JIPK. Volume 16 No 2 November 2024
Sinta 1 (Decree No: 158/E/KPT/2021)
e-ISSN:2528-0759; p-ISSN:2085-5842
Available online at https://e-journal.unair.ac.id/JIPK

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Research Article

High and Low Taxa Specificity of Rhizosphere Bacterial Communities of Mangrove (*Rhizophora mucronata*) from Kuala Langsa and Telaga Tujuh Island, East Aceh

Siti Maysaroh[1], Meutia Samira Ismet[2*], Rita Andini[3], Nurlita Putri Anggraini[2], and Beginer Subhan[2]

[1]Marine Science Study Program, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java. Indonesia
[2]Department of Marine Science and Technology, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java. Indonesia
[3]Genetic Engineering Research Center, Badan Riset and Inovasi Nasional (BRIN), Cibinong, West Java. Indonesia

OPEN ACCESS

ARTICLE INFO

Received:March 10, 2024
Accepted: May 16, 2024
Published: May 26, 20244
Available online: Oct 23, 2024

*) Corresponding author:
E-mail: meutiais@apps.ipb.ac.id

Keywords:
Biodiversity
Ecology Molecular
Ecosystem Mangrove
V3-V4 16S rDNA

Abstract

The focus of this study on understanding the structure and dynamics of rhizosphere bacteria in mangrove ecosystems is driven by the increasing acknowledgment of the crucial roles these microorganisms play in ecosystem functioning. *Rhizophora mucronata*, a key mangrove species, is known for its ecological significance. Investigating the bacteria associated with its rhizosphere offers valuable information about the symbiotic relationships between mangrove vegetation and microbial communities. Bacteria are vital for decomposition and nutrient availability in mangroves. This research examines sediment from rehabilitated and natural areas to understand how human and natural factors shape bacterial communities. The DNA sequence was analyzed using Next-Generation Sequencing (NGS), which targeted the 16S bacterial region in the V3-V4 rDNA. Additionally, environmental factors such as nitrate, phosphate, and sulfur content were also analyzed. Kruskal-Wallis and T-test statistical analyses were used to examine the abundance of bacteria and environmental parameters between study sites. There are differences in the types of bacteria found in Kuala Langsa and Telaga Tujuh. Approximately 7% of the rhizosphere bacterial groups were exclusively detected in Telaga Tujuh, such as Fusobacteriia (Class). Additionally, the abundance of bacteria at both locations differs significantly ($p < 0.05$), as determined by Kruskal-Wallis. The results of the t-test indicate that the observed environmental parameters do not differ significantly from each other. The environmental parameters studied did not significantly impact the types or abundance of detected rhizosphere bacteria.

Cite this as: Maysaroh, S., Ismet, M. S., Andini, R., Anggraini, N. P., Subhan, B. (2024). High and Low Taxa Specificity of Rhizosphere Bacteria Communities of Mangrove (*Rhizophora mucronata*) from Kuala Langsa and Telaga Tujuh Island, East Aceh. *Jurnal Ilmiah Perikanan dan Kelautan*, 16(2):364-376. http://doi.org/10.20473/jipk.v16i2.55882

1. Introduction

Red mangroves (*Rhizophora mucronata)* are often found in coastal areas, including Kuala Langsa and Telaga Tujuh, East Aceh, Indonesia. The mangrove ecosystem in these areas have different characteristics, with Kuala Langsa being a rehabilitated area (Iswahyudi *et al.,* 2019), and Telaga Tujuh being natural (Hanafi *et al.,* 2021). The most common types of mangroves found in these areas belong to the genera *Rhizophora* and *Avicennia* (white mangrove). *Rhizophora* has been widely studied for high adaptability, making it a primary choice for rehabilitation activities (Putri *et al.,* 2015). One of the main characteristics of mangroves is their habitat in tidal areas, which requires adaptation to varying physicochemical conditions (Andriyani *et al.,* 2020; Imamsyah *et al.,* 2020). Environmental variations including pH, salinity, and nutrients significantly impact the high diversity and composition of bacteria found on the soil surface, roots, leaves, and plant stems (Sari *et al.,* 2016; Susilowati *et al.,* 2016). Furthermore, the success of mangrove cultivation in an environment is related to the bacterial communities known as the rhizosphere (Lund *et al.,* 2022), which plays an essential role in converting nutrients from dead mangrove into sources of nitrogen, phosphorus, and other nutrients (Susilowati *et al.,* 2016). The rhizosphere also provides shelter for the bacteria, affecting the composition of the communities (Lu *et al.,* 2022). Bacterial communities are diverse and play an essential role in the success of mangrove growth. However, information regarding their composition is needed, especially in Indonesia. Gomes *et al.* (2010) indicates the importance of understanding the presence of bacteria as they support the success of rehabilitation activities aimed at preventing further forest degradation.

Studies on the mangrove rhizosphere can be carried out using two methods, namely cultivation and DNA metabarcoding. The cultivation method has been widely used, specifically in Indonesia, for bacteria identification. Saputri *et al.* (2021) observed the characteristics of nitrogen-fixing bacteria using Burk's media from mangrove rhizosphere. The results showed that two genera were identified, *Nitrosococcus* and *Bacillus*. Another study focused on the *Avicennia* species and identified five genera including *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Serratia*, and *Vibrio* (Islamiah *et al.,* 2017). Several studies have used molecular identification based on isolation results, as seen in studies conducted by Chrisnawati *et al.* (2023) and Ntabo *et al.* (2018). Previous investigation found that the low number obtained was due to the difficulty in cultivating almost 98% of the bacteria (Sharma *et al.,* 2017). On the other hand, DNA metabarcoding is an alternative that can provide high-resolution profile images (Bulgarelli *et al.,* 2013). This method has been used in studies related to the distribution of bacteria on the surface of mangrove sediments, as shown by Jiang *et al.* (2013) and Basak *et al.* (2015), as well as in aquatic microbiomes (Dhal *et al.,* 2020). Muwawa *et al.* (2021) investigated rhizosphere bacteria communities from four different types of mangrove plants, including *Avicennia marina*, *Ceriops tagal*, *Rhizophora mucronata*, and *Sonneratia alba*, under two different conditions. These included protected and polluted conditions due to degradation by anthropogenic factors, such as plastic pollution, dirt, and the presence of oil spill areas, using DNA metabarcoding. In addition, this study also examined the correlation with the depth of sediment collection. The results showed that the bacteria communities detected were influenced by the type of mangrove rather than the depth of sediment. This raises important questions, specifically about *Rhizophora mucronata*, and its potential environmental influences on the distribution, abundance, and dominance of bacteria. Furthermore, Loganathachetti *et al.* (2016) observed the communities in *Avicennia marina* mangrove type, differentiated by season, and Wu *et al.* (2016) detected rhizosphere based on three types namely *Bruguiera gymnorhiz*a, *Kandelia candel*, and *Aegiceras corniculatum* in the Beliun Estuary. With advancements in technology and science, it has become easier to detect species from genetic traces found in the environment (Fernández *et al.,* 2021). DNA metabarcoding was selected as a tool for bacteria detection due to its effectiveness in monitoring the biodiversity in mangrove sediments (Vilaça *et al.,* 2020). To date, no previous studies have characterized bacteria groups associated with *R. mucronata,* specifically in Indonesia, despite its extensive use as a rehabilitation plant. This information is important to effectively support rehabilitation activities. Therefore, this study was conducted as the first step in determining the characteristic bacteria of *R. mucronata.*

