LOW DO OPERATIONAL IMPACT AND PRELIMINARY QPCR RESULTS AT EAGLES POINT FACILITY

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Metropolitan Council

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Overview

- Plant overview
- Project goals and timeline
- Lessons learned
- Effluent quality
- SVIs and taxonomy
- qPCR
- Microorganism abundance comparison

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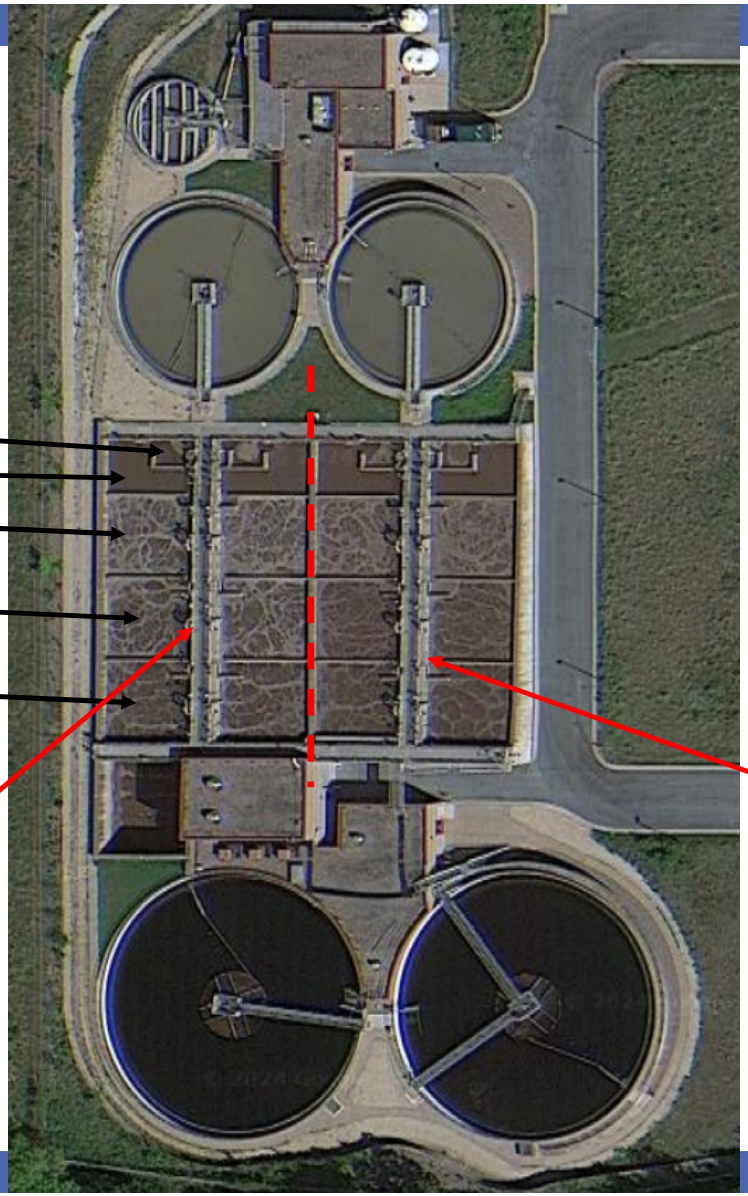
Eagle Point Facility

- 2005 design flow of 10 MGD
- Activated sludge plant with enhanced Bio- P operation
- 6 MGD current flow
- Separate sludge handling
  - GT for primary sludge
  - GBT with polymer for secondary solids
- Solids processing- haul to Metro

Permit limits

Parameter	Limit
Design flow	10 MGD
Current flow	6 MGD
BOD	25 mg/L monthly average
TSS	30 mg/L monthly average
Phos	1.0 mg/L – 12 month rolling average
Ammonia	5 mg/L – from July to September
Nitrogen	Monitor
Fecal Coliform	200 monthly geomean from April to October

Plant Overview



Anoxic
Anaerobic
Zone 1 – Aerobic

Zone 2 – Aerobic

Zone 3 - Aerobic

Aeration Tanks 1&2
High DO = 2.0 mg/L
Individual Clarifiers & RAS

Aeration Tanks 3&4
Low DO = 0.50 mg/L
Individual Clarifiers & RAS

Low DO project started in Nov. 2023

Goals

1. Energy savings
2. Decrease TN in the effluent

Maintain:

1. Nitrification
2. SVI <150
3. Observe airflow savings

Approach:

- Slowly step down the DO setpoints

- Setpoint of 0.5 mg/L DO before ammonia permit season

- Let microbes adapt at each setpoint change ~ 3 weeks

- Troubleshoot when upsets arise

Effluent Quality

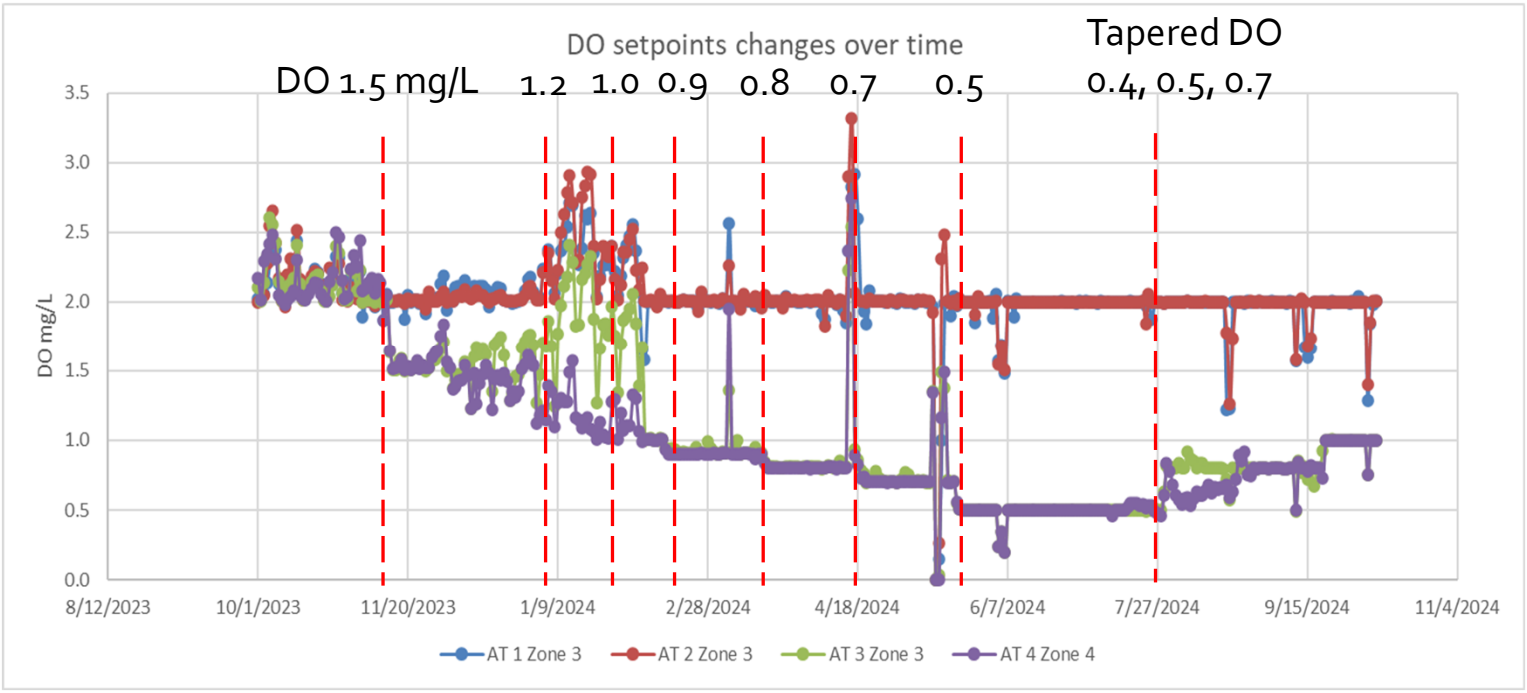
	NH_3-N (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	PO_4-P (mg/L)
Low DO Effluent	1.035	0.384	24.3	0.045

*Use half of non-detect value if below range of Hach kit (PO_4-P <0.05 = 0.025)

