



# Microbial Community Structure and Biogeochemistry of Three Small Eutrophic Lakes

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## Introduction

The three Jackson Lakes within the Bayou Chico Watershed in NW FL, USA were formed from abandoned sand pits. The lakes experienced inundation with marine water during Hurricane Ivan in 2004, and, despite their proximity and similar dimensions, have developed different biogeochemical profiles over time. All the lakes are stratified, and while the NE lake is fully freshwater, the SE and SW lakes have freshwater above the oxycline with salinities below the oxycline ranging between 2.3 and 4.5. This study was undertaken to investigate the microbial communities of the lakes and how microbial communities are shaped over season and depth.

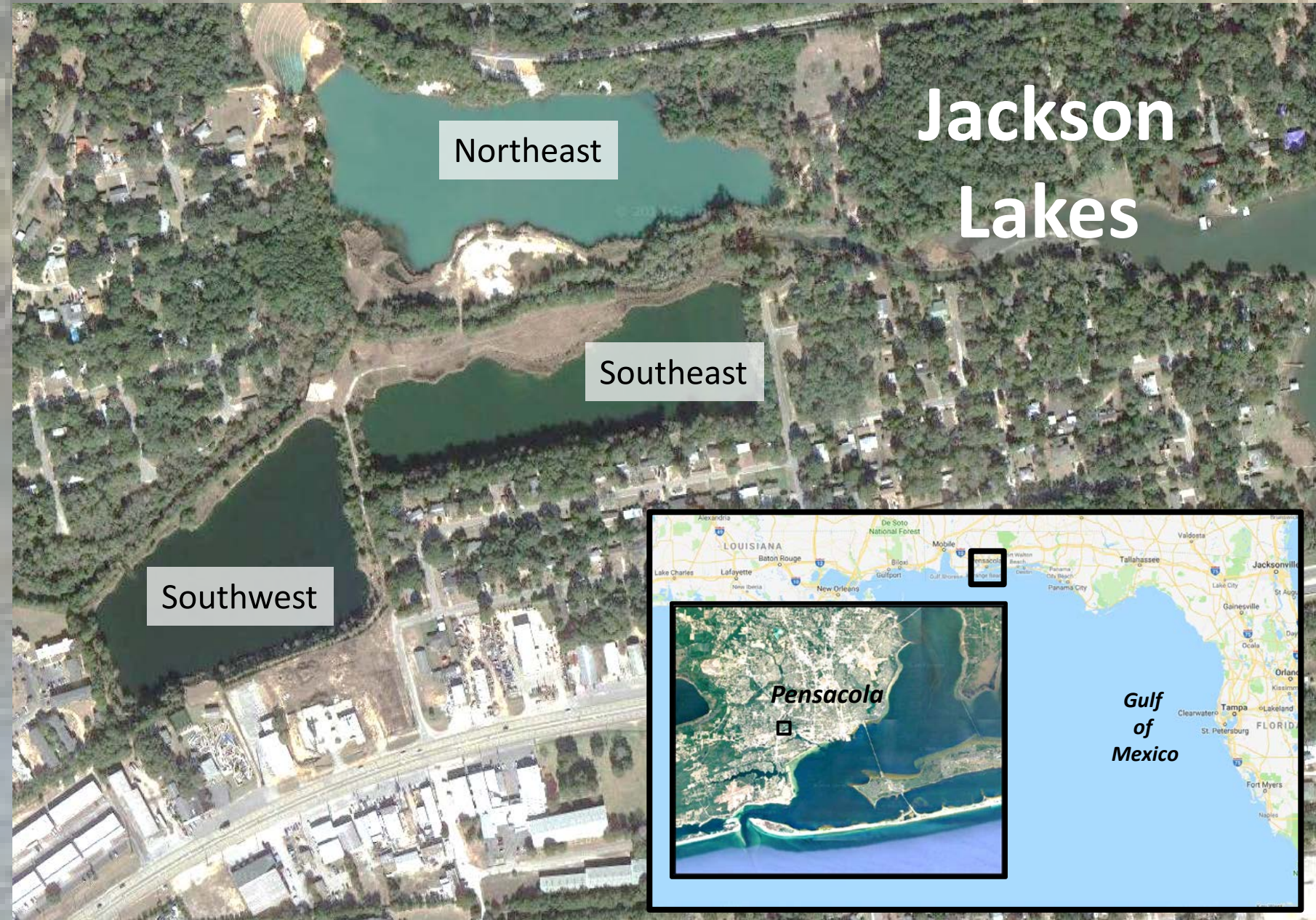


Figure 1. Locations of Jackson Lakes in Pensacola, FL. The lakes were formerly sand pits. Despite their proximity, they have different biogeochemical characteristics.

