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How Does Hummock Creation in Submerging Salt Marshes Alter Nitrous Oxide Fluxes?

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Abstract

Climate change is altering ecosystems and the services they provide. Salt marsh ecosystems typically protect coastal areas and filter nitrogen out of water, but are rapidly submerging due to rising sea levels and human development that prevents landward migration. Recent restoration efforts to preserve salt marshes attempt to build elevation capital and promote vegetation and animal habitat, but it is unclear how such efforts affect salt marsh biogeochemistry and dynamics of nitrous oxide, a potent greenhouse gas. To better understand how adding sediment to submerging salt marshes may alter nitrous oxide fluxes, I leveraged a salt marsh hummock creation experiment to test how elevation gradients and planting treatments alter nitrous oxide fluxes on the hummocks. We found that nitrous oxide fluxes did not differ among vegetation treatments, elevational gradients, or the environmental variables salinity, soil pH, and concentrations of NO_3^- and NH_4^+. However, we found that vegetation cover differed among nitrous oxide flux categories, with greater plant cover associated with nitrous oxide uptake. Our data suggests that hummock creation does not significantly impact nitrous oxide fluxes in salt marshes, which supports the possibility that hummock creation is a viable salt marsh restoration technique.

1.0 Introduction

Human-induced climate change is a driving factor causing many global environmental and ecosystem changes. Over half of the 0.99 ℃ increase in global mean surface temperature since pre-industrial times can be attributed to human activities (IPCC, 2021). This warming causes thermal expansion of ocean water and decreases Arctic sea ice coverage, which contributes to the acceleration of global mean sea level rise.

At the interface between terrestrial and coastal environments in temperate climates around the globe, salt marshes provide multiple ecosystem services that are important to humanity and highly valuable (Barbier et al., 2011, U.S. EPA, 2009). Salt marshes provide vital regulating services such as protecting coastal areas from waves and storm surge, preventing high erosion rates through sediment stabilization, and sequestering millions of tons of carbon annually (Yoskowitz et al., 2016, Morgan et al., 2009, Barbier et al., 2011). Salt marshes also improve water quality and reduce coastal nitrogen loads by filtering up to 93% of anthropogenic nitrogen inputs out of water before they reach estuaries and marine ecosystems (Yang and Silver, 2015, Brin et al., 2010).

Despite the many benefits of salt marshes, they have historically been and continue to be degraded and lost, mostly due to human activities and associated environmental changes. Human development along coastlines has directly caused loss of 25 to 50% of coastal wetlands globally (Kirwan and Megonigal, 2013), with estimates closer to 50% in southern New England salt marshes (Silliman et al., 2009). Indirectly, anthropogenic CO_2 emissions are accelerating sea level rise (IPCC, 2021). Together, developed coastlines that prevent landward migration of salt marshes as sea levels rise are squeezing salt marshes and their services (Mason et al., 2023). This issue is particularly acute along the North American Atlantic coast, with sea level rise rates 3 to 4 times higher than the global average (Sallenger et al., 2012). This increased inundation frequency adds an additional stress to the remaining salt marshes in southern New England.

Salt marsh ecosystem service provision coupled with the threat of deterioration they currently face make wetland restoration an increasingly popular recommendation in policy spheres (Mason et al., 2023). Restoration can take many forms, such as helping marshes regenerate by adding sediment and vegetation to bare or destabilized areas (Mason et al., 2023).

Thin layer sediment placement (TLP) is an increasingly used strategy that aims to restore marshes by adding flat, thin (less than 10 cm thick), homogeneous layers of sediment onto a low elevation or degraded marsh in order to increase its elevation (Raposa et al., 2022). A new method involves the creation of hummocks, which are topographically diverse mounds of sediment added to submerging wetlands. These hummocks facilitate faster elevation gain while also fostering habitats for plant and animal species. Since this is a novel approach, the effects of this strategy on salt marsh biogeochemical processes, particularly on nitrous oxide (N_2O) fluxes, are not yet completely understood.

N_2O is a potent and long-lasting greenhouse gas that can persist in the atmosphere for 150 years after being emitted, with almost 300 times greater global warming potential than CO_2; despite its much smaller concentration in Earth's atmosphere, its potency makes it an important contributor to global warming (IAEA, 1992). Salt marshes can be a potential source of N_2O emissions through two main pathways; as a byproduct of nitrification or incomplete denitrification. N_2O can be produced during nitrification when ammonium (NH_4^+) is oxidized to nitrate (NO_3^-). When certain environmental conditions hinder bacterial oxidation of nitrite (NO_2^-) to NO_3^-, NO_2^- can be oxidized to N_2O instead. Such conditions are more common in high salt marsh elevations with infrequent or no flooding, which creates aerobic conditions favoring this pathway (Yang and Silver, 2015). N_2O can alternatively be produced during incomplete denitrification when NO_3^- is only partially reduced to N_2O instead of completely reduced to nitrogen gas (N_2) due to fluctuating water levels and low pH conditions that are unsuitable for microbes to facilitate complete reduction. These conditions are more common in low salt marsh elevations which experience frequent or prolonged flooding (Yang and Silver, 2015).

The presence of plants in salt marshes is a biological driver that can either suppress or enhance N_2O emissions (Jorgenson et al., 2012, Shieh & Yang, 1997). Plants can alter wetland sediment dynamics by competing for NO_3^-, supplying organic carbon, and transporting oxygen (O_2) (Alldred & Baines, 2015). Photosynthesis and gas transport through plant aerenchyma can bring more O_2 to the rhizosphere, the area surrounding roots in the soil. Since N_2O is produced by incomplete denitrification at low O_2 concentrations, O_2 transported by plants can lower N_2O fluxes in wetland sediments (Jorgenson et al., 2012). Conversely, the introduction of more O_2 to the rhizosphere can create favorable conditions for denitrifying bacteria that can conduct both anaerobic and aerobic denitrification, allowing N_2O to be produced by incomplete denitrification under oxic conditions, which could increase N_2O fluxes (Shieh & Yang, 1997). Plants also influence complete denitrification rates, with vegetated sediments having denitrification rates up to 55% higher than non-vegetated sediments, possibly due to coupled nitrification, an aerobic process, and denitrification, an anaerobic process. (Alldred & Baines, 2015). Microbial communities are another biological driver that influence N_2O fluxes in salt marshes by performing denitrification and nitrification in the soil (Blackwell et al., 2010). These reactions depend on the availability of the substrates NO_3^- and NH_4^+, which are also taken up by plants (Blackwell et al., 2010). Additionally, certain plant species can alter soil microbial community structure and composition, furthering the impact that plants have on the processes that produce N_2O emissions in salt marshes (Gao et al., 2019).