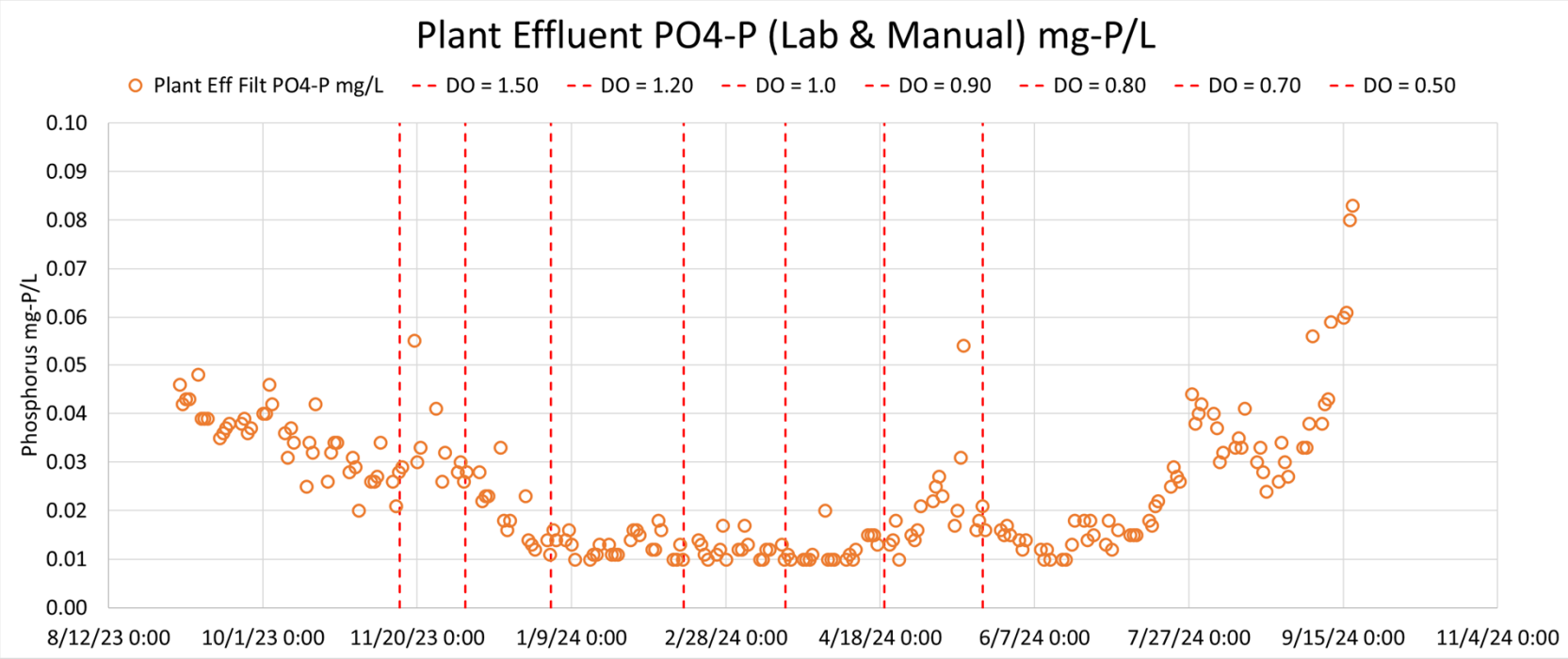
Lessons Learned

- DO probe accuracy is very important
- Identified a leaking check valve
- Power outages affects the plant performance
- Dye test to confirm flows

