ABSTRACT

Title of thesis: METHANE EMISSIONS FROM A TIDAL BRACKISH MARSH ON MARYLAND'S EASTERN SHORE AND THE FACTORS IMPACTING THEM

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Methane is a potent greenhouse gas and may offset a significant portion of the carbon sequestration benefit of many brackish marshes. The objective of this study was to determine whether methane emissions varied across different hydrologic/vegetative communities within a tidal brackish marsh, and if so, what other variables varied with them. We sampled methane emissions from two brackish marshes using static flux chambers, on Maryland's Eastern Shore. Additional data was collected from sampled marsh pore water, water level and soil temperature. We found that there was a significant difference in methane emissions between different hydrologic/vegetative communities. The results of this study help explain the factors that influence methane emissions in a tidal brackish marsh, and the vegetative communities therein; these factors could be used to develop models to better estimate methane emissions at the site-landscape level.

METHANE EMISSIONS FROM A TIDAL BRACKISH MARSH ON MARYLAND'S
EASTERN SHORE AND THE FACTORS IMPACTING THEM

By

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Chapter 1: Introduction and Statement of Research Needs

It has been well documented that marshes sequester vast amounts of carbon that would otherwise be lost to the atmosphere (Chmura et al. 2003). The unique conditions of a marsh that allow these accumulations to occur include high plant productivity, and wet anaerobic soils, which greatly slow the decomposition of organic matter. These same conditions also allow for the production of methane, a potent greenhouse gas, of which wetlands are a major source accounting for the majority of naturally emitted methane to the atmosphere (Wang et al. 1996; Pachauri et al. 2014). Because this greenhouse gas is 28 times more potent than carbon dioxide (Pachauri et al. 2014) over 100 years, emissions from marshes could potentially offset their carbon storage benefits (Poffenbarger et al. 2011). Previous research has indicated that the sulfate present in seawater can greatly suppress methane emissions from saline marshes (salinity >18 ppt); however, emissions vary greatly below this threshold, and this variation increases as salinity decreases from saline to freshwater systems.

Research into the variables that co-vary with methane emissions is needed in order to gain a better understanding of why methane emissions in marshes with <18ppt salinity are highly variable. While previous research has shown a relationship between methane emissions and salinity across sites of varying in salinity (Poffenbarger et al. 2011), causes of within site variations in methane emissions have not been documented. If accurate accounting of greenhouse gas emissions from tidal brackish marshes is to be achieved, additional insight into the parameters that affect methane emissions is needed. No study has yet researched the impact that different plant communities in a tidal brackish marsh have on methane emissions.

Our study was performed at a tidal brackish marsh located on the Deal Island Peninsula on Maryland's Eastern Shore. This exploratory study aimed to elucidate processes occurring in a field setting, in order to ascertain which factors have the greatest impact on methane emissions at a site-specific level. Future research can then focus on the mechanisms behind these factors and their impact on emissions.

As these relationships between methane emissions and other variables such as salinity and plant community become better understood, researchers can better account for methane emissions in tidal brackish marshes, and focus research efforts on the factors that most impact them. Because measuring methane emissions directly is both time intensive and costly, alternatives such as modeling can be used to estimate emissions using minimal measurement from the field. Robust models of emissions are needed to advance tidal marsh restoration supported by carbon crediting (Emmert-Mattox et al. 2010). Current methodologies for wetland greenhouse gas modeling require them to be validated with peer-reviewed data generated from similar ecosystems (Emmer et al. 2015).

The objectives of our study were to determine the differences of methane emissions within different plant communities, or strata, within a tidal brackish marsh. To better understand why methane emissions differed between these strata, we measured additional parameters, including water level, soil temperature, soil pore water sulfate, sulfides, pore water pH and salinity to determine which variables co-varied with methane emissions.

Our hypotheses for this study were:

1) Methane fluxes will differ between plant communities (vegetative/hydrologic strata) that vary hydrologically in terms of flooding frequency and water level.

2) Strata with lower elevation (and therefore higher water level) will have higher methane fluxes

3) Methane flux will be negatively related to pore water salinity, sulfate and hydrogen sulfide concentrations.

This thesis contains three additional chapters, and an appendix of supplementary information. The second chapter is formatted for future submission to a scientific journal and covers the field and laboratory portions of our study. Originally, the third chapter of this thesis was designated to test a new model designed to estimate methane emissions for tidal brackish marshes; however, this model is not yet ready for testing at this time. Instead, this chapter focuses on the factors which can affect the production and emission of methane in tidal brackish marshes. This chapter is not intended for future publication; however, it will serve as a guide for determining which factors should be the focus for future methane modeling efforts. Chapter four serves as a conclusion to this thesis, with future research needs identified. Finally, an appendix of additional data, figures, and statistical code is included.

Chapter 2: Methane Emissions Differ In Four Vegetative/Hydrologic Communities in a Tidal Brackish Marsh

Abstract:

Current research indicates that while salt marshes (salinity > 18ppt) have minimal methane emissions, the lower salinities of tidal brackish marsh systems may allow for increased amounts of methane to be released than previously thought. We sampled methane emissions using static flux chambers from two brackish marshes on the Deal Island Peninsula, located on the Eastern Shore of Maryland. We found that there was a significant difference in methane emissions between different hydrologic/vegetative communities. One community, composed of *Spartina alterniflora*, had emission that were 2.72 times higher than the next highest community. The remaining three treatments, one in *Spartina patens*, and two in *Juncus roemerianus* had much lower emissions. We also found significant differences in the amounts of sulfate remaining in the pore water after reduction, with *S. alterniflora*, the stratum with highest emissions having the least amount of sulfate remaining when compared to all others. This stratum also contained significantly higher amounts of hydrogen sulfide (the byproduct of sulfate reduction) than all other strata. The stratum with the lowest emissions, *S. patens* had significantly higher sulfate, and significantly lower sulfide concentrations as well. Our data show that high rates of both methanogenesis and sulfate reduction co-occurred in a single hydrologic vegetative community, and did not follow previously established relationships between salinity and methane emissions. Accounting for differences in hydrologic/vegetative communities in tidal brackish marshes would improve estimates of greenhouse gas emissions on a site specific scale.

Introduction

Methane is a potent greenhouse gas produced in the anaerobic conditions found in wetland soils. The global warming potential of methane gas is 28 times greater than an equivalent amount of carbon dioxide over a 100-year period (Pachauri et al. 2014), and research shows that this value could be under-reported by up to 40%, depending on how the time period is considered (Neubauer and Megonigal 2015). While the majority of methane emitted to the atmosphere comes from anthropogenic sources, wetlands produce most of the naturally emitted methane (Wang et al. 1996; Solomon et al. 2007). The availability of sulfate from seawater suppresses methane emissions from polyhaline (salinity > 18 ppt) marshes to very low rates (0.2 to 5.7 g CH_4 m^{-2} yr^{-1}). By comparision methane emissions from brackish systems (5-18 ppt salinity) are higher (3.3 to 32.0 g CH_4 m^{-2} yr^{-1}), a pattern that is typically interpreted as a response to the lower availability of sulfate (Poffenbarger et al. 2011). Yet, little research has been conducted on the factors that regulate methane emissions in brackish marsh systems. Athough carbon sequestration rates in brackish marsh soils are very high (Chmura et al. 2003), emitted methane partly or completely offsets the carbon sequestration benefits of tidal marshes (Poffenbarger et al. 2011). There is emerging interest in using tidal marsh restoration as a means to mitigate greenhouse gases in the atmosphere, and as a source of carbon credits (Crooks et al. 2011; Emmer et al. 2015); however to do so will require a better understanding of the factors controlling methane emissions in brackish systems.

Methane is produced in wetlands by methanogenic archaea and bacteria. The production of methane is generally thought to occur when all other electron acceptors have been depleted, leaving carbon dioxide or low molecular weight organic compounds such as

acetates the remaining electron acceptors (Megonigal et al. 2005). Methane production is less energetically efficient than other anaerobic respiration processes, including sulfate reduction (which produces hydrogen sulfide) and iron reduction. The order of these reduction reactions is known as the thermodynamic ladder, since the reactions generate less energy as they progress from aerobic respiration, ultimately down to methanogenesis. The source of electrons for these processes comes from labile organic materials present in the soil, formed from fermentation of organic detritus from plant and animal matter. This fermentation produces both low molecular weight organic compounds and dihydrogen, which are the dominant electron donors for methanogens. Carbon dioxide and organic acids serve as the electron acceptors for methane production. This process is regulated by such factors as the presence of oxygen, pH, temperature, and the presence of alternative electron acceptors. Research suggests a preference for neutral pH ranges for methane production; however methanogens have been shown to be active in acidic pH's below 5.6 (Walker et al. 1998; Garcia et al. 2000; Megonigal et al. 2005). As with most microbial processes, methanogenesis can be temperature dependent, with warmer temperatures stimulating the process (Whalen 2005). Additions of a more energetically favorable electron acceptor, such as sulfate from seawater (in the case of a tidal marsh), has been found to inhibit new methane production in some marshes (Lovley and Klug 1983).

Methane emissions in brackish marshes are influenced by water levels and plant species composition. The variation in water levels in a tidal marsh will change the depth at which the soil profile transitions from aerobic to anaerobic. Fluctuations in the water level also causes some layers of the soil profile to cycle between aerobic and anaerobic conditions. Methane is produced in the anaerobic zone of soils and is transported to the atmosphere by

several mechanisms; however, some barriers exist to its release. Methane diffuses very slowly through water, so high water levels and inundation can prevent methane from easily moving upwards out of the soil profile. Also, if the soil contains a sufficiently thick aerobic zone, methane can be oxidized by methanotrophic bacteria before diffusing into the atmosphere. Tidal waters also bring fresh additions of new compounds to the interior of a marsh, such as sulfate. The introduction of a more favorable electron acceptor can suppress or inhibit methane production which is typically the case as salinities and therefore sulfate availability increases (Poffenbarger et al. 2011; Holm et al. 2016). Ebullition is a sudden release of methane "bubbles" that pass through either inundated soils or open water. They can be released through physical disturbance, such as animals walking along the marsh surface, or from rapidly rising tidal waters. Ebullition can also occur when methane levels in the substrate reach a level that can no longer be contained by the physical forces of the substrate. This process has been found to occur seasonally in some salt marshes, and can vary by plant type (King and Wiebe 1978).

Plant species composition can affect methane emissions through several mechanisms. Methane can be transported to the atmosphere via plants as a byproduct of their normal activity, bypassing the emission barriers of slow water diffusion rates and methane oxidation zones. Via aerenchyma tissue, plants transport methane out of the soil through the rhizosphere, root and stem tissues, then out into the atmosphere (Sorrell et al. 2013). The main driver of this transport is solar heating, which warms leaves exposed to sunlight, drawing air (along with gases such as methane) through plant tissues into the atmosphere. Plants that use C4 carbon fixation, such as *S. patens* and *S. alterniflora,* can emit gasses continuously regardless of varying light conditions, since their stomata are continuously

open. C3 plants, such as *J. roemerianus*, only respire while their stomata are open, therefore the flow of methane from their rhizospheres to the atmosphere is potentially lower. This process also allows oxygen to flow from the atmosphere to the rhizosphere, creating small aerobic zones around plant roots, which are a similar but smaller barrier to methane than the oxidized zone of the soil above. Plants also provide a source of carbon that methanogens use for energy. This carbon comes in many forms, including the dead above-ground biomass which is added to the surface of the soil, below-ground production of biomass (i.e. roots and shoots) which are added directly to the soil, and root exudates, which are simple organic carbon compounds exuded by the roots themselves, some of which can be used directly by methanogens with no need for additional fermentation (Bridgham et al. 2013), unlike the more slowly decomposing plant material. Root distribution can vary between species, and with it, where these deposits of carbon occur within the soil profile.

The objective of this study was to advance the understanding of the effects of water level and plant species composition on methane emissions in brackish marshes at a site-specific scale. We measured methane fluxes in two brackish marshes on the Deal Island Peninsula on the Eastern Shore of Maryland across four different water level/vegetation strata. We also collected data on: elevation, water level, soil temperature, soil pore water sulfate, sulfides, as well as pH and salinity.

We tested three hypotheses during this study: (1) methane fluxes will differ between these vegetative/hydrologic communities (strata), (2) strata with lower elevation (and therefore higher water level) will have higher methane fluxes and, (3) methane flux will be negatively associated with pore water salinity, sulfate, and hydrogen sulfide levels. Our first hypothesis was designed to determine whether different plant communities in a tidal

brackish marsh will impact the emission rate of methane. For hypothesis 2, we reason that higher water tables should make soil conditions more anaerobic closer to the soil surface, which is where the majority of our observed roots were located, which should be more favorable to methanogenesis. According to Poffenbarger et al. (2011), methane production should be lower at higher salinities, and therefore higher sulfate and hydrogen sulfide concentrations in the pore water, which will test our third hypothesis. We also collected soil cores from each sampling plot to be analyzed for potential methane production under anaerobic soil incubations. These incubations allowed us to achieve a basic understanding of the relative potential of each stratum's soil to generate methane, and allowed us to test our first hypothesis in a controlled laboratory setting.

Methods

Study Area

Our study area was located on the Deal Island Peninsula in Somerset County, MD (38.185172N, 75.906279W) (Fig. 1). It consisted of two brackish tidal marshes—one unditched (Unditched) and one restored-ditched marsh (Ditched) located in the same marsh complex. These sites are also being used by others to test restoration technique success in restored ditch marshes (these sites are referred to as Unditched-2 and Ditched-2 in Needelman et al. (2015)). Ditch plugs were installed in April of 2014 by inserting a plastic polyethylene sheet vertically into the ditch approximately 50-m upstream from the tidal source and securing the plug using marsh material sourced from the ditch upstream of the plug. Ditched had an overall lower elevation than Unditched, and was primarily composed of *Juncus roemerianus* (black needlerush). Unditched had a more diverse species community, including *J. roemerianus, Spartina patens* (salt marsh hay), *Spartina*

alterniflora (smooth cordgrass), *Phragmities australis* (common reed), and *Iva frutescens* (marsh elder). Plant productivity trends in tidal marshes in this region include a period of senescence during the late fall through the early spring, with peak plant productivity occurring in late summer. Soils onsite consisted of organic mucky peat; classified as the Mispillion series, a Loamy, mixed, euic, mesic, Terric Sulfihemist, which is a common estuarine marsh soil in this area. This microtidal marsh had a diurnal tidal range of approximately 0.6 meters.
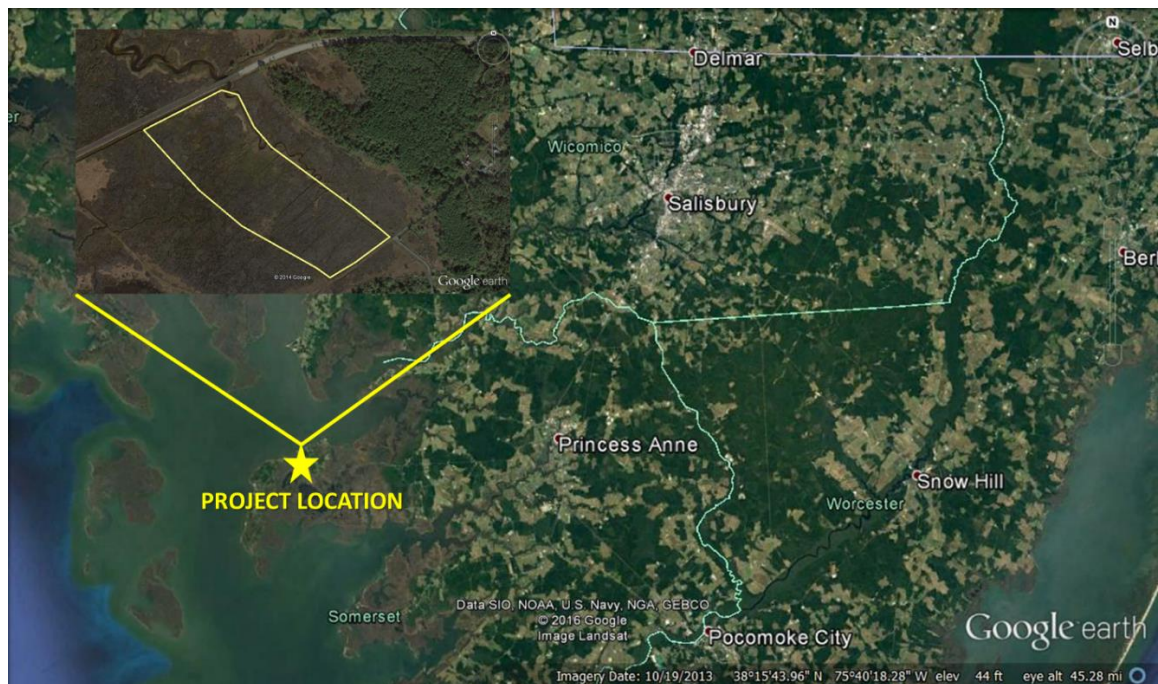
Figure 1 Map of the mid-Delmarva Peninsula, North is to the top of the figure. The project location (38.185172N, 75.906279W) is denoted with a yellow star, with the inset map showing the boundary of both sites.

Design

This project was an observational field study of four different landscapes types, or "strata". The strata differed in their plant community composition and elevation, both of which are closely associated with water levels. The strata corresponded to geographic units that can be used to determine the impact vegetation and water level differences have on methane

emissions in site-specific carbon crediting accounting (Emmer et al., 2015). Water level variability was primarily controlled by elevation in these marshes, with lower elevations having higher water levels. Two of the strata had a common plant community composition dominated by *J. roemerianus*, but differed in elevation with one site at a "High" elevation and the other at a "Low" elevation. The High *J. roemerianus* stratum was located at Unditched (mean elevation 0.334 m; all elevations are reported relative to NAVD88) (Fig. 2), and the Low *J. roemerianus* stratum was located at Ditched (mean elevation 0.305 m) (Fig. 3). The two additional strata consisted of Low *S. alterniflora* (mean elevation 0.299 m) and High *S. patens* (mean elevation 0.409 m), located at Unditched. Five sampling plots were randomly established in each stratum, for a total of 20 plots. Plot placement attempted to capture the variations of elevation and plant community and density which existed within each stratum, while simultaneously being randomly selected and confined to within each stratum. These representative areas were selected prior to plot placement, and once selected, plots were randomly placed within each area. It should be noted that our flux measurements covered a small inference space, since they were not randomly distributed across the entire marsh, only in representative areas of each strata.

Figure 2 Site map of "Unditched", which contains the High J. roemerianus, Low S. alterniflora and High S. patens strata. North is to the top of the photo. All plots are marked with stars, with colors differentiating the different species tested.
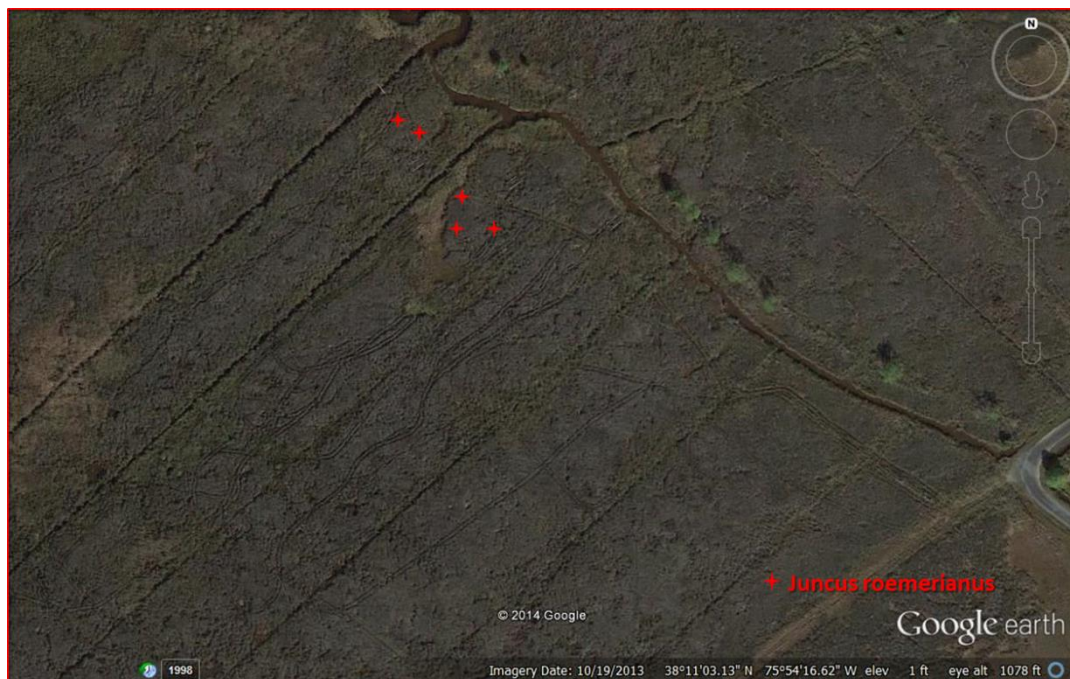
Figure 3 Site map of "Ditched", which contains the Low J. roemerianus stratum. North is to the top of the photo. All plots are marked with red stars, representing the single species being tested at this location.

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Field

We sampled monthly from April to December 2015; samples were not taken from January until March under the assumption that methane production would be neglible due to low temperatures (Whalen 2005). Methane flux, air temperature, and pore water concentrations of phosphate, nitrate, pH, sulfate, hydrogen sulfide, and methane were measured at each plot. Soil temperature (at 10 cm) was recorded at two plots per stratum hourly during the sampling season using HOBO 8k Pendant [®] (Onset Corp., Bourne, MA) sensors. Soil temperature and water levels were not collected during the month of April because loggers were not ready for deployment until May. Water level procedures are described below.

To collect methane flux samples, custom-fabricated square aluminum metal collars were inserted into the marsh to a depth of 10 cm. Flux chambers, constructed of an aluminum frame covered with transparent polycarbonate plastic sheeting, were placed on top of the collar prior to sampling. Chambers were equipped with a closed-cell neoprene strip on the top and bottom, which when clamped to the collar assured an airtight seal (Yu et al. 2013). Two chambers were stacked together in both the *J. roemerianus* strata so the taller plants fit within the chamber without damaging plant stems. Single chambers were used for all *Spartina* plots. Opaque chamber lids with a sampling port were clamped to the top of the chamber to complete the seal. Chambers had a height of 69.5 cm and an interior length and width of 49.5 cm, yielding a total volume of .17 cubic meters for single chambers and .34 cubic meters for double chambers. In order to prevent the weight of the observer from causing ebullition due to soil compression (Sorrell et al. 2013), a 3-m wooden boardwalk was installed in 2014 on which to approach each flux collar.

Methane flux samples were collected over a one-hour period from each sample chamber at each plot. Five samples were collected for each methane flux, an initial sample was taken immediately after the chamber was sealed, and four subsequent samples were taken approximately every 15 minutes thereafter, timed using a watch. Using a 30 mL syringe, the sampling port was opened and then purged three to five times before each sample was taken in order to ensure the sample was pulled from the ambient air inside the chamber, and not the sampling port. Each 18 mL air sample was withdrawn from the chamber from the sampling port with the syringe and injected into a nitrogen-flushed 12-mL vial with rubber septum until analysis using gas chromatography, as described in Yu et al. (2013). After each sample, the sampling port was closed. Air temperature within the sampling chamber was recorded upon the collection of each flux sample from thermometers affixed to the interior of each chamber with tape.