This study aims to determine the bacteria found in *Rhizophora mucronata* mangrove sediments in Kuala Langsa (rehabilitated areas) and Telaga Tujuh (intact or natural areas). Additionally, the study evaluates bacteria communities at both low and high taxonomic levels, as well as the environmental factors affecting their distribution and abundance. The DNA metabarcoding method chosen due to its effectiveness in identifying

bacteria species compared to conventional techniques. The target DNA fragments in this study are located in the V3 and V4 regions of the 16S rDNA gene. The results are expected to provide an overview of environ

2. Materials and Methods

2.1 Materials

The material used in this study consist of

Table 1. Position of sediment sampling from mangrove ecosystem in Kuala Langsa and Telaga Tujuh, East Aceh

Site	Stations	Positions
Kuala Langsa	KL.S1	N 4°31'14.40 " and E 98°0'51.82 "
Kuala Langsa	KL.S4	N 4°31'07.63 " and E 98°0'47.46 "
Telaga Tujuh	T7.S1	N 4°33'26.68 " and E 98°3'32.35 "
Telaga Tujuh	T7.S4	N 4°33'25.81 " and E 98°3'33.76 "

Figure 1. Map of sediment sampling locations in Kuala Langsa and Telaga Tujuh mangrove ecosystem areas, East Aceh

Figure 2. Mangrove area conditions (A) Mangroves resulting from rehabilitation activities in Kuala Langsa and (B) natural in Telaga Tujuh Island, East Aceh

mental changes effecting the condition of *R. mucronata* in both natural and rehabilitated mangrove vegetation.

sediment samples from mangrove ecosystem, and molecular analysis kit from Zymo Research Quick-DNA

Fecal/Soil Microbe Miniprep Kit (USA, Catalog: D6010), agarose gel containing GelRed stain (Biotium, USA), TAE buffer 1x (Vivantis, Malaysia) and 16sDNA primer set from IDT Singapore (341F and 806R). Whilst equipment that were used in this study including PVC core-pore (with a diameter of 5 cm and a length of 10 cm), Uv-Vis (UV-1900) spectrophotometer (Shimadzu Japan), a universal Wee32 PCR machine (HIMEDIA, India), Mupid electrophoresis machine (Taitec Corporatioon, Japan), and Gel Documentation mini-imager.

2.1.1 Ethical approval

This study does not require ethical approval because it does not use experimental animals.

2.2 Study Site

Sediment samples were obtained from the Kuala Langsa and Telaga Tujuh mangrove areas located in the West Langsa District, Langsa City, East Aceh, Indonesia (Table 1, Figure 1, Figure 2). Both areas have extensive mangrove ecosystems with various species, including *R. mucronata*, but differ in terms of environmental conditions. The Kuala Langsa mangrove area has been rehabilitated since 2006 to address land degradation caused by the decreasing mangrove area, which has impacted water availability (Iswahyudi *et al.,* 2019). Meanwhile, mangrove ecosystem on Telaga Tujuh Island developed naturally and is estimated to be centuries old. This finding was supported by Hanafi *et al.* (2021), who observed a dominance of tree stands compared to saplings and seedlings. The mangroves in this region also fall into the climax forest category, where plants have reached a stable and undisturbed position, enabling adaptation to environmental conditions. In this study, environmental parameters were analyzed at the Soil Science Laboratory, Bogor Agricultural University, Bogor. Bacterial DNA isolation was conducted at the Genomics Building of the National Research and Innovation Agency (BRIN), Cibinong, followed by subsequent molecular processes such as Polymerase Chain Reaction (PCR) and DNA electrophoresis, which were performed at PT. Oceanogen Baruga, Bogor, Indonesia. Additionally, DNA sequencing was carried out by sending the samples for assessment to Novogene, Korea.

2.3 Collection of Samples

Sampling was conducted in August 2021, and four sediment samples were collected from Kuala Langsa and Telaga Tujuh mangrove ecosystems. This was achieved using a purposive methodology, in which sediments were collected from areas closest to the *R. mucronata* root system, particularly at depths of 1–10 cm. Specimens were collected using polyvinyl chloride (PVC) pipes with a diameter of 5 cm and a length of 10 cm, following the method described by Giannopoulos *et al.* (2019). Subsequently, the sediments were aseptically packaged and placed into 50 ml falcon containers, preserved on dry ice (room temperature: 25°C) during transportation, and stored at -20°C until further laboratory investigations.

2.4 Environmental Parameters

Various environmental parameters were considered, including acidity (pH), reduction potential (Eh), sediment salinity based on conductivity (EC) values, organic matter, sediment texture, and nutrients. The analysis and measurement procedures for pH, Eh, EC, organic matter, and sediment texture were conducted following the methods describe by (Maysaroh *et al.,* 2023). Other parameters, including nitrate, were assessed using the titrimetric method (Siregar *et al.*, 2022), while the phosphate and sulfur values were obtained from UV-Vis spectrophotometer measurements at a wavelength of 693 nm (nitrate) and 480 nm (phosphate and sulfur) (Huda *et al.,* 2013).

2.5 Molecular Analysis

2.5.1 Extraction and amplification of DNA

The sediment was extracted using the Zymo Research Quick-DNA Fecal/Soil Microbe Miniprep Kit (USA, Catalog: D6010) to isolate bacteria DNA following the manufacturer's instructions. DNA quality was analyzed on a 1.5% agarose gel containing GelRed stain (Biotium, USA). Meanwhile, amplification of the target regions was performed with the 16S primer set 341F (5'CCTAYGGGRBGCASCAG3') and 806R (5'GGACTACNNGGGTATCTAAT3') to optimize the detection of rhizosphere bacteria communities by targeting the V3 and V4 regions at 478 bp (Lund *et al.,* 2022; Muwawa *et al.,* 2021). Vasileiadis *et al.* (2012) mentioned that the V3 region provided higher diversity information than other regions, while V4 had a more conservative nature, useful for identifying evolutionary patterns and phylogenetic relationships with good accuracy. All PCR was performed with 13 µL of 2x Mytaq, 1 µM of forward and reverse primers, and 10 ng of template DNA. The thermal cycle consisted of an initial denaturation at 98°C for one minute, followed by 35 cycles at 95°C for 30 seconds, annealing at 53°C for 30 seconds, elongation at 72°C for 30 seconds (Lund *et al.,* 2022), and a final elongation at 72°C for 5 minutes. To check for contamination, a universal Wee32 PCR machine was used, with a negative control (blank template). Furthermore, PCR product quality was visualized by electrophoresis on a

1% agarose gel (100 µL TAE buffer and 1 g agarose). The electrophoresis machine was run at 50 Volts for 60 minutes, and the results were visualized by UV fluorescence using a Gel Documentation mini-imager.

