Journal of Tropical Forest Science 32(3): 268–282 (2020)
https://doi.org/10.26525/jtfs2020.32.3.268

BACTERIAL COMMUNITIES IN A NEWLY REGENERATED MANGROVE FOREST OF SUNGAI HAJI DORANI MANGROVES IN THE WEST COAST OF SELANGOR, MALAYSIA

Jeyanny V[1,*], Norlia B[1], Getha K[1], Nur-Nabilah A[1], Lee SL[1], Rozita A[1], Nashatul-Zaimah AZ[1], Syaliny G[2], Ne'ryez SR[3] & Tariq-Mubarak H[1]

[1]*Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia*
[2]*Faculty of Science &Natrul Resources, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia*
[3]*Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

**jeyanny@frim.gov.my*

Submitted July 2019; accepted January 2020

The microbial community is dynamic in a mangrove ecosystem and thus controls various nutrient transformation processes in the ecosystem. In the current efforts to rehabilitate mangroves, this study focused on detecting significant variation within the bacterial community distribution in sediments, at two different depths, of an old regenerated mangrove forest stand of *Avicennia marina*. The regenerated mangrove forest site was artificially induced by placing wave breakers. Sediment samples were collected at 0–15 cm and 15–30 cm depth and analysed for soil properties. The bacterial community distribution was investigated using high throughput deoxyribonucleic acid (DNA) sequencing of the 16S rRNA gene. Bioinformatics analysis of the DNA sequence data showed that sediments were dominated by Proteobacteria (mostly Gammaproteobacteria and Deltaproteobacteria), followed by Firmicutes (Clostridia), Chloroflexi (Anaerolinae and Dehalococcoidetes), Actinobacteria (Acidimicrobiia, Coriobacteria), Acidobacteria (RB25 and BPC 102), Bacteroidetes (Saprospirae, Flavobacteriia, Bacteriocedes), Gemmatimonadetes and others. However, the intensity of the bacterial groups differed with respect to sampling depths and soil properties. The DNA sequencing revealed the sustenance of observed phylum in the soils of regenerated mangrove forest as compared to old mangrove stand. This highlights the positive effects of rehabilitation using wave breakers leading to microbial colonisation.

Keywords: **N**ext generation sequencing, microbial diversity, mudflats, tidal, soil properties

INTRODUCTION

Mangroves are among the most productive ecosystems in the world, with a high turnover of organic matter assisted by microbial processes (Alongi 1994). Mangroves serve as nursing grounds for aquatic species as well as to provide food and fodder for the local communities. They are also a renewable resource for wood, provide coastline protection, impede erosion and often accumulate sediments, contaminants and nutrients (Wickramasinghe et al. 2009, Alongi et al. 2005). The ecosystem is an important interface in the exchange of organic matter, gasses and nutrients in the mangrove belts.

Microorganisms in the mangroves are pertinent to maintain primary productivity, conserve vegetation and provide nutrient transformations in the tree rhizosphere. They are actively involved in the biogeochemical cycles that supply nutrients to plants and animals. Soil sediments are usually abundant with organic matter but lack phosphorus and nitrogen prompting the need for microorganisms to take part in nitrogen fixation, phosphate solubilisation, assimilation of photosynthesis, sulphate reduction, enzyme production and hydrocarbon degradation (Ismail et al. 2017, Sahoo & Dhal 2009). A vast suite of microorganisms, mainly bacteria and fungi, constitute 91% of mangrove sediments, however their role and function remain a question (Alongi et al. 2002). Some researchers have shown that plants influence the specific roles of rhizophere microbiome by regulating processes and mechanisms such as production of antibiotic, biofilm formation and symbiosis (Ganguli et al. 2017). However, further understanding of the microbiome of mangroves is lacking.

In order to harness socio-economic benefits, the first step would be to understand the microbial

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diversity in this ecosystem before their functional domains are elucidated. Microbes tend to select their habitat based on environmental conditions, including soil and water chemical properties. For instance, denitrifying bacteria are higher in mangrove soils with higher organic matter, and arbuscular mycorhizal fungi choose low saline mangrove soils (Alongi 1994, Sengupta and Chaudhri 2002). There has been recent interests in mangrove metagenomics (Al Zubaidy et al. 2016, Basak et al. 2015, Ghizelini et al. 2012). However, knowledge is still insufficient on the various taxa of the microbial make up of mangrove sediments, and on how they differ in an original mangrove stand in tidal area and intertidal mudflats, which has accreted with the placement of geotextile materials. This study attempts to analyse the relative abundance of bacteria and the difference in bacterial community of the rhizospheric region of *Avicennia* species in an old growth forest in tidal area (at two different depths), and the newly regenerating *Avicennia marina* stand in mudflats, as a possible measure of soil health indicator for mangrove rehabilitation strategies. This is the first study in Malaysia that aimed to investigate the bacterial diversity in mangrove sediments in a rehabilitated mangrove stand using metagenomics approach.

MATERIALS AND METHODS

Site description

The mangrove forest in Sungai Haji Dorani, Sungai Besar, Selangor (3° 38' N, 101° 01' E) is located to the northwest of Kuala Lumpur (Figure 1), close to the D Muara Marine Park, an ecotourism resort overlooking the Straits of Malacca. It is mainly dominated by the *A. marina* and *Rhizophora apiculata* species. The annual average temperature is 26.9 °C and the annual rainfall is approximately 130 mm. This area faced degradation during early 2005 which prompted the placement of geotextile materials (geotube) by the Department of Irrigation and Drainage (DID), as wave breakers to reduce the impact of eroding waves from the Straits of Malacca. These geotubes are 50 m in length and filled with sand (Raja Barizan 2019). The placement of wave breakers further allowed sedimentation and accretion of muddy substrates behind the geotubes throughout the years that initiated the natural regeneration of *A. marina* species and the survival of artificially planted *R. apiculata* species on the mudflats. The original mangrove belt (tidal) comprising both species were also studied to determine the soil health of the area. These two sites were chosen, mainly to detect the differences or similarities of microbial population that colonise the designated areas, and to provide first-hand information on regenerating forest after severe degradation. The diameter at breast height (DBH) range of mangrove stands on mudflats were 3.7–10.3 cm, whereas the trees on the tidal were 2.8–11.5 cm, in 2017. The attached map (Figure 1) shows the areas of interest of an old growth forest (tidal) and the regenerating forest (mudflats).

