Introduction

•The establishment of coastal salt wetlands helps compensate for the destruction of pre-existing marshes caused by industrial activities such as mining, construction, and excavation (Craft et. al., 2003)
•*Spartina alterniflora* is resistant to flooding, anoxic soils, and high salinities (Mason et. al., 2020)
•Due to its ability to fuel food webs above and below ground, it functions as a keystone species in many salt marsh ecosystems (Wang, Seliskar & Gallagher, 2003)
•In the intertidal zone, S. alterniflora is a substantial source of fixed carbon (Bergen et. al., 2000)
•Spartina alterniflora is negatively affected when high soil pH was combined with high salinity (Li, Shi & Fukuda, 2010)
•Metabarcoding is used to figure out the microorganisms present in a certain ecosystem (Lafferty & Nigro, 2018)
•Soil microbes create a link between plant roots and the soil, recycle soil nutrients, decompose organic matter, and react to changes in the soil ecology (Toor & Adnan, 2020)

Research Question

- How are the microbes present in the roots and surrounding environment of Spartina alterniflora in and around a brackish water pond affected by temporal changes?

Hypothesis

- We expect to find species of bacteria from the phyla of: Acidobacteria, Verrucomicrobia, Cyanobacteria, Bacteroidetes, and many from Proteobacteria (alpha-, beta-, gamma-, delta-, epsilon-, zeta-).
  - In a study assessing microbial communities in S. alterniflora infiltrated areas, different abundances of these phyla of bacteria were present at different locations. However, in all locations sampled, different variants of Proteobacteria were present (Mavrodi, et. al, 2018).
  - In another soil microbiome study, unknown phylogenetic sequences were categorized under the phylum of Proteobacteria and were associated with environments that were plant-based and/or aquatic (Lovell, et. al, 2000).
- We hypothesized that we will find predominantly bacteria from the phylum, Proteobacteria, since it is the most common bacteria found in soil microbiomes (Spain, et. al, 2009).
- If we find high levels of Proteobacteria in soil samples taken, this may be a result of nitrogen fertilization, an abiotic process known to increase organic carbon and nitrogen concentrations while decreasing soil pH; nitrogenous fertilizers can commonly lead to acidification of soil (Dai, et. al, 2018).
  - Proteobacteria plays roles in both the carbon and nitrogen cycles: they take away pollutants from the environment by utilizing excess nitrogen and carbon for their own sources of energy.
  - This relates to how one of the enduring issues in Long Island coastal waters includes nitrogen pollution (Bortman, 2013).
  - It is also important to acknowledge that in more urbanized areas of Long Island, the soil pH is higher than that of rural areas.

Methodology

- Samples were collected from the Long Beach High School pond
- The pond has access to Reynold's channel creating different salinity depending on the part of the pond you're in
- Soil was dug out with a shovel and samples were taken from the soil and placed into snap-cap tubes
- Other metadata was taken and recorded on our table (Future slides)
- We ran soil tests to determine pH, phosphorus, nitrogen, and potassium levels
- Extraction was ran using the Qiagen DNeasy powersoil kit
- PCR was ran on the samples
- PCR product was ran through a gel to check for amplification
- Samples were analyzed using the DNA Subway Purple line

