Analysis of Rhizospheric Denitrifying Microbial Communities Along the Aliso Creek Estuary

Karis Polfer

ABSTRACT

California estuarine ecosystems have faced increased stress due to the effects of climate change. Aliso Creek is a degraded watershed in Laguna Beach, CA that leads to the Pacific Ocean. Polluted waterways are detrimental to the health of humans and aquatic and terrestrial biomes. Excess nutrients such as nitrogen from anthropogenic sources continually pose a threat to water and sediment quality. Water quality must be maintained in the estuary by monitoring total nitrogen (TN), salinity, C:N ratios, and the diversity of the denitrifying microbial community associated with the rhizosphere. I collected rhizospheric sediment samples from *Pulicaria paludosa* along a transect gradient within the Aliso Creek Estuary Restoration Project boundaries. I used an Oakton CON 550 Benchtop Conductivity Meter to obtain the salinity level of each sample. I used an Elemental Analyzer to find TN concentrations and C:N ratios of each sediment sample. I used DNeasy Powersoil Kits for DNA extraction and conducted PCR to prepare a library sample pool, transferring them to the QB3 Sequencing Center at UC Berkeley to analyze the microbial communities in each sequence. I conducted statistical analyses to determine the microbial community diversity of bacteria along the transect gradient. I found that the alpha-diversity of each transect bound was not statistically significant when compared to salinity, TN, and C:N ratios ($p > 0.05$), whereas there was statistical significance in the beta-diversity of each transect bound when compared to salinity ($p = 0.039$). This research will provide valuable insight into the future monitoring of the estuary to improve water quality for years to come.

KEYWORDS

denitrification, 16S rRNA sequencing, salinity, C:N ratios, biogeochemistry

INTRODUCTION

Estuarine ecosystems along the coast of Southern California once had a high biodiversity of flora and fauna, providing habitats to the now endangered Southern Tidewater Goby, Western Steelhead Trout, red-legged frogs, native cattails, pickleweed, and tule (Scarborough et al. 2022, Swift et al. 2016). Due to anthropogenic influences, the health of these coastal estuaries has declined with influxes of fecal bacteria, excess nutrients, and the loss of aquatic mammal diversity (Freeman et al. 2019). Polluted waterways can be detrimental to the health of humans and aquatic and terrestrial ecosystems (Fenn et al. 2003; de Vries 2021) and can cause infectious diseases in both humans and wildlife. Nitrogen is one of the many nutrients that accumulate in excess in coastal estuarine systems. It significantly impacts the water quality due to the detrimental effects eutrophication has on such ecosystems (Freeman et al.).

Nitrogen from point and non-point sources has polluted coastal estuaries by means such as fossil fuel emissions, volatilization of ammonia from chemical fertilizers through air deposition (Skorupka and Nosalewicz 2021), and the sediment acting as an N sink during wet weather runoff (Hawley and Conder 2015). Air pollution affects water quality through excess nitrogen introduced into the water through N deposition contributing to eutrophication and acidification (de Vries 2021). Microbially-assisted phytoremediation is necessary to establish endophytic symbiotic relationships to reduce excess nitrogen through denitrification (Su et al. 2019). Rhizospheric microbial communities are important bioindicators that signify the health of the sediment and aid plants in dealing with abiotic stressors such as overabundance of nitrogen (Pantigoso et al. 2022). The removal of nitrogen into the form of N_2 gas as an end product through the microbial process of denitrification is one of the main pathways (Lehnert 2015). Establishing point and non-point sources of nitrogen is essential to reduce the impact of these sources and to ensure proper management of runoff (Xia et al. 2020). Yet it is unclear what effect salinity has on the quality of sediment due to obstruction of tidal flow by the artificially breached natural barrier in the Aliso Creek Estuary, especially concerning the impacts of nitrogen. This obstruction is caused by skimboarders in the area who dig out the natural barrier for recreational purposes.

Aliso Creek is a 30-square-mile watershed starting from the Santa Ana Mountains to the Pacific Ocean in Laguna Beach, CA (SCWRP 2023). This waterway leads to the Aliso Creek beach where tourists and locals gather to swim and surf, with some local skimboarders consistently

breaching the naturally occurring sand barrier separating the ocean from the creek. This disturbs the ebb and flow of this estuary which may affect the local ecosystem. It is known that when nitrogen concentrations are in excess in polluted groundwater due to agricultural fertilizer runoff, it causes algal blooms by eutrophication and exhausts dissolved oxygen levels (Yousaf et al. 2021). The intersection of variables such as salinity, denitrification rates, and TN are essential environmental factors in estuaries that should be measured side by side with the rhizospheric and surrounding denitrifying microbial community diversity and composition found in the sediment of vegetation along the estuary (Li et al. 2021a). Previous research suggests that high salinity inhibits the bacterial community involved with nitrogen cycling (Li et al. 2021a). This could be a significant factor in the degradation of this estuary due to continuous artificial breaching of the natural barrier separating the brackish water of the estuary from the saline ocean creating a source of stress on the sediment microbial communities, species endemic to the estuary, and vegetation in the local ecosystem. Other studies have shown that denitrification rates in polluted estuaries increase in the presence of increased TN concentrations (Teixeira et al. 2016; Li et al. 2021b). This may support other research suggesting that the diversity of denitrifying bacteria increases with increased concentration of TN (Chen et al. 2021). One issue regarding the water and sediment quality of the Aliso Creek Estuary is the lack of data on the denitrifying microbial communities and comparative analysis on how different environmental variables alter their diversity and function in the area (Hawley and Conder 2015).

