations in each season to optimize the breeding environment, improve water quality, reduce environmental pollution, and increase biological productivity in the lagoon ecosystem.

Keywords: 16S rRNA, bacterial diversity, *Pseudomonadota*, Tam Giang lagoon, V3-V4 regions.

Classification numbers: 3.1.2, 1.4.7, 3.4.2.

1. INTRODUCTION

Lagoon ecosystems are vital coastal habitats renowned for their exceptional biodiversity. Their complex topography, including estuaries, swamps, grasslands, mangroves, and tidal flats, provides a diverse range of niches supporting a rich assemblage of flora and fauna. Lagoon sediments act as substantial repositories for organic matter, essential inorganic nutrients, and potentially harmful metals. These sediments serve as long-term storage sites for nutrients, which are gradually released into the surrounding waters [1-3]. Nutrient-laden conditions within lagoon sediments support dense microbial assemblages [4, 5], which are critical for sustaining the biogeochemical cycling of essential elements (H, C, N, O, S, P). These microorganisms are key drivers of nutrient remineralisation processes [6, 7]. Bacteria in these sediments are integral to nutrient cycling, particularly in processes like nitrogen fixation, nitrification, denitrification, and sulfate reduction. This remineralisation process helps sustain the food web and enhances biological productivity in the lagoon [6]. Moreover, sediment bacteria contribute to detoxification by degrading pollutants such as hydrocarbons and heavy metals, thereby mitigating environmental stressors [7].

As a result, the structure and diversity of lagoon sediment microbial communities significantly impact ecosystem health and management strategies.

Tam Giang-Cau Hai Lagoon spanning over 22,000 hectares, is Southeast Asia's largest lagoon system. Located in Thua Thien Hue province, this ecosystem plays a crucial role in maintaining coastal biodiversity. With its intricate network of river mouths, mangroves, tidal flats, and abundant flora and fauna, Tam Giang Lagoon is one of Vietnam's most sensitive and valuable ecosystems. This lagoon serves as a natural laboratory for studying ecological processes, particularly those related to nutrient cycling and energy flow in coastal environments [8, 9]. It offers invaluable data for researchers investigating biodiversity, climate change, and sustainable resource management. Due to the geographical location and climate, Tam Giang experiences two distinct seasons: the dry season and the wet season. Studies on the fluctuations of temperature, pH, dissolved oxygen (DO), salinity, alkalinity, and ammonia nitrogen (NH_3-N) have shown that these parameters are generally higher during the dry season compared to the wet season. The average values of these environmental factors fluctuate seasonally as follows: 22.8 - 29.3 °C (temperature), 6.09 - 8.87 (pH), 3.76 - 8.25 mg/L (DO), 0.3 - 28.5 ‰ (salinity), 17.9 - 107 mg/L (alkalinity), and 0.019 - 0.725 mg/L (NH_3-N) [10].

Numerous investigations have focused on lagoon ecosystems to characterize biodiversity and explore biotechnological potential. While biodiversity studies in Central Vietnam's lagoons have been conducted, these efforts have primarily concentrated on the water column. To date, the prokaryotic diversity within lagoon sediments remains understudied. This research aims to fill this knowledge gap by examining seasonal variation in microbial diversity within the Tam Giang lagoon ecosystem.

2. MATERIALS AND METHODS

2.1. Study site and sediment sampling

Sediment samples were collected from five distinct locations within Tam Giang Lagoon (100 m from shore in the middle of the lagoon, the intersection between the lagoon and major rivers, lagoon center, the East Sea intersects Tam Giang Lagoon through Thuan An Strait, Ru Cha Mangrove Forest) (Figure 1) during three sampling campaigns corresponding to the dry and wet seasons (middle of February and September, respectively). Samples were collected aseptically, stored on dry ice, and transported to the laboratory. Upon arrival, samples were labelled with detailed information and immediately processed for DNA extraction. Microbial DNA was isolated from sediment samples using the PowerMax® Soil DNA Isolation Kit code

12988.10 (Qiagen) according to the manufacturer's protocol. To represent each season, DNA extracts from individual sites were pooled in equal quantities to create a bulk DNA sample.

