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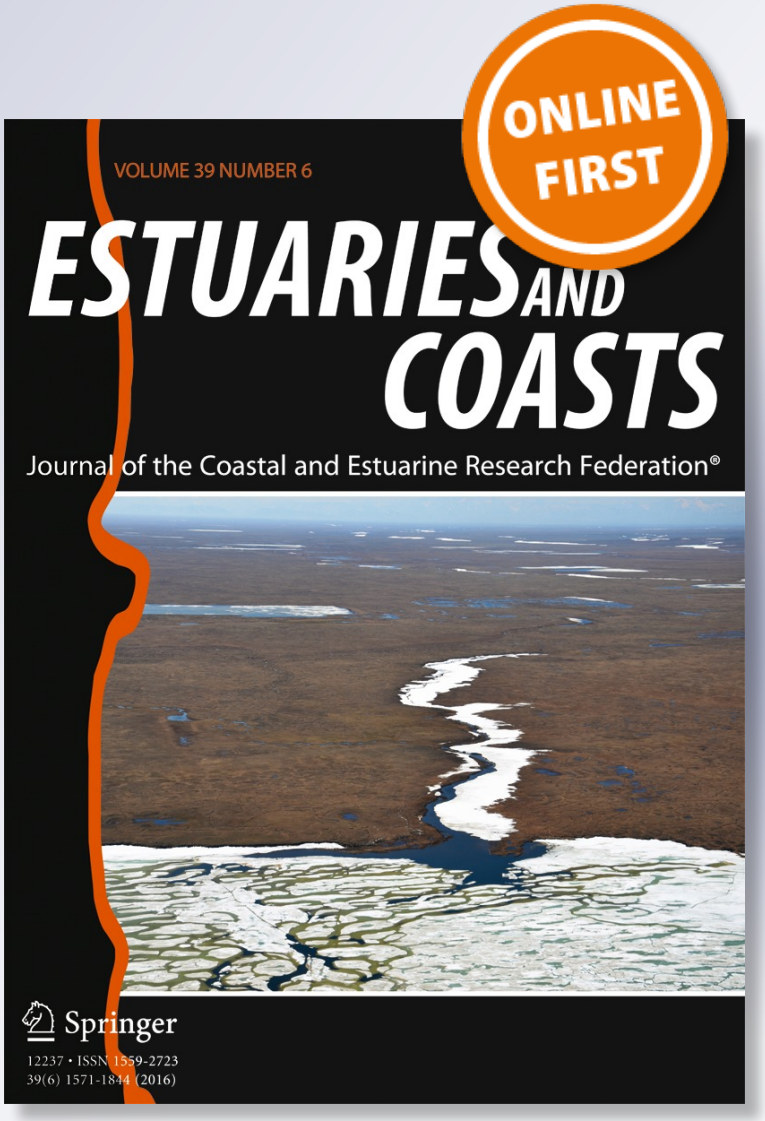

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Gulf Ribbed Mussels (*Geukensia granosissima*) Increase Methane Emissions from a Coastal *Spartina alterniflora* Marsh

Anthony J. Rietl[1,2] · **John A. Nyman**[1] · **Charles W. Lindau**[3] · **Colin R. Jackson**[4]

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Abstract Understanding methane emissions from natural sources is becoming increasingly important with future climactic uncertainty. Wetlands are the single largest natural source of methane; however, little attention has been given to how biota and interactions between aboveground and belowground communities may affect methane emission rates in these systems. To investigate the effects of vegetative disturbance and belowground biogeochemical alterations induced by biota on methane emissions in situ, we manipulated densities of *Littoraria irrorata* (marsh periwinkle snails) and *Geukensia granosissima* (gulf ribbed mussels) inside fenced enclosures within a *Spartina alterniflora* salt marsh and measured methane emissions and sediment extracellular enzyme activity (phosphatase, β-glucosidase, cellobiohydrolase, *N*-acetyl-β-D-glucosaminidase, peroxidase, and phenol oxidase) over the course of a year. Changes in snail density did not have an effect on methane emission; however, increased densities of ribbed mussels significantly increased the emission of methane. Sediment extracellular enzyme activities for phosphatase, cellobiohydrolase, *N*-acetyl-β-D-glucosaminidase, and phenol oxidase were correlated to methane emission, and none of the enzymes assayed were affected by the snail and mussel density treatments. While methane emissions from salt marsh ecosystems are lower than those from freshwater systems, the high degree of variability in emission rates and the potential for interactions with naturally occurring biota that increase emissions warrant further investigations into salt marsh methane dynamics.

Keywords Ribbed mussels · Methane · Salt marsh · Extracellular enzymes

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✉ Anthony J. Rietl
ARietl@Lumcon.edu

[1] School of Renewable Natural Resources, Louisiana State University, Baton Rouge, LA 70803, USA

[2] Present address: Louisiana Universities Marine Consortium, Chauvin, LA 70344, USA

[3] Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

[4] Department of Biology, The University of Mississippi, Shoemaker Hall, University, MS 38677, USA

Introduction

Globally, annual wetland methane (CH_4) emissions ranged from 209 to 245 Tg CH_4 $year^{-1}$ between 1901 and 2012, and wetlands are considered the single largest source of CH_4 (Walter et al. 2001). CH_4 is the second most abundant non-carbon dioxide (CO_2) greenhouse gas in the atmosphere (Montzka et al. 2011) and has contributed 25 % of the total climate forcing over the past 250 years (Shindell et al. 2009). The warming potential of CH_4 is 28 times that of CO_2 (Myhre et al. 2013), and CH_4 levels in the atmosphere have exhibited a persistent increasing trend since 1750, with levels reaching 1803 ppb in 2011 (Ciais et al. 2013). There is a large amount of interannual variability in global emissions with an estimated 50–70 % of the global interannual variability attributed to wetland CH_4 emissions (Bousquet et al. 2006; Chen and Prinn 2006); therefore, changes in CH_4 emission rates from wetlands are important to understand in terms of accounting for this interannual variability and modeling global climate change.

In salt marsh sediments, high amounts of sulfate available for sulfate-reducing bacteria in anoxic sediments present direct competition to CH_4-generating microorganisms or

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methanogens. Consequently, CH_4 emissions from salt marshes are typically much lower than from a freshwater wetland (DeLaune et al. 1983), though the degree to which CH_4 emissions are altered by salinity varies with salt concentration (Poffenbarger et al. 2011). Other major controls over methane emissions in marshes are temperature (King and Wiebe 1978; Bartlett et al. 1985), inundation (Harriss et al. 1982; Moore et al. 1990), net ecosystem production (Whiting and Chanton 1993), and the presence and species of plant (Bergstrom et al. 2007; Kao-Kniffin et al. 2010), though CH_4 emissions from wetlands can only partially be explained by correlations with environmental variables (Hogan 1993; Kettunen et al. 1996; Bousquet et al. 2006).

