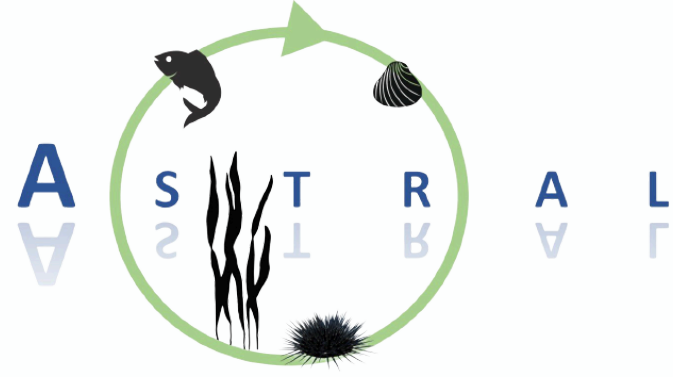


UNDER THE ASTRAL EU PROJECT: MICROBIOME STUDY OF SPECIES IN INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM AT DIFFERENT RECIRCULATION RATES IN SOUTH AFRICA

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INTRODUCTION

Bufflejags Abalone farm is a commercial **land-based IMTA farm** in South Africa, that consistently **recirculates 50%** of the effluent water by using the bioremediation capacity of green seaweed (*Ulva lacunculata*). This saves on pumping costs, provides feed for abalone and reduces nutrient release into the environment. **Increasing recirculation to 75% (long-term) and 100% (short-term)** could further increase circularity and help protect the farm from harmful pathogens. Impacts on the microbiome, role and dynamics of the system remain unknown. **Our aim is to understand the impact of increasing recirculation on the system's microbiome**

METHODS

- We studied IMTA platforms, each comprising **three clusters** with one *Ulva* paddle-raceway and multiple abalone tanks per cluster. Samples of **abalone gut, *Ulva*, inlet, and outlet** water were collected at **50% and 75% recirculation** for a month, and at **100% recirculation** for four days.
- Microbial characterization was conducted through **16S rRNA sequencing of regions V3-V4**.
- Bioinformatic analyses** were performed using **R studio** with the **microeco** and **phyloseq** packages.

RESULTS

Principal component analysis (**PCoA**) of beta diversity revealed that increasing **recirculation impacts** microbial communities differently **based on sample type**. Inlet and outlet samples exhibited significant shifts, while *Ulva* samples showed subtle changes over time. Abalone gut samples remained relatively stable regardless of increasing recirculation levels.

Species richness composition studies showed how increasing recirculation affected the different samples. **Both inlet and outlet samples exhibited a decline in alpha diversity** (Shannon index), indicative of a more specialized community. *Ulva* samples followed a similar trend, though less pronounced. Conversely, abalone samples displayed a slight increase in alpha diversity with recirculation.