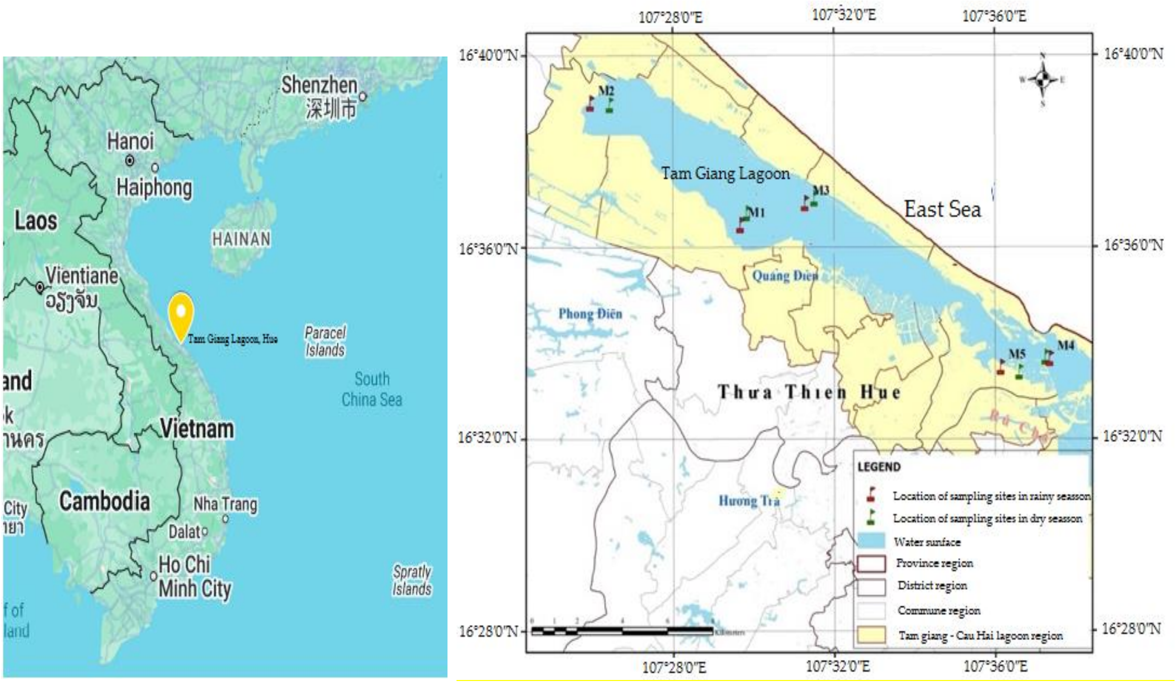

Figure 1. Sampling sites at Tam Giang Lagoon (M1 – 100 m from shore in the middle of the lagoon, M2- The intersection between the lagoon and major rivers, M3- Lagoon center, M4- The East Sea intersects Tam Giang Lagoon through Thuan An Strait, M5- Ru Cha Mangrove Forest)

2.2. 16S V3-V4 amplification

The V3-V4 region of 16S rRNA was amplified by PCR reaction with primers 341F (5'-3': CTACGGGNGGCWGCAG) and 806R (5'-3': GGACTACNNGGGTATCTAAT). Each 10 µL PCR reaction contained 0.5 µL of 10 µM forward primer, 0.5 µL of 10 µM reverse primer, 0.2 µL of DMSO, 4.5 µL of 2x Phusion Master Mix (Thermo Scientific), and 4.3 µL of template DNA. The PCR cycle consisted of pre-denaturation at 98 °C for 2 min, followed by 30 cycles: denaturation at 95 °C for 30s, anneal at 55 °C for 30s, elongation at 72 °C for 30s, and the final extension at 72 °C for 5 min [11]. The size of PCR products, at about 450 bp, was checked by electrophoresis on 2 % agarose, and the target band was extracted from gels using a Qiagen Gel Extraction Kit.

