

Salinity and Biogeochemical Dynamics at Eden Landing Ecological Reserve

Luis Vargas

ABSTRACT

Coastal wetlands are vital ecosystems with a wide array of functions that benefit wildlife and human communities. The purpose of this study was to investigate the ways in which pond salinity influences the biogeochemical dynamics of a coastal wetland system, specifically at Eden Landing Ecological Reserve. To answer this question, I sought to determine whether pH, sediment denitrification capacity, and sediment carbon conversion capacity varied across samples collected from sites of three different salinity levels. To do this, I collected soil and water samples from these three sites for several weeks and conducted batch experiments with the soil to observe the evolution of the concentrations of nitrous oxide (N₂O), carbon dioxide (CO₂), and methane (CH₄) in these experiments. Then, I used ANOVA to determine whether measurements of pH, denitrification capacity, and carbon conversion capacity varied across the salinity groups and used regression analysis to determine whether these measures were correlated with the conductivity that was observed at each sampling point. Regression analysis demonstrated a moderate nonlinear correlation between denitrification capacity and conductivity, with the highest denitrification capacities being observed for low-conductivity samples. No significant differences were observed between salinity levels for all of the metrics and correlations with salinity were weak. However, the medium salinity site exhibited pH levels closest to the center of the ideal range established by the EPA and high salinity values were associated with lower levels of carbon conversion.

KEYWORDS

greenhouse gasses, coastal wetlands, pH, denitrification, carbon sequestration

INTRODUCTION

Though historically overlooked, coastal wetlands are some of the world's most vital ecosystems, providing a wide array of ecosystem services (Clarkson et al. 2014). These include increasing biodiversity, protecting against storm surge, providing recreational space, and improving water quality by filtering out particulate matter and excess nutrients (Clarkson et al. 2014). One of the most crucial functions of wetlands is denitrification, which is the process by which certain species of aerobic bacteria transform nitrates into nitrous oxide (N_2O) (Knowles 1982). Nitrates are a normal part of the nitrogen cycle; in fact, they are one of two forms of nitrogen that are biologically available to plants and algae (Killpack and Buchholz 1993). However, large influxes of nitrates, as well as phosphorus, into water bodies can overstimulate algae, causing algal blooms. When these algae die, microorganisms in the water break them down, consuming much of the dissolved oxygen in the water column in the process. This is called eutrophication and such events have led to the degradation of aquatic ecosystems around the world, occasionally resulting in the mass death of aquatic organisms (Smith and Schindler 2009). Plants and denitrifying bacteria in coastal wetland sediment protect wetlands and the marine ecosystems they feed into from eutrophication by removing nitrates from the water. For this reason, constructed wetlands have become a popular method for mitigating pollution from agricultural and urban runoff (Zhao 2020).

Another crucial aspect of wetlands is their ability to capture and store carbon. The rate of the decomposition of organic matter in soil depends on a variety of factors, including temperature and saturation. The water-logged nature of wetlands creates anoxic conditions in the lower levels of soil that hinders the decomposition of accumulated dead plant matter, effectively capturing and storing carbon in this form (Adhikari et al. 2009). Because of this carbon-storing ability, wetland protection and management has become an important part of strategies proposed by climate scientists to remove carbon from the atmosphere in the face of global climate change. One of the most common metrics used to assess marine health is pH. According to the EPA (2022), the ideal pH range for sustaining marine life is 6.5 to 8.5. This is of particular interest to marine ecologists because of the threat of ocean acidification, which is occurring due to increased atmospheric concentrations of carbon dioxide, which produces acid when dissolved in water. pH fluctuates naturally in wetlands in part due to plant activity. In order to displace cationic nutrients from clay binding sites, plants release hydronium ions, and to displace anionic nutrients, they

release hydroxide ions (Meychik et al. 2021). Thus, fluctuations in local pH can occur due to differences in the availability of cationic and anionic nutrients. pH is also affected by increased organic matter decomposition, as this process breaks down organic molecules into acids and produces carbon dioxide, which dissolves as an acid (Zhang 2017).

Although they are now the subject of extensive scientific interest, wetlands have long been neglected by the land developers and government officials, which has had lasting repercussions on aquatic systems. Globally, up to 87% of wetlands have been lost due to human activities since the 1800s (Davidson 2014). Wetland loss has resulted in decreased biodiversity, decreased land stability, increased vulnerability to storm surge, and poor outflowing water quality (Li et al. 2018). In many modern coastal wetlands, the salinity of pond water has increased dramatically, which has altered the ecology of these regions (Herbert et al. 2015). This is the case in San Francisco Bay, where native wetlands were restructured to increase the salinity of pond water beginning in the 1850s for the purpose of commercial salt production (Takekawa et al. 2006). This change has led to decreased biodiversity and poorer Bay water quality, though it should be noted that increases in pond salinity have created ecological niches that have invited new species to the region (Takekawa et al. 2006).

Some studies have pointed to a correlation between denitrification rate and salinity in wetlands. For example, Neubauer et al. (2018) and Yu et al. (2008) demonstrated that when salt water was introduced to a freshwater marsh, denitrification rates decreased. On the other hand, Fear et al. (2005) observed no change in denitrification rate along a salinity gradient in an estuary. Interestingly, this study also found that variability of denitrification rate increased with salinity. Furthermore, Marton et al. (2012) observed no change in denitrification potential when salinity was increased in soils collected from two sites in a tidal forest and even saw an increase in denitrification potential in soil collected from another site. Other studies have also identified a correlation between respiration or anaerobic digestion rate and salinity. Yang et al. (2018), for example, observed a decrease in respiration activity with increased salinity, which they attributed to changes in microbial community structure. On the other hand, Mottet et al. (2014) observed that a salinity level of 35 g L⁻¹ was most ideal for anaerobic digestion whereas methanogenesis stalled after reaching a salinity of 75 g L⁻¹, suggesting that anaerobic digestion rate slows with increases in salinity above a baseline level. Given these conflicting results, the relationship between salinity and denitrification and carbon sequestration is unclear and appears to be

somewhat dependent on the characteristics of the site under observation. In comparison, fewer studies have been performed analyzing the correlation between salinity and pH in wetlands. Several studies, such as Rugebregt and Nurhati (2020), have examined the correlation between these two measures in seawater, consistently finding a strong negative correlation, to the extent that models have been designed to predict pH using salinity and temperature (Covington and Whitfield 1988). However, the unique dynamics of wetlands beg the question of whether this relation holds true in these systems.

