MANGROVE PEAT IN MEXICO'S YUCATAN PENINSULA: COMPOSITION AND POTENTIAL CARBON SEQUESTRATION PROPERTIES OF MICROBIAL COMMUNITIES

By

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ABSTRACT

Mangroves are woody plants that thrive in the tropics and subtropics in estuaries—regions where freshwater and saltwater meet. Bacteria associated with mangrove peat (plant material unable to decay due to anoxic and acidic environments) with recalcitrant nutrients play an important role in sequestering carbon. In a previous study by Costa *et al.,* bacteria isolated from collection sites at La Paz, Baja California Sur, Mexico, have been shown to sequester carbon in peat aged approximately 5,000 years. Microbial community variation with depth was observed. While microbes have cycled nitrogen deposits, there is little sign of remineralized carbon back into the ecosystem. Nevertheless, the diversity of bacteria differs at each peat level, giving insight into peat carbon density, which is relatively stable. Assuming similar anoxic conditions with recalcitrant material, distinct bacterial communities with decreased carbon turnover according to peat depth are hypothesized in a mangrove forest in Celestun, Yucatan, Mexico. Samples were collected at various depths to examine microbial communities in different sediment types. Microbial DNA was extracted and purified, and the V3 and V4 regions of the 16S rRNA gene were PCR amplified. Results revealed distinct phyla according to sediment type and depth, including *Chloroflexi* in deeper peat deposits and *Pir4* in shallower peat deposits, leaf litter, and roots. The microbial community appears to be remineralizing carbon at a slower rate, allowing for peat to accumulate carbon over time.

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TABLE OF CONTENTS

INTRODUCTION

Mangroves are salt-tolerant and have an extensive root system that protects coastal lands from floods, prevents land erosion, and provides shelter for terrestrial and marine organisms (Jia *et al.* 2020). Besides providing shelter for other animals and protecting the surrounding region from storms, mangroves are essential for biogeochemical cycling (Nagelkerken *et al.* 2008; Nimnoi & Pongsilp, 2022). Mangroves are important sources of carbon sequestration and nutrient cycling by absorbing inorganic particles such as nitrates and phosphates, all of which are made available to be used by mangroves (Costa *et al.* 2022)**.** Habitats such as salt marshes, seagrass beds, and mangroves are carbon sinks, acting through the absorption of large quantities of carbon dioxide from the atmosphere and storing it over hundreds to thousands of years (Howard *et al.* 2017).

Coastal blue carbon refers to carbon sequestration in tidal wetlands in comparison to carbon storage in terrestrial and freshwater counterparts. Coastal wetlands continue drawing carbon dioxide from the atmosphere and are able to accumulate vertically in response to rising sea levels. As a result, new volumes of sediment are created to accommodate more organic matter. Altogether, sedimentation and accelerated sea level rise promote an increased biomass production belowground (Davis *et al.* 2015; McTigue *et al.* 2019). Carbon sequestration in mangroves is on average 168 ± 36 gC m^{-2} yr^{-1}. Blue carbon is mostly stored in waterlogged sediment, preventing the risk of sudden carbon loss and allowing for a steady carbon accumulation. Blue carbon sequestration is estimated from sediment accumulation and organic carbon content, assuming the two parameters remain stable over time.

However, bioturbation, organic carbon remineralization, and pore water discharge deteriorate organic carbon stocks (Taillardat *et al.* 2018). In a study examining compiled soil carbon after mangrove regeneration, long-term stabilization mechanisms are important for the

complete recovery of soil carbon stock. Primary organic carbon stabilization methods may include the recalcitrance of mangrove roots and phenoloxidase inhibition under anoxic conditions (Kida *et al.* 2020). The ability of mangrove forests to act as a carbon sink is due to high primary production and low soil organic matter decomposition rate (Kida *et al.* 2020). A unique characteristic of mangroves is their ability to store carbon by amassing tons of organic carbon in the sediment in the roots (Suello *et al.* 2022). Mangrove soil may accumulate high soil carbon despite high decomposition by large lateral exports. Anoxic conditions and recalcitrant biomass are believed to contribute to the low decomposition rate (Kim *et al.* 2021). Mangroves accumulate peat deposits and can store the peat for millennia (Costa *et al.* 2022). If mangrove forests are disturbed through deforestation, organic carbon is converted into carbon dioxide and released into the atmosphere (Arifanti *et al.* 2022). Because of their ability to store carbon, mangrove peat can be utilized or preserved to mitigate climate change.

Mangrove leaves and wood are mainly composed of lignocellulose components that are degraded by microorganisms. Mangrove bacteria perform biochemical processes involved in nutrient cycling and decomposing complex molecules resulting in detritus, defined as "organic matter in the active process of decomposition" (Holguin *et al.* 2001). Mangrove litter, including fallen vegetation, is transformed into detritus, partly supporting the mangrove food web (Nagelkerken *et al.* 2008). Microbes are largely responsible for carbon and nitrogen cycling in mangrove sediments, displaying enzymatic activity initiating the remineralization of organic matter (Costa *et al.* 2022). Microbial carbonic anhydrase (CA) is an enzyme that can reversibly catalyze the conversion of carbon dioxide to bicarbonate ions to be used for metabolic processes. There is a possibility that microbially sourced CA can be used for carbon sequestration (Zaidi *et al.* 2022). However, microbial enzymatic activity can be disrupted by climate change. Organic carbon accumulation is stopped by phenolic

compounds that suppress the degradation of soil organic matter by inhibiting the activity of enzymes that degrade organic matter. Anoxic conditions from waterlogging have historically blocked the enzymatic removal of phenolic compounds by bacterial tyrosinases. There is compelling evidence that bacterial tyrosinases are one of the key enzymes responsible for influencing carbon cycling in wetland ecosystems by the identification of the TYR gene in the genome (*tyr*$^{+}$) of *Acidobacteria, Actinobacteria, Bacteroidetes Firmicutes, Nitrospirae, Planctomycetes,* and *Proteobacteria.* Climate change can increase the aeration of anoxic wetland soils and increase TYR activity, which is detrimental due to an increased reduction of phenolic compound concentration (Panis & Rompel, 2022).

Soil organic matter formation in most peatland ecosystems occurs slowly primarily due to sub-surface root accumulation. Although the primary mechanism of peat development is the production and accumulation of refractory roots, turf algae, microbial communities, and accumulated litter and detritus can also be contributors (Osland *et al.* 2020). The early stage of peat deposition likely involves the most peat compaction. The actively growing peat surface can be less consolidated than deeper parts of the peat deposit (Costa *et al.* 2022). Costa *et al.* reported a lack of a patterned organic carbon (C_{org}) peat density with age, suggesting undetectable organic material loss. This assumes that changes in the C_{org} are due to organic matter loss due to decomposition. Altogether, there was no carbon loss from the peat in 3,000 years. Understanding microbial interaction in peat gives insight into how carbon is cycled or stored in ecosystems. More knowledge of microbial function can introduce methods of enhancing or maintaining soil carbon storage and carbon sinks to mitigate the effects of climate change.

Costa *et al.* characterized microbial communities by depth and sediment type: calcite, clay, peat, and sand. They found microbial communities differed significantly between

sediment environments. In ancient peats, a distinct microbial community specialized in breaking down recalcitrant matter under anoxic conditions. Based on the study by Costa *et al.*, similar classes including the *Dehalococcoidia* and *Anaerolineae* in the phylum *Chloroflexi* are expected from bacterial communities from peat in mangrove forests in Celestun, Yucatan.

MATERIALS AND METHODS

I. Field Sites

The samples were collected from Celestun, Yucatan, Mexico at four sites: Yaax Ha (20.872998, -90.35884), West Ria (20.883556, -90.364147), North Ria (20.951227, -90.327876), and East Ria (20.90581, -90.34314) (Fig. 1). Hurricanes frequent the Yucatan Peninsula due to its location in the Caribbean Sea's hurricane belt. Climatic patterns vary from semiarid (<400 mm $year^{-1}$) in the north to humid in the south (>1400 mm $year^{-1}$) (Orellana *et al.* 2009). The four collection sites are located in the Ria Celestun Biosphere Reserve, covering 814 km^2, 228 km^2 (22,800 ha) of which are fringe and dwarf mangrove forests (Doyon & Sabinot, 2014; Cinco-Castro & Herrera-Silveira, 2020).