Benthic macrofauna living in or on the surface of marsh sediments can significantly alter many marsh processes, contributing to the physical structure of the marsh, influencing the biogeochemistry of the sediment through carbon inputs, oxygenating the sediment, inducing alterations in the nitrogen cycle (Jones et al. 1994; Kristensen and Kostka 2005; Laverock et al. 2011), and influencing plant production (Bertness 1984b). Salt marshes of the gulf and Atlantic coasts of the USA harbor large populations of bivalves (Honig et al. 2014) and aboveground mollusks (Silliman and Zieman 2001) that have the potential to alter biogeochemical conditions either directly or through a plant intermediary, and thus CH_4 emissions.

The salt marsh bivalve *Geukensia granosissima* (gulf ribbed mussel) inhabits the sediment and anchors to nearby hard substrata or marsh vegetation with strong bissal thread attachments (Franz 1997). These organisms are filter feeders that deposit nutrient-rich feces and pseudofeces into the sediment which could potentially create microniches in which unique microbial communities develop (Fenchel 1996; Kristensen and Kostka 2005). The rapid development of these communities in and around the nutrient-rich substrate provided by the mussels could influence ecosystem-scale biogeochemical cycling (Mermillod-Blondin and Rosenberg 2006; Laverock et al. 2011; Xu et al. 2014) and may influence the emission of CH_4 from sediments (Bonaglia et al. 2014). Alternatively, mussels could influence the production of CH_4 indirectly through alterations in sulfate and sulfide (Dahlback and Gunnarsson 1981; Kraus and Doeller 2004). Microbial symbionts living in the guts of benthic macrofauna (Harris 1993; Stief and Eller 2006) could be responsible for altering local-scale CH_4 emissions, as has been shown for other invertebrates (Brauman et al. 1992). It is also possible that biofilms growing on the exterior of invertebrate shells, which have been shown to increase nitrous oxide production (Stief et al. 2009; Heisterkamp et al. 2010; Welsh et al. 2015), are involved in methane production or consumption.

Aboveground mollusks, such as the marsh periwinkle (*Littoraria irrorata*), also have the potential to alter CH_4 emissions due to their feeding behavior on the stems of plants, which creates small wounds, or radulations, along the stems of *Spartina alterniflora* (Silliman and Zieman 2001), the dominant plant species in many gulf and Atlantic salt marshes. Up to 90 % of total CH_4 emissions from vegetated wetlands may result from emergent aquatic vegetative transport (Sebacher et al. 1985; Van der Nat and Middelburg 1998; Bergstrom et al. 2007), and this snail's feeding behavior could alter gas exchange between the plant stems and roots and the amount of CH_4 actively reaching the atmosphere through the stems of plants, as has been shown for other types of herbivory (Bodelier et al. 2006; Dingemans et al. 2011; Petruzzella et al. 2015). As this species of snail is thought to exert top-down control over *S. alterniflora* production (Silliman and Zieman 2001), given the effects of plants on CH_4 emission and the relationship with CH_4 and net ecosystem production (Whiting and Chanton 1993), *L. irrorata* may be influencing CH_4 indirectly via effects on *S. alterniflora*.

In *S. alterniflora* marshes of coastal southeastern Louisiana, *L. irrorata* and *G. granosissima* are abundant, but their impact in terms of altered CH_4 fluxes from these marshes is unknown. In this study, we conducted a year-long field experiment to elucidate the potential effects of *L. irrorata* and *G. granosissima* on CH_4 emissions and microbial community function via six extracellular enzymes involved in the microbial acquisition of C, P, and N (β-glucosidase, phosphatase, *N*-acetyl-β-D-glucosaminidase, cellobiohydrolase, phenol oxidase, and peroxidase) in a *S. alterniflora* marsh in southeastern Louisiana by field density manipulations of *L. irrorata* and *G. granosissima* within fenced enclosures.

Methods

Sites and Experimental Design

The study took place in a microtidal salt marsh (<15 ppt) dominated by *S. alterniflora* near Cocodrie, LA (29° 13′ 35.85″ N, 90° 40′ 49.03″ W) in the northern Gulf of Mexico. Three field sites were established approximately 150 m apart, each containing six plots. Ambient densities of *L. irrorata* and *G. granosissima* were counted in the area of each site by tossing a 1-m^2 PVC quadrat into the marsh 65 times to determine baseline densities in the area. *L. irrorata* was determined to have a mean (±SE) density of 80 ± 4.2 snails m^{-2}, and *G. granosissima* density was 12 ± 1.5 mussels m^{-2}. Eighteen 0.25-m^2 enclosures were built prior to placing in the field by using four wooden stakes and steel-galvanized hardware cloth with a zinc coating (Wire Cloth Man, Mine Hill, NJ), where the zinc coating is used to deter snail escape (Bertness 1984a). Clear plexiglass base chambers (30 × 30 × 30 cm), as described by Lindau et al. (1991), were installed to approximately 10 cm of depth at each of six plots at each site at the end of May, 2013 and remained

in place for the duration of the experiment. Fenced enclosures were installed around the base chambers at all plots. After installation of the enclosures, all snails and mussels were removed from within the enclosures and base chambers, and after 2 weeks, sediment samples were taken for pretreatment analysis of sediment extracellular enzyme activity. Snails and mussels were then collected from the area outside of the experimental plots; shell sizes were measured by using digital calipers (Table 1), and the animals were added to the 0.25-m^2 enclosures in a 3 × 2 factorial design, with three levels of snail density (0, 20, 60) representing no snails, ambient snail density, and 3× ambient snail density and two levels of mussel density (0, 3) representing no mussels and ambient mussel density. Each site contained these six treatments without replication; therefore, the sites themselves were our replicates. Mussels were collected by carefully cutting the bissal threads attached to *S. alterniflora* roots rather than pulling the animal loose in order to reduce the possibility of injury.

Extracellular Enzyme Assays and Methane Sampling

Samples for extracellular enzyme analysis were collected in sterile 50-ml centrifuge tubes from a depth of 5–10 cm once before manipulating the treatments (time 0) and then once every 3 months thereafter for 1 year (3, 6, 9, and 12 months). Samples were placed on ice and refrigerated upon return to the lab. Each sample consisted of a homogenous mixture of five random sampling points from within each enclosure. The activity of six microbial extracellular enzymes was assayed in each sediment sample. Potential activities of β-glucosidase (EC 3.2.1.21), phosphatase (EC 3.1.3.2), *N*-acetyl-β-D-glucosaminidase (NAG; EC 3.2.1.52), cellobiohydrolase (CBH; EC 3.2.1.91), phenol oxidase (EC 1.10.3.2), and peroxidase (EC 1.11.1.7) were determined by using the protocols demonstrated by Jackson et al. (2013) that have previously been used for wetland sediments (Jackson and Vallaire 2007; Jackson et al. 2009). These specific enzymes were chosen in order to assess microbial community function in terms of microbial acquisition of carbon (β-glucosidase, cellobiohydrolase), nitrogen (NAG), and phosphorus (phosphatase) and the breakdown of large recalcitrant molecules (phenol oxidase, and peroxidase). For each sample, a 10 g subsample was homogenized in pH 5.5 50 mM acetate buffer, and 150 μl aliquots of the slurry were used in individual reactions. Five millimolar 3,4-dihydroxyl-L-phenylalanine (L-DOPA) was used as the substrate for the phenol oxidase and peroxidase assays, and peroxidase assays also received 0.015 % H_2O_2. Five millimolar *p*-nitrophenyl (pNP)-linked substrates were used for the β-glucosidase and phosphatase assays, while 2 mM pNP-linked substrates were used to determine NAG and CBH activity (Jackson et al. 2013). All substrates were dissolved in pH 5.5 50 mM acetate buffer. Assay incubation times were 0.5–1 h for phosphatase and β-glucosidase, 2 h for phenol oxidase and peroxidase, and 3 h for NAG and CBH.

