Microbial ecology of activated sludge system to inform system operation and design

Ali Ling, Becca Vermace, and Trevor Ghylin

MICROBE DETECTIVES

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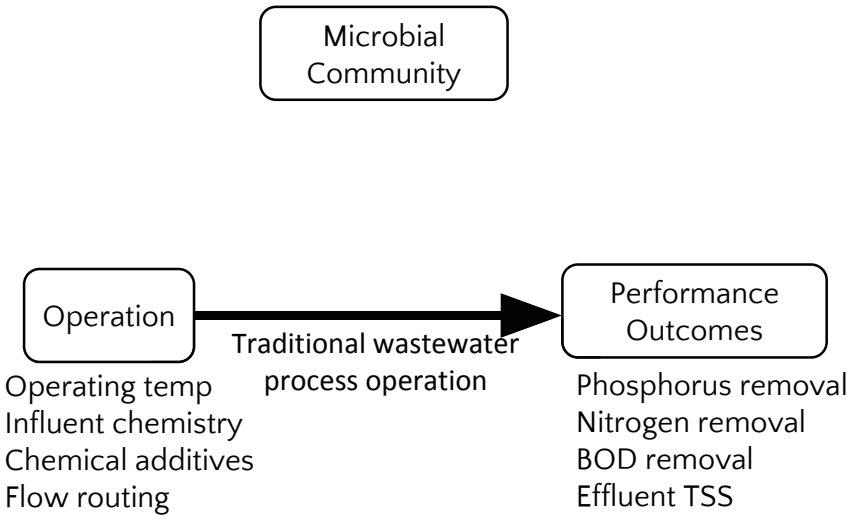
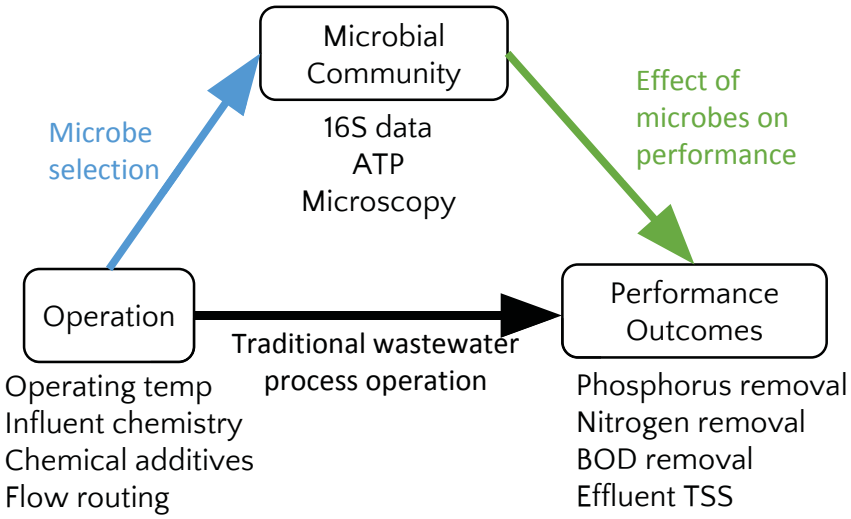
- Context and study goals
- Lessons from just plant data
- Lessons including consideration of microbial ecology
- Key takeaways

Context and Study Goals


Microbial Community
Operation
Traditional wastewater process operation
Performance Outcomes
Operating temp
Influent chemistry
Chemical additives
Flow routing
Phosphorus removal
Nitrogen removal
BOD removal
Effluent TSS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16S data
ATP
Microscopy
Microbe selection
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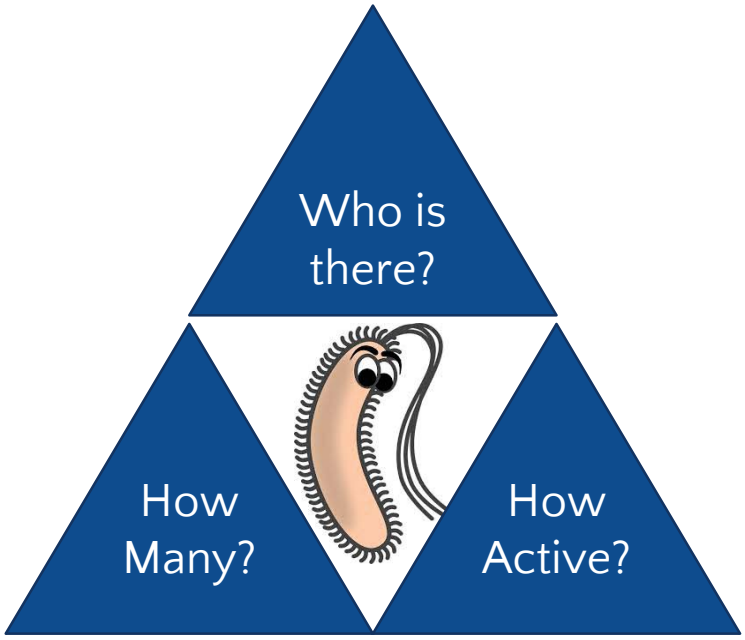
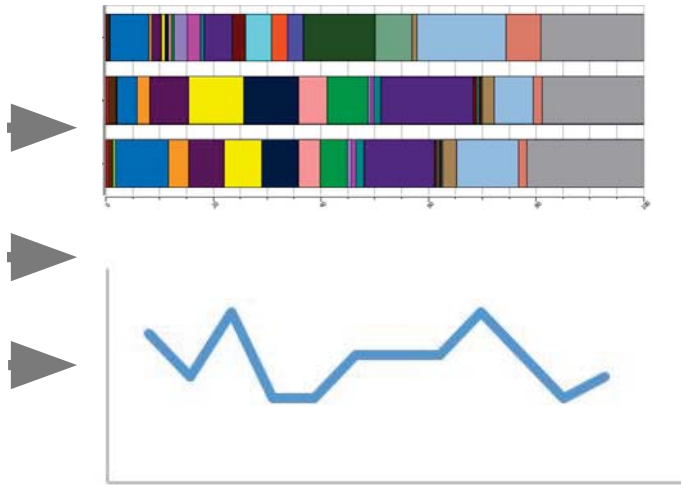
Tools for understanding microbes



