Tidal influences on the structure and function of prokaryotic communities in the sediments of a pristine Brazilian mangrove

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Abstract. Mangrove forests are tidal ecosystems that constitute a large portion of the world's coastline and span tidal zones below, between, and above the tidal waterline. 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. Whereas previous research has characterized microbiomes in pristine versus anthropogenically impacted mangroves, these studies have not addressed differences in tidal microhabitats. The small number of studies that have sought to characterize mangrove tidal zones have all occurred in impacted biomes. Here, we characterize prokaryotic populations and their involvement in nutrient cycling across the tidal zones of a pristine mangrove forest within a Brazilian Environmental Protection Area of the Atlantic Forest. We hypothesize that the tidal zones in pristine mangroves are distinct microhabitats 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 amplicon sequencing, we characterize the prokaryotic communities that inhabit the different tidal zones and the potential differences in their nutrient cycling related functions. We also find specific taxa with varying contribution to functional abundances inhabit different tidal zones. Whereas previous research from anthropogenically impacted mangroves found the intertidal zone to have high prokaryotic diversity and to be functionally

enriched in nitrogen cycling we find that the intertidal zone from pristine mangroves have the lowest prokaryotic diversity and no functional enrichment, relative to the other tidal zones. Our findings suggest that some key aspects of mangrove tidal zone prokaryotic communities may be altered by human activity, especially 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 impact this has on the microhabitat and biodiversity as zonation. 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 Table 1). This has led to an increased understanding in 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., 2019a; 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 sensitivity to perturbation is important to consider, as research in mangrove tidal zone microhabitats (Zhang et al., 2018; Jiang et al., 2013) has so far been done in anthropogenically impacted sites, thus confounding the interpretation of these findings.

In this study we seek 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 amplicon sequencing. The mangrove forests in Brazil 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. The Atlantic Forest and associated ecosystems are highly threatened by anthropogenic disturbances such as logging and farming, as well as habitat loss and fragmentation due to human encroachment, 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 environmental conditions of the area show that preservation efforts initiated in 1998 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.

Considering mangrove zonation as driven, primarily, by tide variation, we hypothesized that sediments of different tidal zones would differ significantly in diversity and composition 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 (Brose and Hillebrand, 2016) as reported previously (Zhang et al., 2018). 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.

Importantly, 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 enabling subsequent research into the effects that anthropogenic effects 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

90   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

95   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

100   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,

105   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 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,

110   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

115   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 (S3 Fig.). We performed a variety of alpha-diversity (S4 Fig., S5 Fig., S6 Fig.,) and beta-diversity (S7 Fig.) and hierarchical correlation clustering (S8 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 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., S2 File), 2. proportional representation between zones, are available as a supplementary files (S2 File), and 3. taxonomy (S3 File). Phylogenetic reconstruction was carried out in QIIME2 using the feature classifier trained on 16S rRNA 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, S2 Fig.).

Vegan (Dixon, 2003) 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 (S3 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 (S9 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 are using 16S rRNA amplicon sequencing a crucial limitation must be considered in evaluating our results (Sun et al. 2020). 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, Supplemental Table 2, Supplemental File 6). 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 6). 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 script (see Supplemental Methods, Supplemental File 1, Supplemental File 6). 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 6). 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 4).

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%). Figure 2 shows the normalized abundances of the orders and families from the 6 dominant phyla in the data set for each tidal zone. It can be observed that the taxonomic profiles are broadly similar across the tidal zones at the family level. However, several families do break from this trend (Supplemental File 4), such as the two families of the order *Alteromonadales* (Fig 2.; 2. *Marinobacteraceae* and *Pseudoalteromonadaceae*) are only abundant in the supralittoral zone. From the Class *Deltaproteobacteria* (Fig 2.; 9. *Desulfarculales*, 10. *Desulfobacterales*, 14. *Myxococcales*, 23. *Syntrophobacterales*) it can be observed that 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) (S4 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 (S7 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 (S3 Table). The results revealed a significant correlation between the prokaryotic populations with salinity and organic matter (Fig. 3, S4 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 taxa, (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, 4 of these families had their lowest population abundances in the intertidal zone, and only one family (*Bacillaceae*) having their highest abundance in this zone. This dominance by *Bacillaceae* and reduced abundance of other families is consistent with our previous observations of the intertidal zone being 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 site. We found 7 families contributing 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, these were mostly associated with assimilatory sulfate reduction (M00176) and dissimilatory sulfate metabolism (M00596). 7 families produce 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.

