Predation, Parasitism, and Performance within EBPRs

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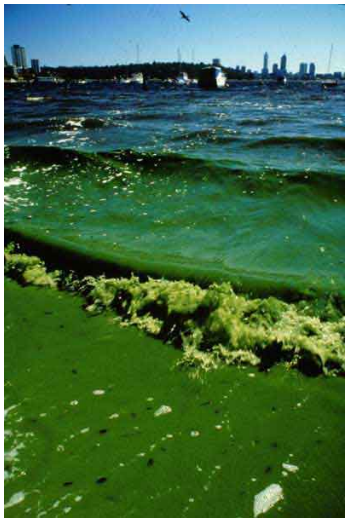
microbes.cae.drexel.edu @SalesLaboratory
Paper #: ENVR 258 jacobrprice.github.io @Jake_in_the_Lab

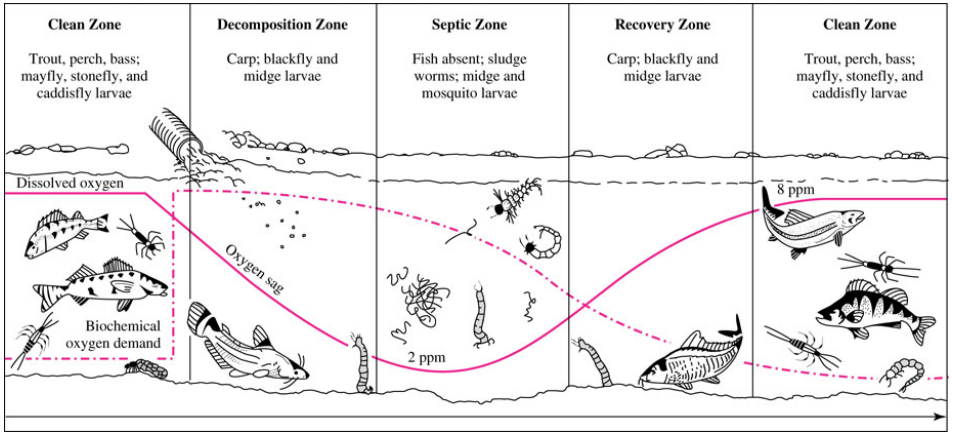
Motivation*

- Release of excess nutrients can lead to eutrophication
  - Hypoxia → fishkills



Raw Wastewater	~ 12 mg P/L
Secondary Treatment	~ 6 mg P/L
Protected Watersheds	1 mg P/L

- Enhanced biological phosphorous removal (EBPR) technologies offer a solution

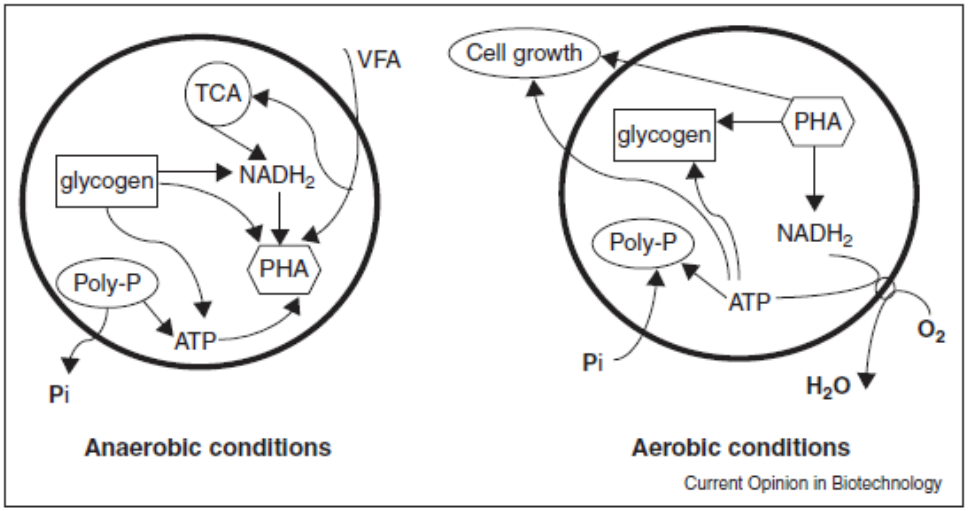


Enhanced Biological Phosphorous Removal

Phosphate Accumulating Organisms (PAO)

- Accumulate 2-5 times as much P as typical heterotrophic bacteria

Anaerobic ←→ Aerobic



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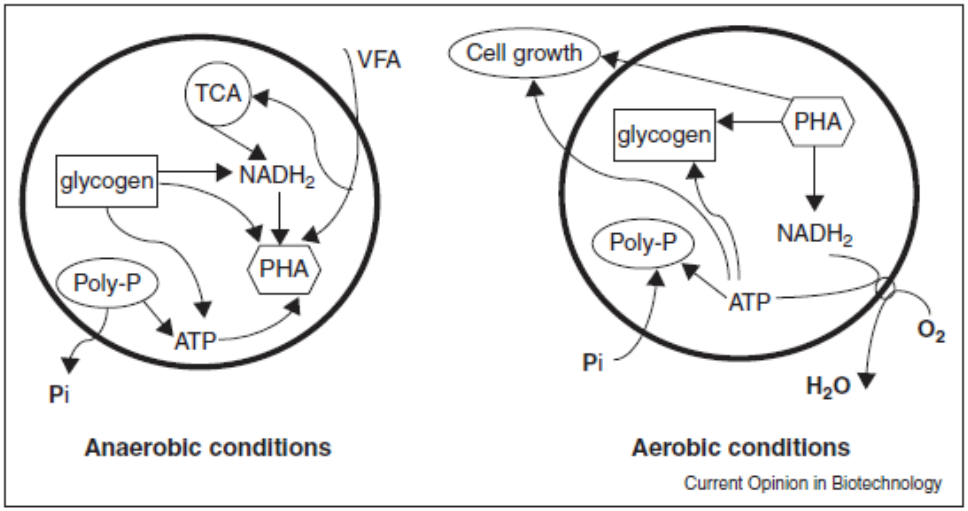
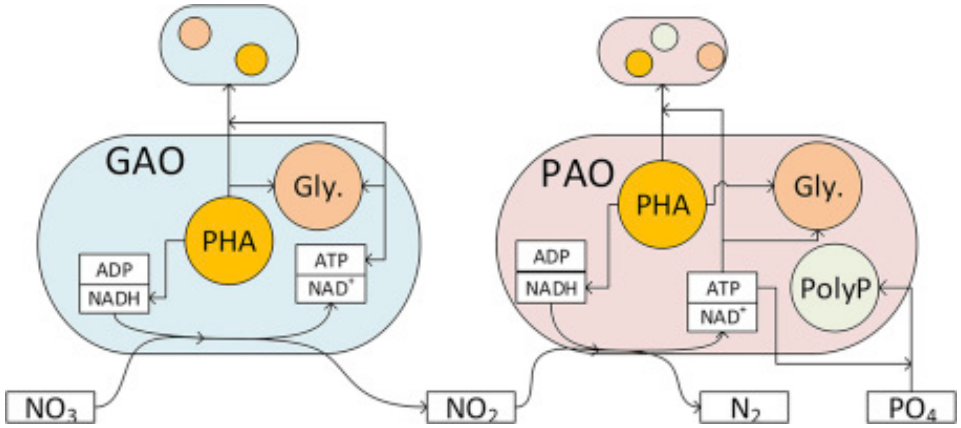


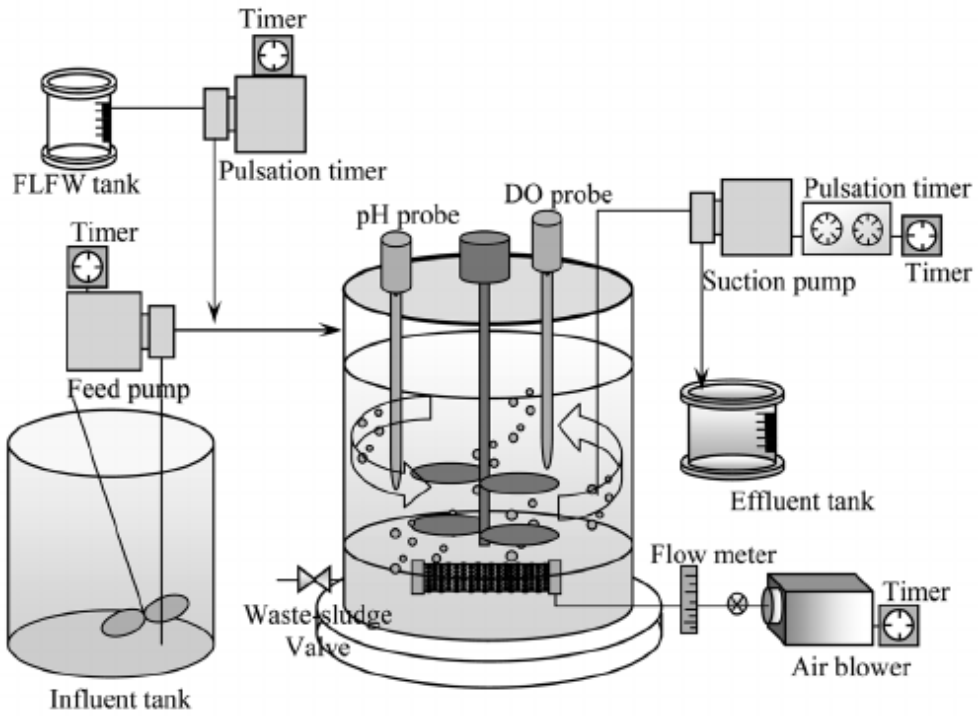


Glycogen Accumulating Organisms (GAO)