2.5.2 Library preparation and sequencing

Sequencing libraries were prepared using the NEB Next® Ultra™ II FS DNA PCR-free Library Preparation Kit (New England Biolabs, USA, Catalog: E7430L) following the manufacturer's recommendations, and indexes were added. Libraries were checked with Qubit and real-time PCR for quantification, and a bioanalyzer was used for size distribution detection. Quantified libraries were collected and sequenced on the Illumina platform according to the effective library concentration and the amount of data required.

2.6 Bioinformatic and Data Analysis

Sequenced nucleotide bases were processed using the bioinformatic pipeline QIIME 2 (Quantitative Insights into Microbial Ecology 2, https://qiime2.org/) (Bolyen *et al.,* 2018). The raw data in the file (fastq.gz) was demultiplexed using the program available in QIIME 2. Each DNA sequence was arranged by cutting nucleotide bases (cut-adapt) in line with the primer sequences used, and its quality was evaluated based on DNA score obtained. Sequences with low quality or score values of <20 were removed (denoising) using the DADA2 feature. This step produced high-quality sequence variants (ASVs) (Chiarello *et al.,* 2022). The final step in data processing was the clustering of ASVs based on the level of similarity at a certain threshold, usually 97-100%. This was achieved using a de novo approach, where the ASVs were clustered in line with the sequence similarity in the data, without the use of a comparison reference (Margareta, 2023). The obtained groups were compared with the SILVA database (http://www.arb-silva.de/, accessed on April 30, 2023), specifically 16S rDNA (Quast *et al.,* 2013). Simultaneously, Operational Taxonomic Units (OTUs) were analyzed with a Venn diagram to obtain common and unique information among different samples or groups. The relative abundance was visualized through the ggplot2 package (Wickham, 2009) in the R software (v4.30, http://r-projekt.org). Furthermore, the Kruskal-Wallis H non-parametric test (IBM SPSS Statistics 25 software) was used to determine whether there was a significant difference between several groups of bacteria based on ordinal variables (Rozi *et al.,* 2022). A p-value of $< \alpha$ (alpha) 0.05 showed significant differences between the tested groups. The post-hoc Mann–Whitney U test was further used to determine which bacterial groups had significant differences. The relationship between environmental parameters and observation stations was analyzed by principal component analysis (PCA) using the ggplot2 package (Wickham, 2009) in R software (v4.30, http://r-projekt.org).

3. Results and Discussion

3.1 Site Differences in Physicochemical Parameters

This study complements the results of Maysaroh *et al.* (2023) by adding environmental parameters in the form of nutrients, including nitrate, phosphate, and sulfur, obtained from the analysis of the Kuala Langsa and Telaga Tujuh sediments (Table 2). Maysaroh *et al.* (2023) mentioned that Kuala Langsa and Telaga Tujuh have high organic matter content (17-35) % and low pH values (<7). The high organic matter content is related to an increased amount of organic acids resulting from bacterial decomposition processes (Elungan *et al.,* 2022), which leads to the accumulation of organic acids and subsequent decrease in sediment pH. The sediment analysis conducted in this study revealed that the nitrate content in Kuala Langsa was 105.95 ± 43.62 mg/L, while in Telaga Tujuh it was 124.10 ± 94.3 mg/L. According to the classification established by Effendi (2003), the nitrate concentrations in both research locations fall into the high category, exceeding 10 mg/L. Additionally, the phosphate content in the sediment at both study sites was high, namely 722.67 ± 137.012 mg/L and 586.67 ± 203.31 mg/L, respectively. Effendi (2003) categorized phosphate concentrations in sediment into four categories: very low (<3 mg/L), low (3-7 mg/L), medium (7-20 mg/L), and high (>20 mg/L). The high levels of nitrate and phosphate can be attributed to the presence of *Rhizophora mucronata*, which inhabits intertidal areas and often receives inputs of organic or mineral materials, including nitrate and phosphate, from both land and sea (Sanders *et al.,* 2014). Sanders *et al.* (2014) stated that mangroves from the genus rhizophora, the main focus of this study, have props roots, which can function as nutrient traps carried by seawater (Ulumuddin, 2019). Furthermore, rhizosphere bacteria activity in mangrove sediment also plays a role in absorbing organic nitrogen, including nitrate (Wang *et al.,* 2019), resulting in high nitrate content in mangrove sediment. Table 2 shows the sulfur content in Kuala Langsa and Telaga Tujuh mangrove sediments, which are 2.05% and 1.38%, respectively. According to Effendi (2003), when the environmental pH decreases and reaches pH 5, sulfur tends to react with other elements and form hydrogen sulfide compounds. Therefore, a decrease in pH can increase the toxicity of hydrogen sulfide (H_2S) in the environment. The presence of sulfur in sediment is caused by seawater input into the mangrove

environment, decomposition processes by sulfur-producing bacteria, and the role of bacteria in oxidizing sulfur compounds into more easily soluble forms.

Station KL.S1 was characterized by a high nitrate content and was dominated by dust with clay fractions, while T7.S1 had a high Eh value with organic

Table 2. Environmental parameters and composition of rhizosphere bacteria in Kuala Langsa and Telaga Tujuh, East Aceh

Environmental parameters	Kuala Langsa	Telaga Tujuh	Reference
pH	5.25±0.31	4.96±0.38	Maysaroh *et al.* (2023)
Organic matter (%)	21.53±4.10	25.47±2.37	Maysaroh *et al.* (2023)
Eh (mV)	120.55±128.62	243.60±35.49	Maysaroh *et al.* (2023)
DHL/EC (mS/cm)	18.58±3.00	17.92±3.08	Maysaroh *et al.* (2023)
Nitrate (mg/L)	105.95±43.62	69.65±1.63	This study
Phospate (mg/L)	619.67±258.00	586.67±203.32	This study
Sulfur (%)	1.63±1.08	1.38±0.82	This study
Clay	22.12±6.05	11.11±3.23	Maysaroh *et al.* (2023)
Silt	21.34±8.81	10.65±2.32	Maysaroh *et al.* (2023)
Sand	56.53±14.87	78.23±5.56	Maysaroh *et al.* (2023)
Sediment texture	Sandy loam	Loamy sand	Maysaroh *et al.* (2023)
Composition of rhizosphere bacteria (High taxa/Phylum)*	Crenarchaeota (31%) Chloroflexi (26%) Acidobacteriota (10%) Proteobacteria (7%) Campylobacterota (5%) Desulfobacterota (4%) Firmicutes (3%) Cyanobacteria (3%) Calditrichota (2%)	Chloroflexi (18%) Proteobacteria (11%) Acidobacteriota 10%) Firmicutes (10%) Crenarchaeota (9%) Nanoarchaeota (4%) Bacteroidota (3%) Desulfobacterota (3%) Actinobacteriota (3%)	This study
Composition of rhizosphere bacteria (Low taxa/Family)*	Sulfurovaceae (18%) Desulfatiglandaceae (11%) Spirochaetaceae (8%) Calditrichaceae (8%) Anaerolineaceae (6%) Psychromonadaceae (4%) Rhodobacteraceae (4%) Vibrionaceae (3%) Pseudoalteromonadaceae (2%) Enterobacteriaceae (2%)	Calditrichaceae (4%) Spirochaetaceae (4%) Desulfatiglandaceae (3%) Anaerolineaceae (3%) Lachnospiraceae (2%) Roseiflexaceae (2%) Pedosphaeraceae (2%) Latescibacteraceae (2%) Desulfosarcinaceae (2%) Oscillospiraceae (2%)	This study