Other tools for understanding microbes

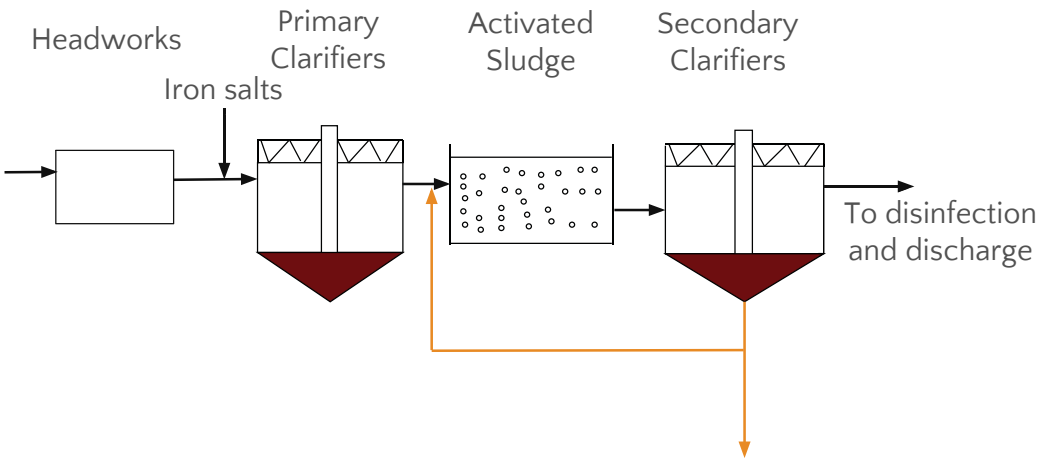
Question	Analysis	Method	Approx Cost/ Sample	Field/ Lab
Who is there?	Characterize entire microbial communities	16S microbiome analysis	$300	Lab
How many?	Detect/quantitate specific genes of interest or total abundance	Quantitative PCR (qPCR)	$150	Lab
How active?	Measure microbial activity	ATP analysis	$20 + equipment	Lab or Field



Why use new methods?

- Microbiological methods typically applied in engineering systems are *centuries* old. Recent technology advances enable more reliable and detailed understanding of microbes and their activities
- Results enable optimization of design and operation practices
- Costs similar to advanced chemical analyses

Process flow



Data used for this study

	Activated Sludge
Operational/Influent Data	• Dissolved Oxygen (DO) • Temperature • Biochemical oxygen demand to ammonia ratio (BOD:NH_3) • Mean cell residence time (MCRT) • Influent ammonia and total phosphorus • Influent flows • F:M and nutrient ratios
Microbial Community Data	• Microbial group abundances
Performance Data	• Effluent Biochemical Oxygen Demand (BOD) • Effluent Ammonia (NH_3) • Effluent Phosphorus (P) • Effluent Total Suspended Solids (TSS) • Sludge volume index (SVI)

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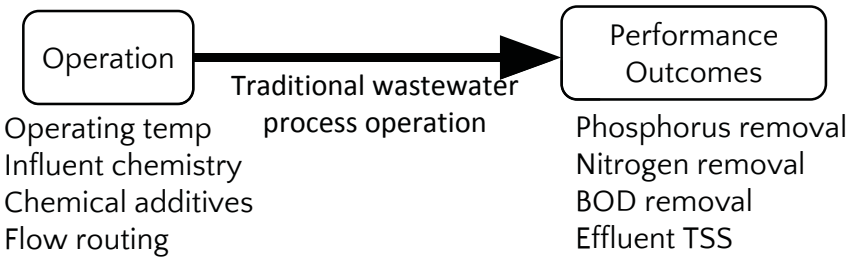
Analysis outcomes with just plant data



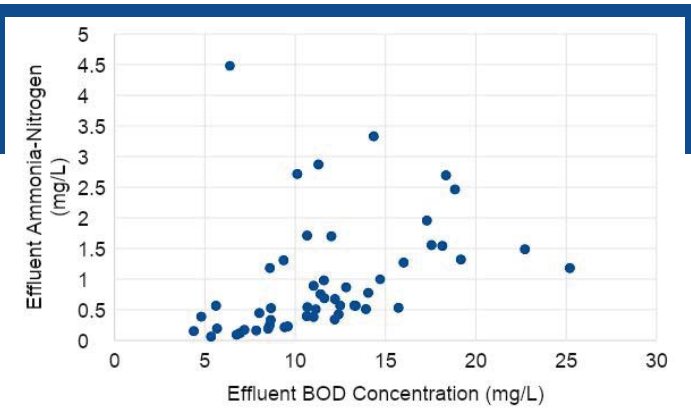
Effluent water quality

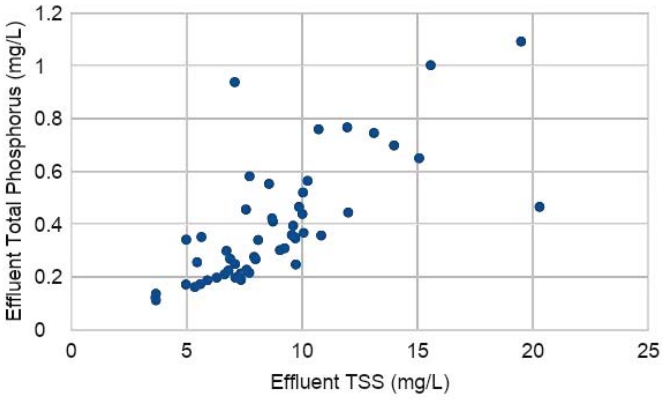
BOD, Ammonia, Total P, TSS all strongly correlated

