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Salinization Impacts on Heterotrophic Respiration, Ecosystem Respiration, and Plant Productivity in Tidal Freshwater Marshes

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Abstract:

Tidal freshwater marshes have preserved substantial stocks of soil carbon, which represents carbon dioxide that is no longer in the atmosphere. There is conflicting evidence in the literature about how disturbances such as sea level rise and associated wetland salinization impact the accumulation and long-term stability of this stored carbon. The goal of this experiment was to quantify how salinization impacts total ecosystem respiration and its component parts, autotrophic and heterotrophic respiration. This was a microcosm experiment using a C4 plant (*Spartina cynosuroides*) grown in soil derived from C3 plant matter and exposed to different salinities (0 to 9 psu). Results indicate that higher levels of salinization changed total ecosystem respiration. The highest salinity caused a decrease in belowground plant biomass and increased aboveground leaf mortality, although aboveground biomass was similar to other treatments. There were some issues with the isotope data, and several of the calculated autotrophic respiration measurements were impossible. These data are reviewed in the Appendix. The results from this study help shed light on changes in carbon fixation and subsequent biomass accumulation in stressed wetland ecosystems. This is a matter of great concern if it is not at a rate that keeps up with sea level rise.

Introduction:

Although many wetlands have been converted for human use such as agriculture, they are now being recognized for both their environmental and economic value. Not only do wetland ecosystems provide habitat for numerous plant and animal species, coastal wetlands have economic values of up to \$194,000 ha^{-1} yr^{-1} for their ecosystem services with things such as flood protection and commercial fishing (Costanza et al., 2014). Further, wetlands have a high capacity for storing carbon in their soils. While the estimated global land area covered by wetlands is only between 5-8%, they hold nearly 30% of the carbon stored in soils (Mitsch and Gosselink, 2007). In the conterminous United States alone, an estimated 11.52 petagrams of carbon are stored in wetland soils (Nahlik and Fennessey, 2016). This is largely owing to their high productivity and generally water-inundated soils that limit oxygen reaching the organic matter to begin decomposition (Bernal and Mitsch, 2012; Mcleod et al., 2011; Qian et al., 2019; Neubauer and Megonigal, 2022). Although climate change poses great risks to all wetlands, those located in coastal regions are particularly threatened due to sea level rise and other factors related to climate change.

Freshwater tidal wetlands, which are characterized by the rise and fall of <0.5 psu tidal waters, are particularly vulnerable to encroaching saltwater due to their linkage to ocean waters downstream. Inland hydrological changes such as drought and decreased river discharge also put many of these freshwater coastal wetlands at risk of salinization (Anderson and Lockaby, 2012; Baldwin, 2007; Hackney et al., 2007). Further threatening the continuing productivity and function of freshwater tidal wetlands along the mid-Atlantic coastal region is land subsidence (Karegar et al., 2016). Although tidal wetlands have the capacity for vertical accretion from plant organic matter and sediment buildup, this does not always occur at a rate that keeps up with sea level rise due to local factors limiting the buildup of sediments and plant growth (Morris et al., 2016; Schuerch et al., 2018). Other risks to coastal wetlands are increasingly severe, such as frequent hurricanes and storms that often result in more storm surges

(Bernstein et al., 2007; Hartmann et al., 2014; Woodruff et al., 2013). This plethora of stressors on coastal wetlands can cause changes to plant community structures and biomass, greenhouse gas emissions such as CO_2 and N_2O, microbial communities, and nitrogen mobilization (Helton et al., 2019; Doroski et al., 2019; Li and Pennings, 2018; Widney et al., 2019; Neubauer et al., 2019). Wetland ecosystems play a vital role on both a local scale by offering diverse species habitats and on a global scale by providing an annual net carbon retention average of approximately 118 g-C m^{-2} yr^{-1} (Mitsch et al., 2013), and these stressors threaten the continued wellbeing and carbon accretion of these wetlands.

Net ecosystem productivity, which is the balance between gross primary productivity and ecosystem respiration, serves as a proxy for the continued increases in carbon stored within wetland soils. Ecosystem respiration is the combined heterotrophic and autotrophic respiration, and any changes in either or both of these would likely impact this rate of carbon storage and, hence, the rate of vertical accretion that allows coastal wetlands to keep pace with sea level rise. Organic matter accumulation accounts for approximately 62% of vertical accretion in freshwater tidal wetlands (Neubauer 2008), meaning that any change in organic matter accumulation may negatively impact this balance of vertical accretion against sea level rise. Previous studies have shown that saltwater intrusion into tidal freshwater wetlands decreases ecosystem respiration (Herbert et al., 2018; Yang et al., 2018; Neubauer, 2013; Neubauer, unpublished). We do not know whether changes in ecosystem respiration are driven by changes in heterotrophic or autotrophic respiration (or both). My research focused on both overall ecosystem respiration and its component parts to measure any potential changes and how they are changing.

Heterotrophic respiration in soils can be from microbes and animals, although microbial activity is the predominant source of heterotrophic respiration in water-inundated soils (Hu et al., 2016). Microbial activity, which is primarily due to bacteria, archaea, and fungi, breaks down available carbon in the soils, thus causing carbon mineralization and subsequent respiration (Mamilov et al., 2004).

Wetland microbial communities have shown varied responses in rates of respiration following saltwater intrusion, with some exhibiting increased respiration (Weston et al., 2011; Chambers et al., 2013; Chambers et al., 2011; Marton et al., 2012), while others exhibit no change or decreased rates of respiration (Marton et al., 2012; Brouns et al., 2014; Minick et al., 2019; Nyman and DeLaune, 1991; Ardon et al., 2018; Doroski et al., 2019). Most changes to heterotrophic respiration have been shown to be from the introduction of sulfates as opposed to simple osmotic and ionic stress (Chambers et al., 2011), although simple osmotic stress has the ability to increase respiration despite an overall loss in microbial biomass (Saviozzi et al., 2011). Elevated salinities stress freshwater-adapted microbes, which can cause them to exhibit increased respiration and decreased growth efficiency, often resulting in them being outcompeted by saltwater-adapted microbes that have a stronger capacity for carbon mineralization (Dang et al., 2018; Bouvier and del Giorgio, 2002; Luo et al., 2019). These factors can create changes in the rates of heterotrophic respiration in different freshwater wetlands.

Certainly, much research has been done on the effects of salinization on heterotrophic respiration, but autotrophic respiration has, up to now, been largely overlooked in wetland environments. Freshwater tidal wetlands experiencing salinization have shown declines in whole ecosystem respiration, which may in part be explained by decreased photosynthesis of plants leading to lower rates of autotrophic respiration (Neubauer 2013) or by the lower plant productivity leading to less reactive carbon in the soils, resulting in lower heterotrophic activity (Kuzyakov and Cheng, 2001). Photosynthesis is closely coupled with rates of autotrophic respiration, with lower plant photosynthesis leading to lower rates of autotrophic respiration (Wertin and Teskey, 2008). Decreased freshwater plant respiration with salinization presents interesting questions regarding their continued productivity and carbon loss in these changing environments. We simply do not know the part autotrophic respiration plays in this marked decrease in ecosystem respiration.

Rising salinities negatively impact productivity of wetland plants with regards to biomass accumulation. Although different plant species have varied sensitivities to elevated salinities, significant decreases in both above- and belowground biomass occur in a wide array of freshwater wetland plant species experiencing salinization (Sutter et al., 2014; Baldwin and Mendelssohn, 1998; Conner et al., 1997). This can be due to osmotic stress, ionic stress, or nutritional deficits of the plants caused by the salinity. Ionic stress occurs as cellular toxicity from the buildup of ions in the plant cells and can result in lower biomass over a longer time period through electrolyte leakage from the plasma membranes (Hniličková et al., 2019).

Freshwater wetlands experiencing salinization can exhibit reduced primary productivity (Herbert et al., 2015), which I hypothesize is in part due to the plants having a decreased carbon use efficiency. Essentially, carbon use efficiency is calculated by dividing autotrophic respiration by gross ecosystem productivity and subtracting that from one. Plants in various upland environments experiencing salinization have exhibited reduced carbon assimilation in their leaves which worsened with leaf age (Vanlerberghe et al., 2020; Cruz et al., 2017). Previous research has also shown that a common response of salt-stressed plants in upland environments is a significant decrease in the photosynthetic efficiency of both glycophytic and halophytic plants (Cha-Um et al., 2010; Hester et al., 2001). This shows a similar response of decreased photosynthetic efficiency of plants exposed to elevated salinities due to osmotic and ionic stress overcoming their defenses. Although previous research on wetland halophytes in their natural mesohaline environments has indicated they have lower photosynthetic efficiency than glycophytes in their freshwater environments (Weston et al., 2014), there is a deficit in knowledge as to how plant photosynthetic efficiency is impacted by rising salinities caused by saltwater intrusion.

It has been a challenge to isolate the rates of autotrophic respiration from whole ecosystem respiration. Two methods used for this are component integration, where the respiration of individual components such as leaves and roots are measured separately, and measurements of isotopic ratios

(Hanson et al., 1999). I focused on the latter by using the naturally occurring isotope ^{13}C and its ratio to ^{12}C to separate autotrophic from heterotrophic respiration. Only about 1.1% of the total atmospheric carbon is ^{13}C, which can vary locally by differences in anthropogenic activity and by season (Farquhar et al., 1989). For the purposes of this study, it must be considered that not all plants utilize the same amount of ^{13}C by their utilization of different photosynthetic pathways. Due to their use of phosphoenolpyruvate (PEP) carboxylase, C4 plants are generally less discriminatory than C3 plants in the incorporation of ^{13}C into biomass (Farquhar et al., 1989). This difference in carbon isotope utilization creates the opportunity for separating autotrophic and heterotrophic respiration. After Wolf et al. (2007), I grew C4 plants in soil that was previously occupied by C3 plants and took advantage of the differences in ^{12}C versus ^{13}C uptake between the two different photosynthetic pathways. Essentially, the CO_2 produced from respiration of the C4 plants has a different $^{12}C/^{13}C$ ratio than CO_2 produced by heterotrophic microbes in the soil (which has a C3-like isotopic value because its organic matter is largely composed of partially-decayed material from the C3 plants that dominated the site). This difference in the isotopic value of CO_2 produced by the plants and soil heterotrophs provided a way to distinguish between autotrophic and heterotrophic respiration.

Hypothesis:

The experiment described below was designed to test the following hypotheses:

1) Salinization of tidal freshwater wetlands will decrease the overall ecosystem respiration (that is, combined autotrophic and heterotrophic respiration).
2) Rates of autotrophic respiration and photosynthesis will decrease with rising salinity, although a greater fraction of plant productivity will be respired at a higher salinity.
3) Heterotrophic respiration will decrease as a result of higher salinity.

Methods:

Microcosm setup:

Soil was collected in August 2020 from a freshwater tidal marsh in the Cumberland Marsh Natural Area Preserve along the Pamunkey River, Virginia (37.557000° N, 76.972878° W). The collection site was within 2-3 m of a small creek and was dominated by C3 plants such as *Peltandra virginica* (arrow arum) and *Pontederia cordata* (pickerelweed). Soils were collected from the root zone (top ~60 cm of soil). Large roots and aboveground plant material were removed in the field with smaller roots removed later by passing the soil through a 2 mm sieve. The soil was mixed with a shovel and further homogenized in a food processor so that all replicate microcosms started with a similar substrate. This soil was used to fill sixty-four individual microcosms (PVC Sewer and Drainpipe, 10.3 cm inner diam × ~30 cm tall) to within 2 cm of the top. These microcosms were capped at their bases to prevent soil from falling through, with small holes in the end caps to allow water movement (e.g., drainage).

In September 2020, thirty-two microcosms were planted with the C4 plant *Spartina cynosuroides* (big cordgrass; note that the genus *Spartina* is also known as *Sporobolus*); the remaining thirty-two microcosms contained only soil. The planted microcosms each had one ~5 cm diameter plant plug of *S. cynosuroides* with a small amount of potting soil from Environmental Concern Plant Nursery. The plants were chosen for their broad tolerance and survival in 9 psu environments (Constantin et al., 2019). Given their salt tolerance, my high salinity treatment (~9 psu) was sublethal and all planted microcosms had living plants at the end of my experiment. These wetland plants offered information on any potential changes in growth, productivity, and rates of autotrophic respiration for plants experiencing increasing salinities. Starter plants were purchased from the nursery, Environmental Concern (St. Michaels, Maryland). This nursery collected all the seeds from one location in Hog Island, North Carolina and starter plants had been grown under freshwater conditions at the nursery.

