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Regulators of coastal wetland methane production and responses to simulated global change

Carmella Vizza[1], William E. West[1, 2], Stuart E. Jones[1], Julia A. Hart[1, 3], and Gary A. Lamberti[1]

[1]Department of Biological Sciences, University of Notre Dame, Notre Dame, 46556, USA
[2]Kellogg Biological Station, Michigan State University, Hickory Corners, 49060, USA
[3]Center for Limnology, University of Wisconsin, Madison, 53706, USA

Correspondence to: Carmella Vizza (cvizza@nd.edu)

Abstract. Wetlands are the largest natural source of methane (CH_4) to the atmosphere, but their emissions vary along salinity and productivity gradients. Global change has the potential to reshape these gradients and therefore alter future contributions of wetlands to the global CH_4 budget. Our study examined CH_4 production along a natural salinity gradient in coastal Alaska wetlands. In the laboratory, we incubated natural sediments to compare CH_4 production rates between freshwater and intertidal wetlands, and quantified the abundances of methanogens and sulfate-reducing bacteria in these ecosystems. We also simulated sea-level rise and enhanced organic matter availability, which we predicted would have contrasting effects on coastal wetland CH_4 production. Intertidal wetlands produced less CH_4 than freshwater wetlands due to high sulfate availability and generally higher abundances of sulfate-reducing bacteria, whereas freshwater wetlands had significantly greater methanogen abundances. Simulated sea-level rise in freshwater sediments, however, did not reduce CH_4 production, perhaps because the 14d incubation period was too short to elicit a shift in microbial communities. In contrast, increased organic matter generally enhanced CH_4 production rates, but this response varied by the macrophyte species added. Our study suggests that CH_4 production in coastal wetlands, and therefore their overall contribution to the global CH_4 cycle, will be sensitive to increased organic matter availability and potentially sea-level rise. To better predict future wetland contributions to the global CH_4 budget, future studies and modeling efforts should investigate how multiple global change mechanisms will interact to impact CH_4 dynamics.

Keywords: Methanogenesis, sea level rise, substrate availability, redox conditions, microbial communities

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1 Introduction

Wetlands contribute about 60% of all natural methane (CH_4) emissions to the atmosphere (Kirschke et al., 2013). As global temperatures continue to increase, some models predict that wetland CH_4 emissions will double by 2100 (Gedney et al., 2004). Since CH_4 traps heat 21 times more effectively than CO_2 (Whalen, 2005), higher wetland emissions could trigger a positive feedback loop that further increases temperatures and CH_4 release. Currently, wetlands at northern latitudes are thought to be on the brink of the "greenhouse compensation point," wherein carbon sequestration is offset by greenhouse gas emissions (Whiting and Chanton, 2001). Warming, an extended growing season, and CO_2 fertilization could upset this balance by converting northern wetlands to net sources of carbon to the atmosphere, especially if the resulting increases in plant productivity could provide additional organic matter to fuel additional CH_4 production (Ringeval et al., 2011). Predicting the response of these ecosystems to global change is challenging because we do not fully understand the sensitivity of the CH_4 cycle to enhanced productivity of wetland plants (McGuire et al., 2009; Ringeval et al., 2011).

Warming associated with increasing CO_2 levels will also lead to sea-level rise in coastal areas and longer growing seasons at northern latitudes (Walther et al., 2002), thus further enhancing the CO_2 fertilization effect (Matthews, 2007; Ringeval et al., 2011), all of which could affect where wetlands stand in relation to their greenhouse compensation point. These elements of global change could alter two of the main factors that influence CH_4 production – substrate availability and redox conditions (Whalen, 2005). Methanogens generally use substrates provided by the fermentation of organic matter, producing CH_4 via two pathways: (1) acetoclastic methanogenesis, where acetate is the substrate of choice, and (2) hydrogenotrophic methanogenesis, where H_2 and CO_2 are the substrates utilized (Conrad, 1999). However, methanogens can be outcompeted for these substrates because carbon is not an energetically favorable electron acceptor in comparison to those used by other microbes. High redox potential and the presence of alternative electron acceptors (e.g., NO_3^-, SO_4^{2-}) can signal intense microbial competition for the fermentative substrates that methanogens utilize (Lovley and Klug, 1983; 1986; Lovley and Phillips, 1987). For example, Winfrey and Ward (1983) observed much greater rates of sulfate reduction than CH_4 production in intertidal sediments until sulfate became depleted. However, an abundant supply of organic matter can reduce competition for methanogens by increasing substrate availability, acting as an electron donor, and lowering redox potential as alternate electron acceptors are consumed (Achtnich et al., 1995). Both redox conditions and substrate availability will therefore play an important role in determining the effects of global change on CH_4 production.