Several environmental drivers contribute to N_2O fluxes in salt marshes; for this study I focused on concentrations of NO_3^- and NH_4^+, salinity, and soil pH. For both denitrification and nitrification, the amount of dissolved inorganic N is the limiting factor in bacterial production of N_2O (Murray et al., 2015). High concentrations of NO_3^- and NO_2 in sediment can allow for higher rates of denitrification to produce N_2O, and higher concentrations of NH_4^+ in sediment can similarly allow for higher rates of nitrification to produce N_2O (Murray et al., 2015). As sea level rise changes flooding patterns in salt marshes, the redox reactions that control nitrification and denitrification are impacted which leads to changes in N_2O fluxes in these ecosystems (Yang and Silver, 2015). Salinity varies along elevational gradients in salt marshes, with lower elevations more prone to inundation by seawater and more saline conditions, and higher elevations less frequently inundated and less saline (Doroski et al., 2018). High salinity has been associated with increased N_2O fluxes, suggesting that saltwater intrusion associated with rising seas could stimulate N_2O emissions from inundated wetland soils (Doroski et al., 2018). Soil pH influences N_2O fluxes in salt marshes, with higher ratios of N_2O to N_2 produced as pH decreases due to the sensitivity of the enzyme N_2O reductase to low soil pH (Baggs et al. 2010). During complete denitrification this enzyme reduces N_2O to N_2, but lower soil pH conditions make incomplete denitrification more likely to occur, causing greater net N_2O emissions out of acidic soils (Baggs et al. 2010).

Hummock creation is a conservation strategy aiming to improve salt marsh resilience against sea level rise by building elevation capital to promote salt marsh habitat. But adding large mounds of sediment to salt marshes may inadvertently impact N_2O fluxes, since flooding regimes and salinity gradients are controlled by elevation and determine whether aerobic or anaerobic processes occur. Normally, salt marshes with low anthropogenic nitrogen inputs can act as N_2O sinks (Yang and Silver, 2015). This leads to the question of whether the construction of hummocks may promote N_2O emissions out of the soil.

A recently implemented, highly-replicated, experimental hummock restoration project in a southern New England salt marsh provided me with an excellent opportunity to examine the effects of NO_3^- and NH_4^+ availability, soil pH, vegetation cover, and elevation-determined salinity gradients on N_2O fluxes. Leveraging this experiment, I asked the question: *How do elevational gradients and restoration planting treatments alter N_2O fluxes from created hummocks*? My overarching hypothesis is that N_2O fluxes will vary among vegetation treatments and along elevation gradients on the hummocks. I also expect that environmental conditions will vary among N_2O flux categories (uptake, no flux, emissions).

2.0 Methodology

2.1 Experimental Design

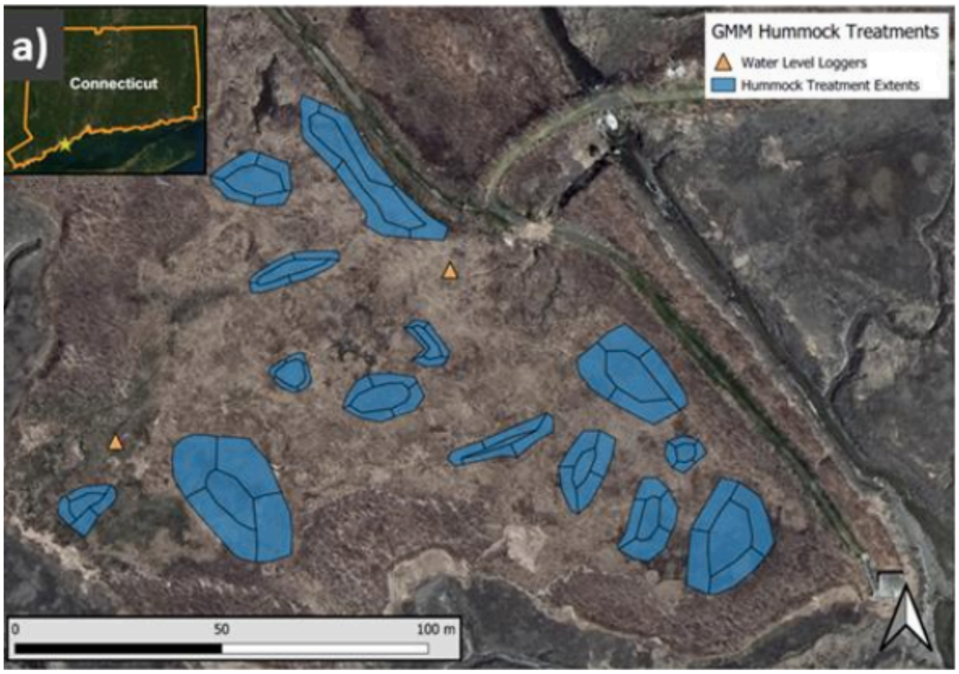
I leveraged a field experiment conducted by CT DEEP and UConn at Great Meadows Marsh (GMM) in Stratford, Connecticut, located along the coast of Long Island Sound. This marsh complex is a part of the Stewart B. McKinney National Wildlife Refuge, and is the site of recent management sediment additions in the form of hummocks.

During winter 2021 to 2022, 14 sediment hummocks were created at GMM using onsite marsh sediment with a targeted height of 1.73 meters (m) (Fig. 1a). Their actual elevations ranged from 1.6 to 1.77 m, with an average height of 1.7 m; relative to the mean high water level, they ranged from 0.48 to 0.66 m, with an average height of 0.58 m above the mean high water level. The hummocks ranged in area they covered from 62 to 775 m^2, covering an average area of 272 m^2.