## Methods

### Water Sampling & Analysis

- Continuous dissolved oxygen and temperature sensors
  - Deployed at least 14 days prior to sampling
  - Deployed continuously
    - 12/14/16-7/27/2017
    - 8/21/2017-12/12/2017
- Sampling Events
  - September/October 2016
  - April 2017
  - July 2017
  - October 2017
- Water collection
  - Same site in each lake
  - 10 depths from surface to bottom
- Filtered using 0.2 µm cartridge filters
- Physical parameters determined at sampling using an In-Situ® Smart Troll meter
  - Salinity
  - pH
  - Temperature
  - Total dissolved solids (TDS)
  - Reduction potential (ORP)
  - Dissolved oxygen (DO)
- Water chemistries
  - Dissolved
    - Total dissolved nitrogen (TDN)
    - Dissolved organic carbon (DOC)
    - Ammonium (NH<sub>4</sub><sup>+</sup>)
    - Total dissolved phosphorus (TDP)
    - Nitrate and nitrite (NO<sub>x</sub>)
    - Nitrite (NO<sub>2</sub><sup>-2</sup>)
  - Ferrous iron (Fe<sup>2+</sup>)
  - Phosphate (PO<sub>4</sub><sup>-3</sup>)
  - Sulfate (SO<sub>4</sub><sup>-2</sup>)
  - Sulfide (HS<sup>-</sup>)
  - Silicate (SiO)
- Particulate
  - Chlorophyll *a*
  - Particulate nitrogen (PN)
  - Particulate carbon (PC)
- Gas
  - Carbon dioxide (CO<sub>2</sub>)
  - Methane (CH<sub>4</sub>)
  - Nitrous oxide (N<sub>2</sub>O)

### Sequencing and Community Structure

- DNA extracted from filters
  - PowerMag DNA Isolation Kit (MoBio)
- 16S rRNA genes
  - Earth Microbiome Project protocols
  - Argonne National Laboratories
- Sequences analyzed and annotated using mothur and the Silva database (Schloss et al., 2009)
- Statistical testing to identify and characterize community structure in the water samples, with relation to environmental parameters performed using PRIMER v7 (Clarke and Gorley 2006)
- Statistical methods included Cluster, BEST, and ANOSIM (Clarke 1993)

*Notice: Mention of trade names, products, or services does not convey official EPA approval, endorsement, or recommendation.*

