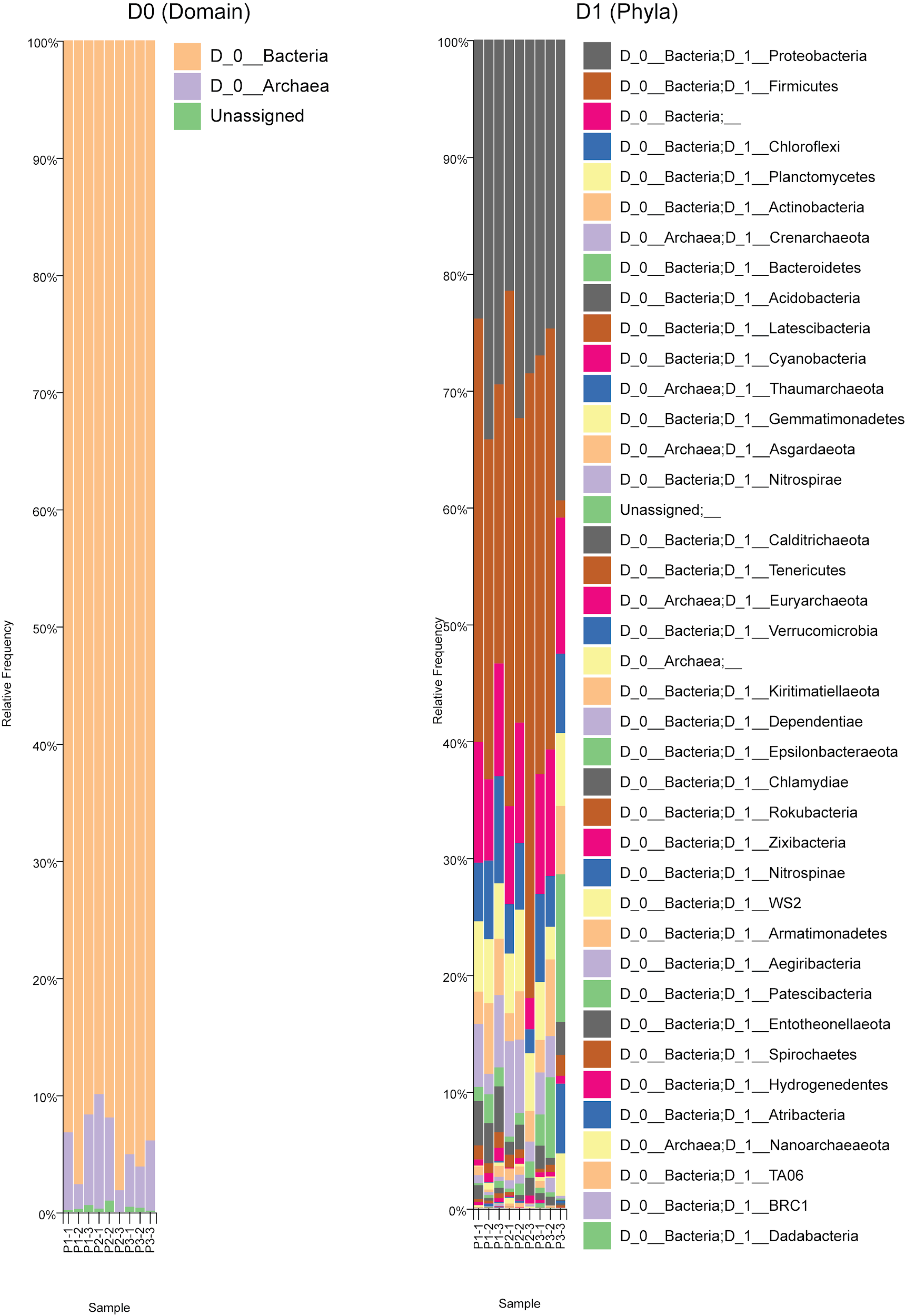
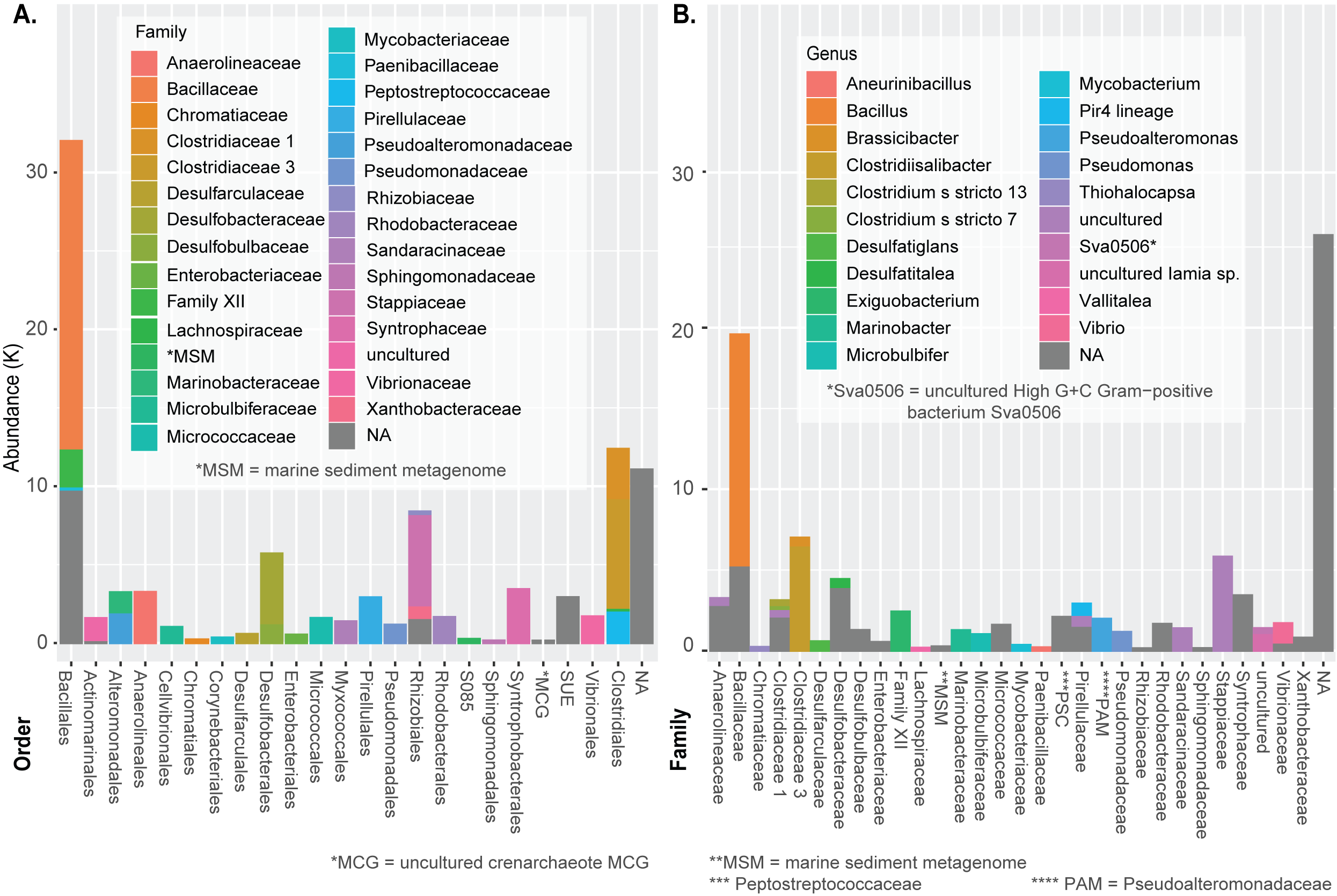
