

# Short-Term Response of Carbon Cycling to Salinity Pulses in a Freshwater Wetland

Lisa G. Chambers\*  
K. Ramesh Reddy  
Todd Z. Osborne

Wetland Biogeochemistry Lab.  
Soil and Water Science Dep.  
Univ. of Florida  
Gainesville, FL 32611-0510

Sea level rise increases the frequency and intensity of storm surges and extreme tidal events in coastal freshwater wetlands. Seawater affects soil biogeochemical processes by inducing osmotic stress and stimulating  $\text{SO}_4^{2-}$  reduction. The objective of this study was to determine the mechanism by which salinity alters C mineralization rates by quantifying the relative importance of ionic stress, compared with the addition of the  $\text{SO}_4^{2-}$  electron acceptor, on the production of  $\text{CO}_2$  and  $\text{CH}_4$ . A batch incubation study measured potential anaerobic respiration and methanogenesis with time in a freshwater wetland soil exposed to varying concentrations (3.5, 14, and 35  $\text{g kg}^{-1}$ ) of seawater or salt (NaCl) solutions. Seawater addition induced a short-term (2-wk) stimulation of  $\text{CO}_2$  production (20–32% greater than the freshwater control) and a continuous suppression of  $\text{CH}_4$  production (up to 94% less than freshwater). Ionic stress (represented by NaCl) did not reduce  $\text{CO}_2$  production at all but did decrease  $\text{CH}_4$  production for 2 wk in both the 14 and 35  $\text{g kg}^{-1}$  NaCl treatments. Our results indicate that microbial populations rebound quickly from ionic stress. The intrusion of dilute seawater (3.5  $\text{g kg}^{-1}$ ) to freshwater wetlands can accelerate organic C mineralization through the short-term increase in  $\text{SO}_4^{2-}$ -induced respiration without inhibiting methanogenesis. Overall, the organic C mineralization rate was 17% higher for 3.5  $\text{g kg}^{-1}$  seawater than the freshwater control. The temporary nature of the microbial response suggests that “pulses” of seawater may have a greater influence on the rate of C cycling in freshwater wetlands than a gradual sea level rise.

**Abbreviations:** MBC, microbial biomass carbon; OC, extractable organic carbon; TOC, total extractable organic carbon.

The soil microbial pool is responsible for many ecosystem processes, including the transfer of C from the organic pool (e.g., biomass) to the inorganic pool (e.g.,  $\text{CO}_2$  and  $\text{CH}_4$ ) (Wetzel, 2001). The rate at which microbes mineralize C is especially important in wetlands, where the high level of primary productivity means changes in the C source–sink potential of wetlands could have implications for the global C cycle. Salinity is a prevalent environmental stressor with the potential to alter the rate of C cycling in wetlands (e.g., Pattnaik et al., 2000; Sangiorgio et al., 2008; Wong et al., 2008). The impact of soil salinity in arid and semiarid regions on crop productivity and nutrient cycling has been studied extensively. Findings indicate that high salt concentrations in upland and paddy soils can decrease the size of the soil microbial community (Muhammad et al., 2006; Pattnaik et al., 2000), decrease the rate of microbial respiration (Gennari et al., 2007; Muhammad et al., 2006; Pathak and Rao, 1998), and decrease the rate of methanogenesis (Pattnaik et al., 2000). Such an overall decrease in the rate of C cycling in these systems is often attributed to osmotic or ionic stress inflicted on the microbial population by increased conductivity in the soil–water environment (Frankenberger and Bingham, 1982; Gennari et al., 2007).

Rising sea level and increasing frequency of saltwater intrusion events also cause stress to microbial populations in freshwater wetlands near the coast. It is estimated that during the 20th century, the sea level rose  $\sim 1.7 \text{ mm yr}^{-1}$ . Since 1993, this rate has increased to  $2.8$  to  $3.1 \pm 0.7 \text{ mm yr}^{-1}$  (Intergovernmental Panel

Soil Sci. Soc. Am. J. 75:2000–2007

Posted online 23 Aug. 2011

doi:10.2136/sssaj2011.0026

Received 20 Jan. 2011.

\*Corresponding author (lisagardner@ufl.edu)

© Soil Science Society of America, 5585 Guilford Rd., Madison WI 53711 USA

All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Permission for printing and for reprinting the material contained herein has been obtained by the publisher.

on Climate Change, 2007). Increasing sea level will amplify the impacts of storm surges and increase the area inundated by extreme tidal events (Michener et al., 1997). In contrast to saline conditions caused by high evaporation rates in arid and semiarid inland soils, seawater contains a relatively uniform mixture of several salts and macro- and micronutrients (Kester et al., 1967). Of particular importance to wetland soils is the abundance of  $\text{SO}_4^{2-}$  in seawater, which can function as an alternative electron acceptor during anaerobic microbial respiration. Sulfate concentrations in seawater are normally between 20 and 30  $\text{mmol L}^{-1}$  compared with concentrations of 0.1 to 0.2  $\text{mmol L}^{-1}$  of  $\text{SO}_4^{2-}$  observed in fresh water (Capone and Kiene, 1988).

Studies investigating the effect of seawater on C cycling have historically been performed along existing salinity gradients in estuaries and coastal zones or using intact soil cores to measure long-term fluxes. In Louisiana coastal wetlands, microbial respiration rates are highest in freshwater wetlands, followed by salt marshes, and lowest in brackish wetlands (Nyman and DeLaune, 1991; Smith et al., 1983). Methane production rates are significantly lower in salt marshes than freshwater wetlands because the abundant  $\text{SO}_4^{2-}$  is more energetically favorable for anaerobic respiration than methanogenesis (Bartlett et al., 1987; King and Wiebe, 1980; Magenheimer et al., 1996). It has been found that within 12 d of 10  $\text{g kg}^{-1}$  seawater addition to a freshwater tidal marsh soil in Georgia, the dominant microbial pathway switched from methanogenesis to  $\text{SO}_4^{2-}$  reduction (Weston et al., 2006). Despite the extensive knowledge regarding competition between methanogens and  $\text{SO}_4^{2-}$ -reducing bacteria, few studies have directly addressed the mechanistic process by which salinity exposure influences microbial mineralization pathways and rates. Specifically, no studies have attempted to distinguish between the importance of ionic stress (increased conductivity) and the addition of the  $\text{SO}_4^{2-}$  electron acceptor in altering C

mineralization rates following a saltwater pulse. This is important for evaluating the relevance of the trends observed in C cycling in agricultural saline soils (i.e., the documented decrease in the size, activity, and composition of the microbial population) and coastal soils subjected to saltwater pulses.

