Effects of tidal influence on the structure and function of prokaryotic communities in the sediments of a pristine Brazilian mangrove

Carolina Oliveira de Santana[1]; Pieter Spealman[2]; Vânia Maria Maciel Melo[3]; David Gresham[2]; Taíse Bomfim de Jesus[4], Fabio Alexandre Chinalia[1]

[1]Programa de Pós-Graduação em Geoquímica: Petróleo e Meio Ambiente. Instituto de Geociências (IGEO), Universidade Federal da Bahia (UFBA), R. Barão de Jeremoabo, s/n - Ondina, Salvador, BA 40170-290, Brazil

[2]Center for Genomics and Systems Biology, Department of Biology, New York University, New York, New York, United States of America.

[3]Laboratório de Ecologia Microbiana e Biotecnologia (LEMBiotech), Departamento de Biologia, Centro de Ciências, Universidade Federal do Ceará, Campus do Pici, Bloco 909, Avenida Mister Hull s/n, 60.455-970 Fortaleza, CE, Brazil

[4]Programa de Pós-Graduação em Modelagem em Ciências da Terra e do Ambiente (PPGM), Universidade Estadual de Feira de Santana (UEFS), Avenida Transnordestina, s/n - Novo Horizonte, Feira de Santana, BA 44036-900, Brazil

Correspondence to: David Gresham (dgresham@nyu.edu)

Abstract. Mangrove forests are ecosystems that constitute a large portion of the world's coastline and span tidal zones below, between, and above the waterline, while the ecosystem as a whole is defined by the health of these tidal microhabitats. However, we are only beginning to understand tidal zone microbial biodiversity and the role of these microbiomes in nutrient cycling. While extensive research has characterized microbiomes in pristine versus anthropogenically impacted mangroves these have, largely, overlooked differences in tidal microhabitats (sublittoral, intertidal, and supralittoral). Unfortunately, the small number of studies that have sought to characterize mangrove tidal zones have occurred in impacted biomes, making interpretation of the results difficult. Here, we characterized prokaryotic populations and their involvement in nutrient cycling across the tidal zones of a pristine mangrove within a Brazilian Environmental Protection Area of the Atlantic Forest. We hypothesized that the tidal zones in pristine mangroves are distinct microhabitats, which we defined as distinct regions that present spatial variations in the water regime and other environmental factors, and as such, these are composed of different prokaryotic communities with distinct functional profiles. Samples were collected in triplicate from zones below, between, and above the tidal waterline. Using 16S rRNA gene amplicon sequencing, we found distinct prokaryotic communities with significantly diverse nutrient cycling functions, as well as specific taxa with varying contribution to functional abundances between zones. Where previous research from anthropogenically impacted mangroves found the intertidal zone to have high prokaryotic diversity and functionally enriched

in nitrogen cycling, we find that the intertidal zone from pristine mangroves have the lowest diversity and no functional enrichment, relative to the other tidal zones. The main bacterial phyla in all samples were *Firmicutes*, *Proteobacteria* and *Chloroflexi* while the main archaeal phyla were *Crenarchaeota* and *Thaumarchaeota*. Our results differ slightly from other studies where *Proteobacteria* is the main phyla in mangrove sediments and *Firmicutes* make up for only a small percentage of the communities. Salinity and organic matter were the most relevant environmental factors influencing these communities. *Bacillaceae* was the most abundant family at each tidal zone and showed potential to drive a large proportion of the cycling of carbon, nitrogen, phosphorus and sulfur. Our findings suggest that some aspects of mangrove tidal zonation may be compromised by human activity, especially in the intertidal zone.

1 Introduction

Mangrove ecosystems exist at the interface of land and sea and constitute a large portion of tropical and subtropical coastlines, spanning 118 countries and approximately 137,000 square kilometers (Giri et al., 2011). Within these ecosystems, the recycling of elements occur via tightly coupled exchanges between plants, microorganisms, and microbes, with bacteria and archaea playing important roles in the biogeochemical cycling of carbon, nitrogen, and phosphate (Imchen et al., 2017; Lin et al., 2019; Alongi, 1988; Reis et al., 2017; Holguin et al., 2001). Mangroves span tidal zones that are characterized by periodic tidal flooding, such that environmental conditions such as salinity vary greatly across small spatiotemporal scales. We refer to the differences in microhabitats and biodiversity in mangrove sediments as zonation and we consider the mangroves to be divided in three main tidal zones, being the sublittoral zone always immerse, while the intertidal zone is exposed to daily variations of water content, and supralittoral zone is the region that presents mangrove vegetation but normally is above the sea level. With each tidal cycle the levels of nutrients, oxygen, and salinity fluctuate, resulting in frequent anaerobic conditions and a wide range of redox potentials in the sediments (Holguin et al., 2001; Andreote et al., 2012; Marcos et al., 2018; Lin et al., 2019). While there has been little research on microbial diversity within mangrove tidal zones, research in non-mangrove tidal ecosystems have found distinct microbiomes with significantly different biogeochemistries (Musat et al., 2006; Boehm et al., 2014; Azzoni et al., 2015). Understanding the interrelationship of mangrove tidal zones, their microbial populations, and their role in nutrient cycling is a key component in understanding mangrove adaptation and conservation (Coldren et al., 2019; Saintilan et al., 2020; Shiau and Chiu, 2020; Allard et al., 2020).

Numerous studies have sought to characterize mangrove microbiomes in both pristine and anthropogenically impacted areas (see Supplemental File 2). This has led to an increased understanding of the sensitivity of mangrove microbiomes to perturbations, such as; pollution (Peixoto et al., 2011; Andreote et al., 2012; Nogueira et al., 2015), environmental degradation (Marcial Gomes et al., 2008, Tong et al., 2019, Cabral et al., 2018), and rising sea levels (Ceccon et al., 2019; Yun et al., 2017; Saintilan et al., 2020). Recently, additional work has sought to understand the diversity of

microbial populations across microhabitats defined by distinct biotic and abiotic characteristics (Luis et al., 2019; Lv et al., 2016; Zhang et al., 2018; Rocha et al., 2016). While the particular effects of these perturbations vary from site to site, broadly these result in significantly altered population structures with substantially different functional profiles. This is important to consider since research in mangrove tidal zone microhabitats (Zhang et al., 2018; Jiang et al., 2013; Zhou et al., 2017) has so far been done in anthropogenically impacted sites, thus confounding the interpretation of these findings.

In Brazil, mangrove forests are primarily composed of the genera *Rhizophora*, *Avicennia*, *Laguncularia* and *Conocarpus* (Pupin and Nahas, 2014) and are part of the Atlantic Forest, one of the most biodiverse biomes on the planet. Unfortunately, the Atlantic Forest and associated ecosystems are highly threatened by anthropogenic disturbances, resulting in a severe decline from its approximate pre-colonial area (Brooks et al., 1999; Ditt et al., 2013). However, in the southern part of Bahia State, Brazil, a significant fragment of the Atlantic Forest remains preserved within the Environmental Protection Area of Pratigi (MMA, Ministério do Meio Ambiente, 2004). Recent studies on the area showed that preservation efforts have been generally effective, resulting in high environmental quality relative to most mangrove forests, both in Brazil and globally (Ditt et al., 2013; Lopes, 2011; Mascarenhas et al., 2019). This preserved area constitutes an increasingly rare site for the understanding of the ecology of unimpacted mangrove forests.

In this study we aimed to characterize the prokaryotic microbiota from sediments of three tidal zones in a pristine mangrove located in the Serinhaém estuary, within the Environmental Protection Area of Pratigi, using 16S rRNA gene amplicon sequencing. Considering that mangrove zonation is driven, primarily, by tide variation, we hypothesized that sediments of different tidal zones would differ significantly in diversity, composition and functioning of prokaryotic communities as a result of differences in the physicochemical properties affected by tides. We also hypothesized that the intertidal zone, being a highly dynamic environment, would have highest diversity as reported previously (Zhang et al., 2018; Brose and Hillebrand, 2016). Several important ecological consequences follow on from high levels of biodiversity, such as increased functional redundancy and increased resilience to environmental perturbations (Girvan et al., 2005). However, unlike the findings from impacted mangroves (Zhang et al., 2018; Jiang et al., 2013) we found the intertidal zone to be the least diverse, suggesting that it may, in fact, be more sensitive to anthropogenic disruption. Our study provides insight into the role of microbes in the functioning of mangrove forests and establishes a baseline for monitoring the health of this important ecosystem, since this information is still scarce for pristine mangrove sites.

This work was conducted before a massive oil spill occurred off the coastline of Brazil in August 2019, impacting hundreds of miles of coastline including the Serinhaém estuary, where this research was conducted. This work therefore serves as a baseline measure of the prokaryotic communities of the tidal zones of what was a pristine mangrove forest. We hope that this will spur subsequent research into the effects that anthropogenic interferences have on mangrove ecosystems.

2 Materials and Methods

2.1 Study area

The Serinhaém Estuary is located in the Low South Region of Bahia State, Brazil (Fig. 1), between the coordinates 13°35'S and 14°10'S and 39°40'W and 38°50'W. The estuary is within the Environmental Protection Area of Pratigi, an Atlantic Forest region with a total area of 85 686 ha, enclosing a 32 km long portion of the Juliana River and emptying directly into Camamu Bay (Corrêa-Gomes et al., 2005).