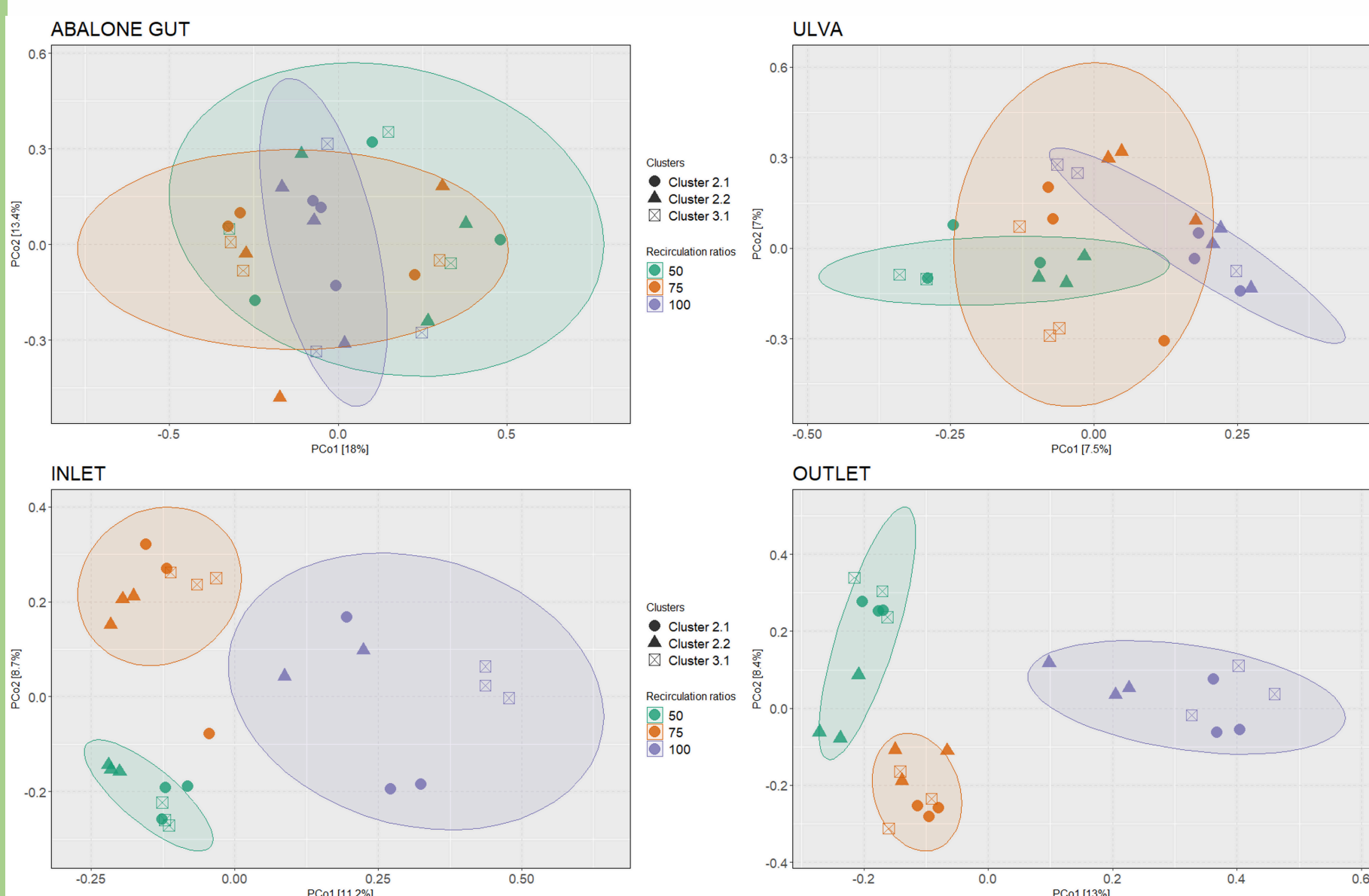


Figure X. Beta diversity Principal Coordinate Analysis (PCoA) of Bray-Curtis index. Plots were divided by sample type: Abalone gut, *Ulva*, inlet and outlet samples. Samples were coloured by recirculation and shaped by cluster.

