Stratifying by Vegetation and Hydrology Improves Tidal Marsh Methane Emission Accounting

Robert Kyle Derby
University of Maryland at College Park

Brian A Needelman (✉ bneed@umd.edu)
University of Maryland at College Park  https://orcid.org/0000-0002-9202-6755

Ana A Roden
University of Wisconsin-Madison

J. Patrick Megonigal
Smithsonian Environmental Research Center

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Abstract

Methane emissions must be directly measured or estimated using methods such as proxies when managing wetlands for greenhouse gas offset activities. Salinity is a useful proxy for tidal marsh CH$_4$ emissions when comparing across a wide range of salinity regimes but does not adequately explain variation in brackish and freshwater regimes where variation in emissions is large. We sought to improve upon the salinity proxy in a marsh complex on Deal Island Peninsula, Maryland, USA by identifying four strata based on hydrology and plant community composition. Mean CH$_4$ chamber-collected emissions measured as mg CH$_4$ m$^{-2}$ hr$^{-1}$ ranked as $S$. alterniora $(1.2 \pm 0.3) >$ High-elevation $J$. roemerianus $(0.4 \pm 0.06) >$ Low-elevation $J$. roemerianus $(0.3 \pm 0.07) = S$. patens $(0.1 \pm 0.01)$. Sulfate depletion generally reflected the same pattern with significantly greater in the $S$. alterniora stratum $(61 \pm 4\%)$ than in the $S$. patens stratum $(1 \pm 9\%)$ with the $J$. roemerianus strata falling in between. We attribute the high CH$_4$ emissions in the $S$. alterniora stratum to sulfate depletion likely driven by limited connectivity to tidal waters. Low CH$_4$ emissions in the $S$. patens stratum are attributed to lower water levels, higher levels of ferric iron, and shallow rooting depth. Moderate CH$_4$ emissions from the $J$. roemerianus strata were likely due to plant traits that favor CH$_4$ oxidation over CH$_4$ production. We concluded that stratification by hydrology and plant community composition can be an effective proxy to estimate CH$_4$ emissions at the site scale.

Introduction

Methane is a potent greenhouse gas produced under the dominantly anaerobic conditions found in wetland soils. The global warming potential of methane (CH$_4$) gas is 32-45 times greater than an equivalent amount of carbon dioxide (CO$_2$) over a 100-year period (Neubauer and Megonigal 2015). While the majority of CH$_4$ emissions come from anthropogenic sources, wetlands produce most of the naturally emitted CH$_4$ (Wang et al. 1996; Solomon et al. 2007) and are the most important source of uncertainty in current global CH$_4$ budgets (Saunois et al. 2020). Coastal wetland CH$_4$ emissions were recently estimated at 5.3-6.2 Tg CH$_4$ yr$^{-1}$, amounting to 60% of the global marine CH$_4$ budget (Al-Haj and Fulweiler 2020), < 7% of global wetland CH$_4$ budget (Saunois et al. 2020), and the largest source of uncertainty in the coastal wetland greenhouse gas budget (Holmquist et al. 2018). There is emerging interest in using tidal marsh restoration and conservation to mitigate greenhouse gases in the atmosphere and as a source of carbon credits (Crooks et al. 2011; Emmer et al. 2015, Needelman et al. 2018, Emmer et al. 2020a, Emmer et al. 2020b), but the high carbon sequestration rates characteristic of tidal wetlands ecosystems with freshwater-to-brackish salinity <18 ppt (Poffenbarger et al. 2011). The sources of this variability remain elusive as there has been relatively little research designed to partition variation. A better understanding of the factors that regulate coastal wetland CH$_4$ emissions is needed to improve global CH$_4$ budgets and to support the implementation of carbon credit methodologies in freshwater and brackish coastal wetlands.

Methane is produced in wetlands by methanogenic archaea and bacteria. The production of CH$_4$ occurs when there is an excess of electron donors over electron acceptors, depleting the availability of alternative electron acceptors such as ferric iron (Fe(III)) and sulfate (SO$_4^{2-}$) (Megonigal et al. 2004). Electron donors are produced from labile organic materials that undergo fermentation to low molecular weight carbon compounds and H$_2$. Electron donors can be present in the soil (e.g. Fe(III)), supplied from external sources such as floodwater (e.g. SO$_4^{2-}$), or provided by plants (e.g. molecular oxygen or oxidized compounds generated by radial oxygen loss). The availability of SO$_4^{2-}$...
from seawater suppresses CH$_4$ emissions from polyhaline (salinity > 18 ppt) marshes to consistently low rates (0.2 to 5.7 g CH$_4$ m$^{-2}$ yr$^{-1}$) (Poffenbarger et al. 2011). Methane emissions from mesohaline brackish systems (5-18 ppt salinity) are greater and more variable (3.3 to 32.0 g CH$_4$ m$^{-2}$ yr$^{-1}$). The process is also regulated by such physiochemical factors as pH (Walker et al. 1998; Garcia et al. 2000) and temperature (Megonigal and Schlesinger 2002; Whalen 2005).

Plant species composition affects CH$_4$ emissions through several mechanisms (Koebisch et al. 2013; Moor et al. 2017; Mueller et al. 2020). The availability of electron donors is largely determined by primary productivity which varies with species composition (Megonigal et al. 2004). Species composition also regulates electron acceptor availability through rhizosphere processes such as root oxygen loss (Calhoun and King, 1997; Colmer, 2003; Jespersen et al. 1998) and rhizosphere regeneration of ferric iron (Neubauer et al, 2005; Sutton-Grier and Megonigal, 2011). Methane can be transported to the atmosphere via aerenchyma tissue, bypassing the emission barriers caused by slow CH$_4$ diffusion rates through soils and soil-surface CH$_4$ oxidation zones (Ding et al. 2005; Le Mer and Roger, 2001; Sorrell et al. 2013; Villa et al. 2020). This is important because CH$_4$ oxidation can consume 70% or more of the CH$_4$ produced in tidal wetland soils (Megonigal and Schlesinger 2002).

