

# Characterization of Microbial Communities with Nitrogen Presence

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Manion Scholar Presentation  
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# Introduction and Purpose

- Elevated nitrogen levels - risk to ecosystems and water quality
- Human input - urban and agricultural:
  - Doubled soil and water nitrogen levels
- Correlations between environmental factors and microbial communities:
  - Agricultural, Urbanized, and Glacial water bodies
  - Central and Northwest Montana
- Benchmark bioindicators → prediction of future water quality



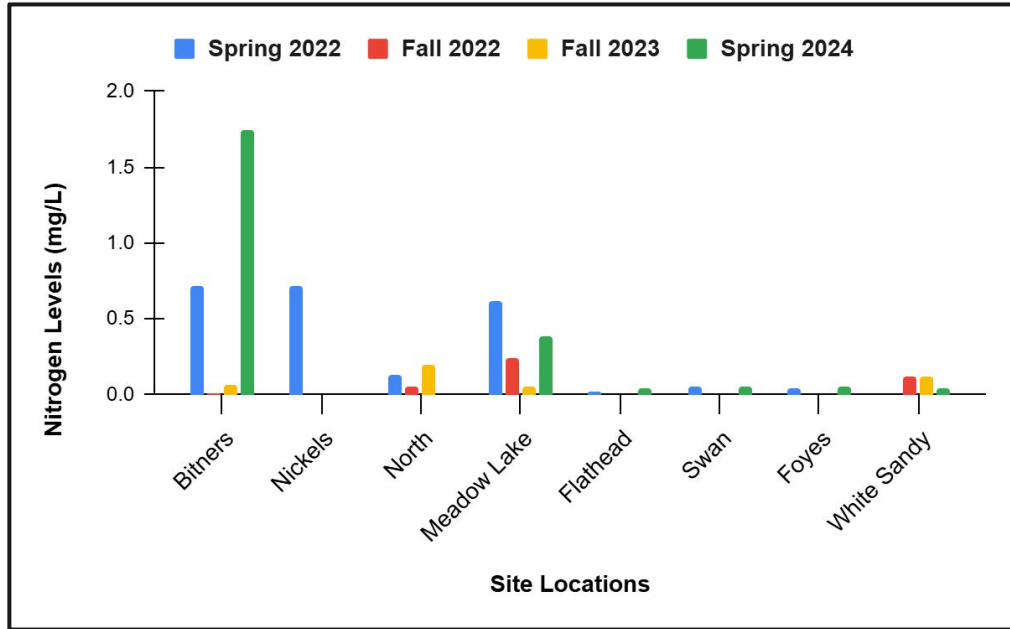


# Overview of Results

- I. General Data
- II. Site
- III. Water Body
- IV. Season
- V. Nitrogen



# General



Nitrogen (nitrate + nitrite) levels in mg/L (ppm) at each sampling location.

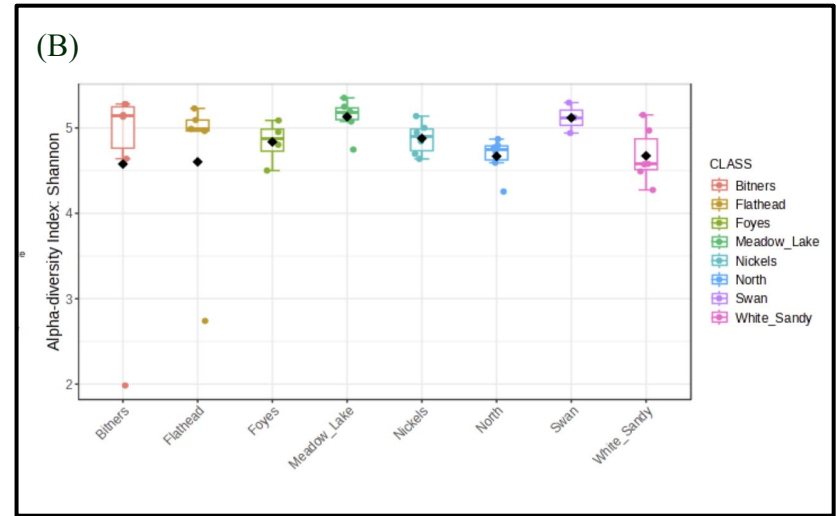
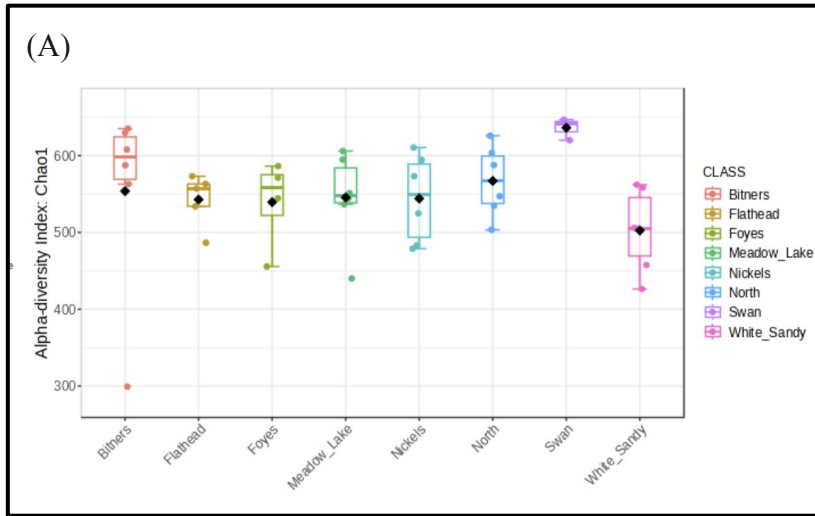
- Spring levels generally trend higher:
  - Runoff
  - Temperature
  - Microbial processes and metabolism
- Levels are not statistically distinguishable between years ( $p = 0.326$ )