In this study, I examined the different environmental variables' contribution to the diversity of rhizospheric denitrifying microbial communities in nitrogen-polluted estuaries. The capabilities of the rhizospheric microbial communities along the estuary to remove nitrogen stored in the sediment and different environmental variables that may alter this natural process are examined. In addition, environmental variables are analyzed to study their effects on the diversity of these denitrifying microbial communities. The three sub-questions are (1) How does the concentration of salinity in the sediment affect the rhizospheric microbial community present along the estuary? (2) How do total nitrogen (TN) concentrations in the sediment affect the rhizospheric microbial community diversity along the estuary? (3) How do the C:N ratios vary with higher levels of diversity of the rhizospheric microbial communities in the sediment along the estuary? For the first subquestion, Based on previous observations, I hypothesized that salinity would significantly affect the diversity of the rhizospheric microbial community present in the sediment. I measured

the salinity and the diversity of the rhizospheric denitrifying microbial communities in each sediment sample. I then hypothesized that the diversity of the rhizospheric denitrifying microbial community would be affected by the amount of TN present in the sediment. To answer this, I measured TN in the sediment and looked at the microbial communities present at different concentrations of TN. For the last subquestion, I hypothesized that higher C:N ratios would be present in more diverse denitrifying microbial communities. To find support for this claim, I measured and compared C:N ratios with the rhizospheric microbial communities.

METHODS

Study site

I focused on the Aliso Creek Estuary, within the set project boundaries of the Aliso Creek Estuary Restoration Project in Laguna Beach, CA. This site gradually became polluted with excess nutrients, heavy metals, and fecal matter accumulating from point and non-point sources. Due to human disturbance from the local resort The Ranch and runoff that accumulates from upstream, the once diverse ecosystem has been low-functioning with native species struggling and has since been taken over by invasive vegetation (SCWRP) such as *Pulicaria paludosa*, *Melilotus albus*, *Carpobrotus edulis*, *Foeniculum vulgare*, among many others observed on my field study. Aside from typical disturbances from fossil fuel emissions and agricultural runoff, this site is also threatened by local skimboarders who illegally breach the natural sand barrier existing between the estuary and the Pacific Ocean (Quarnstrom 2023). This causes drainage of the estuary into the ocean and may indeterminately throw off the natural fluctuations of the watershed (Quarnstrom). My study aims to delve into this issue by analyzing different environmental variables' effects on the rhizospheric microbial diversity along the Aliso Creek Estuary.

Sample collection

To decide on sampling locations and control species for my research, I studied the perimeter of the project boundary lines of the Aliso Creek Restoration Project (Figure 1) to see the most abundantly available vegetation. This ongoing restoration project aims to restore the estuary

to its preindustrial state. The vegetation of abundance for sampling was determined to be *Pulicaria paludosa*, also known as Spanish false fleabane, (Figure 2) as it appeared most often in my observations. This is a naturalized flowering weed that grows in damp and disturbed riparian areas (Calflora 2024), making the Aliso Creek Estuary highly habitable for this species. After locating the species, I set out a sampling strategy that consisted of 4 sample transects, around 70 ft in length, along the north and south bounds of the estuary; I determined 4 transects that gave a robust sample size and distribution along the restoration project boundaries where within each bound of the transects, three replicate rhizospheric and bulk sediment samples were retrieved along with two resin capsules placed in the sediment for an 8-day incubation (Figure 3). In total, 46 sediment samples and 15 resin capsules were collected (one sample location in the second transect could not be determined and one resin capsule was not retrieved). The sampling set-up achieved a transect gradient flow, where environmental variables are compared with the microbial community diversity and abundance data. This incubation captured the flux of elements that flow through the sediment as water fluxes occurred in the estuary during that time. The sampling sites were relatively similar in geomorphological features such as distance to the watershed.

Figure 1. Google Earth image of the Aliso Creek Estuary Restoration Project funded and maintained by the Laguna Ocean Foundation.

Figure 2. *Pulicuria paludosa* was marked with a yellow flag as a sample location for excavation. This plant, Spanish false fleabane, grows remarkably along the Aliso Creek Estuary providing a variable control within my study.

