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

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2018 ACS National Meeting
March 21st, 2018

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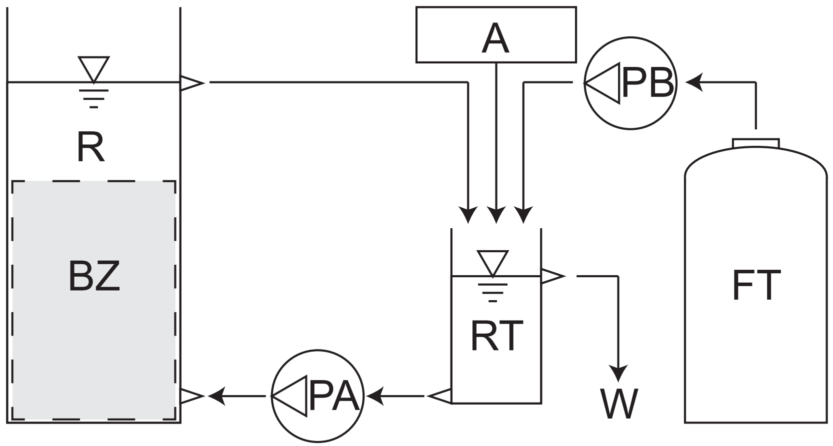
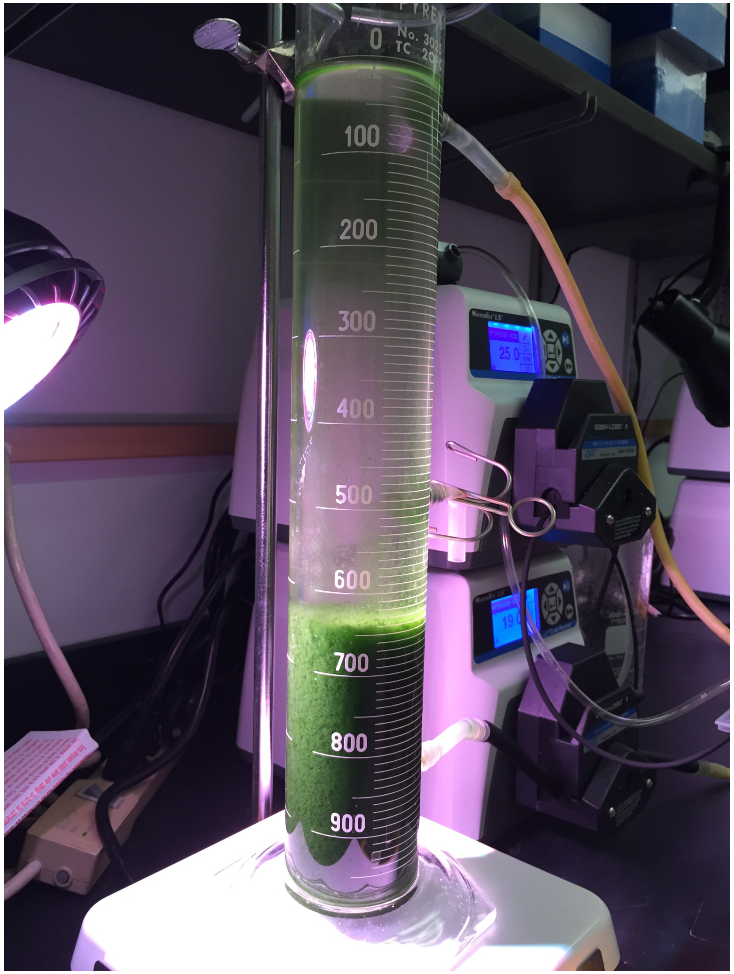
High Density Bioreactor (HDBR)[a]





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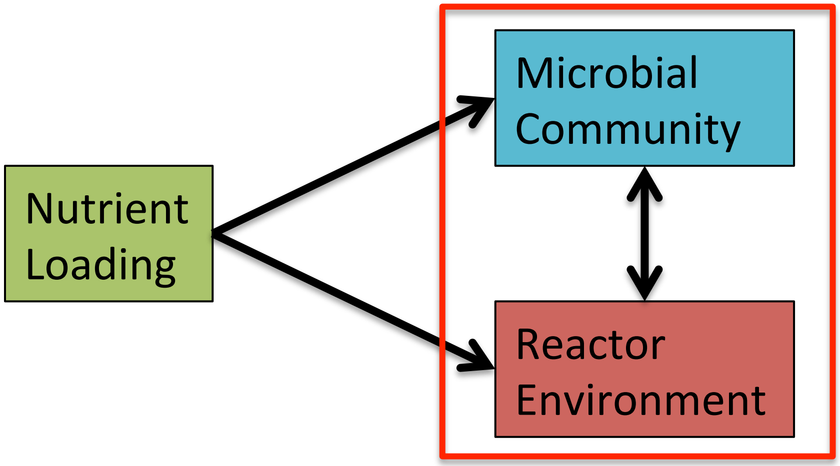
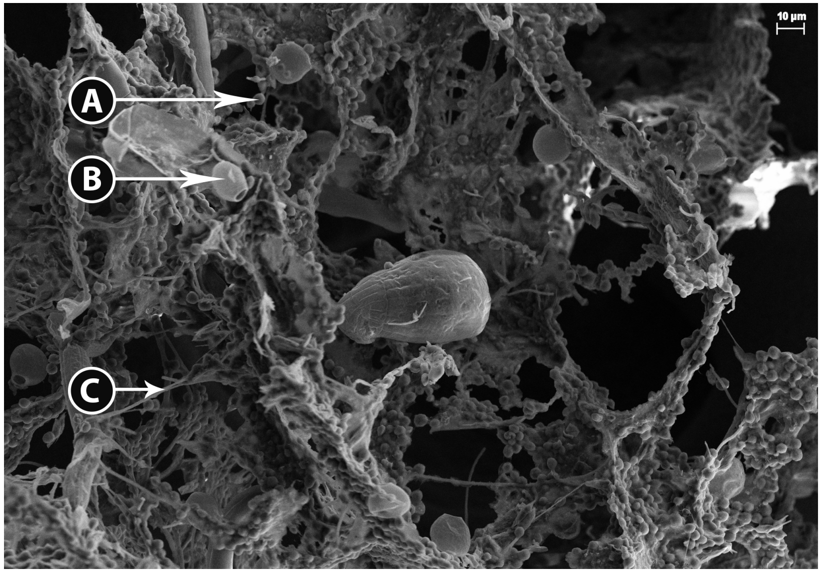
Background





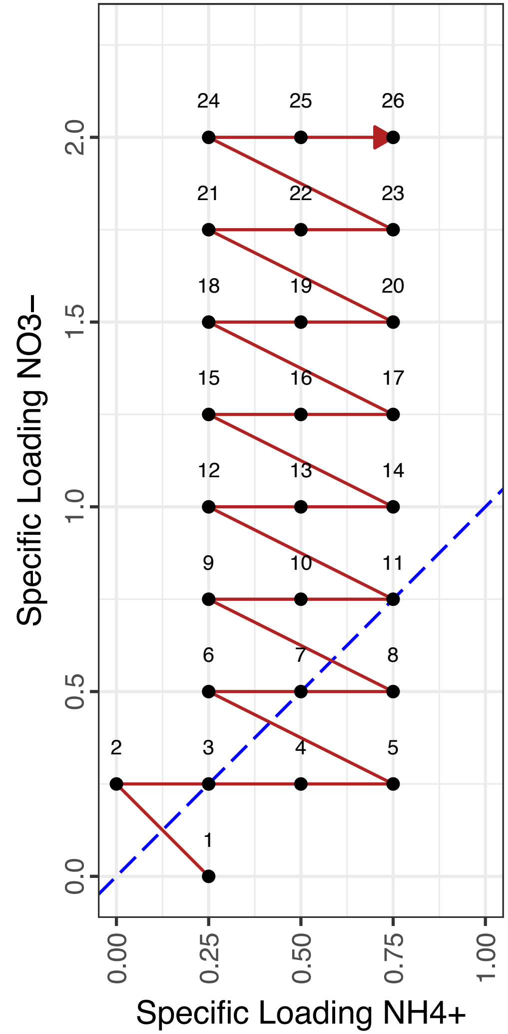
Forces shaping community structure:

- 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.