To accurately forecast the future global CH_4 budget, it is critical that we understand the effects of sea-level rise and increased organic matter availability on CH_4 production in wetlands (Fig. 1), which are likely results of rising global temperatures and CO_2 concentrations. Laboratory studies and field surveys report increased CH_4 production and emissions with warming (Moore and Dalva, 1993; Klinger et al., 1994; Lofton et al., 2014). However, despite their potential importance in regulating CH_4 emissions from wetlands, especially those at northern latitudes, few studies have attempted to

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simulate the effects of sea-level rise or increased substrate availability on CH_4 production. Both of these global change mechanisms are likely to disrupt coastal wetland biogeochemical cycles, especially at northern latitudes where their effects are likely to be stronger and more abrupt.

We studied wetland ecosystems in the Copper River Delta of Alaska, an area vulnerable to global change because of its northern location and proximity to the ocean. Over the past 50 years, average annual temperatures in Alaska have increased 1.9 °C, with winter temperatures rising 3.6 °C (U.S. Global Climate Change Program, 2009), which is extending the growing season. In addition, the projected global sea-level rise of 100 cm by 2100 (Vermeer and Rahmstorf, 2009) will be exacerbated along the southcentral Alaskan coast where tectonic subsidence is prominent (Freymueller et al., 2008). For example, the Copper River Delta, which is subsiding at about 8.5 mm per year (Freymueller et al., 2008), is at risk of a relative sea-level rise of about 1.7 m by 2100.

Our study objectives were to (1) compare CH_4 production rates and microbial community abundances in sediments from freshwater and intertidal wetlands in the Copper River Delta, (2) simulate sea-level rise for freshwater wetlands, and (3) simulate increased organic matter availability in freshwater wetlands. We hypothesized that (1) intertidal marsh sediments will have lower CH_4 production rates than those from the freshwater wetlands, (2) intertidal marsh sediments will have higher abundance of sulfate-reducing bacteria, but lower numbers of methanogens than freshwater wetlands, (3) simulating sea-level rise in freshwater sediments will decrease CH_4 production rates, with sulfate availability largely being responsible for this effect, and (4) increasing the amount of organic matter available will enhance CH_4 production, but substrate quality will moderate this effect. Our conceptual model for these interactions is depicted in Fig. 1.

2 Materials and Methods

2.1 Study area

The Copper River in southcentral Alaska is the eighth largest river in the United States (U.S. Geological Survey, 1990). Draining a large region of the Chugach Mountains and the Wrangell Mountains into the Gulf of Alaska, the Copper River and its sediment deposits have shaped the largest contiguous wetland on the Pacific Coast of North America. The Copper River Delta (CRD) encompasses about 283,000 hectares of wetland habitat and supports extraordinary biodiversity (Bryant, 1991) in a largely pristine landscape. Wetlands and shallow ponds (< 2 m in depth) were created and modified by the Great Alaska earthquake in 1964 that elevated the CRD by 1−4 m depending on location (Thilenius, 1995). A natural succession of wetlands thereby emerges from the ocean to the uplands (Fig. 2). Our study focused on the intertidal marsh and freshwater pond habitats, collectively referred to as CRD “wetlands.”

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2.2 Experimental design

2.2.1 Sample collection

Using a handheld bucket auger, sediment samples (~ 250 mL) were collected from nine freshwater wetlands and five intertidal marsh sites varying in physicochemical parameters (Table 1). Due to extensive habitat heterogeneity within the freshwater wetlands (i.e., open water and several different macrophyte zones), we collected at least five sediment samples representative of the different habitats at each wetland (n = 9) along with at least 1 L of hypolimnetic water during each sampling period, so that the average CH_4 production rates from each system could be accurately assessed. In contrast, the intertidal marsh sites generally exhibited less habitat heterogeneity than the freshwater wetlands (i.e., we observed only mudflat sites dominated by *Carex* spp.), but we observed temporal fluctuations in salinity with a YSI Pro Plus multiparameter water quality meter indicative of tidal influence. We, therefore, collected 1 L of water and one sediment sample at five different sites along a salinity gradient.

2.2.2 Freshwater and intertidal wetland comparison

To assess CH_4 production, laboratory incubations were conducted using sediment and water samples collected during two sampling periods (June and August 2014). Specifically, we conducted five incubations for each freshwater wetland (n = 9) and five incubations for intertidal marsh (n = 5 separate locations in the continuous intertidal zone). We then we used the average CH_4 production rates from each freshwater wetland as a replicate in comparing CH_4 production rates between freshwater (n = 9) and intertidal (n = 5) systems at each sampling period.

2.2.3 Sea-level rise simulation

To assess the effects of sea-level rise on CH_4 production, additional sediments were collected in June from a single site in five of the freshwater wetlands (n = 5) and then incubated with intertidal water (6.3 mM sulfate). We then compared them to the average CH_4 production rates of the five sediment samples incubated with freshwater from that same subset of freshwater wetlands (n = 5) during June 2014.

