

Sinigalliano & Chun - CRCP Project # 1114

Metagenomics Results as of July 2015

Summary of Data from 16S rDNA Next-Generation-Sequencing & Metagenomic Bioinformatics Analysis from Water and Coral Samples that have Completed Illumina Hi-Seq Sequencing as of July 2015

- Comparisons of bacterial constituents of these samples, between sites and sampling dates, have been determined by examining the numbers and types of Operational Taxonomic Units (OTUs). Species diversity, and richness, evenness, and rarefaction analysis were also determined.
- 131 samples collected during 2014 have been sequenced so far, including:
 - Coastal Inlet Surface Water Samples
 - Sewage effluent samples from two WWTP
 - Surface water expression boils from the oceanic outfalls of treated effluent from two WWTP
 - Coastal water from surface and depth of four reefs offshore of Miami-Dade & Broward Counties
 - Coral samples (coral polyp tissue and mucus) from two species of coral at these 4 reefs
- Approximately 300 more samples to be sequenced by the conclusion of this project

Illumina Sequencing and Analysis Methods

- DNA extracts were normalized to a concentration of $2.5 \text{ ng } \mu\text{l}^{-1}$ by dilution in nuclease-free water and used as template for a PCR assay targeting the hypervariable V4 region of the 16S rDNA.
- Each sample was amplified with a reverse primer containing a unique 6 bp nucleotide sequence on the 5'-terminus to allow samples to be pooled for sequencing and separated later. Library construction and paired-end sequencing ($2 \times 100 \text{ nt}$) of amplicons was performed by the BioMedical Genomics Center at the University of Minnesota (Saint Paul, MN) using the Illumina HiSeq platform (U. Minn. PI: Chanlan Chun).
- Raw sequence reads were pair-end aligned, separated by sample, and trimmed for quality as described previously using mothur software v. 1.36.0. Sequences of abundance < 2 over the entire dataset were excluded from analysis. Sequences were aligned to the RDP taxonomic database, and analysis of OTUs was performed at a sequence cutoff of 0.02. The number of sequence reads associated with each group was subsampled to that of the smallest group for comparisons of beta diversity (total species diversity and turnover among samples) and relative taxonomic abundance.
- Alpha diversity indices (Chao, Ace, Shannon, non-parametric Shannon, and Simpson indices) were calculated using Mothur. The Bray-Curtis measure of dissimilarity was used to construct distance matrices among sites. Weighted and unweight UniFrac analyses were also calculated using Mothur software package.

- A total of 5,999 OTUs were identified among all samples. Coverage for all samples was estimated at $99.3 \pm 0.1\%$. Among land-based pollution sources, more OTUs were generally identified in ocean outfall surface boils and treated wastewater effluent samples than in coastal inlet samples and with higher diversity as estimated by Ace, Chao, Shannon, and non-parametric Shannon indices
- Significant difference in microbial diversity of coral tissues were also observed in coral tissues relative to that of water samples at coral reefs.
- Eighty four (1.4%) of OTUs could not be assigned to phyla. Among all samples, 58 phyla were identified and *Proteobacteria*, *Bacteroidetes* and *Cyanobacteria* were the predominant phyla identified.
- The phylum *Firmicutes* was more dominant in coral tissues in comparison to water samples. Abundance of Archaea (*Crenarchaeota*, *Euryarchaeota*, and *Parvarchaeota*) is 0 to 13.9% of all samples, but the *Euryarchaeota* were relatively more dominant in samples collected in cold season (November and January).
- Weighted UniFrac and principal coordinate analyses (PCoA) revealed that, among individual samples, the bacterial community of coral tissues was significantly different than water samples in coral reefs and in coastal inlets/outfalls.

- Among land-based pollution sources (costal inlets and outfalls), the community composition and structure of the costal outfalls (two treated sewage effluent oceanic wastewater outfalls) from Miami Central and Miami North varied depending on location and season. In contrast, the bacterial community of coastal inlets was similar, regardless of spatial and temporal variations. Moreover, coastal inlet communities were closely related with those in water samples in coral reefs.
- Based on Principle Coordinate Analysis, the microbial community structure and composition (based on 16S rRNA gene sequences) for the treated wastewater effluent were significantly distinct from all other samples, including even the seawater surface expression boils of the effluent oceanic outfalls.
 - Previous studies have shown there is a very large dilution factor from the effluent discharge pipes (approximately 30+ meters in depth) to the surface. Our metagenomic study here indicates that there is a substantial change in microbial community composition from discharge at depth to the surface expression of these oceanic outfalls.
 - Microbial community composition of the water at the surface of these oceanic WWPT outfalls far more closely resembles the population of open ocean, coastal, and reef water samples than it does the treated wastewater effluent samples or the coastal inlet samples.
- Previous studies suggest most of the effluent-associated bacteria detected in outfalls are dead or inactivated, while bacteria from coastal inlets are presumed viable and active.

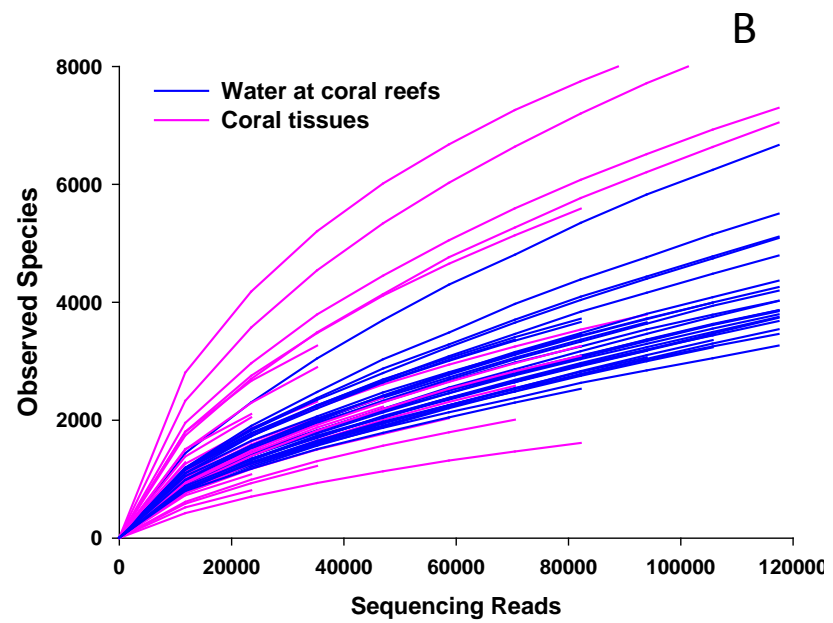
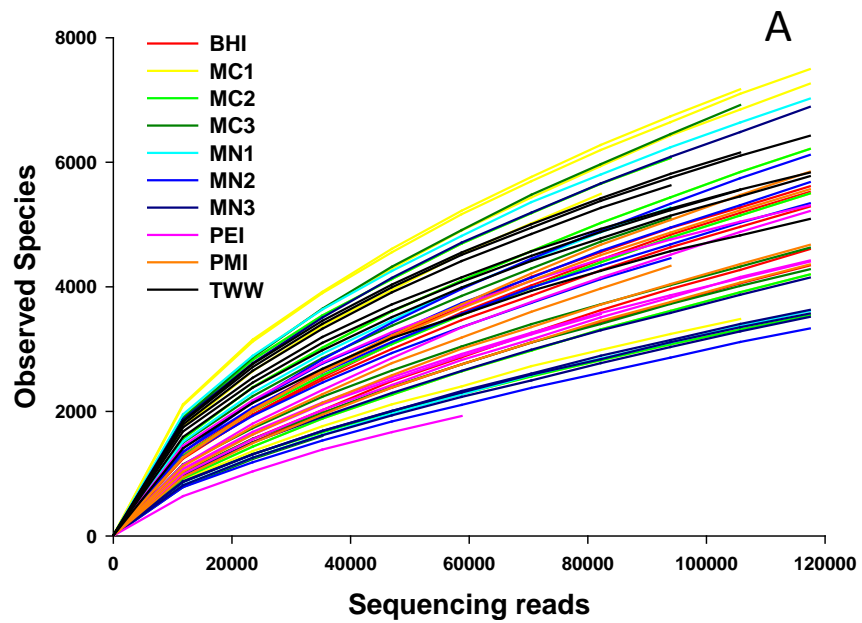