<u>Sites</u>	<u>2022</u> # of replicates (barcode identifiers)	<u>2023</u> # of replicates (barcode identifiers)	<u>2024</u> # of replicates (barcode identifiers)	<u>Total</u> <u>Replicates</u>
Bitners (Agricultural)	2 (55, 57)	3 (31, 32, 33)	1 (7)	6
Nickels (Agricultural)	1 (54)	2 (28, 30)	3 (4, 5, 6)	6
North (Agricultural)	3 (49, 50, 51)	0	3 (25, 26, 27)	6
Meadow Lake (Urbanized)	2 (59, 60)	3 (34, 35, 36)	1 (12)	6
White Sandy (Urbanized)	2 (71, 72)	1 (47)	3 (22, 23, 24)	6
Flathead (Glacial)	1 (65)	1 (42)	3 (16, 17, 18)	5
Foyes (Urbanized)	0	1 (37)	3 (13, 14, 15)	4
Swan (Urbanized)	0	0	3 (19, 20, 21)	3

Number of replicates remaining for each sampling location and year after rarefaction.

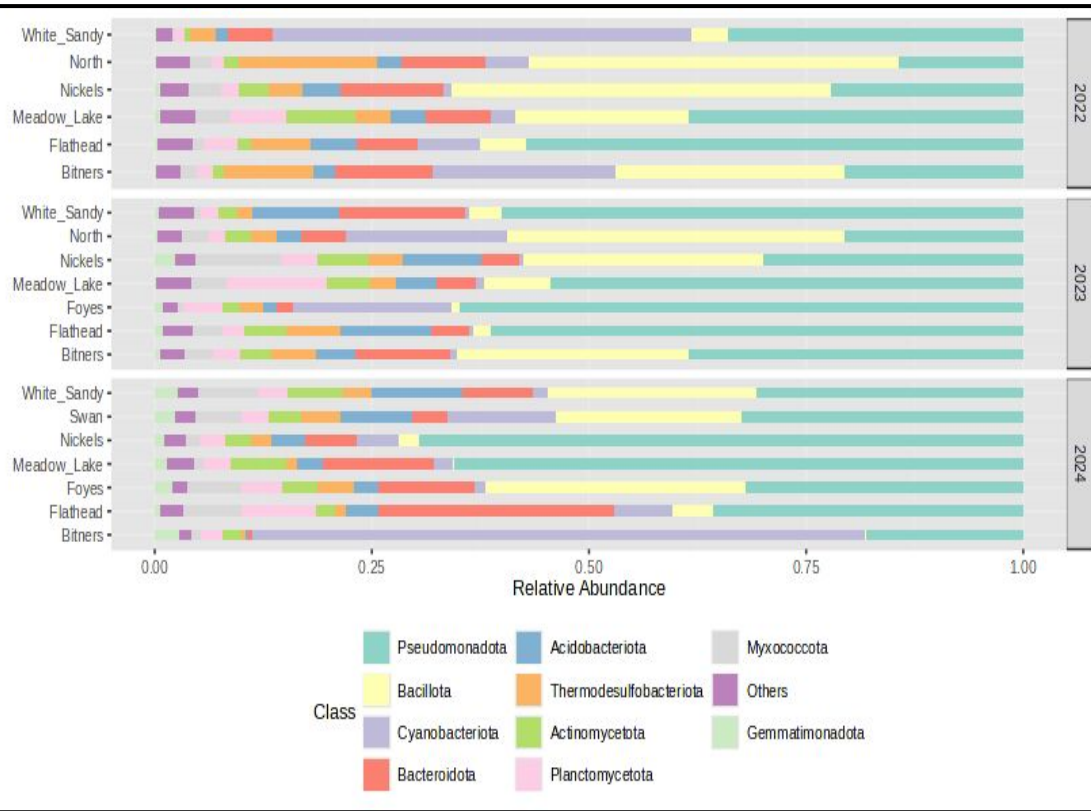
- 1-1.6 million sequences/year
- 42 replicates
- 6/9 highest replicates:
  - Swan lowest (3/9)
- 3 Agricultural - 18
- 4 Urbanized - 19
- 1 Glacial - 5