## Community Structure based on 16S rRNA gene Sequences and Environmental Influences by Lake

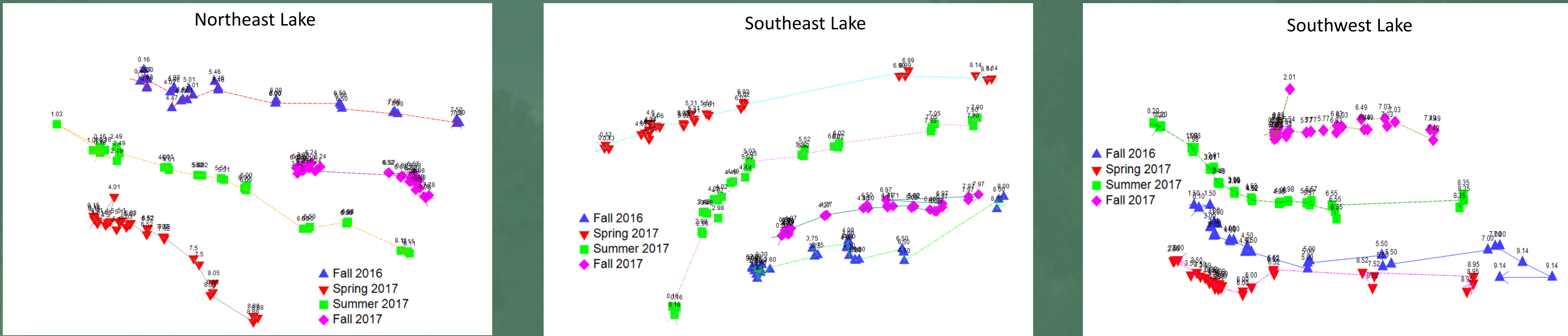


Figure 2. Non-metric multidimensional scaling of samples based on similarity of 16S rRNA gene sequences; each lake separated by season. Samples are labeled by depth in meters below lake surface. Samplings in the Spring and Fall of 2017 occurred after mixing events, and the communities are not as stratified as they are during the other sampling periods. However, the Southeast and Southwest lakes become stratified more quickly after a mixing event than the Northeast lake.

## Continuous Monitoring Data of the Lakes – Temperature and Dissolved Oxygen

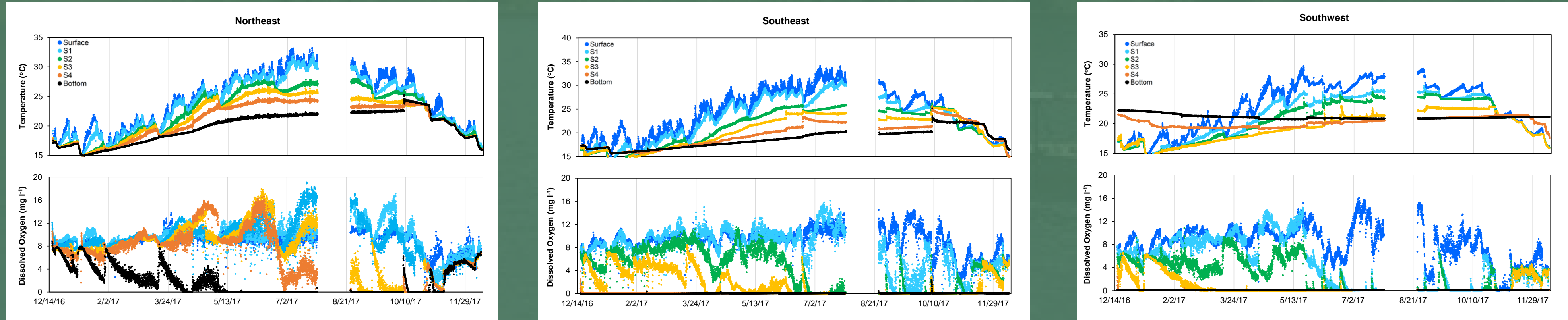


Figure 3. Continuous vertical profiles of temperature (°C) and dissolved oxygen (mg/l) recorded every 60 seconds using PME MiniDOTs. Vertical spacing of the sensors varied based on the total depth and position of the pycnocline in each lake at the time of deployment.