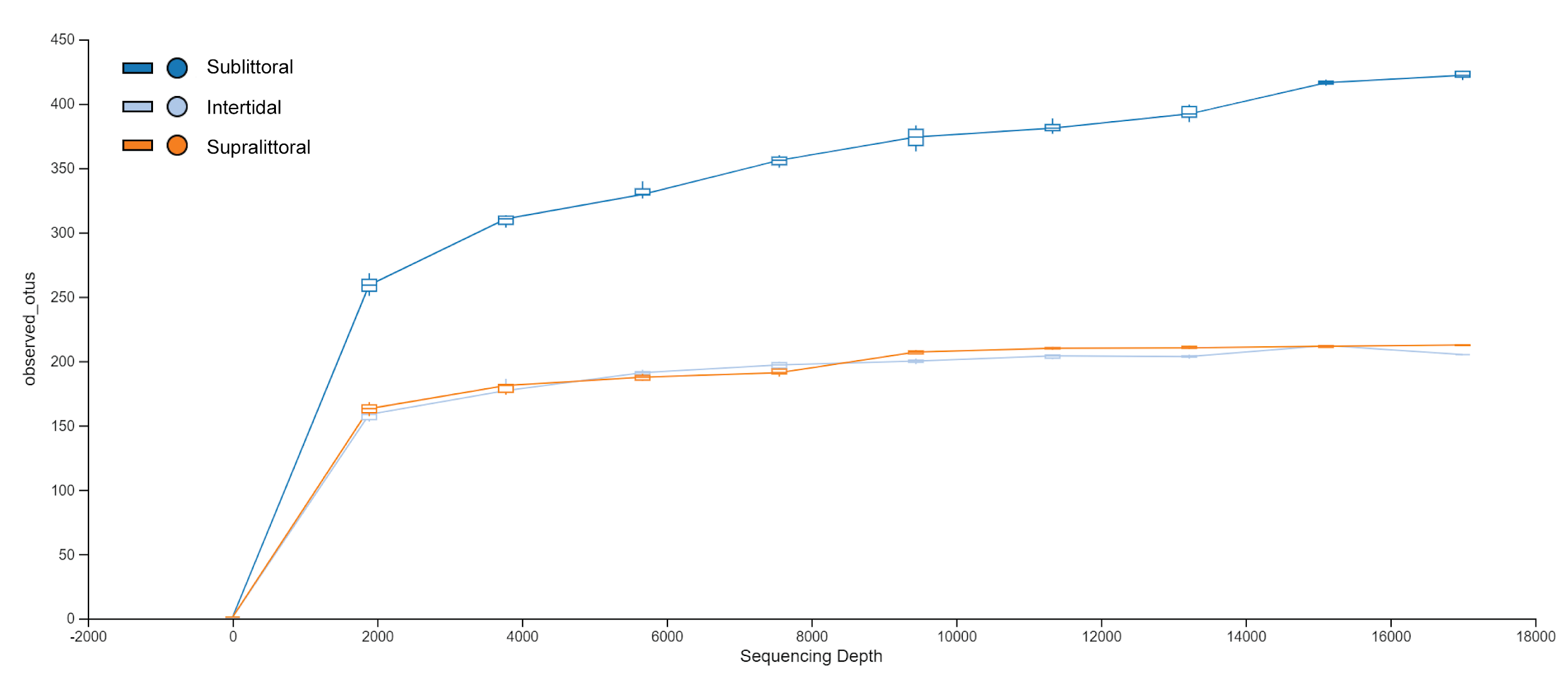
# Supplemental Sections