After incubation, assays were centrifuged (4000×*g*, 10 min) and 100 μl supernatant transferred to reading microplates containing 200 μl 0.067 M NaOH (pNP-linked substrates) or 200 μl H_2O (L-DOPA substrates). Absorbance was read at 410 nm for pNP-linked substrates and 460 nm for L-DOPA substrates by using a Synergy HT microplate spectrophotometer (BioTek, Winooski, VT). Enzyme activity was calculated after accounting for control absorbance and expressed as μmol substrate consumed h^{-1} g^{-1} dry weight of sediment.

CH_4 emissions were measured monthly for 1 year beginning in July, 2013 by using clear Plexiglas chambers (30 × 30 × 30 cm) that were sampled following the common protocol of Lindau et al. (1991) for closed chamber measurements. Each chamber top consisted of a sampling port (rubber septum), battery operated 12 V fan, pressure control tube, and a thermometer. The pressure control consisted of 1 m of plastic tubing (1.5 mm I.D.) that maintained equilibrium gas pressure between the outside and inside of chambers. Prior to sampling, Vacutainers were placed on a high-vacuum preparation line to remove any residual gases and then re-sealed with silicone rubber. For each headspace sample, 15 ml was removed from the flux chambers with a syringe and injected into a 10-ml

Table 1 Snail (*Littoraria irrorata*) (n = 20–80) and mussel (*Geukensia granosissima*) (n = 3–6) shell sizes for density manipulation treatments at three sites in a southeastern Louisiana salt marsh vegetated with *Spartina alterniflora*

Site	Snail-mussel density (m^{-2})	Snail shell size (mm)	Mussle shell size (mm)
1	0–12	0	88.13 ± 2.2
	80–0	23.11 ± 0.22	0
	80–12	23.18 ± 0.23	78.9 ± 2.8
	240–0	23.33 ± 0.30	0
	240–12	22.87 ± 0.16	78.77 ± 2.5
2	0–12	0	85.63 ± 1.9
	80–0	22.03 ± 0.17	0
	80–12	22.03 ± 0.13	82.10 ± 6.1
	240–0	22.48 ± 0.16	0
	240–12	22.36 ± 0.30	83.77 ± 2.7
3	0–12	0	78.13 ± 1.2
	80–0	23.07 ± 0.25	0
	80–12	22.40 ± 0.16	76.93 ± 2.2
	240–0	22.98 ± 0.21	0
	240–12	23.20 ± 0.24	79.03 ± 1.5

Values are means ± SE

evacuated gas Vacutainer. Twelve samples were taken per site (two from each of six plots), and a slight over pressure of headspace gas was injected into each Vacutainer to prevent atmospheric contamination (Lindau et al. 1991). After collecting each sample, 100 % silicone rubber sealant was spread over the puncture in the rubber septum. All gas samples were analyzed on Shimadzu 14-A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD) fitted with a flame ionization detector within 24 h of collection, and 1 ml of sample was injected for analysis.

At each plot during each sampling event, we measured pH, water temperature, salinity, and conductivity by using a YSI handheld probe (YSI Incorporated, Yellow Springs, OH). Water depth within the base chamber was also measured, along with the height of the stacked chambers for calculation of headspace volume. Air temperature was recorded periodically during sampling, and internal chamber temperatures were monitored and recorded in order to adjust for temperature in our CH_4 calculations (IAEA 1992). A subset of sediment taken from samples collected for extracellular enzyme analysis was dried and combusted (500 °C, 2 h) to determine organic content as ash free dry weight (AFDW). At the end of the experiment, aboveground biomass within each base chamber was clipped, dried in an oven (80 °C, 4 days), and weighed.

Statistical Analysis

All data analyses were performed in R version 3.0.1 (http://www.r-project.org). CH_4 emissions were square root transformed in order to bring our observations as close to normality as possible and then analyzed via a mixed effects model by using package nlme. Due to the seasonal nature of our CH_4 emission data and inherent autocorrelation, we used a correlation structure consisting of a continuous auto regressive model of order 1 (corCAR1 in the gls function of the nlme package as described in Zuur et al. 2009). Therefore, our mixed effects model tested the effects of snail and mussel density treatments on CH_4 emission while taking into account the known autocorrelation in CH_4 emission over time. Sediment enzyme activity was analyzed for each enzyme to test for differences in snail and mussel density treatments and sampling month by using three-way ANOVA followed by a Tukey's honest significance test. Enzyme activities were either square root or log transformed in order for data to meet the assumptions of normality. We tested for correlations between individual enzymes, enzyme activities, and CH_4 emission and tested for relationships between CH_4 emission and enzyme activities with our measured environmental variables by using linear regression.

Results

Environmental Variables

None of the measured environmental variables (Table 2) differed by density treatment over the course of the study. Since environmental variables were not significantly different in terms of treatments within individual sites, we also pooled these variables together to examine differences between sites. Sediment carbon content (C %) was both higher and more variable at site 3 than at sites 1 and 2 (C % was 21.85 ± 0.9, 23.07 ± 0.6, and 27.15 ± 1.1 for sites 1, 2, and 3, respectively). Salinity levels over the course of the study ranged between 6 and 14.75 ppt (Table 2). Aboveground biomass for each plot sampled at the end of the experiment ranged between 80.4 and 180.3 g DW (Table 2), and mean aboveground biomass for sites 1, 2, and 3 were 127.28 ± 10.5, 126.87 ± 12.9, and 130.40 ± 13.4 g DW, respectively.

Extracellular Enzyme Activities

Extracellular enzyme activity ranged between 0.04 and 8.44 μmol h^{-1} g DW^{-1} with phosphatase exhibiting the highest range in activity and phenol oxidase the lowest (Fig. 1). Enzyme activities for CBH and NAG exhibited a similar temporal pattern, where a spike in activity was observed in the September, 2013 sample, which corresponded to the first enzyme sampling after applying the snail and mussel density treatments. Phosphatase activity showed a general increasing trend from June, 2013 to January, 2014 for all treatments, at which point activity leveled off and partially declined, and phenol oxidase activity showed a general decrease in activity level for all treatments over the course of the experiment (Fig. 1). In terms of correlations, phosphatase activity was correlated to β-glucosidase ($r = 0.53$), β-glucosidase was correlated to CBH and NAG ($r = 0.52$ and 0.54, respectively), CBH correlated to NAG and phenol oxidase ($r = 0.93$ and 0.46, respectively), and NAG was correlated to phenol oxidase ($r = 0.43$).