In tidal marshes, water table position and periods of soil inundation are controlled by hydrologic factors such as soil hydraulic conductivity, distance from open water, and soil surface elevation relative sea surface elevation. Elevation zonation subdivides tidal marshes into low marsh areas that are frequently inundated by the tides, and high marsh areas that are infrequently influenced by the tides. Water table depth influences soil oxygen availability (Epp and Chanton, 1993; Gilbert and Frenzel, 1995), and hence the potential for aerobic processes such as CH$_4$ oxidation (Grünfield and Brix, 1999; Megonigal and Schlesinger 2002). Soils in low marsh areas that are permanently or frequently inundated experience low rates of O$_2$ diffusion and sustain anaerobic environments where methanogenesis can occur (Ding et al. 2010). Tidal wetland studies have documented correlations between elevation, water level, and CH$_4$ emissions (Altor and Mitsch, 2006; Audet et al. 2013; Ding et al, 2010; Grünfield and Brix, 1999), implicating hydroperiod as a dominant influence on wetland CH$_4$ emissions.

Both plant community composition and water table depth have proven to be effective proxies for predicting CH$_4$ emissions in wetland ecosystems such as peatlands (Audet et al. 2013; Bubier et al. 1995; Couwenberg et al. 2001; Dias et al. 2010). Wetland plant species exhibit different tolerances to inundation (Sorrell et al. 2000; Vann and Megonigal, 2003), leading to varying plant community composition across elevation gradients (Perry and Hershner 1999). Wetland vegetation is well suited for serving as a proxy to predict CH$_4$ fluxes due to direct and indirect influences of plant species on labile soil organic carbon (i.e. root exudates), soil moisture, and CH$_4$ gas transport via plant aerenchyma tissue (Couwenberg et al. 2001). Previous studies have established direct links between plant species composition and CH$_4$ fluxes (Audet et al. 2013; Bhullar et al. 2014; Shäefer et al. 2011) and have used plant species composition to accurately predict CH$_4$ fluxes from peatlands (Couwenberg et al. 2001; Dias et al. 2010). Water table depth, as influenced by relative elevation, has also proven to be a good proxy for predicting CH$_4$ emissions in peatlands as water table level determines aerobic/anaerobic zones and redox states in the soil profile (Ding et al. 2010).

The objective of this study was to advance our understanding of the effects of water level and plant species composition on CH$_4$ emissions in brackish marshes at a site scale. We measured CH$_4$ fluxes in two brackish marshes on the Deal Island Peninsula on the Eastern Shore of Maryland, USA across four different strata defined by water level and plant community composition (Needelman et al. 2018). We collected field data on elevation, water
level, soil temperature, and soil pore water $\text{SO}_4^{2-}$, sulfides, pH and salinity, and laboratory soil incubations using field-collected soil cores to assess potential CH$_4$ production.

**Methods**

**Study Area**

Our study area was located on the Deal Island Peninsula in Somerset County, Maryland, USA (38.185172N, 75.906279W) (Fig. 1). It consisted of two brackish tidal marshes, one unditched (Unditched) and one that had been ditched then restored (Ditched) located in the same marsh complex (referred to as Unditched-2 and Ditched-2 in Needelman et al. 2015). Ditch plugs were installed at the Ditched site in April of 2014 by inserting a plastic polyethylene sheet vertically across the ditch approximately 50 m upstream from the tidal source and securing the plug using sediment sourced from the ditch upstream of the plug. The Ditched site had an overall lower elevation than the unditched site, and was primarily composed of *Juncus roemerianus* (black needlerush). The Unditched site had a more diverse species community including *J. roemerianus*, *Spartina patens* (salt marsh hay), *Spartina alterniora* (smooth cordgrass), *Phragmites australis* (common reed), and *Iva frutescens* (marsh elder). Plant productivity 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 July through August. Soils on site consist of thick moderately to highly decomposed organic horizons overlying loamy mineral horizons; within Soil Taxonomy they classify as the Mispillion series, Loamy, mixed, euic, mesic, Terric Sulphhemists, which are common estuarine marsh soil in this area. This microtidal marsh had a diurnal tidal range of approximately 0.6 meters as measured in the adjacent tidal creek.

**Design**

Four strata that differed in their plant community composition and elevation, both of which are closely associated with water levels, were identified prior to the study from onsite observations and overhead satellite imagery. The strata corresponded to geographic units that may be used to estimate CH$_4$ emissions when engaged in site-specific carbon crediting accounting methodologies (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 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 the unditched site and had a mean elevation of 0.334 m relative to NAVD88, while the Low *J. roemerianus* stratum was located at the ditched site with a mean elevation 0.305 m. The two additional strata consisted of one dominated by *S. alterniora* at a relatively low mean elevation of 0.299 m, and one dominated by *S. patens* at a relatively high mean elevation of 0.409 m. Both the Low *S. alterniora* and High *S. patens* strata were located at the Unditched site. A representative area was selected within each stratum that included a range of elevation and plant diversity. Five sampling plots were randomly established within the representative area in each stratum, for a total of 20 plots. It should be noted that our flux measurements covered a small inference space, since they were only in representative areas of each strata and not randomly distributed across the entire marsh. Three of the strata were located within 25-50m of a tidal creek, while one (*S. alterniora*) was located in a more central location in the marsh complex at approximately 100 m from a tidal creek. The 20 plots occupied an approximate area of 0.06 km$^2$, with each 5-plot strata encompassing an approximate area of 1,000 m$^2$.