Soil sampling and analysis

Soil sampling was carried out in two sites, i.e. the tidal and the mudflat areas. At both plots, sampling was done in areas with *A. marina* to avoid bias due to species variability. Soil samples for the metagenomic study were collected at 0–15 cm and 15–30 cm depths to detect bacterial population gradient in the top soil. Studies have shown that microbial population differs with depth where higher counts of microbes are found on the top soil layer due to availability of organic residue for mineralisation, and lower depths are correlated with metabolic processes that occur at the root region (Fierer et al. 2003, Huang et al. 2011). Each depth consists of 5 replications totalling up to 20 subsamples. After collection, the soil samples were transported in a cooler box filled with ice within the same day and stored at -20 °C until processing. For soil sediment analysis, soil samples were collected at 0–15 and 15–30 cm at both tidal and mudflat areas. These samples were tested for dry pH, electrical conductivity (EC), nitrogen (N), organic carbon (C) and cation exchange capacity (CEC). The complete profile up to 120 cm depth is available in Jeyanny et al. (2019). Soil pH was determined using a pH meter in 1:2.5 soil to water slurry, whereas electrical conductivity was determined using a 1:5 soil to water ratio using a conductivity meter. Organic carbon was analysed according to method of Walkley and Black (1934), and soil nitrogen was determined using Kjeldahl digestion. Available P was determined by the method of Bray & Kurtz (1945). The CEC was extracted using 1 M ammonium acetate (NH_4OAc), calibrated at pH 7.

Figure 1 Sampling site at the mudflats and the tidal areas in Sungai Haji Dorani, Selangor

DNA extraction and sequencing

Four environmental samples (TD1, TD2, MF1 and MF2) with five replicates each were analysed. The TD1 and TD2 represented 0–15 and 15–30 cm soil depth in tidal respectively, while MF1 and MF2 representd 0–15 and 15–30 cm depth in mudflats respectively. Genomic DNA of soil microbe from the 5 replicates of each sample were extracted from 0.25 g of soil using Power Soil DNA Isolation Kit (QIAGEN). The extracted DNA was quantified using NanoDrop 2000 Spectrophotometer. The DNA purity was further confirmed by electrophoresis on 1% agarose gels using Qubit@ 2.0 fluorometer. Further analysis were carried out on the extracted genomic DNA samples with a concentration of more than 20 ng uL^{-1} in 35 uL volume; the optical density of 260/280 were within 1.8–2.0. Once completed, the five replicates of each sample were further pooled to represent each depth at each site, representing a composite biological replicate that may comprehensively characterise the microbial community. Using pooled samples to characterise populations is not only cost-effective, but also leads to more precise and less biased parameter estimation of individual samples (Caudill 2010). Before pooling, precautionary measures were taken to ensure all DNA concentration of pooled samples were of similar values, to avoid biasness. To meet the requirements of the analysis, a consistent amount of volume per replicate was used to yield the required total volume (60 µL). Thus, 4 samples were analysed where TD1 and TD2 represented 0–15 and 15–30 cm soil depth in tidal respectively, and MF1 and MF2 represented 0–15 and 15–30 cm depth in mudflats respectively. These samples were outsourced for amplicon sequencing.

Barcoded amplicon sequencing

Region V3 and V4 of 16S rRNA gene were amplified using specific primers with barcode. All PCR reactions were carried out with Phusion® high-fidelity PCR master mix. For PCR amplification, sample volume was mixed with 1X loading buffer (containing SYB green) and operated on 2% agarose gel for detection. Samples with bright bands between 400–450 bp were chosen for purification with Qiagen

gel extraction kit. Sequencing libraries were generated using TruSeq® DNA PCR-free sample preparation kit, and index codes were added. The library quality was assessed on the Qubit@ 2.0 fluorometer and Agilent bioanalyser 2100 system. Finally, the library was sequenced on the Illumina HiSeq 2500 platform and 250 bp paired-end reads were generated.

Amplicon sequencing data analysis

The raw reads were assigned to their paired-end reads based on their unique barcode, and were truncated by removing the barcode and primer sequence. Sequence assembly was carried out using FLASH (version 1.2.7) to merge paired-end reads when at least some of the reads overlapped the reads generated from the opposite end of the same DNA fragment; the splicing sequences were called raw tags. The raw tags went through data filtration to attain high-quality clean tags according to Qiime (version 1.7.0) quality controlled process. The chimeric sequences were removed by comparing the tags with the reference Gold database using UCHIME algorithm to obtain the effective tags.

Analysis of each soil sample were carried out using Uparse version 7.0.1001 where all effective tags with 97% DNA sequence similarity were assigned to same operational taxonomic units (OTUs). GreenGene database was used based on RDP classifier version 2.2 algorithm to annotate the representative sequence for each OTU as taxonomic information. Data normalisation was carried out using a standard sequence number corresponding to the sample with the least sequences. Further, alpha diversity analysis was applied to analyse complexity of species diversity, while beta diversity analysis evaluated differences of samples in species complexity.

RESULTS AND DISCUSSION

Soil chemical characteristics

The aim of the study was to assess the bacterial community structure and composition in sediments of a regenerated mangrove forest in Sungai Haji Dorani mangroves, as opposed to an old growth mangrove forest, at different depths. Previous studies have shown high microbial diversity in mangrove environments, indicating that they may play an important role in nutrient cycling (Reef et al. 2010). However, considering that mangrove ecosystems are very dynamic, in this study, different sediment samples were collected at the rhizosphere region of *A. marina,* and subjected to physical and chemical analyses. The results showed that some factors were relatively different according to the plots or depth.