2.2 Sampling and DNA extraction

For clarity, here we refer to the location of a sample as a 'site' and the collection of sites within a tidal zone as a 'zone'. Samples were collected from 3 tidal zones (centered around 13°42'59.0"S, 39°01'35.9"W) in the Serinhaém estuary in July 2018 during the morning low tide period. No sites exhibited signs of anthropogenic disturbance or pollution. The 3 collection zones were chosen based on tidal influence; sublittoral, intertidal, and supralittoral regions (Fig. 1). We evaluated tidal zones based on water level at morning low-tide, additional factors were also considered to differentiate between the intertidal and supralittoral zones, such as the presence of surface soil moisture, proximity to restinga vegetation, and the direction of a local guide. From each tidal zone, 3 samples of superficial sediments (top 10 cm of the surface layer) were collected with a cylindrical sediment core sampler. Precautions were taken to avoid the disruption of rhizospheres associated with vegetation, which could otherwise lead to contamination of the soil by rhizosphere specific microbes. Sample sites were located a minimum of 15m from each other in a triangle. Plant and other organic material were subsequently removed from core samples.

Physical-chemical parameters were measured using a multiparameter monitoring system (YSI model 85, Columbus). Metal concentration analysis was previously performed by our lab (da Silva Pereira, 2016) and found no significant difference in metal concentrations relative to background within the Serinhaém estuary. For each sample an aliquot was taken for DNA extraction while the remainder was used for measuring organic matter content using 'loss-on-ignition' (Nelson and Sommers, 1996). The total genomic DNA was extracted from 0.25 g of sediment using the PowerSoil DNA Isolation Kit (Qiagen, Carlsbad, CA, USA).

2.3 Library preparation and sequencing

After DNA extraction, we amplified the V4 region of the prokaryotic 16S rRNA gene using PCR with the primer pair 515F-Y (Parada et al., 2016) and 806R-XT (Caporaso et al., 2011) using KAPA HiFi for 25 cycles (see Supplemental Methods, Supplemental File 1). Illumina sequencing adapters and dual-index barcodes were added to the amplicon target using Nextera XT according to manufacturer's directions (Illumina, San Diego, CA, USA). DNA sequencing was performed using Illumina MiSeq platform, V2 kit (300 cycles).

2.4 Data analysis

2.4.1 Sequence Trimming, Denoising and ASV Clustering

Trimmomatic (Bolger et al., 2014) was used to filter and trim demultiplexed sequences. Paired reads were combined using QIIME2 for reads with an overlap greater than 9 while paired reads with an overlap greater than 6 were combined using a custom script (see Supplemental Methods, Supplemental File 1). Reads were denoised using DADA2 (Callahan et al., 2016) in QIIME2 (Bolyen et al., 2019) and then clustered into amplicon sequence variants (ASVs). Alpha-rarefaction was calculated using QIIME2 (S4 Fig.). We performed a variety of alpha-diversity (S5 Fig., S6 Fig., S7 Fig.,) and beta-diversity (S8 Fig.) and hierarchical correlation clustering (S9 Fig.) tests using QIIME2.

2.4.2 Taxonomic assignment, community visualization and environmental tests

Taxonomic assignment was performed using QIIME2's naive Bayes scikit-learn classifier (Bokulich et al., 2018) trained using the 16S rRNA gene sequences in SILVA database (Silva SSU 132), (McDonald et al., 2012), (see Supplemental Methods, Supplemental File 1). Additional QIIME2 visualizations for 1. ASV abundance (S1 Fig., Supplemental File 3), 2. proportional representation between zones, are available as a supplementary files (Supplemental File 3), and 3. taxonomy (Supplemental File 4). Phylogenetic reconstruction was carried out in QIIME2 using the feature classifier trained on 16S rRNA gene sequences. All groups were required to be present within at least 2 samples with a minimum of 3 reads each. In one event a taxonomic family was manually relabelled from 'Uncultured eukaryote' to 'Uncultured Bathyarchaeia' (Fig. 2, S3 Fig.).

The Vegan R package (Dixon, 2003; Oksanen et al., 2015; R Core Team, 2019) was used to test correlations between community structure and environmental variables. Distances were calculated using metaMDS (distance used was Bray-Curtis) and environmental variables were fit using envfit (S2 Table). Additionally, we also tried a constrained ordination analysis using Vegan's capscale method. However, the linear model fit of the complete data set using this approach did not identify a statistically significant correlation (S10 Fig.).

2.4.3 Functional analysis using PICRUSt2

Functional analysis was performed using PICRUSt2 (Douglas et al., 2019; Barbera et al., 2019; Czech et al., 2020; Louca and Doebeli, 2018; Ye and Doak, 2009) with default settings. As we used 16S rRNA gene amplicon sequencing a crucial limitation must be considered in evaluating our results (Sun et al., 2020), which is that all KEGG ortholog (KO) abundances (and subsequent pathway analysis) are derived from the inferred abundance of genes from the closest reference taxa that matches our supplied taxa generated by QIIME2. As such, there is the potential for disagreement between the actual organism and the reference we rely on for inference. To address this, we use QIIME2's nearest sequenced taxon index (NSTI) as a heuristic cut-off in the evaluation of taxon level functional analysis (Section 3.3, S1 Table, Supplemental File 7). Although we are using taxonomic families in this study, we will continue to use the NSTI cut-off of 0.15, despite it being

derived for species level comparisons. Where relevant families with median NSTI scores above one standard deviation are labelled with an asterisk (*), families with NSTI outside of this range were not included in this analysis. A zone-specific analysis of significant differential abundances was performed using the general-linear model method of the ALDEx2 package (see Supplemental Methods, Supplemental File 1, Supplemental File 7). A heatmap of KOs with significant differential abundance between zones was then generated (Fig. 6).

2.4.4 Zone-specific taxonomic and functional enrichment

In order to identify which species were significantly different in abundance in each zone we performed taxa enrichment analysis (Fig. 4), using a custom python (Van Rossum and Drake, 2009) script (see Supplemental Methods, Supplemental File 1, Supplemental File 7). To determine which taxa were driving the differences in functional abundance, we calculated taxa specific KO enrichment (Fig. 4) using the relative functional abundances from PICRUSt2 (see Supplemental Methods, Supplemental File 1, Supplemental File 7). KOs were limited to the KEGG metabolic pathways (ko00030, ko00195, ko00680, ko00710, ko00720, ko00910, ko00920). KOs were deemed enriched in a taxa if that taxa accounted for at least 10% of the total relative functional abundance for that KO. A taxa was deemed to be enriched for an metabolic pathway (Fig. 4) if it were enriched for three KOs within that pathway. A more detailed analysis of metabolic pathway modules was performed using KEGG Mapper Reconstruction to identify instances of taxa enrichment that included complete pathway modules (Fig. 5, Supplemental File 5).

2.5 Accessibility

The entire computational workflow is available on github: https://github.com/pspealman/COSantana_2020.

The data has been deposited as PRJNA608697 in the NCBI BioProject database: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA608697.

3 Results

3.1 Structural composition of prokaryotic communities

Sequence clustering yielded a total of 2,030 ASVs, of which 94.3% were assigned to Bacteria, 5.3% were assigned to Archaea and 0.4% were not assigned to any prokaryotic kingdom (S1 Fig.). All sites combined resulted in 36 phyla, 158 orders, 79 classes, 164 families, 135 genera and 95 species. Approximately 89% of all the sequences belonged to 6 phyla: *Firmicutes* (33.7%), *Proteobacteria* (32%), *Chloroflexi* (7%), *Planctomycetes* (5.9%), *Actinobacteria* (5.7%) and *Crenarchaeota* (4.4%). *Crenarchaeota*, *Thaumarchaeota* and *Asgardaeota* were the representatives of Archaean groups with

more than 1% abundance in the samples (Fig. 2). Taxonomic profiles are broadly similar across the tidal zones at the family level (S2 Fig.). However, several families do break from this trend (Supplemental File 5), such as the two families of the order *Alteromonadales* (S2 Fig.; 2. *Marinobacteraceae* and *Pseudoalteromonadaceae*) are only abundant in the supralittoral zone. From the Class *Deltaproteobacteria* (S2 Fig; 9. *Desulfarculales*, 10. *Desulfobacterales*, 14. *Myxococcales*, 23. *Syntrophobacterales*), the majority of orders and families are either more abundant or only present at the sublittoral zone as well.

Alpha-diversity analysis showed a trend of the intertidal zone having the lowest prokaryotic diversity (Shannon's index: 3.8) while the sublittoral zone had the highest (Shannon's index: 4.8) and the supralittoral region had intermediary diversity (Shannon's index: 4.2) (S5 Fig). However, these differences between diversity indices were not statistically significant between the tidal zones, suggesting that the communities are similar in terms of biodiversity. Similarly, the beta-diversity analysis revealed no statistical differences in the taxonomic structure of prokaryotic populations between zones (S8 Fig.).

3.2 The influence of environmental variables

We also aimed to determine if population structures across mangrove tidal zones correlated with the abiotic environmental variables of salinity, water content, organic matter and temperature from each tidal zone (S2 Table). The results revealed a significant correlation between the prokaryotic populations with salinity and organic matter (Fig. 3, S3 Table). Specifically, increased organic matter was positively correlated with sublittoral population abundances, while increased salinity was positively correlated with supralittoral population abundances. Neither water content or temperature measures reflect a significant difference in community structure between zones.

3.3 Tidal zone-specific taxa enrichment and their contributions to metabolic functional potentials

To determine which specific taxa were significantly different in abundance between zones, we performed a zone-specific enrichment test for each taxon, (Fig. 4). At the taxonomic level of family, taxa that were a substantial component of the population of at least one zone (at least 10% of the population) and whose abundance in another zone was significantly different were defined as zone-specific enriched taxa. Additionally, these taxa were also evaluated for their role in metabolic processes, those with enrichment of metabolism associated KOs, (accounting for more than 10% of the total of a given KO for at least 3 KOs of a single metabolic pathway) were then labelled for that pathway. We also performed pathway analysis wherein a pathway module would be defined as enriched if the complete set of KOs for that pathway were enriched (Fig. 5).