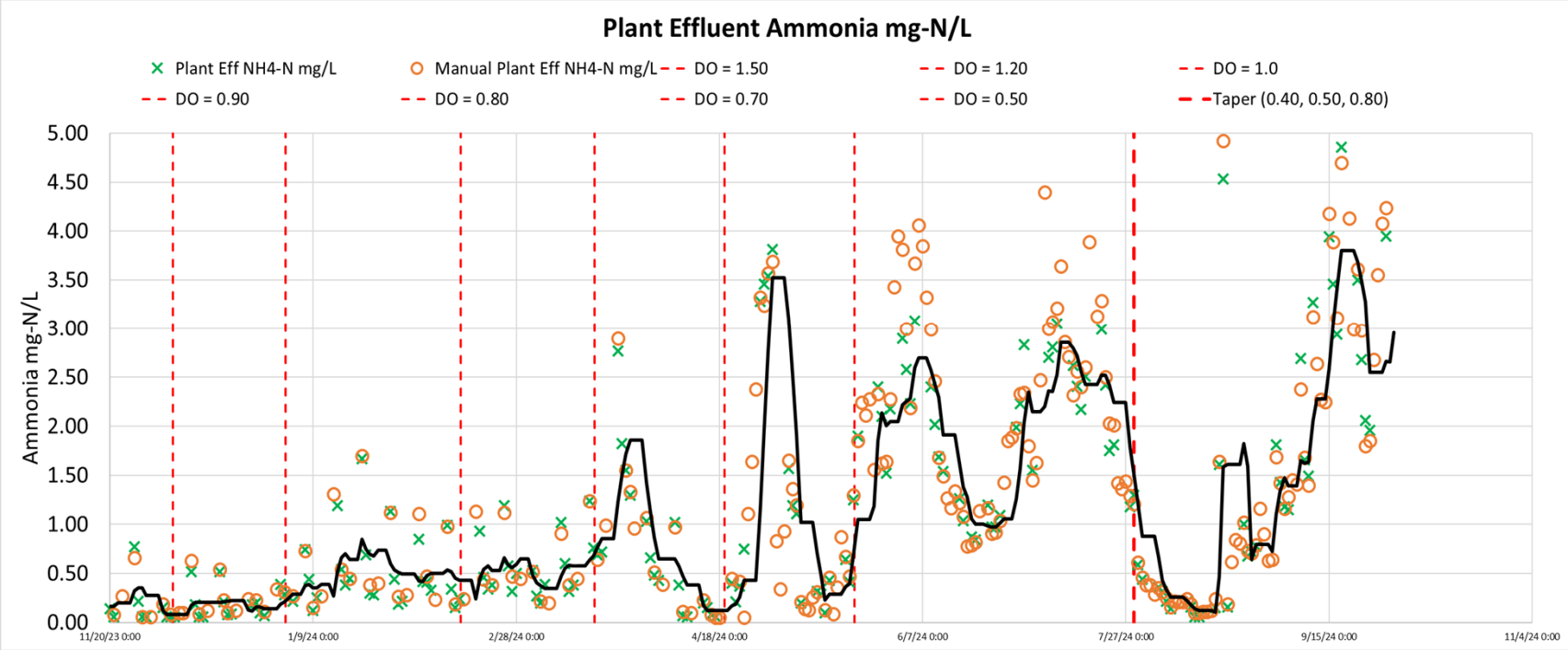
Step down DO slowly



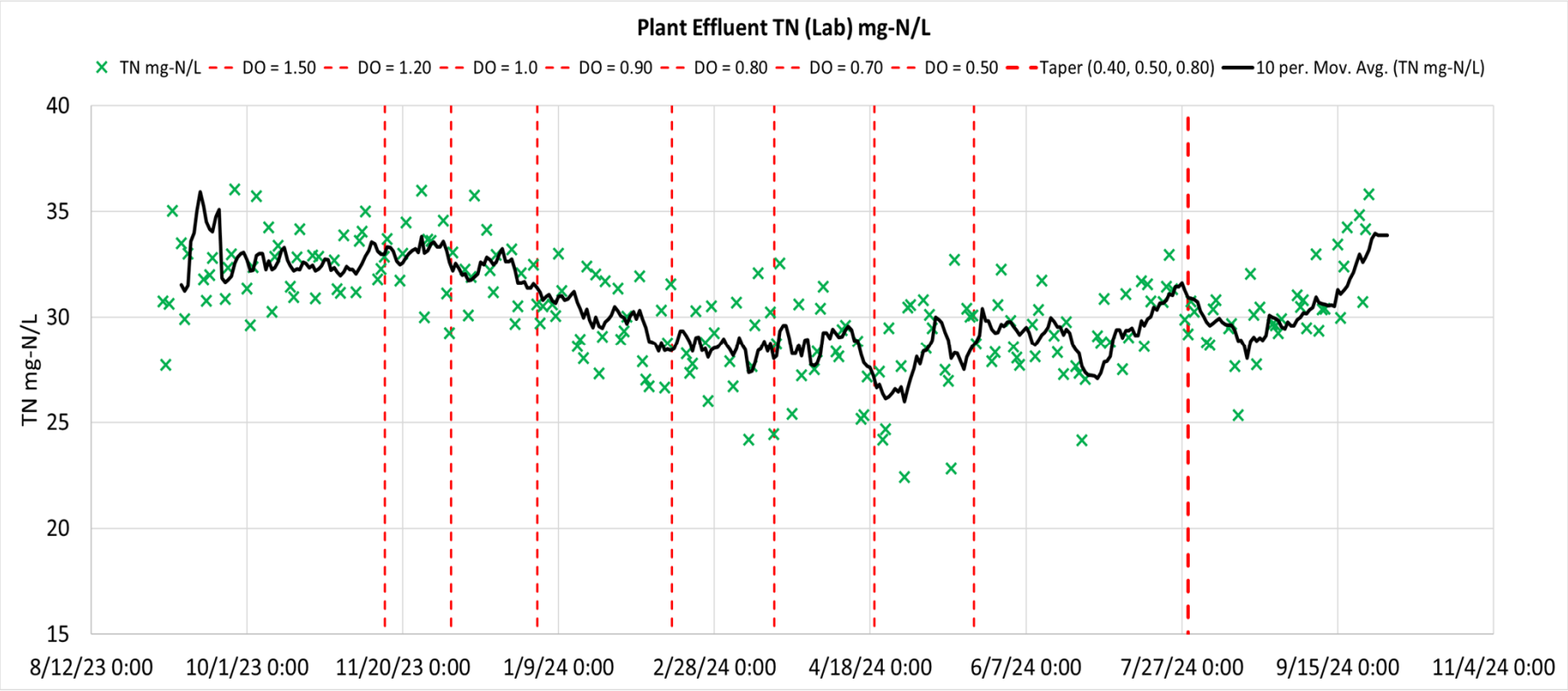
Phos during step changes



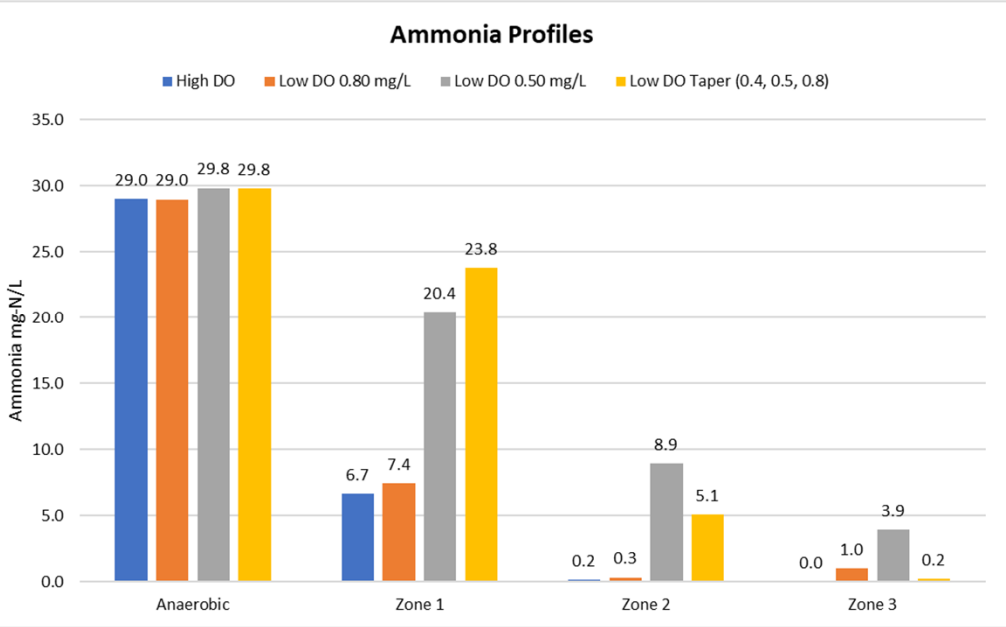
Ammonia during step changes

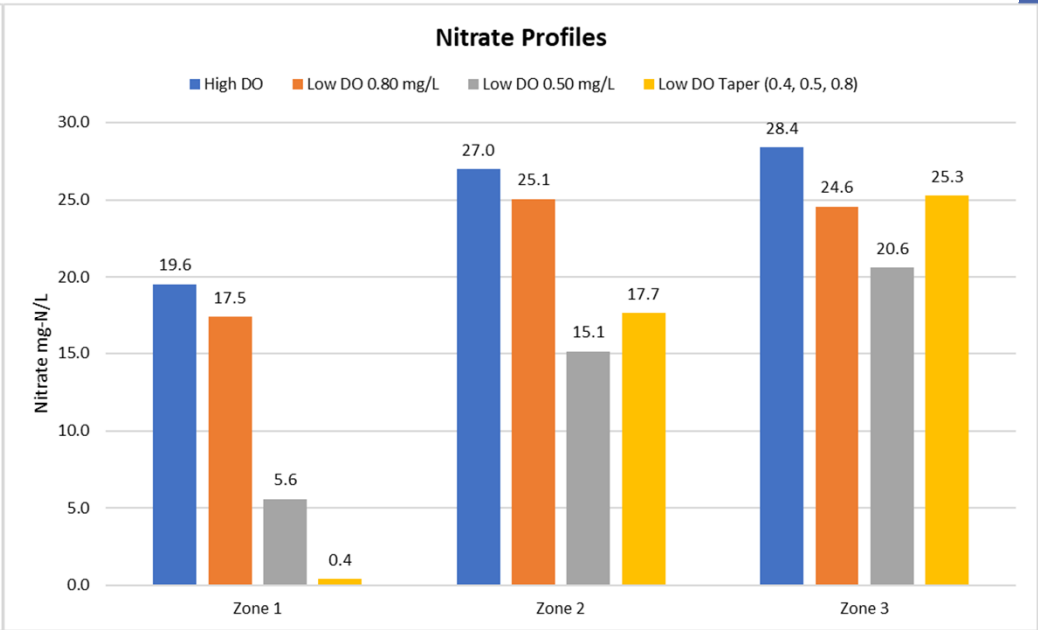


TN during step changes

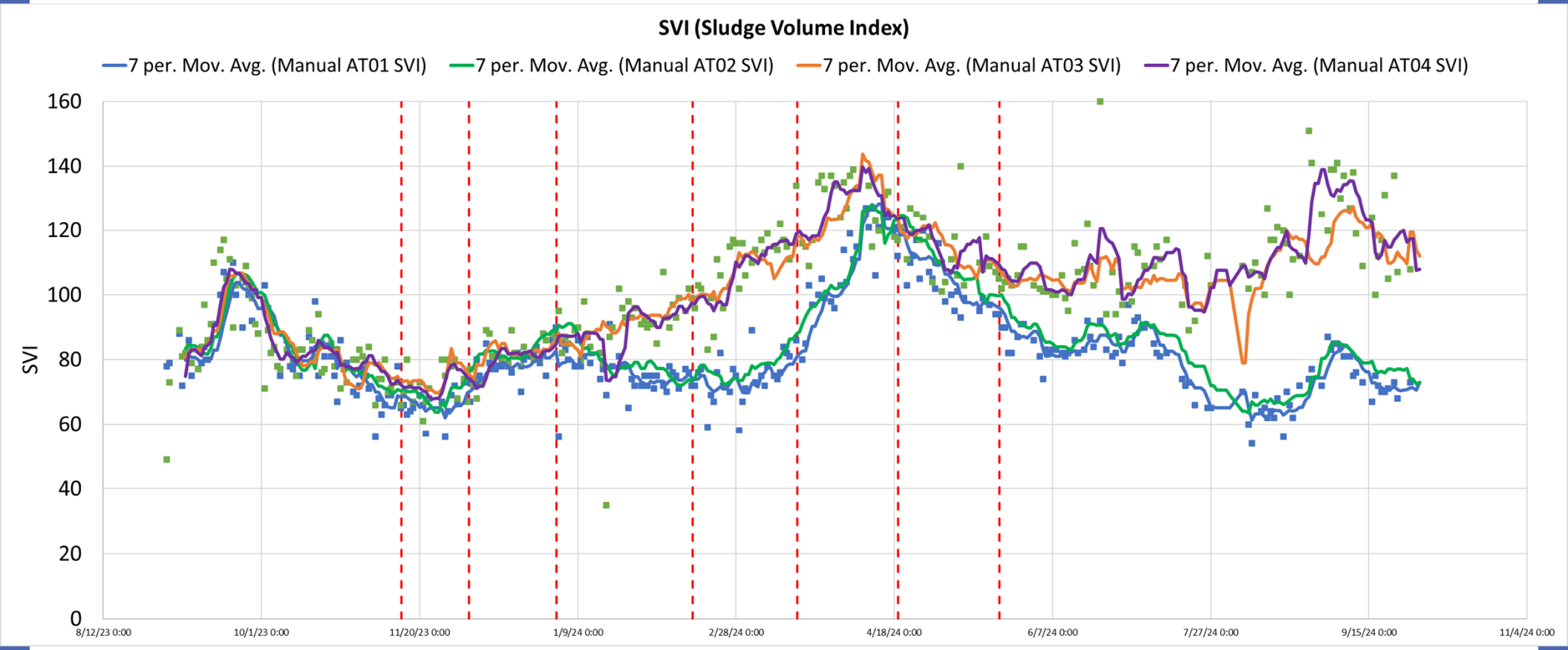


SND

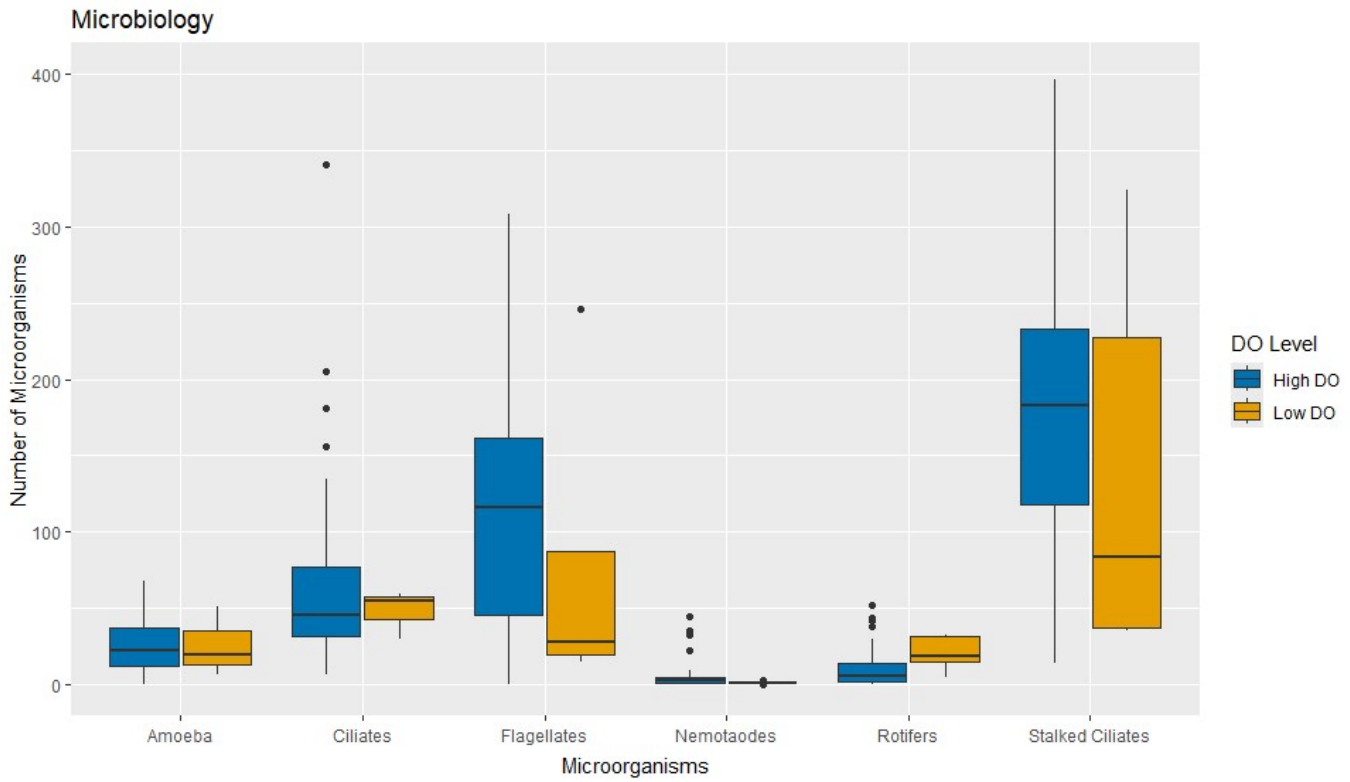

Ammonia Profiles
High DO
Low DO 0.80 mg/L