**Field Methods**
We sampled monthly from April to December 2015; samples were not taken from January until March under the assumption that CH$_4$ production would be negligible due to low temperatures (Marsh et al. 2005). Methane flux, air temperature, and pore water concentrations of pH, SO$_4^{2-}$, hydrogen sulfide, and CH$_4$ 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 sensors (Onset Corp., Bourne, MA). Soil temperature and water level data were not collected during the month of April because loggers were not ready for deployment until May.

Each of the 20 sample plots received a custom-fabricated square aluminum metal collar that was permanently inserted into the marsh to a depth of 10 cm nine months prior to the first sample. Flux chambers were constructed of an aluminum frame made of 2.5-cm wide angle stock covered with transparent polycarbonate plastic film. Chambers were placed on top of the collar about 10 minutes 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). The taller plants in the $J$. roemerianus strata were accommodated without damaging plant stems by stacking chambers. 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 0.17 m$^3$ for single chambers and 0.34 m$^3$ for double chambers. In order to prevent the weight of the observer from causing ebullition due to soil compression (Sorrell et al. 2013), each plot had a 3 m wooden boardwalk suspended above the soil surface by PVC legs for approaching the flux collar.

Methane flux samples were collected over a 1-hr period from the 5 replicate flux plots in a given strata. An initial sample was taken immediately after each chamber was sealed with four subsequent samples taken at approximately 15-min intervals for a total of 25 samples (5 per plot) over the 1-hr period. Using a 30 mL syringe, the sampling port was opened and then expelled back into the chamber three to five times before each sample was taken. Each 18 mL air sample was withdrawn from the chamber and injected into a N$_2$-flushed 12-mL Exetainer vial with rubber septum until analysis (Yu et al. 2013). Air temperature within the sampling chamber was recorded upon the collection of each flux sample from thermometers affixed to the interior of each chamber.

Porewater samples were taken at 10 cm depth using a porewater sipper and syringe (Fisher et al. 2013) and analyzed for pore water CH$_4$, hydrogen sulfide (unfiltered), pH (unfiltered), salinity (unfiltered), and SO$_4^{2-}$ (filtered through a 0.45-µm filter) as described by Keller et al. 2009. Porewater CH$_4$ was collected by withdrawing 15 mL of pore water, after which 15 mL of ambient air was drawn into the syringe and the syringe capped. The sample was then agitated for 1-2 minutes for the CH$_4$ to be stripped into the drawn air, the stripped water was expelled, and the gas sample was stored in N$_2$-flushed Exetainers for analysis (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 (Koch et al. 1990). Hydrogen sulfide and pH samples were analyzed the same day as sample collection; salinity was analyzed within two weeks in the laboratory using a YSI Model 3100 conductivity meter; and 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 a peak CH$_4$ emission period. We collected porewater at 20 cm depth in addition to 10 cm and analyzed it for the same analytes excluding CH$_4$ but including ferrous iron (Fe$^{2+}$).

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; one was also installed 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 Networking Global Positioning System (RTN GPS) unit, which provides elevation data with approximately 2 cm accuracy (http://www.keynetgps.com).

Soil cores were collected during the July sampling event and analyzed for potential anaerobic CH$_4$ and CO$_2$ production. Cores were collected from approximately 0-40 cm depth using a circular metal gouge corer. The corer was inserted into the marsh, with careful attention paid to minimize compaction of the soft peat. The core was removed and cut at a depth of 20 cm, yielding two depth increments per plot. Cores were placed into sample bags 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, stored on ice during transport, and placed in a 4 °C cold room until processing. Water for these incubations was collected from the bore hole from which the core was removed, stored on ice for transport back to the lab, purged with nitrogen gas to remove oxygen before being sealed and placed in a cold room at 4 °C. Soil cores and water samples were stored in the cold room within 8 hours of their collection and incubated within 5 days.

**Laboratory Analyses**

Flux chamber headspace samples were measured on a Varian 450 gas chromatograph using a Combi-Pal autosampler and corrected for dilution of 18 ml of sample into 12 ml of N$_2$ in the Exetainer. Flux rate was calculated as the linear increase in headspace [CH$_4$] over time based on measurements of chamber temperature and volume and assuming atmospheric pressure (n=147 fluxes). The linear slope was calculated in Excel using the Regression function. Data points were excluded from the regression if they indicated an ebullition event (large spike in [CH$_4$]) or an Exetainer leak (large drop in [CH$_4$]). Most fluxes were calculated from five points, but never from fewer than three points. No flux measurements were excluded based on arbitrary regression R$^2$ or p-value limits but fluxes were excluded in several cases where an ebullition event or leak was large compared to the CH$_4$ flux rate rate (n=17). In cases where there was no significant trend in headspace [CH$_4$] and no evidence of ebullition or leaks the flux was assigned a value of zero. Because most of the excluded fluxes were collected during periods of low CH$_4$ flux they had relatively little influence on the annual flux calculation.

To estimate annual emissions averaged rates from each measurement campaign. We assumed that the fluxes in the unsampled months of January, February, and March were equal to our observed values from April. The twelve monthly values were averaged and converted to annual units. While this method likely overestimated CH$_4$ emissions, overestimation is the conservative and therefore preferable approach for carbon credit accounting (Needelman et al. 2018).