Considering the taxa contributions to metabolic modules in the sediments, *Bacillaceae* is the most abundant family at each site and as such has the potential to drive a large proportion of functional activity. A pathway analysis of the KOs

reveals *Bacillaceae* is enriched in 17 metabolic pathways including several carbon metabolism (M00003, M00004, M00005, M00006, M00007, M00011, M00168, M00579, M00580) as well as, methane (M00345), nitrogen (M00531), and sulfur metabolism (M00176, M00616). Notably, some of these pathways are only enriched in the sublittoral site, such as sulfur metabolism (M00176, M00616), or are absent from the supralittoral site (M00004, M00007, M00580), suggesting that these roles are occupied by other families such as *Flavobacteriaceae* (M00580, M00176) and *Pirellulaceae* (M00176). Other contributions made by the enriched taxa to the metabolic modules are represented on Figure 5.

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., 2019a; El-Tarabily, 2002; Imchen et al., 2017; Lin et al., 2019; Zhang et al., 2018), 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. 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 leads 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., 2019b).

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. 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 difference in diversity indices were not statistically significant, the data shows a clear trend of differentiation between zones that suggests 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 of mangroves in anthropogenically impacted areas 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 the observed intertidal mangrove zone of our study area.

In this study, we assessed the functional profiles of taxa identified using 16S rRNA sequences. It is important to note that these functional profiles are not the result of Whole Genome Sequencing (WGS), 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. As such, the functional profiles here are best understood as a measure of potential metabolic activity. That said, functional profiling 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).

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 in mangrove sediments is in agreement with previous observations of the microbial diversity in these environments (Rocha et al., 2016); (Ceccon et al., 2019a; Cabral et al., 2016; Mendes and Tsai, 2014; Zhao and Bajic, 2015). Conceptually, one could interpret 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 were one of their number to be removed.

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 this, as well as a further exploration of whether different groups of organisms actively participate in element cycling in mangrove sediments is the subject of future studies.

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.

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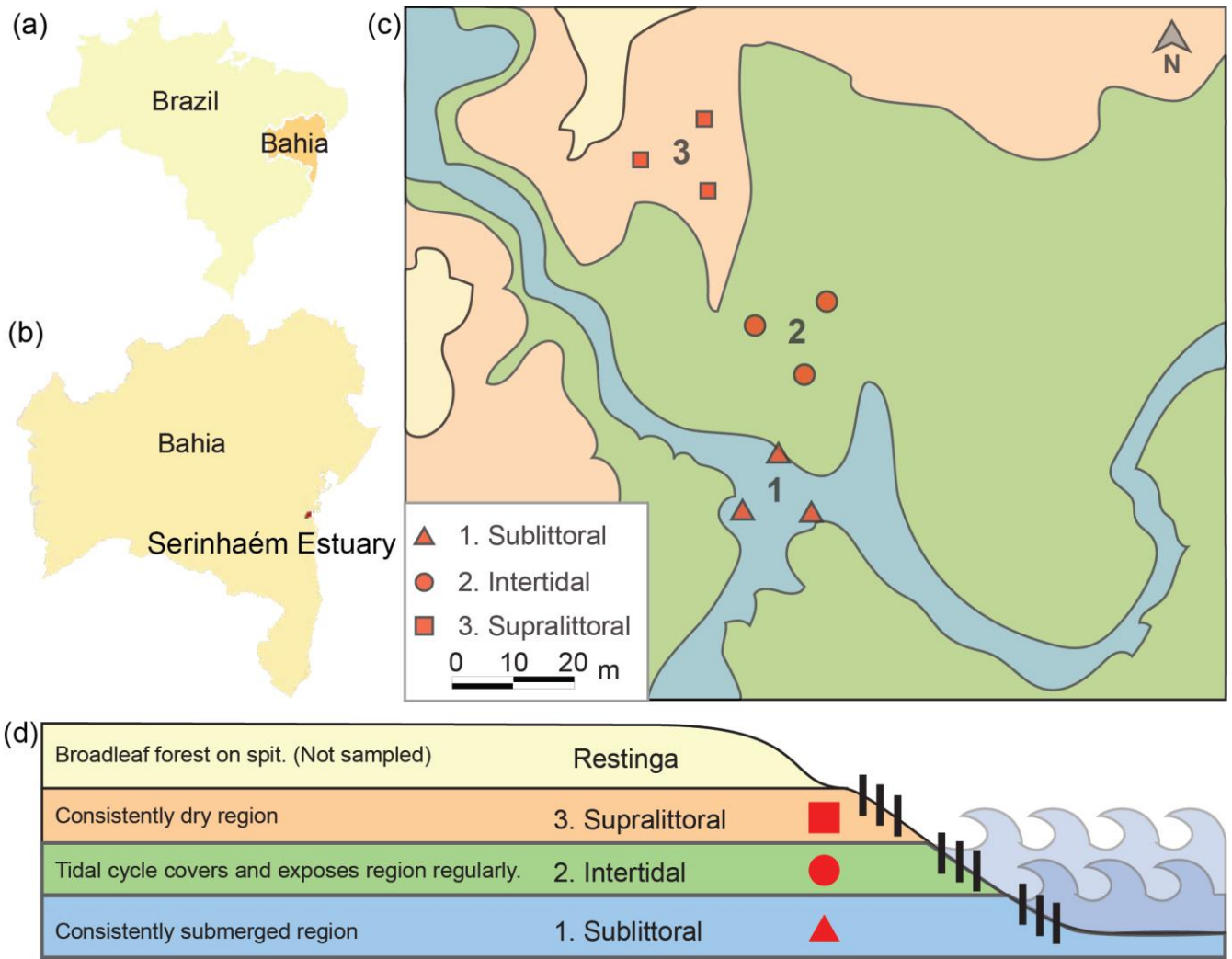
Fig 1. Map and schematic of sediment sampling sites. Here we show the locations of the sampling sites relative to Brazil (A) and Bahia (B). A picture shows the relation of the three sampling sites within each zone (1. sublittoral, 2. intertidal, 3. supralittoral, (C)). A schematic shows the topographic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