** Top 10 rhizosphere bacterial groups*

PCA explained the distribution of environmental parameters obtained from *R. mucronata* sediments in the study sites. PCA analysis accounted for 98.20% variation in the total variables used, with Factors 1 and 2 (F1 and F2) explaining 61.10% and 37.90%, respectively (Figure 3). The independent sample t-test showed significance > 0.05 (Table 2) for pH ($p = 0.493$), organic matter ($p = 0.361$), Eh ($p = 0.322$), EC ($p = 0.484$), nitrate ($p = 0.362$), phosphate ($p = 0.761$), and sulfur ($p = 0.442$). In general, the environmental parameters observed in Kuala Langsa and Telaga Tujuh were not significantly different from each other.

matter and sand fraction (Figure 3). Station KL.S4 was distinguished by pH, DHL, phosphate, and sulfur values, while T7.S4 was not characterized by any environmental parameters. Furthermore, station T7.S1 was dominated by the sand fraction, with the sand, dust, and clay content reaching 75%, 12%, and 13%, respectively. This occurred due to the direct exposure of the location to the marine environment, leading to the sedimentation process being influenced by oceanographic factors, such as currents and waves. Suwoyo *et al.* (2015) mentioned that current and wave speeds determine the size of the carried sediment particles.

Generally, areas with strong currents have sand-dominant substrates (Nybakken, 1993). The current speed around Telaga Tujuh Island ranged from 0.25 - 0.33 m/s (Purba *et al.*, 2017). Mason (1981) divided the current speed of water into very slow (<0.1 m/s), slow (0.1 - 0.25 m/s), medium (0.25 - 0.5 m/s), fast (0.5 - 1.0 m/s), and very fast (>1.0 m/s). The current velocity in Telaga Tujuh was classified as moderate, indicating the possibility of carried particles having a larger size than the clay and dust fractions.

3.2 Species Identification and Taxonomy Composition

A total of ~131,000 sequence reads or ~3,000 OTUs were successfully obtained from Kuala Langsa and Telaga Tujuh mangrove sediments. Among these, 2,353 OTUs were found at both study sites, with 774 unique to Kuala Langsa and 777 exclusives to Telaga Tujuh. The total number of OTUs obtained was 3,974, of which 154 (4%) were identified as species taxa,

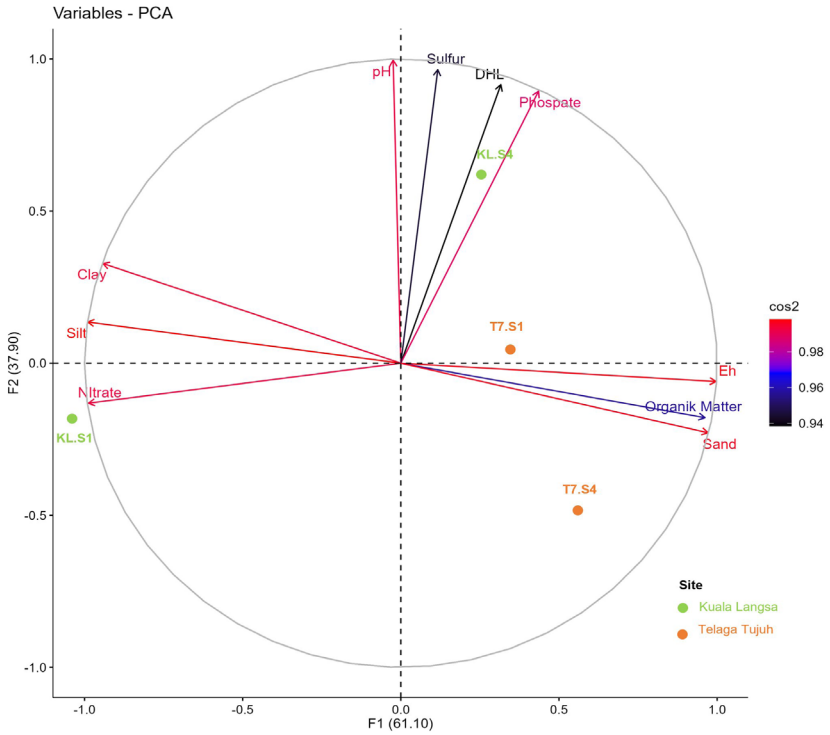

Figure 3. Principal component analysis (PCA) between variables in mangrove environments in Kuala Langsa and Telaga Tujuh

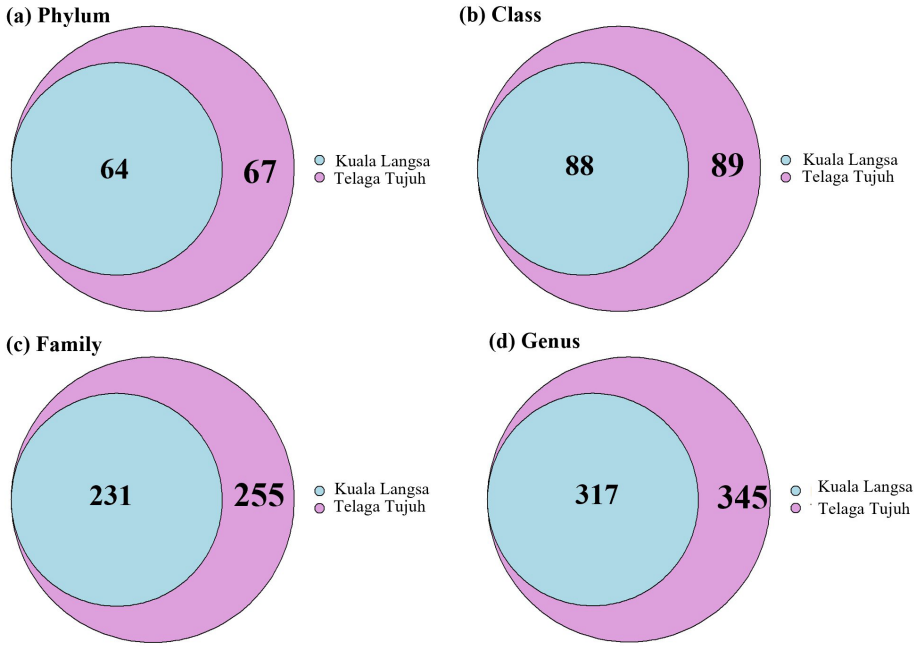

Figure 4. Groups of rhizosphere bacteria at high (a,b) and low (c,d) taxa levels from Kuala Langsa and Telaga Tujuh mangrove sediments based on Operational Taxonomic Units (OTUs)

