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Localized Downcore Variation of Microbial Profiles in Mangrove Sediments and Its Role in Blue Carbon Storage

(Variasi Kedalaman Setempat Profil Mikrob dalam Sedimen Bakau dan Peranannya dalam Penyimpanan Karbon Biru)

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ABSTRACT

The profiling of the composition and diversity of microbial communities in mangrove sediments allow for the understanding of blue carbon storage. This study investigated the downcore variation of microbial profiles and related carbon fixation pathways in mangrove sediments of Pulau Seribuat, Malaysia. 16s RNA sequencing was carried out on replicate cores in three depth layers. The bacterial phyla of Proteobacteria, Chloroflexi and Planctomycetota showed significant downcore variation within the sediment depths. Proteobacteria showed highest abundance in the top layer while Chloroflexi dominated the bottom layer. Statistical analysis indicated that Proteobacteria and Chloroflexi abundance were both significantly different between top and bottom layers, while the middle layer acted as a transition zone for the microbial community. Further analysis of predicted functional pathways indicated the prevalence of the Calvin-Benson-Bassham (CBB) cycle and the reverse tricarboxylic acid (rTCA) cycles throughout the three sediment layers. Although trends were observed in their abundance, the PERMANOVA tests showed no significant differences. These findings suggest that sediment microbial profile is influenced by other factors such as oxygen and nutrient availability, which in turn may affect the rates of carbon fixation. This suggests that the microbial community composition may serve as a potential indicator for processes within the blue carbon cycle, aiding in management of these ecosystems.

Keywords: Carbon fixation; carbon release; KEGG pathway; mangrove sediment; microbial profiles

ABSTRAK

Komposisi dan kepelbagaian komuniti mikrob dalam tanah bakau membolehkan pemahaman mengenai penyimpanan karbon biru. Penyelidikan ini mengkaji variasi profil mikrob mengikut kedalaman dan laluan penetapan karbon yang berkaitan dalam sedimen bakau di Pulau Seribuat, Malaysia. Penjujukan 16s RNA telah dijalankan pada tiga teras replikasi yang dikumpulkan dari tapak pensampelan. Tiga filum bakteria, iaitu Proteobakteria, Chlorofleksi dan Planctomycetota menunjukkan variasi ketara mengikut kedalaman sedimen. Proteobacteria menunjukkan kelimpahan tertinggi di lapisan atas sedimen, manakala Chlorofleksi mendominasi lapisan bawah yang bersifat anaerobik. Analisis statistik menunjukkan bahawa kelimpahan Proteobakteria dan Chlorofleksi adalah berbeza secara signifikan antara lapisan atas dan bawah, manakala lapisan tengah berfungsi sebagai zon peralihan bagi profil tersebut. Analisis lanjut terhadap laluan fungsian yang diramalkan menunjukkan kehadiran kitaran Calvin-Benson-Bassham (CBB) dan kitaran asid trikarboksilik songsang (rTCA) di seluruh lapisan sedimen. Walaupun terdapat trend dalam kelimpahan laluan ini, ujian PERMANOVA utama menunjukkan bahawa tiada perbezaan yang signifikan. Penemuan ini mencadangkan bahawa profil mikrob sedimen dipengaruhi oleh faktor lain seperti ketersediaan oksigen dan nutrien yang seterusnya boleh mempengaruhi kadar penetapan karbon. Ini mencadangkan bahawa komposisi komuniti mikrob mungkin berfungsi sebagai penunjuk berpotensi bagi proses dalam kitaran karbon biru, sekali gus membantu dalam pengurusan ekosistem ini.

Kata kunci: Laluan KEGG; pelepasan karbon; penetapan karbon; profil mikrob; sedimen bakau

INTRODUCTION

In recent years, blue carbon - the carbon stored in marine and coastal areas such as mangroves, seagrasses and salt marshes - has gained attention with its potential role in mitigating climate change. Carbon fixation is among the fundamental natural process in blue carbon cycling whereby inorganic carbon is converted into organic compounds via photosynthesis (Li et al. 2024). This conversion is

important in sustaining life on Earth but also in regulating the global carbon cycle and mitigating climate change (Lee et al. 2025). Related processes involve the usage of carbon dioxide to form organic matter (OM) that acts as an energy source for multiple organisms. Therefore, understanding carbon fixation is essential in uncovering the dynamics of an ecosystem and furthermore, in climate regulation and life sustainability (Sharma et al. 2022).

Blue carbon ecosystems are highly effective at sequestering CO_2 from the atmosphere, storing it in biomass and in sediments beneath them as OM. This carbon storage not only contributes to climate change mitigation but also offsets greenhouse gas emissions (Stankovic et al. 2021). In a past study, mangroves were shown to sequester carbon at rates higher than terrestrial forests, reaching up to 226 gCm^{-2} per year (Chatting et al. 2022). Blue carbon ecosystems also supports marine life; for instance, fish catches can be up to 70% higher in areas close to mangroves compared to those without, highlighting the multiple ecological and economic benefits of these environments (von Hammerstein et al. 2024). Therefore, preservation of these systems is crucial for maintaining the stability of marine ecosystems.