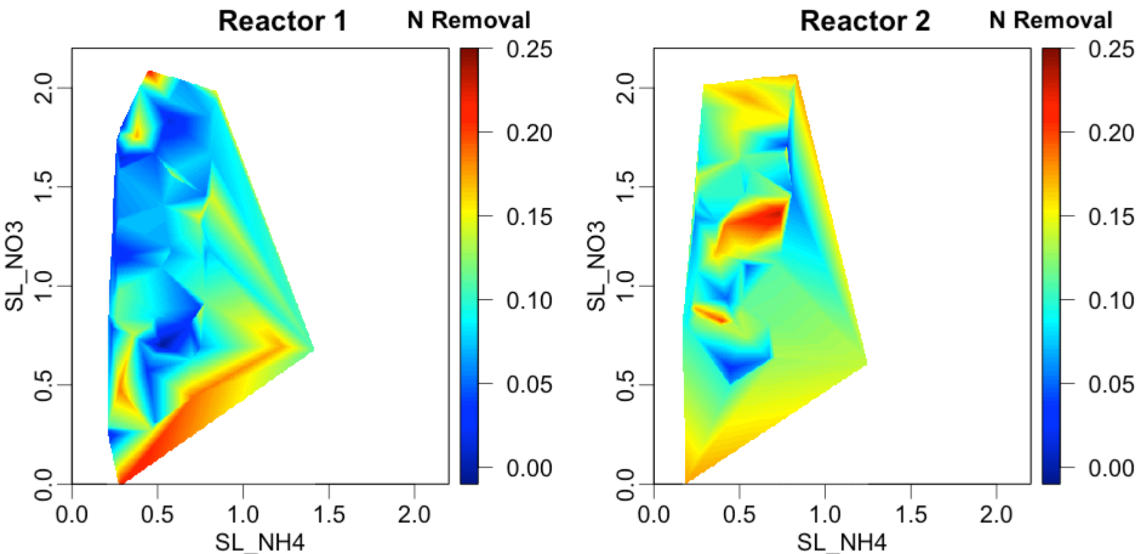
Experimental Design*

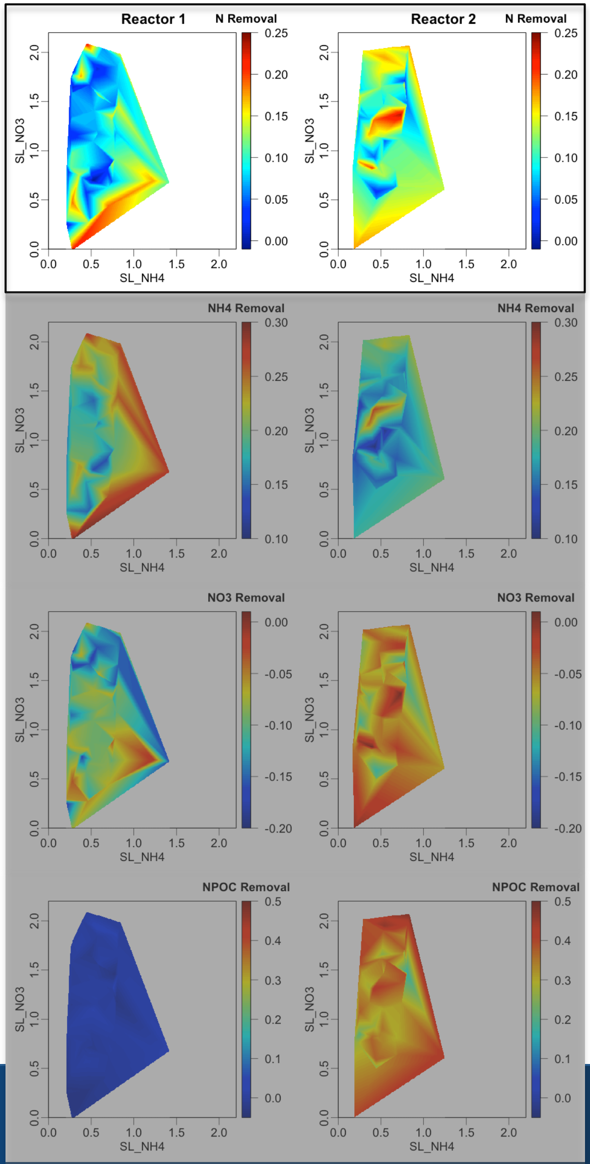
- Two reactors:
  - R1: no supplemental OC
  - R2: 10 mg/L TOC (as glucose)
- 26 loading conditions
  - Vary NH_4^+ & NO_3^-
  - 7 days/condition (generally)
  - COMBO media[c]
- Data/samples collected:
  - Influent/effluent samples
    - Nutrient composition
  - Reactor operational parameters
    - 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

