SAND2023-04951C

Sandia National Laboratories

Trait drift in the field and laboratory: Characterizing microbial community composition and genotype shifts through time

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International Conference on Algal Biomass, Biofuels and Bioproducts 2023

13th June | **Waikoloa Beach, Hawaii, USA**

U.S. DEPARTMENT OF ENERGY NNSA National Nuclear Security Administration

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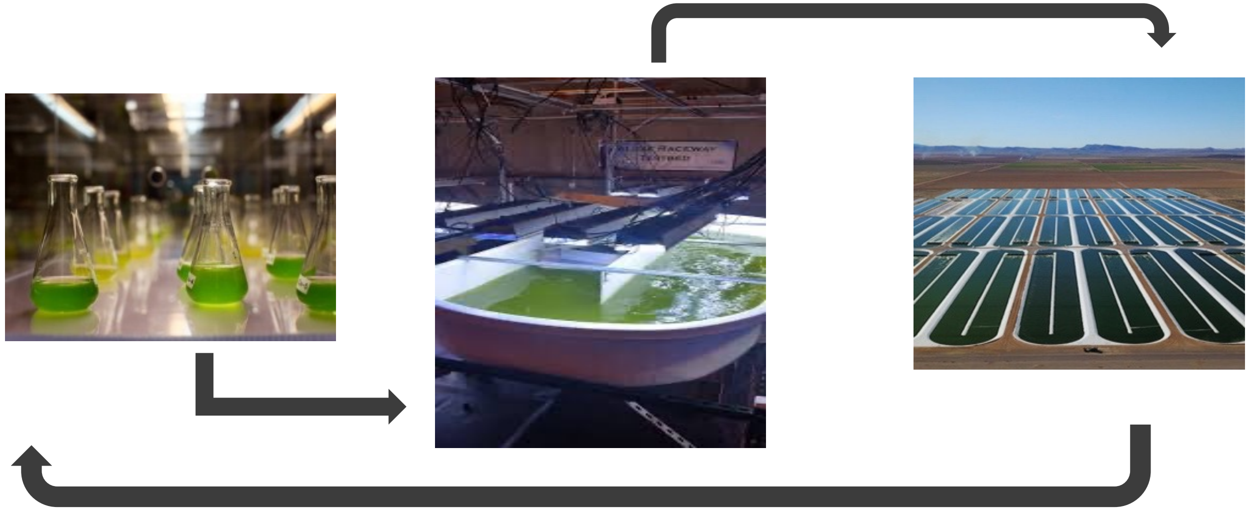
Optimizing Selection Pressures and Pest Management to Maximize Algal Biomass Yield (OSPREY)

What drives desirable traits?

1) Balancing Indoor and Outdoor Selection Pressures
    1) Abiotic – Temperature, pH, Salinity
    2) Biotic- Pests/predators, Competitors
2) Improvement of Field Strain Performance, Resiliency, and Composition



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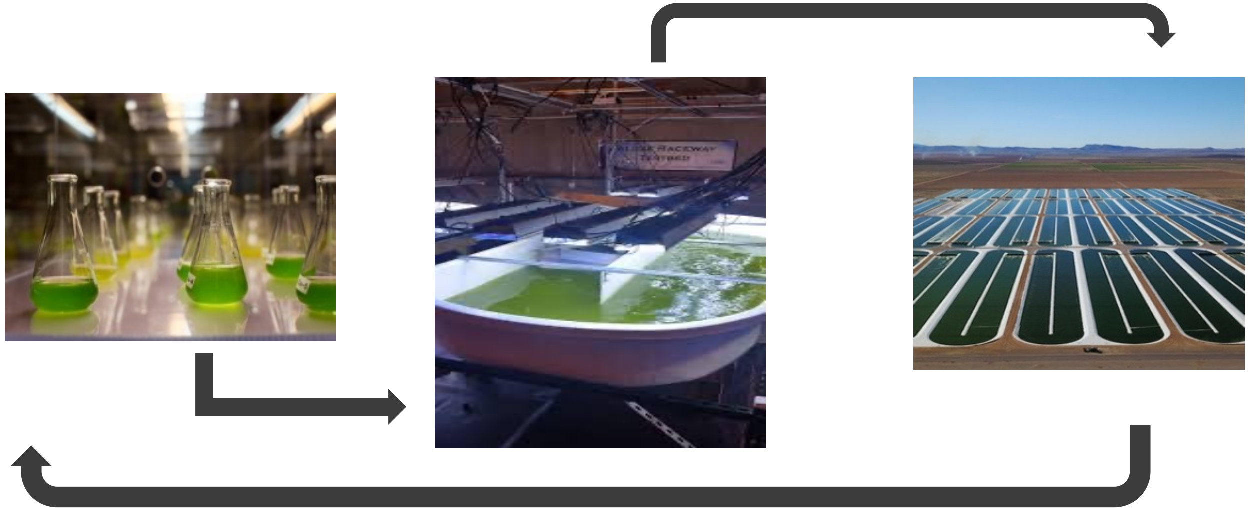

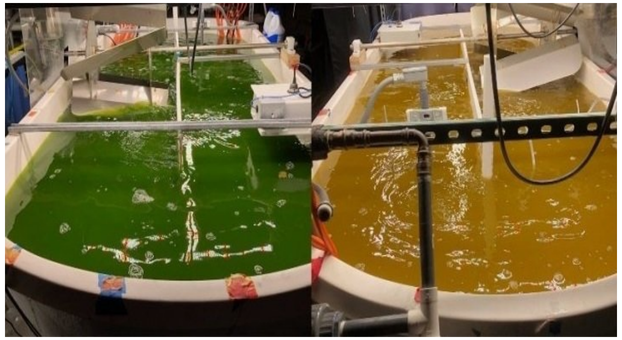

Healthy **Sick**

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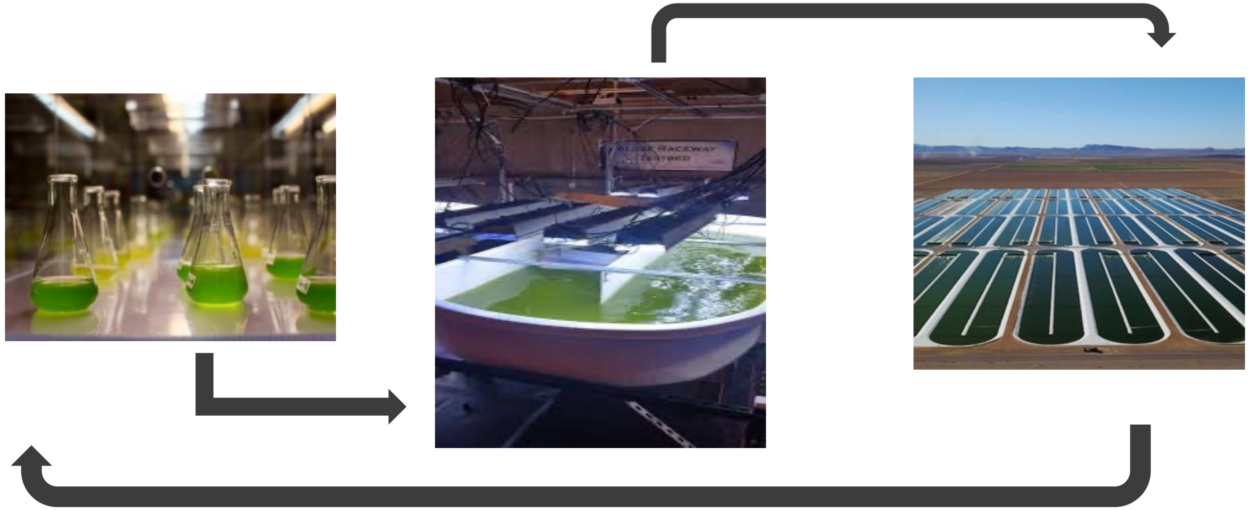