Inversely, the remineralization of organic carbon (OC) in these systems is also equally important to maintain a healthy environment. Once these carbon sources are stored in the geosphere, microbial communities break down these compounds into simple inorganic forms, such as CO_2, and dissolved inorganic carbon (DIC) (Garritano, Song & Thomas 2022). This process is vital in regulating carbon storage especially in mangrove sediments. In an ideal scenario, mangroves would act as long-term carbon sinks, removing circulating CO_2 from the atmosphere and storing it long-term (Alongi 2020), depending on the balance between carbon inputs (OC and OM) and outputs (remineralization). If remineralization rates are high, a larger proportion of the stored carbon would be released back into the atmosphere, reducing the net carbon fixation potential of the mangrove (Yamuza-Magdaleno et al. 2024). Hence, efficient sequestration by marine plants may be empirically quantified if it can be shown that carbon fixation rates are high within the blue carbon ecosystem.

To understand how the rates can be enhanced, microbial communities should be studied first as they are among the drivers of carbon fixation processes (Rodríguez-Ramos et al. 2022). These organisms facilitate the biochemical pathways that leads to the stabilization of OC (Abdullah & Tsutsumi 2018). There are several biochemical pathways involved in the process of carbon fixation with the Calvin-Benson-Bassham (CBB) cycle as the most common pathway. This cycle primarily occurs in photosynthetic organisms such as seagrasses and mangroves. cbbM-carrying bacteria in sediments such as Proteobacteria convert atmospheric CO_2 into organic compounds, where it is then assimilated by other microbes or plants (Lannes et al. 2019). The cbbM gene encodes the enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCo). This enzyme then facilitates the incorporation of CO_2 into ribulose-1,5-biphosphate. Studies have suggested that cbbM-carrying bacteria are vital in intertidal sediments where its utilization of CBB is effective in carbon fixation (Zarzycki & Fuchs 2011).

Another pathway for blue carbon fixation is the reverse tricarboxylic acid (rTCA) cycle I and II. These cycles are important in anaerobic conditions such as deep sea and anoxic environments as they allow microorganisms such as Chloroflexi to convert CO_2 into OM. The ATP citrate lyase (ACL) enzyme in the microbe catalyzes the cleavage of citrate into oxaloacetate and acetyl-CoA. This then continues onto the formation of stable carbon compounds. The effectiveness of these microbes, especially in dark conditions, also makes it one of the drivers of carbon cycling (Wahlund & Tabita 1997).

The 3-HP cycle and 3HP superpathway are also a carbon fixation drivers where CO_2 is reduced to form acetyl-CoA, which is then used in energy generation and biosynthesis (Shih, Ward & Fischer 2017). This anaerobic pathway is influenced by the availability of CO_2 and with optimal substrate availability, would form stable OC. Unlike the CBB cycle, the 3-HP cycle requires fewer ATP for carbon fixation and can also produce heavier biomass (Hügler & Fuchs 2005).

These microbial pathways do not only facilitate direct carbon fixation but also enhances carbon sequestration efficiency in blue carbon ecosystems (Rodriguez et al. 2025). Past studies have shown that enhancing the efficiency of these pathways increases carbon flow towards biomass production and reduces CO_2 emission (Liang, Zhao & Yang 2020). Optimizing microbial carbon use efficiency (CUE) would contribute to long-term storage of blue carbon. Therefore, there is a dynamic interplay between OM, primary producers and decomposers that sustains ecosystem health. Understanding these microbial contributions opens opportunities to enhance blue carbon management strategies and maintaining ecological balance.

In this study, finding on the microbial characteristics of carbon fixation in sediments is presented for the mangrove forest found in Pulau Seribuat (Pahang, Malaysia). The work was conducted to study the localized variation of microbial profiles downcore of mangrove sediments and how it is related to the expression of blue carbon fixation pathways. The data provided would act as a proxy representing the profiles of younger, intermediate-aged and older sediment layers, and can be used to review the potential capacity of blue carbon storage in mangroves. The outcome will be beneficial in understanding the movement of carbon and the importance of microbial profile to monitor the carbon capture capacity of blue carbon ecosystems.

MATERIALS AND METHODS

SITE DESCRIPTION

Samples were collected from Pulau Seribuat (1°24'10.86"N, 103°31'56.58"E; Figure 1) in March and June of 2022. This site is located off the coast of Pahang, Malaysia and is chosen due to its relatively pristine environment, untouched by anthropogenic activities (Zairfornoor et al. 2024). It is in the Tioman Marine Park area and relatively remote compared to other coastal areas. The location is also known for its dense mangrove forests and seagrass meadows, hence suitable for ecological baseline studies. The water quality and oxygen levels of the area are favourable for marine life, allowing for environmental studies where multiple algal and coral research has also been conducted (Arina, Rozaimi & Zainee 2019; Ismail, Zaidnuddin & Ismail 2021).