We found 8 families with statistically different population abundances between tidal zones (*Bacillaceae*, *Flavobacteriaceae*, *Micrococcaceae*, *Clostridiaceae*, *Peptostreptococcaceae*, *Desulfobacteraceae*, *Rhodobacteraceae* and *Vibrionaceae*), 4 of these families (*Flavobacteriaceae*, *Micrococcaceae*, *Clostridiaceae* and *Vibrionaceae*) had their lowest population abundances in the intertidal zone, and only one family (*Bacillaceae*) had their highest abundance in this zone. No archaea satisfied the statistical criteria necessary for inclusion. This dominance by *Bacillaceae* and reduced abundance of

other families is consistent with our previous observations of the intertidal zone having a low diversity. Furthermore, these differences in population abundance can also affect the functional profile of these zones. Each family also contributed substantially (>= 10%) to the total potential functional abundance of at least one of the metabolic pathways at one zone (Fig. 4). Of those 8 families with the exception of *Peptostreptococcaceae*, we found each contributed significantly to carbon metabolism, which includes photosynthesis (Ph, 2 families), carbon fixation in photosynthetic (Co, 6 families) or heterotrophic organisms (Cp, 7 families), and methane metabolism (M, 7 families). All families contributed to sulfur metabolism associated KOs, and these were mostly associated with assimilatory sulfate reduction (M00176) and dissimilatory sulfate metabolism (M00596). 7 families produced substantial amounts of nitrogen metabolism associated KOs. Only 6 families contributed importantly to the general phosphorus metabolism associated KOs. Taken together, this suggests that zone-specific taxa enrichment may also contribute to differential metabolic activities at these zones.

We also analysed KO enrichment at a higher resolution by considering taxa enrichment of metabolic modules (Fig 5). We found greater taxa enriched KOs in the sublittoral zone, where 24 out of 29 (82.8%) taxa enriched pathways are found, versus 21 (72.4%) and 22 (75.9%), in the intertidal and supralittoral zones, respectively. Notably, *Bacillaceae* is the most enriched family at each zone and, as such, has the potential to drive a large proportion of functional activity, being enriched in 17 metabolic modules in 8 pathways. These include modules from the carbohydrate metabolism pathways (citrate cycle, pentose phosphate), as well as sulfur and nitrogen metabolism. Notably, some of these are enriched at only one zone, such as sulfur metabolism and the citrate cycle suggesting that these roles are occupied by other families at these tidal zones.

3.4 Zone specific differences in metabolism associated KEGG Orthologs

To determine if there were significant differences in potential metabolic activity between tidal zones, we calculated the potential functional abundance of metabolic KOs for each zone. KOs with significantly different functional abundances between zones are shown in Figure 6. Overall, the functional profiles showed that the majority of metabolic pathways for the transformations of carbon, nitrogen, phosphorus and sulfur are more abundant in the sediments of the sublittoral zone. Taken together, the results suggest a positive correlation between biodiversity and potential metabolic functional abundances.

4 Discussion

In this work, we extended the study of preserved mangrove areas to characterize the variance in prokaryotic populations within microhabitats defined by the influence of the tidal regime. Previous research has shown that mangrove forests exhibit zonation driven by different biotic and abiotic factors which influence microbial community structure. However, most of these have been conducted in anthropogenically impacted areas (Pupin and Nahas, 2014; Marcial Gomes et al., 2008; Alzubaidy et al., 2016; Rocha et al., 2016; Ceccon et al., 2019; El-Tarabily, 2002; Imchen et al., 2017; Lin et al., 2019; Zhang et al., 2018; Zhou et al., 2017), thus confounding the makeup of the microbial population structures.

Conversely, studies that sought to identify differences induced by pollution and urbanization on mangroves found broad differences in prokaryotic populations in impacted areas compared to preserved mangrove areas (Pupin and Nahas, 2014; Nogueira et al., 2015), but did not study the population differences of distinct microhabitats within mangroves.

Our work shows that the different tidal zones of the Serinhaém estuary's mangrove have similar community structures with distinct functional profiles. We observed a prevalence of *Firmicutes* in the sediments of all tidal zones, accounting for 34% of all reads. This differs from previous studies of mangroves, both around the world and in Brazil (Alzubaidy et al., 2016; Andreote et al., 2012; Mendes and Tsai, 2014; Nogueira et al., 2015; Ceccon et al., 2019; Imchen et al., 2017, Cheung et al., 2018, Zhou et al., 2017) which rarely found the relative frequency of *Firmicutes* to be above 10%. Instead, these studies found *Proteobacteria* to be the dominant phyla and, while *Proteobacteria* was also abundant in our samples (32% of all reads), it never exceeded the abundance of *Firmicutes*. Notably, *Firmicutes* were mainly represented by the family *Bacillaceae* which in our potential functional analysis was an important driver of metabolic cycles. We also compared our observations to coastal marine sediments (Supplemental File 2) and here too, we found *Proteobacteria* to be the dominant taxa at anthropogenically impacted sites. However, in one study performed at a marine sanctuary there was also the observation of high abundance *Firmicutes* (18 - 31%).

Previous work on contaminated mangroves in Brazil has suggested that *Gammaproteobacteria* is a bioindicator of an anthropogenically impacted mangrove (Andreote et al., 2012a). Comparing normalized reads across our samples for all *Firmicutes* and *Gammaproteobacteria* (S4 Table) and find that the two taxa are significantly negatively correlated (Pearson's R = -0.84, p-value = 0.004), consistent with *Firmicutes* as being a bioindicator of a pristine environment. However, *Firmicutes* were also found at elevated levels at several anthropogenically impacted sites (Torres et al., 2019; Haldar and Nazareth, 2018; Tiralerdpanich et al., 2018). While it could be that their presence is due to the pristine nature of the area, it may also be due to the local climate (Nogueira et al., 2015), an unmeasured environmental factor (Tong et al., 2019), or because of the presence of another organism such as a non-prokaryote or prokaryote which is difficult to detect using 16S rRNA gene amplicon sequencing (Eloe-Fadrosh et al., 2016; Zhang et al., 2021).

The results show that both salinity and organic matter were significantly correlated with communities in different tidal zones, confirming the hypothesis that changes in physicochemical parameters lead to changes in the prokaryotic communities inhabiting these sediments. It has been previously observed that tidal variations can cause nutrient washout or erosion (Behera et al., 2019; Zhang et al., 2020), which in turn can change the bioavailability patterns for organic matter and essential nutrients along the distinct tidal zones of a mangrove. Salinity levels are also influenced by the tidal variations and it has been previously determined that the increased salinity as well as the prevalence of humic-like substances can interfere with the accessibility of the microbial community to the sediment organic matter (Wang et al., 2010; Ceccon et al., 2019).

It is important to note that other environmental variables such as granulometry, vegetation and pollutant distributions may also impact mangrove sediment communities, as observed previously (Peixoto et al., 2011; Colares and Melo, 2013; Rocha et al., 2016). Furthermore, it is also possible that they are influenced by additional biological factors, such as fungi and other eukaryotic microbes (Simões et al., 2015), and plant rhizome contamination (Bennett and

Klironomos, 2019; Miller et al., 2019), as observed in previous work (Rocha et al., 2016; Zhang et al., 2017). Thus, our understanding of prokaryotic community structures will be greatly increased if complimented with rhizome and eukaryotic populations information.

It is also important to notice that the use of 16S rRNA gene amplicons potentially impairs the identification of some important prokaryotic groups, especially from the domain Archaea, which in some environments can reach 10% of the total microbial community (Eloe-Fadrosh et al. 2016). Recent research has emphasized how the use of 16S rRNA gene amplicon sequencing can lead to failure in detecting some archaeal populations which can play important roles in nutrient cycling (Zhang et al., 2021). Taken together, however, this work suggests that a variety of environmental factors, potentially driven by tidal cycles, can generate niche variations that influence the structure and function of communities.

Although the differences in diversity indices were not statistically significant, the data shows a clear trend of differentiation between zones that suggests that further investigation, with a larger number of samples, would be sufficient to resolve differences with statistical significance. The highest biodiversity was found in the sublittoral mangrove sediments, while the intertidal zone had the lowest biodiversity. The findings of taxonomic groups that were more abundant and or exclusive to specific zones is also suggestive of the importance of the physical-chemical parameters on shaping the microbial communities. The prevalence of the groups of *Deltaproteobacteria* in the sublittoral zone, for example, could be associated with the prevalent anaerobic conditions of such sediments, which could result in selection of specific prokaryotic groups such as sulphate-reducing bacteria (Andreote et al. 2012), as seen with the increased abundance of *Desulfobacteraceae*, *Desulfobulbaceae*, and *Syntrophaceae*.

Our data suggests that the intertidal sediments of mangrove forests have lower prokaryotic diversity than those in the constant environments, such as the supralittoral and sublittoral regions. Models of biodiversity in dynamic environmental regions, such as intertidal zones, have suggested that oscillating environments may support higher biodiversity than that observed in static environments (Brose and Hillebrand, 2016). Furthermore, studies in anthropogenically impacted mangroves have often found substantial diversity in the intertidal zones as well (Lin et al., 2019; Zhang et al., 2018). An alternative model is that the constant shifts in environmental parameters can create harsh environmental conditions that select for resistant taxa thus reducing the relative biodiversity (Statzner and Moss, 2004), the latter of which is consistent with our results. Considering other surrounding environments such as the shallow marine sediments (Supplemental File 2), we may expect the diversity to decrease in comparison to the mangrove sediments, potentially with an increase in *Proteobacteria*, a trend that has been reported in previous studies which found similar groups prevailing in mangrove sediments (Wang et al., 2012; Behera et al. 2019).