TSS contains

- Biomass
- Phosphorus precip

Treatment efficacy is primarily limited by settling

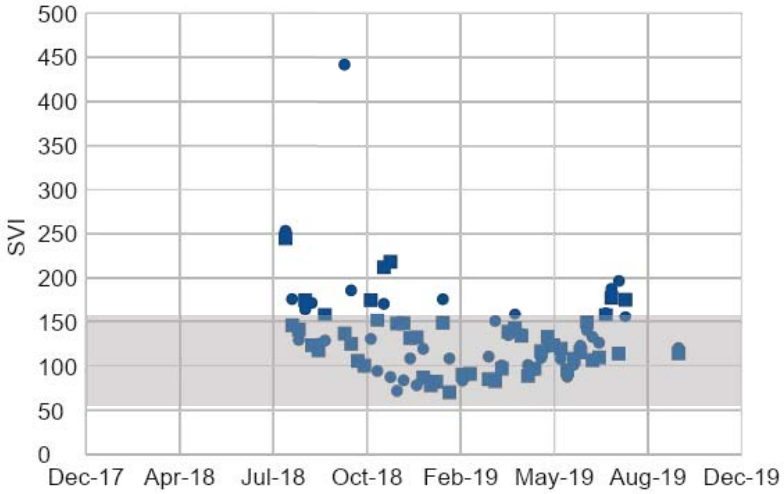


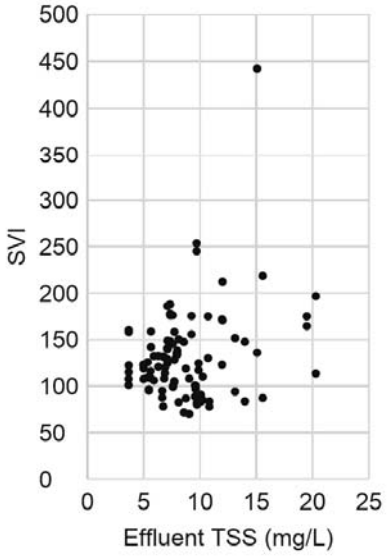


Sludge settleability

Higher SVI (poorly settling)

- Mostly during fall
- Correlated to high TSS

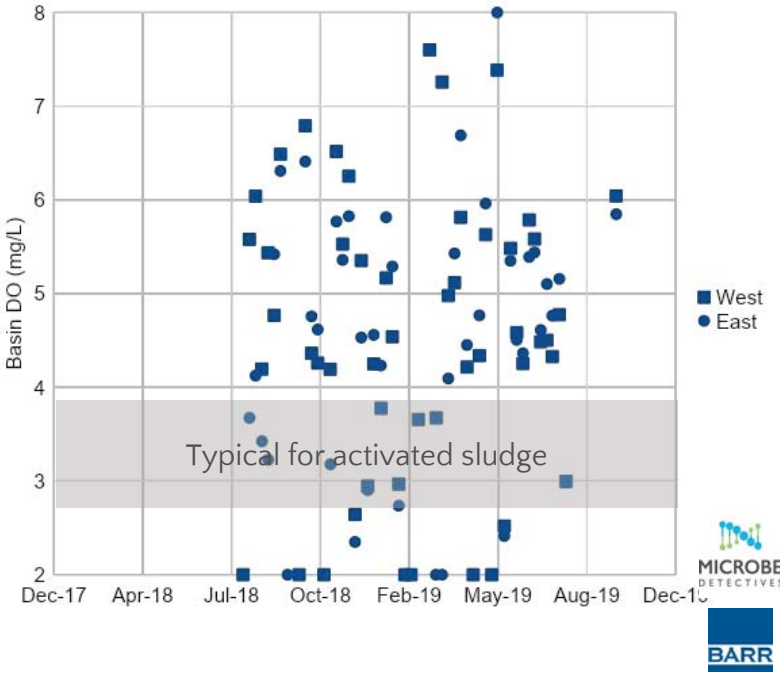




Effect of dissolved oxygen

Dissolved oxygen (DO) concentrations higher than typically used in activated sludge.