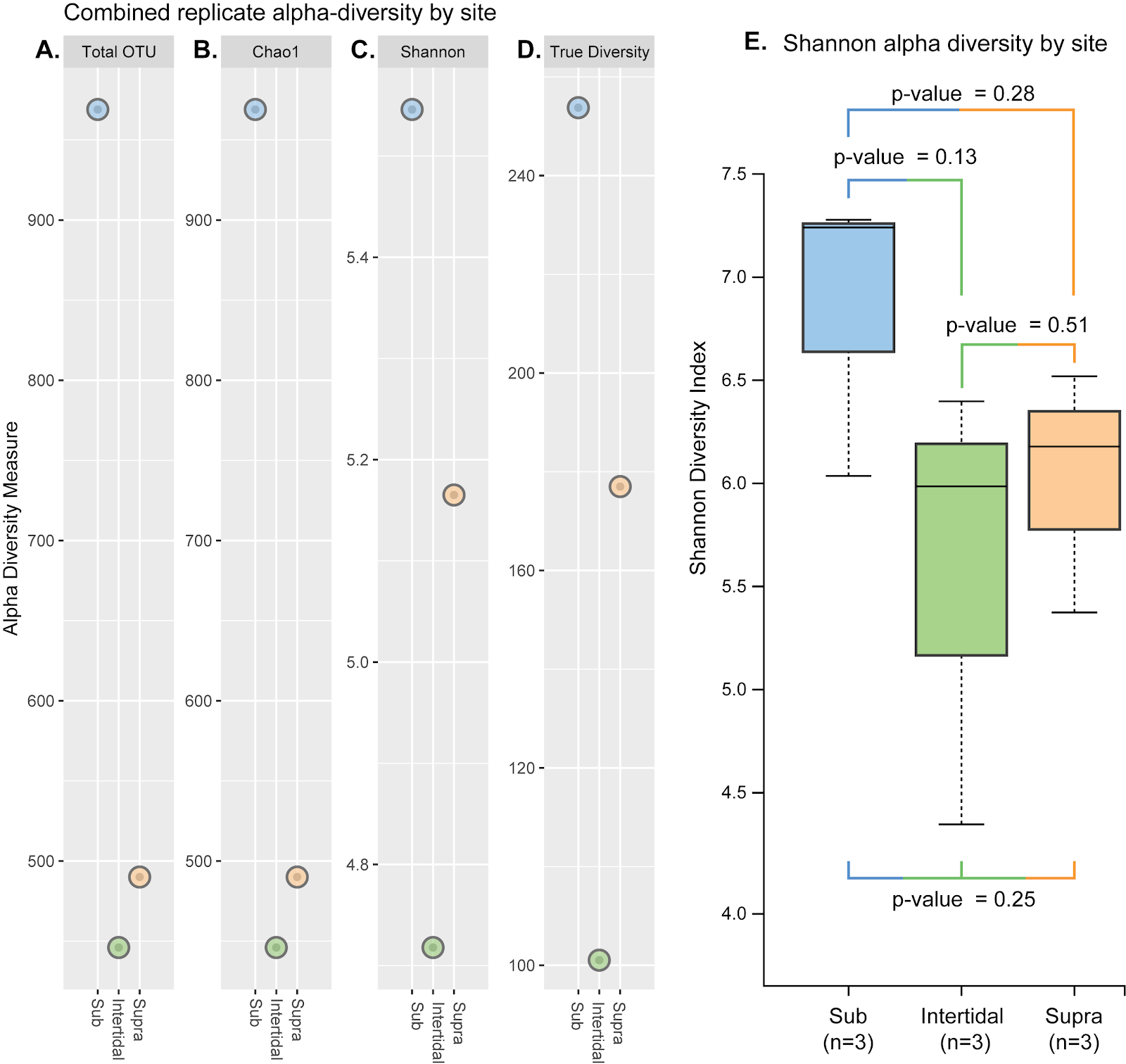
**Supplemental Figure 1. Bar charts of Domain and Phyla taxonomic proportion per site and replicate.** Figures generated using the taxa-bar-plots.qzv (Supplemental File 1) in QIIME2 View.