Volunteers completed planting the hummocks in Spring 2022, with five planting treatments of varying species compositions and planting densities per hummock. The planting treatments were 1) *Spartina patens* with 30 centimeter (cm) spacing, 2) *Spartina patens* with 60 cm spacing, 3) a mix of *Spartina patens* and *Distichlis spicata* with 30 cm spacing, 4) a mix of

Spartina patens and *Distichlis spicata* with 60 cm spacing, and 5) *Juncus gerardii* with 60 cm spacing (Fig. 1b). We compared these treatments to the naturally-occurring vegetation in our unmanipulated controls in the inter-hummock marsh area.

a.



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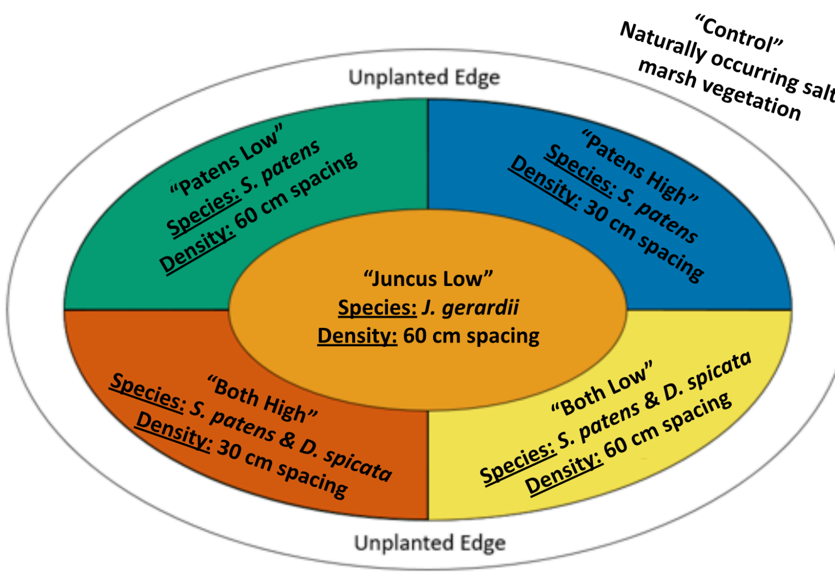

Figure 1.
a. *GMM restoration site in coastal Connecticut; in blue, the 14 experimental hummocks.*
b. *Generalized planting design for the hummocks: outer treatments were randomized.*

2.2 Field and Laboratory Measurements

All data were collected in August 2023 in the middle of the growing season. To investigate how N_2O fluxes varied along elevational gradient flooding patterns and planting density and species compositions on the 14 GMM hummocks, I leveraged ongoing research funded by the EPA Long Island Sound Study being conducted by Drs. Beth Lawrence, Ashley Helton, and Chris Elphick on environmental and biological responses to hummock creation. Established field and laboratory protocols in accordance with the standard biogeochemical methods of the larger EPA project were used to complete this research. To investigate our research questions we quantified N_2O fluxes, elevation, vegetation percent cover, NO_3^- concentration, NH_4^+ concentration, soil salinity, and soil pH by collecting and processing data.

N_2O Fluxes

N_2O fluxes were quantified using opaque static chambers (Holland et al. 1999) made of PVC sections ("collars"), which were installed in the hummocks prior to the growing season. The gas sampling campaign occurred on August 21-22, 2023 within 3 hours of low tide in order to limit tidal and temperature variability. We clipped any plants growing in the collar and placed petroleum jelly on the cut stem to minimize gas diffusion from the cut stems. During sample collection, a PVC cap with a vent tube, sampling port, and septa was sealed onto the collar with petroleum jelly (Holland et al. 1999) (Fig. 2a). To measure gas fluxes, gas samples were collected every 15 minutes for 45 minutes (T0, T15, T30, T45) with a gas-tight syringe (Fig. 2b). One field air sample was taken per hummock during the sampling period, and barometric pressure, soil and air temperatures, the presence of crab holes, and the height of the chamber from the soil surface were recorded as well. After collecting gas samples, the contents of the syringe were injected into evacuated 23 mL glass vials. These were transported to the lab to analyze for CO_2, CH_4, and N_2O using a Perkin Elmer Clarus 580 Gas Chromatograph (GC). Gas standards were run every 40 samples for quality assurance.

a. b.

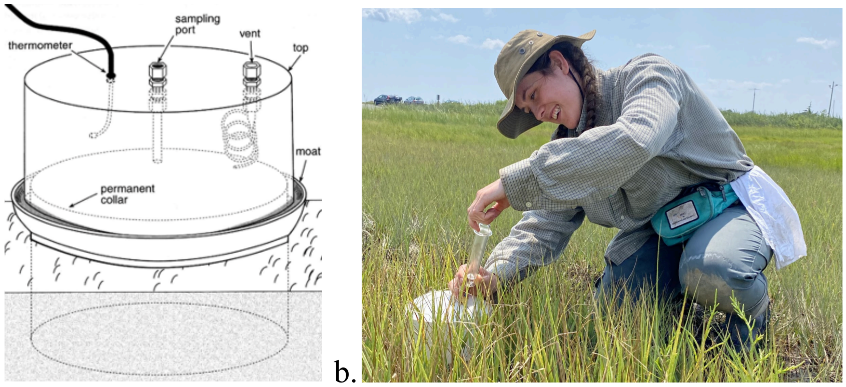

Figure 2.
a) *Diagram of a "collar" (opaque static chamber) used to collect gas samples ([Holland et al. 1999](#)).*
b) *A member of the lab group collecting a static gas flux sample from a GHG collar.*

Quantifying Elevation

Elevation was quantified using a Real-Time Kinematic Positioning (RTK) GPS device; these measurements were collected in the field during vegetation and soil data collection. By placing the RTK at each of the collars we quantified the precise elevations (m) where we collected N_2O fluxes. In addition to the absolute (continuous) elevation, we established categorical elevation values based on how high on the hummock collars were placed (Fig. 3)