# Site



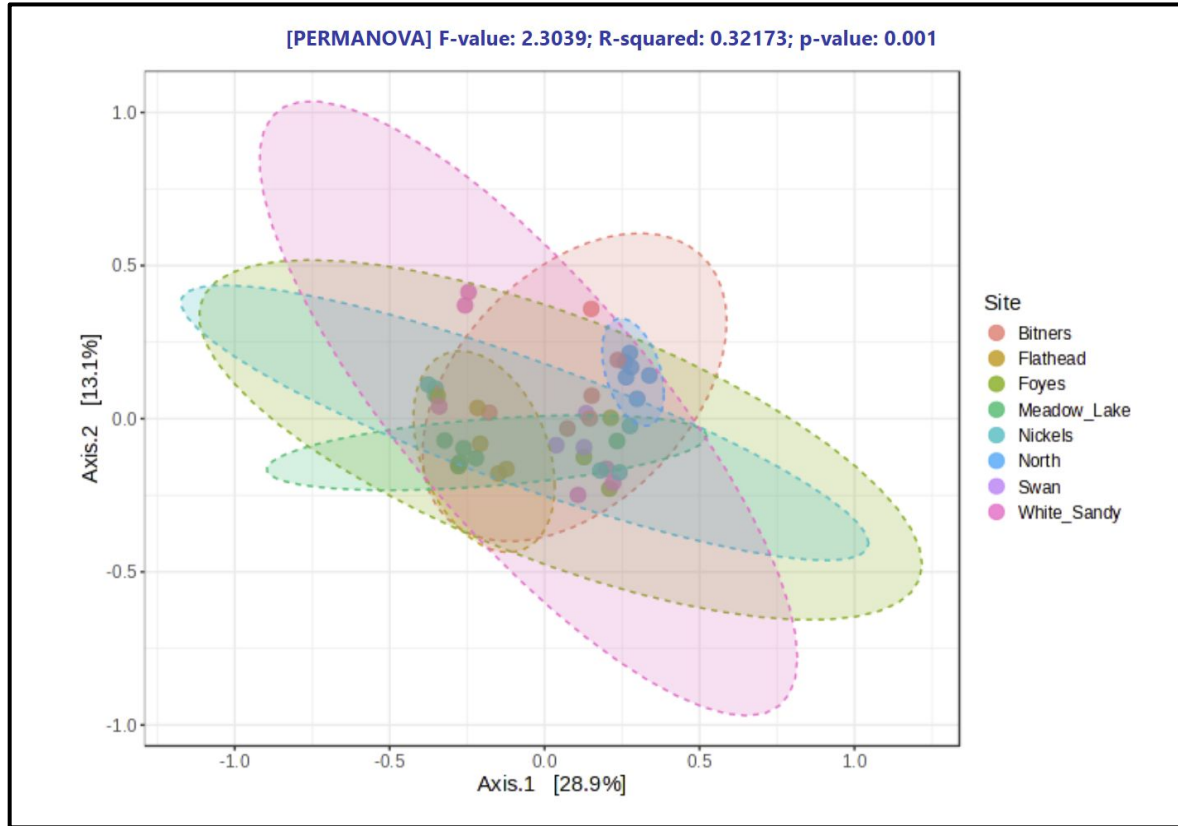
Box and whisker plots of the sediment sample alpha diversity at the genus level using the Chao1 index (A) and the Shannon index (B), grouped by site. Black points represent the average diversity for each site.