**Supplemental Figure 2. Taxonomic abundances from all sample sites.** Taxa identified within the samples 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 with the Family being the stack (A) and Family is the horizontal with Genus being the stack (B).



**Supplemental Figure 3. Alpha-rarefaction.** Progressive downsampling of sequences grouped by sampling site reveals early saturation for Intertidal and Supralittoral sediment sites but slow and incremental growth for the Sublittoral site.



**Supplemental Fig 4**. **Alpha diversity by sampling site**. Alpha diversity (mean diversity of species per site) measured using the absolute number of OTUs (**A**), Chao1 estimator (**B**), Shannon’s diversity index (**C**), Shannon’s true diversity (**D**). The results of the Kruskal-Wallis test on the distribution of Shannon’s alpha-diversity indices across all sites and pair-wise (**E**).

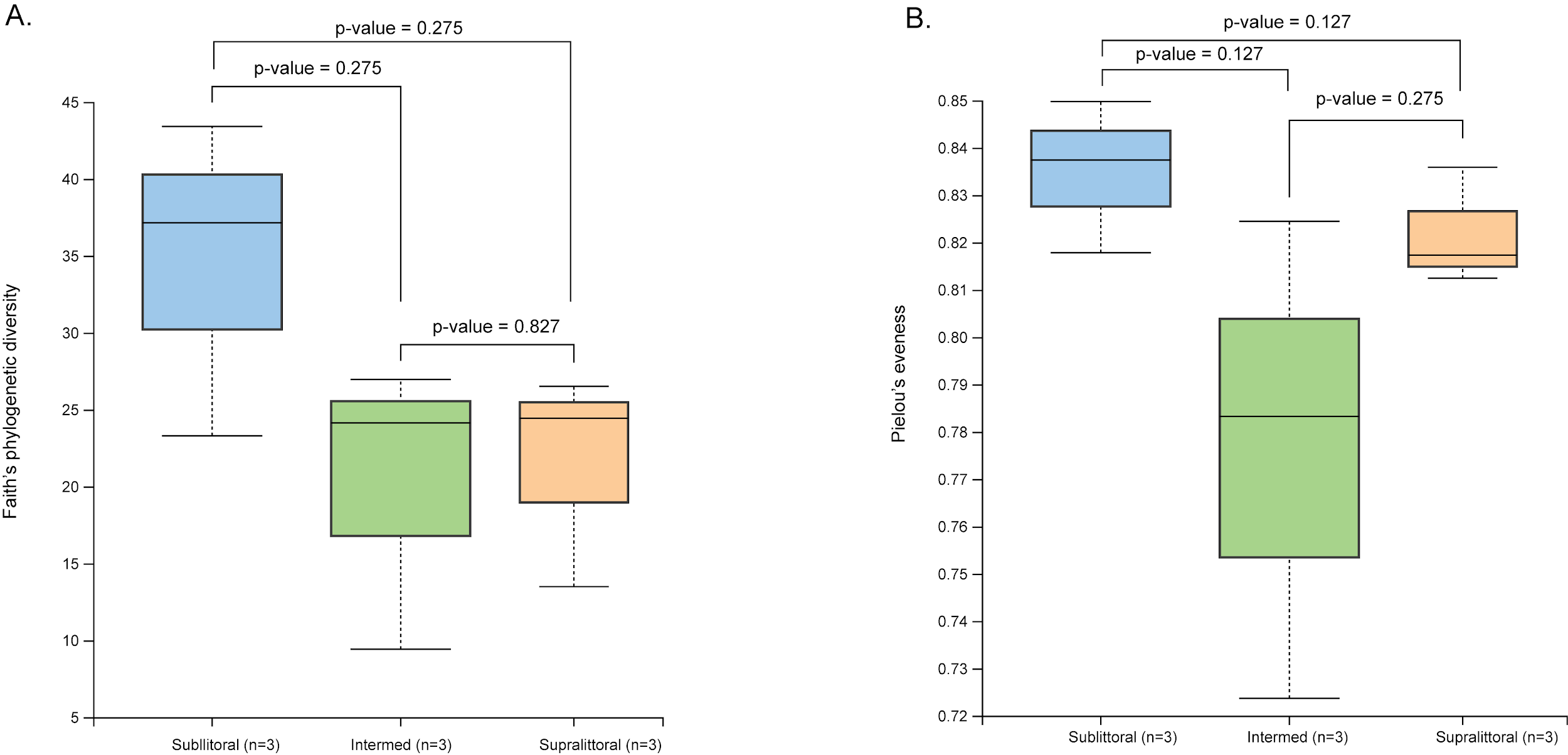
## **Alpha diversity indices show communities at sublittoral sites to be the most diverse**

The alpha diversity indices for each zone are shown in S4 Fig. Overall the sediments from the sublittoral zone had higher richness and diversity indices, while the intertidal zone exhibited the lowest alpha diversity indices (S5, S6, S7 Fig.). This is consistent with alpha-rarefaction calculation which found the OTU diversity in both Intertidal and Supralittoral sites saturated early relative to the Sublittoral site (S3 Fig.). However, neither the pairwise or joint Kruskal-Wallis test found that the differences between zones as significant (S4E). A previous study of mangrove sediments [[1]](https://paperpile.com/c/jFsWqT/xHeQ) also observed substantial but not significant differences between group diversities. It is important to note that alpha-diversity is a local measure of richness and is not a taxonomic comparison, therefore sites with distinct taxa can have the same diversity index if the distribution of the abundances is similar [[2]](https://paperpile.com/c/jFsWqT/72ij), [[3]](https://paperpile.com/c/jFsWqT/2W0n).

The Total OTU column shows the richness within each zone (the absolute number of OTUs found in the site samples), not considering the abundance of the taxa (S4A Fig). We found 969 OTUs in the sublittoral zone, while the supralittoral had 490 OTUs and the intertidal 446 Considering these numbers, the richness in the sublittoral samples is much greater than the richness found for the two other tidal zones of the mangrove.