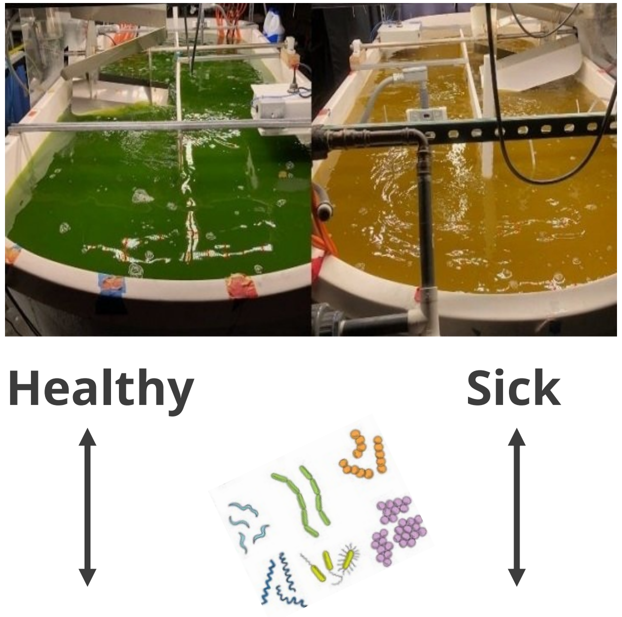

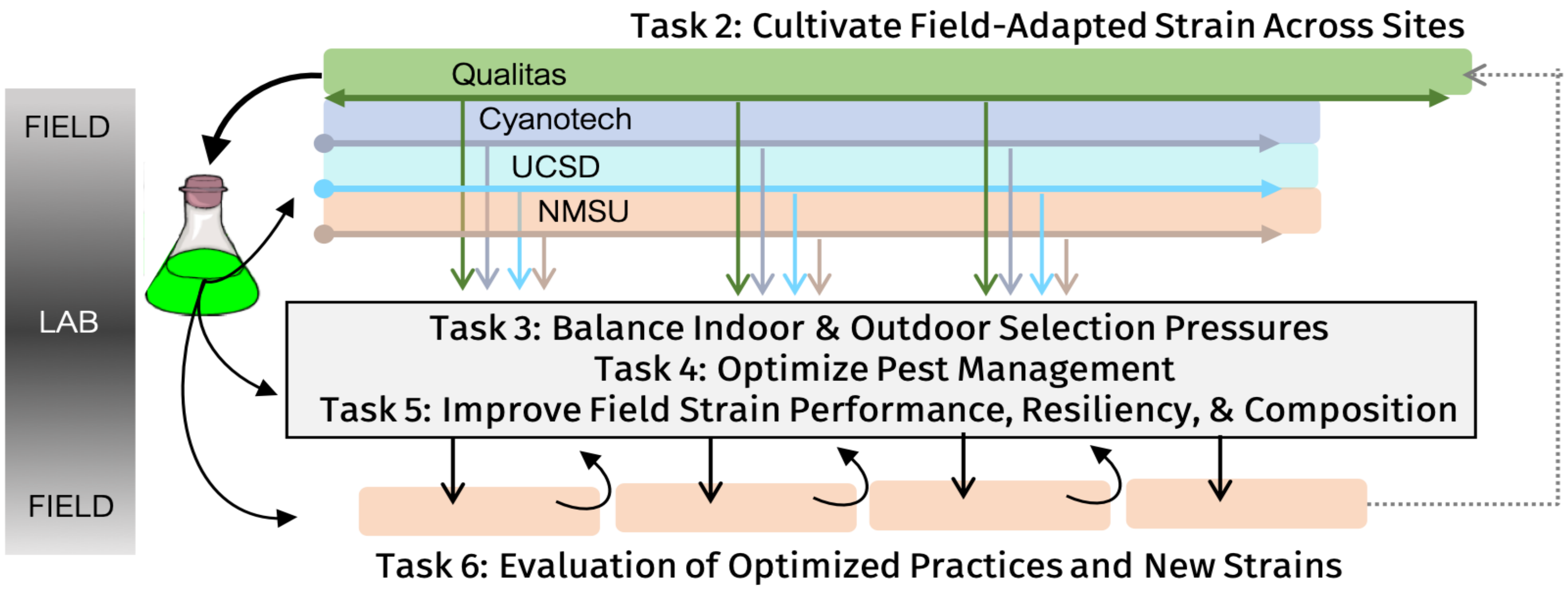

Task 2: Cultivate Field-Adapted Strain Across Sites

Task 3: Balance Indoor & Outdoor Selection Pressures
Task 4: Optimize Pest Management
Task 5: Improve Field Strain Performance, Resiliency, & Composition

Task 6: Evaluation of Optimized Practices and New Strains

Tracking microbial diversity and genotypic changes

What drives changes in productivity at all scales of cultivation over time?

1) Maintenance in the lab – Changes in phenotype
    1) Plates
    2) Flasks
    3) Bioreactors

1) Field cultivation
    1) Pests
    2) Environmental effects

1. Acquire growth rates and phenotypically characterize over time
2. Characterize changes in microbial diversity
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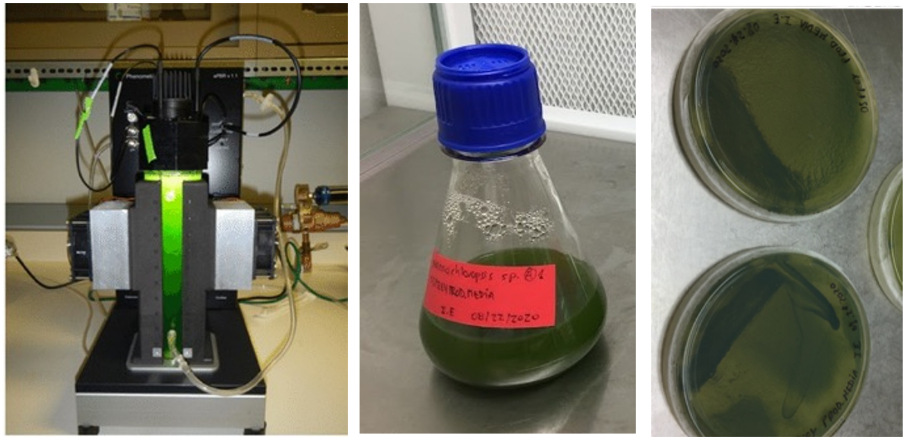
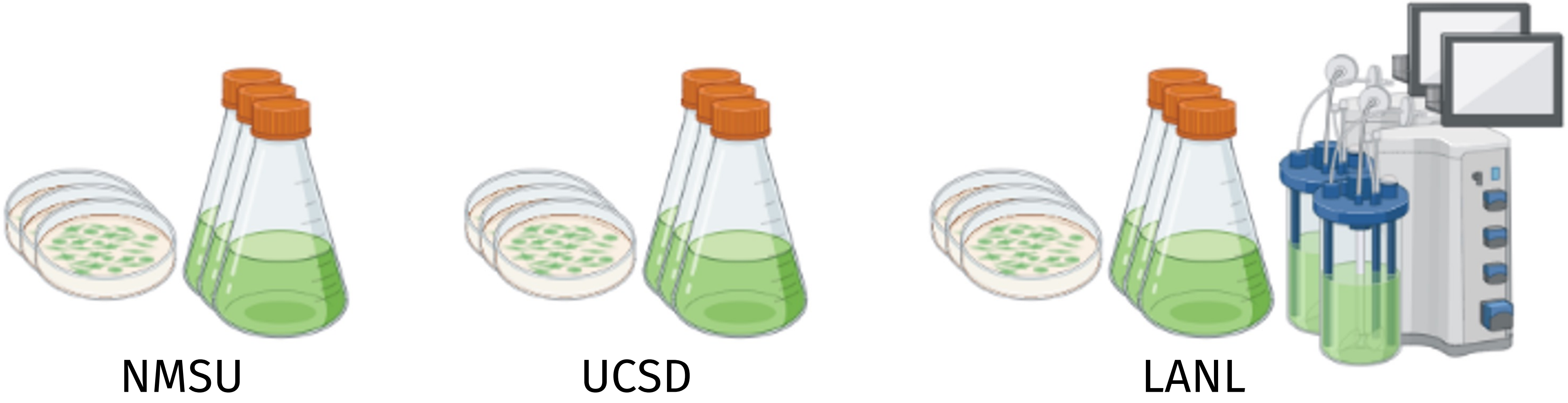
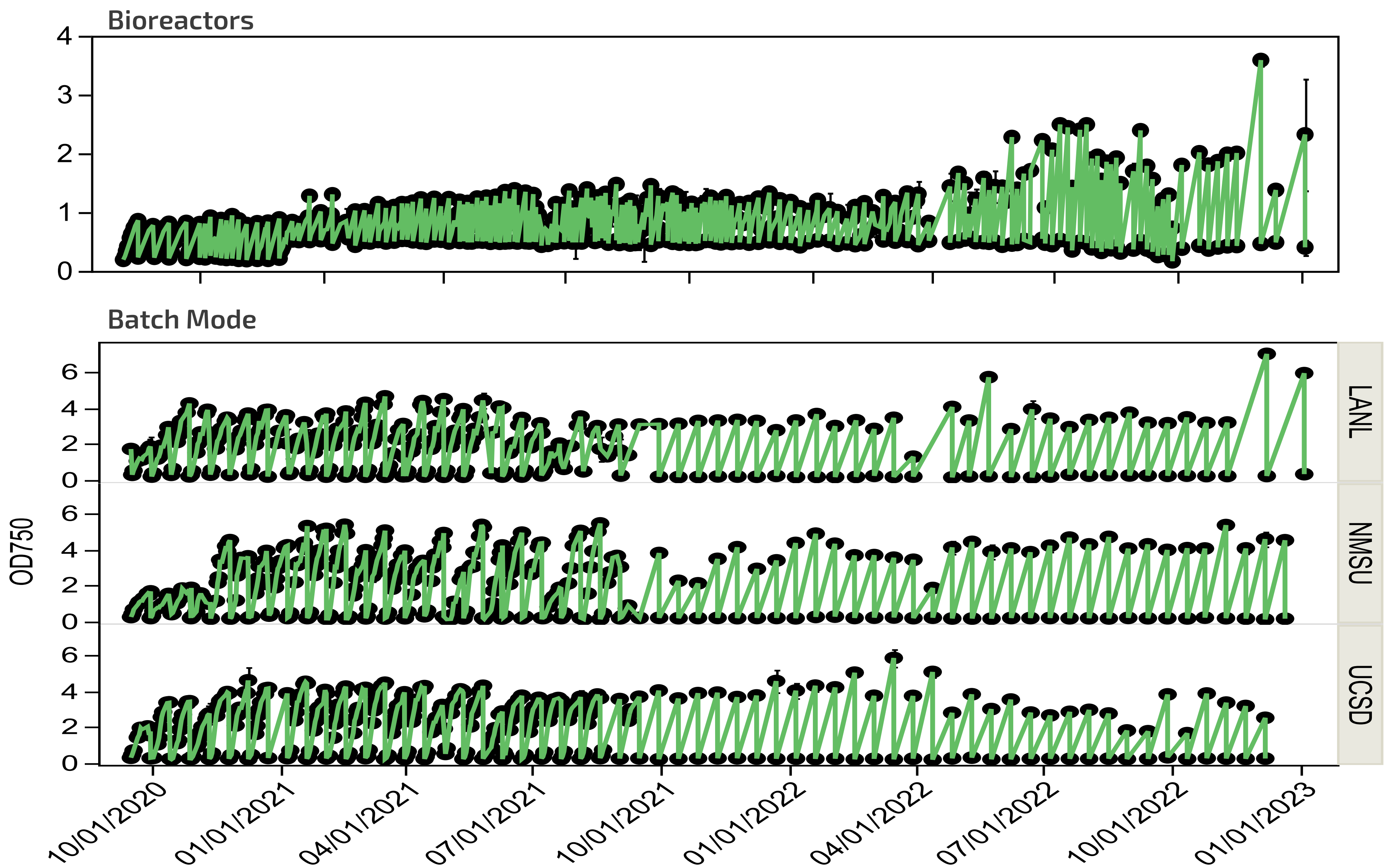
Maintenance of field-adapted strain across three labs using three different culturing methods