Hydrogen sulfide was determined with a Lazar Laboratory model 146S sulfide electrode. Sulfide antioxidant buffer was prepared the day before sample collection with deoxygenated (N$_2$-stripped) distilled water, sodium salicylate, sodium hydroxide, and ascorbic acid according to Koch et al. (1990). A standard curve created from a serial dilution of a Na$_2$S/buffer solution prepared on the day of each sampling event and readings were complete within 4 hours of sample collection. The pH was measured with a YSI Pro Plus (https://www.ysi.com) pH meter calibrated with standards at pH 7.0 and 10.0. Salinity was measured with a calibrated conductivity/salinity electrode. The remaining analytes SO$_4^{2-}$ and Fe$^{2+}$ were quantified at the Chesapeake Biological Laboratory, Solomons Island, MD. Reduced
iron was analyzed according to EPA method 200.1 and SO$_4^{2-}$ was analyzed according to the National Environmental Methods Index Standard Methods: 4110B for ions in water by ion chromatography (www.nemi.gov).

Sulfate depletion was calculated by assuming the SO$_4^{2-}$ concentration in the absence of sulfate reduction was that of full-strength marine seawater (Canfield 2004) diluted to the observed salinity of our sample. We then divided our observed SO$_4^{2-}$ concentration by this expected concentration of SO$_4^{2-}$ to estimate the consumption of SO$_4^{2-}$ (i.e. depletion) in the porewater.

Soil cores collected for incubations were removed from cold storage within 5 days of collection and placed into an anaerobic hood containing a N$_2$/H$_2$ mixture (Megonigal and Schlesinger 2002). Two sections were removed from each core, yielding a 8-12 cm depth sample and a 28-32 cm depth sample. The outer 10 mm (approximately) of the resulting disks were removed to expose the center of the core, which was assumed to have had minimal O$_2$ exposure from collection to processing. We then removed as many live roots as feasible. Five grams of wet soil material was placed in a 35-mL serum bottle with 5 mL of the degassed water from the core hole. Headspace samples of 0.5 mL were injected directly into a Shimadzu gas chromatograph with a flame ionization detector for CH$_4$ or a LI-COR LI-7000 (LiCor, Lincoln, NE) for CO$_2$. Methanogenesis generally slowed dramatically after 5 days, so our calculations of potential anaerobic CH$_4$ production rates are based on incubation days 1-5.

**Statistical Analysis**

Data were analyzed using SAS 9.3 (SAS Institute Inc. Cary, NC). Regression analyses were performed on flux data using Proc Reg to determine the slope of CH$_4$ or CO$_2$ concentration change over time. All variables were evaluated for normality using PROC UNIVARIATE and those that required transformation were log transformed to improve normality. Parameters transformed were: CH$_4$ flux, pore water hydrogen sulfide concentration, pore water SO$_4^{2-}$ concentration, and pore water CH$_4$ 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**

**Antecedent Water Levels**

Water level data collected during the 2 weeks prior to and during sampling events varied significantly between strata (p<0.0001) and month (p<0.0001). Water levels of the *S. patens* stratum was significantly lower than all other strata, with a mean of 9 cm below the soil surface, while the other strata had similar mean water levels approximately 1 cm below the soil surface (Table 1). We were unable to test for a strata by month interactive effect because only two wells were deployed in each strata (and only one in the High *J. roemerianus* stratum); however, *S. patens* had a lower mean water level in all months (Derby 2016). Mean water levels were highest in July, August, and October and lowest in May, June, September, and December.

**Methane Emissions and Porewater Chemistry by Strata**

Average CH$_4$ flux over the study varied significantly between strata (p<0.0001) and month (p<0.0001), and had a significant interactive effect (p=0.018). Methane emissions from the four strata ranked *S. alterniflora* >> High *J. roemerianus* > Low *J. roemerianus* = *S. patens* (Table 1). Mean CH$_4$ emissions from the *S. alterniflora* stratum was
2.72 times greater than the next highest CH$_4$ emitter (Table 1) despite similar inundation regimes among the three highest emitting strata. High CH$_4$ emissions in the _S. alterniflora_ stratum coincided with significantly higher porewater CH$_4$ concentrations and lower SO$_4^{2-}$ concentrations than the other three strata (Table 1). Porewater salinity in the _S. alterniflora_ stratum was similar to other strata (Table 1) suggesting that relatively low SO$_4^{2-}$ concentrations were due to high rates of SO$_4^{2-}$ consumption. Indeed, SO$_4^{2-}$ was depleted by 61% in the _S. alterniflora_ stratum. Sulfate depletion was significantly different between strata (p<0.0001) and month (p<0.0001) and ranked _S. alterniflora_ = High _J. roemerianus_ > Low _J. roemerianus_ > _S. patens_. Sulfate depletion rates in the _S. alterniflora_ stratum were over two times greater than those seen in low _J. roemerianus_ (Table 1). Low SO$_4^{2-}$ concentrations in the _S. alterniflora_ stratum were accompanied by significantly higher amounts of hydrogen sulfide (72 mg L$^{-1}$) as compared to the other three strata (all < 20 mg L$^{-1}$).

The _S. patens_ stratum had the lowest mean porewater CH$_4$ concentrations and SO$_4^{2-}$ depletion rates, with only 1.4% SO$_4^{2-}$ depleted. _S. patens_ also exhibited the highest mean concentrations of reduced iron (i.e. ferrous iron, Fe$^{2+}$) during the single campaign when it was measured, with 72 mg L$^{-1}$ of ferrous iron in porewater collected 10 cm below the soil surface (Table 1). None of the other three strata had reduced iron porewater concentrations exceeding 0.8 mg L$^{-1}$. _S. patens_ also had a significantly lower mean porewater salinity (12.3 ppt) than the other three strata, which ranged in mean salinity from 14.2 to 14.8 ppt. The High and Low _J. roemerianus_ and _S. alterniflora_ strata were not significantly different in porewater iron concentrations or salinity (Table 1).