This study sought to determine the process by which salinity affects C cycling in a freshwater wetland soil. Specifically, is the microbial community inhibited by ionic stress or the addition of  $\text{SO}_4^{2-}$ , and what effect does this have on the overall rate of C cycling? This was done by comparing how microbial respiration, methanogenesis, and microbial population size responded to different concentrations of seawater (containing  $\text{SO}_4^{2-}$ ) and salt (strictly NaCl) additions. We hypothesized that potential respiration rates would be reduced by additions of both seawater and NaCl due to increased ionic stress to the microbial community, but potential methanogenesis would be reduced to a much greater extent in the seawater treatments due to competition with  $\text{SO}_4^{2-}$  reducers. We also anticipated a reduction in the size of the microbial population with increasing concentrations of both seawater and NaCl.

## MATERIALS AND METHODS

### Experimental Design

A bulk field composite peat soil sample (0–10 cm) was collected from St John's Marsh Conservation Area (27.91833° N, –80.77389° W), a freshwater wetland dominated by an even mix of *Typha* spp. and *Salix* spp. (Fig. 1). Following return to the laboratory, the soil was homogenized and approximately 15 g (wet weight) of soil was added to 70-mL glass serum bottles. Seven treatments were evaluated. They consisted of seawater at concentrations of 35, 14, and 3.5  $\text{g kg}^{-1}$ , NaCl at concentrations of 35, 14, and 3.5  $\text{g kg}^{-1}$ , and a deionized (DI) water treatment to serve as the freshwater control. The seawater and NaCl treatments functioned as discrete analogs of ionic stress (measured in  $\text{g kg}^{-1}$ ) while allowing isolation of the  $\text{SO}_4^{2-}$  reduction effect on C mineralization. All treatments were prepared in triplicate. Fifteen milliliters of a randomly assigned treatment solution was added to each bottle to form a soil slurry.

Three concentrations of seawater were made using Neomarine Reef Salt mix (Brightwell Aquatics, Elysburg, PA). Thirty-five grams of salt mix was diluted in 1 L of DI water to create 35  $\text{g kg}^{-1}$  seawater. The solution was purged with ambient air for several hours to establish the  $\text{CO}_2/\text{HCO}_3$  equilibrium, and then the pH and specific conductivity ( $\mu\text{S m}^{-1}$ ) were measured. The seawater solution was further diluted with DI water to 14 and 3.5  $\text{g kg}^{-1}$  and the pH and specific conductivity were again noted. The ionic content of the artificial seawater mimicked that of natural seawater without any additional nutrients or C. Sulfate was the only available electron acceptor in the seawater treatments.

Three concentrations of NaCl were made by diluting 35 g of crystalline NaCl in 1 L of DI water. The solution was purged with ambient air for several hours to establish the  $\text{CO}_2/\text{HCO}_3$  equilibrium, and then the pH and specific conductivity were measured. The NaCl solution was further diluted with DI water to 14 and 3.5  $\text{g kg}^{-1}$  and the pH and specific conductivity were again noted.

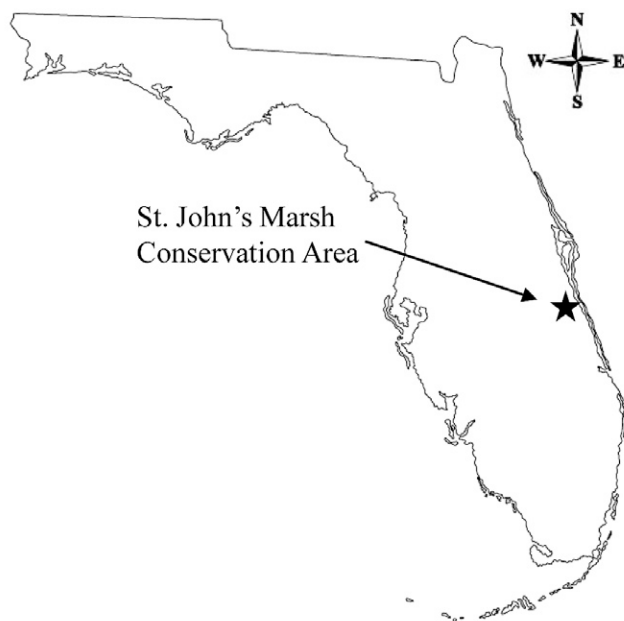


Fig. 1. Location of freshwater marsh soils collected for this manipulative laboratory experiment.

The bottles were capped with butyl stoppers and aluminum crimp caps, evacuated to  $-75$  kPa, and flushed with  $O_2$ -free  $N_2$  gas for 1 min to create anaerobic conditions. The incubation bottles were then placed in the dark on a circulating shaker at  $30^\circ C$ . Headspace was extracted and measured on a gas chromatograph (GC 8A, Shimadzu Scientific Instruments, Columbia, MD) fitted with a thermal conductivity detector and flame ionization detector to determine the concentrations of  $CO_2$  and  $CH_4$ , respectively. Headspace samples were measured after 1, 2, 4, and 6 d to produce a daily rate of production. All bottles were then purged with  $O_2$ -free  $N_2$  gas for 1 min to prevent  $CO_2$  accumulation in the headspace, and the sampling cycle was repeated again. The sampling and purging sequence was repeated for 3 wk and the rates of potential  $CO_2$ -C and  $CH_4$ -C production were calculated with time.

## Soil Properties

Bulk density was determined after oven drying a known volume of subsample at  $70^\circ C$  until constant weight. The organic matter content was determined using the loss-on-ignition method (Nelson and Sommers, 1996). Three grams of triplicate ground, dried soils were placed in a muffle furnace at  $550^\circ C$  for 4 h, cooled, and reweighed. The percentage weight loss was calculated as the difference between the soil weight before and after ashing, multiplied by 100.

Soil pH and specific conductivity were measured on all samples after completion of the 3-wk incubation period. A 2:1 (water/soil) suspension was created and allowed to equilibrate for 30 min before measurement (Thomas, 1996; USEPA, 1982). The pH was measured using an Accumet Research AR50 pH meter (Fisher Scientific, Waltham, MA) and the specific conductivity was measured on a Markson Model 1054 electrical conductivity meter (Amber Scientific, Eugene, OR).

Total extractable organic C (TOC), extractable organic C (OC), and microbial biomass C (MBC) were determined for all samples following the 3-wk incubation period using the fumigation-extraction method of Vance et al. (1987). The TOC was defined as the extractable organic C extracted from the fumigated samples and OC was defined as the extractable organic C extracted from the unfumigated samples. Microbial biomass C (MBC) was determined by subtracting the extractable C of an unfumigated sample from the corresponding fumigated sample. Duplicate 5-g (wet weight) samples were prepared in 25-mL centrifuge tubes. One set was fumigated with chloroform for 24 h and the other set served as the unfumigated control. Following the chloroform treatment, both fumigated and unfumigated samples were extracted with 25 mL of  $0.5 \text{ mol L}^{-1} K_2SO_4$  agitated for 30 min on a circulating shaker, and centrifuged at 5000 rpm for 10 min. The supernatant was vacuum filtered through Whatman no. 42 filter paper and stored at  $4^\circ C$  until analysis for total organic C (TOC 5050A, Shimadzu Scientific Instruments, Columbia, MD). An extraction efficiency coefficient of  $k_{EC} = 0.37$  was applied to all samples (Sparling et al., 1990).