The microcosms were maintained in the greenhouse at Virginia Commonwealth University's Eugene and Lois Trani Center for Life Sciences in Richmond, Virginia. Because the experiment lasted throughout the winter of 2020/2021, natural sunlight was supplemented with eight full spectrum, 1000W growing lights that were hung ~76 cm above the microcosms. The lights were on timers set for twelve hours of light per day for the plants.

The microcosms were placed into tidal systems that simulated semidiurnal tides (two high and two low tides per day), with two planted and two soil-only microcosms per tidal system. The tidal systems were made of four 5-gallon buckets (each 36.8 cm tall): two stacked on top of two below to create an upper and a lower level. The upper buckets held the planted and soil-only microcosms, whereas the lower buckets served as water reservoirs. The "high tide" for the microcosms was initiated by the water pumps running for six-hour intervals and pumping water from the lower to the upper buckets such that the soil surface became submerged by roughly 2 cm of water. The "low tide" was simulated by the six-hour intervals where the pumps were turned off by the timer, allowing water to drain from the upper to the lower buckets through small holes in the sides of the upper buckets that were at a level of approximately 5 cm above the base of the bucket.

All tidal systems were initially filled with freshwater, and plants were allowed to acclimate to their new environment for two weeks. The forty liters of water used to fill each tidal system were tap water, which is classified as fresh water (<0.5 psu). The forty-eight treatment planted microcosms and soil-only microcosms, being grown in saline environments, had enough Instant Ocean aquarium salt (Spectrum Brands, Blacksburg, VA) stirred in to gradually increase the salinity over a two-week period until each system reached the desired salinities for its respective treatment; those being 2, 6, and 9 psu (97.10 g, 292.66 g, and 440.55 g of salt added per 40 liters, respectively). The salinity treatments had the salt stirred into the water for a minimum of ten minutes using stir bars to dissolve the salt. Each system reached its target salinity at the same time, so the incremental increase for the 2 psu treatment was

smaller than for the 6 or 9 psu treatments (1 psu increase per week versus an increase of 3 or 4.5 psu per week, respectively). The sixteen control group microcosms (combined planted and soil-only microcosms) were continually maintained in freshwater, without any added salt. The water for all microcosms was changed every other week, although there were several building closings due to the pandemic and air circulation problems which prevented regular changes of water for several months through the latter part of 2020 and early 2021.

There were several points in which aphids were observed on the plants, so diatomaceous earth and a Spectrum Brand aphid treatment were applied to all microcosms four times between December 2020 and March 2021.

Autotrophic and Heterotrophic Respiration:

Wetland plants were grown at various salinities in microcosms using a C4 plant : C3 soil design (after Wolf et al., 2007). By measuring the ^{13}C values of CO_2 emitted from the microcosms and taking advantage of the isotopic difference between the plants and soil, I was able to use an isotope mixing model (Equations 1 and 2, below) to determine how much respiration came from the plants versus the soil and how those varied with salinity. While heterotrophic respiration occurred in all microcosms, to minimize confusion I will use the phrase "soil CO_2 emissions" when talking about heterotrophic respiration in the soil-only microcosms and "heterotrophic respiration" when referring to heterotrophic portion of ecosystem respiration in the planted microcosms. I focused on the release of CO_2 as opposed to CH_4 because CH_4 production is less likely to occur under elevated salinities since it is less energetically favorable reaction than sulfate reduction from the sulfates that are present in seawater (Capone & Kiene, 1988). Another reason methane was not measured for this study is that, although it is a much more potent greenhouse gas, 38 times more CO_2 was released than CH_4 in a freshwater tidal wetland (Neubauer 2013). Li et al. (2022) found that, under elevated salinities, 6400 times more CO_2 was emitted

than CH_4 in mud flats and vegetated salt marshes, which highlights the higher energetic favorability of sulfate reduction.

I directly measured the rates of soil CO_2 emissions (soil-only microcosms) and ecosystem respiration (planted microcosms) . The rates of autotrophic and heterotrophic respiration were calculated using the measured rates of CO_2 emissions and the ^{13}C value of that emitted CO_2. As detailed below, the laboratory sampling procedure followed Bernal et al. (2017), with calculations using equations presented in Wolf et al. (2007). These measurements were taken between the times of late May and early June 2021 in ambient greenhouse temperatures (average = 34˚C). Each microcosm was fitted with an opaque PVC chamber (10.2 cm inner diameter, 77 and 152 cm tall for soil-only and planted microcosms, respectively) with a pipe joint (Figure 1). Once the chamber was fitted onto each microcosm with the pipe joint, the microcosm and pipe joint was submerged in water to prevent outside air from entering the chambers. The chamber volumes were then flushed with Airgas Ultra Zero Air for a minimum of five minutes, with the exhaust gas vented through a LI-7000 CO_2 analyzer (LI-COR Biosciences, Lincoln Nebraska) until the CO_2 concentration in the chamber reached a low and stable value (median [CO_2] = 2.43 ppm). This ensured that the CO_2 samples collected were emitted by the microcosms and were not a mix of CO_2 from the microcosms and from ambient air. The CO_2 was allowed to accumulate in the chambers for 6 hours (planted microcosms) or 16 hours (soil-only microcosms) due to their lower rate of respiration. Once the incubations were complete, a small battery-operated fan was run inside each chamber for a minimum of 5 minutes to circulate the air. The final CO_2 concentrations were measured by collecting 40 ml of air from the chambers in syringes and pushing the air through the LI-7000 CO_2 analyzer. Using a syringe, an additional 60 ml was collected from each chamber and all except 5 ml of this air was pushed through each 12 ml Exetainer borosilicate vial (Labco, Buckinghamshire, England) with a vent needle being inserted to allow air to escape. The vent needle was then removed, and the remaining 5 ml was added to each vial, causing the vials to be slightly over-

pressurized and preventing outside air from entering them. Each Exetainer vial had previously been flushed for at least 5 minutes with Airgas ultra-high purity nitrogen gas, which was injected using a syringe and vent needle that allowed air to escape. The ^{13}C isotope values of CO_2 emitted from each microcosm were determined by sending gas samples to the University of Connecticut Isotope Facility (Groton, CT) that is managed by the Tobias Lab.

Isotope Calculations:

The equations for determining rates of autotrophic and heterotrophic respiration are listed below:

$$\mathbf{F_{Total} = F_{Plant} + F_{Soil}} \quad \text{(Eq. 1)}$$

$$\mathbf{F_{Plant} = F_{Total}\,[(\delta_{Total} - \delta_{Soil}) / (\delta_{Plant} - \delta_{Soil})]} \quad \text{(Eq. 2)}$$

Equation 1 has the autotrophic and heterotrophic respiration, F_{Plant} and F_{Soil}, respectively, that is in units of µmol CO_2 m^{-2} h^{-1}. The total ecosystem flux of CO_2 (F_{Total}; µmol CO_2 m^{-2} h^{-1}) was calculated from the long-term (multi-hour) incubations of CO_2 in each planted microcosm, the samples of which were run through the LI-COR LI-7000. Equation 2 was to determine autotrophic respiration (F_{plant}). The δ_{Soil} shown in Equation 2 represents the ^{13}C value of the CO_2 emitted from the soil-only microcosms during the 16-hour incubations. The δ_{Total} in Equation 2 represents the ^{13}C value of the CO_2 emitted from the planted microcosms during the 6-hour incubations. The δ_{Total} was determined by the samples collected from each microcosm, while the δ_{Soil} was used only for determining F_{plant} for the planted microcosms in the same tidal system. The δ_{Plant} were determined by the ^{13}C values from the weighted averages of the pulverized aboveground and belowground biomasses from each planted microcosm. Once autotrophic

respiration was calculated, Equation 1 could be used to determine heterotrophic respiration (F_{Soil}) for the planted microcosms.

Gross Ecosystem Productivity:

Measurements for whole-microcosm photosynthesis and (short-term) respiration were taken between late May and early June 2021 to calculate gross ecosystem production using the LI-COR LI-7000 on sunny days, with grow lights supplementing natural light. Transparent acrylic chambers measuring 147 cm tall were placed onto the planted microcosms (n= 2 per salinity treatment) with a seal at the base and top to prevent gas entering or escaping. I then measured changes in CO_2 every 15 seconds during the ~10 min incubations with the transparent chambers.

After removing the chamber for venting and then reinstalling it on the microcosm, the chamber was tightly covered with black plastic to prevent any light from entering the chambers and the rate of ecosystem respiration (ER) was measured over 10 minutes using the LI-COR LI-7000 analyzer. The slope of CO_2 vs. time was calculated from these data to determine the rates of net photosynthesis (transparent chamber) and ecosystem respiration (dark chambers). The slopes (in ppm min^{-1}) were converted to molar fluxes (µmol m^{-2} min^{-1}) using greenhouse temperatures, chamber volumes, and the ideal gas law. Soil distance from the top of each microcosm was measured in three places for each microcosm to determine the volume of airspace for each individual microcosm. These data were added to chamber volume to determine total volume of microcosm + chamber airspace per microcosm. The ER, when subtracted from the NEP, was used to find gross ecosystem productivity (GEP; Figure 1).

With the autotrophic respiration data (from the isotope measurements) being divided by the GEP, the fraction of GEP respired by the plants can be calculated. The fraction of GEP respired can then be subtracted from one to calculate the carbon use efficiency (Figure 1).

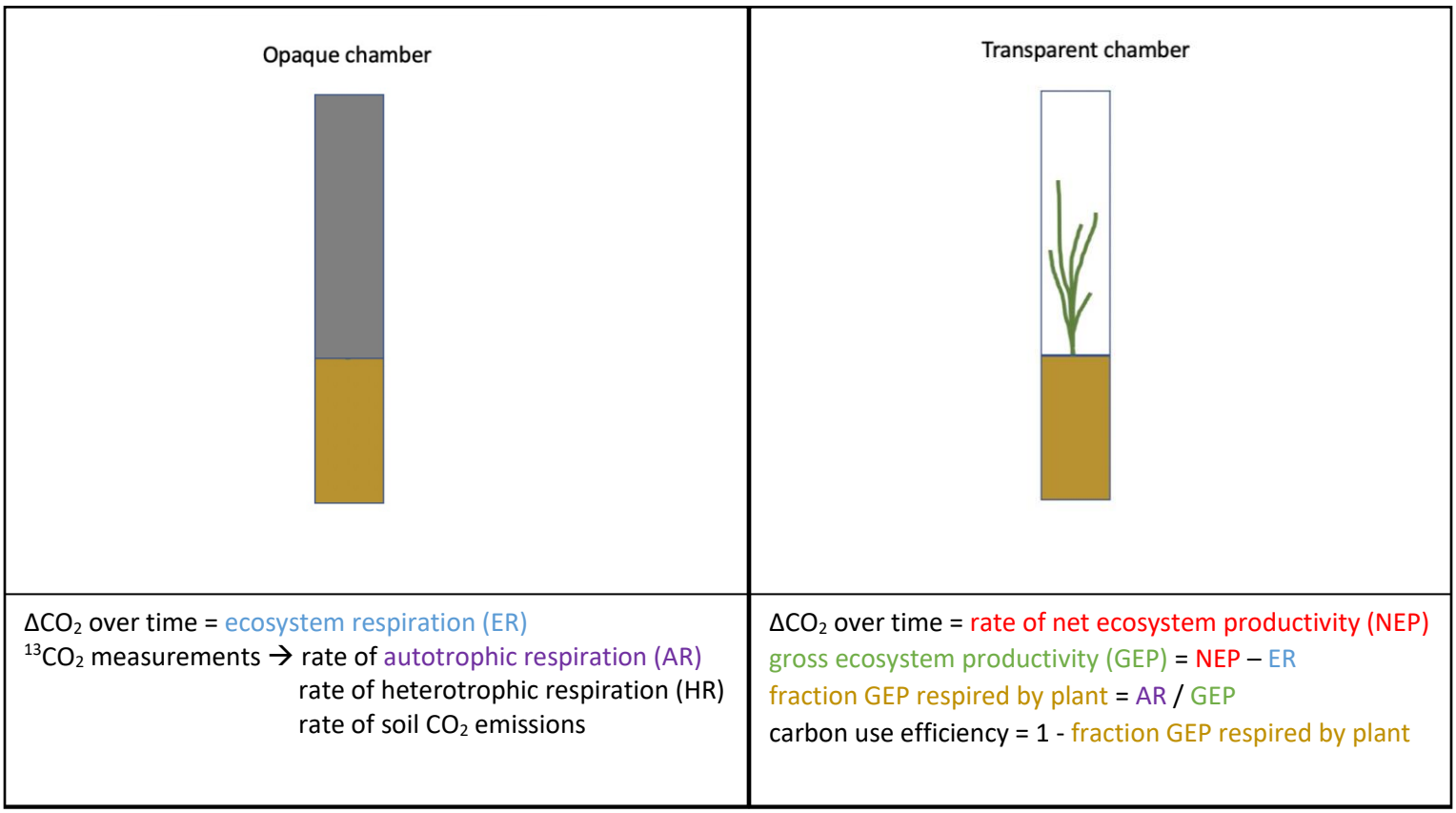

Figure 1. Overview of measurements made using opaque and transparent chambers.