Pore water samples were taken at 10 cm depth using a pore water sipper and syringe (Fisher et al. 2013). One pore water sample was collected at each plot during each field sampling event to be analyzed for pore water methane, hydrogen sulfide (unfiltered), pH (unfiltered), salinity (unfiltered), and sulfate (filtered). Filters used were size 0.45µm. Pore water methane was collected by withdrawing 15 mL of pore water using a pore water sipper inserted into the marsh at 10 cm depth, after which 15 mL of ambient air was drawn into the syringe and the syringe capped. The sample was then agitated for approximately 1-2 minutes for the methane to diffuse into the drawn air, and then the gas sample was injected and stored in nitrogen flushed exetainers for analysis with the methane flux samples (Keller et al. 2009). Hydrogen sulfide samples were diluted in a 1:1 ratio of sample to sulfide antioxidant buffer in the field to prevent sulfide volatilization and oxidation. A filtered

composite sample, consisting of approximately 5 mL of pore water from each plot, was collected during each field sampling event for each of the four strata conditions and analyzed for ammonium and phosphate concentrations (ammonium and phosphate data are located in the appendix). Hydrogen sulfide and pH samples were analyzed the same day as sample collection; salinity was analyzed within two weeks in the laboratory; all other pore water samples were frozen and analyzed during the winter of 2016.

Additional data were collected during the July 2015 sampling event, which was predicted to be during the peak methane emission period. We collected all pore water at 20 cm depth in addition to 10 cm and analyzed these samples for ferrous iron (Fe^{2+}).

Soil cores were collected during July sampling event from each sample plot to be analyzed for potential methane and carbon dioxide production. Cores were collected to approximately 40 cm using a circular metal "punch" corer, or gouge auger. The corer was inserted into the marsh, with careful attention being paid to minimize compaction of the soft peat. The core was removed, measured, and bifurcated at 20 cm, yield two pieces of core per plot. The top of the core was marked with a poker chip for easy identification back at the laboratory. Cores were photographed and placed into a labeled sample bag in which as much air as possible was removed. The cores were then placed in a cooler with ice and transported back to the laboratory, where each bag was flushed three times with nitrogen gas to remove oxygen. Cores were then returned to a cooler with ice and stored in a four-degree Celsius cold room until processing. Water for these incubations was collected from the hole from which the core was removed. It was placed in the iced cooler alongside the cores for transport back to the lab, and bubbled with nitrogen gas to remove oxygen before being placed in the cold room along with the cores.

Water level was measured at each stratum in order to determine water levels at the time of sampling and antecedent water level conditions during the two-week period leading up to the sampling period. Water level recorders (HOBO U20-L, Onset Corp, Bourne, MA) were installed adjacent to the chamber transects to continuously record water levels in the marsh and in the tidal creek adjacent to the field site during the field season. We deployed two water level loggers in each stratum, except for the low water table *J. roemerianus* stratum, which had one water level logger due to its small area relative to the other strata. Barometric pressure was collected onsite to correct the unvented loggers (HOBO U20-L, Onset Corp, Bourne, MA). We surveyed the elevation of all 20 plots and water level logger locations using a Real-time Kinematic Global Positioning System (RTK GPS) unit, which provides elevation data with approximately 2 cm accuracy.

Laboratory Analyses

Gas flux samples were measured in the laboratory using gas chromatography (GC) with a Varian 450-GC using a Combi-Pal autosampler. This GC used a flame ionization detector (FID) with helium as a carrier gas to detect methane concentrations present in the sample. Serial dilutions were made using a 10.509 ppm methane standard to give a standard set of 7.506, 5.004, 3.003, 1.501 and 0.500 ppm. Standards were prepared in nitrogen-flushed exetainer vials. Additional standards of 2000, 1000, 500 and 100 ppm were prepared in evacuated vials in order to generate a standard curve for the pore water methane samples, as they were assumed to have a higher methane concentration than the flux samples.

Once the standard curves were generated, flux samples were corrected for dilution (by multiplying GC output by 30/18) and then concentrations for each sample were generated using the standard curve. Concentrations for each flux were plotted with concentration

(parts per million methane) on the y axis and time (minutes) on the x axis. Using these graphs, trend lines were created, and the slope of this line was used to calculate methane flux per hour per square meter (n=147). If the observed methane results did not trend upwards (as it would be expected as concentration increases), but instead remained near the initial concentration (time = zero), fluxes were assumed to be zero (n=16), and the concentration was entered as the minimum observed flux vale, 0.002 mg methane per hour per square meter, for use in analysis (needed due to the log normal transformation of these data). If the trend indicated an ebullition event (characterized by a large peak of methane concentration when compared to previous measurements) then it was discarded and not used in analysis (n=3). If fluxes had data inconsistent with any of these described trends, it was assumed that the flux was collected incorrectly, and it was not used in analysis (n=14).

Hydrogen sulfide pore water samples were analyzed using a Lazar Laboratory model 146S sulfide electrode. Sulfide antioxidant buffer was prepared the day before sample collection with deoxygenated (nitrogen-gassed) distilled water, sodium salicylate, sodium hydroxide, and ascorbic acid. A standard curve created from a serial dilution (0.1, 1, 10, 100 and 1000 ppm) of a Na_2S/buffer solution was prepared for each sampling event; and the electrode millivolt reading was transformed into hydrogen sulfide concentration for each pore water sample (Koch et al. 1990).

The day of collection, pore water pH was measured with a calibrated pH meter. Salinity was measured with a calibrated conductivity/salinity electrode in the days following collection. Additional parameters (NH_4^+, PO_4^{3-}, SO_4^{2-} and Fe^{2+} concentrations) were frozen and sent to the Chesapeake Biological Laboratory for analysis. Reduced iron was analyzed according to EPA method 200.1 and sulfate was analyzed according to the National

Environmental Methods Index Standard Methods: 4110B for ions in water by ion chromatography (www.nemi.gov).

In order to estimate the percent of sulfate remaining after reduction from brackish tidal waters, we calculated the percent sulfate expected at our observed salinity using the value for sulfate concentration of undiluted marine seawater found in Canfield 2004, and dividing our observed sulfate concentration by the expected value of sulfate.

Cores collected for incubation and their associated waters were stored in a cold room in a cooler with ice until processing. When processed, cores were removed from the cold room, and placed into an anaerobic hood containing a nitrogen/hydrogen gas mixture similar to Megonigal and Schlesinger (2002). We then cut two sections from each core: the 0-20 cm core was cut from 8-12 cm and the outsides of the resulting "patty" were sliced away in order to expose the inner sections of the core, which were assumed to have had minimal oxygen exposure from collection to processing. The same patty was removed from 28-32 cm in the 20-40 cm core. We then removed as many live roots as feasible to then accrue 5 grams of wet soil material, which was placed in a 35-mL serum bottle, along with 5 mL of the degassed water from the core hole. The serum bottles were then capped inside the hood and removed from the hood for sampling.

Methane samples were taken by withdrawing 0.5 mL of headspace gas and injecting directly into a Shimadzu gas chromatograph with a flame ionization detector. Peaks were recorded and compared to a standard curve generated from standards of 10.51, 100, 500, 1000 and 2000 ppm methane. Carbon dioxide was sampled by withdrawing .5 mL of headspace gas and injecting it into a LI-COR LI-7000 (LiCor, Lincoln, NE). Peaks were recorded and compared to a standard curve generated from carbon dioxide standards of

250, 1000, 2500, 5000 and 10000 ppm carbon dioxide. Samples were taken daily for approximately two weeks. Mols of each gas present at day five were used for statistical analysis.

Statistical Analysis

Data were analyzed using SAS 9.3 (SAS Institute Inc. Cary, NC). All variables were evaluated for normality using PROC UNIVARIATE and those that required transformation were log transformed to improve normality. Parameters transformed were: methane flux, pore water hydrogen sulfide concentration, pore water sulfate concentration, and pore water methane concentrations. All parameters were analyzed using PROC MIXED with strata and month in the model statement with repeated measures. Post-hoc Tukey mean comparisons were used with $\alpha = 0.05$ used to indicate significance.

Results

Methane Flux

Methane flux varied significantly between strata (p<0.0001), month (p<0.0001), and had a significant interactive effect (p=0.0182). *S. alterniflora* had the significantly highest mean emissions, followed by High *J. roemerianus*, which was significantly higher than Low *J. roemerianus* and *S. patens*, which were not significantly different from one another (Fig. 4).
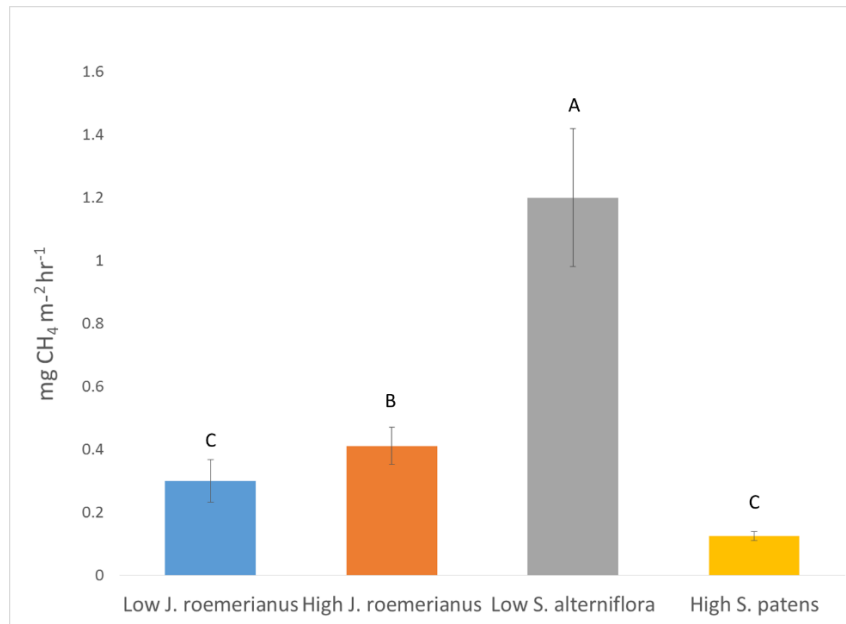
Figure 4 Mean hourly methane flux by four strata in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Error bars signify standard error, and significant differences are noted by letters

July had the highest observed methane fluxes, and was not significantly different than June, August, September and October. April and December had significantly lower emissions than all other months, but were not significantly different than May, June and November. May was also not significantly different than June, August, September, October, and November (Fig. 5).

Figure 5 Mean hourly methane flux during the sampling period in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Inset table shows ANOVA results of the log transformed data. Error bars signify standard error and significant differences are shown with letters.

Significant within month differences were observed in May, June and September. In May and June, *S. alterniflora* was not significantly different from any strata other than the low *J. roemerianus* stratum, and all other strata were not significantly different from one another. In September, *S. alterniflora* was not significantly different from any strata other than the *S. patens* stratum, and all other strata were not significant from one another. No significant within month differences were observed in April, July, August, October, November and December (Note: Full means comparisons for all tested variables are available in the appendix).

Antecedent Water Levels

Water level data collected during the 2 weeks prior to, and during the sampling event varied significantly between strata (p<0.0001) and month (p<0.0001). Because these data consisted of just two readings for each strata (and only one reading in the High *J. roemerianus* stratum), we were unable to test for interactive effects. The mean water level of the *S. patens* stratum was significantly lower than all other strata, with a mean water level of 9 cm below the soil surface. Low *J. roemerianus* was significantly different from all other strata with a mean water level of 1.1 cm below the soil surface. High *J. roemerianus* and *S. alterniflora* were not significantly different than one another, with mean water levels of 0.7 cm and 1 cm below the soil surface (Fig. 6).
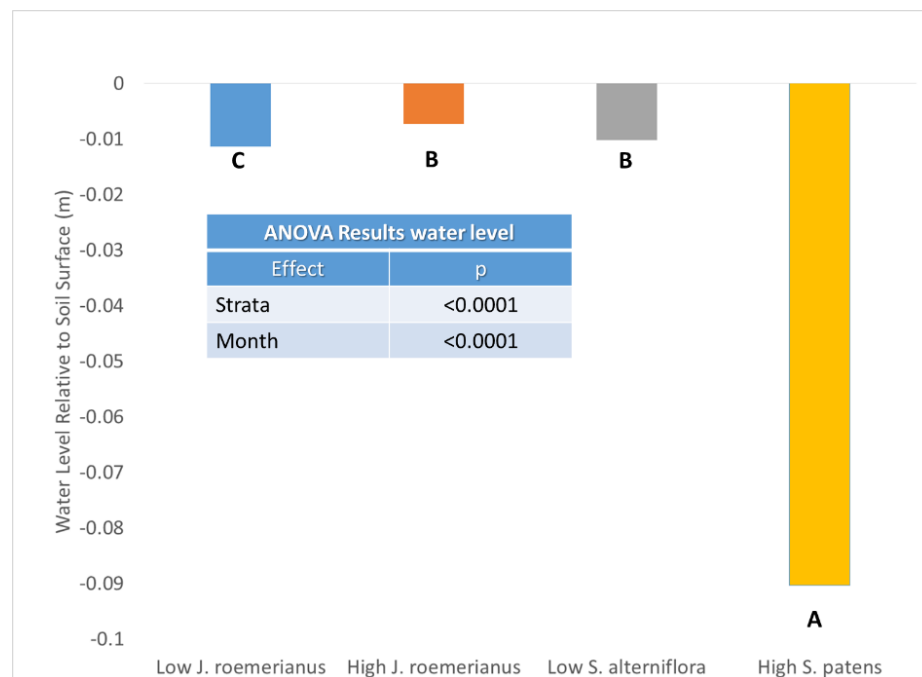
Figure 6 Mean water levels for the time period of two weeks prior to and during methane sampling by four strata in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. "Zero" on the Y-axis indicates the surface of the marsh. The inset table shows results of the ANOVA of this data. Significant differences are represented by letters.

Water level did not differ significantly in the months of May (mean water level 9 cm below the surface) and December (8 cm). June (5 cm) and September (4 cm) also were not significantly different from one another. The months of August and October (both 1 cm) had similar water levels, and the months of November (3 cm) and July (which had water levels within 1 cm of the marsh surface) had water levels that were significantly different than any other month (Fig. 7).
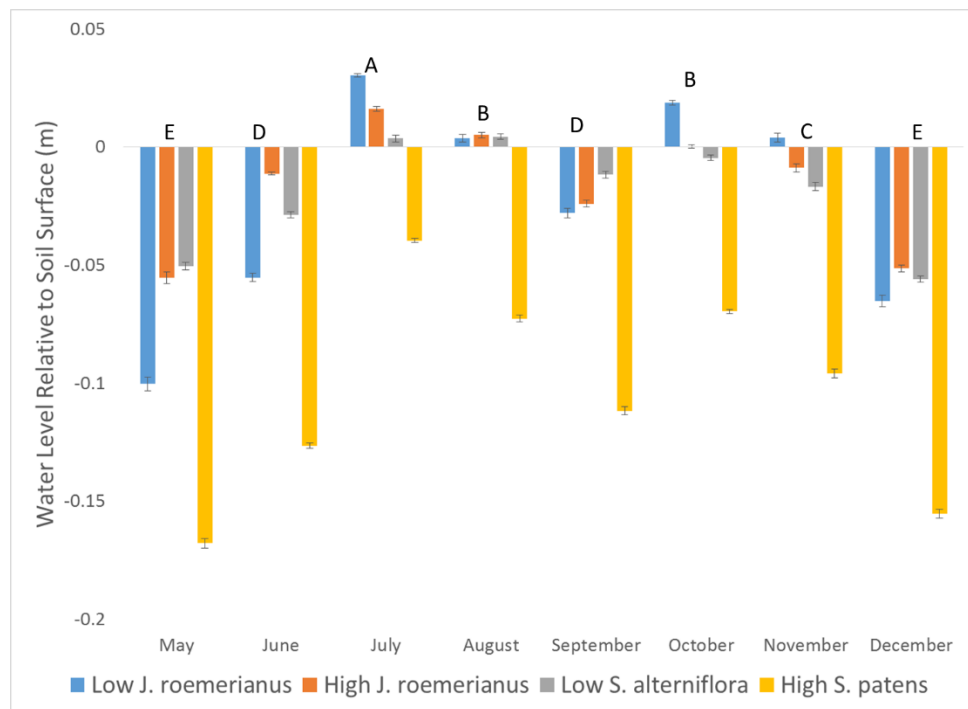
Figure 7 Mean water levels for the time period of two weeks prior to and during methane sampling by month in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. "Zero" on the Y-axis indicates the surface of the marsh. Significant differences are represented by letters.

Soil Temperature

Soil temperature at 10 cm depth varied significantly between strata (p<0.0001) and month (p<0.0001). Since these data were an average of two readings for each strata, we were unable to test for interactive effects. The mean soil temperature of the Low *J. roemerianus* stratum was significantly higher than all other strata, with a mean soil temperature of 20.5°

Celsius. High *J. roemerianus* (19.0º Celsius) was not significantly different than to *S. alterniflora* (19.5º Celsius). *S. patens* was significantly different than all other strata, with a mean soil temperature of 20.1º Celsius at 10 cm below the soil surface (Fig. 8). All months had significantly different soil temperatures than each other, with none being statistically similar (Fig. 9).
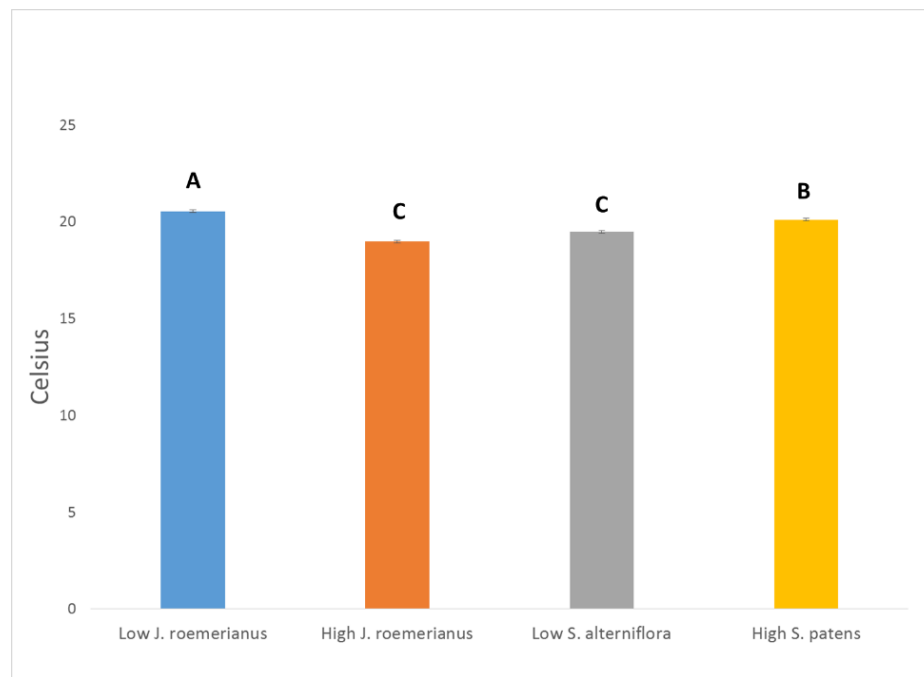
Figure 8 Mean soil temperature in degrees Celsius by four strata in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Error bars signify standard error. Significant differences are shown with letters.

Figure 9 Monthly mean soil temperatures in degrees Celsius in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Error bars signify standard error. Significant differences are shown with letters.

Salinity

Salinity varied significantly between strata (p<0.0001) and month (p<0.0001) but no interactive effect was observed (p= 0.54). By strata, both High and Low *J. roemerianus* and *S. alterniflora* did not differ significantly from one another with mean salinities ranging from 14.2 to 14.8 ppt. *S. patens* had significantly lower measured pore water salinity, with a mean of 12.3 ppt (Fig. 10).

Figure 10 Pore water salinity in parts per thousand by four strata in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Significant differences are shown with letters. Error bars represent standard error.

We observed a general trend of increasing salinity over the sampling period. May had significantly lower salinities than all other months (mean 11.4 ppt). June (mean 13.0) and July (13.5 ppt) did not have significantly different salinities from each other, and July was not significantly different than August through November. December's salinities (mean 15.3 ppt) were not significantly different than August through November as well, but was significantly higher than May through July (Fig. 11).
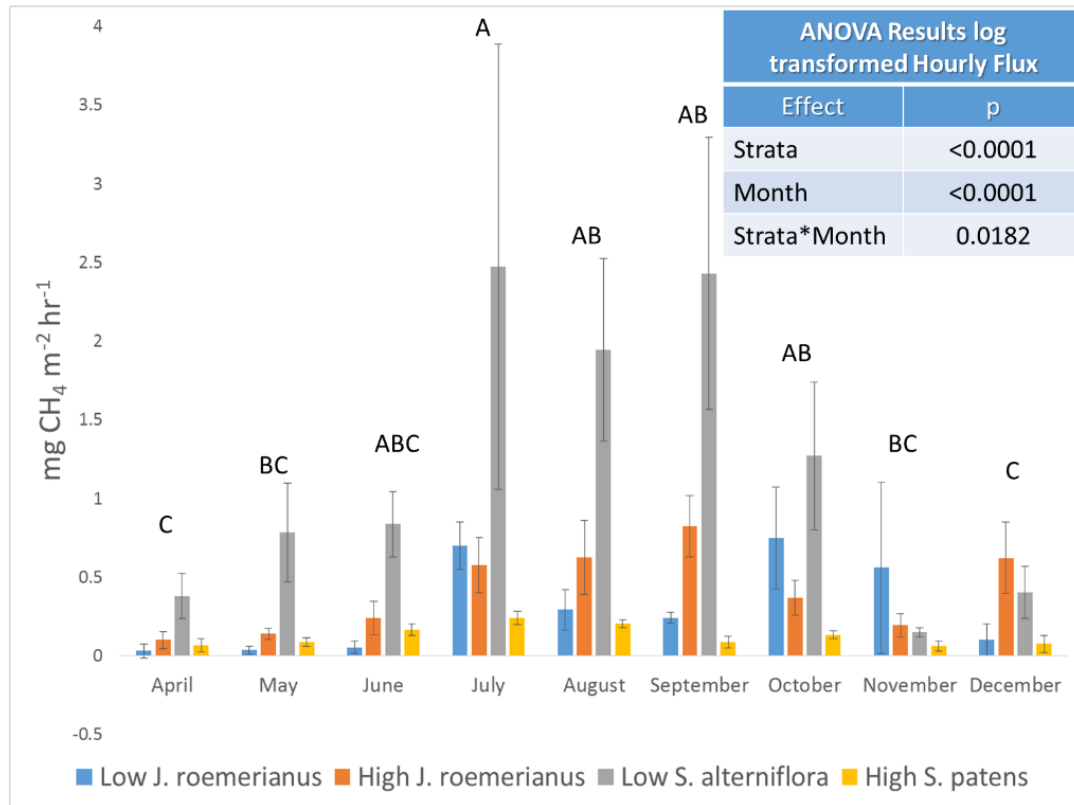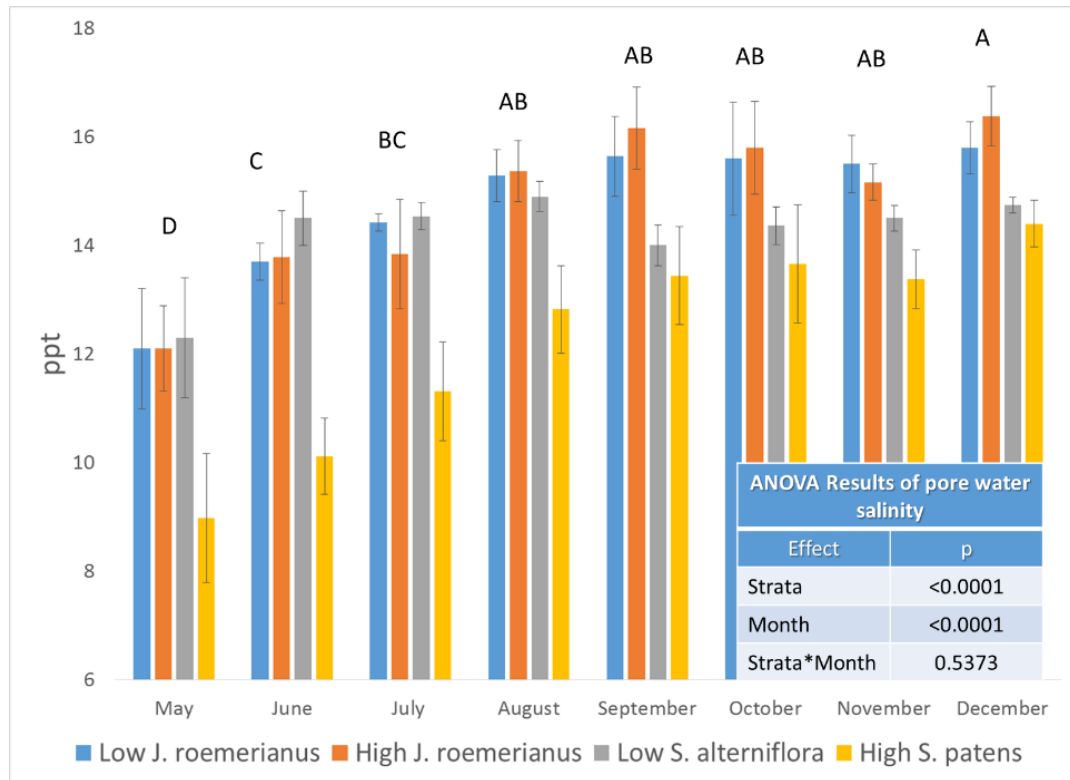
Figure 11 Pore water salinity in parts per thousand during the sampling period in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Inset table shows ANOVA results of the data. Error bars signify standard error and significant differences are shown with letters