SAMPLE COLLECTION

Sampling was performed during low tides when the mangrove substrate was exposed from submersion. The sediment cores (n=3) were taken randomly by using an open auger corer (5.5 cm diameter, 120 cm length) with no obvious sediment compaction of the extruded cores. To mitigate contamination of the samples, aseptic technique was used where spatula was sanitized before collecting the next sample. Gloves and masks were worn to also reduce contamination. Each core was 100 cm in length and divided into three layers: top (1 - 20 cm), middle (40 - 60 cm) and bottom (80 - 100 cm).

DNA EXTRACTION AND SEQUENCING

Sediment cores were extruded from the core barrel and cut into 10 cm thick slices, up to 100 cm along the core length. The sediment core depths were chosen to represent indicative oxic, suboxic and anoxic layers of the sediment. Samples were then stored in 50 mL centrifuge tubes and transported in 4 °C for DNA extraction. Total DNA was extracted and purified from each sediment sample using the DNeasy PowerSoil DNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's manual. The bacterial 16S rRNA gene was selected for amplification with primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC), targeting the V3-V4 regions (Thijs et al. 2017). These regions are hypervariable, which allows for distinguishing the microbes between different taxa. Furthermore, the selected primers have been optimized for specificity to bacterial sequences, avoiding eukaryote or virus interference to provide higher quality data (Mori et al. 2014). 1st and 2nd stage PCR was conducted, and quality was measured via Agilent Bioanalyzer 2100 System (Liu et al. 2019a). The PCR products were paired-end sequenced using the Illumina 300 TM MiSeq sequencing platform (Illumina, United States).

DATA ANALYSIS

Amplicon sequence variant (ASV) generation using the DADA2 workflow was used in this analysis (Callahan et al. 2016). Raw read quality assessment was conducted with FastQ (Andrews 2010), followed by Cutadapt 3.5 (Martin 2011) for primer and adaptor removal. Paired-end reads were processed and merged with DADA2 V1.18 (Callahan et al. 2016). After inferencing, ASVs and chimeric sequences generated from PCR artifacts were eliminated to avert false positive findings. Next, taxonomic classification was conducted using the SILVA database (V138), enabling the linking of ASVs to establish the profile of microbial taxa. A phylogenetic tree was created

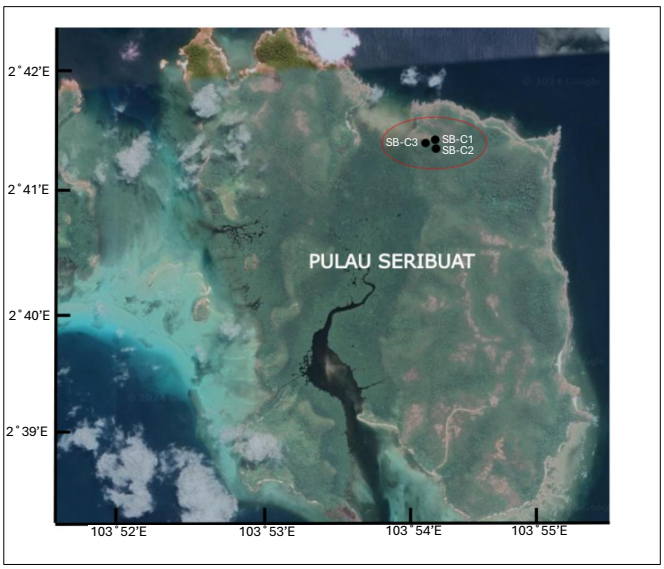

FIGURE 1. Core sampling location

with MUSCLE for aligning sequences (Edgar 2004) and FastTree2 for constructing the tree (Price, Dehal & Arkin 2010). The ASVs present in each core were represented as relative abundance and generated into a table for statistical analyses (Table A1). PICRUSt2 were used to predict the functional potential of the microbe community. It inferred gene family abundances like KEGG orthologs (KOs) from phylogenetic placement of ASVs and predicted functional pathways. These pathways were categorized into broader functional pathways using mapping files that were created based on the MetaCyc database. For a clearer view of the data, pathways were transformed into log values and presented in a clustered bar chart. To investigate the significance of downcore trends, PERMANOVA and SIMPER analyses (Primer-E Ver. 7) were carried out.

RESULTS

SEDIMENT BACTERIAL COMMUNITIES

ASVs from across 53 phyla were recovered from the mangrove sediments. Bacterial communities were grouped distinctly by depth: wherein top of the sample represented the top 20 cm of the core, middle represented the middle 40-60 cm of the core and bottom represented the bottom 80-100 cm of the core (Figures 2-4). Depth-wise analysis indicated that Protobacteria was most abundant at top layers of sediment (Figure 2). In contrast, Chloroflexi was the most abundant in the bottom layer of the sediment. Meanwhile, the middle layer reflects this change as Protobacteria numbers decline while Chloroflexi abundance starts to increase. For Desulfobacterota and Acidobacteriota, these phyla remain in moderate abundance downcore while Planctomycetota shows a decreasing trend downcore.