Demultiplex Reads

Minimum	280
Median	273102.5
Mean	234496.5
Maximum	479967
Total	4220937

Table 2: Sequence Counts Summary

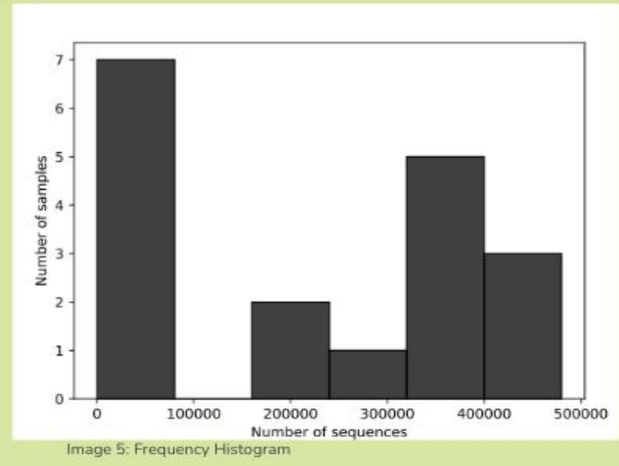

Image 5: Frequency Histogram

Results

- Relatively strong positive correlations were observed between air temperature and biodiversity and water temperature and biodiversity showing higher air and water temperatures yield better biodiversity.
- There was a relatively moderate correlation observed between salinity and biodiversity however the p-value was too large to deem the correlation significant.
- Along with many other temporal variables pH showed relatively little correlation with biodiversity meaning they showed no effect on biodiversity.
- The samples were mainly dominated by Actinobacteria and Alphaproteobacteria.

Discussion

- There was a large gap between February and July where samples didn't work so changes occurring in those months were not seen
- Changes were observed in the relative abundance of *Gammaproteobacteria*, *Actinobacteria*, and *BPC102* (*Acidobacteria*). These bacteria may have relative abundances correlated with changes in variables observed.
- Alpha rarefaction leveled off at 6000 reads. Levelling off indicates that a larger sampling depth will likely still yield the same biodiversity
- Demultiplex showed a large number of reads for all samples with July through October having a much greater number of reads (~150000-500000) and January and February having much fewer (~20000-60000). This means that the July through October samples have more sound results.
- All samples passed through the DADA2 filter with the majority of reads passing through in each sample meaning the majority was quality DNA.

Conclusion

- Earlier samples were dominated by *Gammaproteobacteria* while later samples were dominated by *Actinobacteria*
- There was correlation observed between air temperature and biodiversity, water temperature and diversity, and salinity and biodiversity indicating that higher air and water temperatures yield more biodiversity
- Fairly little correlation between the other variables and biodiversity means they don't cause changes in biodiversity
- This means that people using *Spartina* in wetland restoration should prioritize growing it in warm temperatures and low salinities over other variables.

Experimental Error Sources

- Nitrogen test didn't look like it was working properly
- DNA Degradation was possible due to the DNA being processed long after some samples were taken
- Contamination was possible during the PCR prep causing the DNA to not amplify well
- Nutrient tests aren't precise enough to detect small changes in nutrient levels that may cause changes in biodiversity.

Further Research

- Researching specific bacteria that we noticed changes with to determine their role in the Microbiome to gain more insight into the effect of the temporal changes on the *Spartina*

Assessing Temporal Variability in the Rhizosphere Microbiome in *Spartina alterniflora*

Methodology

Image 1: Image of the pond showing salinity differences and Spartina locations

Image 2: Satellite view of collection location

PCR Recipe/Protocol

12.5µL PCR MasterMix
6.5µL Pure Water*
1µL DNA*
5µL Primer
25µL PCR Mix
(Note: Water and MasterMix are added to a separate tube and split between the samples)
*Could be changed for better results

The primers have at their core primers that amplify the V4 region on 16S ribosomal RNA.