- Cooperate?
- Compete?
  - (for VFA)

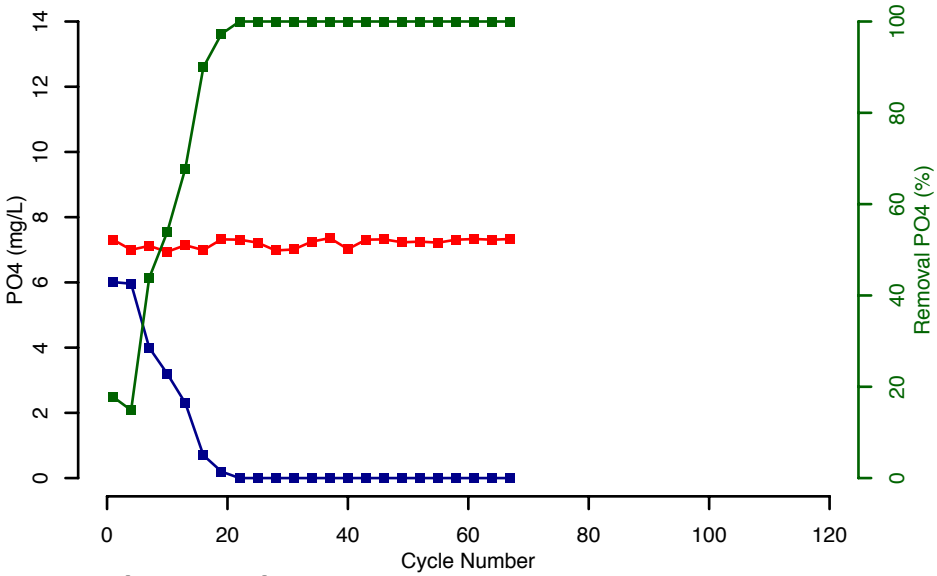
Experimental Approach / Methods*

- 2 x SBRs
  - Experiment S
    - 23 days = 67 cycles
  - Experiment O
    - 40 days = 120 cycles
- 16S rRNA
  - Experiment S
    - 2 sampling events (beginning/ end)
  - Experiment O
    - 7 sampling events
- Statistical analysis
  - Ordination (PCoA)
  - Clustering (UPGMA)
  - Differential Abundance Testing



Kinetic Behavior

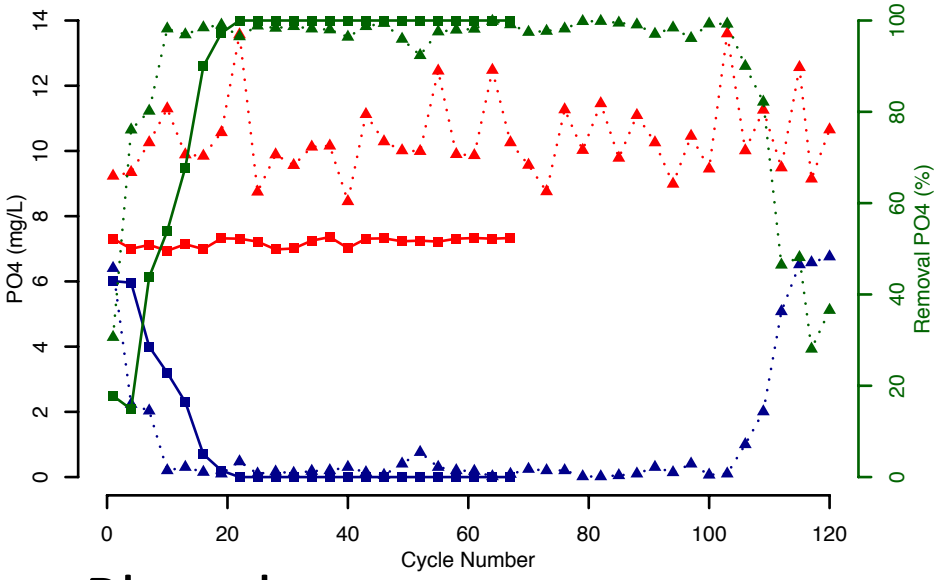
- Experiment S
  - Ideal startup and operation
  - Near complete removal after 7 days (21 cycles)



- Phosphate
  - Initial
  - Final
  - Removal (%)

Kinetic Behavior

- Experiment S
  - Ideal startup and operation
  - Near complete removal after 7 days (21 cycles)
- Experiment O
  - Similar startup
  - Crash in performance on 35th day



- Phosphate
  - Initial
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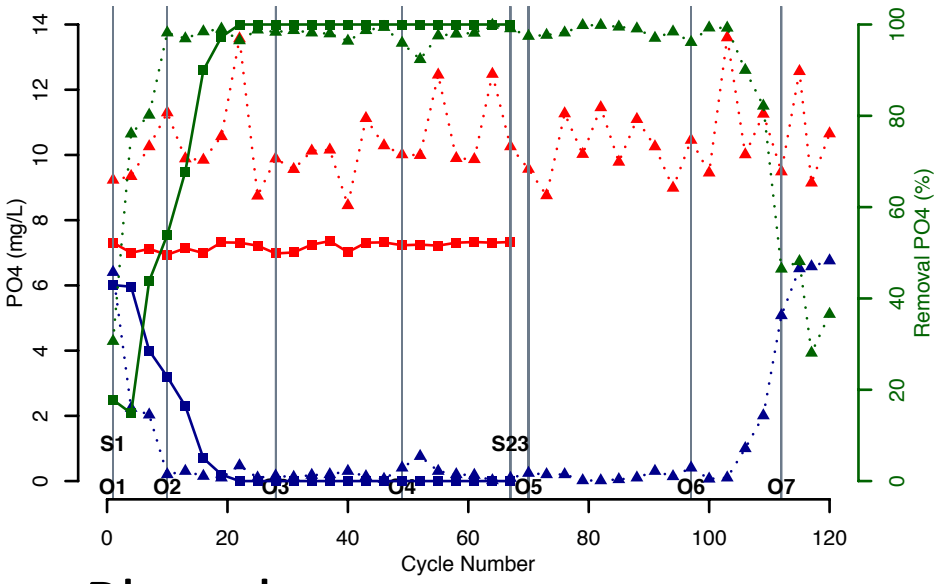
  - Removal (%)

Kinetic Behavior*

- Experiment S
  - Ideal startup and operation
  - Near complete removal after 7 days (21 cycles)
- Experiment O
  - Similar startup
  - Crash in performance on 35th day
- Use sequencing results to investigate the cause of the crash.