1) QT001 established under 3 different growth modes
    1) Storage on plates (3 reps each at of 3 labs)
    2) Batch w/ transfers every 2 weeks (3 reps at each of 3 labs)
    3) Bioreactors to simulate Imperial, TX (2 reps at LANL)





Cultivation across lab sites and culture modes



Alina Corcoran

Distributed test beds across four sites

Nannochloropsis sp. QT001 ponds in Texas, New Mexico, California and Hawaii

Different environmental pressures

Changes in microbial diversity

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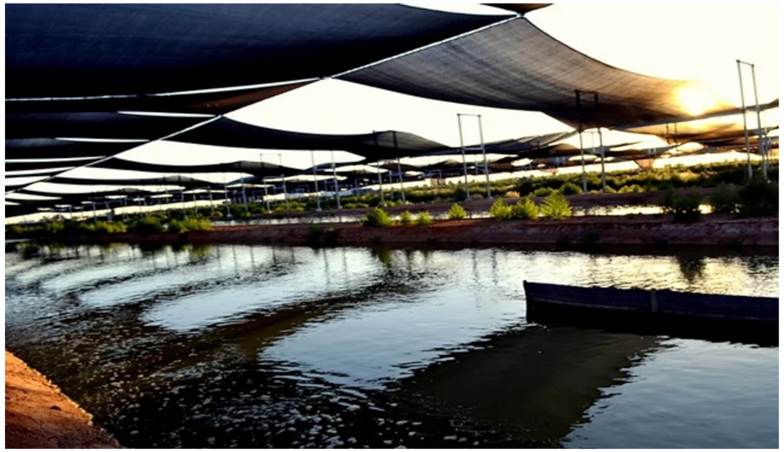
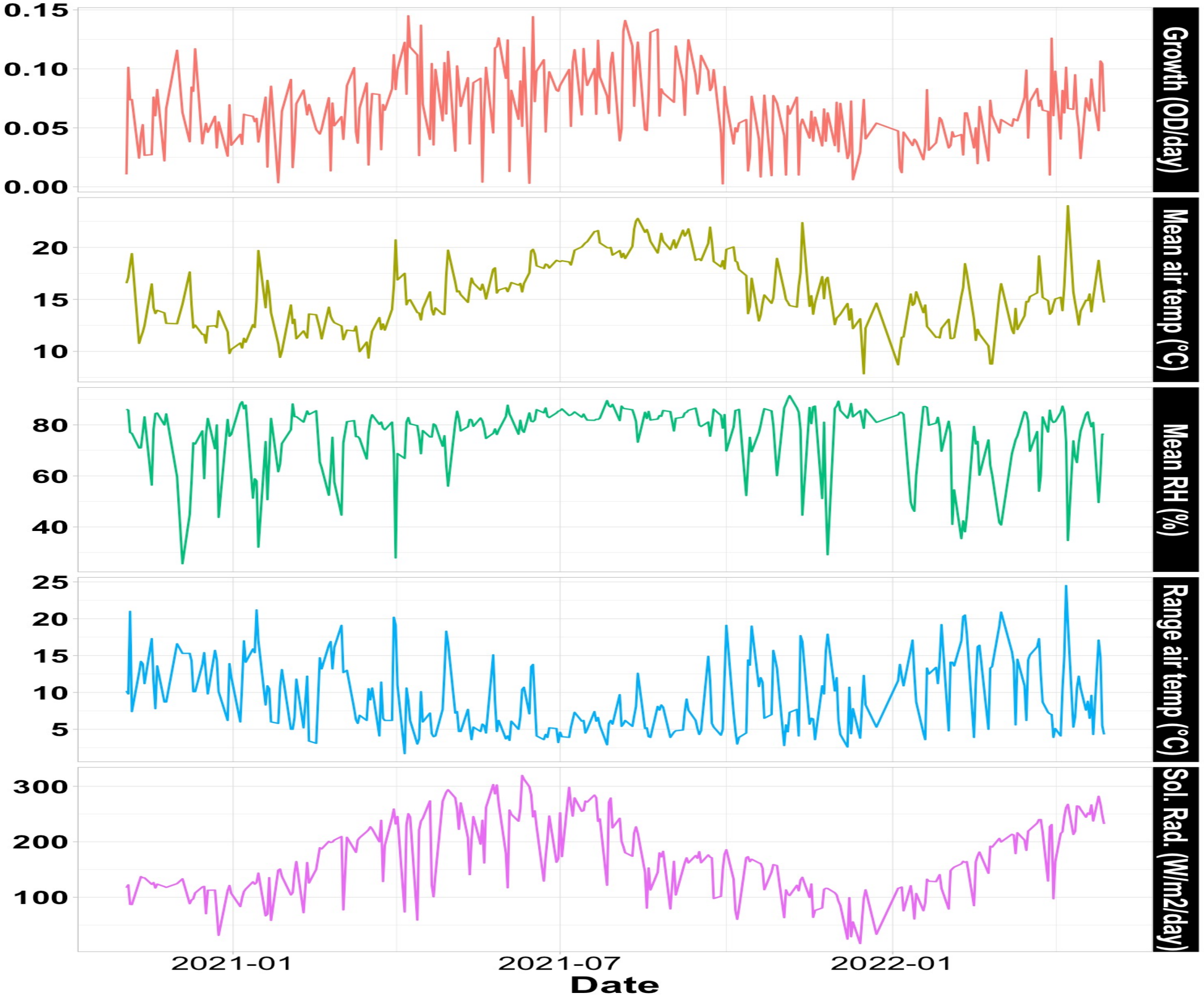


UCSD Growth and environmental parameters

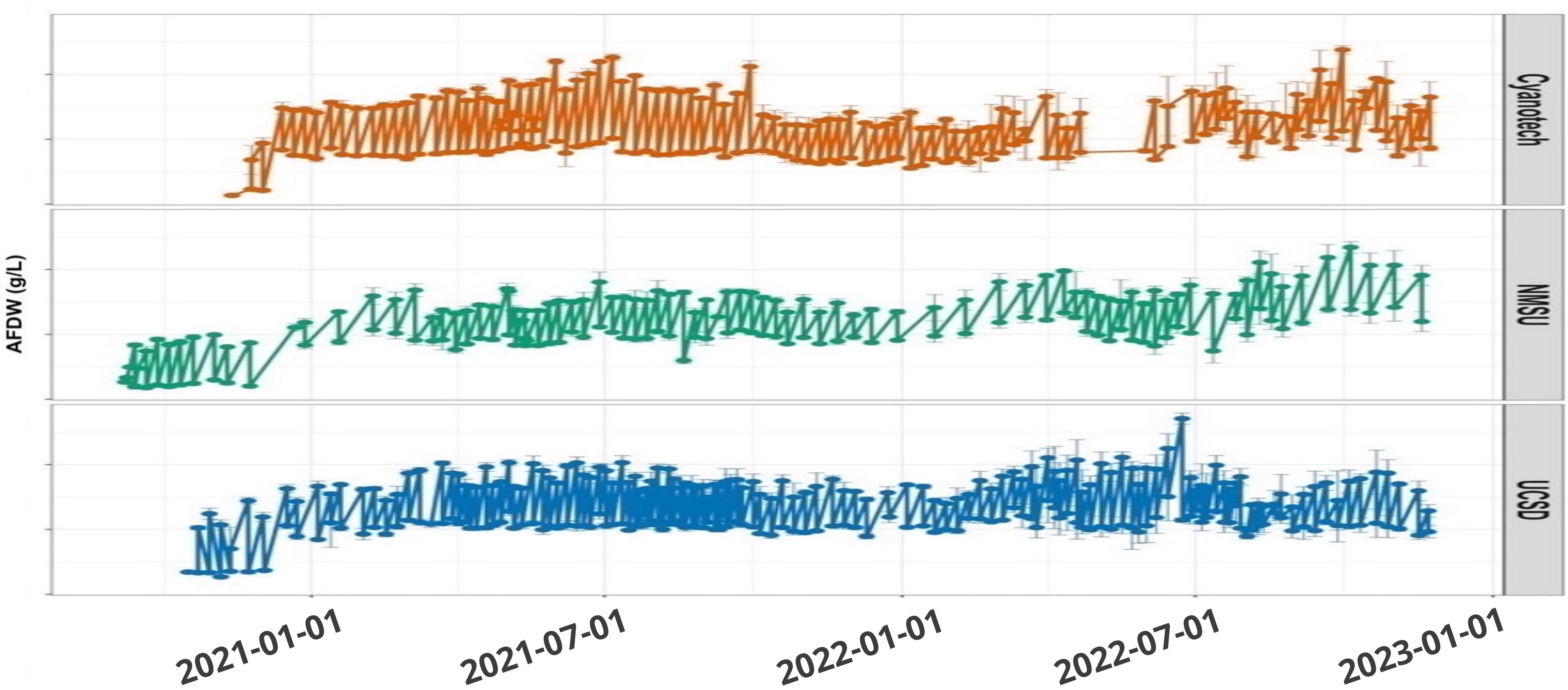
1) Growth monitoring over time
2) Environmental data
3) 16S sequencing
4) ProxiMeta Analysis
5) Metadata (CO2, bleach dosing, etc.,)