Sulfate

Sulfate varied significantly between strata (p<0.0001), month (p<0.0001) and had a significant interactive effect (p=0.0185). By strata, *S. alterniflora* was significantly lower than all other strata, with a mean sulfate concentration of 434.3 mg/L (Fig. 12). *S. patens* had the highest mean sulfate concentration at 852.2 mg/L, but was not significantly different than the Low *J. roemerianus* stratum, which had a mean sulfate concentration of 693 mg/L. The High *J. roemerianus* stratum was not statistically different than the Low *J. roemerianus*, which had a mean sulfate concentration of 601.8 mg/L. Monthly statistical differences are shown in Figure 13.
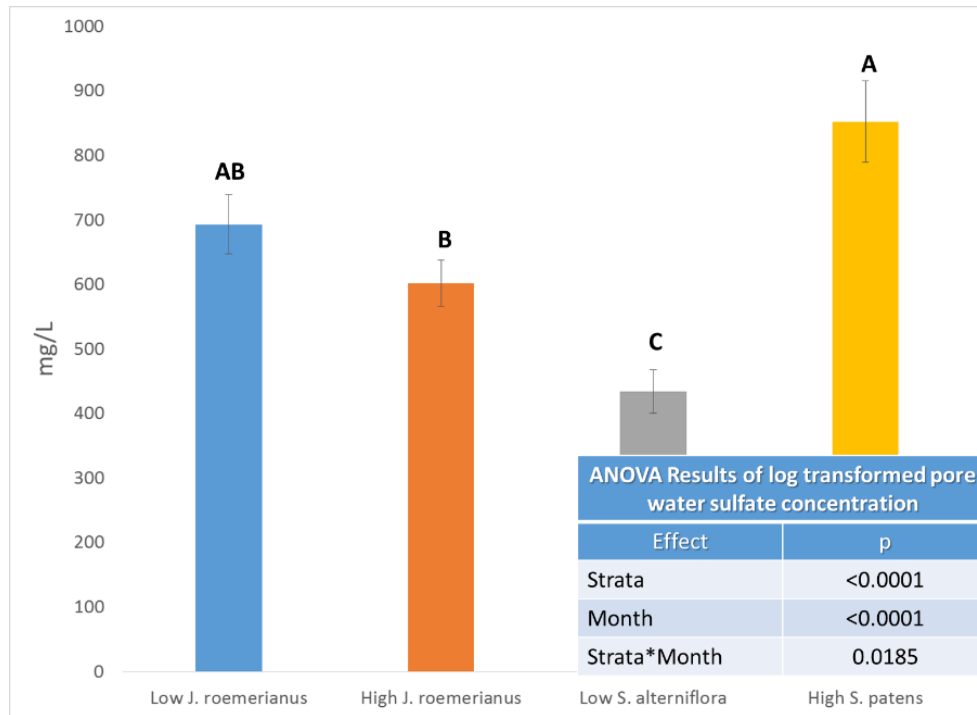
Figure 12 Pore water sulfate concentration in mg/L by four strata in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Inset table shows ANOVA results of the log transformed data. Error bars signify standard error and significant differences are shown with letters.
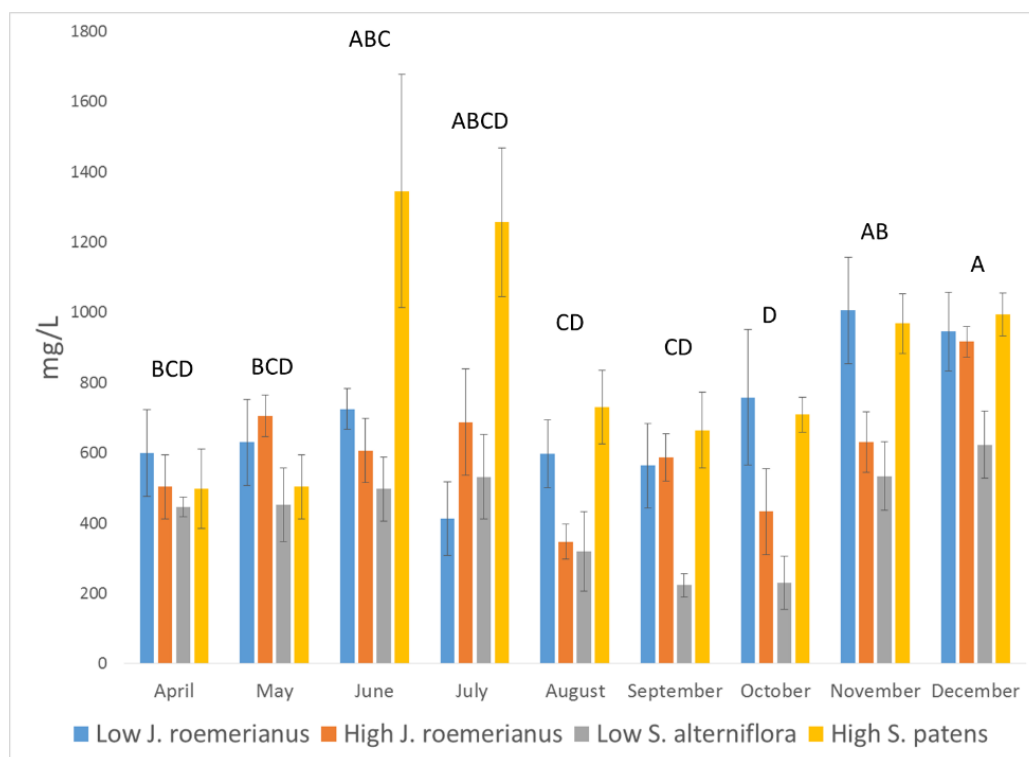
Figure 13 Pore water sulfate concentrations by month, in mg/L in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Error bars signify standard error and significant differences are shown with letters.

The within month significant differences for sulfate were only confined to two months, July and October, within all other months, each strata did not differ significantly from one another (see Appendix). In July, sulfate concentration in the *S. patens* stratum (1257.1 mg/L) was significantly greater than Low *J. roemerianus* (412.9 mg/L); both of these strata were not significantly different than the *S. alterniflora* or High *J. roemerianus* stratum. In October, *S. alterniflora* (230.3 mg/L) was not significantly different than High *J. roemerianus* (432.5 mg/L); however, Low *J. roemerianus,* and *S. patens* were not significantly different from High *J. roemerianus*.

Sulfate Remaining

Sulfate remaining (measured as the percent sulfate remaining relative to the expected sulfate at the given salinity) was significantly different between strata (p<0.0001) and

month (p<0.0001), and had a significant interactive effect (p=0.0001). This measurement can be changed to sulfate depletion by subtracting the percent sulfate remaining from 100. By strata, *S. alterniflora* had the lowest percent sulfate remaining, with a mean 39.4% sulfate remaining. This was not significantly different than the High *J. roemerianus* stratum, which had a mean 54.3% sulfate remaining. Low *J. roemerianus* had a mean percent remaining of 61.7%, which was not statistically different than High *J. roemerianus,* but statistically different than *S. alterniflora*. *S. patens* had the highest percent sulfate remaining, 98.6%, which was significantly higher than all other strata (Fig. 14).
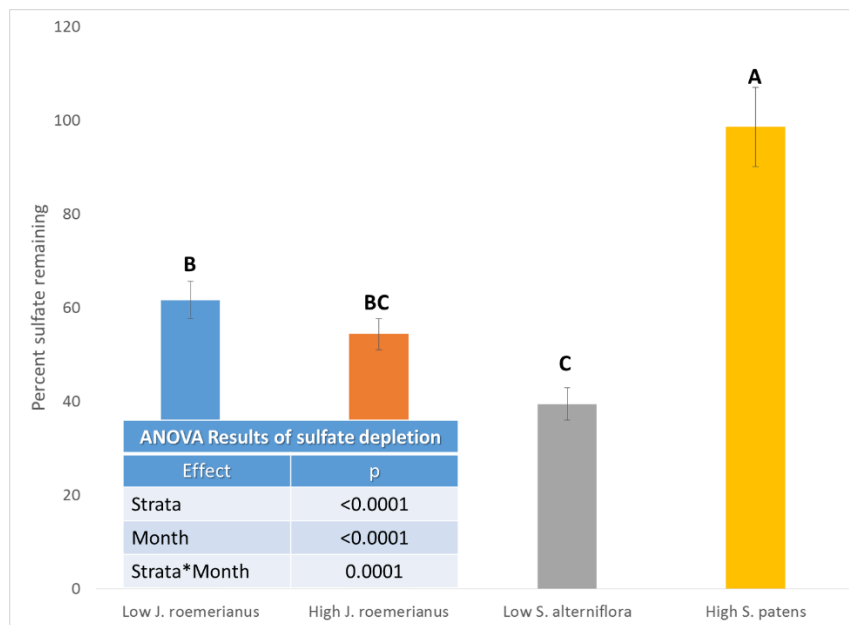
Figure 14 Percent sulfate remaining in pore water by four strata in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Inset table shows ANOVA results of the data. Error bars signify standard error and significant differences are shown with letters.

Significantly higher percentages of sulfate remaining were observed in June (85.5%), July (73.5%) and December (73.8%). Significantly lower percentages were observed in August (45.7%), September (44.9%) and October (46.4%). May and November had similar percentages to all months (Fig. 15).
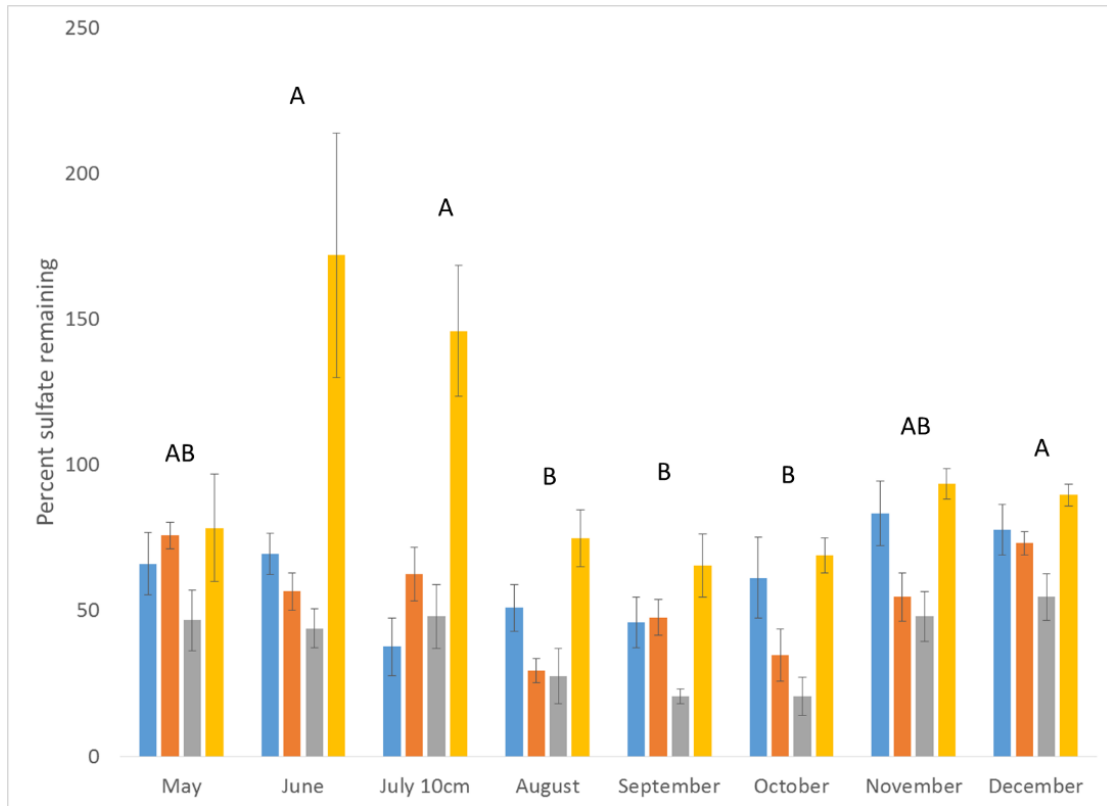
Figure 15 Percent sulfate remaining in pore water by month in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Error bars signify standard error and significant differences are shown with letters.

Hydrogen Sulfide

Hydrogen sulfide concentrations were significantly different between strata ($p<0.0001$) and month ($p<0.0001$), and had no significant interactive effect ($p=0.2229$). By strata, *S. alterniflora* had the significantly highest mean hydrogen sulfide concentration, 2119.3 μM (Fig. 16). All other strata were not significantly different than each other. Monthly statistical differences are shown in Figure 17.
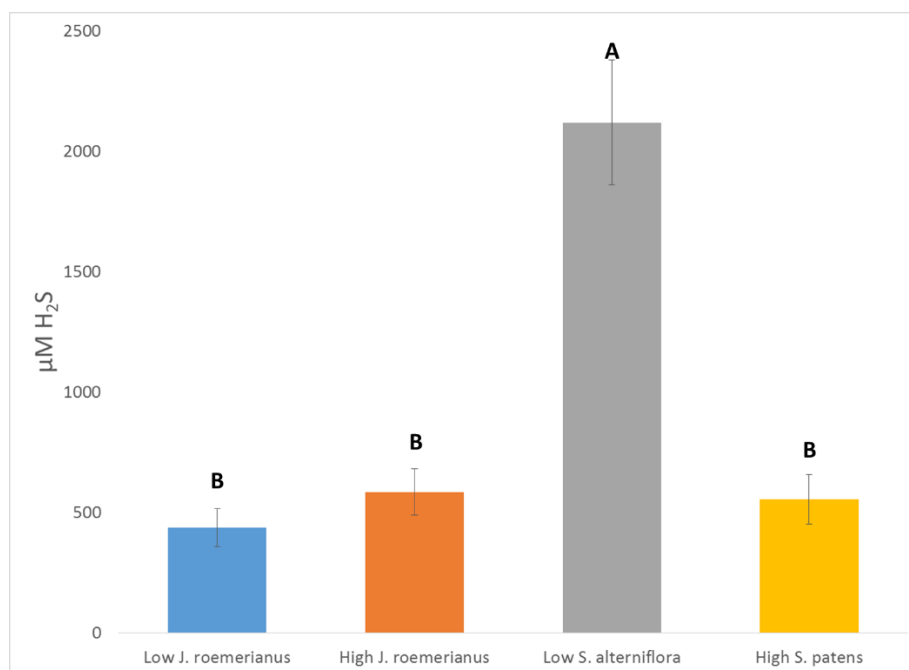
Figure 16 Pore water hydrogen sulfide concentration in µM by four strata in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Error bars signify standard error and significant differences are shown with letters.
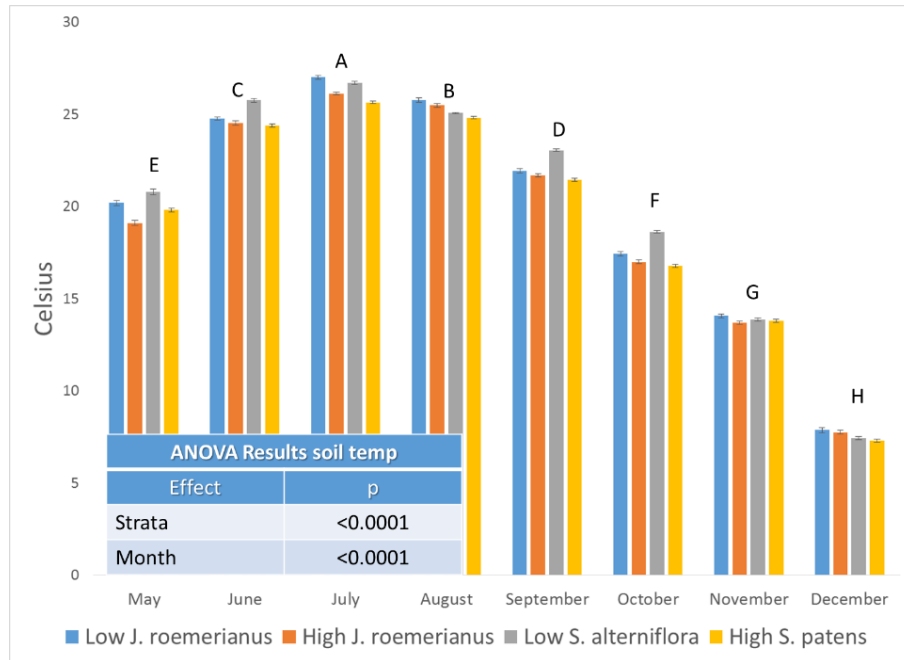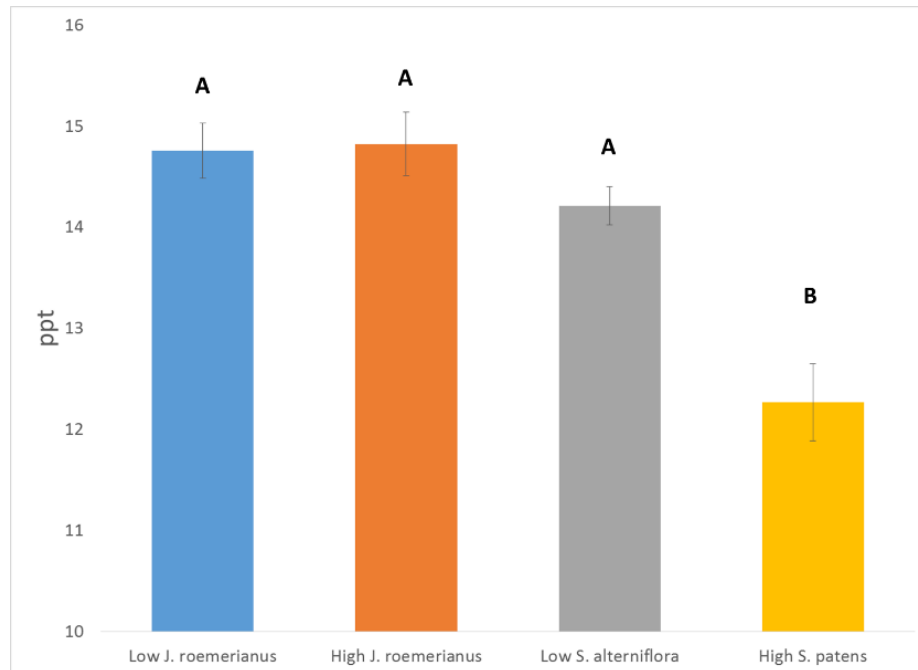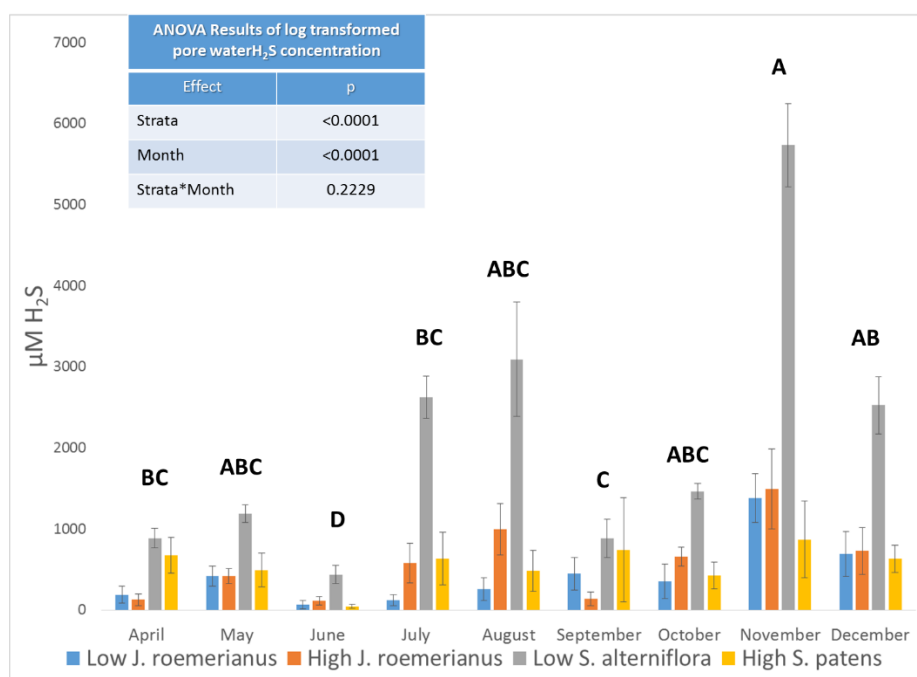

Figure 17 Pore water hydrogen sulfide concentration in µM by month in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Inset table shows ANOVA results of the log-transformed data. Error bars signify standard error and significant differences are shown with letters.

pH

pH was significantly different between strata (p=0.0327) and month (p<0.0001) and had a significant interactive effect (p=0.0199). Mean pH for *S. patens* was 6.4, *S. alterniflora* had a mean pH of 6.6, while High and Low *J. roemerianus* had mean pH's of 6.6 and 6.5, respectively. Only *S. patens* differed significantly from *S. alterniflora*, all other strata were not significantly different from one another. Monthly comparisons are shown in Figure 18.
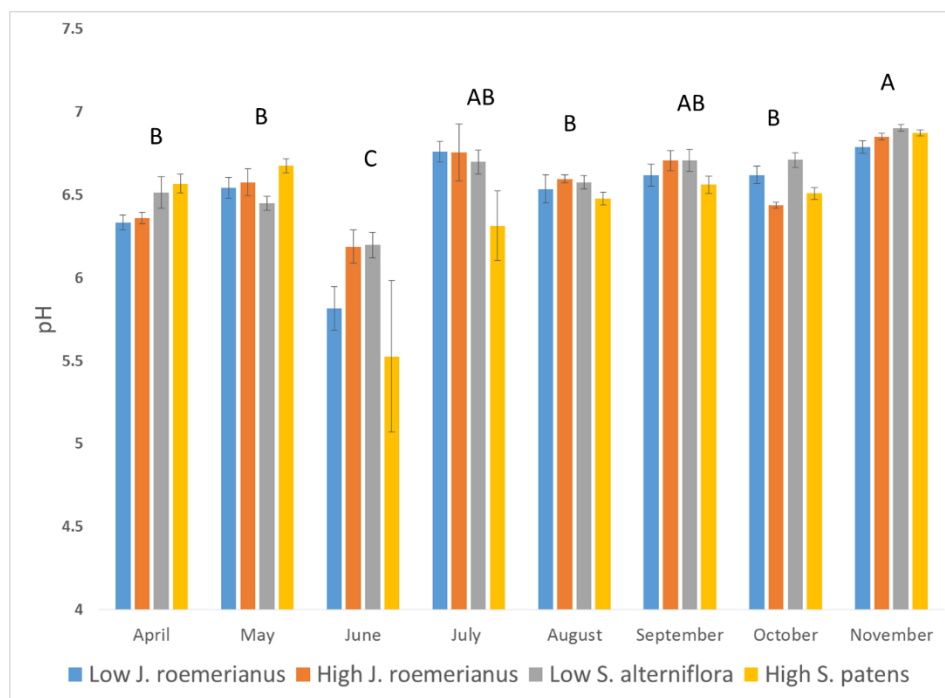
Figure 18 Pore water pH by month in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Error bars signify standard error and significant differences are shown with letters.