- Phosphate
  - Initial
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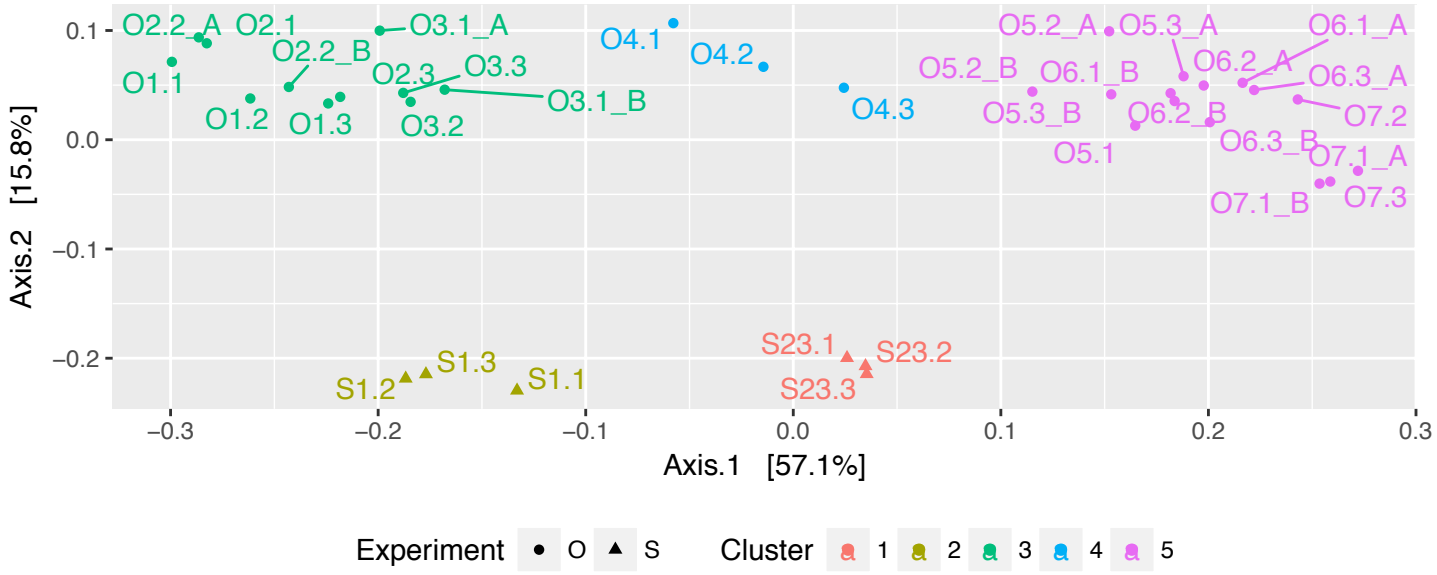
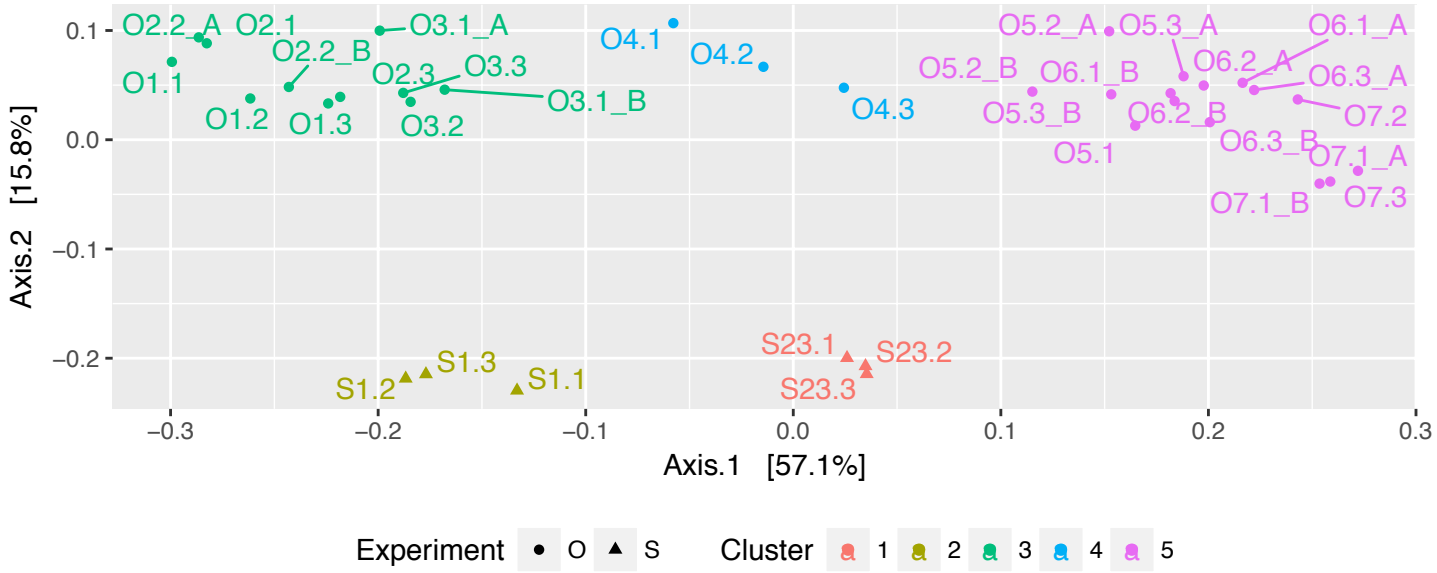
Ordination and Clustering



PCoA + UPGMA

- 5 distinct clusters

Ordination and Clustering*



PCoA + UPGMA

- 5 distinct clusters

Differential Abundance Testing

- ~ Experiment + Status

Status	Exp. O	Exp. S
Startup	O1	S1
Attenuation	O2 – O3	
Operation	O4	S23
Failing	O5 – O6	
Crashed	O7	

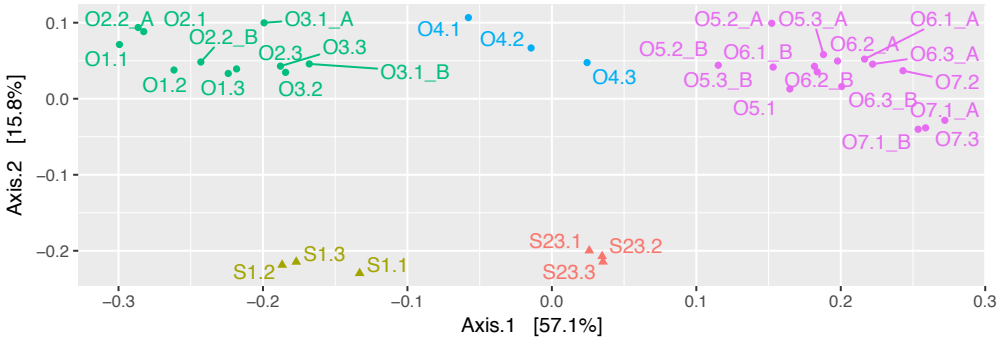
Differential Abundance & Cohort Plot

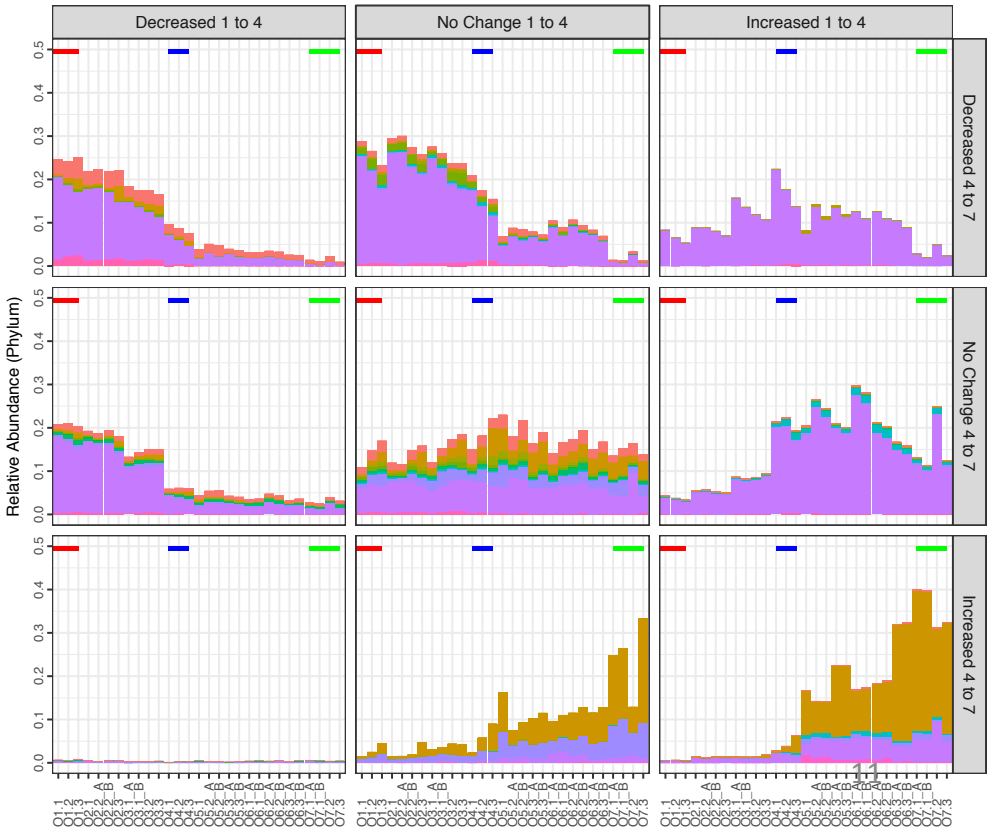
Differential Abundance

~ Experiment + Status

Contrasts

- Startup
  - Startup → Operation
- Crash
  - Operation → Crashed
- ASV/OTU State:
  - Decreased
  - No (statistically sig.) change
  - Increased