Figure 1. Rarefaction analysis curve of all coastal inlets, outfalls, and wastewater effluents (A) and water and coral tissues at coral reefs (B)

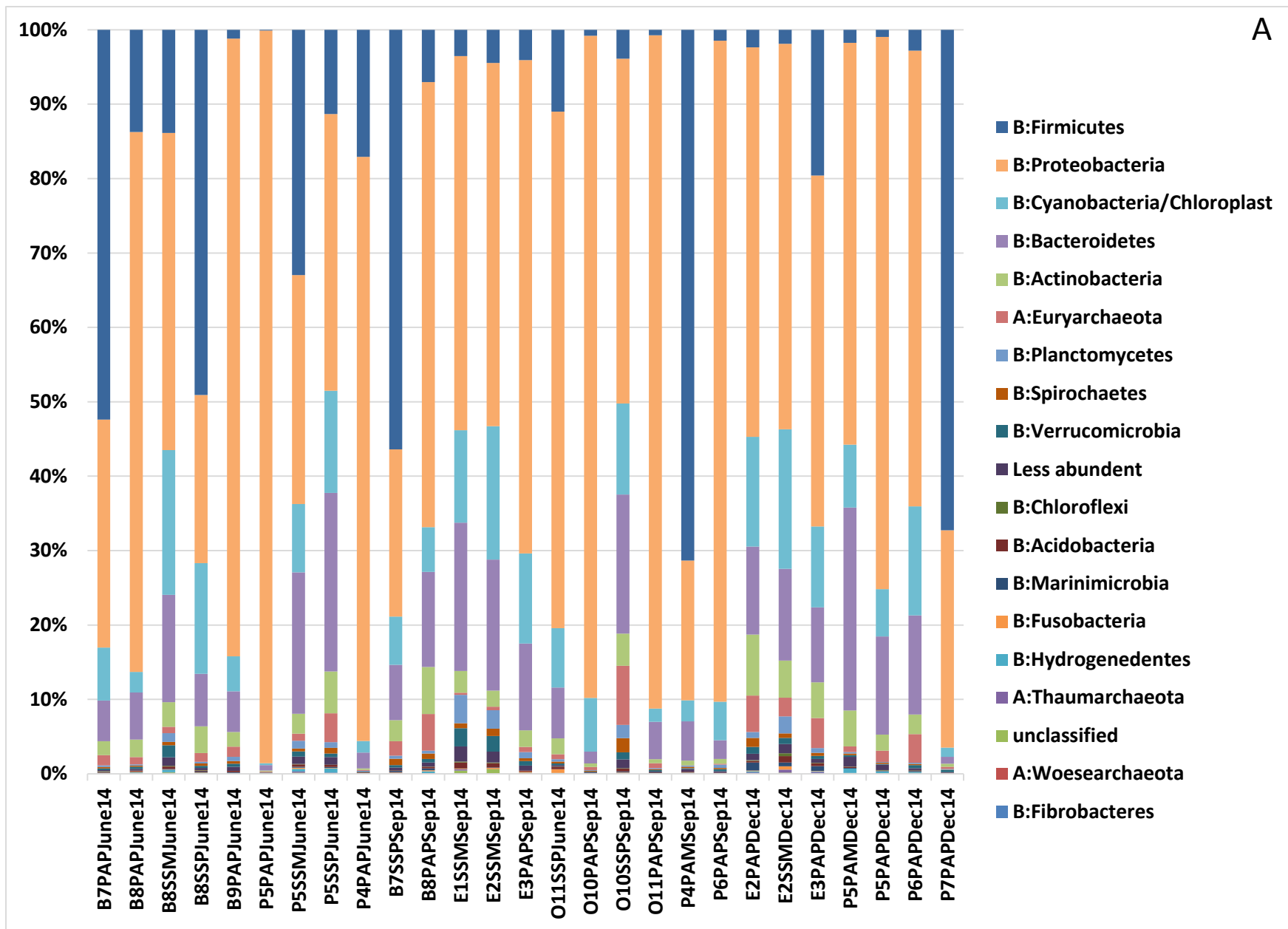


Figure 2. Most abundant phyla identified in among all samples (A. Coral tissues; B. Water at coral reefs ; C. coastal inlets; and D. outfalls and wastewater effluents)