Pore water Methane

Pore water methane concentrations varied significantly between strata (p<0.0001), month (p<0.0001) and had a significant interactive effect (p<0.0001). *S. alterniflora* had a mean pore water methane concentration significantly higher than all other strata at 4796 ppm. High *J. roemerianus* had a mean concentration of 1243 ppm, which was not significantly

different than Low *J. roemerianus* (1175 ppm). *S. patens* had a mean pore water methane

concentration of 264 ppm and was significantly lower than the other three strata (Fig. 19).
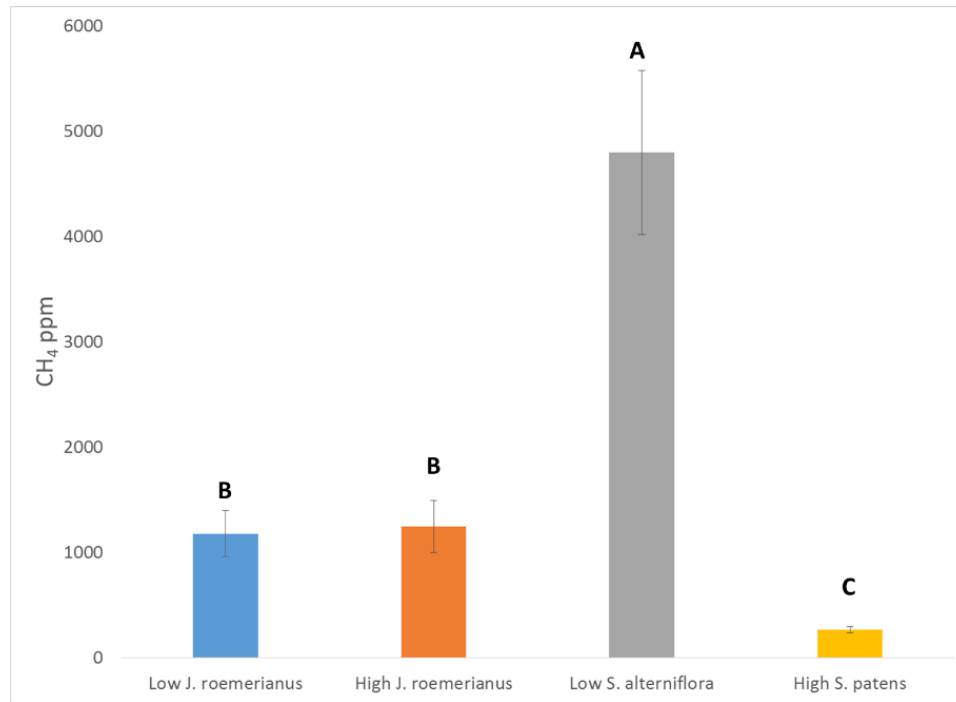
Figure 19 Pore water methane concentration in parts per million by four strata in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Error bars signify standard error and significant differences are shown with letters.

The months of April (mean concentration 257 ppm), May (309 ppm), and June (269 ppm)

did not have significantly different pore water methane concentrations. The months of July

(1047 ppm) through November (1047 ppm) were all not significantly different than one

another, and had significantly higher concentrations than April through June. December

(617 ppm) was not significantly different than any other month (Fig. 20).
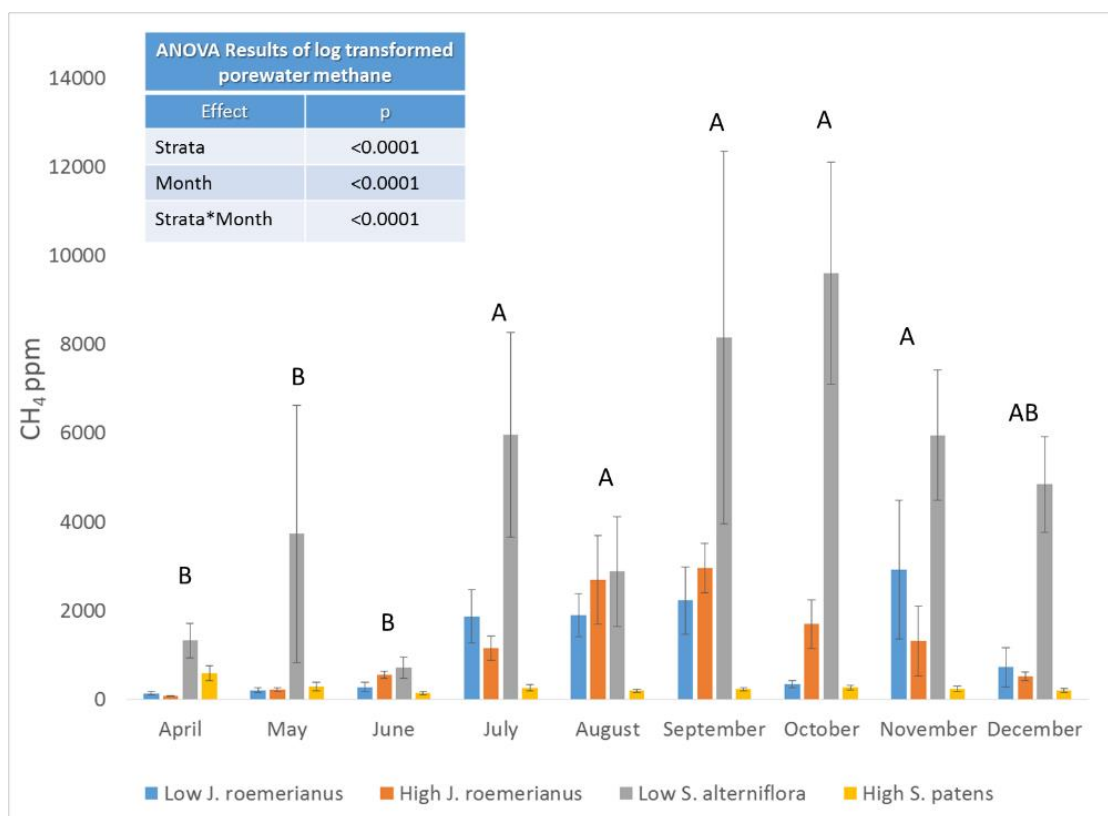
Figure 20 Pore water methane concentration by month in parts per million in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Inset table shows ANOVA results of the log-transformed data. Error bars signify standard error and significant differences are shown with letters.

Reduced Iron (July Only)

Reduced iron concentrations were taken only in the month of July. At 10 cm, *S. patens* had significantly higher mean concentrations of reduced iron, 72 mg/L. *S. alterniflora* had a mean reduced iron concentration of 0.02 mg/L, High *J. roemerianus* had a mean concentration of 0.3 mg/L, and Low *J. roemerianus* had a mean concentration of 0.8 mg/L (Fig. 21). All other strata were not significantly different from one another. Data from 20 cm can be found in the appendix.
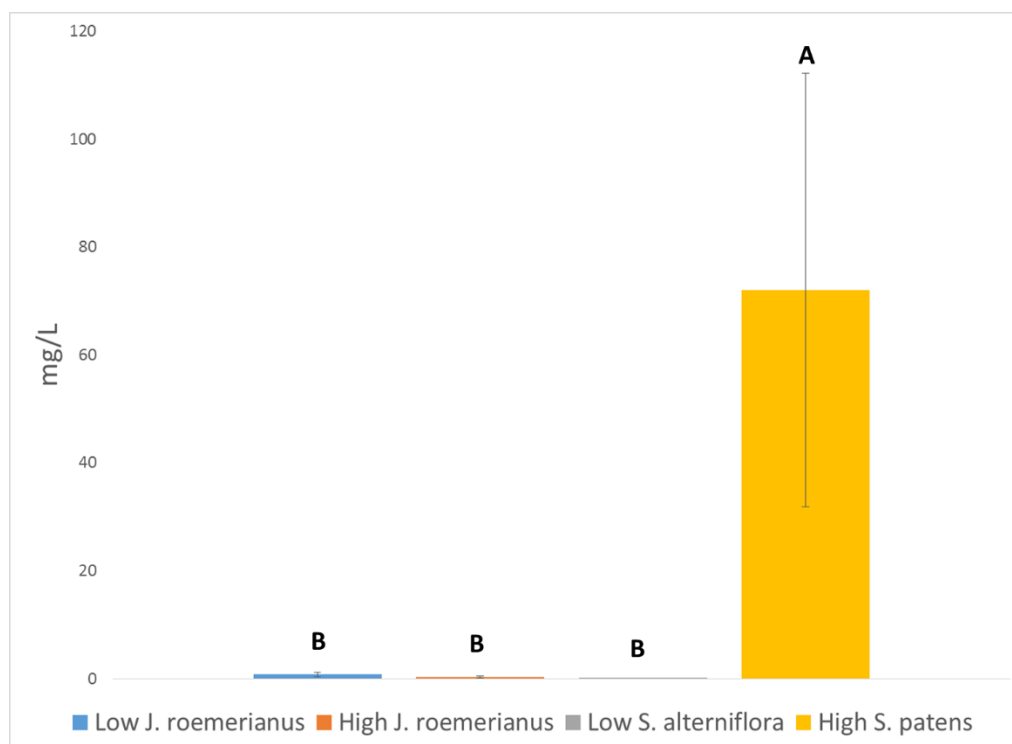
Figure 21 Reduced iron concentration in mg/L by four strata at 10 cm depth in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Error bars signify standard error. Significance differences are shown with letters.

Anaerobic Incubations

Incubations were measured for carbon dioxide and methane for a period of two weeks. The rate of methanogenesis slowed after 5 days; and minimal production occurred after this time (example shown in Figure 22). For this reason, methane production was determined using days 1-5, as production at time zero was assumed to be negligible.
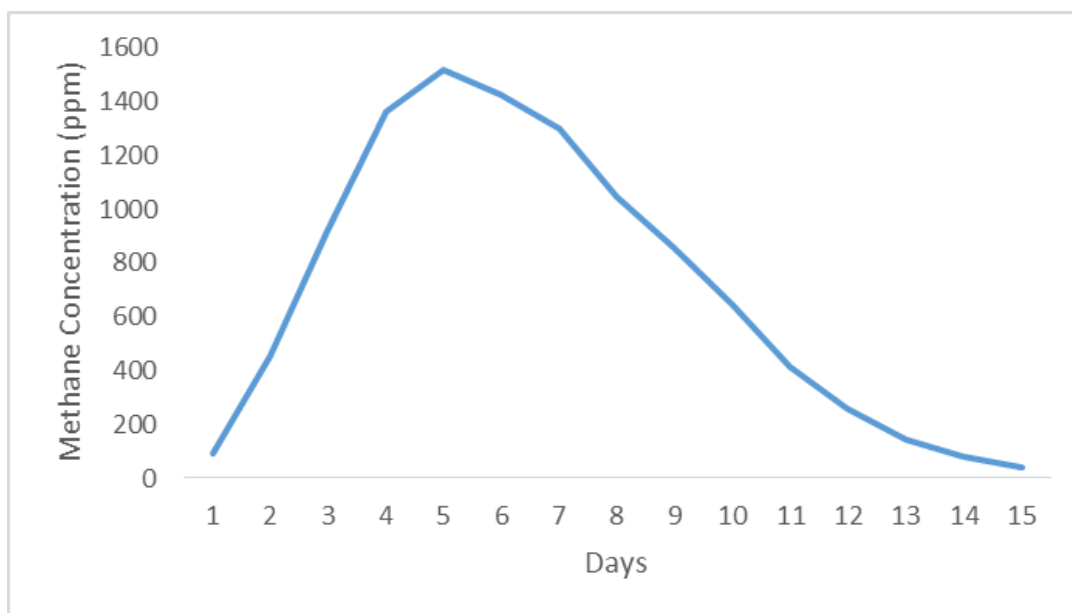
Figure 22 Methane concentration in parts per million, from 10 cm depth, from soil cores collected from S. alterniflora plot 3SA-2 from a tidal brackish marsh on the Deal Island Peninsula located on Maryland's Eastern Shore. Note the peak emissions observed at day 5, with declining concentrations observed thereafter.

The mean amount of mols of methane produced by the *S. alterniflora* incubations was 13.8 mol at 10 cm, and 2.7 mol at 30 cm. *S. alterniflora* produced 101.5 and 96.5 mol of carbon dioxide at 10 and 30 cm, respectively. *S. patens* produced 0.4 mol of methane at 10 cm and 0.6 mol methane at 30 cm, and 229.5 mol and 115.0 mol of carbon dioxide at 10 and 30 cm, respectively. Low *J. roemerianus* produced 0.9 mol and 0.7 mol of methane at 10 and 30 cm respectively and 171.8 and 101.1 mol of carbon dioxide at 10 and 30 cm respectively. Finally, the High *J. roemerianus* stratum produced 0.8 mol of methane at 10 cm, and 0.6 mol of methane at 30 cm, while producing 146.5 and 85.4 mol of carbon dioxide at 10 and 30 cm, respectively.

At 10 cm, *S. alterniflora* produced significantly more methane than all other strata (Fig. 23); and no other stratum was significantly different than one another. For carbon dioxide production, all strata were not significantly different, except for *S. patens*, which produced significantly more carbon dioxide than *S. alterniflora*. At 30 cm, we observed no

significant differences in methane or carbon dioxide production. The 10 cm incubations produced significantly more methane and carbon dioxide than those from 30 cm depth (p=0.0431).
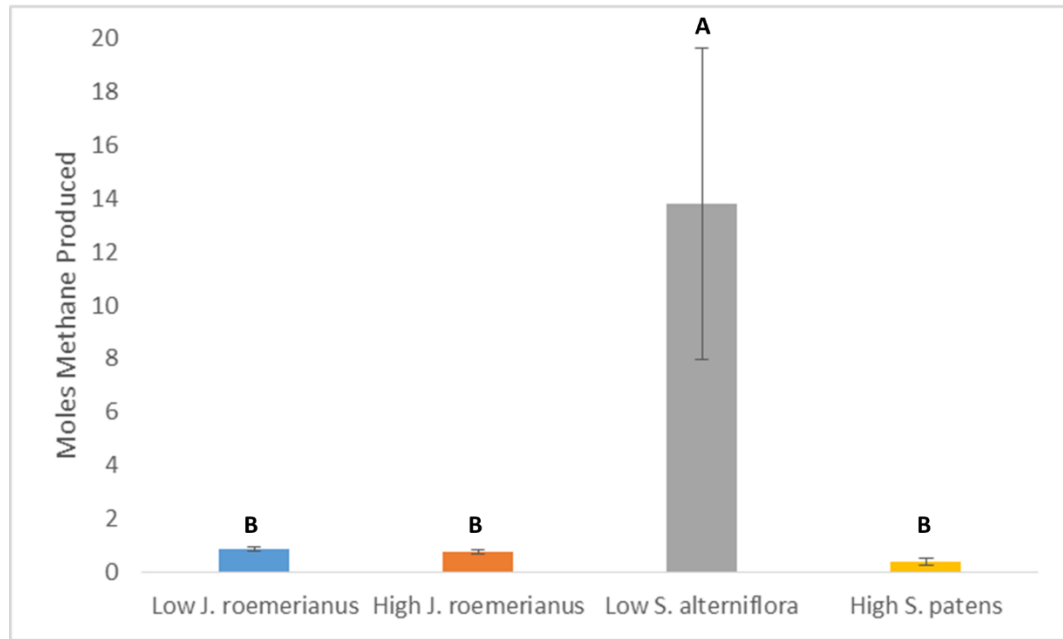
Figure 23 Mean methane production rate at 10 centimeter depth by anaerobic incubation of soil material by four strata in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore. "Low" vs "High" refers to comparative elevations within the site. Error bars signify standard error, significant differences are signified with letters.

Discussion

We found that vegetative/hydrologic strata within our study marsh had different methane emissions (Fig. 4). The low elevation *S. alterniflora* strata had the highest mean emission rate, approximately 2.7 times higher than the next highest stratum, high elevation *J. roemerianus*. The high and low *J. roemerianus* strata did not have significantly different emissions from each other. High elevation *S. patens* had the lowest overall methane emission rate of the four tested strata, with less than half of the mean hourly flux of the next highest stratum, the low elevation *J. roemerianus*.

Monthly Methane Flux, Plant Community, Water Level and Soil Temperature Changes

Methane fluxes followed a monthly trend in our study marsh similar to other temperate mid-latitude marshes (DeLaune et al. 1983; Prieme 1994; Alford et al. 1997). Emissions were lowest during the early spring and late winter months, and highest during the summer and early fall. Differences in the plant communities, soil temperature and water levels appear to have been the driving forces behind these seasonal differences. Higher methane emissions rates occurred when periods of high plant productivity, soil temperature, and water level coincided together.

It appears that an availability of labile carbon from the plant species *S. alterniflora* was a dominant driver of methane emissions in our marsh system. This stratum accounted for the largest amount of methane produced. The highest emissions occurred during the summer and early fall when plant productivity is highest. High plant productivity should stimulate methane emissions, as during these times, plant gas transport would be assumed to be high. Additionally, the amounts of root exudates being generated within the plant rhizosphere would be higher during these periods, which could generate energy substances for use by methanogens.

During the months of April, May and June we observed uniformly low methane emissions. While we lack water level and soil temperature data for April, these data for May and June showed comparatively low water levels across all four strata. Soil temperatures in May were approximately 5º C lower than June, and while methanogens have been found to be active at temperatures within this temperature range (Megonigal et al. 2005), Q_{10} values for methanogens have been reported to vary between 4.1 (Segers 1998) and 2 (Megonigal

et al. 2005), with higher values reported (Whalen 2005), so this temperature could have impacted emissions. However, soil temperatures rose to nearly 25° C in June across all strata, yet methane emissions were not significantly higher during this month than in May. Low plant productivity and low water levels in May were likely responsible for the lower emissions of methane relative to the later summer months. Water levels in June were also below the soil surface; however, for all strata except *S. alterniflora*, water levels were, on average, less than or approximately 5 cm below the soil surface. While this is significantly different from the following months with "high water", these conditions (aside from *S. patens*) are not truly "low water" (Fig. 7). Figure 24 shows the monthly fluctuations of one of the water level loggers placed in the *S. alterniflora* strata, showing the variation in water levels over the course of the experiment.
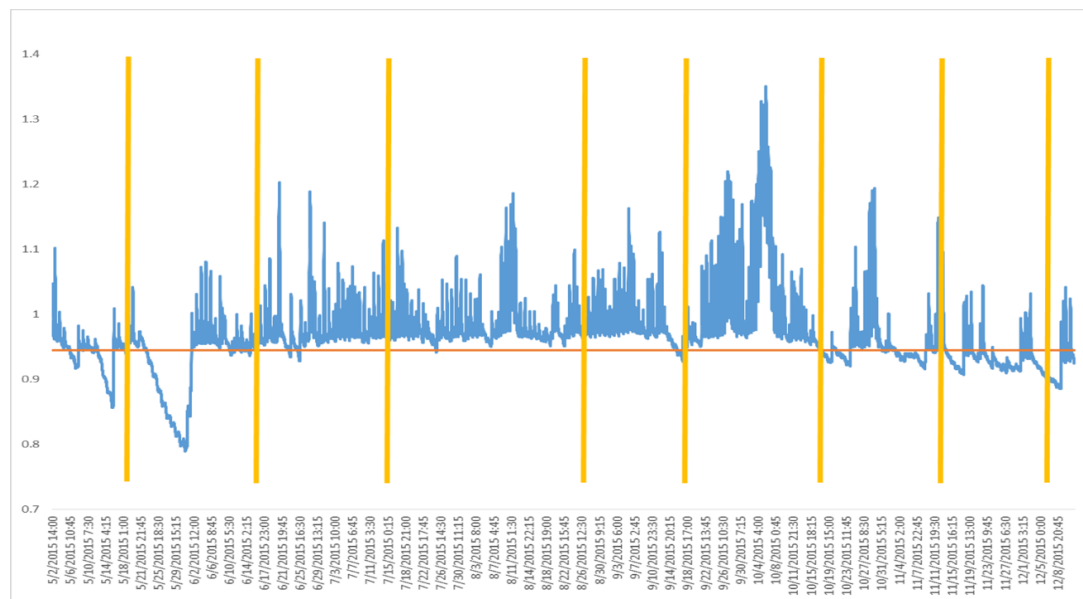
Figure 24 Relative water levels at water level logger adjacent to flux plot 3SA-4 located in a tidal brackish marsh on the Deal Island Peninsula on Maryland's Eastern Shore, recorded every 15 minutes from May to December 2015. The red horizontal line indicates the marsh surface while the gold vertical lines indicate sampling events, from May to December. The Y-axis is in meters, and represents the depth of the water to the sensor, the X-axis is the Date and Time each measurement is taken.

Emissions rose to their highest levels in July, August and September, when soil temperatures and water tables were also at their highest during the entire sampling period. This methane emission increase was seen most strongly in the *S. alterniflora* stratum whose emission rate more than doubled from June; however, the other three strata also had increases in methane emission rates. The mean water level of all strata except *S. patens* showed the soil was completely inundated during the two weeks leading up to and during the sampling period. Plant productivity at this time should be at its highest levels of the year as well, as these sampling points occurred during the peak of the growing season. These conditions should all be favorable to methanogenesis, which was reflected in the higher emission rate data.

During the month of September, emissions were comparable to July and August; however, both soil temperature and water level began to decrease. It should be noted that soil temperatures in September (21-24^oC) were lower than June (24-26^oC); however, fluxes were much higher in September. For example, *S. alterniflora* and high *J. roemerianus* each had mean emission rates over three times higher in September than June. This was likely due to productivity in plants in the marsh complex not at yet at their peak levels in June, and while productivity was likely beginning to decline in September, the remnants of labile carbon produced during the summer was likely still available for methanogens.

Beginning in October, as soil temperatures began to decline and plants began to senesce, mean methane emission rates began to decrease, although they were not statistically different than the summer months. Soil temperature within the strata were lower during October, with all strata declining by 3-5° C; however, the water level was higher than in September, with all strata except *S. patens* at or near complete inundation. Flux rates

between October, September and August were not significantly different, however the overall trend exhibits a decrease.