Differential Abundance & Cohort Plot*

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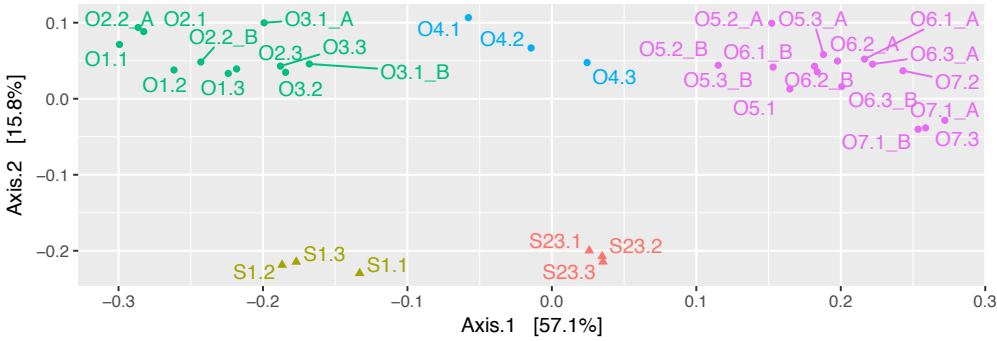
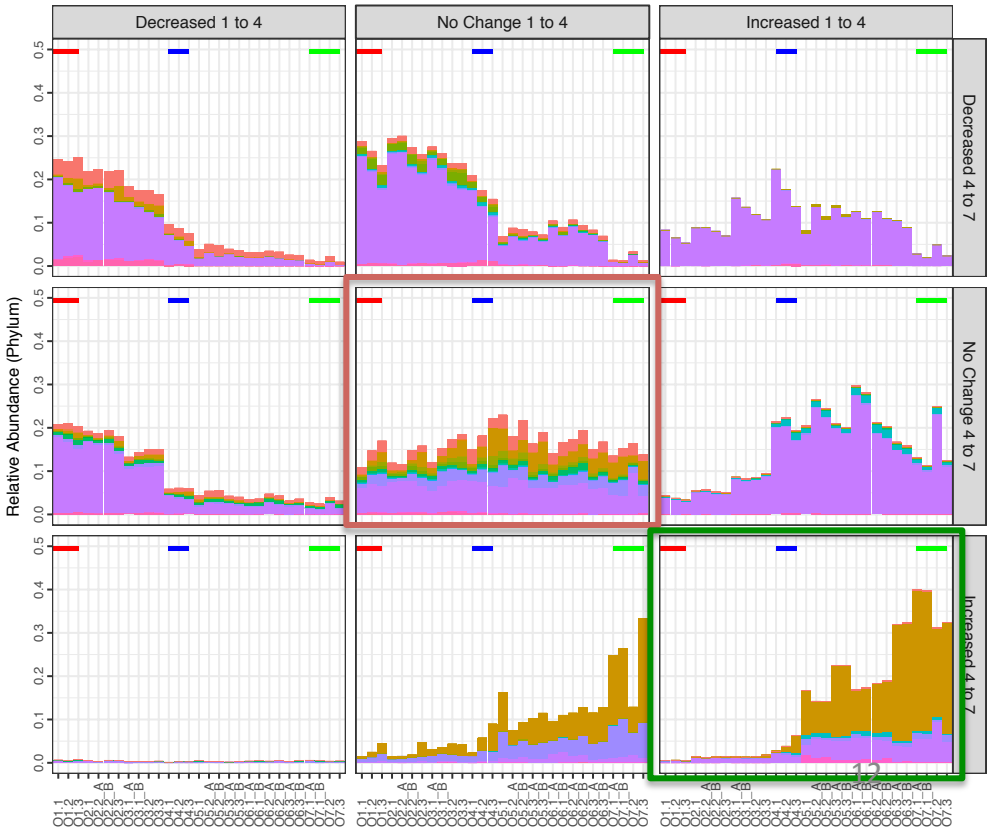
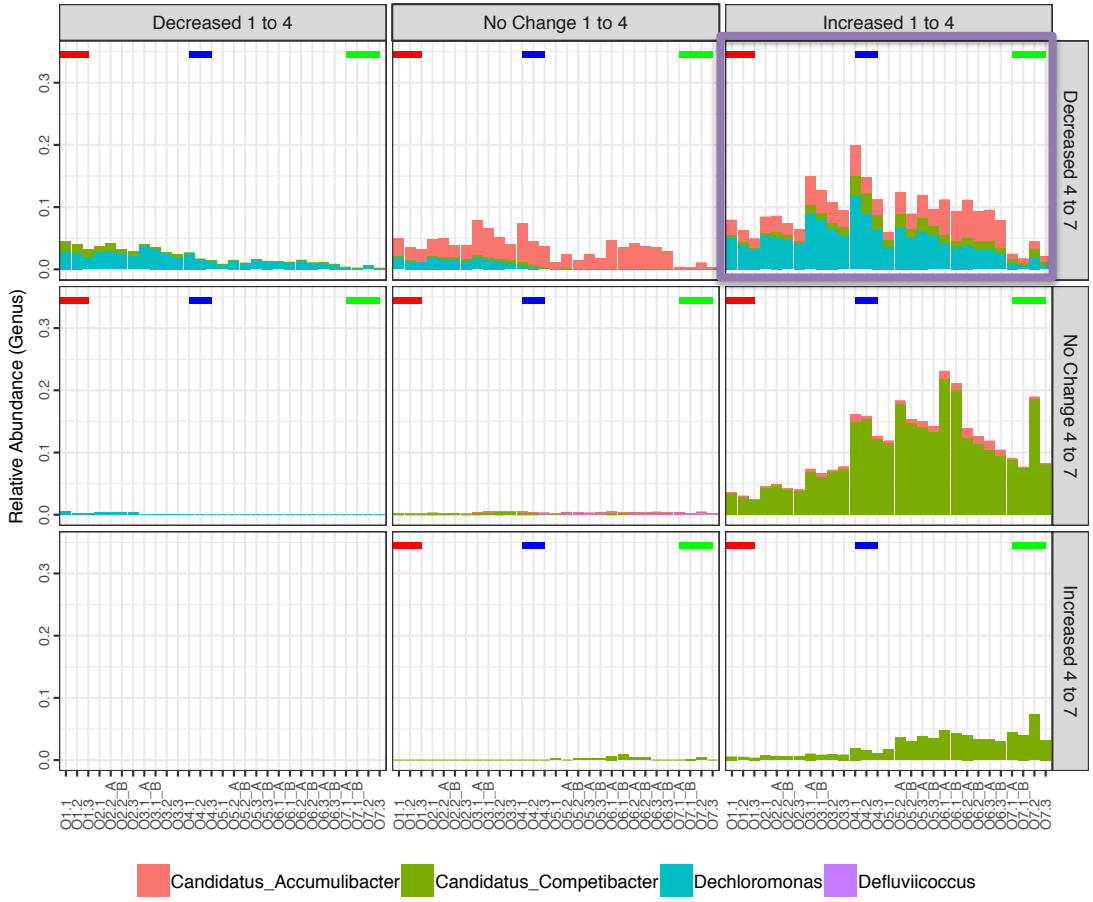




Differential Abundance

- PAO
  - *Ca. Accumulibacter*
  - *Dechloromonas* spp.
- GAO
  - Ca. Competibacter
  - *Difluviicoccus* spp.



