

Examining nutrient uptake and transformation within photosynthetic microbial communities using a high density bioreactor

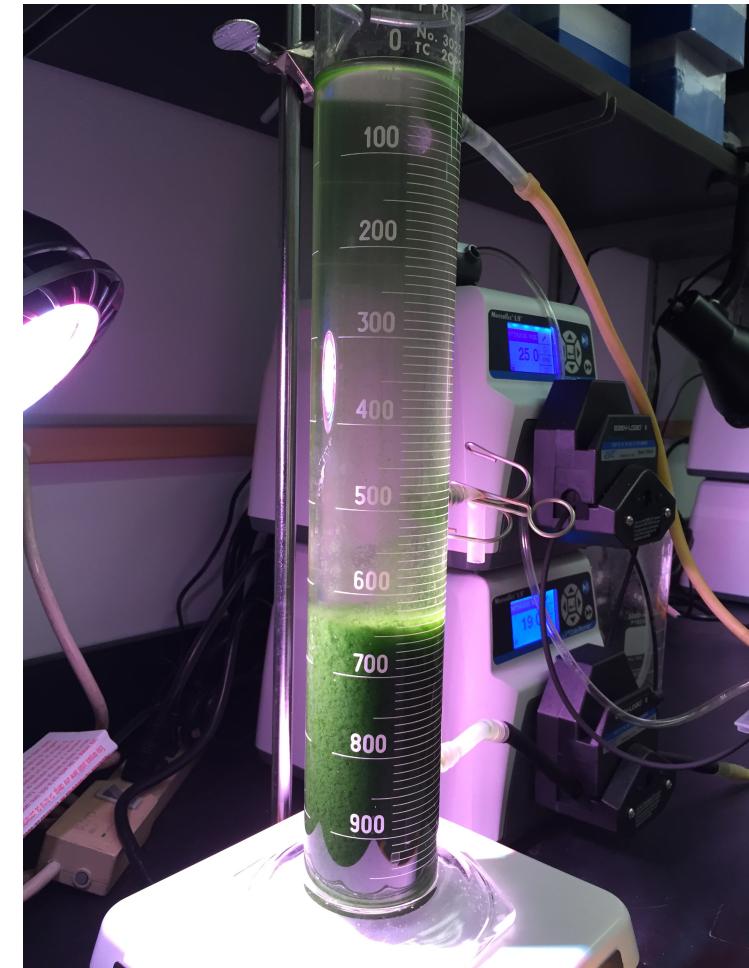
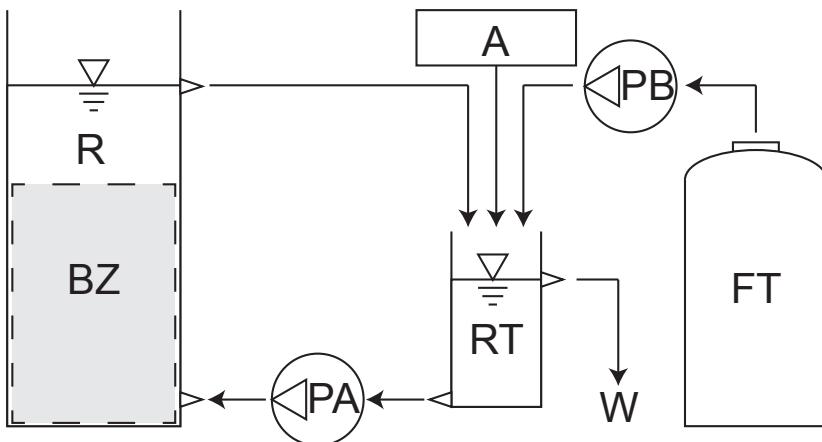
Jacob R. Price & Christopher M. Sales.

2018 ACS National Meeting
March 21st, 2018

microbes.cae.drexel.edu @SalesLaboratory
jacobrprice.github.io @Jake_in_the_Lab

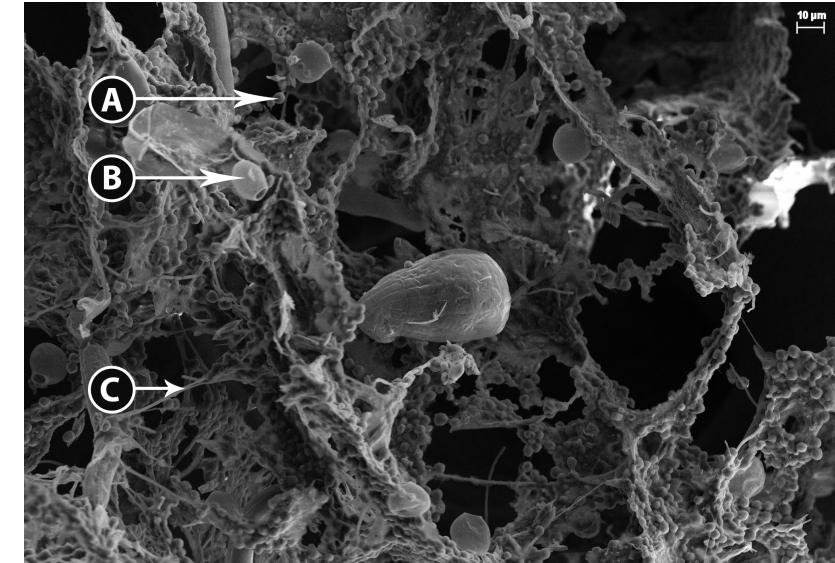
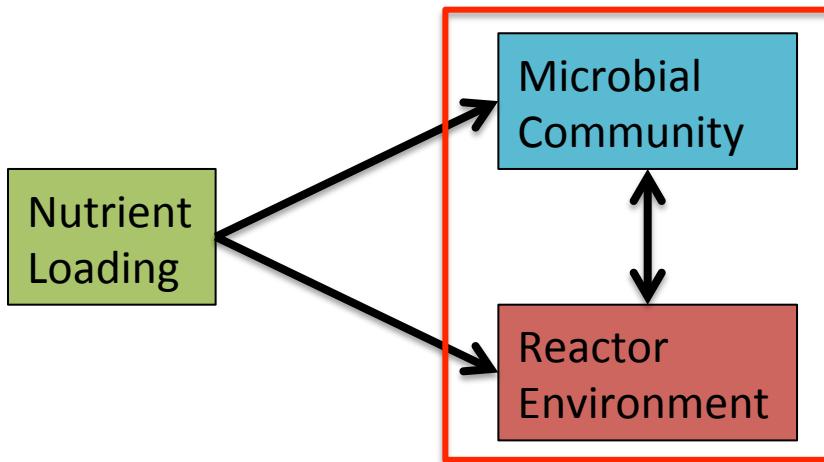


High Density Bioreactor (HDBR)^a



^a Price, J.R., Shieh, W.K., Sales, C.M. 2015. A novel bioreactor for high density cultivation of diverse microbial communities. *J. Vis. Exp.* (106), e53443, doi:10.3791/53443.

Background



Forces shaping community structure:

- Stochastic
- Deterministic
- Ecological
 - Natural selection
 - Genetic drift

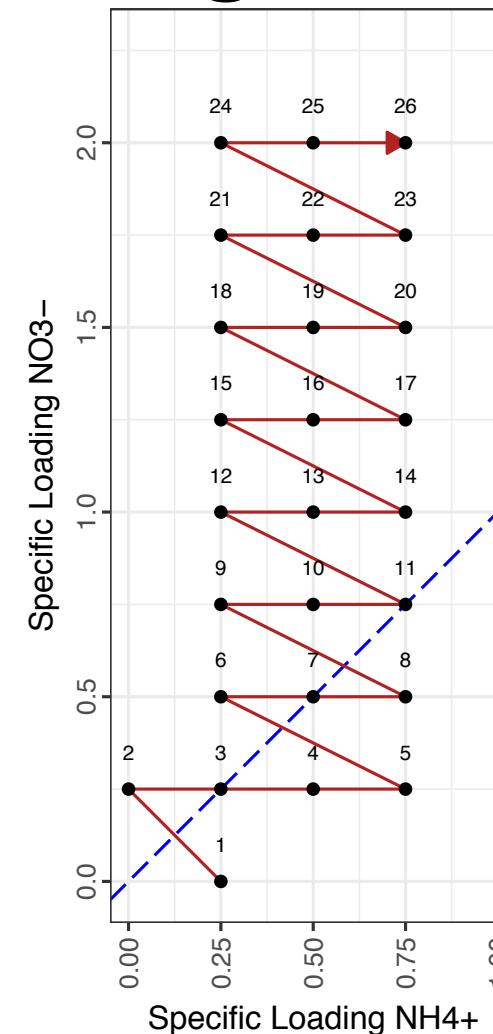


Figure Credits: ^b Price,J.R., Keshani Langroodi, S., Lan, Y., Becker, J.M., Shieh, W.K., Rosen, G.L., and C.M. Sales. 2016. Untangling the microbial ecosystem and kinetics in a nitrogen removing photosynthetic high density bioreactor. Environ. Sci.: Water Res. Technol. doi: 10.1039/C6EW00078A.



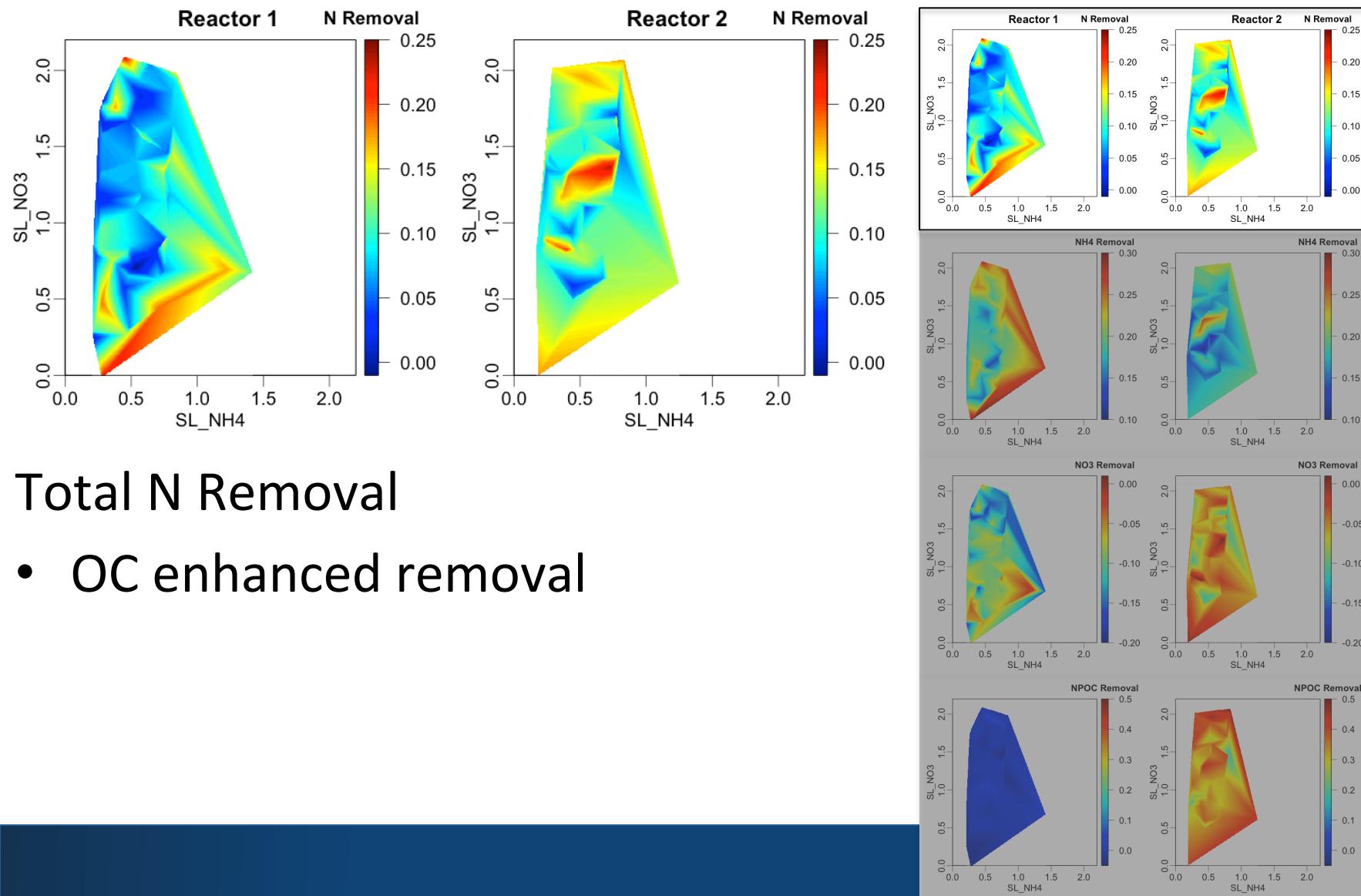
Experimental Design*

- Two reactors:
 - R1: no supplemental OC
 - R2: 10 mg/L TOC (as glucose)
- 26 loading conditions
 - Vary NH_4^+ & NO_3^-
 - 7 days/condition (generally)
 - COMBO media^c
- Data/samples collected:
 - Influent/effluent samples
 - Nutrient composition
 - Reactor operational parameters
 - Biomass, suspended solids, flow rate, etc.
 - Biological samples
 - Amplicon sequencing
 - V4-v5 16s rDNA
 - MiSeq 2x250 bp