Table 1 displays the soil chemical characteristics on both plots examined up to 30 cm depth. Soil data revealed that the soil pH of the mudflats was more alkaline (> 7.6) compared to the tidal belt which is acidic, especially the top soil (6.59) (Table 1). The range of EC for mudflats was 11.99 to 15.85 µs cm^{-1}, but markedly higher in the tidal area at 0–15 cm depth (20.92 µs cm^{-1}). The availability of total nitrogen (N) and carbon (C) were 49 and 32% higher, respectively, in the tidal compared to mudflats of the top soil at 0–15 cm depth. There were no distinctive changes for soil CEC between depths within plots, but values for tidal were relatively 1.5 to 2 folds higher compared to mudflats.

Several studies support that pH, salinity and attributes related to soil acidity are the best predictors of the richness and diversity of microbial communities in soils (Xi et al. 2014, Tam and Wong 1998). Based on past reports, soil acidity in the mangroves of Haji Dorani fell between 6.0 to 7.5 due to frequent intertidal exchange of sea water that regulates the pH (Mohamad Fakhri et al. 2017, Jeyanny et al. 2018). The amount of increased C and N is attributed to the increase in organic matter in the tidal belt, which correlates with increased litter production of mangroves despite hosting a few tree species (Mukherjee et al. 2009). In the present study, the tidal mangrove soils appeared to exhibit considerably higher CEC values as compared to mudflats (Table 1). This phenomenon could be largely attributed to the occurrence of higher amount of organic matter (i.e N and C). Besides, finer particles of soil such as clay fraction, which was reported to be elevated in the tidal, could have increased the CEC values of mangrove soils, in addition to the effects of organic matter (Mohamad Fakhri et al. 2017). Clay is able to hold more cations due to increased surface area as compared to sand and silt. The visual observations confirmed that root proliferation of *A. marina* pneumatophores was intensified in the tidal area, thus trapping finer particles (i.e. clay) for nutrient transfer and displaying higher CEC values.

Table 1 Soil chemical analysis in tidal and mudflats area in Sg. Haji Dorani, Sungai Besar, Selangor

Soil profile	Dry pH	EC (mS cm^{-1})	N (%)	OC (%)	CEC (cmol kg^{-1})
Tidal					
0–15	6.59	20.92	0.35	3.62	25.58
15–30	6.70	10.96	0.17	1.93	21.34
Mudflats					
0–15	7.63	11.99	0.18	2.45	12.68
15–30	7.72	15.85	0.16	1.88	13.84

EC: electrical conductivity, N: nitrogen, OC: organic carbon, CEC: cation exchange capacity

Amplicon sequencing analysis

A total of 604,914 raw reads were obtained from the 4 environmental samples (MF1, MF2, TD1 and TD2) (Table 2). After quality trimming, a total of 473,917 reads were obtained. A total of 102,522 reads were obtained for MF1 (average read length of 421 bp). The reads for MF2, TD1 and TD2 were 113,286, 103,101 and 155,008 respectively with an average read length of 419 (Table 2).

The OTUs were defined at a cut-off of 97% sequence similarity, a commonly recognised level for comparative analysis of 16S rRNA gene (Uroz et al. 2010). Based on the number of OTU, the total amount for mudflats were higher, i.e. 7206, as compared to 6602 for tidal samples (Table 3). It was also noticed that deeper depths (15–30 cm) recorded higher amounts of OTU. Samples MF2 and TD2 displayed higher percentages, 1.7 and 5.6% respectively, compared to upper layers. These differences of diversity between depths were confirmed with the display of Shannon diversity index. For species richness (Chao1), it was shown that MF1 displayed the greatest richness. Goods coverage estimate for each sample ranged from 98.4 to 98.9% indicating that the sampling was sufficient to cover almost all bacterial communities (Zhang et al. 2017).

Rarefaction curve analysis, estimating degree of coverage of bacterial diversity in the four soil samples, is shown in Figure 2. Rarefaction indicated that the number of detected OTUs increased with the number of sequences sampled in each of the soil samples. The left-side of the steep slope indicated that a large fraction of the species diversity remains to be discovered (Figure 2). However, the curve became plateau to the right, indicating that a reasonable number of individual samples have been taken and only the rarest species remain to be sampled. The distinction between the prokaryotic communities of different samples was further augmented at the genus level, as shown by the rarefaction curves. The curve highlighted the greater species richness of the rhizosphere soil sediment of mudflat samples (3572–3634) compared to tidal samples (3211–3291).

Although microbial diversity was expected to be enhanced in the tidal area, due to the existing mangrove belt, the highest number of observed species were found in the mudflats (Table 3). The increased bacterial diversity (10–11%) in mudflats is shown in Table 3 and Figure 2. It suggested the formation of new communities at the rhizosphere of the regenerating vegetation that provided root exudates (i.e. carbon) for microbe metabolism. The tidal samples which maybe at equilibrium or influenced by other anthropogenic factors, such as agricultural residues, inorganic fertilisers, pesticides and human activities (Al Zubaidy et al. 2016, Chen et al. 2016, Jeyanny et al. 2018).

Shared OTUs between the samples were determined via Venn diagram. Figure 3 shows the Venn diagram based on 16S rRNA gene from both areas. The number of shared OTUs between MF1 and MF2 was 2951, which was more than 4-fold higher than the unique OTUs of MF1 and MF2 alone (621–683). For tidal samples, the shared OTUs was 2349, more than 2-fold higher as compared to TD1 and TD2, respectively. Generally, deeper depths (MF2 and TD2) showed higher number of sequences.