Low DO 0.50 mg/L
Low DO Taper (0.4, 0.5, 0.8)
Ammonia mg-N/L
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
29.0 29.0 29.8 29.8
6.7 7.4 20.4 23.8
0.2 0.3 8.9 5.1
0.0 1.0 3.9 0.2
Anaerobic
Zone 1
Zone 2
Zone 3


Nitrate Profiles
High DO
Low DO 0.80 mg/L
Low DO 0.50 mg/L
Low DO Taper (0.4, 0.5, 0.8)
Nitrate mg-N/L
30.0
25.0
20.0
15.0
10.0
5.0
0.0
19.6 17.5 5.6 0.4
27.0 25.1 15.1 17.7
28.4 24.6 20.6 25.3
Zone 1
Zone 2
Zone 3

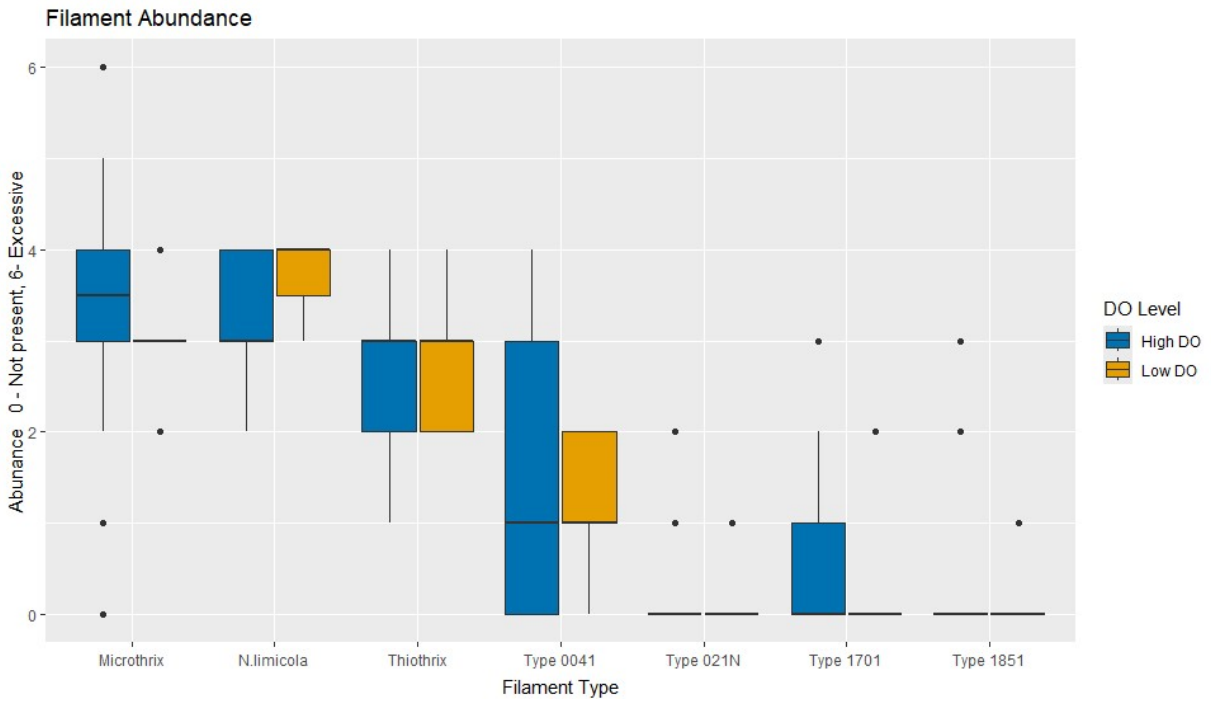
SVI


SVI (Sludge Volume Index)
7 per. Mov. Avg. (Manual AT01 SVI)
7 per. Mov. Avg. (Manual AT02 SVI)
7 per. Mov. Avg. (Manual AT03 SVI)
7 per. Mov. Avg. (Manual AT04 SVI)
SVI
160
140
120
100
80
60
40
20
0
8/12/23 0:00
10/1/23 0:00
11/20/23 0:00
1/9/24 0:00
2/28/24 0:00
4/18/24 0:00
6/7/24 0:00
7/27/24 0:00
9/15/24 0:00
11/4/24 0:00