Eden Landing Ecological Reserve (ELER) in southeast San Francisco Bay has been purposefully partitioned into wetland ponds of various salinities in order to attract a wide variety of species and enhance biodiversity (De La Cruz et al. 2018). This is unique among other wetland restorations in the region in that it creates ideal conditions to observe differences in ecosystem dynamics between sites of different pond salinities while reducing the variability of other factors by nature of the proximity of the sites. This begs the question of how these inherent differences in salinity affect the biogeochemical processes discussed thus far. Given that results in other systems vary so widely, investigating the impact of salinity on biogeochemical dynamics within the ELER system could provide new insights on the ecosystem dynamics of the region.

The goal of this project is to quantify differences in biogeochemical dynamics between ponds of varying salinities at ELER. To accomplish this goal, I will specifically ask: (1) Does the pH of pond water differ between sites of different salinities?, (2) Does sediment denitrification capacity differ between sites of different salinities?, and (3) Does sediment carbon conversion capacity differ between sites of different salinities? Here, sediment denitrification capacity refers to the maximum fraction of original soil nitrogen appearing as N_2O that is observed in the batch experiments described below. Similarly, sediment carbon conversion capacity refers to the maximum fraction of original soil carbon appearing as CO_2 and CH_4 that is observed. To answer these questions, I collected water and soil samples from several locations at ELER, processed these samples and recorded measurements in the lab, and transformed the data to perform statistical analysis. Based on the results of the previous studies above, I predicted that pH, denitrification capacity, and carbon conversion capacity would differ between salinity sites and that all three metrics would be inversely correlated with salinity.

METHODS

Study Site

The site I chose to study is Eden Landing Ecological Reserve, a complex of various coastal salt marshes in Hayward and Union City, California that is operated by the California Department of Fish and Wildlife. From the late 1800s until the late 20th century, Eden Landing was the site of an expansive saltworks, which transformed the ecology of the wetland region. The extensive development of the land and the increased salinity level of pond water forced out many native species and led to a decrease in ecosystem productivity (“Background Report” 2002). The South Bay Salt Pond Restoration Project was created to address these issues, and since 2000, has restored many wetlands throughout the Bay Area, including ELER. The project team took inspiration from the saltworks in designing the reserve, moving Bay water through a series of ponds of increasing salinity, not to harvest salt, but to cater to a diverse community of birds and other wildlife that require a wide array of salinity conditions (“Eden Landing Ecological Reserve” 2022). This built-in salinity gradient provides a unique opportunity to study the influence of salinity on wetland dynamics, and the proximity of the ponds reduces the influence of confounding variables. Twenty four sampling locations were under initial consideration, all at the edges of ponds accessible via the loop trail in the northern portion of the reserve. To narrow these down to three sites, I sampled water from each site and measured the electrical conductivity of the samples in the lab. Then I selected a site of low conductivity, a site of medium conductivity, and a site of high conductivity to sample from for the duration of the experiment (Figure 1).



Figure 1. Map of sampling locations under initial consideration. Sampling locations under initial consideration are represented by the yellow stars. The low, medium, and high salinity sites chosen are colored in green, orange, and red respectively. Source: South Bay Salt Pond Restoration Project

pH Measurements and Analysis

To collect pH and DO measurements, I used 50 ml Falcon tubes to sample 50 ml of water from each of the three sample sites each week. I kept these samples on ice and then in the refrigerator until I could take the water quality measurements. In the lab, I first measured the electrical conductivity of the samples as a proxy for salinity using a conductivity meter. Then I measured pH using a pH meter and took readings after the electrode had been submerged in the sample for one minute. I repeated this process for five weeks, yielding five pH and conductivity measurements for each of the three sampling sites.

Sediment Collection and Batch Experiments

To collect sediment for denitrification capacity and carbon conversion capacity analysis, I took duplicate 15 cm soil core samples from the three sites each week. I collected these cores using plastic tubes that measured 6 cm in diameter and 30 cm in length. The soil cores were stored on ice and then air-dried on plastic sheets until the sediment was dry to the touch, which took about one week. Once it was dry, I crushed the sediment using a mortar and pestle and sieved it through 2 mm mesh. Then, I transferred 50g of each soil sample into 250 ml bottles

with 200 ml of deionized water, which were flushed with nitrogen gas for 1 hour, and crimped the bottles shut. I incubated these samples in the dark at 30°C on a shaker for 7 days and sampled headspace gas at the following time points: 0 hr, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 24 hr, 48 hr, 72 hr, 96 hr, 120 hr, and 144 hr. To sample gas, I used a syringe to extract 10 ml of gas from the bottles through the rubber stopper and injected it into 20 ml headspace vials, which were previously flushed with nitrogen and crimped. These vials were refrigerated until chromatography analysis. I repeated this process three times, producing three time series for each of the sampling sites samples.

Gas Chromatography and Elemental Analysis

To find the concentrations of N_2O , CO_2 , and CH_4 in the headspace vials, I used a Shimadzu GC-14A gas chromatograph (GC) and measured the peak areas of these target gasses. I built a regression model for each day that I used the GC to estimate gas concentrations based on measured peak area values. To generate this model, I first I ran several samples of a standard gas mix and outdoor air through the GC and chose 3 samples of the standard whose peak area results had a coefficient of variation of less than 3% and 3 samples of the outdoor air whose peak results had a coefficient of variation of less than 5%. I then performed linear regression on these points to derive an equation for the prediction line. In addition, I determined the concentrations of carbon and nitrogen in the original soil samples using a CN analyzer, to be used in calculating the fraction of nitrogen present as N_2O and the fraction of carbon present as CO_2 . These quantities were used to standardize the quantities of the product gasses instead of original nitrate organic carbon concentrations because of difficulties accessing an ion chromatograph and a TIC/TOC analyzer. Thus, I make the assumption that nitrate and organic carbon form equal fractions of total nitrogen and carbon respectively in the original soil samples.