B

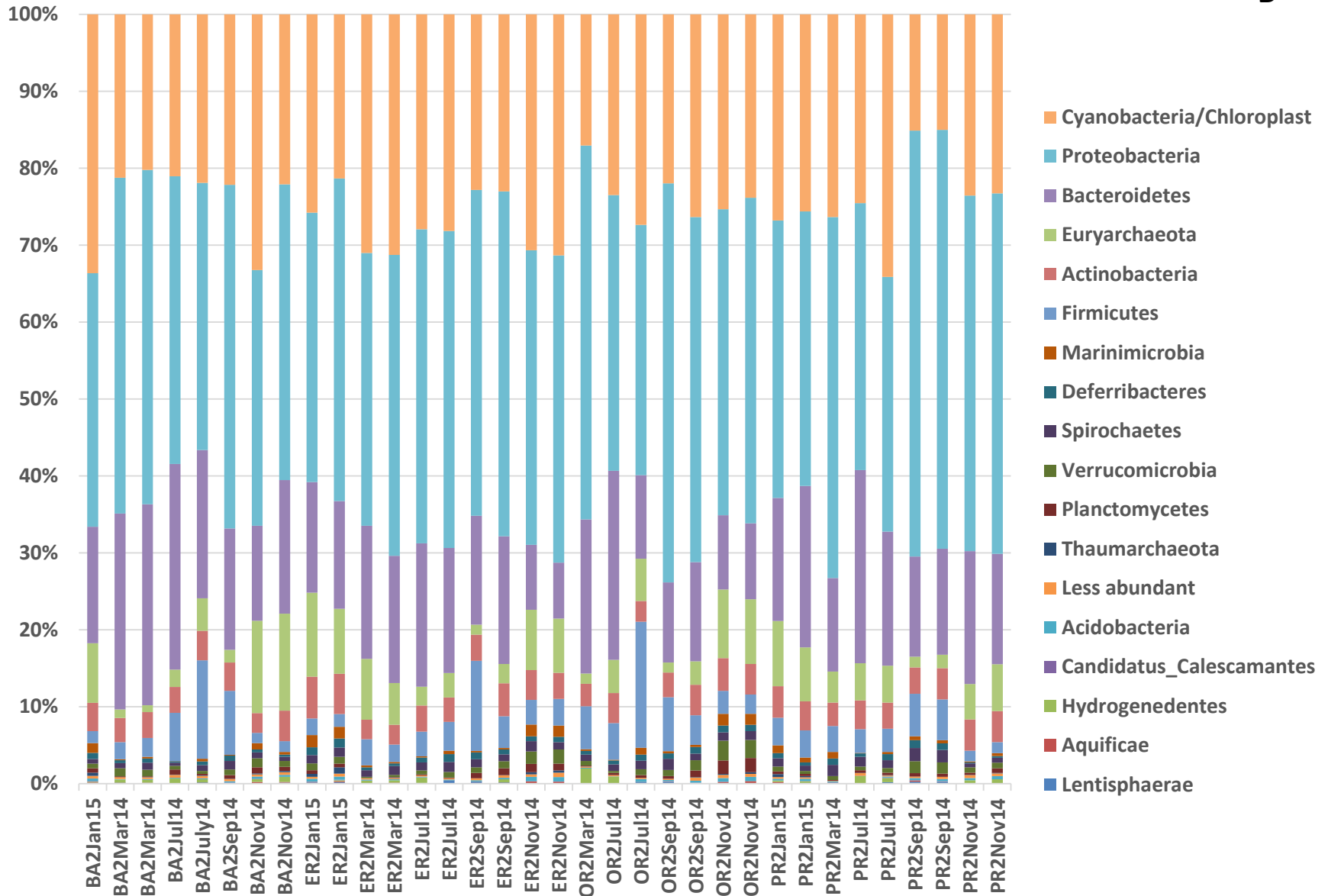


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C

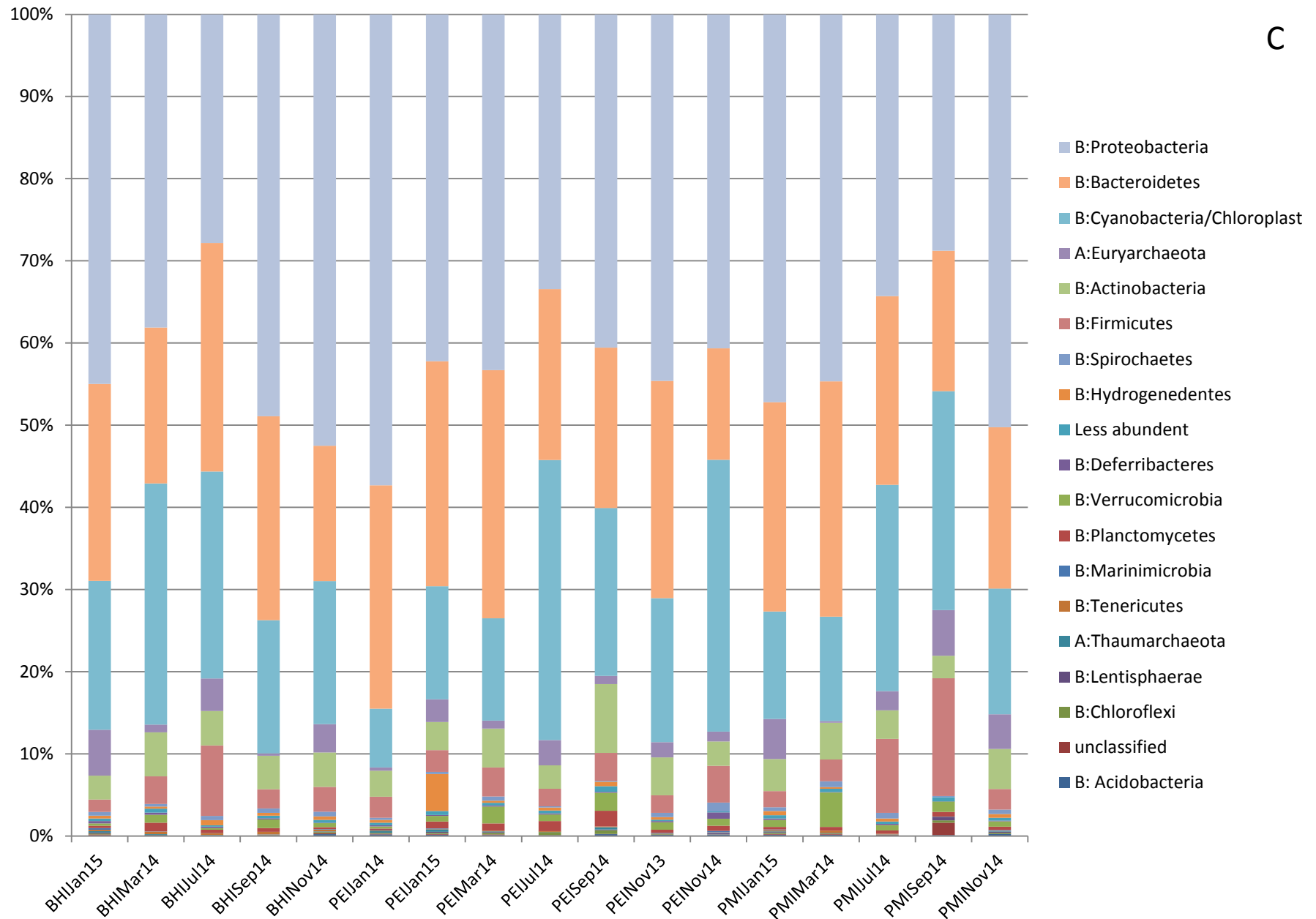