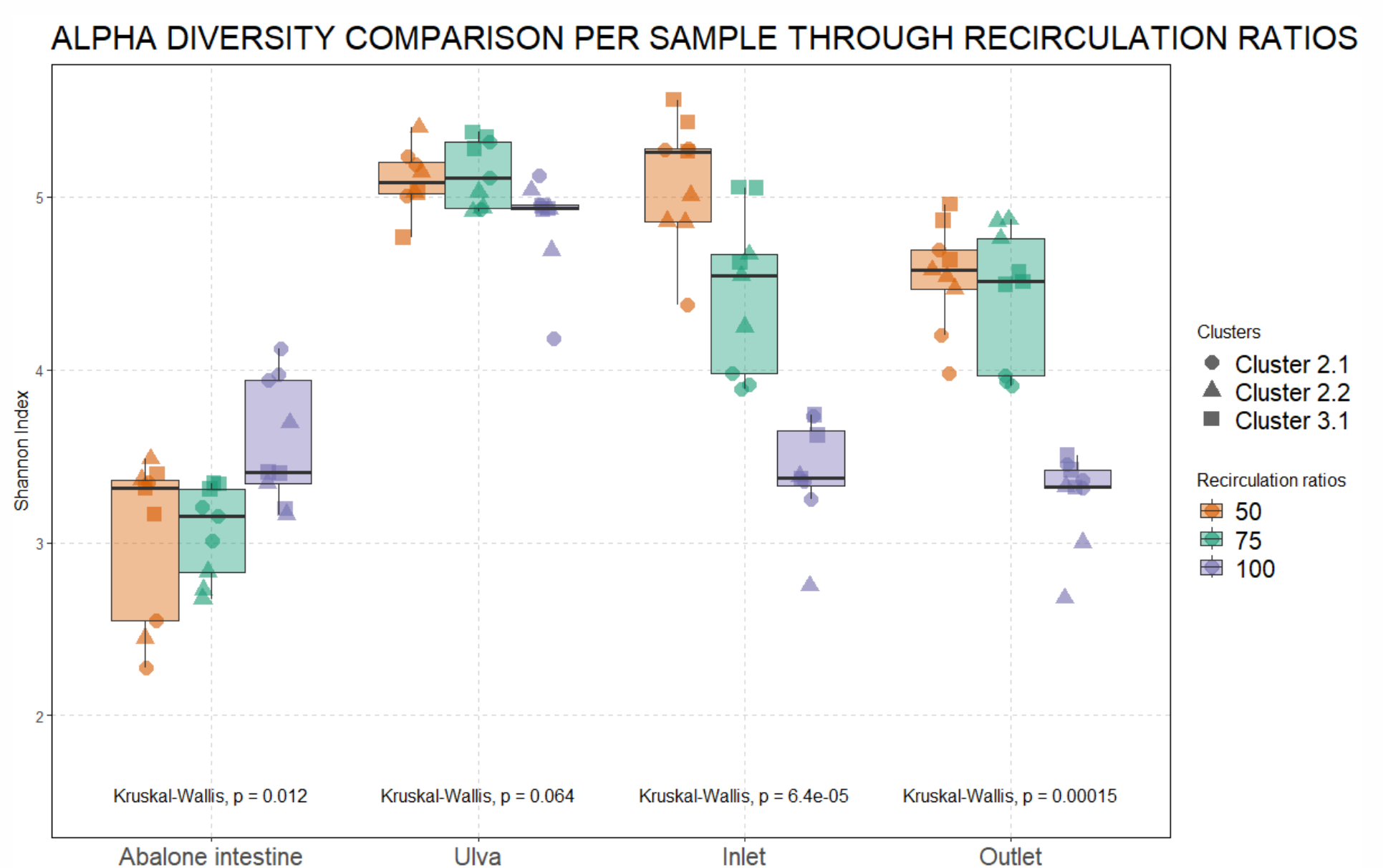


Figure Y. Alpha diversity plot with Shannon Index. Plots were divided by sample type: Abalone gut, *Ulva*, inlet and outlet samples. Samples were coloured by recirculation and shaped by cluster.

Analysis of differential genera revealed potential interrelationships between sample types. The increase in ***Glaciecola*** within the *Ulva* microbiome suggests a potential role for this genus in modulatory **interactions in the surrounding environment**. Moreover, the presence of ***Planctomycetes*** in both abalone gut and *Ulva* samples indicates potential interspecies interactions. Despite observing no direct correlation between *Vibrio* abundance and recirculation, the system demonstrated **resilience to dysbiosis under increased recirculation conditions**.

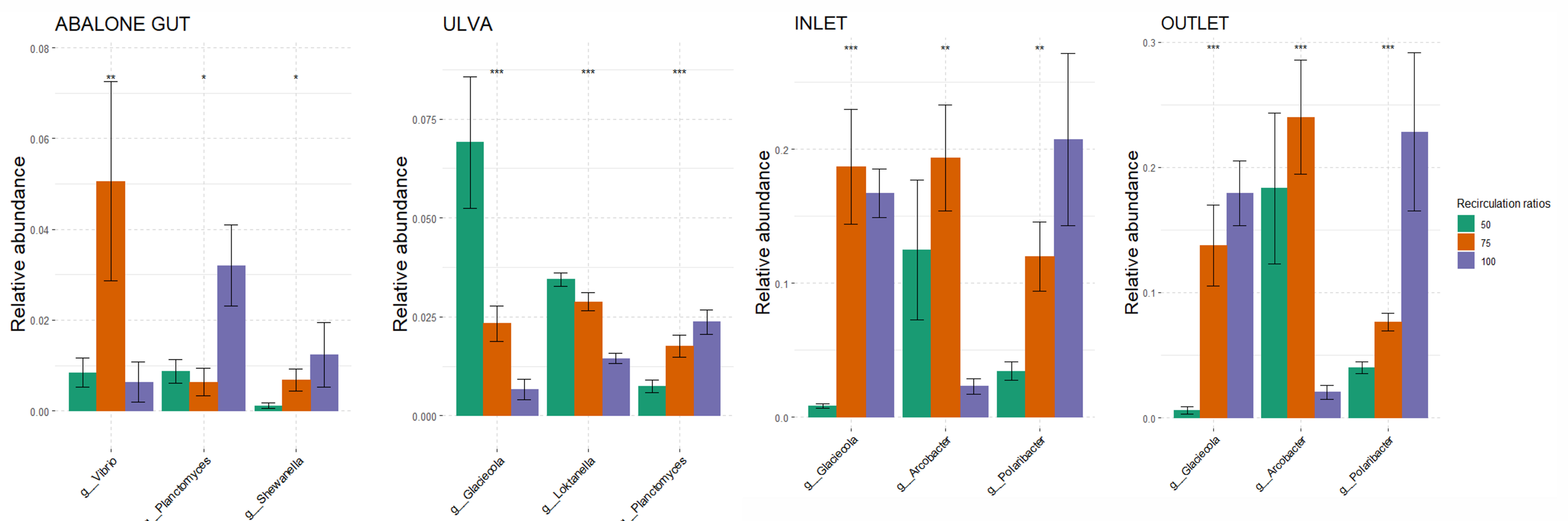


Fig Z. Differential genera per samples through increasing recirculation levels (50, 75, 100). Calculated with *lefse*, showing significant differences between abundances.

CONCLUSIONS

- Recirculation differentially impacted microbial communities, with inlet and outlet showing significant changes, *Ulva* showing slight changes, and abalone gut minimally.
- Ulva* microbiome influences surrounding environment through genera like *Glaciecola*, potentially mediating interactions with other components of the surrounding water's ecosystem.
- Implement microbiome analysis for pathogen detection and early warning systems using metagenomics to species level.



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