Community functional profiling was identified using 16S rRNA gene sequences and identified higher metabolic potentials for the cycles of C, N, P and S in the sublittoral region. The higher abundances of KOs found in the sublittoral zone is possibly due to the greater taxonomic diversity that was also observed for this region. Notably, this result is in agreement with the understanding that many microbiome functions in ecosystems are redundant (Tolkkinen et al., 2020; Barnes et al., 2020). It is important to note that these functional profiles are not the result of Whole Metagenome Sequencing

(WMS), as such it is not necessarily true that all members of the taxa have the same genome as the reference holotype, nor is it necessarily true that these genes would be expressed equally, even if present and this should best be understood as a measure of potential metabolic activity. Studies that have performed functional profiling using metagenome sequencing on mangrove sediments resulted in comparatively greater resolution (Zhang et al., 2021), although the use of 16S rRNA gene sequencing has been proved to provide comparable results for the recovery of general patterns of pathway abundances (Jovel et al., 2016).

Generally, diverse communities with organisms possessing redundant metabolic functions may be more stable against environmental perturbations, as the organisms will respond differently to stressors, increasing the likelihood of survival of some taxa (Girvan et al., 2005). Thus, the identification of the intertidal zone as having the lowest diversity suggests it may be in a more precarious position. This concern is amplified as the water itself is frequently the carrier of contamination; from rivers, as is the case for urban waste (Yunus et al., 2011) and from the oceans through the tides, as the case with oil spill contamination (Cabral et al., 2016). Thus, it is important to consider that different parts of the mangrove tidal zone could be exposed to different levels of contamination and that this could, in turn, affect the organisms in a zone-specific manner.

The identification of enriched taxa with similar functional potentials for the diverse nutrient metabolisms is in agreement with previous observations of the microbial diversity in mangrove sediments (Rocha et al., 2016; Ceccon et al., 2019; Cabral et al., 2016; Mendes and Tsai, 2014; Zhao and Bajic, 2015). Conceptually, one could interpret that this higher order redundant biodiversity could grant the microbiome a higher level of resilience when challenged by environmental perturbation, as organisms with similar metabolic pathways have the potential to maintain the ecological functions even if some taxa were lost.

In conclusion, the results suggest that communities with distinct diversity and functional profiles occupy the sediments of the mangrove tidal zones. This variance is significantly associated with the abiotic environmental variables of salinity and organic matter. Our functional potential analysis suggests there could be significant variation of metabolic potentials between tidal zones. We observed that the intertidal region, the most biophysically dynamic zone in the mangrove forest, presents the least biodiversity. One possible explanation of this is that the dynamic, cyclic, tidal environment is itself harsher than that found in the other tidal zones. This study of pristine mangroves tidal zones suggests that some microhabitats may be more sensitive to anthropogenic impact than others. Preservation, conservation, and remediation measures of mangroves should be aware of the fragility of the intertidal zone and the increased threat this zone faces from soluble pollutants, such as urban sewage, fertilizer and pesticide run-off, as well as buoyant pollution such as plastics and oil spills. However, it remains an open question if other microhabitats, such as the sublittoral zone, have greater resilience to environmental perturbations given their greater abundance of taxonomic diversity and metabolic function.

Conceptually, this property of a dynamic environment defining a selective niche, similar to a physical barrier, is worthy of further study. Further exploration of whether different groups of organisms found to have metabolic potentials are actively participating in the element cycles in these mangrove sediments will also benefit the field.

5 Funding

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 (https://www.capes.gov.br/) and by the Fundação de Amparo à Pesquisa do Estado da Bahia – Fapesb (http://www.fapesb.ba.gov.br/) [grant number PNE0021/2014]. Additional funding provided by National Science Foundation (NSF) [MCB1818234].

6 Acknowledgments

The authors would like to thank the sequencing facility of the Microbial Ecology and Biotechnology Laboratory (LEMBiotech).

7 Conflict of Interest

The authors declare that they have no conflict of interest.

8 References

Allard, S. M., Costa, M. T., Bulseco, A. N., Helfer, V., Wilkins, L. G. E., Hassenrück, C., Zengler, K., Zimmer, M., Erazo, N., Mazza Rodrigues, J. L., Duke, N., Melo, V. M. M., Vanwonterghem, I., Junca, H., Makonde, H. M., Jiménez, D. J., Tavares, T. C. L., Fusi, M., Daffonchio, D., Duarte, C. M., Peixoto, R. S., Rosado, A. S., Gilbert, J. A. and Bowman, J.: Introducing the Mangrove Microbiome Initiative: Identifying Microbial Research Priorities and Approaches To Better Understand, Protect, and Rehabilitate Mangrove Ecosystems, mSystems, 5(5), doi:10.1128/msystems.00658-20, 2020.

Alongi, D. M.: Bacterial productivity and microbial biomass in tropical mangrove sediments, Microb. Ecol., 15(1), 59–79, doi:10.1007/BF02012952, 1988.

Alzubaidy, H., Essack, M., Malas, T. B., Bokhari, A., Motwalli, O., Kamanu, F. K., Jamhor, S. A., Mokhtar, N. A., Antunes, A., Simões, M. F., Alam, I., Bougouffa, S., Lafi, F. F., Bajic, V. B. and Archer, J. A. C.: Rhizosphere microbiome metagenomics of gray mangroves (Avicennia marina) in the Red Sea, Gene, 576(2 Pt 1), 626–636, doi:10.1016/j.gene.2015.10.032, 2016.

Andreote, F. D., Jiménez, D. J., Chaves, D., Dias, A. C. F., Luvizotto, D. M., Dini-Andreote, F., Fasanella, C. C., Lopez, M. V., Baena, S., Taketani, R. G. and de Melo, I. S.: The microbiome of Brazilian mangrove sediments as revealed by metagenomics, PLoS One, 7(6), e38600, doi:10.1371/journal.pone.0038600, 2012.

Azzoni, R., Nizzoli, D., Bartoli, M., Christian, R. R. and Viaroli, P.: Factors Controlling Benthic Biogeochemistry in Urbanized Coastal Systems: an Example from Venice (Italy), Estuaries Coasts, 38(3), 1016–1031, doi:10.1007/s12237-014-9882-6, 2015.

Barbera, P., Kozlov, A. M., Czech, L., Morel, B., Darriba, D., Flouri, T. and Stamatakis, A.: EPA-ng: Massively Parallel Evolutionary Placement of Genetic Sequences, Syst. Biol., 68(2), 365–369, doi:10.1093/sysbio/syy054, 2019.

Barnes, E. M., Carter, E. L. and Lewis, J. D.: Predicting Microbiome Function Across Space Is Confounded by Strain-Level Differences and Functional Redundancy Across Taxa, Front. Microbiol., 11, 101, doi:10.3389/fmicb.2020.00101, 2020.

Behera, P., Mohapatra, M., Kim, J. Y., Adhya, T. K., Pattnaik, A. K. and Rastogi, G.: Spatial and temporal heterogeneity in the structure and function of sediment bacterial communities of a tropical mangrove forest, Environmental Science and Pollution Research, 26(4), 3893–3908, doi:10.1007/s11356-018-3927-5, 2019.

Bennett, J. A. and Klironomos, J.: Mechanisms of plant-soil feedback: interactions among biotic and abiotic drivers, New Phytol., 222(1), 91–96, doi:10.1111/nph.15603, 2019.

Boehm, A. B., Yamahara, K. M. and Sassoubre, L. M.: Diversity and transport of microorganisms in intertidal sands of the California coast, Appl. Environ. Microbiol., 80(13), 3943–3951, doi:10.1128/AEM.00513-14, 2014.

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A. and Gregory Caporaso, J.: Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin, Microbiome, 6(1), 90, doi:10.1186/s40168-018-0470-z, 2018.

Bolger, A. M., Lohse, M. and Usadel, B.: Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics, 30(15), 2114–2120, doi:10.1093/bioinformatics/btu170, 2014.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., Cope, E. K., Da Silva, R., Diener, C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang, L., Kaehler, B. D., Kang, K. B., Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M. G. I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B. D., McDonald, D., McIver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C., Morton, J. T., Naimey, A. T., Navas-Molina, J. A., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples, S. L., Petras, D., Preuss, M. L., Pruesse, E., Rasmussen, L. B., Rivers, A., Robeson, M. S., 2nd, Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear, J. R., Swafford, A. D., Thompson, L. R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., van der Hooft, J. J. J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., et al.: Reproducible, interactive, scalable and

extensible microbiome data science using QIIME 2, Nat. Biotechnol., 37(8), 852–857, doi:10.1038/s41587-019-0209-9, 2019.

Brooks, T., Tobias, J. and Balmford, A.: Deforestation and bird extinctions in the Atlantic forest, Anim. Conserv., 2(3), 211–222, doi:10.1111/j.1469-1795.1999.tb00067.x, 1999.

Brose, U. and Hillebrand, H.: Biodiversity and ecosystem functioning in dynamic landscapes, Philos. Trans. R. Soc. Lond. B Biol. Sci., 371(1694), doi:10.1098/rstb.2015.0267, 2016.

Cabral, L., Júnior, G. V. L., Pereira de Sousa, S. T., Dias, A. C. F., Lira Cadete, L., Andreote, F. D., Hess, M. and de Oliveira, V. M.: Anthropogenic impact on mangrove sediments triggers differential responses in the heavy metals and antibiotic resistomes of microbial communities, Environ. Pollut., 216, 460–469, doi:10.1016/j.envpol.2016.05.078, 2016.

