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# Biobanking Media Affects the Microbiome Diversity of Kelp Gametophytes

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# Abstract

Kelp canopy resilience is partly influenced by their microbial partners, yet the stability of these associations in biobank-stored germplasm is unknown. We investigated how storage duration and media composition drive changes in the microbiome of *Macrocystis pyrifera* gametophytes. Our data suggest a shift in microbiome composition, characterized by a decline in overall microbial biodiversity. Media type significantly modulated this effect, with L1 formulation better preserving a diverse community structure than F/2 formulation. Our work here indicates that biobanking practices inadvertently select for specific microbial consortia, with implications for the viability and physiological function of preserved kelp germplasm.


# BACKGROUND

Kelp gametophytes are microscopic, free-living stages ideally suited for long-term storage in biobanks, which are essential for aquaculture, restoration, and conservation [1, 2]. Wild kelp microbiomes shift in response to environmental fluctuations [3], but the trajectory of these changes under stable, artificial biobank conditions remains unclear and understudied. Specifically, the combined effect of storage duration and media formulation on microbiome composition has not been systematically tracked. Standard practice involves maintaining gametophytes in nutrient-enriched media under controlled light, nutrients and temperature. There are no global standard procedures for gametophyte biobanking [4], and different types of media are currently in use, most popularly PES [5] and F/2 [6, 7]. Long-term storage in PES has shown to cause a reduction in native microbial community richness in *Macrocystis pyrifera* gametophytes and shifts the dominant taxon from *Parasphingorhabdus* (Sphingomonas) to *Stutzerimonas (*Pseudomonas) over time (Bernie Tolentino, personal communication). Skewing of the microbiome may favor copiotrophs in PES media due to the possible oxidation of β-glycerophosphate into glycerol and phosphate during storage [8, 9]. Here, we advance this line of inquiry and document gametophyte microbiome shifts across times through different media types. We compared F/2, the derivative of PES against L1, a formulation that contains additional trace metals and no external sugars. We hypothesize that storage media type drives successional changes in the associated bacterial community over time. Uncovering these dynamics is vital for developing biobanking protocols that preserve not only the host, but also its functional microbiome.

# METHODS

*M. pyrifera* sporophytes were collected from Leo Carrillo (coordinates 34°2'40"N 118°56'2"W), on low tide, under an emergency permit (CDFW) from coastal areas affected by the 2025 Palisades fire runoff (Los Angeles, CA USA). Dark, sorus (reproductive) tissue was collected from sporophylls. Zoospore release was induced by a three-step process: cleaning with a 1% iodine solution to remove epiphytes, drying it in napkins, and then rehydrating it in filtered seawater [10]. Following spore release, the resulting gametophytes were cultured across two media treatments (n = 6), in normal conditions (11C, red light (~ 5 µmol $m^{-2}s^{-1}$)): F/2 and L1 medium to minimize exogenous carbon and selectively favor kelp-derived

bacteria. Si was omitted from both treatments, and $GeO_2$ (0.5 mg/L) was added to discourage diatom growth. After a growth period of 3 months in 6-well plates, biomass from each treatment was split for parallel biobank preservation with media replenishment once every six months for minimal gametophyte maintenance, for a total of 18 months.

Sample DNA was extracted using the NucleoSpin Plant II DNA kit (Macherey-Nagel, USA), according to manufacturer protocol, with a modification in the homogenization step: instead, samples were placed in 200 µL lysis solution PL1, followed by bead-beating with 0.2–0.5 mm Si/Zir beads in a bead-mill for 4.0 m/s, for 1 cycle of 1:30. Sequencing and amplification of the V5-V7 region of the 16S rDNA gene was done using the endophytic 799F and 1193R primer set [11] at Novogene (Beijing, China) using the Illumina NovaSeq 6000 platform, resulting in 250-bp paired-end reads. Paired end reads were then demultiplexed by the sequencing facility and quality checked with FastQC [12].