## Data Analysis

Statistical analysis was performed using SAS version 9.1 (SAS Institute, Cary, NC). All data sets were first tested to determine if the assumptions of homogeneity and normality were met using Levene's test and the Shapiro-Wilk test, respectively. Where these assumptions were not met, the raw data were logarithmically transformed and further statistical analysis was conducted using the data set that fulfilled the assumptions of homogeneity and normality. A two-way repeated measures ANOVA ( $\alpha = 0.05$ ) was used to determine the interaction between  $CO_2$  and  $CH_4$  production, treatment, and time. Significant differences were identified using the least square means post-hoc test. One-way ANOVA models ( $\alpha = 0.05$ ) were also used to identify significant differences between pH, conductivity,  $SO_4^{2-}$  concentration, NaCl concentration, extractable C indicators, and microbial biomass. Pearson's product correlations were performed to determine if correlations existed between  $CO_2$  and  $CH_4$  production and  $SO_4^{2-}$  concentration, NaCl concentration, pH, conductivity, extractable C indicators, and microbial population indicators.

## RESULTS

### Soil Properties

The soil consisted of a flocculant peat and had a bulk density of  $0.097 \pm 0.01 \text{ g cm}^{-3}$  and an organic matter content of  $55 \pm 0.6\%$ . The initial soil pH was  $6.82 \pm 0.02$ ; at the conclusion of the experiment, the soil pH was highest for the  $35 \text{ g kg}^{-1}$  seawater treatment ( $7.15 \pm 0.03$ ) and lowest for the  $35 \text{ g kg}^{-1}$  NaCl treatment ( $6.41 \pm 0.04$ ). The pH of the  $3.5 \text{ g kg}^{-1}$  seawater,  $3.5 \text{ g kg}^{-1}$  NaCl, and freshwater control treatments did not differ from the initial pH (Table 1). Specific conductivity ranged from  $285 \pm 4 \mu S m^{-1}$  to below the detection limit ( $3 \mu S m^{-1}$ ) for the  $35 \text{ g kg}^{-1}$  NaCl and the freshwater control, respectively (Table 1). Specific conductivity was significantly different for all treatments ( $P < 0.01$ ) except the  $3.5 \text{ g kg}^{-1}$  seawater and  $3.5 \text{ g kg}^{-1}$  NaCl treatments. Sulfate and NaCl concentrations were significantly different for all treatments (Table 1).

**Table 1. Soil properties according to treatment. Soil pH, specific conductivity and MBC measured following destructive sampling after the 3-wk incubation.**

Treatment	pH	Conductivity	$SO_4^{2-}$	NaCl	Microbial biomass C
— $g \text{ kg}^{-1}$ —		— $\mu S m^{-1}$ —	— $g \text{ kg}^{-1}$ dry soil —		— $mg \text{ kg}^{-1}$ —
Seawater					
35	$7.15 \pm 0.03$ a†	$250 \pm 12$ a	$30.4 \pm 3.9$ a	$120.4 \pm 15.5$ a	$371 \pm 53$
14	$6.9 \pm 0.06$ b	$113 \pm 4$ b	$12.2 \pm 0.6$ b	$48.1 \pm 2.4$ b	$533 \pm 84$
3.5	$6.78 \pm 0.05$ c	$24 \pm 5$ c	$3.5 \pm 0.3$ c	$13.0 \pm 1.0$ c	$415 \pm 118$
NaCl					
35	$6.41 \pm 0.04$ d	$285 \pm 4$ d	BD‡ d	$421.7 \pm 9.4$ d	$447 \pm 97$
14	$6.54 \pm 0.01$ e	$130 \pm 5$ e	BD d	$165.8 \pm 14.2$ e	$596 \pm 171$
3.5	$6.72 \pm 0.01$ c	$364 \pm 1$ c	BD d	$4.2 \pm 0.3$ f	$498 \pm 138$
Freshwater control	$6.82 \pm 0.01$ c	BD f	BD d	BD f	$467 \pm 32$

† Means  $\pm$  standard deviations followed by different letters are significantly different at  $P < 0.01$ .

‡ BD, below detection limit.

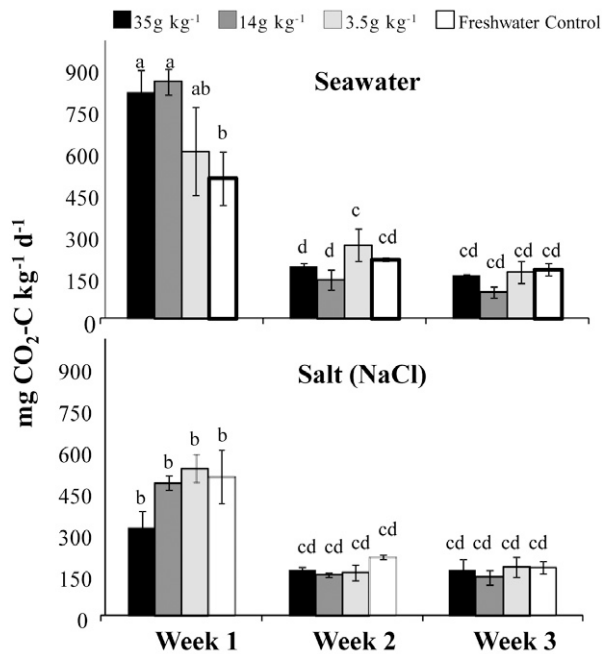


Fig. 2. Anaerobic microbial respiration rate by treatment during the 3-wk incubation period. Error bars represent standard deviations;  $n = 3$  for all treatments; different letters indicate significant differences at  $P < 0.01$ .