The main PERMANOVA analysis indicates that overall Chloroflexi, Planctomycetota and Proteobacteria have significant variation downcore of the sediments. Conversely, Acidobacteriota, Actinobacteriota and Desulfobacterota do not exhibit significant differences. In Table 1(B), pairwise PERMANOVA tests showed the depths of which the phyla are significantly different. For both Chloroflexi and Proteobacteria, there is a difference in abundance between the top and bottom layers of sediments. The abundance of Planctomycetota is significantly different in between middle and bottom layers.

The SIMPER analysis highlights the taxa contributing to the highest dissimilarity values in the sediments microbial community downcore. Proteobacteria contributed the highest percentages in top-middle (5.4%) and top-bottom comparisons (11.35%), suggesting its dominance in shaping microbial community differences. Meanwhile, Chloroflexi (6.77%) contributed the most differences in the middle-bottom comparison (Table 2).

The Shannon diversity index showed slight variations across depths. The middle depth exhibited highest median diversity, followed by top layer while the bottom layer showed lowest diversity. However, even with these differences, no significant difference was detected among the sediment depths. This suggests that the alpha diversity profile remains relatively stable downcore. Phyla richness showed a different trend compared to alpha diversity. The top layer displayed the highest richness with greater variability, reflecting a more heterogenous community structure. The middle layer showed intermediate richness with less variability while the bottom layer had the lowest richness and narrowest range. However, statistical tests showed no significant differences in richness between sediment depth layers ($p > 0.05$).

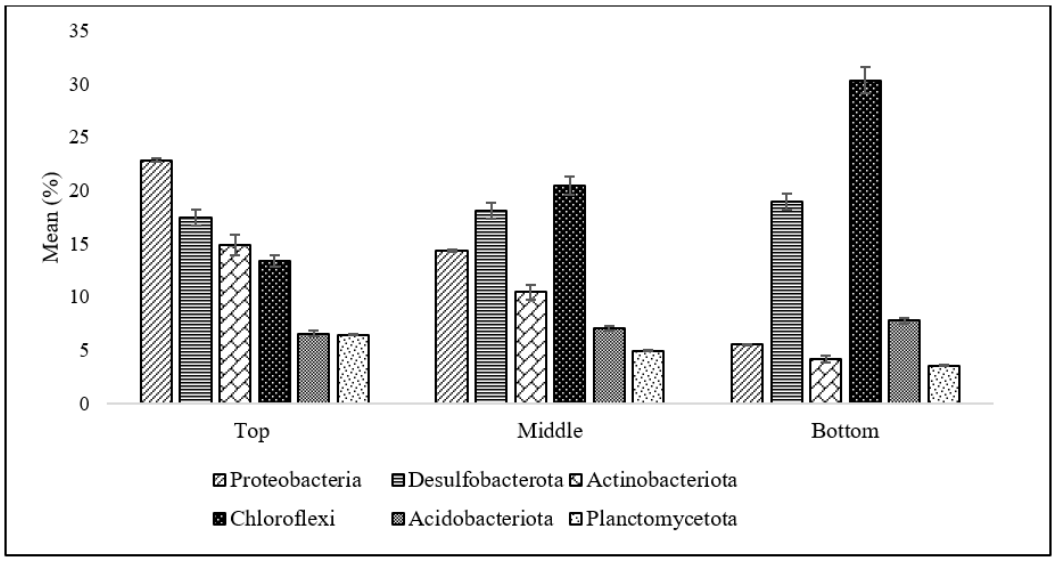

FIGURE 2. The percentage abundance (mean ± standard error) of the six most common taxa across different depths in Pulau Seribuat sediments

TABLE 1. Statistical analysis on the common taxa consistently found across different sediment depths in Pulau Seribuat. A) main PERMANOVA test for each taxa (df = 2) and B) PERMANOVA test (pairwise) for each taxa in different depths

(A)				
Source of variation	SS	MS	F	P
Acidobacteriota	3.021	1.510	1.82	NS
Actinobacteriota	4.476	2.238	3.81	NS
Chloroflexi	5.723	2.861	7.54	*
Desulfobacterota	0.124	0.006	0.04	NS
Planctomycetota	5.342	2.672	6.03	*
Proteobacteria	5.224	2.612	5.65	*

(B)			
Source of variation	Chloroflexi	Planctomycetota	Proteobacteria
Top - Middle	NS	NS	NS
Top - Bottom	**	*	**
Middle - Bottom	NS	*	NS

* - significant differences ($p < 0.05$); ** - significant differences ($p < 0.01$); NS – no significant differences ($p > 0.05$)

TABLE 2. SIMPER analysis on the common taxa consistently found across different depths in Pulau Seribuat