Cabral, L., Pereira de Sousa, S. T., Júnior, G. V. L., Hawley, E., Andreote, F. D., Hess, M. and de Oliveira, V. M.: Microbial functional responses to long-term anthropogenic impact in mangrove soils, Ecotoxicol. Environ. Saf., 160, 231–239, doi:10.1016/j.ecoenv.2018.04.050, 2018.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A. and Holmes, S. P.: DADA2: High-resolution sample inference from Illumina amplicon data, Nat. Methods, 13(7), 581–583, doi:10.1038/nmeth.3869, 2016.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N. and Knight, R.: Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample, Proc. Natl. Acad. Sci. U. S. A., 108 Suppl 1, 4516–4522, doi:10.1073/pnas.1000080107, 2011.

Ceccon, D. M., Faoro, H., Lana, P. da C., Souza, E. M. de and Pedrosa, F. de O.: Metataxonomic and metagenomic analysis of mangrove microbiomes reveals community patterns driven by salinity and pH gradients in Paranaguá Bay, Brazil, Sci. Total Environ., 694, 133609, doi:10.1016/j.scitotenv.2019.133609, 2019.

Colares, G. B. and Melo, V. M. M.: Relating microbial community structure and environmental variables in mangrove sediments inside Rhizophora mangle L. habitats, Appl. Soil Ecol., 64, 171–177, doi:10.1016/j.apsoil.2012.12.004, 2013.

Coldren, G. A., Langley, J. A., Feller, I. C. and Chapman, S. K.: Warming accelerates mangrove expansion and surface elevation gain in a subtropical wetland, edited by B. Silliman, J. Ecol., 107(1), 79–90, doi:10.1111/1365-2745.13049, 2019.

Corrêa-Gomes, L. C., Dominguez, J. M. L., Barbosa, J. S. F. and da Silva, I. C.: PADRÕES DE ORIENTAÇÃO DOS CAMPOS DE TENSÃO, ESTRUTURAS, HERANÇA DO EMBASAMENTO E EVOLUÇÃO TECTÔNICA DAS BACIAS DE CAMAMÚ E PORÇÃO SUL DO RECÔNCAVO, COSTA DO DENDÊ, BAHIA, Revista Brasileira de Geociências, 35(4), 117–128, doi:10.25249/0375-7536.200535s4117128, 2005.

Czech, L., Barbera, P. and Stamatakis, A.: Genesis and Gappa: processing, analyzing and visualizing phylogenetic (placement) data, Bioinformatics, doi:10.1093/bioinformatics/btaa070, 2020.

Ditt, E., Zysman, N., Cunha, R. S. da and Rocha, R. B. da: Conservação Da Biodiversidade Por Meio Da Atividade Extrativista Em Comunidades Quilombolas, Revista Brasileira de Ciências Ambientais, 27, 2013.

Dixon, P.: VEGAN, a package of R functions for community ecology, Journal of Vegetation Science, 14(6), 927, doi:10.1658/1100-9233(2003)014[0927:vaporf]2.0.co;2, 2003.

Douglas, G. M., Maffei, V. J., Zaneveld, J., Yurgel, S. N., Brown, J. R., Taylor, C. M., Huttenhower, C. and Langille, M. G. I.: PICRUSt2: An improved and extensible approach for metagenome inference, Bioinformatics, 497, 2019.

El-Tarabily, K. A.: Total microbial activity and microbial composition of a mangrove sediment are reduced by oil pollution at a site in the Arabian Gulf, Can. J. Microbiol., 48(2), 176–182, doi:10.1139/w01-140, 2002.

Giri, C., Ochieng, E., Tieszen, L. L., Zhu, Z., Singh, A., Loveland, T., Masek, J. and Duke, N.: Status and distribution of mangrove forests of the world using earth observation satellite data: Status and distributions of global mangroves, Glob. Ecol. Biogeogr., 20(1), 154–159, doi:10.1111/j.1466-8238.2010.00584.x, 2011.

Girvan, M. S., Campbell, C. D., Killham, K., Prosser, J. I. and Glover, L. A.: Bacterial diversity promotes community stability and functional resilience after perturbation, Environmental Microbiology, 7(3), 301–313, doi:10.1111/j.1462-2920.2005.00695.x, 2005.

Holguin, G., Vazquez, P. and Bashan, Y.: The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview, Biol. Fertil. Soils, 33(4), 265–278, doi:10.1007/s003740000319, 2001.

Imchen, M., Kumavath, R., Barh, D., Azevedo, V., Ghosh, P., Viana, M. and Wattam, A. R.: Searching for signatures across microbial communities: Metagenomic analysis of soil samples from mangrove and other ecosystems, Sci. Rep., 7(1), 8859, doi:10.1038/s41598-017-09254-6, 2017.

Jiang, X.-T., Peng, X., Deng, G.-H., Sheng, H.-F., Wang, Y., Zhou, H.-W. and Tam, N. F.-Y.: Illumina sequencing of 16S rRNA tag revealed spatial variations of bacterial communities in a mangrove wetland, Microb. Ecol., 66(1), 96–104, doi:10.1007/s00248-013-0238-8, 2013.

Lin, X., Hetharua, B., Lin, L., Xu, H., Zheng, T., He, Z. and Tian, Y.: Mangrove Sediment Microbiome: Adaptive Microbial Assemblages and Their Routed Biogeochemical Processes in Yunxiao Mangrove National Nature Reserve, China, Microb. Ecol., 78(1), 57–69, doi:10.1007/s00248-018-1261-6, 2019.

Lopes, N. S.: Análise da paisagem com base na fragmentação - caso APA do Pratigi, baixo sul da Bahia, Brasil, Revista Eletrônica do Prodema, 6(1), 53–67, 2011.

Louca, S. and Doebeli, M.: Efficient comparative phylogenetics on large trees, Bioinformatics, 34(6), 1053–1055, doi:10.1093/bioinformatics/btx701, 2018.

Luis, P., Saint-Genis, G., Vallon, L., Bourgeois, C., Bruto, M., Marchand, C., Record, E. and Hugoni, M.: Contrasted ecological niches shape fungal and prokaryotic community structure in mangroves sediments, Environ. Microbiol., 21(4), 1407–1424, doi:10.1111/1462-2920.14571, 2019.

Lv, X., Ma, B., Yu, J., Chang, S. X., Xu, J., Li, Y., Wang, G., Han, G., Bo, G. and Chu, X.: Bacterial community structure and function shift along a successional series of tidal flats in the Yellow River Delta, Scientific Reports, 6(1), doi:10.1038/srep36550, 2016.

Marcial Gomes, N. C., Borges, L. R., Paranhos, R., Pinto, F. N., Mendonça-Hagler, L. C. S. and Smalla, K.: Exploring the diversity of bacterial communities in sediments of urban mangrove forests, FEMS Microbiol. Ecol., 66(1), 96–109, doi:10.1111/j.1574-6941.2008.00519.x, 2008.

Marcos, M. S., Barboza, A. D., Keijzer, R. M. and Laanbroek, H. J.: Tide as Steering Factor in Structuring Archaeal and Bacterial Ammonia-Oxidizing Communities in Mangrove Forest Soils Dominated by Avicennia germinans and Rhizophora mangle, Microb. Ecol., 75(4), 997–1008, doi:10.1007/s00248-017-1091-y, 2018.

Mascarenhas, R. B., Aragão, I. R., Reis, P. and de Jesus Bomfim, T.: ANÁLISE DE METAIS-TRAÇOS EM SEDIMENTOS DA APA DO PRATIGI, BAHIA, Sitientibus , 0(53), doi:10.13102/sitientibus.v0i53.4467, 2019.

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Andersen, G. L., Knight, R. and Hugenholtz, P.: An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea, ISME J., 6(3), 610–618, doi:10.1038/ismej.2011.139, 2012.

Mendes, L. W. and Tsai, S. M.: Variations of Bacterial Community Structure and Composition in Mangrove Sediment at Different Depths in Southeastern Brazil, Diversity , 6(4), 827–843, doi:10.3390/d6040827, 2014.

Miller, E. C., Perron, G. G. and Collins, C. D.: Plant-driven changes in soil microbial communities influence seed germination through negative feedbacks, Ecol. Evol., 9(16), 9298–9311, doi:10.1002/ece3.5476, 2019.

MMA, Ministério do Meio Ambiente: Plano de Manejo da APA do Pratigi - Encarte II Zoneamento e Plano de Gestao, http://www.inema.ba.gov.br/wp-content/uploads/2011/09/PM_APA_Pratigi_Encarte-II.pdf, 2004.

Musat, N., Werner, U., Knittel, K., Kolb, S., Dodenhof, T., van Beusekom, J. E. E., de Beer, D., Dubilier, N. and Amann, R.: Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Rømø Basin, Wadden Sea, Syst. Appl. Microbiol., 29(4), 333–348, doi:10.1016/j.syapm.2005.12.006, 2006.

Nelson, D. W. and Sommers, L. E.: Total Carbon, Organic Carbon, and Organic Matter: Part 3 Chemical Methods, in Methods of Soil Analysis, edited by D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, and M. E. Sumner, pp. 961–1010, Soil Science Society of America, American Society of Agronomy, Madison, WI, USA., 1996.

Nogueira, V. L. R., Rocha, L. L., Colares, G. B., Angelim, A. L., Normando, L. R. O., Cantão, M. E., Agnez-Lima, L. F., Andreote, F. D. and Melo, V. M. M.: Microbiomes and potential metabolic pathways of pristine and anthropized Brazilian mangroves, Regional Studies in Marine Science, 2, 56–64, doi:10.1016/j.rsma.2015.08.008, 2015.

Parada, A. E., Needham, D. M. and Fuhrman, J. A.: Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples, Environ. Microbiol., 18(5), 1403–1414, doi:10.1111/1462-2920.13023, 2016.

Peixoto, R., Chaer, G. M., Carmo, F. L., Araújo, F. V., Paes, J. E., Volpon, A., Santiago, G. A. and Rosado, A. S.: Bacterial communities reflect the spatial variation in pollutant levels in Brazilian mangrove sediment, Antonie Van Leeuwenhoek, 99(2), 341–354, doi:10.1007/s10482-010-9499-0, 2011.