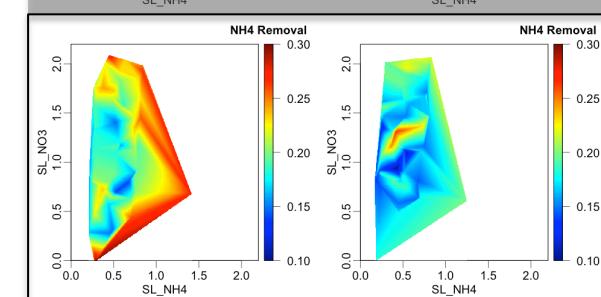
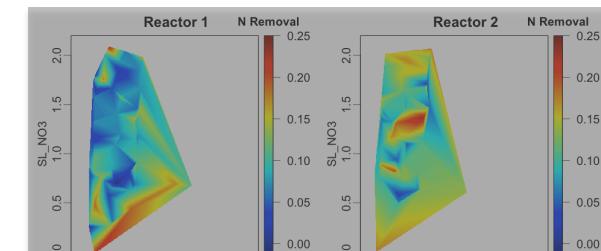
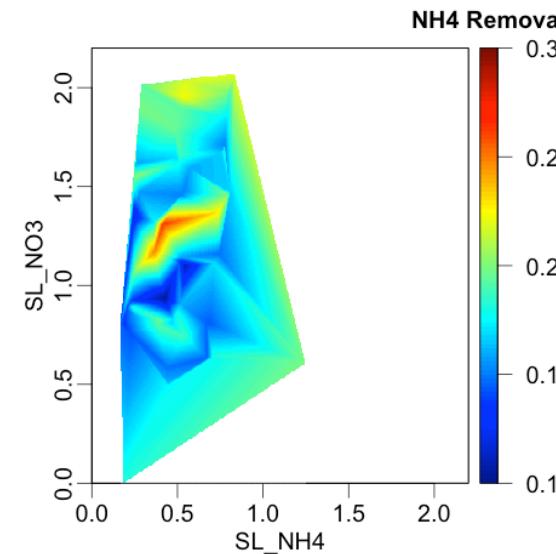
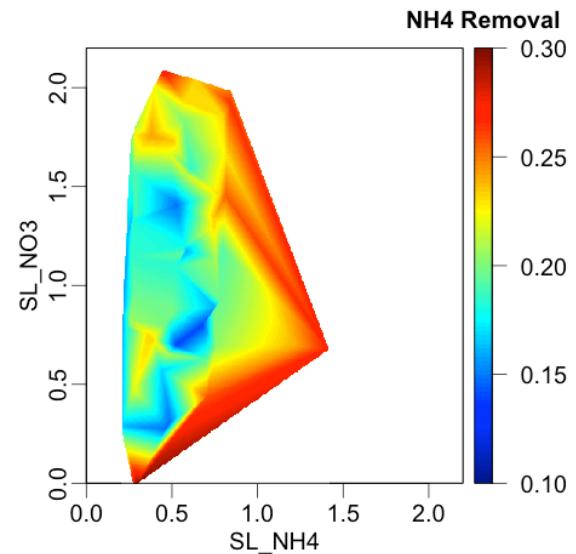


^c Kilham, S., Kreeger, D., Lynn, S., Goulden, C., Herrera, L. 1998. COMBO: a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia*. doi:10.1023/A:1003231628456

Nutrient Kinetics

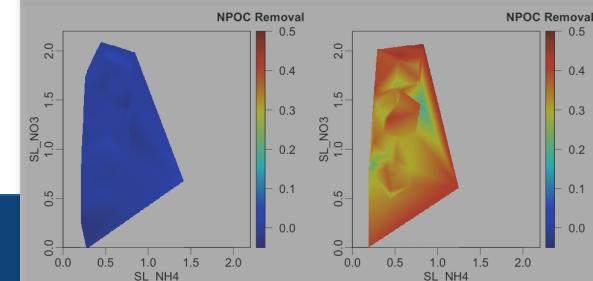
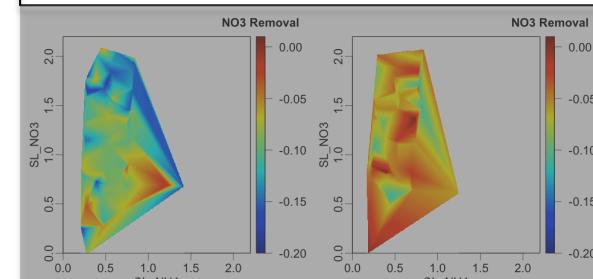


Nutrient Kinetics

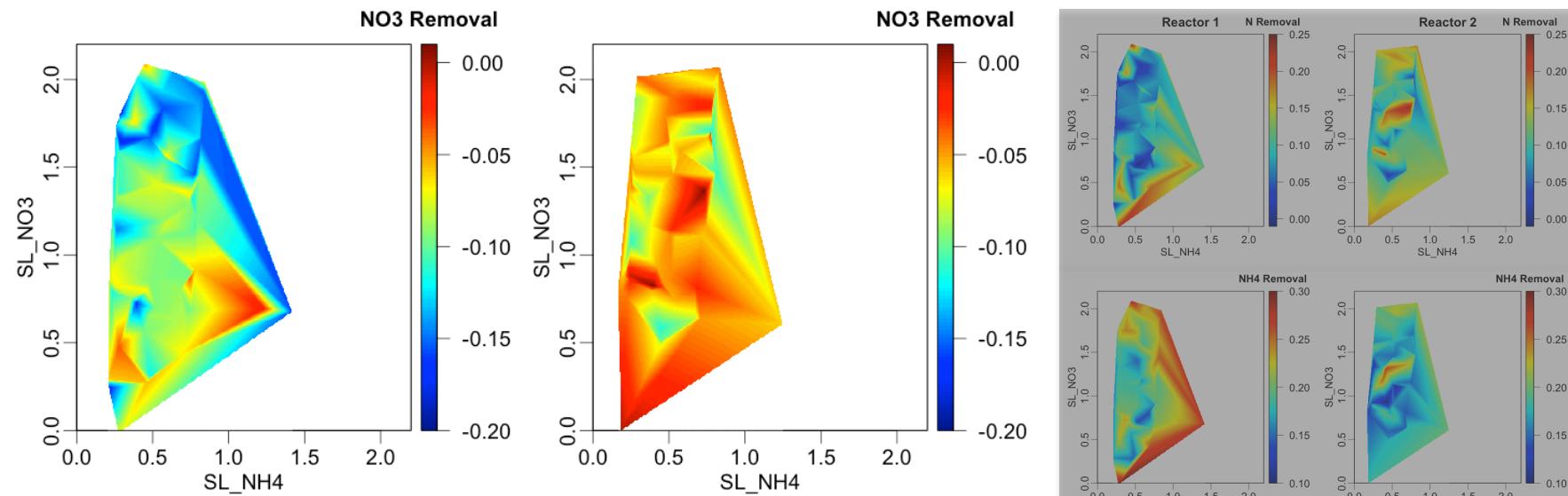


NH_4^+ Removal

- R1 & R2
 - increased with NO_3^- loading
- R1
 - achieved higher NH_4^+ removal

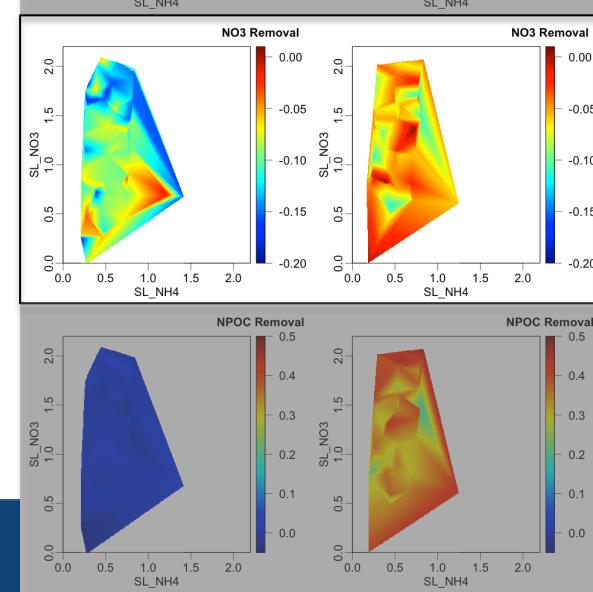


Nutrient Kinetics

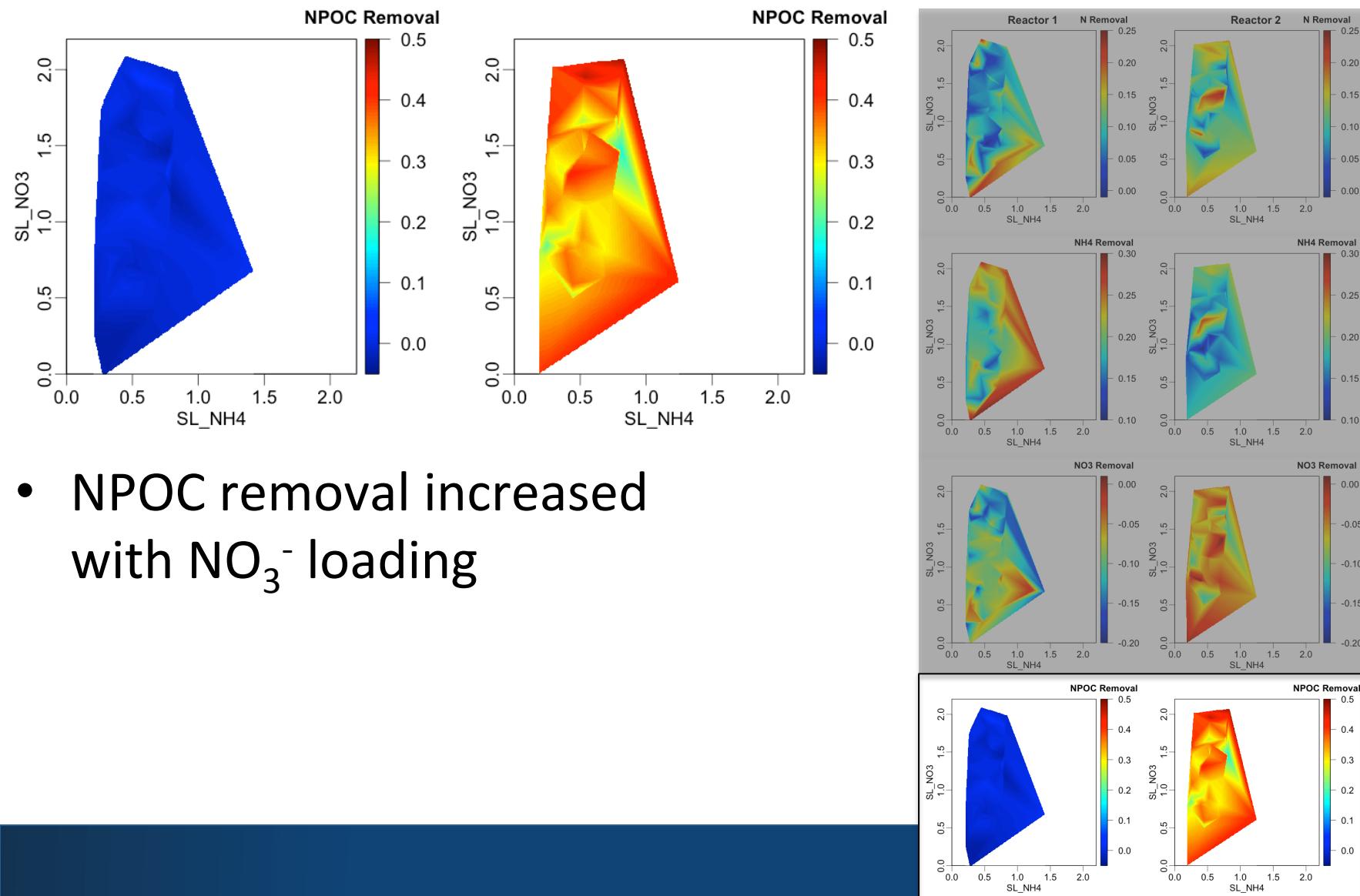


NO₃⁻ removal

- Globally negative
 - Nitrification is taking place within both reactors
- “Less negative” for R2