Calculating the Fraction of Soil Nitrogen and Carbon Present as Target Gasses

Once I had the concentrations of each target gas in the headspace vials, I transformed these values to calculate the fraction of nitrogen present as N_2O and the fraction of carbon present as CO_2 and CH_4 . I first calculated the moles of gas that occupied each headspace vial

using known pressure, temperature, and volume values. Here, pressure was calculated using the fact that 10 ml of gas were injected into 20 ml vials containing gas at atmospheric pressure. Then, I multiplied the moles of gas in the vial by the concentrations of the target gasses, which were in parts per million (ppm), to yield the moles of each gas that were in the 10 ml transfer syringe. I divided this quantity by the syringe volume to yield the relevant concentration and multiplied this value by the sum of the volumes of the headspace of the batch experiment bottles and the transfer syringe to yield the moles of each gas that were in the headspace before each gas sample was taken.

To account for the gas that was dissolved in the aqueous phase of the batch experiment bottles, I applied Henry's Law using the partial pressures of each target gas in the headspace as well as Henry's Constants for each gas from Sander (2015). To calculate the partial pressures of each gas in the headspace of the bottles, I first had to determine the bottle pressure and headspace gas concentration at each sampling point. To calculate bottle pressure, I used the pressure and volume of the bottle before each sample was taken and treated each headspace gas sampling as an isothermal expansion. To calculate the headspace gas concentration, I used the headspace pressures that I had calculated and the temperature and volume of the bottle to find the moles of gas in the headspace and divided the moles of each target gas in the headspace by this value.

Once I had the partial pressures of the target gasses in the headspace, I derived the aqueous concentrations of the gasses and multiplied this value by the volume of water to find the moles of each gas that were dissolved in water. Then, I took the sum of the quantity of each gas in the headspace and in the water to determine the total moles of each gas that were produced. To account for gas that was lost in headspace sampling, I added the quantity of each target gas in the sampling syringe from each previous headspace sample to the quantity of gas observed at each sampling point. To account for the amount of target gas originally in the headspace, I calculated the amount of the gasses using their ambient concentrations and headspace volume and subtracted this quantity from each gas measurement.

To determine the fraction of nitrogen present as N_2O , I divided the quantity of N_2O in the bottle at each point by the amount of nitrogen that was originally observed in each soil sample. To determine the fraction of carbon present as CO_2 and CH_4 , I added the quantities of these gasses at each sampling point and divided this value by the amount of carbon that was originally

observed in each soil sample. Finally, I averaged the values yielded by the duplicate soil samples to produce one time series for each target gas for each batch experiment set. The maximum fraction of nitrogen present as N_2O in each time series was labeled as denitrification capacity for that sample (Figure 4) and the maximum fraction of carbon present as CO_2 and CH_4 in each time series was labeled as the carbon conversion capacity (Figure 7).

Data Analysis

The data analysis for the pH, denitrification capacity, and carbon conversion capacity data consisted of repeated-measures ANOVA as well as linear regression. In repeated measures ANOVA, the predictor variable was the salinity level and the response variable was the pH of the water, the sediment denitrification capacity, or the sediment carbon conversion capacity. In linear regression, the predictor variable was the pond salinity at the time the sample was taken and the response variable was the pH of the water, the sediment denitrification capacity, or the sediment carbon conversion capacity. I performed repeated-measures ANOVA in Python using the `anovaRM` function from the `statsmodels` library and chose the standard significance level of 0.05. I performed regression analysis using the regression tools in Excel and aggregated data across salinity levels and sampling dates.

RESULTS

pH Measurements

The results of the repeated-measures ANOVA show that pH measurements do not differ significantly between salinity groups. The average pH for the low, medium, and high salinity groups respectively were as follows: 7.032 (90%CI [6.9598, 7.1042]), 7.198 (90%CI [7.047, 7.349]), and 7.096 (90%CI [7.0156, 7.1764]) (Figure 2). There was no statistically significant difference in pH between salinity groups, $F(4,2) = 1.7578$, $p = 0.2329$.

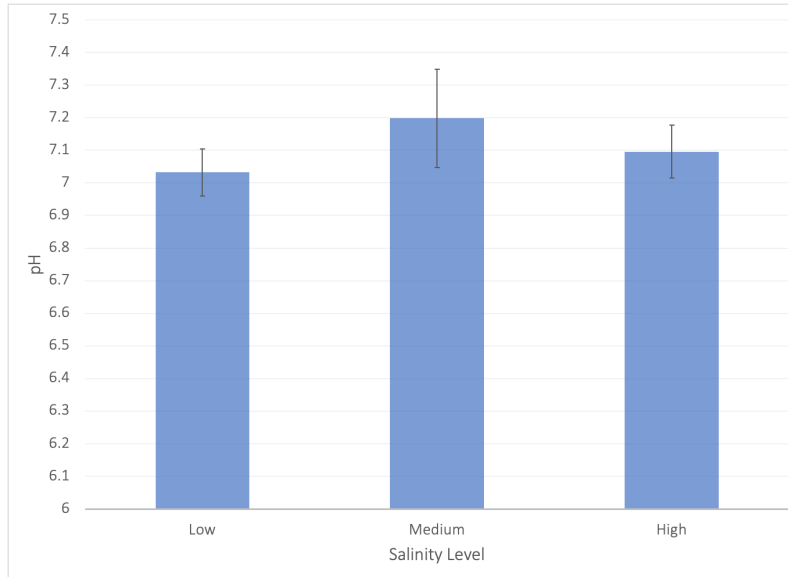


Figure 2. Comparison of mean pH between salinity groups. pH was similar between salinity levels, with significant overlap of the 90% confidence intervals of the mean pH between groups.

The results of linear regression between pond conductivity and pH yielded an R^2 value of 0.031, indicating a weak correlation between pH and conductivity (Figure 3).