Sequencing resulted in 1.06M total reads, averaging about 205k reads per sample, prior to processing. All analysis was conducted using R [13]. Amplicon sequence variants were generated in DADA2 [14] using default parameters, with a modification for the FilterandTrim function (truncLen = 220,200), given the length of our endophytic primers. Taxonomy was assigned using the SILVA SSU v138.1 database (DOI: 10.5281/zenodo.4587955). Chloroplast, mitochondrial removal and data analysis was done using Phyloseq [15]. Zero count sequences were removed along with reads that did not occur in at least 3x in 17% of the total samples and the data was rarefied to 112, 013 reads. Differential abundance analysis (Wilcox test and FDR adjustments) was conducted using Metacoder [16]. Statistical analysis to determine alpha diversity was done using vegan [17]. Additional visualizations were conducted in ggplot2 [18].

## RESULTS

# L1 favors the rare microbiome, promoting maintenance of higher bacterial diversity

Alpha-diversity analysis (Fig. 1) utilizing the Chao1 metric revealed that L1-maintained samples harbored significantly higher species richness compared to treatment F/2 ($p = 9.37 e-05$, one-way ANOVA). However, the Shannon index did not differ between groups ($p = 0.61$, one-way ANOVA), indicating that while L1 contains more total taxa, the distribution of abundances is similar between conditions. To compare taxa (order and genera) between L1 and F/2 media, relative abundances were visualized as a heatmap (Fig. 2). Higher taxon richness was observed in L1 samples, while F/2 samples showed a greater reduction in taxa, they also supported a microbial community compositionally distinct from that of L1. At the order/family levels, F/2 displayed high abundances of Enterobacterales and Rhodobacterales (*Sulfitobacter*, *Tateyamaria)* as well as members of the Alteromonodales (*Pseudoalteromonas*) and Oceanospirillales (*Cobetia*, *Alcanivorax*). In contrast, L1 maintained an additional 15 more orders, including bacteria previously marked as potential Seaweed Beneficial

Microbes [19], specifically those from the Flavobacteriales (*Owenweeksia, Vicingus*, *Muricauda*), Actinomycetes (*Micrococcales)* and Rhodobacterales (also seen in F/2 media).

The increase in species richness without a corresponding change in Shannon diversity or order-level community composition indicates that L1 media selectively promotes the rare microbiome, those low-abundance microorganisms that are often ecologically important but numerically minor [20, 21]. This pattern is consistent with studies showing that nutrient-rich or complex media can support greater taxonomic diversity by allowing rare taxa to persist, while the dominant community framework remains resistant to change [22–24].

It is also worthwhile to explore whether alpha diversity and differential abundance in samples may vary due to interaction and effects surrounding other factors, like sex or “tank effects”. For example, the outlier sample in our F/2 samples was a male (sample ID= G24) gametophyte, which showed a different microbiome than our F/2 female gametophytes, an effect that may be enhanced by media type maintenance. However, we did not notice a similar trend in our L1 male gametophyte (sample ID= G17), where a microbiome composition was consistent, regardless of sex.

Given the small sample size (n = 3 per group) of our exploratory study, we emphasize effect sizes (log2 fold change) over statistical significance. Differential abundance analysis between L1 and F2 media types identified no taxa with statistically significant differences after FDR correction (FDR < 0.05), likely due to the limited sample size (DOI: 10.5281/zenodo.21926483). However, examination of the top 25 taxa by log2 fold change revealed substantial biological variation between the two conditions. The most enriched taxa in L1 media are shown in Fig. 3, while taxa depleted in L1 (enriched in F2) highlighted members of the Enterobacterales order. The directionality and magnitude of these log2 fold changes provide preliminary evidence that L1 selects for distinct microbial community composition. These taxa represent priority candidates for targeted validation in future studies with larger sample sizes, and the effect sizes observed here can inform power calculations for subsequent experimental designs.