## Physical and Chemical Parameters

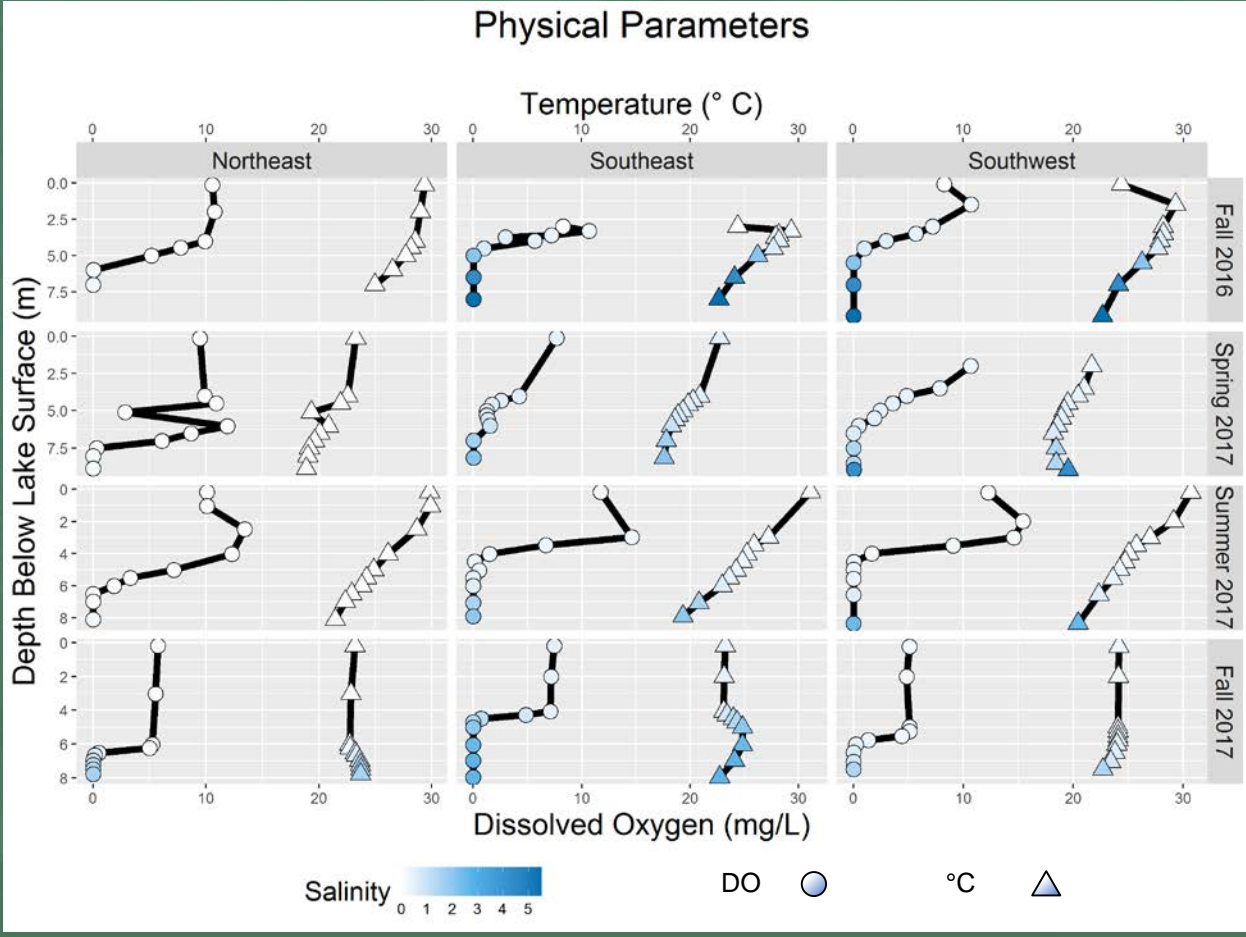


Figure 4. Physical conditions measured by Smart Troll, including dissolved oxygen (DO), temperature (°C), and salinity