Source of Variation	Taxa	Av. Abu %	Diss. %
Top - Middle	Proteobacteria	18.66	5.40
	Chloroflexi	16.96	4.51
	Actinobacteriota	12.68	2.79
	Planctomycetota	5.78	0.10
	Desulfobacterota	17.81	0.38
	Acidobacteriota	6.84	0.32
Top - Bottom	Proteobacteria	14.25	11.35
	Chloroflexi	21.90	11.12
	Actinobacteriota	9.56	7.02
	Planctomycetota	5.09	1.90
	Desulfobacterota	18.27	0.98
	Acidobacteriota	7.18	0.79
Middle - Bottom	Chloroflexi	25.48	6.77
	Proteobacteria	9.98	6.02
	Actinobacteriota	7.34	4.31
	Planctomycetota	4.33	0.96
	Desulfobacterota	18.58	0.62
	Acidobacteriota	7.44	0.48

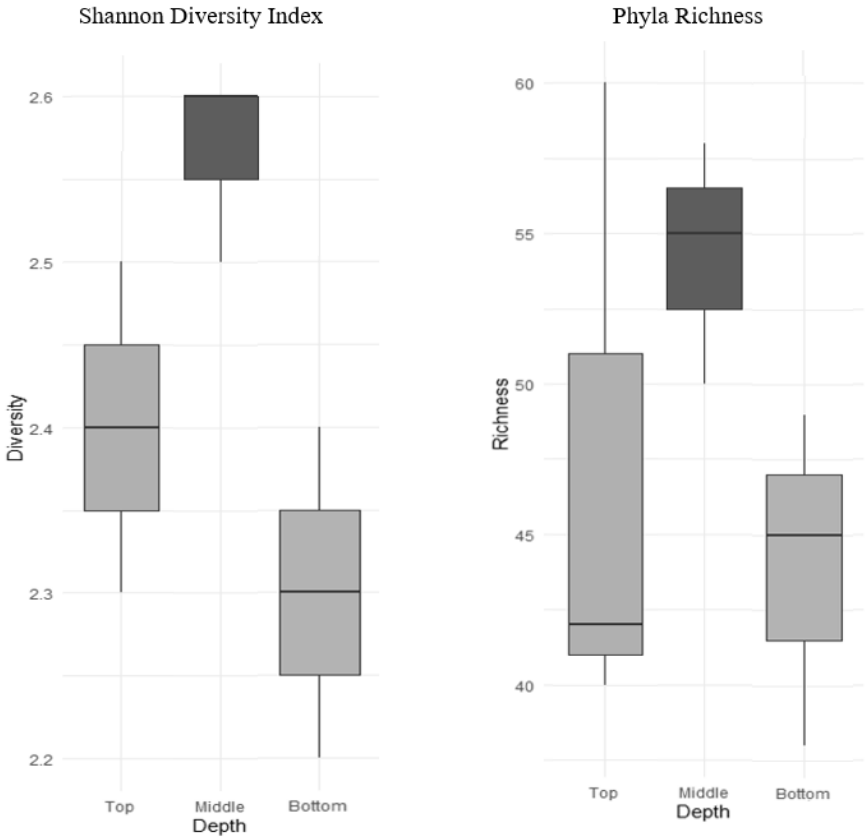

FIGURE 3. Diversity metrices of overall taxa composition across different sediment depths at Pulau Seribuat

CARBON FIXATION PATHWAYS IN DIFFERENT CORE DEPTHS

The five most common pathways related directly to carbon fixation is presented in Figure (4). The most predicted functional pathway was the CBB cycle and rTCA cycle I with related abundance across all depths. Pathways related to rTCA cycle II only started to be present in the middle and decreased in operational taxonomic unit counts towards the bottom layer. For 3-HP cycle and superpathway, they are observed to only be present in the middle layers. The main PERMANOVA tests indicate that overall, there are no significant differences in the presence of the carbon fixation pathways (Table 3).

The SIMPER analysis highlights the pathways that are most dissimilar between depths. Overall, the CBB pathway contributes the most to differences in the layers with the top versus middle showing a 14.21% dissimilarity, top versus bottom at 14.83% and middle versus bottom at 16.21%. This is followed by rTCA cycle I with dissimilarity of 6.41% between top and middle layers. This is also reflected in top versus bottom and middle versus bottom comparisons, with 11.35% and 10.26% dissimilarity respectively. For the remaining pathways, relatively minor (< 0.01%) dissimilarities were observed (Table 4).

DISCUSSION

In this study, site-specific data is provided on microbial profiles of mangrove sediments and carbon fixation related pathways expressed in the sediments. The information gathered allows for a localized understanding of the microbial drives of blue carbon storage. Pulau Seribuat was selected as a sampling site due to its relatively pristine environment, located away from anthropogenic activities. However, the data collected here would only reflect baseline data and does not represent other blue carbon areas that are mostly close to human settlements. When compared with past studies in close range with anthropogenic sites such as samples taken from surface sediments of river mangrove estuaries, Proteobacteria was still found to have an abundance of around ~20% (Liu et al. 2024, 2019b; Mai et al. 2021). This is similar to the abundance of the taxa in our samples. Chloroflexi was also described as the predominant phyla in another study (Blazejak & Schippers 2010; Yahaya et al. 2024). This suggests that dominant bacteria may maintain its abundance in anthropogenic areas although their functional roles and interactions within the microbial community may shift in response to environmental stressors.