## Conclusion and Future Work

This study examined the effect of L1 vs F2 media on the microbial community of *M. pyrifera* gametophytes, where we observed higher species richness over time in gametophytes maintained in L1 media, but no significant changes in evenness. While no taxa showed significant differential abundance after FDR correction, log2 fold changes identified the top taxa, providing preliminary insights into media-driven community shifts and generating hypotheses for future validation studies with larger cohorts. Here, we provide valuable preliminary insights into the stability of native microbiomes in stored gametophytes but are constrained by low statistical power. Future studies with increased replicates, additional species and mediums (like PES), will enhance both the robustness and generalizability of these findings. Finally, we anticipate that the taxa identified in this study will warrant further functional characterization, which hold value for biobanking organizations and researchers working with similar phycobiome systems.

# Declarations

*Funding*

This work was supported by the Grantham Foundation for the Protection of the Environment.

*Competing Interests*

The authors have no relevant financial or non-financial interests to disclose.

*Author Contributions*

**MMR**: Methodology (supporting); Validation (equal); Formal Analysis (supporting); Investigation (equal); Writing (Review & Editing)(equal); Visualization (supporting); Funding Acquisition (equal); Writing- Original Draft (equal); Writing- Review & Editing (lead). **CO**: Formal Analysis (supporting); Data Curation (supporting); Visualization (supporting); Writing- Review & Editing (supporting). **JB:** Investigation (supporting); Writing- Review & Editing (supporting). **LR:** Investigation (supporting); Project Administration (supporting); Writing- Review & Editing (supporting). **EGA**: Conceptualization (lead); Methodology (lead); Software (lead); Formal Analysis(lead); Investigation(lead); Data Curation(lead); Visualization(lead); Supervision(lead); Project Administration(lead); Funding Acquisition(equal); Writing- Original Draft (lead)

*Data Availability*

Demultiplexed sequences are available at the National Center for Biotechnology Information (NCBI) Sequence Read Archives (SRA) under accession code: **PRJNA1508979.**

*Ethics Approval*

No ethical approval was required for this study.