Pupin, B. and Nahas, E.: Microbial populations and activities of mangrove, restinga and Atlantic forest soils from Cardoso Island, Brazil, Journal of Applied Microbiology, 116(4), 851–864, doi:10.1111/jam.12413, 2014.

Reis, C. R. G., Nardoto, G. B. and Oliveira, R. S.: Global overview on nitrogen dynamics in mangroves and consequences of increasing nitrogen availability for these systems, Plant Soil, 410(1), 1–19, doi:10.1007/s11104-016-3123-7, 2017.

Rocha, L. L., Colares, G. B., Nogueira, V. L. R., Paes, F. A. and Melo, V. M. M.: Distinct Habitats Select Particular Bacterial Communities in Mangrove Sediments, Int. J. Microbiol., 2016, 3435809, doi:10.1155/2016/3435809, 2016.

Saintilan, N., Khan, N. S., Ashe, E., Kelleway, J. J., Rogers, K., Woodroffe, C. D. and Horton, B. P.: Thresholds of mangrove survival under rapid sea level rise, Science, 368(6495), 1118–1121, doi:10.1126/science.aba2656, 2020.

Shiau, Y.-J. and Chiu, C.-Y.: Biogeochemical Processes of C and N in the Soil of Mangrove Forest Ecosystems, For. Trees Livelihoods, 11(5), 492, doi:10.3390/f11050492, 2020.

da Silva Pereira, M.: Espacialização geoquímica dos sedimentos do estuário do Rio Serinhaém, Baixo Sul da Bahia – Brasil, PROGRAMA DE PÓS-GRADUAÇÃO EM MODELAGEM EM CIÊNCIAS DA TERRA E DO AMBIENTE – PPGM, Universidade Estadual de Feira de Santana., 2016.

Simões, M. F., Antunes, A., Ottoni, C. A., Amini, M. S., Alam, I., Alzubaidy, H., Mokhtar, N.-A., Archer, J. A. C. and Bajic, V. B.: Soil and rhizosphere associated fungi in gray mangroves (Avicennia marina) from the Red Sea—a metagenomic approach, Genomics Proteomics Bioinformatics, 13(5), 310–320 https://www.sciencedirect.com/science/article/pii/S1672022915001382, 2015.

Statzner, B. and Moss, B.: Linking ecological function, biodiversity and habitat: a mini-review focusing on older ecological literature, Basic Appl. Ecol., 5(2), 97–106, doi:10.1078/1439-1791-00221, 2004.

Tolkkinen, M. J., Heino, J., Ahonen, S. H. K., Lehosmaa, K. and Mykrä, H.: Streams and riparian forests depend on each other: A review with a special focus on microbes, Forest Ecology and Management, 462, 117962, doi:10.1016/j.foreco.2020.117962, 2020.

Tong, T., Li, R., Wu, S. and Xie, S.: The distribution of sediment bacterial community in mangroves across China was governed by geographic location and eutrophication, Mar. Pollut. Bull., 140, 198–203, doi:10.1016/j.marpolbul.2019.01.046, 2019.

Wang, Z.-Y., Yuan-Zheng, X. I. N., Dong-Mei, G. A. O., Feng-Min, L. I., Morgan, J. and Xing, B.-S.: Microbial Community Characteristics in a Degraded Wetland of the Yellow River Delta, Pedosphere, 20(4), 466–478, doi:10.1016/s1002-0160(10)60036-7, 2010.

Ye, Y. and Doak, T. G.: A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes, PLoS Comput. Biol., 5(8), e1000465, doi:10.1371/journal.pcbi.1000465, 2009.

Yun, J., Deng, Y. and Zhang, H.: Anthropogenic protection alters the microbiome in intertidal mangrove wetlands in Hainan Island, Appl. Microbiol. Biotechnol., 101(15), 6241–6252, doi:10.1007/s00253-017-8342-1, 2017.

Yunus, K., Yusuf, N. M., Shazili, N. A. M., Chuan, O. M., Saad, S., Chowdhury, A. J. K. and Bidai, J.: Heavy metal concentration in the surface sediment of Tanjung Lumpur mangrove forest, Kuantan, Malaysia, Sains Malays., 40(2), 89–92 http://journalarticle.ukm.my/2457/1/02_Kamaruzzaman.pdf, 2011.

Zhang, H., Liu, F., Zheng, S., Chen, L., Zhang, X. and Gong, J.: The differentiation of iron-reducing bacterial community and iron-reduction activity between riverine and marine sediments in the Yellow River estuary, Marine Life Science & Technology, 2(1), 87–96, doi:10.1007/s42995-019-00001-6, 2020.

Zhang, X., Hu, B. X., Ren, H. and Zhang, J.: Composition and functional diversity of microbial community across a mangrove-inhabited mudflat as revealed by 16S rDNA gene sequences, Sci. Total Environ., 633, 518–528, doi:10.1016/j.scitotenv.2018.03.158, 2018.

Zhang, Y., Yang, Q., Ling, J., Van Nostrand, J. D., Shi, Z., Zhou, J. and Dong, J.: Diversity and Structure of Diazotrophic Communities in Mangrove Rhizosphere, Revealed by High-Throughput Sequencing, Front. Microbiol., 8, 2032, doi:10.3389/fmicb.2017.02032, 2017.

Zhao, F. and Bajic, V. B.: The value and significance of metagenomics of marine environments, Genomics Proteomics Bioinformatics, 13(5), 271 https://www.ncbi.nlm.nih.gov/pmc/articles/pmc4678774/, 2015.

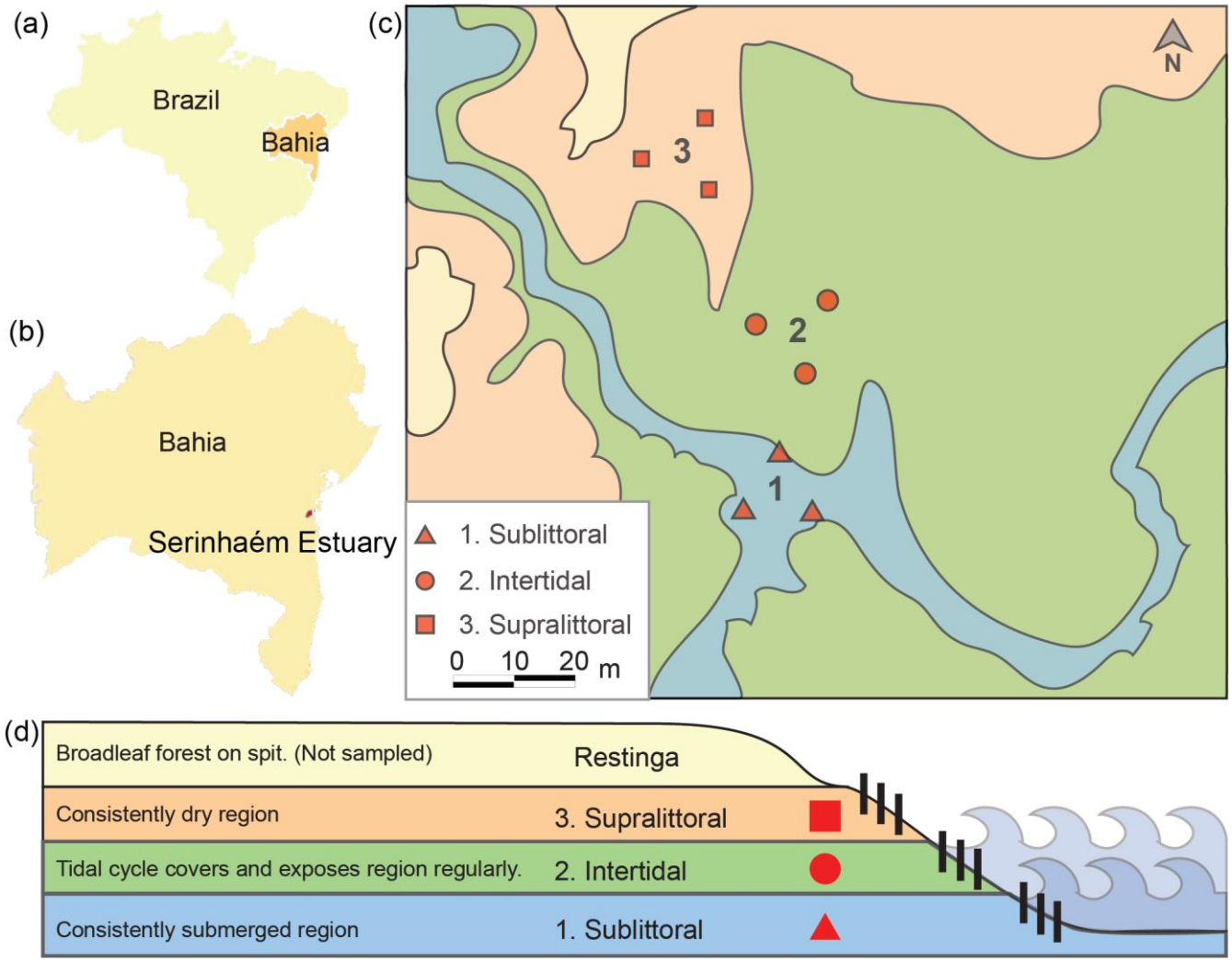

Fig 1. Map and schematic of sediment sampling sites. Here we show the locations of the sampling sites relative to Brazil (a) and Bahia (b). A picture shows the relation of the three sampling sites within each zone (1. sublittoral, 2. intertidal, 3. supralittoral, (c)). A schematic shows the topographic and tidal relation of each sampling site (d).