2.3. Sequencing and data analysis

A sequencing library was constructed using the NEBNext Ultra DNA Library Prep Kit for Illumina according to the manufacturer's protocol. Unique index codes were incorporated during library preparation. Library quality was assessed using a Qubit 2.0 Fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system. Paired-end sequencing (250 bp read length) was performed on an Illumina NovaSeq 6000 platform.

Paired-end reads are assembled using the make.contig command in Mothur [12]. Sequences with ambiguous bases or contigs exceeding 500 bp were excluded. The cleaned reads were aligned with the Silva database version 138.1, which includes 128,884 bacterial, 2,846 archaeal, and 14,871 eukaryotic sequences [13]. Classification of the aligned reads was performed with the classify.seqs, cluster.split, and classify.otus commands. To assign sequences to Operational Taxonomic Units (OTUs) with 97% similarity, the 16S Silva 138.1 database and the 16S rRNA RPD version 19 training set (July 2023) were used. Chimera and non-bacterial sequences, such as Chloroplast, Mitochondria, Eukaryota, and unknown sequences, were removed from the dataset.

3. RESULTS AND DISCUSSION

3.1. 16S rRNA amplification and sequencing characteristics

The V3-V4 sequence region of the 16S rRNA gene was amplified using primers 341F and 806R (Figure 2).

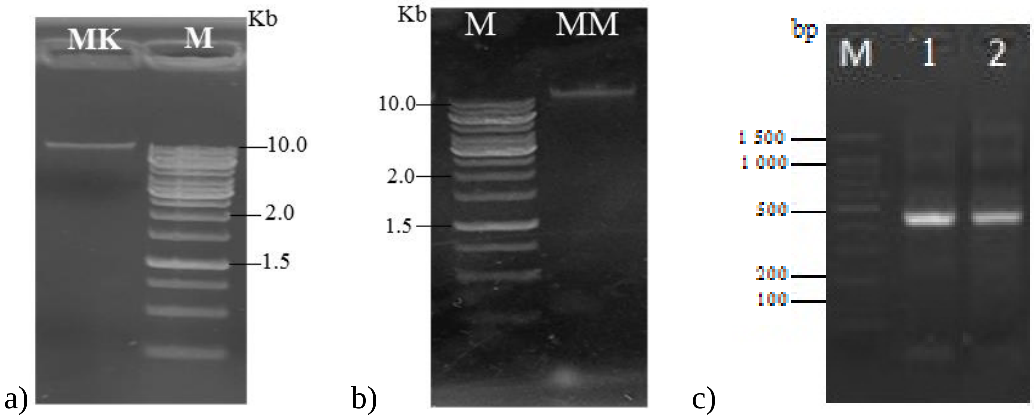

Figure 2. Electrophoresis of DNA products (a, b) and PCR products (c) of MM and MK samples on 1% agarose gel. (a): M: 1 Kb marker from Invitrogen, USA; MK: DNA product of MK sample. (b): M: 1 Kb marker from Invitrogen, USA; MM: DNA product of MM sample. (c): M: 100 bp marker from Novogene (Singapore); Well 1: PCR product of MK sample; Well 2: PCR product of MM sample

After sequencing, about 205,000 sequence reads were obtained for the dry season sample (MK) and wet season sample (MM). These sequence reads were cleaned by removing adapters, primers, PhiX contamination, and trimming low quality resulting in 42,363 and 34,958 clean reads for MK and MM, respectively. Then they were assigned to OTU using Mothur for each sample (Table 1).

Table 1. Characteristics of 16S rRNA sequencing.

