

# Assessing the Impact of Ocean Alkalinity Enhancement on Benthic Microbial Communities Using eDNA Metabarcoding

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## INTRODUCTION

- One of the proposed methods of mitigating climate change is the Ocean Alkalinity Enhancement (OAE) project.
  - pH is one of the main factors that determine how much CO<sub>2</sub> the ocean can absorb.
  - Increasing the pH (or enhancing the alkalinity) allows the ocean to sequester more CO<sub>2</sub>.
- Ecological studies on the Impact of OAE on natural ecosystems are lacking.
- Fully operational OAE plant implemented in Halifax Harbour
  - Has been dosing magnesium hydroxide in the harbour continuously since November 2024
- This study focuses on assessing the impact of OAE on benthic microbes (i.e. Bacteria and Archaea).
- This study could help provide necessary insight into the ecological effects of OAE before future implementation.

## HYPOTHESIS AND PREDICTION

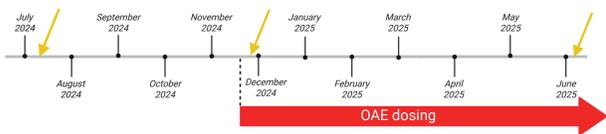
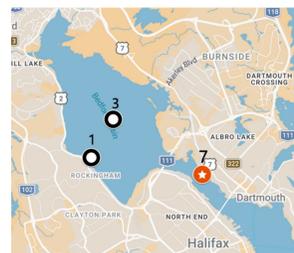
**Hypothesis:** Adding magnesium hydroxide at Tuft's cove will affect the benthic microbial community composition. We suspect that some of the undissolved OAE particles will settle on the sediment surface before dissolving, releasing additional alkalinity, leading to a disturbance and inducing community shift.

**Prediction:** There will be a significant changes in community composition and diversity after the OAE dosing starts.

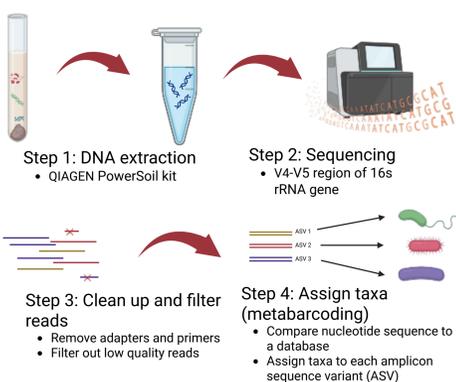
## METHODS

### Sample Collection

- Samples collected from 3 stations:
  - Station 1 ~30 m depth
    - Control site
  - Station 3 ~70 m depth
    - Control site
  - Station 7 ~18 m depth
    - Tuft's cove - OAE dosing



### eDNA Analysis



## RESULTS

- No obvious difference in community composition between dates for stations 1 (fig. 1a) and 3 (fig. 1b).
- Noticable shift in community composition in June 2025 at station 7 (fig. 1c).
  - Certain taxa seemed to become more dominant

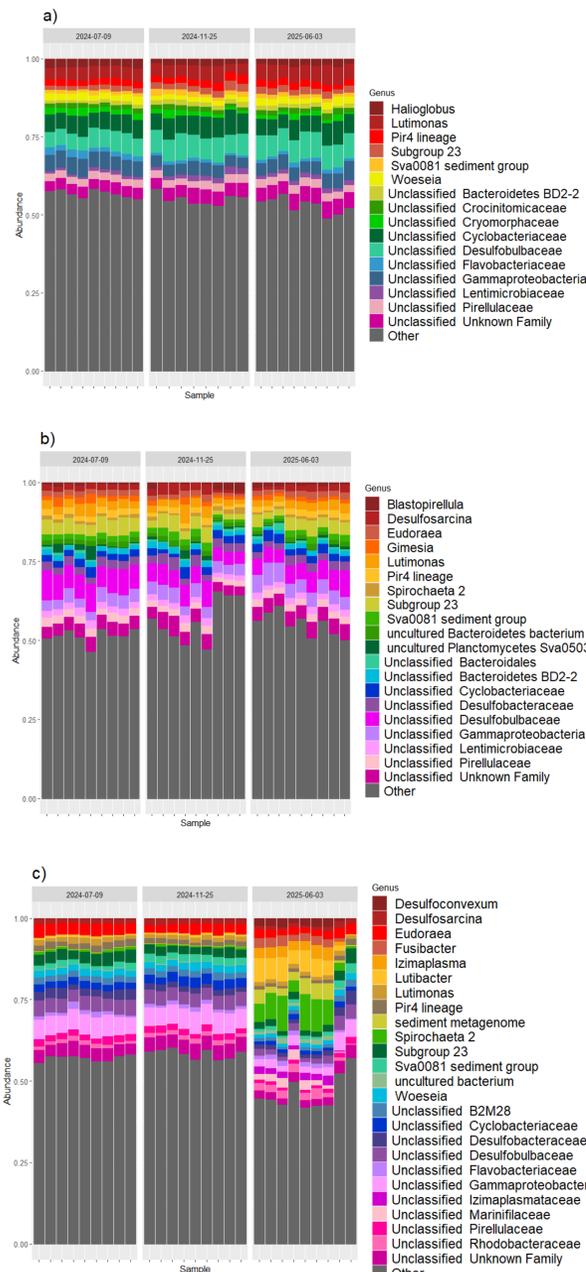


Figure 1. Relative abundance of bacteria and archaea genera within samples at a) station 1, edge of the Bedford basin; b) station 3, deepest part of the Bedford basin; and c) station 7, at Tuft's cove where the OAE dosing is occurring (Halifax, NS). Samples were taken in July 2024 (before the dosing started), November 2024 (a week after it started) and in June 2025 (several months after it started) (n=9 per station per date). "Other" represents any ASV that constitute less than 0.02 of all ASVs.