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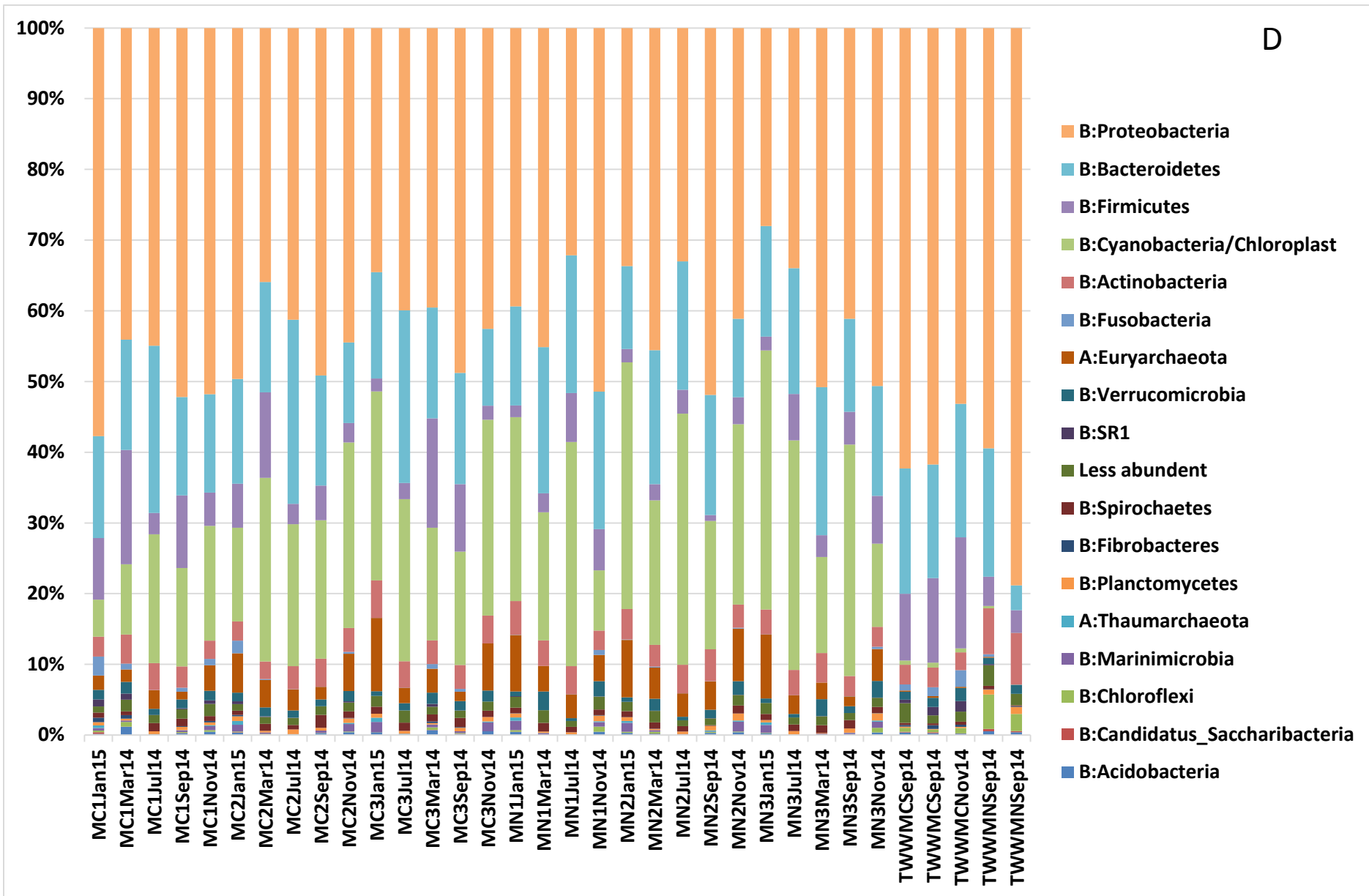


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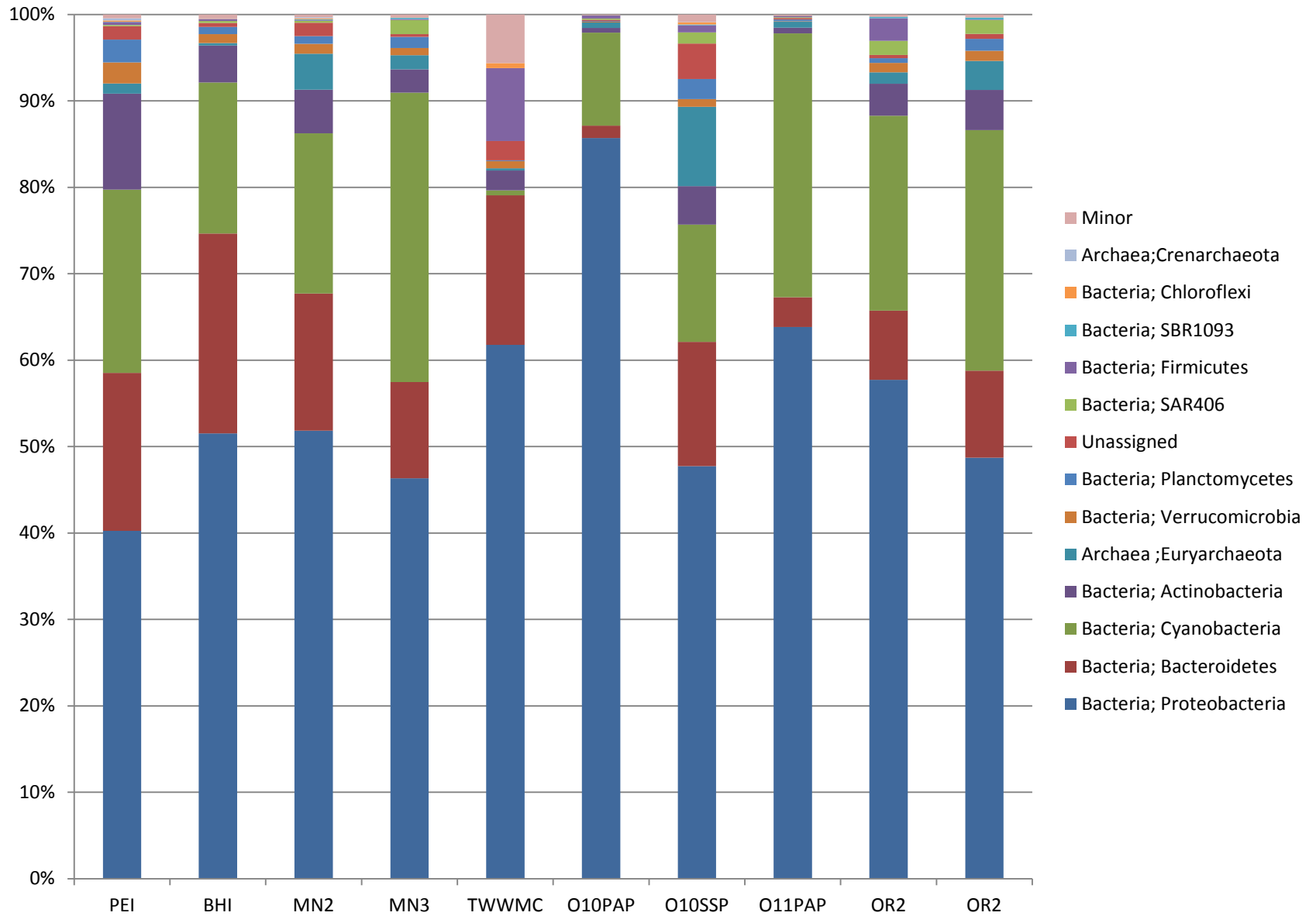