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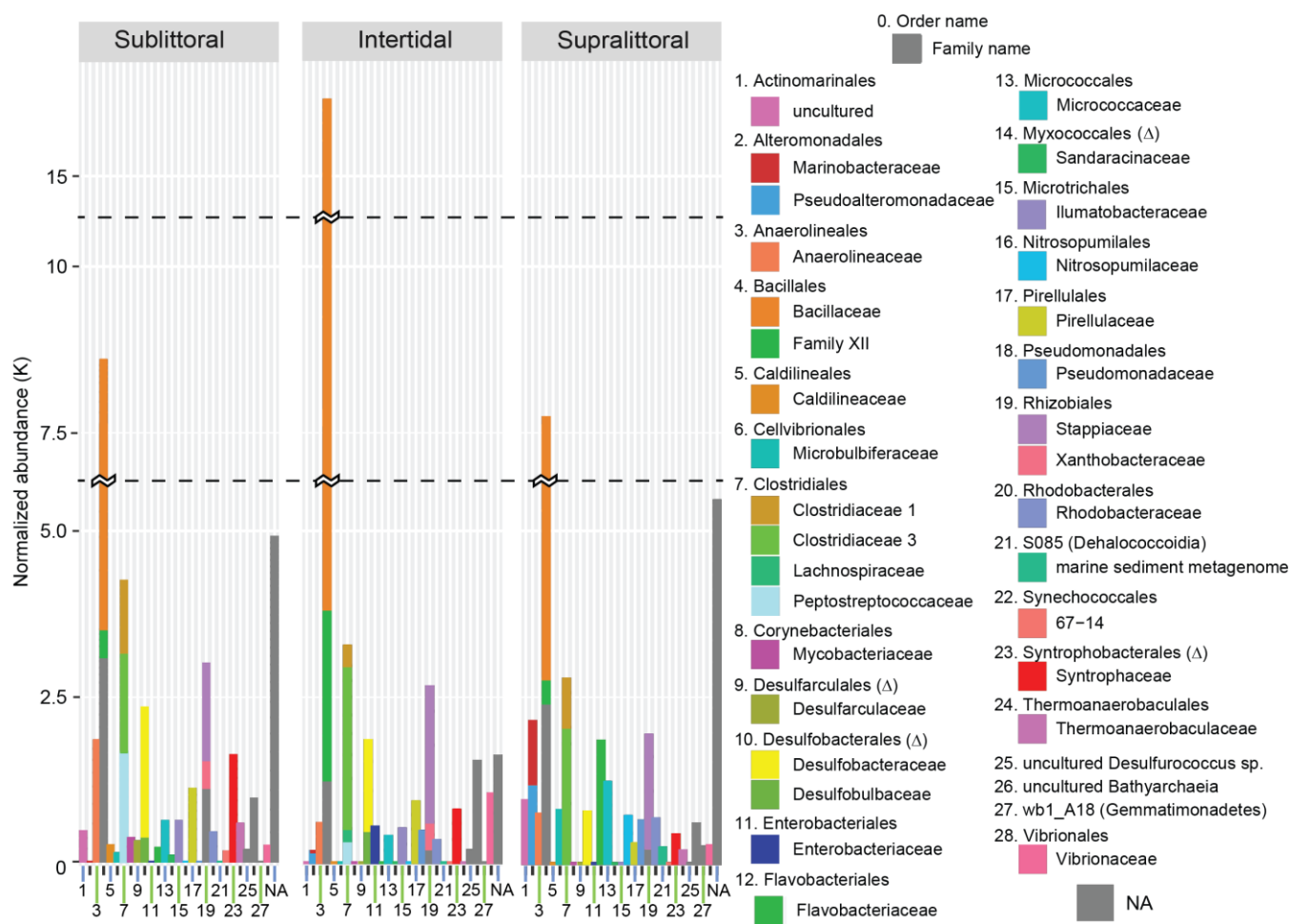
Fig 2. Normalized taxonomic abundances from each tidal zone. Taxa identified are shown as stacked bar plots, the horizontal axis is the higher taxonomic level while the stacked bars are the lower level. Order is the horizontal axis with Family being the vertical stack. Taxa abundance (Y-axis, thousands (K) of reads) has been normalized by downsampling sequence depth. For visualization purposes only high abundance families are shown. Members of Deltaproteobacteria are labelled with a delta (Δ).