During November, we observed lower fluxes across all strata, although emissions this month were not significantly different than October. *S. alterniflora*, had a mean emission rate of 0.15 mg CH_4 m^{-2} hr^{-1}, which was over eight times lower than the rate observed in October. Soil temperatures again decreased by 3 to 5° Celsius; however, water levels were similar to those observed in August. Plant productivity at this point was likely very minimal, again contributing to low methane emission rates. This month demonstrated that high water levels alone were not sufficient to generate methane emissions. Pore water methane concentrations in the *S. alterniflora* were not significantly different than the months in which we observed higher emissions during the late summer and early fall months. Methane was still present in the soil during this month; the lack of plant gas transport may have prevented the majority of this methane from being released in a manner other than ebullition, which we did not measure.

In the final month of sampling we saw low emission rates, comparable to April, May, June and November. Soil temperatures continued to decrease, with our lowest recorded temperatures (approximately 7° Celsius). December also had the second lowest observed water levels, second only to the May sampling period. The combination of low water levels (and therefore larger aerobic zones in the soil profile) and low temperatures (which slow microbial activity) along with the marsh plant community being in full senescence (or nearly so), explains the low level of emissions for this last sampling.

Anaerobic Incubations

In order to gain a basic understanding of the amount of available carbon available to methanogens in the soil, we performed anaerobic incubations from soil cores collected from the area immediately adjacent to each flux plot at 10 and 30 cm. These incubations also allowed us to test the emission rates from each strata in a more controlled laboratory environment. After five days of measurement, the methane produced in each bottle was calculated. *S. alterniflora* produced over 16 times more methane at 10 cm than the next closest incubation, low *J. roemerianus.* This trend was the same as the ones we observed during our field measurements. We also measured carbon dioxide production, and the numbers exhibited less variability between strata, and CO_2 was produced by all strata across depth. The production carbon dioxide was likely due to the decomposition of whatever root material remained in the incubation soils, as well as from other anaerobic respiration occurring using alternate electron acceptors. Much less carbon dioxide was produced in cores taken from *S. alterniflora,* which also produced much more methane than the other strata. This result suggests that a source of carbon was present in the *S. alterniflora* stratum that was more directly available to methanogens or in a larger quantity, and/or that the *S. alterniflora* had a greater population of methanogens than the other strata. This also suggests that the relationship between emissions and plant productivity may not be related to gas transport through plant aerenchyma, but rather with the amount of labile carbon these plants are producing.

Since this method required manual separation of roots from within wetland soil material, some limitations need to be addressed. Remnants of root material left in the soil could inflate the amount of carbon dioxide produced, as the remaining roots left in the incubations

would decompose into carbon dioxide as they are consumed by other microorganisms. This source of carbon (roots) would not be usable for methanogens until it had already undergone fermentation into the lower molecular weight compounds these microorganisms use. This issue was especially likely in the *S. patens* cores, since the fine roots in this stratum were much denser and more difficult to remove than the larger roots of the other two species.

Pore Water and Methane Flux

Overall, we observed that salinity and sulfate concentrations did not appear to influence methane emissions in our study marsh. In addition, we did not find the hypothesized negative relationship between months with high methane emissions and high hydrogen sulfide levels, and in fact there was a positive relationship in some months. Interestingly, there was a negative relationship between the mean amount of sulfate remaining after reduction (Fig. 14), and methane emissions (Fig. 4), indicating that methane was being produced, as sulfate was also being reduced into hydrogen sulfide.

The stratum with the lowest methane emissions, *S. patens,* had significantly lower (12.3 ppt) salinity than the three other strata (between 14.2 and 14.8 ppt). The three remaining strata all had similar mean salinity, yet varied significantly in their methane emission rates. Significantly lower salinities were observed in May, June and July, however methane emissions were significantly lower in May and June than in July. Salinities from August until December were not significantly different; however, emissions peaked in August and September before declining. This suggests that additional controls beyond salinity affected methane production and emission in this tidal brackish marsh, and that the salinity proxy for methane emissions described in Poffenbarger et al. (2011) and further investigated by

Holm et al. (2016) may overlook the importance of other factors of methane production at this site-specific scale.

Sulfate reduction has been assumed to inhibit methanogenesis, since it is a more energetically favorable reaction. We inferred sulfate depletion in all strata from other data; however, the strongest depletion was observed at the *S. alterniflora* stratum. Here, mean pore water sulfate concentrations were the lowest, 344.3 mg/L. Conversely, *S. patens* had a mean concentration of 852.2 mg/L, over twice as high. Both of the *J. roemerianus* strata had higher sulfate concentrations than *S. alterniflora*, with the "high" strata having a mean concentration of 601.8 mg/L and the "low" strata slightly higher at 693.0 mg/L. We found that the *S. patens* stratum had a mean value of 98.6% sulfate remaining after reduction. In the months of June and July, this stratum had mean sulfate remaining percentages of 172% and 146%, respectively, indicating that sulfate was actually accumulating in the system, possibly from pyrite oxidation, or upward diffusion of deep hydrogen sulfide being oxidized. *S. alterniflora* had a mean value of 39.4% sulfate remaining. High *J. roemerianus* and Low *J. roemerianus* had mean values of 54.3% and 61.6%, respectively. *S. patens* had the highest amount of sulfate remaining after reduction, with the lowest mean methane emissions, while the opposite was true of *S. alterniflora*. This trend was the same in *J. roemerianus;* however, the differences in sulfate remaining as well as methane emissions were not statistically significant between these two strata.

We also measured hydrogen sulfide concentrations, a byproduct of sulfate reduction, in the pore water of each stratum. During months with high methane emissions, we hypothesized that we would find low concentrations of this compound because it was assumed methanogens would not outcompete highly active sulfate-reducing bacteria for the

available carbon, therefore sulfate reduction should not be producing this compound during periods of methane emissions, and we should not observe high levels concurrent with high methane emissions. However, we found that the stratum with the highest mean concentration, 2119.3 µM, *S. alterniflora*, also had the highest emission rate. *S. patens* had a much lower hydrogen sulfide concentration, 555.0 µM, along with the lowest rate of methane emission. Both *J. roemerianus* strata had similar mean sulfide concentrations to *S. patens*.

In July only, we measured the concentration of reduced iron in the pore water (Fig. 21). The presence of reduced iron indicates active iron reduction, because reduced iron is highly mobile in soils. In the *S. patens* stratum, we observed a mean reduced iron concentration of 72.0 mg/L, which was 93 times higher than the next highest mean concentration, 0.77 mg/L in the low *J. roemerianus* stratum. The concentration in the remaining two strata were even lower. The significantly higher reduced iron concentration indicates active iron reduction occurring in this stratum, which is even more energetically favorable than sulfate reduction. This iron may be entering this system through mineral material being deposited along this section of the marsh complex, since it is directly adjacent to the tidal creek that flows through this marsh. This, along with the lower water levels in the *S. patens* stratum, were likely the primary driver behind its much lower methane emissions when compared to other strata. Even though reduced iron data was only available for July, the water table data suggest that reduced iron levels may have been higher in the *S. patens* stratum across the entire year.

While we observed statistically significant main effects and interactions in the measured pore water pH in both strata and month, it is unlikely that pH had a substantial impact on

methane production in our tidal marsh. Methanogens have been shown to prefer pH conditions which are close to neutral, as well as conditions at pH's below 5.6 (Walker et al. 1998; Garcia et al. 2000; Megonigal et al. 2005), and our measured values fall well within this range (Fig. 18).

In order to gauge the production of methane in the soil itself, we measured pore water methane concentrations. Concentrations followed the same general trends as the measured fluxes (Fig. 20). *S. alterniflora* had the highest measured pore water methane concentrations for each month we sampled (mean 4796 ppm). *S. patens* had consistently minimal pore water methane concentrations, with a mean of 264 ppm of methane over the sampling period. Both *J. roemerianus* strata had similar pore water methane concentrations, which were slightly higher than *S. patens*, with the low *Juncus* having a mean methane concentration of 1175 ppm and the high *Juncus* having a mean concentration of 1243 ppm. Over the course of the study period, we observed two distinct groupings for this data. The months of April, May and June all had statistically significantly lower methane concentrations in the pore water than July through November, which were all statistically similar. December was not statistically significantly different from any month. It is important to note that we measured these concentrations at a 10 cm depth in the soil profile, so it is possible that methane being generated below this depth could be consumed via methanotrophic bacteria if water levels in the soil column were below this point (such as the *S. patens* stratum). These results show that while methane was still present within the pore water in the later months of the study period, our concurrent flux measurements do not show a similarly high amount of methane reaching the atmosphere through plant aerenchyma, possibly due to lack of plant productivity after the marsh plant

community had undergone senescence. This methane could be released through ebullition, however continuous monitoring would be required to accurately estimate this process.

Conclusions and Implications

Our hypothesis that methane emissions would be different within hydrologic and vegetative communities (strata) was confirmed. We found that the low elevation *S. alterniflora* stratum had significantly higher methane emissions than the remaining three strata. The high elevation *J. roemerianus* stratum was lower than *S. alterniflora;* however, both the high elevation *S. patens* and low elevation *J. roemerianus* had statistically similar emissions which were lower than the other two strata. These plant communities have evolved over time to tolerate the changing conditions in a tidal brackish marsh, and those conditions can have an impact on the ability of methanogens to produce methane. Our four strata comprised four very different environments within one marsh complex, and our methane emission reflect this spatial variation. *Spartina alterniflora*, had the highest emissions, and is one of the few wetland species that can tolerate the high sulfide concentrations and is located in lower elevation areas of our marsh (King et al. 1982; Koch et al. 1990). In fact, our mean annual hydrogen sulfide concentration in this stratum (2119.3 μM) approaches the levels of deemed stressful (1.2-2 mM) and lethal (>3 mM) by Seliskar et al. (2004) in a media growth study, and we exceeded this level in some of our monthly measurements (Fig. 17). This same study also found that *Spartina patens* was less tolerant to increased hydrogen sulfide concentrations. Since we found that high methane emission rates can occur concurrently to high sulfide concentrations; marshes with large areas of *S. alterniflora* may contribute more to methane emissions than those with other species.

Differences between strata in methane emissions appears to have been driven by a varying combination of plant productivity, water levels and, to a lesser extent, soil temperature. The strata with the lowest emissions, *S. patens* seems to be driven by its lower water levels, and therefore more aerobic conditions. These conditions seem to favor a system driven by iron reduction, if levels we observed in July are any indication of what the system is like throughout the year. The low water levels and more aerobic conditions, coupled with iron reduction, likely suppressed both methanogenesis and sulfate reduction when compared to the other strata. Research has also shown that *S. patens* decays at a rate slower than *S. alterniflora* (Valiela et al. 1985), so it is possible carbon usable to methanogens accumulates slower in this plant community. Both the high and low *J. roemerianus* strata were very similar in regards to methane emissions, and despite an approximate 3 cm difference in elevation, had similar water levels throughout the year. Similar to the differences between *S. patens* and *S. alterniflora, S. alterniflora* has been found to degrade faster than *J. roemerianus* (Haines and Hanson 1979), so a similar carbon accumulation mechanism may explain the difference between these two plant communities as well. When compared to *S. alterniflora*, both *Juncus* strata had similar conditions, including salinity, water level and temperature. The short form of *S. alterniflora* (which is the form in our marsh) has been found to have lower productivity than *J. roemerianus* (Giurgevich and Dunn 1982) in previous research; however, this does not explain our differences in emissions in these strata.

We propose that plant productivity is a primary driver of variation of methane emissions in this tidal brackish marsh. While we did not measure productivity or plant gas transport directly, we visually observed that plant productivity peaked in the mid to late summer

(July through October), which is when we also observed our highest methane emissions. This increased plant productivity likely both increased the rate of active transport of methane through plant aerenchyma, as well as increasing the amount of labile carbon available for methanogens in the soil. Root exudates can be a source of carbon for methanogens (Bridgham et al. 2013); although additional research is needed to determine how the rates at which these compounds are produced and accumulated within the rhizosphere during the growing season differ for tidal marsh species. Such research could explain the differences in the rates of methanogenesis that we observed during the growing season, such as the lower emissions during May and June, when compared to September and October. The differences between the rates of production and accumulation of exudates between different species (such as *S. alterniflora* and *J. roemerianus*) could also explain the differences we observed between strata with similar temperatures and water levels. The incubation data we collected suggests that some differences in the types of organic matter and microbial communities present in the soil affects the production of methane in an anaerobic incubation, with *S. alterniflora* producing much more than all other strata (Fig. 23).

We observed this difference relative to methane fluxes from the field data in the methane concentrations of the pore water as well. Early in the sampling season from April until June, all pore water methane concentrations were significantly lower, until they increased and remaining not significantly different than from July through November (December was not significantly different from any month). These two different levels we observed show a change occurring in early summer, which we attribute to the increased plant productivity, higher water levels and increasing soil temperatures within our marsh. The continuing high

emissions and pore water methane concentrations into the late fall likely resulted from an excess of carbon remaining in the soil after plants began to senesce in the fall. *S. alterniflora* had the highest pore water methane concentrations, similar to its high methane emissions. *S. patens* again had the lowest; however, since we collected this data at 10 cm depth only, we do not know how much methane is being produced lower in the soil profile in this or any other stratum. In the case of *S. patens* we hypothesize that any methane being produced deeper in the soil profile is being consumed by methanotrophy, since water levels for this strata were often 10 cm below the soil surface during the sampling period (Megonigal and Schlesinger 2002).

During our study, we attempted to minimize ebullition from our flux measurements, since these events are random and unpredictable. We accomplished this in two ways; first, we installed boardwalks to avoid disturbing any methane bubbles lurking below the surface, secondly, after measurements were completed, we discarded fluxes that showed indications that an ebullition event may have occurred during sampling. Other methane emissions studies such as Holm et al. (2016) are able to take ebullition into account through the use of eddy covariance towers. These towers can measure fluxes over a much larger area, and can account for large uncontrolled releases that smaller static chamber methods aim to avoid. Therefore, we may have underestimated our annual emissions since we did not take these irregular emission events into account.

Since some strata supported higher methane emissions than others within a single marsh, simple predictive relationships between methane emissions to a single variable such as salinity (Poffenbarger et al. 2011), while useful for large scale estimates of emissions, may not be applicable at this higher resolution of emissions. We found that in our tidal brackish

marsh salinities varied significantly both within strata and by month. These differences would have been enough to impact emission estimates if a single value had been used for the entire system estimate. Our mean salinity range throughout the entire site across months and strata was between 9 and 16.4 ppt with our maximum and minimum observed salinities being 19.3 and 7.2 ppt, respectively. If using salinity as a single predictor for methane emissions, a substantial over or under-estimation of emissions could have occurred. For example, using the formula for methane flux estimation from Poffenbarger et al. (2011) (log(CH4) = -0.055*salinity+1.36) with the mean salinities from our site, we would calculate an emission range of 7.5 to 2.9 g CH$_4$ m^{-2} yr^{-1}, which is an uncertainty of 4.6 g CH$_4$ m^{-2} yr^{-1}. It is unknown whether this level of error is acceptable at a region or site-level scale. Marsh complexes with similar types of hydrologic/vegetative communities exist throughout the world, and ignoring the impact these differences may have on fluxes could be problematic for site-level greenhouse gas accounting.

Poffenbarger et al. (2011) used salinity as a proxy for sulfate concentrations to predict methane emissions. Our data show that in some strata, higher sulfate concentrations can occur in areas with lower salinity. While we state that additional factors are at work in regards to methane emissions, we note that our values for each strata do agree well overall with the relationship established in Poffenbarger et al. (2011) when plotted along with the data they present (Fig. 25). However, our estimated values do represent substantial error from their measured values; particularly for the *S patens* and *S. alterniflora* strata. *S. patens* emitted 3.9 g CH$_4$ m^{-2} yr^{-1} less methane than predicted, High *J. roemerianus*, 0.7 g CH$_4$ m^{-2} yr^{-1} less, and Low *J. roemerianus* 2.1 g CH$_4$ m^{-2} yr^{-1} less than predicted. *S. alterniflora* emitted 4.8 g CH$_4$ m^{-2} yr^{-1} more methane than what is estimated by the curve.

To calculate our yearly flux estimate, we used the mean flux rate for each strata in April to estimate each strata's emissions in January, February and March. Research in created wetlands in Ohio has shown that emissions during winter months are not always negligible (nearly 40% of annual emissions over two years) (Morin et al. 2014), so this assumption may potentially underestimate our annual emission. When these data are added to the default values for methane emissions in mesohaline marshes (salinities 5-18 ppt) presented in Poffenbarger et al. (2011), the mean value for these wetland systems drops, from 16.4 to 12.1 g CH$_4$ m^{-2} yr^{-1}.

The values from our measurements, as well as the predicted values from Poffenbarger et al. (2011), can be converted to carbon equivalents (Mg C ha^{-1} yr^{-1}), they then can be compared to default carbon sequestration rates for tidal wetland marshes. This value, 1.46 Mg C ha^{-1} yr^{-1} is accepted as the default rate at which marshes sequester carbon (Emmer et al. 2015). Actual emissions from the *S. alterniflora* stratum are equivalent to 0.67 Mg C ha^{-1} yr^{-1}, while the predicted value is 0.29 Mg C ha^{-1} yr^{-1}. When compared to the default rate of carbon sequestration, methane emissions from this stratum offset 45.9% of their sequestration benefits, compared to only 19.9% when using its predicted value. The actual *S. patens* carbon equivalent was 0.07 Mg C ha^{-1} yr^{-1}, which offsets only 4.8% of the carbon sequestration of this stratum and the predicted value is actually an overestimation of its emissions, 0.37 Mg C ha^{-1} yr^{-1} which would account for 25.3% of this stratums emissions. The High *J. roemerianus* stratum emissions, when converted to carbon equivalents, was 0.22 Mg C ha^{-1} yr^{-1}, versus a predicted value of 0.27 Mg C ha^{-1} yr^{-1}, these would account for 15.1% (actual) or 18.5% (predicted) of this stratums emissions, respectively. The Low *J. roemerianus* stratum emissions, when converted to carbon equivalents, was 0.16 Mg C

ha^{-1} yr^{-1}, versus a predicted value of 0.27 Mg C ha^{-1} yr^{-1}, which would account for 11%

(actual) or 18.5% (predicted) of this stratums emissions. These percentages show the

importance of accurate accounting for methane emissions, as two of our strata, *S.*

alterniflora and *S. patens* have substantial differences between their predicted and actual

offsets. *S. alterniflora* underestimates its carbon offset by 26%, and *S. patens* overestimates

its carbon offset by 20.5%.
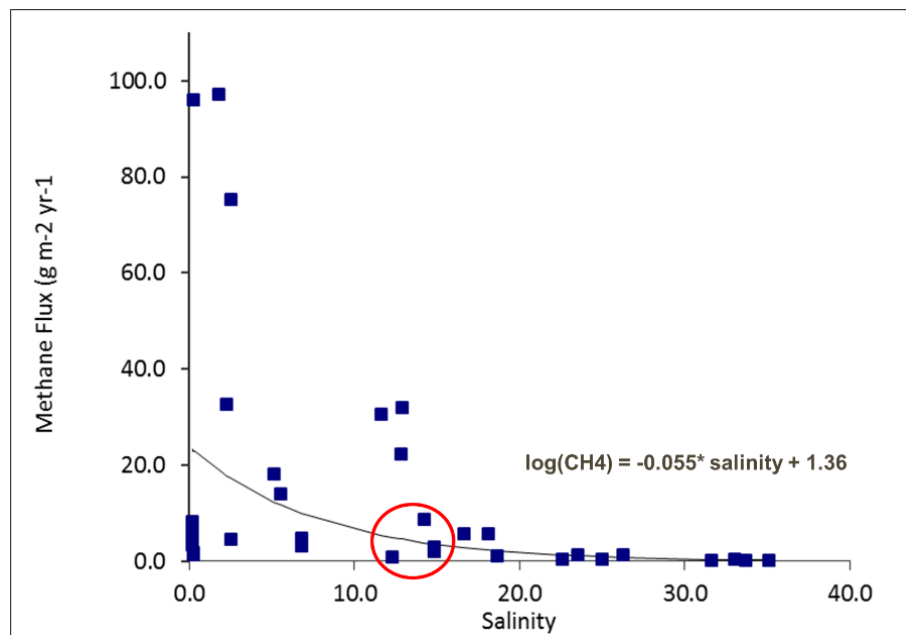
Figure 25 Tidal marsh methane emissions versus salinity reproduced from data from Poffenbarger et. al. (2011). Data from this study circled in red. Within this circle, the single value above the curve is the Low S. alterniflora stratum, the lower value is the S. patens stratum, and the two values below the curve to the right side are both J. roemerianus strata. This graph omits data points with emissions greater than 100 g CH4 m−2 yr−1 for clarity

Our research indicated that the percent of sulfate remaining after reduction seemed to be a

better indicator of methane flux than salinity or sulfate concentration alone. The strata with

the highest mean sulfate remaining, *S. patens* had the lowest mean emissions over the entire

sampling period. The opposite was true with *S. alterniflora*, which showed the least amount

of sulfate remaining after reduction, which indicated that sulfate reduction was occurring

in this stratum, along with the highest rates of methane emission. Measuring sulfate, in addition to salinity, could be an efficient means to improve the accuracy of methane emission estimates in tidal brackish marshes.

Within our strata, methane production did not follow conventional thinking in regards to the thermodynamic ladder. Methanogenesis is the least energetically favorable of the reduction reactions, except for hydrogen gas production, and therefore is expected to occur only when all other electron acceptors are fully (or nearly) depleted. This chain has been well studied, including in slurries of anoxic rice paddy soils (Achtnich et al. 1995). Our data show that peak active sulfate reduction was occurring concurrently with methane production in our marsh. *S. alterniflora* had the highest mean pore water hydrogen sulfide concentrations, while having the lowest sulfate concentrations. This indicates active sulfate reduction occurring in the soil profile of the stratum, which was also indicated by the low percent of sulfate remaining; however, this stratum also has the highest observed methane emissions in our study site.

This co-production of methane and hydrogen sulfide goes against our third hypothesis, that methane production would be lower when sulfate and hydrogen sulfide concentrations were high. There are two possible mechanisms that could be responsible for this co-production of methane and hydrogen sulfide: excess labile carbon availability, and microsite differentiation of methane producing and sulfate-reduction zones. An excess of labile carbon could reduce competition between sulfate-reducing bacteria and methanogens and allow for co-existence between these two communities. Previous research in bioreactors has indicated that mutualistic relationships can develop between iron and sulfate reducers, in turn narrowing the gap of energetic favorability between iron

reduction, sulfate depletion, and methanogenesis (Bethke et al. 2011). Additional research into the microbial communities associated with different plant communities could shed light on the complex relationship between methanogens, sulfate-reducing bacteria, and the plants that grow alongside them.

Microsites, or small areas of highly reducing conditions within the soil profile, could allow for methanogenesis to occur in areas where sulfate reduction is not occurring. Microsites have been shown to produce small amounts of methane in upland forested systems, originally thought to be too dry and too aerobic to produce this greenhouse gas (Megonigal and Guenther 2008). Small areas within the soils in tidal brackish marshes may also have microsite areas wherein the rate at which electron acceptors with higher energetically favorable reactions are depleted faster than they can be replenished (Poffenbarger et al. 2011). Other research had defined microsites differently, describing them as micro-landscape positions within a landscape, such as hummocks and lower lawn areas within fens and peatlands (Kettunen 2003; Galand et al. 2003). A more clear definition that distinguished between smaller within-soil microsites, and larger landscape microsites (which are similar to our strata) would be beneficial to furthering research on this topic.

The thermodynamic ladder of reduction reactions has been assumed to hold true across field and lab environments; however, our data shows that it may not be as simple a relationship as it is normally shown. If this ladder is not accurate at the site-specific level that we have sampled, then reevaluation of its applicability may be necessary before it is used in generating methane emission estimates in tidal brackish marshes.