Sample	Raw reads	Clean reads	OTU
MK	205,519	42,363	19,426
MM	206,788	34,958	18,631

Taxonomic classification was performed by Mothur and the database ‘Silva 138.1’ (Figure 3). The OTUs were classified as 99.99 % at the Kingdom level, 95.86 % at the Phylum level, 84.93 % at the Class level, 70.24 % at the Order level, 54.41 % at the Family level, 26.69 % at the Genus level, 2.2 % at the Species level, 0.29 % ASVs at Species_exact level.

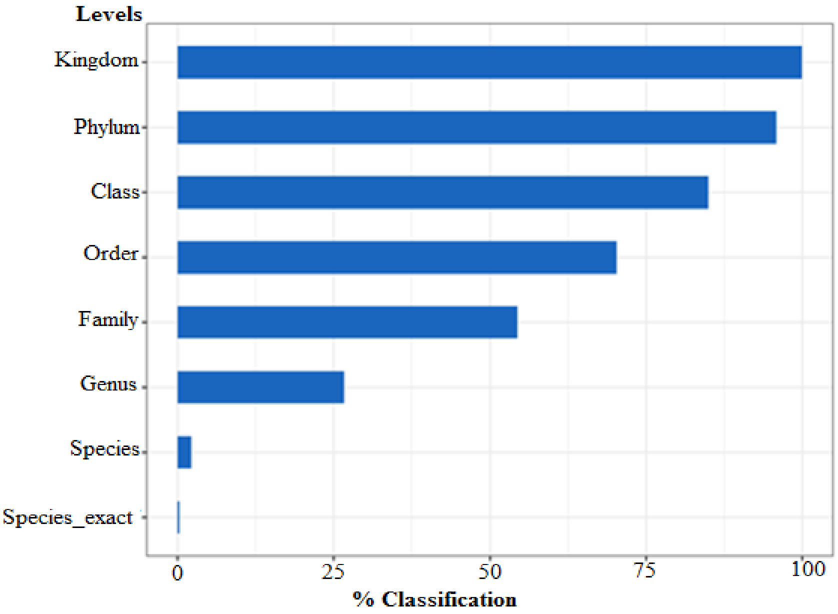

Figure 3. The taxonomic classification against the Silva database

The rarefaction curves show that all of the samples qualified for further analysis, and the MM and MK samples are at the same diversity (Figure 4).

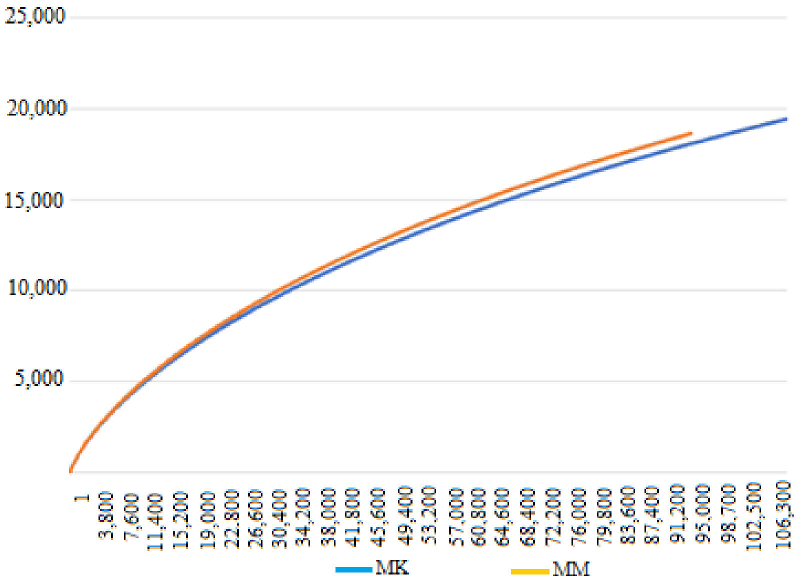

Figure 4. Rarefaction curves showing the number of Species with respect to the Sample Size of two seasons.