Figure 3. Comparison of microbial composition in phyla level among different types of samples collected in September.

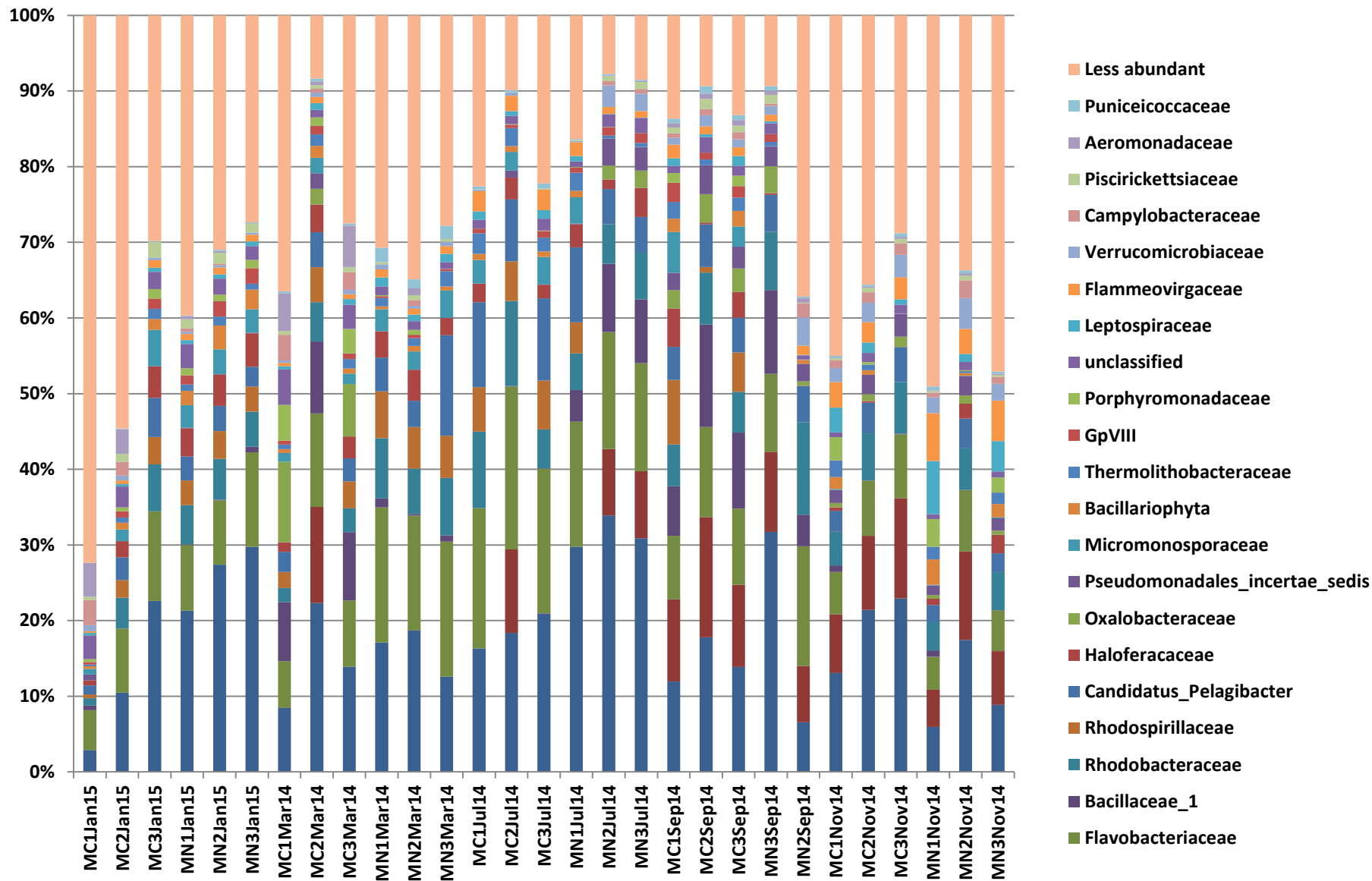


Figure 4. Twenty most abundant families identified among coastal outfalls.