- Different calculations:
  - Diversity within samples
- Swan lowest variability in diversity:
  - Lower replicate count
- White Sandy and Bitners displayed the greatest variability per sample site
- $p = 0.4811$  for Chao1,  $p = 0.7610$  for Shannon:
  - Richness not affected by location



Relative abundance of the top 10 phyla averaged by sample locations across the three collection years.

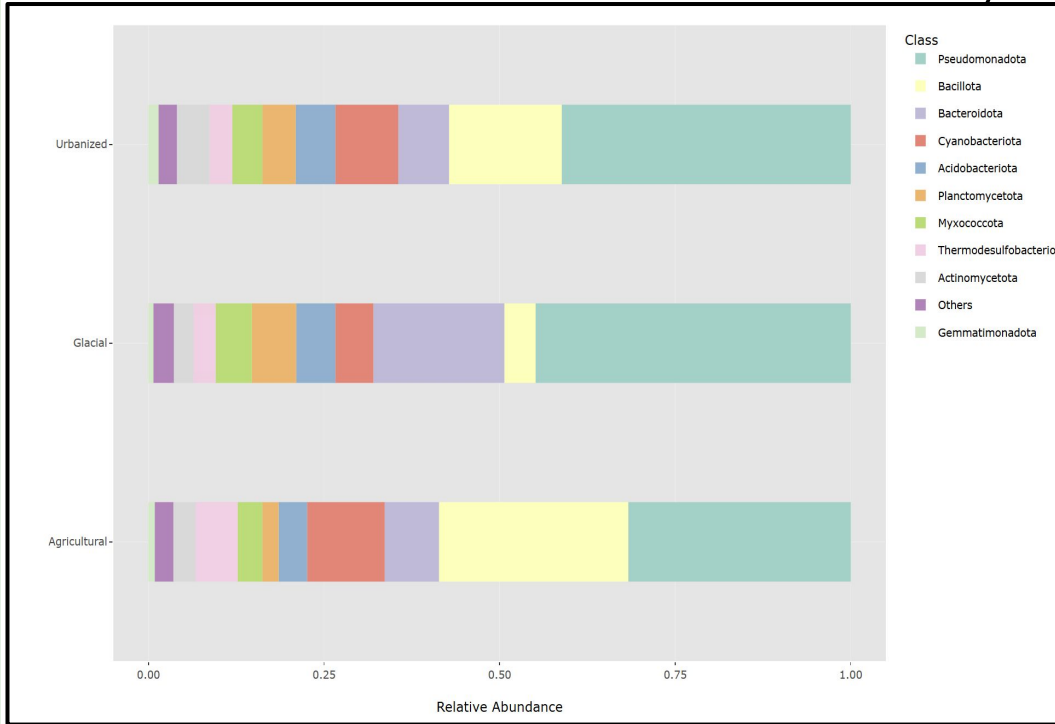
- Pseudomonadota ↑2022 →2023
  - Cyanobacteriota decreased
- Highest Cyanobacteriota:
  - Bitners in 2024
  - White Sandy in 2022
- Glacial - Bacteroidota:
  - Cold nutrient-poor environments
- Agricultural - Bacillota:
  - Livestock



PERMANOVA analysis resulting in a p-value of 0.001.