Of the six enzymes tested, β-glucosidase was the only enzyme to show differences based upon experimental treatments where an interaction between snail and mussel density significantly ($p < 0.05$) increased β-glucosidase activity; however, this was only seen in our treatments consisting of ambient (control) animal densities. Since the other five enzymes did not show differences based upon experimental treatments within sites, we again looked at site differences. NAG and phenol oxidase showed differences based upon site, with NAG exhibiting higher activity at site 3 and phenol oxidase a reduction in activity at site 3. Phosphatase, CBH, and peroxidase showed no differences based upon site.

Relating extracellular enzyme activities to our measured environmental variables showed that β-glucosidase,

Table 2 Environmental characteristics of three sites undergoing treatment combinations of snail (*Littoraria irrorata*) and mussel (*Geukensia granosissima*) density in a southeastern Louisiana salt marsh vegetated with *Spartina alterniflora*

Site	Snail-mussel density (m^{-2})	Salinity (ppt)	pH	Water depth (cm)	Carbon %	Aboveground biomass (g DW)
1	0–0	6.60 ± 1.2	6.87 ± 0.1	7.22 ± 3.1	25.26 ± 1.7	125.3
	0–12	13.81 ± 1.3	7.02 ± 0.1	6.67 ± 3.0	17.77 ± 1.2	133.5
	80–0	9.64 ± 1.3	6.94 ± 0.1	6.89 ± 3.2	19.55 ± 0.6	162.4
	80–12	11.03 ± 1.0	6.83 ± 0.1	6.17 ± 2.9	27.57 ± 1.4	98.3
	240–0	11.84 ± 1.2	6.94 ± 0.1	7.56 ± 3.4	17.81 ± 0.4	98.2
	240–12	10.15 ± 0.9	6.94 ± 0.1	7.44 ± 3.3	23.12 ± 1.9	146
2	0–0	6.00 ± 1.8	6.80 ± 0.1	6.22 ± 3.1	22.86 ± 0.6	112.6
	0–12	6.56 ± 1.7	6.89 ± 0.2	6.78 ± 3.2	23.46 ± 1.0	89.3
	80–0	13.25 ± 1.6	7.09 ± 0.1	7.00 ± 3.4	20.81 ± 1.5	129.3
	80–12	13.00 ± 1.7	6.98 ± 0.2	6.33 ± 3.1	27.99 ± 1.2	163.6
	240–0	14.75 ± 1.4	7.01 ± 0.1	6.67 ± 3.2	20.89 ± 1.8	164.1
	240–12	8.57 ± 1.8	7.16 ± 0.1	6.86 ± 4.0	22.44 ± 1.0	102.3
3	0–0	11.60 ± 1.2	6.82 ± 0.1	6.22 ± 3.1	27.11 ± 1.1	180.3
	0–12	11.64 ± 1.4	6.95 ± 0.1	5.56 ± 2.8	24.66 ± 2.6	129.3
	80–0	11.84 ± 1.4	6.83 ± 0.1	6.44 ± 3.2	26.23 ± 2.2	146.6
	80–12	11.23 ± 1.9	7.01 ± 0.1	5.67 ± 2.8	26.74 ± 2.4	119.2
	240–0	11.18 ± 1.5	6.94 ± 0.1	6.11 ± 3.1	29.70 ± 4.6	80.4
	240–12	12.70 ± 1.0	6.98 ± 0.1	6.11 ± 3.0	28.45 ± 3.3	126.6

Values were measured monthly between July, 2013 and August, 2014 and are means ± SE, except for aboveground biomass which was measured at the end of the experiment (August, 2014)

phosphatase, CBH, and NAG all tended to have higher activity levels in treatments with higher C % ($r = 0.78$, 0.51, 0.47, and 0.57, respectively), which also corresponded to treatments that contained mussels. We also observed that CBH, NAG, and phenol oxidase were influenced by salinity; however, the relationship was weaker for phenol oxidase ($r = 0.53$, 0.64, and 0.33, respectively).

Methane Emissions

CH_4 emissions ranged between 0 and 32.25 mg CH_4 m^{-2} h^{-1} with a mean annual emission rate of 4.53 ± 0.41 mg CH_4 m^{-2} h^{-1} (39.68 g CH_4 m^{-2} $year^{-1}$) for all treatments combined. The emission of CH_4 from our sites showed a distinct seasonal pattern that followed our temperature measurements (Fig. 2), where emission rates were highest between July and October of 2013, at which point emission rates dropped in November, 2013 (Fig. 2). CH_4 emissions stayed low throughout the winter months (November, 2013–March, 2014) at which point emission rates began to rise again until the end of the experiment (August, 2014; Fig. 2). We did not detect any correlations between CH_4 emission and our measured environmental variables, other than a weak negative correlation between CH_4 emission and salinity ($r = -0.36$). However, extracellular enzyme activity from four of the six enzymes assayed was correlated to CH_4 emission ($r = -0.41$, 0.60, 0.62, and 0.46 for phosphatase, CBH, NAG, and phenol oxidase, respectively).

CH_4 emissions showed a significant response ($p < 0.05$) to our mussel treatments, where CH_4 emissions were elevated when mussels were present (Fig. 3). We did not, however, see a significant effect due to snail density in our treatments. The differences observed in CH_4 emission with respect to mussel density were more pronounced in the summer and spring months as opposed to the winter months when CH_4 emissions were relatively low (Fig. 3). On an annual basis, CH_4 emissions from treatments with and without mussels were 5.42 ± 0.67 and 3.67 ± 0.45 mg CH_4 m^{-2} h^{-1} or 47.48 and 32.15 g CH_4 m^{-2} $year^{-1}$, respectively.

Discussion

In this study, data gathered on the effects of differing snail and mussel densities on CH_4 emissions and extracellular enzyme activities in a *S. alterniflora* salt marsh suggests that snail density did not influence the emission of CH_4; however, the presence of mussels was related to an increase in CH_4 emission. β-glucosidase exhibited a treatment effect where an interaction between snail and mussel densities increased activity; however, this was only seen in our treatments consisting of ambient (control) animal densities, and none of the five other enzymes tested showed effects based upon density treatments.