### Potential Microbial Respiration

The rate of potential CO<sub>2</sub> production was significantly higher in the 35 and 14 g kg<sup>-1</sup> seawater treatments than the freshwater control and NaCl treatments during Week 1 ( $P < 0.01$ ) but did not differ significantly during Weeks 2 and 3 (Fig. 2). There were no significant differences in the CO<sub>2</sub> production rate between the NaCl treatments throughout the entire study. Considering all the treatments, time was a significant factor for CO<sub>2</sub> production ( $P < 0.001$ ) as well as the time  $\times$  treatment interaction ( $P < 0.001$ ). Significantly greater rates of microbial respiration occurred during Week 1 than Weeks 2 and 3 for all treatments (Fig. 2). The contribution of SO<sub>4</sub><sup>2-</sup> reduction to respiration decreased with time, however, as seen by the difference in CO<sub>2</sub> production between the seawater treatments and the freshwater control. During

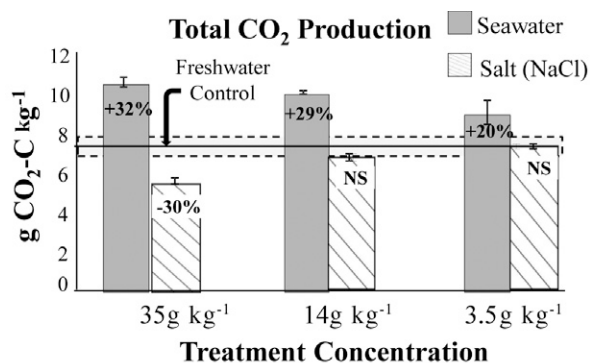


Fig. 3. Mean total anaerobic CO<sub>2</sub> produced during the 3-wk incubation period by treatment and concentration. Horizontal lines represent the mean (solid line) and standard deviation (dotted lines) of total production by the freshwater control. Percentages represent differences in mean total production compared with the freshwater control ( $P < 0.05$ ). Error bars represent standard deviations; NS = not significant;  $n = 3$  for all treatments.

Week 1, it can be estimated that 44% of anaerobic respiration was mediated by SO<sub>4</sub><sup>2-</sup> reduction, while SO<sub>4</sub><sup>2-</sup> reduction accounted for 21 and 15% of respiration during Weeks 2 and 3, respectively.

The total amount of CO<sub>2</sub> produced during the 3-wk incubation period was significantly greater ( $P < 0.01$ ) for all the seawater treatments than the freshwater control (Fig. 3). Total CO<sub>2</sub> production was 32% higher in the 35 g kg<sup>-1</sup> seawater treatment than the freshwater control, 29% higher in the 14 g kg<sup>-1</sup> seawater treatment, and 20% higher in the 3.5 g kg<sup>-1</sup> seawater treatment. Total CO<sub>2</sub> produced by the NaCl treatments did not differ from the freshwater control (Fig. 3).

The variables that correlated with CO<sub>2</sub> production differed between the seawater and NaCl treatments. Seawater respiration rates were positively correlated ( $P < 0.01$ ) with indicators of extractable C (TOC and OC) (Table 2). Respiration in the NaCl treatments was positively correlated with pH and negatively correlated with conductivity and OC ( $P < 0.01$ ) (Table 2).

### Potential Methanogenesis

The rate of potential CH<sub>4</sub> production was significantly lower ( $P < 0.001$ ) for the 35 and 14 g kg<sup>-1</sup> seawater treatments than the 3.5 g kg<sup>-1</sup> seawater treatment and the freshwater control for all 3 wk (Fig. 4). The 35 and 14 g kg<sup>-1</sup> NaCl treatments also had a significantly lower rate of CH<sub>4</sub> production during Weeks 1 and 2 than the 3.5 g kg<sup>-1</sup> NaCl treatment and the freshwater control but did not differ significantly during Week 3 (Fig. 4). Time was not a significant factor for the CH<sub>4</sub> production rate.

The total amount of CH<sub>4</sub> produced during the 3-wk incubation period was significantly less for the 35 and 14 g kg<sup>-1</sup> seawater and NaCl treatments than the freshwater control (Fig. 5). Total CH<sub>4</sub> production was 94 and 79% lower in the 35 and 14 g kg<sup>-1</sup> seawater treatments, respectively, than the freshwater control. Total methane production was reduced by 55 and 23% in the 35 and 14 g kg<sup>-1</sup> NaCl treatment, respectively, compared with the freshwater control (Fig. 5). Neither the seawater nor the 3.5 g kg<sup>-1</sup> NaCl treatments differed from the freshwater control in CH<sub>4</sub> production.

In contrast to respiration, CH<sub>4</sub> production in the seawater treatments was not correlated with indicators of extractable C but

Table 2. Pearson's product correlation coefficients ( $r$ ) for the correlation of anaerobic respiration and methanogenesis rates with soil parameters; for all values,  $n = 9$  and  $df = 7$ .

Parameter	Treatment	CO <sub>2</sub> production	CH <sub>4</sub> production
pH	seawater	NS	-0.75*
	NaCl	0.84**	0.94**
Conductivity	seawater	NS	-0.81**
	NaCl	-0.90**	-0.96**
Total extractable organic C (fumigated samples)	seawater	0.76*	NS
	NaCl	NS	-0.72*
Extractable organic C (unfumigated samples)	seawater	0.85**	NS
	NaCl	-0.82**	-0.93**
Microbial biomass C	seawater	NS	NS
	NaCl	NS	NS

\* At  $r = 0.67$ ,  $P = 0.05$ ; NS = not significant.

\*\* At  $r = 0.80$ ,  $P = 0.01$ .

did show a significant negative correlation with pH and conductivity (Table 2). The NaCl treatments showed a significant positive correlation with pH and a negative correlation with conductivity ( $P < 0.01$ ) (Table 2). The negative correlation between NaCl and OC suggests methanogens were not C limited (Table 2).

### Sulfate vs. Sodium Chloride

Sulfate (i.e., seawater treatments) and NaCl additions had significantly different effects on potential respiration, potential methanogenesis, and other variables of interest. Sulfate in high concentrations produced a short-term increase in microbial respiration (Fig. 2). This relationship between  $\text{SO}_4^{2-}$  and  $\text{CO}_2$  was also observed as a significant positive correlation between these variables ( $r = 0.64$ ,  $P < 0.01$ ) (Table 3). In contrast, methanogenesis decreased as the  $\text{SO}_4^{2-}$  concentration increased (Fig. 4). A significant negative correlation existed between  $\text{CH}_4$  and  $\text{SO}_4^{2-}$  concentration ( $r = -0.80$ ,  $P < 0.01$ ) (Table 3). Sulfate also showed a strong ( $P < 0.01$ ) positive correlation with the  $\text{CO}_2/\text{CH}_4$  production ratio and pH (Table 3). The  $\text{CO}_2/\text{CH}_4$  ratio was similar for all NaCl treatments, the  $3.5 \text{ g kg}^{-1}$  seawater treatment, and the freshwater control ( $2.0 \pm 0.5$ ), but significantly higher for the  $14 \text{ g kg}^{-1}$  seawater ( $11.2 \pm 1.4$ ) and  $35 \text{ g kg}^{-1}$  seawater ( $43.3 \pm 4.0$ ) treatments.