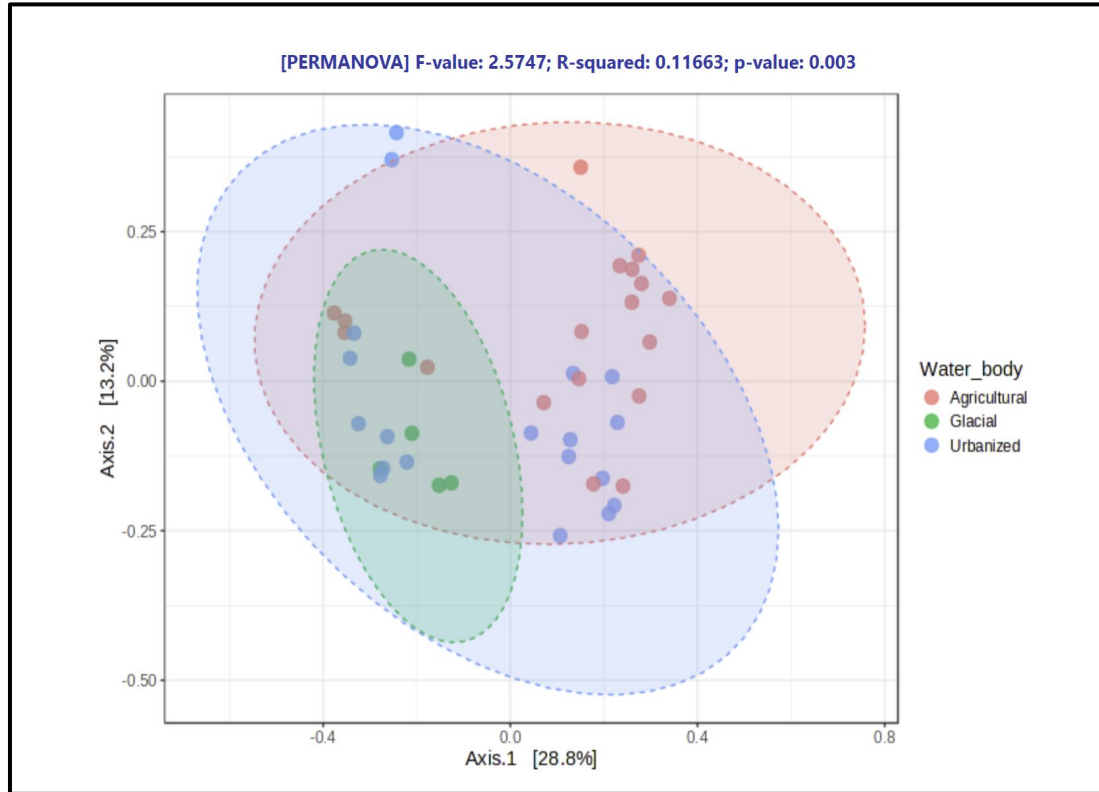
- Clustering indicates similarity among samples from a given site:
  - White Sandy
- Site is a statistically significant variable ( $p = 0.001$ )

# Water Body Type



- Agricultural: High Bacillota (livestock impact)
- Glacial: Higher Pseudomonadota and Bacteroidota
- Urbanized: Intermediate profile:
  - Cyanobacteriota-Intermediate
  - Intermediate level of anthropogenic input

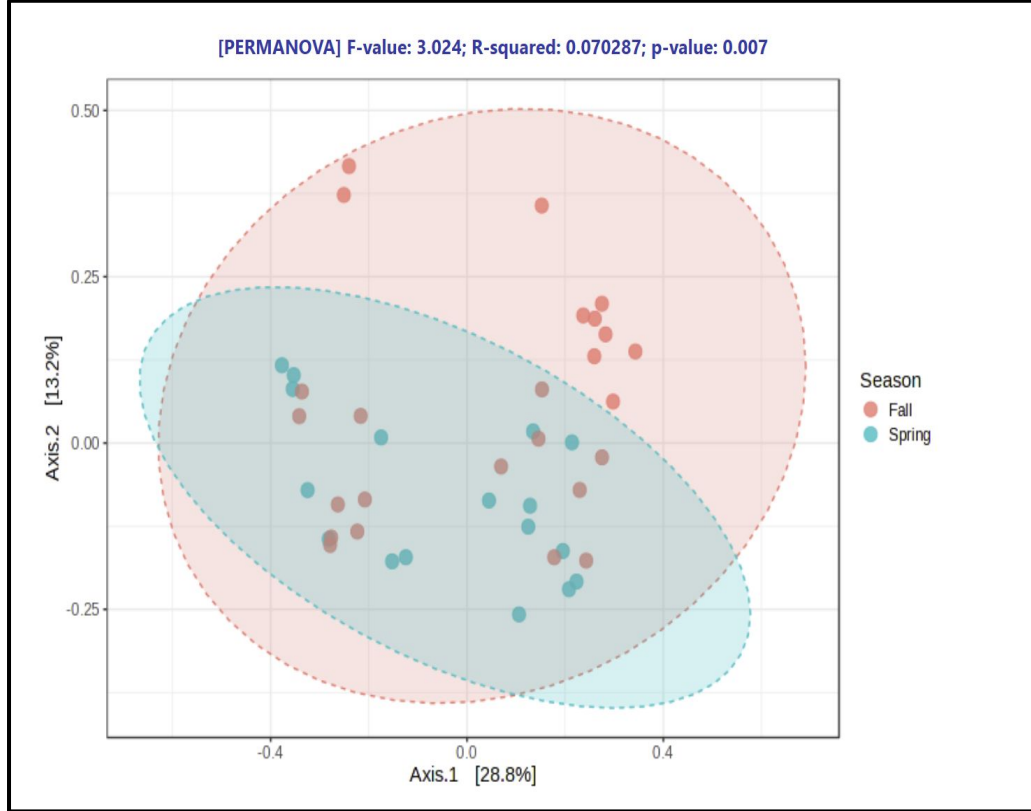
Relative abundance of the top 10 phyla averaged by water body type (Agricultural, Glacial, and Urbanized).