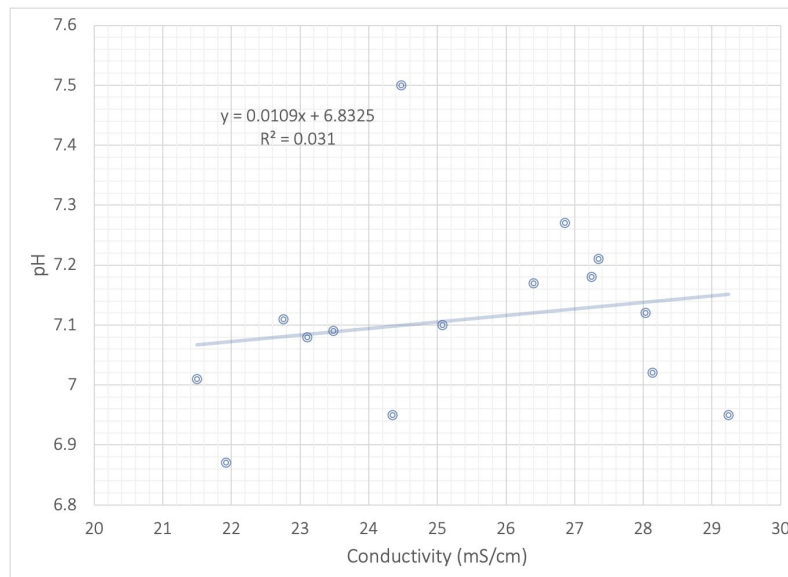


Figure 3. Correlation between pH and conductivity. The correlation between pH and conductivity was weak. The highest pH values and variation were observed in the medium salinity range.

Denitrification Capacity

Repeated measures ANOVA demonstrated that denitrification capacity did not differ significantly between salinity groups, although the p-value was the lowest among the three metrics under analysis. The fraction of nitrogen present as N_2O peaked at 24 hours for the low and high salinity levels and at 6 hours for the medium salinity level. The values at these peaks were used in the data analysis.

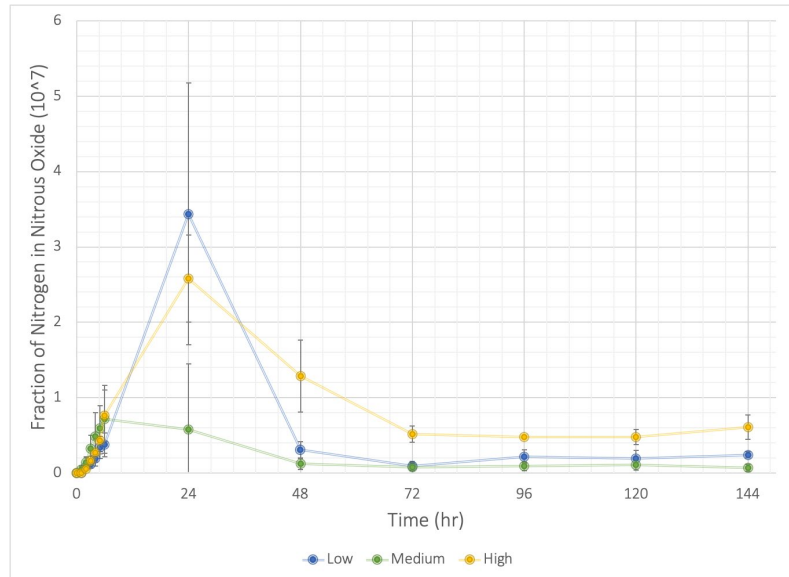


Figure 4. Time series of the fraction of nitrogen present as N_2O . The fraction of nitrogen in N_2O peaked early in the batch experiment for all salinity groups.

The average denitrification capacity for each of the salinity groups was as follows: 3.44×10^{-7} (90%CI [1.79×10^{-7} , 5.09×10^{-7}]), 1.01×10^{-7} (90%CI [3.71×10^{-6} , 1.65×10^{-7}]), and 2.58×10^{-7} (90%CI [2.03×10^{-7} , 3.13×10^{-7}]) (Figure 5). No statistically significant difference in denitrification capacity was observed between salinity groups, $F(2,2) = 5.2955$, $p = 0.0752$.

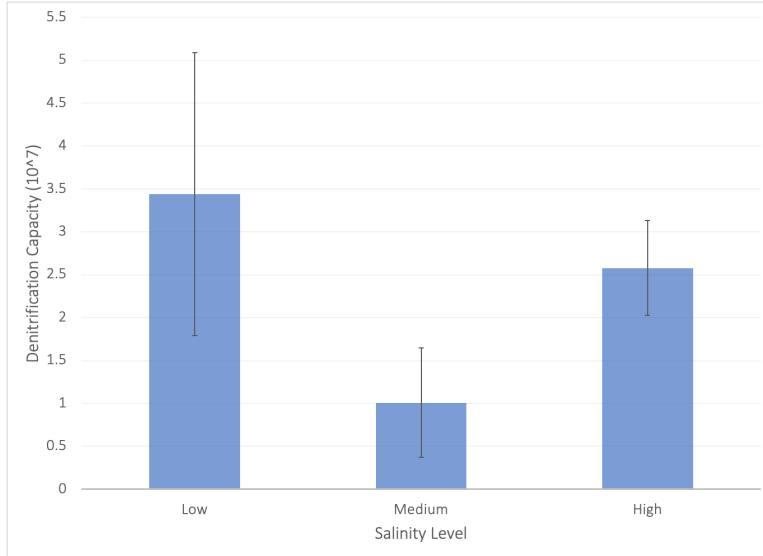


Figure 5. Comparison of the mean denitrification capacity between salinity groups. Denitrification capacity did not differ significantly between salinity levels, with a slight overlap between the 95% confidence intervals of the groups.

The results of linear regression between pond conductivity and pH yielded an R^2 value of 0.091, indicating a weak correlation between pH and conductivity (Figure 6).

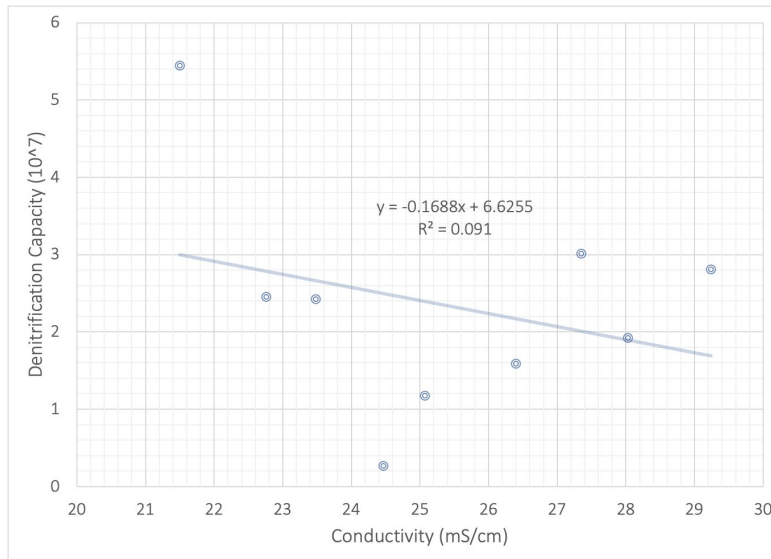


Figure 6. Correlation between denitrification capacity and conductivity. Denitrification capacity and conductivity exhibited a relatively weak correlation.