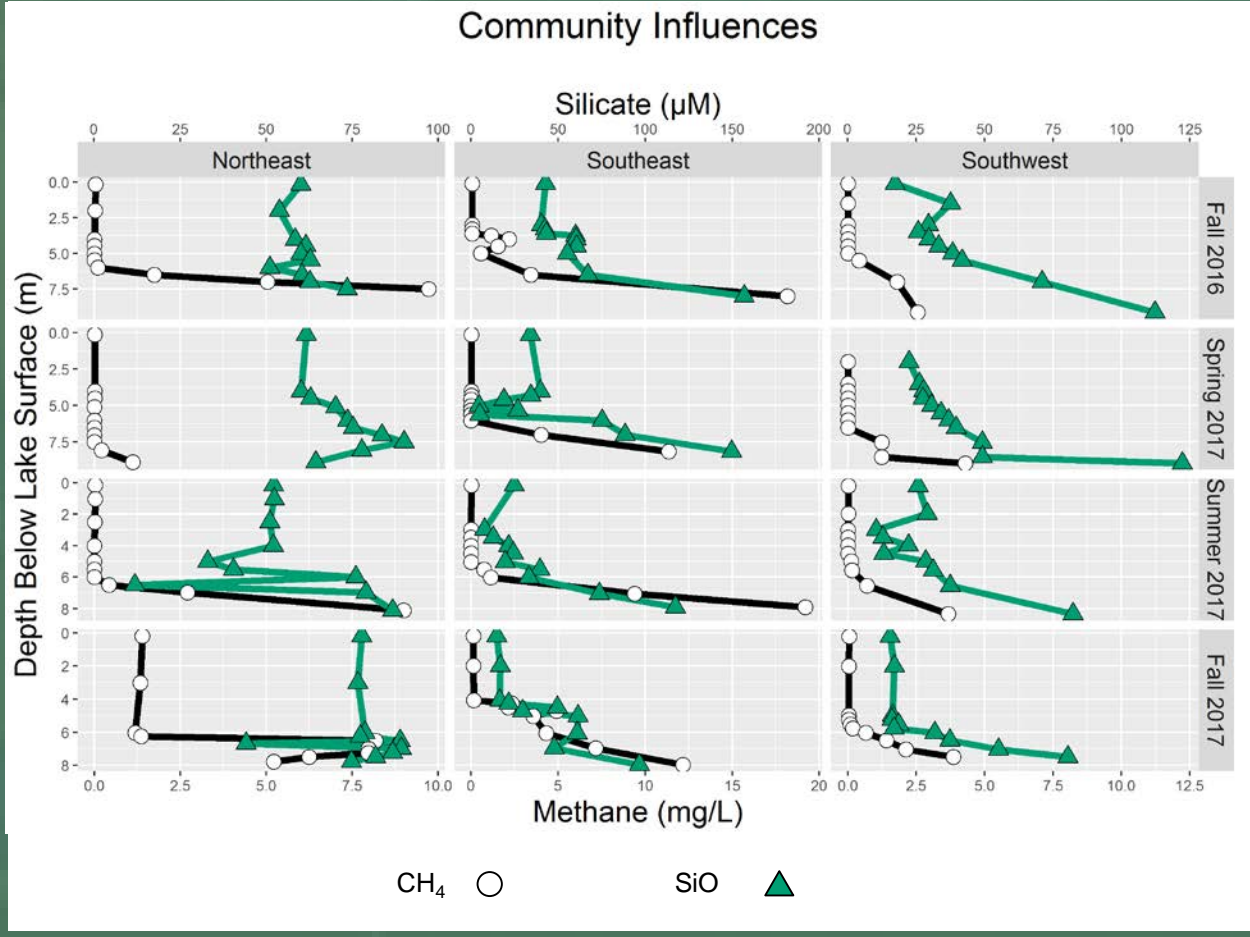


Figure 5. Environmental parameters determined to most influence the microbial communities of the lakes included methane (CH<sub>4</sub>) and silicate (SiO).