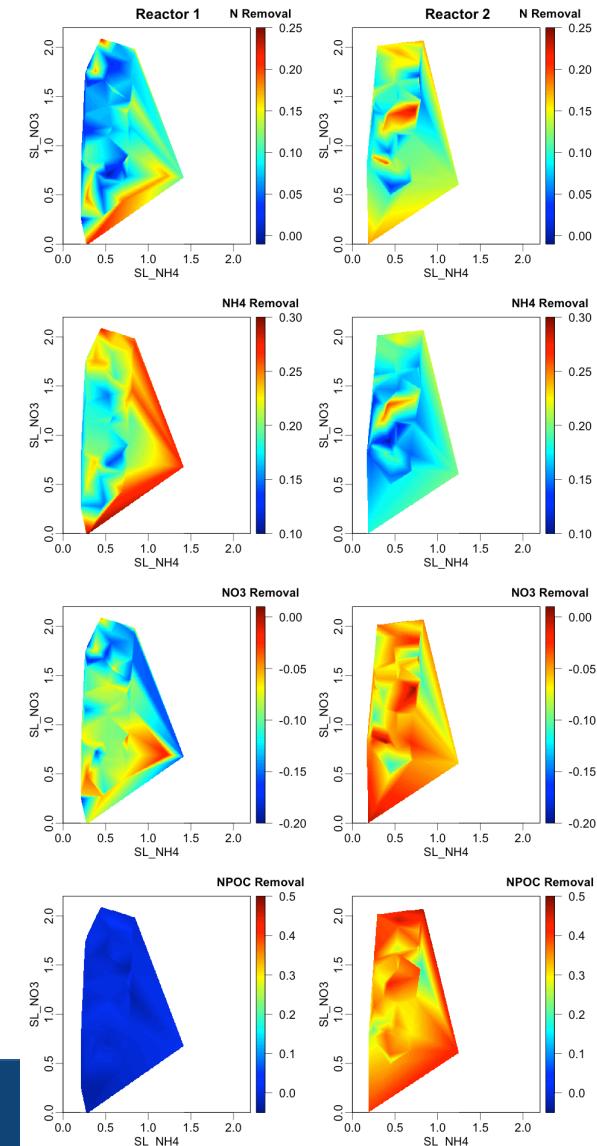


Nutrient Kinetics*



Motivation for dimensional reduction

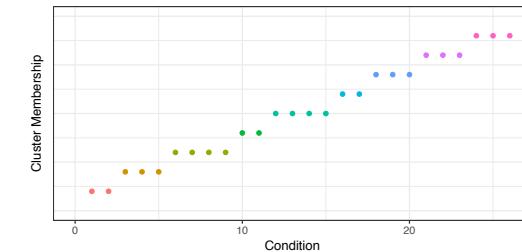
- Each of the nutrients (or operational parameters) could be compared in a pairwise fashion.
 - Tedious
 - Increases false discovery rate
 - Does not take other nutrients (P, S, etc) into account
- Need to simultaneously consider the influence of all variables
- **Microbial community structure is not being taken into account...**



Machine Learning Techniques

Temporally constrained clustering

- Hierarchical clustering method
- Cluster formation is constrained to objects/clusters which neighbor each other (temporally adjacent)
- Identify structural discontinuities within dataset



^d Palmer, M. Webpage: Ordination Methods – An overview. <http://ordination.okstate.edu/overview.htm>

^e Buttigieg PL, Ramette A (2014) A Guide to Statistical Analysis in Microbial Ecology: a community-focused, living review of multivariate data analyses. FEMS Microbiol Ecol. 90: 543–550.

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Ordination

- Principal Components Analysis
 - Creates projection of samples onto axes which maximize their variance

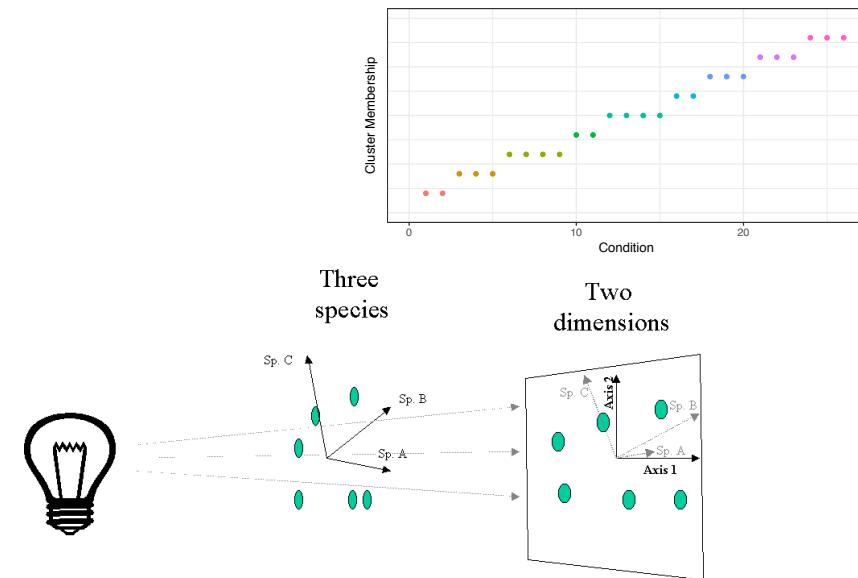


Figure credit: M. Palmer ^d

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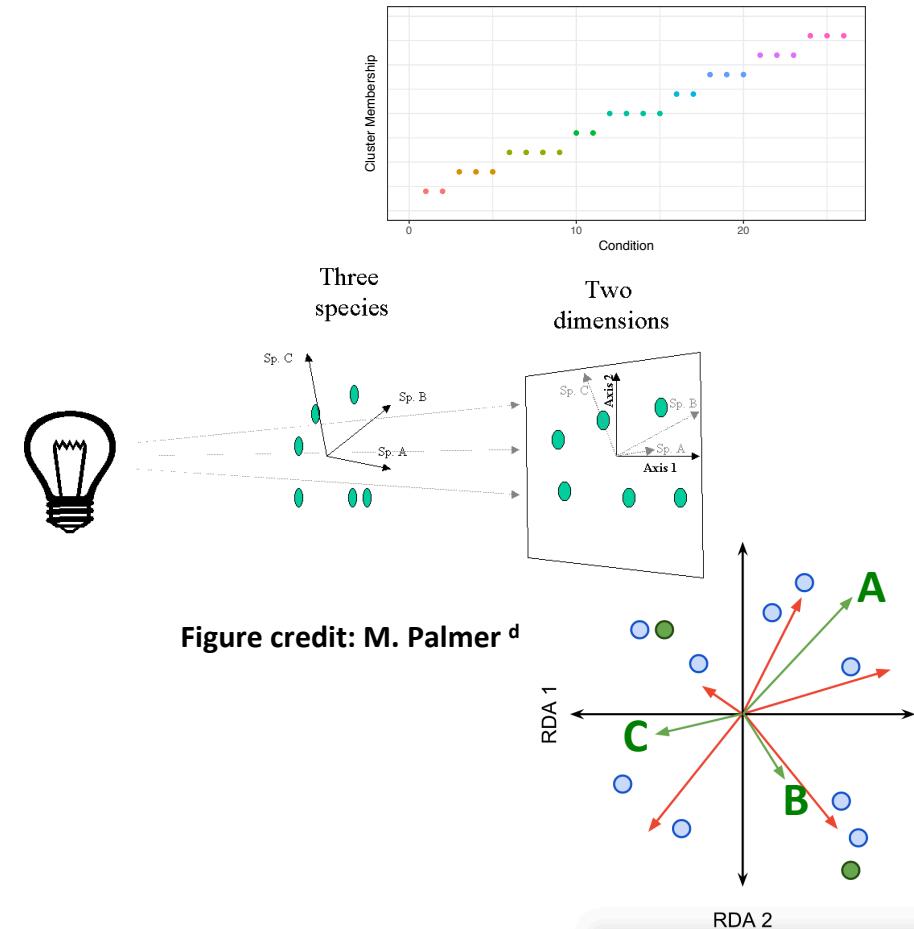
Machine Learning Techniques

Temporally constrained clustering

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Ordination

- Principal Components Analysis
 - Creates projection of samples onto axes which maximize their variance
- Redundancy Analysis
 - Constrained version of PCA
 - Analog to multivariate multiple regression



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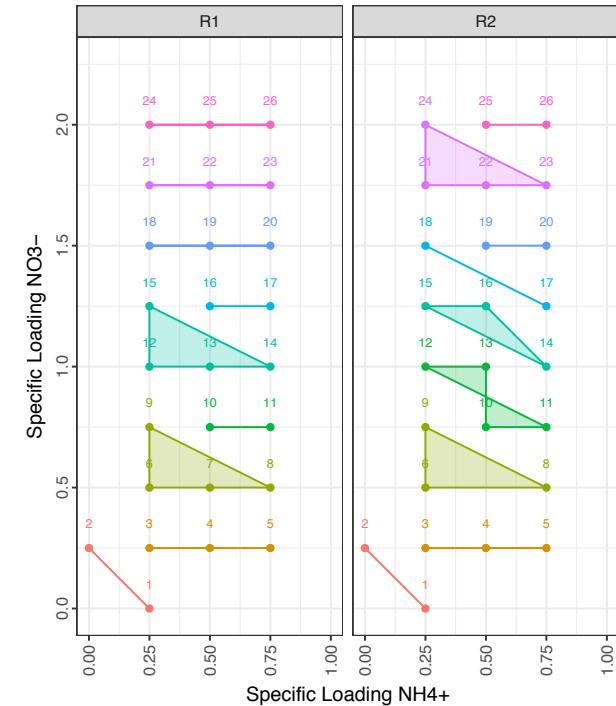
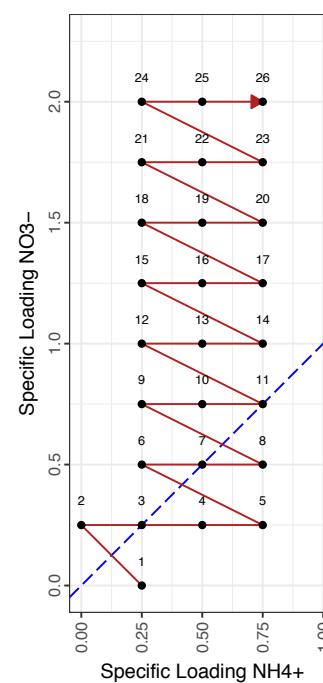
Hierarchical Clustering

NO_3^- loading

- dominates the microbial community structure

NH_4^+ loading

- No substantial impact on community structure
 - Nitrification already at its maximum rate
 - ? Small gradient length
 - ? O_2 limitation



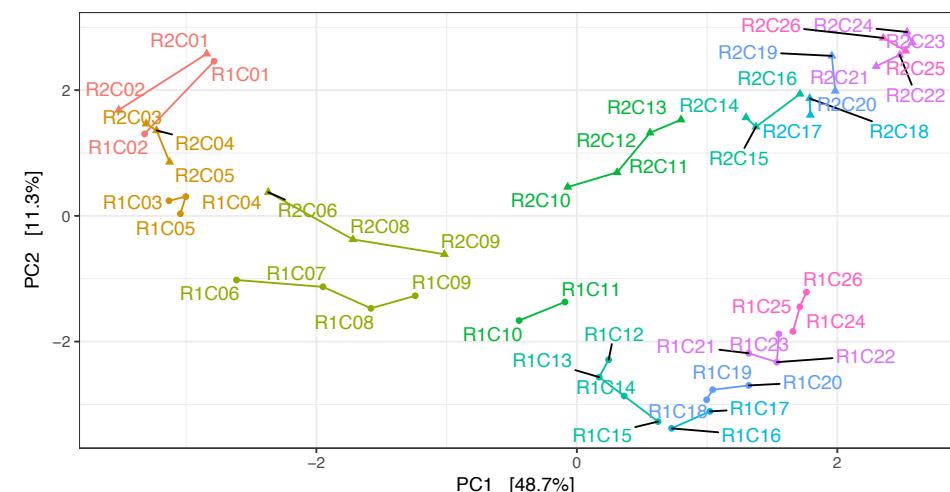
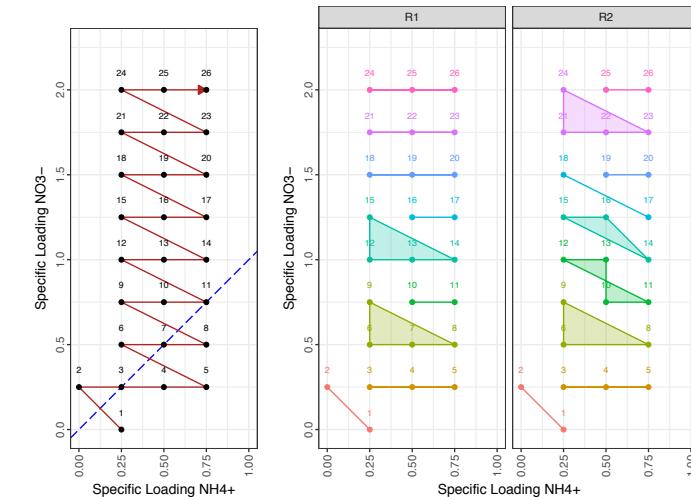
Principal Component Analysis (PCA)