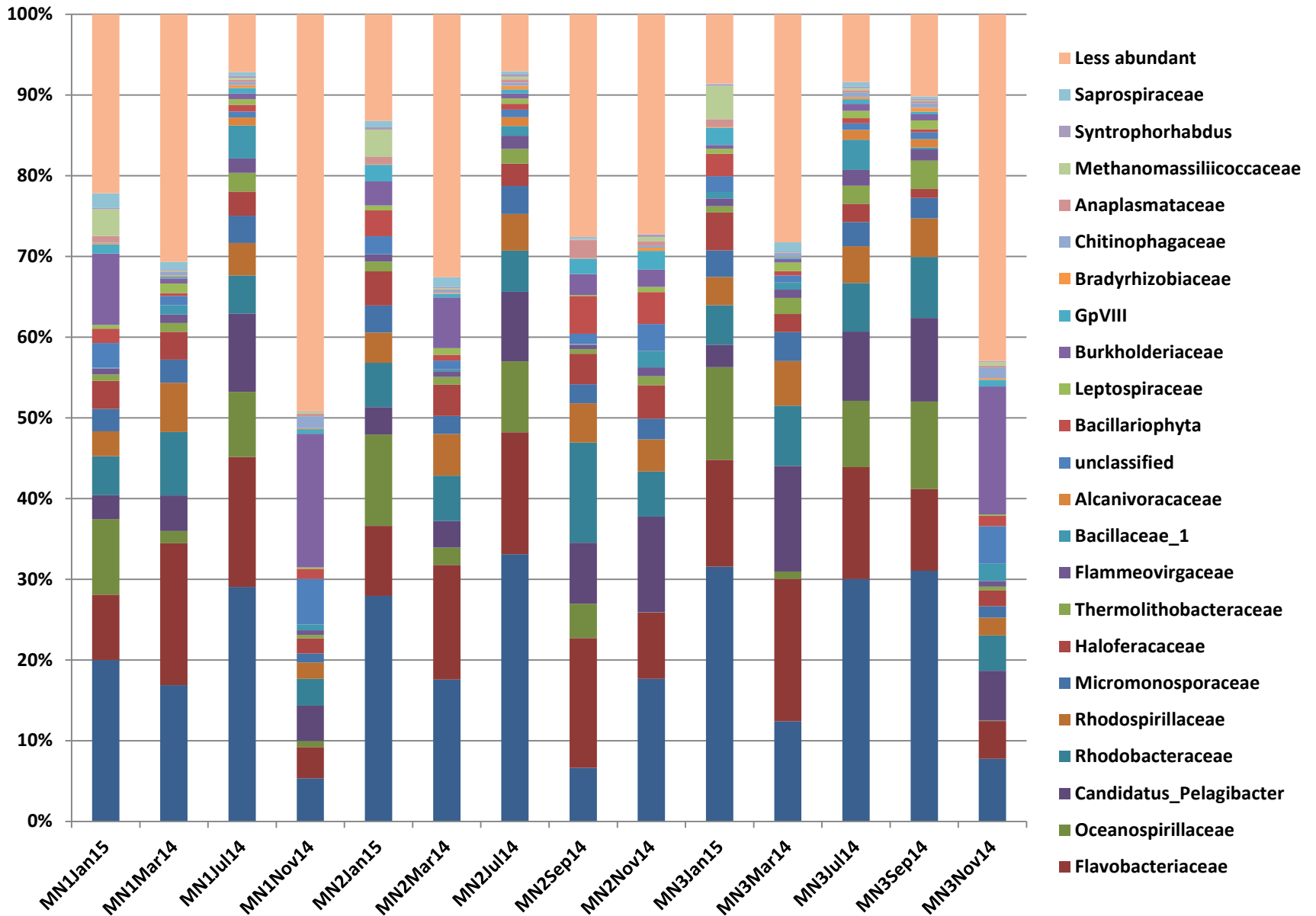


Figure 5A. Most abundant families identified in among Miami North outfalls

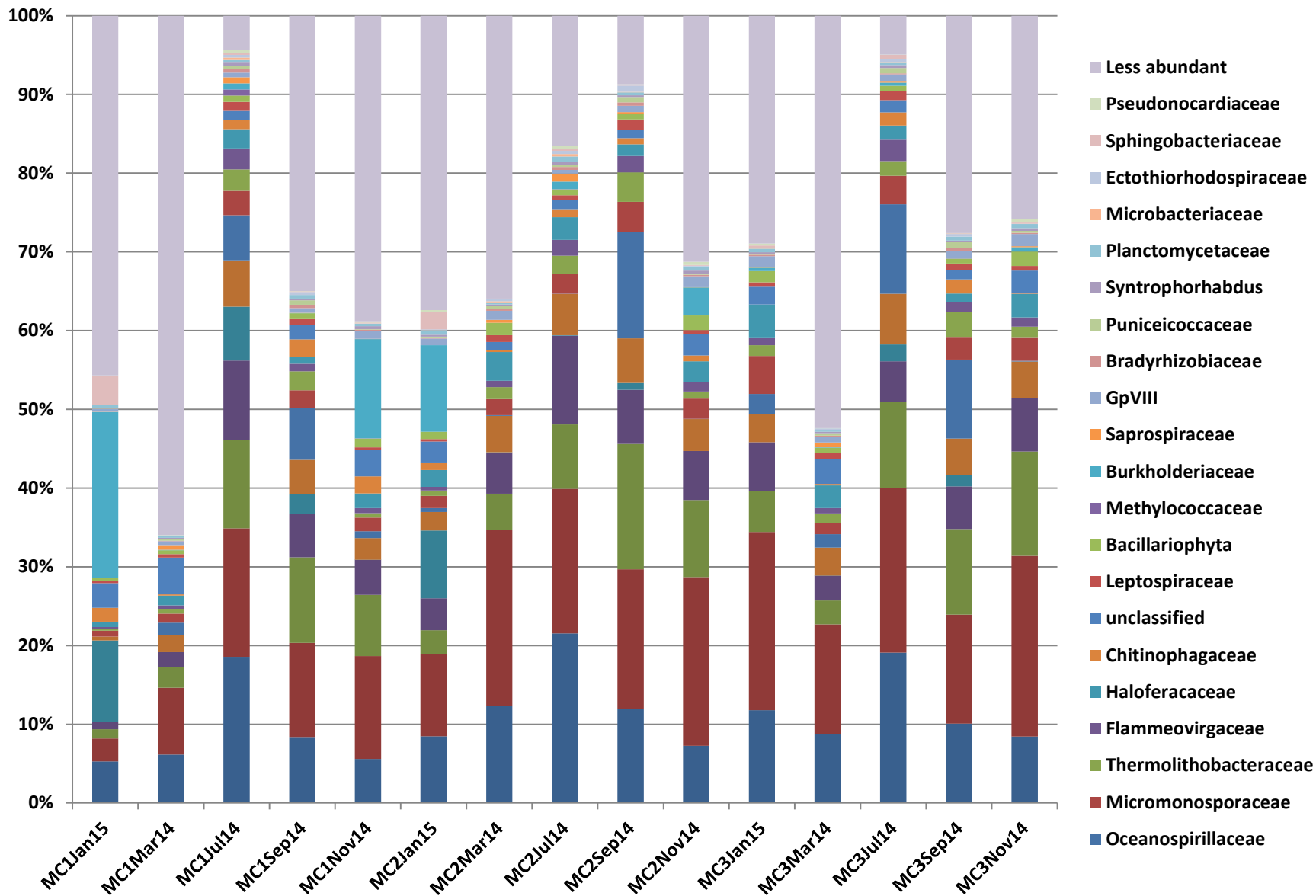