Carbon Conversion Capacity

The results of ANOVA demonstrated that carbon conversion capacity did not differ significantly between salinity groups. The fraction of carbon in CO₂ and CH₄ increased steadily throughout the batch experiments, so the value at 144 hours was used in repeated-measures ANOVA.

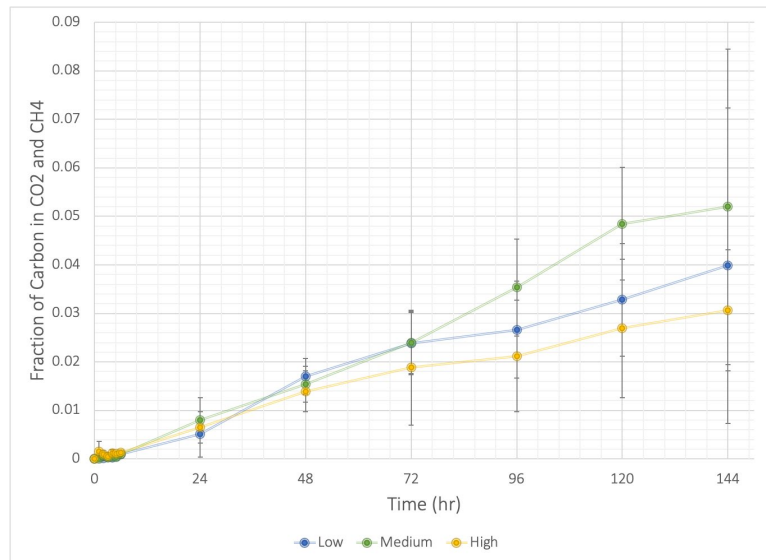


Figure 7. Time series for the fraction of carbon in CO₂ and CH₄. The fraction of carbon present as CO₂ and CH₄, as well as the variation in these values, grew steadily for all salinity groups. The values at 144 hours were used in ANOVA.

The average carbon conversion capacity for each of the salinity groups was as follows: 3.99×10^{-2} (90%CI [2.14×10^{-2} , 5.84×10^{-2}]), 5.20×10^{-2} (90%CI [2.11×10^{-2} , 8.29×10^{-2}]), and 3.06×10^{-2} (90%CI [1.88×10^{-2} , 4.24×10^{-2}]) (Figure 8). No statistically significant difference in denitrification capacity was observed between salinity groups, $F(2,2) = 5.2955$, $p = 0.0752$.

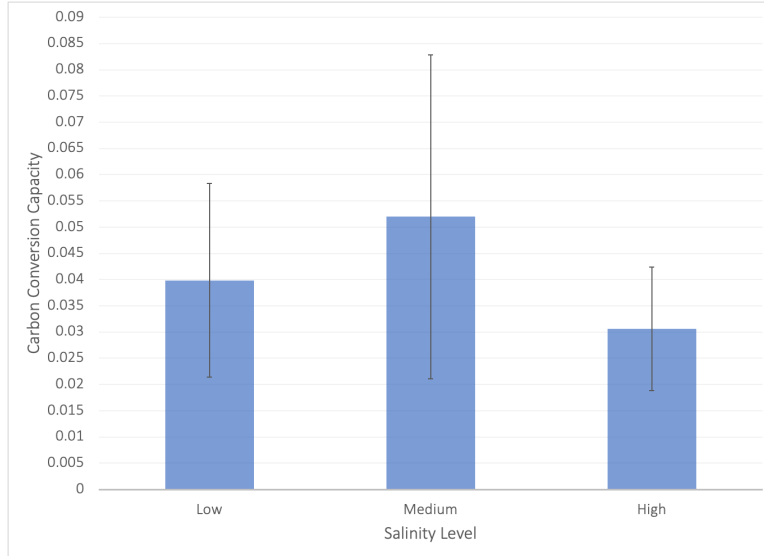


Figure 8. Comparison of the mean carbon conversion capacity between salinity groups. Carbon conversion capacity did not differ significantly across salinity levels. There is significant overlap between the 90% confidence intervals of the groups.

The results of linear regression between pond conductivity and pH yielded an R^2 value of 0.1125, indicating a weak correlation between pH and conductivity (Figure 9).

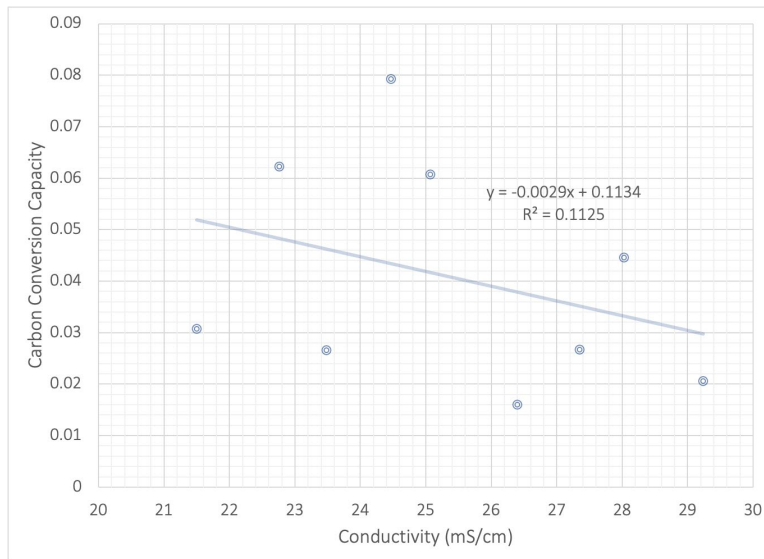


Figure 9. Correlation between carbon conversion capacity and conductivity. There was a slight negative correlation between carbon conversion capacity and conductivity.