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# Figures

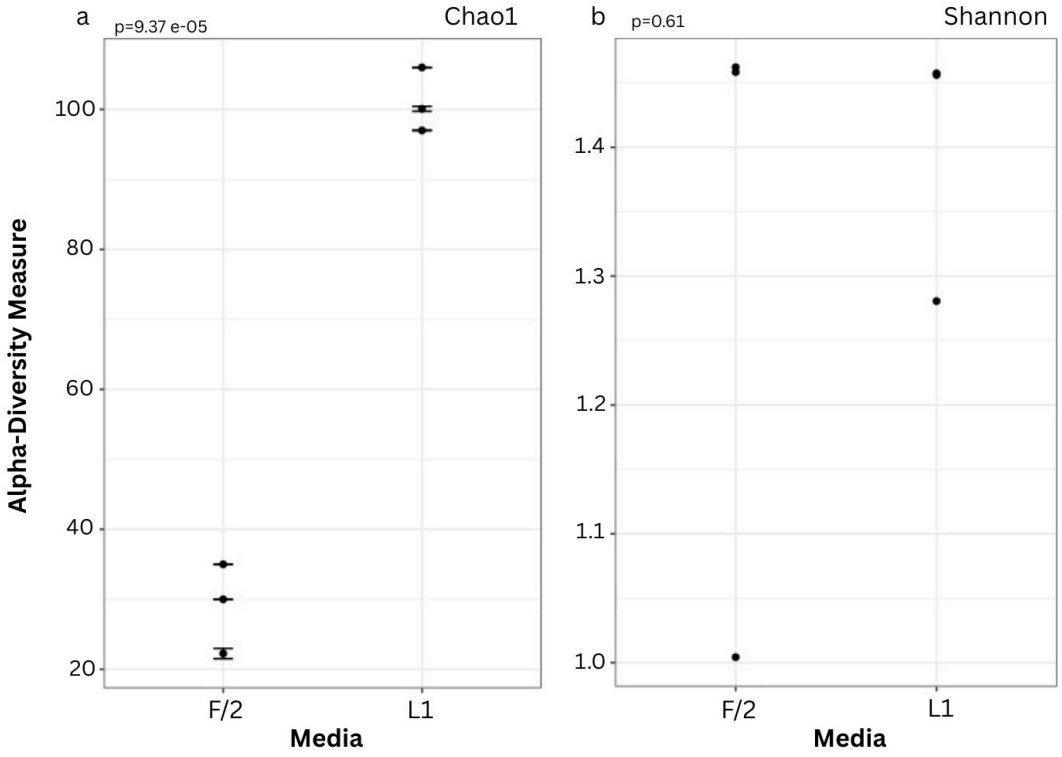



**Figure 1**

Alpha diversity plots using the a) Chao1 metric (p=9.37e-05) to estimate bacterial richness and b) Shannon Index to estimate both richness and evenness (p=0.61).