Diversity indices are mathematical measures based on taxa richness and abundance. Shannon’s diversity index gives the same weight to the richness and abundance data and ranges from a high of 5.54 in the sublittoral region, 4.71 in the intertidal, and 5.18 for the supralittoral samples (S4B Fig). To check for statistical differences in the diversity by tidal zone, we performed the Kruskal-Wallis test on the Shannon’s diversity measures and the results show no significance for the compared groups, indicating that the great differences in local richness could be the result of limited sampling of these mangrove regions (S4E Fig).

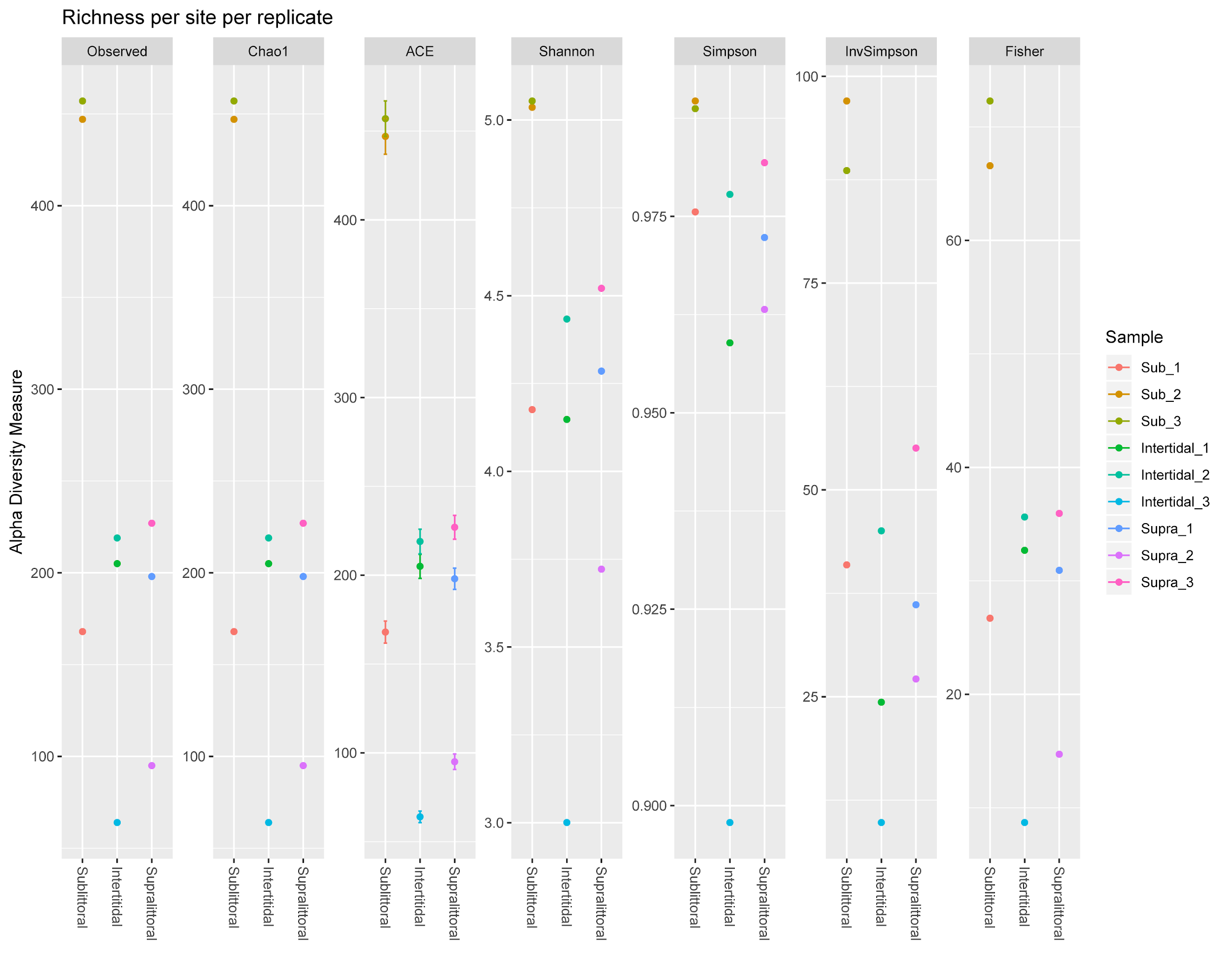
Because diversity indices involve non-linear scales with different sensitivities to rare and common species, the diversity index is not an intuitive measure. By transforming diversity indices into true diversities [[2]](https://paperpile.com/c/jFsWqT/72ij) we can explore the information using the more intuitive measure of number equivalents [[4]](https://paperpile.com/c/jFsWqT/DhbF). Transforming Shannon’s index into true diversity, we see that the sublittoral zone has a diversity equivalent of 254 species if each were evenly abundant. The number equivalent for Shannon's index of the intertidal zone shows a diversity equivalent to 111 equally frequent species. For the supralittoral zone, the number equivalent to the Shannon’s index shows the same diversity as a location having 177 equally frequent species. This suggests that the diversity in the sublittoral zone is more than 50% greater than the diversity in the intertidal zone and at least 30% more diverse than the supralittoral zone of the mangrove forest (S4D Fig).