Table 2 Next generation sequencing (NGS) statistics where the reads were generated by Illumina HiSeq 2500

Sample	Total reads	Combined reads	Uncombined reads	Percent combined (%)	Combined base (bp)	Minimum length (bp)	Maximum length (bp)	Average length (bp)
MF1	137,127	102,522	34,605	74.76	43,144,408	122	443	421
MF2	148,455	113,286	35,169	76.31	47,522,803	95	441	419
TD1	137,015	103,101	33,914	75.25	43,149,706	122	441	419
TD2	182,317	155,008	27,309	85.02	64,952,360	64	442	419
Total	604,914	473,917	130,997	78.34	198,769,277	122	441	419

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Table 3 Comparison of alpha diversity index of 16S rRNA sequencing from mangrove samples

Sample	OTU	Shannon	Simpson	Chao1	ACE	Goods
MF1	3572	9.455	0.994	4197.146	4243.793	0.984
MF2	3634	9.616	0.995	3935.505	3967.137	0.989
TD1	3211	9.765	0.997	3625.062	3682.577	0.987
TD2	3391	9.925	0.997	3736.894	3770.398	0.988

All the indices of the samples were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3); OTU: operational taxonomic units, Shannon: diversity index, Simpson: diversity index, Chao1: species richness, ACE: abundance-based coverage estimator

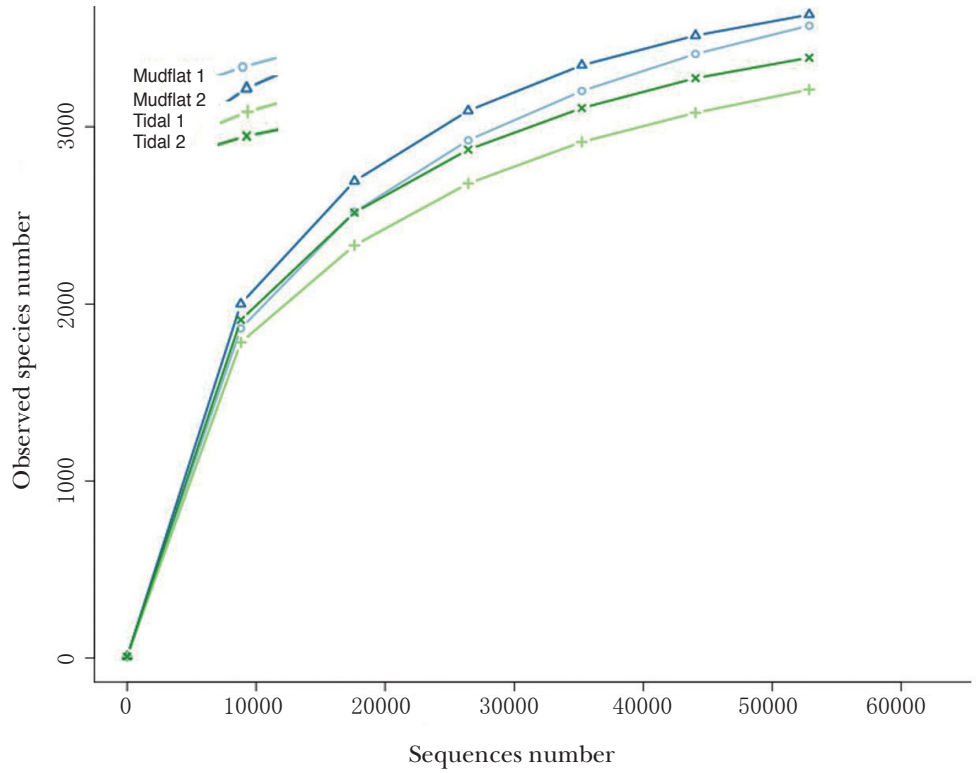

Figure 2 Rarefaction curves generated for 16S rRNA pyrosequence from samples collected at mudflats and tidal at two different depths; sequences were grouped into operational taxonomic units (OTUs) based on a distance sequence similarity of 97%

However, the results were contrary to Mendes and Tsai (2014) who reported that low percentage of OTUs were shared between shallow and deeper samples. Deeper depths correlates with a more profound anoxic (deficit of oxygen) environment. Most facultative microbes in mangroves are anaerobic, such as the sulfate reducing bacteria from the Proteobacteria phyla, which efficiently transforms nutrients and reduce sulfate in anaerobic mud (Ghizelini et al. 2012). The current data was consistent with Reef et al. (2010) who showed that organic matter transformation is more profound in rhizosphere at deeper layers. Bacterial diversity measurements in the study also indicated highest number of observed species in deeper samples. Thus, the findings indicated that mangrove holds a diverse bacterial community, shaped by the variations of the ecosystem such as sediment properties and depth.

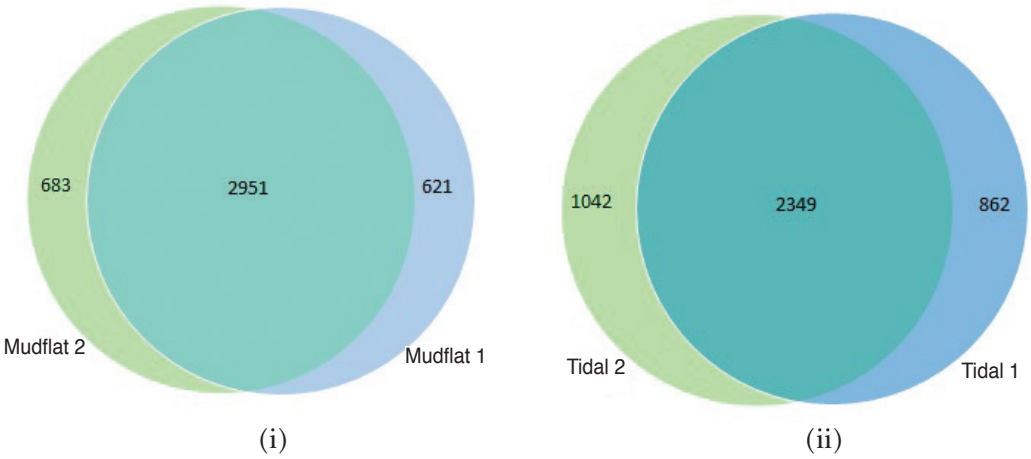

Figure 3 Venn diagram for (i) MF1 and MF 2 and (ii) TD1 and TD2

Bacterial abundance analysis at phylum level

The mudflat samples at both depths showed population of Proteobacteria (53%), Firmicutes (6–10%), Chloroflexi (5–6%), Actinobacteria (4–5%), Acidobacteria (4–5%), Bacteroidetes (4%), Gemmatimonadetes (3%), Verucomicrobia (2–3%), WS3 (2–3%) and Nitrospirae (1%). Cyanobacteria (1%) was only present in 0–15 cm samples, and Archaea (2–8%) recorded the highest percentage in 15–30 cm depth (Figure 4).

