MICROBIAL COMMUNITY DYNAMICS IN SHRIMP *Litopenaeus vannamei* INTEGRATED MULTITROPHIC AQUACULTURE SYSTEMS

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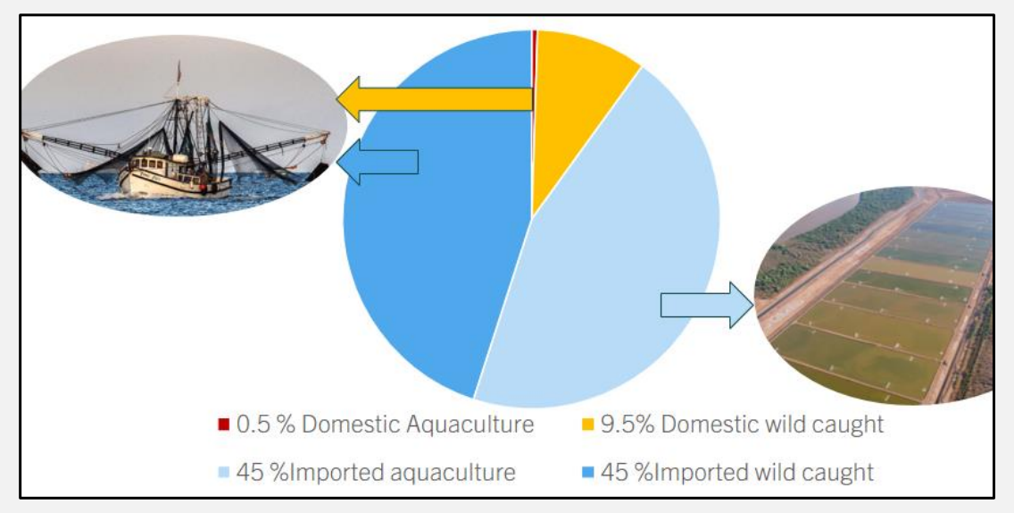
White shrimp (*Litopenaeus vannamei*) were grown in a land-based, integrated multi-trophic aquaculture system (IMTA) at University of New Hampshire's Coastal Marine Lab. Three treatment types were analyzed for nitrogen dynamics over a 4-week period. No biofilter or mechanical filter were used.

Background

Current shrimp aquaculture practices typically involve high-intensity monoculture and large-scale production for maximum revenue. This leads to mangrove habitat destruction, densely populated growing areas and high concentrations of nitrogen produced by animal waste. Shrimp is the highest valued seafood import in the United States, at US $7.8 billion in 2022. The consumption of imported shrimp creates potential for the development of domestic aquaculture practices that utilize oysters and seaweed to remove nitrogen from shrimp wastewater.



Shrimp Consumption in the United States

Study Goals

1. Compare dissolved nitrogen across treatment type and time.
2. Analyze the relationship between microbial community composition and aquaculture treatments.
3. Analyze water samples for *Vibrio spp.* to understand human health implications associated with species and system design.

Study Design- Treatments:

All systems included Red seaweed (*Gracilaria vermiculophylla)* and White shrimp (*Litopenaeus vannamei*). One row of tanks varied by having Oysters (O): Eastern oysters (*Crassostrea virginica*), Air(A): Air stones in place of oysters, or had neither- Settle(SE).