Yaax Ha (YH) contained soft peat from 0 to 55 cm; coffee-colored clay with roots from 50-55 cm; coffee-colored clay with roots and shells from 125 to 150 cm; and brown peat with clay, shells, and roots from 165 to 237 cm. West Ria (WR) contained roots and detritus from 5 to 10 cm; clayey, rooty peat from 25 to 30 cm; peaty clay with roots from 45 to 90 cm; and peaty clay with shells from 105 to 116 cm. North Ria (NR) contained leaf litter from 5 to 10 cm; brown peat with large roots from 25 to 50 cm; tan clay with roots from 65 to 70 cm; cream/tan colored clay with roots from 88 to 93 cm; and tan/gray clay with roots and shells from 105 to 230 cm. East Ria (ER) contained clayey, crumbly peat from 5 to 10 cm; light coffee-colored peaty clay with roots from 25 to 70 cm; coffee-colored clay with occasional roots from 82 to 86 cm, tan/gray clay with shells and occasional roots from 105 to 130; cream-colored loose clay with shells from 145 to 170 cm; and shells with loose clay from 185 to 190 cm.

II. Field Collection

Mangrove sediments were sampled with a Russian peat corer (Aquatic Research instruments) attached to a blade, collecting semi-cylindrical sections of sediments 5 cm in diameter and up to 50 cm in length. Extension rods were subsequently added upon return to the collection hole to obtain deeper 50 cm of sediment. The process was repeated to rejection—when the corer tip hit hard substratum—to capture the entire sediment column. Each core segment was subsampled with depth at 5 cm intervals (0-5 cm, 10-15 cm, etc.). A knife and a measuring tape were used to collect samples every 5 cm vertically. Each core was visually identified as peat or clay, with occasional roots, shells, and litter. Carbon analysis samples were taken adjacent in the core to microbial samples.

Figure 1: Mangrove forest and site collection in the Ria Celestun Biosphere Reserve (Celestun, Yucatan, Mexico).

III. Carbon and Nitrogen Analysis

Percent N and C_{org}, $\delta^{15}N$, $\delta^{13}C$, and C_{org} density were used to measure the carbon and nitrogen age and quantity in the sediments. Each peat sample was placed in a drying oven at 60℃ until dry (≥ 24 h). Dried samples were weighed and homogenized with a ball mill and mortar and pestle until they were able to pass through a 500 μm sieve. To ensure the remaining carbon was organic, samples were HCl fumigated to remove $CaCO_3$. 6-9 mg was weighed from each sample and analyzed by GC-IRMS (Carlo Erba NA 1500 elemental analyzer) to yield $\delta^{15}N$, $\delta^{13}C$, and percent carbon and nitrogen by mass. Percent carbon (or nitrogen) times the sample's measured bulk density gives the carbon (or nitrogen) mass per unit volume.

IV. DNA Extraction, Quantification, PCR

Samples were stored at -20℃ in Celuestun, Yucatan, and transferred in dry ice to UC Riverside for molecular analysis. 0.25 g of each sample was weighed for DNA extraction. Microbial DNA was extracted with a QIAGEN DNeasy® Powersoil Pro Kit following the manufacturer's instructions. The MoBio homogenizer was used for the bead-beating stage. NanoDrop2000/2000c UV-Vis spectrophotometer (ThermoFisher Scientific) was used for quantification. After quantification, PCR was conducted with primers targeting the V3 and V4 regions of the 16S rRNA gene of bacteria. Microbial genomic DNA (2.5 μl) was combined with forward and reverse primers (5 μl each) and 2x KAPA HiFi HotStart ReadyMix (KAPA Biosystems) (12.5 μl). A Bio-Rad MJ Research PTC 200 Thermocycler was used for sample amplification with the following: 95℃ for 3 min; 34 cycles of 95℃ for 30 s, 55℃ for 30 s, 72℃ for 30 s, and 72℃ for 5 min; and hold at 4℃. Amplified DNA extract was sequenced using an Illumina Miseq instrument. Dual indices and Illumina

sequencing adaptors were attached to the amplicon using the Nextera XT Index Kit (Illumina). Amplicon DNA (5 µl) was combined with 2x KAPA HiFi HotStart ReadyMix (25 µl), Index 1 and 2 primers (5 µl each), and PCR grade water (10 µl). The same thermocycler settings were used for the subsequent PCR cycles after cleanup. The samples were verified with gel electrophoresis with visible banding of the original PCR and post-cleanup amplicons.

V. Sequence Analysis

Analyses of bacterial communities were conducted in RStudio with the vegan package. To perform a whole genome alignment, the DADA2 pipeline was used to recover ASVs (amplicon sequence variants) from amplicon data. After the target region of the 16SrRNA gene was sequenced, the DADA2 pipeline was used to filter, trim, and remove chimeric sequences. The biodiversity of the microbial community was quantified with the Shannon-Weiner Species Diversity Index. Beta diversity was quantified by PERMANOVA (permutational ANOVA) to compare microbial communities by site using Bray-Curtis dissimilarity.

RESULTS

I. Diversity by Site

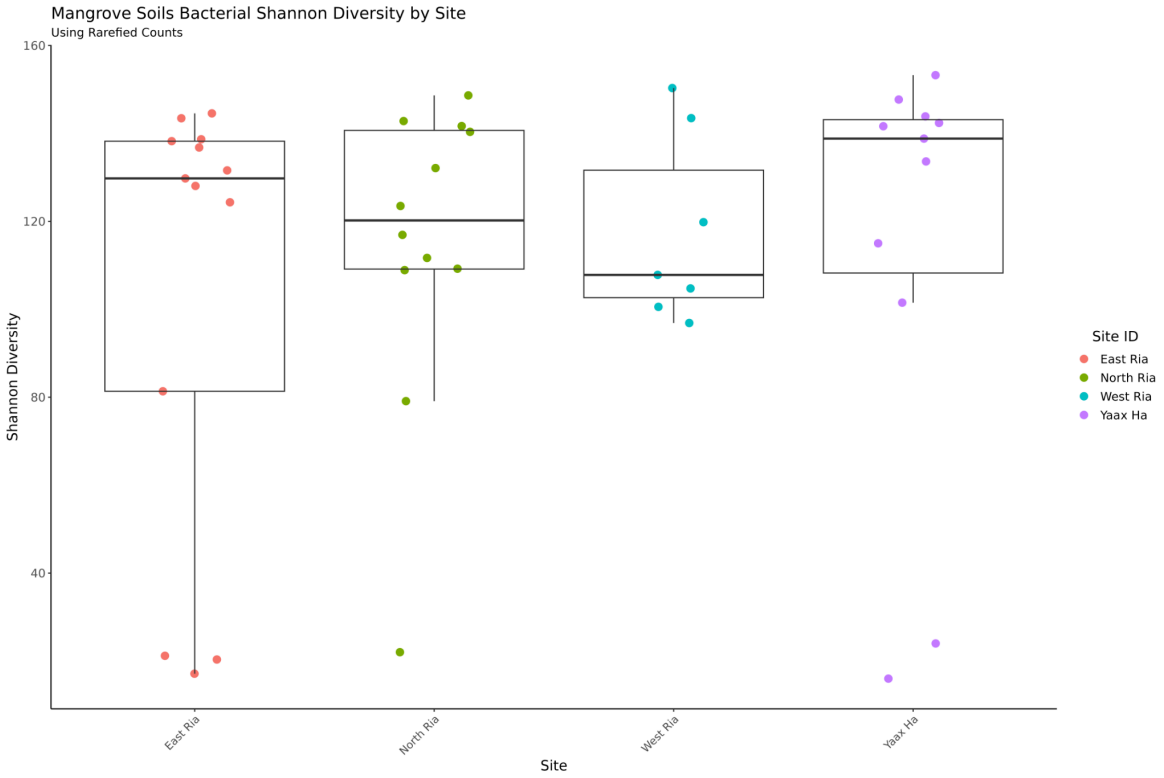

Figure 2: Shannon Diversity Index—the number of species in a habitat (richness) and relative abundance (evenness)—in peat samples at ER, NR, WR, YH (Kruskal-Wallis chi-squared = 1.0813, df = 3, p-value = 0.7816)

Shannon Wiener Index did not differ significantly by site ($P > 0.05$), indicating that richness and evenness are similar across all four sites. Species richness did not differ significantly by site (Kruskal-Wallis chi-squared = 1.0893, df = 3, p-value = 0.7797) (Fig. 2). Significant differences between depths could not be compared due to different groups that skewed results.