Figure 5B. Most abundant families identified in among Miami Central outfalls

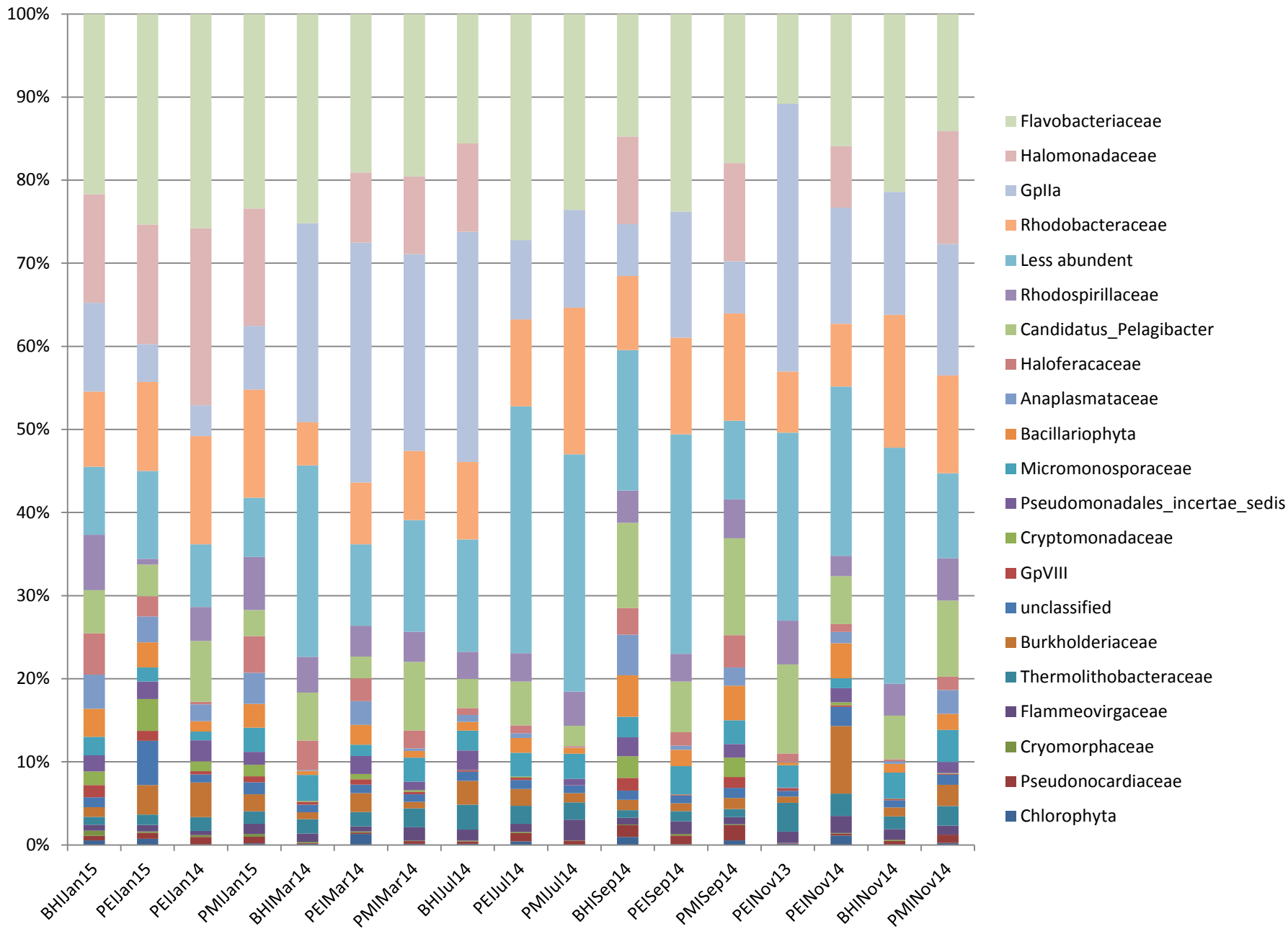


Figure 6. Twenty most abundant families identified among coastal inlets.