The pattern of CH_4 emission observed in our study clearly shows the effects of seasonality on marsh CH_4 gas production and interestingly also shows that the high variability in

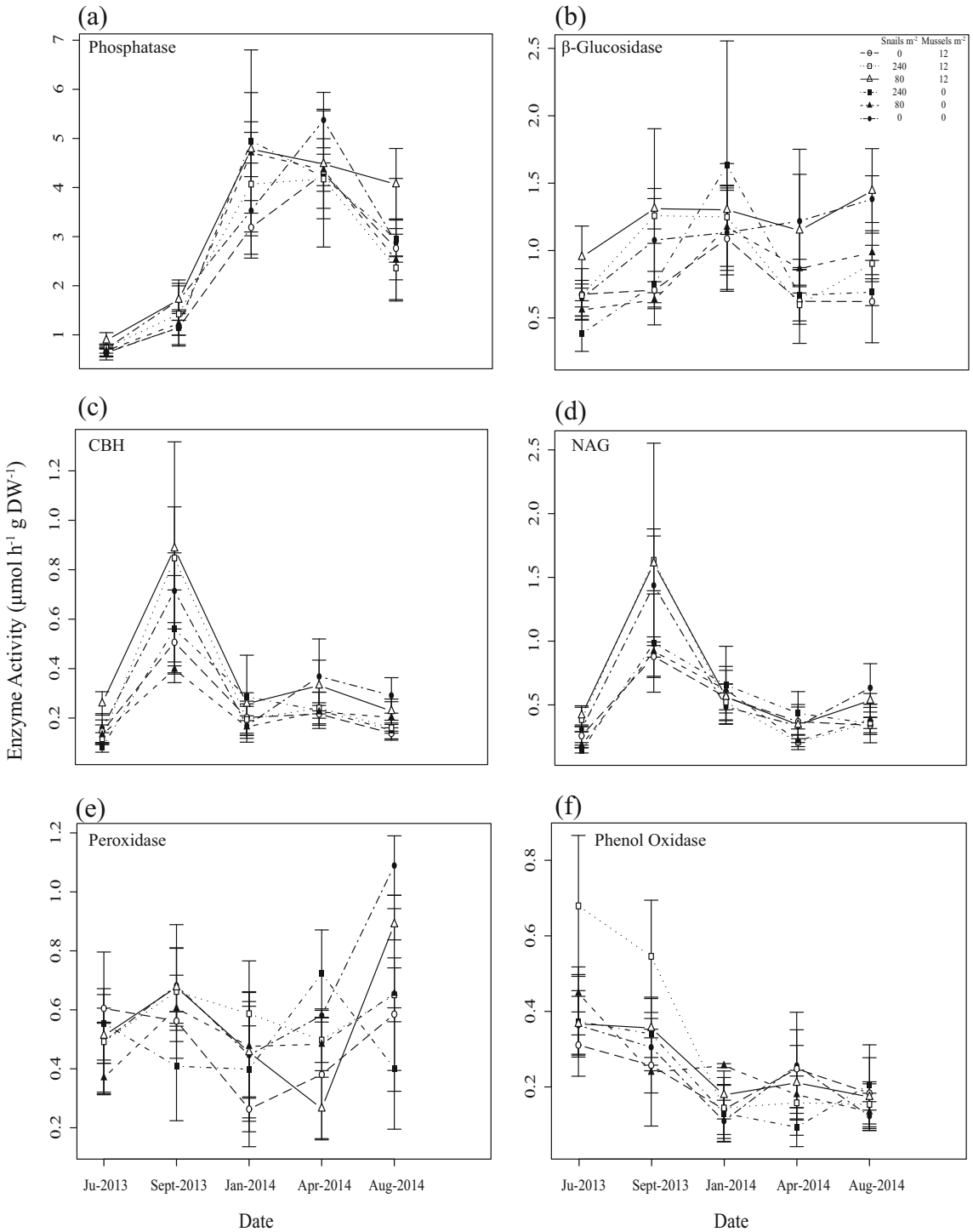

Fig. 1 Potential extracellular enzyme activity (μmol h^{-1} g DW^{-1}) for **a** phosphatase, **b** β-glucosidase, **c** cellobiohydrolase (CBH), **d** *N*-acetyl-β-D-glucosaminidase (NAG), **e** peroxidase, and **f** phenol oxidase in sediments taken from three sites in a southeastern Louisiana salt marsh vegetated with *Spartina alterniflora*, in each of six snail (*Littoraria irrorata*) and ribbed mussel (*Geukensia granosissima*) density treatments. Samples were taken at 3-month intervals between June, 2013 and August, 2014. Values are means ± SE for each treatment with sites averaged together. *Open circles* represent treatments with 0 snails and 12 mussels m^{-2}, *open squares* 240 snails and 12 mussels m^{-2}, *open triangles* 80 snails and 12 mussels m^{-2}, *black squares* 240 snails and 0 mussels m^{-2}, *black triangles* 80 snails and 0 mussels m^{-2}, and *black circles* 0 snails and 0 mussels m^{-2}

emissions from treatments that had the highest number of snails and mussels at the beginning of our experiment completely disappears with the arrival of winter, indicating changes in CH_4 production and/or consumption, as well as

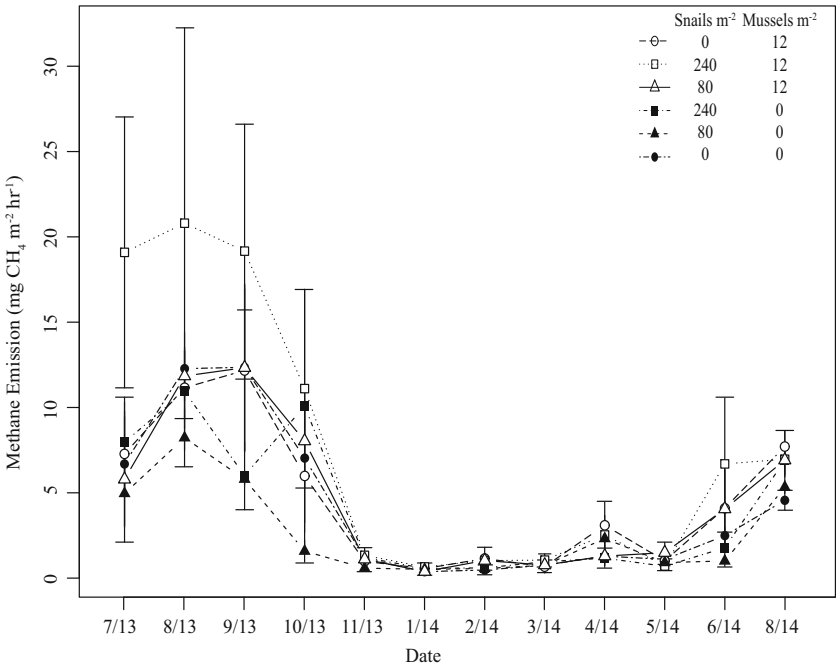

Fig. 2 Methane emission (mg CH_4 m^{-2} h^{-1}) from three sites in a southeastern Louisiana salt marsh vegetated with *Spartina alterniflora* in each of six snail (*Littoraria irrorata*) and ribbed mussel (*Geukensia granosissima*) density treatments. Measurements were taken monthly from July, 2013 to August, 2014. *Open circles* represent treatments with 0 snails and 12 mussels m^{-2}, *open squares* 240 snails and 12 mussels m^{-2}, *open triangles* 80 snails and 12 mussels m^{-2}, *black squares* 240 snails and 0 mussels m^{-2}, *black triangles* 80 snails and 0 mussels m^{-2}, and *black circles* 0 snails and 0 mussels m^{-2}. Values are means ± SE

possible changes in the activities of the mussels used in this study. One probable explanation for this temporary response in CH_4 emission that we observed is that our treatment consisting of the highest snail and mussel densities created an initial temporary shock to the system, where two organisms known to influence *S. alterniflora* production (Bertness 1984b; Silliman and Zieman 2001) are introduced at high densities and thereby affect CH_4 emissions through the intermediary of the plant. However, even as CH_4 emissions were not related to snail density, we did see a consistent significant trend with respect to elevated CH_4 emissions related to mussel density, a trend that continued even after the winter drop in CH_4 emission rates.