Initial Step: 94°C - 3min

Denaturation
94°C - 45s

Annealing
50°C - 1min

Extension
72°C - 1.5min

x35

Final Step: 72°C - 10min
12°C - ∞

Data

Alpha Rarefaction

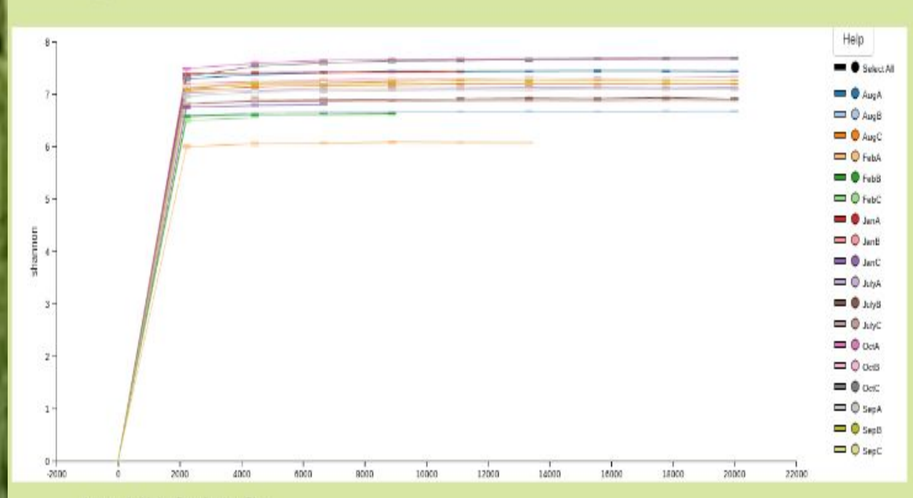

Image 4: Alpha Rarefaction Plots

DADA2 Stats

sample-id (#q2:types)	input (numeric)	filtered (numeric)	percentage of input passed filter (numeric)
SetA-F1	233785	189644	81.12
SetA-F10	366369	329340	89.89
SetA-F11	371225	322418	86.85
SetA-F12	450946	396253	87.87
SetA-F2	422614	377126	89.24
SetA-F3	204681	184756	90.27
SetA-F4	366737	301700	82.27
SetA-F5	312420	255442	81.76
SetA-F6	479967	422042	87.93
SetA-F7	365950	324225	88.6
SetA-F8	337620	294134	87.12
SetA-F9	280	111	39.64
SS1A	60200	33525	55.69
SS1B	60799	30881	50.79
SS1C	50852	26224	51.57
SS2A	55750	29081	52.16
SS2B	46260	24948	53.93
SS2C	34482	17892	51.89