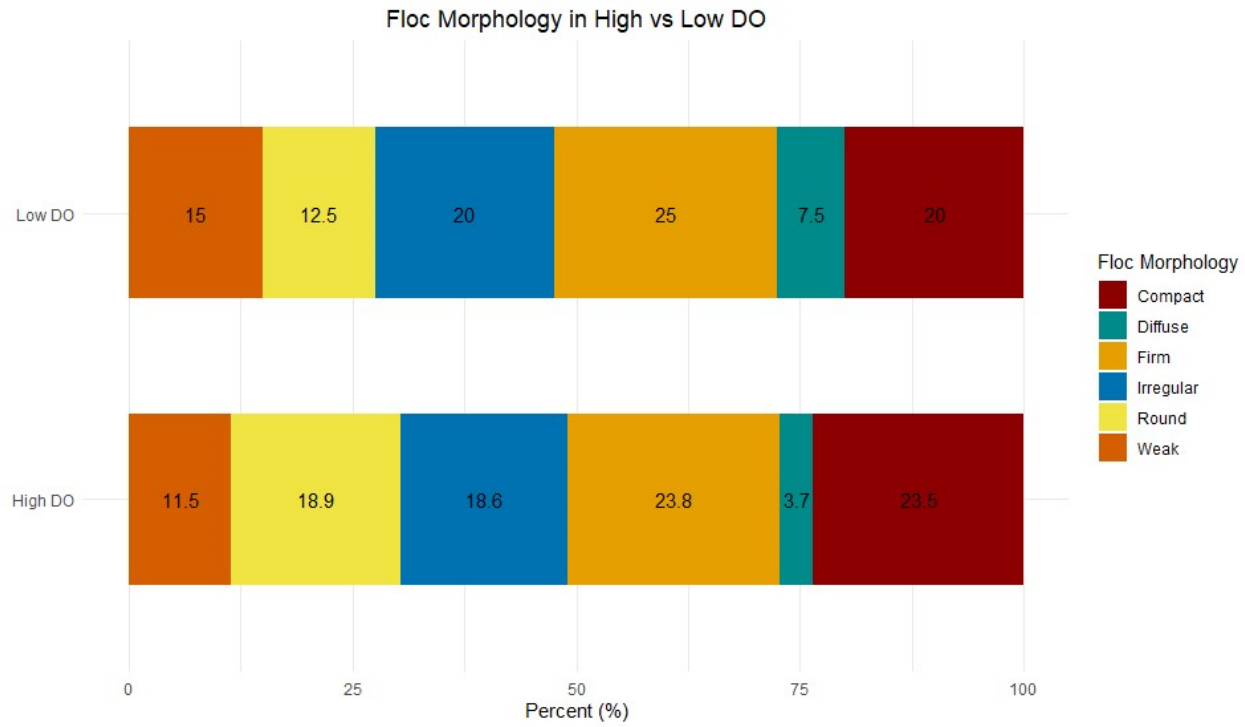
Tax Reports General Microbiology



Relative filament abundance comparison



Tax reports – Floc characteristics



Low DO

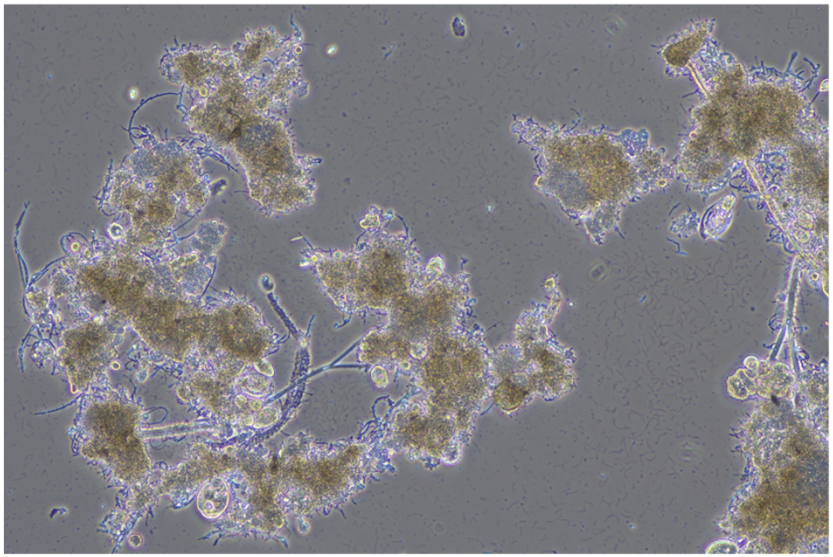
- Compact
- Firm
- Irregular

High DO

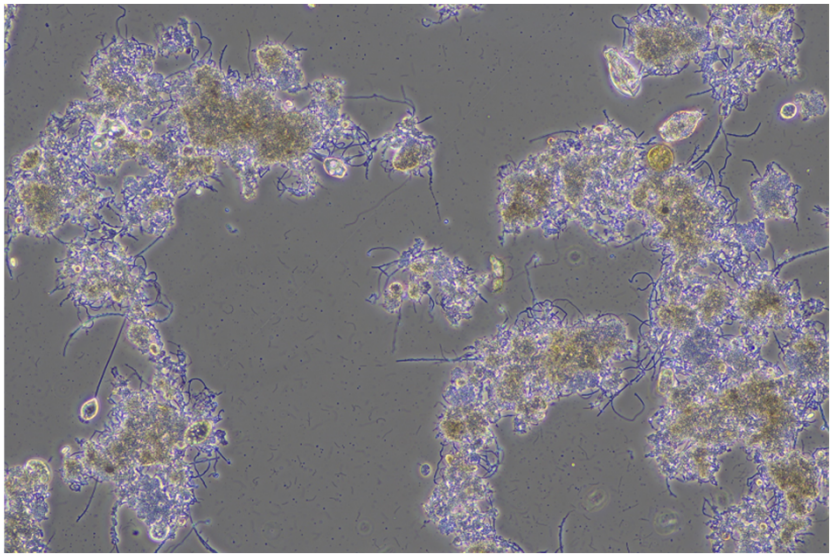
- Compact
- Firm
- Round

Tax report images

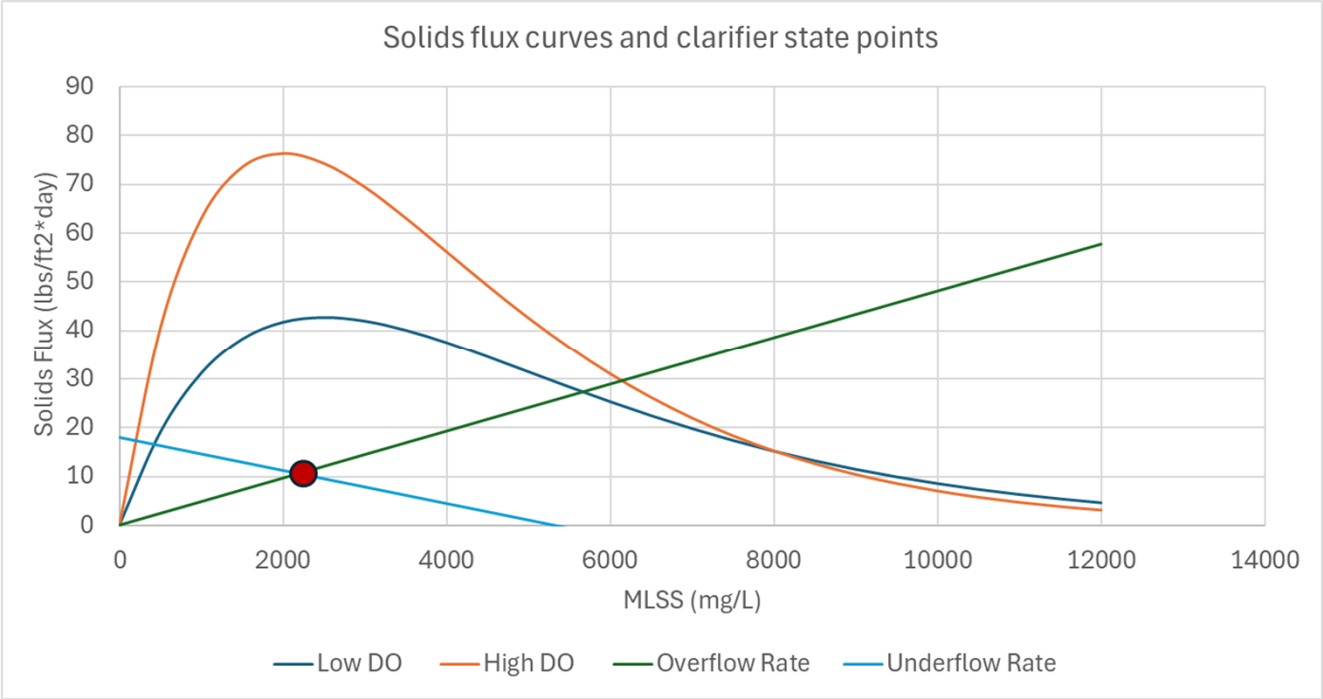
High DO



Low DO



Flux curve and state point



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Biological Nutrient Removal

Microbes (bugs) that are chemotrophic can remove various nutrients

- Phosphorous
- Ammonia

Microbial community can depend on:

- Influent composition
- Process set-up
- **Operating parameters**
- Environmental conditions

Slide 19

AC0 Don’t need

Chesley, Alexa, 2024-08-19T15:37:36.111

What is qPCR

- Quantitative polymerase chain reaction (qPCR)
- Molecular biology technique to amplify DNA
- Thermocycler allows for real-time analysis of reaction
  - Allows for exact quantification of initial DNA amounts
- Use in wastewater treatment: can be used to quickly to get quantitative values of different bug populations ranging from broad classes to specific species

Microbes of Interest

Looking at 3 broad classes of microbes that help remove ammonia

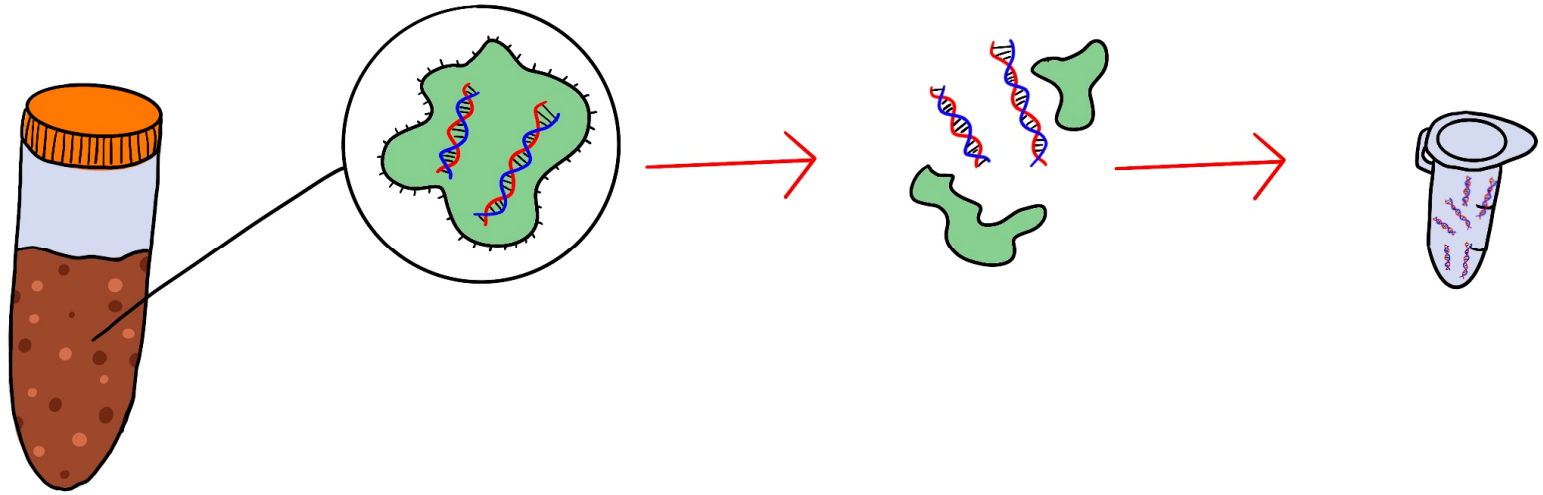
- Ammonia Oxidizing Bacteria (AOB)
- Ammonia Oxidizing Archaea (AOA)
- Complete Ammonia Oxidizing Bacteria (CMX)

Additionally looking at classes of microbes that remove phosphorous and their competitors

- Phosphorous accumulating organisms (PAOs)
- Glycogen accumulating organisms (GAOs)