The tidal samples, at both depths, showed the population of Proteobacteria (44–47%), Chloroflexi (11%), Firmicutes (8%, only present in 15–30 cm depth), Actinobacteria (5–8%), Acidobacteria (6%), Bacteroidetes (3–4%), Gemmatimonadetes (6–7%), WS3 (3–4%) and Nitrospirae (1–3%). Cyanobacteria (1%) and Archaea (3%) were only present in 0–15 cm samples. Verucomicrobia was absent in these samples (Figure 5).

Majority of the assigned reads from the samples were of the Proteobacteria (44–53%) domain, while the other dominant phyla were Firmicutes (6–10%), Chloroflexi (5–11%), Actinobacteria (4–8%), Acidobacteria (4–6%), Bacteroidetes (3–4%), Gemmatimonadetes

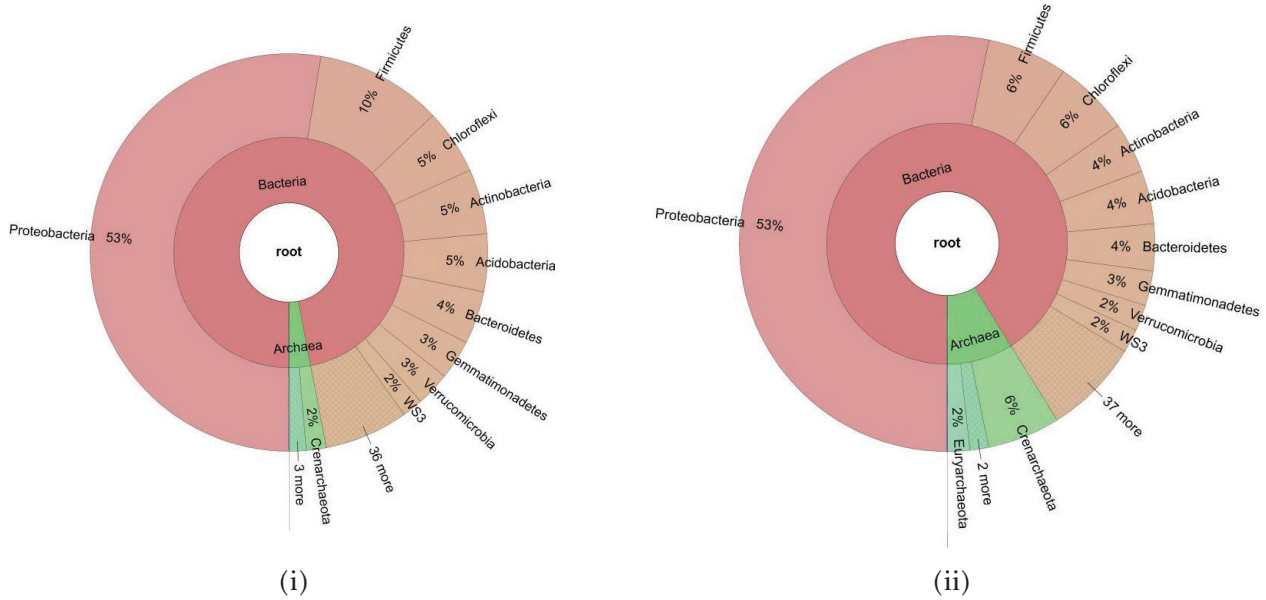

Figure 4 Bacterial phyla composition distribution in mangrove sediments (i) mudflats 0–15 cm and (ii) mudflats 15–30 cm of Sungai Haji Dorani, Selangor

(3–7%), Verucomicrobia (2–3%), WS3 (1–3%), Nitrospirae (1–3%), Cyanobacteria (1%), Planctomycetes (1–2%) and Archaea (2–8%).

Generally, the bacterial abundance in mangrove sediments are highly influenced by levels of salinity, soil pH, soil depth, organic matter, aeration, moisture, tree species and many other factors (Alongi et al. 1994, Ghizelini et al. 2012, Xi et al. 2014). This was ascertained by the results where soil chemical properties and soil depth played important roles in influencing the makeup of microbial community. One of the biggest phyla in mangrove sediments is none other than Proteobacteria. Proteobacteria are anaerobic microbes that assist in various biogeochemical cycles namely denitrification, carbon assimilation, reduction of sulphides and nutrient transformation (Mendes and Tsai 2014, Pureza et al. 2012). The second biggest phyla was Firmicutes. Firmicutes is the most common bacteria in the human gut system, thus found nearer to human settlements of the mangrove belts (Koliada et al. 2017). The absence of Firmicutes in the 0–15 cm depth of tidal samples may be indicative of its penchant for deeper depths, which are more anoxic compared to surface samples. However, its presence at both depths in the tidal samples might be due to the site specific environment, that needs further investigation. Pupin & Nahas (2014) reported that denitrifying bacteria such as Firmicutes prefer anoxic conditions. This is due to its role in catalysing the conversion of nitrate via nitrite in nitrogen dioxide and nitrogen gas under anaerobic conditions, in the presence of organic matter.

Chloroflexi was abundant in all samples. This is due to its critical role in organic matter decomposition at both sites (Wu et al. 2016). Previous studies have shown predominant bacterial phylotypes in mangrove sediments, clustering within Proteobacteria, Firmicutes, Chloroflexi and Actinobacteria (Ismail et al. 2017, Zhang et al. 2017). Although Actinobacteria is an important phyla for bioprospecting, cellulose degradation and metal oxidation, its abundance is smaller as compared to the total bacteria community in a mangrove forest in China (Fernandez et al. 2014, Getha et al. 2014, Lu et al. 2016). This phyla constituted about 8% in the tidal as compared to the mudflats (5%).

The metagenome study on characterisation of microbial population in mangrove soil collected from Rantau Abang, Terengganu, Malaysia via NGS showed that Proteobacteria (43.72%) was the dominant phyla, followed by Acidobacteria (17.68%), Firmicutes (13.45%), Actinobacteria (4.55%), Nitrospirae (4.22%), Planctomycetes (3.06%), Chloroflexi (2.88%), Verrucomicrobia (2.69%), Spirochaetes (1.70%), Chlamydiae (1.32%), and Bacteroidetes (1.31%) (Ismail et al. 2017). The current results were similar except that Acidobacteria did not have higher colonisation in the west coast samples compared to the east coast of Peninsular Malaysia. Nevertheless, the study was able to differentiate the variations with respect to depths and site, which was not accounted for in previous studies.