1st axis

- appears to be dominated by:
 - Time
 - NO_3^- loading
- Large discontinuity between clusters 3 & 4
 - Shut down between C6 & C7 for filming ^a

2nd axis

- Differentiation between reactors
 - Possible NPOC influence

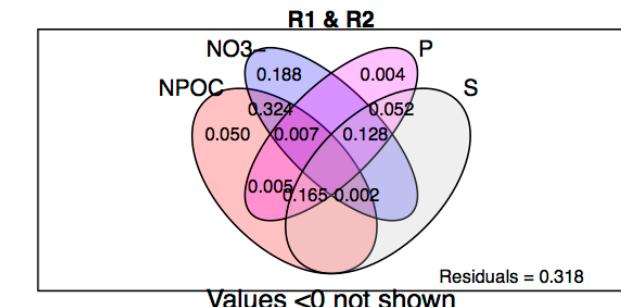
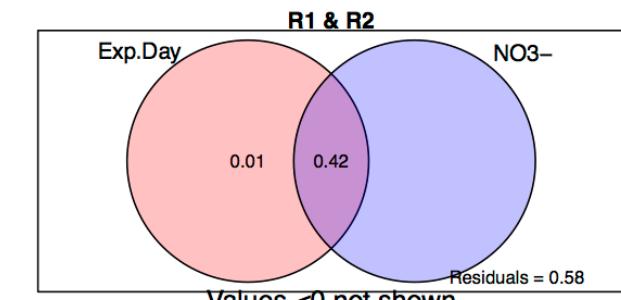
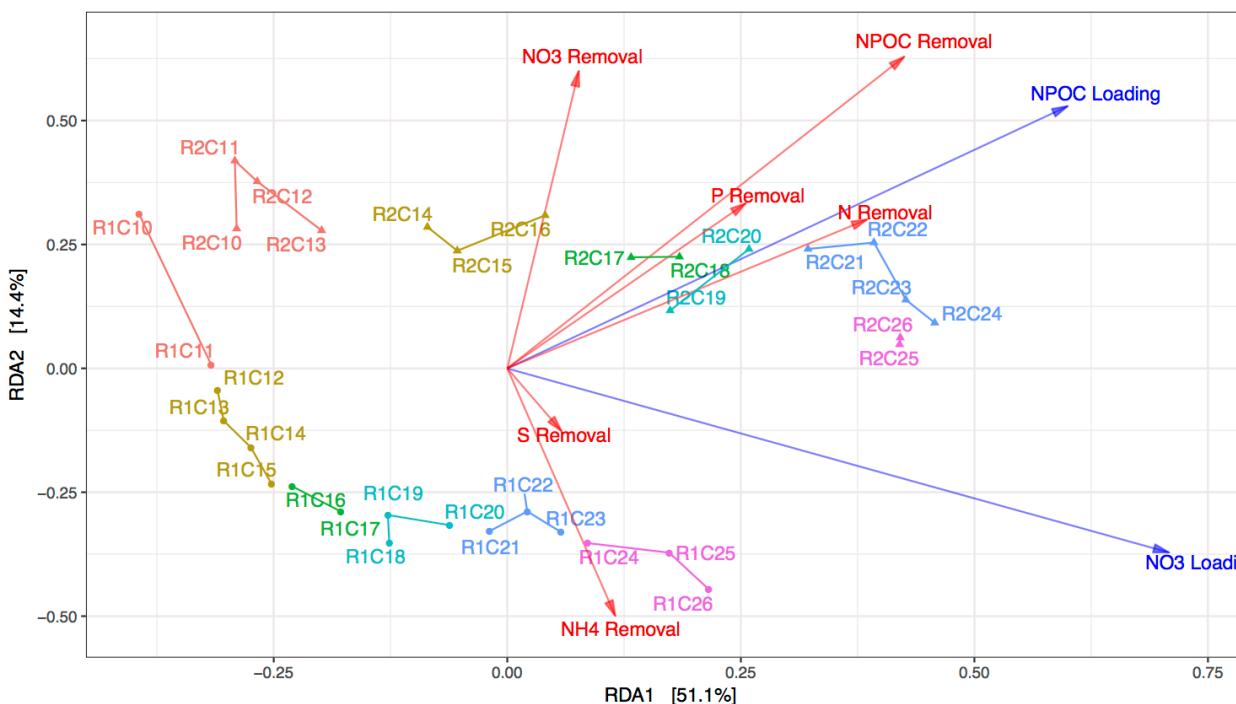


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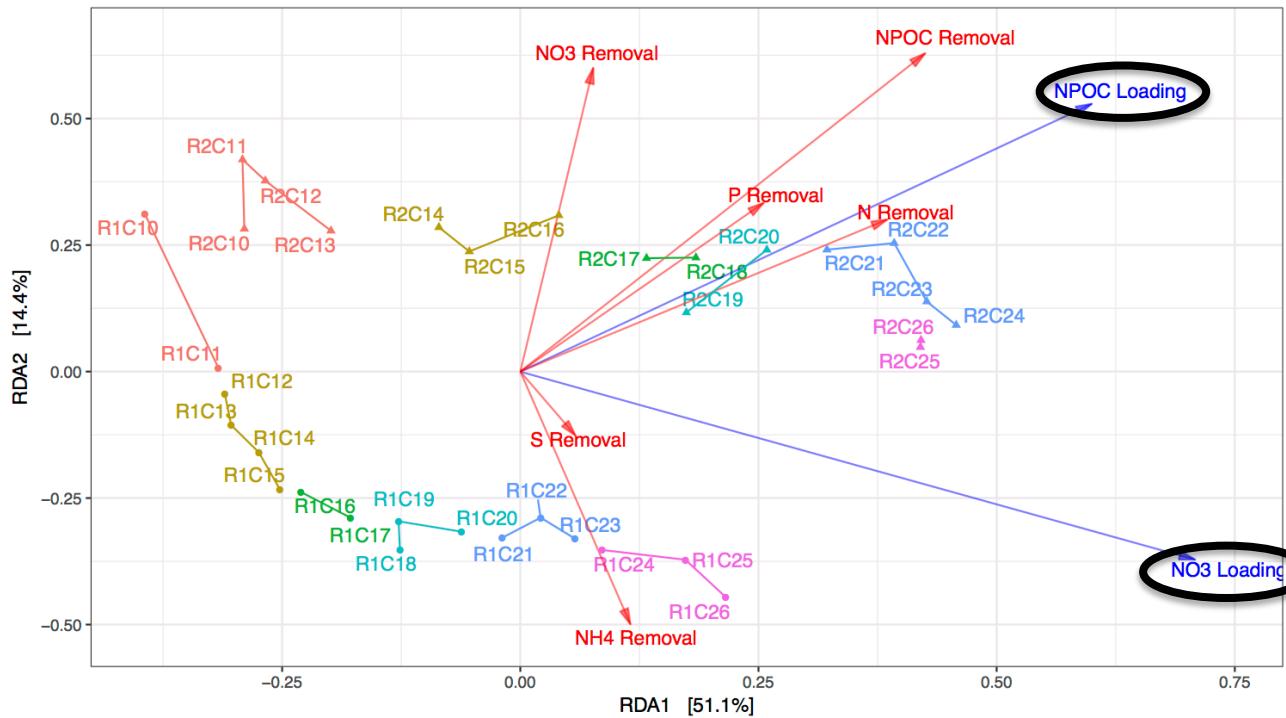
Redundancy Analysis (RDA)

Parsimonious model: ($r^2 = 0.40$, adj. $r^2 = 0.37$)

- Community $\sim \text{NO}_3^-$ loading + NPOC loading
- Removal rates fitted to constrained model after it was constructed
- RDA model
 - confirms conclusions from kinetics and PCA
 - and expands upon them



Redundancy Analysis



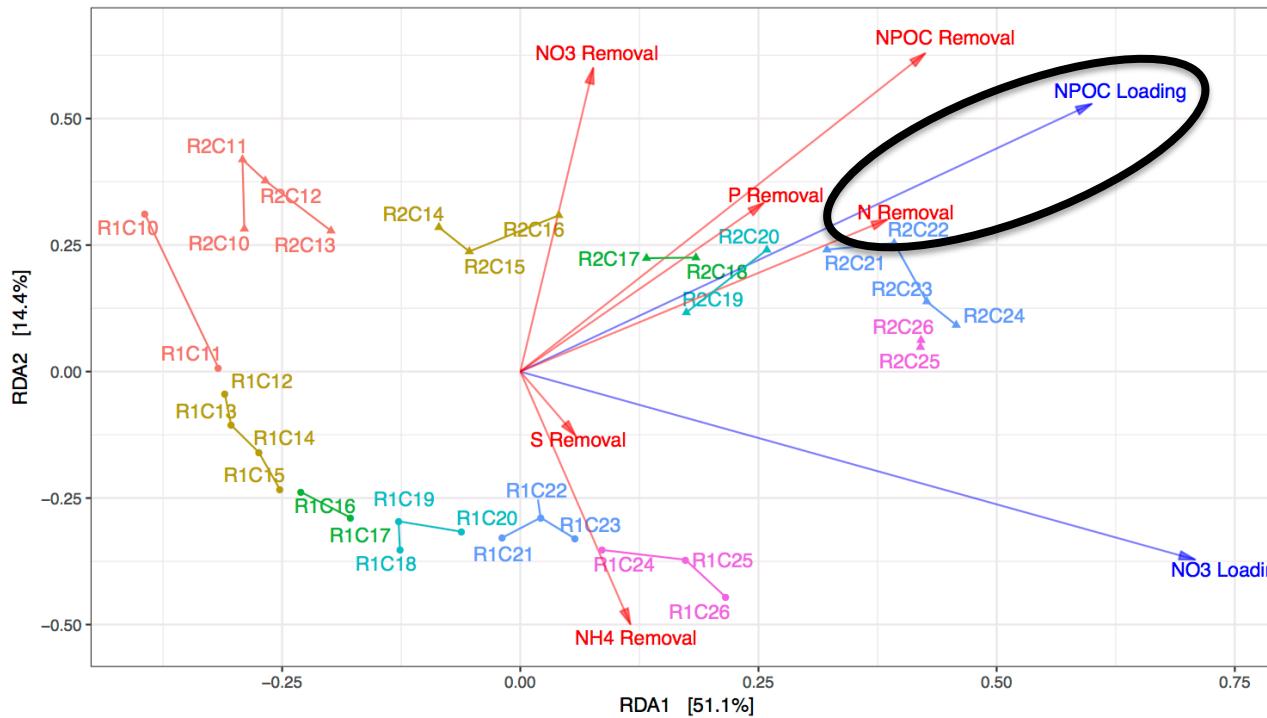
PCA

- 1st axis dominated by NO₃⁻ loading
- NPOC drives differentiation between reactors via 2nd axis

Temporally-constrained clustering

- NO₃⁻ loading determines cluster membership

Redundancy Analysis



PCA

- 1st axis dominated by NO_3^- loading
- NPOC drives differentiation between reactors via 2nd axis