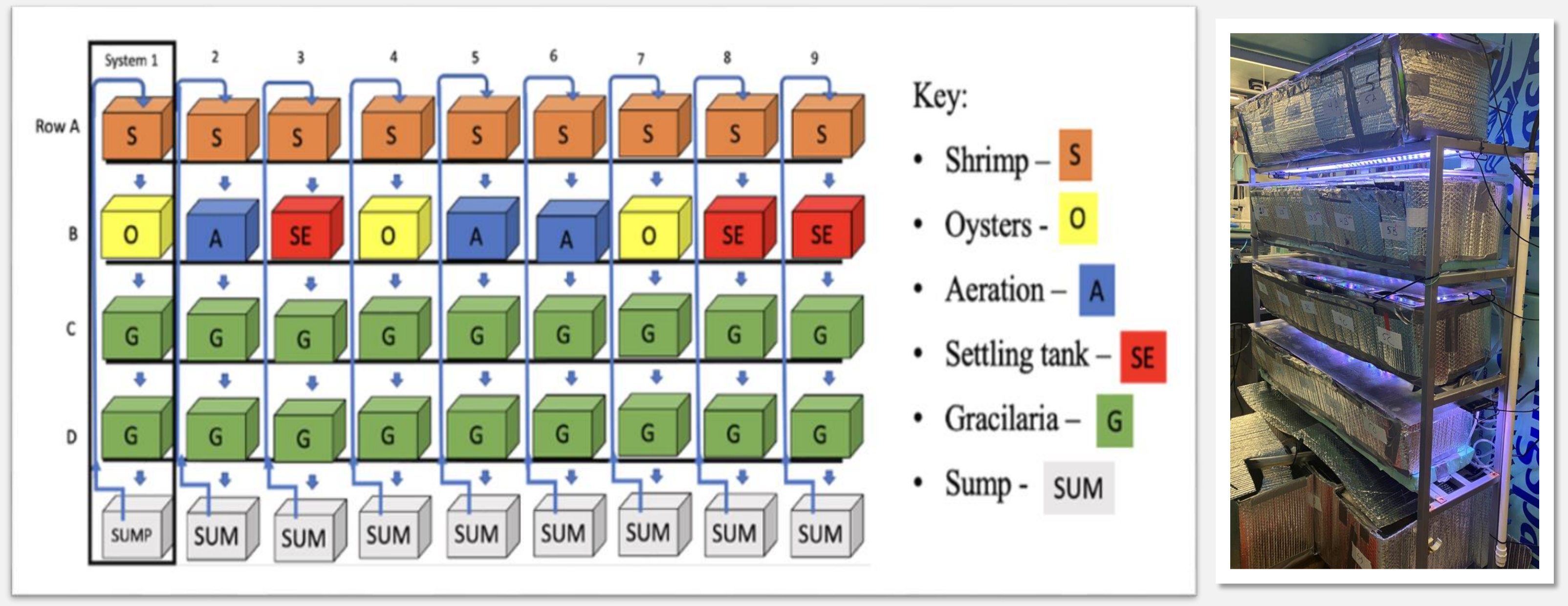


Illustration (left) and photo (right) of the experimental AHAB system used for the land-based IMTA experiment containing *Litopenaeus vannamei, Gracilaria vermiculophylla* and *Crassostrea virginica*

Methods

- Shrimp were fed once per day with Shrimp PL 40-9 1.5mm Ziegler 40 % protein feed. The feed amount was determined by the Zeigler feed chart provided with the feed order (~1.5-3.5 g/day/system).
- Light level in the *Gracilaria* tanks was 60 µmol/m$_2$/s with daylength set to 12:12 light/dark.

Nitrogen

- Water samples for dissolved nitrogen were collected every morning before feeding from water flowing out of row D into the sump.
- Total Ammonia Nitrogen (TAN) and *(NO2+NO3)* samples were processed using the LaMotte Test Kit and a spectramax plate reader.
- Total nitrogen was calculated by taking the sum of TAN and *(NO2+NO3)* for each sampling period.

Microbial

- Water samples for microbial analyses were collected from row B of each system once a week and filtered through a S-Pak 0.47um 47mm wide filter to collect bacteria.
- Illumina sequencing reads were processed in QIIME2 (v2020.2) using Cutadapt for primer removal and DADA2 for quality filtering, denoising, and amplicon sequence variant (ASV) inference.
- Sequence count tables, taxonomic assignments, sample metadata, and phylogenetic information were integrated into a single experiment-level object using the R package *phyloseq* (v1.40.0).
- Taxonomy for 16S rRNA gene libraries were assigned at the genus level and *Vibrio*-centric *hsp60* libraries were classified to the species level.
- Differential abundance and variability analyses were performed on non-normalized count data.
- Comparisons were conducted across treatments and sampling dates
- Differentially abundant taxa between groups were further evaluated as biomarkers using linear discriminant analysis of effect size (LEfSe)
- Indicator species analyses were conducted by transforming non-normalized count data to presence–absence matrices and applying the *indicspecies* R package to identify taxa.

Shifts in the microbial communities were examined over the 4-week trial as a function of treatment type and time.