DISCUSSION

Through this study, I determined whether each of the variables under consideration varied with salinity. Contrary to my hypothesis, none of the variables under consideration varied significantly between salinity levels. I found that the pH of water samples did not differ significantly between salinity groups and the correlation between pH and salinity was weak. In addition the denitrification capacity of the soil samples did not differ significantly between the groups and there appeared to be a weak correlation between salinity and denitrification capacity. Furthermore, the carbon conversion capacity of soil samples did not differ significantly between soil samples and the correlation between salinity and carbon conversion capacity was also weak. There was a high amount of variation in the results, which may be due to the influence of environmental factors other than salinity. This level of variation suggests that the relationship between salinity and these biogeochemical processes is somewhat particular to the wetland system under observation.

Relationship between salinity and pH

The results of repeated measures ANOVA indicated that the pH of water samples did not differ significantly between salinity groups. pH was also very weakly correlated with salinity. There was a small difference in pH between the salinity groups, but it was not as strong as I had expected based on previous studies, such as Rugebregt and Nurhati (2020), which demonstrated a negative correlation between the values in seawater. These results are evident in the analysis of differences between means (Figure 2), where the 90% confidence intervals of the means in the different salinity groups overlap significantly despite differences in the means. I hypothesized that I would observe a positive correlation between pH and salinity, but this was not strongly demonstrated in the results of the linear regression, which yielded an R^2 value of 0.031, indicating a weak correlation. One reason that pH was observed to be higher in the medium conductivity range might be because of potential differences in plant activity at this sampling location. As Meychik et al. (2021) demonstrates, plant uptake of nutrients can influence the pH of the surrounding soil. Thus, the high pH levels observed at this site could be due to higher levels of nitrate compared to ammonium at this sampling location. Although the results did not

support my hypothesis that pH differs between salinity levels and that pH is positively correlated with salinity, the difference in vegetation levels and potential differences in the ratio of nitrate and ammonium concentrations between sampling sites may have confounded the data. Further studies could take care to select sites with similar levels of vegetation and nitrate and ammonium concentrations.

Also observed in the data was high variation in pH at the medium salinity site. This variability could be due to observed variation in the amount of organic matter present in the water samples upon collection. Because water samples were refrigerated for several weeks before pH measurements were taken, this may have allowed time for further decomposition and the release of organic acids in some samples. Among the three salinity levels observed, the average salinity closest to the middle of the ideal pH range identified by the EPA was the medium salinity level, which had an average pH of 7.198 (90%CI [7.047, 7.349]). This fact suggests that the medium salinity level is the most ideal for ecosystem productivity, however wetland managers should take note of the high level of variation when making decisions regarding salinity levels of wetland ponds.

Relationship between salinity and sediment denitrification capacity

Repeated measures ANOVA results revealed that denitrification capacity did not differ significantly between salinity groups. These results are visible by the slight overlap of the 95% confidence intervals for the mean denitrification capacities of the medium salinity level with that of the low salinity level. Denitrification capacity was also weakly correlated with salinity level, with a generally negative trend observed. This trend was also observed in Yu et al. (2008), which studied the effect of the introduction of seawater on denitrification in freshwater sediment, and Meng et al. (2020), which investigated the influence of soil salinity and moisture on nitrification and denitrification in a riparian wetland. In both of these studies, a negative correlation between sediment denitrification and salinity was observed. Neubauer et al. (2018) also observed a similar correlation in studies of the effect of saltwater intrusion on microbial community structure and denitrification in a tidal freshwater marsh. In this case, the researchers attributed the trend to a decrease in the population and activity of denitrifying bacteria at high salinity

levels. This effect of salinity on microbial communities might explain the slight negative trend observed in this experiment.

There was significant variation in denitrification measurements, particularly in the low salinity group, which could be due to the influence of a wide range of variables on the denitrification process. The time series for the fraction of nitrogen present as N_2O behaved differently than expected. In all samples, the fraction of nitrogen present as N_2O peaked towards the beginning of the incubation, with the low and high salinity groups peaking at 24 hours and the medium salinity level peaking at 6 hours. This peaking behavior could be due to two processes occurring at the same time: the slowing of denitrification due to the depletion of sediment nitrate, and the conversion of N_2O gas into N_2 , which is the next step in the nitrogen cycle (Stein & Klotz 2016). To remedy this, future studies could spike batch experiments with equal quantities of nitrate, because the amount of nitrate present in the soil samples was insufficient to support more conversion of NO_3^- to N_2O than conversion of N_2O to N throughout the batch experiment. Future studies could also analyze the amount of N_2 produced, which was not analyzed in this study due to lack of access to the appropriate instrument. All things considered, the low salinity level appears to be the most ideal for maximizing denitrification capacity and removing excess nitrate from the Eden Landing system. As Neubauer et al. (2018) suggests, the decrease in denitrification at high salinity levels could be due to the decrease in microbial activity with increased salinity.

Relationship between and sediment carbon conversion capacity

The results of ANOVA revealed that carbon conversion capacity did not differ significantly between salinity groups. There were subtle differences in mean carbon conversion capacity between groups, however, the 90% confidence intervals of the mean carbon conversion capacities overlap significantly (Figure 8), indicating no statistical difference in carbon conversion capacity between groups. A weak negative correlation was also observed between conductivity and carbon conversion capacity (Figure 9). These results are in agreement with Qu et al. (2018), which investigated the effects of soil salinity on the rate of soil organic carbon decomposition in a tidal wetland and observed a negative correlation between salinity and organic matter decomposition. Fei Xi et al. (2014) similarly observed decreased rates of

microbial respiration with increased salinity in studies of the effect of salinity on soil respiration in an estuarine wetland, which they attributed to a restricting effect of salinity on heterotrophic bacteria. On the other hand, in studies of the effect of simulated salt water intrusion on greenhouse gas production in incubated tidal forest sediment, Marton et al. observed an increase in CO₂ production and a decrease in CH₄ production with higher salinity. This difference in effects on decomposition products might explain the weak correlation observed in my results, as the ratio of the production of CO₂ to production of CH₄ seemed to differ between sample sites. This discrepancy in CO₂ and CH₄ production could be investigated in future studies.