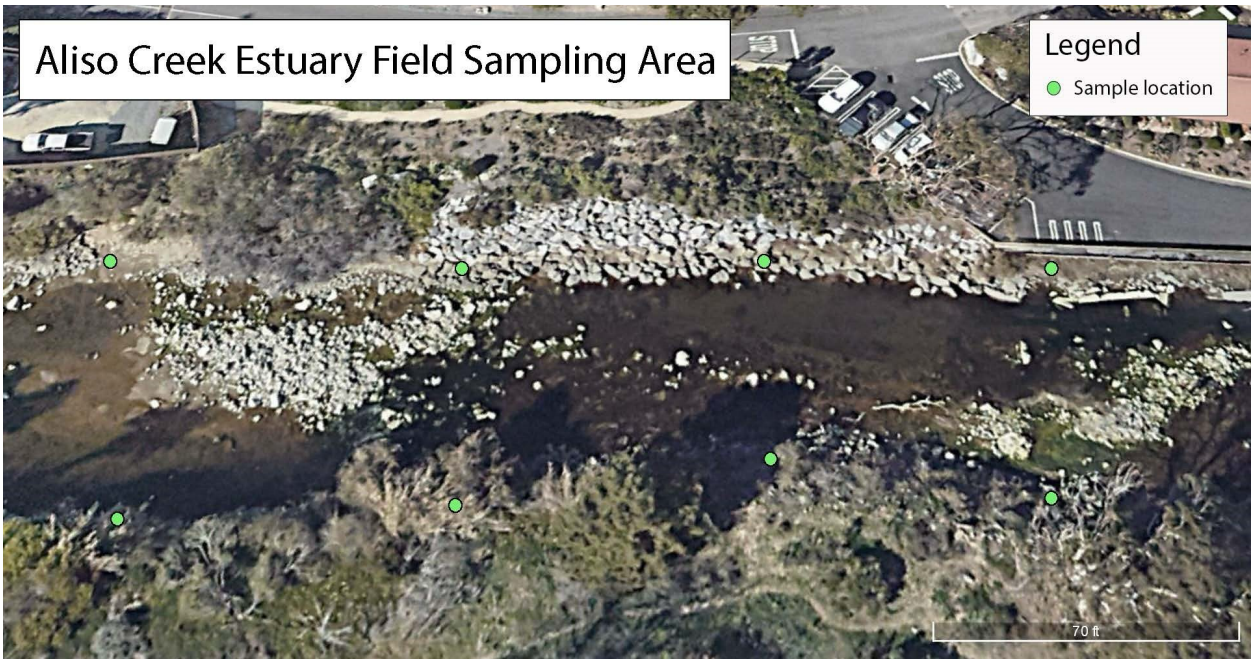

Figure 3. Aliso Creek Estuary field sampling area. The bright green dot represents the locations where rhizospheric and bulk sediment samples were collected, and incubated resin capsules were placed. These four transects garner a downstream gradient set up within the restoration project's boundaries. Transect 1 is on the left leading to transects 2, 3, and 4 on the right. The southbound contains sediment samples A-C and the northbound D-F.

Resin capsules

Resin capsules in the sediment provide valuable information on the elements that flow in the water through the estuary. In addition to sediment sampling, I placed 2 UNIBEST PST-1 Capsules at each sample bound at a uniform depth of 10 inches in the sediment for an 8-day incubation. This method collected data on the elemental composition giving further insight into the quality of water and sediment to be determined in the lab through ICP-MS analysis. After the 8-day incubation, the capsules were retrieved in allocated Falcon Tubes and placed in a refrigerator where I then shipped the capsules back to the Lawrence Berkeley Lab. The element components in each resin capsule obtained provide valuable insight into heavy metals contaminating the estuary as well as important non-metallic elements that affect estuarine ecosystems (Table 1).

Table 1. Raw data results from incubated resin capsules analyzed by an ICP-MS. Elements in this table include the heavy metals lead, chromium, and nickel, and important nonmetals sodium and phosphorus. Sub-samples A and B represent the southbound and C and D represent the northbound of each sample transect. There is no 4D resin capsule as it was unable to be retrieved in the field.

	Pb		**Cr**		**Ni**		**Na**		**P**	
Sample ID	Conc [ppb]	Conc SD	Conc [ppb]	Conc SD	Conc [ppb]	Conc SD	Conc [ppb]	Conc SD	Conc [ppb]	Conc SD
FB_1	1.19	0.02	8.74	0.18	4.12	0.13	4215.84	122.28	3.68	2.67
FB_2	0.49	0.01	8.42	0.09	5.21	0.12	3581.01	35.01	31.79	7.34
FB_3	0.46	0.01	8.19	0.09	5.42	0.13	3747.75	40.76	23.37	8.22
4A	28.42	1.06	19.16	1.81	183.94	2.10	171175.20	5103.06	1933.14	69.00
4B	15.23	0.22	18.97	0.32	62.85	1.05	425479.36	16760.35	4046.25	82.54
4C	41.08	1.04	12.72	0.20	245.40	3.74	264813.96	10510.55	4120.46	69.88
3A	64.60	1.11	62.46	1.26	282.71	5.06	442001.10	9589.43	7034.96	73.84
3B	27.86	0.52	24.29	0.35	87.94	1.27	258403.48	4323.73	4704.24	137.43
3C	12.95	0.21	19.28	2.69	73.46	1.41	591126.72	19568.65	2976.05	50.69
3D	16.23	0.31	14.35	0.75	66.75	1.26	759310.53	14472.43	3137.99	54.50
2A	73.38	1.54	16.59	0.25	115.51	1.25	676331.76	14543.86	12880.92	127.96
2B	72.35	2.86	21.24	0.28	170.68	1.58	450519.33	5614.33	7300.92	71.68