Chapter 3: Factors Effecting Methane Emissions in Tidal Brackish Marshes

Introduction

With a global warming potential 28 times greater than carbon dioxide (Pachauri et al. 2014), methane is a greenhouse gas with worldwide implications. Methane is produced by both natural and anthropogenic sources, with wetlands being the largest single natural source of methane entering the atmosphere. While wetlands can sequester large amounts of carbon in their soils (Chmura et al. 2003), the production of methane in these ecosystems can potentially offset those carbon storages. The unique conditions of tidal brackish marshes can be ideal for methane generation. The high amounts of organic matter present in brackish marsh soils, as well as anaerobic conditions from flooding create conditions favorable for methanogenesis. However, previous research indicates that increasing salinity can suppress methane production in these systems (Poffenbarger et al. 2011), and once salinity reaches 18 ppt, methane production becomes consistently low. High variability of methane emissions in lower salinity (<18 ppt) marshes has been observed; the major factors that control these fluctuations, such as hydrology, salinity, microbial communities, plant communities, and labile soil organic matter availability are not well understood. These factors contribute not only to the production of methane in the soil, but also to its transport to the atmosphere, either through plant transport (via aerenchyma tissue), passive diffusion, and ebullition (methane bubbles). During passive diffusion, methane can be oxidized (methanotrophy) by microorganisms both aerobically and anaerobically (Valentine and Reeburgh 2000).

Accurate estimation of methane emissions is important, both for global greenhouse gas accounting, and carbon financing. In the field of tidal marsh restoration and conservation,

new carbon financing opportunities may help offset the costs of preserving and restoring these important ecosystems (Crooks et al. 2011; Emmer et al. 2015). In order for carbon credits to be issued for a particular project with a site salinity less than the 18 ppt cut-off, current methodologies require either its methane emissions to be estimated via direct measurement of a system, which is generally outside the ability of a project, in scope, cost and time; published data on a similar system (which rarely exists); or through published models (i.e. proxies), of which none currently exist at a resolution that would be suitable or appropriate for a restoration project (i.e. a project level model).

In order for methane models to be used for carbon financing in a tidal brackish marsh system, they must be validated for that system, or one similar. This requires the model to be validated with published field data from a site with similar conditions, such as vegetation, salinity and climate. This validation must also be published with peer-review. No current models are available at this time that fulfill these requirements.

Due to the complex nature of methanogenesis in marshes and wetlands, their large spatial area globally, and the importance of methane as a greenhouse gas, attempts have been made to quantify their methane emissions using models. Large-scale "top-down" models use available knowledge and emissions of methane generation in marsh systems, and apply those rates across large areas. While they are useful for worldwide accounting, issues plague their accuracy, and not all of these models are in agreement with one another, partly due to the lack of full understanding of methane emissions at the site-specific, or higher resolution level (Melton et al. 2013). On the opposite end of top-down models are process-based models, which generally are mechanistic, sampling intensive, estimations of methane production and consumption under either controlled or selected field conditions,

and may test individual factors of methane generation. Neither these top-down, nor smaller process-based models are useful for project level modeling. While significant research has been previously done by others in regards to how improvements in methane emission models could be accomplished (Bridgham et al. 2013), a study specific to project-level modeling is necessary.

The objective of this chapter is to analyze the factors necessary for a successful methane emissions model that could be utilized in a tidal brackish marsh system, in order to provide cost effective emission estimation for project level greenhouse gas accounting. We will briefly discuss how methane is produced and moves through a brackish tidal marsh system. Then we will review the factors of methane generation which could be used in modeling.

Methane Production, Consumption and Transport in Tidal Brackish Marshes

Methane Production

Methane (CH_4) is produced in the anaerobic zones of a soil profile. Its production, called methanogenesis, is undertaken by methanogens, both archaea and bacteria, and is the least energetically favorable of the reactions undertaken in the reducing zones of a soil profile. The process of methanogenesis is described in thorough details by others (Megonigal et al. 2005); however, a review of the basic production of this greenhouse gas will aid in the understanding of the factors that will be necessary in order to produce an accurate model of the process in a tidal brackish marsh.

The microorganisms which create methane generate this gas as a byproduct of the breakdown of organic material. In tidal brackish marshes, aboveground biomass is produced by plants living in the system. When these plants produce roots, or die, the carbon present in their living biomass does not oxidize readily, due to the flooded nature of the

soil. Instead, microorganisms that have adapted to these low oxygen conditions consume the organic carbon in increasingly less efficient reactions, ending with methanogenesis.

The two primary pathways by which methane is produced are known as hydrogenotrophic methanogenesis (or CO_2/H_2 reduction) and acetate fermentation. Each use different compounds as the energy source (electron donor) and electron sink (electron acceptor) to generate methane. In CO_2/H_2 reduction, carbon dioxide provides the carbon, as well as acting as an electron sink, while the hydrogen provides the energy necessary for the reaction to occur, and is generally the limiting agent. The general reaction of this pathway is:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$
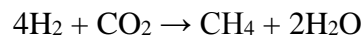
Acetate fermentation is unique and less common than CO_2/H_2 reduction, in that one reactant (acetate) provides *both* the electron source, and the electron sink for methanogenesis. The microorganisms responsible follow this reaction:

$$CH_3COOH \rightarrow CO_2 + CH_4$$
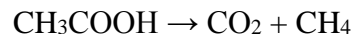
Other pathways exist for the production of methane; however, the above account for the majority of methane production in anaerobic environments, such as those found in tidal brackish marshes.

Methane Consumption

Methane consumption, or methanotrophy, occurs when microorganisms consume methane as an energy source. It can occur both in the aerobic zone of the soil profile, or anaerobically. The aerobic consumption occurs both on the edge of the aerobic and anaerobic zone and in the rhizosphere of wetland plants as they transport oxygen to their roots. This barrier to the release of methane to the atmosphere can oxidize 20-40% of

methane before it diffuses into the atmosphere (Whalen 2005). Some methane can be also be consumed by anaerobic methane oxidation, or "reverse methanogenesis". This process is not well understood; however, it involves a consortium relationship with sulfate reducing bacteria (Valentine and Reeburgh 2000; Megonigal et al. 2005).

Methane Transport to the Atmosphere

Once methane is produced in the anaerobic zone, and makes it past the methanotrophic microorganisms, it then moves into the atmosphere. The three major ways in which methane reaches the atmosphere are diffusion, ebullition, and plant-mediated transport. Diffusion is a passive process in which methane slowly percolates through both the soil and any water present on its surface from areas of higher concentration to lower (Bazhin 2010). Ebullition, or "methane bubbles," occur when methane builds up in a subsurface environment, until it reaches a critical point and releases itself uncontrollably, either by physically breaking through the substrate, or by some external disturbance (such as a rising water level) which allows for the release (Whalen 2005). Plant transport of methane occurs as plants respire. Wetland plants require gas exchange to their roots, otherwise they would become waterlogged and die. As this gas is brought down through plant tissue to their roots via aerenchyma structures, methane enters and moves outward, bypassing the aerobic zone in the soil. Research has shown that certain plants can account for higher methane transport rates, due to different rates of photosynthesis, biomass (and therefore available carbon) production, and the efficiency of rhizome gas exchange (Whalen 2005).

Factors Which Could Be Used as Measurable Inputs for Methane Modeling

In order for a methane emissions model to be useful for carbon financing in tidal brackish marshes, it must be relatively easy to use. Additionally, a model must also be accurate, and

utilize the proper input factors in order to represent project specific conditions. Each factor a model uses should be easily measurable in the field, and be important to the production and emission of methane. The following sections discusses potential factors related to methanogenesis that could be measured by scientists in order to estimate its production in a tidal brackish marsh, and how that factor could affect methane production.

Vegetation

Tidal brackish marshes can have large variation in their vegetation communities. These plant species have evolved over time to withstand the constantly changing conditions present in a tidal brackish marsh system. Differing periods of inundation and drying, varying salinity, and generally wet conditions make plant life in this environment live literally on the edge. These changes within a marsh complex can lead to very different plant communities within a tidal brackish marsh. These species differences can have large impacts on the ability of that community to produce methane. For instance, recent research has shown that plant species changes due to anthropogenic disturbance can change availability of electron acceptors in wetland soils, impacting their ability to produce methane (Sutton-Grier and Megonigal 2011). In a rewetted brackish fen in northern Germany, vegetation type was the only variable that changed with regards to methane flux, suggesting some plant community control on methane emissions (Koebsch et al. 2013); however, little research has been done in natural tidal brackish marshes, as most studies do not separate plant communities within a single marsh complex.

Since methanogenesis is dependent on organic matter availability, plant biomass production is an important factor in methane modeling. As plants grow, they also die, and their biomass is added to the soil. However, plant biomass is not only what is seen above

the ground. Below ground production of roots, rhizomes and their secretions, or root exudates, provide a source of labile organic matter as well. This biomass is broken down in the soil by microorganisms through primary and secondary fermentation into forms utilized by methanogens, including acetate, H_2 and CO_2 (Bridgham et al. 2013). These more bioavailable forms of carbon can be utilized by other terminal electron acceptors as well; and in some cases, methanogens are outcompeted by them.

The measure of above and below ground biomass production can indicate the amount of organic matter available for methanogenesis, since it is the source of electrons for the process. Additionally, plant root exudates provide an additional source of labile organic matter which is easily accessible to soil microorganisms (Megonigal et al. 1999). Root exudates can also increase the turnover rate of recalcitrant sources of soil organic matter (de Graaff et al. 2010; Basiliko et al. 2012), making more available for methanogens. The differences in the rates of plant material litter breakdown has been studied previously both in the field and laboratory (Haines and Hanson 1979; Valiela et al. 1985; Windham 2001), and has determined that species such as *Spartina alterniflora* and *Spartina patens* decompose more readily than others such as *Juncus roemerianus* or *Phragmites austrailis.* However, rates at which this litter and detritus decompose to compounds useful for microorganisms such as methanogens is lacking; additional research in this area with regards to common wetland plant species would further the knowledge of this process.

In addition to the biomass provided by plant growth (both below and above the soil surface), the act of photosynthesis allows wetland plants to move oxygen through their aerenchyma tissues to their roots. This process oxygenates the roots, keeping them alive and respiring in the waterlogged soil of a tidal brackish marsh. This process also

oxygenates the area around the roots and rhizomes, forming a small barrier to methanogenesis, some methane can pass through these aerenchyma, and can be transported through to the atmosphere, bypassing any aerobic barrier in the soil profile above the anaerobic zone. This rate of gas flow could impact the rate at which methane is pumped into the atmosphere. Certain models (http://www.waquoitbayreserve.org/research-monitoring/salt-marsh-carbon-project/) have utilized the measurement of light (PAR) reaching the plants themselves as a proxy for photosynthesis, in order to avoid measuring the process itself.

Different plants also utilize different pathways for photosynthesis, the two main pathways are C3 and C4 photosynthesis. The primary difference between these two pathways is that C3 plants can only generate energy while their stomata are open during the day. C4 plants can continue generating energy at night, when their stomata are closed, and they are not actively respiring. While the majority of plants on Earth are C3 (including *J. roemerianus*), some important wetland species, such as *S. alterniflora*, and *S. patens*, utilize the C4 pathway. Research conducted at Sapelo Island, Georgia found that short-form *S. alterniflora* (the form found in our marsh) and *J. roemerianus* had differing rates of respiration and productivity, with *Juncus* being slightly higher (Giurgevich and Dunn 1982). The difference in the rates of respiration between these two pathways could potentially impact their methane emissions, however additional research into the impact these pathways have on trace gas fluxes could be addressed.

Hydrology

Hydrology is one of the most important determining factors that makes a wetland a wetland. Without high water levels, the conditions necessary to determine a wetland

(hydrophytic vegetation, hydric soils) would not exist. In a tidal brackish marsh, the water level is constantly changing, as the tide goes in (floods) and out (ebbs) over the course of the day, and as water enters the wetland from upland areas or precipitation. Additionally, increased plant respiration has also been found to lower the water table in tidal brackish marshes containing *S. alterniflora,* potentially increasing the aerobic zone of the soil profile (Dacey and Howes 1984). The water levels within a tidal brackish marsh can determine which plant communities occur, since some species are more likely to be found in the drier "high" marsh (such as *S. patens*) or the wetter "low" marsh (*S. alterniflora*).

As the marsh soil becomes increasingly waterlogged, most of the oxygen in the soil is quickly consumed, and the soil becomes anaerobic. The longer this condition occurs, the more anaerobic (or reduced) the soil becomes, as other microorganisms reduce organic matter, including iron reducers, and sulfate reducers. Methanogenesis occurs in soils that are highly reducing, since other forms of anaerobic respiration are more energetically favorable. The higher in the soil profile the water level occurs, the smaller the aerobic (or oxygen rich) zone of the soil profile remains. Our research showed that the three strata with higher water levels all had higher mean rates of methane emissions than the driest stratum, *S. patens.* In a tidal brackish marsh, this zone can fluctuate over time, as tides ebb and flood. It is important to note; however, that regardless of the water level in the soil profile, a small zone of aerobic conditions will exist. This aerobic zone can reduce methane emissions through aerobic methane oxidation, as well as removing the anaerobic conditions that methanogenesis requires.

In tidal brackish systems, the influx of saline water brings more than anaerobic conditions, dissolved within this water are minerals not found in large quantities in fresh water,

specifically salts, including chloride and sulfate. These compounds and their impacts to methanogenesis will be discussed in the following section.

Salinity, Sulfate and Hydrogen Sulfide

Tidal brackish floodwaters are a mixture of fresh and sea (salt) water. The salinity of this water varies depending on numerous factors; however, of the salinity present in this water, approximately 7.8% of the dissolved ions will be comprised of sulfate, since the current concentration of sulfate present in seawater is approximately 28mM (Canfield 2004). Previous research has indicated that increased salinity (and therefore increased sulfate concentration) can reduce methane emissions from wetlands (Poffenbarger et al. 2011).

Since sulfate reduction is a more energetically favorable reaction than methanogenesis, increased concentration of hydrogen sulfide would indicate that sulfate reduction is occurring in a tidal brackish marsh. Measurements of both sulfate and hydrogen sulfide concentration, in addition to salinity in the pore water of a tidal brackish marsh may allow for improved estimations of the amount of sulfate reduction occurring within the soil. In chapter 2, we discuss that this measurement may actually be a better indicator of potential limits to methanogenesis, as opposed to salinity.

Redox and pH

Methanogenesis is not an energetically favorable reaction, and requires strongly reducing conditions in order to be energetically worthwhile. The measurement of reduction-oxidation potential (redox, or Eh) can be done in the field with platinum electrodes. There are limitations to this technique, as the electrodes only measure redox at one, small point. Also, this technique can be highly variable, as shown in the appendix, and methanogenesis

can still occur in areas where according to reduction oxidation estimations, it should not be. Additionally, pH is necessary in order for this data to be useable.

Methanogens have been shown to prefer neutral pH conditions (Megonigal et al. 2005), however additional studies have shown their activity in acidic conditions below 5.6 (Walker et al. 1998; Garcia et al. 2000). In chapter 2, we found significant differences in pH by month and strata, but we do not consider these differences impactful on our methane emissions. For methane modeling, pH is a necessary measurement if recording Eh, and would be necessary otherwise only in areas with extreme pH's, since areas with extreme pH's may inhibit certain methanogens, or the processes involved in the formation of their sources of energy.

Temperature

Like all microbial processes, methanogenesis is affected by temperature. Observed Q_{10} rates vary from 1 to 25 (Whalen 2005), with other studies stating an average rate of 4.1 (Segers 1998), while others assume a rate of 2 is more typical (Megonigal et al. 2005). This wide range is thought to occur due to the underlying processes responsible for the depletion of alternate electron acceptors. Measuring soil temperatures would be beneficial for modeling, as higher temperatures should produce more methane emissions than cooler ones.

Soil Characteristics

In order for significant methanogenesis to occur, large amounts of labile carbon need to be present. Additionally, a lack of oxygen is necessary for anaerobic conditions to develop, since methanogenesis is an anaerobic process. Wetland soils are generally rich in organic matter, with a low bulk density, and are by nature, wet. These two conditions make these

soils ideal for methanogenesis. Therefore, soils with a lower bulk density (and therefore higher organic matter content), and a higher water content, should be more prone to methanogenesis. These types of conditions would not readily exist in most upland mineral soils; however, methanogenesis has been found to occur in upland forested soils during periods of higher water content in the soil (Megonigal and Guenther 2008). Soils with a higher bulk density also contain more mineral elements, such as iron. Iron reduction is an even more energetically favorable reaction than methanogenesis, so it would be presumed that soils with high iron content would have lower emission rates.

Microbial Communities

Field measurements of methanogen populations and the amounts present within a tidal brackish marsh would be a valuable source of information in methane modeling. In chapter two, anaerobic incubations indicated that either *S. alterniflora* contained more labile carbon than the other strata, or possibly had a more varied and active community of methanogens able to make use of the carbon available. One methane emissions model in development, the MEM-CH$_4$ model (the original, non-methane version of MEM is available online at: jellyfish.geol.sc.edu/model/marsh/mem.asp), uses anaerobic incubations to determine how microbial communities decompose organic matter within the soil and generate methane.

Nutrient Loading From External Sources

Increased nutrient availability can increase plant productivity, yet it is unknown if this increase can impact the rate of methane flux through plants. However, certain nutrients, such as nitrate, which is used in fertilizer, are alternate electron acceptors and are energetically favorable to iron reduction, sulfate reduction and methanogenesis. It is well known that nitrate runoff from agricultural operations into coastal waters, and tidal

wetlands is a source of excess nutrients. Nitrate is reduced to ammonium, and research into whether this process affects methane emissions could determine if its measurement would be necessary for methane modeling.

Conclusions

The potential for modeling methane emissions in tidal brackish marshes exist, and some models are beginning to be released for use, or are currently in development. However, the varying factors that can impact the process of methanogenesis require that any model generated for site-level methane emissions take into account the unique features of that site, as broader generalizations which are appropriate for larger scale estimates may not work at models with a higher resolution.

Chapter 4: Final Conclusions and Future Research Needs

Our research has shown the complexity of methane emissions from tidal brackish marshes. While these ecosystems sequester carbon as one of their benefits, the unique conditions present in their soils can allow for substantial emissions of greenhouse gases, which can offset the benefits of carbon sequestration.

We hypothesized that the different vegetative and hydrologic communities within this system would have different methane emissions rates, and we confirmed this hypothesis. We also hypothesized that the stratum with the lowest elevation, and therefore highest water level (in our case *S. alterniflora*) would have the highest methane emissions. While this stratum did have the highest methane emissions of the four strata tested, it did not have the highest mean water levels. Conversely, the opposite of this hypothesis was validated, as the high elevation *S. patens* stratum had the lowest water levels, and the lowest mean emissions of all four strata, although it was not significantly different from the low elevation *Juncus* stratum. Finally, we hypothesized that strata with high levels of salinity, sulfate and hydrogen sulfide concentrations (and therefore greater indicators of sulfate reduction) would have the least amount of methane emissions. We found the opposite to be true at our site. *S. patens* had the lowest mean salinity, while all other strata were not significantly different. *S. patens* also had the lowest mean methane emissions, and the highest sulfate concentrations observed during the study. Our results show that methane emissions and sulfate reduction are co-occurring in some certain hydrologic/vegetative communities and not in others. The intricacies of this process require further explanation in order to fully understand its mechanisms.

Future carbon crediting and greenhouse gas accounting will need to take the methane emission differences of different vegetative and hydrologic communities into consideration when estimating net carbon flux to the atmosphere. If default methane emissions values (such as those used for marshes about 18 ppt) were derived and then used for an entire marsh complex, it may not be sufficient enough to account for the differences between different communities within a marsh. Current methodologies can take these different communities into account (Emmer et al. 2015) for tidal marsh restoration; however, differences in each marsh complex may make default values difficult to establish without a better understanding of the process within each community. Unfortunately, modeling of methane emissions from tidal brackish marshes with different communities may be extremely difficult if the unique characteristics of each marsh do not follow easily predictable trends. More research of this type is needed to determine if these differences in communities are found in similar systems, as well as others in different climates, in order to validate this finding.

Additional work that could assist in this endeavor is vast, and covers a broad range of potential topics, limited only by time and funding. In order to get a complete site-level estimate of emissions, installation of an eddy-covariance tower in our marsh complex would assist us in gathering nearly continuous emission data, across the entire year, as well as capturing unpredictable ebullition events. If done concurrently with chamber sampling, a true picture of both point and site wide measurements could be compared to get a much better picture of the carbon cycling of this site to the atmosphere, especially if chamber measurements were taken during both the daytime, and nighttime to capture any diurnal

variations in flux rates, and during the winter, when we assumed minimal methane production and release.

Additional pore water depths could be sampled throughout the soil profile, in order to determine if there was a decrease in belowground methane production as depth increased, as found in Megonigal and Schlesinger (2002). Additional depths would be especially helpful in the *S. patens* stratum, as the water level was often just above the depth sampled, and methanogenesis may possibly have been taking place in earnest lower in the profile, but being consumed before release. Reduced iron concentrations over the course of the growing season may confirm that iron reduction is suppressing both methanogenesis and sulfate reduction in this stratum.

We did not complete a full analysis of the soil present in each of these strata. Information such as bulk density, organic matter percentages, and iron content may have shed some insight into the differences we observed in both the pore water and methane fluxes. Iron content is especially important, given how high the reduced iron concentrations observed in the *S. patens* stratum were in July. If the other strata have far less available iron, it is likely that iron reduction is not a factor to consider in the majority of our marsh, as the *J. roemerianus* and *S. alterniflora* occupy much more area than *S. patens* in our marsh complex.

A research need we have identified within the literature is the types of labile organic carbon generated by different plant species in the form of root exudates. Our data show that there is a difference between emission rates in different plant communities; however, our data does not include the different types of carbon that these species are providing to the methanogens in the soil, although our anaerobic incubation data suggests that differences

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may be occurring. If, for example, *S. alterniflora* generates a form of labile carbon more accessible to methanogens, this may explain the higher rates we observed. Additionally, analyses of the microbial communities themselves could be performed between strata. It is possible that our highest emitting stratum *S. alterniflora* has a higher proportion of methanogens present in its microbial community; and all other strata lack such a community, there could be fewer emissions from them simply because the communities are not as well established.

Finally, additional incubations could answer the questions our initial results have proposed. It is obvious that soil from *S. alterniflora* is producing more methane than any other, and the remaining strata had far less methane production. Attempts to either suppress methanogenesis in the *S. alterniflora* by adding sulfate or iron, or bolster methanogenesis in the *Juncus* or *S. patens* by adding additional labile carbon, could verify that either sulfate competition is not important in *S. alterniflora* for the former, or that methanogenic communities are not present in the latter two species types. Additional destructive sampling after incubations are complete could compare the loss of carbon from each soil on a dry weight basis as well.

Our study has generated some interesting answers, along with some very interesting questions. In order to further our understanding of this complex process, additional experiments in tidal brackish marshes could lead to increased understanding of the contributions of these ecosystems to the global greenhouse gas budget, and open site-level restoration projects to carbon crediting opportunities in the future.

Additional Field Analysis, Methods and Results

These field analyses were collected during the field season, however they were not used in the analysis of in Chapter 2. Their methods are below.

Creek pH and Salinity

An additional pH and salinity measurement was taken in the middle of the tidal creek water column adjacent to each site during each field visit using a calibrated combination pH/conductivity probe, in the approximate middle of the water column.

Ammonium and Phosophate Analysis Methods

Ammonium was analyzed according to EPA method 350.1 and phosphate was analyzed according to EPA method 365.1 (www.epa.gov).