- Statistically significant relationship between community composition and water body ( $p = 0.003$ )

PERMANOVA test to assess separation by water body type, resulting in a p-value of 0.003.

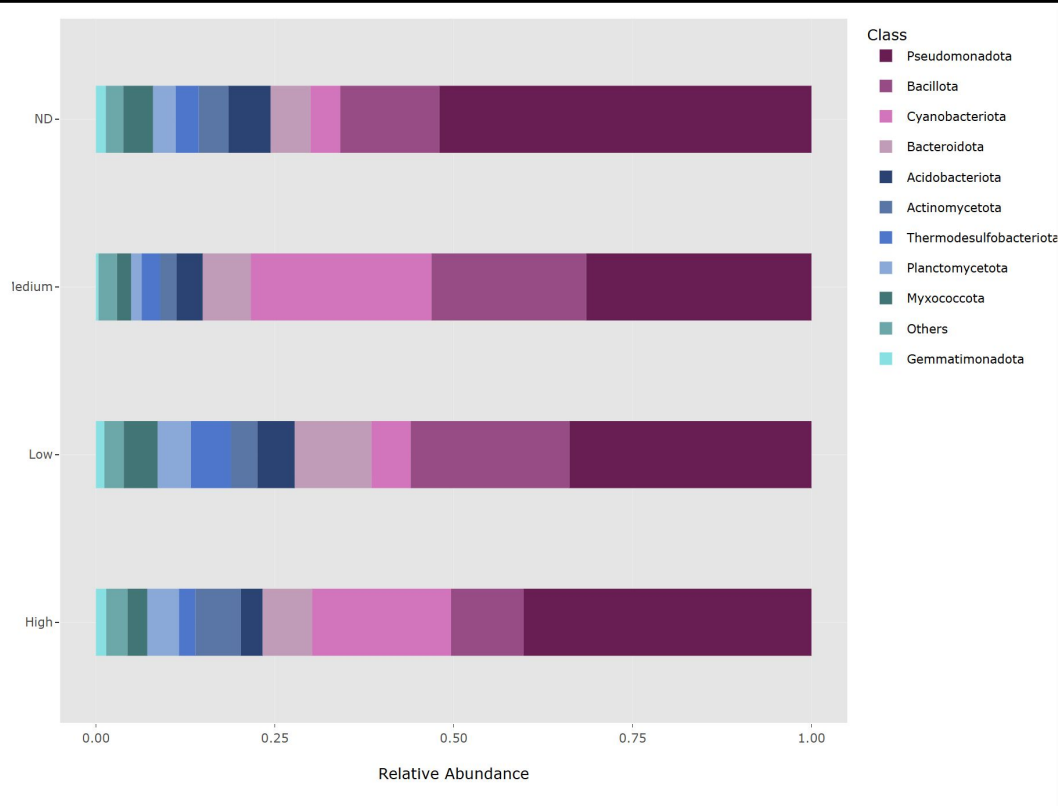
# Season



PERMANOVA test to assess the separation between Fall and Spring samples, resulting in a p-value of 0.007.