Correlate periods of low productivity with various factors



Isidora Echenique

Cultivation Across Sites and Culture Modes over



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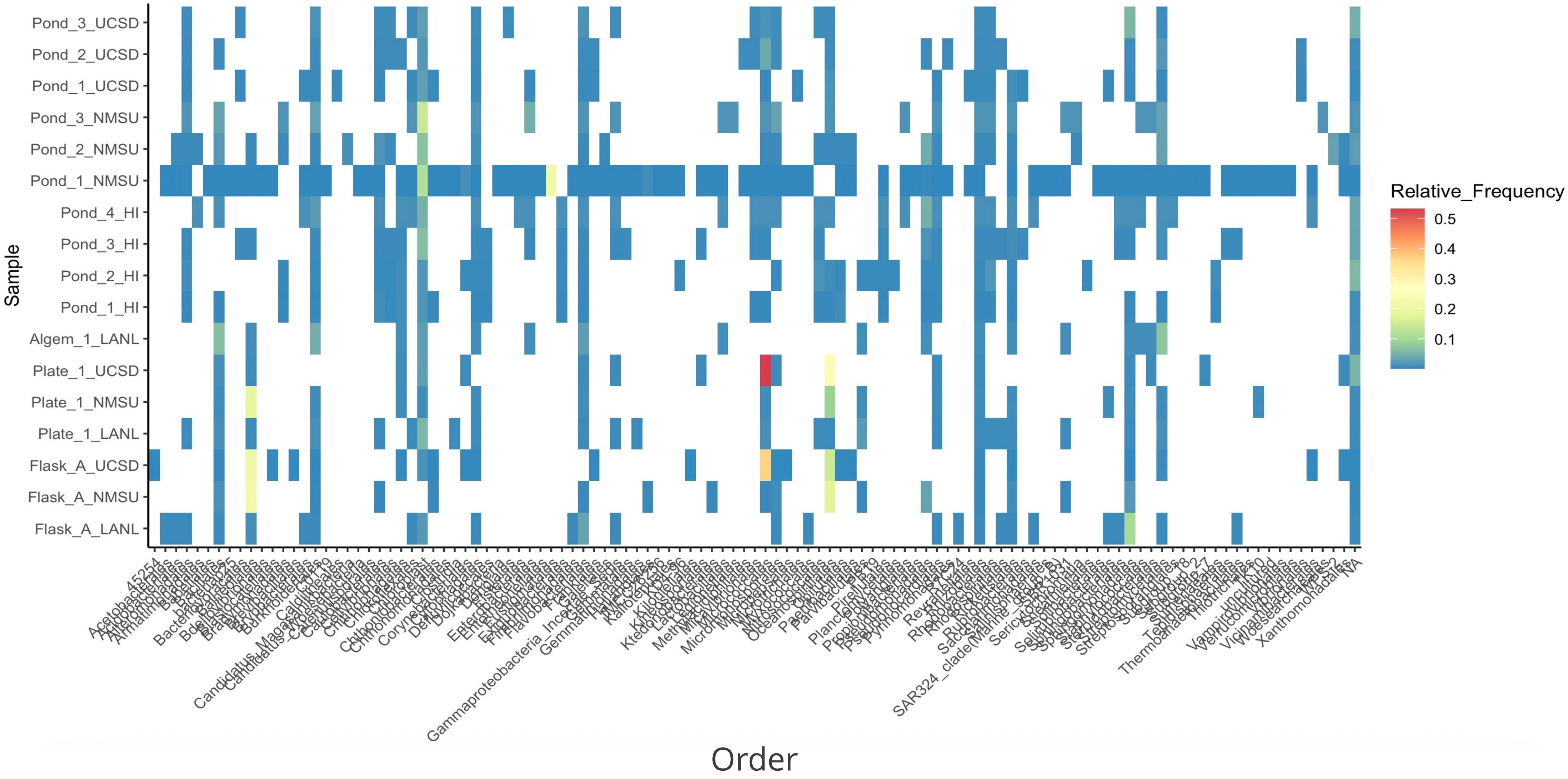
16S Relative abundance after three months of cultivation



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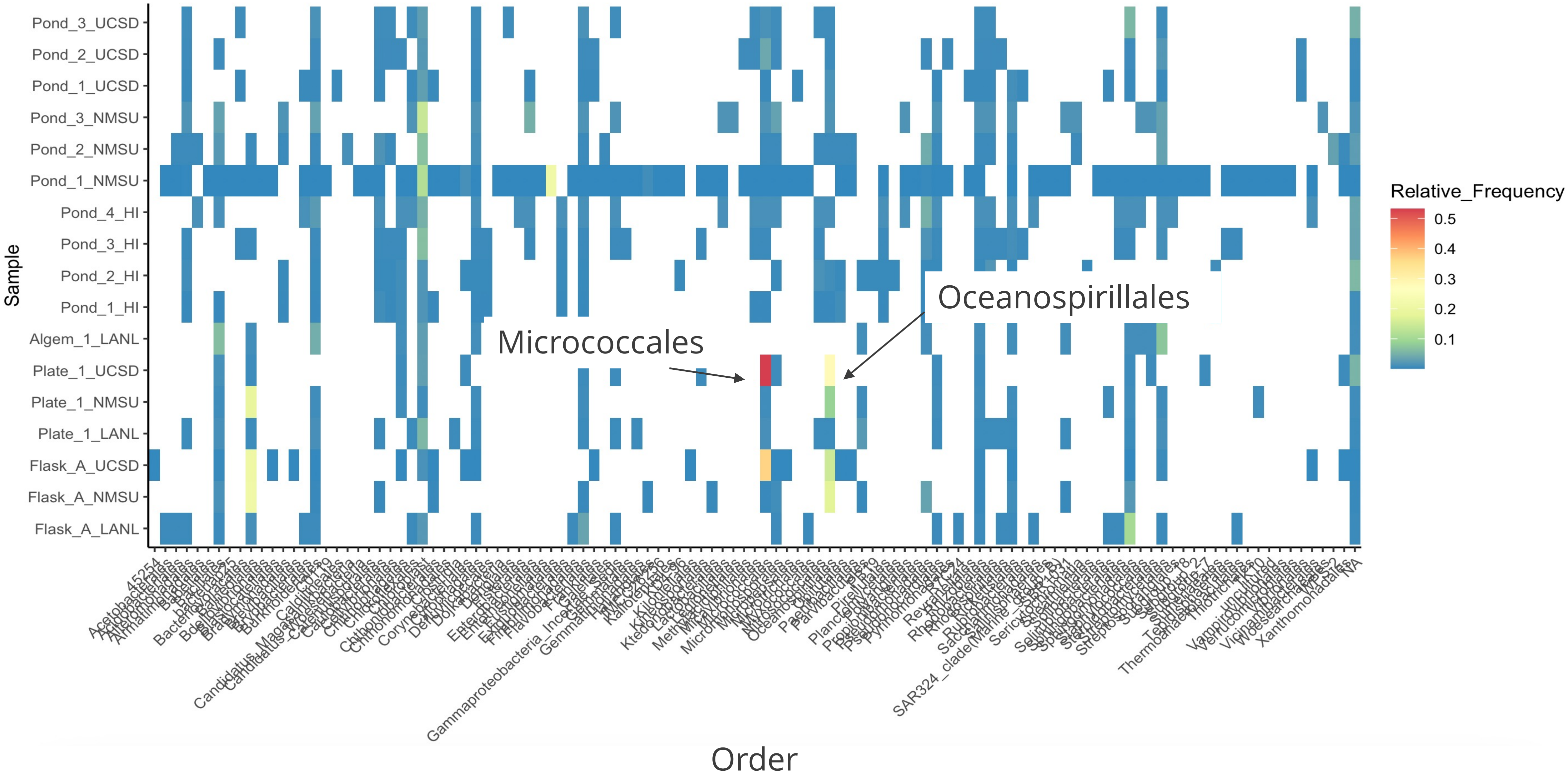