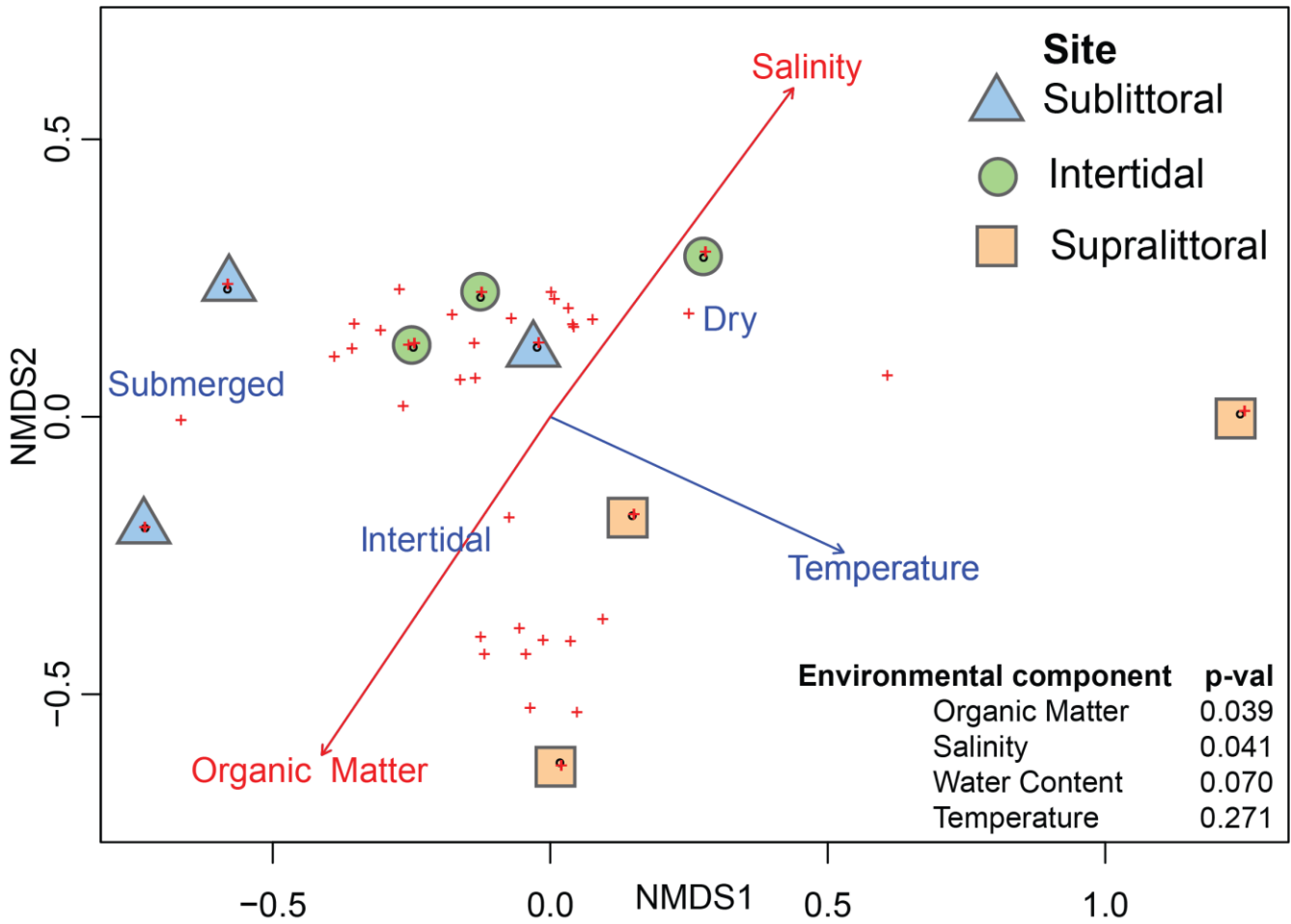
Fig. 3. Correlation between environmental variables and prokaryotic communities. Dots represent taxonomic abundances per site as plotted by Vegan metaMDS using nonmetric multidimensional scaling (NMDS). Arrow length is a representative of the predictor strength of environmental variable vectors, with red arrows having statistical significance as calculated by envfit (p-value <= 0.05).