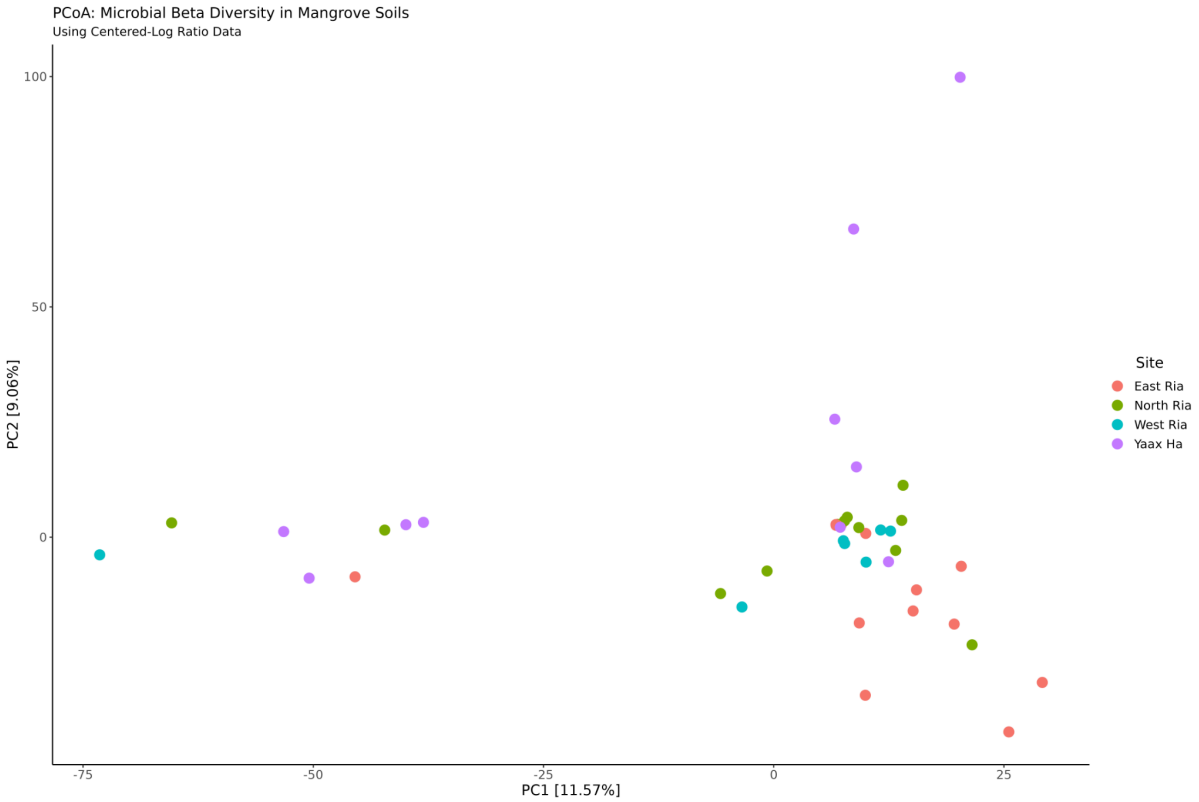

Figure 3: Principal Coordinate Analysis (PCoA) plot of site ID and microbial diversity (beta diversity) in mangrove soils at ER, NR, WR, YH (Adjusted p-value = 0.00509949). YH vs WR: P= 0.148, YH vs. ER: P=0.001, YH vs. NR: P=0.079, WR vs. NR: P=0.283, WR vs. NR: P=0.098, ER vs. NR: P=0.014.

There is a significant difference in beta diversity by site in contrast to the Shannon-Weiner Diversity Index. A PCoA plot of site ID and microbial diversity revealed an 11.57% variance in PC1 (Site ID) and a 19.06% variance in PC2. ER and YH (P=0.001) and ER and NR (P=0.014) have the least cluster overlap, indicating that the members in their microbial communities are the most diverse (P < 0.5). NR and WR (P=0.0283) and YH and NR (P=0.079) have similar microbial compositions (P > 0.5) (Fig. 3).

II. Relative Abundance of Microbial Communities

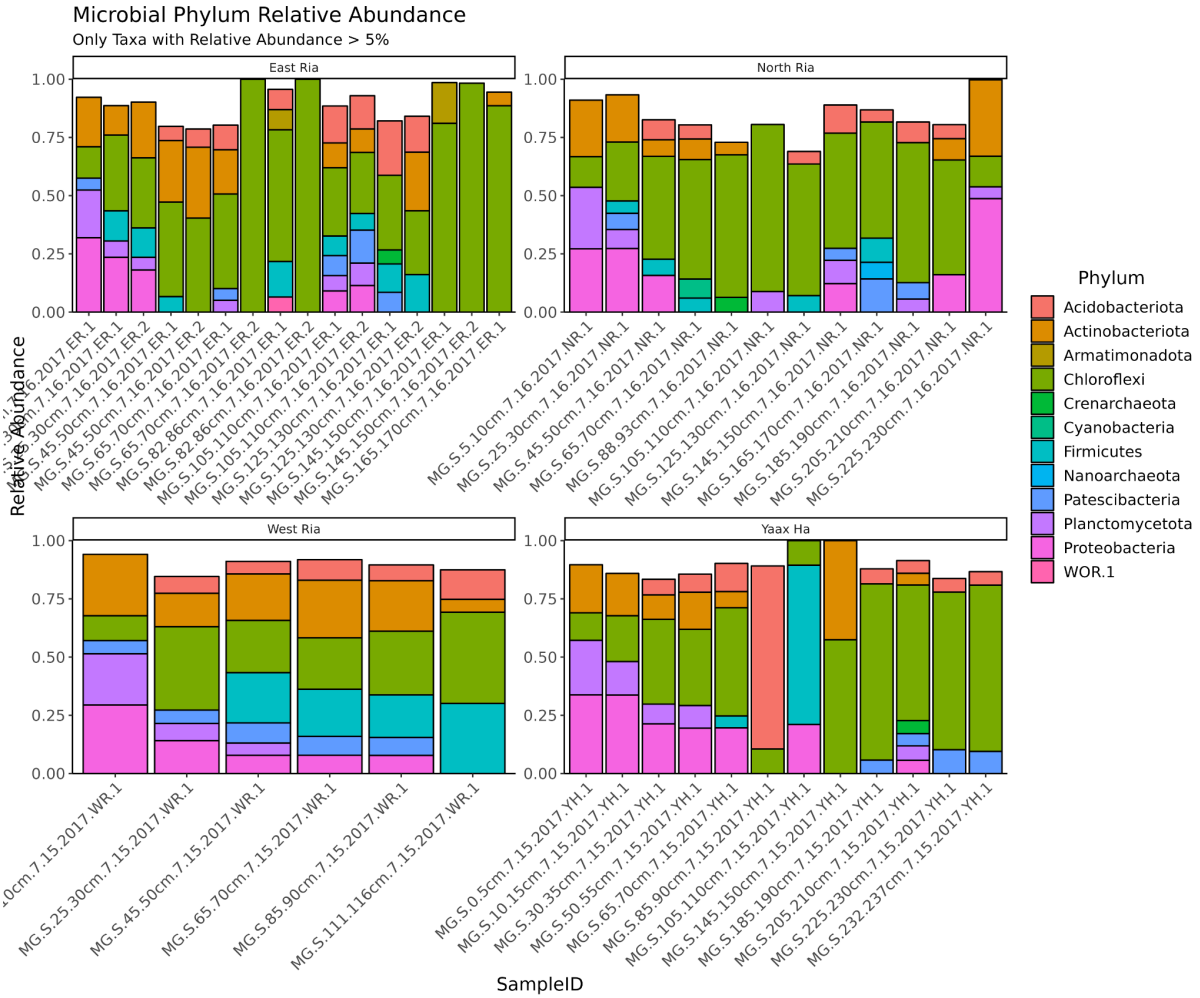

Figure 4: Relative abundance plots of microbial phyla > 5%.

Taxonomic assignment revealed twelve phyla across the four sites: *Acidobacteria, Actinobacteria, Armatimonadota, Chlorflexi, Crenarchaeota, Cyanobacteria, Firmicutes, Nanoarchaeota, Patescibacteria, Planctomyectota, Proteobacteria,* and *WOR.1* (Fig. 4). There is a greater relative abundance of *Chloroflexi* across ER, NR, and YH at and below 145 cm. In ER, *Chloroflexi* has the greatest abundance between 65 to 86 cm, and 145 to 170 cm. NR has the most abundance from 25 to 210 cm (Fig. 4). However, WR has an even abundance of *Chloroflexi* across depths, correlating with peaty clay from surface level to deeper samples. The uppermost 50 cm of ER and NR favor *Proteobacteria*, encompassing a mixture of leaf litter, peat, and clay. *Firmicutes* in WR are abundant from 45 to 116 cm, with

105 cm in YH (Fig. 4). *Firmicutes* are associated with clayey material in WR and YH. *Firmicutes*, including genera *Bacillus* and *Paenibacillus*, are an integral part of plant microbiomes, and the selection of microbes from the soil pool is influenced by the host plant (Borriss, 2020). Thus, there is a possibility that an increase in *Firmicutes* members is essential for mangrove microbiomes. Similar to the study by Costa *et al.*, surface samples were dominated by *Proteobacteria*, known for metabolic diversity (Ghose *et al.* 2024). The microbial community varies across sediment types, giving insight into anoxic recalcitrant peat metabolism (Costa *et al.* 2020).