Leaf-Level Respiration:

Leaf-level measurements were also taken at the beginning and end of the experiment (October 2020 and June 2021) using the LI-COR LI-6400 XT using a dark chamber with ambient temperatures, humidity, and CO_2 levels.

Plant Processing:

Upon completion of the project (June 2021), aboveground biomass was collected to compare overall plant growth under normal versus salt-stressed conditions. The height of the tallest plant in each microcosm was measured, and the number of leaves per microcosm was counted prior to cutting the aboveground biomass at the soil surface. Leaves were classified as either live (≥40% green) or senesced (<40% green) and counted separately. In addition to the *S. cynosuroides* in the plant microcosms, several

Sparganium americanum (American bur-reed) plants were found in the 0 and 2 psu plant microcosms. The aboveground biomass was counted, clipped, and weighed separately from the *S. cynosuroides* after drying. The plant material from each microcosm was then dried at 45 °C for ~24 hours in a drying oven.

The belowground biomass was separated from the soil using a wet separation method involving copious water and a 1 mm sieve. The microcosms were placed in a cold room immediately after the aboveground biomass was clipped and processed for belowground biomass in July through August 2021. The belowground biomass from the *S. americanum* was also separated as best as possible from the *S. cynosuroides.* This was possible since the roots of the smaller *S. americanum* were bright reddish in color as opposed to the deep orange of the larger *S. cynosuroides* roots. The belowground biomass was then dried for a minimum of 7 days in a drying oven at 45 °C. The dried aboveground and belowground biomass samples were immediately weighed upon removal from the drying oven and ground in an IKA Works MF 10 grinder with a 2 mm sieve. The pulverized plants from each microcosm were shipped to the University of Arkansas Stable Isotope Laboratory (Fayetteville, AR) to measure ^{13}C values and percents carbon and nitrogen.

Pre-experiment plant samples (both aboveground and belowground biomass) were also pulverized and sent to the University of Arkansas Stable Isotope Laboratory. This was to determine the carbon isotope ratio in the plant material prior to the experiment being conducted on the plants.

Soil Samples:

Between July and August 2021, soil samples were collected from all but three of the planted microcosms in the 2 psu treatment. A total of approximately 5 grams of soil was cut from 1-2 cm below the soil surface in three separate areas in each microcosm to ensure the carbon isotope values were representative of the whole microcosm. All visible roots were removed, and the soil was dried at 45 °C for at least five days. The soil samples were pulverized then in a mortar and pestle. These soil samples

were collected to determine if the carbon isotope ratios were similar to those of the gas samples. All soil samples were shipped to the University of Arkansas Stable Isotope Laboratory to measure ^{13}C values. The carbon isotope ratio of the plug soil was also tested to determine if its carbon isotope ratio was statistically similar to that of the wetland soil. The plug soil was a small amount of potting soil around each plant that was left intact when planting the microcosms.

Statistics:

One-way ANOVAs were used to determine if there were significant differences in total ecosystem respiration, autotrophic respiration, photosynthesis, and soil CO_2 emissions for the different salinity treatments over time. ANOVA tests were also used to find any significant differences in the final aboveground, belowground, total biomass, root to shoot ratios, height of tallest plants per microcosm, and number of leaves of the plants subjected to the different salinity levels. When an ANOVA revealed a significant treatment effect, Tukey post hoc tests were run. Statistical tests were conducted using R (RStudio PBC, Version 1.3.1093, Boston, MA, USA) at an $\alpha = 0.05$.

Results:

The tallest plant height average for all the treatments was 96 cm, and there was no significant difference in leaf height among the treatments, whether including the *S. americanum* in these calculations ($p = 0.297$) or only looking at the *S. cynosuroides* ($p = 0.536$). The average length of the *S. americanum* plants in the control group was 68.7 cm, while the single *S. americanum* plant that grew in the 2 psu treatment was 56.5 cm tall. There were no significant differences in the total number of leaves per microcosm ($p = 0.788$) or with the leaves classified as live leaves ($p = 0.434$) among the different salinity levels (Table 1). There were no significant differences between the total number of senesced leaves (both *S. cynosuroides* and *S. americanum*; $p = 0.141$) among the salinity levels and there was no

significant difference in the number of senesced leaves among different salinity levels for only *S. cynosuroides* (p = 0.079; Table 1). The lowest average number of senesced leaves (only *S. cynosuroides*) in any treatment was in the control group, which was ~33 leaves per microcosm. The highest average number of senesced *S. cynosuroides* leaves was ~42 leaves per microcosm in the 6 psu treatment.

Table 1. Tallest plant height and number of leaves (total, live, senesced) among the different salinity levels. Rows split the data between data looking at both *S. cynosuroides* and *S. americanum* together and when looking at each species individually. Values are averages, with standard deviations (±) shown in parentheses. Asterisks (*) indicate that a single plant was observed in the entire 2 psu treatment so no standard deviation was available. Four microcosms in the control group contained *S. americanum*, plants, while only one microcosm in the 2 psu treatment contained *S. americanum.* No *S. americanum* plants were found in the 6 or 9 psu microcosms. *S. cynosuroides* was found in all microcosms.

	Salinity	**Tallest Plant Height (cm)**	**Total Leaves (# per microcosm)**	**Live Leaves (# per microcosm)**	**Senesced Leaves (# per microcosm)**
All plants	**0**	92.6 (±42.0)	80 (±16)	46 (±14)	34 (±8)
	2	89.3 (±46.9)	78 (±17)	36 (±10)	41 (±10)
	6	100.3 (±17.2)	79 (±7)	37 (±7)	42 (±5)
	9	103.5 (±16.5)	74 (±12)	39 (±7)	35 (±7)
S. cynosuroides	**0**	100.5 (±10.6)	70 (±15)	37 (±10)	33 (±8)
	2	96.5 (±7.5)	77 (±18)	36 (±10)	41 (±10)
	6	100.3 (±18.4)	79 (±7)	37 (±7)	42 (±5)
	9	103.5 (±17.7)	74 (±12)	39 (±7)	35 (±7)
S. americanum	**0**	68.7 (±14.9)	10 (±16)	9 (±14)	2 (±3)
	2	56.5	1 (*)	1 (*)	0 (*)

Salinity affected belowground biomass, but not aboveground biomass. When combined, salinity significantly impacted total above- and belowground biomass (p = 0.020), with the total biomass of the 9

psu treatment being significantly lower than the 2 psu treatment. No significant difference was found between total aboveground biomass ($p = 0.250$) at the various salinity levels. Belowground biomass was significantly impacted at the various salinities, with belowground biomass in the 2 psu treatment being greater than in the 6 and 9 psu treatments. Belowground biomass was ~40% lower in the 9 psu treatment compared to the 2 psu treatment (Figure 2).

The root:shoot ratios were also significantly different between salinity treatments ($p = 0.002$), with the 2 psu treatment having a significantly greater root:shoot ratio than the 9 psu treatment (2.97:1 and 1.73:1, respectively).

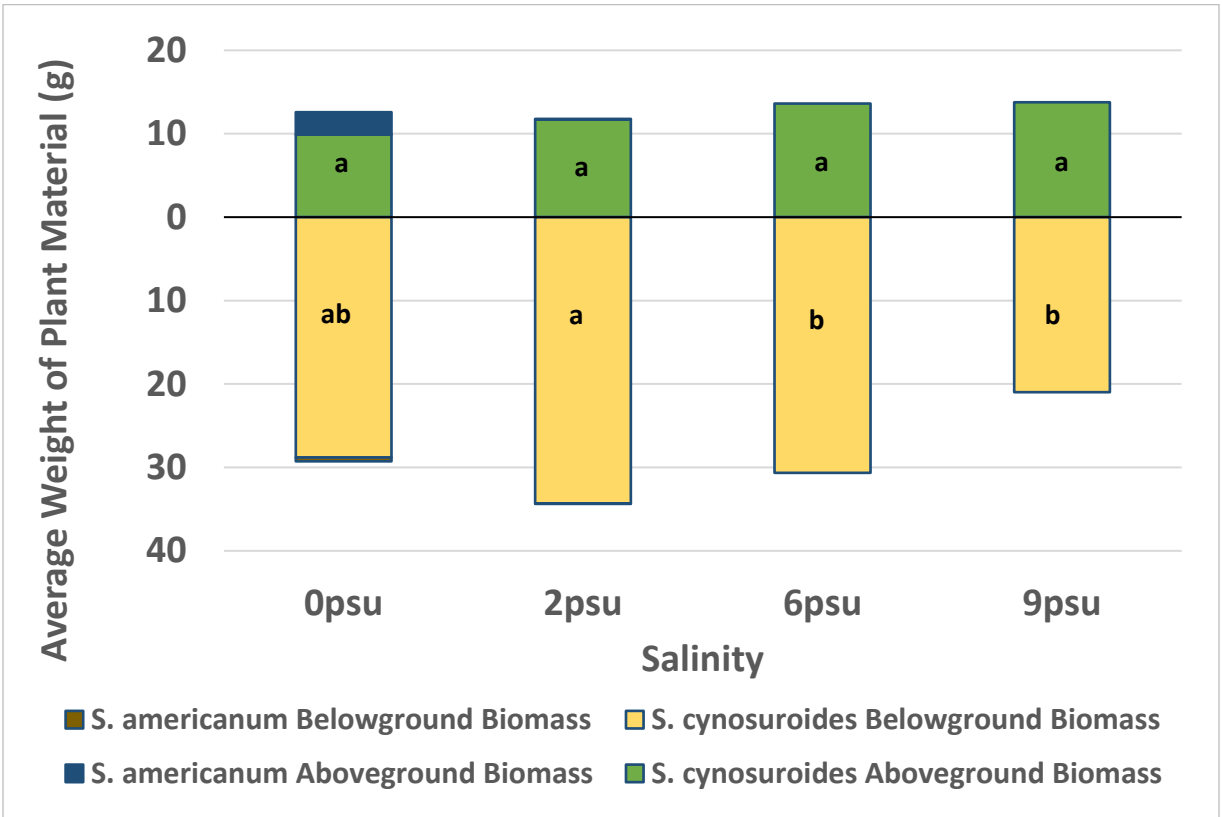

Figure 2. Average per-microcosm aboveground biomass was measured at the end of this experiment (June 2021) and belowground biomass was weighed once it was separated from the soil (August 2021). There were eight replicates per treatment. The values above the zero line represent aboveground biomass whereas those values below are belowground biomass. The letters indicate statistically similar or different treatments.

For the ecosystem respiration (µmol CO_2 m^{-2} hr^{-1}) that was measured through the long-term respiration measurements, there were no significant difference in combined plant and soil respiration among the salinity treatments (p = 0.345). The lowest mean ecosystem respiration was in the 9 psu treatment, which was ~80% that of the control group (Figure 3). Soil CO_2 emissions in the long-term respiration measurements differed significantly among the different salinity levels (p = 0.016), with soil CO_2 emissions in the 2 psu treatment being ~60% that of the control group and causing significant differences between the treatments (Figure 4). The mean CO_2 emission rates were ~10-15 times greater from the planted microcosms compared with the soil-only microcosms (Figures 3 vs. 4).