High DO concentrations were correlated to growth of filaments

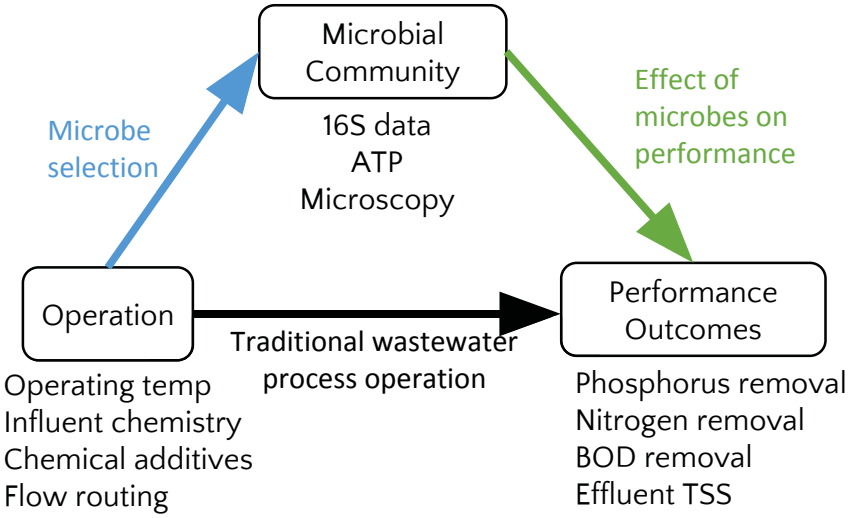


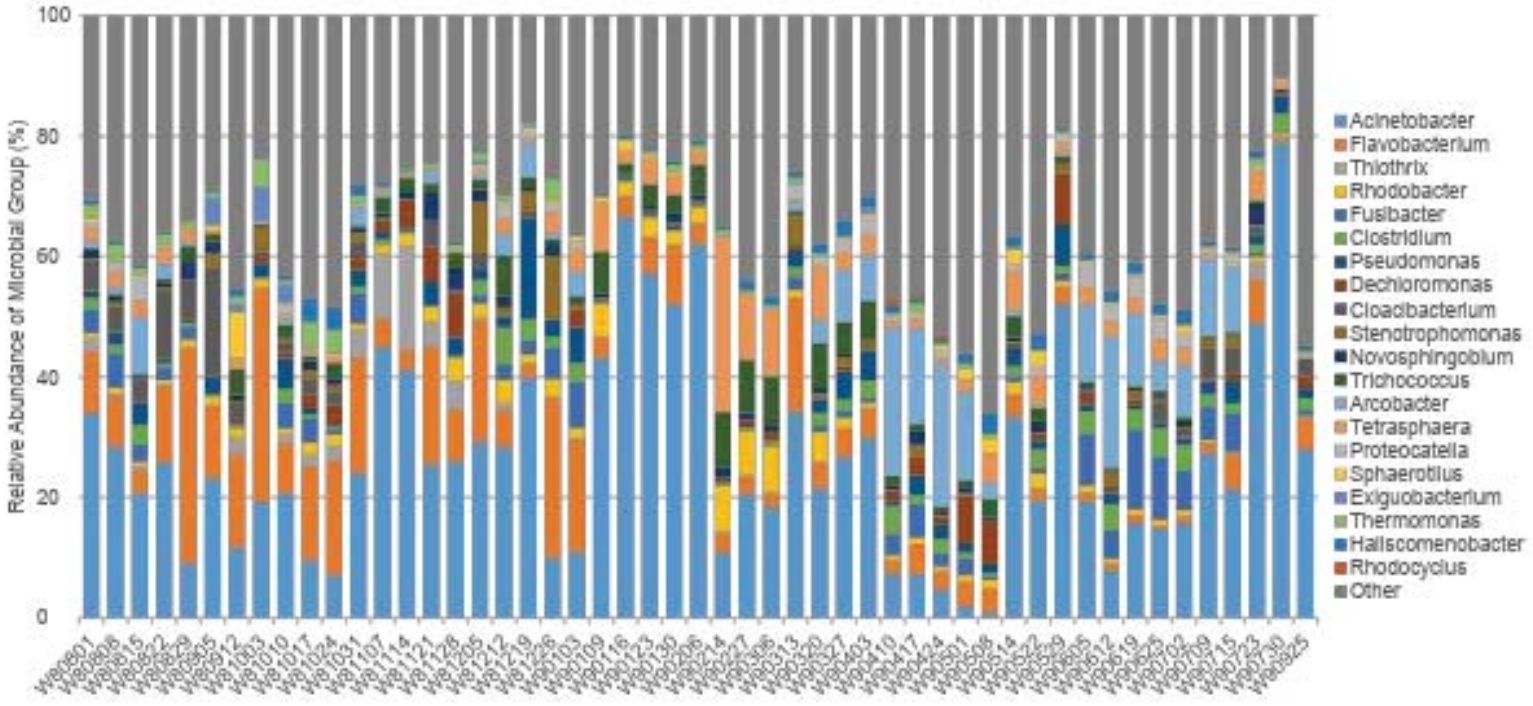
Potential operational changes

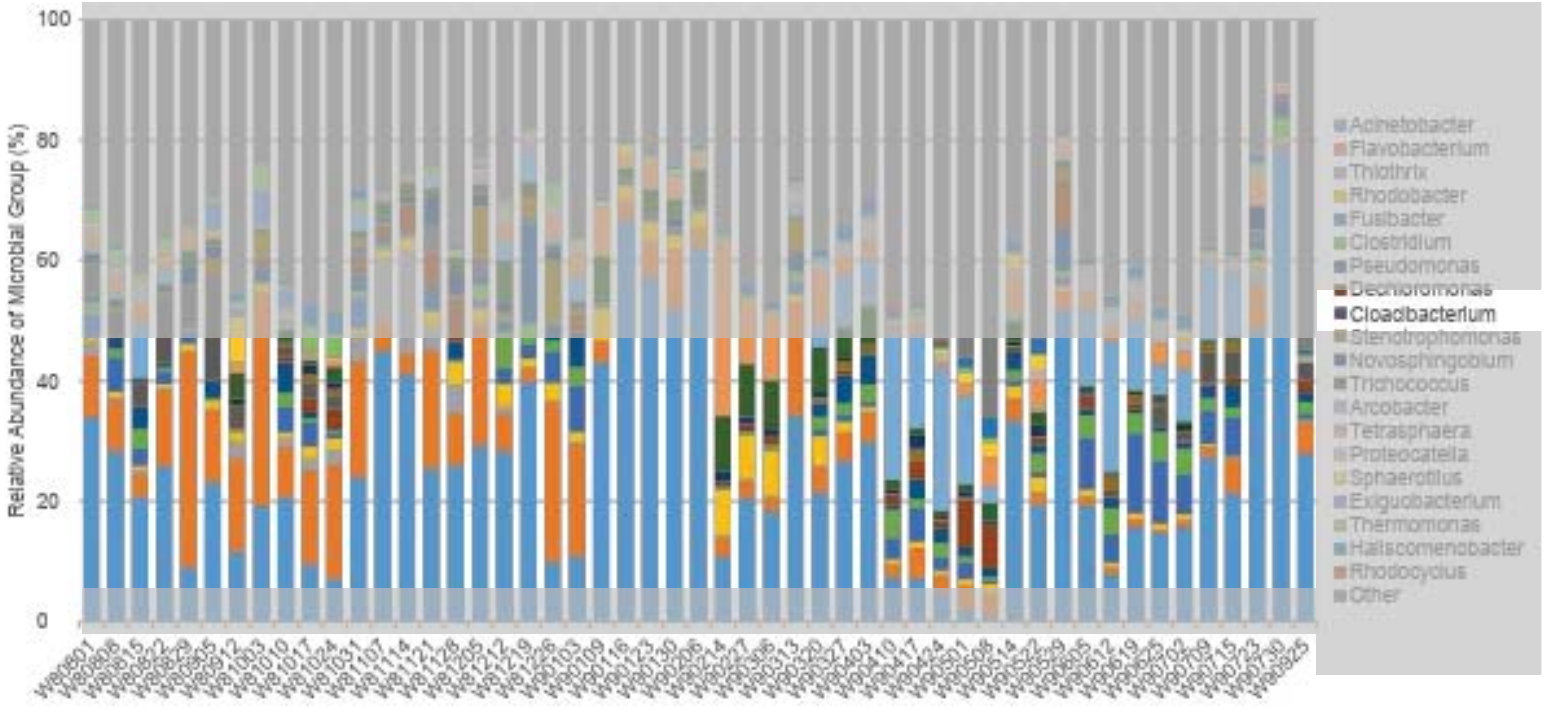
Upgrade blowers to provide more targeted oxygen supply to aeration basin