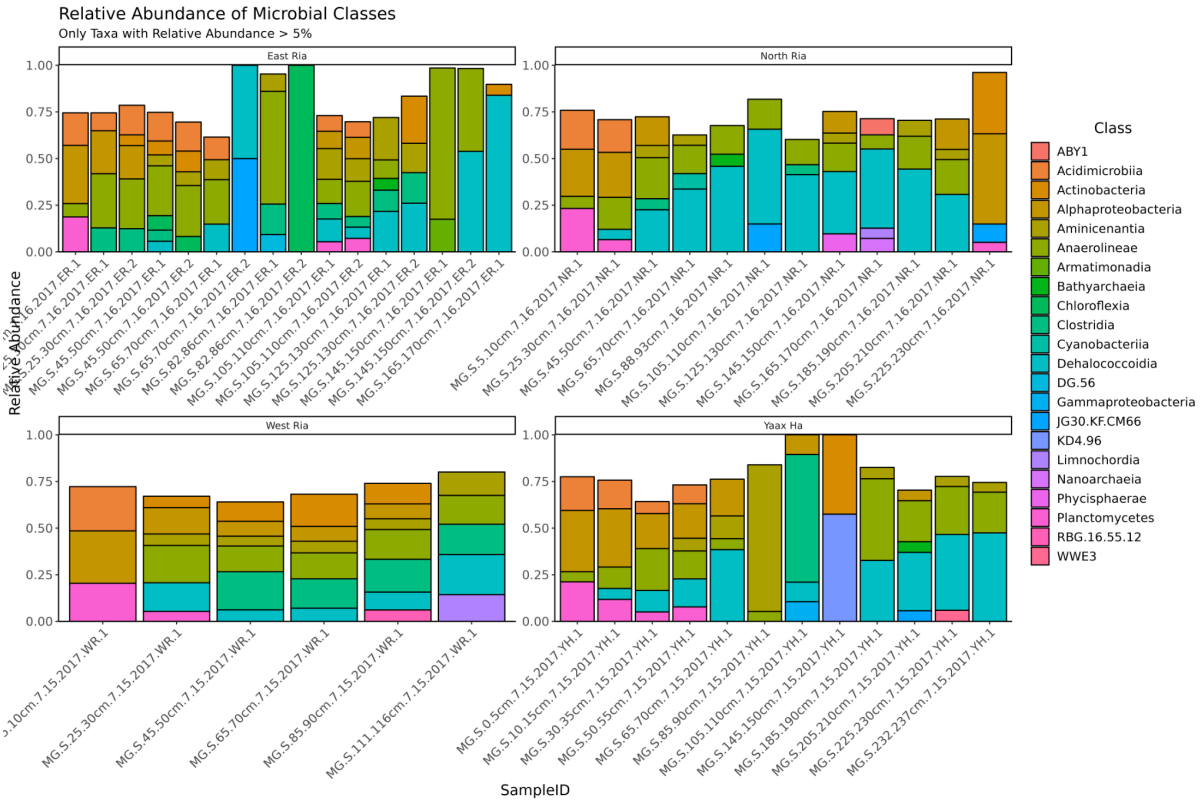

Figure 5: Relative abundance plots of microbial classes > 5%.

There is a pattern of greater relative abundance of classes according to depth between sites, primarily between YH and ER. *Dehalococcidia* and *Anaerolineae* are more prominent in the deeper peaty clay in ER (145 to 170 cm) and peat YH (185 to 237 cm) (Fig. 5). Both

classes are known to be present in anoxic conditions (Costa *et al.* 2020). Previously, the *Anaerolineae* lineage of *Chloroflexi* was identified as one of the core microbial populations in anaerobic digesters (Xia *et al.* 2016). There is a greater abundance of *Acidomirobiia, Planctomycetes,* and *Alphaproteobacteria* in the shallower peat across all four sites at least from 0 to 10 cm. Interestingly, NR displayed a greater abundance of *Actinobacteria* and *Alphaproteobacteria* at 225 to 230 cm not found at any other depth at the three other sites. In contrast, YH had a greater abundance of *Dehalococcidia* and *Anaerolineae* at the same depth. Although NR and ER consist of clayey material with roots and shells with depth, ER has a greater abundance of *Anaerolineae* at 145 to 150 cm. WR maintains a more consistent class abundance, especially 25 to 90 cm compared to the other sites at similar depths (Fig. 5).

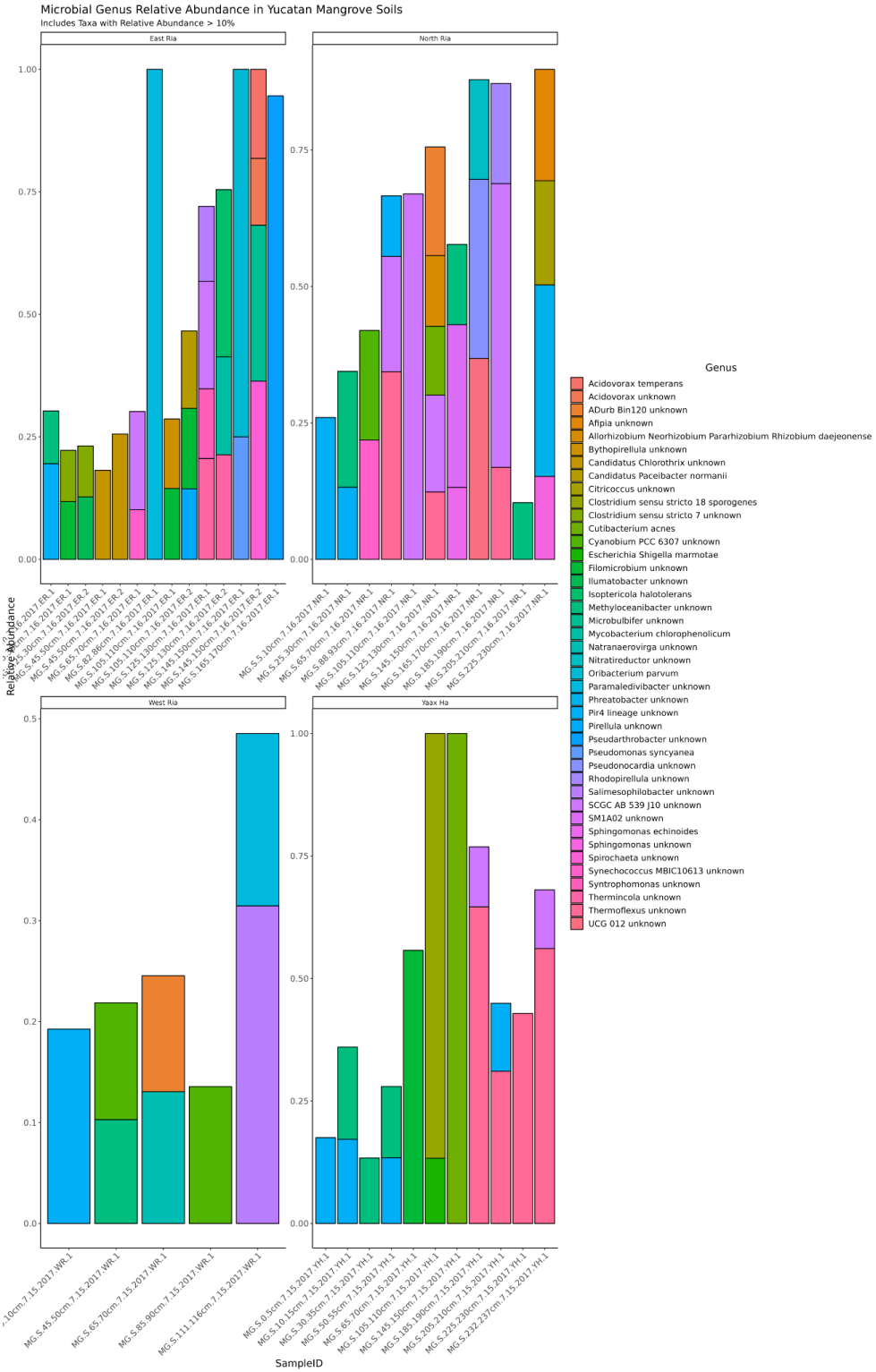

Figure 6: Relative Abundance of microbial genera > 10% by site.

Some genera were unique to a specific site or were less abundant in other sites. ER has a large relative abundance of *Oribacterium parvum* at 82 cm and *Pseudoarthrobacter* has

an increased abundance at 165 to 170 cm (Fig. 6). NR and WR have varying relative abundances with depth. However, WR has an increase in abundance of *Salimesophilobacter* at 111 to 116 cm (Fig. 6). *Salimesophilobacter* in the phylum *Firmicutes* is a strictly anaerobic mesophile whose sole carbon source can be carbohydrates, alcohols, and carboxylic acids (Zhang *et al.* 2013). NR has changes in composition starting from 205 cm showing variable changes in relative abundance. The shift of relative abundance with increasing depth is most apparent at NR with *Microbulbifer* at 205 cm. *Thermoflexus* is more present in relative abundance between 185 to 237 cm in YH (Fig. 6).