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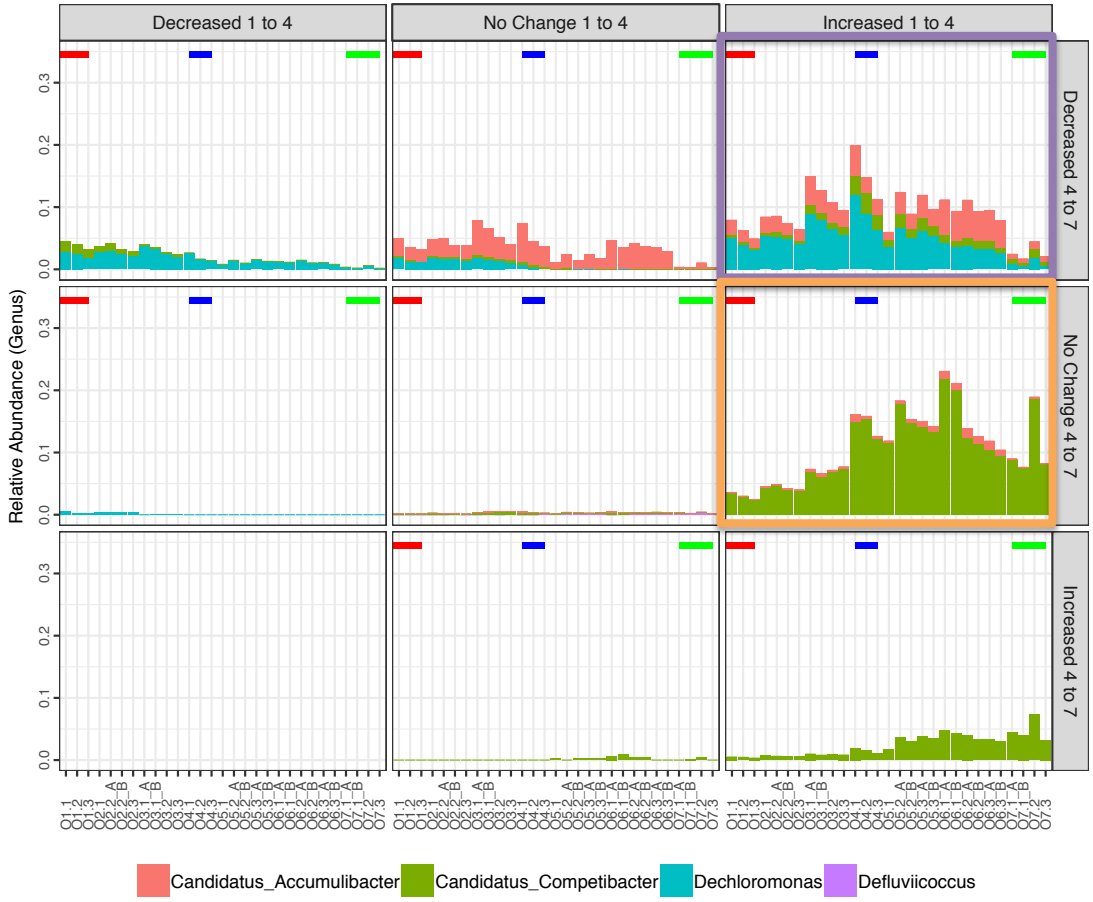

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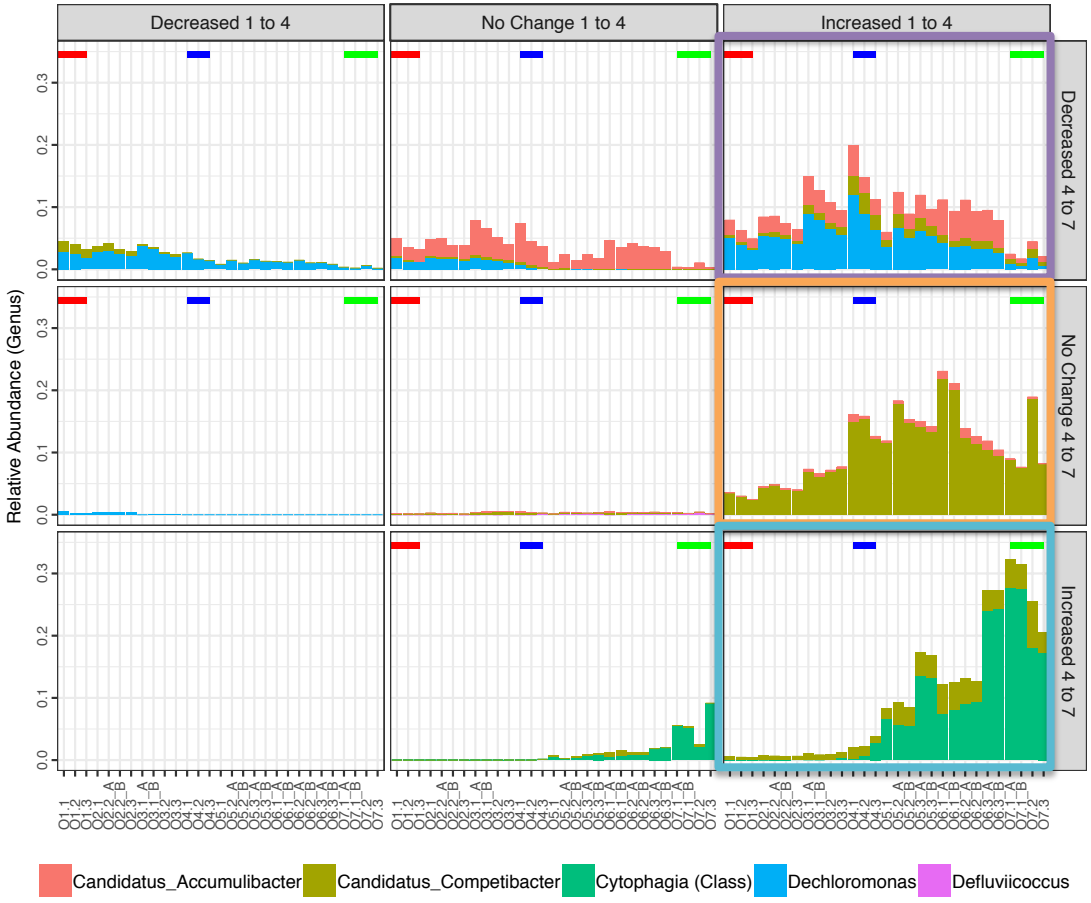
- Organic C degraders
  - Cytophagales (Class)



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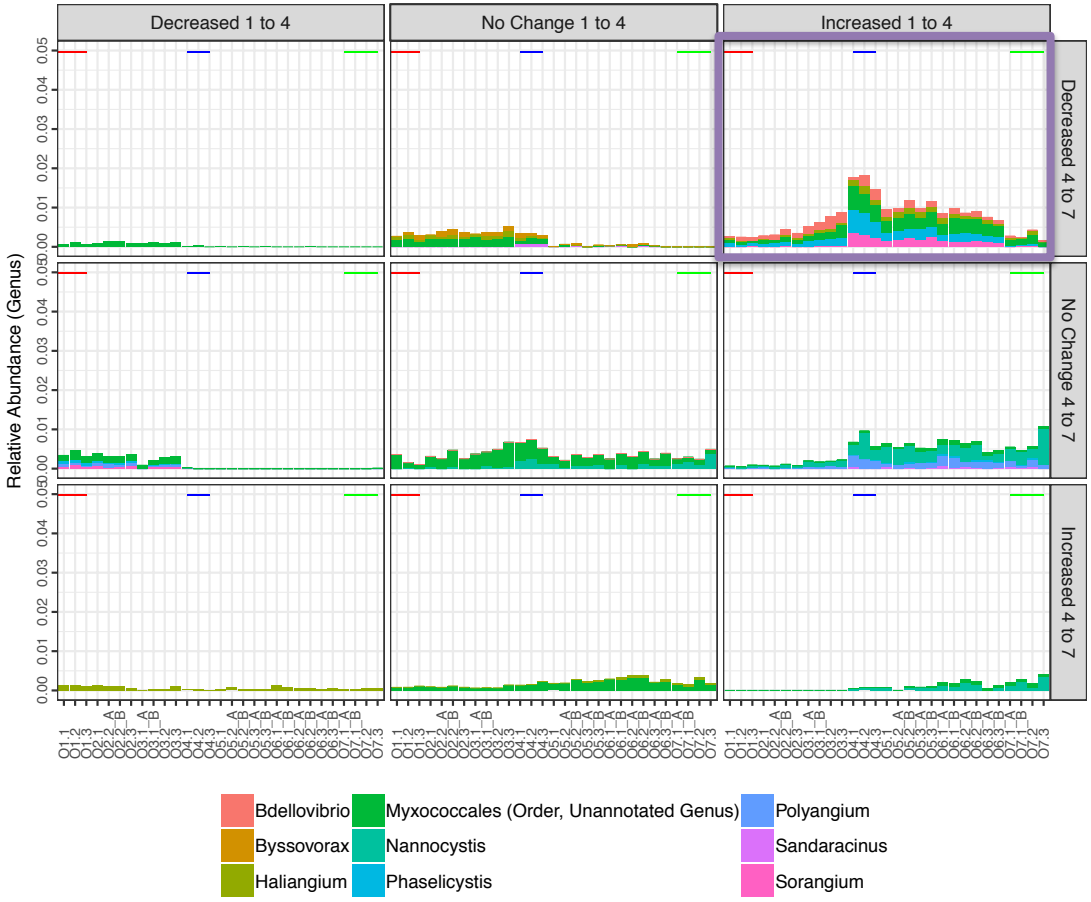
- Organic C degraders
  - Cytophagales (Class)
- Predatory bacteria
  - *Bdellovibrio* spp.
  - Myxococcales (Order)