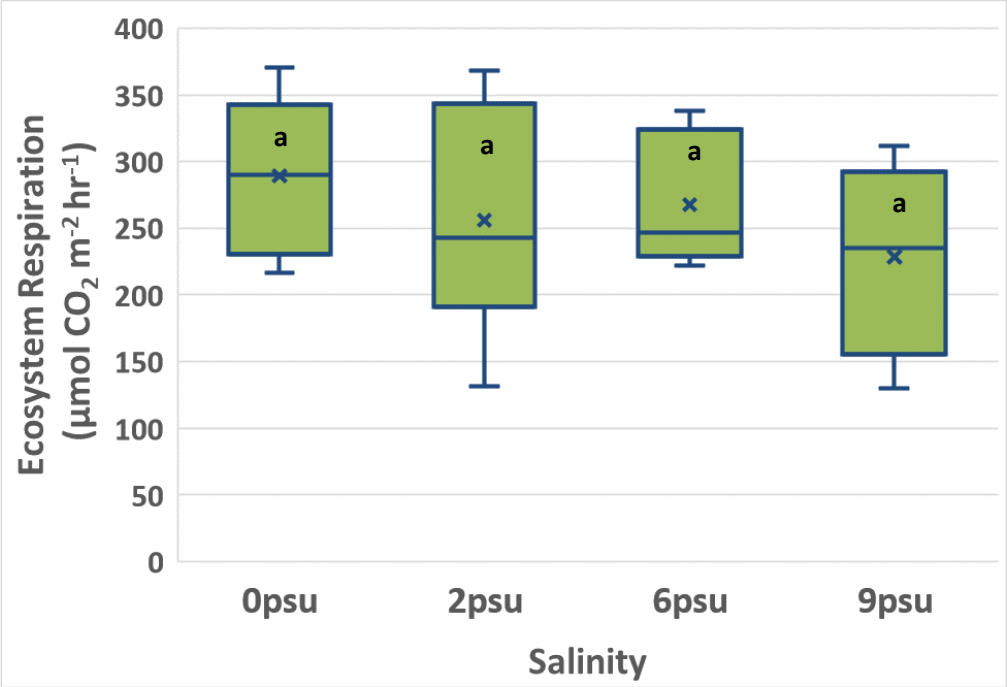

Figure 3. Box and whisker plot for long-term ecosystem respiration measurements (µmol CO_2 m^{-2} hr^{-1}) between salinity treatments. The x in the boxes indicate the mean, while the median is shown by the solid lines within each box. The whiskers indicate the minimum and maximum values of the data collected. There were eight replicates per treatment. The letters indicate statistically similar treatments.

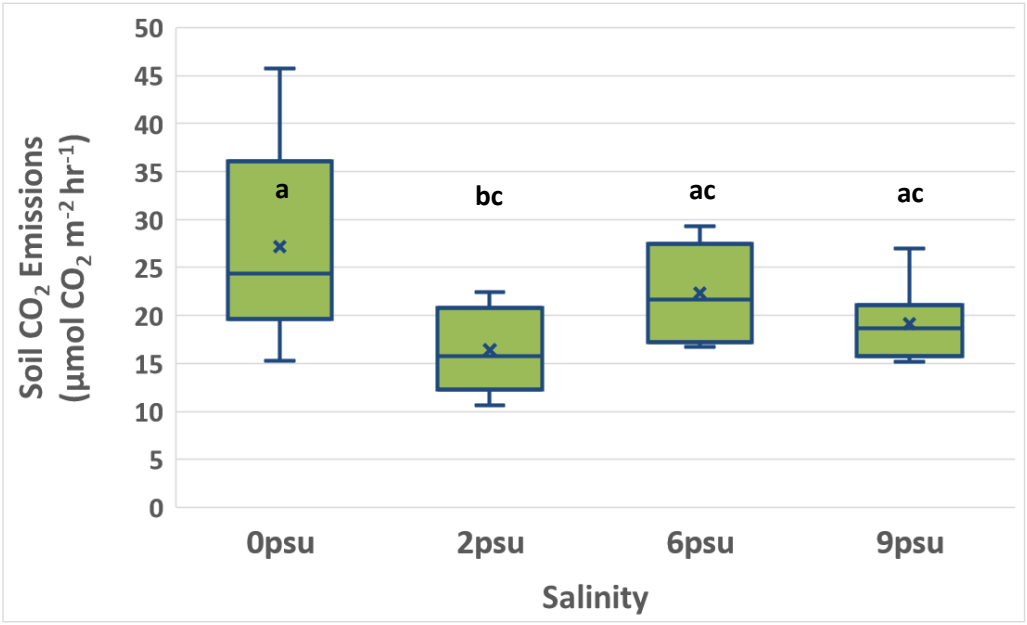

Figure 4. A box and whisker plot for soil CO_2 emissions (μmol CO_2 m^{-2} hr^{-1}) among the salinity treatments. The soil CO_2 emissions were heterotrophic respiration measured from the soil-only microcosms. The x in the boxes indicate the mean, while the median is shown by the solid lines within each box. The whiskers indicate the minimum and maximum values of the data collected. There were eight replicates per treatment. The letters indicate statistically similar or different treatments.

There were significant differences in post-project leaf-level respiration (μmol CO_2 m^{-2} sec^{-1}) in the *S. cynosuroides* among the different salinity levels ($p = 0.007$). The 6 and 9 psu treatment leaf respiration was significantly higher than the 2 psu treatments, with means of 0.79 μmol m^{-2} sec^{-1} versus 0.34 and 0.31 μmol m^{-2} sec^{-1}, respectively (Figure 5). No significant difference in leaf-level respiration before salinity treatments were added to the microcosms was found ($p = 0.058$). The pre-salinization leaf-level respiration rates in these plants ranged from -2.91 to 1.78 μmol m^{-2} sec^{-1}, with a mean of -0.36 μmol m^{-2} sec^{-1}.

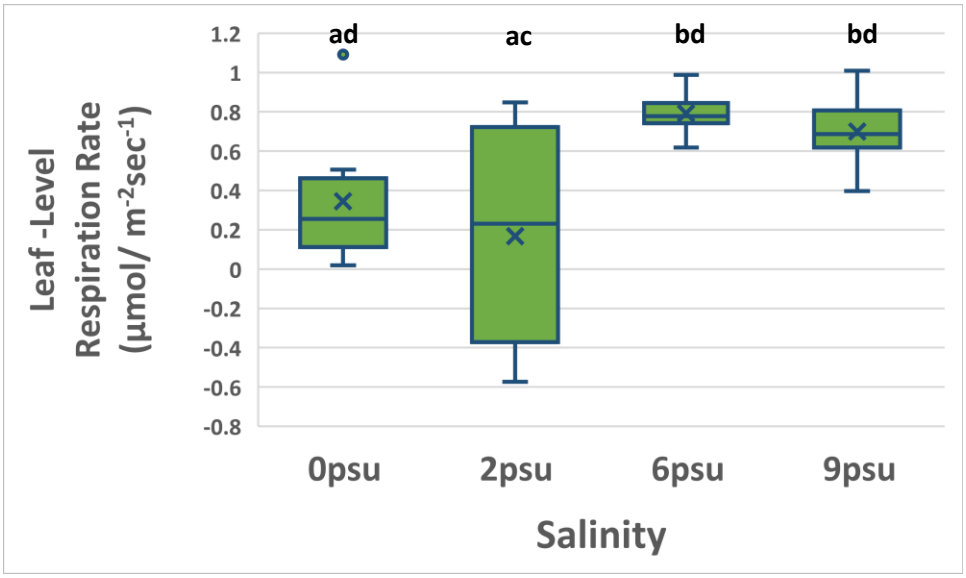

Figure 5. A box and whisker plot for leaf-level respiration (µmol CO_2 m^{-2} sec^{-1}) of the *S. cynosuroides* among salinity treatments at the end of the experiment. The x in the boxes indicate the mean, while the median is shown by the solid lines within each box. The whiskers, except in cases of outliers, indicate the minimum and maximum values of the data collected. There was an outlier from an *S. cynosuroides* plant in the control group, as indicated by the blue point at 1.09 µmol m^{-2} sec^{-1}. There were eight replicates per treatment.

There was no significant difference in Gross Ecosystem Productivity (GEP; µmol CO_2 m^{-2} min^{-1}) between the treatments (Figure 6; $p = 0.346$). The average GEP for the control group was the highest, at 971.79 µmol CO_2 m^{-2} min^{-1}, while the lowest average GEP was measured in the 2 and 6 psu treatment (456.29 and 577.14 µmol CO_2 m^{-2} min^{-1}, respectively), meaning that the microcosms in these treatments were fixing approximately half the carbon than that of the control group plants (Figure 6). Due to a data logger issue that caused the loss of the majority of the GEP data, these patterns in GEP vs. salinity are based on only two replicates per treatment.

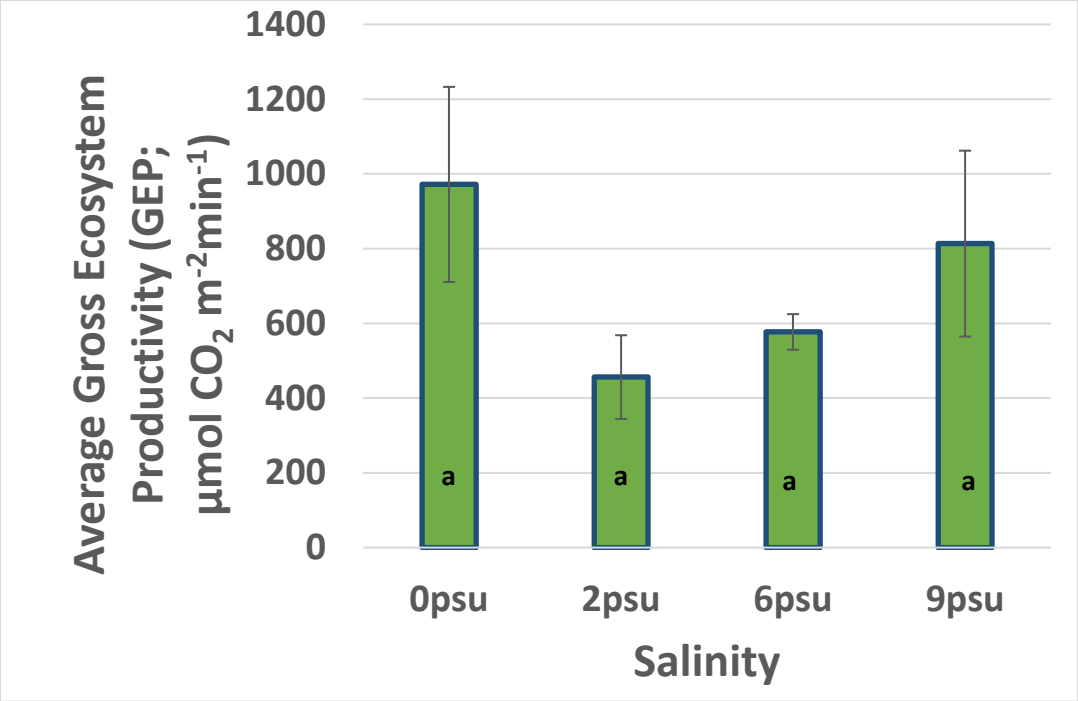

Figure 6. Bar graph of the average gross ecosystem productivity (GEP; µmol CO_2 m^{-2} min^{-1}) at the different salinity levels in the short-term incubations. The bars included the standard deviation of GEP at each salinity level. There were two replicates per treatment.

Discussion:

Rising salinities negatively impact wetland plant growth, plant productivity, and microbial processes. Compared with freshwater marsh plants such as *S. americanum*, halophytic plants such as *S. cynosuroides* have higher salinity tolerances than freshwater plants and experience less stress due to elevated salinities. Plant productivity of *S. cynosuroides* with regards to accumulated aboveground biomass was not significantly impacted by elevated salinities, although there was significantly lower belowground biomass in the mid- and high-level salinities (6 and 9 psu, respectively) than in the low salinity treatment (2 psu). Lower total biomass and senescence of less salt-tolerant plants may be expected based on the low survival observed in the *S. americanum.* Some suppression of ecosystem

respiration was observed, but it was not significant. Significantly suppressed soil CO_2 emissions suggest that autotrophic respiration was higher at elevated salinities in the planted microcosms, although this inference may be an imperfect representation of the actual trend of heterotrophic respiration due to the effects the plants themselves have on microbial processes that result in CO_2 emissions. Leaf-level respiration was increased when *S. cynosuroides* plants were exposed to mid- and high-level salinities. Salinity is also expected to have similar effects on freshwater marsh plants, although this effect may be more pronounced in glycophytes. Gross ecosystem productivity was suppressed at all elevated salinity levels, which may be a better indicator to the effects of elevated salinities on glycophytes.