Results

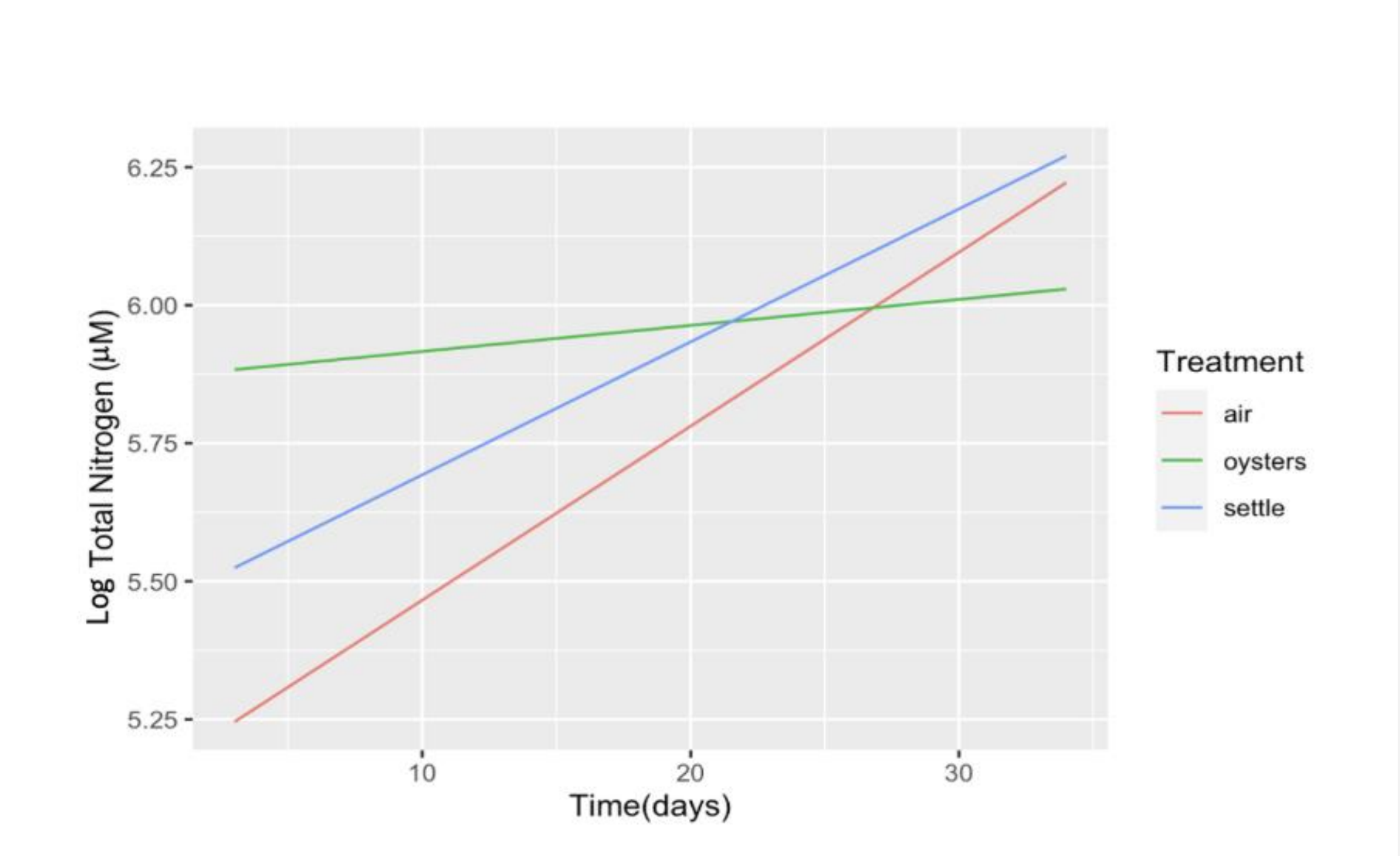


Figure 1: Log total nitrogen as a function of time.
Oysters had a significant impact on maintaining low nitrogen levels in the system compared to treatments with no oysters ($p < 0.032$).