Although the observed OTU of the dry season was lower than that of the wet season, the *Chao1* and *ACE* indexes were vice versa (Figure 5). The *Chao1* is an indicator of species richness (total number of species in a sample) that is sensitive to rare OTUs (singletons and doubletons). Higher values indicate higher diversity. The *ACE* (Abundance-based Coverage

Estimator) index, proposed by Chao, serves as a metric for estimating the number of OTUs within a community. The *ACE* stands as one of the prominent indices for estimating species richness, distinguishing itself from the *Chao1* algorithm. The *Shannon* and *Simpson* indexes were in opposite manner, they were lower in the dry season than in the wet season (Figure 5). The *Shannon* index provides a quantitative measure of diversity or uncertainty, with higher values indicating greater diversity. *Simpson's* Diversity Index is a measure of diversity that takes into account the number of species present, as well as the relative abundance of each species. As species richness and evenness increase, so does diversity increases.

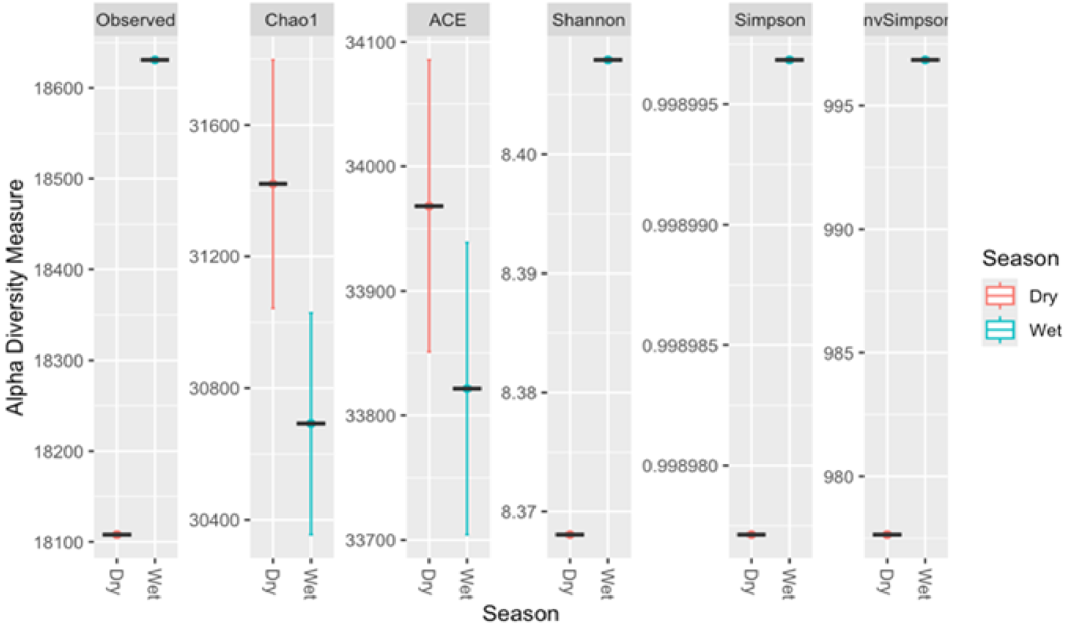

Figure 5. Alpha diversity with different indexes of Chao1, ACE, Shannon, Simpson, and invSimson for each of the samples