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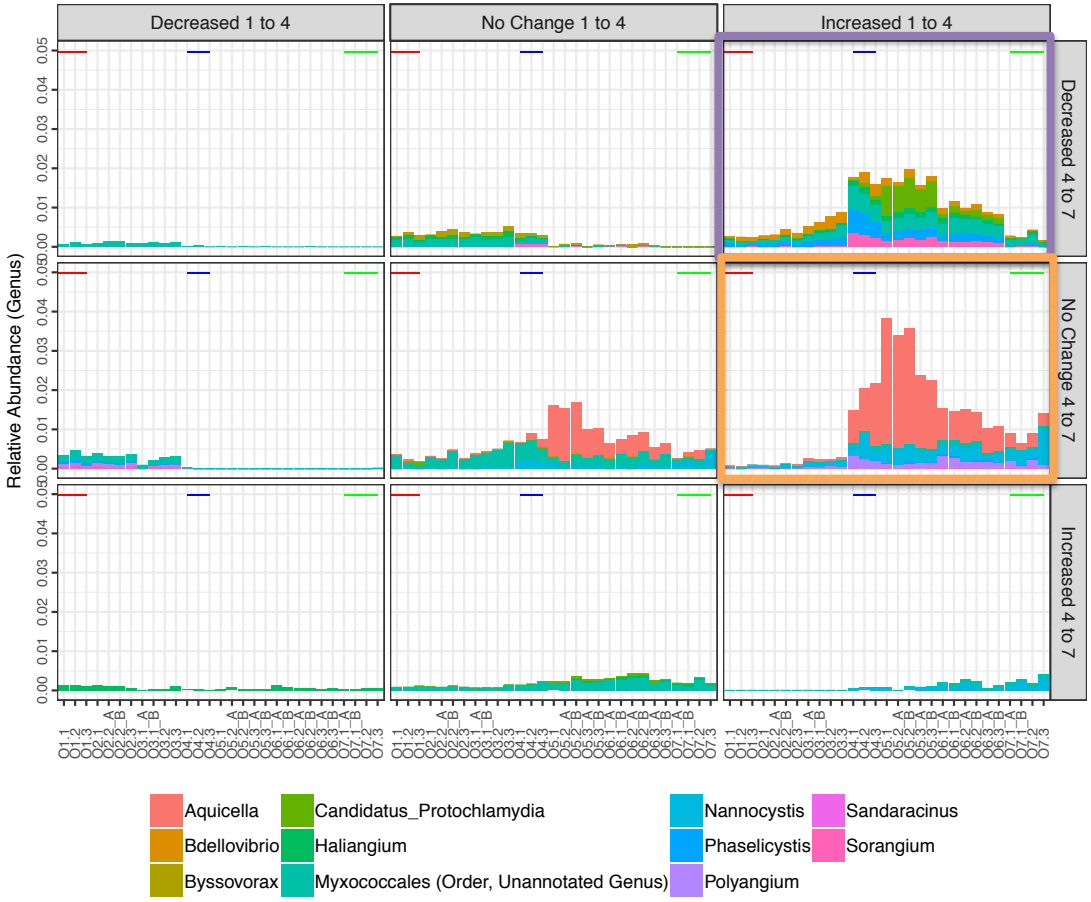
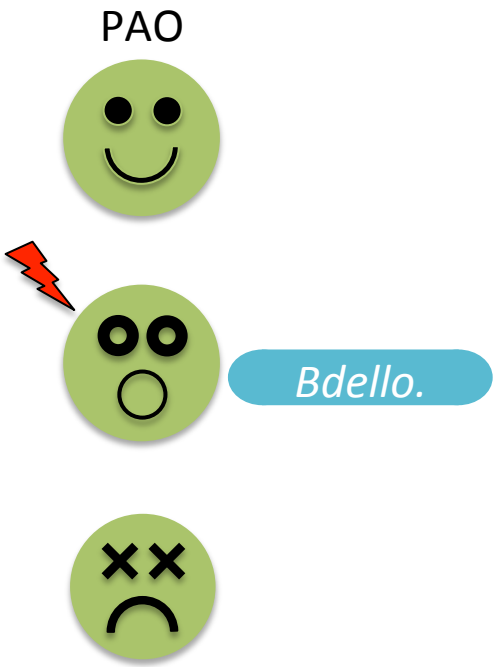
  - Ca. *Competibacter*
  - *Difluviicoccus* spp.
- Organic C degraders
  - Cytophagales (Class)
- Predatory bacteria
  - *Bdellovibrio* spp.
  - Myxococcales (Order)
- Protist-associated
  - *Aquicella* spp.
  - *Ca. Protochlamydia*



Discussion & Conclusions



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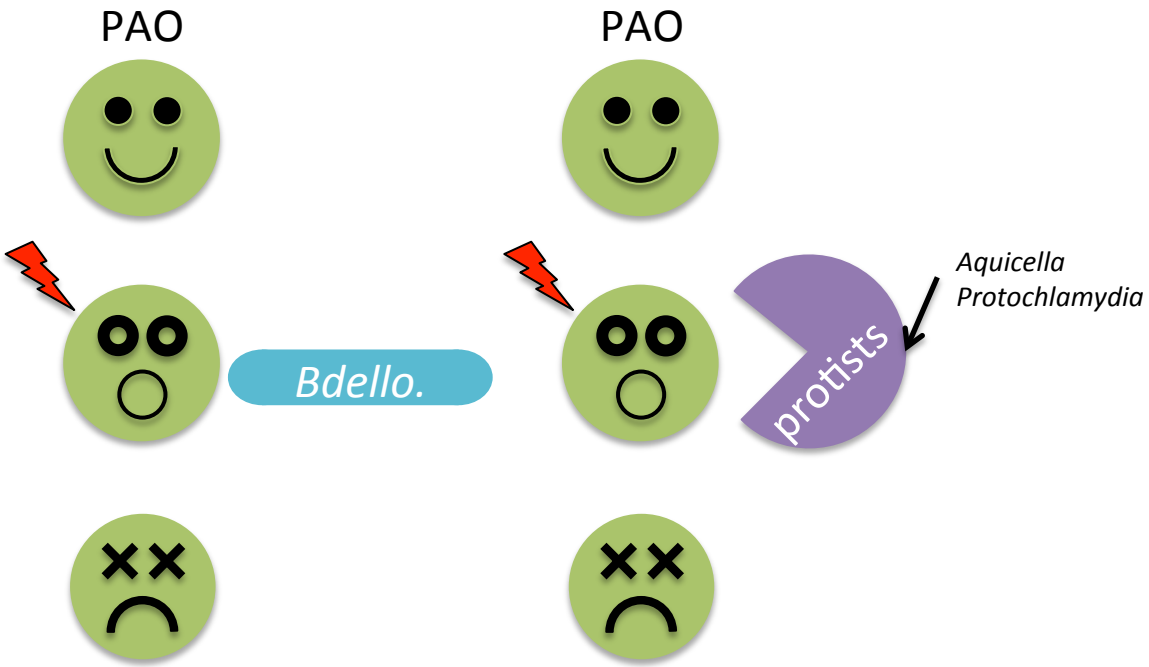