July Redox Profile and Indicator of Reduction in Soils Tube Deployment

To complement the pore water hydrogen sulfide data in July, additional hydrogen sulfide were assessed visually using Indicator of Reduction in Soil (IRIS) tubes (Rabenhorst et al. 2010; Rabenhorst et al. 2013). A redox profile was collected at each stratum using platinum electrodes, and Eh was recorded with a laboratory voltmeter, with a calomel (+244mV) reference. We collected redox measurements at 10, 20 and 30 cm depth. IRIS (Rabenhorst et al. 2010, Rabenhorst et al. 2013) tubes were installed at each stratum to get a visual estimation of sulfide distribution in the soil profile. IRIS tubes were installed on the same day as redox electrodes, removed 5 minutes after installation, cleaned with creek water to remove any attached sediment, and photographed in the field.

July Pore Water Measurements

During the July sampling, we collected pore water at 20 cm depth in addition to 10 cm. Also, individual pore water samples were analyzed for ammonium and phosphate rather than analyzing composited samples; all samples were also analyzed for ferrous iron (Fe^{2+}).
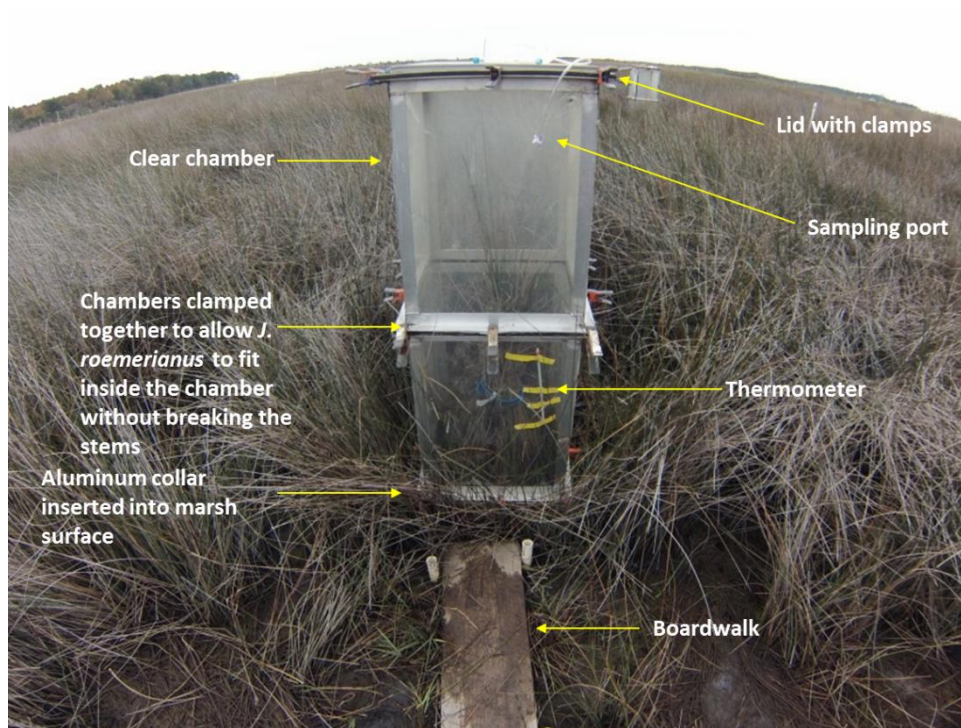
Statistical Analysis Code Example (SAS)