The difference in elevation between the two _J. roemerianus_ strata was not highly apparent in the water level and CH$_4$-related attributes we measured. The two strata were not significantly different from one another in, salinity, SO$_4^{2-}$ concentrations, sulfide concentrations, percent SO$_4^{2-}$ depleted, porewater CH$_4$ concentrations, and reduced iron concentrations (Table 1).

The highest CH$_4$ emissions were observed in July, August, and September; the lowest were observed in April, November, and December (Fig. 2). Significant strata by month interactions were observed in May, June, and September. In May and June, _S. alterniflora_ was not significantly different from any strata other than Low _J. roemerianus_; all other strata were not significantly different from one another. In September, _S. alterniflora_ was not significantly different from any strata other than _S. patens_; all other strata were not different from one another. No significant within-month differences were observed in April, July, August, October, November and December (Derby 2016). Porewater CH$_4$ exhibited a similar seasonal trend as CH$_4$ emissions, with the highest concentrations in the months July through November (Derby, 2016).

_Anaerobic incubations_

Surficial soils (8-12 cm) from the _S. patens_ stratum had the lowest CH$_4$ production, highest CO$_2$ production and a significantly higher ratio of CO$_2$:CH$_4$ production (ratio=993) that the other strata. At the other extreme was the _S. alterniflora_ stratum which produced substantially more CH$_4$ and less CO$_2$ than the other strata, and therefore had the lowest CO$_2$:CH$_4$ ratio (ratio=40) among the four sites (Table 2). The two _J. roemerianus_ strata fell in between these extremes with a CO$_2$:CH$_4$ ratio of about 200 (Table 2), though there were no significant differences in CO$_2$:CH$_4$ ratio between these strata and _S. alterniflora_. The 10 cm incubations produced significantly more CH$_4$ and CO$_2$ than those from 30 cm depth (p=0.04, data not shown, Derby 2016).
Discussion

Methane emissions varied by plant community type and hydrogeomorphic setting, suggesting that these variables are useful for dividing tidal marshes into strata to optimize the costs of sampling effort with the need to reduce parameter uncertainty when estimating CH$_4$ emissions. Mean emissions across strata ranked as $S. \text{alterniflora} >$ High-elevation $J. \text{roemerianus} >$ Low-elevation $J. \text{roemerianus} > S. \text{patens}$ (Table 1). There is a need to understand the mechanisms that lead to such differences among strata in order to advance proxies and models of CH$_4$ emissions from tidal wetlands.

We attribute the low emissions from the $S. \text{patens}$ stratum to a combination of relatively low water levels, shallow rooting depth, and higher mineral inputs, all of which have the capacity to suppress CH$_4$ production and promote CH$_4$ oxidation. It is well established that CH$_4$ production is suppressed by alternative electron acceptors such as O$_2$, Fe(III) and SO$_4^{2-}$ (Holm et al. 2016; Neubauer et al. 2005; Poffenbarger et al, 2011; Roden and Wetzel, 2003). Relatively high O$_2$ flux into the upper soil surface (0-10 cm depth) would be favored by both the relatively thick aerobic zone (i.e. deeper water-table) and shallow distribution of root biomass that is characteristic of $S. \text{patens}$ communities (Bernal et al. 2016). Mean antecedent water depth was 9 cm deep in this stratum compared to other strata with water levels near the soil surface.

Because the majority of $S. \text{patens}$ roots are in the top 10 cm of the soil profile (Windham 2001), it is likely that root oxygen loss was also an O$_2$ source in this community. O$_2$ suppresses methanogenesis via electron donor competition by two mechanisms, directly as an electron acceptor for aerobic bacteria and indirectly by regenerating poorly crystalline iron oxides on the root surface (i.e. iron plaque). Root-deposited iron plaque is rapidly consumed by iron-reducing bacteria (Weiss et al. 2003; Weiss et al. 2004), suppressing both SO$_4^{2-}$ reduction and methanogenesis (Neubauer et al. 2005). This mechanism is consistent with our observation that the $S. \text{patens}$ community had dramatically higher concentrations of reduced iron (measured only in July) and SO$_4^{2-}$ than the other strata, and the lowest SO$_4^{2-}$ depletion (Table 1). The close proximity of this site to the tidal creek may have also allowed for greater mineral inputs during flooding events to support iron cycling. Finally, if rates of methanotrophy are O$_2$-limited as studies suggest (King, 1996; Lombardi et al, 1997; Megonigal and Schlesinger 2002), then relatively high rates of CH$_4$ oxidation would be expected to further decrease the amount of CH$_4$ emitted through passive diffusion through plants, as documented in other tidal wetlands (Megonigal and Schlesinger 2002).