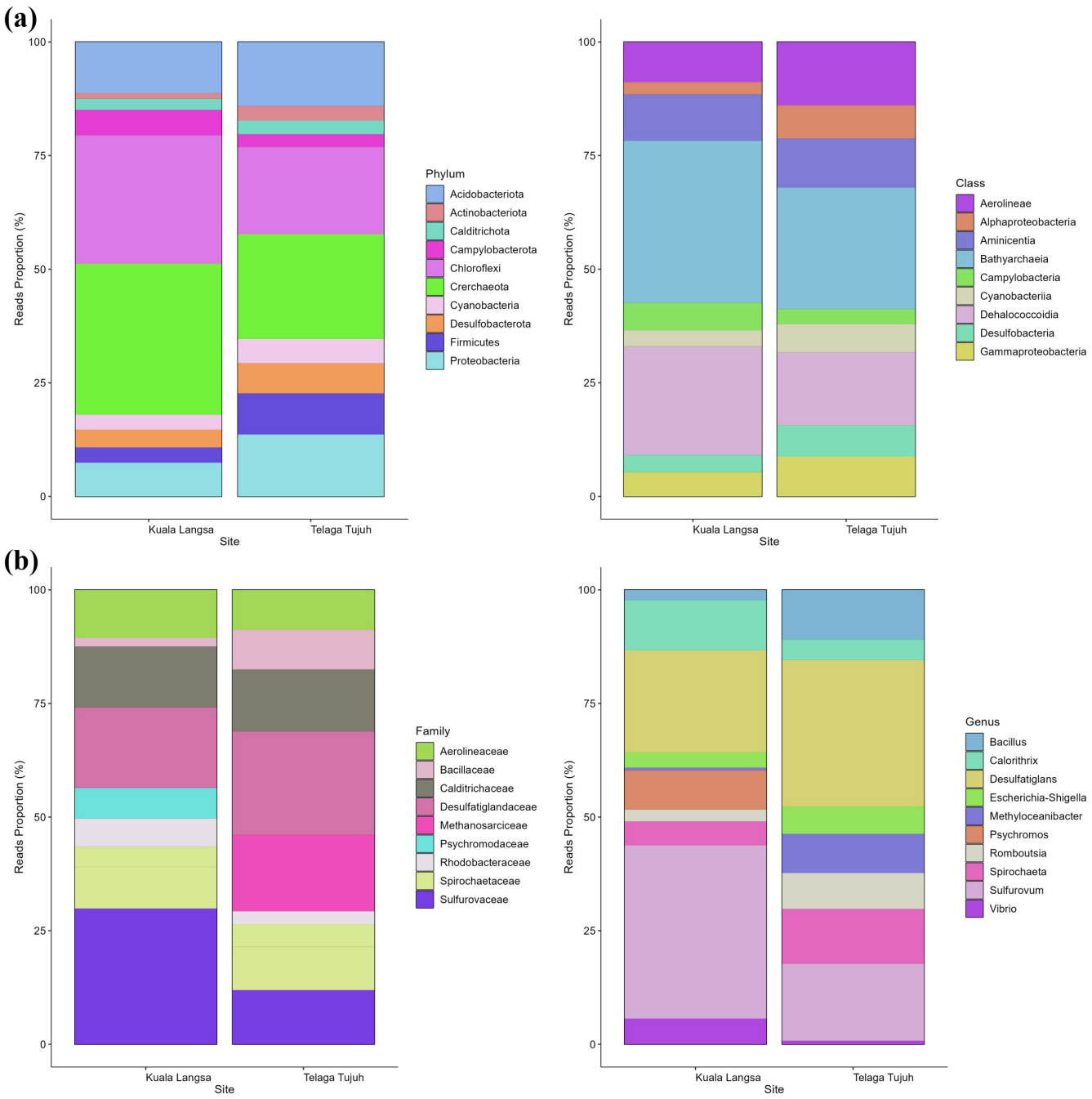

Figure 5. Relative abundance of rhizosphere bacteria at (a) high and (b) low taxa levels based on sequence variant sequences (ASVs)

while 3,750 OTUs (96%) were only identified at higher taxa, including phylum, class, and order. A total of 552 OTUs (14%) belonged to the kingdom Archaea, and the unidentified ones could not be read based on the SILVA database. Casas *et al.* (2017) suggested that unrecognized taxa in the database would be classified as unknown. This suggests the need to enrich the genetic database (Lear *et al.*, 2018) both in terms of the taxonomy and geography of each species (Bucklin *et al.*, 2016). Lear *et al.* (2018) also stated that many OTUs were not defined down to species taxa due to the limited information in the gene reference lists used, considering the high genetic diversity of bacteria in sedimentary environments (Lear *et al.*, 2018). Bacteria have large genetic variations as a form of self-adjustment to environmental factors, and the changes that occur have not been fully identified (Lear *et al.*, 2018).

Based on the types of bacteria detected, the Telaga Tujuh mangrove ecosystem harbors a greater diversity of rhizosphere bacteria compared to Kuala Langsa across all taxonomic levels (Figure 4). This is attributed to the stability of the Telaga Tujuh ecosystem, which provides a consistent environment for

bacteria to thrive and proliferate effectively (Hanafi *et al.,* 2021). The bacteria groups found at the high taxa level included 64 phyla and 88 classes, while at the low taxa levels, 213 families and 317 genera representing were recorded in both study areas. Approximately 93% of the rhizosphere bacteria groups found in Kuala Langsa were also detected in Telaga Tujuh. Meanwhile, Telaga Tujuh had some groups that were not present in Kuala Langsa, such as Candidatus, Fusobacteriota, and Poribacteria at the phylum level and Fusobacteriia at the class level. About 128 species were detected in the two study sites, with 14 only found in Telaga Tujuh. Additionally, the study also reveals that the abundance of rhizosphere bacteria found exhibits significant differences based on the Kruskal-Wallis test results ($p < 0.05$). Post hoc Mann Whitney U Test results also yielded p-values < α (alpha) = 0.05.