2.2.4 Increased organic matter simulation

To assess the effects of increased organic matter on CH_4 production, four sediment samples from different sites were used from five of the freshwater wetlands (n = 20). An aliquot of each sediment sample from each wetland was incubated with fresh macrophyte tissue from one of four species (treatment) and then compared to an aliquot that served as a paired control sediment sample (total pairs = 20; 5 wetlands x 4 treatments). This paired design controlled for "within pond" sediment heterogeneity to better capture the response of the methanogens to adding organic matter, or ΔCH_4 production (treatment–control). Our four organic matter treatments were based upon the four dominant aquatic macrophyte species on the CRD – buckbean (*Menanythes trifoliata*), horsetail (*Equisetum variegatum*), lily (*Nuphar polysepalum*), and marestail (*Hippuris*

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vulgaris). All vegetation for each species was collected from the same plant individual to ensure minimal difference in quality within each treatment.

2.3 Laboratory analyses

2.3.1 Sediment slurry incubations

For each incubation, approximately 60 mL of sediment and 60 mL of water were incubated in a 250mL serum bottle in the dark at approximately 14.0 °C. Since ambient temperature was generally lower than average pond temperature (June: 17.2 ± 0.9 °C, August: 18.4 ± 1.3 °C), estimated rates of CH_4 production potential were considered conservative. Each bottle was made anoxic by purging it with N_2 gas for five minutes. Headspace samples (10 mL) were removed at 2, 5, 8, 11, and 14 days, injected into a 2mL serum vial (pre-evacuated with a vacuum pump), sealed with silicone, and stored upside down in water for less than three months until the samples could be analyzed using gas chromatography. To maintain atmospheric pressure in the slurry incubations, 10 mL of N_2 gas was added after each sampling point. CH_4 concentrations were measured using an Agilent 6890 gas chromatograph equipped with a flame-ionizing detector (Agilent Technologies, Santa Clara, CA, USA) as detailed by West et al. (2015). After accounting for headspace dilution due to sampling, CH_4 production rates were inferred from the slope of the linear regressions of CH_4 concentrations over time and are reported as bottle rates (i.e., μmol per bottle per day; West et al., 2015)

2.3.2 Physicochemical measurements

Temperature, pH, dissolved oxygen, specific conductivity, and salinity were measured at each sampling location using a YSI Pro Plus multiparameter water quality meter (YSI, Yellow Springs, OH, USA). Dissolved organic carbon was analyzed using a Shimadzu TOC-VCSH (Shimadzu Scientific Instruments, Kyoto, Japan). Acetate, nitrate, and sulfate concentrations were analyzed using a Dionex ICS-5000 (Thermo Fisher Scientific, Sunnyvale, CA, USA), but only sulfate was detectible in the water column. Water chemistry analyses were performed using instrumentation at the University of Notre Dame Center for Environmental Science and Technology.

2.3.3 Sediment organic matter and porewater chemistry

To examine starting conditions for each CH_4 production assay, a subsample of sediment was frozen at the start of the incubation for later analysis. A portion of each subsample was dried for at least 48 hours at 60 °C, and the dry weight was recorded. Subsequently, the organic matter in the sediment was combusted at 500 °C for four hours, and the sediment was re-wetted and then dried at 60 °C for at least 48 hours before re-weighing (Steinman et al., 2011). Organic matter was estimated as the percent of sediment material lost during combustion and scaled up to estimate the total sediment organic matter (g) in each incubation bottle. To extract porewater from the sediment, another portion (~ 50 mL) was centrifuged for 45 minutes at 4 °C at ~ 4000 RCF. The total volume of supernatant per volume of sediment was recorded, and a subsample

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of the porewater was also analyzed on the Dionex ICS-5000 for acetate, nitrate, and sulfate. Porewater concentrations were scaled to the total amount of each anion (μmol) in each incubation bottle.

2.3.4 Microbial analyses

DNA was extracted from frozen sediments used in other analyses, including multiple June intertidal sediments (n = 10), the freshwater sediments used in the sea-level rise simulation (n = 5), and a composite of the five sediment samples (1 g sediment per sample was added to make a 5-g composite) from the nine wetlands for the June time period (n = 9) according to the manufacturer's protocol with a PowerSoil DNA isolation kit (Mo Bio, Carlsbad, CA, USA). Extracted DNA served as a template for quantitative PCR (qPCR) targeting of two genes – the alpha subunit of methyl coenzyme reductase (*mcrA*) and the alpha subunit of dissimilatory sulfite reductase (*dsrA*). The *mcrA* gene catalyzes the reduction of a methyl group to CH_4 (Thauer, 1998), and is possessed by all known methanogens thereby making it ideal for quantifying methanogen abundance (Luton et al., 2002; Earl et al., 2003; Castro et al., 2004). The *dsrA* gene catalyzes the final step in sulfate respiration, and its ubiquity in sulfate-reducing bacteria makes it powerful at assessing their abundance (Wagner et al., 1998; Klein et al., 2001; Zverlov et al., 2005).