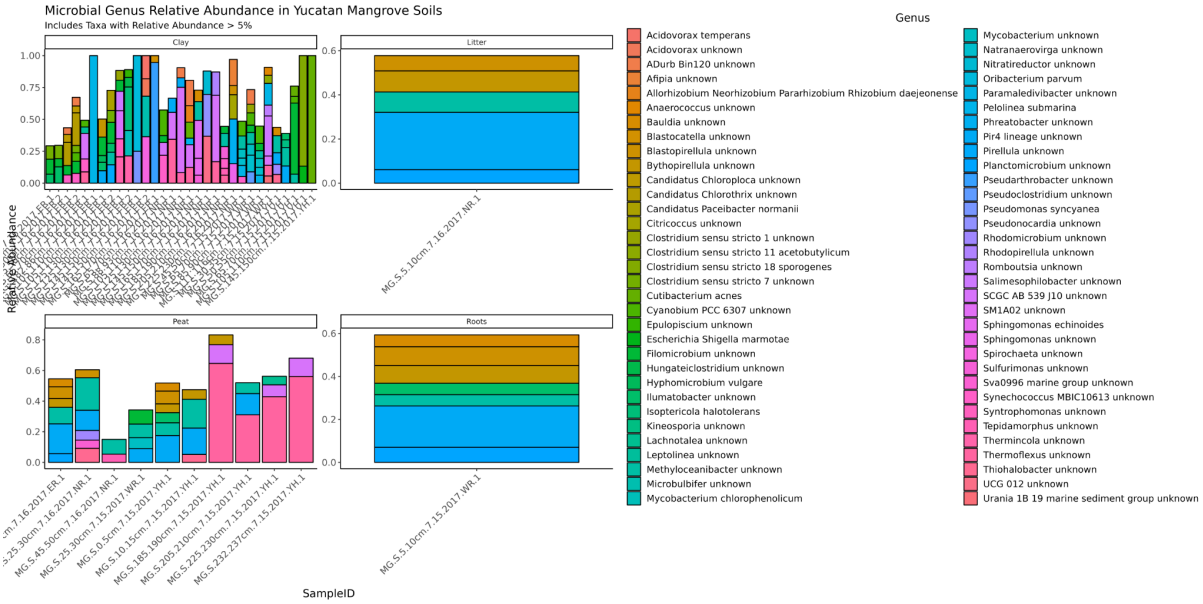

Figure 7: Relative abundance plots of microbial genera > 5% by clay, litter, peat, and roots.

Clay samples displayed a greater diversity of genera across all four sites (Fig. 7). Peat samples are more diverse with depth with *Thermoflexus* in the deeper, more compacted YH peat from 185 to 237 cm. Roots in WR at 5 cm, litter in NR at 5 cm, and peat at 0 to 5 cm have a similar microbial composition with a greater abundance of *Pir4* with an unknown lineage (Fig. 7). By 16S rRNA gene sequencing, the Pirellula-related Pir4 clade of aquatic bacteria is detected in various anoxic and micro-oxic habitats (Dedysh *et al.* 2020). *Pir4*

correlates with more water flowing through the root, layer, and uppermost peat segments in marine environments. The surface zone likely includes less compacted peat formation with roots present selecting for microbes that are not as specific to recalcitrant material and anoxic conditions.

III. Bulk Density, %N, and %C

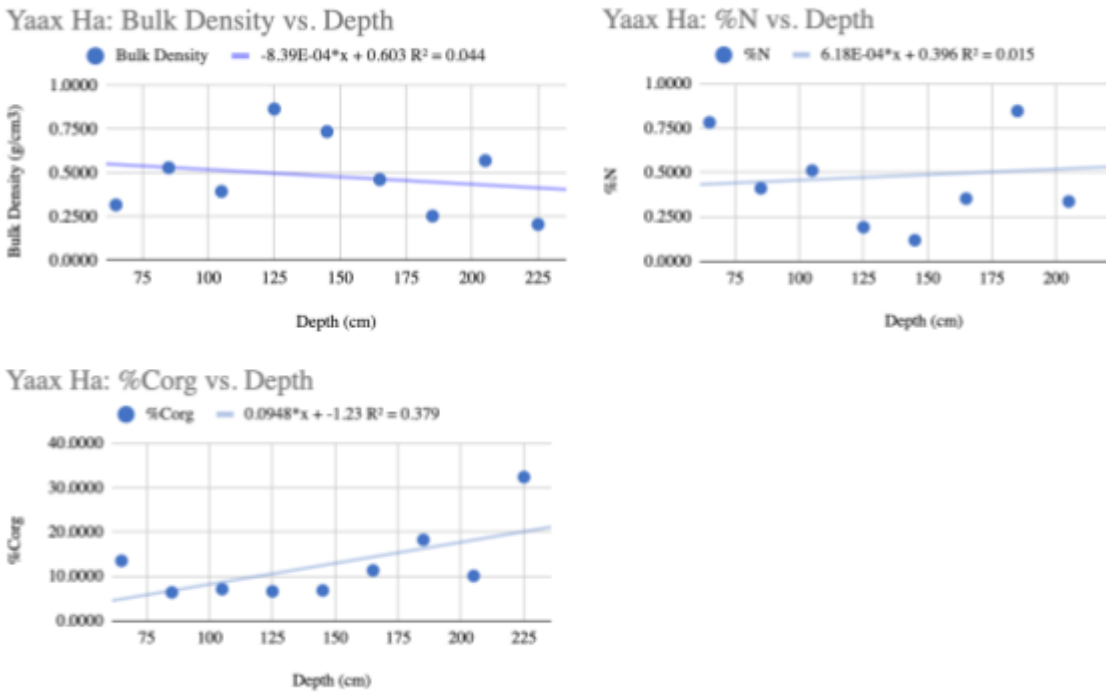

Figure 8: Linear regression plots of YH bulk density (R^2=0.044), $\%C_{org}$ (R^2=0.379), and %N (R^2=0.015) by depth.

There is a weak correlation between decreasing bulk density and %N with depth and a moderate correlation with $\%C_{org}$ with depth at YH (Fig. 8). Therefore, bulk density remains relatively stable with depth. According to $\delta^{15}N$ and $\delta^{13}C$ radiometric analyses, YH peat is aged 1626 ± 15 years old at the deepest segment (232-237 cm). *Chloroflexi* is most abundant and associated with peat samples at YH ≥ 1,000 years old. Similarly, *Chloroflexi* was the most commonly overrepresented phylum in Baja California with peat samples ≥ 1,000 years

old. *Dehalococcoidetes* in the phylum *Chloroflexi* include taxa that can undergo sulfate reduction and anaerobic dehalogenation of aliphatic organic compounds (Costa *et al.* 2020). Therefore, peat samples in YH have a distinct community that develops with older peat deposits (Fig. 6, Fig. 7). However, there is not a strong correlation between increasing % C_{org} with older peat, suggesting that carbon remains relatively stable with depth.

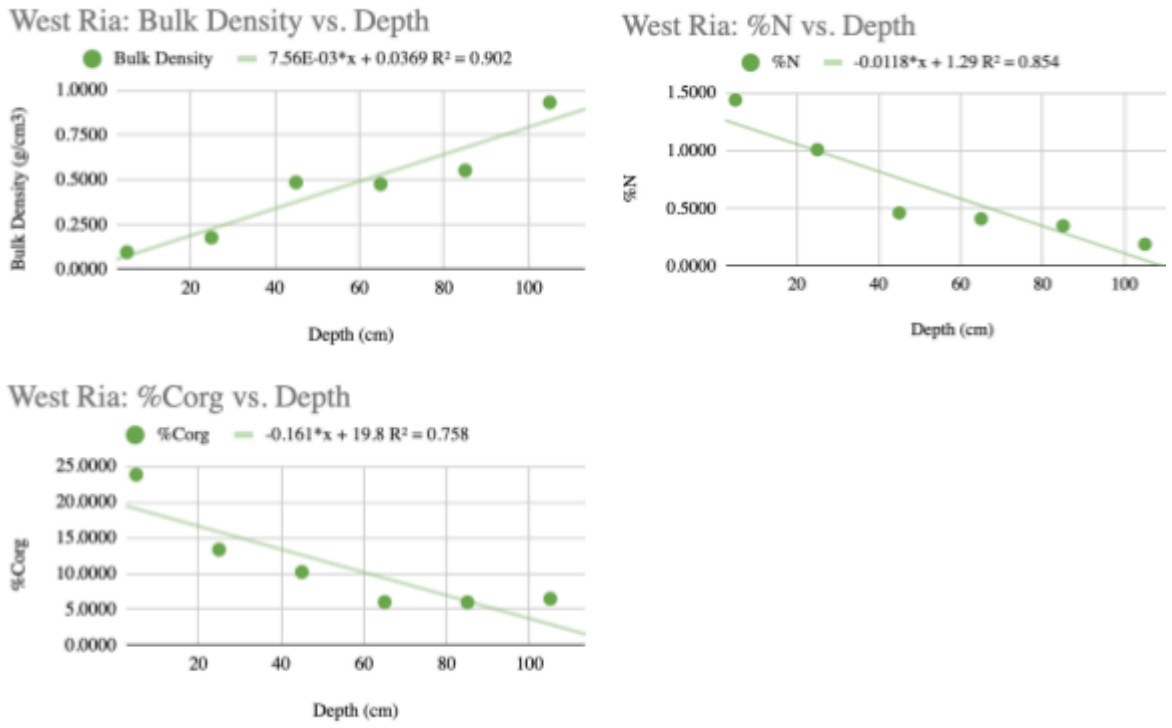

Figure 9: Linear regression plots of WR bulk density (R^2=0.902), %C_{org} (R^2=0.854), %C_{org} (R^2=0.758) by depth.