Micrococcales

Oceanospirillales

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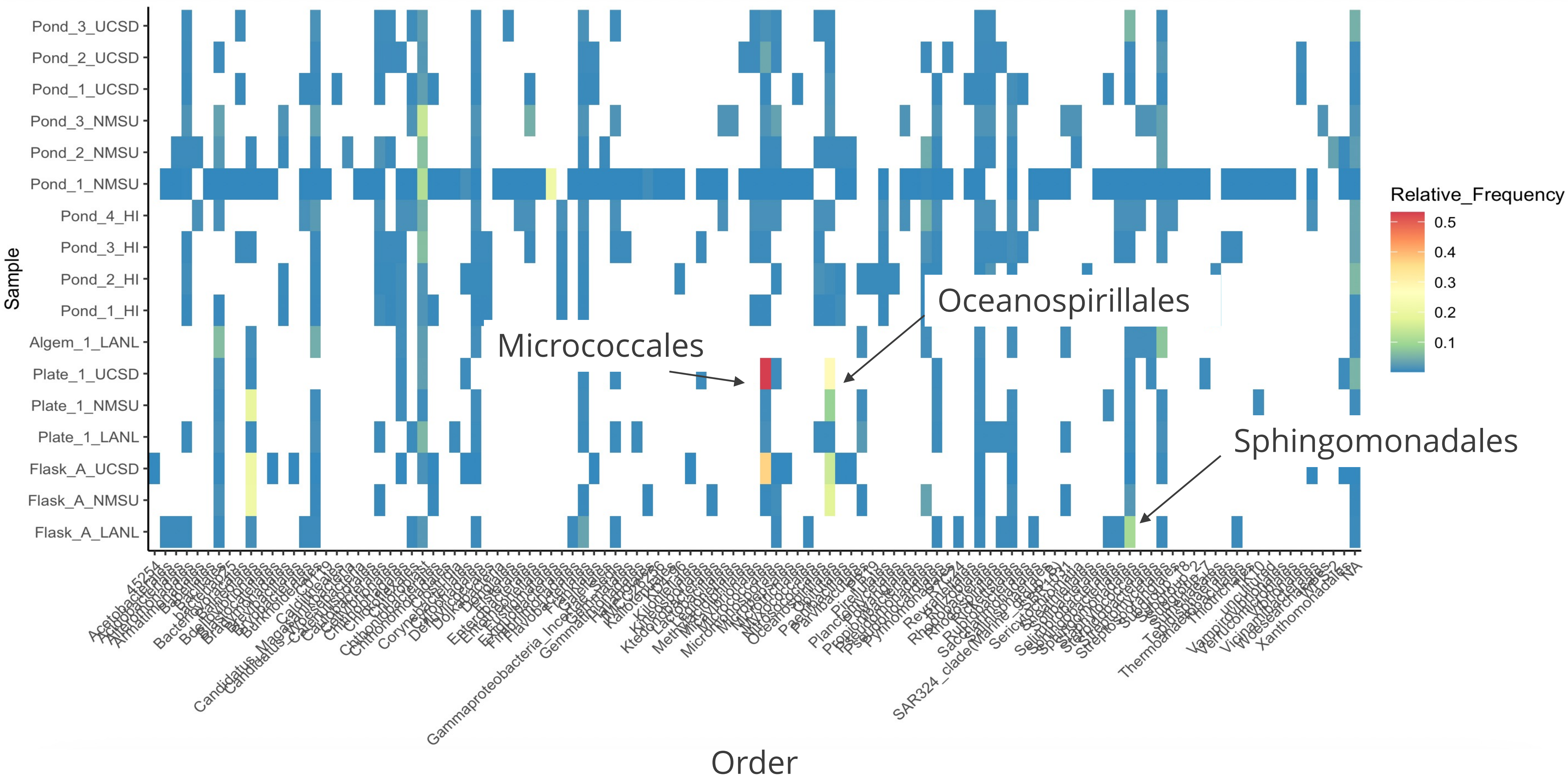
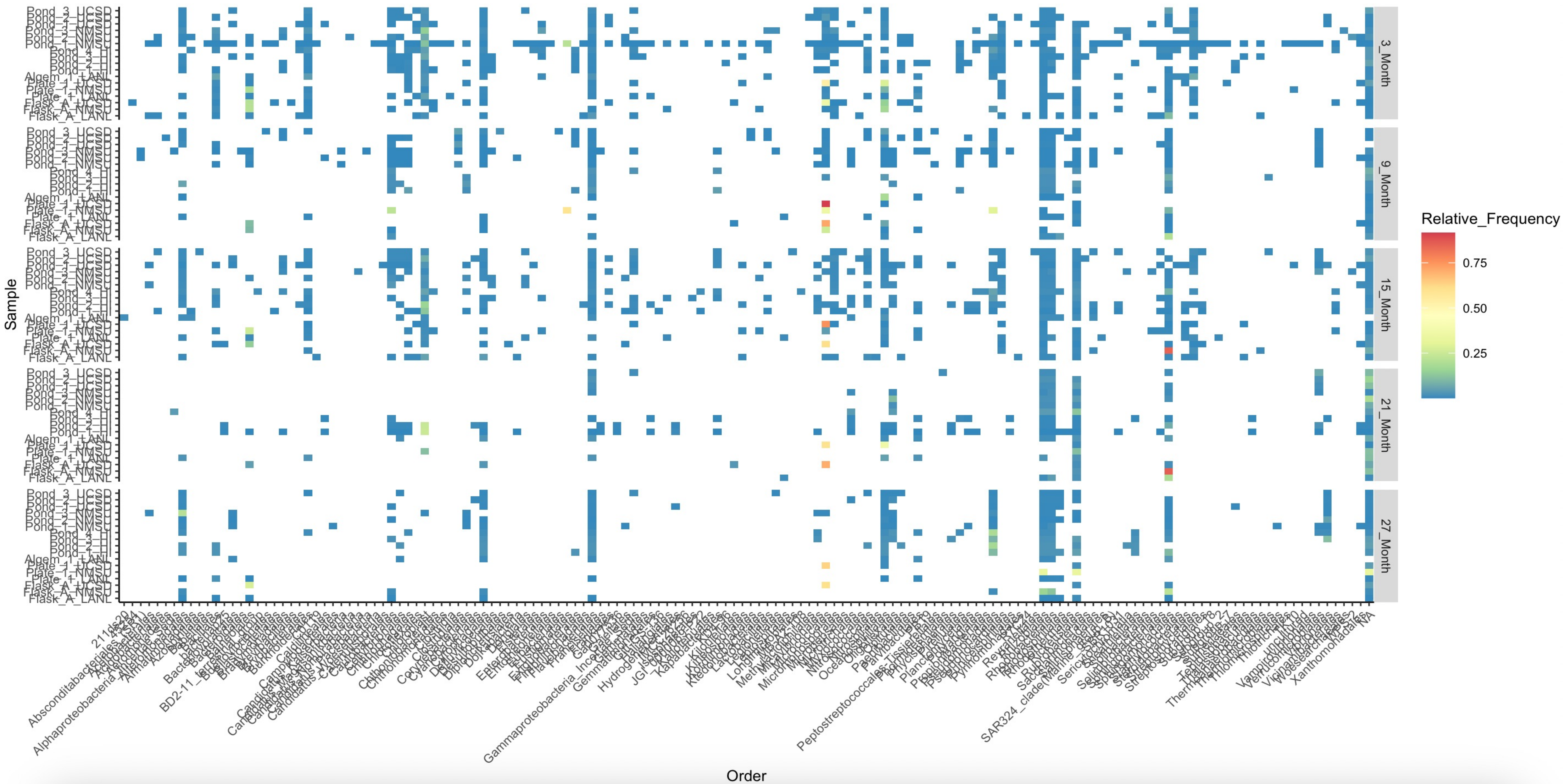


Oceanospirillales

Micrococcales

Sphingomonadales

16S Relative abundance over twenty-seven months



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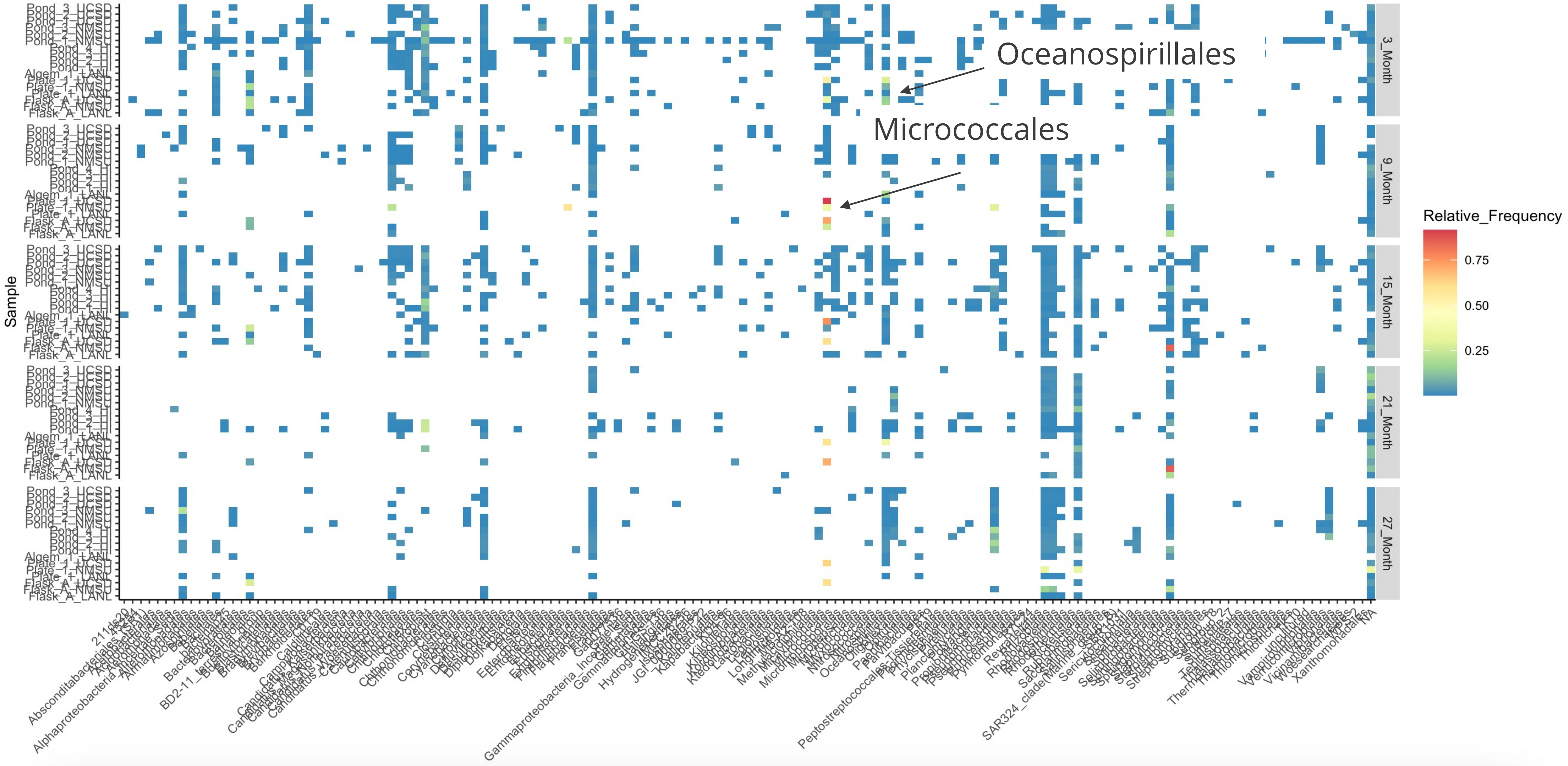
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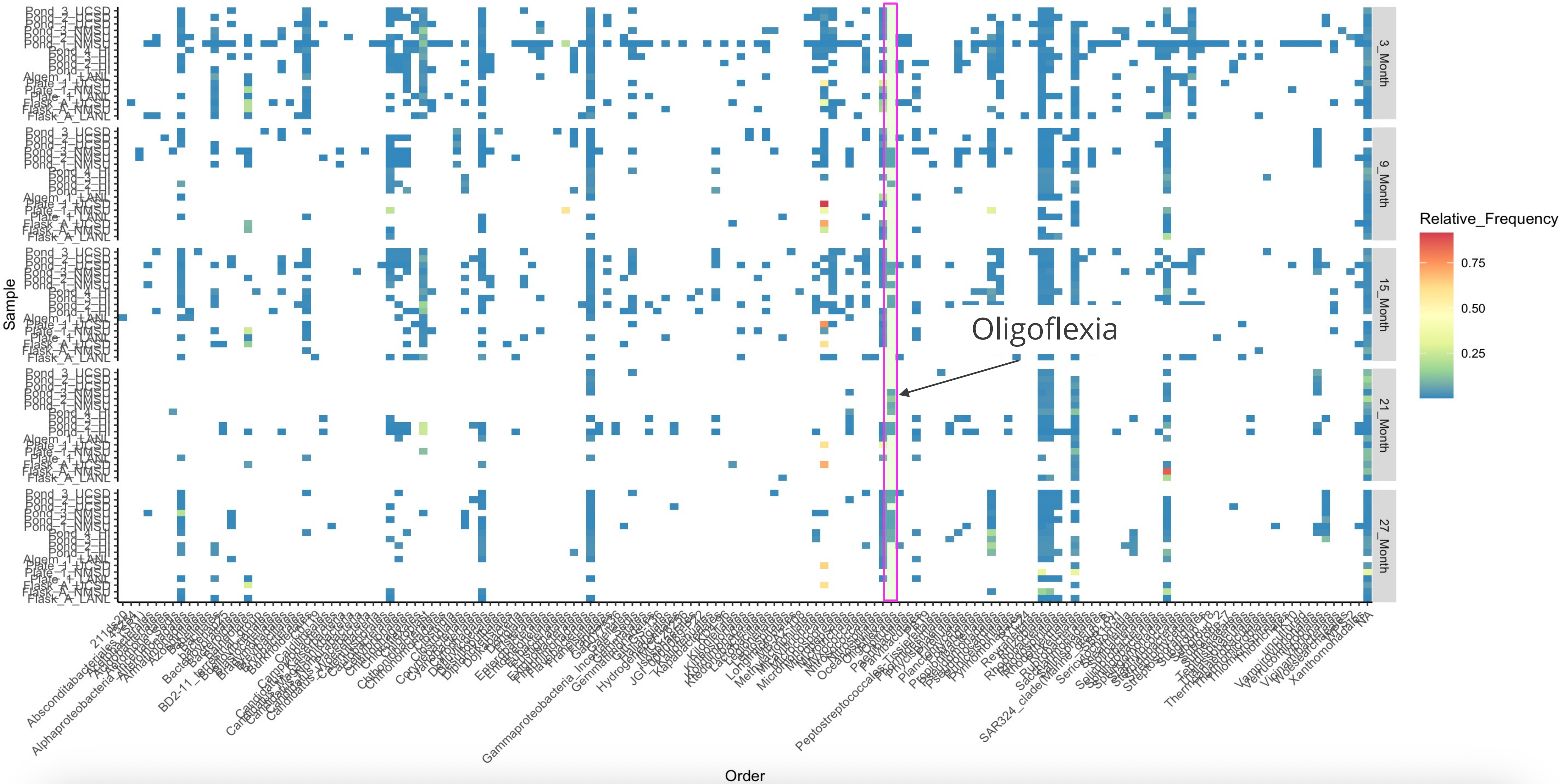