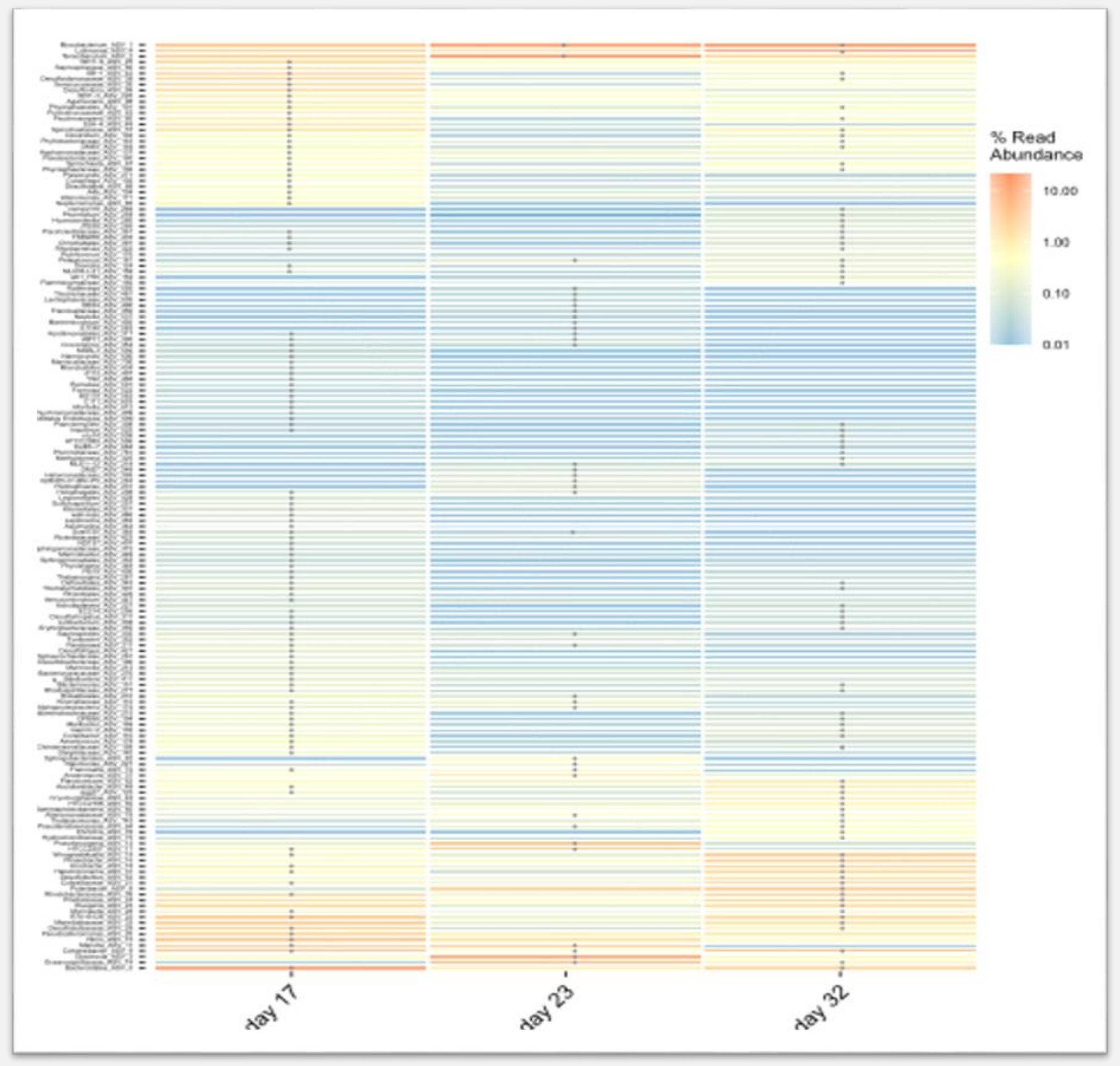


Figure 2. Relative abundance of differentially abundant genera across IMTA systems on day 17, 23, and 32). The days with significantly higher abundance of certain genera are marked by a grey circle. 23% of community members showed differential abundance over time, with many genera trended downward, particularly those involved in carbon cycling, nitrogen cycling, sulfur cycling, environmental adaptation, and pathogenesis. These temporal patterns offer insights into ecosystem disturbances and the functional dynamics of microbial communities.