Below is an example of the statistical code used to analyze collected variables. Code was the same between variables, only the variable tested differed.
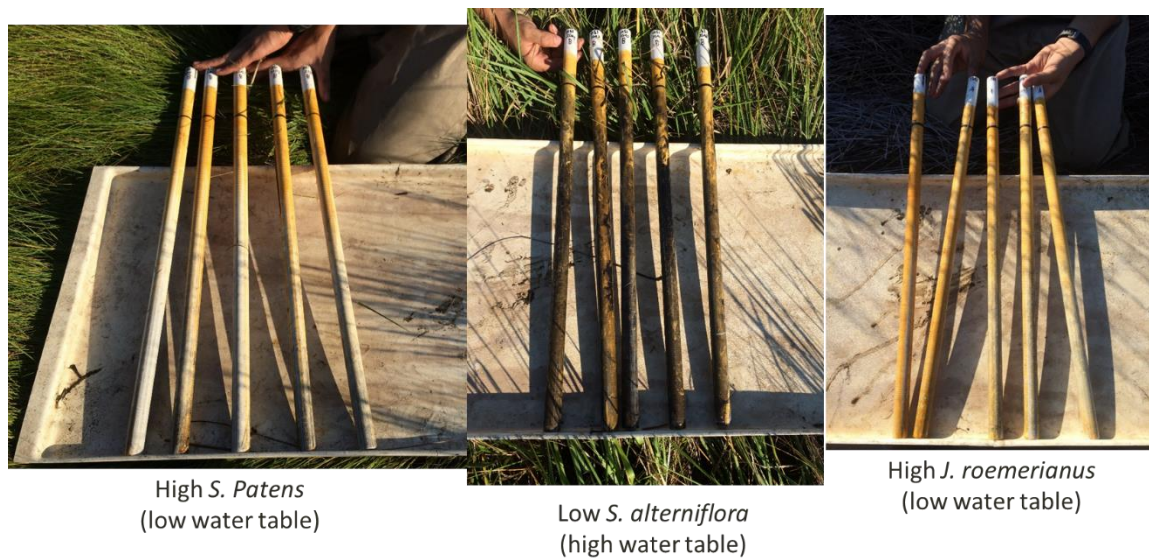
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Proc Mixed Data=methane;
Class site month; Model logflux=site|month;
REPEATED site / SUBJECT=plot TYPE=cs r rcorr;
lsmeans month site/pdiff;
lsmeans month site/adjust=tukey;
        *site 3jr 3sa 3sp 4jr;
estimate '3jr vs 4jr' site 1 0 0 -1;
estimate '3sa vs 3sp' site 0 1 -1 0;
estimate '3jr vs 3sp' site 1 0 -1 0;
estimate '3jr vs 3sa' site 1 -1 0 0;
estimate '4jr vs 3sp' site 0 0 1 -1;
estimate '4jr vs 3sa' site 0 1 0 -1;
run;
```
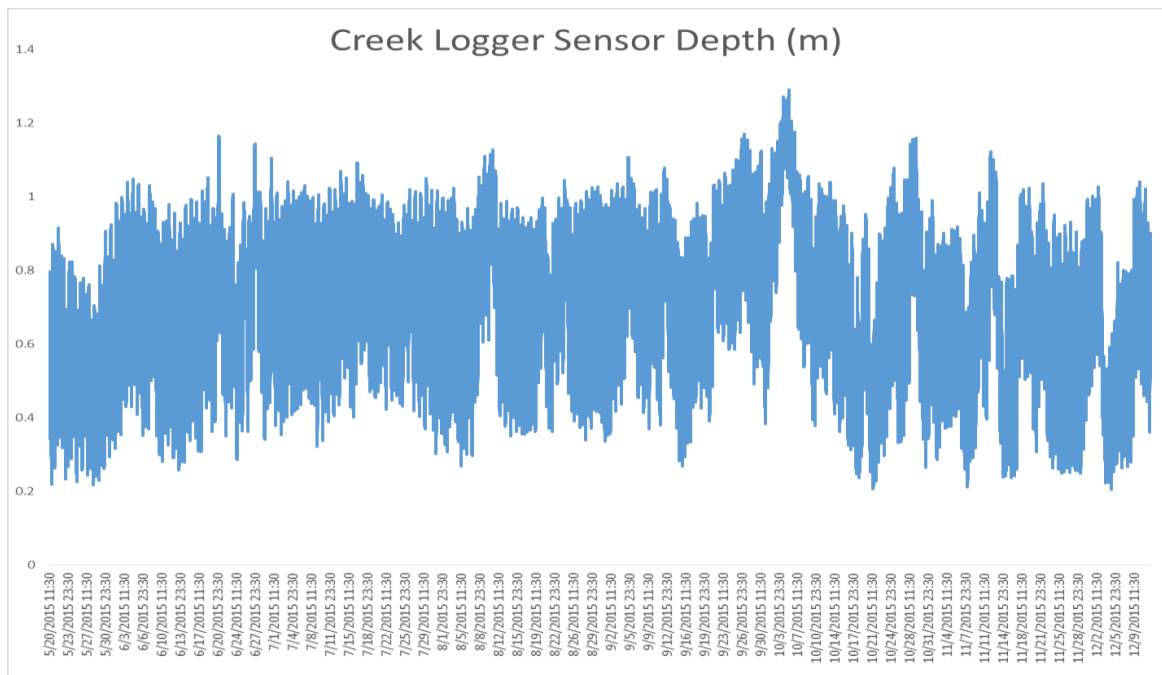
Additional Figures
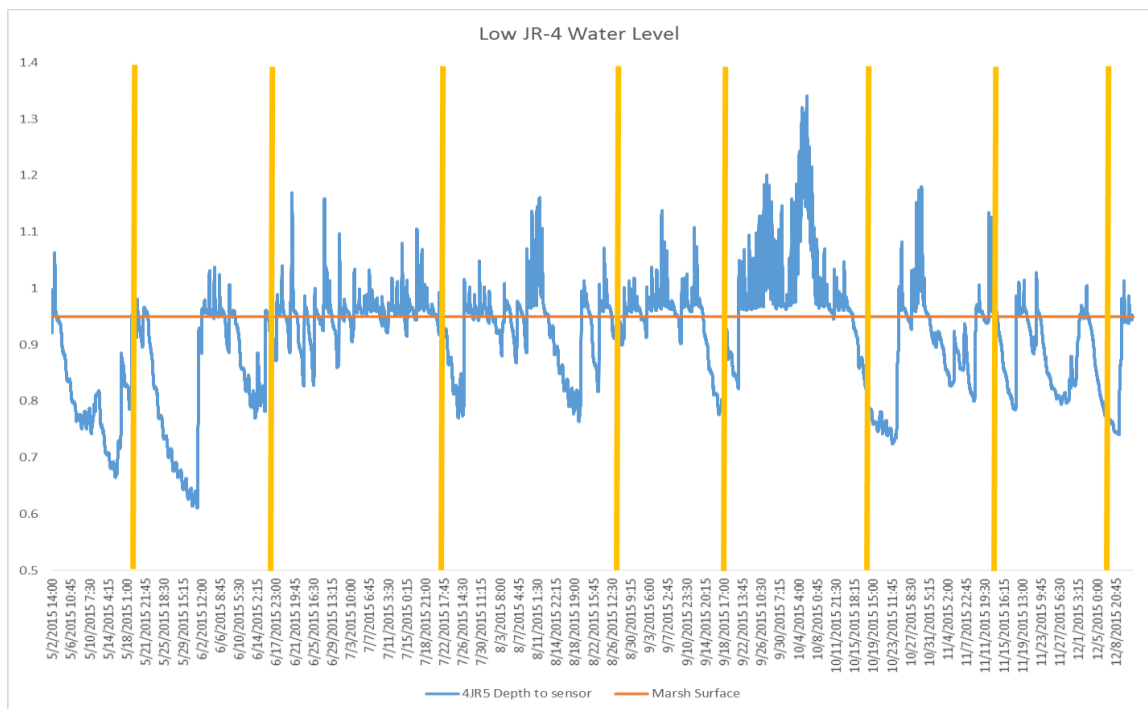
Methane Flux Chamber

IRIS Tubes, 5 Minute Exposure

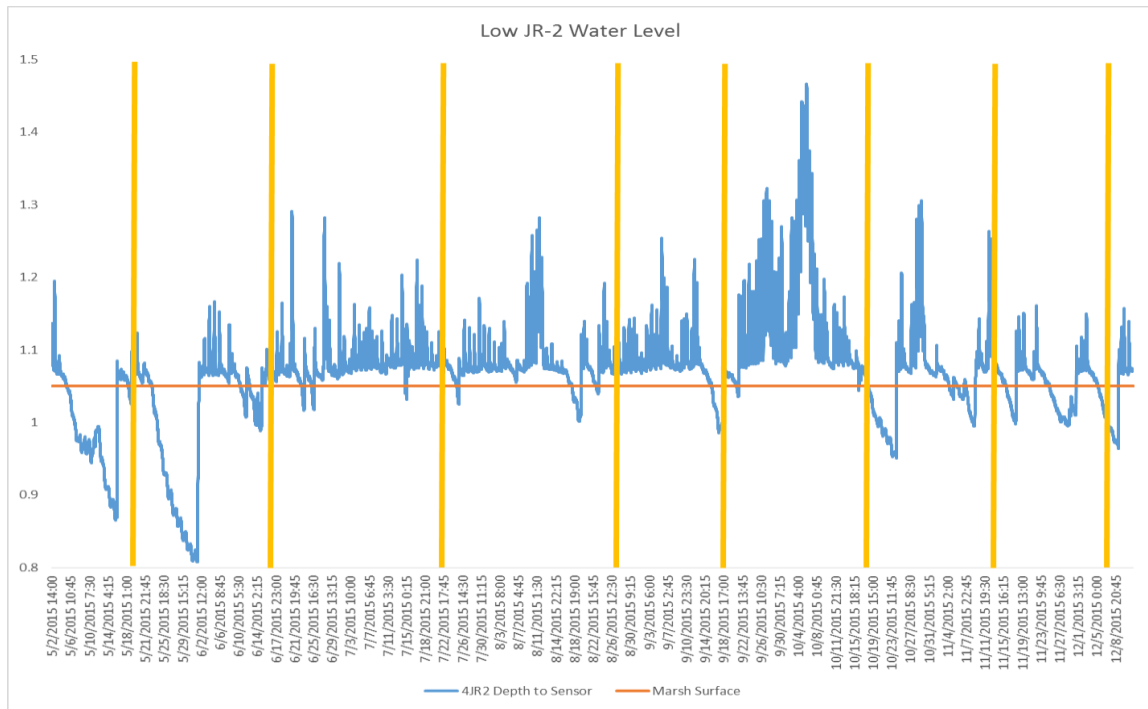High *S. Patens*
(low water table)
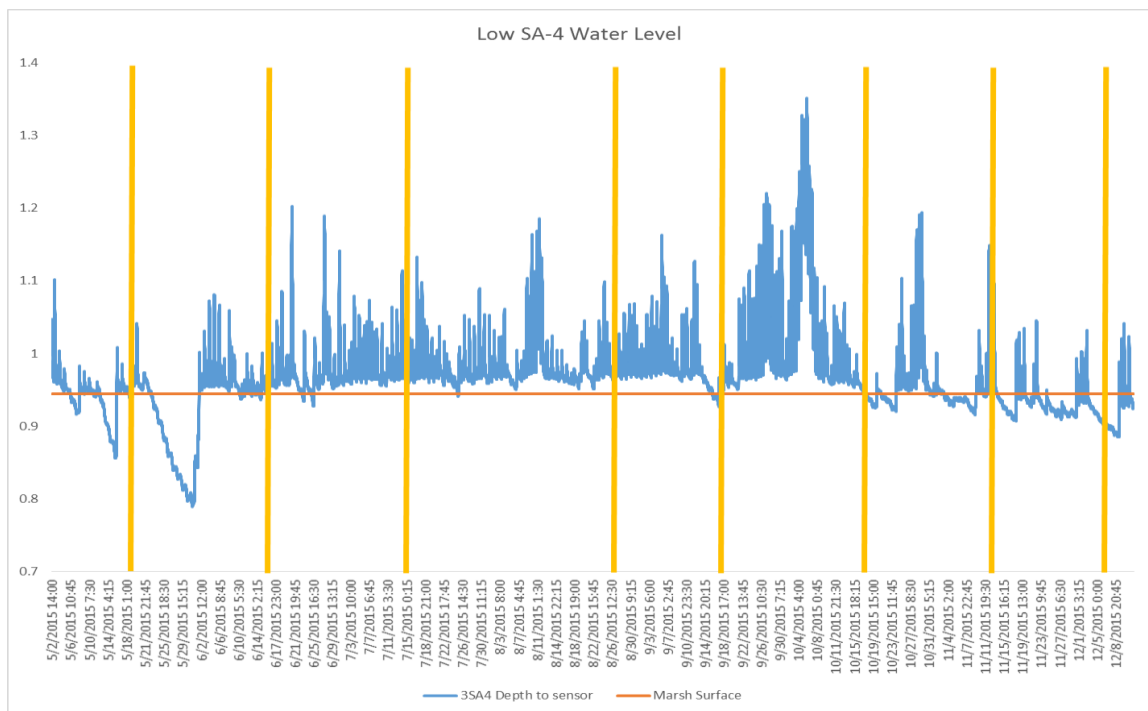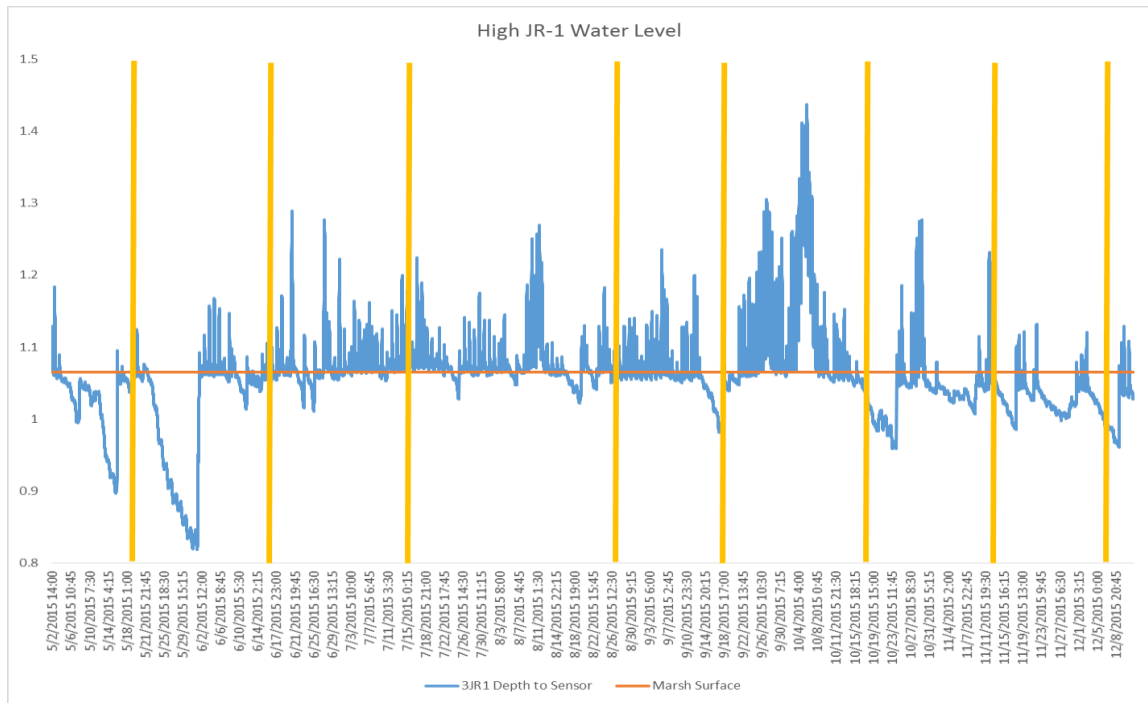
Low *S. alterniflora*
(high water table)

High *J. roemerianus*
(low water table)

Redox Profile: July 2015
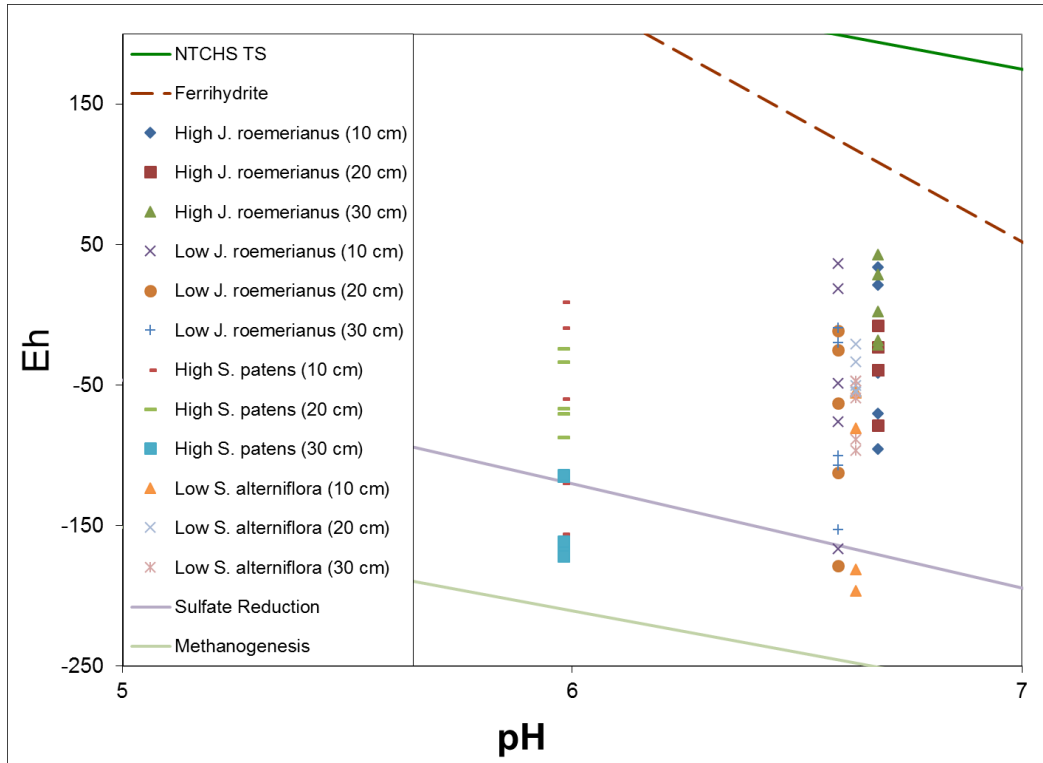
Yearly Water Level Graphs

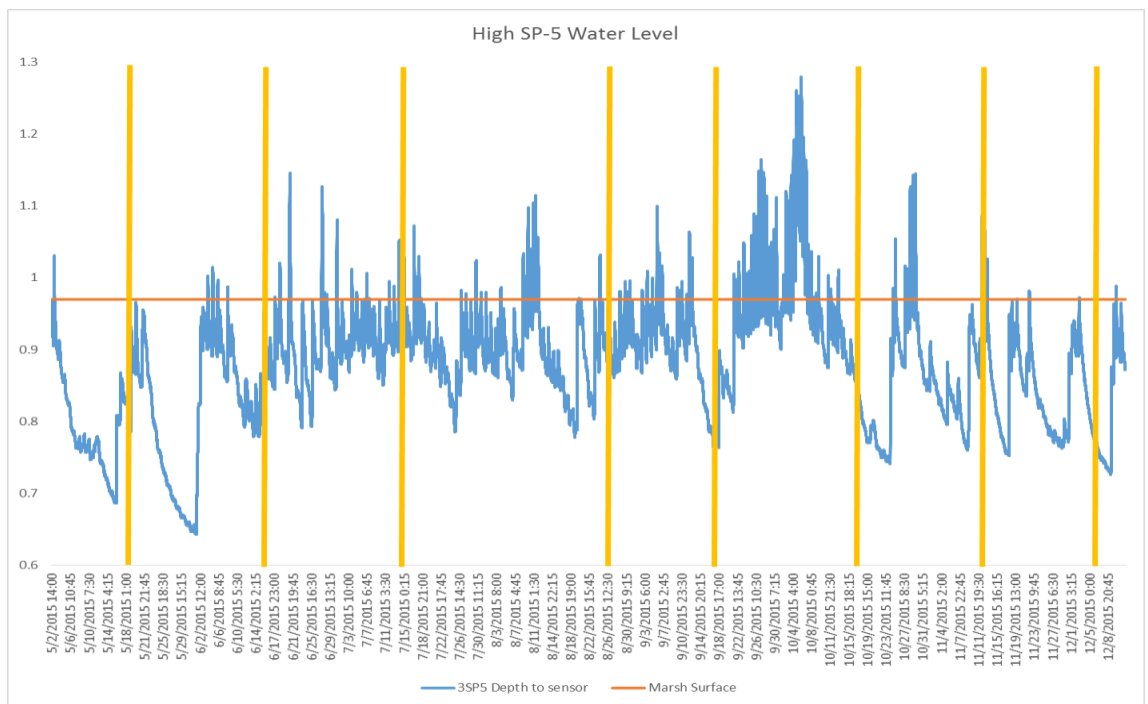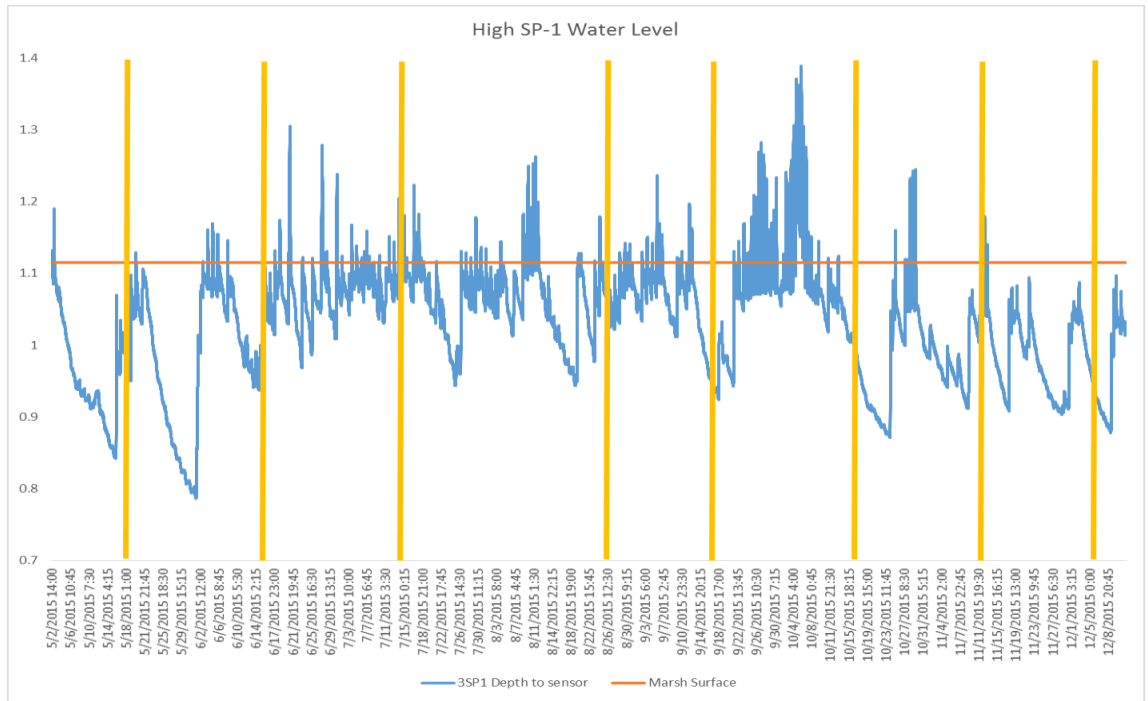Low JR-2 Water Level

Low JR-4 Water Level
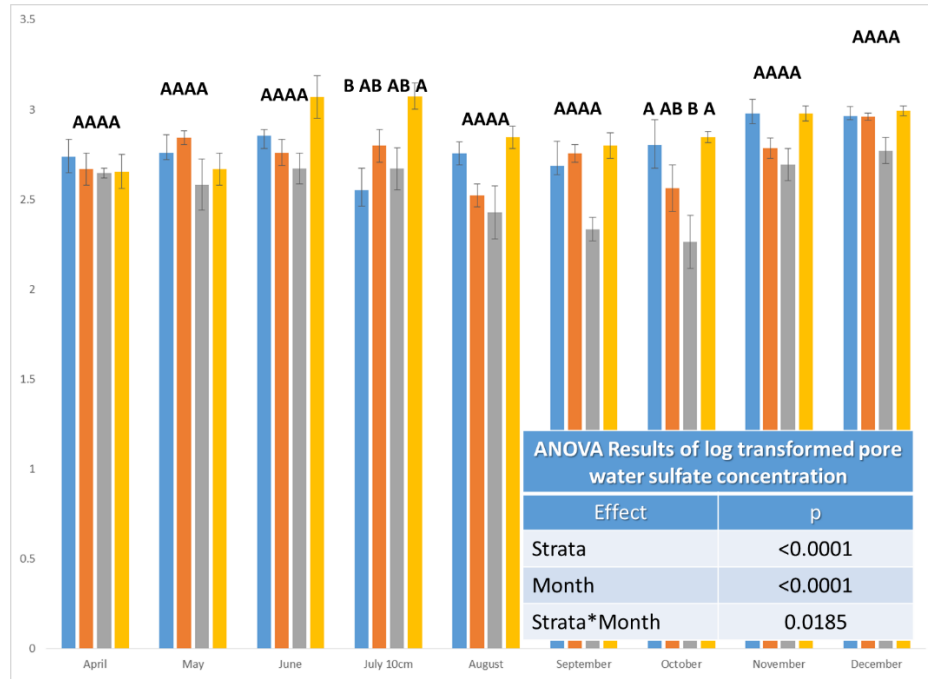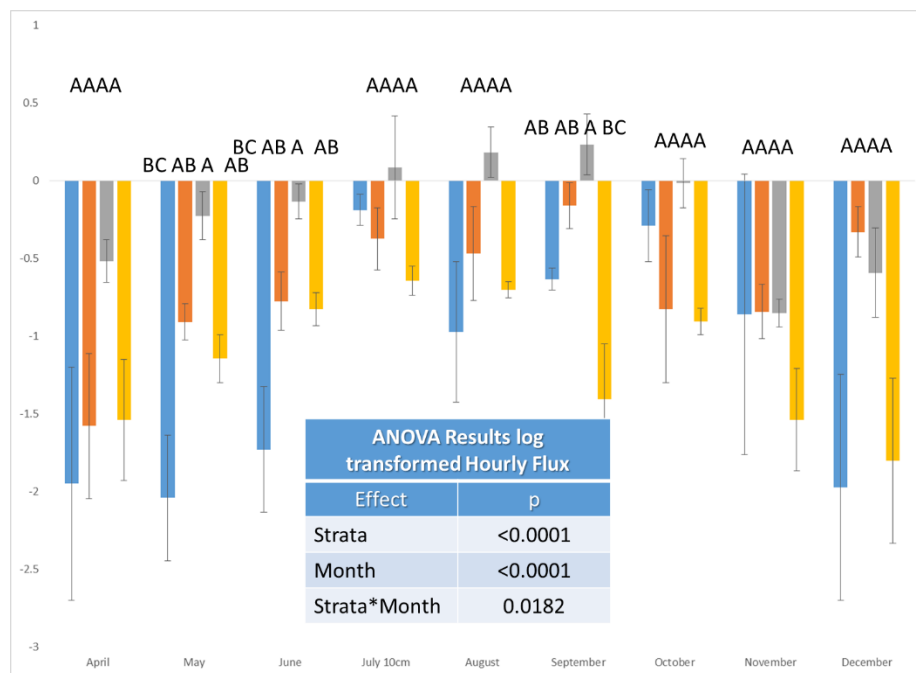
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High SP-1 Water Level

High SP-5 Water Level

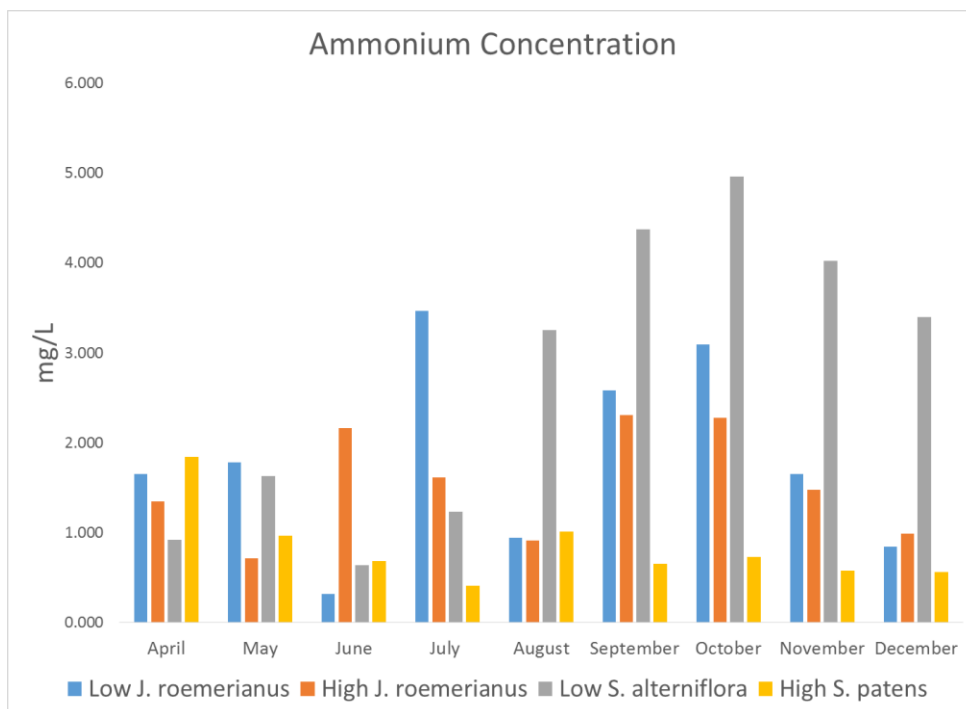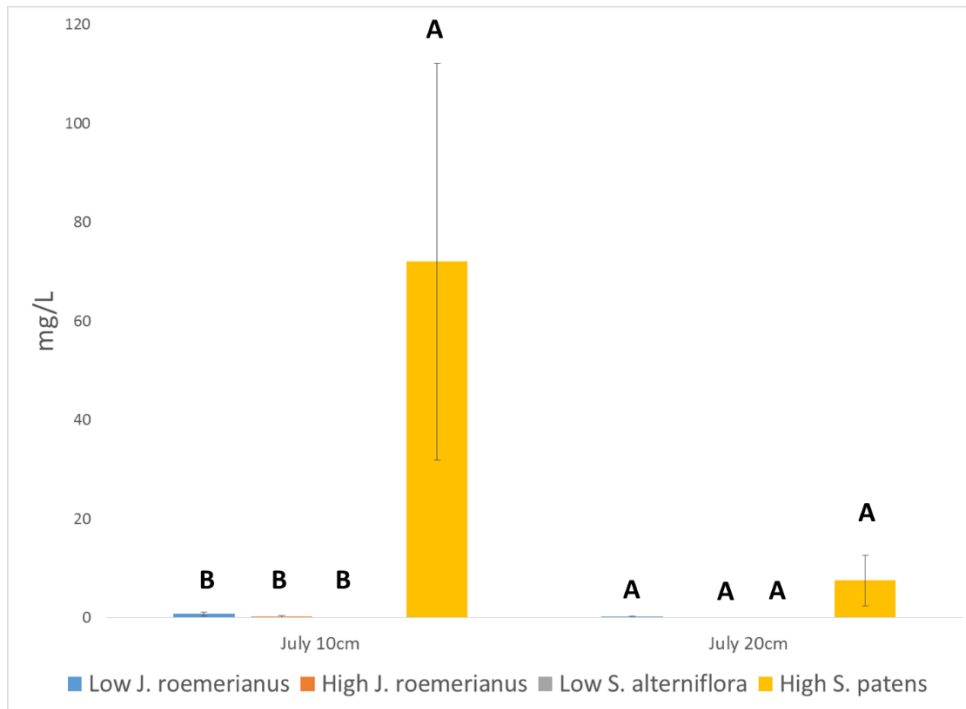Additional Flux and Pore Water Histograms

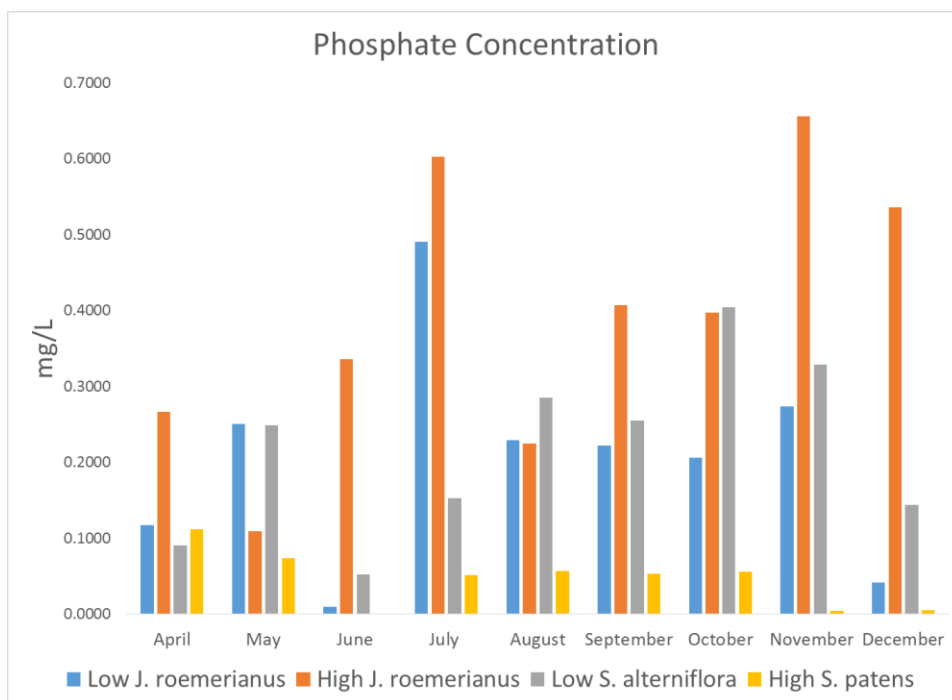Within-month pore water sulfate comparisons (log transformed values plotted)
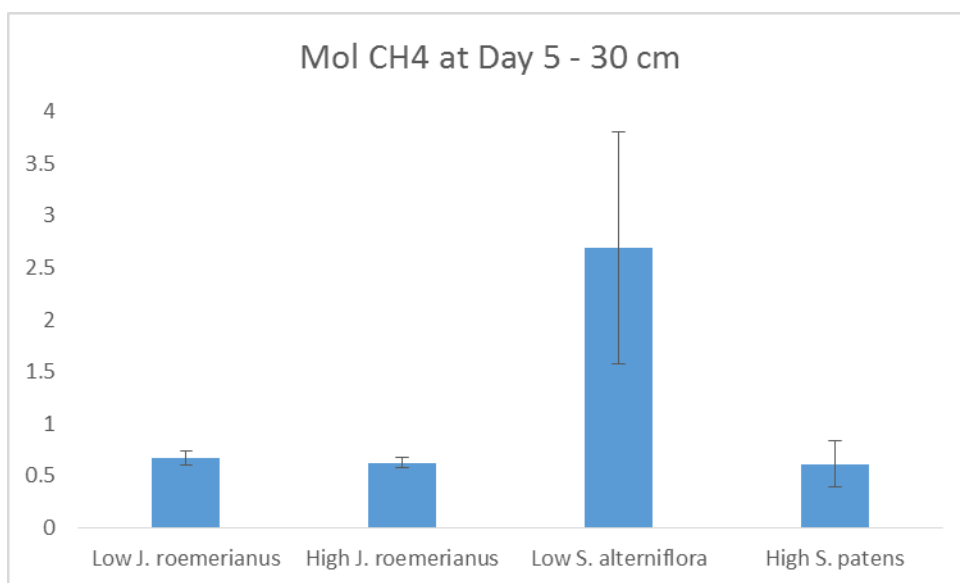

Within-month methane flux comparisons (log transformed values plotted)

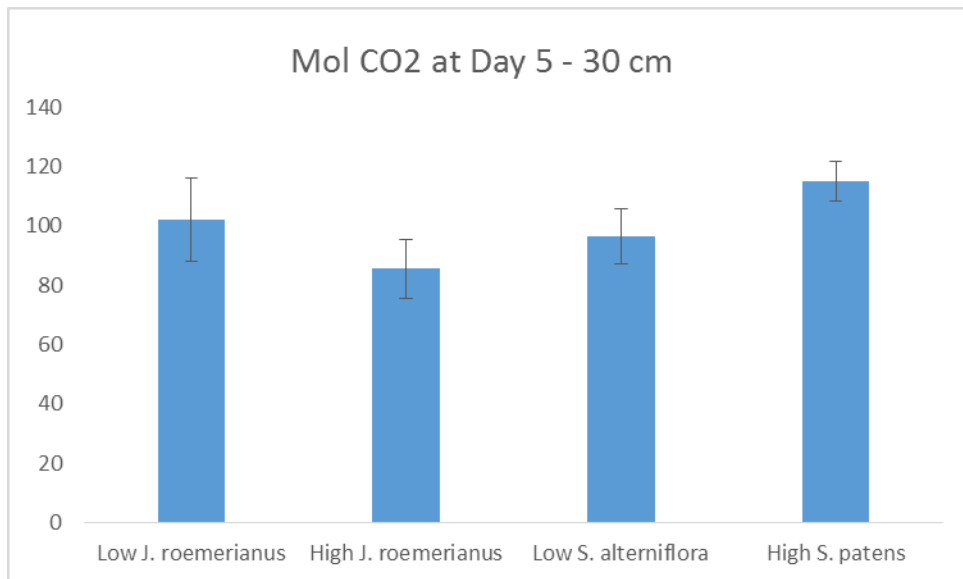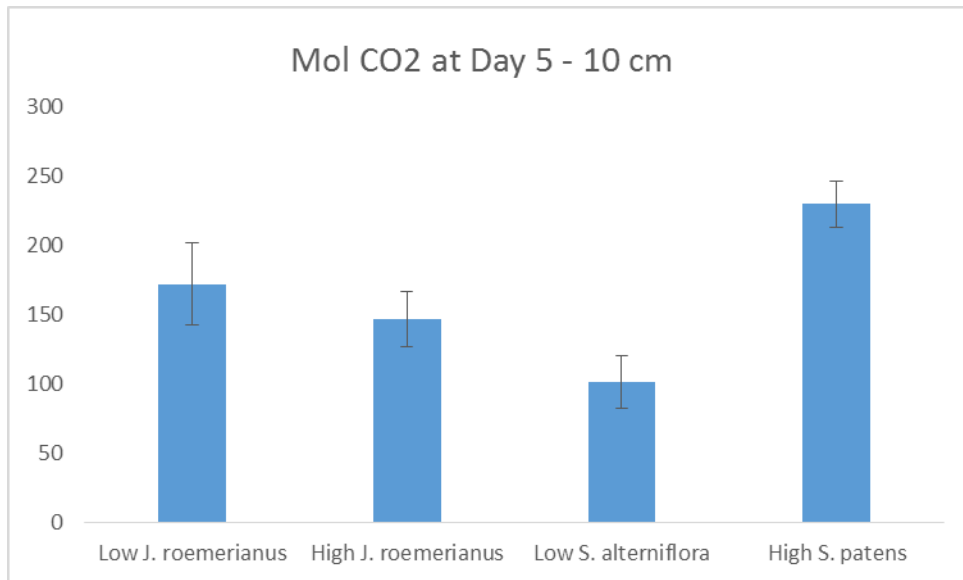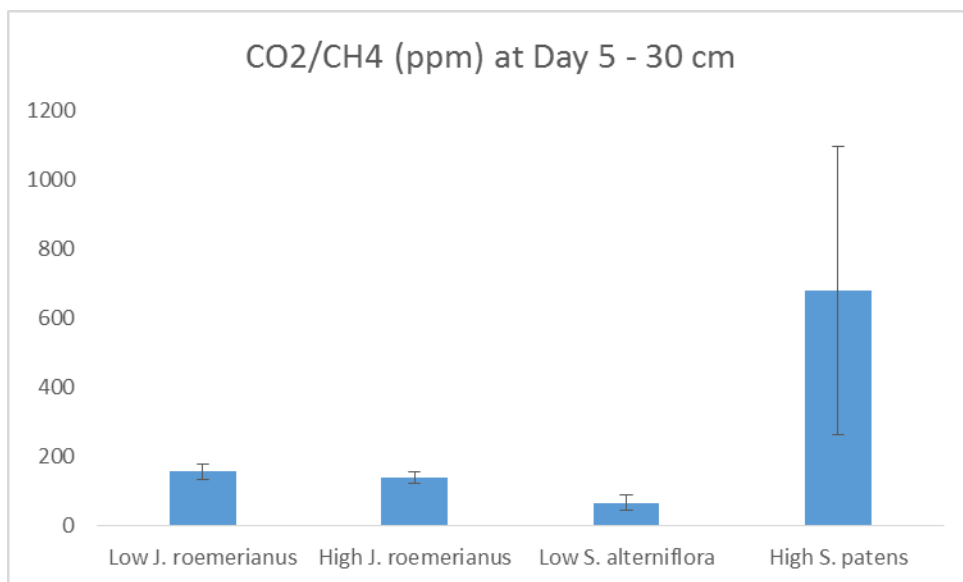Reduced Iron, July 2015, 10 and 20 cm

Phosphate Concentration

Additional Incubation Histograms

Mol CH4 at Day 5 - 30 cm

Mol CO2 at Day 5 - 10 cm

Mol CO2 at Day 5 - 30 cm

CO2/CH4 (ppm) at Day 5 - 10 cm

CO2/CH4 (ppm) at Day 5 - 30 cm
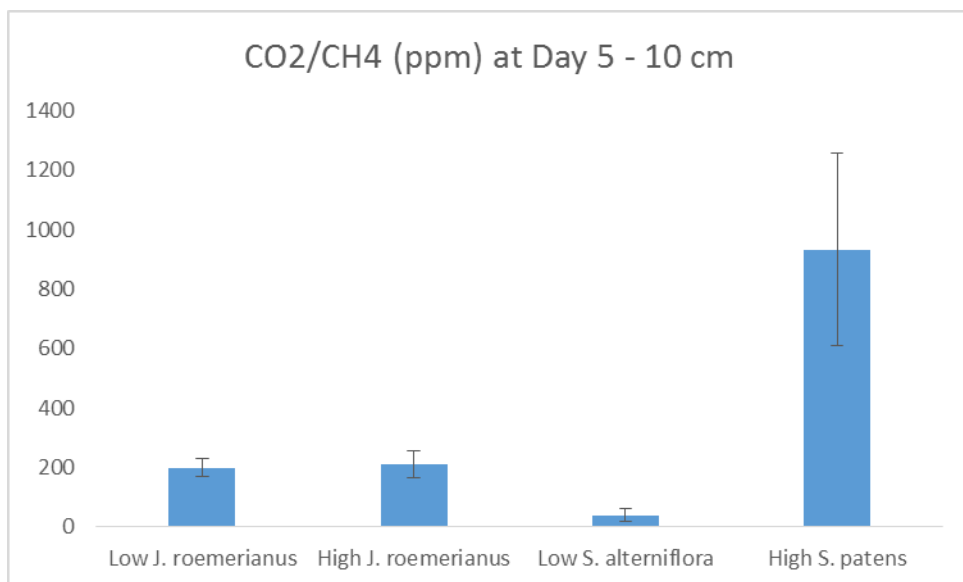
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