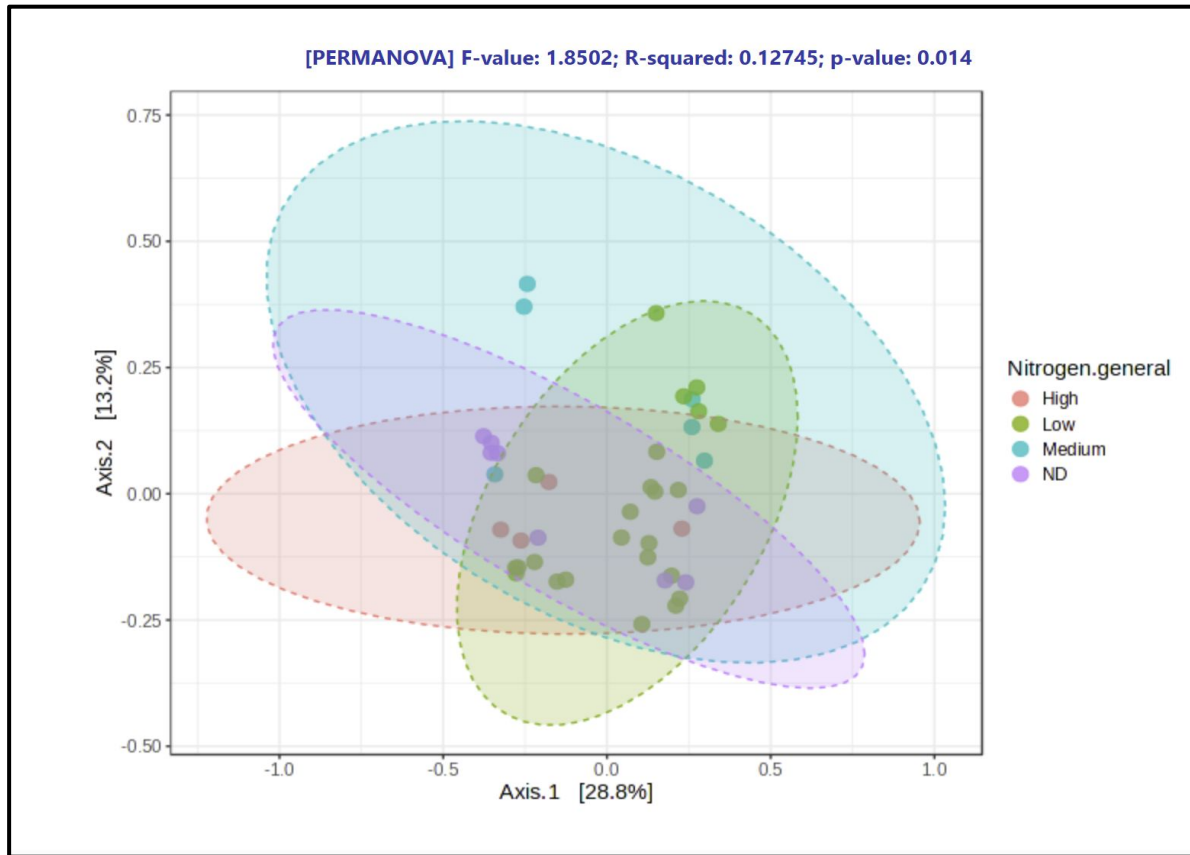
- Spring sample communities similar
- Fall samples show more variability:
  - Two years of Fall samples
- PERMANOVA analysis ( $p = 0.007$ ):
  - Season is a statistically significant factor
  - Season correlates with microbial composition

# Nitrogen



Relative abundance of the top 10 phyla averaged by nitrogen levels: ND (Not-Detectable; n = 8), Low (0-0.099 mg/L; n = 24), Medium (0.10-0.199 mg/L; n = 6), and High (> 0.20 mg/L; n = 4).

- 4 Nitrogen levels
- Same top 4 phyla in all levels
  - Pseudomonadota
  - Bacillota
  - Cyanobacteriota
  - Bacteroidota
- Pseudomonadota 52.1% - ND
  - 32% - Medium & Low
- Cyanobacteriota 25.4% - Medium
  - 4-5% Low and ND
  - Aligns with literature
  - Not enough High Replicates
- Nitrogen level shapes abundances, not identities



PERMANOVA test to assess the separation between nitrogen levels, resulting in a p-value of 0.014.