-> expected to save energy costs and improve settling characteristics

Analysis outcomes with microbial ecology




Relative Abundance of Microbial Group (%)
100
80
60
40
20
0
Acinetobacter
Flavobacterium
Thiothrix
Rhodobacter
Fusibacter
Clostridium
Pseudomonas
Dechloromonas
Cloacibacterium
Stenotrophomonas
Novosphingobium
Trichococcus
Arcobacter
Tetrasphaera
Proteocatella
Sphaerotilus
Exiguobacterium
Thermomonas
Hallscomenobacter
Rhodocyclus
Other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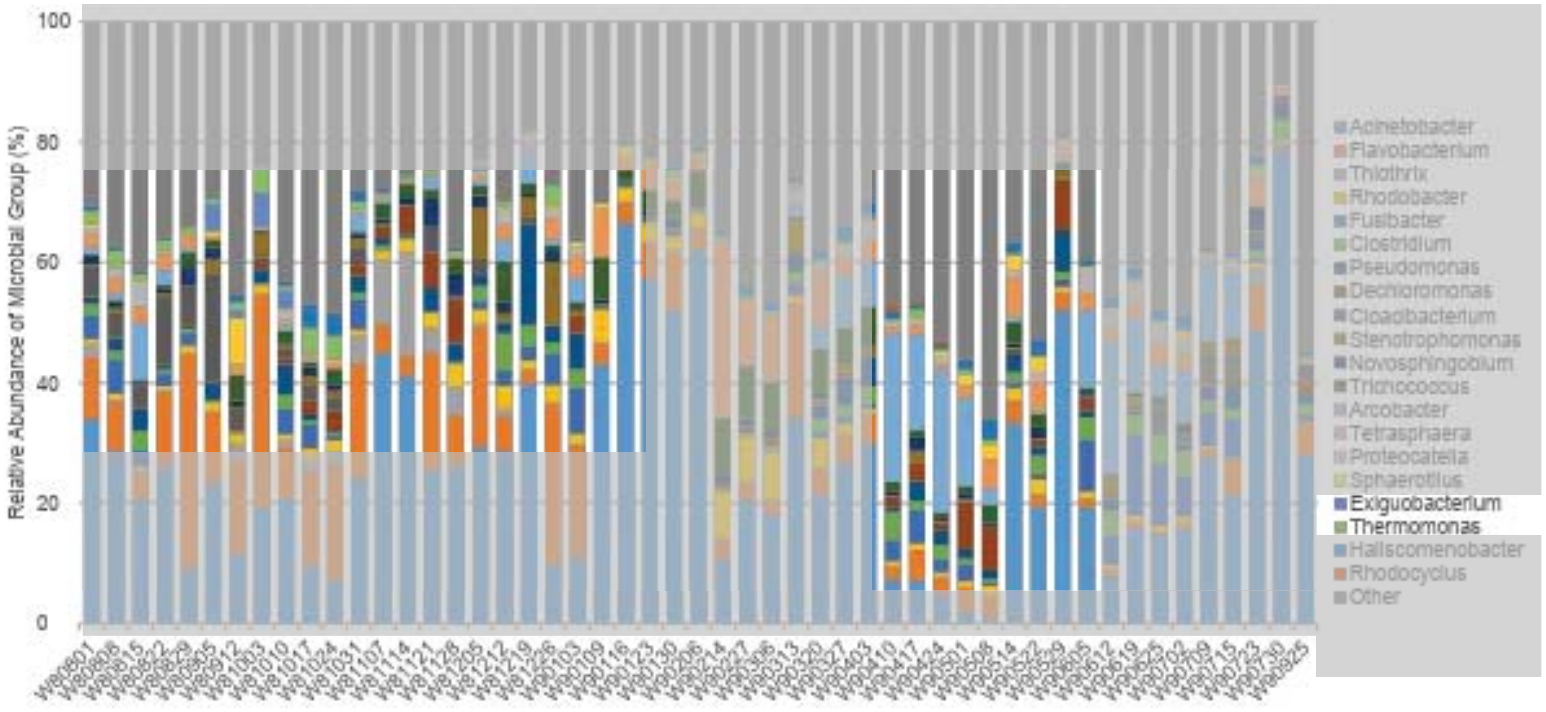
Proteocatella
Sphaerotilus
Exiguobacterium
Thermomonas
Halomonobacter
Rhodocyclus
Other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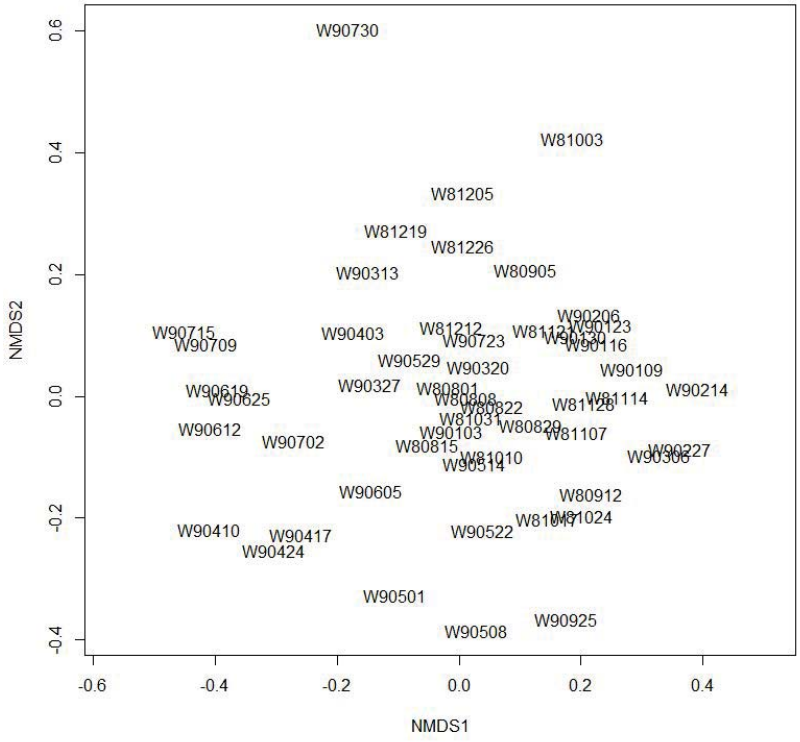
Halliscomenobacter
Rhodocyclus
Other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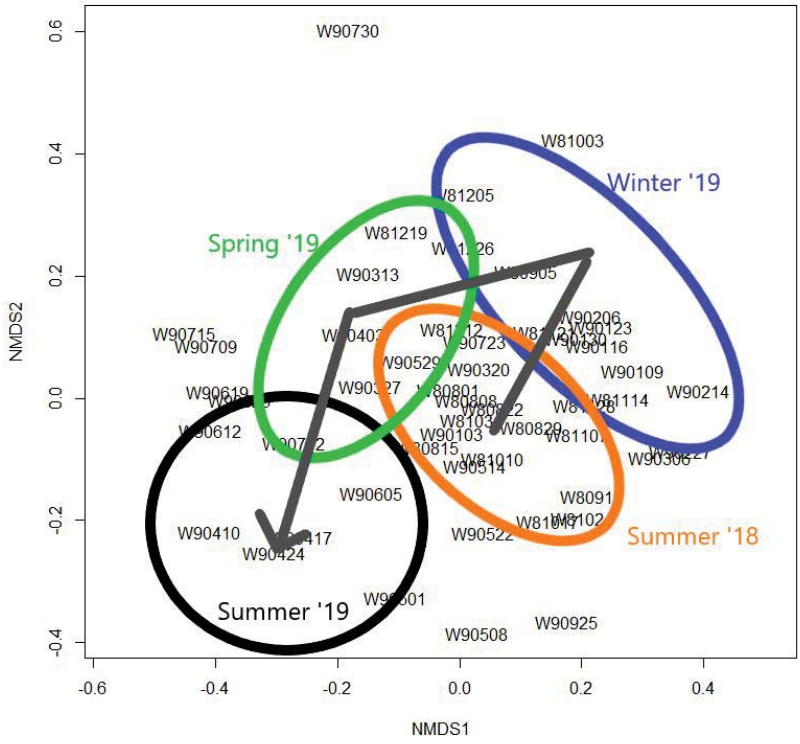
W90522
W90424
W90501
W90925
W90508
NMDS1
NMDS2
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6