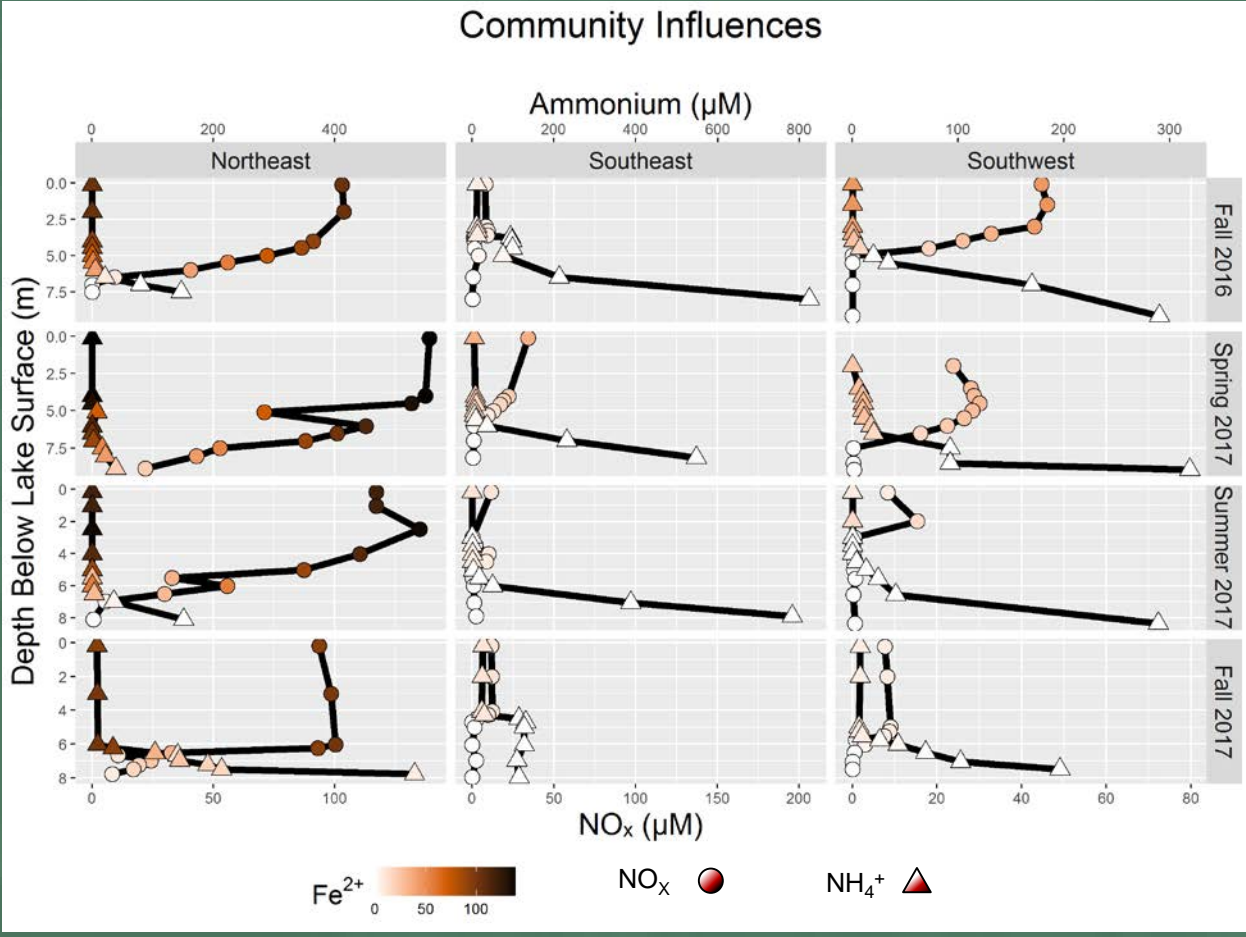


Figure 6. Environmental parameters determined to most influence the microbial communities of the lakes included NO<sub>x</sub>, ammonium (NH<sub>4</sub><sup>+</sup>), and Fe<sup>2+</sup>.

## Correlating Community Structure with Environmental Variables – Within Lakes

Northeast Lake BEST Analysis – Linking Community Assemblage to Environmental Variables		
Factor	Environmental Variables	Correlation
Ordered Depth	NO <sub>x</sub>	.783
Ordered Depth	NO <sub>x</sub> , pH	.858
Season	NO <sub>x</sub>	.890
Season	NO <sub>x</sub> , C:N	.905

Table 1. BEST analysis of correlations of microbial community structures in the NE Lake, across seasons, and ordinal depths, based on the environmental parameters. NO<sub>x</sub> is highly influential in this lake.

Table 2. BEST analysis of correlations of microbial community structures in the SE Lake, across seasons, and ordered depths, based on environmental parameters. The community structure is less easily explained than the NE Lake. The SE Lake is deeper and temperature has a large effect on depth. Dissolved carbon dioxide correlates strongest with season.

Southeast Lake BEST Analysis – Linking Community Assemblage to Environmental Variables		
Factor	Environmental Variables	Correlation
Ordered Depth	°C	.664
Ordered Depth	°C, PC	.753
Ordered Depth	°C, Chlorophyll <i>a</i> , PC	.771
Ordered Depth	°C, Chlorophyll <i>a</i> , PC, Sulfide	.792
Season	CO <sub>2</sub>	.838
Season	NO <sub>x</sub> , CO <sub>2</sub>	.853
Season	°C, CO <sub>2</sub> , SO <sub>4</sub> <sup>-2</sup>	.856
Season	°C, Fe <sup>2+</sup> , CO <sub>2</sub> , SO <sub>4</sub> <sup>-2</sup>	.869