Bacterial abundance analysis at class level

In mudflat samples of both depths (Figure 5), the biggest group was Gammaproteobacteria (34–33%), followed by Deltaproteobacteria (13–15%) and Alphaprotebacteria (5%). Clostridia (6–10%) was the only group detected under Firmicutes. Anaerolineae (4%) and Dehalococcoidetes (0.6–1%) under Chloroflexi were detected, while 8 or 9 more classes were undetected. Actinobacteria had 2 distinctive classes which were Acidimicrobiia (3–4%) and Coriobacteria (1%). Both depths hosted RB25 and BPC 102 (0.8–1%) under Acidobacteria. Saprospirae, Flavobacteriia and Bacterodia were 1% or lesser in the Bacteriocedes group. Gemm-1, 2 and 4 were found in 0–15 cm soil depth (< 1%) but only Gemm-2 in deeper depths of soil. Verrucomicrobiae (2%) under Verucomicrobia was detected. In the mudflats, 1% Cyanobacteria was detected. There were 2 main groups detected under Archaea, which were Crenarchaeota (4.6%) and Euryarchaeota (1%) in 15–30 cm depth, but only Crenarchaeota (0.7%) was detected on the top layer (Figure 6).

In tidal samples of both depths (Figure 7), the biggest group detected was Gammaproteobacteria (16–19%), followed by Deltaproteobacteria (17–18%) and Alphaprotebacteria (10%). Firmicutes was not detected on the upper layer of tidal samples. Clostridia (8%) was the only group detected under Firmicutes in the 15–30 cm samples. Anaerolineae (7–8%) and SO85 (1–2%) were detected under Chloroflexi. Actinobacteria had 1 distinctive class which was Acidimicrobiia (4–6%), while more than 6 groups were undetected. Both depths hosted Acidobacteria-6 (2%) and BPC 102 (2%) under Acidobacteria. Bacteroidia

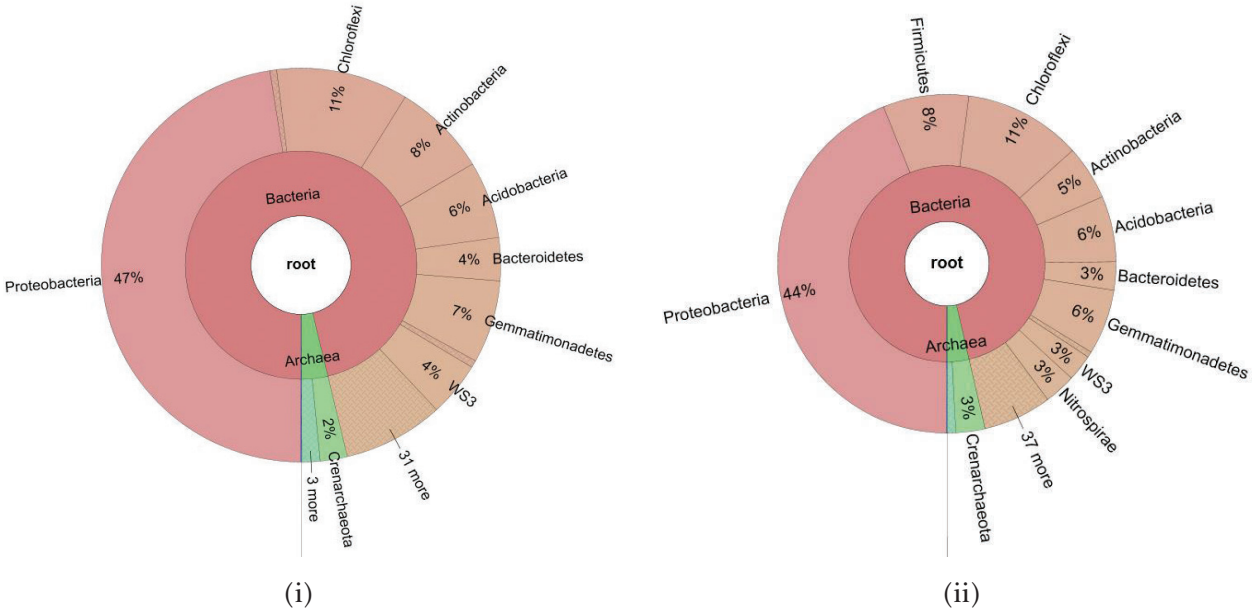

Figure 5 Bacterial phyla composition distribution in mangrove sediments (i) tidal 0–15 cm and (ii) tidal 15–30 cm of Sungai Haji Dorani, Selangor