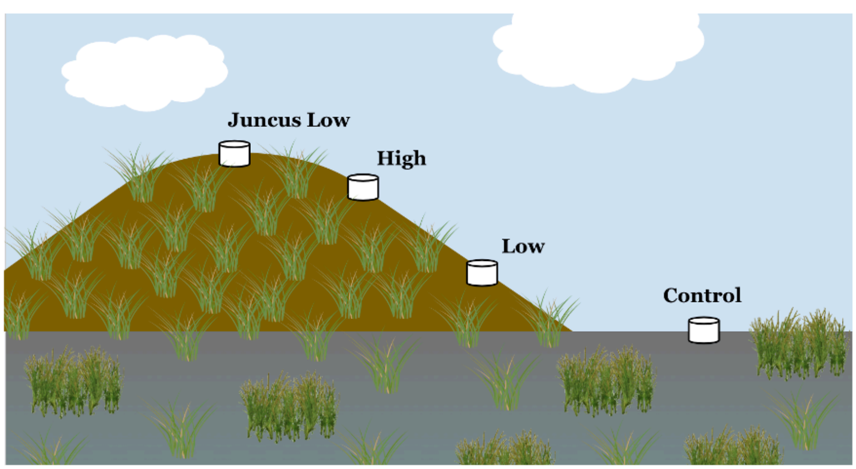

Figure 3. *Categorical elevations were "Control" (located at native marsh elevation), "Low" (located lowest on each hummock), "High" (located higher on each hummock), and "Juncus Low" (located at the top of each hummock within the Juncus vegetation treatment).*

Quantifying Vegetation Cover

Vegetation percent cover was quantified using 1x1 m quadrats in each of the five planting treatments on each hummock, and in one random control point close to each hummock to compare hummock values to unrestored conditions. Quadrats were placed next to the collars and oriented to be parallel to the elevational transect (Fig. 4). This allowed us to visually estimate what percentage of each plot was covered by different plant species, bare ground, and standing water. Percent cover was estimated from above so that the cover values for all plant species and any non-vegetated areas added up to one-hundred percent. A duplicate percent cover estimation was completed every twenty quadrats to ensure sampling consistency. For our study we were interested in the total vegetation percent cover value that allowed us to compare the relative amount of vegetation in each of the plots we sampled.

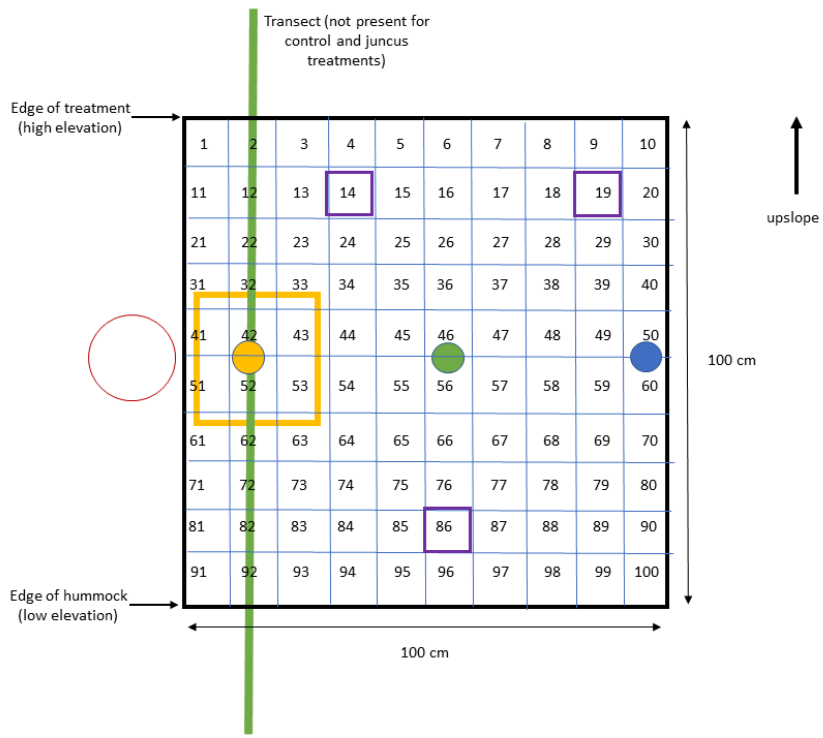

Figure 4. *Example of a 1x1 m quadrat and the setup for sampling vegetation and soil characteristics; the quadrat was placed next to the GHG collar (red circle) and parallel to the elevational transect (green line).*

Quantifying Soil Core-Derived Variables

Three soil cores were taken from evenly spaced cells in line with the GHG collar, within the sampled quadrat area (Fig. 4). One per plot was used to test for the soil characteristics NO_3^- and NH_4^+ concentration, soil salinity, and soil pH. To take these soil samples, a 5 cm diameter bulb planter was put in the ground to a depth of 8 cm. We used a shovel to bring up the soil and keep the core intact, then placed the soil in a plastic bag, labeled it, and squeezed the air out of the bags before sealing to prevent atmospheric pressure from influencing the chemical processes. These samples were transported on ice in a cooler back to the laboratory and stored in the lab fridge until being sieved and processed within ten days.

NO_3^- and NH_4^+ concentrations were derived from fresh, field-moist soil core samples that we sieved through a 2 mm screen and weighed into centrifuge tubes. We then extracted NO_3^- and NH_4^+ by mixing 2.5 g of the sieved soil with 25 mL of 2 mL/L KCl solution in 50 mL falcon tubes. Each tube was vortexed for five seconds and then shaken at 200 rpm for 30 minutes. After the mixture settled, we filtered the supernatant through Whatman 589/1 filters which we pre-rinsed with 2 M KCl, rinsed with DI water, then dried at 38 ℃ in an oven. We used the Gallery™ Discrete Analyzer colorimetric determination to quantify KCl-extractable NO_3^- (through enzymatic reduction following Campbell et al. (2004)) and NH_4^+ (EPA Method 350.1 Rev 2). We report the concentrations of these extracts in mg N/L here as bulk density estimates are not available at this time.