The *mcrA* and *dsrA* genes were amplified using a 20μL qPCR reaction in a Mastercycler ep realplex^2 gradient S (Eppendorf, Hamburg, Germany), using SYBR Green as the reporter dye. Each reaction contained 1 μL of intertidal or pond DNA template and was conducted using the PerfeCTa SYBR Green FastMix (Quanta BioSciences). For the *mcrA* qPCR, primer details and thermocycling conditions in West et al. (2012) were replicated except that we employed a fluorescent detection step at 78 ºC for 20 seconds. For the *dsrA* qPCR primer, details and thermocycling conditions in Kondo et al. (2008) were replicated. Melting curves for both *mcrA* and *dsrA* were run to ensure absence of non-specific amplification. Amplification, fluorescence data collection, and initial data analysis were all performed by the Eppendorf realplex^2 software.

Standard qPCR curves for *mcrA* and *dsrA* were generated by pooling gel-extracted amplicons containing our qPCR primer sites from a subset of our pond and intertidal samples. We amplified *mcrA* using primers detailed in Luton et al. (2002) and thermocycling conditions in West et al. (2012), and *dsrA* by replicating primer details and thermocycling conditions in Kondo et al. (2008). After amplification, we used gel electrophoresis and an Invitrogen PureLink Quick Gel Extraction Kit (Invitrogen, Carlsbad, CA, USA) to isolate the *mcrA* and *dsrA* amplicons. Following clean-up, we quantified the purified amplicons using Invitrogen's Qubit technology. We then used serial ten-fold dilutions of these genes to generate standard curves for qPCR. Our detection limit for each gene was approximately 1000 copies per g of sediment. Samples below detection were assigned a value of 999 copies per g for further analysis. We ran triplicate analyses of all samples for both the *mcrA* and *dsrA* qPCR, the averages of which were used in summary statistics and analyses.

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2.4 Statistical analyses

For the freshwater pond and intertidal marsh comparison, we analyzed how four factors influenced CH_4 production rates (log-transformed) using additive general linear models (GLM). The four factors were: (1) ecosystem type (freshwater or intertidal), (2) time period, (3) porewater acetate availability, and (4) total sulfate and nitrate present. A total of 16 candidate models (all possible additive combinations of the four factors including the null model) were compared based on criteria in Burnham and Anderson (2002). A subset of those models, excluding the null model and those with considerably less support ($\Delta_i > 4$), were then used to determine model-averaged parameter estimates and to estimate the relative importance of variables (Burnham and Anderson, 2002). To compare the abundance of methanogens and sulfate-reducing bacteria, we first used a chi-squared test for each gene to determine whether the presence/absence of *mcrA* or *dsrA* was independent of ecosystem type. We then used a non-parametric Kruskal-Wallis tests to determine whether the number of copies of *mcrA* or *dsrA* varied by ecosystem type.

For the sea-level rise simulation, we conducted a paired *t*-test to determine whether CH_4 production rates in freshwater pond sediments were affected by being flooded with intertidal brackish water instead of freshwater from their respective wetlands. Pearson correlations were computed (Zar, 2010) to determine whether porewater acetate or total sulfate levels were related to CH_4 production rates during this experiment.

To determine whether adding organic matter affected CH_4 production rates, we first used an analysis of variance (ANOVA) with treatment (i.e., macrophyte species) as the factor of interest and freshwater wetland as a blocking variable. Then we analyzed how three factors influenced the response of each sediment, or ΔCH_4 production (treatment–control), using additive GLMs. The three factors were: (1) macrophyte species added, (2) total acetate available in the porewater, and (3) total amount of sulfate and nitrate present. A total of eight candidate models (all possible additive combinations of the three factors including the null model) were compared as described above. All statistical analyses were conducted in the R software environment using the base and MuMIn packages (R Development Core Team, 2016).

3 Results

3.1 Freshwater and intertidal wetland comparison

3.1.1 Water column and porewater chemistry

Water column and porewater chemistry of the incubations varied more by ecosystem type than by time period. Total sulfate levels in freshwater incubations (June: 5.5 ± 4.1 µmol; August: 2.8 ± 2.1 µmol; mean ± sd) were about two orders of magnitude lower than in intertidal incubations (June: 316 ± 329 µmol; August: 264 ± 275 µmol) and did not vary between time periods. In comparison to total sulfate levels, porewater nitrate availability was low, with pond and intertidal marsh

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averaging 0.10 ± 0.06 µmol and 0.01 ± 0.02 µmol across both time periods, respectively. The total amount of acetate available in the pond incubations was similar in June (1.9 ± 1.5 µmol) and August (2.0 ± 1.2 µmol), while levels in the intertidal marsh incubations were generally higher and more variable especially in August (15.1 ± 18.0 µmol) than in June (9.2 ± 4.9 µmol).