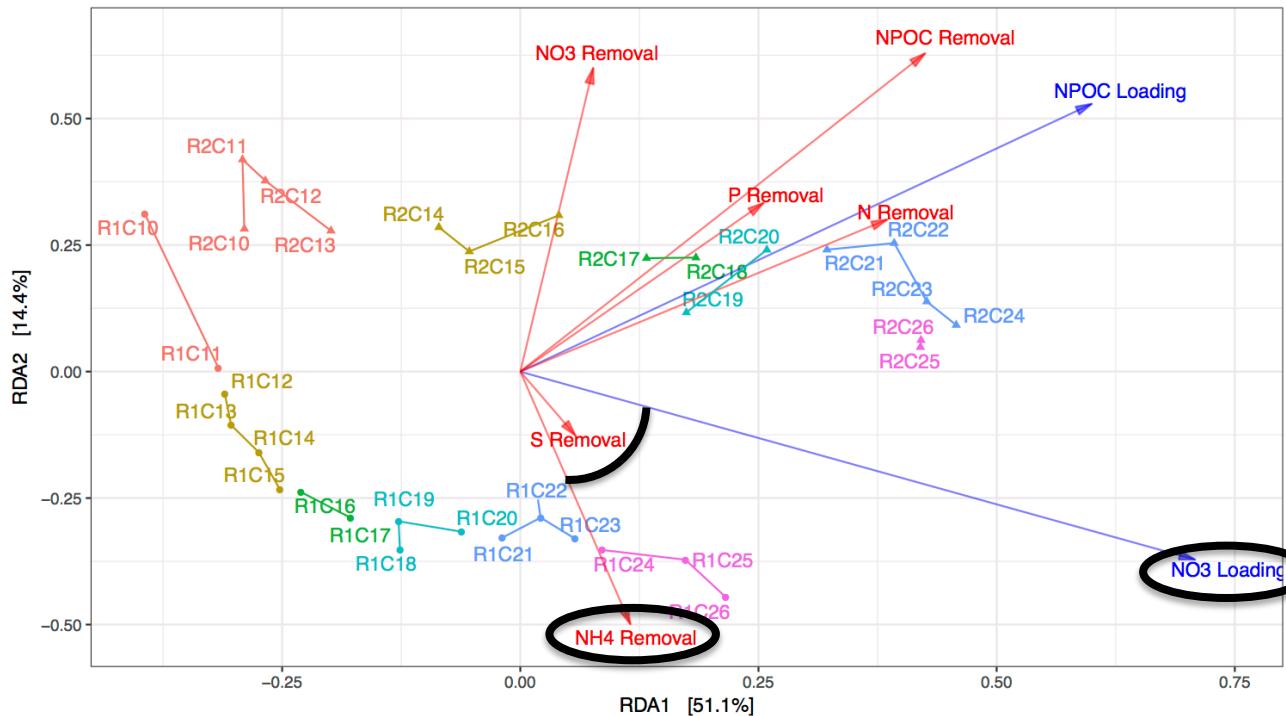
Temporally-constrained clustering

- NO_3^- loading determines cluster membership

Kinetics

- **NPOC increases total N removal**

Redundancy Analysis



PCA

- 1st axis dominated by NO_3^- loading
- NPOC drives differentiation between reactors via 2nd axis

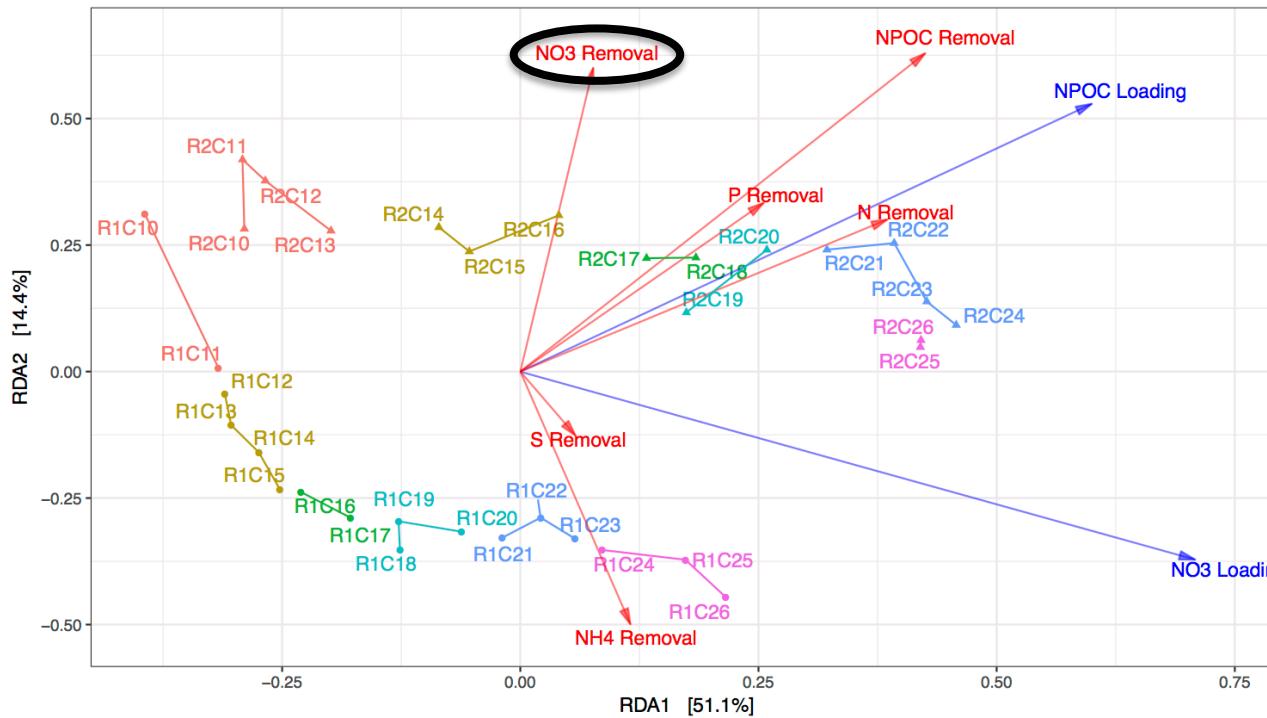
Temporally-constrained clustering

- NO_3^- loading determines cluster membership

Kinetics

- NPOC increases total N removal
- **NH₄⁺ removal**
 - R1 more removal than R2
 - Removal increased with NO_3^- loading

Redundancy Analysis



PCA

- 1st axis dominated by NO_3^- loading
- NPOC drives differentiation between reactors via 2nd axis

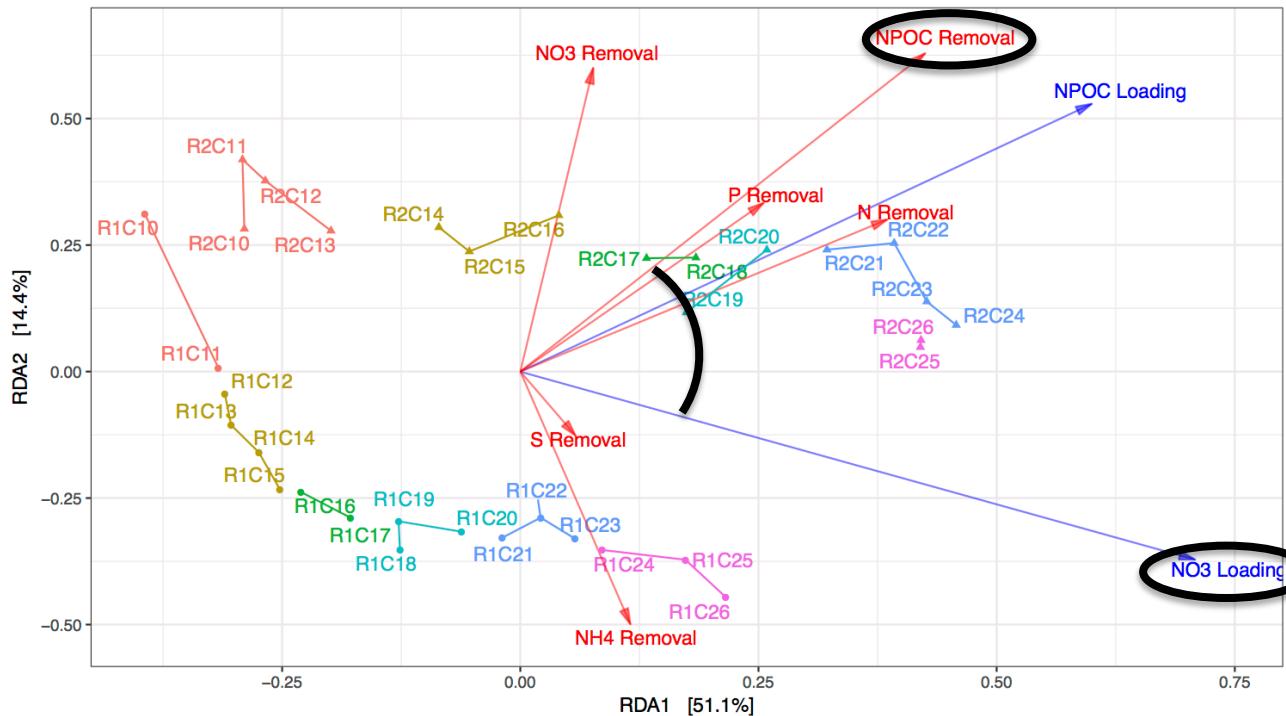
Temporally-constrained clustering

- NO_3^- loading determines cluster membership

Kinetics

- NPOC increases total N removal
- NH_4^+ removal
 - R1 more removal than R2
 - Removal increased with NO_3^- loading
- **Higher NO_3^- removal in R2**

Redundancy Analysis*



PCA

- 1st axis dominated by NO_3^- loading
- NPOC drives differentiation between reactors via 2nd axis

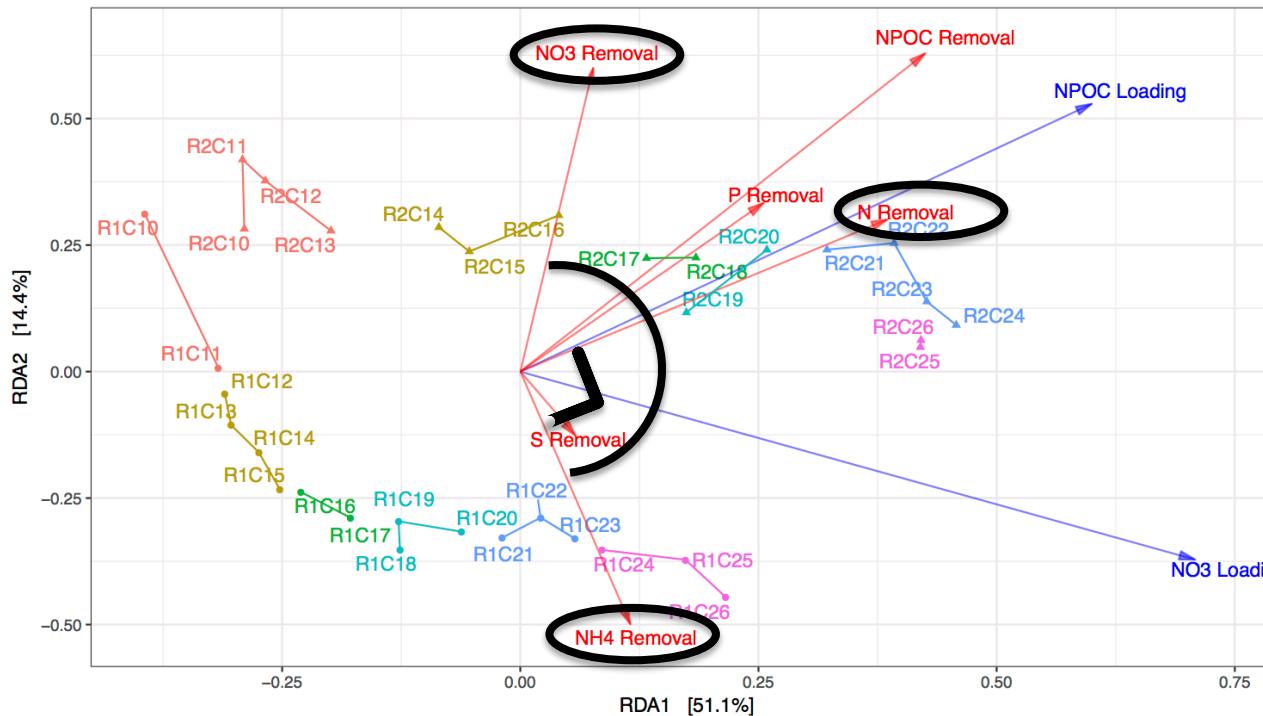
Temporally-constrained clustering

- NO_3^- loading determines cluster membership

Kinetics

- NPOC increases total N removal
- NH_4^+ removal
 - R1 more removal than R2
 - Removal increased with NO_3^- loading
- Higher NO_3^- removal in R2
- **NPOC removal increased with NO_3^- loading**