We measured pH and salinity by adding 20 g of sieved soil sample to 80 mL of water (1:4 soil to water) to create a soil slurry, then shook these solutions on a shaker table at 200 rpm for 10 minutes. Once the mixture equilibrated 30 minutes later, we measured pH and salinity values using an Orion ROSS Ultra Refillable ph/ATC Triode.

2.3 Data Analysis

All N_2O sample concentrations were first compared to the GC's detection limit, which was 0.05 ppm; none of our samples were below the detection limit. The measured gas sample concentrations (ppm-v) were converted to mass (μg-m^3) and corrected to air temperature and barometric pressure conditions in the field using the Ideal Gas Law. The converted values were then used to calculate N_2O fluxes, equivalent to the rate of N_2O exchange represented by the slope of the best fit line for the regression of μg N_2O-N accumulation per m^2 per minute. N_2O fluxes were assigned a slope of zero if their concentration difference over time was less than the minimum detectable concentration difference (MDCD) (Yates et al. 2006). For N_2O fluxes whose concentration differences were above the MDCD but whose linear accumulation of N_2O over time had an $R^2 < 0.8$, we recalculated the flux when the linear fit could be improved by dropping one or two samples (e.g. if the linear accumulation had an asymptote, we removed the T45 sample value and kept the N_2O flux estimate if the R^2 value improved). Of 140 total flux measurements, 98 were below the MDCD and set to zero, and nine did not satisfy the linear criteria and were excluded from analysis.

In order to test our hypotheses, I used R Studio for all statistical analysis. To test the hypothesis that N_2O fluxes would vary among the manipulated factors (vegetation treatments and categorical elevations), I used Kruskal-Wallis tests due to the non-normal (non-parametric) distribution of the N_2O data. Due to the predominance of N_2O fluxes equal to zero, I used boxplots to visualize differences between N_2O emission values from the vegetation treatments separately from N_2O uptake values. Similarly, I used boxplots to visualize differences between N_2O emission values from the categorical elevations separately from N_2O uptake values.

To test the hypothesis that environmental variables (continuous elevation, vegetation percent cover, concentration of NO_3^- and NH_4^+, salinity, and soil pH) would vary among the three N_2O flux categories (uptake, no flux, emission), I again used Kruskal-Wallis tests for non-parametric data. For statistically significant ($p < 0.05$) differences in environmental variable values between N_2O flux categories, I used the Dunn test to perform pairwise comparisons.

3.0 Results

Of the 131 N_2O fluxes, approximately three quarters were zero, and one quarter were evenly split between uptake and emission. (Fig. 5).

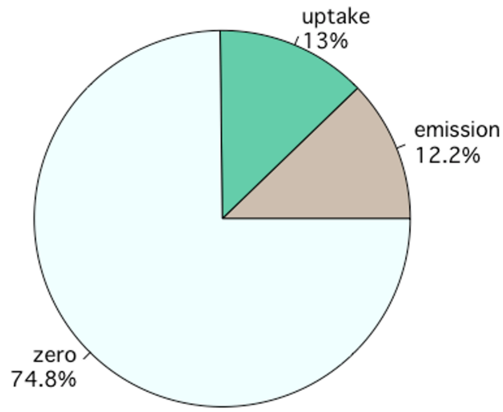

Figure 5. *<u>Distribution of N_2O Fluxes:</u> most (~75%) of the observed N_2O fluxes were equal to zero. The remaining (~25%) were evenly split between N_2O uptake and emission.*

N_2O Fluxes from Vegetation Treatments and Categorical Elevations

We found no differences among vegetation planting treatments in either N_2O emission ($p = 0.86$, Kruskal-Wallis test; Fig. 6a) or N_2O uptake ($p = 0.59$, Kruskal-Wallis test; Fig. 6b). Likewise, we found no differences among categorical elevations in either N_2O emission ($p = 0.33$, Kruskal-Wallis test; Fig. 6c) or N_2O uptake ($p = 0.84$, Kruskal-Wallis test; Fig. 6d).