The 16S rDNA gene classification results showed the abundance of bacteria groups, with high taxa such as phylum and class (Figure 5a), along with low taxa, represented by family and genus (Figure 5b). This study visualized at least 10 bacteria groups, with four phyla having the highest abundance based on sequence variant reads (ASVs), namely Crenarchaeota (26%), Chloroflexi (22%), Acidobacteriota (9%), and Proteobacteria (6%). Zhu *et al.* (2022) reported that the phyla Proteobacteria, Chloroflexi, and Actinobacteria were predominantly found in mangrove sediments. Similarly, (Muwawa *et al.,* 2021) mentioned that the phylum with the highest abundance found in *Rhizophora mucronata* roots was Proteobacteria. Another study mentioned that besides Proteobacteria, bacteria groups from the Bacteroidetes, Actinobacteria, Cyanobacteria, and Verrucomicrobia phyla were also distributed in mangrove sediments (Lu *et al.,* 2022). The differences in the phylum and abundance found in this study were caused by variations in environmental factors in the studied mangrove ecosystem (Kurniawan *et al.,* 2020).

The results showed that rhizosphere bacteria communities found in mangrove sediments of *R. mucronata* in Kuala Langsa and Telaga Tujuh were dominated by the Crenarchaeota group from the kingdom Archaea. Meanwhile, other studies cited by Zhu *et al.* (2022) and Lu *et al.* (2022) reported a dominance of the phylum Proteobacteria. Lu *et al.* (2022) mentioned that the abundance and distribution were influenced by organic carbon, pH, temperature, sulfur, and nitrate. The prevalence of the Crenarchaeota group was due to the ability to perform metabolic functions, specifically the carbon, nitrogen, and sulfur cycles (Zhang *et al.,* 2019). Baskaran *et al.* (2023) also reported that most of the archaea communities found in China were from the phylum Crenarchaeota. This is because Rhizophora stands have a supporting root shape that facilitates the accumulation of organic matter from the land or sea (Ulumuddin, 2019). The higher the accumulation of organic matter, the greater the decomposition process carried out by bacteria and archaea, including sulfate reduction. According to Thatoi *et al.* (2013), the sulfate-reducing microbial communities were more abundant under the roots of Rhizophora due to the higher level of sediment and organic matter accumulation compared to the Avicennia mangrove. Chloroflexi, a group of bacteria with the second highest order after Crenarchaeota can photosynthesize under aerobic and anaerobic conditions. Anaerobic conditions occur when mangrove substrate is inundated with water, resulting in low oxygen levels. This uniqueness provides a competitive advantage over other phyla (Islam *et al.,* 2019). Wu *et al.* (2021) also mentioned that bacteria from Chloroflexi played an important role in decomposing organic matter. The organic matter content found in Kuala Langsa and Telaga Tujuh was in the high category and dominated by the mud substrate texture (Table 2). The ability to facilitate organic matter decomposition whether in water or mud, positions the bacteria as contributors to nutrient cycling.

At the low taxa level, rhizosphere bacteria communities in the two mangrove areas consisted of at least 24 families and 28 genera found only in Telaga Tujuh. The bacteria and archaeal communities were detected at both study sites (Figure 5b). At the family level, the highest abundance was observed for the Desulfatiglandaceae and Sulfurovaceae groups, while at the genus level, *Desulfatiglans* and *Sulfurovum* were more abundant. *Desulfatiglans* is a genus of Desulfatiglandaceae, and *Sulfurovum* belongs to the Sulphurovaceae family. Both groups are widely found in marine sediments and act as sulfate reducers (Li *et al.,* 2018). In addition, *Sulfurimonas* genera were also found in Kuala Langsa and Telaga Tujuh. This bacteria is still in the same group as Sulfurimonadaceae (Muwawa et al., 2021). In the study, bacteria members of the genus were found in the roots of *Rhizophora mucronata* in polluted and unpolluted areas. *Sulfurimonas* is a facultative anaerobic bacterium capable of oxidizing sulfur compounds in mangrove sediments. This process requires the use of oxygen as an electron acceptor, facilitating a reduction in the concentration of hydrogen sulfide, which can be toxic to some organisms (Sam and Maria, 2019). The presence of the genera under various environmental conditions underlines the significance of characterizing the *Rhizophora mucronata* ecosystem.

4. Conclusion

The diversity of rhizosphere bacteria detected

in Telaga Tujuh is greater than that in Kuala Langsa. Significant differences ($p < 0.05$) were observed in bacterial abundance between the two locations at all taxonomic levels. Environmental parameters did not vary significantly between observation sites ($p > 0.05$). This indicates that there are other factors beyond those studied that influence bacterial abundance and diversity. Information on vegetation age and climatic conditions during sediment sampling can further our understanding of rhizosphere bacterial communities and their impact on mangrove growth, aiding in more effective conservation and rehabilitation efforts.

Acknowledgement

The authors are grateful to the Directorate of Higher Education Research and Technology for the grant awarded with the derivative contract number 18910/IT3.D10/PT.01.02/M/T/2023, as well as to Mrs. Rita Sastrawan from Rettet and Regenwald e.V. from Germany, Prof. Darusman and Mr. Yusmadi from Aceh Wetland Foundation (AWF) for providing support and funds.

Authors' Contributions

The contributions of each author are as follows: May drafted the manuscript, processed the data, and visualized it in the form of diagrams. Meutia designed the main conceptual idea and critically revised the article. Begin and Andini revised the manuscript and Lita collected field samples. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Funding Information

This study was partially supported by the Directorate of Higher Education Research and Technology with grant number: 18910/IT3.D10/PT.01.02/M/T/2023.ts.

References

Andriyani, L. W., Geoffrey, B. D., & Samira, I. M. (2020). Relationship Between Gastropods (*Cassidula nucleus* and *Cassidula vespertilionis*) and Mangroves (*Avicennia marina* and *Sonneratia alba*) in Rehabilitated Mangrove Ecosystem in Pantai Indah Kapuk, Jakarta, Indonesia. *AACL Bioflux*, 13(4):2327-2335.

Basak, P., Majumder, N.S., Nag, S., Bhattacharyya, A., Roy, D., Chakraborty, A., SenGupta, S., Roy, A., Mukherjee, A., Pattanayak, R. & Ghosh, A. (2015). Spatiotemporal Analysis of Bacterial Diversity in Sediments of Sundarbans Using Parallel 16S rRNA Gene Tag Sequencing. *Microbial Ecology*, 69(1):500-511.

Baskaran, V., Mahalakshmi, A., & Pravavathy, V. R. (2023). Mangoves: A Hotspot for Novel Bacterial and Archaeal Diversity. *Rhizosphere*. 27(1): 1-16.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al¬ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, T., Callahan, B. J., Caraballo¬rodríguez, A. M., Chase, J., … Caporaso, J. G. (2018). QIIME 2: Reproducible, Interactive, Scalable, and Extensible Microbiome Data Science. *PeerJ*. 37(1):852-857.

Bucklin, A., Lindeque, P. K., Rodriguez-Ezpeleta, N., Albaina, A., & Lehtiniemi, M. (2016). Metabarcoding of Marine Zooplankton: Prospects, Progress and Pitfalls. *Journal of Plankton Reseacrh,* 38(3):393-400.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver Loren van Themaat, E., & Schulze-Lefert, P. (2013). Structure and Functions of The Bacterial Microbiota of Plants. *Annual Review Plant Biology*, 64(1):807-838.