Southwest Lake BEST Analysis – Linking Community Assemblage to Environmental Variables		
Factor	Environmental Variables	Correlation
Ordered Depth	CH <sub>4</sub>	.717
Season	NH <sub>4</sub> <sup>+</sup>	.883
Season	Salinity, CO <sub>2</sub>	.894
Season	NO <sub>x</sub> , CO <sub>2</sub> , NH <sub>4</sub> <sup>+</sup>	.912

Table 3. BEST analysis of the correlations of the microbial community structures in the SW Lake, across seasons, and ordinal depths, based on the environmental parameters. The community structure by depth is best correlated with methane. Ammonium has the largest single correlation with community structure across the seasons, but, when two variables are allowed, salinity and carbon dioxide correlate better. Only three variables are required to achieve a correlation above 0.9; NO<sub>x</sub>, which is important during season across all lakes, carbon dioxide and ammonium.

## Community Structure based on 16S rRNA gene Sequence All Lakes, All Sampling Events

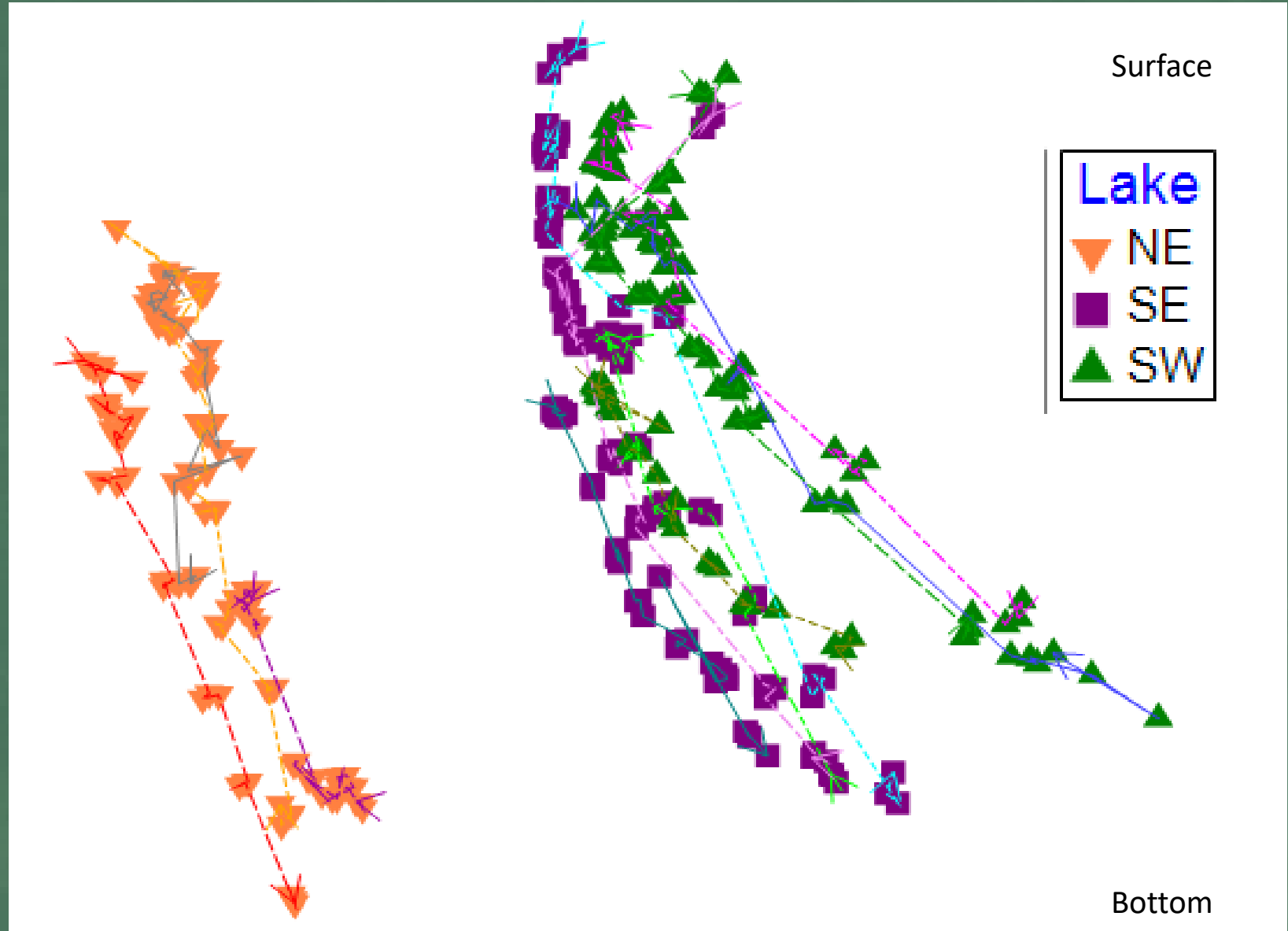


Figure 7. Non-metric multidimensional scaling of samples for all three lakes based on Bray-Curtis similarity of transformed abundance of 16S rRNA gene sequences (Log (X+1)). Communities from the lakes' surfaces are largely found at the top of the figure, and likewise for the bottom.

## Correlating Community Structure with Environmental Variables Across Lakes

### BEST Analysis – Linking Community Assemblage to Environmental Variables

Factor	Environmental Variables	Correlation
Lake	CH <sub>4</sub>	.640
Lake	NO <sub>x</sub> , NH <sub>4</sub> <sup>+</sup>	.645
Lake	NO <sub>x</sub> , NH <sub>4</sub> <sup>+</sup> , Fe <sup>2+</sup>	.664
Season	SiO	.546
Ordered Depth	NO <sub>x</sub> , Fe <sup>2+</sup>	.730

Table 4. BEST analysis of the correlations of the microbial community structures across all the lakes, seasons, and depths, based on the environmental parameters.

## Results

The communities were more similar within a lake, and between sampling times with depth being a major determinant within lakes, than among lakes. In agreement with the chemical data, the sulfidic SW and SE lakes were more like one another than either was to the NE lake. Continuous data monitors demonstrated that these lakes are polymictic. The community structure data suggests that after mixing events, the SW and SE lakes established redox gradients more rapidly than in the NE lake, where DOC concentrations were lowest and salinity is negligible. The community structures of the lakes correlated to different environmental drivers.