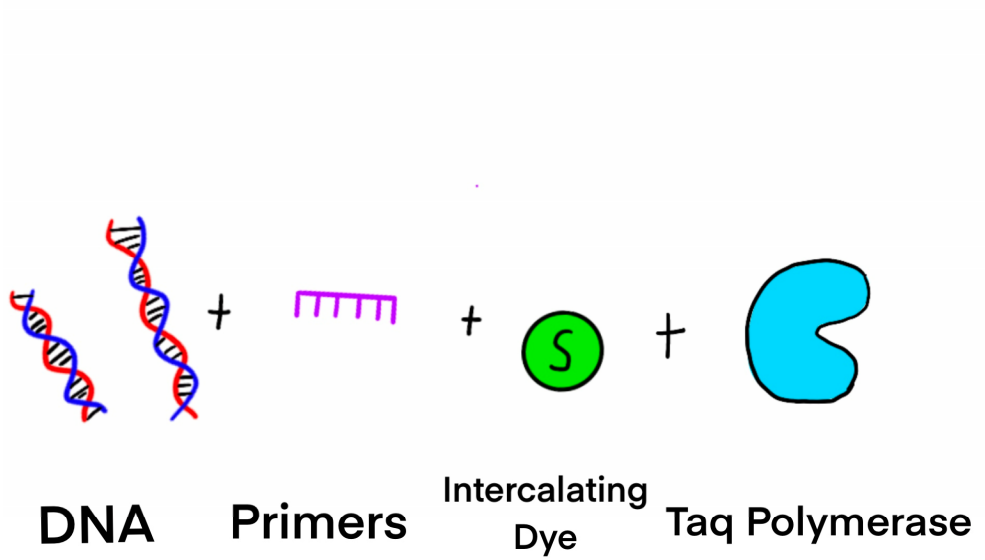
Sample Collection

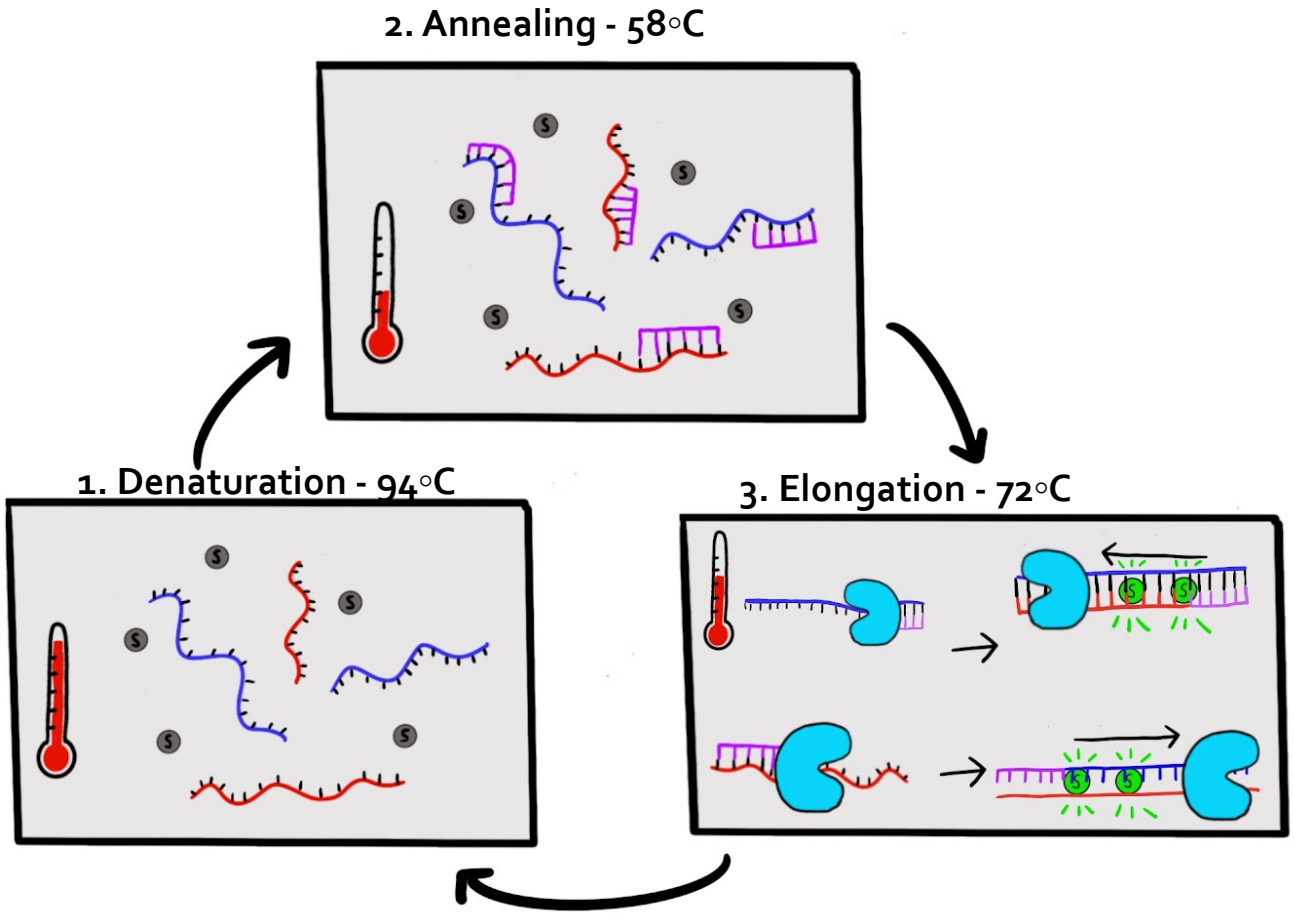
- Mixed liquor samples collected from anoxic zones
- DNA must be extracted within 24 hours
- Bug cells are lysed to release DNA into liquid matrix
  - Lysed either through beating apart or freeze/thaw cycles that degrade cell structure
- Various buffers used to wash away impurities while leaving DNA
- With DNA now isolated, stored at -80 ◦C to avoid degradation



qPCR Components

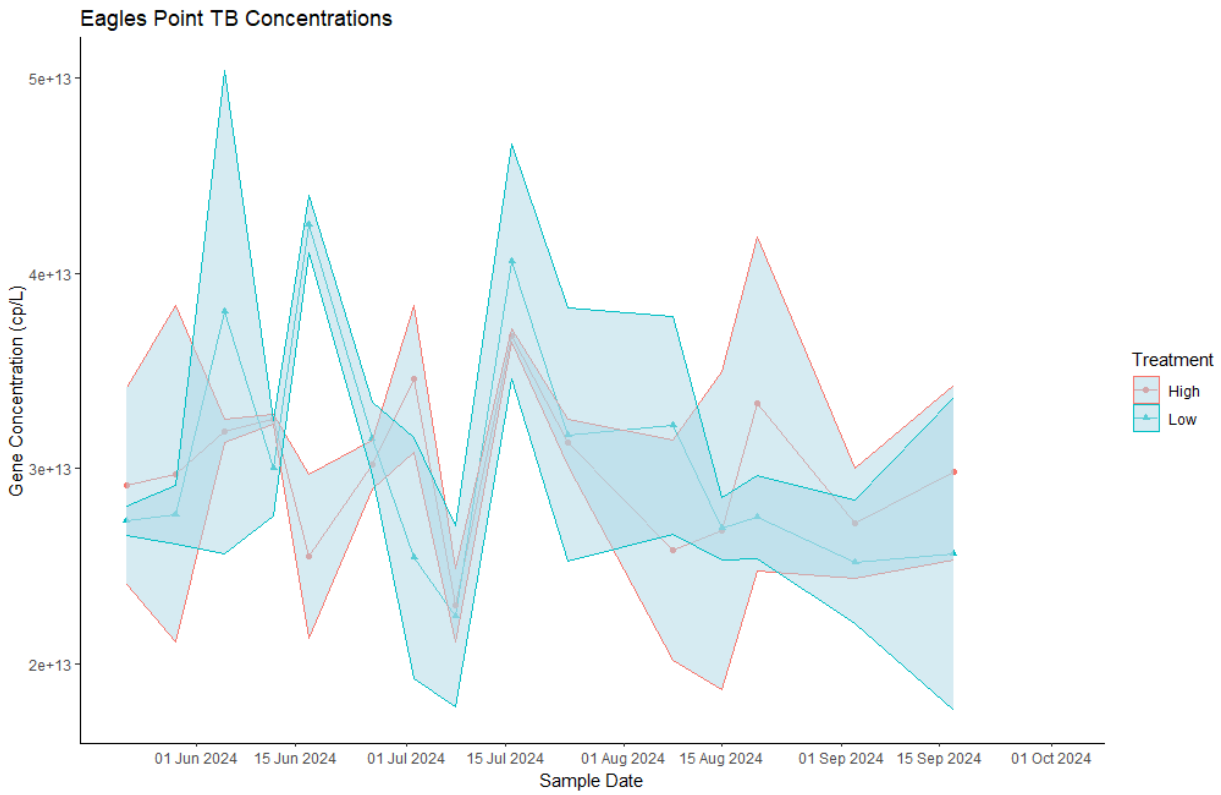
- **DNA**
  - All DNA in sample, that of interest and that which is not
- **Primers**
  - Small nucleotide fragments that match to a specific part of DNA of the microbe of interest
  - Flags what part of the DNA to amplify to Taq polymerase
- **Intercalating Dye**
  - A dye that inserts itself in the DNA structure and fluoresces when amplification occurs
- **Taq Polymerase**
  - Enzyme that causes DNA replication