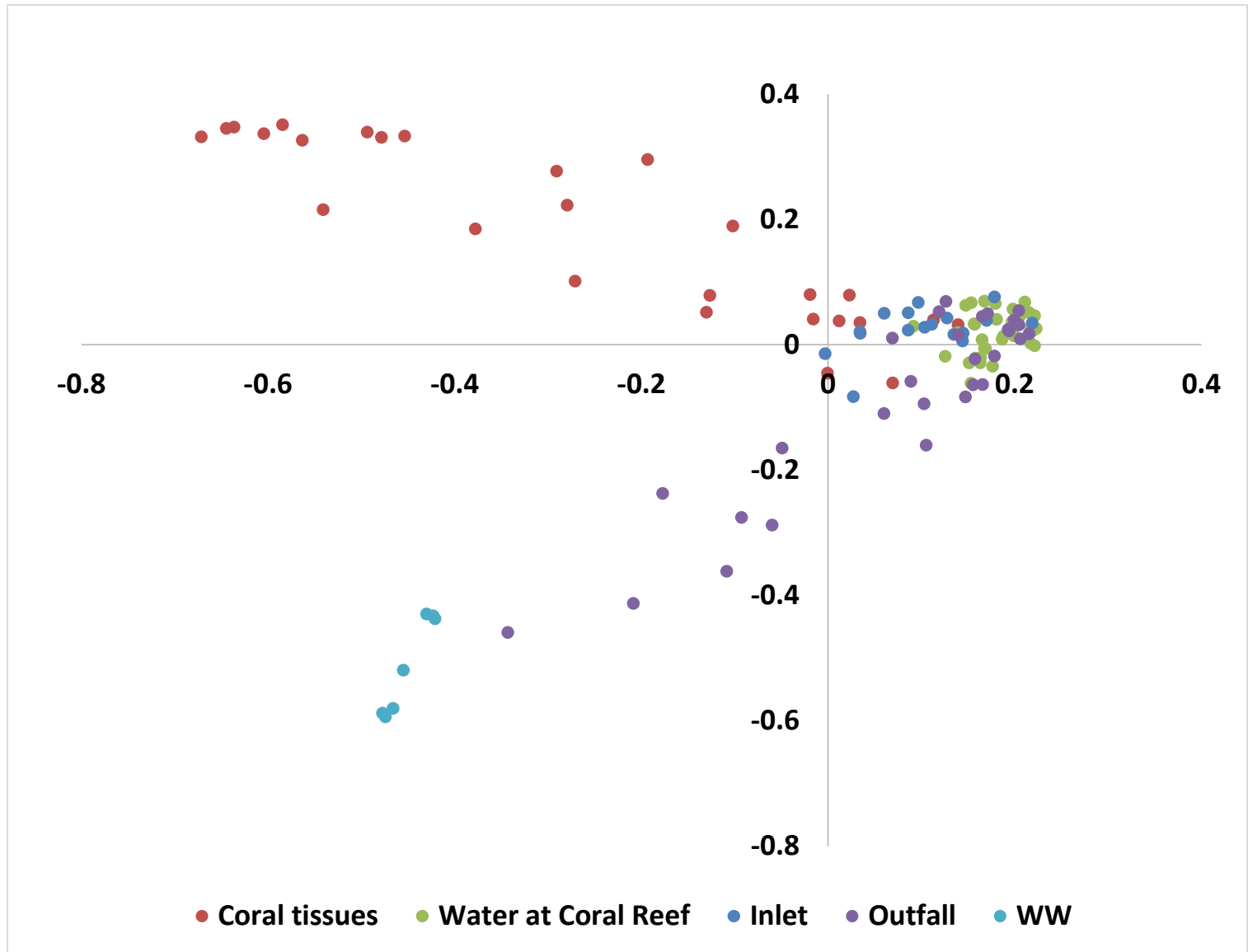


Figure 7. Principal coordinate analysis of community structure. A total of fifteen axis accounted for all differences among samples.

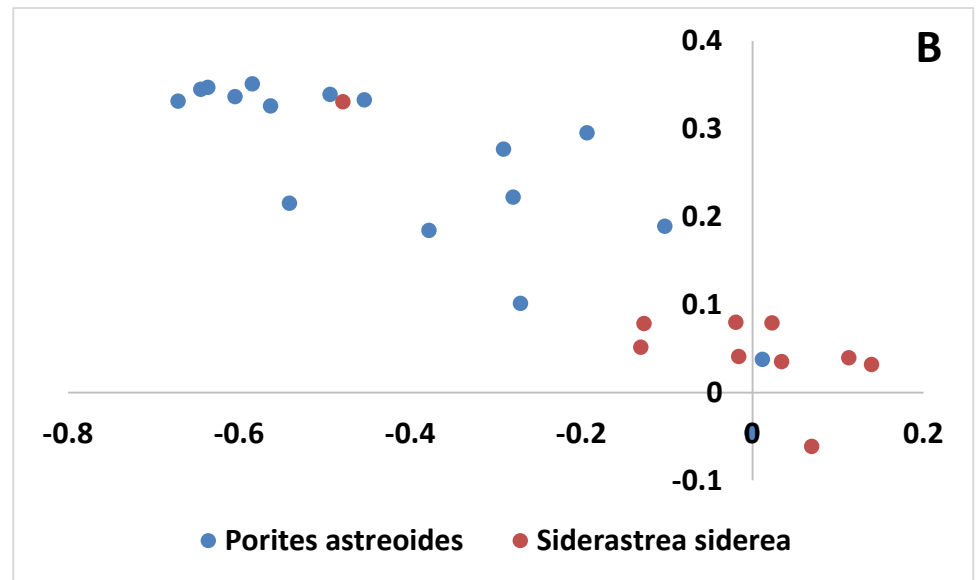
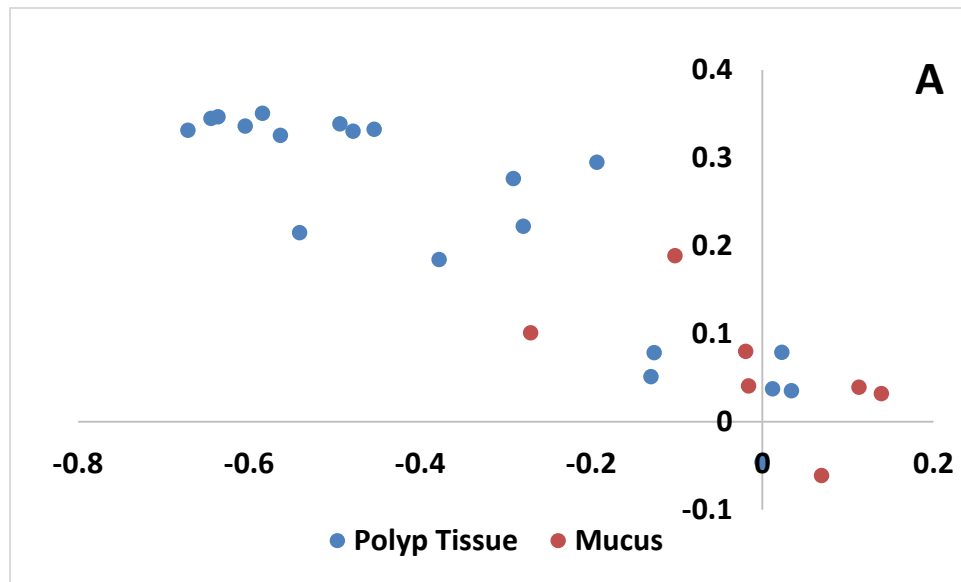


Figure 8. Principal coordinate analysis of community structure in coral tissues by tissue types (A) and by coral species (B).

Preliminary Conclusions so far....

Please note: definitive conclusions are held in reserve until the end of the project after all sampling, sequencing, and data analysis is completed and the entire dataset is available for assessment of status and trends

- Microbial compositions of coral samples and wastewater effluent appear distinct in comparison to those in coastal inlet, outfalls, and water samples at coral reefs.
- Greater variability in microbial community structure among outfall samples was seen among the 346 families identified
- Generally, the microbial community compositions of outfall samples appear to be dependent on season rather than outfall location or distance from outfall. Particularly, the microbial compositions of samples collected in warm season (July and September) were quite similar among them despite sampling location but significantly different from those in cold season (January, March, and November).
- In contrast to outfall samples, the microbial community composition of coastal inlets in the family level has less variability, and does not show the same seasonal effect.

Preliminary Conclusions so far.... (continued)

- The microbial community of coastal inlets and water samples at coral reefs was relatively similar among them regardless of sampling sites and month. Their community structure was significantly different from those of wastewater effluent. In contrast, the microbial community of individual outfall samples varied among them in relation to season and location.
- The microbial community of coral tissues appears to be dependent on coral species (*Porites asteroides* vs *Siderastrea sidereal*) and tissue types (Polyp vs Mucus). Unweighted Unifrac analysis show similar trends, suggesting differences in community structure are driven by shifts in relative abundance of shared OTUs rather than or as well as by presence or absence of particular OTUs.
- Preliminary data so far suggests that microbial community composition of reef waters and corals may be more likely influenced by land based sources of bacterial pollution from coastal inlets and urbanized runoff. When combined with stressors of increasing water temperatures as seen from the metadata of this project and observations of increased bleaching in the vicinity of these coral genomic observatory sites, it suggests that further management attention needs to be paid to the water quality of coastal inlet discharge and urbanized stormwater runoff as at least one set of stressors that might be mitigated in the face of increasing thermal stress to the reefs.