There is a strong correlation between bulk density, %N, %Corg, and depth in WR (Fig. 9). Bulk density strongly increases with depth, likely due to increased decomposition of peat that generally increases with depth (Rezanezhad *et al.* 2016). The trends of strongly decreasing %N and %Corg are likely due to increased amounts of carbon and nitrogen cycling through exportation.

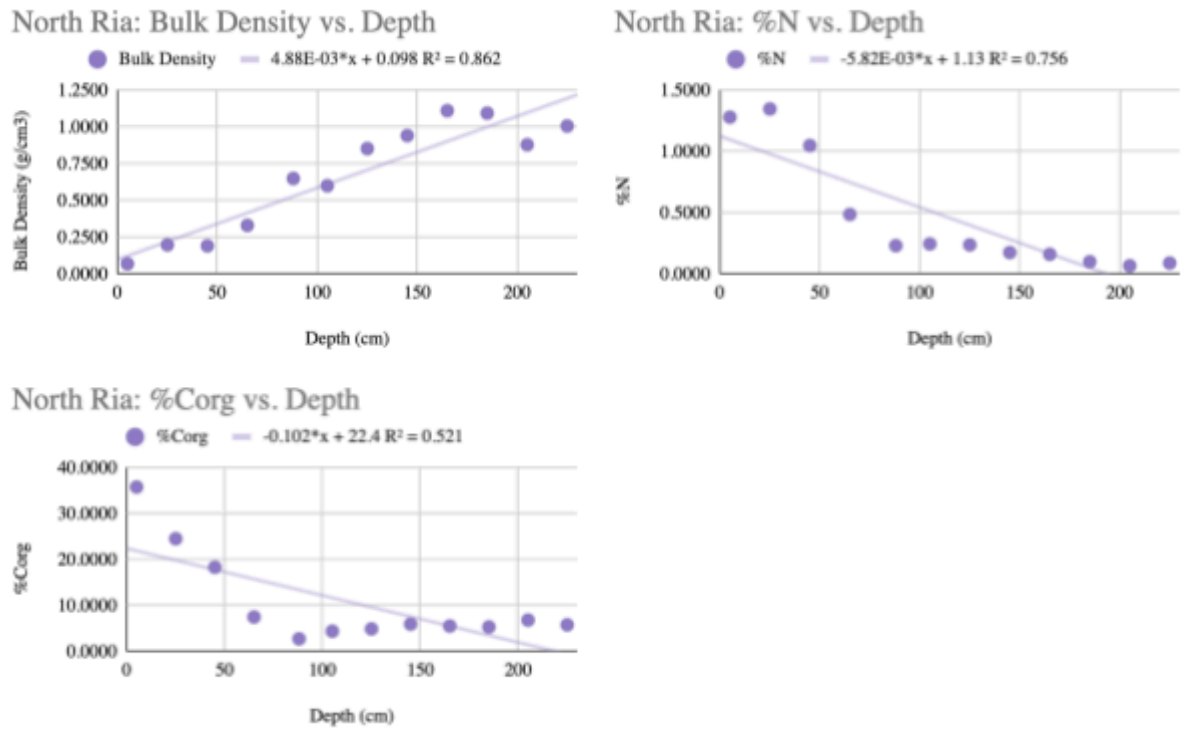

Figure 10: Linear regression plots of NR bulk density (R^2=0.862), %N (R^2=0.756), $\%C_{org}$ (R^2=0.521).

There is a strong correlation between bulk density and %N and a moderate correlation between $\%C_{org}$ and depth. Bulk density strongly increases with a strong decrease in $\%C_{org}$ and %N (Fig. 10). Considering the trends with WR (Fig. 9), NR has a faster carbon and nitrogen turnover with an increased bulk density likely due to increased decomposition.

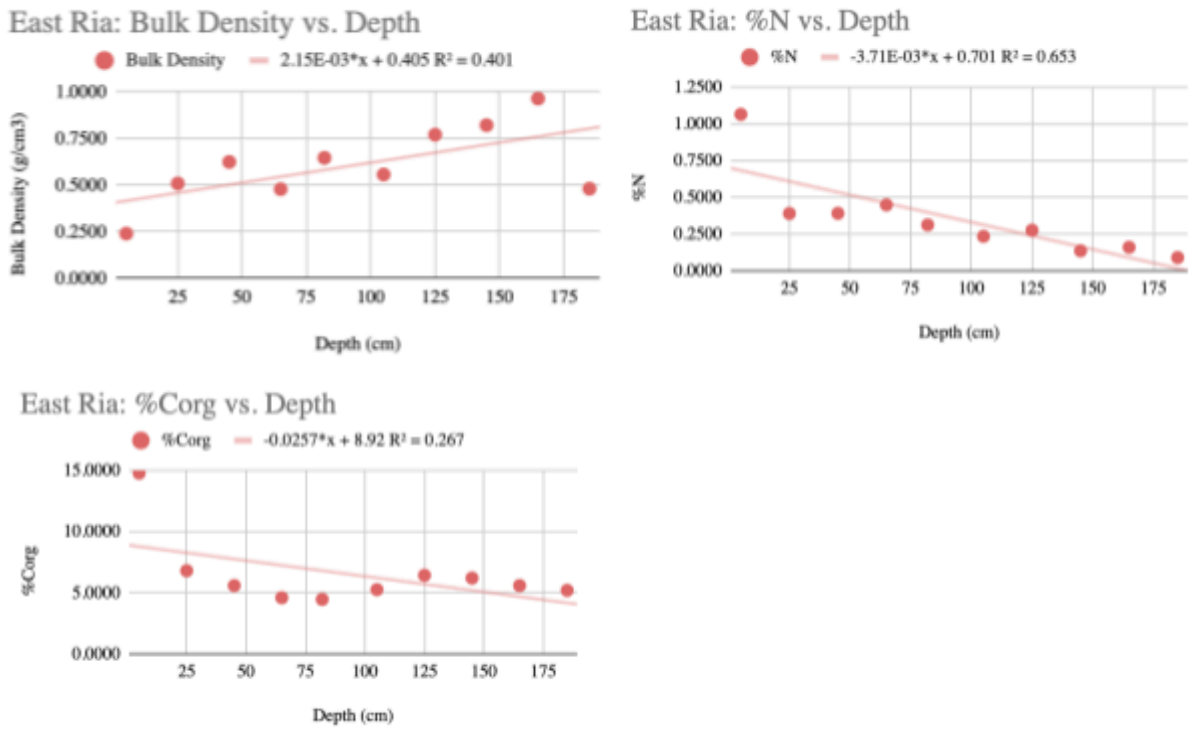

Figure 11: Linear regression plots of ER bulk density (R^2=0.401), %N (R^2=0.653), and $\%C_{org}$ (R^2=0.267).

There is a moderate correlation between bulk density and %N by depth and a weak correlation between $\%C_{org}$ and depth. Bulk density moderately increases with depth, likely indicating that microbial decomposition is moderate (Fig. 11). The decreasing %N reveals a greater exportation of nitrogen compared to carbon, which is more stable with depth. As a result, the carbon turnover is slower in ER.

DISCUSSION

Alpha diversity did not differ significantly by site or depth. An individual site shared similar taxa with depth, including *Actinobacteria, Chloroflexi, Proteobacteria,* and *Firmicutes.* YH appeared to have a small rise in the relative abundance of *Acidobacteria* but was not enough to be significant.