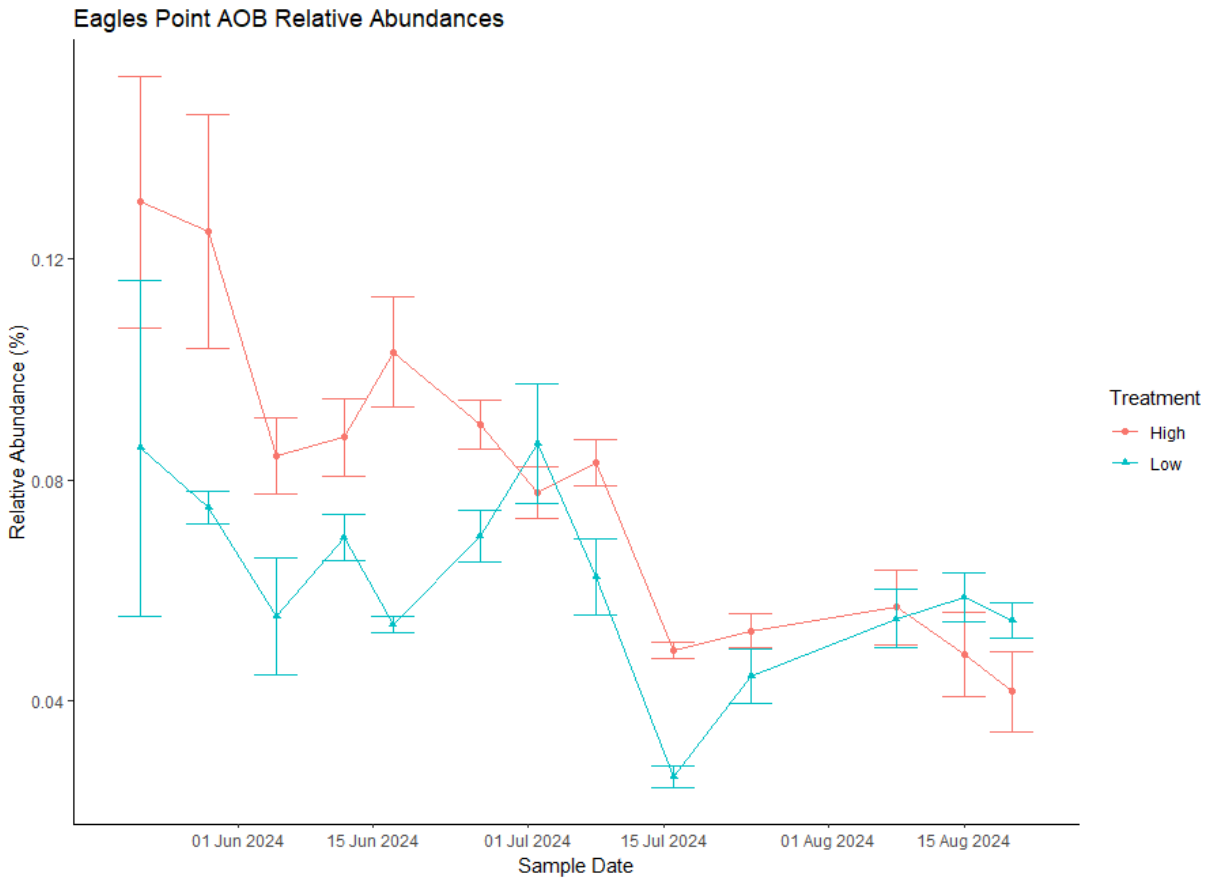


qPCR Steps

- **Denaturation**
  - High temperatures make double stranded DNA (dsDNA) split apart into single stranded DNA (ssDNA)
- **Annealing**
  - Lower temperatures allow for primers to attach
- **Elongation**
  - At a medium temperature, Taq Polymerase moves along the ssDNA and copies the DNA
  - When DNA amplification occurs, SYBR green dye fluoresces
- **Cycle Repeats**
  - Taq polymerase dissociates at high temperatures
  - Amount of DNA increases exponentially until primers or Taq is depleted



TOTAL BACTERIA RESULTS


Eagles Point AOB Relative Abundances
Relative Abundance (%)
0.12
0.08
0.04
01 Jun 2024
15 Jun 2024
01 Jul 2024
15 Jul 2024
01 Aug 2024
15 Aug 2024
Sample Date
Treatment
High
Low

AOB RESULTS

What does this mean?

Expected:

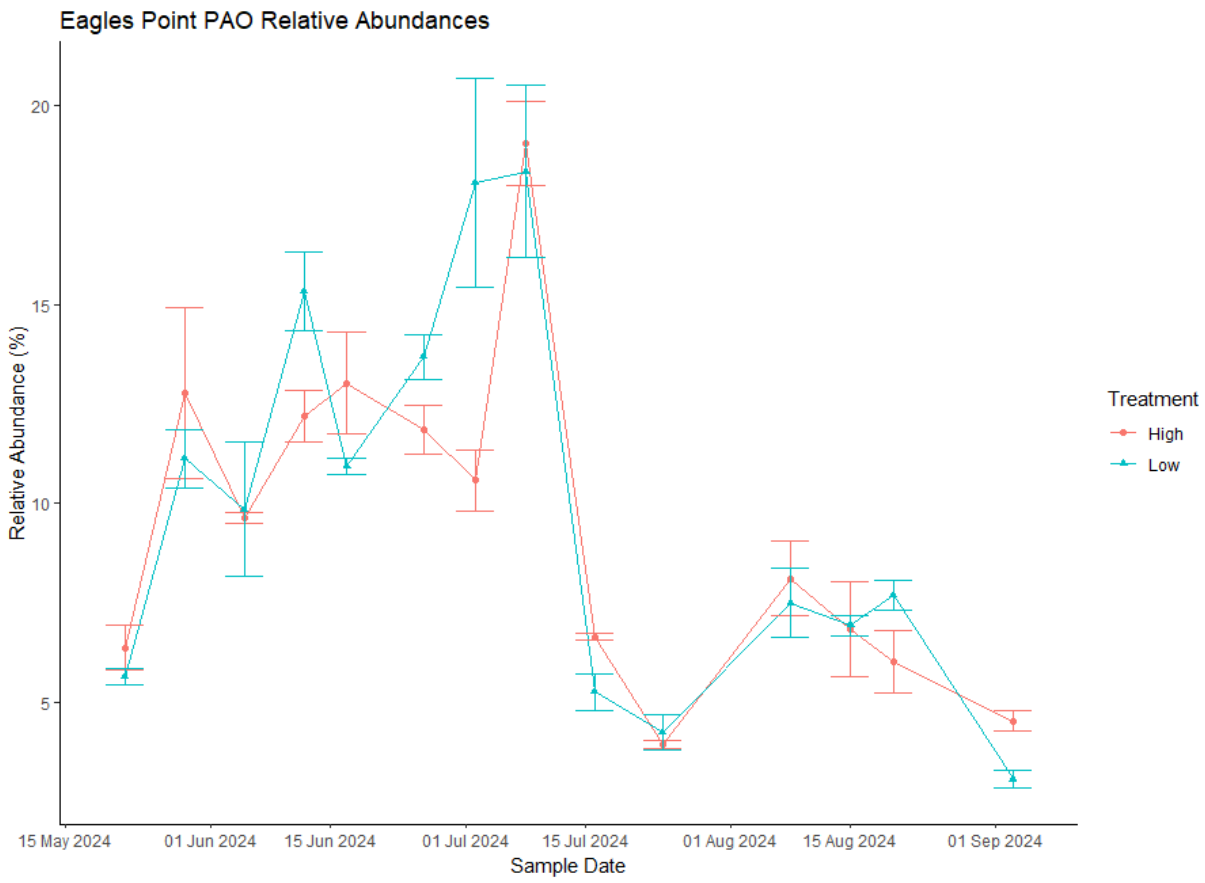
- Thought that AOB populations would decrease under low DO and be replaced by other ammonia removing bugs

Reality:

- Both tanks had low levels of AOBs indicating that a lot of ammonia removal is done by other bugs
- AOB population trends were consistent between tanks
- Something else is changing that is affecting ammonia – AOAs or CMX?

Other Bugs of Interest: AOAs and CMX

- Ammonia differences due to AOAs and CMX?
- Kansas University Results: found both AOAs and CMX to be significant parts of the bug community
- Currently having issues with methods to determine these bug populations in our samples
  - Need to optimize methods to get quality data


Eagles Point PAO Relative Abundances
Relative Abundance (%)
20
15
10
5
15 May 2024
01 Jun 2024
15 Jun 2024
01 Jul 2024
15 Jul 2024
01 Aug 2024
15 Aug 2024
01 Sep 2024
Sample Date
Treatment
High
Low

PAO RESULTS

What does this mean?

Expected:

- PAO populations increase due to being able to out-compete other bugs in accessing oxygen
- Increased PAO populations responsible for better P removal

Reality:

- Distinct anoxic zone led to higher levels of PAOS
- Observed a difference in ability to remove P, Low DO faster
- PAO populations the same between tanks
- Are different types of specific PAOs present? Are proportions of specific PAOs different?

Ongoing Work

Sludge Characterization

- Polymer binding
- Floc formation
- Settleability

Microbiology characterization

- AOAs and Comammox methods
- Continuing population studies

Phosphorous uptake studies

- Use with PAO abundance data

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QUESTIONS?