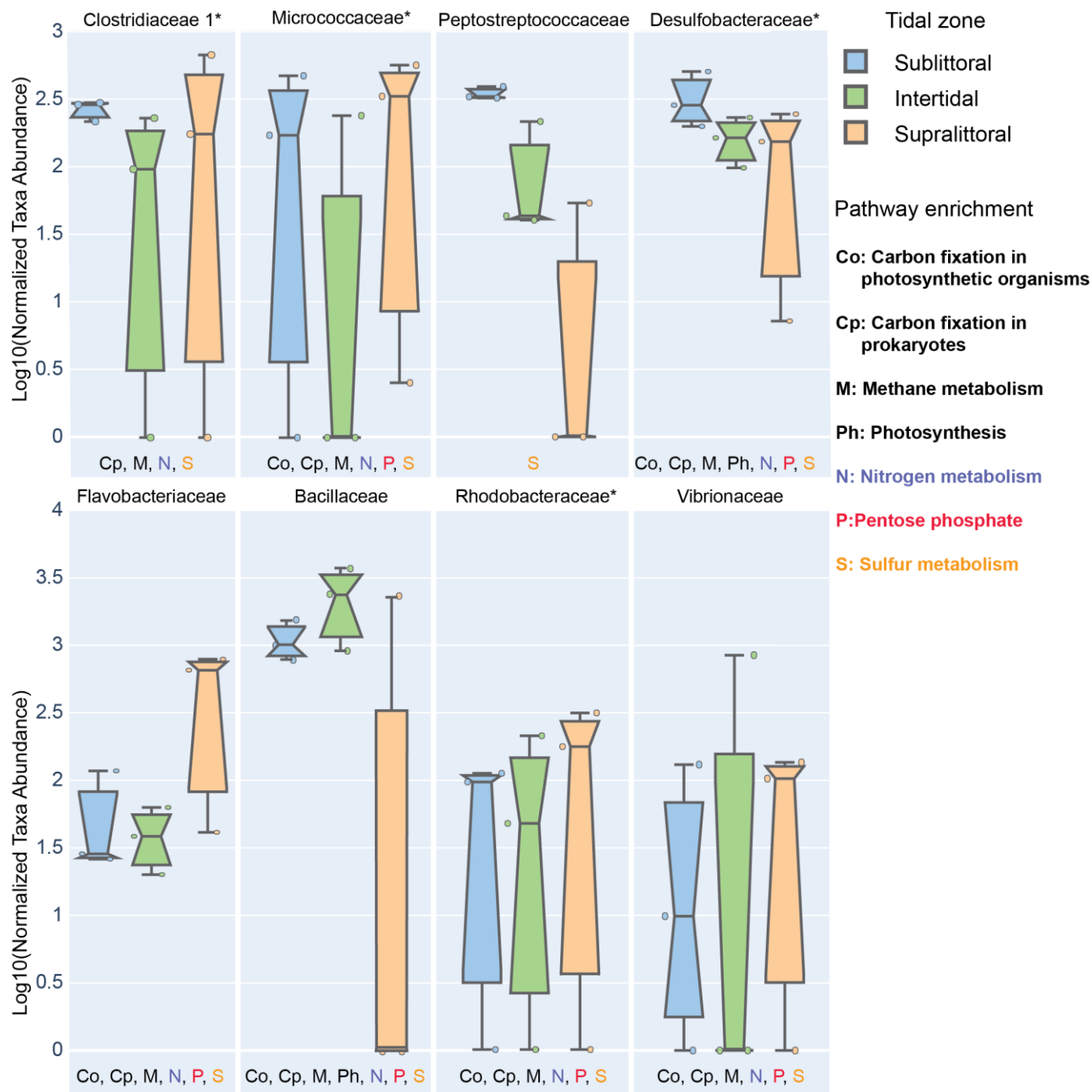
Fig. 4. Zone specific measures of taxa find significant enrichment in sediments of different tidal zones. Here we show prokaryotic families that have significantly different abundances between zones, and whose mean effect size exceeded 10% in at least one zone. To have been labelled with a metabolic 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 *.
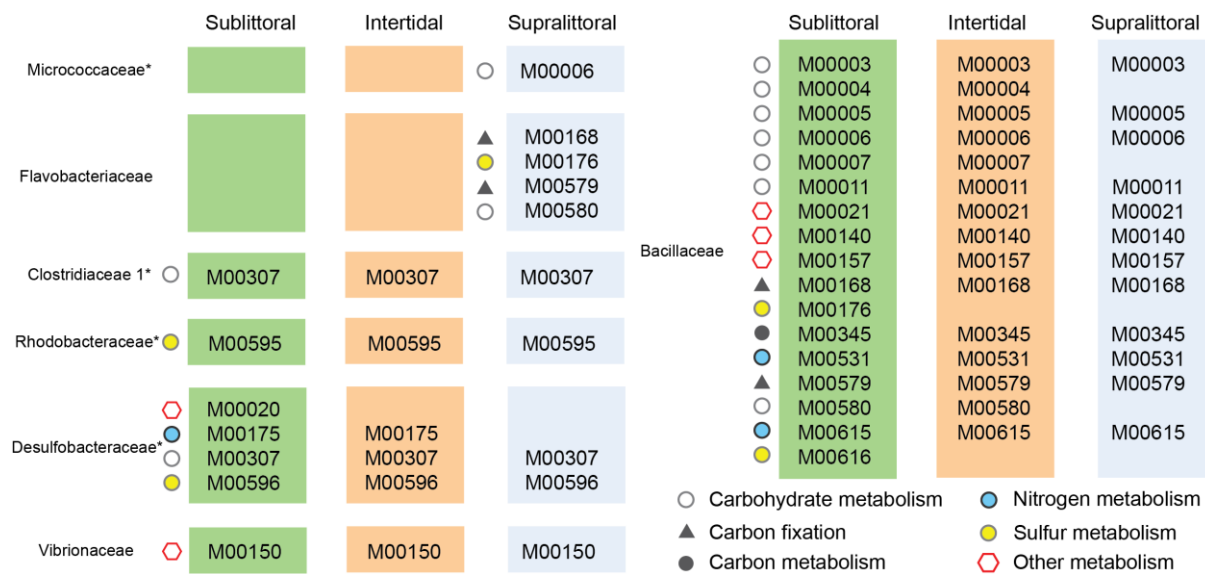
Fig. 5. Zone specific enrichment of pathway modules. Here, we show the impact that the