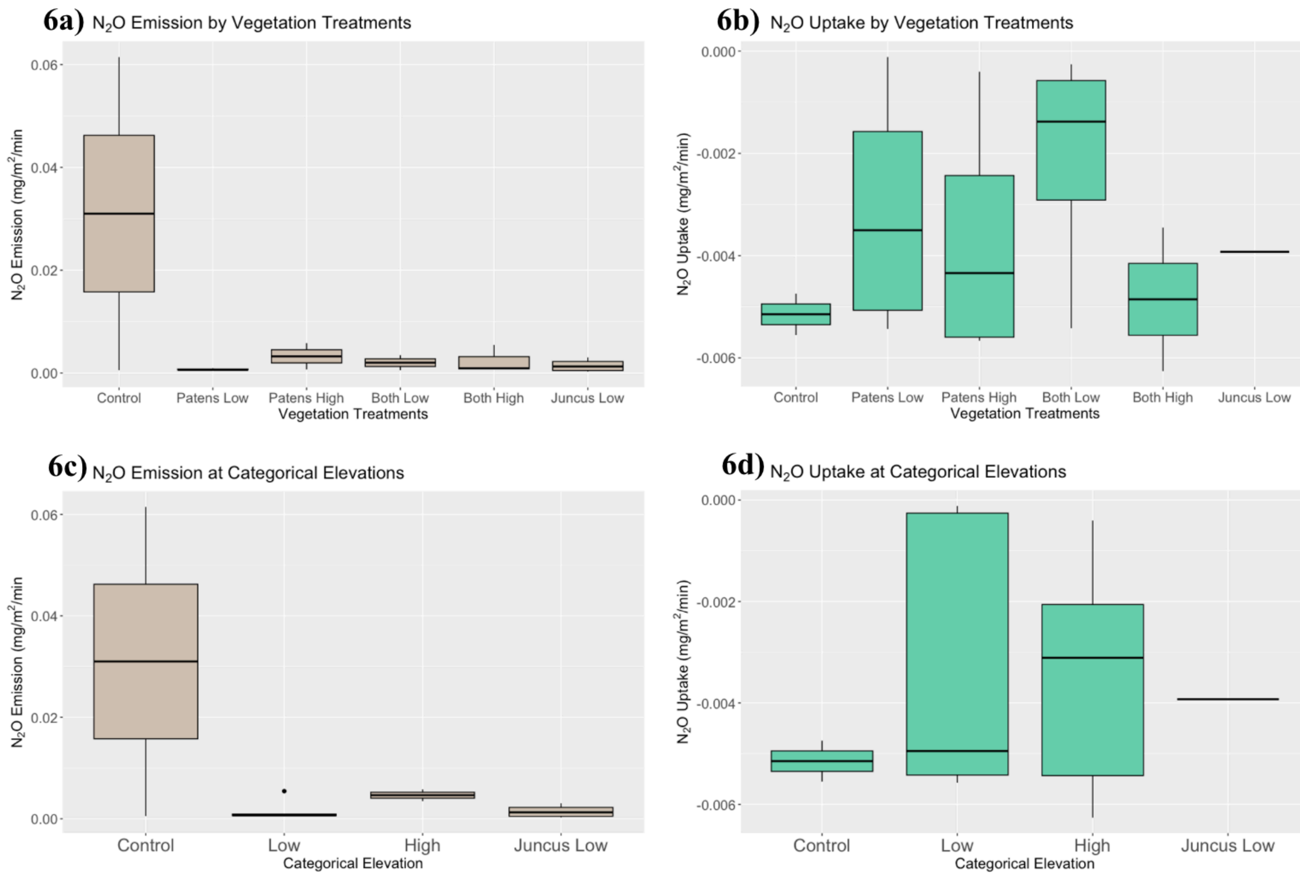

Figure 6. *Boxplots comparing **a)** N_2O emission fluxes across vegetation treatments, **b)** N_2O uptake fluxes across vegetation treatments, **c)** N_2O emission fluxes across categorical elevation plots, and **d)** N_2O uptake fluxes across categorical elevation plots.*

Biological Variable Levels of N_2O Flux Categories

We found that there was a marginally significant difference between the vegetation percent covers of N_2O flux categories ($p = 0.07$, Kruskal-Wallis test; Fig. 7). The vegetation percent covers of N_2O emission varied from those of N_2O uptake ($p = 0.0407$, Dunn test), and the vegetation percent covers of N_2O emission varied from those of N_2O flux equal to zero ($p = 0.08$, Dunn test).

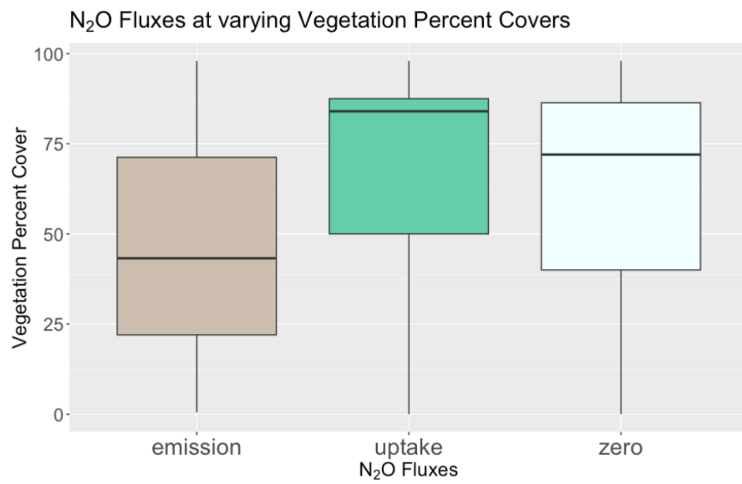

Figure 7. *Boxplot comparing non-transformed vegetation percent cover values between the three N_2O flux categories.*

Environmental Variable Levels of N_2O Flux Categories

We found that there was no significant difference between the continuous elevations, (p = 0.47, Kruskal-Wallis test; Fig. 8a), salinities (p = 0.58, Kruskal-Wallis test; Fig. 8b), soil pH (p = 0.8889, Kruskal-Wallis test; Fig. 8c), concentrations of NO_3^- (p = 0.714, Kruskal-Wallis test; Fig. 8d), or concentrations of NH_4^+ (p = 0.5678, Kruskal-Wallis test, Fig. 8e) of N_2O flux categories.