3.1.2 CH_4 production

CH_4 production rates were higher in freshwater wetlands than in intertidal marsh and approximately an order of magnitude higher in both ecosystems in August compared to June (Fig. 3). Porewater acetate positively influenced CH_4 production rates, while total sulfate and nitrate availability negatively influenced CH_4 production rates (Table 2). The most likely model contained all four factors – ecosystem type, time period, acetate, and total sulfate/nitrate (Table 2). Based upon model averaging of the top three models (Table 2), all four factors appeared to influence CH_4 production with the relative importance of these variables being 1.00 for ecosystem, 1.00 for porewater acetate, 0.90 for total sulfate and nitrate availability, and 0.73 for time period.

3.1.3 Functional group abundances

Intertidal sediments generally had higher abundances of sulfate-reducing bacteria, while freshwater sediments were characterized by higher numbers of methanogens. In the intertidal marsh, three out of ten samples were below the detection limit for the *dsrA* gene, our proxy for sulfate-reducing bacteria abundance, but we detected this gene in all nine freshwater wetland composite samples. The presence or absence of the *dsrA* gene was independent of ecosystem type (χ^2 = 3.21, df = 1, P = 0.07). Intertidal sediments (n = 10) and freshwater wetland sediments (n = 9) had 3.52 ± 5.39 x 10^5 and 5.20 ± 5.08 x 10^4 copies of *dsrA* per gram, respectively. Due to high variability, the number of copies of *dsrA* did not differ significantly by ecosystem (Kruskal-Wallis: H = 1.31, df = 1, P = 0.25). In contrast, we detected the *mcrA* gene, our proxy for methanogen abundance, in only two out of ten intertidal samples, but in all nine freshwater wetland samples. The presence or absence of the *mcrA* gene was dependent on ecosystem type (χ^2 = 12.44, df = 1, P = 0.0004). Intertidal samples had 2.14 ± 5.78 x 10^4 copies of the *mcrA* per gram of sediment, while freshwater wetlands had 1.84 ± 1.25 x 10^5 copies of *mcrA* per gram of sediment. Methanogen abundance therefore differed significantly between ecosystem types (Kruskal-Wallis: H = 11.24, df = 1, P = 0.0008).

3.2 Sea-level rise simulation

Flooding of pond sediments with brackish water did not affect CH_4 production rates (Fig. 4). Even though total sulfate levels increased from 4.2 ± 2.4 to 385 ± 6 µmol with the addition of intertidal water, CH_4 production rates did not differ between treatment and control incubations (paired t-test: t = 0.44, df = 4, P = 0.68). However, CH_4 production rates were significantly correlated with porewater acetate levels (r = 0.89, t = 5.56, df = 8, P = 0.0005), but not with total sulfate levels (r = 0.08, t =

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0.22, df = 8, P = 0.83). The pond sediments used in this sea-level rise simulation (n = 5) had about an order of magnitude higher number of copies of *mcrA* (3.12 ± 4.40 x 10^5) than *dsrA* (5.32 ± 6.33 x 10^4) per gram of sediment.

3.3 Increased organic matter simulation

The organic matter treatments significantly influenced CH_4 production rates ($F_{4, 16}$ = 4.52, P = 0.01), but this effect varied with macrophyte species (Fig. 5). Adding buckbean and marestail had little effect on CH_4 production, while the lily and horsetail treatments generally increased methanogen activity (Fig. 5). The most likely model for predicting ΔCH_4 production (treatment – control) included acetate availability, which had a negative effect on the response (Table 3). The next best models included porewater acetate and species (Model 2) or porewater acetate and total sulfate and nitrate availability (Model 3), which had a positive effect on the response (Table 3). Models 1–4 (Table 3) were averaged to determine parameter estimates with the relative importance of the variables being 0.89 for porewater acetate, 0.36 for macrophyte species, and 0.13 for total sulfate and nitrate availability. Using the model-averaged parameters, our predictions of the response of CH_4 production rates to increased substrate availability closely followed the observed results (Fig. 6).