3.2. Taxonomic composition of the bacterial communities

Tam Giang Lagoon, situated in Thua Thien-Hue Province, is Vietnam's largest lagoon with geographic coordinates of 16°15′00″–16°42′00″N and 107°22′00″–107°57′00″E. Encompassing an area of 216 km² and extending 68 km in length with a width ranging from 2 to 10 km, the lagoon exhibits a maximum depth of 4.2 m and an average depth of 1.6 m. Water exchange with the sea occurs through the Thuan An inlet in the north and the Tu Hien inlet in the south. The lagoon's hydrographic characteristics include a semi-closed, stratified system with brackish water conditions. A total of 45 Bacterial phyla were found by DNA sequence homology with reference genomes from the Silva database. The major Bacterial phyla present in the two samples MK and MM were *Acidobacteriota*, *Actinomycetota*, *Bacteroidota*, *Chloroflexota*, *Nitrospirota*, *Pseudomonadota*, and *Verrucomicrobiota*. In which, the *Pseudomonadota* phylum accounts for the largest percentage, at 42.8 % and 47.2 % in dry and rainiy seasons, respectively. The phylum *Amicenantes* had a higher number in the dry season than in the rainy season. In the rainy season, *Amicenantes* was determined to exist at less than 1 %. Comparing the number of bacteria in the same phylum between the two seasons showed that the phyla *Acidobacteriota*, *Aminicenantes*, and *Verrucomicrobiota* were dominant in the dry season, while the phyla

Pseudomonadota and *Acidobacteriota* were dominant in the rainy season. Meanwhile, the percentage of the remaining phyla were mostly similar in both seasons (Figure 6).

At the Class level, among the 18 major classes, *Deltaproteobacteria* is the most dominant class out of the 17 identified classes. This is followed by *Gammaproteobacteria* and *Betaproteobacteria*. The remaining classes have lower proportions. The results also indicated that *Acidobacteria*, *Aminicenantes*, *Bacteroidota*, *Betaproteobacteria*, *Pseudomonadota*, and *Subdivision*3 had larger proportions than the others in the dry season. *Actinomycetota*, *Chloroflexota*, *Deltaproteobacteria*, *Flavobacteriia*, whereas *Gammaproteobacteria* had the largest proportions in the rainy season. The remaining classes had similar proportions (about 1 %) between the two seasons.

Interestingly, there are bold variations of abundance between MK and MM samples at all taxonomic levels. At the phylum level, the abundance of *Acidobacteriota* in the dry season sample at 2.6 %, which was twice as higher as the figure in the wet season (1.2 %. Similarly, the percentage of *Verrucomicrobiota* in the dry season (at 3.3 %) was also over 2-fold higher than the number in the rainy season (at 1.6 %). In contrast, the abundance of *Chloroflexota* phylum in wet season MM is higher than that in dry season MK (3.8 % vs. 3.0 %). Within the phylum of *Pseudomonadota*, the abundance variation of classes between dry and wet seasons are back and forth. The class *Deltaproteobacteria* and *Gammaproteobacteria* increased from dry to wet season (119 % and 138 %). Meanwhile, the class *Betaproteobacteria* decreased by 176 %.