The NaCl addition had no effect on potential respiration (Fig. 2) nor was there a correlation between NaCl concentration and respiration (Table 3). The effect of the NaCl addition on methanogenesis was slightly greater than on respiration, with the 35 and  $14 \text{ g kg}^{-1}$  NaCl treatments reducing  $\text{CH}_4$  production for 2 wk (Fig. 4). Sodium chloride concentrations  $> 13 \text{ mg L}^{-1}$  may negatively impact methanogenesis but concentrations below this (as seen in the  $3.5 \text{ g kg}^{-1}$  seawater and NaCl treatments) were

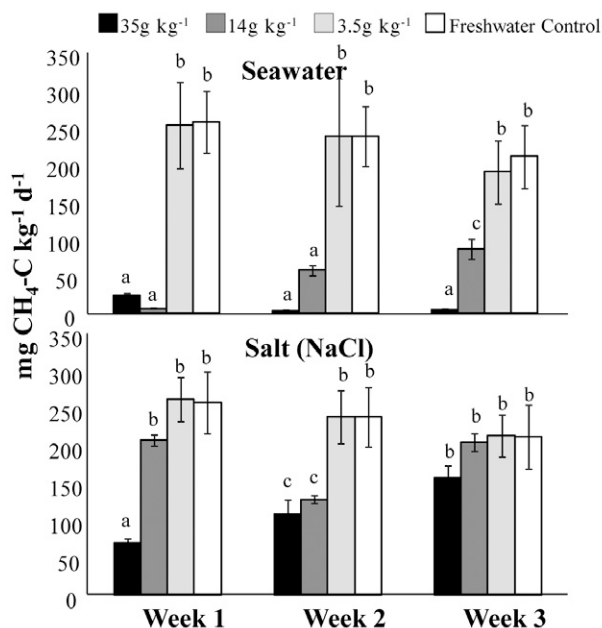


Fig. 4. Methanogenesis rate by treatment during the 3-wk incubation period. Error bars represent standard deviations;  $n = 3$  for all treatments; different letters indicate significant differences at  $P < 0.01$  for Week 1,  $P < 0.05$  for Weeks 2 and 3.

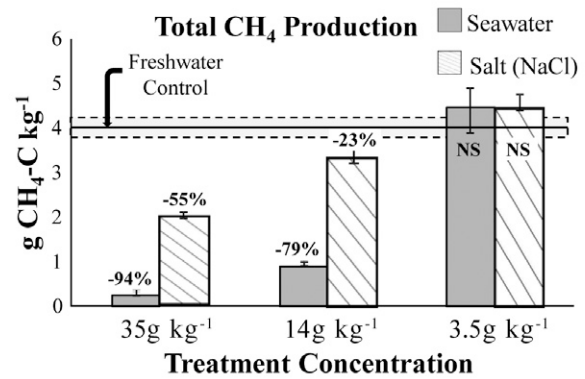


Fig. 5. Mean total  $\text{CH}_4$  produced during the 3-wk incubation period by treatment and concentration. Horizontal lines represent the mean (solid line) and standard deviation (dotted lines) of total production by the freshwater control. Percentages represent differences in mean total production compared with the freshwater control ( $P < 0.05$ ). Error bars represent standard deviations; NS = not significant;  $n = 3$  for all treatments.

not correlated with methanogenesis (Table 3). The main effect of NaCl was a significant increase in conductivity, TOC, OC, and pH (Tables 1 and 3).

### DISCUSSION

High concentrations of seawater ( $14$  and  $35 \text{ g kg}^{-1}$ ) caused a significant increase in pH, while high concentrations of NaCl ( $14$  and  $35 \text{ g kg}^{-1}$ ) decreased pH (Table 1). The increase in pH caused by seawater was probably a result of the high  $\text{CaCO}_3$  content of the seawater mix and a product of  $\text{SO}_4^{2-}$  reduction, while strictly NaCl additions may have displaced  $\text{H}^+$  ions from the cation exchange complex and caused the pH to decrease. A similar displacement of  $\text{NH}_4^+$  ions from sediments by NaCl has been observed (Baldwin et al., 2006) and is further supported by the strong negative correlation between pH and NaCl concentration in this study (Table 3). While it is unlikely that this near-neutral range in pH between treatments ( $6.4$ – $7.2$ ) could have caused microbial inhibition, it is an interesting side effect of salinization that could select for specific microbial species in

Table 3. Pearson's product correlation coefficients ( $r$ ) for the correlation of treatment water properties with soil parameters; for all values,  $n = 21$  and  $df = 19$ .

Parameter	$\text{SO}_4^{2-}$	NaCl	Conductivity	pH
	$\text{g kg}^{-1}$		$\mu\text{S m}^{-1}$	
$\text{CO}_2$ production	0.64**	NS	NS	0.71**
$\text{CH}_4$ production	-0.80**	NS	-6.1**	NS
$\text{CO}_2/\text{CH}_4$ production ratio	0.97**	NS	5.4*	0.76**
Conductivity	0.48*	0.83**	—	NS
pH	0.82**	-0.59**	NS	—
Total extractable organic C (fumigated samples)	NS	0.69**	5.4*	NS
Extractable organic C (unfumigated samples)	NS	0.84**	8.2**	NS

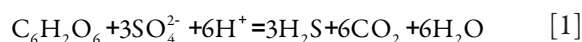
\* At  $r = 0.44$ ,  $P = 0.05$ ; NS = not significant.

\*\* At  $r = 0.55$ ,  $P = 0.01$ .

the long term. Specific conductivity significantly increased in all 14 and 35 g kg<sup>-1</sup> treatments (Table 1) but did not mirror the conductivity of the added solution due to a dilution effect by the soil pore water. Conductivity was most strongly correlated with NaCl concentration (Table 3).

### Potential Microbial Respiration

In general, seawater additions had a stimulatory effect on the potential CO<sub>2</sub> production rate (Fig. 2), and the total amount of CO<sub>2</sub> produced was directly related to the concentration of seawater added (Fig. 3). During the 3-wk incubation, the total CO<sub>2</sub> production was 32% higher in the 35 g kg<sup>-1</sup> seawater treatment, 29% higher in the 14 g kg<sup>-1</sup> seawater treatment, and 20% higher in the 3.5 g kg<sup>-1</sup> seawater treatment than the freshwater control. The stimulation of the respiration rate was short lived, however; all seawater treatments returned to a CO<sub>2</sub> production rate similar to the freshwater control by Week 3 (Fig. 2). The difference in the magnitude of the increase in CO<sub>2</sub> production in the seawater treatments can be attributed to the increased availability of SO<sub>4</sub><sup>2-</sup> to serve as a terminal electron acceptor in anaerobic microbial respiration. Using the theoretical relationship of 1 mol SO<sub>4</sub><sup>2-</sup>/2 mol CO<sub>2</sub>,



indicates that only ~20% of the SO<sub>4</sub><sup>2-</sup> added was reduced. Therefore, SO<sub>4</sub><sup>2-</sup> depletion was also not responsible for the decline in activity over time in the seawater treatments.