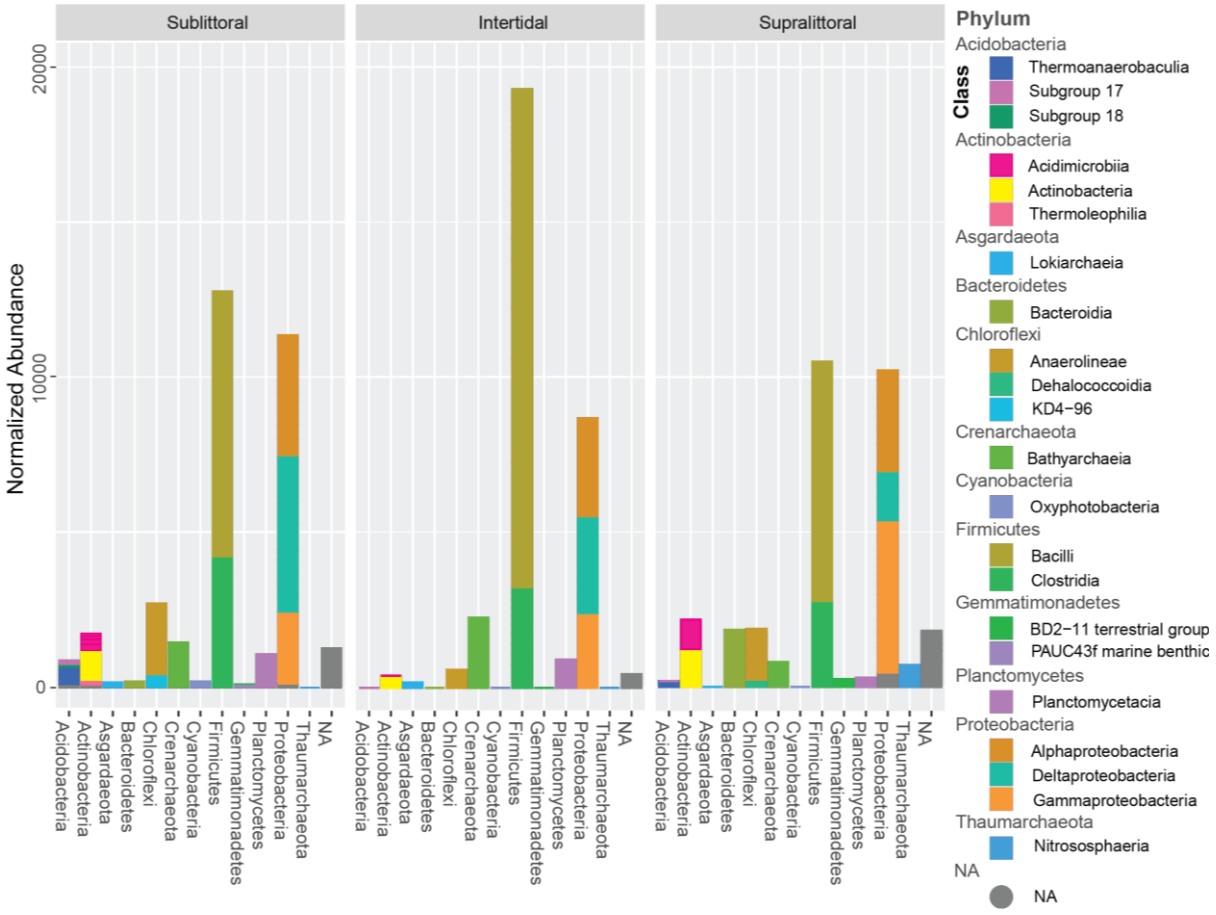

Fig 2. Normalized taxonomic abundances from each tidal zone. Taxa identified with abundance higher than 1% are shown as stacked bar plots, the horizontal axis is the Phylum level while the stacked bars are the Class level. Taxa abundance has been normalized by downsampling sequence depth.

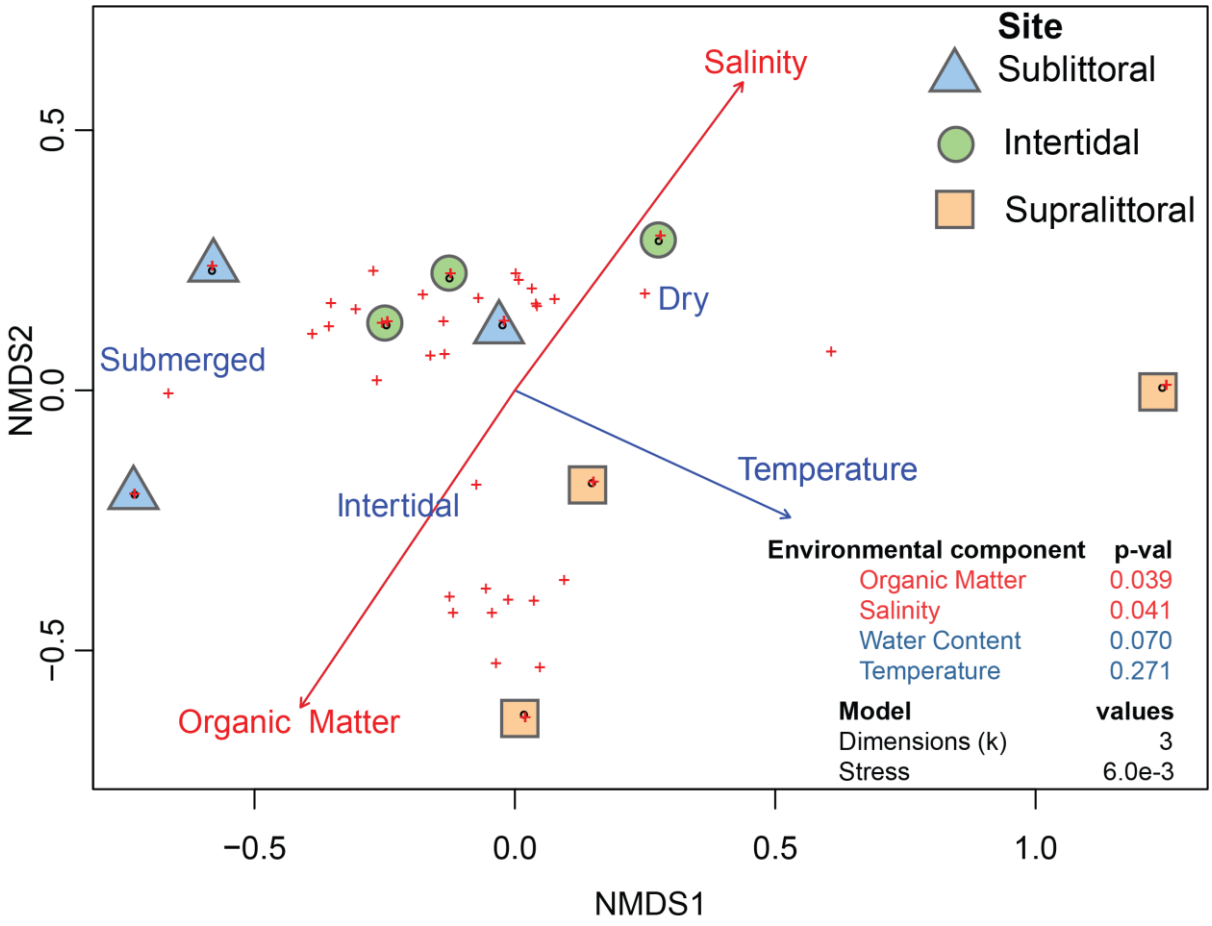

Fig. 3. Correlation between environmental variables and prokaryotic communities. Dots represent taxonomic abundances per site as plotted by Vegan metaMDS using nonmetric multidimensional scaling (NMDS). Arrow length is a representative of the predictor strength of environmental variable vectors, with red arrows having statistical significance as calculated by envfit (p-value <= 0.05).