- A significant drop in Shannon diversity can be observed in June 2025 at stations 1 and 7.
  - The decrease is more pronounced at station 7
- No significant difference between July 2024 and November 2024 for any of the stations.

## RESULTS

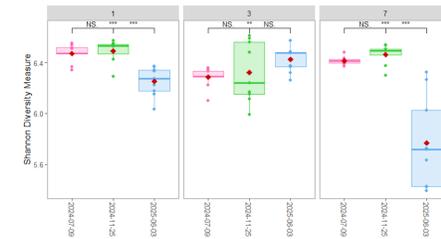


Figure 2. Boxplot of the Shannon diversity index within samples at station 1, edge of the Bedford basin; station 3, deepest part of the Bedford basin; and station 7, at Tuft's cove where the OAE dosing is occurring (Halifax, NS). Samples were taken in July 2024 (before the dosing started), November 2024 (a week after it started) and in June 2025 (several months after it started) (n=9 per station per date). The red point represents the mean of each group. Asterisk represent a significant difference between dates while 'NS' represent no significant difference. Red diamond represents the mean.

- NMDS plot supports prior results showing the samples taken from station 7 in June 2025 to be much further than the rest of the samples
  - A PERMANOVA analysis revealed those samples to be statistically different from the other ones taken from station 7

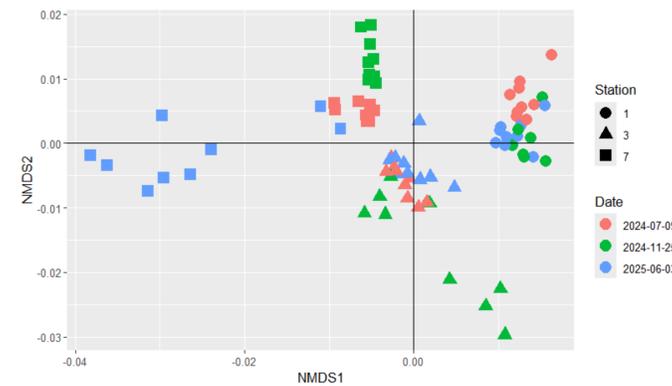


Figure 3. Non-metric Multidimensional Scaling (NMDS) plot of all samples (n=86) using the Weighted UniFrac distance. Color of the point represent the date when the samples were taken and the shape of the dot represents which station the samples were taken from.

- ANCOMBC-II and ALDEx2 were used to determine which taxa changed in abundance significantly.
- Some taxa such as Gammaproteobacteria and *Draconibacterium* decreased in abundance significantly in June 2025 relative to July and November 2024.
- Others such as *Bacteroidales* and *Lutibacter* increased significantly in June 2025 compared to July and November 2024 according to the ANCOMBC-II
  - ALDEx2 shows no significant increase in *Lutibacter* in June 2025 compared to July 2024

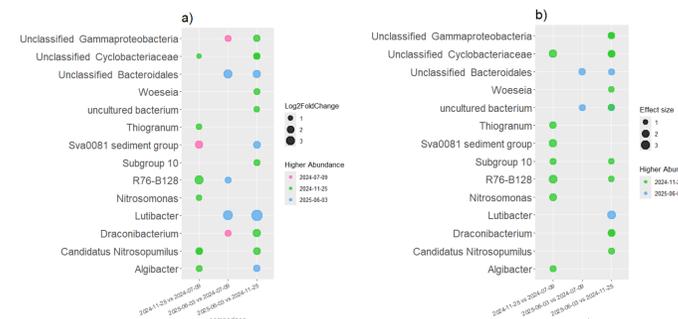


Figure 4. Differential abundance plots for samples taken at station 7 using the ANCOMBC-II (a) and ALDEx2 (b) analyses. On the x axis is the pairwise comparison of each dates the samples were taken from, the size of the point represents the size of the change for each genera (log<sub>2</sub> fold change for ANCOMBC-II, effect size for ALDEx2), and the color represents which of the two dates being compared has a higher abundance relative to the other.

## DISCUSSION

- Change in composition and drop in diversity in June 2025 at station 7 and drop in diversity seems to indicate that something has been affecting the microbial community between before and after the OAE dosing.
- This could mean that the dosing of OAE foodstuff (magnesium hydroxide) has a negative effect on benthic microbial.
- Differential abundance analysis revealed an increase in the *Lutibacter* genus.
  - Flavobacteria* (family in which *Lutibacter* belongs to) has been shown to be more resilient to disturbances [1]
- However, from May 5 to June 2, 2025 (The day before the June 2025 samples were collected) maintenance was being done on the wastewater treatment system.
  - Wastewater not fully treated was released directly in the harbour on the Dartmouth side.
- Flavobacteria* is also common in wastewater and nutrient rich water [2]
  - Domestic wastewater is expected to be nutrient rich.
- Increased nutrients (especially Nitrogen) is also associated with decreased diversity in sediment microbes [3]

## CONCLUSION

- It is likely that a certain disturbance occurred near station 7 between July 2024 and June 2025 that changed the community composition and decreased the diversity
  - May indicate that OAE dosing is affecting the community.
  - However, it is hard to attribute it to the OAE dosing with certainty.
  - Could be a result of more than one factor.

### Future directions:

- Samples taken in November 2025 are undergoing analysis currently and once done, will hopefully provide more insight into the cause of this community shift
- Analyzing how eukaryotic organisms have been affected may also provide more insight.

## ACKNOWLEDGEMENTS

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