2C	45.71	0.58	21.33	0.12	174.66	1.15	304207.78	10933.30	7385.81	88.80
2D	43.39	0.76	31.43	0.12	317.96	5.04	350529.85	7757.59	2493.77	45.29
1A	19.60	0.35	15.19	0.19	75.61	1.43	493981.40	7153.47	5102.88	71.67
1B	54.21	1.11	40.78	0.58	507.13	5.13	450525.36	6288.62	6961.34	19.92
1C	16.04	0.37	23.33	0.20	70.07	1.50	83549.38	3662.15	586.15	21.29
1D	29.53	0.38	36.96	0.36	140.90	2.15	692232.68	13339.81	1183.35	8.59

Rhizospheric and bulk sediment sampling and lab analysis

Sediment samples I collected in the field give valuable information once analyzed in the lab. I obtained rhizospheric sediment samples using a spade disinfected with 70% ethanol wipes to excavate the plant, where the shaking method was utilized which entails shaking the plant manually to remove any remnant bulk sediment attached to the roots (Micallef et al. 2009). I scraped the roots using sterilized scalpels to collect the rhizospheric sediment, (Micallef et al.) and placed them into respective collection bags. I obtained bulk sediment samples from the areas of rhizospheric sediment collection. The sampling sites were relatively similar in geomorphological features such as distance to the watershed whereas other features such as percent moisture and sediment makeup varied greatly between some sample bounds and transects. I then put the samples in a cooler with ice packs until I transported them to a -30-degree freezer. Then I shipped the sediment samples over dry ice to Lawrence Berkeley National Laboratory and placed them in an ultralow freezer for further lab analysis. All sediment samples were obtained from within 10 inches below the sediment surface.

DNA extraction and sequence processing. I extracted the rhizospheric and bulk sediment DNA by following the protocol provided in the DNeasy Powersoil Kit (Wu 2022). After the DNA

extractions, the DNA in the samples was amplified, normalized, and purified using PCR and then sent to the QB3 Sequencing Facility at UC Berkeley, then retrieved for further processing. For further analysis, I followed the dada2 pipeline in R to create a phyloseq object using the Phyloseq package (Gabri 2022, McMurdie and Holmes 2013).

Salinity, TN, C:N ratios. I measured TN in the sediment in the lab after thawing the samples in a refrigerator. Following lab protocol, I dried, sieved, and ground the sediments in a Tissue-Lyzer. Then, I placed about 30 mg of sediment in 10.5 x 9mm tin capsules and sent them to be analyzed in an elemental analyzer-isotope ratio mass spectrometer (EA-IRMS). This gives insight into the TN levels in the sediment samples obtained. The EA-IRMS also gave us information about C:N ratios in the sediment which offers valuable information on terrestrial inputs within the ecosystem (Plummer et al. 2015). I used the bulk sediment samples collected to test the environmental variables to be analyzed (Table 1). When looking at boxplots of the environmental variable data, I found that all sampling sites were quite dissimilar in measurements of salinity (Figure 4). For example, the ranges between samples in 2A-2C within the field site have a minimum of 9.01 ppt and a maximum of 14.93 ppt. Within the same sample bound, we can see that TN, C:N ratios, pH, and percent moisture were all fairly consistent, whereas microbial biomass in µg N/g soil was highly variable. These variabilities were examined to determine what causes such fluctuation between each transect and within transect sample bounds via statistical analysis.

Table 2. Summary of environmental variables used in this study. Salinity in ppt, TN, C:N ratios, pH, percent moisture (%m), and microbial biomass in µg N/g soil analyzed in the lab of each bulk sediment sample surrounding the roots of *Pulicaria paludosa* along the transect gradient of the Aliso Creek Estuary.

Sample ID	Salinity (ppt)	TN	C:N	pH	%m	M. Biomass N (µg N/g soil)
1A	8.73	0.06	14.68	8.36	18.13	6.52
1B	5.23	0.06	14.23	8.31	16.97	1.95
1C	8.87	0.03	13.08	8.4	15.77	2.60
1D	11.90	0.07	16.92	8.48	13.35	21.78

1E	18.40	0.05	17.26	8.31	10.56	20.50
1F	9.17	0.06	16.02	8.23	12.57	21.72
2A	9.01	0.06	16.69	8.73	20.87	7.10
2B	14.93	0.05	18.64	8.32	21.60	2.16
2C	10.88	0.06	16.42	8.45	20.87	8.97
2D	7.92	0.11	18.45	8.3	19.28	26.95
2F	9.79	0.06	16.98	8.85	14.71	12.00
3A	10.77	0.07	15.11	8.63	17.93	6.21
3B	12.34	0.04	14.52	8.66	10.80	20.35
3C	10.66	0.06	14.56	8.4	18.16	22.76
3D	8.35	0.09	16.77	8.13	17.56	15.41
3E	8.06	0.11	14.97	8.21	17.69	17.39
3F	17.73	0.09	15.70	8.18	14.77	14.41
4A	15.12	0.05	18.20	7.71	9.18	5.53
4B	19.67	0.04	17.74	7.75	8.58	20.19
4C	13.02	0.11	13.12	7.6	26.19	20.64
4D	8.61	0.05	17.78	8.45	11.80	8.20
4E	9.55	0.09	16.14	8.4	20.08	15.81