Salinity Effects on Plant Productivity:

Aboveground biomass of *S. cynosuroides* in this study was not significantly impacted by the elevated salinities, likely due to their high salinity tolerance. Previous research has shown that significant decreases in aboveground biomass occurred in *S. cynosuroides* that were grown in conditions >14 psu when compared to plants grown in <14 psu (White and Alber, 2009). The high salinity treatment for this study only had a goal salinity of 9 psu, which may be the reason no significant difference in aboveground biomass was observed. Li and Pennings (2018) found that freshwater tidal plants such as *Polygonum hydropiperoides* and *Pontederia cordata* that have a lower salinity tolerance than *S. cynosuroides* had significantly lower aboveground biomass and survival of the plants when exposed to a continuous porewater salinity of 5 psu.

I found that belowground biomass was lowest at the highest salinity, while the mid-salinity treatment had only a slightly higher belowground biomass than the aforementioned highest salinity treatment. This is consistent with the findings of Chen et al. (2017), who measured significant decreases belowground biomass of marsh plants at elevated salinities. Although belowground biomass was

generally greater at the lower salinity treatment, I unexpectedly found that the greatest belowground biomass was for plants grown in the low salinity treatment, not in freshwater. This is consistent with previous research where there was a stimulatory response of some *Spartina* species growth in low salinity environments versus freshwater environments (Wu et al., 2005). I hypothesized that greater belowground biomass in the *S. cynosuroides* grown in low salinities than in the control group is due to a vacuolar H^+-ATPase subunit c1 (*SaVHAc1*) gene previously found in *S. alterniflora* that allows for increased salt tolerance by causing the plants to store large levels of K^+ in their vacuoles which is balanced by the low levels of Na^+ entering the cell (Baisakh et al. 2012). Essentially, the K^+ in the vacuoles activates many necessary enzymatic processes in the plant cells and more K^+ being stored in the vacuoles means that they are more likely to outcompete the Na^+ for the binding sites (Bhandal and Malik, 1988), but the plants also need some Na^+ to create a cytosolic balance within the cells. The high-level salinity treatment did have significantly lower belowground biomasses than the low salinity treatment, indicating that the aforementioned threshold of stimulated growth had been surpassed. I hypothesized this was the Na^+ volume in the vacuole exceeding the balance of K^+/Na^+, preventing necessary cellular function and causing ionic stress in the plants.

Additionally, sulfates may have some fertilization effects on *S. cynosuroides* and may contribute to plant growth and productivity at low salinities. Sulfur is a necessary component in plant growth and biomass production. Stribling (1997) found that *S. cynosuroides* exhibited faster growth at mid-levels of sulfate inputs when compared to low-levels of (80 vs. 8 µmol SO_4^{2-} l^{-1}, respectively), but growth rates declined at even higher sulfate concentrations (800+ µmol SO_4^{2-} l^{-1}).

The different responses of aboveground versus belowground biomass caused lower root to shoot ratios at higher salinities. The lowest root to shoot ratio was observed in the high salinity treatment, with less than two times as much roots as shoots. The high salinity treatment root to shoot ratio was significantly lower than the low salinity treatment. Previous research found no significant

difference when comparing freshwater and 7 psu conditions for *S. cynosuroides* root to shoot ratio (McHugh and Dighton, 2004). I hypothesized that the different responses in roots and shoots that significantly altered the root to shoot ratio were for two reasons: 1.) the root biomass was lower at the highest salinity due to the root cells being submerged in the high concentration of ions that inhibited root growth from ionic stress, and 2.) the leaf biomass was unaffected due to salt glands in the leaves excreting some of the salt prior to it entering the vacuoles. *S. cynosuroides* has salt glands on the adaxial subsidiary cells of leaves, which is one method *S. cynosuroides* has evolved to escape some of the negative effects of excess ions in their cells (Maricle et al., 2009). Secreted salt was visually observed on the leaves in the high salinity treatment, so this further supports my hypothesis. Although some salt-tolerant plants have the adaptation to prevent salt ions from entering the root cells (Katschnig et al., 2015), I was unable to find research that indicated that *S. cynosuroides* contain this adaptation. Whether or not *S. cynosuroides* do possess this adaptation, the resulting lower root biomass at high salinities suggests that the plants were negatively impacted by the salt.

Salinization can also impact availability of various nutrients such as nitrogen, which may in turn cause nutrient limitations that negatively impact plant biomass production. Several ways in which nitrogen balances can be affected as a result of salinization are by displacement of sedimentary ammonium cations (van Dijk et al., 2015), decreased nitrification (Rysgaard et al., 1999), and decreased denitrification (Neubauer et al., 2019). For example, previous research has found that exposing a freshwater marsh to two years of elevated salinities (4.5 and 9 psu) caused mobilization and loss of ammonium from the sediment (van Dijk et al., 2019). For my research, however, plant nitrogen concentration ranged from 0.73 (sd ±0.1) %N for roots to 0.71 (sd ±0.1) %N for leaves (Figures 12 and 13) and did not vary among the salinity treatments ($p = 0.577$ and $p = 0.242$, respectively), suggesting that differences in soil N availability ($p < 0.0001$, with significantly lower %N in soils at elevated salinities) did not drive the differences in observed root biomass (Figure 14).

Tidal wetland plant species have varied sensitivities to elevated salinity based on their adaptations to salty conditions, which will create varied responses to elevated salinities such as that which were observed between *S. cynosuroides* and *S. americanum*. Even though this experiment focused on *S. cynosuroides*, a plant that is typically found in brackish marshes, the presence of *S. americanum* in some of the microcosms provided an opportunity to contrast the responses of a halophyte (*S. cynosuroides*) and glycophyte (*S. americanum*) to the same salinity stressor. Several *S. americanum* plants were found in the control and low salinity treatment plant microcosms, but not in the mid- and high salinity treatments. No *S. americanum* plants were observed in the soil-only microcosms, so I think that these plants grew from the plugs and not from seeds present in the natural wetland soil. None of the *S. americanum* were observed upon visual inspection of the plugs prior to planting. For the mid- to high-level salinity treatments, I think that either 1.) there had been *S. americanum* seeds in the plugs that did not germinate in the higher salinity environments, 2.) *S. americanum* seeds that were present in the higher salinity environments germinated but then died off before I observed them, or 3.) there were *S. americanum* plants which were not initially observed and died off once the salinity treatments were applied. These suppositions are supported by previous research that has shown that *Sparganium* are freshwater plants with extremely limited survival rates even at low salinities of 0.2 psu (Kaijser et al., 2019). The lack of freshwater plant growth from salinization, such as that which I observed with the *S. americanum*, is likely to occur with many plants in freshwater marshes with rising sea levels, although some plant species have greater salt tolerance than others.

Although GEP was not significantly impacted by elevated salinities, the plants grown in the freshwater fixed an average of twice as much carbon as the low salinity treatment. One of those microcosms grown in the freshwater systems did have an *S. americanum* plant growing in it, so it is possible that this single plant had some disproportionate impact on the GEP in the freshwater

microcosms. The microcosm with the *S. americanum* had a GEP that was over 70% higher than the microcosm without the *S. americanum* plant. The ratio of GEP to aboveground biomass provides an estimate of rates of C fixation (photosynthesis) per unit of aboveground plant biomass and supports the idea that elevated salinities stressed the plants. Under freshwater conditions, the GEP to aboveground biomass ratio averaged 76.98 μmol CO_2 g^{-1} m^{-2} min^{-1} in the control group versus 30.82, 33.27, and 49.07 μmol CO_2 g^{-1} m^{-2} min^{-1} for the salinity treatments for the 2, 6, and 9 psu treatments, respectively (Figure 15). These values did not differ by treatment ($p = 0.248$).

Salinity Effects on CO_2 Respiration

Although ecosystem respiration was statistically similar at all salinity levels, there was some suppression of CO_2 emissions in elevated salinities. Given that there was a significant difference in soil CO_2 emissions this suggests that autotrophic respiration offset the lower soil CO_2 emissions at elevated salinities. There were issues with the isotopic autotrophic respiration calculations in my study, however, and I was unable to use that data in my study. For example, the calculations indicated that autotrophic respiration sometimes accounted for either more than 100% or less than 0% of the measured ecosystem respiration for those respective microcosms. Either of these outcomes is impossible and I will review the potential causes for these results in the Appendix.

Since I am unable to use the isotopic autotrophic respiration data, I will review the leaf-level respiration measurements as a way to observe any potential changes in respiration at different salinities. The low salinity treatment resulted in the lowest average leaf-level respiration with the mean respiration of the control being slightly higher, while the mid- and high-level salinities exhibited significantly increased leaf-level respiration (Figure 5). This provides an interesting look at the potential trend in the autotrophic respiration of these plants. Given that there was no significant difference in the

ecosystem respiration, this would mean that the heterotrophic respiration of the planted microcosms may have been decreased, similar to that which was observed in the soil CO_2 emissions, while the autotrophic respiration may have been increased.

Soil CO_2 emissions were significantly lower in the low salinity than the control group that indicates there was sensitivity in the microbial communities to the elevated salinity, which is in line with previous research that had similar results (Brouns et al., 2014; Minick et al., 2019; Nyman and DeLaune, 1991; Ardon et al., 2018; Doroski et al., 2019). Although the mid- and high salinity treatments did not exhibit significantly lower soil CO_2 emissions than the control group, microbial respiration was suppressed. It should also be noted that many lab saltwater intrusion studies are short term studies, lasting only several hours to several days. My study was on a longer time scale (spanning one year), meaning that this may be more representative of the long-term effects of saltwater intrusion on microbial activity. For example, a four-hour incubation of freshwater wetland soils by Marton et al. (2012) found that there were higher microbial emissions of CO_2 on average in soil samples that were exposed to 2 and 5 psu than those in freshwater. In my study, I found that salinity suppressed soil CO_2 emissions, which is directly in opposition to what Marton et al. (2012) found in their research. Research by Weston et al. (2011) found that there were higher emissions of CO_2 in the elevated salinity treatment (5 psu) than in the freshwater environment for the entire year-long experiment, although CO_2 emissions were decreasing in the last six months. This suggests that the elevated CO_2 emissions observed by Weston et al. (2011) may have been leveling out in the last months of their research. Thus, it is possible that higher CO_2 emissions had initially occurred in my elevated salinity microcosms but decreased once most of the labile carbon had been mineralized, which was also similar to the findings of Neubauer et al. (2013). One shortcoming of my long-term experiment was that the unplanted microcosms did not include labile carbon inputs which would naturally occur in a tidal wetland setting.

Salinity Effects on Vertical Accretion:

Freshwater tidal wetlands receive mineral material inputs (e.g., sand, silt, clay) with the tides, but many wetlands still need to accumulate organic matter to help keep pace with sea level rise. This means that suppressed above- or belowground biomass accumulation from rising salinities may pose a problem for wetlands already at risk of becoming inundated when water levels rise faster than a wetland can accrete soil. Salinity can also impact decomposition of plant material. Over the long term, the production of CO_2 by soils generally decreases with elevated salinities of ~2 psu when compared to freshwater conditions (Neubauer et al., 2013). This may be beneficial in terms of retaining plant organic materials in the soil, but the plants are still at risk of decreased biomass production in the first place due to water inundation from rising sea levels and salt stress. Neubauer (2013) found that there was generally a significantly decreased net ecosystem productivity in the elevated salinity plots than in freshwater plots. Herbert et al. (2018) also found that freshwater tidal wetland sites exposed to press treatments of brackish water (resulting in 2-5 psu porewater salinity) exhibited decreased gross ecosystem productivity and caused the senescence of a large portion of the plants within the treatment site. All of these factors from salinity work together to produce complex effects on the carbon balance in freshwater tidal wetlands that bring into question the future wellbeing of these existing ecosystems.