The $S. \text{alterniflora}$ stratum had the highest average CH$_4$ emissions and porewater chemistry that differed from the $S. \text{patens}$ community in several respects (Table 1). The $S. \text{alterniflora}$ stratum was in the center of the marsh complex (Fig. 1). Because hydrologic fluxes generally decrease with increasing distance from the tidal creek (Jordan et al. 1985), it is likely that soils in the $S. \text{alterniflora}$ stratum had relatively slow rates of advection compared to the other strata. Indeed, water table depths decreased relatively slowly after floods in the $S. \text{alterniflora}$ stratum (Derby 2016). This hydrologic difference likely decreased rates of advective transport of O$_2$ and SO$_4^{2-}$ to the soil profile to replenish these electron acceptors. We propose that the relatively low inputs of SO$_4^{2-}$ to the $S. \text{alterniflora}$ stratum led to SO$_4^{2-}$ limitation of SO$_4^{2-}$ reduction rates, allowing methanogens to compete more effectively with sulfate-reducing bacteria for electron donors. Porewater evidence supporting this interpretation includes low concentrations of Fe$^{2+}$, high SO$_4^{2-}$ depletion, and high concentrations of both hydrogen sulfide and CH$_4$. This interpretation is also consistent with the results of the July anaerobic incubations showing that the CO$_2$:CH$_4$ ratio was lowest in $S. \text{alterniflora}$ soils and highest in $S. \text{patens}$ soils. Because aerobic respiration, sulfate reduction, and iron reduction generate CO$_2$ rather than
CH$_4$, these data suggest that methanogens had relatively little competition for organic carbon and H$_2$ in the S. alterniflora stratum.

Water table depths in the High and Low J. roemerianus strata were similar to the S. alterniflora stratum but metrics related to CH$_4$ emissions were consistently lower than the S. alterniflora and higher than the S. patens strata, namely CH$_4$ emissions, porewater SO$_4^{2-}$ concentrations, and anaerobic incubation CO$_2$:CH$_4$ ratio. The difference in CH$_4$ emissions between S. alterniflora and the J. roemerianus strata cannot be explained by water levels, salinity, pH, or reduced iron because they were not significantly different between these strata. For example, mean water table depth in the S. alterniflora stratum and the two J. roemerianus strata were within 1 cm of each other, while emissions were 2.5 times greater in the S. alterniflora stratum. The J. roemerianus strata were closer to the tidal creek and presumably CH$_4$ production was not limited by tidal inputs of SO$_4^{2-}$-supply as we suspect was the case in the S. alterniflora stratum. However, the relatively low CH$_4$ emissions in the J. roemerianus strata compared to S. alterniflora may also have been related to plant traits that regulate CH$_4$ emissions by influencing the balance between CH$_4$ production and oxidation (Sutton-Grier and Megonigal 2011, Mueller et al. 2020), which itself is influenced by traits that affect CH$_4$ transport through plants (Komiya et al. 2020). Mueller et al. (2020) proposed that plant traits vary across species such that some push the balance between these opposing processes toward net CH$_4$ production while others favor net CH$_4$ oxidation. We hypothesize that among the dominant species present at the site, S. alterniflora favors net CH$_4$ production while J. roemerianus favors net CH$_4$ oxidation, and that the lower CH$_4$ emissions rates in the J. roemerianus strata may have been due to relatively high rates of root oxygen loss by J. roemerianus. This interpretation is supported in part by data on porewater [SO$_4^{2-}$], which mediates the outcome of competition between sulfate-reducing bacteria and methanogens. Porewater [SO$_4^{2-}$] was 4.5 mM in the S. alterniflora stratum but exceeded 6 mM in the J. roemerianus strata where CH$_4$ emissions rates were relatively low. Although this difference in porewater [SO$_4^{2-}$] seems small, the relationship between these variables is non-linear and displays a threshold value above which sulfate reduction dominates and below which methanogenesis dominates (Megonigal et al. 2004). There is a general lack of CH$_4$-relevant porewater data for coastal wetlands, but a robust record from a brackish marsh located 100 km from the study site in Chesapeake Bay observed that porewater [CH$_4$] declined abruptly when SO$_4^{2-}$ concentrations exceeded approximately 4 to 6 mM (Keller et al. 2009). We propose that the S. alterniflora and J. roemerianus strata fell on opposite sides of this threshold.

Although floodwater salinity can be an effective proxy for porewater SO$_4^{2-}$ concentrations when comparing sites across large spatial scales (Poffenbarger et al. 2011), our data demonstrate the limitations of using the salinity proxy at local scales. Variation among strata in SO$_4^{2-}$ concentration and SO$_4^{2-}$ depletion may have been caused by variation in rates of SO$_4^{2-}$ transport from tidal floodwater into soils, sulfide oxidation related to O$_2$ diffusion from water table depth or root O$_2$ loss, or primary production (i.e. carbon availability). We cannot distinguish among these mechanisms with the present dataset. Sulfate depletion was a better indicator of CH$_4$ flux than salinity or SO$_4^{2-}$ concentration alone and may prove to be a superior proxy for CH$_4$ emissions in tidal brackish marshes.

Within our strata, CH$_4$ production did not strictly follow the conventional interpretation that differences in the free energy yield among competing microbial respiration processes means that just one process dominates microbial respiration at a time, with methanogenesis expected to occur only when all other electron acceptors are fully (or nearly) depleted. Our data suggest that peak sulfate reduction activity was occurring concurrently with peak CH$_4$ production in the S. alterniflora stratum which had both the highest mean pore water hydrogen sulfide concentrations
and the lowest SO_4^{2-} concentrations. We attribute this to spatial variation in electron donors and acceptors that creates microsites of high SO_4^{2-} depletion and methanogenesis. Microsites have been shown to produce small amounts of CH_4 in upland forested systems, originally thought to be too dry and too aerobic to produce this greenhouse gas (Megonigal and Guenther 2008). Microsites also exist in tidal marsh soils due to local (i.e. rhizosphere) consumption of electron acceptors at rates faster than they can be replenished (e.g. Rabenhorst et al. 2010).