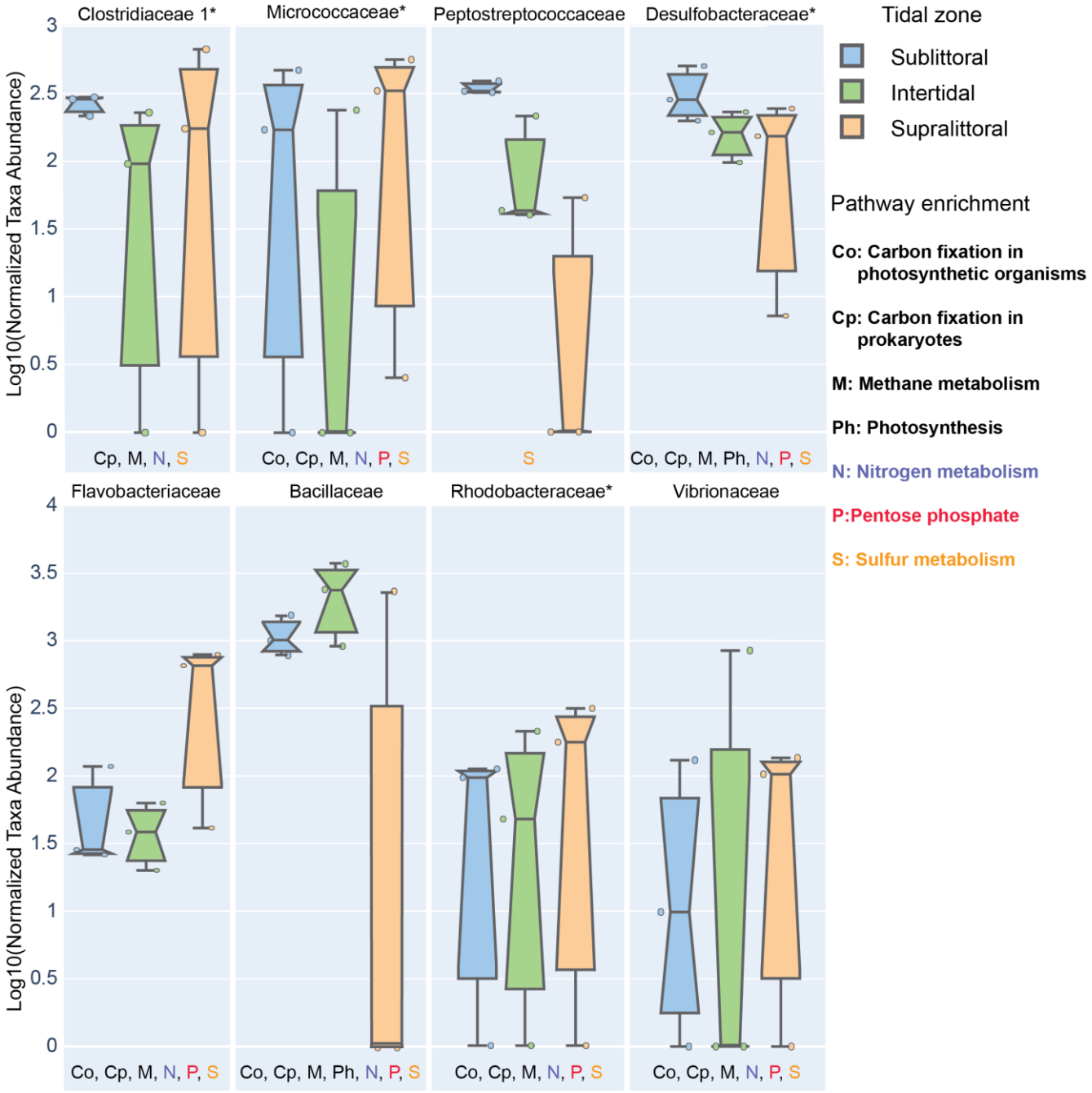

Fig. 4. Zone specific measures of taxa find significant enrichment in sediments of different tidal zones. Here we show prokaryotic families that have significantly different abundances between zones, and whose mean effect size exceeded 10% in at least one zone. To have been labelled with a metabolic pathway, taxa were required to have at least 10% of three KOs in that pathway in any zone. Families with a median NSTI within 1 standard deviation of 0.15 are labelled with *.

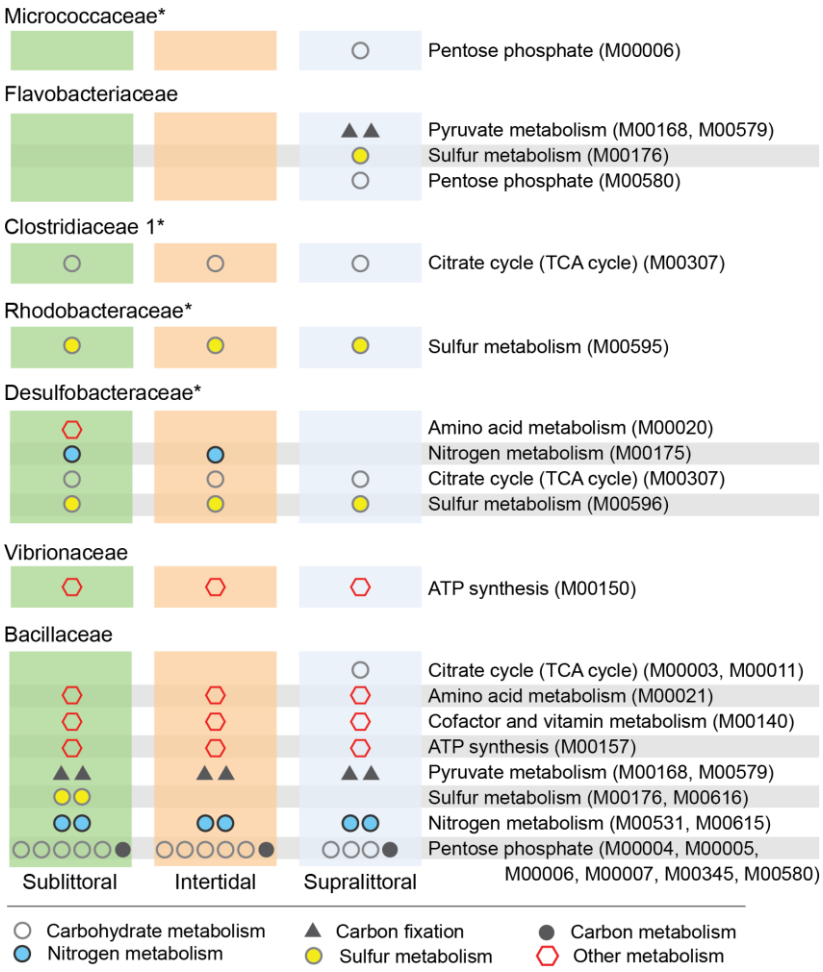

Fig. 5. Zone specific enrichment of pathway modules. Here, we show the impact that the differential abundances of major families (Figure 4) have on potential functional profiles at each zone at the level of pathway modules. To be included here each module must be completely enriched within a single taxon in a single zone, such that the taxa accounts for at least 10% of the total potential functional abundance for each KO involved in the pathway. Families with a median NSTI within 1 standard deviation of 0.15 are labelled with *.

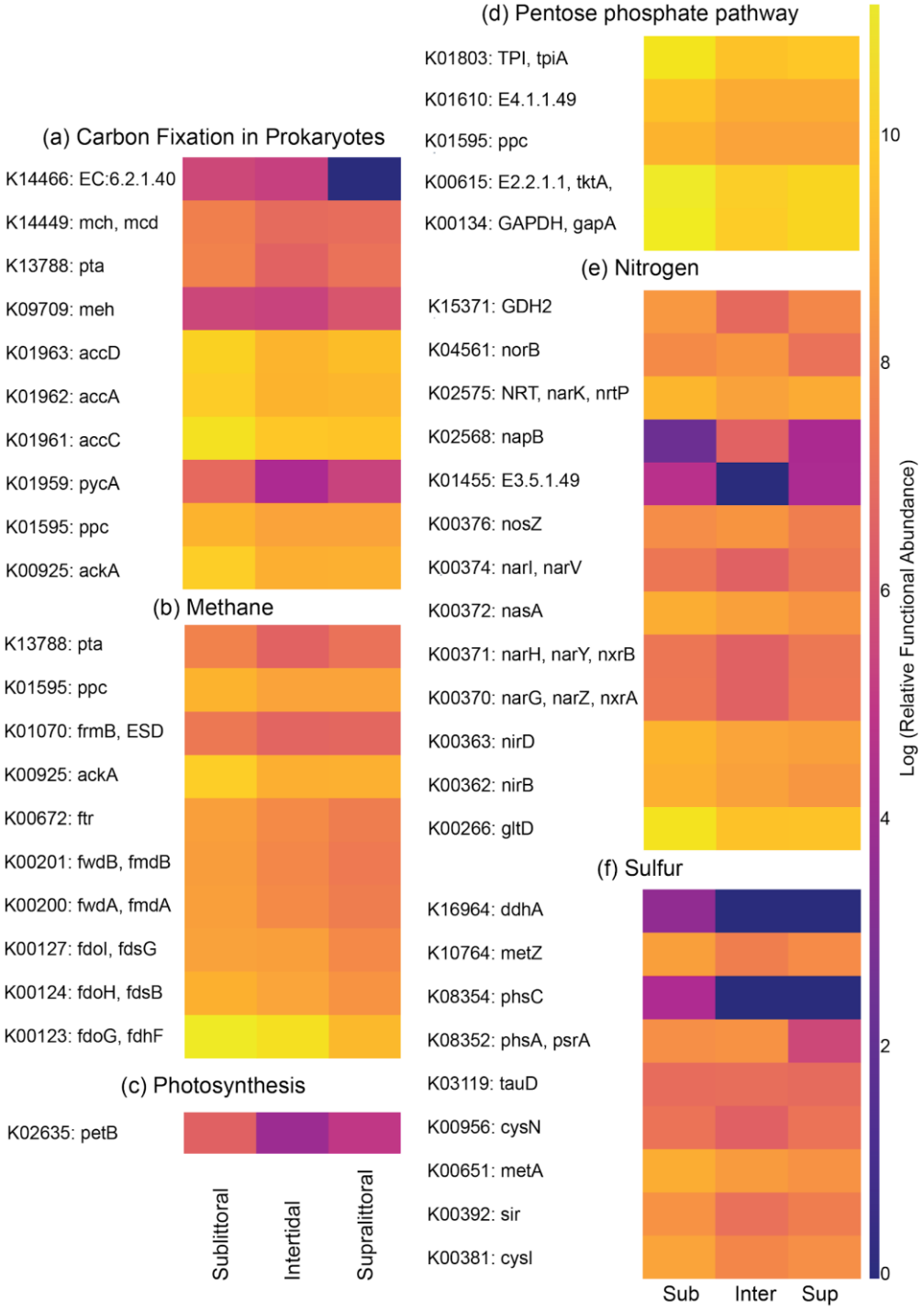

Fig. 6. Zone specific measures of metabolism associated KEGG Orthologs. The heatmaps show the metabolism associated KOs that have significant differences of functional abundance between zones for carbon, phosphorus, sulfur and nitrogen pathways. Notably, the majority of KOs (45/54, 83%), have their highest relative functional abundance in the sublittoral zone, with the intertidal (5/54, 9.3%) and supralittoral (4/54 7.4%) as near equal minorities.