Microbial community members did significantly differ by site. Some phyla appeared to increase in relative abundance of *Chloroflexi* the most with depth in YH and ER. There were similar genera in shallow peat, leaf litter, and roots, revealing specific taxa in shallower and deeper material. Mid-range material showed a range of taxa that could be a transition of adaptations from aerobic to anaerobic conditions or material type (shells, clay, roots) with depth.

YH has the most relative abundance of *Chloroflexi* associated with older and more developed peat layers. The peat layers with increased organic matter are reflected with stable bulk density. The mid-range depths consist mostly of clay, which could explain the change in the relative abundance of *Acidobacteria* and *Actinobacteria* while transitioning back to a greater relative abundance of *Chloroflexi* at deeper segments. At YH, both %N and $\%C_{org}$ increase steadily, indicating a slower nitrogen and carbon turnover associated with carbon sequestration in the peat.

WR has an even abundance of microbial community members throughout its depth segments. The surface material at the site consists mostly of peaty clay with roots and detritus. Since there is less recalcitrant material and more water at the surface, it is most likely that the surface environment has not been selected for the presence of anaerobic bacteria (*Chloroflexi*). In addition, *Chloroflexi* does not show prominence over other taxa at

each sample depth, demonstrating that the peat at WR is younger and has less recalcitrant organic matter.

NR consists of litter and peat at shallower depths and clay at deeper depths. NR has an increase in bulk density with depth and a steady increase in $\%C_{org}$. Nitrogen is cycled at a high rate back into the environment, but the steady increase in carbon could be due to increased carbon sequestration due to peat formation. Thus, it is likely that there might be an increase in the relative abundance of *Chloroflexi* due to more peat depositing in the deeper layers in NR.

ER has a mixture of peat and clay in its shallower depths and contains less peat than YH, but still has a greater relative abundance of *Chloroflexi* than WR and NR. Decreased peat decomposition due to anoxic conditions mostly likely contributes to the rise of *Chloroflexi* in ER and YH. The environments of WR and NR have not selected for microbes that can thrive in more anoxic conditions due to more dissolved oxygen present. In addition, this indicates that the site is potentially in the process of peat deposition at a different rate than WR and NR. NR and WR will possibly have a rise of *Chloroflexi* abundance similar to YH and ER once more peat is deposited over time.

Altogether, WR and NR have greater bulk densities associated with more decomposition and greater remineralization of carbon exported back to the environment. In contrast, YH and ER have less and more stable bulk densities associated with increased storage in peat due to decreased decomposition. These differences select for different microbes, with aerobic microbes at the surface layers and anaerobic microbes at the deeper layers. Across all sites, the $\%C_{org}$ varies with depth, with the strongest correlation in WR and NR and the weakest correlation in YH and ER. At WR, NR, and ER, %N decreases at a faster rate with depth than %C. The observed trends support that carbon content and bulk density

can be functionally related to peat formation (Sternberg-Rodriguez *et al.* 2022). Peat has higher organic matter content, lower bulk density, and can contain pore space as high as 100% with dead end or isolated pores. Microporosity increases during peat decomposition and degradation; however, a higher macroporosity can be observed in strongly decomposed and less decomposed peat. (Liu & Lenartz, 2018). Together, microporosity and macroporosity are a contributing factor to lower bulk density and can be tied to the stable bulk density in YH. In contrast, WR, NR, and ER have strongly increasing bulk densities, which might be due to a combination of more mineralized soils, less organic matter content, or pore structure.

Vegetated coastal ecosystems, such as mangrove forests, sequester carbon tens of times faster than terrestrial forests (Kida *et al.* 2020). Blue carbon ecosystems have significant potential for carbon sequestration and greenhouse gas offsets. Reforestation efforts can be focused on areas suffering from degradation or deforestation by anthropogenic or natural factors to increase blue carbon potential (Song *et al.* 2023).

Interestingly, *Chloroflexi, Acidobacteria, Proteobacteria,* and *Actinobacteria* were phyla present in Yucatan mangrove core samples and treatment peatlands in Northern Finland. Peatlands are used to purify mining-affected waters for contaminant removal. Microorganisms have specialized to tolerate high contaminant concentrations of metals and metalloids. Diverse microbial communities exist with prolonged mine water inflow and are potentially involved in nitrogen and sulfate turnover. Thus, microbes play an important role in contaminant removal (Kujala *et al.* 2018).

Two dominant taxa (*Chloroflexi* and *Proteobacteria*) in shallower and deeper peat in Yucatan are found in the mangrove habitats of Campal and Panaji in India. Ghose *et al.* reported similar observations of dominance of *Proteobacteria* and *Chloroflexi*. The dominant taxa included groups contributing to the biogeochemical cycling of organic matter sediment.

Shotgun metagenomic analysis revealed carbohydrate metabolism and glycoside hydrolases degrading plant-based starch, pectin, and cellulosic organic matter. In addition, the presence of xenobiotic biodegradation pathways acts as a natural way to degrade high amounts of pollutants in the area (Ghose *et al.* 2024). Xenobiotic contaminants such as azodyes, phenolics, polycyclic aromatic hydrocarbons, pesticides, and chlorinated compounds show long-term persistence with little biodegradation. Xenobiotics are a major environmental threat and cause harmful effects at each trophic level in the food chain once discharged into the environment. (Miglani *et al.* 2022). As a result, taxa found in Yucatan can potentially engage in bioremediation due to anthropogenic activities.

Microorganisms perform most decomposition reactions, and carbon which is more complex and does not decompose as quickly becomes stored over longer time scales. Below mangroves, peat is theorized to sequester carbon due to an absence of fungi capable of degrading lignin in woody material (Costa *et al.* 2022). Therefore, mangrove debris can only be partially decomposed. There are still gaps in knowledge on where fungi fit into energy pathways and how they recycle nutrients back to the environment. The various enzymes associated with fungi can be used to infer their community function. Since some of these enzymes are specific to high salt environments, researching more into their roles can provide insights into the role of fungi in nutrient cycling (Thatoi *et. al* 2013). Identifying fungal taxa through metagenomics and ascertaining the biochemical aspects that certain fungal species share in common is fundamental to understanding the community function as a whole. As a result, learning about the community function has greater implications for environmental processes, such as carbon sequestration within mangrove roots. Altogether, the roles of these enzymes can be examined together and compared between species to see if fungi share similar processes as a whole or are vastly different. The biochemical processes are complex

and are hardly studied in the field of mycology; there is generally more knowledge about bacteria in certain ecological aspects while virtually little is known about fungi in a similar lens (Hyde *et al.* 1995).

Future work can examine any metabolic functions of bacteria in the phylum involved in carbon cycling at shallower sediment types. Species richness by depth did not produce any significant differences due to too many groups in question. Grouping the sample IDs into shallow, mid, and deep soils would allow for a better comparison. A shotgun metagenomic analysis can be conducted to examine the predominance of metabolic pathways contributing to pollutant degradation in dominant taxa in Celestun. The ratio of peat to clay can be examined for any significant differences in the microbial composition of the community. Additional research can be conducted to determine if a core mangrove microbiome is present in mangrove forests that are separated geographically.

In conclusion, there is a significant difference in microbial community members by site, most notably in YH and ER. A stabilized peat formation and decreased composition with depth in YH supports *Thermoflexus* genera in the *Chloroflexi* phylum. There is a greater relative abundance of *Pir4* in ER, NR, and WR within roots, litter, and surface samples in peat. Further, the microbial community appears to be turning over the carbon in the mangrove peat very slowly, allowing peat to accumulate and sequester carbon.

REFERENCES

1. Arifanti, V. B., Novita, N., Subarno, N., & Tosiani, A. (2021). Mangrove deforestation and CO2 emissions in Indonesia. *IOP Conference Series. Earth and Environmental Science*, *874*(1), 012006. https://doi.org/10.1088/1755-1315/874/1/012006
2. Borriss, R. (2020) *Beneficial microbes in Agro-Ecology*. ScienceDirect. https://www.sciencedirect.com/book/9780128234143/beneficial-microbes-in-agro-ecology
3. Cinco-Castro, S., & Herrera-Silveira, J. A. (2020). Vulnerability of mangrove ecosystems to climate change effects: The case of the Yucatan Peninsula. *Ocean & Coastal Management*, *192*, 105196. https://doi.org/10.1016/j.ocecoaman.2020.105196
4. Costa, M.T., Ezcurra, E., Aburto-Oropeza, O., Maltz, M., Arogyaswamy, K., Botthoff , J., Aronson, E. (2022). Baja California Sur mangrove deep peat microbial communities cycle nitrogen but do not affect old carbon pool. Mar Ecol Prog Ser 695:15-31. https://doi.org/10.3354/meps14117
5. Davis, J. L., Currin, C. A., O'Brien, C., Raffenburg, C., & Davis, A. (2015). Living Shorelines: Coastal Resilience with a Blue Carbon Benefit. *PloS One*, *10*(11), e0142595. https://doi.org/10.1371/journal.pone.0142595
6. Dedysh, S. N., Kulichevskaya, I. S., Beletsky, A. V., Ivanova, A. A., Rijpstra, W. I. C., Damsté, J. S. S., Mardanov, A. V., & Ravin, N. V. (2020). Lacipirellula parvula gen. nov., sp. nov., representing a lineage of planctomycetes widespread in low-oxygen habitats, description of the family Lacipirellulaceae fam. nov. and proposal of the orders Pirellulales ord. nov., Gemmatales ord. nov. and Isosphaerales ord. nov.