Community changes seasonally, but not identical between years



Hypotheses from Microbial Data

- Similar community members over time, but abundances vary on a weekly basis.
- 10–70% *Acinetobacter* spp.
  - No significant correlations to outcomes or influent/operation conditions
  - This one microbial group has a significant effect on microbial diversity and evenness

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More trends seen through microbial groups

What microbes are good versus bad?

	Microbial Group (Genus)	Likely Role in Community	Outcomes				
			BOD	NH3	SVI	P	TSS
Good - Microbes correlated to higher performance	*Haliscomenobacter*	Filamentous	X		X	X	X
	Proteocatella					X	
	Fusibacter					X	X
	Arcobactor					X	X
	Clostridium	Varied				X	X
	Cloacibacterium	Anaerobe		X			
Bad - Microbes correlated to lower performance	*Flavobacterium*	Varied				X	X
	Thermomonas	Not Identified					X
	Thiothrix	Filamentous			X		X
	Cloacibacterium			X			
	Novosphingobium	Aromatic degrader	X			X	X

More trends seen through microbial groups

What microbes are good versus bad?

How to select for the right microbes?

	Microbial Group (Genus)	Likely Role in Community	Outcomes					Operation/influent			
			BOD	NH3	SVI	P	TSS	RAS Cl	MLSS	DO	Temp
Good - Microbes correlated to higher performance	*Haliscomenobacter*	Filamentous	X		X	X	X				
	Proteocatella					X					
	Fusibacter					X	X				
	Arcobactor					X	X	↑	↑		↓
	Clostridium	Varied				X	X			↓	
	Cloacibacterium	Anaerobe		X						↓	
Bad - Microbes correlated to lower performance	*Flavobacterium*	Varied				X	X				↓
	Thermomonas	Not Identified					X				↓
	Thiothrix	Filamentous			X		X		↑		↓
	Cloacibacterium			X							↓
	Novosphingobium	Aromatic degrader	X			X	X				

Again, lower DO is beneficial

What microbes are good versus bad?