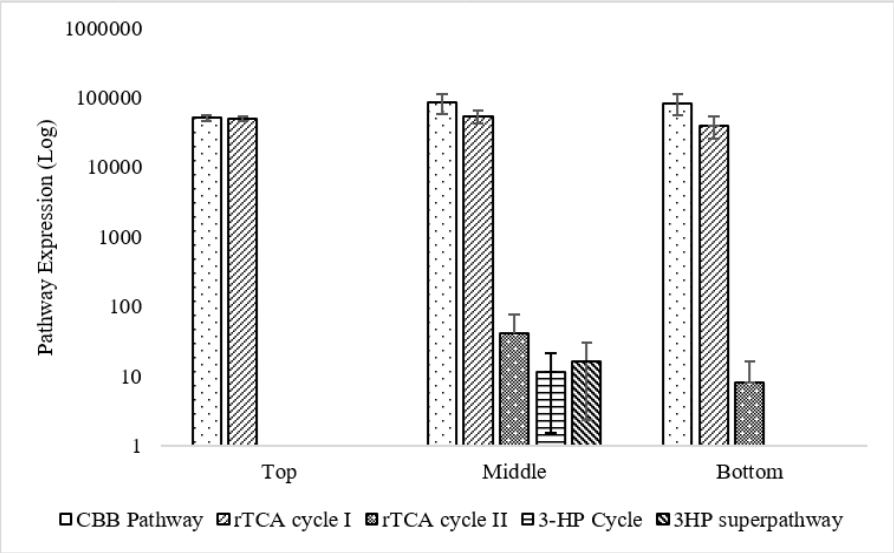

FIGURE 4. Pathway abundance levels in mangrove sediments of Pulau Seribuat

TABLE 3. PERMANOVA analysis on the five-carbon fixation related pathways present downcore in Pulau Seribuat

Source of variation	SS	MS	F	P
CBB	1.546	0.773	0.72	NS
rTCA cycle I	1.252	0.626	0.56	NS
rTCA cycle II	1.383	0.692	0.63	NS
3 - HP cycle	2.000	1.000	1.00	NS
3 - HP superpathway	2.000	2.000	1.00	NS

(df = 2). * - significant differences ($p < 0.05$); ** - significant differences ($p < 0.01$); NS – no significant differences ($p > 0.05$)

TABLE 4. SIMPER analysis on the five-carbon fixation related pathways downcore of Pulau Seribuat

Source of Variation	Taxa	Av. OTU Count	Diss. %
Top - Middle	CBB	6.92×10^4	14.21
	rTCA cycle I	5.18×10^4	6.41
	rTCA cycle II	2.38×10^1	0.04
	3 - HP cycle	5.70	0.01
	3 - HP superpathway	8.27	0.01
Top - Bottom	CBB	6.75×10^4	14.83
	rTCA cycle I	4.47×10^4	11.35
	rTCA cycle II	4.08	0.003
	3 - HP cycle	NA	NA
	3 - HP superpathway	NA	NA
Middle - Bottom	CBB	8.53×10^4	16.21
	rTCA cycle I	4.68×10^4	10.26
	rTCA cycle II	2.45×10^1	0.14
	3 - HP cycle	5.70	0.01
	3 - HP superpathway	8.27	0.004

DEPTH DEPENDENT MICROBIAL COMMUNITIES IN MANGROVE SEDIMENTS

From the top 10 taxa that were abundant in the sediments, we looked at six taxa that was common across each layer of the cores. Downcore variation of microbial profiles were found to exist as reflected in the trends of Proteobacteria, Chloroflexi and Planctomycetota. This may be due to the role of oxygen and nutrient availability as limiting factors (Mun & Ling 2022; Zhou et al. 2017). In Figure 2, we saw that Protobacteria was the most abundant in top layers of sediment where the access to more aerobic conditions allows them to utilise oxygen in ATP production. A fall in oxygen levels would decrease microbial activity, in which was reflected by the decrease in abundance with depth (Jeyanny et al. 2021; Mohiddin Mohd Ngesom et al. n.d.). This was confirmed by the results in Tables 1 and 2 that indicated significant differences of Proteobacteria in the top and bottom layers. The following dissimilarity results further strengthen the argument for this difference. This is consistent with several regional studies where Proteobacteria has been found to significantly decrease in numbers, especially when the environment is anaerobic (Qiao et al. 2018). The argument that oxygen is one of the limiting factors in microbial composition, as mentioned in Zhang et al. (2021) is therefore strengthened.