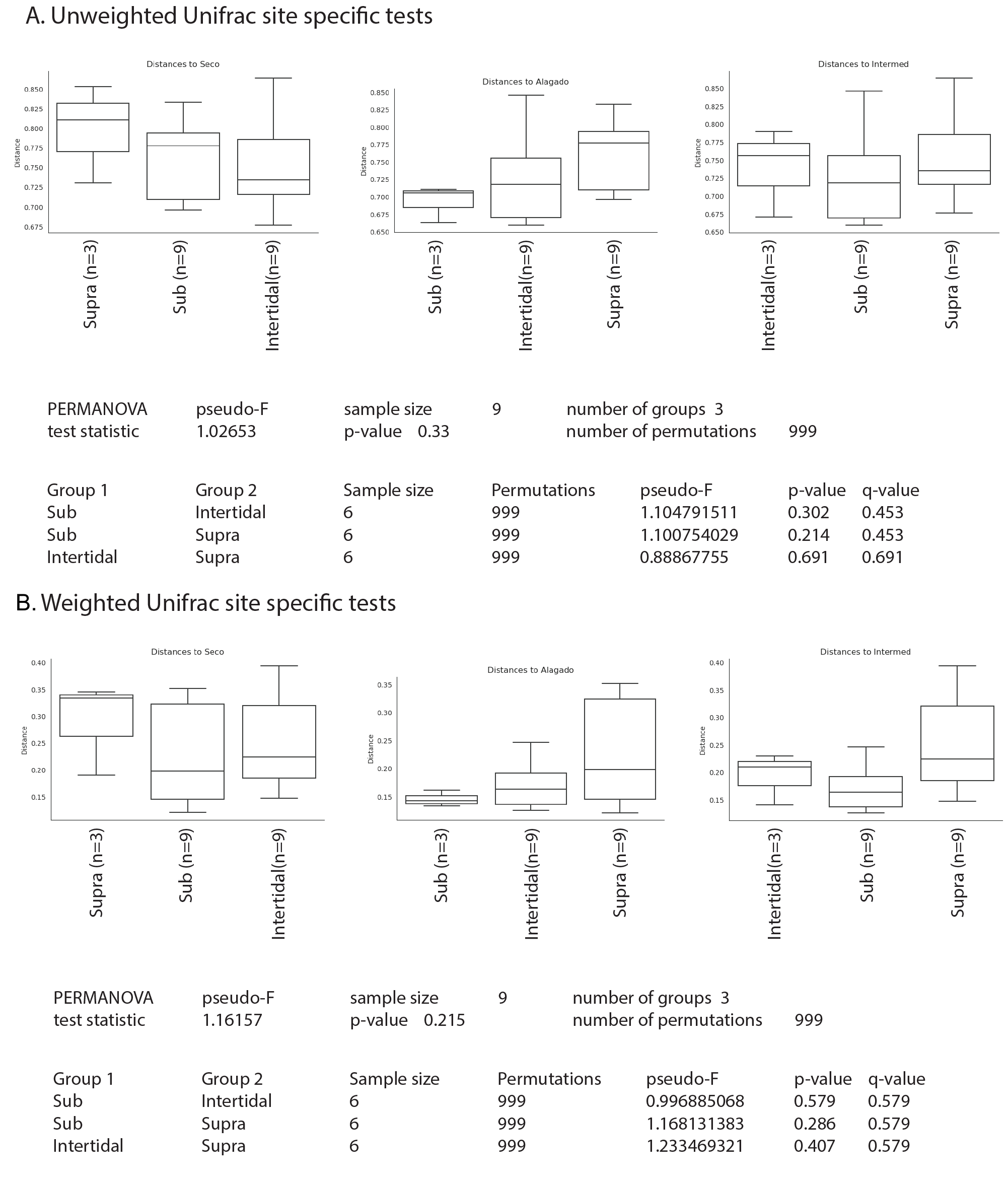
**Supplemental Figure 5. Statistical tests for Faith and Pielou evenness.** Box plots showing the statistical results of alpha-diversity for Faith’s phylogenetic diversity and Pielou’s evenness, as calculated by QIIME2.

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**Supplemental Figure 6. Additional alpha-diversity measures.** Box plots showing the results of additional alpha-diversity measures: abundance-based coverage estimator (ACE), Simpson, and Inverse Simpson, as calculated by QIIME2.