Conclusion

Belowground plant productivity was suppressed by salinity stress. In addition to the amount of fixed carbon by the plants being decreased, there was a decrease in ecosystem respiration at elevated salinities. Soil CO_2 emissions were also suppressed under elevated salinity while leaf-level respiration increased. This suggests plants and microbes are less productive under salt-stressed conditions. Less carbon fixation that results in lower plant biomass means wetlands would likely not accumulate as much

carbon. Analysis of the percent soil carbon between the different salinity treatments in my experiment supports this hypothesis, with the control and 2 psu treatment having higher percent carbon than the 6 and 9 psu treatments ($p=0.0006$; means: 6.04 and 5.94 versus 5.78 and 5.72 %C, respectively; Figure 16). Decreased percent soil carbon was also observed by Neubauer et al. (2013) following a three and a half year salinization study. Less carbon fixation and subsequent biomass accumulation in stressed wetland ecosystems is a matter of great concern if it is not at a rate that keeps up with sea level rise. Although tidal wetlands do receive sedimentary inputs with the tides, plant biomass plays a key role in vertical accretion of wetlands (Boyd and Sommerfield 2016).

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Appendix.

The purpose of this section is to review the isotopic calculations for autotrophic and heterotrophic respiration rates, to explore some of the potential reasons for the impossible results from these calculations, and to include some additional figures of various data. I feel that it is both necessary and beneficial to include this isotopic autotrophic respiration data since it was a core part of my thesis proposal and I want to explore it further.

Autotrophic Respiration:

This project was designed so that CO_2 produced by respiring plants would be isotopically different from CO_2 produced by soil heterotrophs, therefore allowing me to calculate how much ecosystem respiration is due to autotrophic vs. heterotrophic processes (Equation 2). Using this approach, I calculated that autotrophic respiration accounted for a median of ~64% of ecosystem respiration (and, therefore, heterotrophic respiration was ~36% of ecosystem respiration) across all planted microcosms. However, for 7 of the 32 planted microcosms, autotrophic respiration was calculated to account for >100% of ecosystem respiration (range: 107 to 6872%), a result that is impossible and meaningless. Additionally, for 5 of 32 planted microcosms, autotrophic respiration accounted for <0% of ecosystem respiration (range: -6 to -114%), which is another biologically impossible result. If I exclude these 12 microcosms in which autotrophic respiration was >100% or <0% of ecosystem respiration, the remaining 20 microcosms had an autotrophic respiration rate that was a median of ~60% of ecosystem respiration.

There are several possible experimental causes for these biologically impossible isotope results: 1.) The plants were contaminated with soil, affecting the resulting plant $\delta^{13}C$. 2.) The *S. americanum* affected the $\delta^{13}C$ values or CO_2 respiration measurements in the microcosms where this species was

mixed with the *S. cynosuroides*. 3.) The exetainers used for storing the gas samples for $\delta^{13}C$ analysis leaked during transit/storage. 4.) The isotope lab/s made an error in running the samples. 5.) There was another source of CO_2 in the chambers such as algae which produced a different $\delta^{13}C$. 6.) One or more of the chambers leaked enough atmospheric CO_2 that this impacted the autotrophic respiration calculations. 7.) The soil $\delta^{13}C$ values are not representative of CO_2 derived from heterotrophic respiration. 8.) The plant $\delta^{13}C$ values are not a good representation of plant $\delta^{13}C$ end member values.

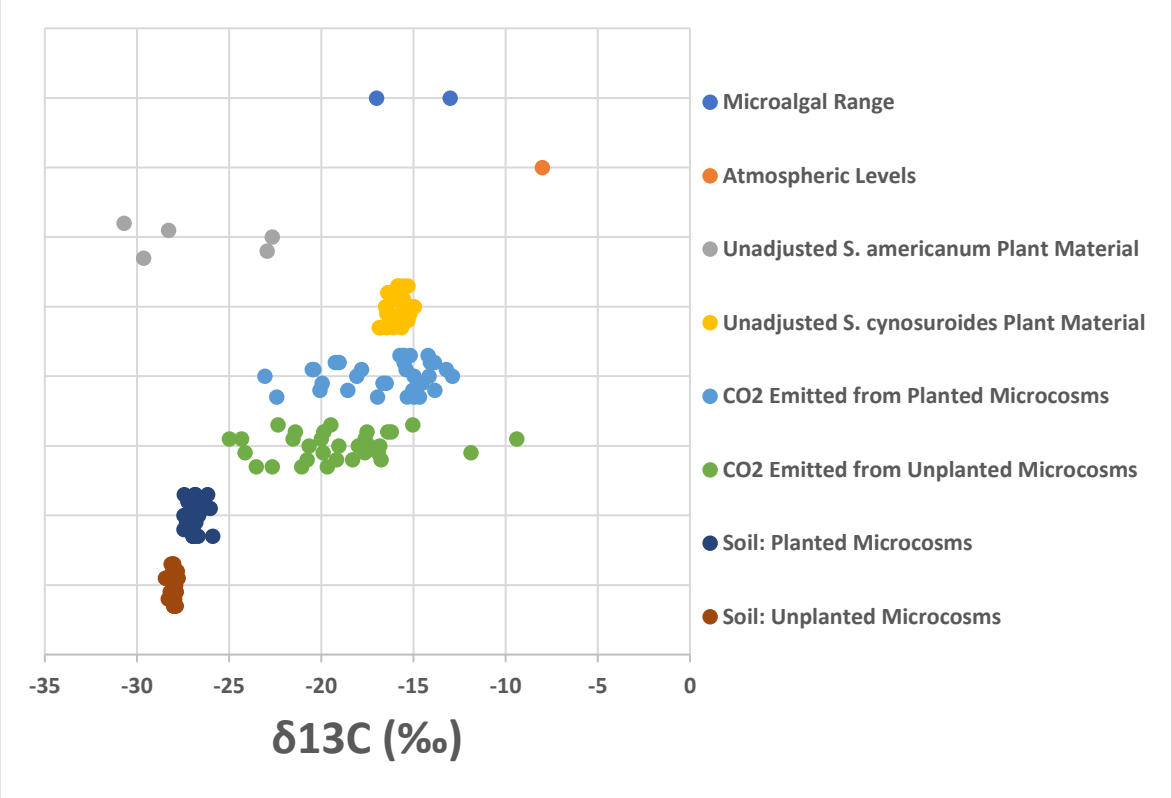

Figure 7. A scatterplot of the isotope data collected from this study. The microalgal range was measured by Currin et al. (1995). The atmospheric $\delta^{13}C$ levels of approximately -8 ‰ were as estimated by Farquhar et al. (1989). The $\delta^{13}C$ of the *S. americanum* and *S. cynosuroides* samples were measured from plant material and include the datapoints that were unadjusted for contamination of the plant samples with soil. The CO_2 emitted from the planted and unplanted microcosms were gaseous samples collected from incubations. The soil samples from planted and unplanted microcosms were measured from physical soil samples.

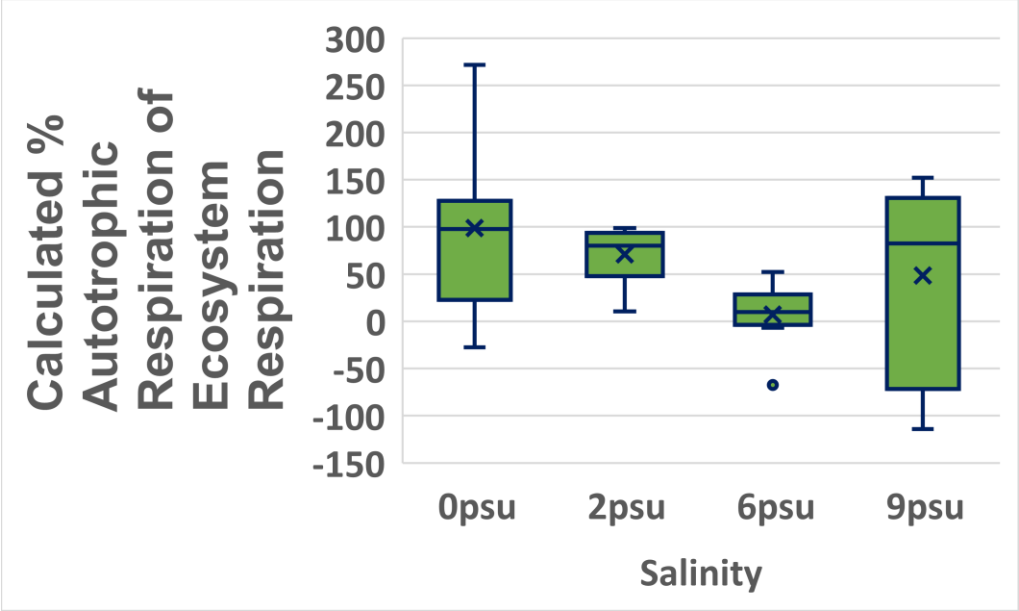

Figure 8. A box and whisker plot of the percent autotrophic respiration from ecosystem respiration in each respective microcosm. This was calculated using gas samples from long-term incubations and isotope values from plant samples that were adjusted for contamination with soil. The x in the boxes indicate the mean, while the median is shown by the solid lines within each box. The whiskers, except in the case of outliers, indicate the minimum and maximum values of the data collected. Outliers are depicted by the blue dots. This plot had an outlier with a value in the 0 psu group with a value of 6872% that was removed for visual clarity. There were eight replicates per treatment.

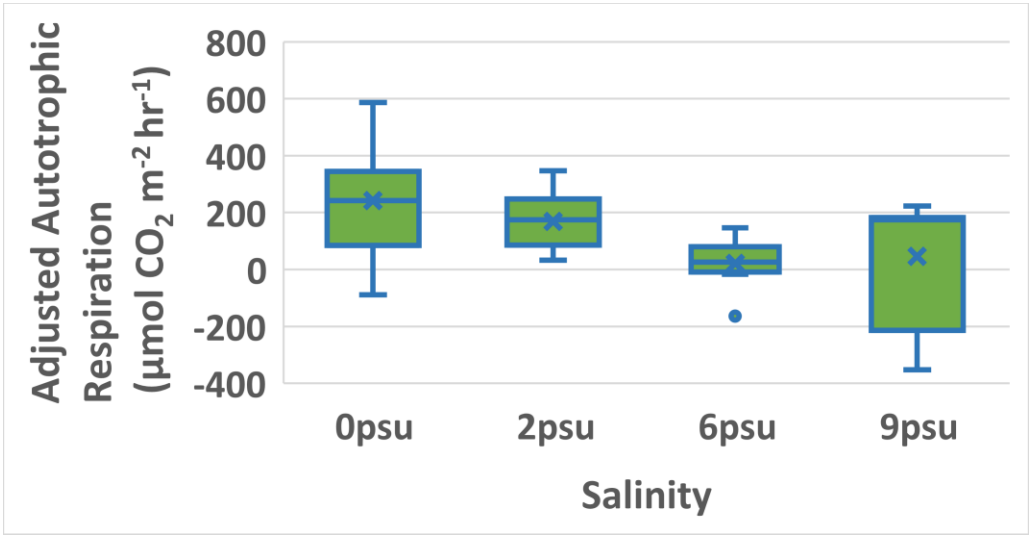

Figure 9. A box and whisker plot of the autotrophic respiration (µmol CO_2 m^{-2} hr^{-1}) as calculated from the long-term incubations and isotope values from the plant samples adjusted for the contamination with soil. This plot has one outlier with a value of 24169 µmol CO_2 m^{-2} hr^{-1} removed. The x in the boxes indicate the mean, while the median is shown by the solid lines within each box. The whiskers indicate the minimum and maximum values of the data collected. The outlier is depicted by a blue dot in the 6 psu treatment, which was -164 µmol CO_2 m^{-2} hr^{-1}. There were eight replicates per treatment.