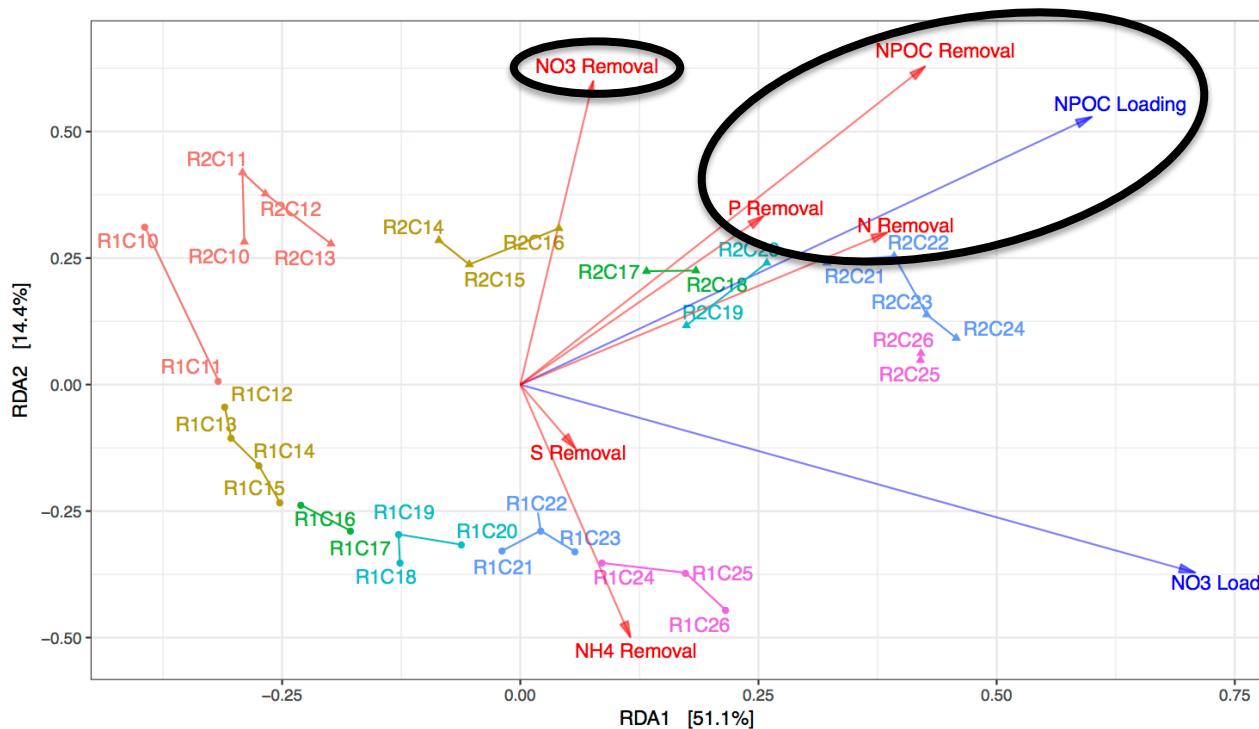
Redundancy Analysis



Expanding upon results

- Nitrification occurs in both reactors
 - Negative correlation between NH_4^+ removal and NO_3^- removal
- No correlation between NH_4^+ removal and total N removal
 - Limited nitrification rates

Redundancy Analysis*



Expanding upon results

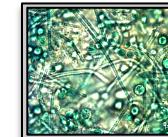
- Nitrification occurs in both reactors
 - Negative correlation between NH₄⁺ removal and NO₃⁻ removal
- No correlation between NH₄⁺ removal and total N removal
 - Limited nitrification rates
- **Heterotrophic growth drives nutrient removal**
 - Heterotrophs may be relying on NO₃⁻ as e- acceptor
- **Implies anaerobic/micro-aerobic conditions**
 - Supports O₂ limitation as cause for limited nitrification rates

Summary & Conclusion

- Many mixed community studies rely on a combination of theoretical considerations and results from axenic culture studies
 - May limit analysis by not accounting for a number of ecologically relevant forces
- Using the described methods, results are
 - Quantitative
 - Statistically rigorous
- We demonstrate that a number of competing and complementing forces shape the variation and structure of microbial communities within PBRs including:
 - Stochastic effects
 - Due to time (autocorrelation)
 - Deterministic effects
 - Due to nutrient loading / availability
 - Divergent forces
 - Genetic drift (? Time ?)
 - Environmental pressures causing selection (NPOC)

Acknowledgements

- Christopher Sales
 - Aspen Walker
 - Thomas Thompson
 - Jonas Becker
- theseus – An R package for the analysis and visualization of microbial community data
 - Stephen Woloszynek
 - Gail Rosen
 - <https://cran.r-project.org/web/packages/theseus/>



Sales Laboratory



References

- ^a Price, J.R., Shieh, W.K., Sales, C.M. 2015. A novel bioreactor for high density cultivation of diverse microbial communities. *J. Vis. Exp.* (106), e53443, doi:10.3791/53443.
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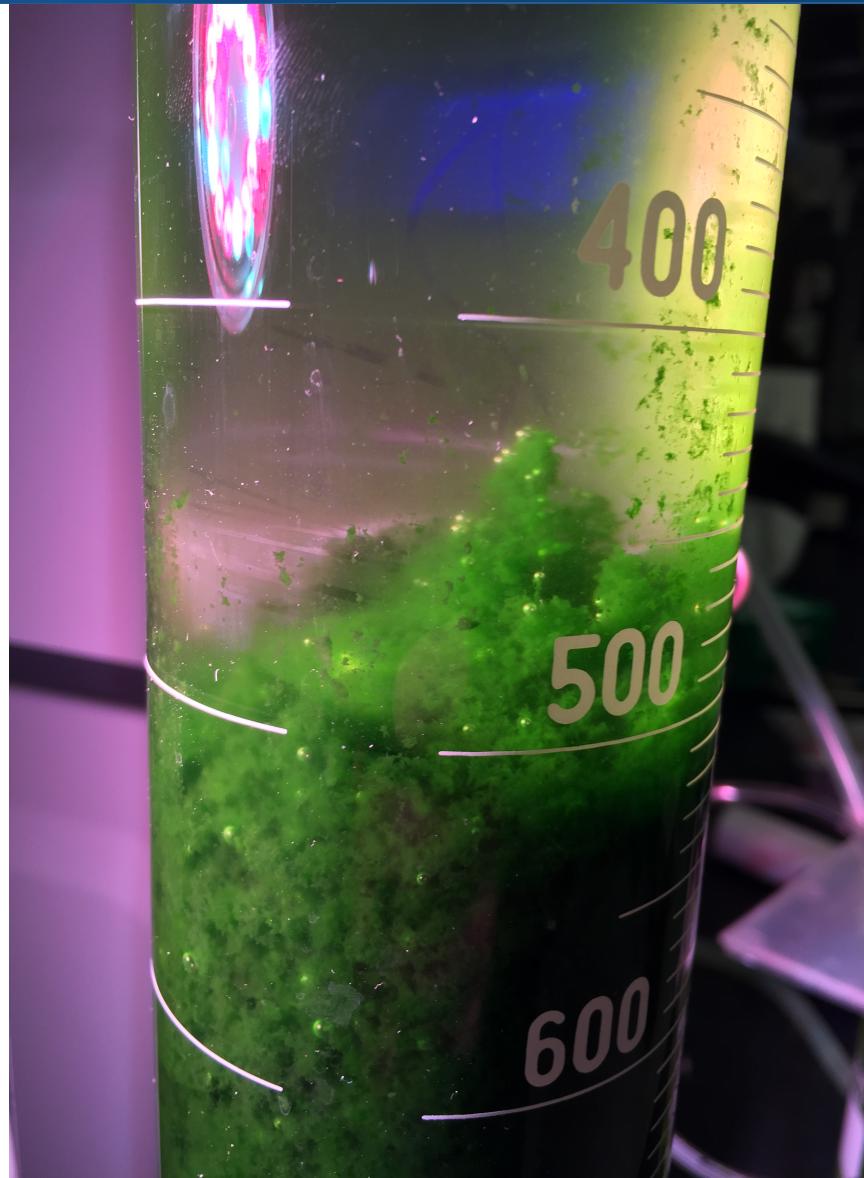


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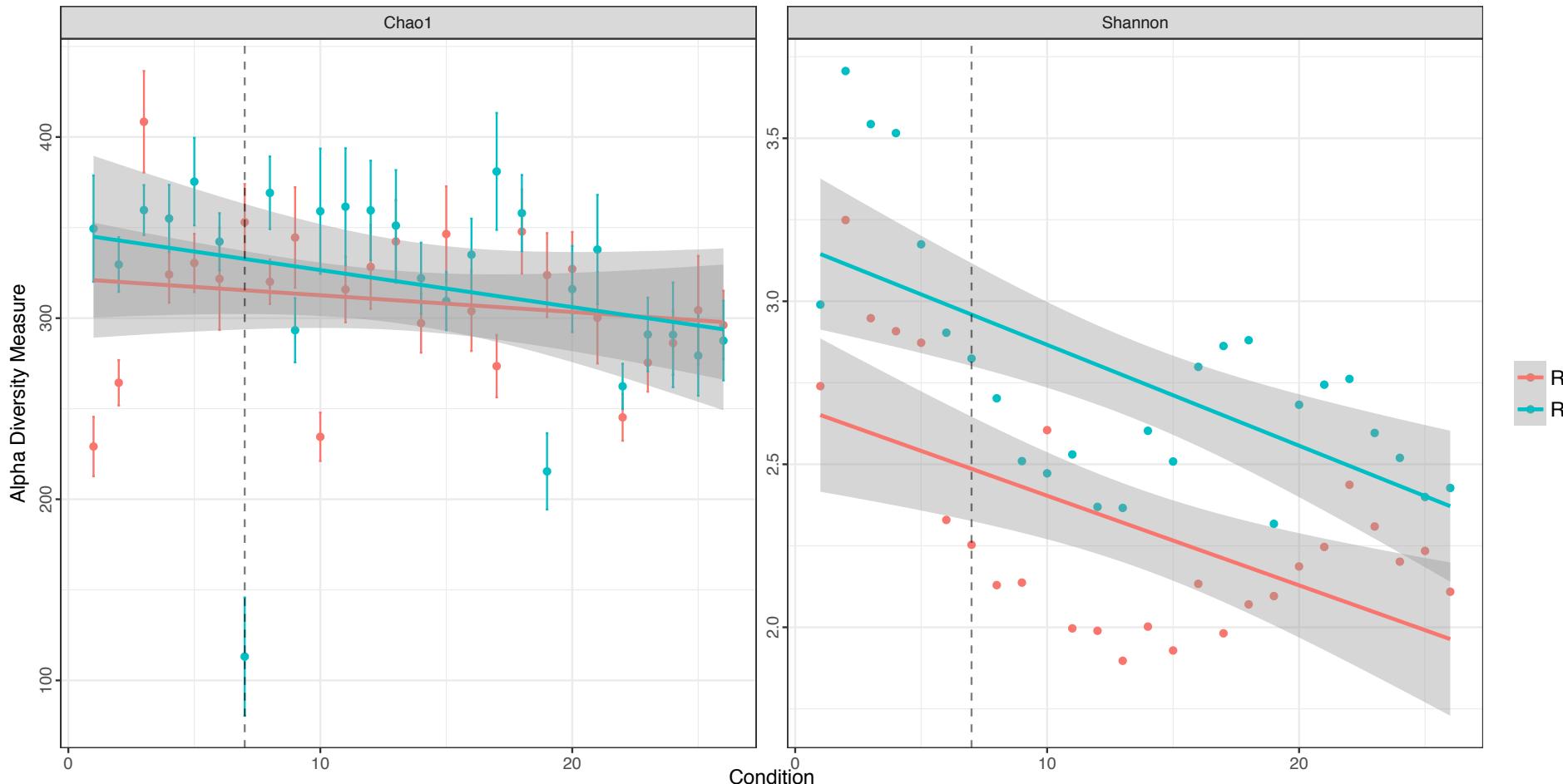
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Environmental Engineering