The method by which *G. granosissima* influenced CH_4 emissions in this study are unknown and to our knowledge has not been investigated previously. It is likely that changes in CH_4 emissions in our treatments with mussels are due to biogeochemical changes induced by the mussels

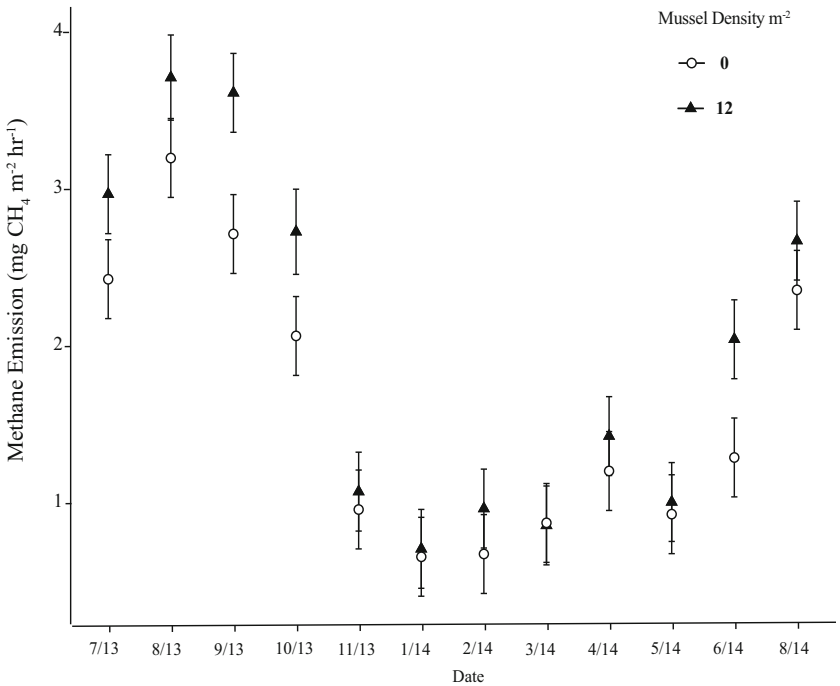

Fig. 3 Least-squared means of methane emission (mg CH_4 m^{-2} h^{-1}) from a mixed effects model from three sites in a southeastern Louisiana salt marsh vegetated with *Spartina alterniflora* for each of two mussel density treatments. *Open circles* represent 0 mussels m^{-2}, and *black triangles* are 12 mussels m^{-2}. Values are means ± SE

belowground. *Geukensia dimissa* (the Atlantic ribbed mussel) is known to increase *S. alterniflora* production in Atlantic salt marshes (Bertness 1984b), as well as altering soil nutrient levels via filter feeding and biodeposition (Jordan and Valiela 1982; Bertness 1984b). It is possible that as plant production increases, CH_4 emissions could be affected by increased plant transport to the atmosphere; however, we did not detect any changes in aboveground plant biomass due to our treatments and cannot say that plant production, as influenced by mussel density, had any affect on our measured CH_4 emissions. Another possible explanation is that temperature increases due to the closed chamber method of measuring gases in wetlands had an effect on mussel activity.

Ribbed mussels filter a large portion of water volume in an inundated salt marsh, and the result of this feeding behavior is that particulate organic matter and waste products from the organisms that are high in N and P are consolidated and deposited in the marsh sediment (Jordan and Valiela 1982). While these belowground changes in nutrient concentration could potentially alter CH_4 emissions, it is likely that we would have observed a corresponding change in extracellular enzyme activities related to mussel-induced nutrient loading. Since no treatment effect was observed for any of the N and P active extracellular enzymes we tested, we cannot conclude that nutrient changes due to mussels are implicated in the observed changes in CH_4 emission. The effects of nutrient addition on CH_4 dynamics under field conditions are not clear, and experimental effects tend to vary with the type, quantity, and method of nutrient application. CH_4 oxidation has been shown to be inhibited by nitrogen fertilizers (Crill et al. 1994; Alam and Jia 2012), and urea fertilization has been shown to enhance CH_4 production (Wang et al. 1992), which are possible mechanisms by which the mussels in our treatments could have affected CH_4 production or consumption.

One possible explanation for our observation of increased CH_4 emission rates in the presence of mussels is that biodeposition of feces and pseudofeces from *G. granosissima* into the sediment could potentially create microzones or small localized areas that differ in biogeochemistry from the surrounding regions (King and Wiebe 1980) that may have been missed in our enzyme sampling. These microsites of nutrient-rich biodeposition could lead to rapid localized microbial growth, thereby depleting O_2 concentrations in the immediate vicinity. This O_2 depletion surrounding mussel biodeposits could lead to increased CH_4 emissions simply by creating a more hospitable anaerobic environment for methanogens in the rhizosphere. However, in a sulfate-rich salt marsh, the increased anoxia would likely lead to increased rates of sulfate reduction, thereby depleting the microzone of available sulfate first (King and Wiebe 1980), at which point a switch to methanogenesis becomes favorable. Even as sulfate reduction is known to preclude methanogenesis in marshes with ample sulfate, it has also been shown that these processes can occur simultaneously (Oremland et al. 1982; Purvaja and Ramesh 2001). Relatedly, there could also be an indirect effect of mussels on the production of CH_4 through known alterations in sulfate and sulfide (Dahlback and Gunnarsson 1981; Kraus and Doeller 2004).

Extracellular enzyme activities observed in this study were within the range of activity observed in other salt marsh enzyme studies (Ravit et al. 2003; Rietl et al. 2016), and activity levels varied over the course of the year, revealing patterns that are useful in interpreting the seasonal fluxes of nutrients at our study sites. Phosphatase activity has been shown to decrease in response to phosphate additions (Allison and Vitousek 2005), indicating that possibly for our sites, phosphorus was readily available in the warmer months but then quickly began to deplete in winter; however, many other factors could also influence phosphatase activity. This could be a tidal response, as water levels at our sites were much lower for the winter months than for spring and summer, indicating that rising tides bring in the majority of available phosphorus to our sites. We also observed a late summer to autumn spike in CBH and NAG activity, which preceded the rapid increase in phosphatase activity in January, 2014; however, due to the 3-month interval between sampling for enzyme activities, we cannot conclude that these patterns in activity are related.