All of the treatments produced significantly more CO<sub>2</sub> in the first week than in Weeks 2 and 3 (Fig. 2). This could be a limitation of the experimental design. Because CO<sub>2</sub> measurements were taken from a closed system with a finite supply of bioavailable C, with time the substrate limitation would have surpassed the alternative electron acceptor availability in regulating the rate of microbial activity. The agitation of the soil slurry may have exaggerated the initial pulse of available electron donors by releasing C compounds previously protected within soil aggregates. Regardless, because all incubations were composed of replicate soil substrate, the difference in response among treatments can be attributed to the concentration of SO<sub>4</sub><sup>2-</sup> electron acceptors.

The positive correlation between CO<sub>2</sub> production and indicators of extractable C ( $P < 0.05$ ) supports the hypothesis of a C limitation. A significant decrease in the C flux rate with time has also been observed in studies of intact soil cores, with the decline in CO<sub>2</sub> and CH<sub>4</sub> production attributed to progressive C limitation (Weston et al., 2011).

Previous work has indicated that the percentage of microbial respiration mediated by SO<sub>4</sub><sup>2-</sup> reduction increases as total respiration increases (Howarth, 1984). The present study also found a logarithmic increase in the percentage of respiration attributed to SO<sub>4</sub><sup>2-</sup> reduction as the respiration rate increased. When respiration rates were highest (Week 1), 44% of respiration was mediated by SO<sub>4</sub><sup>2-</sup> reducers, and when respiration was low (Week 3), only 15% of respiration was mediated by SO<sub>4</sub><sup>2-</sup>

reducers. This was owing to the assumption that SO<sub>4</sub><sup>2-</sup> was the only alternative electron acceptor present in the seawater that was not present in the freshwater control or NaCl treatments and the fact that the soils were maintained under anaerobic conditions from the time of collection to the conclusion of the study.

In contrast to the seawater, NaCl addition had no effect on CO<sub>2</sub> production (Fig. 2). Because NaCl does not function as an electron carrier the way SO<sub>4</sub><sup>2-</sup> does, we can conclude that ionic stress alone does not affect microbial community respiration. These findings, however, do not address whether the microbial community structure or diversity was altered by the NaCl addition. Baldwin et al. (2006) performed a detailed analysis of microbial community structure using phospholipid fatty acid biomarkers and discovered that NaCl > 50 μS m<sup>-1</sup> decreased the microbial diversity but did not alter the microbial biomass. No significant differences in MBC among the NaCl treatments were identified in the present study either (Table 1). Our findings suggest that the tolerance of anaerobic microbes to ionic stress may be higher than initially anticipated, and the microbial community may have the ability to adapt to increased ionic stress within the period of 1 wk (Fig. 2). The high species richness of freshwater sediments is believed to allow the community to switch biochemical pathways in a matter of days (Edmonds et al., 2009).

### Potential Methanogenesis

Sulfate reduction is thermodynamically preferred over methanogenesis because of the higher net energy yield for obligate anaerobes (Capone and Kiene, 1988). The higher concentrations of SO<sub>4</sub><sup>2-</sup> in seawater led to the hypothesis that CH<sub>4</sub> production would be lower in seawater wetlands than freshwater wetlands. This has been confirmed by numerous studies (e.g., Abril and Iversen, 2002; Purvaja and Ramesh, 2001; Reeburgh and Heggie, 1977). Some research, however, has found that maximum CH<sub>4</sub> emissions occur at intermediate salinities (Bartlett et al., 1987; Sotomayor et al., 1994) and may still be substantial in saltwater wetlands with high C inputs (Biswas et al., 2007; Purvaja and Ramesh, 2001).

By comparing the repression of CH<sub>4</sub> in seawater and NaCl treatments, this study was able to differentiate between the effect of SO<sub>4</sub><sup>2-</sup> competition and ionic stress on methanogenic microbes. A low concentration (3.5 g kg<sup>-1</sup>) of seawater or NaCl did not affect CH<sub>4</sub> production (Fig. 4). Seawater additions of 14 g kg<sup>-1</sup> and above did significantly, and persistently, reduce methanogenesis (Fig. 4). Other work has proposed that a salinity of ≥ 13 g kg<sup>-1</sup> is required to alter CH<sub>4</sub> flux (Bartlett et al., 1987). Total CH<sub>4</sub> production in this study was 94 and 79% lower in the 35 g kg<sup>-1</sup> and 14 g kg<sup>-1</sup> seawater treatments, respectively, than the freshwater control. A strong negative correlation ( $P < 0.01$ ) between the SO<sub>4</sub><sup>2-</sup> concentration and CH<sub>4</sub> production (Table 3) suggests that SO<sub>4</sub><sup>2-</sup> reduction replaced methanogenesis as the main form of anaerobic respiration. The increase in CO<sub>2</sub> cannot be directly calculated from the decrease in CH<sub>4</sub>, however, because of the use of competitive and noncompetitive substrates between the two groups of anaerobes (Capone and Kiene, 1988).

An inverse correlation between conductivity and CH<sub>4</sub> production was found previously by Magenheimer et al. (1996).

Sodium chloride decreased CH<sub>4</sub> production but to a lesser extent than the seawater did (Fig. 4). Overall, the 35 g kg<sup>-1</sup> NaCl reduced CH<sub>4</sub> production by 55% and 14 g kg<sup>-1</sup> NaCl reduced CH<sub>4</sub> production by 23% relative to the freshwater control. This repression of methanogenesis by NaCl is slightly less than found by Baldwin et al. (2006), where as little as 10 μS m<sup>-1</sup> decreased CH<sub>4</sub> production by 30% during approximately 1 mo.

Although the soil slurry design used in this study limits the interpretation of the CH<sub>4</sub> flux rates to an estimation of potential methanogenesis, it does provide evidence for a differential sensitivity of methanogens to salt. The fact that the decline in CH<sub>4</sub> production was not directly correlated with the increase in CO<sub>2</sub> production suggests that heterotrophic methanogens, rather than autotrophic (CO<sub>2</sub> or H<sub>2</sub> using) methanogens, dominate in this soil and were most strongly affected by salt additions. Other work has suggested that high concentrations of NaCl will inhibit acetoclastic (heterotrophic) methanogens (Baldwin et al., 2006), which may have driven the short-term decrease in CH<sub>4</sub> production in the 35 and 14 g kg<sup>-1</sup> NaCl treatments (Fig. 4).