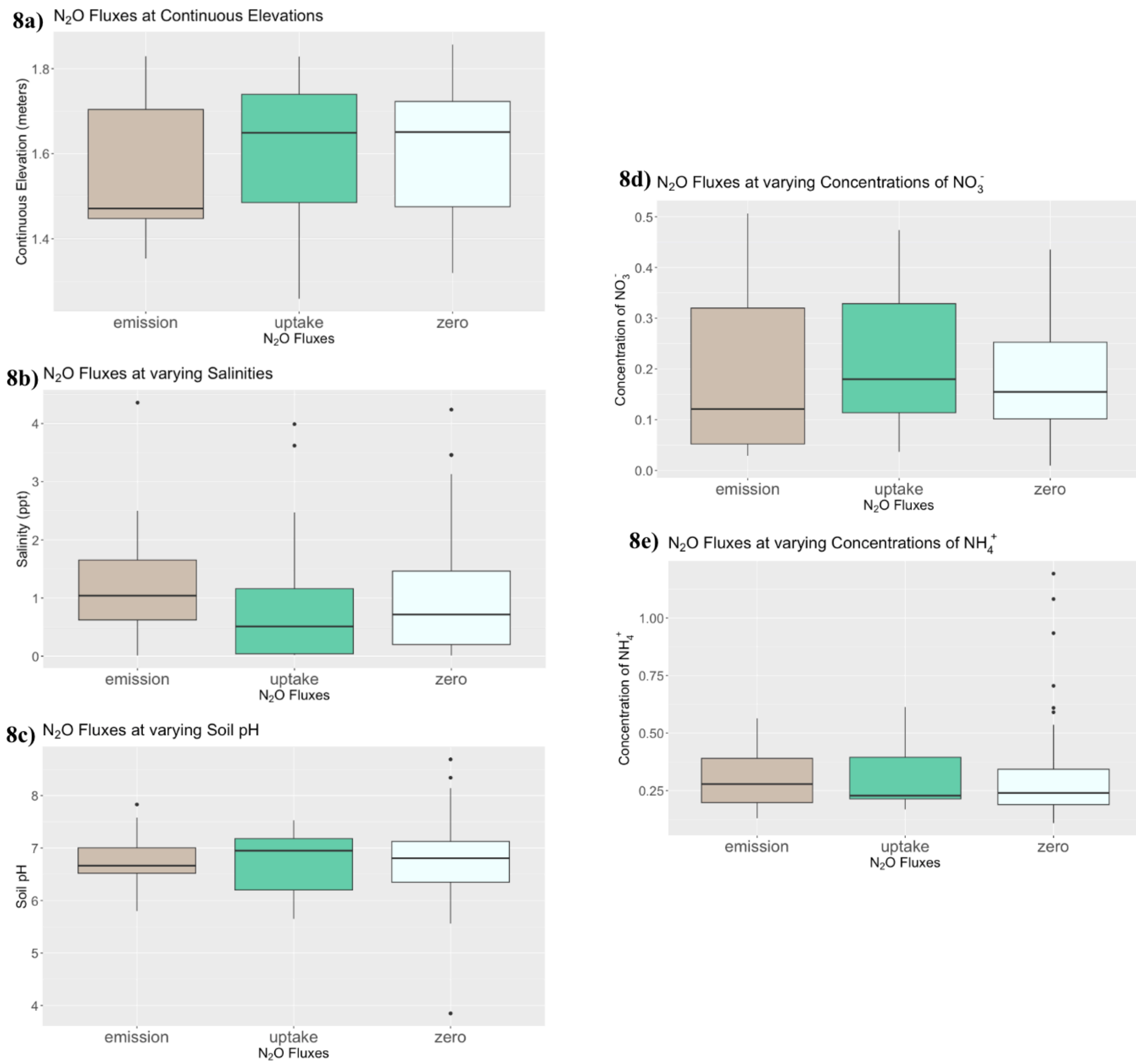

Figure 8. *Boxplots comparing* ***a)*** *continuous elevation,* ***b)*** *salinity,* ***c)*** *soil pH,* ***d)*** *NO_3^- concentration, and* ***e)*** *NH_4^+ concentration between the three N_2O flux categories.*

4.0 Discussion

As sea levels rise and submerge salt marshes, conservation interventions are needed to preserve the services provided by these ecosystems. Restoration techniques are capable of addressing this issue, but information is needed to characterize whether these techniques are achieving their goals or if there are unintentional tradeoffs, such as N_2O emissions. Hummock creation in a recently restored salt marsh added different sediment depths and experimentally manipulated vegetation species and densities with the objective of increasing elevation and providing habitat for the endangered salt marsh sparrow. But as a novel approach, this technique has unknown effects on salt marsh biogeochemistry and N_2O fluxes. Interestingly, I did not find evidence to support my hypothesis that N_2O fluxes would vary among vegetation treatments and along elevation gradients on the hummocks. However, I did find evidence that environmental conditions varied among N_2O flux categories. Vegetation cover was higher in plots where N_2O uptake occurred, and lower in plots with N_2O emission (Fig. 7), but there were no differences in many environmental variables (salinity, soil pH, NO_3^-, NH_4^+) between plots where N_2O emission, uptake, or no flux occurred (Fig. 8).

4.1 Biological Variables

I hypothesized that N_2O uptake or an N_2O flux equal to zero (no net movement of N_2O into or out of the soil) would occur at plots with higher vegetation percent covers. Vegetated sediments have been found to have denitrification rates up to 55% higher than non-vegetated sediments, with this complete denitrification removing NO_3^- from wetland sediments to enter the atmosphere as N_2 instead of N_2O (Alldred & Baines, 2015). But the mechanism underlying this correlation between vegetation cover, denitrification, and N_2O uptake is complicated by the many factors that influence denitrification rates of plants. Plants can alter sediment denitrification by competing with microbes for NO_3^-, supplying organic carbon, and transporting O_2 to typically anoxic sediments via diffusion from their roots, with different plant functional characteristics driving altered denitrification rates (Alldred & Baines, 2015). Prior to restoration activities, the vegetation cover of GMM consisted primarily of the invasive, non-native

Phragmites australis (common reed) species. Once a controlled burn was performed to clear out this species, the inter-hummock native marsh sediment became dominated by *Spartina alterniflora* (smooth cordgrass) (Young, 2023). Ehrenfeld (2003) observed changes in microbial enzyme activity in salt marsh sediments where *Phragmites australis* replaced *Spartina alterniflora*; the transition from *Phragmites australis* to *Spartina alterniflora* domination in GMM could have caused a similar change. Alldred & Baines (2015) found that *Spartina alterniflora* had the highest observed denitrification rates compared to other salt marsh species, which could explain why the lowest N_2O emission values we observed occurred in *Spartina alterniflora*-dominated control plots. Additionally, nitrifying microbes are weak competitors for NH_4^+ against plant roots (Verhagen et al., 1994), suggesting higher plant density on the hummocks could be reducing the amount of NH_4^+ available to be nitrified to N_2O by microbes. Additional information on species traits that promote uptake of NO_3^- and NH_4^+ (above and below ground biomass, rooting area and depth, composition of plant tissues, and litter quality) are needed (Alldred & Baines, 2015). To answer my research question, I looked at how total vegetation cover altered N_2O fluxes; to further investigate the mechanism behind our findings that higher vegetation cover correlates with N_2O uptake, exploring above and below ground biomass in conjunction with specific species identities may give a clearer picture as to what is driving the relationship between abundance and uptake.