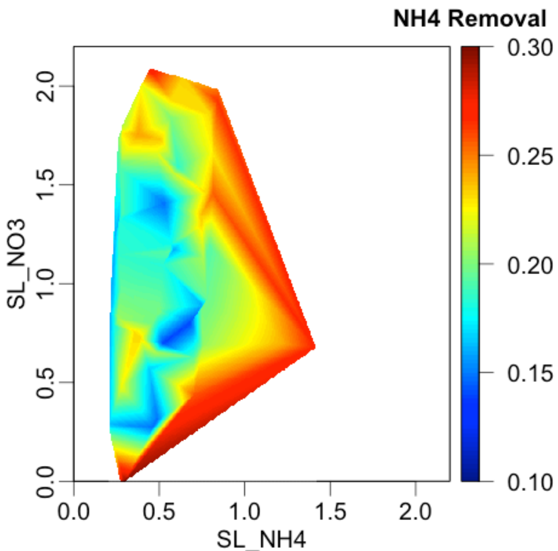


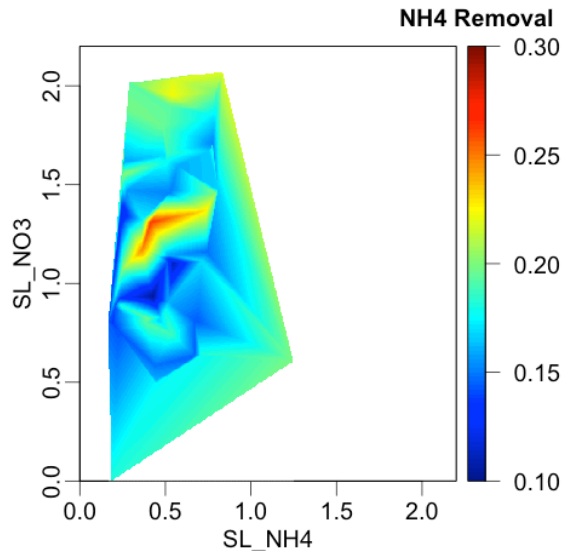


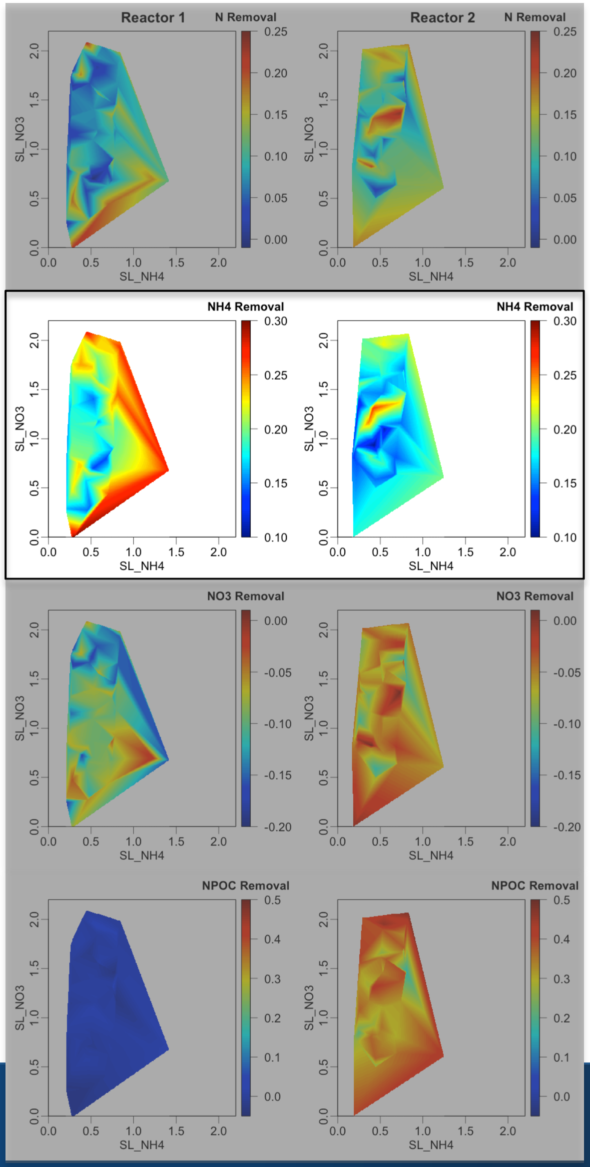
Total N Removal

- OC enhanced removal

Nutrient Kinetics



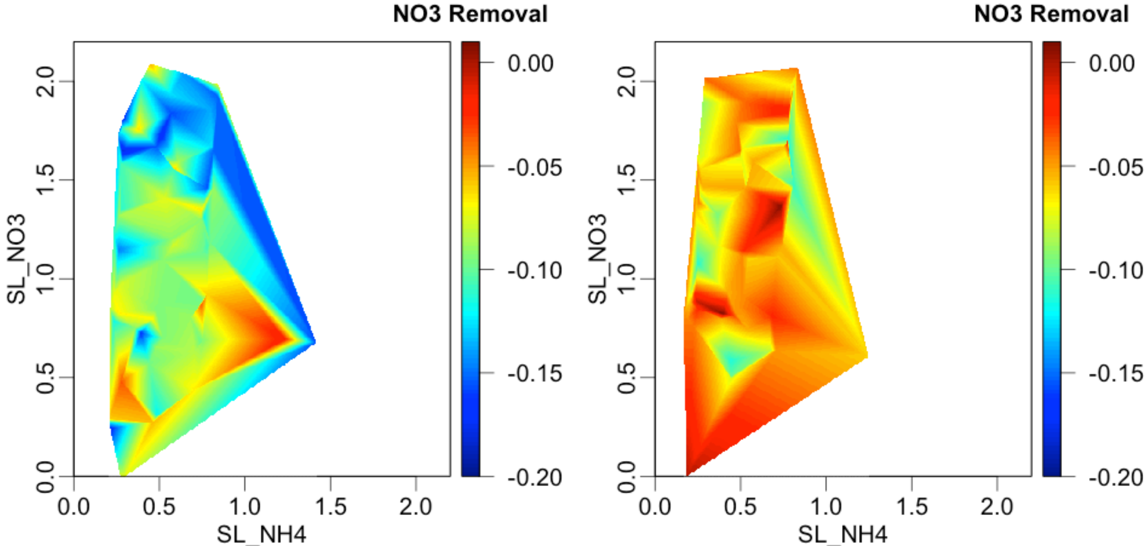


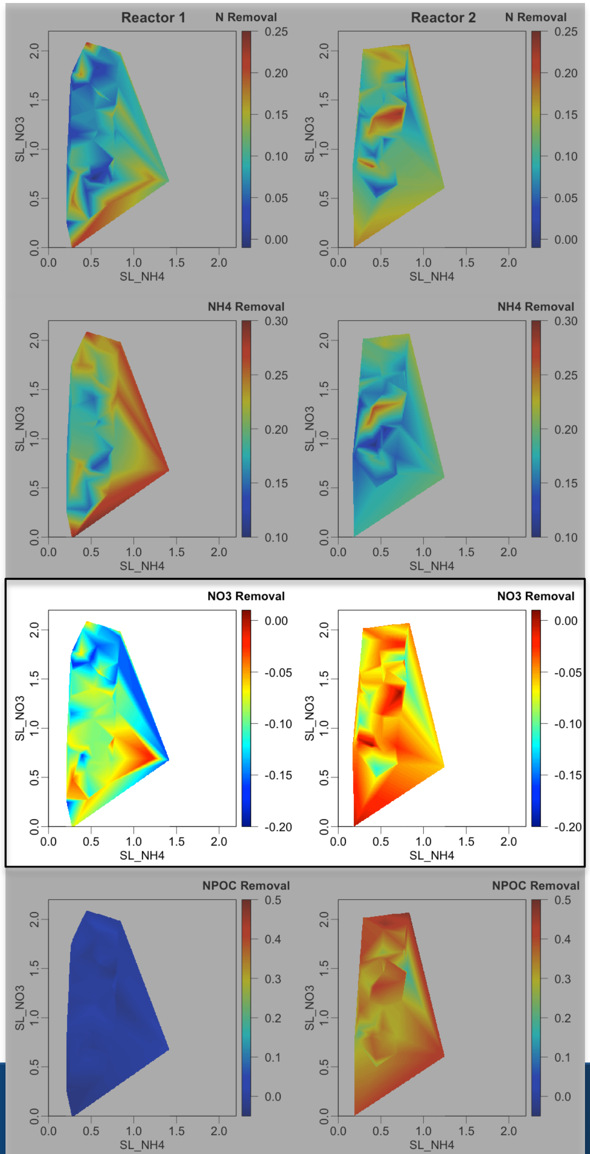


NH_4^+ Removal

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

Nutrient Kinetics

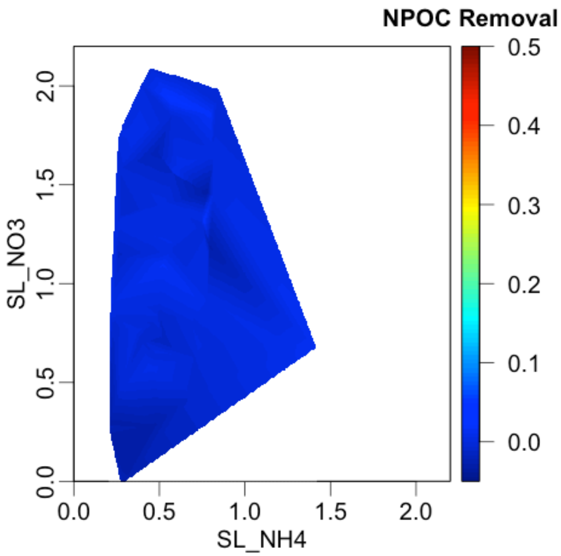


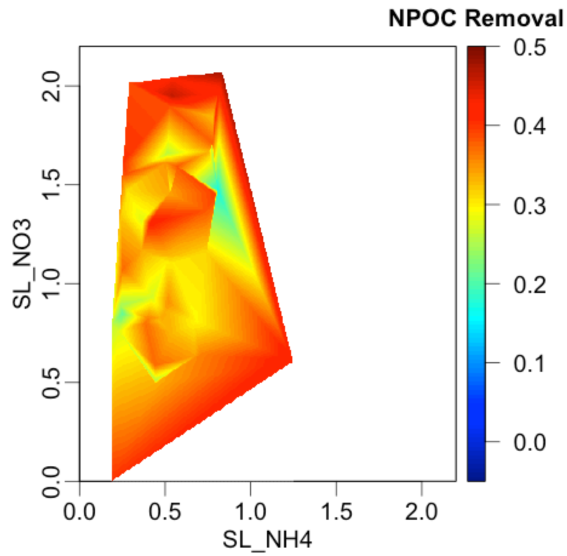


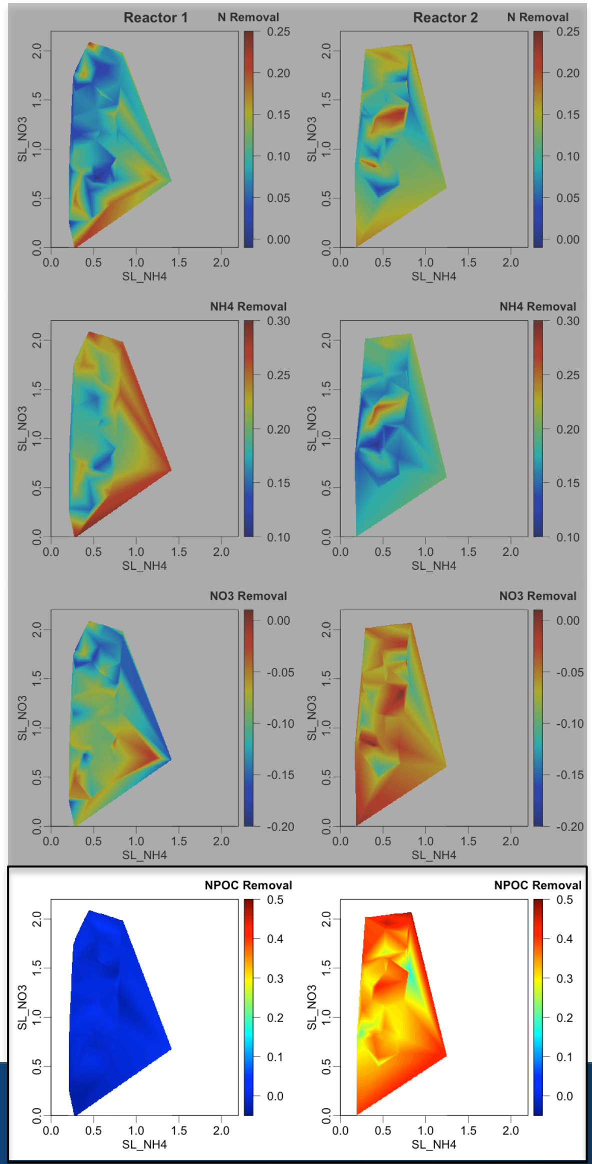
NO_3^- removal

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

Nutrient Kinetics*



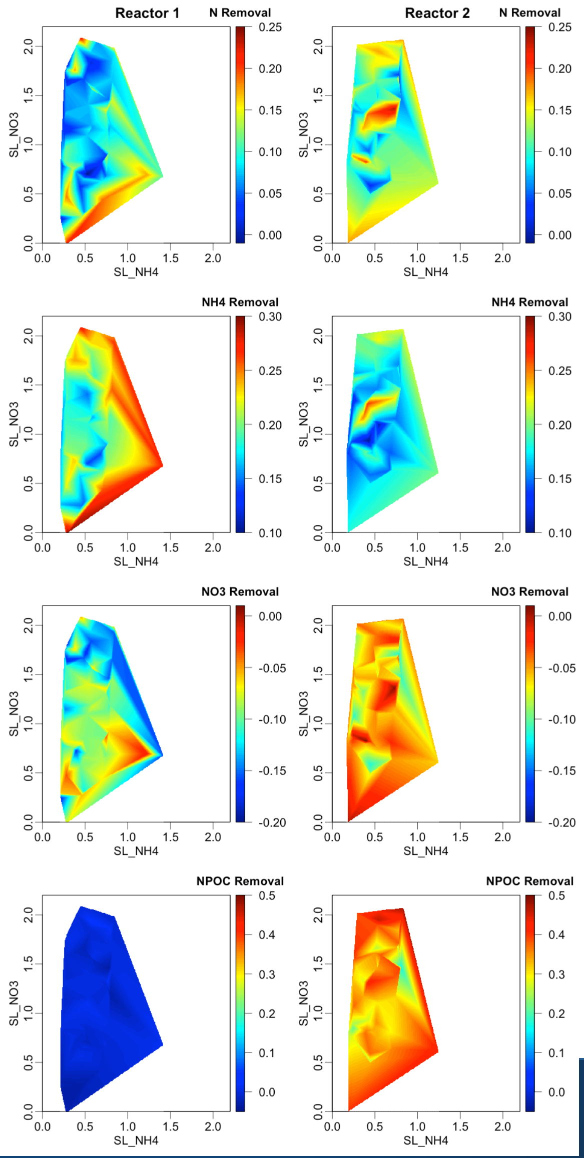




- NPOC removal increased with NO_3^- loading

Motivation for dimensional reduction

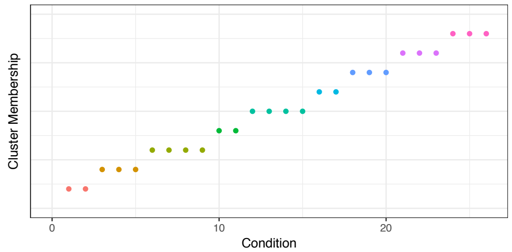
- Each of the nutrients (or operational parameters) could be compared in a pairwise fashion.