CH_4 emissions from salt marsh ecosystems are often thought to be of negligible importance in terms of global annual CH_4 estimates; however, the high degree of variability in emission rates and the potential for interactions that increase the emission of CH_4 from a salt marsh warrant further investigations into salt marsh CH_4 dynamics. Sasser et al. (2014) have estimated that saltwater marsh occupies a land area of approximately 2954 km^2 in coastal Louisiana. Using this estimate and our mean rate of CH_4 emission, we find that without mussels present in the sediment, these marshes will emit approximately 0.10 Tg CH_4 $year^{-1}$, and with mussels present at the ambient density we observed, that number increases 33.3 % to 0.14 Tg CH_4 $year^{-1}$. Along the the Atlantic coast, *G. demissa* densities have been found at >2000 individuals m^{-2} (Chintala et al. 2006), highlighting the importance of further research into salt marsh CH_4 dynamics and the role that benthic macrofauna may play across differing systems and regions.

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References

Alam, M.S., and Z. Jia. 2012. Inhibition of methane oxidation by nitrogenous fertilizers in a paddy soil. *Frontiers in Microbiology* 3: 1–13.

Allison, S.D., and P.M. Vitousek. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry* 37: 937–944.

Bartlett, K.B., R.C. Harriss, and D.I. Sebacher. 1985. Methane flux from coastal salt marshes. *Journal of Geophysical Research* 90: 5710–5720.

Bergstrom, I., S. Makela, P. Kankaala, and P. Kortelainen. 2007. Methane efflux from littoral vegetation stands of southern boreal lakes: an upscaled regional estimate. *Atmospheric Environment* 41: 339–351.

Bertness, M.D. 1984a. Habitat and community modification by an introduced herbivorous snail. *Ecology* 65: 370–381.

Bertness, M.D. 1984b. Ribbed mussels and *Spartina alterniflora* production in a New England salt marsh. *Ecology* 65: 1794–1807.

Bodelier, P.L.E., M. Stomp, L. Santamaria, M. Klaassen, and H.J. Laanbroek. 2006. Animal-plant-microbe interactions: direct and indirect effects of swan foraging behavior modulate methane cycling in temperate shallow wetlands. *Oecologia* 149: 233–244.

Bonaglia, S., F.J.A. Nascimento, M. Bartoli, I. Klawonn, and V. Bruchert. 2014. Meiofauna increases bacterial denitrification in marine sediments. *Nature Communications* 5: 5133.

Bousquet, P., P. Ciais, J.B. Miller, E.J. Dlugokencky, D.A. Hauglustaine, C. Prigent, G.R. Van der Werf, P. Peylin, E.-G. Brunke, C. Carouge, R.L. Lagenfelds, J. Lathiere, F. Papa, M. Ramonet, M. Schmidt, L.P. Steele, S.C. Tyler, and J. White. 2006. Contribution of anthropogenic and natural sources to atmospheric methane variability. *Nature* 443: 439–443.

Brauman, A., M.D. Kane, M. Labat, and J.A. Breznak. 1992. Genesis of acetate and methane by gut bacteria of nutritionally diverse termites. *Science* 257: 1384–1387.

Chen, Y.-H., and R.G. Prinn. 2006. Estimation of atmospheric methane emissions between 1996 and 2001 using a three-dimensional global chemical transport model. *Journal of Geophysical Research: Atmospheres* 111: –25.

Chintala, M.M., C. Wigand, and G. Thursby. 2006. Comparison of *Geukensia demissa* populations in Rhode Island fringe salt marshes with varying nitrogen loads. *Marine Ecology Progress Series* 320: 101–108.

Ciais, P., C. Sabine, G. Bala, L. Bopp, V. Brovkin, J.G. Canadell, A. Chhabra, R. DeFries, J. Galloway, M. Heinmann, C. Jones, C. Le Quere, R.B. Myneni, S. Piao, and P. Thornton. 2013. Carbon and other biogeochemical cycles. In: Stocker, T.F., Qin, D., Plattner, G-K et al., (eds) Climate Change 2013: the physical science basis. Contribution of Working Group 1 to the Fifth Assesment Report of the Intergovernmental Panel on Climate Change, Cambridge, UK, New York, NY

Crill, P.M., P.J. Martikainen, H. Nykanen, and J. Silvola. 1994. Temperature and N fertilization effects on methane oxidation in a drained peatland soil. *Soil Biology Biochemistry* 26: 1331–1339.

Dahlback, B., and L.A.H. Gunnarsson. 1981. Sedimentation and sulfate reduction under a mussel culture. *Marine Biology* 63: 269–275.

DeLaune, R.D., C.J. Smith, and W.H. Patrick. 1983. Methane release from gulf coast wetlands. *Tellus* 35: 8–15.

Dingemans, B.J.J., E.S. Bakker, and P.L.E. Bodelier. 2011. Aquatic herbivores facilitate the emission of methane from wetlands. *Ecology* 92: 1166–1173.

Fenchel, T. 1996. Worm burrows and oxic microniches in marine sediments. 1. Spatial and temporal scales. *Marine Biology* 127: 289–295.

Franz, D. 1997. Resource allocation in the intertidal salt-marsh mussel *Geukensia demissa* in relation to shore level. *Estuaries* 20: 134–148.

Harris, J.M. 1993. The presence, nature, and role of gut microflora in aquatic invertebrates: a synthesis. *Microbial Ecology* 25: 195–231.

Harriss, R.C., D.I. Sebacher, and F.P. Day. 1982. Methane flux in the great dismal swamp. *Nature* 297: 673–674.

Heisterkamp, I.M., A. Schramm, D. de Beer, and P. Stief. 2010. Nitrous oxide production associated with coastal marine invertebrates. *Marine Ecology Progress Series* 415: 1–9.

Hogan, K.B. 1993. *Current and future methane emissions from natural sources*. Office of Air and Radiation, Washington: US Environmental Protection Agency.

Honig, A., M.K. La Peyre, and J. Supan. 2014. Effects of low and high salinity regimes on seasonal gametogenesis of the ribbed mussel *Geukensia granosissima* in coastal Louisiana, USA. *Sexuality and Early Development in Aquatic Organisms* 1: 75–82.

International Atomic Energy Agency (IAEA), 1992. Manual on measurement of methane and nitrous oxide emissions from agriculture. IAEA, Vienna, IAEA-TECDOC-674

Jackson, C.R., and S.C. Vallaire. 2007. Microbial activity and decomposition of fine particulate organic matter in a Louisiana cypress swamp. *Journal of the North American Benthological Society* 26: 743–753.

Jackson, C.R., K.C. Liew, and C.M. Yule. 2009. Structural and functional changes with depth in microbial communities in a tropical Malaysian peat swamp forest. *Microbial Ecology* 57: 402–412.

Jackson, C.R., H.L. Tyler, and J.J. Millar. 2013. Determination of microbial extracellular enzyme activity in waters, soils, and sediments using high throughput microplate assays. *Journal of Visualized Experiments* 80: e50399. doi:10.3791/50399.

Jones, C.G., J.H. Lawton, and M. Shachak. 1994. Organisms as ecosystem engineers. *Oikos* 69: 373–386.

Jordan, T.E., and I. Valiela. 1982. A nitrogen budget of the ribbed mussel, *Geukensia demissa*, and its significance in nitrogen flow in a New England salt marsh. *Limnology and Oceanography* 27: 75–90.