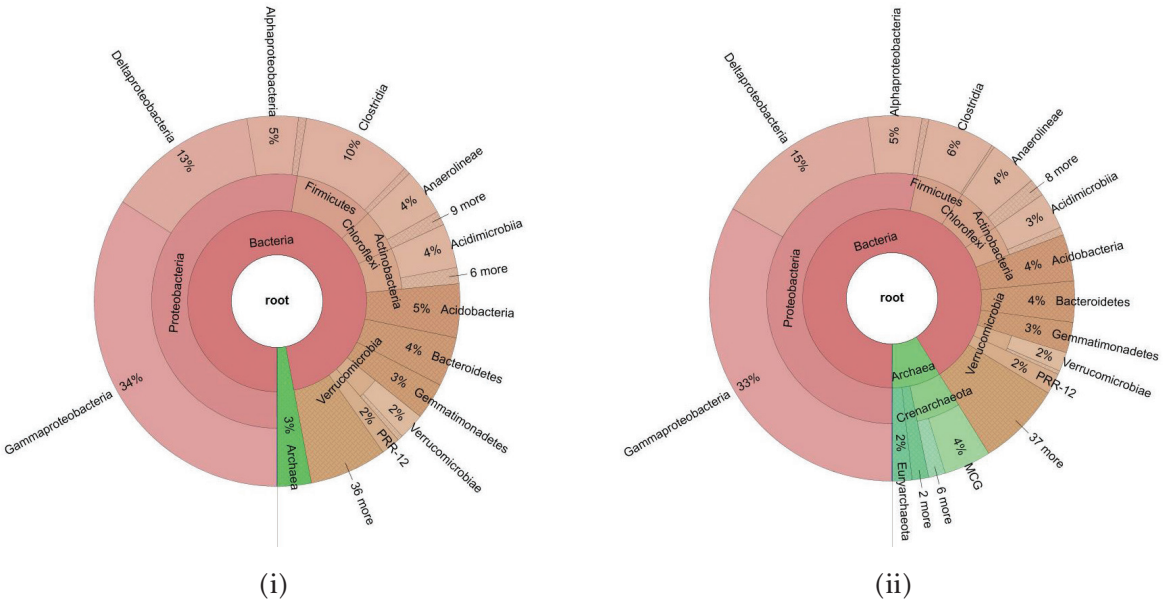

Figure 6 Bacterial taxa composition distribution in mangrove sediments (i) mudflats 0–15 cm and (ii) mudflats 15–30 cm of Sungai Haji Dorani, Selangor

was 1% or lesser in the Bacteriocedes group. Gemmatimonadetes had several unclassified groups accounting to 6–7%. The tidal samples of the upper layer had 1% Cyanobacteria. There were 2 main groups under Archaea, which were Crenarchaeota (2–3%) and Parvachaeota (1%), which were only present in the 0–15 cm depth.

The bacterial relative abundance detected in the samples showed a predominance of Gammaproteobacteria (Figure 8). This group was relatively higher (0.34–0.33) in the mudflats compared with the tidal samples (0.16–0.18). Other groups such as Deltaproteobacteria and Alphaproteobacteria were relatively higher in the

tidal samples, 0.18–0.17 and 0.10 respectively. Clostridia were highly dominant in all samples except for the 0–15 cm tidal samples. It was markedly reduced to 95% (0.005) in the mudflats compared to tidal. Collectively, the population of Clostridia was 83% higher in the mudflats (0.06–0.09) compared to tidal samples. The 0–15 cm layer of tidal samples were almost absent (0.005) of Clostridia. However, the relative abundance of Anaerolinae was relatively higher in tidal (0.07–0.08) compared to mudflats. Whereas, Acidimicrobiia were higher in tidal samples (0.07–0.04) compared to mudflats at similar depths. The population of PRR-12, MCG, Gemm-2 and Nitrospirae did not follow any significant trends with relation to different sites or depths. Almost all of them fell between the range of 0.011–0.045. The group which was not accounted for (i.e. others) had a relative abundance from 0.248–0.285.

It was observed that the Gammaproteobacteria class was higher in the mudflat samples (Figures 6 and 8) compared to tidal. This is due to their role as active mediators for nitrogen, sulphur and carbon cycles (Fernandes et al. 2014). The lower values obtained for soil N and C in the mudflats could be contributed by this class which actively transforms elements for biological and chemical processes. Gammaproteobacteria are active denitrifying bacteria, and assist in organic matter breakdown (Greer et al. 2010). The pristine conditions of tidal area of an old growth forest displayed increased population of Deltaproteobacteria (17–18%) as compared to mudflats. Deltaproteobacteria participate in iron and sulphur reduction, to be utilised as nutrients at the rhizospheric region of the old growth forest, compared to the newly regenerating forests (Dos Santos et al. 2011). Furthermore, they assist in the degradation of cellulose, hydrocarbon, metal oxidation and nitrate reduction (Fernandez et al. 2014). The abundance of Alphaproteobacteria is well noted in the tidal samples, compared to the mudflats (Figures 7 and 8). With the availability of higher organic matter (Table 1) and vegetation of the old growth forest, it was likely that the members of this class participated in nitrogen fixation, decomposition of organic matter and photosynthesis (Yokokawa and Nagata 2010, Nair 2015). Clostridia was the only class under Firmicutes that was detected in both areas, but only in deeper samples of the old growth forest. Clostridia's selectivity towards deeper depths was not comprehendible. It is assumed that Clostridia may serve as an indicator of hydrocarbon pollution of boat traffic, close to a marine tourist resort (Dos Santos et al. 2011). Clostridia is also shown to actively degrade organic substances from anthropogenic activities (i.e agricultural residue and faeces), as well as plant microbial interactions. Its abundance is common in a mangrove ecosystem. *Clostridium* clusters and their diverse consortiums dominate the bacterial communities during anaerobic decomposition process in water and sediments (Gomes et al. 2014). The class Anaerolineae was found to be higher in old growth forest (tidal) (Figures 7 and 8). This indicates its key role in electron transfer to anodes to produce metabolic intermediates from root exudates for plant productivity (Wu et al. 2016). This class scavenge for organic compounds from decaying cell residues from organic litter for catabolic processes, and involves in carbon cycling (Hug et al. 2013). The class Acidimicrobiia may have been elevated in tidal samples due their ability to withstand heavy metal contamination, acidic and other extreme environments, closer to human settlements as compared to the mudflats (Barns et al. 2007). This marine actinobacterial group is comprised of a few cultivable representative species that were mostly isolated from extremely acidic environments, and plays an important role in biogeochemical cycling (Hu et al. 2018).

In general, it was found that soil properties such as C, N, pH and CEC could influence the microbial community of the tidal and mudflats, as the alpha diversity index differed in both tidal and mudflats owing to the organic matter levels and nutrient retention. The highest OTUs were found in the more alkaline environment with lower CEC as compared to then acidic environment of the mudflats. The close proximity of mudflats to the open sea and the distance from the old growth forest may facilitate the colonisation of microbes as reported by Dias et al. (2010) in a Brazilian mangrove.