Organisms were identified as common across lakes, endemic to particular lakes, or as present during single sampling events (in progress).

*The views expressed in this poster are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.*

## 30 Seconds? Read This!

- Despite their proximity, the three Jackson Lakes have different biogeochemical characteristics and have different microbial communities.
- The Southeast and Southwest lakes have salinity gradients, sulfidic bottom water, and are more alike to one another than to the Northeast Lake. The NE Lake is fully freshwater.
- While these lakes are all polymictic, the microbial communities in the Southeast and Southwest lakes become stratified more quickly, likely due to saline bottom waters.
- BEST analysis shows that methane concentration most strongly correlates with differences in the microbial communities between lakes when considering only one parameter; NO<sub>x</sub> and NH<sub>4</sub><sup>+</sup>, and then Fe<sup>2+</sup> explain most of the variability in communities when considering up to three parameters.
- NO<sub>x</sub> and Fe<sup>2+</sup> (but not NH<sub>4</sub><sup>+</sup>) correlate best with differences in the microbial community structure among the ordered depths across the lakes, while silicate correlated best with microbial community changes with seasons.
- Considering the lakes individually, BEST analysis demonstrates that the microbial community structures correlate with different environmental parameters in each lake, with NO<sub>x</sub> important in all three lakes.

## Future Directions

- Quantify nitrogen cycling genes (*amoA*, *nosZ*, *nirK*, *nirS*, *nrfA*, *hzo*, *napA*) and genes encoding sulfur and methane cycling enzymes.
- Sequence 18S rRNA gene for algae and diatom community structure.
- Identify species and functional groups indicative of biogeochemical changes within and across lakes.
- Develop indicator species indices for chemicals of interest to stakeholders (e.g., nutrient pollution).

## References

- Clarke, KR (1993) Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117-143.
- Clarke, KR, Gorley, RN, 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth, 192pp.
- Schloss, PD, et al. (2009) Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75(23): 7537-41.

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