Systematic and applied microbiology, *43*(1), 126050. https://doi.org/10.1016/j.syapm.2019.126050

7. Doyon, S., & Sabinot, C. (2014). A new 'Conservation Space'? protected areas, environmental economic activities and discourses in two Yucatán biosphere reserves in Mexico. *Conservation & Society/Conservation & Society*, *12*(2), 133. https://doi.org/10.4103/0972-4923.138409
8. Ghose, M., Parab, A. S., Manohar, C. S., Mohanan, D., & Toraskar, A. (2024). Unraveling the role of bacterial communities in mangrove habitats under the urban influence, using a next-generation sequencing approach. *Journal of Sea Research*, *198*, 102469. https://doi.org/10.1016/j.seares.2024.102469
9. Holguin, G., Vazquez, P., & Bashan, Y. (2001). The role of sediment microorganisms in the productivity, conservation, and rehabilitation of Mangrove ecosystems: An overview. *Biology and Fertility of Soils*, *33*(4), 265–278. https://doi.org/10.1007/s003740000319
10. Howard, J., Sutton-Grier, A., Herr, D., Kleypas, J., Landis, E., Mcleod, E., Pidgeon, E., & Simpson, S. (2017). Clarifying the role of coastal and marine systems in climate mitigation. *Frontiers in Ecology and the Environment*, *15*(1), 42–50. https://doi.org/10.1002/fee.1451
11. Hyde, K. D., & Lee, S. Y. (1995). Ecology of mangrove fungi and their role in nutrient cycling: What gaps occur in our knowledge? *Hydrobiologia*, *295*(1-3), 107–118. https://doi.org/10.1007/bf00029117
12. Jia, S.-L., Chi, Z., Liu, G.-L., Hu, Z., & Chi, Z.-M. (2020). Fungi in mangrove ecosystems and their potential applications. *Critical Reviews in Biotechnology*, *40*(6), 852–864. https://doi.org/10.1080/07388551.2020.1789063

13. Kida, M., & Fujitake, N. (2020). Organic carbon stabilization Mechanisms in mangrove soils: a review. *Forests*, *11*(9), 981. https://doi.org/10.3390/f11090981
14. Kujala, K., Mikkonen, A., Saravesi, K., Ronkanen, A., & Tiirola, M. (2018). Microbial diversity along a gradient in peatlands treating mining-affected waters. *FEMS Microbiology Ecology/FEMS Microbiology, Ecology*, *94*(10). https://doi.org/10.1093/femsec/fiy145
15. Liu, H., & Lennartz, B. (2018). Hydraulic properties of peat soils along a bulk density gradient—A meta study. *Hydrological Processes*, *33*(1), 101–114. https://doi.org/10.1002/hyp.13314
16. McTigue, N., Davis, J., Rodriguez, A. B., McKee, B., Atencio, A., & Currin, C. (2019). Sea level rise explains changing carbon accumulation rates in a salt marsh over the past two millennia. *Journal of Geophysical Research. Biogeosciences*, *124*(10), 2945–2957. https://doi.org/10.1029/2019jg005207
17. Miglani, R., Parveen, N., Kumar, A., Ansari, M. A., Khanna, S., Rawat, G., Panda, A. K., Bisht, S. S., Upadhyay, J., & Ansari, M. N. (2022). Degradation of Xenobiotic Pollutants: An Environmentally Sustainable Approach. *Metabolites*, *12*(9), 818. https://doi.org/10.3390/metabo12090818
18. Nagelkerken, Ivan & Blaber, S. & Bouillon, Steven & Green, P. & Haywood, Michael & Kirton, L.G. & Meynecke, Jan-Olaf & Pawlik, Joseph & Penrose, Helen & Sasekumar, A. & Somerfield, Paul. (2008). The habitat function of mangroves for terrestrial and marine fauna: A review. Aquatic Botany. 89. 10.1016/j.aquabot.2007.12.007.
19. Nimnoi, P., & Pongsilp, N. (2022). Insights into Bacterial Communities and Diversity of Mangrove Forest Soils along the Upper Gulf of Thailand in Response to

Environmental Factors. *Biology*, *11*(12), 1787. https://doi.org/10.3390/biology11121787

20. Orellana, R., Espadas, C., Conde, C., & Gay, C. (2009). Atlas escenarios de cambio climático en la Península de Yucatán. *Mérida: Centro de Investigación Científica de Yucatán (CICY)*, *43*, 191-193.
21. Osland, M. J., Feher, L. C., Spivak, A. C., Nestlerode, J. A., Almario, A. E., Cormier, N., From, A. S., Krauss, K. W., Russell, M. J., Alvarez, F., Dantin, D. D., Harvey, J. E., & Stagg, C. L. (2020). Rapid peat development beneath created, maturing mangrove forests: ecosystem changes across a 25‐yr chronosequence. *Ecological Applications*, *30*(4). https://doi.org/10.1002/eap.2085
22. Panis, F., & Rompel, A. (2022). The Novel Role of Tyrosinase Enzymes in the Storage of Globally Significant Amounts of Carbon in Wetland Ecosystems. *Environmental science & technology*, *56*(17), 11952–11968. https://doi.org/10.1021/acs.est.2c03770
23. Rezanezhad, F., Price, J. S., Quinton, W. L., Lennartz, B., Milojevic, T., & Van Cappellen, P. (2016). Structure of peat soils and implications for water storage, flow and solute transport: A review update for geochemists. *Chemical Geology*, *429*, 75–84. https://doi.org/10.1016/j.chemgeo.2016.03.010
24. Song, S., Ding, Y., Li, W., Meng, Y., Zhou, J., Gou, R., Zhang, C., Ye, S., Saintilan, N., Krauss, K. W., Crooks, S., Lv, S., & Lin, G. (2023). Mangrove reforestation provides greater blue carbon benefit than afforestation for mitigating global climate change. *Nature Communications*, *14*(1). https://doi.org/10.1038/s41467-023-36477-1
25. Sternberg-Rodríguez, P., Ezcurra, P., Costa, M. T., Aburto-Oropeza, O., & Ezcurra, E. (2022). Precision of mangrove sediment blue carbon estimates and the role of coring

and data analysis methods. *Ecology and evolution*, *12*(12), e9655. https://doi.org/10.1002/ece3.9655

26. Suello, R. H., Hernandez, S. L., Bouillon, S., Belliard, J.-P., Dominguez-Granda, L., Van de Broek, M., Rosado Moncayo, A. M., Ramos Veliz, J., Ramirez, K. P., Govers, G., & Temmerman, S. (2022, March 18). *Mangrove sediment organic carbon storage and sources in relation to forest age and position along a deltaic salinity gradient*. Biogeosciences. Retrieved March 22, 2023, from https://bg.copernicus.org/articles/19/1571/2022/
27. Taillardat, P., Friess, D. A., & Lupascu, M. (2018). Mangrove blue carbon strategies for climate change mitigation are most effective at the national scale. *Biology Letters*, *14*(10), 20180251. https://doi.org/10.1098/rsbl.2018.0251
28. Thatoi. H, Beher, B. & Mishra, R. (2013) Ecological role and biotechnological potential of mangrove fungi: a review, *Mycology*, 4:1, 54-71, DOI: 10.1080/21501203.2013.785448
29. Xia, Y., Wang, Y., Wang, Y., Chin, F. Y. L., & Zhang, T. (2016). Cellular adhesiveness and cellulolytic capacity in Anaerolineae revealed by omics-based genome interpretation. *Biotechnology for Biofuels*, *9*(1). https://doi.org/10.1186/s13068-016-0524-z
30. Zaidi, S., Srivastava, N., & Khare, S. K. (2022). Microbial carbonic anhydrase mediated carbon capture, sequestration & utilization: A sustainable approach to delivering bio-renewables. *Bioresource Technology*, *365*, 128174. https://doi.org/10.1016/j.biortech.2022.128174
31. Zhang, Y., Fang, M., Zhang, W., Li, T., Wu, M., & Zhu, X. (2013). Salimesophilobacter vulgaris gen. nov., sp. nov., an anaerobic bacterium isolated from

paper-mill wastewater. *International Journal of Systematic and Evolutionary Microbiology*, *63*(Pt_4), 1317–1322. https://doi.org/10.1099/ijs.0.040915-0