## CONCLUSIONS

This study used laboratory soil slurry incubations to assess the short-term effects of NaCl and seawater on anaerobic C cycling in a freshwater wetland soil. These idealized conditions (lack of diffusion barriers, constant redox conditions, and the exclusion of alternative electron acceptors) allowed isolation of the two opposing biogeochemical forces that act on coastal wetland soils subjected to a pulse of seawater: ionic stress and SO<sub>4</sub><sup>2-</sup>-induced respiration. Findings indicate that the concentration (g kg<sup>-1</sup>) of the seawater being introduced to the freshwater soil is the critical factor in determining its impact on soil C cycling. Oligohaline seawater (3.5 g kg<sup>-1</sup>) accelerates the overall C mineralization through the combined production of CO<sub>2</sub>-C and CH<sub>4</sub>-C (Fig. 6), thus enhancing the rate of organic C decomposition. This occurs as a result of the short-term acceleration of SO<sub>4</sub><sup>2-</sup> reduction without the inhibition of methanogenesis. The overall C mineralization rate was 17% higher in the 3.5 g kg<sup>-1</sup> seawater treatment than the freshwater control (Fig. 6). Mesohaline and haline concentrations of seawater (14 and 35 g kg<sup>-1</sup>) also produced a short-term stimulation of anaerobic respiration, but the effect was offset by a decrease in methanogenesis (Fig. 6). Although the effects on the C cycle observed in this study were temporary (1–2 wk), the increased frequency of storm surges and extreme tidal events in coastal wetlands that are expected to accompany sea level rise makes these findings significant. Additionally, the fact that the microbial response was temporary indicates that dynamic changes and “pulses” of seawater may be more influential to the C cycle in coastal wetland soils than gradual sea level rise.

The change in the CO<sub>2</sub>/CH<sub>4</sub> production ratio following seawater intrusion may have significant implications for global warming. Assuming a CO<sub>2</sub>-equivalent radiative forcing of 25 for CH<sub>4</sub> (Intergovernmental Panel on Climate Change, 2007), mid-salinity wetlands (14 g kg<sup>-1</sup>) have a 72% lower global warming

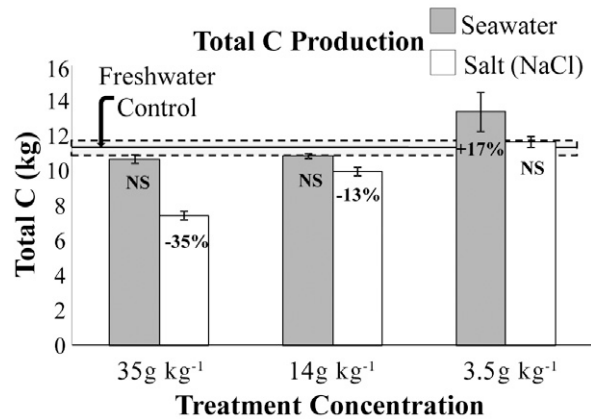


Fig. 6. Total C (CH<sub>4</sub> and CO<sub>2</sub>) produced during the 3-wk incubation period by treatment and concentration. Horizontal lines represent the mean (solid line) and standard deviation (dotted lines) of total production by the freshwater control. Percentages represent differences in mean total production compared with the freshwater control ( $P < 0.05$ ). Error bars represent standard deviations; NS = not significant;  $n = 3$  for all treatments.

potential (GWP) than freshwater wetlands, and high-salinity wetlands (35 g kg<sup>-1</sup>) have 86% lower GWP than freshwater wetlands.

Coastal wetlands in the contiguous United States are estimated to sequester 10.2 Tg C yr<sup>-1</sup>, equivalent to 31% of the total C sequestered in all contiguous U.S. wetlands (Bridgman et al., 2006). With a sea level rise occurring at ~3 mm yr<sup>-1</sup> (Intergovernmental Panel on Climate Change, 2007), the gently sloping U.S. coastal zone of the Atlantic Ocean and Gulf of Mexico are already experiencing seawater encroachment into previously fresh and low-salinity wetlands (e.g., Donnelly and Bertness, 2001; Hussein, 2009; Williams et al., 1999). The results of this study suggest that the biochemical effects of seawater intrusion, especially pulsing events, on organic C mineralization in coastal wetlands may require a reevaluation of the C balance of coastal wetlands in light of the predicted sea level rise.

## REFERENCES

- Abril, G., and N. Iversen. 2002. Methane dynamics in a shallow non-tidal estuary (Randers Fjord, Denmark). *Mar. Ecol. Prog. Ser.* 230:171–181. doi:10.3354/meps230171
- Baldwin, D.S., G.N. Rees, A.M. Mitchell, G. Watson, and J. Williams. 2006. The short-term effects of salinization on anaerobic nutrient cycling and microbial community structure in sediment from a freshwater wetland. *Wetlands* 26:455–464. doi:10.1672/0277-5212(2006)26[455:TSEOSO]2.0.CO;2
- Bartlett, K.B., D.S. Bartlett, R.C. Harriss, and D.I. Sebacher. 1987. Methane emissions along a salt-marsh salinity gradient. *Biogeochemistry* 4:183–202. doi:10.1007/BF02187365
- Biswas, H., S.K. Mukhopadhyay, S. Sen, and T.K. Jana. 2007. Spatial and temporal patterns of methane dynamics in the tropical mangrove dominated estuary, NE coast of Bay of Bengal, India. *J. Mar. Syst.* 68:55–64. doi:10.1016/j.jmarsys.2006.11.001
- Bridgman, S.D., J.P. Megonigal, J.K. Keller, N.B. Bliss, and C. Trettin. 2006. The carbon balance of North American wetlands. *Wetlands* 26:889–916. doi:10.1672/0277-5212(2006)26[889:TCBONA]2.0.CO;2
- Capone, D.G., and R.P. Kiene. 1988. Comparison of microbial dynamics in marine and fresh-water sediments: Contrasts in anaerobic carbon catabolism. *Limnol. Oceanogr.* 33:725–749. doi:10.4319/lo.1988.33.4\_part\_2.0725
- Donnelly, J.P., and M.D. Bertness. 2001. Rapid shoreward encroachment of salt marsh cordgrass in response to accelerated sea-level rise. *Proc. Natl. Acad.*