4 Discussion

We found that CH_4 production was lower in intertidal than in freshwater wetlands, likely due to differences in redox state (i.e., higher sulfate levels in the intertidal) and in microbial communities (i.e., lower methanogen abundances in the intertidal). Short-term simulation of sea-level rise in freshwater sediments (~14 days), however, did not influence CH_4 production rates. In contrast, higher organic matter availability generally enhanced CH_4 production rates, but this response varied by macrophyte species and the amount of substrate already available. Overall, these results demonstrate that the interaction of global change mechanisms must be considered in modeling the future contribution of coastal wetlands to the global CH_4 budget (Fig. 1).

4.1 Freshwater and intertidal wetland comparison

CH_4 production rates in intertidal marsh were substantially lower than those of freshwater wetlands, as predicted. Many studies have attributed the decrease in wetland CH_4 emissions along increasing salinity and sulfate concentrations to sulfate-reducing bacteria outcompeting methanogens for substrates (DeLaune et al., 1983; Bartlett et al., 1987; Magenheimer et al., 1996; Poffenbarger et al., 2011), but none of these directly assessed whether lower CH_4 emissions resulted from reduced CH_4 production or higher CH_4 oxidation. In contrast, our study quantified CH_4 production along a similar spatial gradient and directly linked lower CH_4 production to higher sulfate and nitrate concentrations and differences in microbial

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communities. The presence of these alternative electron acceptors likely negatively impacted methanogens via competition for organic substrates with denitrifiers and sulfate-reducing bacteria (Oremland and Polcin, 1982; Lovley and Klug, 1986; Achtnich et al., 1995). Our study also demonstrates that intertidal sediments had generally higher sulfate-reducing bacteria (*dsrA*) abundances, but significantly lower levels of methanogens (*mcrA*) than freshwater sediments. Collectively, these results suggest that shifts in the relative abundance of functional microbial guilds between intertidal and freshwater wetlands contribute to differences in CH_4 production between these ecosystems.

In addition to the influences of microbial communities and redox conditions on CH_4 production, acetate availability appeared to be an important factor. Substrate availability regulates CH_4 production (Whalen, 2005), and acetate is one of the major precursors for methanogenesis (Conrad, 1999). However, in other Alaskan wetlands, hydrogenotrophic methanogenesis is thought to be the primary pathway of methane production (Hines et al., 2001), with August 2001 methanogenesis rates ranging from about 0.1 to 1.6 µmol per day (Hines et al., 2008). Despite conservative incubation temperatures, CRD freshwater wetlands exhibited CH_4 production rates an order of magnitude greater than those observed in nearby Alaskan wetlands 13 years earlier (Hines et al., 2008). CH_4 production rates therefore appear to be increasing over time, possibly due to warming, since higher temperatures have been shown to increase overall methanogenesis rates in shallow Alaskan lake sediments (Lofton et al., 2014). Additionally, the role of the acetoclastic pathway is likely to grow more important in northern wetlands as vascular plant growth increases (Hines et al., 2008; Klady et al., 2011), since the fermentation of vascular plant matter facilitates the production of acetate.

CH_4 production rates often vary seasonally as a function of temperature, but we observed August rates that were an order of magnitude higher than those conducted in June. Other factors affecting CH_4 production that could vary seasonally include (1) availability of organic matter such as acetate for CH_4 production (Whiting and Chanton, 1993; Walter et al., 2001), (2) redox conditions including sulfate concentrations (Sinke et al., 1992), (3) microbial population densities (Yannarell and Triplett, 2005), or (4) the pathway by which CH_4 is produced (Avery et al., 1999). In our study, we did not observe large seasonal differences in porewater acetate or sulfate availability in CRD wetlands, but we did not assess seasonal variation in the abundances of methanogens and sulfate-reducing bacteria, their per-cell activity rates, or availability of H_2 or methanogenic substrates other than acetate. Therefore, it is possible that the observed seasonal differences in CH_4 production rates were the result of microbial community shifts, decreased per-cell activity of methanogens in June, greater CH_4 produced from the hydrogenotrophic pathway during August as acetate levels did not change, or some combination of these potential explanations.

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4.2 Sea-level rise simulation

Despite our finding that CH_4 production rates were significantly lower in intertidal marsh sites, simulating sea-level rise in freshwater sediments surprisingly did not affect CH_4 production rates. In contrast, DeLaune et al. (1983) found that CH_4 production was inhibited with the addition of ~10 mM sulfate, which is higher than the sulfate concentration (~6 mM) used in this study. However, their study examined the effects of high sulfate levels on brackish sediments (DeLaune et al., 1983), where methanogen abundances are likely to be lower and sulfate-reducing bacteria are probably primed for activity. The freshwater wetland sediments that we used for this simulation had methanogen abundances an order of magnitude higher than sulfate-reducing bacteria. Although the presence of sulfate-reducing bacteria was detectible, we do not know whether these taxa were active or dormant. In fact, dormant taxa can account for almost 40% of taxon richness in nutrient-poor systems (Jones and Lennon, 2010), such as the CRD freshwater wetlands. Additionally, we conducted 14d incubations, which may have been too short to allow for shifts in the relative abundance of sediment microbial populations (Hoehler and Jørgensen, 2013).