Casas, L., Pearman, J. K., & Irigoien, X. (2017). Metabarcoding Reveals Seasonal and Temperature-Dependent Succession of Zooplankton Communities in The Red Sea. *Frontiers in Marine Science*, 4(1):1-15.

Chiarello, M., McCauley, M., Villéger, S., & Jackson, C. R. (2022). Ranking The Biases: The Choice of OTUs vs. ASVs in 16S rRNA Amplicon Data Analysis Has Stronger Effects on Diversity Measures Than Rarefaction and OTU Identity Threshold. *PLoS One*, 17(2):1-19.

Chrisnawati, S.D., Sabdaningsih, A., Jati, O.E. & Ayuningrum, D. (2023). Isolation and Molecular Identification of Rhizosphere Bacteria from *Rhizopora* sp. Mangrove Sediments in The Tapak Mangrove Ecosystem, Semarang. Indonesian *Journal of Marine Science and Technology*, 16(2):117-124.

Effendi, H. (2003). Study of Water Quality for Resource Management and Water Environment. Yogyakarta: Kanisius.

Dhal, P.K., Kopprio, G.A. & Gärdes, A. (2020). Insights on Aquatic Microbiome of The Indian

Sundarbans Mangrove Areas. *PLoS One*, 15(2): 1-18.

Fernández, Marques, V., Fopp, F., Juhel, J. B., Borrero-Pérez, G. H., Cheutin, M. C., Dejean, T., González Corredor, J. D., Acosta-Chaparro, A., Hocdé, R., Eme, D., Maire, E., Spescha, M., Valentini, A., Manel, S., Mouillot, D., Albouy, C., & Pellissier, L. (2021). Comparing Environmental DNA Metabarcoding and Underwater Visual Census to Monitor Tropical Reef Fishes. *Environmental DNA*, 3(1):142-156.

Giannopoulos, G., Lee, D. Y., Neubauer, S. C., Brown, B. L., & Franklin, B. R. (2019). A Simple and Effective Sampler to Collect Undisturbed Cores from Tidal Marshes. *BioRvix*, 1(1):1-18.

Gomes, N. C. M., Cleary, D. F. R., Pinto, F. N., Egas, C., Almeida, A., Cunha, A., Mendonça-Hagler, L. C. S., & Smalla, K. (2010). Taking root: Enduring Effect of Rhizosphere Bacterial Colonization in Mangroves. *PLoS One*, 5(11):1-10.

Hanafi, I., Subhan, & Basri, H. (2021). Analysis of Mangrove Vegetation (Case Study In The Mangrove Forest of Telaga Tujuh Island, West Langsa District). *Jurnal Ilmiah Mahasiswa Pertanian,* 6(4):740-748.

Huda, M. K., Latifah, A., & Prasetya, A. T. (2013). Making Liquid Organic Fertilizer from Cow Urine with Molasses Additives Fermentation Method. *Indonesian Journal of Chemical Science,* 2(3):1-6.

Imamsyah, A., Geoffrey B. D., & Meutia, S. I. (2020). Mangrove Vegetation Structure Based on Biophysical Environmental Quality in Bali Ngurah Rai Forest Park. *Ecothrophic*, 14(1):88-99.

Islam, Z. F., Cordero, P. R. F., Feng, J., Chen, Y. J., Bay, S. K., Jirapanjawat, T., Gleadow, R. M., Carere, C. R., Stott, M. B., Chiri, E., & Greening, C. (2019). Two Chloroflexi Classes Independently Evolved The Ability to Persist on Atmospheric Hydrogen and Carbon Monoxide. *ISME Journal*, 13(7):1801-1813.

Islamiah, D.N. & Rahmawati, R.L., (2017). Types of Rhizosphere bacteria in the Avicennia Mangrove Soil Area in Kanan Village, Mempawah District Hilir, West Kalimantan. *Protobiont Journal*, 6(3):165-172.

Iswahyudi, Kusmana, C., Hidayat, A., Pramudya Noorachmat, & Bambang. (2019). Environment Biophysical of Mangrove Forest in Langsa City, Aceh. *Journal of Natural Resources and Environmental Management,* 10(1):98-110.

Jiang, X.T., Peng, X., Deng, G.H., Sheng, H.F., Wang, Y., Zhou, H.W. & Tam, N.F.Y. (2013). Illumina Sequencing of 16S rRNA Tag Revealed Spatial Variations of Bacterial Communities in A Mangrove Wetland. *Microbial Ecology*, 66(1):96-104.

Kurniawan, A., & Asriani, E. (2020). Review: Quorum sensing bacteria and Their Role in Changes in pH Values in Kolong Pascatambang Timah with Different Ages. *Jurnal Ilmu Lingkungan,* 18(3):602-609.

Lear, G., Dickie, I., Banks, J., Boyer, S., Buckley, H. L., Buckley, T. R., Cruickshank, R., Dopheide, A., Handley, K. M., Hermans, S., Kamke, J., Lee, C. K., Macdiarmid, R., Morales, S. E., Orlovich, D. A., Smissen, R., Wood, J., & Holdaway, R. (2018). New Zealand Ecological Society Methods for The Extraction, Storage, Amplification and Sequencing of DNA from Environmental Samples. *New Zealand Journal of Ecology,* 42(1):1-50.

Li, Y., Tang, K., Zhang, L., Zhao, Z., Xie, X., Chen, C. T. A., Wang, D., Jiao, N., & Zhang, Y. (2018). Coupled Carbon, Sulfur, and Nitrogen Cycles Mediated by Microorganisms in The Water Column of A Shallow-Water Hydrothermal Ecosystem. *Frontiers in Microbiology*, 9(1):1-13.

Loganathachetti, S.D., Sadaiappan, B., Poosakkannu, A. & Muthuraman, S. (2016). Pyrosequencing-Based Seasonal Observation of Prokaryotic Diversity in Pneumatophore-Associated Soil of *Avicennia marina*. *Current Microbiology*, 72(1):68-74.

Lu, K., Yang, Q., Jiang, Y., & Liu, W. (2022). Changes in Temporal Dynamics and Factors Influencing the Environment of The Bacterial Communities in Mangrove Rhizosphere Sediments in Hainan. *Sustainability,* 14(12):7415.

Lund, M., Agerbo Rasmussen, J., Ramos-Madrigal, J., Sawers, R., Gilbert, M. T. P., & Barnes, C. J. (2022). Rhizosphere Bacterial Communities Differ Among Traditional Maize Landraces. *Environmental DNA*, 4(6):1241-1249.

Margareta, A. (2023). Bioinformatics Insights Next Generation Sequencing in Intestinal Mycobiome Metagenomic Samples. *Jurnal Medical Laboratory*, 2(1):41-58.

Maysaroh, S., Ismet, M. S., Subhan, B., Andini, R., Sembiring, E. R., & Anggraini, N. P. (2023).

Effective DNA Extraction Method for Metagenomic Analysis of Rhizosphere Bacteria from Mangrove Sediments. *Depik,* 12(2):198-209.