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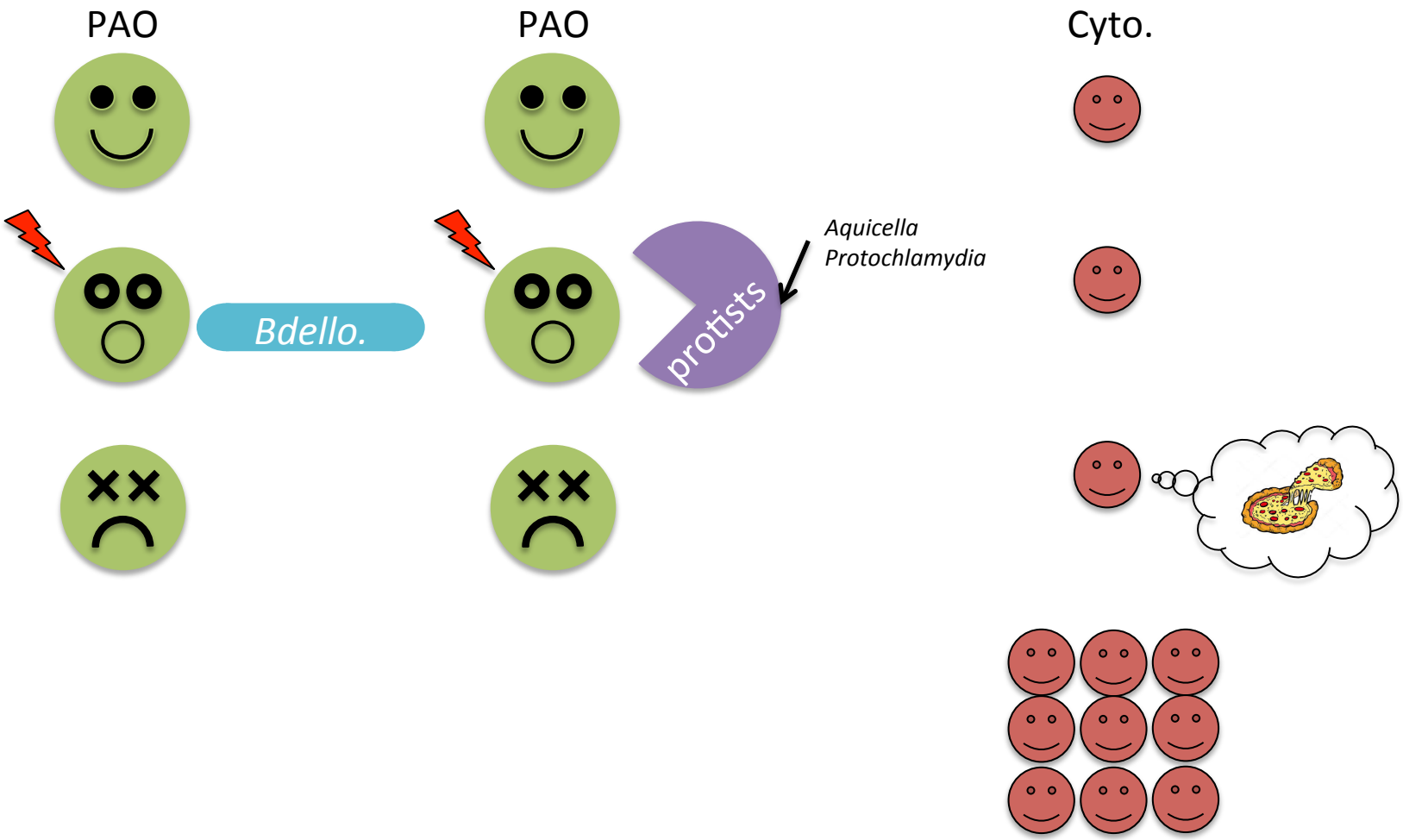
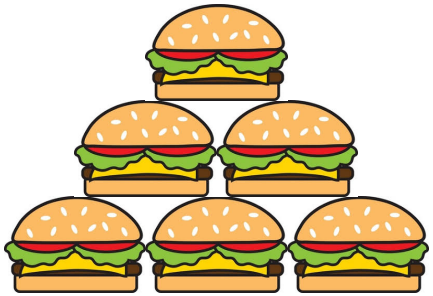


Discussion & Conclusions

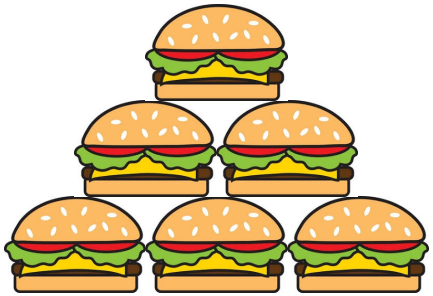
PAO



GAO

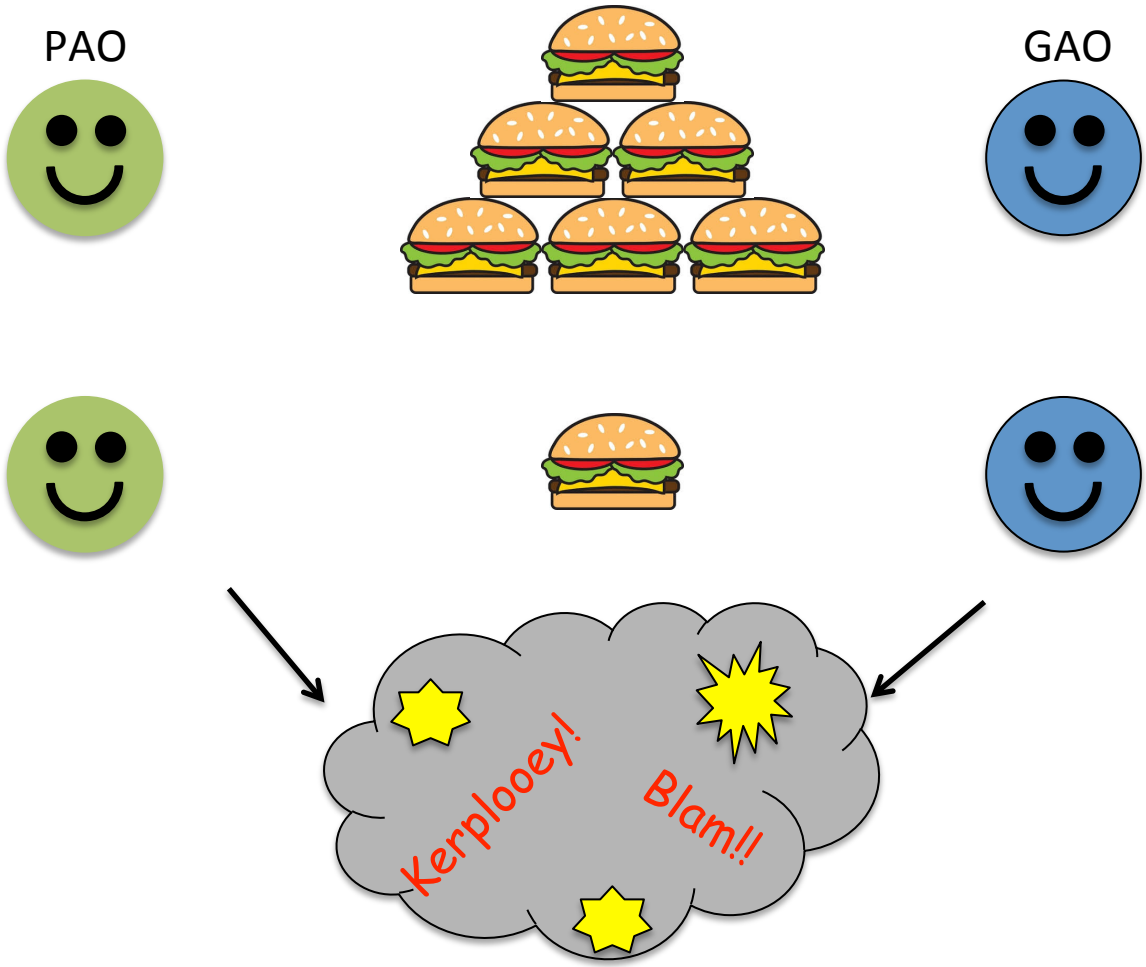
Discussion & Conclusions

PAO



GAO

Discussion & Conclusions**



Future Work*

- Expand differential abundance testing methods
- Metagenomic Sequencing
  - Integrate WGS and Amplicon sequencing results
  - Recover MAGs

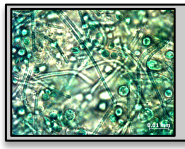
Acknowledgements

Drexel University

- Christopher Sales
  - Saeed Keshani
- Gail L. Rosen
  - Stephen Woloszynek

Xi'an University of Architecture & Technology

- Yaping Nan
  - Yachao Wang
  - Min Cheng
  - Linjiang Yuan



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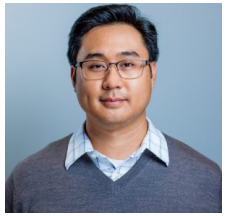

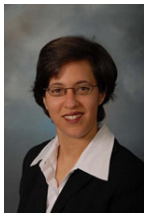

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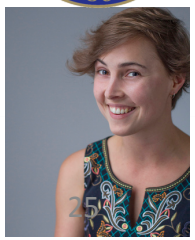