4F	9.26	0.08	16.84	8.41	15.77	13.99

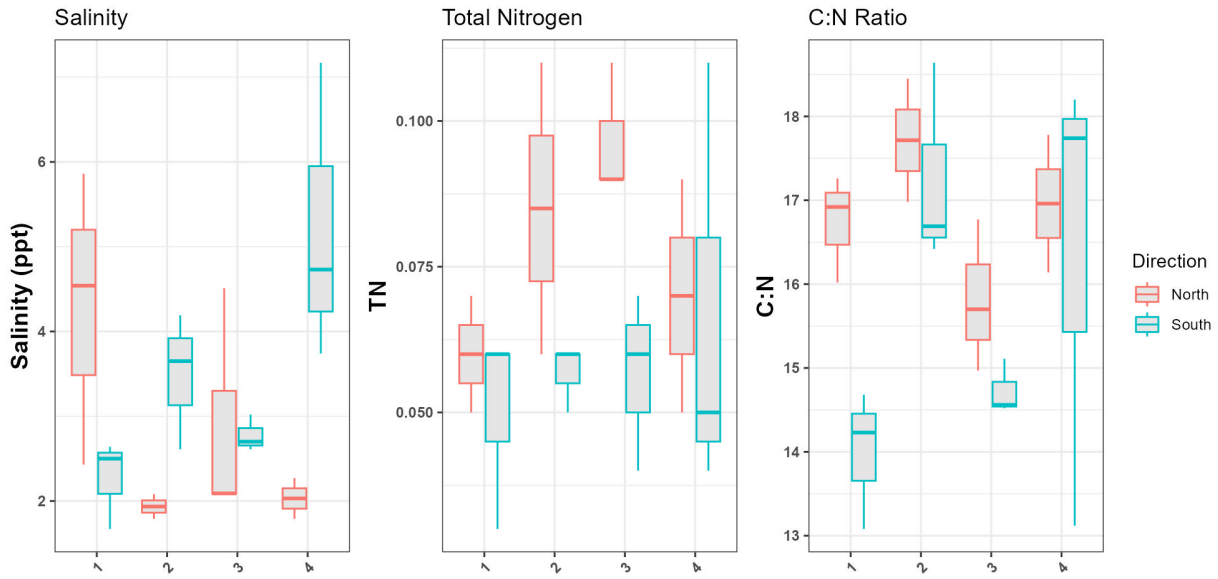

Figure 4. Boxplot of environmental variables measured (salinity, TN, C:N ratios) against each transect subsetted into north and south bounds. Environmental variables within each transect and bound are highly variable. This variability may be attributed to human disturbance in the flow of water in the estuary.

RESULTS

Data analysis summaries

Rhizospheric and bulk sediment statistical analysis

To analyze the sequences obtained from QB3 Sequencing Center, the phyloseq package in R was utilized to obtain Shannon Index scores, NMDS, and relative abundances from the compiled library sample pool (Berry 2016). After determining the rhizospheric microbial communities' alpha- and beta-diversity scores from Shannon diversity index scores and NMDS, they were compared to the environmental variables salinity, TN, and C:N ratios. These variables were statistically analyzed using linear regression and the Adonis test using the vegan package (Oksanen et al. n.d.) in R. I used relative abundance to observe differences in community composition between each sample transect and within the sample bounds (Figure 5). By observation, I can see that *Proteobacteria*, *Bacteroidota*, *Actinobacteria*, and *Acidobacteria* make up a large portion of the microbial communities in each sample transect and transect bound. These phyla of bacteria are known to play crucial roles in denitrification pathways for nitrogen removal (Pan et al. 2022, Li et al. 2023, Wang et al. 2024, Kalam et al. 2020, Pessi et al. 2022). Smaller portions of the phyla composed in the relative abundance graph such as *Chloroflexi*, *Planctomycetota*, and *Verrucomicrobiota* are also found to be involved in the denitrification pathway (Schwartz et al. 2022, Cheung et al. 2024). The high abundance scores of phyla associated with denitrification within each sampling transect suggest that there are high levels of nitrogen removal through this pathway that occur in the Aliso Creek Estuary sediment.