Muwawa, E. M., Obieze, C. C., Makonde, H. M., Jefwa, J. M., Kahindi, J. H. P., & Khasa, D. P. (2021). 16S rRNA Gene Amplicon-Based Metagenomic Analysis of Bacterial Communities in The Rhizospheres of Selected Mangrove Species from Mida Creek and Gazi Bay, Kenya. *PLoS One*, 16(3):1-22.

Ntabo, R.M., Nyamache, A.K., Lwande, W., Kabii, J. and Nonoh, J. (2018). Enzymatic Activity of Endophytic Bacterial Isolates from Selected Mangrove Plants in Kenya. *The Open Microbiology Journal*, 12(1):354-363.

Nybakken, J. W. (1993). Basics of Mangrove Ecology. Jakarta: Gramedia.

Purba, F. A., Fikri, A., Rasuldi, riza, Wilianti, M. I., & Febri, S. P. (2017). The Relationship of Biological and Physical Parameters of Waters to The Growth of Oyster Oysters in Kuala Langsa Waters. *Jurnal Ilmiah Samudera Aquatika,* 1(1):64-71.

Putri, L., Yulianda, F., & Wardianto, Y. (2015). Mangrove Zoning Pattern and Macrozoobenthos Association in Pantai Indah Kapuk Area, Jakarta. *Bonorowo Wetlands,* 5(1):29-43.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools. *Nucleic Acids Research*, 41(1):590-596.

Rozi, F., & Maulidiya, D. (2022). Analysis of Changes in Inflation in Several Major Cities in Indonesia Using the Kruskal-Wallis Test. Multi Proxymity: *Jurnal Statistika Universitas Jambi,* 1(2):103-115.

Rusianti, C.R., Saleh, F.I.E., Talakua, S., Alianto, A., Mangando, S., Demena, Y. E., Manalu, E., Eldiester, F. C., Rumbino, F. N. Y., & Raharjo, S. (2022). Mangrove Vegetation Structure and *Sonneratia alba* Mangrove Litter Production in Telaga Wasti, Manokwari Regency, West Papua. *Jurnal Pembangunan Berkelanjutan*, 4(2): 93-103.

Sam, K., & Maria, J. G. (2019). Role of Sulfur-Oxidizing Bacteria on The Ecology in Tropical Mangrove Sediments. *Regional Studies in Marine Science.* 28(1):1-9.

Sanders, C. J., Eyre, B. D., Santos, I. R., MacHado, W., Luiz-Silva, W., Smoak, J. M., Breithaupt, J. L., Ketterer, M. E., Sanders, L., Marotta, H., & Silva-Filho, E. (2014). Elevated Rates of Organic Carbon, Nitrogen, and Phosphorus Accumulation in A Highly Impacted Mangrove Wetland. *Geophysical Research Letters*, 41(7):2475-2480.

Saputri, K.E., Idiawati, N.S., & Sofiana, M. S. J. (2021). Isolation and Characterization of Nitrogen-Fixing Bacteria from Mangrove Rhizosphere in Kuala Singkawang. *Jurnal Laut Khatulistiwa*, 4(2):80-84.

Sari, M. A., Purnomo, P. W., & Haeruddin. (2016). Analysis of Oxygen Demand for Sediment Organic Matter Decomposition in The Mangrove Area of Bedono Demak Village. *Management of Aquatic Resource,* 5(4);285-292.

Sharma, A., & Lal, R. (2017). Survey of (Meta)genomic Approaches for Understanding Microbial Communities Dynamics. *Indian Journal of Microbiology*, 57(1):23-38).

Siregar, R. M., Anggita Widodo Program, P., Kimia, S., Matematika, F., Ilmu, D., & Alam, P. (2022). Titrimetric Determination of Nitrogen Nutrients from Oil Palm Leaves. *Sains dan Teknologi,* 1(1):1-5.

Susilowati, D. Ni., Fauziah, F., Setiawati, M. R., Parnoto, E., Hidayati, E., Setyowati, M., & Rachmiati, Y. (2016). Analysis of Tea Plant Rhizosphere Bacterial Communities in Hatcheries Using Terminal Restriction Fragment Length Polymorphism (TRFLP) technique). *Jurnal Penelitian Teh dan Kina*, 19(2):147-156.

Suwoyo, H. S, D., Sri, M. (2015). Dominan Factors Affecting Sediment Oxygen Consumpsion Level in Intensive White Shrimp. *Jurnal Ilmu dan Teknologi Kelautan Tropis*. 7(2):639–654.

Thatoi, H., Behera, B. C., Mishra, R. R., & Dutta, S. K. (2013). Biodiversity and Biotechnological Potential of Microorganisms from Mangrove Ecosystems: A Review. *Annals of Microbiology,* 63(1):1-19.

Ulumuddin, Y. I. (2019). Methane: Greenhouse Gas Emissions from Blue Carbon Ecosystems, Mangroves. *Jurnal Ilmu Lingkungan,* 17(2):359-372.

Vasileiadis, S., Puglisi, E., Arena, M., Cappa, F., Cocconcelli, P. S., & Trevisan, M. (2012). Soil Bacterial Diversity Screening Using Single 16S rRNA Gene V Regions Coupled With Multi-Million Read Generating Sequencing Technologies. *PLoS One*, 7(8):1-11.

Vilaça, S. T., Grant, S. A., Beaty, L., Brunetti, C. R., Congram, M., Murray, D. L., Wilson, C. C., & Kyle, C. J. (2020). Detection of Spatiotemporal Variation in Ranavirus Distribution Using eDNA. *Environmental DNA*, 2(2):210-220.

Wang, F., Chen, N., Yan, J., Lin, J., Guo, W., Cheng, P., Liu, Q., Huang, B., & Tian, Y. (2019). Major Processes Shaping Mangroves as Inorganic Nitrogen Sources or Sinks: Insights From A Multidisciplinary Study. *Journal of Geophysical Research: Biogeosciences*, 124(5):1194-1208.

Wickham, H. (2009). ggplot2. Elegant Graphics for Data Analysis. Newyork: Springer Nature.

Wu, S., You, F., Hall, M., & Huang, L. (2021). Native Plant Maireana brevifolia Drives Prokaryotic Microbial Communities Development in Alkaline Fe Ore Tailings Under Semi-Arid Climatic Conditions. *Science of the Total Environment,* 760(1):1-11.

Zhang, Z., Zhang, P., Lin, Q., Cha, Z., & Luo, W. (2019). Response of Bacterial Communities in Rubber Plantations to Different Fertilizer Treatments. *3 Biotech*, 9(8):1-9.

Zhu, D. H., Song, Q. L., Nie, F. H., Wei, W., Che, M. M., Zhang, M., Lin, H. Y., Kang, D. J., Chen, Z. B., Hay, A. G., & Chrn, J. J. (2022). Effect of Environmental and Spatial Variables on Bacteria in Zhanjiang Mangrove Sediments. *Current Microbiology,* 79(4):1-11.