4.2 Environmental Variables

I hypothesized that N_2O uptake or no flux would occur at lower salinities, and N_2O emission would occur at higher salinities. Doroski et al. (2018) found that N_2O emission is associated with higher salinities in salt marshes, but we did not find any salinity patterns among different N_2O flux categories. It is still possible that N_2O varies along salinity gradients found in salt marshes, but that this variation was not reflected in our data. Nielsen et al. (2009) found that elevated N_2O production occurs during abrupt changes in salinity due to the sensitivity of N_2O-reductases. Our experimental design may have been insufficient to capture N_2O pulses associated with rapid shifts in salinity.

I hypothesized that N_2O uptake or no flux would occur with higher soil pH, and N_2O emissions would occur at lower soil pH. While Baggs et al. (2010) found greater N_2O emissions in acidic soils due to the higher likelihood of incomplete denitrification, we did not see N_2O flux patterns along soil pH gradients. However, their research observed these differences between soils with pHs of 4.5 to 7.0 (Baggs et al. 2010), while our soils tended to be circumneutral, ranging between a pH of 5.56 to 8.69, which may explain why we did not observe relationships between soil pH and N_2O fluxes.

I did not find evidence that N_2O fluxes were correlated with availability of NO_3^- and NH_4^+ in the soil. According to Murray et al. (2015), high concentrations of dissolved inorganic nitrogen in soil promotes N_2O emission, while low concentrations combined with low amounts of O_2 in soil promotes N_2O consumption by denitrifiers and therefore N_2O uptake occurs. One explanation for this is that the salt marsh we studied had relatively low inorganic nitrogen levels. Meyer et al. (2008) utilized microsensors to investigate N_2O production in subtropical estuarine sediments and found a similar relationship to Murray et al. (2015) (N_2O emissions were produced under higher inorganic nitrogen conditions); but Meyer et al. (2008) found that N_2O production under low nutrient conditions was below their sensor's detection limit. Meyer et al. (2008) also simulated eutrophication scenarios by creating high nutrient conditions in their soil core incubations; this involved adding NO_3^- and NH_4^+ in 500 µmol / L concentrations. These sudden increases in inorganic nitrogen caused a substantial escalation in denitrification and nitrification activity, which Meyer et al. (2008) states could have exacerbated N_2O production. The concentrations of inorganic nitrogen in our study were much lower than those used by Meyer et al. (2008), which could be why a majority of the N_2O fluxes we measured were equal to 0; there might not have been enough inorganic nitrogen substrate in the salt marsh soils to undergo denitrification and nitrification to produce N_2O. Salt marshes subjected to low amounts of anthropogenic nitrogen loading from surrounding developed areas can act as N_2O sinks because N_2O consumption is limited by NO_3^- or anoxic conditions (Yang and Silver, 2015); it is possible that GMM is one such salt marsh.

4.3 Experimental Drawbacks

Data collection at the GMM hummocks will continue during Summer 2024, allowing for a more ample dataset to further inform scientists and managers. After sampling and processing the data collected during Summer 2023, several sources of error could be addressed to improve the accuracy of our results and the conclusions we draw from them.

There were several aspects of the experimental design that could have impacted the N_2O flux values. The methodology for measuring N_2O fluxes required that we remove any plants within the collar, so we cut the stems and covered the ends with vaseline to measure N_2O fluxes from the soil only. But our findings suggest that plant cover plays an important role in N_2O fluxes on the hummocks, suggesting that it may be beneficial to include plant contributions to N_2O fluxes in our measurements. This could be accomplished by using tall chambers that allow entire plants to remain inside the collar. Another part of the N_2O flux measurement methodology that could be altered is the time over which samples were collected. As we analyzed the N_2O flux graphs we saw a tendency for the points to either follow a zigzag pattern (the T0 point is low, T15 has a higher value, T30 has a lower value again, and T45 has a higher value again) or reach an asymptote between T30 and T45. These patterns often require that we drop entire fluxes, or that we drop one or two points to make the N_2O flux linear. Based on this tendency, it may be advantageous to measure N_2O fluxes on a different time scale than methane and CO_2. Taking four N_2O measurements over 30 or 20 minutes instead of 45 could give us a more accurate picture of the initial movement of N_2O either into or out of the soil. It would also aid with N_2O flux analysis since less points would have to be dropped, giving us a more accurate idea of the slope from four data points rather than two or three.

4.4 Future Outlook

Our results suggest that hummock construction in GMM does not risk a tradeoff between restoring a degraded salt marsh at the cost of greater N_2O emissions. It is important to measure the effect of this management strategy on GHG emissions because salt marshes provide many ecosystem services and habitat for endangered species such as the salt marsh sparrow. If N_2O emissions were higher from the hummocks than from the native marsh sediment, then hummocks would be contributing to the positive feedback loop of global warming and rising sea levels, “squeezing” and submerging them, endangering the services and habitat they provide.

Conclusions drawn from sampling during 2023 should be used to inform future data collection and analysis, particularly during the summer 2024 sampling season. More intensive sampling from the hummocks may be required to better understand the nitrogen cycling that occurs within them; this may include collecting more gas and plant sample replicates, since vegetation cover was found to have the most significant impact on N_2O fluxes. Preliminary data shows that the hummocks have a negligible effect on N_2O fluxes, with no significant differences in N_2O fluxes along the elevational gradient or between levels of environmental variables. However, the variables that were tested (elevation, vegetation cover, salinity, soil pH, NO_3^-, and

NH_4^+) should be further investigated to determine whether they interactively impact N_2O fluxes; salt marsh biogeochemical processes are highly dynamic and interdependent and therefore do not always exhibit clear one-to-one relationships (Mitsch & Gosselink, 2015).

As a novel salt marsh management approach in its primary years of data collection, this project provides a foundation for future research into N_2O dynamics on hummocks. Salt marsh restoration projects also offer unique opportunities to managers, policy-makers, and individuals invested in protecting their coastal communities. Restoration of GMM was no exception, where the efforts of volunteers, local high school students, contractors, and project partners facilitated the planting of 165,000 grasses on the hummocks (Young, 2023).

In the face of accelerating sea level rise, hummock creation is a mitigation strategy to address salt marsh degradation and submersion. But to prevent further salt marsh loss, the causes of climate change will need to be addressed.

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