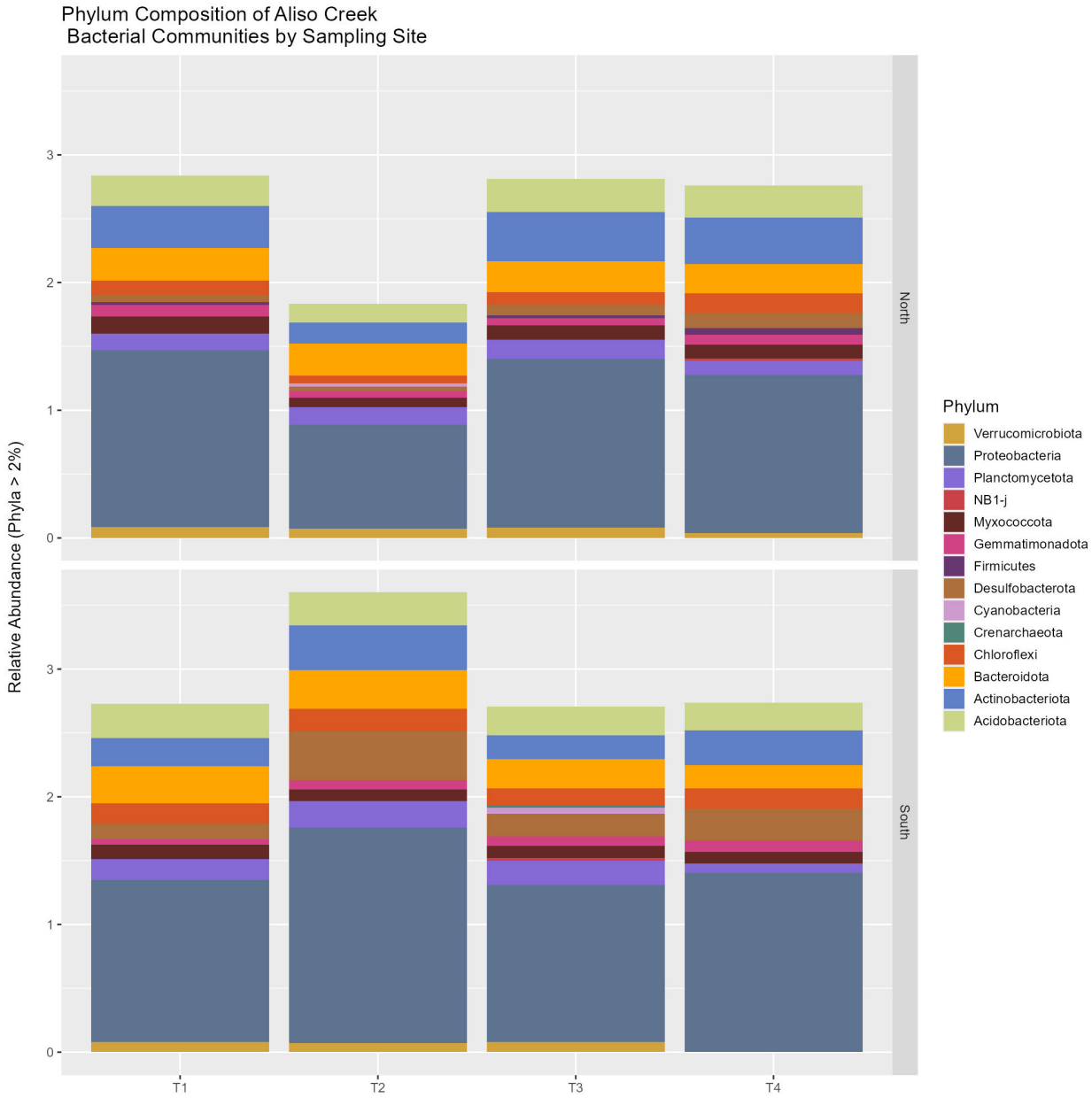

Figure 5. Relative abundance of phyla in the Aliso Creek Estuary bacterial communities by sampling transect and bounds. Relative abundance gives information on the differences in microbial community composition. The lower total relative abundance score for T2 on the northbound can be attributed to only having obtained two samples in this area, whereas the southbound of this transect has a higher relative abundance due to having a duplicate rhizospheric sample in the DNA extraction and sequencing process.

Comparing environmental variables with microbial community diversity. Concentrations of salinity, TN, and C:N ratios in the sediment samples when compared with alpha-diversity measurement, the Shannon Diversity Index, showed no significant difference through linear regression analysis ($p > 0.05$) (Figure 6). Although there was no significant difference, there is a slight negative correlation between Shannon Diversity scores and each environmental variable

(Figure 6). When I looked at beta-diversity measurements through NDMS, there was a significant difference between salinity and microbial community diversity in the sediment ($p < 0.05$) upon performing the Adonis test (Figure 7). All other variables when analyzed through this method were insignificant.