How to select for the right microbes?

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			BOD	NH3	SVI	P	TSS	RAS Cl	MLSS	DO	Temp
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	Proteocatella					X					
	Fusibacter					X	X				
	Arcobactor					X	X	↑	↑		↓
	Clostridium	Varied				X	X			↓	
	Cloacibacterium	Anaerobe		X						↓	
Bad - Microbes correlated to lower performance	*Flavobacterium*	Varied				X	X				↓
	Thermomonas	Not Identified					X				↓
	Thiothrix	Filamentous			X		X		↑		↓
	Cloacibacterium			X							↓
	Novosphingobium	Aromatic degrader	X			X	X				

Increasing RAS chlorination selects for good microbes

What microbes are good versus bad?

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			BOD	NH3	SVI	P	TSS	RAS Cl	MLSS	DO	Temp
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	Proteocatella					X					
	Fusibacter					X	X				
	Arcobactor					X	X	↑	↑		↓
	Clostridium	Varied				X	X			↓	
	Cloacibacterium	Anaerobe		X						↓	
Bad - Microbes correlated to lower performance	*Flavobacterium*	Varied				X	X				↓
	Thermomonas	Not Identified					X				↓
	Thiothrix	Filamentous			X		X		↑		↓
	Cloacibacterium			X							↓
	Novosphingobium	Aromatic degrader	X			X	X				

Winter conditions (lower wasting) saw better performance

What microbes are good versus bad?

How to select for the right microbes?

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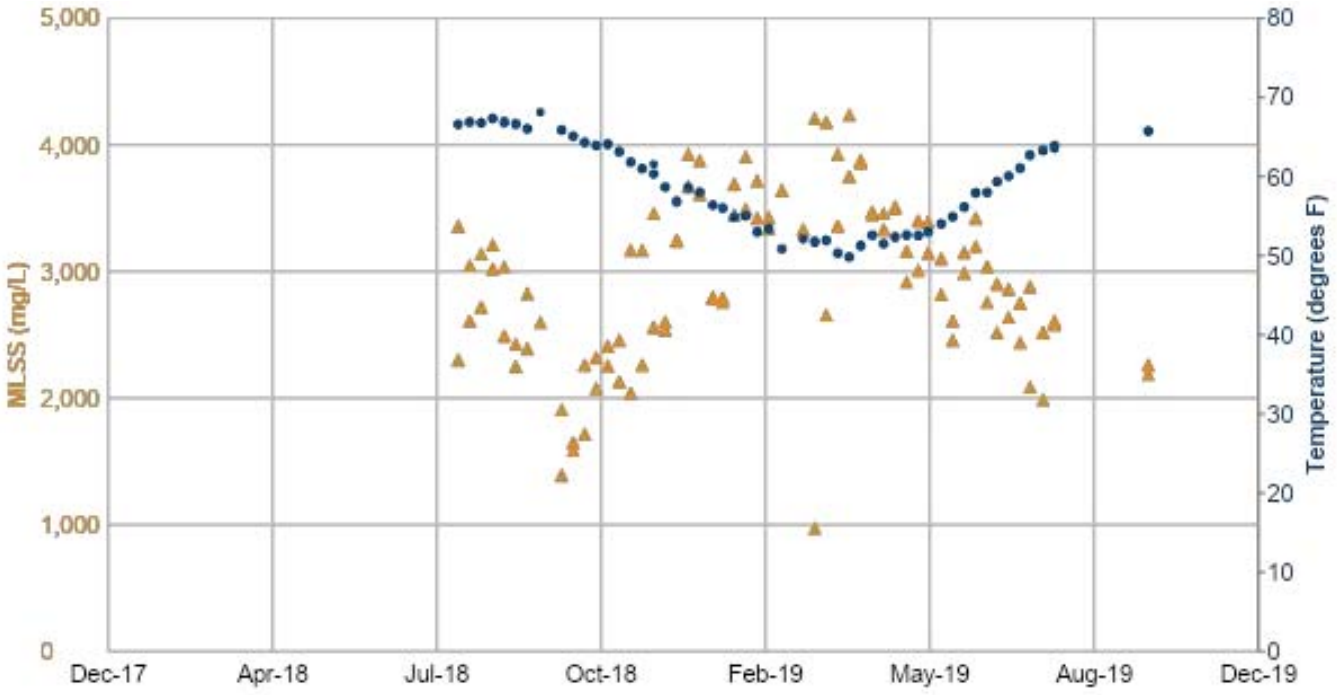
	Proteocatella					X					
	Fusibacter					X	X				
	Arcobactor					X	X	↑	↑		↓
	Clostridium	Varied				X	X			↓	
	Cloacibacterium	Anaerobe		X						↓	
Bad - Microbes correlated to lower performance	*Flavobacterium*	Varied				X	X				↓
	Thermomonas	Not Identified					X				↓
	Thiothrix	Filamentous			X		X		↑		↓
	Cloacibacterium			X							↓
	Novosphingobium	Aromatic degrader	X			X	X				

Winter and summer conditions



Hypotheses from Microbial Data

- Better indicator of effluent TSS than SVI, DO or any other parameter traditionally measured
- No trends observed relating F:M ratio or MCRT directly to specific microbe abundances.
- Winter conditions grow more favorable microbes, and are reflected by low temperatures and high MLSS and MCRT
  - Methods to retain MLSS during summer months has the potential to improve treatment