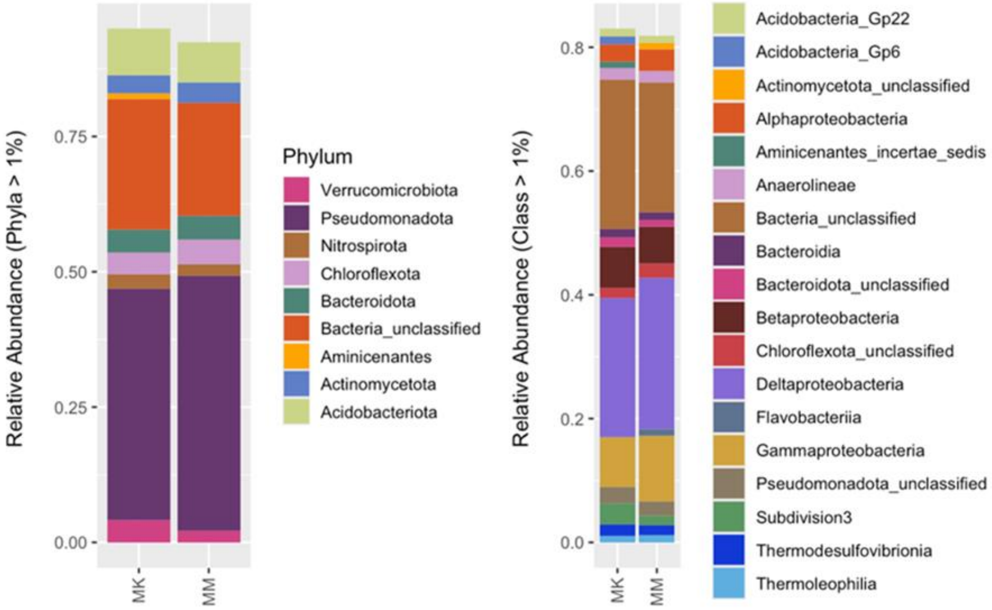

Figure 6. Histogram representing the distribution of major taxonomic groups for Bacteria at the Phyla and Class levels in different types of climate.

Pseudomonadota are ubiquitous microorganisms within terrestrial ecosystems. As key microbial constituents, they contribute significantly to ecosystem function through processes including nutrient mineralization, carbon sequestration, organic matter decomposition, and atmospheric nitrogen fixation. *Desulfobacterales*, a prominent member of this phylum, plays a critical role in nitrogen cycling, encoding approximately 12 % of nitrogen pathway genes on average [14]. This underscores the intricate coupling of nitrogen, sulfur, and carbon cycles within mangrove environments. *Gammaproteobacteria* exhibit remarkable metabolic diversity and redundancy in hydrothermal sediments, driving organic carbon turnover and facilitating nitrogen and sulfur cycling [15]. *Betaproteobacteria* are essential for nitrogen fixation in various plant species, catalyzing the oxidation of ammonium to nitrite, a crucial plant nutrient. In addition to their ecological importance, betaproteobacteria contribute to soil pH regulation and elemental cycling, thereby holding significant economic value [16]. The detailed composition of bacterial communities at different taxonomic levels (phylum, class, order, and Family) was visualized using the Krona pie charts (Figure 7 and Figure 8).

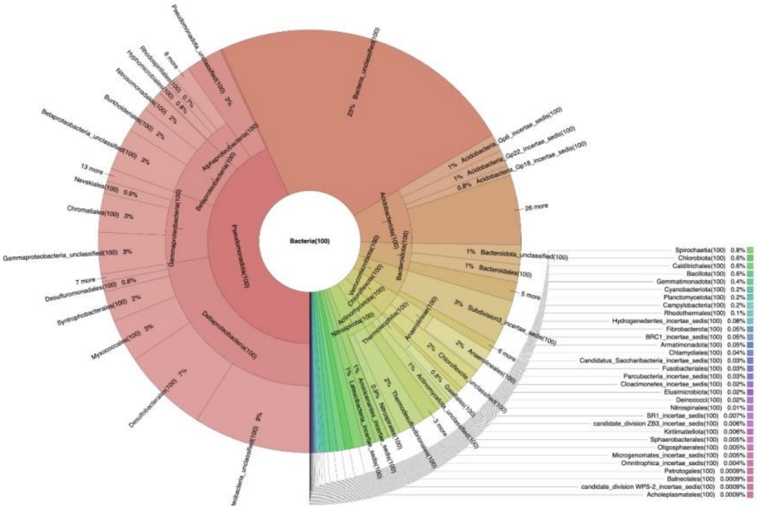

Figure 7. Krona chart visualizing the taxonomic composition of the bacterial community associated MK sample.