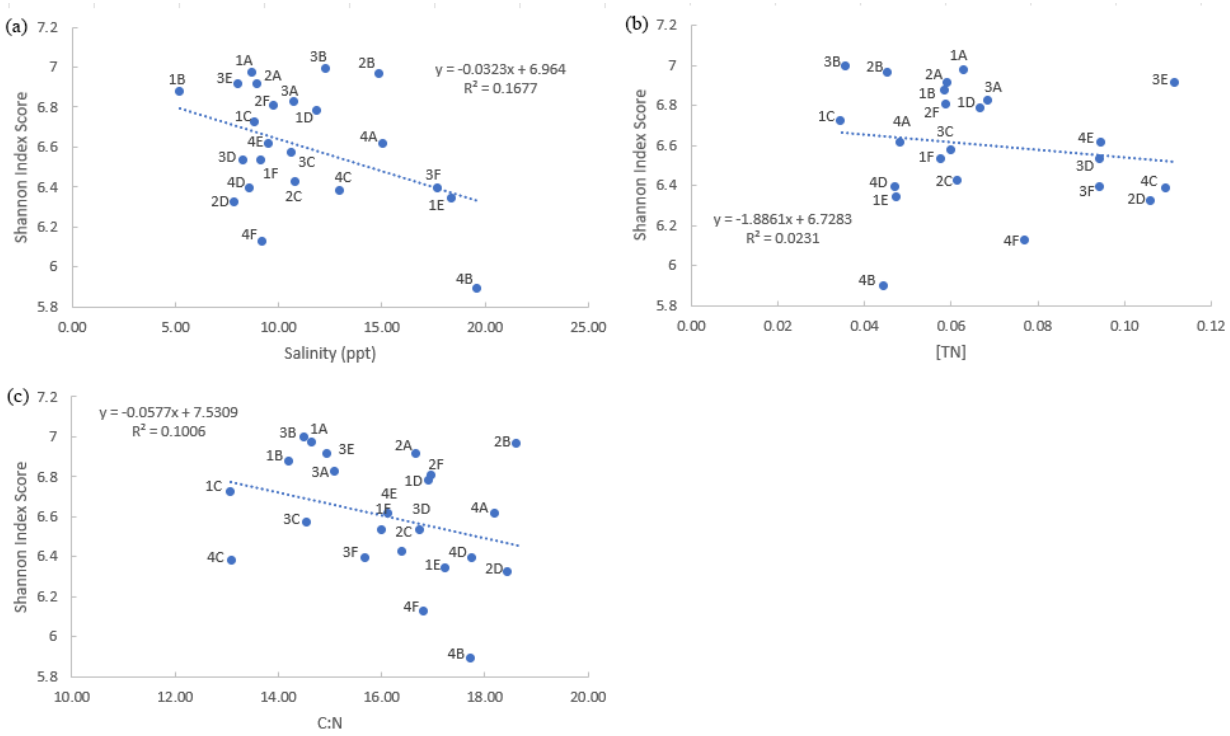

Figure 6. Shannon diversity index scores plotted against salinity in parts per trillion, TN, and C:N ratios. Linear regression graphs were obtained in Excel where statistical tests were performed in R. A weak negative correlation is seen across each variable comparison ($p > 0.05$).

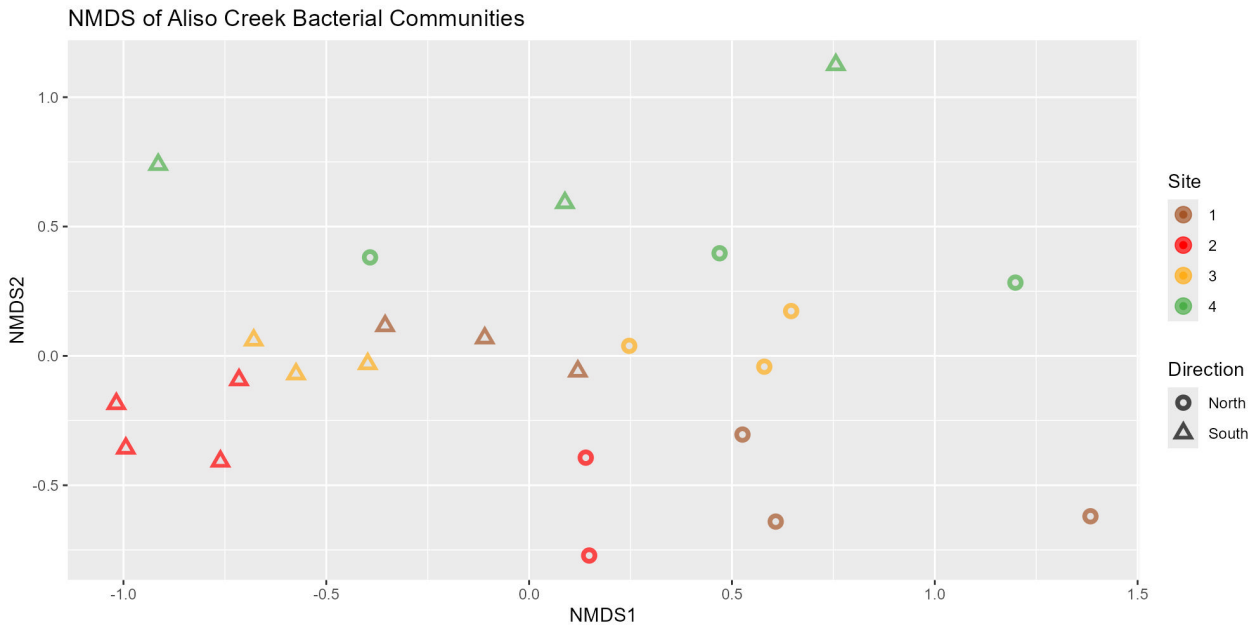

Figure 7. NMDS plot of rhizospheric sediment samples grouped by transect site and direction. After statistical analysis through the Adonis test, a p-value < 0.001 was obtained when testing the significance of direction. Therefore, the null hypothesis is rejected and there is a significant difference between the direction of sites. A p-value of 0.039 was obtained where there is a significant difference when testing for salinity.