- ND and Low nitrogen levels cluster
- No clear clustering between Medium and High classifications:
  - More variability in composition
  - Increased nutrient availability
- Nitrogen is significant variable ( $p = 0.014$ )

# Limitations

- Sampling
  - Spring 2022 pilot study → only water samples, 7 locations vs. 8 in main study (added White Sandy)
  - Sediment collection → Fall of 2022
- DNA extraction
  - Varying sediment types → poor extraction efficiency
- Experimental variables
  - Variables not entirely independent:
    - Year & Season
    - Site & Water Body
- Site accessibility
  - White Sandy road was inaccessible in 2023 → 0.5 mile from sample site

## Sediment type- DNA extraction

- 66.7% Agricultural
- 52.8% Urbanized
- 55.6% Glacial

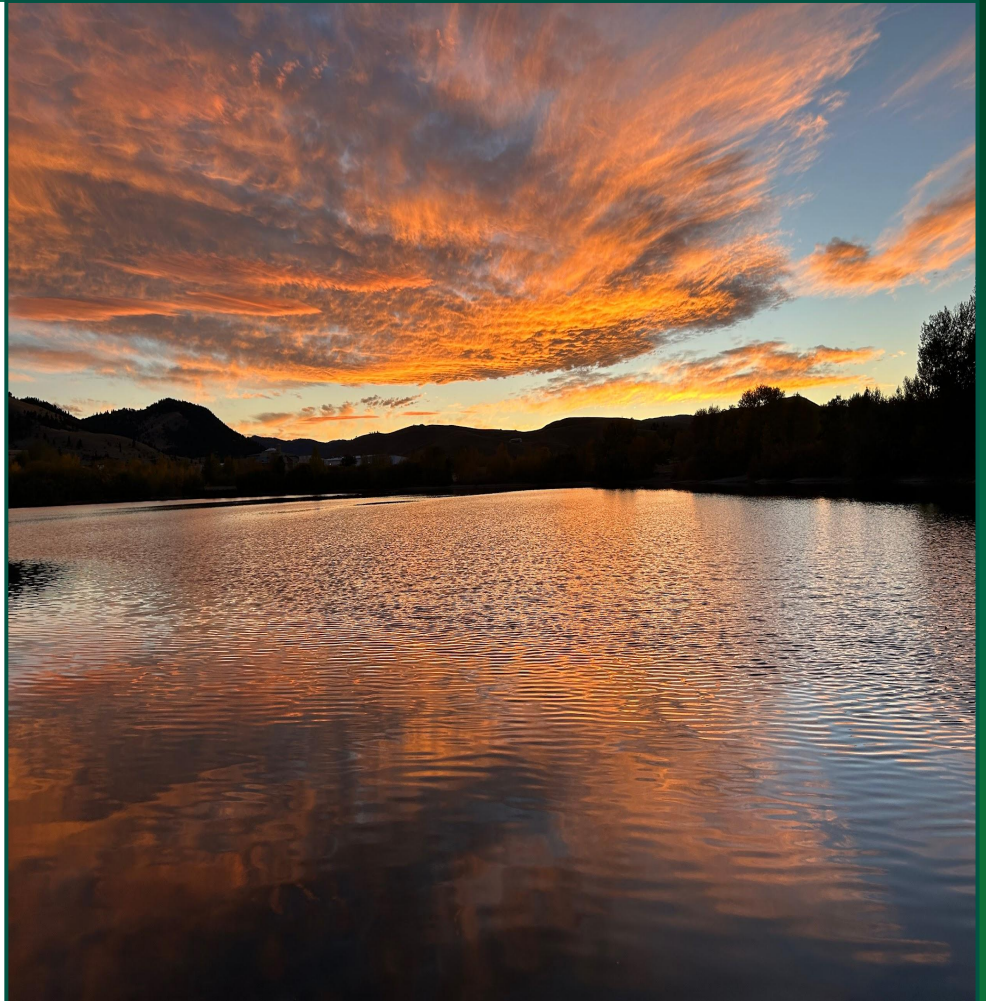
# Future Directions

- Further sampling and microbial analysis
- Spring and Fall samples
- Sample additional glacial water bodies
- Collect environmental information:
  - Temperature, precipitation, water movement, algae levels
  - Chemical analysis
- Expression of metabolic genes associated with nitrogen/nutrient cycling



# Conclusion

- Site ( $p = 0.001$ )
  - Water body type ( $p = 0.003$ )
  - Season ( $p = 0.007$ )
  - Nitrogen level ( $p = 0.014$ )
- 
- Nitrogen level is significant but has a lesser effect on composition than other variables
  - Aquatic microbial community composition in Montana is shaped by site, season, water body type, and nitrogen level





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Questions?

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