**Carbon offset implications**

Salinity is a useful proxy for CH_4 emissions from tidal marshes with salinity regimes > 18 ppt because CH_4 emissions are low compared to their soil carbon sequestration rates and variation among marshes is small. However, tidal brackish marshes at lower salinities may emit enough CH_4 to offset a significant fraction of their radiative cooling and variation in CH_4 emissions among marshes within a given salinity regime is large. Such uncertainty is accommodated in carbon offset programs such as the Verified Carbon Standard by requiring the project to estimate CH_4 emissions by direct monitoring or by using published data, a model, or a proxy that can be demonstrated to be valid for the project site (Needelman et al. 2018). Stratification by hydrology and vegetation characteristics may provide a more effective proxy than salinity to estimate CH_4 emissions. Stratification also offers a means to constrain variability within direct monitoring schemes.

We compared the CH_4 offset estimates produced through our direct measurements to those predicted by the salinity-based proxy equation of Poffenbarger et al. (2011). For this comparison we assumed a single soil carbon sequestration rate of 1.46 Mg C ha\(^{-1}\) yr\(^{-1}\) for all strata in the marsh complex, which is the default rate in the carbon crediting methodology of Emmer et al. (2015), derived as the median value from Chmura et al. (2003). In three of the four strata the salinity proxy overestimated emissions by about 20%, 40%, and 80% (Table 3). Overestimation is preferable to underestimation to avoid awarding carbon credits that exceed actual greenhouse gas benefits, but overestimation decreases the financial viability of carbon offset projects. These errors caused the positive radiative balance to be underestimated by just 4-8% in the *J. roemerianus* strata, suggesting that incorporating vegetation and hydrology proxies would not be a substantial improvement over the salinity proxy alone. In addition, the cost of *in situ* emission measurements would not be rewarded by a meaningful increase in carbon credits in the *J. roemerianus* strata. However, the positive radiative balance of the *S. patens* stratum was underestimated by >20%, suggesting that a proxy based on vegetation and hydrology would improve CH_4 emission estimates, and that the expense of collecting *in situ* data may be worthwhile. The salinity proxy overestimated CH_4 emissions in the *S. alterniflora* stratum where the positive radiative balance was overestimated by about 25% (Table 3), indicating that a salinity-based proxy would award carbon credits exceeding actual climate benefits in this stratum. Improved proxies are needed to incentivise carbon offsets projects by reducing monitoring costs while ensuring that projects achieve estimated climate benefits.

Our results suggest that proxies for CH_4 emissions from tidal wetlands with salinity regimes < 18 ppt can be improved by incorporating metrics related to hydrology such as flooding frequency and duration or the position of the soil surface relative to the tidal frame, and metrics related to plant traits such as species identity, plant functional type, or biomass. Ideally these metrics would be identifiable at high spatial resolution for low cost, such as through analysis of freely available remote sensing data. Currently there is no widely accepted method to remotely sense surface water salinity, but robust methods exist for remote sensing of plant cover, biomass, primary production, and elevation (Buffington et al. 2016, Byrd et al. 2018, Feagin et al. 2020).
Conclusions

Tidal wetland restoration and conservation projects have the potential to mitigate greenhouse gas emissions to the atmosphere and generate carbon credits, but a better understanding of the factors influencing wetland CH$_4$ emissions in brackish and freshwater systems (salinity < 18 ppt) is required to lower carbon crediting project costs and estimation uncertainty at site-specific scales. We found significantly different methane emission rates across four strata defined by hydrology and plant community composition that otherwise had similar salinity regimes. We inferred that they deviated from the rates predicted by a salinity proxy due to processes that regulate the availability of competing terminal electron acceptors such as O$_2$, Fe(III), and SO$_4^{2-}$ and due to plant traits that regulate CH$_4$ emissions. Low CH$_4$ emission rates in the high-elevation _S. patens_ stratum was attributed to relatively high inputs of Fe(III) through tidal inputs and oxidation of reduced iron and O$_2$ through diffusion across the soil surface and root O$_2$ loss, both of which maintain high [SO$_4^{2-}$] by suppressing microbial reduction of SO$_4$. By contrast, the _S. alterniflora_ stratum was relatively isolated from tidal inputs of Fe(III) and SO$_4$, so it produced large amounts of CH$_4$ once SO$_4^{2-}$ had been sufficiently depleted. The greater CH$_4$ emissions from the low-elevation _S. alterniflora_ stratum than the low and high elevation _J. roemerianus_ strata could not be explained by water table depth, salinity, pH, and [Fe$^{2+}$], suggesting an important role for plant traits such as root O$_2$ loss regulating CH$_4$ emissions at local scales. The mechanisms driving these patterns were not measured directly but likely involve variations in rates of SO$_4^{2-}$ diffusion from tidal floodwater based on site hydrologic connectivity; rates of sulfide oxidation as influenced by O$_2$ diffusion or root O$_2$ loss; and differing plant primary productivity. Our results suggest that stratification by vegetation and hydrologic setting improves estimates of CH$_4$ emissions from tidal marshes. Our findings illustrate the need to better understand controls over CH$_4$ emissions at site-specific scales to improve carbon sequestration offset estimates in brackish and freshwater coastal wetland ecosystems.

Declarations

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Conflicts of interest

None.

Availability of data and material (data transparency)

All data from this manuscript will be made available through the Coastal Carbon Research Coordination Network.