- Sci. 98:14218–14223. doi:10.1073/pnas.251209298
- Edmonds, J.W., N.B. Weston, S.B. Joye, X. Mou, and M.A. Moran. 2009. Microbial community response to seawater amendment in low-salinity tidal sediments. *Microbiol. Aquat. Syst.* 58:558–568.
- Frankenberger, W.T., and F.T. Bingham. 1982. Influence of salinity on soil enzyme activities. *Soil Sci. Soc. Am. J.* 46:1173–1177. doi:10.2136/sssaj1982.03615995004600060011x
- Gennari, M., C. Abbate, V. La Porta, A. Baglieri, and A. Cignetti. 2007. Microbial response to Na<sub>2</sub>SO<sub>4</sub> additions in a volcanic soil. *Arid Land Res. Manage.* 21:211–227. doi:10.1080/15324980701428732
- Howarth, R.W. 1984. The ecological significance of sulfur in the energy dynamics of salt marsh and coastal marine sediments. *Biogeochemistry* 1:5–27. doi:10.1007/BF02181118
- Hussein, A.H. 2009. Modeling of sea-level rise and deforestation in submerging coastal Ultisols of Chesapeake Bay. *Soil Sci. Soc. Am. J.* 73:185–196. doi:10.2136/sssaj2006.0436
- Intergovernmental Panel on Climate Change. 2007. *Climate change 2007: A synthesis report.* Cambridge Univ. Press, Cambridge, UK.
- Kester, D.R., I.W. Duedall, D.N. Connors, and R.M. Pytkowic. 1967. Preparation of artificial seawater. *Limnol. Oceanogr.* 12:176–178.
- King, G.M., and W.J. Wiebe. 1980. Regulation of sulfate concentrations and methanogenesis in salt marsh soils. *Estuar. Coast. Mar. Sci.* 10:215–223. doi:10.1016/S0302-3524(80)80059-4
- Magenheimer, J.F., T.R. Moore, G.L. Chmura, and R.J. Daoust. 1996. Methane and carbon dioxide flux from a macrotidal salt marsh, Bay of Fundy, New Brunswick. *Estuaries* 19:139–145. doi:10.2307/1352658
- Michener, W.K., E.R. Blood, K.L. Bildstein, M.M. Brinson, and L.R. Gardner. 1997. Climate change, hurricanes and tropical storms, and rising sea level in coastal wetlands. *Ecol. Appl.* 7:770–801. doi:10.1890/1051-0761(1997)007[0770:CCHATS]2.0.CO;2
- Muhammad, S., T. Muller, and R.G. Joergensen. 2006. Decomposition of pea and maize straw in Pakistani soils along a gradient in salinity. *Biol. Fertil. Soils* 43:93–101. doi:10.1007/s00374-005-0068-z
- Nelson, M.L., and L.E. Sommers. 1996. Total carbon, organic carbon, and organic matter: Loss-on-ignition method. p. 1004–1005. In D.L. Sparks (ed.) *Methods of soil analysis. Part 3. Chemical methods.* SSSA Book Ser. 5. SSSA, Madison, WI.
- Nyman, J.A., and R.D. DeLaune. 1991. CO<sub>2</sub> emission and soil Eh responses to different hydrological conditions in fresh, brackish, and saline marsh soils. *Limnol. Oceanogr.* 36:1406–1414. doi:10.4319/lo.1991.36.7.1406
- Pathak, H., and D.L.N. Rao. 1998. Carbon and nitrogen mineralization from added organic matter in saline and alkali soils. *Soil Biol. Biochem.* 30:695–702. doi:10.1016/S0038-0717(97)00208-3
- Pattanaik, P., S.R. Mishra, K. Bharati, S.R. Mohanty, N. Sethunathan, and T.K. Adhya. 2000. Influence of salinity on methanogenesis and associated microflora in tropical rice soils. *Microbiol. Res.* 155:215–220.
- Purvaja, R., and R. Ramesh. 2001. Natural and anthropogenic methane emission from coastal wetlands of South India. *Environ. Manage.* 27:547–557. doi:10.1007/s002670010169
- Reeburgh, W.S., and D.T. Heggie. 1977. Microbial methane consumption reactions and their effects on methane distributions in freshwater and marine environments. *Limnol. Oceanogr.* 22:1–9. doi:10.4319/lo.1977.22.1.0001
- Sangiorgio, F., A. Basset, M. Pinna, L. Sabetta, M. Abbiati, M. Ponti, et al. 2008. Environmental factors affecting Phragmites australis litter decomposition in Mediterranean and Black Sea transitional waters. *Aquat. Conserv. Mar. Freshwater Ecosyst.* 18:S16–S26. doi:10.1002/aqc.955
- Smith, C.J., R.D. DeLaune, and W.H. Patrick, Jr. 1983. Carbon dioxide emission and carbon accumulation in coastal wetlands. *Estuarine Coastal Shelf Sci.* 17:21–29. doi:10.1016/0272-7714(83)90042-2
- Sotomayor, D., J.E. Corredor, and J.M. Morell. 1994. Methane flux from mangrove sediments along the southwestern coast of Puerto Rico. *Estuaries* 17:140–147. doi:10.2307/1352563
- Sparling, G.P., C.W. Feltham, J. Reynolds, A.W. West, and P. Singleton. 1990. Estimation of soil microbial C by a fumigation–extraction method: Use on soils of high organic matter content, and a reassessment of the kEC factor. *Soil Biol. Biochem.* 22:301–307. doi:10.1016/0038-0717(90)90104-8
- Thomas, G.W. 1996. Soil pH and soil acidity. p. 475–490. In D.L. Sparks (ed.) *Methods of soil analysis. Part 3. Chemical methods.* SSSA Book Ser. 5. SSSA, Madison, WI.
- USEPA. 1982. Method 120.1: Conductance by conductivity meter. In *Methods for the chemical analysis of water and wastes.* EPA/600/4-79/020. USEPA Environ. Monitoring Syst. Lab., Cincinnati, OH.
- Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19:703–707. doi:10.1016/0038-0717(87)90052-6
- Weston, N.B., R.E. Dixon, and S.B. Joye. 2006. Ramifications of increased salinity in tidal freshwater sediments: Geochemistry and microbial pathways of organic matter mineralization. *J. Geophys. Res.* 111:G01009. doi:10.1029/2005JG000071
- Weston, N.B., M.A. Vile, S.C. Neubauer, and D.J. Velinsky. 2011. Accelerated microbial organic matter mineralization following salt-water intrusion into tidal freshwater marsh soils. *Biogeochemistry* 102:135–151. doi:10.1007/s10533-010-9427-4
- Wetzel, R.G. 2001. *Limnology: Lake and river ecosystems.* Academic Press, San Diego.
- Williams, K., K.C. Ewel, R.P. Stumpf, F.E. Putz, and T.W. Workman. 1999. Sea-level rise and coastal forest retreat on the west coast of Florida, USA. *Ecology* 80:2045–2063. doi:10.1890/0012-9658(1999)080[2045:SLRA CF]2.0.CO;2
- Wong, V.N.L., R.C. Dalal, and R.S.B. Greene. 2008. Salinity and sodicity effects on respiration and microbial biomass of soil. *Biol. Fertil. Soils* 44:943–953. doi:10.1007/s00374-008-0279-1