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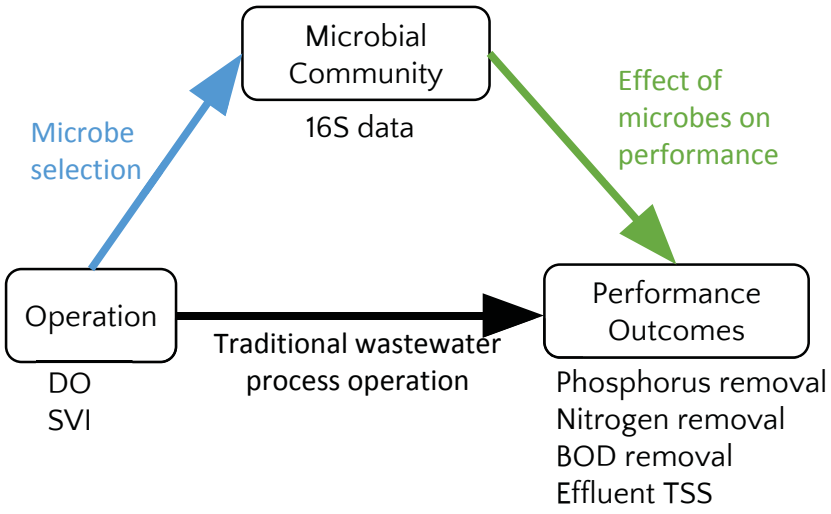
Key Takeaways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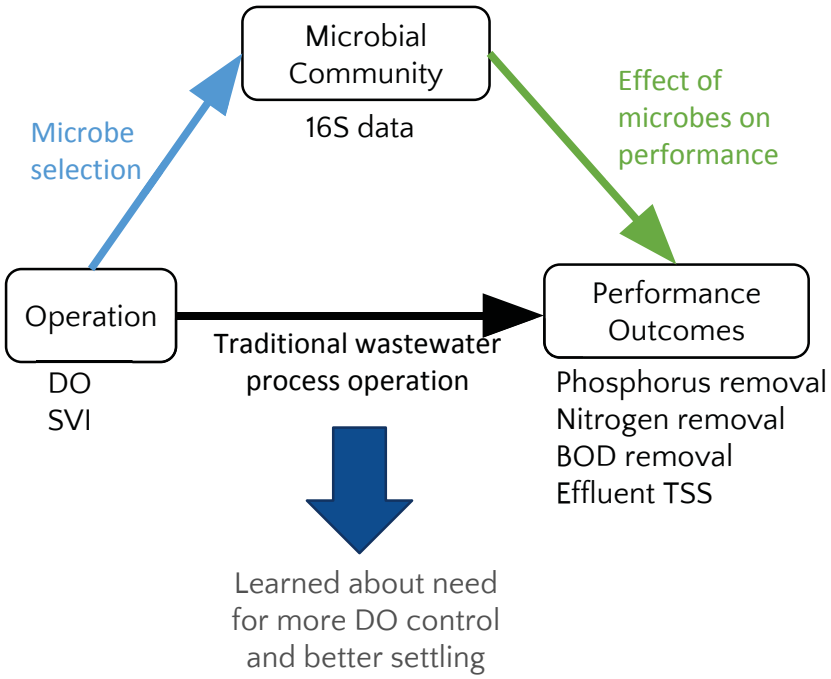
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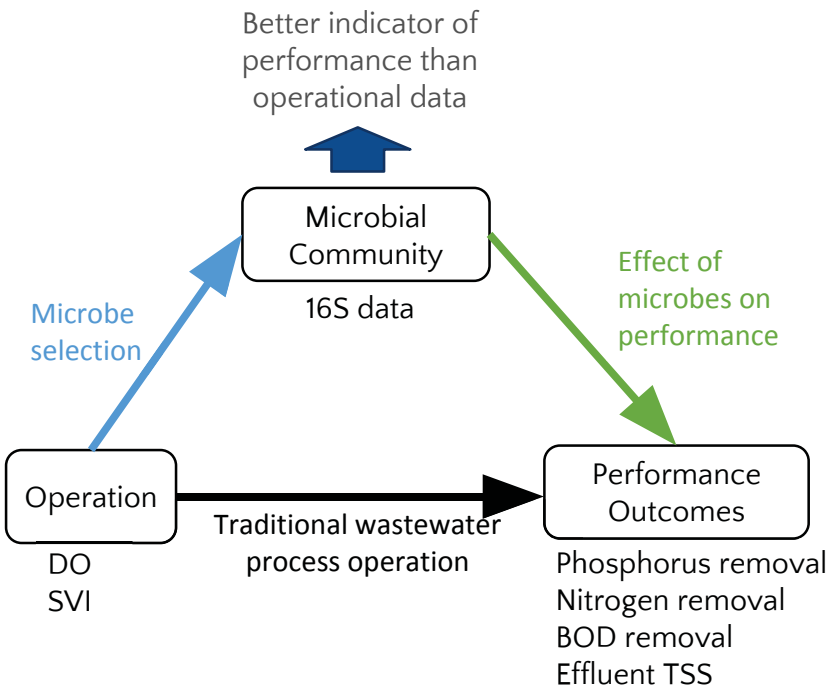
Learned about need for more DO control and better settling


Better indicator of performance than operational data
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Takeaways

- Can learn useful trends and insight from more detailed analysis of plant data
- **Microbial community data can identify additional correlations**

Use of Microbial Methods

- System Understanding
  - Provide an **operational baseline** and to help understand potential causes of system upsets
  - Specific growth preferences of **microbial groups** can be used to select or inhibit targeted abundant microbial groups
- Operational Input
  - **Real-time measurement (ATP or cell count)** data may be a useful of to understand microbial activity and reactor performance.
  - Upcoming advances in qPCR and 16S turnaround times

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Thanks to

Microbe Detectives

- Trevor Ghylin
- John Tillotson

Barr

- Becca Vermace
- Don Richard