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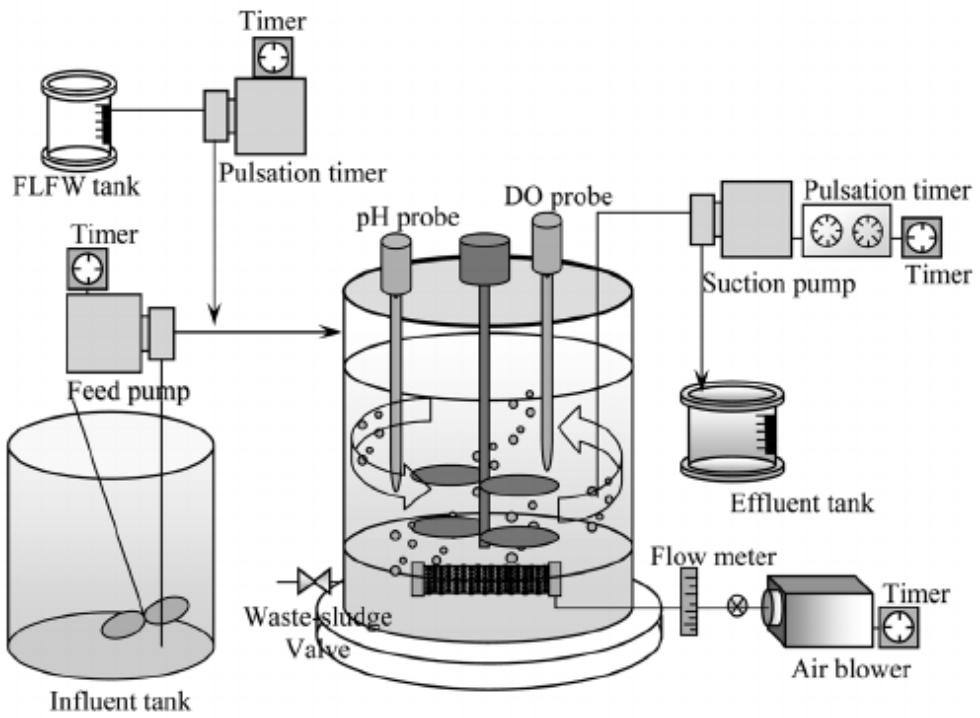
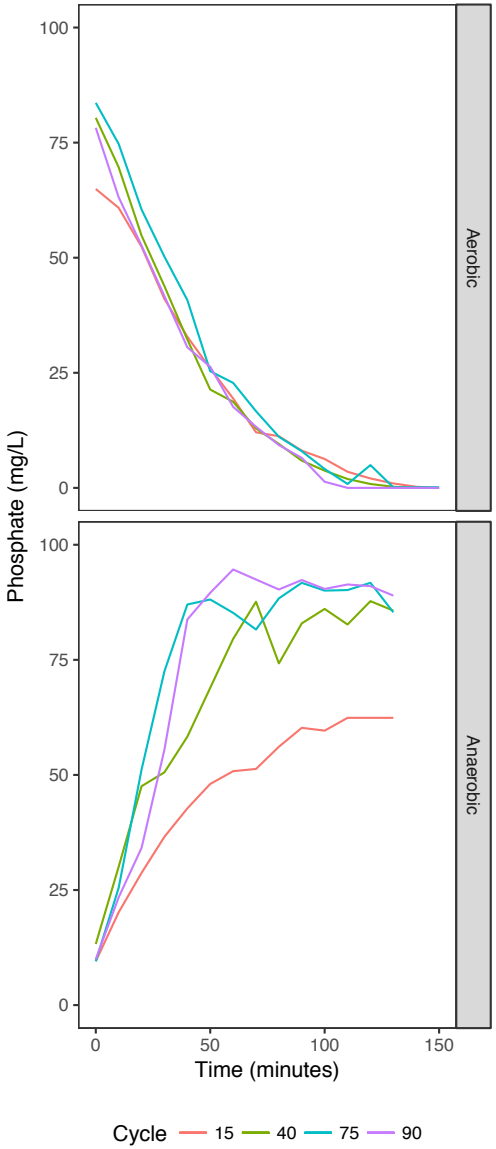
Supplemental

Methods

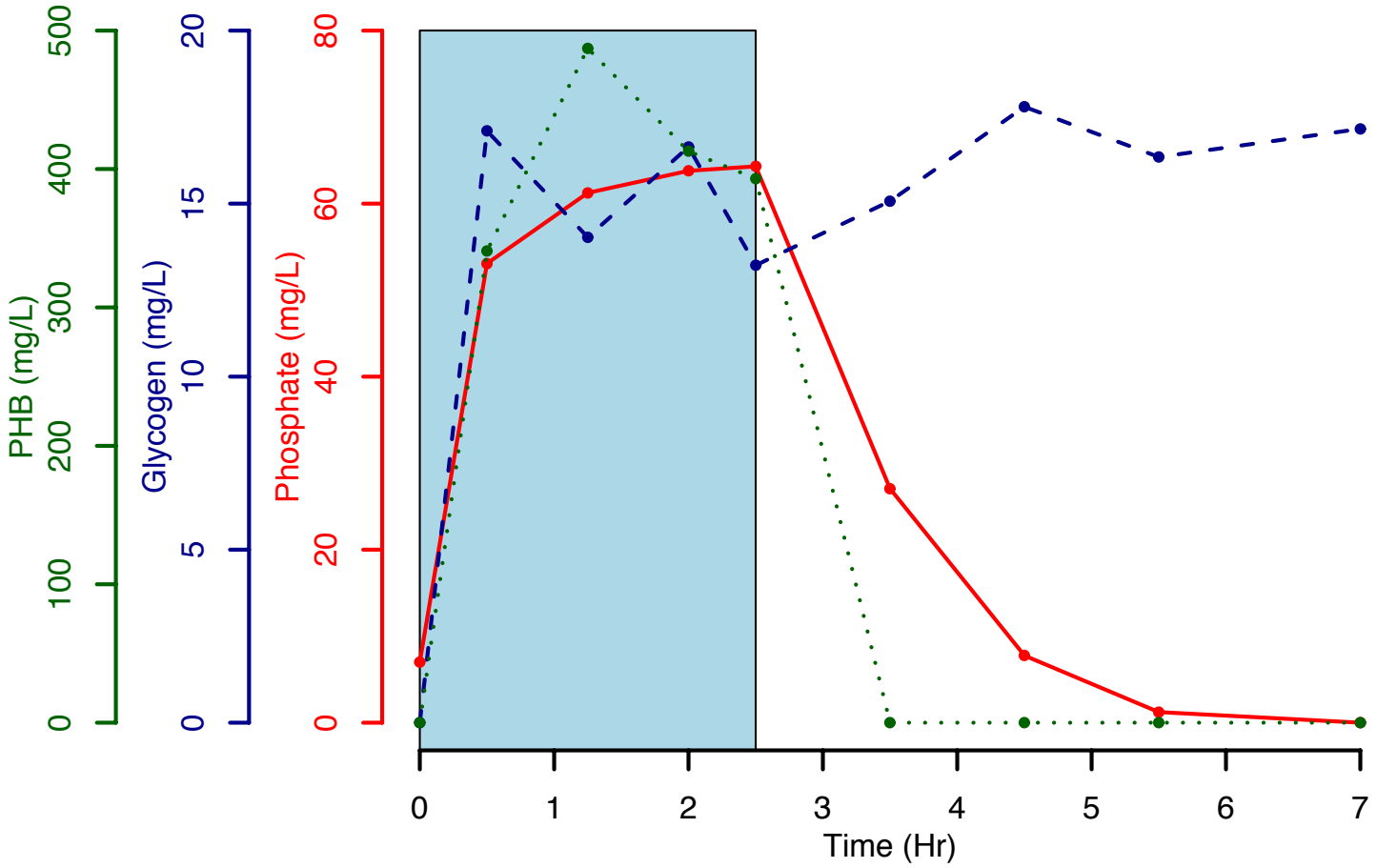
- 2 x SBRs
  - Experiment O
    - 40 days = 120 cycles
  - Experiment S
    - 23 days = 67 cycles
- 8-hour cycles
  - Anaerobic - 2.5 hrs
  - Aerobic – 4.5 hrs
  - Settling – 1 hr
- Synthetic influent
  - C – acetate
  - N – NH_4^+
- Chemical Data
  - PO_4^{3-}, COD, Gly, PHB
- 16S rRNA
  - Experiment O
    - 7 sampling events
  - Experiment S
    - 2 sampling events (beginning/end)



Kinetics


Phosphate (mg/L)
100
75
50
25
0
Aerobic
Anaerobic
0
50
100
150
Time (minutes)
Cycle
15
40
75
90

Kinetics


PHB (mg/L)
0
100
200
300
400
500
Glycogen (mg/L)
0
5
10
15
20
Phosphate (mg/L)
0
20
40
60
80
0
1
2
3
4
5
6
7
Time (Hr)

Alpha Div & High-level Abundances