4.3 Increased organic matter simulation

Higher availability of organic matter generally increased CH_4 production rates, but this effect varied with the species of macrophyte added to the incubations. Differences in litter quality is known to influence methanogen communities and CH_4 production (Yavitt et al., 1990; 2000; Valentine et al., 1994). For example, West et al. (2012) found that adding algal carbon significantly enhanced CH_4 production relative to terrestrial carbon. Although aquatic macrophyte carbon may be of lower quality than that of algae, aquatic macrophytes are likely more labile than terrestrial plants (Schlickeisen et al., 2003). For example, Tiegs et al. (2013) found that terrestrial plants decomposed more slowly than aquatic macrophytes in CRD wetlands. Of these aquatic macrophyte species, buckbean and lily leaves decomposed at about the same rate, but faster than marestail and horsetail. The rate of decomposition of different plant species was correlated with phosphorus content, and therefore indicative of litter quality differences (Tiegs et al., 2013). However, our CH_4 production response did not follow the decomposition pattern documented by Tiegs et al. (2013); we observed higher CH_4 production for the lily and horsetail treatment relative to the control, but not for buckbean and marestail.

Other measures of litter quality beyond elemental composition could explain differences in the methanogen response. West et al. (2015), for example, found that higher lipid content of phytoplankton enhanced CH_4 production rates. Alternatively, certain properties may influence the fermentative microbial communities associated with vegetation during decomposition (Boon et al., 1996), which are responsible for providing methanogenic substrates. For example, in a survey of 209 plants, Bishop and MacDonald (1951) reported that buckbean was one of the 10 most active species for antibacterial substances, while horsetail did not possess such properties. Specifically, buckbean extracts include aucubin, a defensive

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compound that can inhibit many strains of anaerobic bacteria (Weckesser et al., 2007). Marestail also contains aucubin as well as a verbascoside, another antimicrobial compound (Damtoft et al., 1994). In contrast, the only part of lily linked to potential antimicrobial properties is the rhizomes, which have been used in folk medicine (Padgett, 2007) and are more likely to require defensive compounds because of competition with the sediment microbial community than the floating leaves we used for this experiment. Therefore, we hypothesize that CH_4 production varied as a function of a different measure of litter quality than previously put forward (e.g., C:N:P, percent lignin, or lipid content), whereby the negative effects of the antimicrobial properties of buckbean and marestail on the fermentative bacteria superseded the positive effect of increasing the amount of organic matter.

Many other studies have documented that CH_4 production is enhanced by the addition of direct substrates such as acetate and H_2 (Williams and Crawford, 1984; Bachoon and Jones, 1992; Amaral and Knowles, 1994; Coles and Yavitt, 2002; Yavitt and Seidman-Zager, 2006), or the addition of indirect substrates such as dextrose and glucose (DeLaune et al., 1983; Williams and Crawford, 1984; Coles and Yavitt, 2002), which would need to be broken down by fermentative bacteria before methanogens could utilize them. Fewer studies have examined the effects of more biologically realistic, indirect substrates such as plant or algal matter (but see Valentine et al., 1994; West et al., 2012; 2015). Interestingly, in our study, the amount of acetate already available in the sediment appeared to moderate the methanogen response to enhanced substrate availability. The negative relationship between ΔCH_4 production and porewater acetate concentration suggests that methanogenic substrate concentrations can become saturated, which is expected from traditional Michaelis-Menten enzyme kinetics.

Another indication of substrate limitation is the positive relationship between the methanogenic response to added organic matter and the total amount of sulfate and nitrate available in the incubation. These alternative electron acceptors provide more energy than either methanogenic pathway (acetoclastic or hydrogenotrophic) when coupled to the oxidation of organic matter (Stumm and Morgan, 1996; Schlesinger and Bernhardt, 2013). For example, Westermann and Ahring (1987) found that inhibiting sulfate reduction stimulated CH_4 production in an alder swamp, suggesting that methanogens and sulfate-reducing bacteria compete for common substrates, and that nitrate addition reduced both sulfate reduction and CH_4 production. Sulfate and nitrate availability, therefore, may signal strength of competition for electron donors (organic matter) that methanogens must overcome to produce CH_4. The higher the competition, the more likely that methanogens respond positively to the addition of organic matter. The response of methanogens to increased substrate availability, therefore, is likely regulated by the quality of the substrate (e.g., C:P, lipid content, antimicrobial compounds), strength of competition for substrate (e.g., redox conditions, microbial community assemblages, per-cell activity rates), and whether substrate availability is limiting or saturated in the environment. Although total sulfate and nitrate availability played a less significant

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role than acetate and macrophyte species, the model using averaged estimates from all three parameters allowed us to accurately predict the response in CH_4 production for this experiment.