A high level of variation in carbon conversion capacities was observed across salinity levels. This variability could be due to variation in the amount of organic carbon originally present in the samples. Lai et al. (2013) studied the effects of the addition of organic matter and water on CO₂ flux in an arid soil and observed that respiration rates in soil increased with an increased level of organic material originally present in the sample. Thus, although I standardized my data using the amount of carbon originally present in each sample, the amount of organic carbon originally present may have confounded this data. The medium salinity sampling site was more vegetated than the low and high salinity sites, and individual samples from this site appeared to have different amounts of organic matter mixed in. This discrepancy in the amount of organic matter originally present in soil samples may explain the higher carbon conversion capacity and higher level of variation observed in the data for this site.

The time series for the fraction of carbon converted to CO₂ and CH₄ increased fairly linearly across salinity groups, and variation in the fraction of carbon converted to CO₂ and CH₄ also increased with time. This increase in variation may be due to some samples depleting their organic carbon faster than others, leading to a slower increase in the fraction of carbon in CO₂ and CH₄ as the batch experiments went on. Thus, future experiments could spike samples with a high amount of organic carbon to ensure that decomposition rate remains fairly steady. All things considered, high salinity levels appear to be the most ideal for minimizing respiration and anaerobic digestion and retaining carbon in the Eden Landing system, which are common goals in preventing greenhouse gas emissions. As Fei Xi et al. (2014) suggests, lower sediment respiration and anaerobic digestion rates at high salinity could be attributed to the restricting effect of increased salinity on microbial activity.

Limitations

The results of previous experiments show that the relationship between salinity and wetland biogeochemistry is highly dependent on the region under observation and is affected by many other factors such as nutrient supply, microbial community structure, and soil composition. Thus, the results of this experiment apply only to the Eden Landing system and do not characterize the relationship between salinity and biogeochemical processes in all wetlands. In addition, the conclusions of this study rely on the assumption that all factors other than salinity are the same at each sample location. This assumption was made due to the inherent similarities between sample sites due to their geographic proximity. However, there exist differences between sites that were out of my control and did not fit in the scope of the study. Although statistical differences and correlations were found throughout the study, significant variation was present in the data, which can be attributed to this lack of control over independent variables in the study. This is characteristic of all observational studies. The results are further limited by the number of sampling locations and sample points chosen for the study, which was kept minimal due to the lack of a research team. To build a more complete model of the influence of salinity on biogeochemical processes at Eden Landing, more sampling locations could be selected and more samples could be taken. In addition, several human errors occurred during the process which may have confounded the data slightly. For example, a few data points were lost from the gas concentration measurements. These were accounted for by using the average of the data points adjacent to the lost measurement.

Future Directions

The goal of this study was to characterize the relationship between salinity and pH, denitrification capacity, and carbon conversion capacity at Eden Landing Ecological Reserve. Future studies could expand on this study design by adding sample sites and sampling more times in order to build more robust models. There were many variables that were not incorporated in the scope of the project, such as seasonality, microbial community structure, soil characteristics, and limiting nutrients. These could be the focus of further studies in the region. In

addition, studies could be conducted using a similar design in other coastal wetland regions, particularly those undergoing restoration.

Conclusion

The results of this experiment indicate that the salinity of ponds at Eden Landing has a subtle influence on the biogeochemical processes that occur in the water and sediment of the reserve. All measures under consideration varied to some extent with salinity, but none of them varied with statistical significance. Medium salinity levels appeared to be conducive to maintaining pH well within the range established by the EPA. Meanwhile, low salinity appears to be related with high denitrification capacity, which is crucial in reducing eutrophic events. Finally, high salinity seemed to be related with low levels of carbon conversion, which is important for retaining carbon in wetlands. My hope is that these findings can be used by wetland scientists to gain insight on the way salinity influences these processes and perhaps influence recommendations on future wetland restorations. In particular, the knowledge gained from experiments like this can help us better design wetland restorations that best serve local wildlife as well as our communities.

ACKNOWLEDGEMENTS

This project would not have been possible without the efforts and contributions of the following people. Thank you to Celine Pallud for guiding me in designing my experiment and opening her lab to me when others were hesitant to. It was only because of the high-quality equipment and materials that I was given access to that I was able to collect all of my data. I am grateful for Pranjal Dwivedi, a PhD candidate in the Pallud Lab, who spent time guiding me in developing my methods and teaching me how to use lab equipment. I also appreciate the ESPM 175 teaching team for their dedication to guiding us in developing our projects. I would like to thank Patina Mendez in particular for her thoughtful advice and recommendations on my experimental design and writing and for putting so much effort into developing and presenting learning materials to our class every week for three semesters. I am also grateful to the Silver Lab for opening up their space for me to use for gas chromatography and elemental analysis. I would like

to thank Gisela Gonzalez Pagan and Tibisay Perez in particular for training me on the GC. Thank you to Tibisay again for sending me an Excel sheet that made processing GC data so much easier. I'm also thankful to Geoffrey Logan for setting up the GC every day before I used it, sometimes on short notice, and for always offering help and advice. I'm especially grateful for my family: Elizabeth Solis, Jose Vargas, Maria Vargas, Anna Vargas, and Leonardo Vargas for accompanying me on sampling trips and offering their unconditional support throughout this project. Finally, I'd like to thank my friends, especially Kendall Wade, who never hesitated to offer support when I felt stressed or overwhelmed. And thanks again to my family and friends for being there and cheering me on at the Environmental Sciences Senior Thesis Symposium.

REFERENCES

- Adhikari, S., R. Bajracharya, and B. Sitaula. 2009. A Review of Carbon Dynamics and Sequestration in Wetlands. *Journal of Wetlands Ecology* 2:42–46.
- Background Report on the Cargill Salt Ponds. 2002.
<https://www.southbayrestoration.org/document/background-report-cargill-salt-ponds>.
- Bertoldi, G. L., and W. C. Swain. (n.d.). California Wetland Resources. U.S. Geological Survey.
- Clarkson, B. R., A.-G. E. Ausseil, and P. Gerbeaux. 2013. Wetland ecosystem services. *Ecosystem services in New Zealand – conditions and trends*:192–202.
- Covington, A. K., and M. Whitfield. 1988. Recommendations for the Determination of pH in Sea Water and Estuarine Waters. *Pure & Applied Chemistry* 60:865–870.
- Dahl, T. E., and G. J. Allord. (n.d.). History of Wetlands in the Conterminous United States. *Technical Aspects of Wetlands*:8.
- Davidson, N. 2014. How much wetland has the world lost? Long-term and recent trends in global wetland area. *Marine and Freshwater Research* 65:936–941.
- De La Cruz, S. E. W., L. M. Smith, S. M. Moskal, C. Strong, J. Krause, Y. Wang, and J. Y. Takekawa. 2018. Trends and habitat associations of waterbirds using the South Bay Salt Pond Restoration Project, San Francisco Bay, California. Page 5 Open-File Report. U.S. Geological Survey.
- Eden Landing Ecological Reserve. 2023.
<https://wildlife.ca.gov/Lands/Places-to-Visit/Eden-Landing-ER#10541120-history>.
- Fear, J. M., S. P. Thompson, T. E. Gallo, and H. W. Paerl. 2005. Denitrification rates measured along a salinity gradient in the eutrophic Neuse River estuary, North Carolina, USA.