College of Engineering

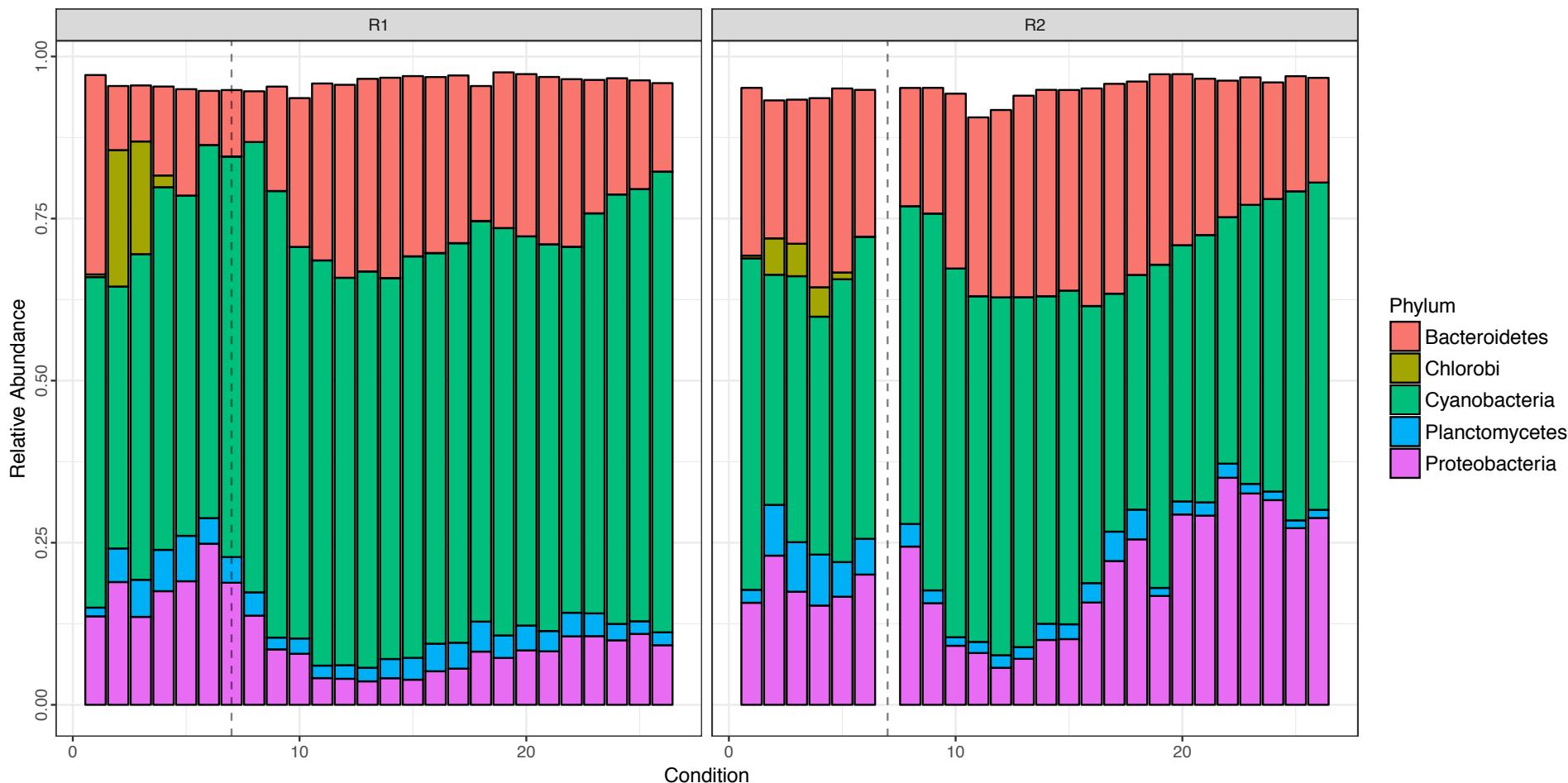
Supplemental Figures



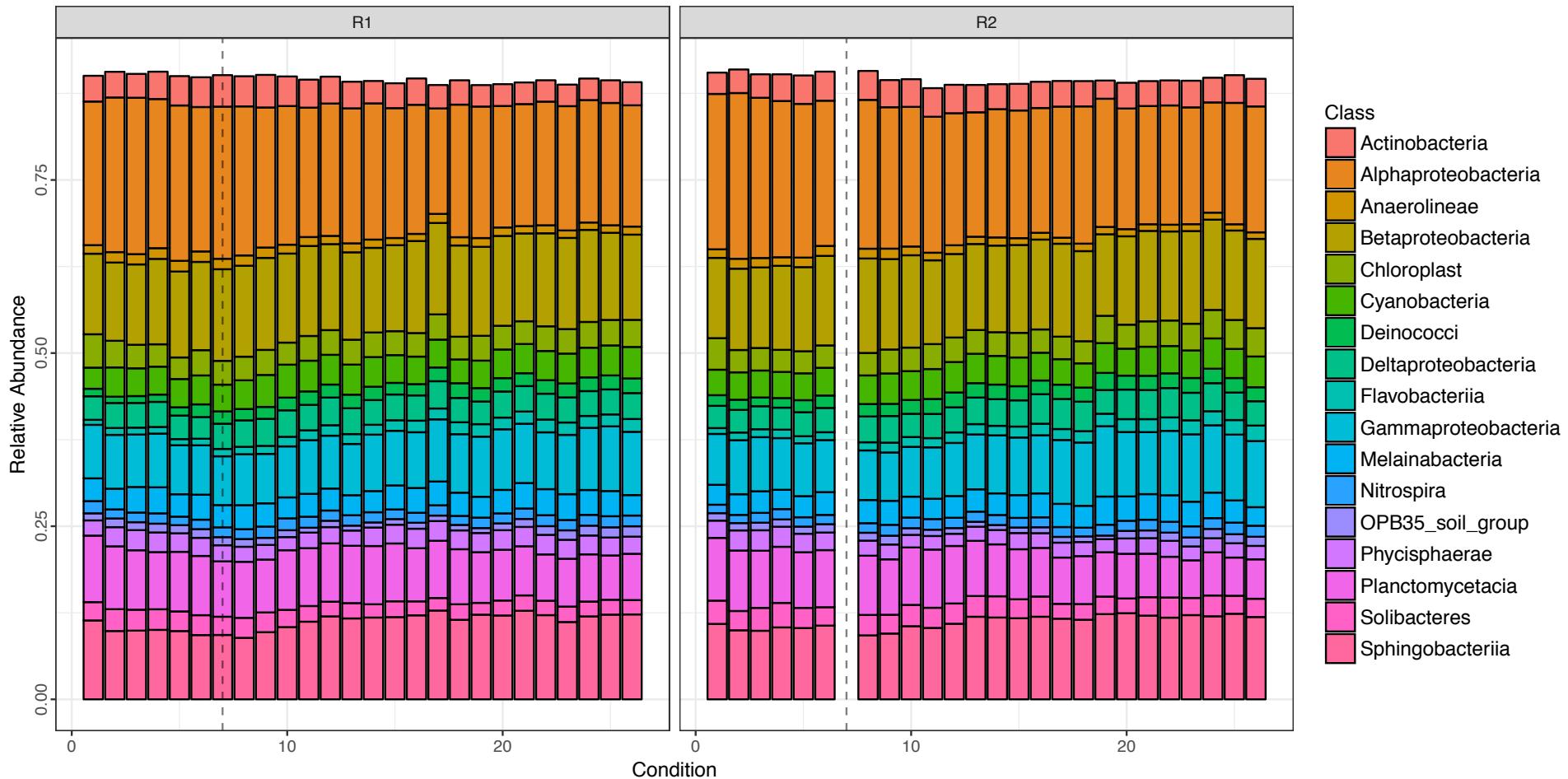
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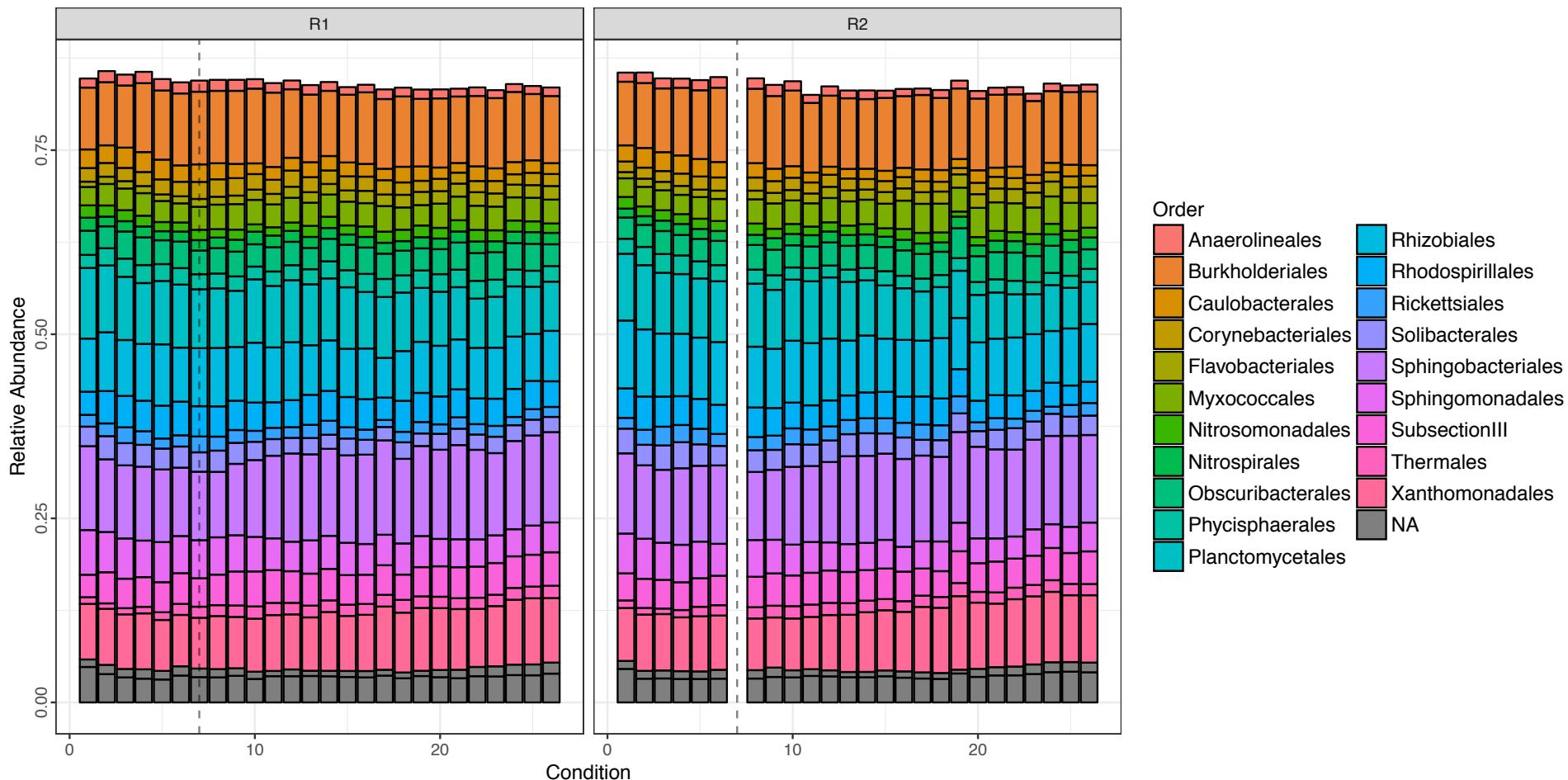
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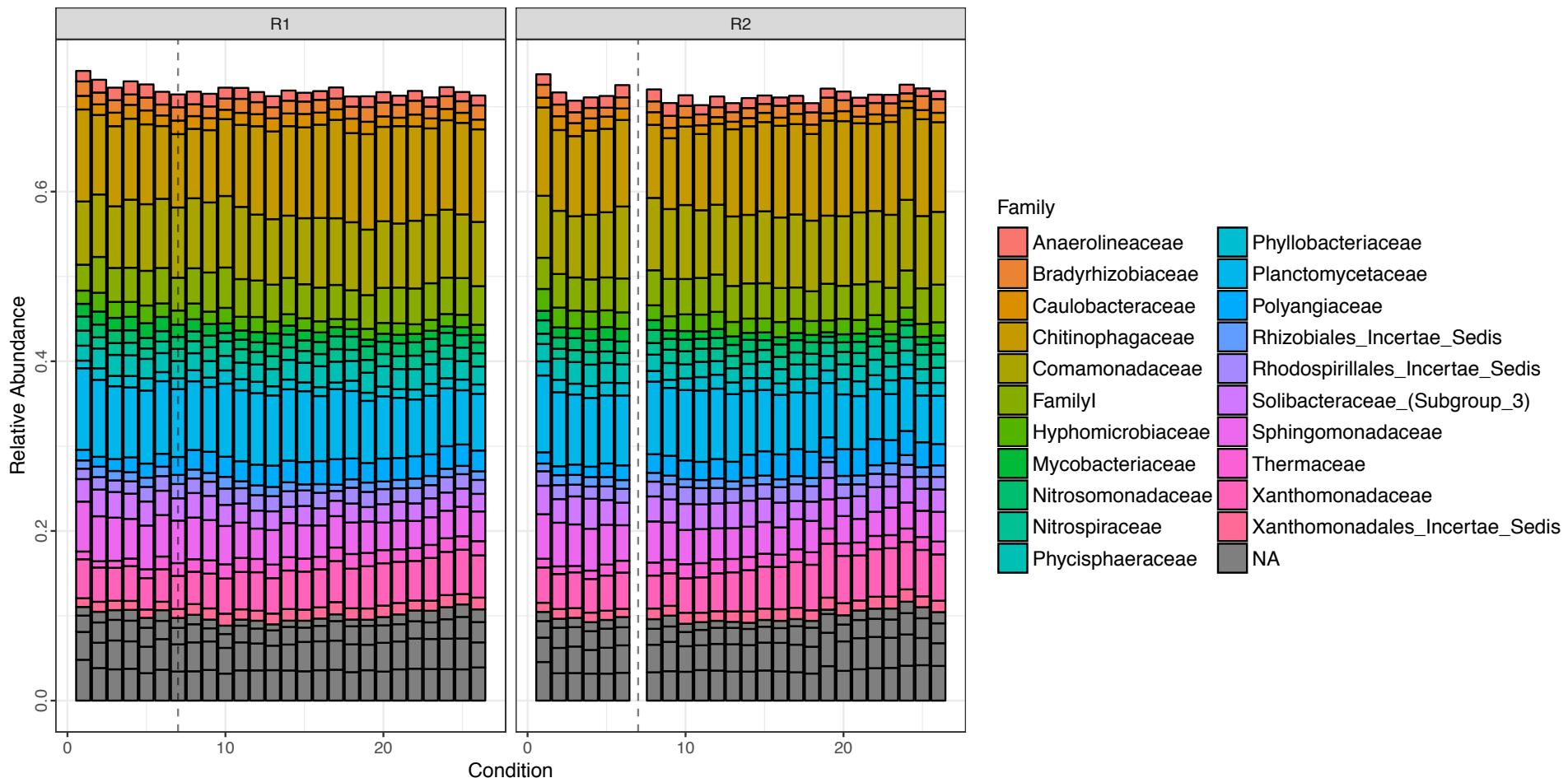
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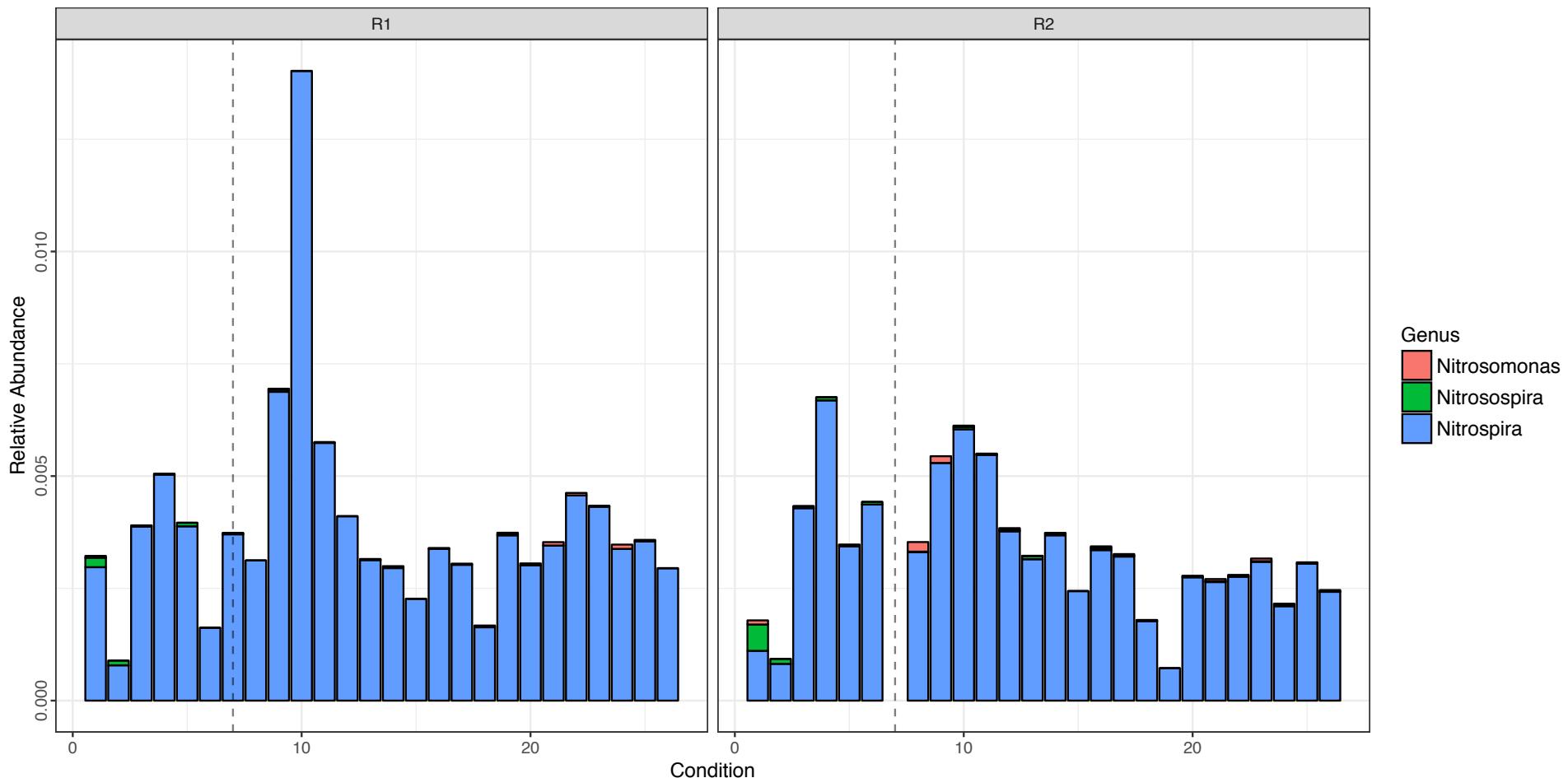