Kao-Kniffin, J., D.S. Freyre, and T.C. Balser. 2010. Methane dynamics across wetland plant species. *Aquatic Botany* 93: 107–113.

Kettunen, A., V. Kaitala, J. Alms, J. Silvola, H. Nykanen, and P.J. Martikainen. 1996. Cross correlation analysis of the dynamics of methane emissions from a boreal peatland. *Global Biogeochemical Cycles* 10: 457–471.

King, G.M., and W.J. Wiebe. 1978. Methane release from soils of a Georgia salt marsh. *Geochimica et Cosmochimica Acta* 42: 343–348.

King, G.M., and W.J. Wiebe. 1980. Regulation of sulfate concentrations and methanogenesis in salt marsh soils. *Estuarine and Coastal Marine Science* 10: 215–223.

Kraus, D.W., and J.E. Doeller. 2004. Sulfide consumption by mussel gill mitochondria is not strictly tied to oxygen reduction: measurements using a novel polarographic sulfide sensor. *Journal of Experimental Biology* 207: 3667–3679.

Kristensen, E., and J.E. Kostka. 2005. Macrofaunal burrows and irrigation in marine sediment: microbiological and biogeochemical interactions. In *Coastal and estuarine studies: interactions between macro- and microorganisms in marine sediments*, ed. E. Kristensen, R.R. Haese, and J.E. Kostka, 125–157. Washington, DC: American Geophyiscal Union.

Laverock, B., J. Gilbert, K. Tait, A.M. Osborn, and S. Widdicombe. 2011. Bioturbation: impact on the marine nitrogen cycle. *Biochemical Society Transactions* 39: 315–320.

Lindau, C.W., P.K. Bollich, R.D. DeLaune, W.H. Patrick Jr., and V.J. Law. 1991. Effect of urea fertilizer and environmental factors on methane emission from a Louisiana, USA rice field. *Plant and Soil* 136: 195–203.

Mermillod-Blondin, F., and R. Rosenberg. 2006. Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. *Aquatic Sciences* 68: 434–442.

Montzka, S.A., E.J. Dlugokencky, and J.H. Butler. 2011. Non-CO_2 greenhouse gases and climate change. *Nature* 476: 43–50.

Moore, T., N. Roulet, and R. Knowles. 1990. Spatial and temporal variations of methane flux from subarctic/northern boreal fens. *Global Biogeochemical Cycles* 4: 29–46.

Myhre, G., D.T. Shindell, F.-M. Breon, W. Collins, J. Fuglestvedt, J. Huang, D. Koch, J.-F. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura, and H. Zhang. 2013. Anthropogenic and natural radiative forcing. In *Climate*

change 2013: the physical science basis. Contribution of working group 1 to the fifth Assesment. Report of the intergovernmental panel on climate change, ed. T.F. Stocker, D. Qin, G.-K. Plattner, et al. Cambridge: Cambridge University Press.

Oremland, R.S., L.M. Marsh, and S. Polcin. 1982. Methane production and simultaneous sulphate reduction in anoxic, salt marsh sediments. *Nature* 296: 143–145.

Petruzzella, A., R.D. Guariento, A. da Rocha Gripp, C.C. Marinho, M.P. Figueiredo-Barros, and F. de Assis Esteves. 2015. Herbivore damage increases methane emission from emergent aquatic macrophytes. *Aquatic Botany* 127: 6–11.

Poffenbarger, H.J., B.A. Needelman, and J.P. Megonigal. 2011. Salinity influence on methane emissions from tidal marshes. *Wetlands* 31: 831–842.

Purvaja, R., and R. Ramesh. 2001. Natural and anthropogenic methane emission from coastal wetlands of South India. *Environmental Management* 27: 547–557.

Ravit, B., J.G. Ehrenfeld, and M.M. Haggblom. 2003. A comparison of sediment microbial communities associated with *Phragmites australis* and *Spartina alterniflora* in two brackish wetlands of New Jersey. *Estuaries* 26: 465–474.

Rietl, A.J., M.E. Overlander, J.A. Nyman, and C.R. Jackson. 2016. Microbial community composition and extracellular enzyme activities associated with *Juncus romerianus* and *Spartina alterniflora* vegetated sediments in Louisiana saltmarshes. *Microbial Ecology* 71: 290–303.

Sasser, C., J. Visser, E. Mouton, J. Linscombe, and S. Hartley. 2014. Vegetation types in coastal Louisiana. *US Geological Survey Scientific Investigations Map* 3290(1).

Sebacher, D.I., R.C. Harriss, and K.B. Bartlett. 1985. Methane emissions to the atmosphere through aquatic plants. *Journal of Environmental Quality* 14: 40–46.

Shindell, D.T., G. Faluvegi, D.M. Koch, G.A. Schmidt, N. Unger, and S.E. Bauer. 2009. Improved attribution of climate forcing to emissions. *Science* 326: 716–718.

Silliman, B.R., and J.C. Zieman. 2001. Top-down control of *Spartina alterniflora* production by periwinkle grazing in a Virginia salt marsh. *Ecology* 82: 2830–2845.

Stief, P., and G. Eller. 2006. The gut microenvironment of sediment-dwelling *Chironomus plumosus* larvae as characterized with O_2, pH, and redox microsensors. *Journal of Comparative Physiology B* 176: 673–683.

Stief, P., M. Poulson, L.P. Nielson, H. Brix, and A. Schramm. 2009. Nitrous oxide emission by aquatic macrofauna. *Proceedings of the National Academy of Sciences* 106: 4296–4300.

Van der Nat, F.-J.W.A., and J.J. Middelburg. 1998. Effects of two common macrophytes on methane dynamics in freshwater sediments. *Biogeochemistry* 43: 79–104.

Walter, B.P., M. Heimann, and E. Matthews. 2001. Modeling modern methane emissions from natural wetlands. 1. Model description and results. *Journal of Geophysical Research* 106: 34,189–34,206.

Wang, Z., R.D. Delaune, C.W. Lindau, and W.H. Patrick. 1992. Methane production from anaerobic soil amended with rice straw and nitrogen fertilizers. *Fertilizer Research* 33: 115–121.

Welsh, D.T., D. Nizzoli, E.A. Fano, and P. Viaroli. 2015. Direct contribution of clams (*Ruditapes philippinarum*) to benthic fluxes, nitrification, denitrification and nitrous oxide emission in a farmed sediment. *Estuarine, Coastal and Shelf Science* 154: 84–93.

Whiting, G.J., and J.P. Chanton. 1993. Primary production control of methane emission from wetlands. *Nature* 364: 794–795.

Xu, X., W. Zhao, M. Xiao, J. Huang, C. Fang, B. Li, and M. Nie. 2014. Snails promote methane release from a freshwater lake ecosystem. *Frontiers in Environmental Science* 2: 1–6.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., and Smith, G. M. 2009. Mixed effects models and extensions in ecology with Gail R.M., Krickeberg K., Samet J.M., Tsiatis A., Wong W., editors. *New York, NY: Spring Science and Business Media*.