  - Tedious
  - Increases false discovery rate
  - Does not take other nutrients (P, S, etc) into account
- Need to simultaneously consider the influence of all variables
- Microbial community structure is not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



Machine Learning Techniques

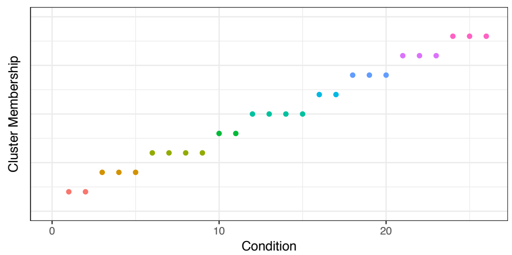
<u>Temporally constrained clustering</u>

- 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.

Machine Learning Techniques



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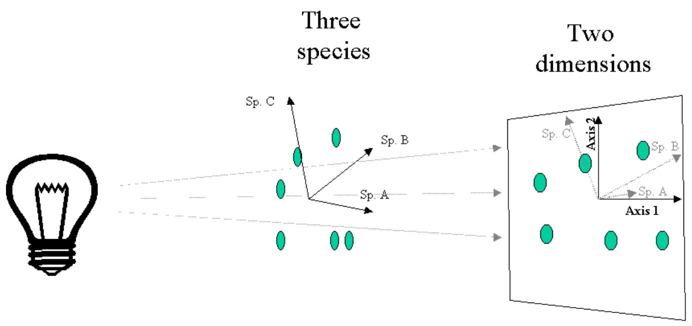
Ordination

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



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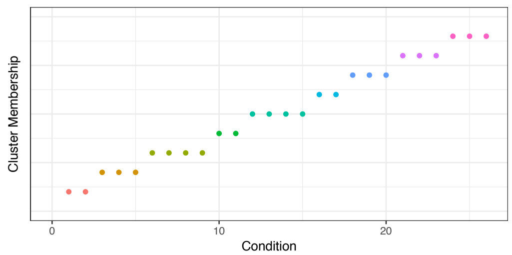
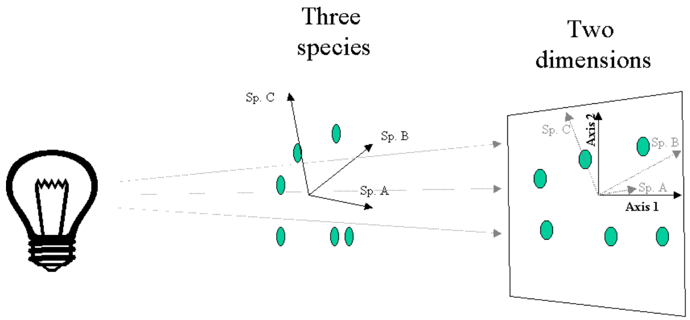
<u>Ordination</u>