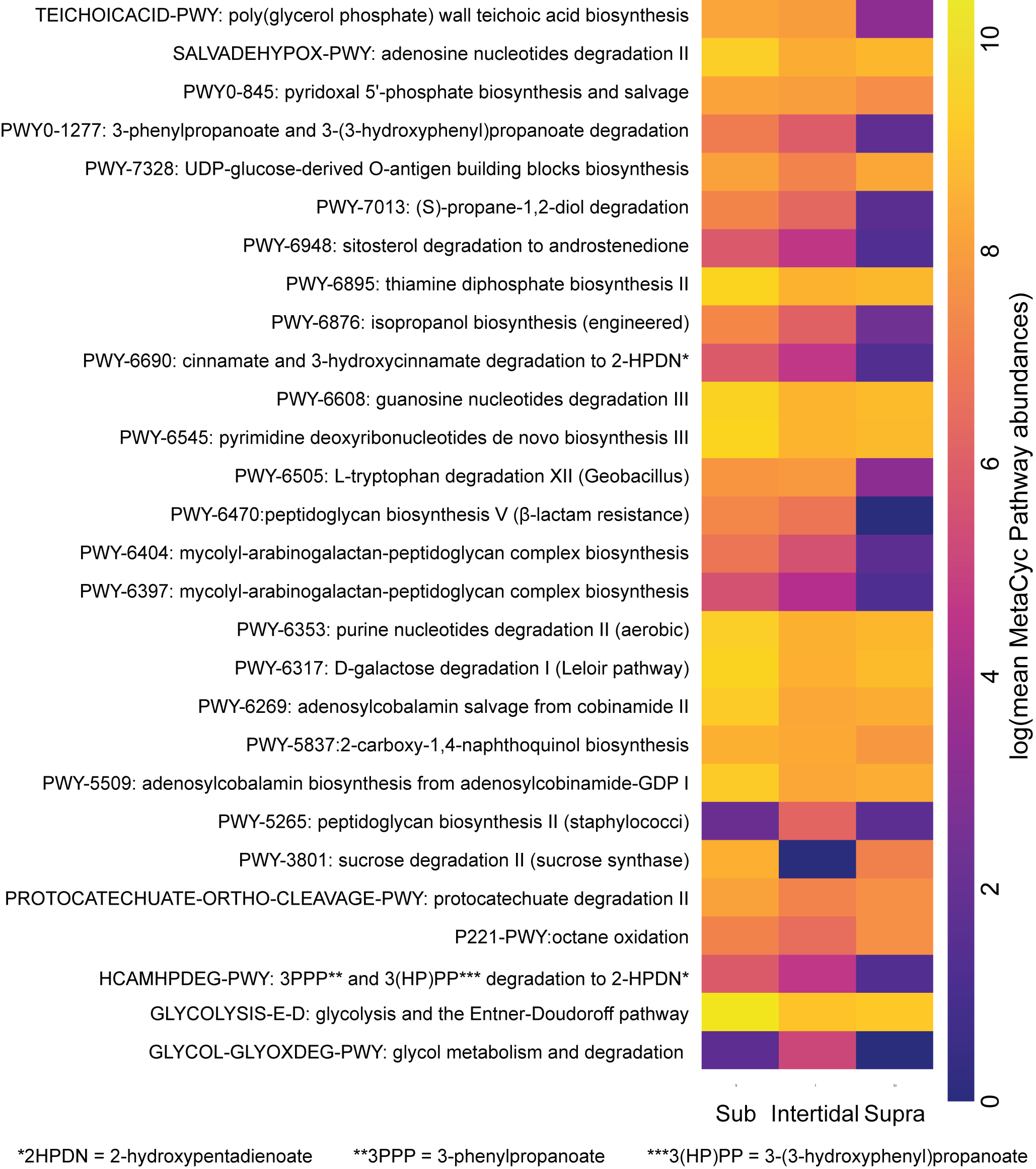
**Supplemental Figure 7. Alpha-diversity by site by replicate.** Alpha-diversity measures for each site with each replicate represented, as calculated by QIIME2.



**Supplemental Figure 8. Unweighted and Weighted Unifrac measures and PERMANOVA statistical tests.**

In order to analyze the data using phylogenetic approaches, we also performed PERMANOVA using the Weighted and Unweighted Unifrac distance metrics, although these found no significant difference between phylogenetically weighted groups (S2 Table, S8 Fig.). Given the large amount of phylogenetic variability observed between replicates, our experiment is potentially underpowered for the application of this phylogenetic test.

The Unifrac distance analyses (Fig. 4; S8A Fig.) take into consideration the abundances and phylogenetic relations between the taxa in each sample. Intriguingly, while qualitatively different population abundances were observed between tidal zones, these were not found to be significant. These results are consistent with previous studies in mangrove sediments that show the dominance of some specific taxa in the upper levels [[5]](https://paperpile.com/c/jFsWqT/F7hs), [[6]](https://paperpile.com/c/jFsWqT/PTSU), [[7]](https://paperpile.com/c/jFsWqT/wAhV), [[8]](https://paperpile.com/c/jFsWqT/tiRT), [[9]](https://paperpile.com/c/jFsWqT/axvI), [[10]](https://paperpile.com/c/jFsWqT/apDn). Taken together with the results of the non-phylogenetic distance metrics, this could be interpreted as higher taxonomic level phylogenetic correlations and abundances being relatively stable, with the majority of diversity between zones occurring within the lower level taxa.

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**Supplemental Figure 9. Heatmap of site specific enriched MetaCyc pathways.** This heatmap shows MetaCyc pathways that are significantly enriched at specific sites (see Methods). Note, unlike the Kegg Object enrichment (Figure 7), these pathways are not a subset limited to metabolism associated pathways. Also, unlike the Kegg Object enrichment the patterns of enrichment at the level of pathways is mainly in only two modes, inverse to elevation and lowest in intertidal. There are 12 pathways whose functional abundance is inverse to elevation (TEICHOICACID-PWY, PWY0-845, PWY0-1277, PWY-7013, PWY-6948, PWY-6876, PWY-6690, PWY-6505, PWY-6404, PWY-6470, PWY-6397, HCAMHPDEG-PWY), including 4 of the 5 pathways involved in Cell Wall Biosynthesis and 4 of the 5 pathways involved in Aromatic Compound Degradation. Of the 14 pathways whose functional abundance is lowest in intertidal (SALVADEHYPOX-PWY, PWY-7328, PWY-6608, PWY-6545, PWY-6317, PWY-6353, PROTOCATECHUATE-ORTHO-CLEAVAGE-PWY, PWY-3801, PWY-6269, PWY-5509, GLYCOLYSIS-E-D, PWY-6895, PWY-6608, PWY-6545), these include 4 of 4 pathways involved in Nucleoside and Nucleotide Degradation and 2 of 2 involved in Nucleoside and Nucleotide Biosynthesis.