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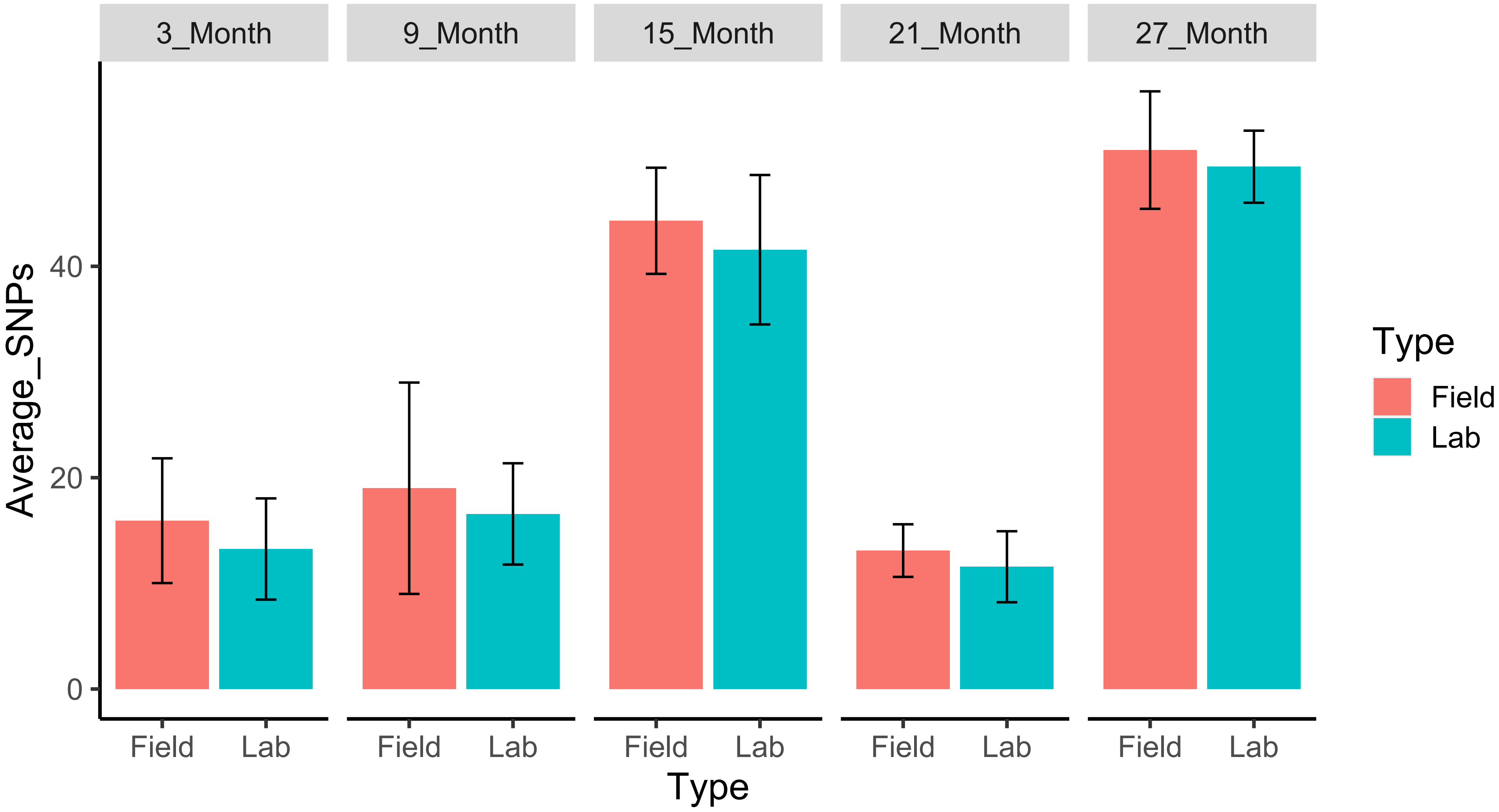
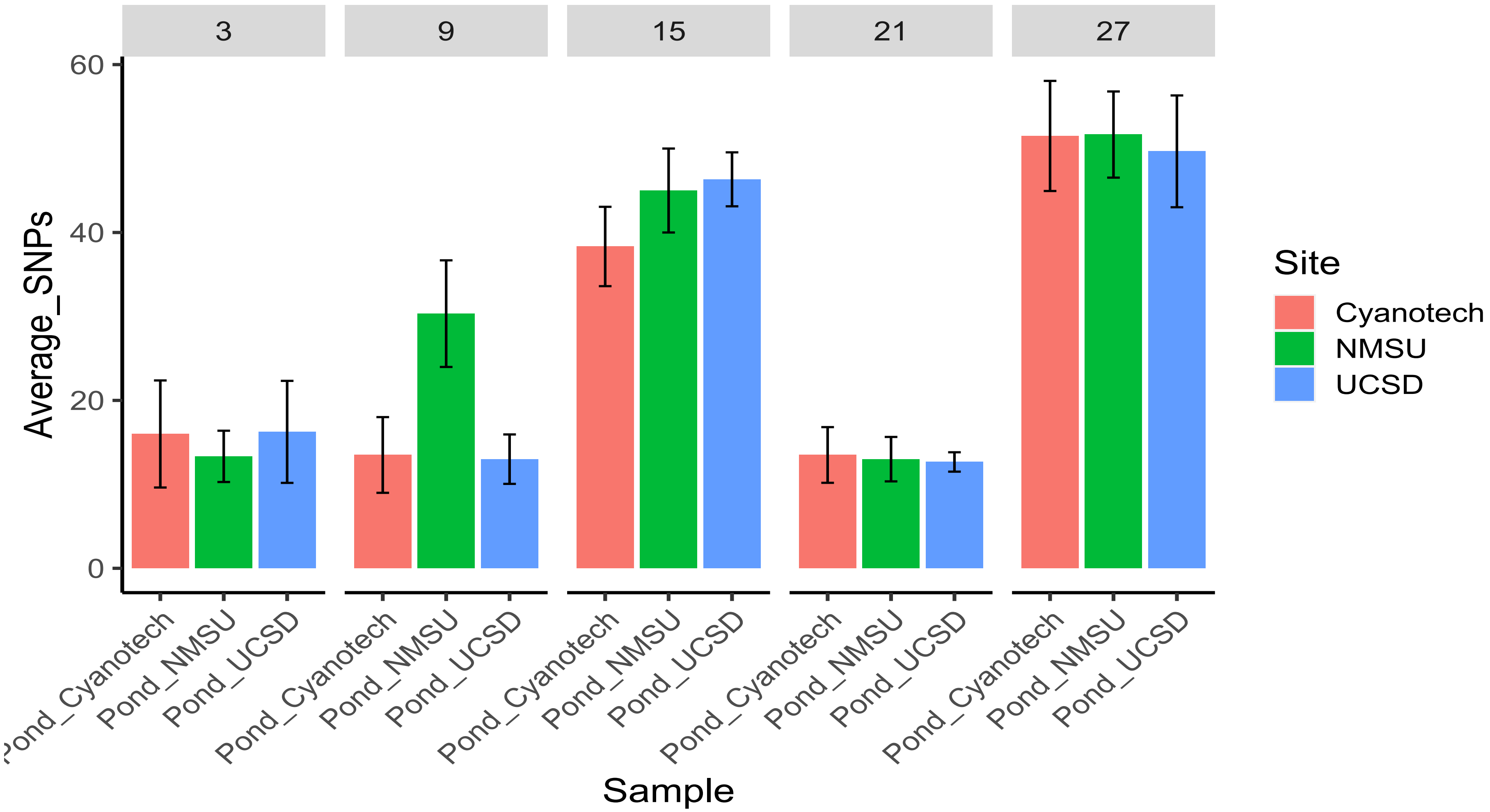
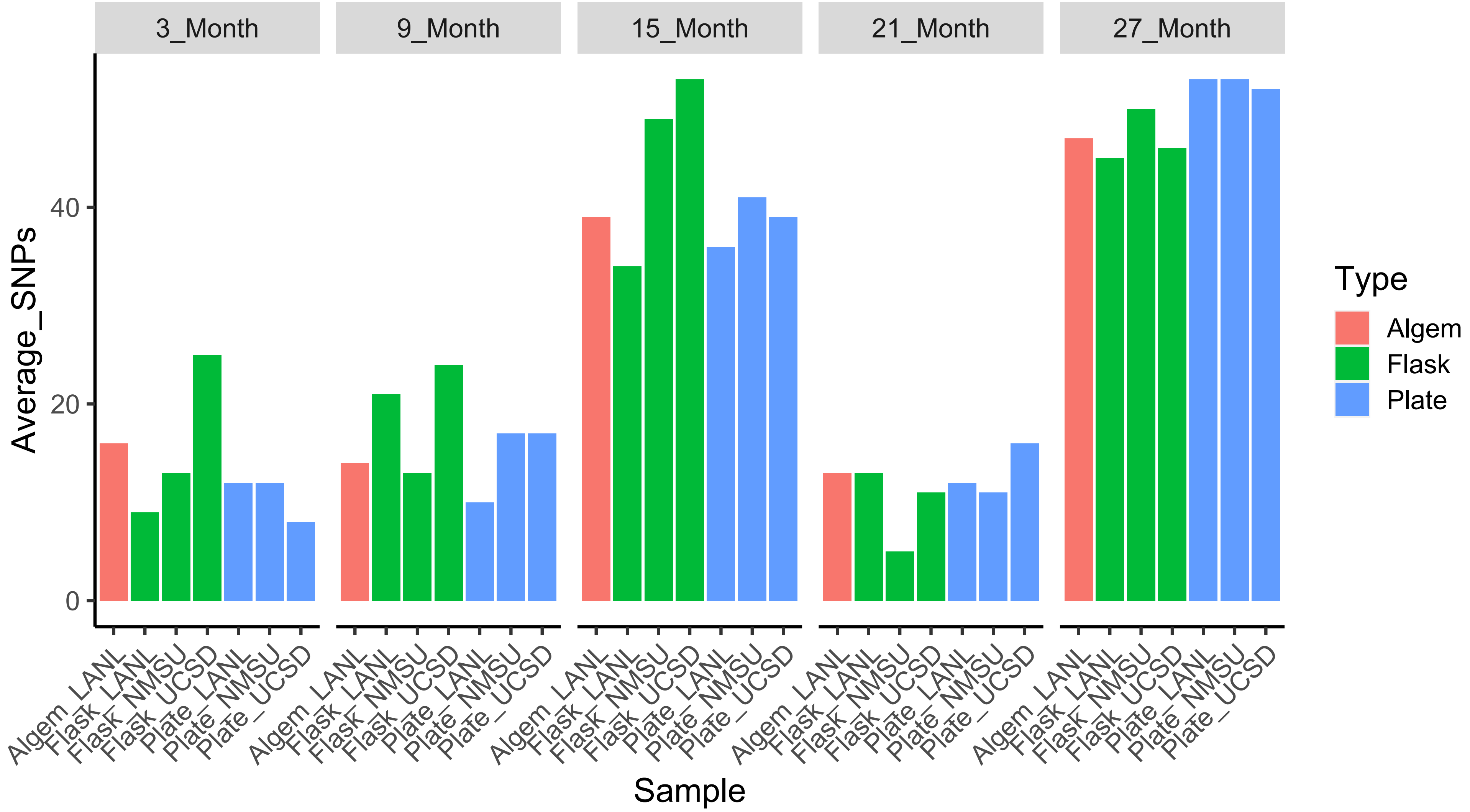
Average number of SNPs over time: Field vs Lab