- 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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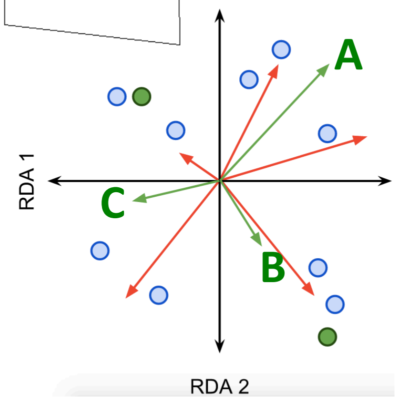

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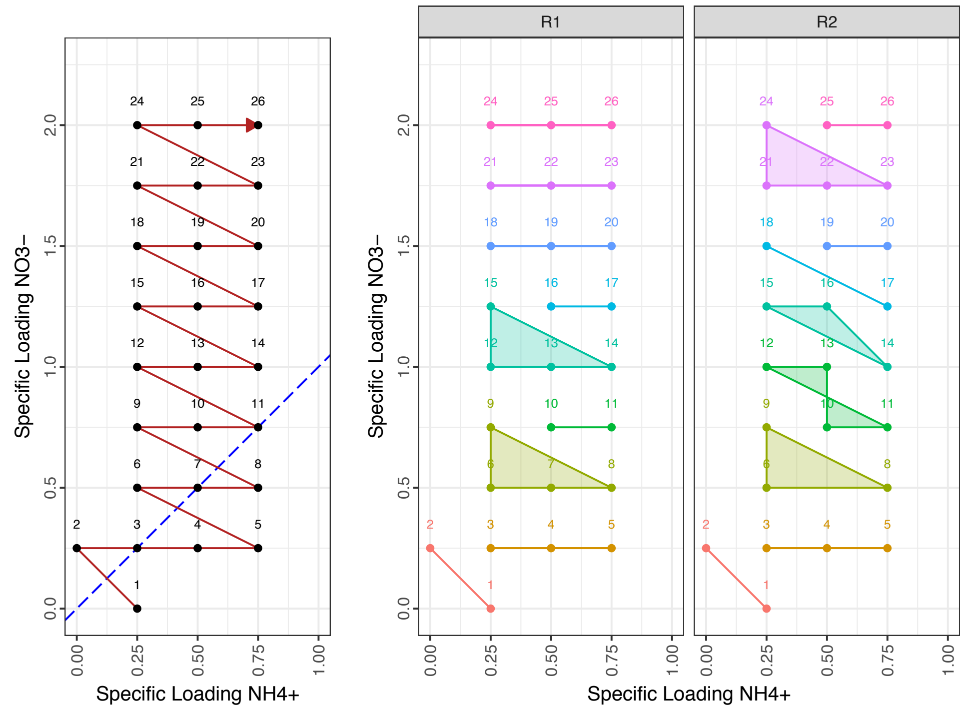
Hierarchical Clustering

NO_3^- loading

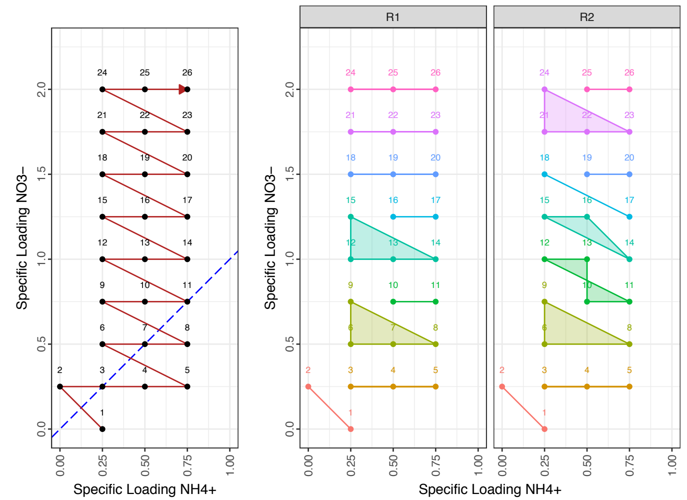
- dominates the microbial community structure

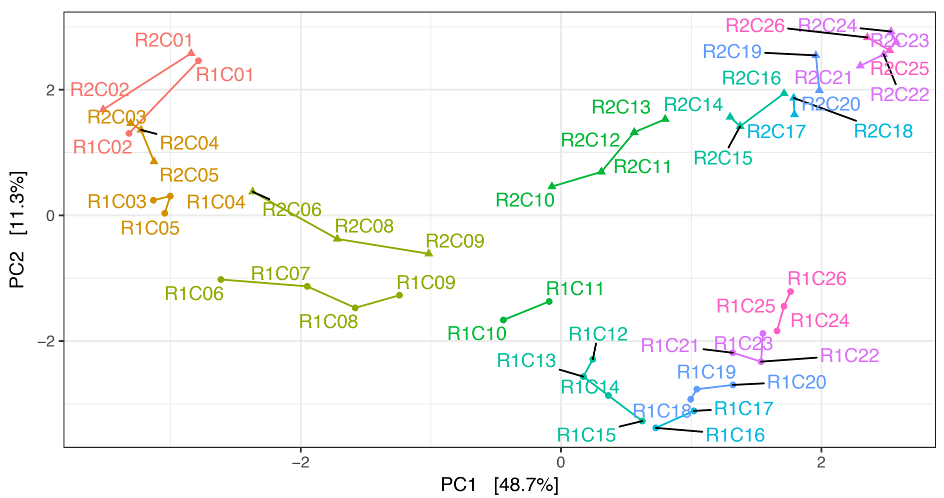
NH_4^+ loading

- 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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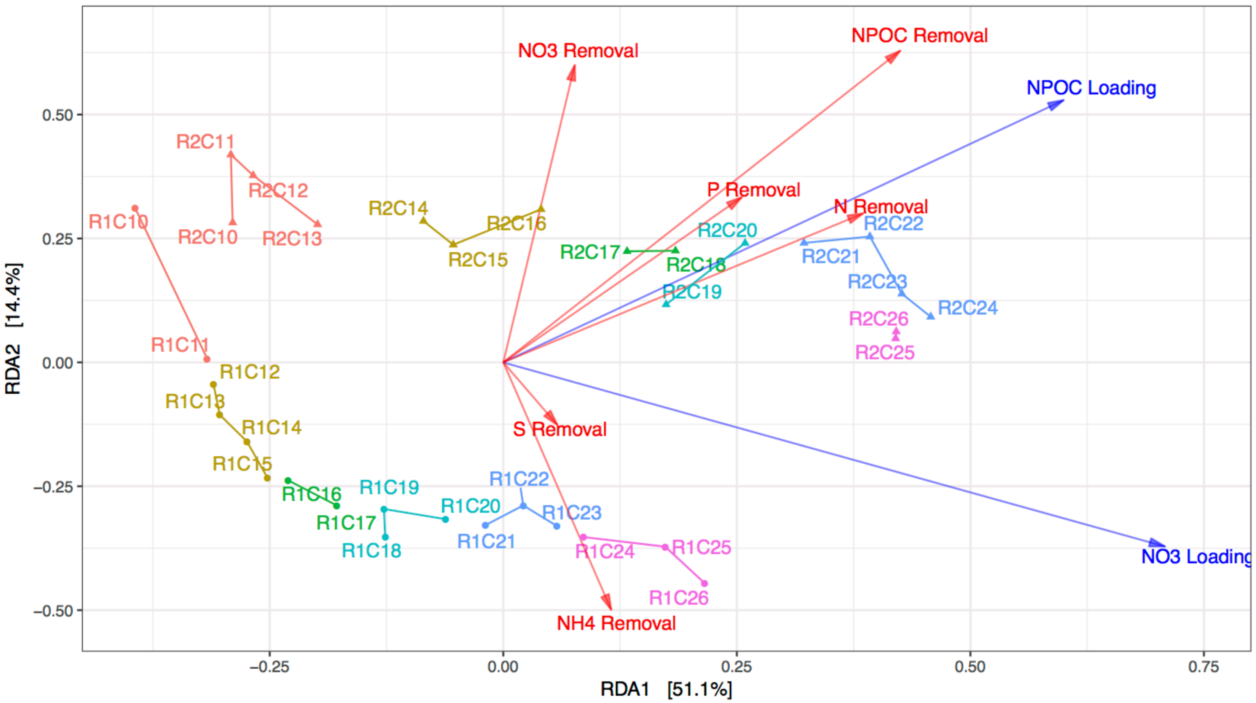
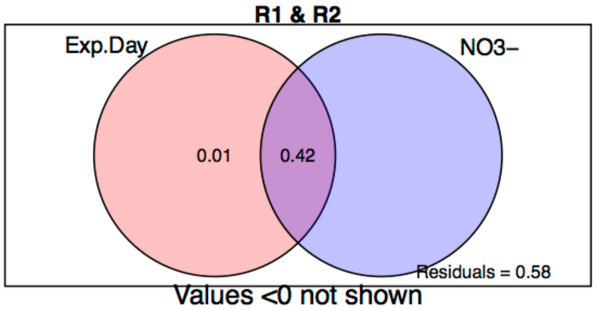
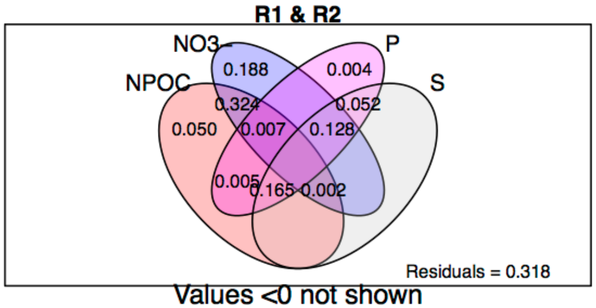
Redundancy Analysis (RDA)