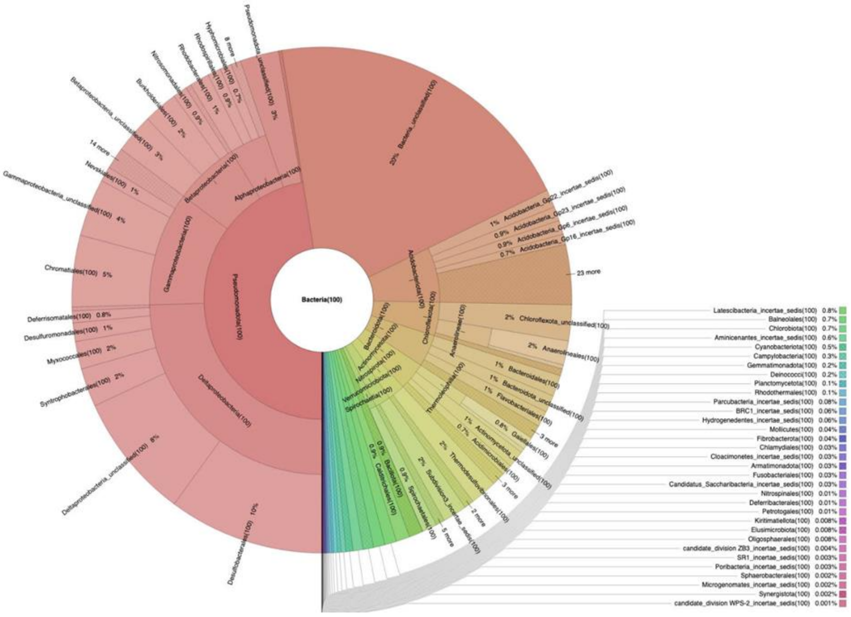

Figure 8. Krona chart visualizing the taxonomic composition of the bacterial community associated MM sample.

Several studies have shown that coastal lagoons are unstable ecosystems, strongly affected by physical and chemical agents caused by human activities [17, 18]. They are the intersection of freshwater environments (from rivers and streams) and saltwater environments (sea, mangrove forests). This has created biodiversity here, especially the diversity of bacterial community structures. In recent years, seasonal bacterial diversity has been considered important by many scientists and has begun to be studied. Lin et al. (2018) hypothesize that the seasonal variability observed in lagoon community composition is driven by seasonal changes in environmental and trophic conditions [17]. This indicated that different environmental variables could significantly explain the variance in community composition across the different seasons. In this study, we also predicted that the rainy and dry seasons in the Tam Giang lagoon may change the chemical composition and other factors in the lagoon, leading to changes in the bacterial community structure in each season. Our research results are similar to some other research results, showing that *Proteobacteria* is the dominant group in both research seasons [19-22]. However, according to the study of Lin et al. (2018), in Fengxian Lagoon, China, the *Alpha* subclass fluctuated strongly between seasons. In Tam Giang Lagoon, Vietnam, all four subclasses *Alpha*, *Beta*, *Gamma*, and *Delta* changed significantly in the two seasons, in which the *Delta* subclass was the most dominant in terms of quantity.

4. CONCLUSIONS

For the first time, the diversity of microbial communities in Tam Giang Lagoon’s sediments through two distinct seasons of the year was investigated. The *Pseudomonadota* phylum was the most dominant phylum followed by *Acidobacteriota*, *Verrucomicrobiota*, and *Chloroflexota* phyla. Meanwhile, the composition of the remaining phyla was more stable during the two study seasons. There were strong changes in the relative abundance of classes within the *Pseudomonadota* phylum. The *Deltaproteobacteria* and *Gammaproteobacteria* classes increased from the dry season to the rainy season, by 119 % and 138 %, respectively. Meanwhile, the *Betaproteobacteria* class decreased by 176 %. *Pseudomonadota* are ubiquitous microorganisms in terrestrial ecosystems. As key microbial constituents, they contribute significantly to ecosystem function through processes including nutrient mineralization, carbon sequestration, organic matter decomposition, and atmospheric nitrogen fixation. Bacterial resources in the lagoon in each season can help assess the situation in order to find the right solution to overcome the environmental pollution of this ecosystem, and at the same time, it is possible to exploit useful bacteria with valuable biological activity to serve human life.

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CRediT authorship contribution statement. TTKD and PVC designed the research study and processed the data. TTKD performed the research. TTH and DHG analyzed the data and wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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