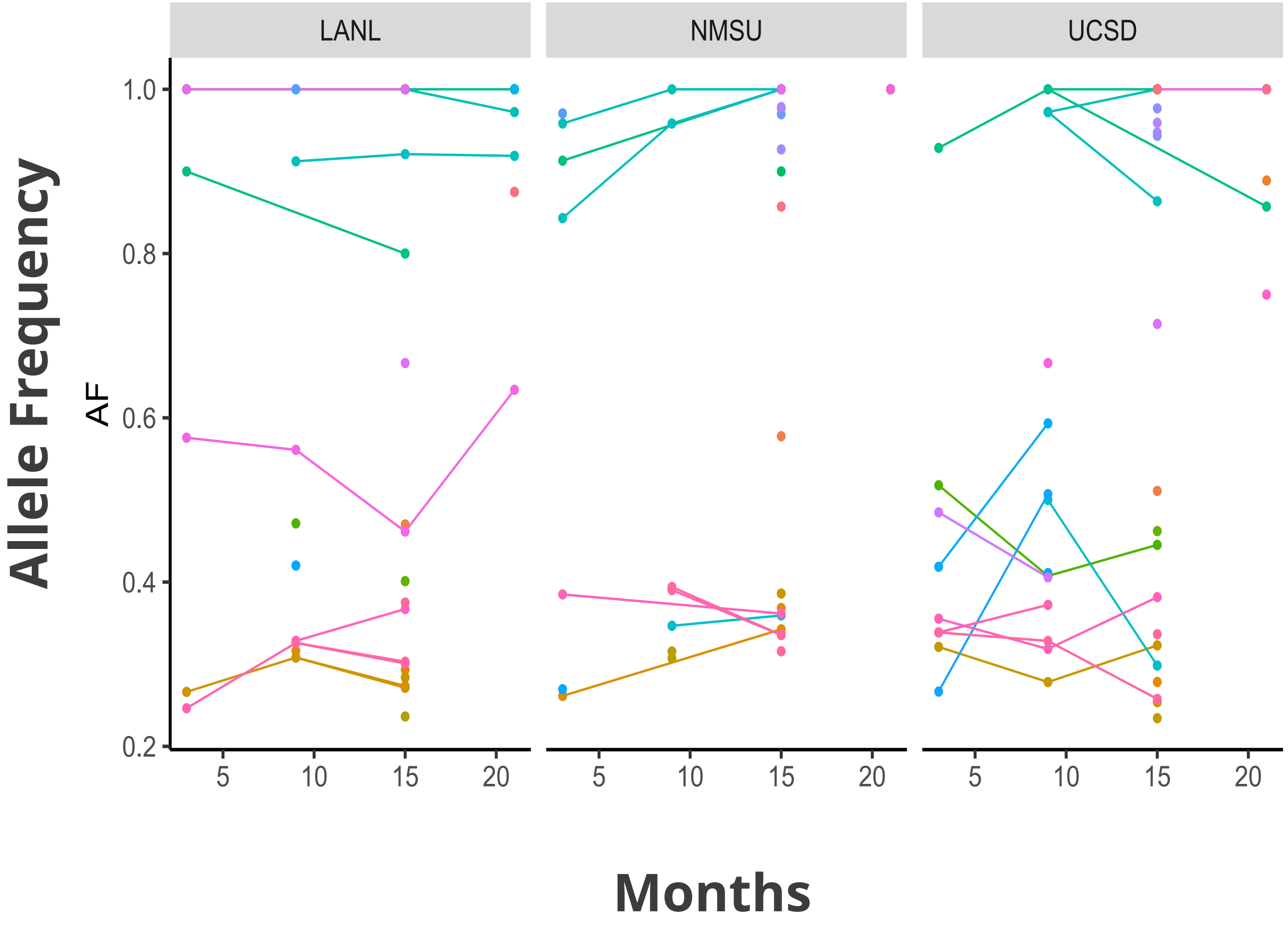
Average number of SNPs over time: Ponds



Average number of SNPs over time: Lab



Tracking allele frequencies of lab-maintained strains (Flasks)