<u>Parsimonious model:</u> ($r^2 = 0.40$, adj. $r^2 = 0.37$)

- Community ~ 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







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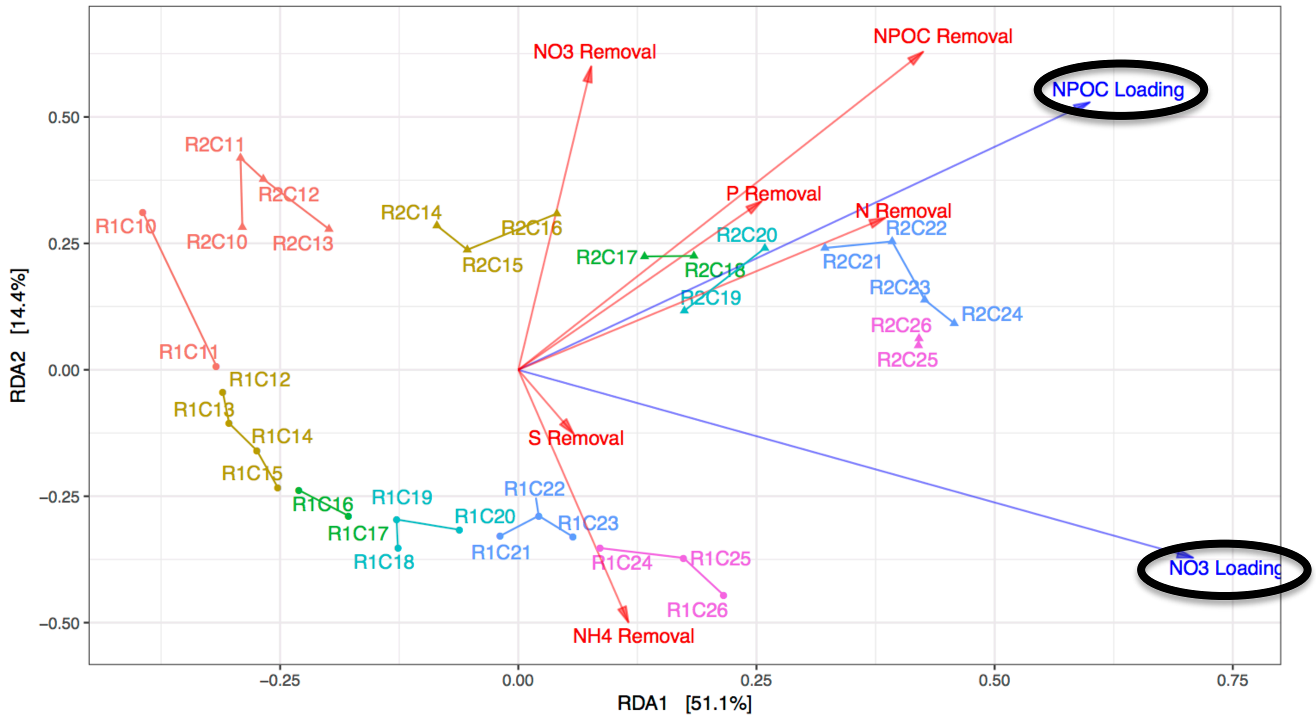
Redundancy Analysis