differential abundances of major families (Figure 4) has on potential functional profiles at each zone at the level of pathway modules. To be included here each module must be completely enriched within a single taxa 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. Notably 24 out of 29 (82.8%) taxa enriched pathways are found in the sublittoral zone versus 21 (72.4%) and 22 (75.9%), in the intertidal and supralittoral zones, respectively. Families with a median NSTI within 1 standard deviation of 0.15 are labelled with *.
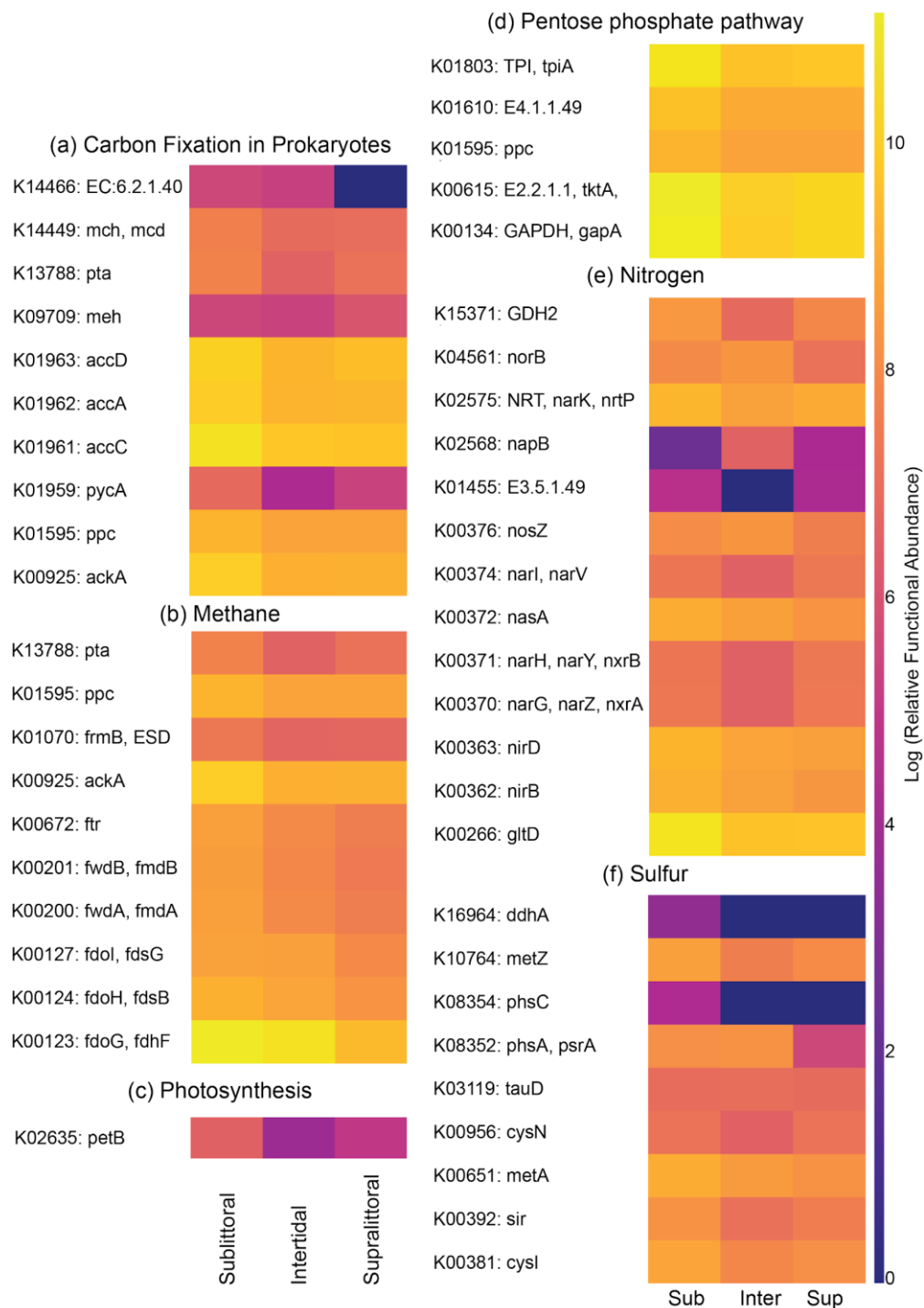
Fig. 6. Zone specific measures of metabolism associated KEGG Orthologs. The heatmaps show the metabolism associated KOs that have significant differences of functional abundance between zones for carbon, phosphorus, sulfur and nitrogen pathways. Notably, the majority of KOs (45/54, 83%), have their highest relative functional abundance in the sublittoral zone, with the intertidal (5/54, 9.3%) and supralittoral (4/54 7.4%) as near equal minor contributors.