Possibility 1.) I noticed that some of my plant samples had carbon contents that were lower than expected for "pure" plant matter, raising the possibility that some of the plant samples were contaminated with soil, lowering the measured plant carbon content while also skewing the δ_{Plant} values used in Equation 2 since the plants and soil had distinct ^{13}C isotopic values. To address whether accounting for this would improve the calculations of autotrophic vs. heterotrophic partitioning of ecosystem respiration, I calculated what the δ_{Plant} values would have been in the absence of soil contamination. To do this, the %C values of the soil samples were used in conjunction with literature values for %C of various *Spartina* roots and shoots to determine the fraction of soil to the fraction of plant that were present. This assumed the fraction of roots and soils were the entire sample (that is, there were no other possible sources of contamination). A simple algebraic solving for two variables was used for Equations 3 and 4. With Equations 3 and 4, the fraction_soil and fraction_plant were determined, and these were then used in Equation 5. The $\delta^{13}C$_measured was determined by the plant root and shoot samples that were sent into University of Arkansas Stable Isotope Laboratory. The δ^{13}_soil had also been determined by sending the soil samples into the University of Arkansas Stable Isotope Laboratory. With these, the $\delta^{13}C$_plant for the roots and shoots were calculated. Prior to the corrections, the *S. cynosuroides* had an average δ^{13} of -15.89‰ and after adjustments they had an average δ^{13} of -14.17‰ (Figure. 7), which is closer to previous leaf measurements of *S. anglica* taken by Hemminga et al. (1996) that averaged -13.65‰. These data did not correct the issue, however, because there were still 12 of the planted microcosms that had autotrophic respiration that was >100% or <0% of ecosystem respiration.

%C_measured = fraction_plant * %C_plant + fraction_soil * %C_soil (Eq. 3)

1 = fraction_plant + fraction_soil (Eq. 4)

δ^{13}C_measured = fraction_plant * δ^{13}C_plant + fraction_soil * δ^{13}C_soil (Eq. 5)

δ^{13}C_plant = (δ^{13}C_measured - fraction_soil * δ^{13}C_soil) / fraction_plant (Eq. 5)

Possibility 2.) The presence of *S. americanum* in some of the microcosms could have thrown off the plant $\delta^{13}C$ end member because this C3 plant has a ^{13}C value that is distinct from the C4 S. cynosuroides (medians of 28.19‰ vs. -15.89‰, respectively). However, I accounted for the more negative isotopic ratios of the *S. americanum* when I calculated biomass-weighted plant end-member isotope values for each planted microcosm. Also, while S. americanum was found in the control group and one microcosm of the 2 psu treatment, this does not explain the 7 microcosms in the 6 and 9 psu treatments in which autotrophic respiration was >100% or <0% of ecosystem respiration but no *S. americanum* was present.

Possibility 3.) There was some contamination of the ^{13}C value of CO_2 air samples taken from long-term incubations of the soil-only microcosms. These samples, once injected in the vials, were mailed to the University of Connecticut Isotope Facility where they remained for several months before being analyzed. I determined that the leakage of ambient air into the vials was likely not the case since the CO_2 concentration (ppm) of the gas I added to each vials (measured with the LI-COR LI-7000 at the time of sample collection) was strongly and positively correlated ($r^2 = 0.95$) with the mass of C (ug C as CO_2) measured at the isotope lab (Figure 10). If some of the vials had become contaminated with ambient air during shipping and storage prior to analysis, I would have expected that there would have been much more scatter in this relationship. There were a few apparent outliers from the overall trend line. However, upon further examination, there was no clear pattern (Figure 10) in terms of how close a point was to the CO_2 mass vs. concentration trend line and whether the calculated %autotrophy values

were plausible (0 < % autotrophy < 100%) or impossible (% autotrophy > 100% or <0% of ecosystem respiration). Thus, I do not believe that this was the cause of the aberrant results in the autotrophic respiration calculations.

Possibility 4.) The isotope lab(s) made an error. But, these labs also ran standards, which gave the actual values and ensured that the given values did not significantly deviate from these. The University of Connecticut Isotope Facility to which the gas samples were sent, had an R^2 of 0.9978 for Run 1 and 0.9992 for Run 2 between the accepted standards and measured values, indicating that there was a high degree of accuracy for these values once a slight offset from the standards was accounted for. A comparison of the measured values versus the standards done by the University of Arkansas Stable Isotope Laboratory also indicated a high level of accuracy and precision (sd: 0.10 and 0.12‰ $\delta^{13}C$) in their instrumentation. Thus, I do not believe that this was the cause of the issues in the autotrophic respiration calculations.

Possibility 5.) There was another source of CO_2 produced in the chambers. Some algal growth was observed on the surface of the soil, and, although I attempted to remove all of it, there likely was still some on the soil surface producing CO_2. Previous research into salt marsh benthic algae, however, has shown that the $\delta^{13}C$ were roughly -17 to -13‰ (Currin et al., 1995), meaning that a great deal of the algae would have needed to be emitting the CO_2 to bring the $\delta^{13}C$ up to throw off the autotrophic respiration calculations from the *S. cynosuroides* $\delta^{13}C$. The unplanted microcosms did emit an unusually high $\delta^{13}C$ from CO_2, however, when compared to the solid samples of that same soil (Figure 7). This means that if there was a large amount of algae in those unplanted microcosms releasing a higher $\delta^{13}C$, it could have caused the aberrant autotrophic respiration calculations.

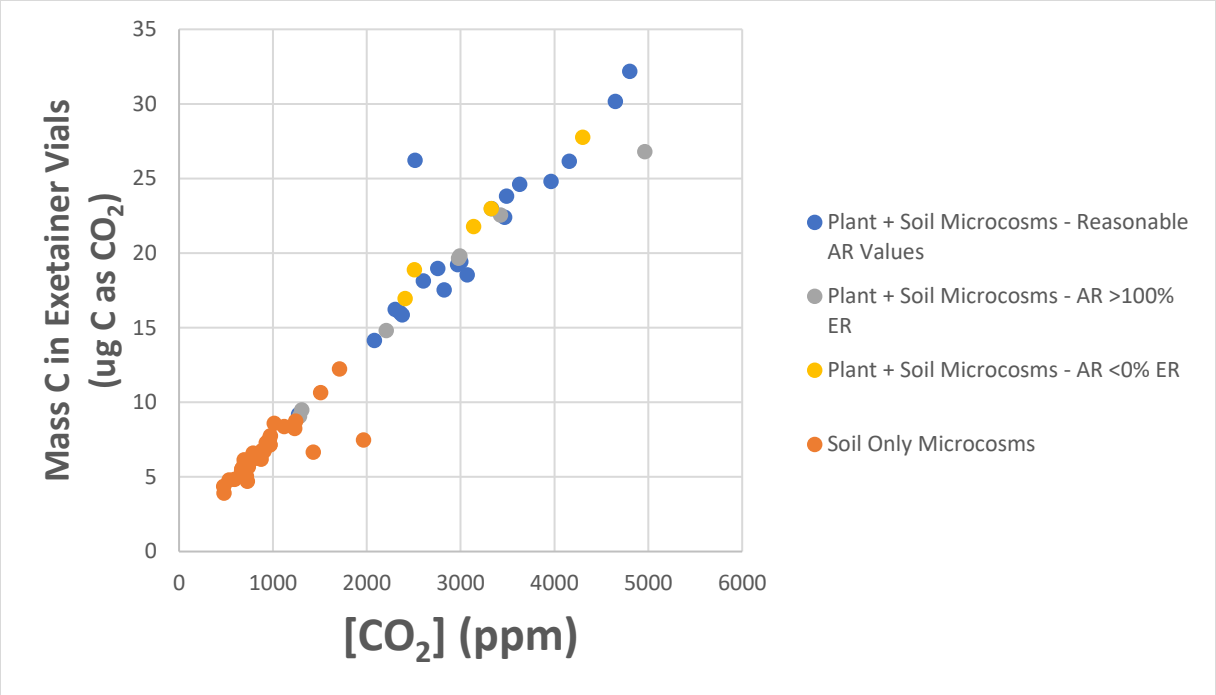

Figure 10. A scatterplot with the x-axis showing the CO_2 (ppm) that was measured with the LI-COR LI-7000 and the y-axis showing the Mass C in Exetainer Vials (ug C as CO_2) as measured at the University of Connecticut Isotope Facility. The Plant + Soil Microcosm data points are split into three categories: the first (in blue) being the reasonable autotrophic respiration (AR) measurements (that is, AR is 0-100% of ecosystem respiration, ER)). The second category (in gray) contains the Plant + Soil Microcosm data points where calculations indicated that autotrophic respiration accounted for >100% ecosystem respiration. The third category (in yellow) contains the Plant + Soil Microcosm data points where calculations indicated that autotrophic respiration accounted for <0% of ecosystem respiration. The Soil Only Microcosm data points are depicted in orange.

Possibility 6.) There was leakage of ambient air into one or more of the chambers. Although there were seams in which there could have been air leakage, these were also caulked that would have prevented this from happening. At several points while sampling, I did notice that there were compromised seams and I retook the measurements after recaulking the chambers. Although the pipe fittings that attached the chambers to the microcosms were not caulked, the seams were submerged below water to prevent air from coming into the chambers. I attempted to consistently use two of the sixteen chambers with their respective salinity treatments and the control, so it is possible that one or more specific chambers leaked air from outside the chambers. Specifically, based on the difference in

$\delta^{13}C$ values between the physical soil samples and the soil CO_2 emissions (Figure 7), it is possible that the Soil Only Microcosm chambers leaked air from outside the chambers.

Possibility 7.) The soil $\delta^{13}C$ values are not representative of CO_2 derived from heterotrophic respiration. In my project, the physical soil samples from the unplanted microcosms had a much more negative $\delta^{13}C$ than the CO_2 emitted from the unplanted microcosms (mean: -28 versus -19‰, respectively), meaning that these samples may have caused the issues in the autotrophic respiration calculations. Bernal et al. (2017) measured the $\delta^{13}C$ respiration signature and the $\delta^{13}C$ of solid samples of C3 and C4 plant derived soils, however, and their values were similar (-13.7 ‰ respiration from C4 plant derived cores and those solid soil samples had a $\delta^{13}C$ of -14.2 ‰). Being that the $\delta^{13}C$ of the soil CO_2 emissions and solid soil samples was similar in this previous research, however, I believe that the gas samples from the unplanted microcosms were somehow contaminated by another source of CO_2.

Possibility 8.) The plant $\delta^{13}C$ are not a good representation of plant $\delta^{13}C$ end member values. Bernal et al. (2017), however, tested this specifically and found that plant $\delta^{13}C$ respiration is similar to the $\delta^{13}C$ of the plant material. Thus, I do not believe that this was the cause of the impossible calculated autotrophic respiration measurements.

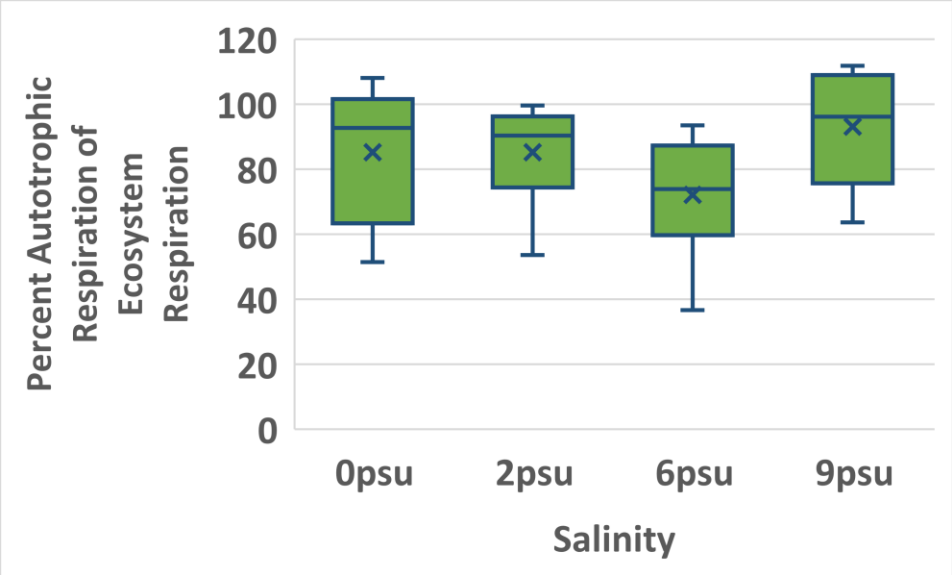

Figure 11. A box and whisker plot of the percent autotrophic respiration from ecosystem respiration in each respective microcosm. This was calculated from adjusted plant samples and solid soil samples from long-term incubations. The x in the boxes indicate the mean, while the median is shown by the solid lines within each box. The whiskers indicate the minimum and maximum values of the data collected. There were eight replicates per treatment.