DISCUSSION

The results of my research conclude that salinity, TN, and C:N ratios are insignificant ($p > 0.05$) when compared to the alpha-diversity measurements obtained by the Shannon Diversity Index scores of microbial community diversity through linear regression analysis in R. When compared to beta-diversity scores obtained through NMDS in R, I found that concentrations of salinity showed a significant difference ($p < 0.05$) where the other variables were also statistically significant ($p > 0.05$). In addition, when looking at the relative abundance of each rhizospheric sediment sample, there were significant abundances of phyla that contribute to the removal of nitrogen through the denitrification pathway. The removal of nitrogen (N) through denitrification is a key process of mediating N concentrations in coastal watersheds. The rest of this discussion looked at the implications of these results on the Aliso Creek Estuary and their implications for future management and research on this estuary and reflecting coastal estuarine ecosystems across California.

Salinity effect on microbial community diversity

Salinity concentrations, based on previous findings in the literature, have been shown to have a significant effect on the composition of bacterial communities (Li et al. 2021) thus affecting the overall diversity and richness of the microbial community. I observed a significant difference between beta-diversity measurements of rhizospheric sediment samples and salinity concentrations ($p < 0.05$) showing that salinity does affect microbial community beta-diversity. Past literature has solidified this observation found in my study (Zhao et al. 2020, Chen et al. 2022). On the other hand, there was no significant difference observed when compared to alpha diversity ($p > 0.05$). This is contradictory to other observations in previous research that have suggested salinity does have a significant effect on the alpha diversity of microbial communities (Chen et al.). The results I obtained between salinity concentration and beta diversity are expected based on the observations in previous studies, whereas finding no significant difference between salinity and alpha diversity was surprising.

TN concentration effect on microbial community diversity

I examined TN concentrations compared with alpha and beta diversity of rhizospheric microbial communities and found no significant differences ($p > 0.05$). In previous studies, increased loads of TN decreased alpha diversity significantly supports the negative correlation observed in my obtained linear regression graph (Figure 6) (Xu et al. 2022). My lack of significant results in this research suggests that increased or decreased TN does not affect the diversity of microbial communities in rhizospheric sediment. These results are surprising based on previous literature on the subject but can be attributed to my study looking at native TN loads in comparison to loads of increased TN in other studies.

C:N ratio effect on microbial community diversity

I analyzed C:N ratios in bulk sediment and compared them with the alpha and beta diversity of rhizospheric microbial communities and found no significant differences ($p > 0.05$). In previous studies this observation is also made as C:N ratios did not affect the alpha diversity of microbial

communities (Horton et al. 2019). However, a contrasting result of the C:N ratio significantly affecting beta diversity was found (Gawol et al. 2022). My lack of significant results in this research suggests that increased or decreased C:N does not affect the alpha and beta diversity of microbial communities in rhizospheric sediment. These results are surprising based on previous literature on the subject.

Environmental variable effects on microbial community diversity

After going through the statistical analyses of environmental variable effects, salinity was the only variable resulting in a significant difference with beta diversity of microbial communities based on the obtained p-value ($p = 0.039$). All other results obtained suggest that TN and C:N ratios have no significant effect on the alpha and beta microbial community diversity. These results conflict with other research findings as these variables are typically significant. To answer my CRQ asking which environmental variables affect the diversity of microbial communities in the Aliso Creek Estuary, I conclude that salinity is a significant driver in the beta diversity of denitrifying microbial communities.

Limitations

The state of the estuary lacks abundant vegetation to be sampled leaving only nonnative and invasive species in the area. This introduced a sampling design challenge where in the second transect chosen, the north end contained only two targeted macrophyte species where I had hoped to retrieve three samples per location. I lost a resin capsule within the fourth transect due to the estuary flooding from wet-weather runoff and high tides. Due to constraints of time, not all of the lab data I had hoped to achieve were able to be done such as measuring the denitrification potential of each bulk sediment sample. I also was unable to isolate denitrifying microbial community diversity in comparison with the obtained environmental variables which would have further strengthened the results and implications of my study.

Future directions

This research should be further explored with a strengthened sampling design to account for variability in sample locations that may arise due to the state of the estuary. Denitrification potential should be measured in future studies valuing the objective of looking at denitrifying microbial communities to implement stronger arguments in the data analysis. In future studies, the isolation of denitrifying microbial community diversity with obtained environmental variables by binning denitrifying functional genes according to each sample should be implemented as well.

Broader Implications

My research in this study hopes to improve future management considerations of not just the Aliso Creek Estuary restoration efforts (SCWRP), but also the greater majority of estuarine ecosystems along the coast of California. This study also aims to provide sediment management suggestions to the Aliso Creek Restoration efforts and other estuaries facing anthropogenic stress. The conclusions drawn from this research can help to better serve the needs of California's estuarine ecosystem by understanding how these variables affect each other and the sediment quality associated with emergent macrophytes. Through understanding the relationship between environmental variables determining sediment quality and microbial communities associated with the nonnative and invasive vegetation being studied, vegetation management can be pursued based on salinity and nitrogen levels (Ysebaert et al. 2016). A greater source of knowledge on environmental variables affecting the estuarine sediments may be drawn from this research in hopes of allowing further inquiries to be developed and pursued.

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