Table 3: DADA2 Table

Alpha Diversity

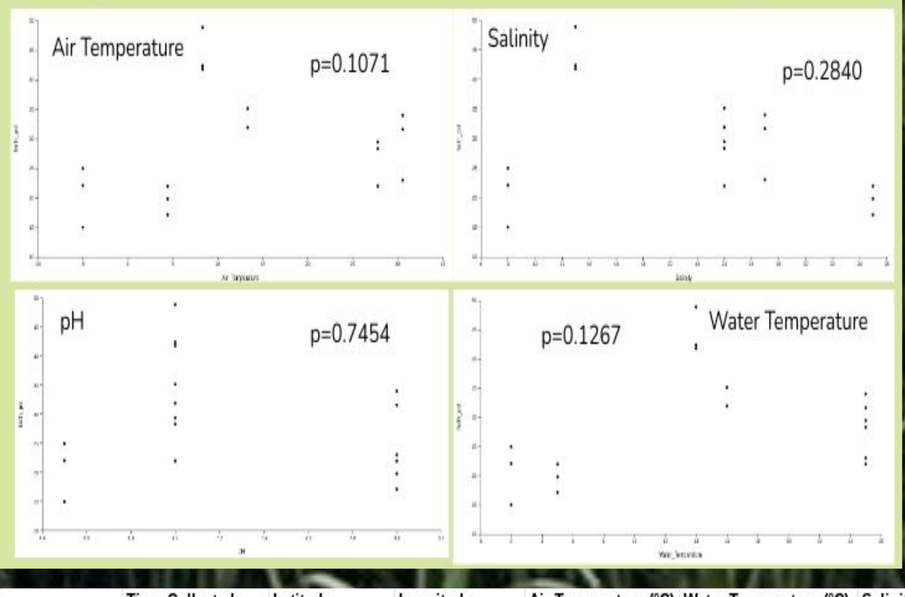

Image 3: Alpha Diversity Plots

Bar Plots

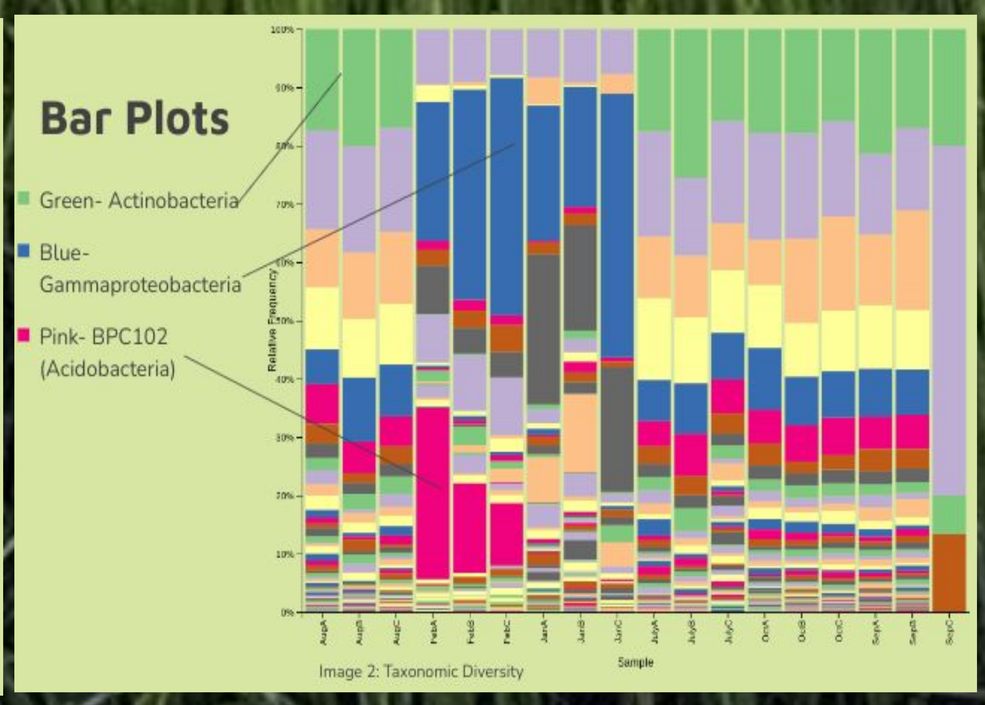

Image 2: Taxonomic Diversity

	Time Collected	Latitude	Longitude	Air Temperature (°C)	Water Temperature (°C)	Salinity (ppt)	Water Depth (cm)	Depth to Rhizosphere (cm)	Soil pH	Phosphorus (lb/acre)	Potassium (lb/acre)	Nitrogen (lb/acre)
January	9:02 AM	40.593529	-73.634294	-5	2	8	unsubmerged	15.24	6.5	0-50	>200	Very trace
February	9:07 AM	40.593557	-73.634274	4.44	5	35	partially submerged	10.16	8	0-50	>200	Very trace
March	9:50 AM	40.593557	-73.634274	10.56	10	34	submerged	10	7	0-50	>200	Very trace
April	9:00 AM	40.593451	-73.634258	10	12	33	unsubmerged	10	8	0-50	>200	Very trace
May	9:00 AM	40.59354	-73.63433	26	20	29	unsubmerged	7	6	0-50	>200	Very trace
June	1:38 PM	40.59354	-73.63434	25	23	11	unsubmerged	13.97	7	0-50	>200	Very trace
July	5:38 PM	40.59355	-73.63436	27.78	25	24	unsubmerged	15	7	50-100	>200	Very trace
August	1:48 PM	40.59351	-73.63435	30.56	25	27	partially submerged	10	8	0-50	>200	Very trace
September	7:11 AM	40.59358	-73.63436	13.33	16	24	partially submerged	23	7	50-100	>200	Very trace
October	7:09 AM	40.59356	-73.63434	8.33	14	13	unsubmerged	10	7	0-50	>200	Very trace
Summary	January 27, 2022		February 28, 2022		March 31,2022		April 28, 2022		May 31, 2022			
High Tide	2:59 AM		6:53 AM		9:02 AM		7:09 AM		9:36 AM			
Low Tide	9:35 AM		1:05 PM		3:02 PM		12:54 PM		3:28 AM			
Weather	3mph winds/fair sky		16 mph winds/cloudy		13mph winds/mostly cloudy		22 mph winds/mostly cloudy and windy		12 mph winds/partly cloudy			
Notes:	Pond was frozen at collection location		Other collection location was submerged		Rained earlier that day							
	June 30, 2022		July 31, 2022		August 30, 2022		September 29, 2022		October 28, 2022			
High Tide	11:19 AM		12:05 PM		10:47 AM		10:58 AM		10:37 AM			
Low Tide	4:49 PM		5:46 PM		4:47 PM		4:34 PM		4:13 AM			
Weather	13 mph winds/mostly cloudy		13 mph winds/partly cloudy		18 mph winds/partly cloudy		14 mph winds/partly cloudy		14 mph winds/fair sky			

Table 1: Table of collected metadata