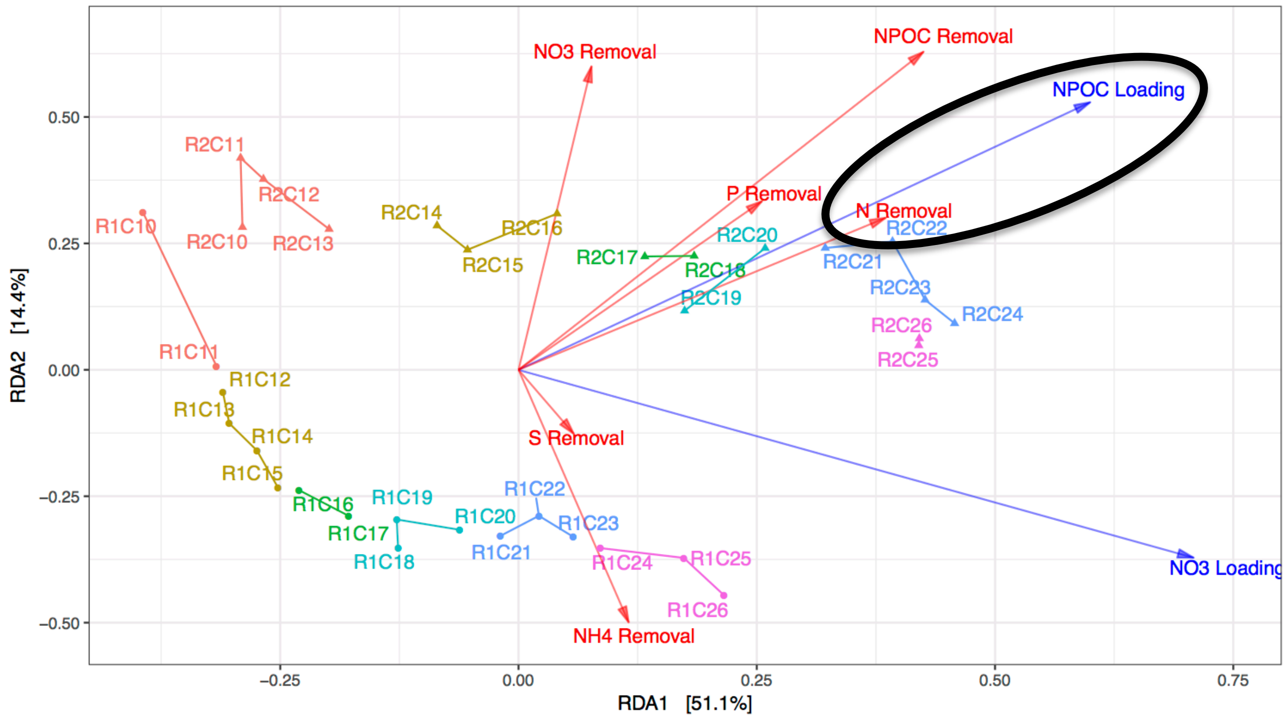
<u>PCA</u>

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

<u>Temporally-constrained clustering</u>

- NO_3^- loading determines cluster membership

Redundancy Analysis



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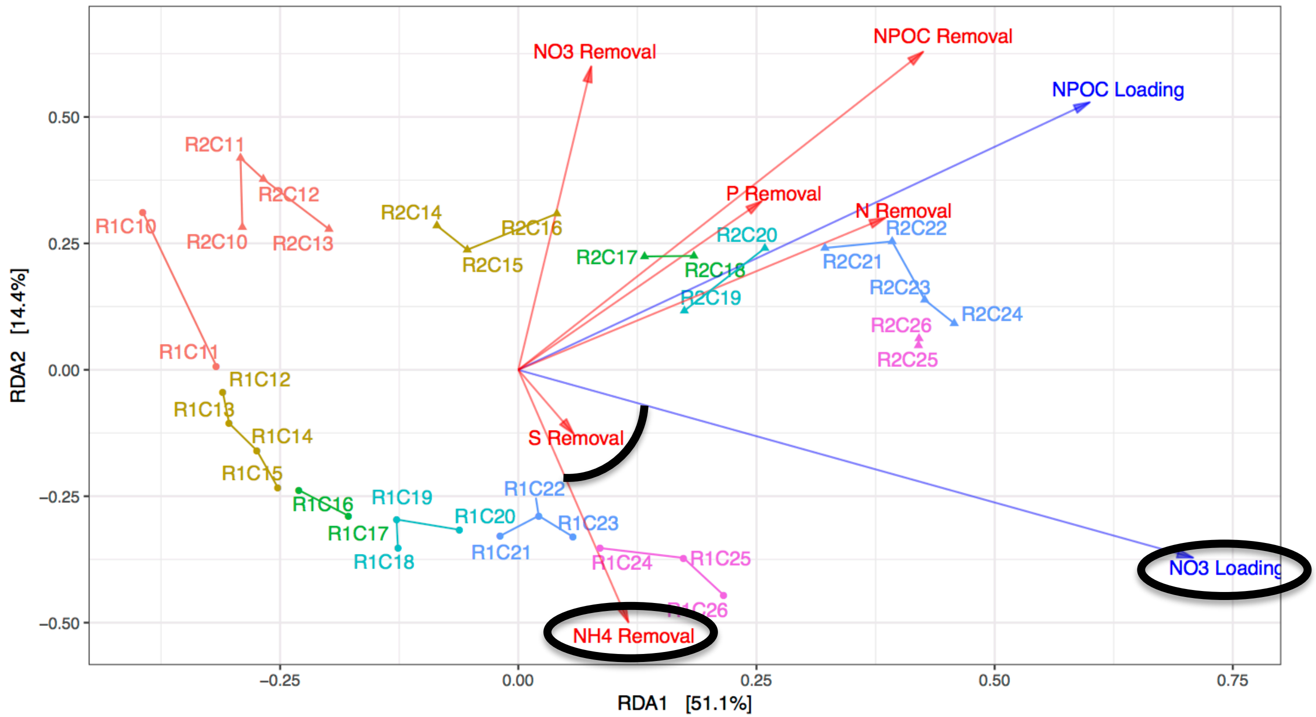
<u>Kinetics</u>

- **NPOC increases total N removal**

Redundancy Analysis



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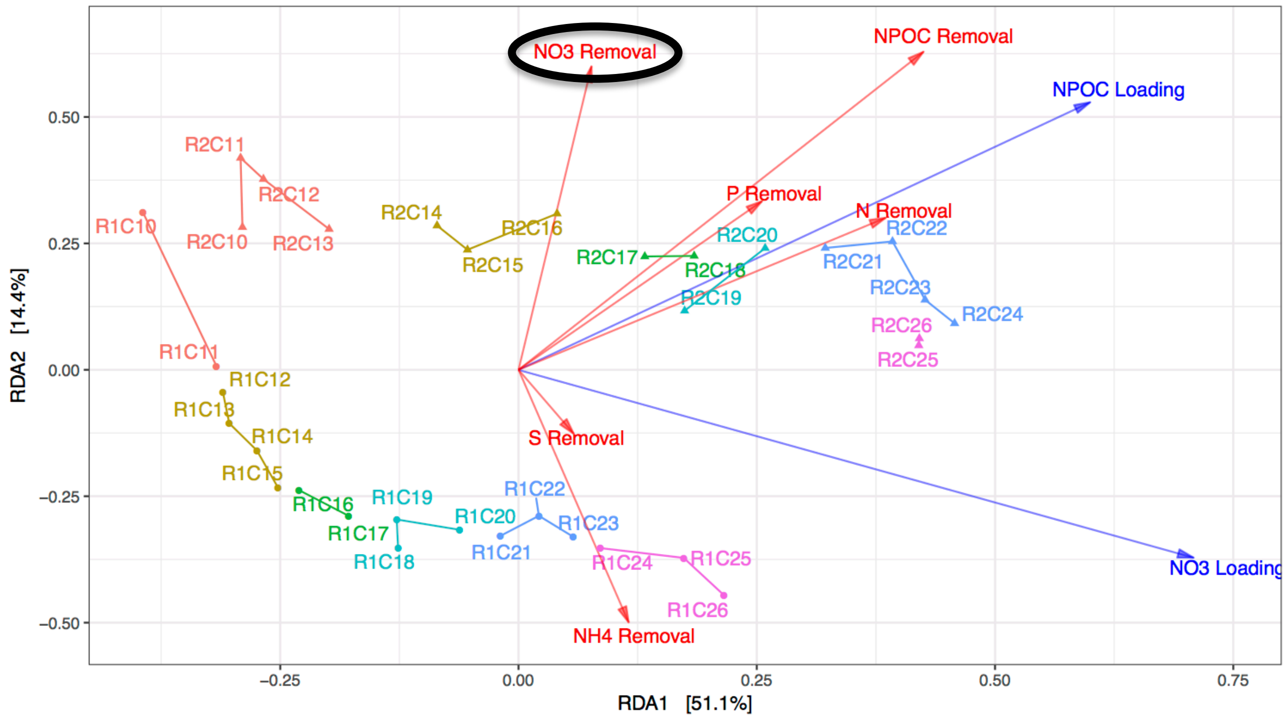
<u>Temporally-constrained clustering</u>

- NO_3^- loading determines cluster membership

<u>Kinetics</u>

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

Redundancy Analysis



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<u>Kinetics</u>

- 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**

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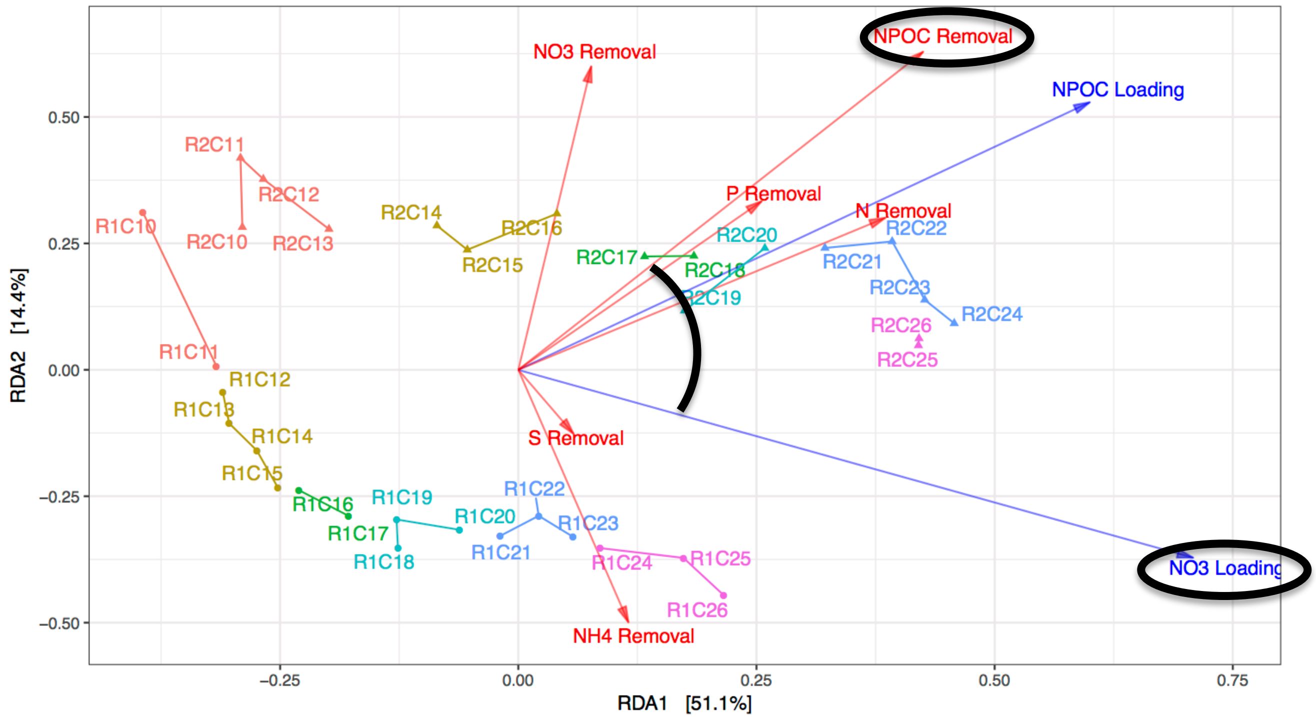
Redundancy Analysis*