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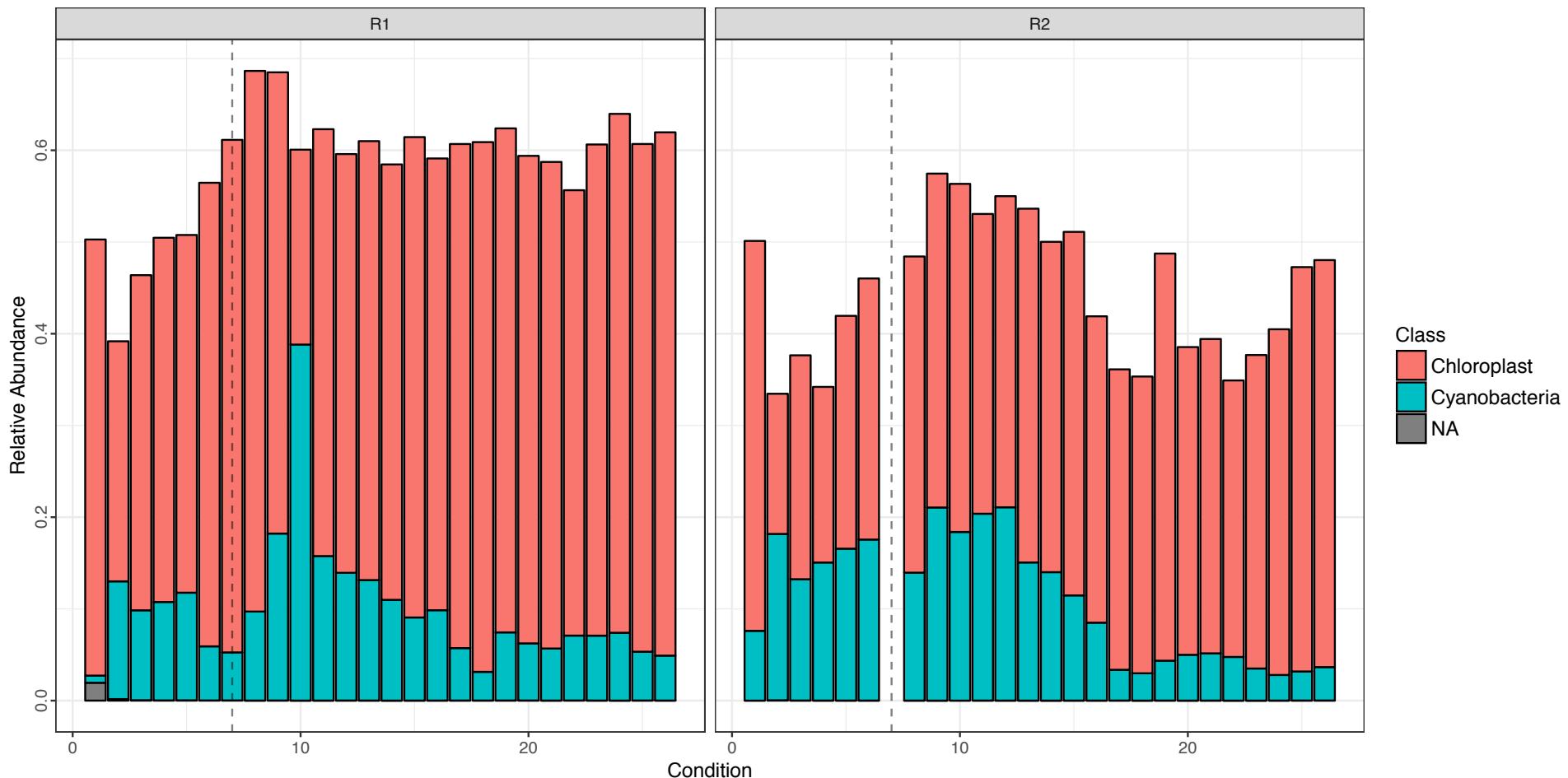
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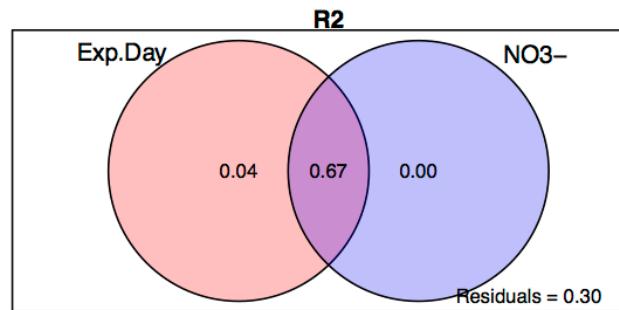
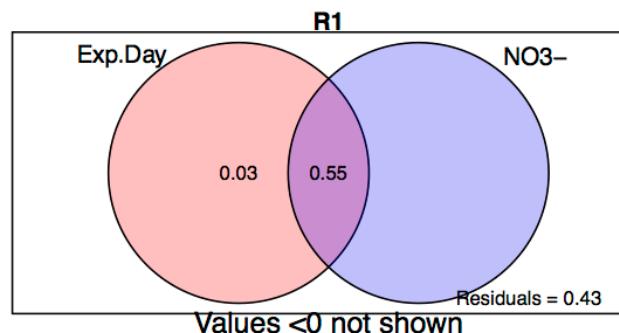
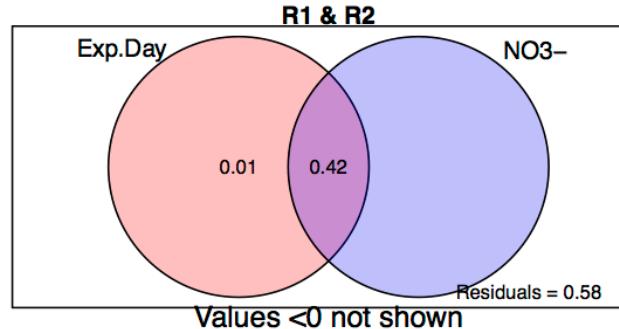
AOB & NOB



Algae & Cyanobacteria



Variance Partitioning: Time/NO₃



Variance Partitioning: Nutrients