As the depth of sediments move towards the middle layer, we saw a decline in the Proteobacteria abundance and an increase in Chloroflexi. This again highlights that oxygen is a limiting factor of microbial composition. The shift from a fully aerobic environment to microaerophilic provide a less viable condition for Proteobacteria to thrive in. Instead, anaerobic Chloroflexi has evolved to utilise surrounding organic compounds for energy production (Bovio-Winkler et al. 2023). Both PERMANOVA and SIMPER analyses showed that the change in abundance was significant for Chloroflexi, especially between top and bottom layers. Chloroflexi was described to significantly increase in deeper sediments (Vuillemin et al. 2020), therefore the present study confirms the incline of growth in deeper sediment layers. The trends of Proteobacteria and Chloroflexi continues to the bottom layers where statistical analyses indicated that the differences in abundance were significantly different.

In Planctoymycetota, we saw a decrease downcore in the chemoautotroph that thrives in oxygen rich environments (Klimek, Herold & Calusinska 2024). Statistical tests imply that the abundance of the taxa is relatively stable between the top and middle layers but declines significantly at the bottom layer. This could indicate that Planctomycetota is more prevalent or adapted to the upper sediment layers (Ivanova & Dedysh 2012). With the limited availability of nutrients in the lower layers of the sediment, Planctomycetota is unable to perform anammox (anaerobic ammonium oxidation). This process utilizes CO_2 as a carbon source, being involved in carbon fixation. Therefore, the significant decline in Planctomycetota abundance suggest a reduced capacity for carbon fixation by the taxa in deeper sediments.

A recent study suggested that the increase in depth would lead to substrates becoming more recalcitrant, making them less accessible to microbes hence shifting microbial composition (Bruni et al. 2022). This connects to another study that highlights bacteria in lower depths tend to degrade OM at a slower rate, producing stable compounds (Chang et al. 2023). Prolonged exposure to this activity produces sediment OC that becomes increasingly resistant to microbial breakdown. Therefore, the variation of taxa abundance may be due to nutrient availability and remineralization rates of OM.

PROTEOBACTERIA AND CHLOROFLEXI AS MAIN BACTERIAL DRIVERS IN BLUE CARBON FIXATION OF SEDIMENTS

Our study also explored the relationship between microbial composition and the predicted functional potential of blue carbon fixation pathways. We found that microbial pathways could be used to gauge the related carbon fixation processes. Although PERMANOVA did not reveal statistically significant variations in pathway profiles at different depths, the predicted existence of these pathways still provides valuable insight into the dataset. Consequently, their possible activity within sediment layers was examined to better understand how microbial communities might influence carbon cycling and long-term storage (Ayala-Muñoz et al. 2022). We focused on the CBB cycle, rTCA cycle and the 3-HP pathway as they are recognized as primary drivers of carbon fixation in the environment. While other metabolic routes exist, these five pathways represent the most significant mechanism by which the microbes convert inorganic carbon into stable carbon compounds.

In Proteobacteria, ATP is often generated by the consumption of oxygen. However, many species also possess the capability of carbon fixation under microaerophilic or anaerobic conditions using diverse metabolic pathways (Santos et al. 2022). The ATP produced is then utilized in the Calvin-Benson-Bassham cycle for carbon fixation. In this process, CO_2 is fixed into ribulose biphosphate (RuBP) by the enzyme RuBisCo, leading to formation a 3-carbon sugar phosphate that is then deposited into the sediment as a carbon source. However, in this study the Proteobacteria found in sediments are heterotrophic, relying on the consumption of organic carbon rather than atmospheric CO_2 fixation. As such, photorespiration - where RuBisCO binds to O_2 instead of CO_2 - resulting in inefficient carbon fixation, does not occur as postulated by Xu et al. (2021). Heterotrophic Proteobacteria rapidly degrade organic matter and recycle carbon compounds thereby limiting their ability to synthesize new biomass from CO_2. This reaction leads to inefficient fixation of carbon, as it not only reduces the overall yield of long-term carbon but also diverts resources away from productive carbon fixation

pathways. This aligns with the findings of Du et al. (2023) that suggested that Proteobacteria contributes significantly to immediate carbon cycling processes but having less pronounced contributions to long-term carbon fixation. Other phyla such as Chloroflexi was highlighted to have a more critical role in stabilizing OC over extended periods.

Although there is lower Proteobacteria abundances in deeper sediments, the CBB pathway is predicted to remain active alongside the rTCA cycle I, which is more efficient in low O_2 levels (Mitchell et al. 2024). The rTCA cycle facilitates the recycling of inorganic carbon into organic forms, making it readily available for long-term storage. The presence of genes involved in the rTCA indicates that there has been a functional adaptation of microbial communities to the prevailing environmental conditions (Kitadai, Kameya & Fujishima 2017). However, these predictions are based on the taxonomic profiles and inferred functional potential. To confirm this, actual gene expression analysis should be carried out. Therefore, we can deduce that in the top and middle layers of the sediment, both the CBB and rTCA pathways are active at comparable levels, reflecting a balance in carbon fixation strategies.