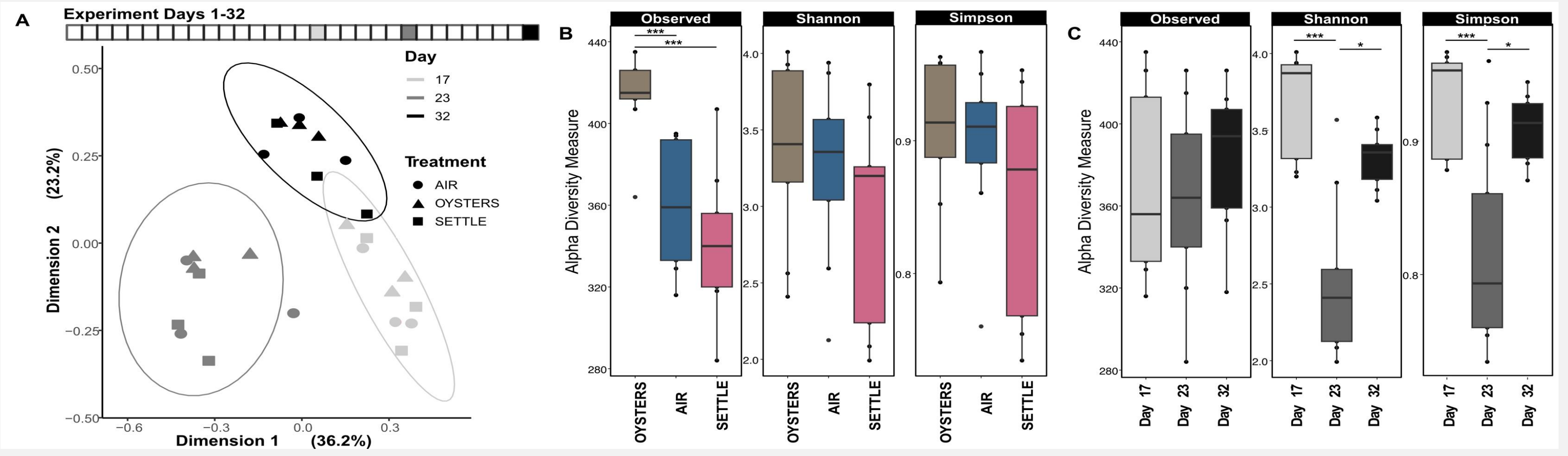


Figure 3: Schematic Summary of IMTA Systems and Community Compositions
(**A**) Beta-diversity of microbiome communities, analyzed using Bray-Curtis dissimilarity and visualized through principal coordinate analysis (PCoA), *revealed distinct clustering by sample collection day (light grey, grey, and black points), independent of treatment type (depicted by shape)*. (**B**) Alpha-diversity metrics (Observed, Shannon, and Simpson indices) showed that *systems with oyster treatments exhibited greater observed diversity, or unique membership, compared to air or settle treatments*. (**C**) Temporal trends in alpha-diversity metrics indicated a significant decrease in Shannon and Simpson indices on day 23, *suggesting a potential environmental stressor or disturbance that was alleviated by day 32.*

Microbial communities shifted across treatment type and system age.