**Supplemental Tables**

|  |  |
| --- | --- |
| **Phylum** | **Abundance (sequences)** |
| Acidobacteria | 5754 |
| Actinobacteria | 9390 |
| Aegiribacteria | 14 |
| Armatimonadetes | 14 |
| Asgardaeota | 1015 |
| Atribacteria | 7 |
| Bacteroidetes | 6245 |
| BRC1 | 6 |
| Calditrichaeota | 748 |
| Chlamydiae | 67 |
| Chloroflexi | 13225 |
| Crenarchaeota | 7921 |
| Cyanobacteria | 1365 |
| Dadabacteria | 4 |
| Dependentiae | 96 |
| Entotheonellaeota | 8 |
| Epsilonbacteraeota | 86 |
| Euryarchaeota | 298 |
| Firmicutes | 60307 |
| Gemmatimonadetes | 1267 |
| Hydrogenedentes | 7 |
| Kiritimatiellaeota | 133 |
| Latescibacteria | 1939 |
| Nanoarchaeaeota | 7 |
| Nitrospinae | 20 |
| Nitrospirae | 808 |
| Patescibacteria | 8 |
| Planctomycetes | 10888 |
| Proteobacteria | 62135 |
| Rokubacteria | 35 |
| Spirochaetes | 7 |
| TA06 | 6 |
| Tenericutes | 349 |
| Thaumarchaeota | 1299 |
| Verrucomicrobia | 258 |
| WS2 | 18 |
| Zixibacteria | 30 |

**S1 Table. Phyla Abundances** Total phyla abundances obtained from QIIME2 pipeline using the Open Reference method with a 97% similarity (--p-perc-identity 0.97) against the reference 16S rRNA sequences in SILVA database (Silva SSU 132).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Compared sites** | **Bray-Curtis** | | | **Jaccard** | | | **Weighted Unifrac** | | | **Unweighted Unifrac** | | |
| Pseudo-F | p-value | q-value | Pseudo-F | p-value | q-value | Pseudo-F | p-value | q-value | Pseudo-F | p-value | q-value |
| Sub- to Intertidal | 1.209269 | 0.103 | 0.159 | 1.033214 | 0.207 | 0.207 | 0.996885 | 0.579 | 0.579 | 1.104792 | 0.302 | 0.453 |
| Sub- to Supra- | 1.368294 | 0.106 | 0.159 | 1.075842 | 0.109 | 0.207 | 1.168131 | 0.286 | 0.579 | 1.100754 | 0.214 | 0.453 |
| Intertidal to Supra- | 0.923174 | 0.782 | 0.782 | 1.021887 | 0.187 | 0.207 | 1.233469 | 0.407 | 0.579 | 0.888678 | 0.691 | 0.691 |
| all groups | 1.15787 | **0.064** | - | 1.04359 | **0.035** | - | 1.16157 | 0.215 | - | 1.02653 | 0.33 | - |

**S2 table. Statistical results of beta-diversity tests.** Statistical results (pseudo-F, p-value, q-value) for PERMANOVA beta diversity analysis.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sampling Site** | **Water content** | **Salinity** | **Temperature (Celsius)** | **Organic Matter (%)** |
| **Sublittoral** | Submerged | 665.9 | 25 | 3.71 |
| **Intertidal** | Intertidal | 677.6 | 29 | 3.86 |
| **Supralittoral** | Dry | 1047 | 29 | 1.4 |

**S3 table.** **Environmental variables from collection sites.**

# 

# 

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Vectors: |  |  |  |  |
|  | NMDS1 | NMDS2 | r2 | Pr(>r) |
| Salinity | 0.62494 | -0.78067 | 0.6987 | 0.009 |
| Temperature.celsius | 0.69345 | -0.72051 | 0.2881 | 0.309 |
| Organic.Matter | -0.62066 | 0.78408 | 0.6787 | 0.023 |
| Permutation: free | |  |  |  |
| Number of permutations: 999 | | |  |  |

# 

|  |  |  |
| --- | --- | --- |
| Centroids: |  |  |
|  | NMDS1 | NMDS2 |
| Sublittoral-1 | -0.2428 | 0.1346 |
| Sublittoral-2 | -0.0548 | 0.0533 |
| Sublittoral-3 | -0.2062 | 0.0758 |
| Intertidal-1 | -0.2928 | -0.2077 |
| Intertidal-2 | -0.0439 | 0.0292 |
| Intertidal-3 | 0.1154 | 0.3729 |
| Supralittoral-1 | -0.0116 | -0.0511 |
| Supralittoral-2 | 0.0499 | -0.3573 |
| Supralittoral-3 | 0.6869 | -0.0496 |
| Water.content\_dry | 0.2417 | -0.1527 |
| Water.content\_intertidal | -0.0738 | 0.0648 |
| Water.content\_submerged | -0.1679 | 0.0879 |
|  |  |  |
| Goodness of fit: | |  |
|  | r2 | Pr(>r) |
| Sample | 1 | 1 |
| Water.content | 0.3736 | 0.168 |
| Permutation: free | |  |
| Number of permutations: 999 | | |

**S4 table. Results of Vegan envfit.** For Environmental variables and taxonomic abundances.

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