Besides soil properties, soil depth is also an important factor in determining microbial diversity. The gradient of oxygen in soils determine the zonation and functioning of the microbial communities, as deeper samples were richer in terms of anaerobic microbes (Reef et al. 2010). Although the analysis was limited to

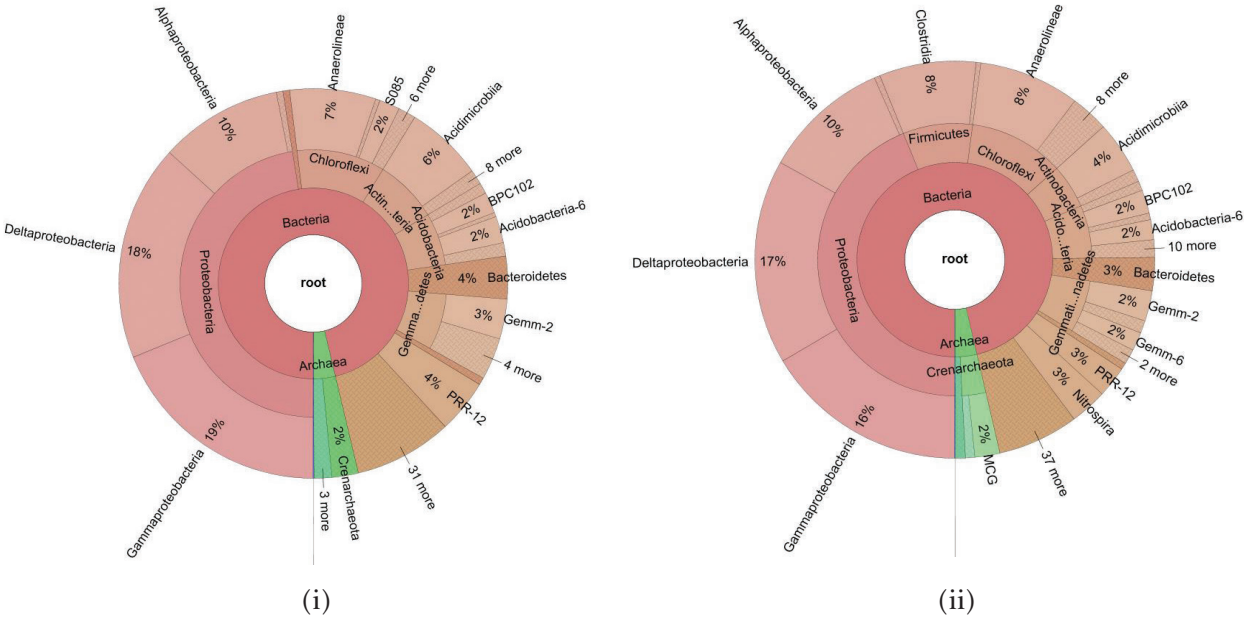

Figure 7 Bacterial taxa composition distribution in mangrove sediments (i) tidal 0–15 cm and (ii) tidals 15–30 cm of Sungai Haji Dorani, Selangor

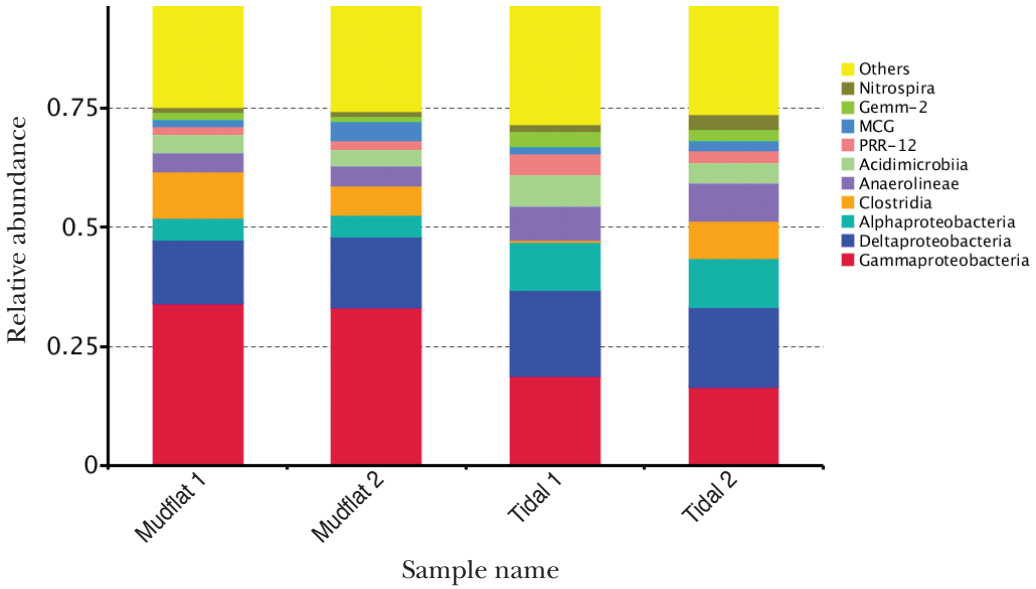

Figure 8 Relative bacterial abundance of the most dominant class (OTU)

single sediment samples at two depths from two different locations, the amplicon sequencing technique was useful in revealing sustenance of observed species number at mudflats, compared to tidal. This is important to highlight the positive effects of rehabilitation efforts of mangroves leading to microbial colonisation.

CONCLUSION

Shifts in the soil bacterial populations found in this study were directly attributed to ecosystem characteristics of the old growth forest, and newly regenerated mangrove forest which was artificially induced with wave barrier breakers.

Future research should take into account the functional analysis of microbes with relation to nutrient transformation, that may assist forest managers in isolating and culturing novel bacteria, mainly for increasing vegetative productivity, bioprospecting, hydrocarbon degradation and various other applications. The recovery of the soil microbial communities in the regenerated mangrove will take time to reach equilibrium. However, the information on pioneer specific bacterial colonisation is important for future restoration/replanting strategies to expedite rehabilitation. This is the first study on microbial diversity in mangroves soils of Malaysia using NGS metagenomics approach to compare a newly regenerated mangrove site which was artificially induced.

ACKNOWLEDGEMENTS

The authors would like to thank FRIM Steering Committee, Department of Forestry Malaysia and the Ministry of Water, Land and Natural Resources Malaysia for the research funding (vot no. 23410207001). The field and technical assistance of the Soil Management Branch of FRIM are duly acknowledged. The authors extend their gratitude to Hamdan Omar for preparing the map of the study site.

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