My conclusions based on these analyses are that there was some issue with the gas samples from the unplanted microcosms. The soil samples from the unplanted microcosms had an average of -28.02‰, that is more similar to the expected $\delta^{13}C$ of C3 plant material than the $\delta^{13}C$ of the gas samples from incubations in the unplanted microcosms (average: -19.07‰). Given that I believe there was some form of contamination of the gas samples from the unplanted microcosms, I recalculated the data using the solid soil samples to represent the $\delta^{13}C$ soil in Equation 2. Although this did produce more reasonable results, there were still five microcosms in which calculations showed autotrophic respiration was >100% ecosystem respiration (range: 102 – 111%), which are physically impossible. Further, there was a great deal of variability in these data, with one microcosm having a calculated 36% autotrophic respiration of ecosystem respiration while other <100% microcosms had a calculated 99% autotrophic respiration (Figure 11). Thus, I do not believe that this recalculation of the autotrophic respiration is correct either.

Additional Figures:

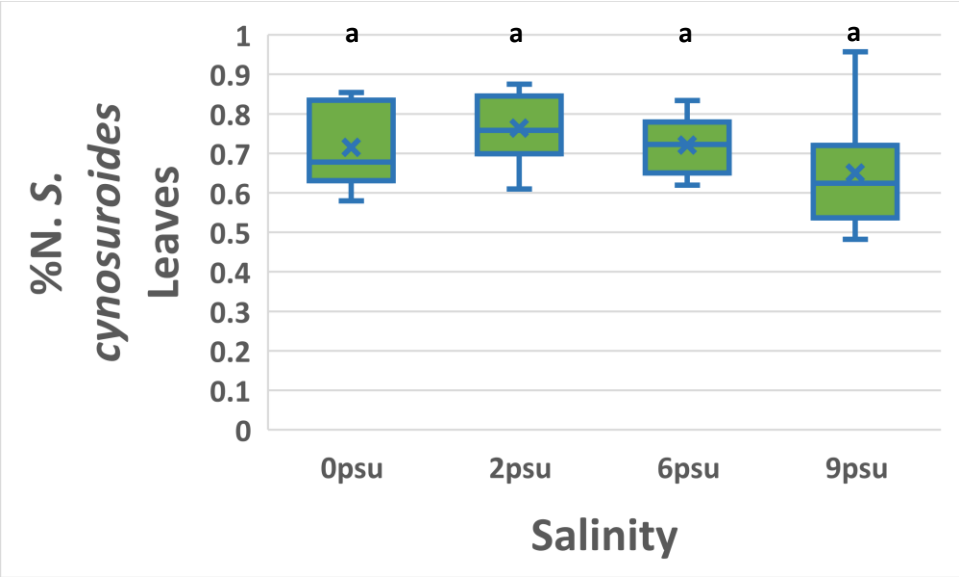

Figure 12. A box and whisker plot for percent nitrogen in the *S. cynosuroides* leaves among salinity treatments at the end of the experiment. The x in the boxes indicate the mean, while the median is shown by the solid lines within each box. The whiskers indicate the minimum and maximum values of the data collected. There were eight replicates per treatment. The letters indicate statistically similar or different treatments.

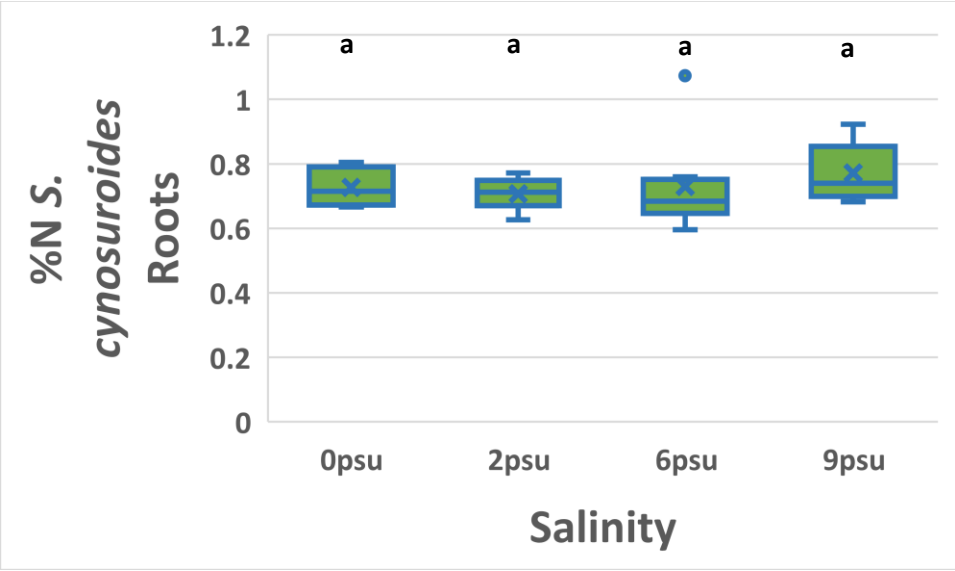

Figure 13. A box and whisker plot for percent nitrogen in the *S. cynosuroides* roots among salinity treatments at the end of the experiment. The x in the boxes indicate the mean, while the median is shown by the solid lines within each box. The whiskers, except in cases of outliers, indicate the minimum and maximum values of the data collected. The outlier is depicted by a blue dot of 1.07% N above the 6 psu treatment. There were eight replicates per treatment. The letters indicate statistically similar or different treatments.

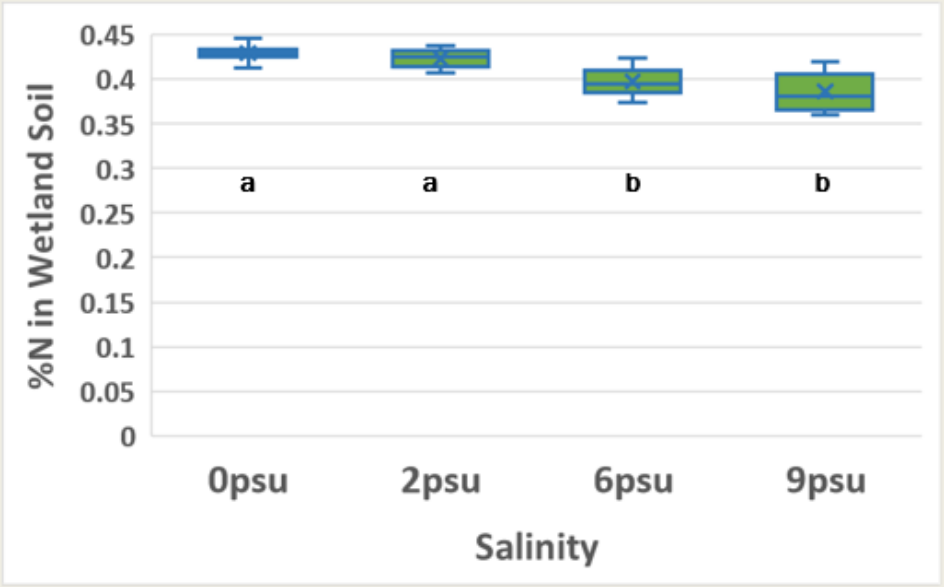

Figure 14. A box and whisker plot for percent nitrogen in the soils among salinity treatments at the end of the experiment. The x in the boxes indicate the mean, while the median is shown by the solid lines within each box. The whiskers indicate the minimum and maximum values of the data collected. There were sixteen replicates per treatment. The letters indicate statistically similar or different treatments.

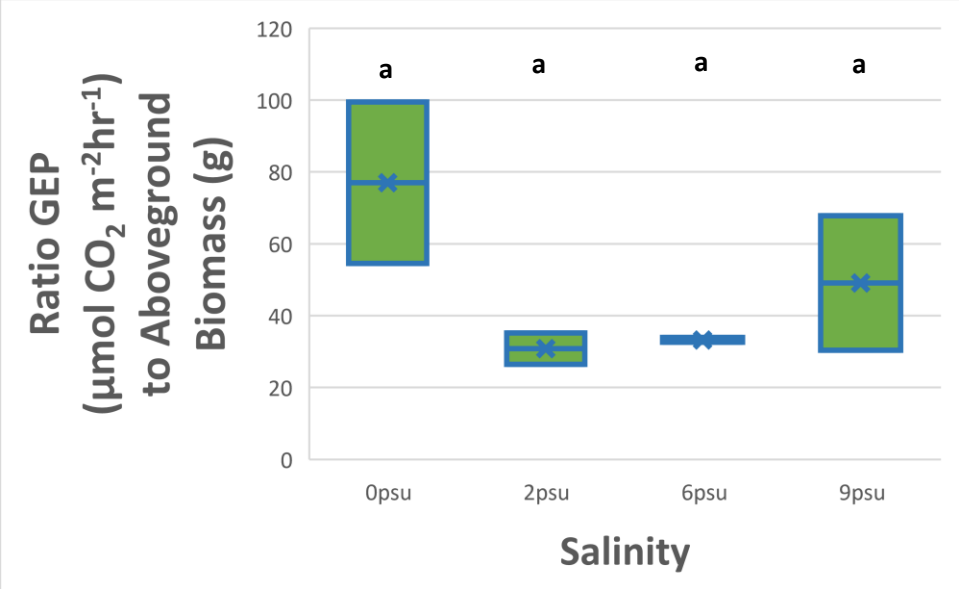

Figure 15. A box and whisker plot of the gross ecosystem productivity (GEP; µmol CO_2 m^{-2} min^{-1}) for every gram of Aboveground Biomass (g). The x in the boxes indicate the mean, while the median is shown by the solid lines within each box. There were two replicates per treatment. The letters indicate statistically similar or different treatments.

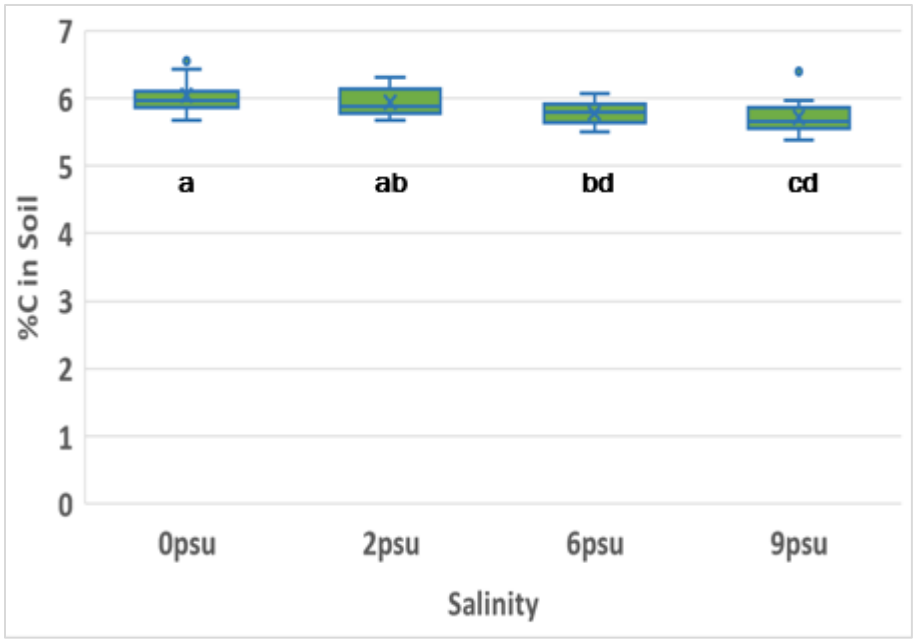

Figure 16. A box and whisker plot for percent carbon in the soils among salinity treatments at the end of the experiment. The x in the boxes indicate the mean, while the median is shown by the solid lines within each box. The whiskers, except for outliers, indicate the minimum and maximum values of the data collected. The outlier in the 0 psu group was 6.65% C from a planted microcosm, while the outlier in the 9 psu treatment was 6.40% C from an unplanted microcosm. There were sixteen replicates per treatment. The letters indicate statistically similar or different treatments.