Code availability (software application or custom code)

Not applicable

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Table 1. Mean values by strata of Methane Flux, Porewater Salinity, Porewater Sulfate concentration, Porewater Sulfate Depletion, Porewater Hydrogen Sulfide, Porewater Methane, Porewater Reduced Iron (*Reduced Iron was collected in July only), and Antecedent Water Levels (defined as mean water level below soil surface for the two weeks prior to sampling) in a brackish marsh complex. Superscript letters denote statistical differences between strata. Means with the different letters have statistically significant differences. Standard error is presented below each mean.
| Strata               | Methane Flux (mg CH\(_4\) m\(^2\) hr\(^{-1}\)) | Salinity (ppt) | Sulfate (mg/L) | Sulfate Depletion (%) | Hydrogen Sulfide (mg/L) | Porewater Methane (mg/L) | Reduced Iron* (mg/L) | Antecedent Water Level (cm) |
|----------------------|-----------------------------------------------|----------------|----------------|-----------------------|-------------------------|--------------------------|-----------------------|----------------------------|
| S. alterniflora      | 1.2\(^A\) ±0.3                              | 14.2\(^A\) ±0.2| 434.3\(^C\) ±33.7| 60.6\(^C\) ±3.5       | 72.2\(^A\) ±8.8        | 4795.6\(^A\) ±781.2     | 0.02\(^B\) ±0.009       | 1\(^B\) cm                   |
| S. patens            | 0.1\(^C\) ±0.01                             | 12.3\(^B\) ±0.4| 852.2\(^A\) ±63.0| 1.4\(^A\) ±8.5        | 18.9\(^B\) ±3.5        | 263.7\(^C\) ±29.7       | 72.0\(^A\) ±40.1         | 9\(^A\) cm                   |
| J. roemerianus (Low Elevation) | 0.3\(^C\) ±0.07                           | 14.8\(^A\) ±0.3| 693.0\(^AB\) ±45.7| 38.3\(^B\) ±4.0       | 14.9\(^B\) ±2.7        | 1174.7\(^B\) ±217.4     | 0.78\(^B\) ±0.4           | 1.1\(^B\) cm                |
| J. roemerianus (High Elevation) | 0.4\(^B\) ±0.06                           | 14.8\(^A\) ±0.3| 601.8\(^B\) ±35.8| 54.3\(^BC\) ±3.3      | 19.9\(^B\) ±3.3        | 1243.0\(^B\) ±247.8     | 0.30\(^B\) ±0.2           | 0.7\(^B\) cm                |

Table 2. Mean Values of Mol CO\(_2\) and CH\(_4\) Produced on Day Five of Anaerobic Soil Incubations and the Ratio of CO\(_2\) to CH\(_4\) produced from soils collected from a brackish marsh complex. Superscript Letters Denote Statistical Differences Between strata. Means with the different letters have statistically significant differences. Standard error is presented below each mean.

| Strata               | Mol of CH\(_4\) Produced at 10cm in Anaerobic Incubations | Mol of CO\(_2\) Produced at 10cm in Anaerobic Incubations | Ratio of CO\(_2\):CH\(_4\) Produced at 10cm in Anaerobic Incubations |
|----------------------|------------------------------------------------------------|----------------------------------------------------------|---------------------------------------------------------------|
| S. alterniflora      | 13.8\(^A\) ±5.8                                           | 101.5\(^A\) ±19.0                                        | 39.8\(^A\) ±22.1                                              |
| S. patens            | 0.4\(^B\) ±0.1                                            | 229.5\(^B\) ±16.6                                        | 933.1\(^B\) ±324.2                                            |
| J. roemerianus (Low Elevation) | 0.9\(^B\) ±0.1                                           | 171.8\(^AB\) ±29.7                                       | 199.1\(^A\) ±31.1                                             |
| J. roemerianus (High Elevation) | 0.8\(^B\) ±0.1                                           | 146.5\(^AB\) ±19.5                                       | 209.2\(^A\) ±43.5                                             |

Table 3. Field-measured salinity and methane flux mean values from four strata in a tidal marsh complex as compared to predicted flux (based on observed salinity) from Poffenbarger et al. 2011, with percent differences of actual and predicted carbon sequestration offsets.
| Strata                  | Salinity (ppt) | Methane Flux (Mg C ha\(^{-1}\) yr\(^{-1}\)) | Predicted Methane Flux from Poffenbarger et. al (Mg C ha\(^{-1}\) yr\(^{-1}\)) | Percent Difference from Poffenbarger et. al | Percent of Carbon Sequestration Offset (actual) | Percent of Carbon Sequestration Offset (predicted) |
|------------------------|----------------|--------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------------|-------------------------------------------------|--------------------------------------------------|
| \textit{S. alterniflora} | 14.2           | 0.67                                       | 0.29                                                                            | 131.0%                                    | 45.9%                                           | 19.9%                                             |
|                        |                |                                            |                                                                                 | **More than Predicted**                   |                                                 |                                                   |
| \textit{S. patens}     | 12.3           | 0.07                                       | 0.37                                                                            | 81.0%                                     | 4.8%                                            | 25.3%                                             |
|                        |                |                                            |                                                                                 | **Less than Predicted**                   |                                                 |                                                   |
| \textit{J. roemerianus} (Low Elevation) | 14.8           | 0.16                                       | 0.27                                                                            | 40.7%                                     | 11.0%                                           | 18.5%                                             |
|                        |                |                                            |                                                                                 | **Less than Predicted**                   |                                                 |                                                   |
| \textit{J. roemerianus} (High Elevation) | 14.8           | 0.22                                       | 0.27                                                                            | 18.5%                                     | 15.1%                                           | 18.5%                                             |
|                        |                |                                            |                                                                                 | **Less than Predicted**                   |                                                 |                                                   |