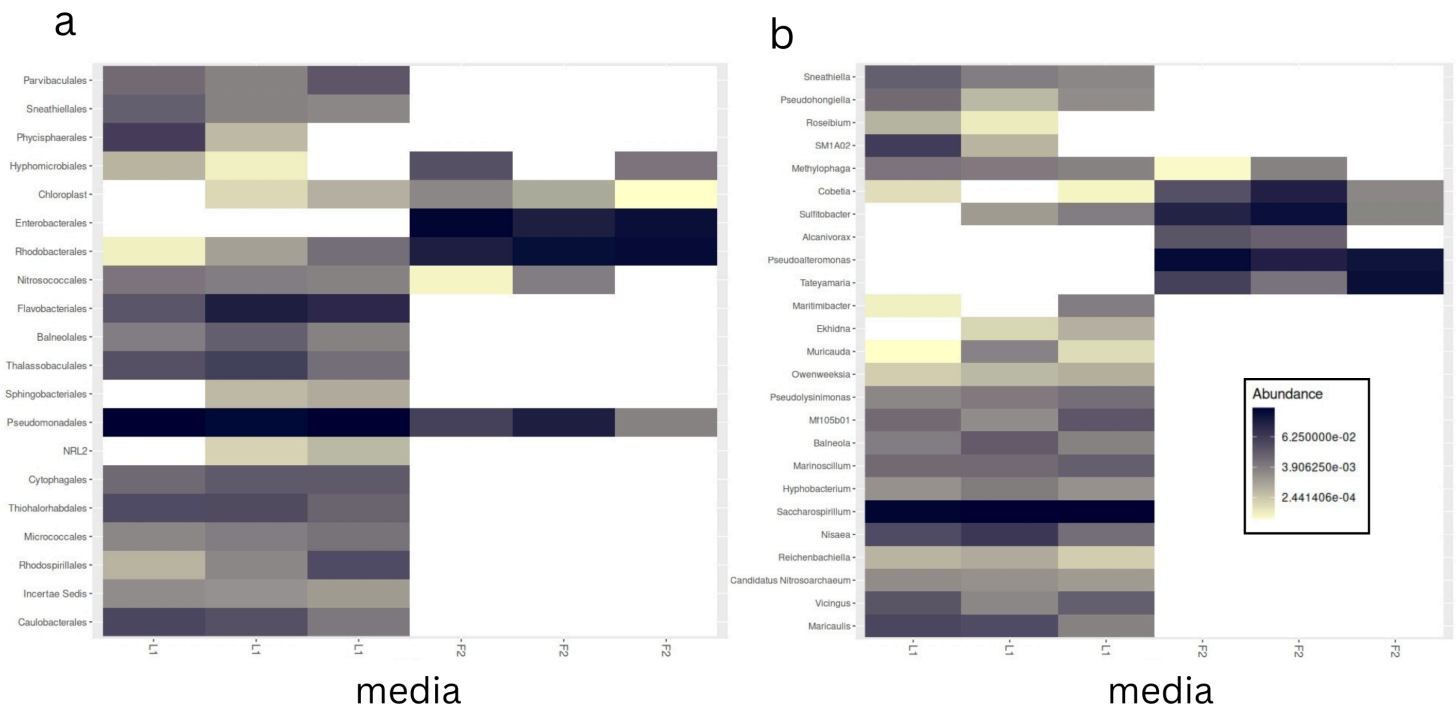



**Figure 2**

Relative abundance heatplots of the microbial communities of *M. pyrifera* gametophytes in F/2 vs L1 media, classified by a) order and b) genera.

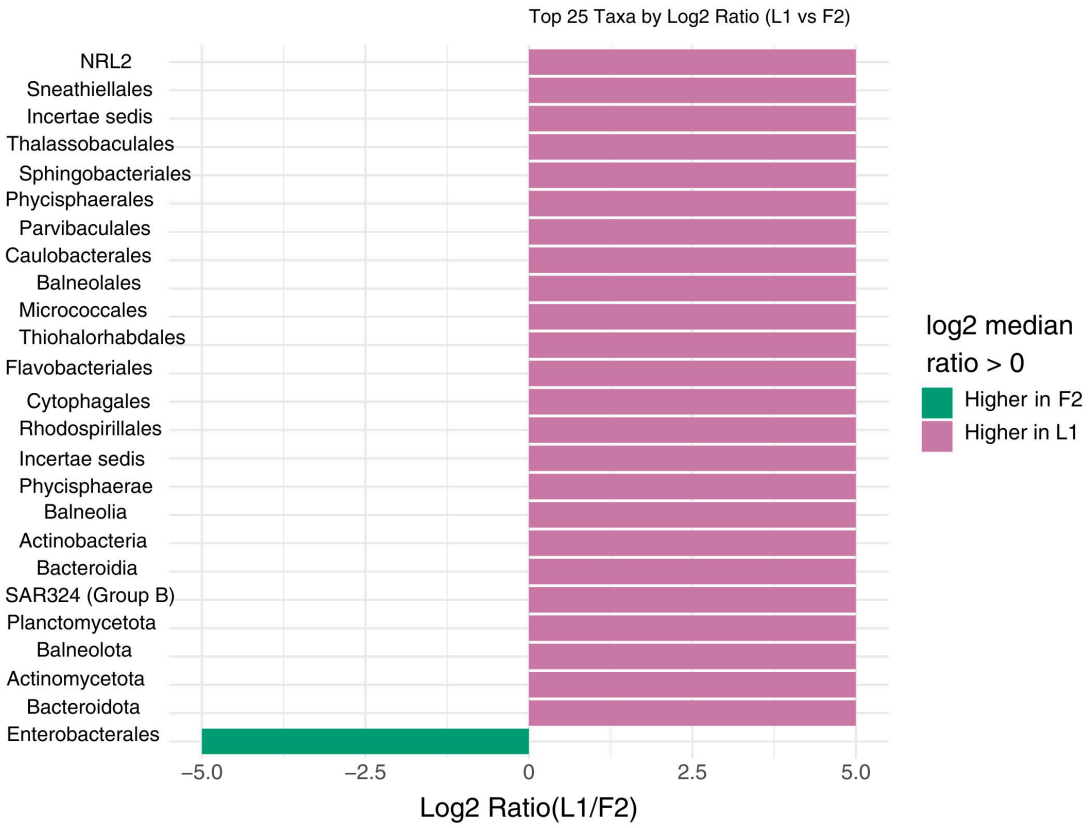



**Figure 3**

Top 25 bacterial taxa, as determined by log2 ratio (using the R package, Metacoder) for F/2 vs L1 groups. Full statistical results can be found at Zenodo (DOI:10.5281/zenodo.21926483).