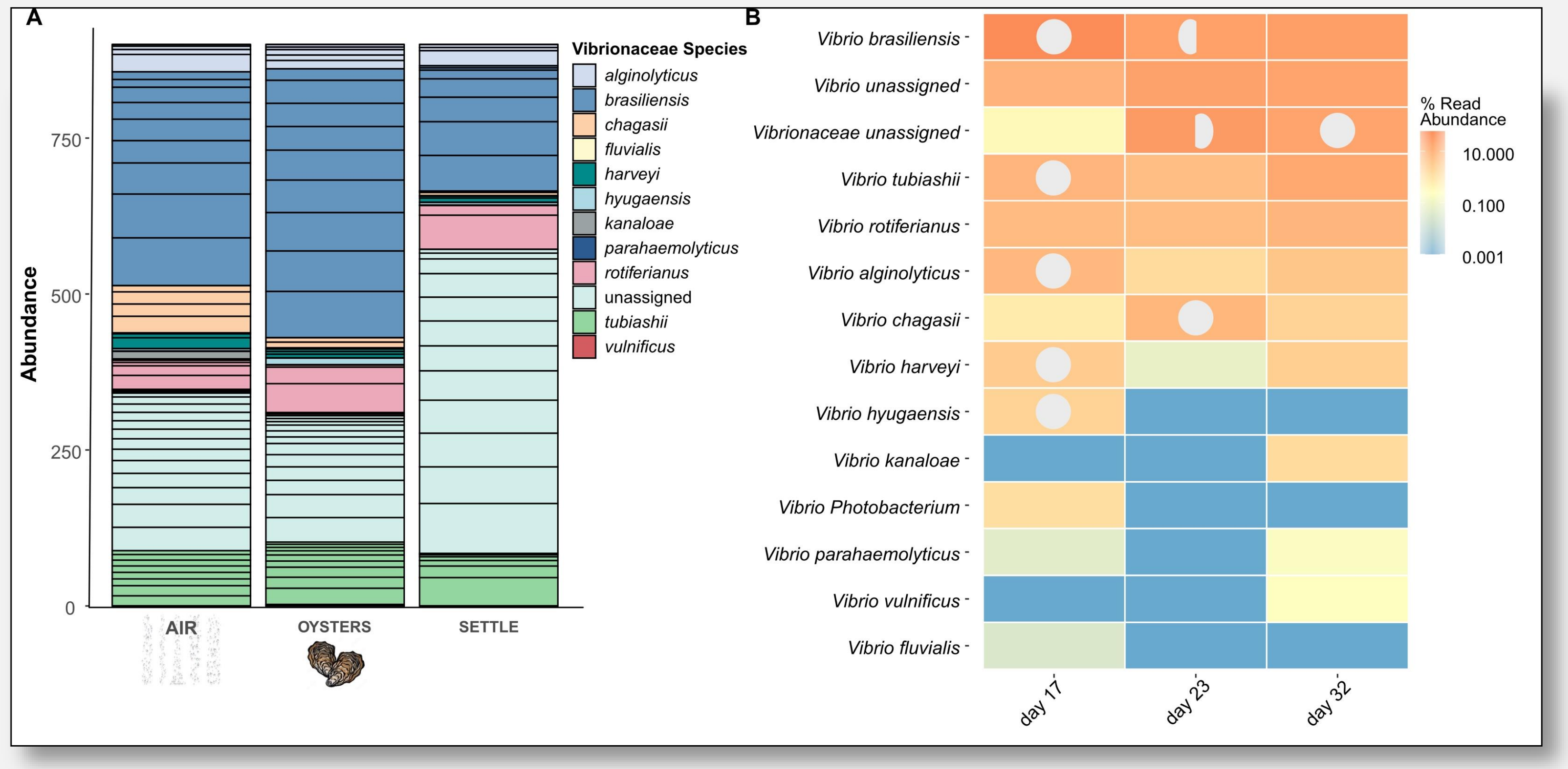


Figure 4: Vibrionaceae taxonomic composition of microbial communities across sample groups and temporal abundance patterns. A) The relative abundance of Vibrionaceae at the species level in IMTA systems with air, oysters, or settling tanks. **B**) The relative abundance of Vibrionaceae on days 17, 23 and 32. Circle indicates higher abundance when compared the two other days, partial circle means abundance is greater compared to one sampling day (cut circle in direction of which day).

Conclusions

The nitrogen concentration in the oyster treatment (Fig. 1) increased at a significantly slower rate than the air treatment, which contained an air stone but no oysters. This finding suggests that oysters played a key role in mitigating the increase in nitrogen concentration during the experiment. Labrie et al., (2023) additionally found that areas of an estuary with oysters had an increased capacity to remove nitrogen despite their production of TAN as a waste product (Labrie et al., 2023).

The findings of this study highlight the significant impact of system treatments—namely aeration, oysters, and settling tanks—and temporal dynamics on the of microbial communities in shrimp aquaculture systems (Figs. 2 & 3). Across the three treatment types, microbial community composition varied substantially, with distinct patterns of differential abundance observed over time. Notably, aeration and oyster-based systems were associated with higher microbial diversity and a greater number of biomarkers, suggesting that these treatments promote a more dynamic microbial environment compared to settling tanks.

Interestingly, the temporal patterns observed in Vibrio species were also strongly influenced by system age. While Vibrio populations were diverse across all treatments (Figure 4A), certain species, such as *Vibrio tubiashi*, were more abundant in systems with oysters and settling tanks. These findings suggest that Vibrio populations may be shaped not only by system treatment but also by the maturation of the aquaculture environment (Fig. 4B).

Several nitrogen-associated taxa exhibited consistent trends in abundance. Some genera, including *Rhodospirillaceae*, *Hyphomicrobiaceae*, *Ruegeria*, and *Planctomycetes*, declined steadily from day 17 through day 32, while a smaller subset, including Pseudanabaenales and Pseudoruegeria, increased over the same period. This reflects the persistent shifts in nitrogen availability and cycling in the system. The recovery of transient taxa alongside the consistent upward and downward trends of others indicates that the IMTA microbiome exhibits both resilience and successional restructuring.

In conclusion, the temporal dynamics of microbial communities, particularly regarding nitrogen cycling and Vibrio populations, emphasize the importance of system age and treatment conditions in influencing aquaculture ecosystem functions. Our results suggest that systems with oysters and aeration are more capable of supporting diverse and active microbial communities.