At the bottom sediment layers, Chloroflexi is the most abundant phyla while Proteobacteria is the lowest. With O_2 levels being absent or very low, the CBB and rTCA cycle I remains ongoing. This suggests that pathways are more stabilized throughout the different layers of sediments and that both long- and short-term storage of carbon occurs throughout the sediment (Shoemaker et al. 2024). However, there is the question of 3-HP cycle and superpathway occurring in the middle layers. Despite being anaerobic pathways, they are also dependent on CO_2 availability (Alber, Kung & Fuchs 2008). Hence, their expression in middle layers indicates that these conditions may favor the activity of more specific microbial communities capable of utilizing available substrates for carbon fixation.

The data in this study does not account for the other underlying effects of the sediment environment itself on the microbial community. For instance, shallow areas which are more exposed to drying could be a reason for differences in composition (Paranaíba et al. 2020). This contrasts with waterlogged conditions of sediments, especially for deeper sediment layers of mangrove or seagrass areas (Yin et al. 2024). Additionally, this study was done under the assumption that the soils have not been disturbed, and no major soil upheaval has occurred (Liu et al. 2022). Therefore, changes in these factors may also be the factors for the variation seen in the profiles. It has also been mentioned that microbial variation was influenced by factors such as nitrogen and pH, varying with depth (Bertolet, Louden & Jones 2022). Therefore, it is crucial to understand that sediment microbial communities are not only shaped by oxygen availability, but also physical changes and environmental gradients that in turn affects remineralization and carbon fixation rates.

The data has important implications for future blue carbon planning especially in the capacity for short- and long-term carbon storage locally and regionally where a similar blue carbon habitat profile occurs. Unstable carbon forms are susceptible to result from environmental disturbances or rapid microbial respiration (Mason-Jones et al. 2023), which would release the blue carbon back into the atmosphere, indicating the limited permanence of the blue carbon. Conversely, stable carbon forms have a long-term storage capacity and enhances the permanence of blue carbon (Hilmi et al. 2021). This in turn translates into more credible and valuable blue carbon credits. Therefore, understanding the role of microbial composition in carbon fixation is essential to prioritize the conservation of blue carbon ecosystems.

CONCLUSIONS

In this study we addressed two main questions in view of blue carbon: What is the variation seen in microbial profiles of mangrove cores across different depth layers? And are the differences reflected in carbon fixation rates? First, we have established that there are significant differences in microbial profiles as the depth of sediments increase. Proteobacteria would make up most of the top layer of sediments while Chloroflexi was more abundant in the deeper layers. All the pathways maintained their abundance throughout the studied cores. Although the results suggest that the carbon fixation pathways may be conserved at least within the 100 cm depths of mangrove sediments from Pulau Seri-Buat, the lack of statistically significant differences means further work is required to confirm this hypothesis. Overall, these findings highlight the stability of carbon fixation potential despite the shift in microbial profiles, suggesting that deeper sediment layers may serve as long-term carbon storage reservoirs. This would help progress the understanding of the roles of microbes in mangrove sediments with respect to blue carbon storage. The identified microbial characteristics provide a foundation as to where further research should be focused on. To enable a more thorough review of the findings, future work is required to further confirm spatial and temporal consistency in view of the current findings. Moreover, the design of the study can be improved by expanding the scope of investigation to include other microbial phyla beyond those directly involved in carbon fixation. It is important to note that further transcriptomic and proteomic analyses would also yield insights on whether predicted functional potential would translate into actual ecosystem function.

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TABLE A1. (A,B,C) The top 10 most abundant phyla across all sediment layers

(A) Top Level		
Top 10 Pylum	Mean (%) of Abundance in Sediment	SE ± (%)
Proteobacteria	22.9	2.2
Desulfobacterota	17.5	1.7
Actinobacteriota	14.9	4
Chloroflexi	13.4	2.5
Acidobacteriota	6.6	0.3
Planctomycetota	6.5	1
Bacteroidota	3.5	0.4
Verrucomicrobiota	2.7	0.1
Myxococcota	1.6	0.1
Gemmatimon-adota	1.6	0.1

(B) Middle Level		
Top 10 Pylum	Mean (%) of Abundance in Sediment	SE ± (%)
Chloroflexi	20.5	4.1
Desulfobacterota	18.1	2.9
Proteobacteria	14.4	6.5
Actinobacteriota	10.5	4
Acidobacteriota	7.1	1
Planctomycetota	5	0.2
Bacteroidota	3.2	0.3
Firmicutes	2.6	0.9
Calditrichota	2.2	0.7
Crenarchaeota	1.9	1.2

(C) Bottom Level		
Top 10 Pylum	Mean (%) of Abundance in Sediment	SE ± (%)
Chloroflexi	30.4	0.9
Desulfobacterota	19	3.7
Acidobacteriota	7.8	0.4
Proteobacteria	5.6	0.8
Crenarchaeota	4.3	1.1
Actinobacteriota	4.2	0.4
Planctomycetota	3.6	0.4
Sva0485	3.6	0.4
Firmicutes	3.1	1.4
Caldirichota	2.9	0.3