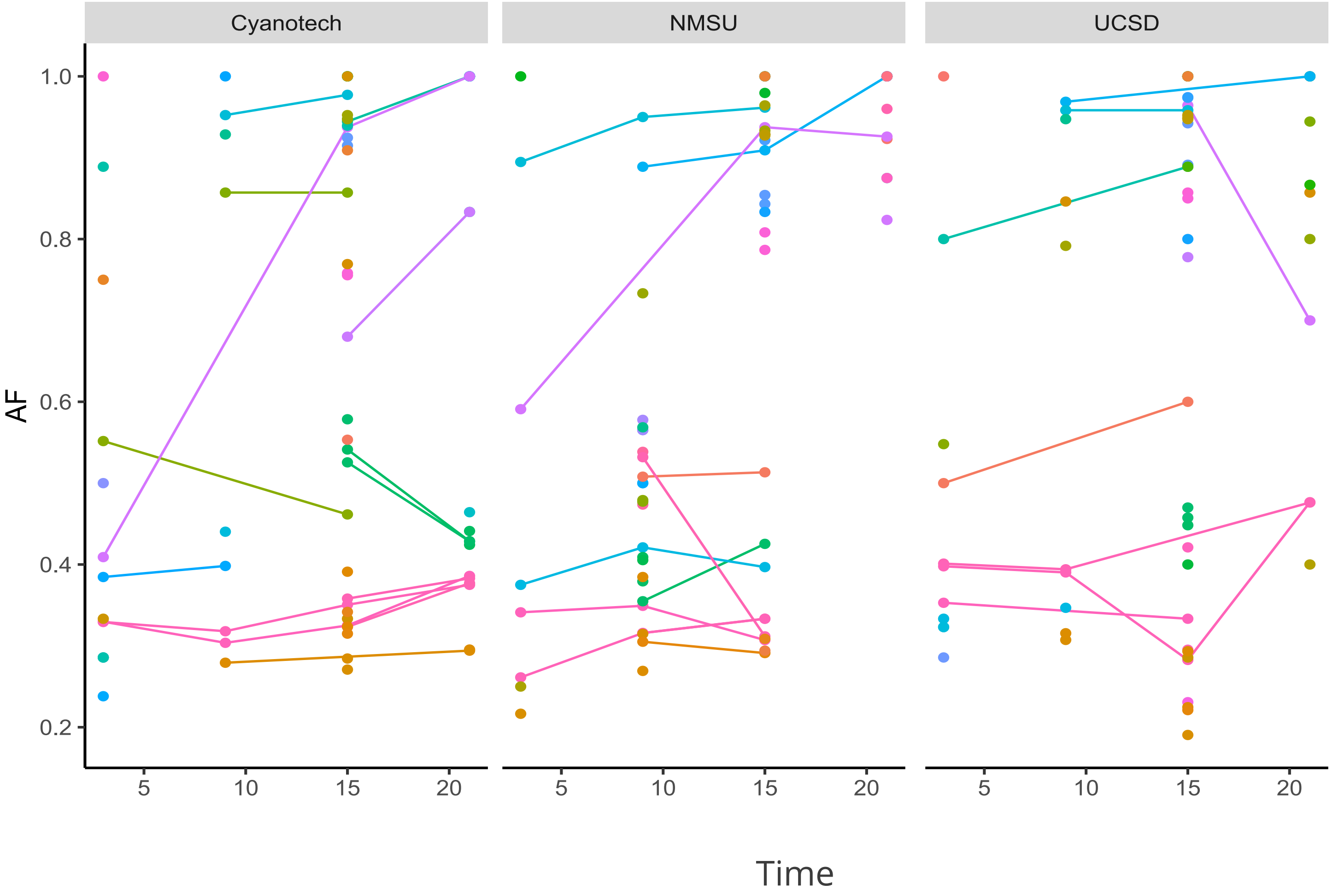


Variant	Number of sites	Gene
contig_127:30432	3	Intergenic
contig_36:356361	3	MNS4: Alpha-mannosidase I MNS4
contig_37:1225154	3	Intergenic
contig_5:2139	3	Intergenic
contig_51:24400	3	Intergenic
contig_52:37100	3	Intergenic
contig_6:562714	3	Hypothetical protein
contig_65:150702	2	Intergenic
contig_65:150790	2	Intergenic
contig_77:68452	3	Transcription elongation factor spt6
contig_38:2084	1	ATP-dependent DNA/RNA helicase DHX36

- Some alleles started out at fixation or quickly approached fixation

- Others changed through time

Tracking allele frequencies of field-maintained strains (Ponds)



Variant	Number of sites	Gene
contig_92:64681	1	Intergenic
contig_86:1412	2	Intergenic
contig_85:91908	4	Intergenic
contig_78:253958	3	TLP1: Thaumatin-like protein 1
contig_77:67295	2	Intergenic
contig_65:150818	2	Intergenic
contig_65:150790	2	Intergenic
contig_65:150649	2	Intergenic
contig_62:68084	1	HAC1 Histone acetyltransferase HAC1
contig_6:562715	4	g08222.t1_hypothical protein
contig_52:37100	1	Intergenic
contig_51:24400	3	Intergenic
contig_5:2140	2	Intergenic
contig_43:19160	1	Intergenic
contig_38:1663	2	Intergenic
contig_37:1225154	4	Intergenic
contig_36:356362	3	Intergenic
contig_30:955002	1	Intergenic
contig_25:148216	2	Intergenic
contig_18:1266	3	g06934.t1
contig_15:211972	4	Intergenic
contig_15:211844	2	Intergenic
contig_127:30432	2	Intergenic
contig_125:9903	2	Intergenic
contig_111:6540	4	Intergenic
contig_111:12114	1	SYT3 Synaptotagmin-3
contig_11:1129	1	Intergenic
contig_105:1444	1	Intergenic

In Summary

1) Cultivate field adapted strain across sites and cultivation type

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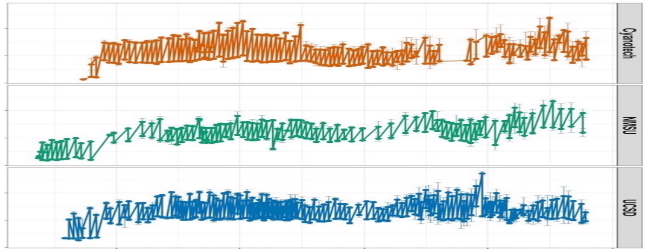
2) Acquired growth and metadata across ~3 year time span



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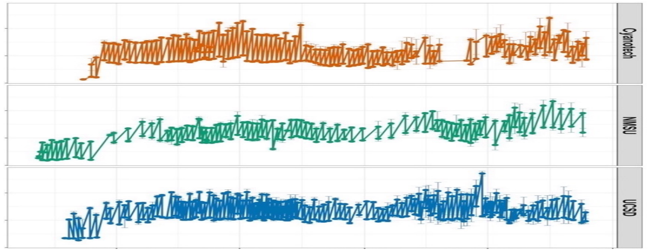

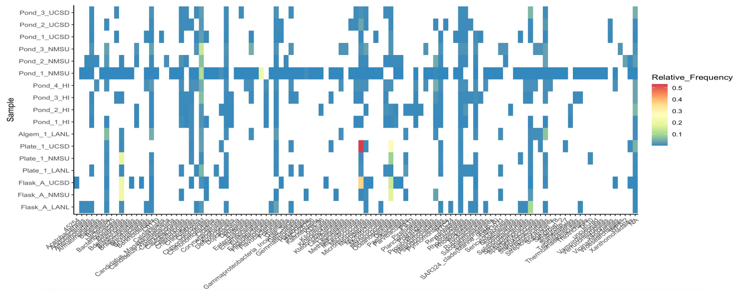

1) Cultivate field adapted strain across sites and cultivation type

2) Acquired growth and metadata across ~3 year time span

3) Characterize microbial dynamics in lab and field maintained cultures
    1) Differences in microbial diversity across labs
    2) Identification of potential pest

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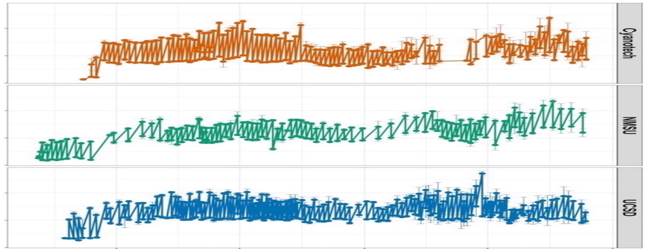

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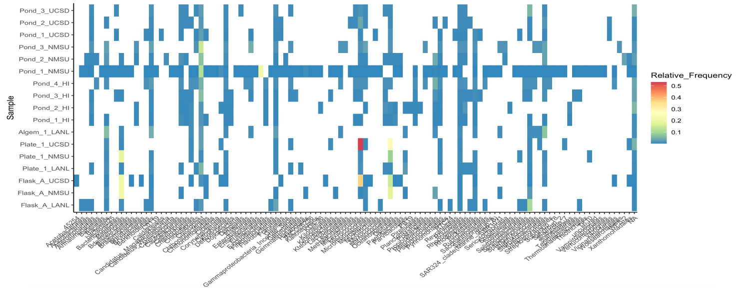

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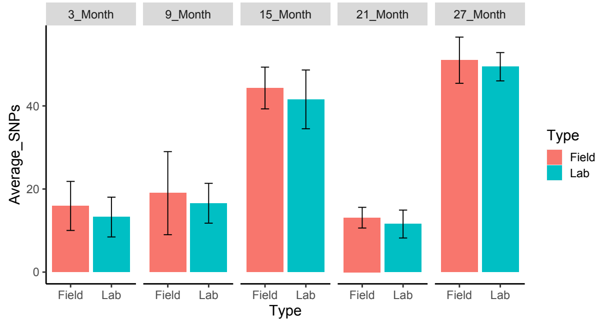

1) Track algal genotypic changes through time
   1) General trend of increase in mutations over time
   2) Associate mutations with increases in productivity

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