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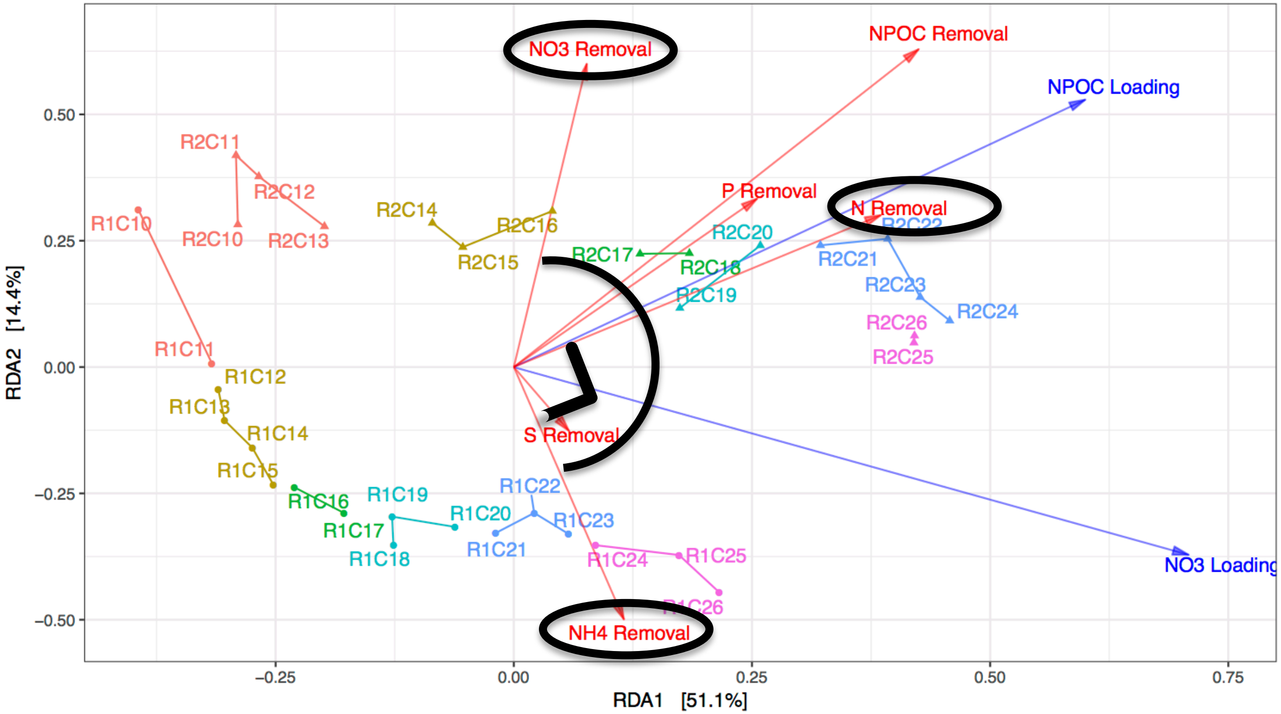
- NPOC increases total N removal
- NH_4^+ removal
  - R1 more removal than R2
  - Removal increased with NO_3^- loading
- Higher NO_3^- removal in R2
- **NPOC removal increased with NO_3^- loading**

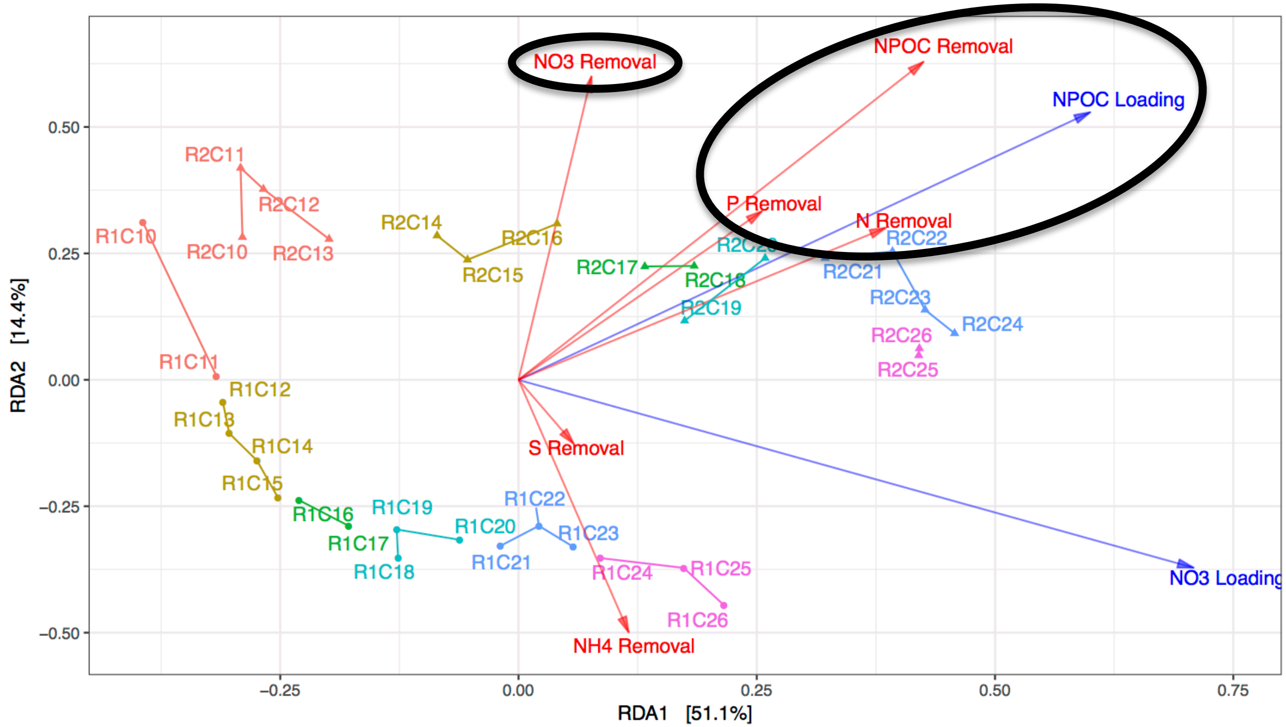
Redundancy Analysis



<u>Expanding upon results</u>

- 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*



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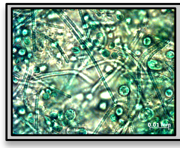
- **Heterotrophic growth drives nutrient removal**
  - **Heterotrophs may be relying on NO_3^- as e-acceptor**
- **Implies anaerobic/micro-aerobic conditions**
  - **Supports O_2 limitation as cause for limited nitrification rates**

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/



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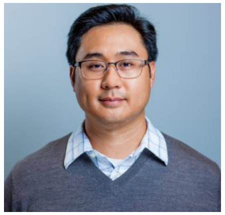

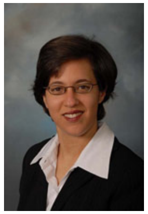

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References

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