Estuaries 28:608–619.

- Fei Xi, X., L. Wang, J. Jun Hu, Y. Shu Tang, Y. Hu, X. Hua Fu, Y. Sun, Y. Fai Tsang, Y. Nan Zhang, and J. Hai Chen. 2014. Salinity influence on soil microbial respiration rate of wetland in the Yangtze River estuary through changing microbial community. *Journal of Environmental Sciences* 26:2562–2570.
- Herbert, E. R., P. Boon, A. J. Burgin, S. C. Neubauer, R. B. Franklin, M. Ardón, K. N. Hopfensperger, L. P. M. Lamers, and P. Gell. 2015. A global perspective on wetland salinization: ecological consequences of a growing threat to freshwater wetlands. *Ecosphere* 6:art206.
- Hey, D. L., A. L. Kenimer, and K. R. Barrett. 1994. Water quality improvement by four experimental wetlands. *Ecological Engineering* 3:381–397.
- Jinglie, Z., S. M. Theroux, B. de M. C. P, W. H. Hartman, T. Ye, and T. S. G. 2022. Microbial drivers of methane emissions from unrestored industrial salt ponds. *The ISME Journal* 16:284–295.
- Killpack, S. C., and D. Buchholz. 1993. Nitrogen in the environment : nitrogen's most common forms. University of Missouri Extension:1.
- Knowles, R. 1982. Denitrification. *Microbiological Reviews* 46:43–70.
- Lai, L., J. Wang, Y. Tian, X. Zhao, L. Jiang, X. Chen, Y. Gao, S. Wang, and Y. Zheng. 2013. Organic Matter and Water Addition Enhance Soil Respiration in an Arid Region. *PLOS ONE* 8:e77659.
- Li, X., R. Bellerby, C. Craft, and S. E. Widney. 2018. Coastal wetland loss, consequences, and challenges for restoration. *Anthropocene Coasts* 1:1–15.
- Marton, J. M., E. R. Herbert, and C. B. Craft. 2012. Effects of Salinity on Denitrification and Greenhouse Gas Production from Laboratory-incubated Tidal Forest Soils. *Wetlands* 32:347–357.
- Meng, Y., Z. He, B. Liu, L. Chen, P. Lin, and W. Luo. 2020. Soil Salinity and Moisture Control the Processes of Soil Nitrification and Denitrification in a Riparian Wetlands in an Extremely Arid Regions in Northwestern China. *Water* 12:2815.
- Meychik, N., Y. Nikolaeva, and M. Kushunina. 2021. The significance of ion-exchange properties of plant root cell walls for nutrient and water uptake by plants. *Plant Physiology and Biochemistry* 166:140–147.
- Mottet, A., F. Habouzit, and J. P. Steyer. 2014. Anaerobic digestion of marine microalgae in different salinity levels. *Bioresource Technology* 158:300–306.

- Neubauer, S. C., M. F. Piehler, A. R. Smyth, and R. B. Franklin. 2019. Saltwater Intrusion Modifies Microbial Community Structure and Decreases Denitrification in Tidal Freshwater Marshes. *Ecosystems* 22:912–928.
- Qu, W., J. Li, G. Han, H. Wu, W. Song, and X. Zhang. 2019. Effect of salinity on the decomposition of soil organic carbon in a tidal wetland. *Journal of Soils and Sediments* 19:609–617.
- Rugebregt, M. J., and I. S. Nurhati. 2020. Preliminary Study of Ocean Acidification: Relationship of pH, Temperature, and Salinity in Ohoililir, Southeast Maluku. *IOP Conference Series: Earth and Environmental Science* 618:012004.
- Sander, R. 2015. Compilation of Henry's law constants (version 4.0) for water as solvent. *Atmospheric Chemistry and Physics* 15:4399–4981.
- Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? *Trends in Ecology & Evolution* 24:201–207.
- Stein, L. Y., and M. G. Klotz. 2016. The nitrogen cycle. *Current Biology* 26:R94–R98.
- Takekawa, J. Y., A. K. Miles, D. H. Schoellhamer, N. D. Athearn, M. K. Saiki, W. D. Duffy, S. Kleinschmidt, G. G. Shellenbarger, and C. A. Jannusch. 2006. Trophic structure and avian communities across a salinity gradient in evaporation ponds of the San Francisco Bay estuary. *Hydrobiologia* 567:307–327.
- US EPA, O. 2013, November 21. Indicators: Acidification. Overviews and Factsheets. <https://www.epa.gov/national-aquatic-resource-surveys/indicators-acidification>.
- Visiting the Pond Complexes. (n.d.). <https://www.southbayrestoration.org/pond-complexes/eden-landing>.
- Yang, J., C. Zhan, Y. Li, D. Zhou, Y. Yu, and J. Yu. 2018. Effect of salinity on soil respiration in relation to dissolved organic carbon and microbial characteristics of a wetland in the Liaohe River estuary, Northeast China. *Science of The Total Environment* 642:946–953.
- Yu, K., R. Delaune, and D. Seo. 2008. Influence of salinity level on sediment denitrification in a Louisiana estuary receiving diverted Mississippi River water. *Archives of Agronomy and Soil Science* 54:249–257.
- Zhang, H. 2017, April 1. Cause and Effects of Soil Acidity - Oklahoma State University. <https://extension.okstate.edu/fact-sheets/cause-and-effects-of-soil-acidity.html>.
- Zhao, Y., B. Ji, R. Liu, B. Ren, and T. Wei. 2020. Constructed treatment wetland: Glance of development and future perspectives. *Water Cycle* 1:104–112.