5 Conclusions

Our study demonstrates that potential interactions between elements of global change, specifically sea-level rise and increased organic matter availability, could have competing effects on CH_4 production in coastal wetlands (Fig. 1). Sea-level rise is likely to decrease long-term CH_4 production rates, but there may be a delayed response. Determining the timescale required for processes at the microbial scale to shift towards sulfate reduction is challenging, and the magnitude of seawater intrusion needed to induce this shift is currently unclear. As others have noted, the global carbon cycle is inextricably linked to other elemental cycles (i.e., sulfur) by processes taking place at the microbial scale (Schimel, 2004; Burgin et al., 2011).

In contrast to sea-level rise, longer growing seasons and CO_2 fertilization will likely enhance carbon substrate supply and in turn CH_4 production. Our study demonstrates that the effect of increased organic matter depends on plant species, the availability of other methanogenic substrates, and the presence of alternative electron acceptors. It is possible that longer growing seasons and CO_2 fertilization could reduce competition between methanogens and other microbial communities by providing more substrates, as we saw in freshwater wetlands with higher sulfate concentrations, thereby superseding the effect of sea-level rise. Additionally, the CO_2 fertilization effect could increase organic matter accretion of marsh plants, which could physically counteract sea-level rise by raising marsh elevation (Langley et al., 2009). Future studies should consider how the interaction of sea-level rise, increased organic matter, and warming will affect both the microbial and ecosystem processes of the global methane cycle. This intersection of global change processes will be particularly important for projecting the position of coastal wetland ecosystems relative to their greenhouse compensation point.

6 Data availability

The data will be freely accessible through the international repository, Knowledge Network for Biocomplexity (KNB) at: https://knb.ecoinformatics.org doi:10.5063/F1125QKN.

Author contributions. CV designed the study as sparked from discussions with SEJ. CV and JAH conducted the fieldwork and laboratory analyses. WEW played a key role in methodology and analysing methane samples with the GC. SEJ and

GAL played advisory roles in shaping this research. CV wrote prepared the manuscript with contributions from all co-authors.

Competing interests. The authors declare that they have no conflict of interest.

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Table 1. Physical and chemical characteristics (mean ± sd) of the wetlands sampled in the Copper River Delta including elevation, maximum depth, temperature, pH, dissolved oxygen (DO), specific conductivity (SpC), salinity, dissolved organic carbon (DOC), water column sulfate concentration, and sediment organic matter (SOM). Freshwater physicochemical parameters, with the exception of elevation, DOC, sulfate and SOM, are from spot measurements of the hypolimnion conducted throughout summer 2014 (n = 4 per freshwater wetland). Intertidal marsh parameters are from spot measurements of the surface layer (n = 10). DOC, sulfate, and SOM are from June and August 2014 (n = 10 per wetland).

Wetland	Elevation (m)	Max Depth (m)	Temp (°C)	pH	DO (mg/L)	SpC (µs/cm)	Salinity (ppt)	DOC (mg/L)	Sulfate (µmol/L)	SOM (%)
Eyak N	5.2	0.60	15.3	5.5	7.4 ± 2.1	13.3 ± 3.0	0.01	6.5 ± 1.9	1.6 ± 0.3	2.0 ± 0.5
Eyak S	5.5	0.61	16.1	7.0	6.9 ± 2.0	11.3 ± 2.2	0.00	5.6 ± 0.5	2.0 ± 0.2	1.8 ± 0.5
Lily	8.2	0.65	13.1	5.9	3.2 ± 1.5	60.1 ± 18.6	0.03	3.5 ± 1.0	6.0 ± 2.2	2.1 ± 0.5
Rich Hate Me	18.3	0.57	11.6	6.1	2.2 ± 1.4	56.4 ± 6.9	0.03	2.1 ± 0.5	24.2 ± 4.5	3.1 ± 3.9
Scott S	13.4	0.81	14.2	6.3	8.6 ± 3.8	61.0 ± 37.1	0.03	2.1 ± 0.6	54.4 ± 16.6	1.5 ± 2.2
Storey N	4.6	0.56	16.8	6.9	8.2 ± 0.3	74.1 ± 11.3	0.04	11.4 ± 0.6	4.5 ± 0.7	1.8 ± 0.2
Storey S	2.1	0.60	16.6	7.3	9.3 ± 0.3	69.5 ± 6.4	0.03	4.2 ± 0.3	7.9 ± 0.5	1.9 ± 3.1
Tiedeman N	5.5	0.66	16.6	6.0	8.4 ± 2.1	12.8 ± 2.8	0.01	6.7 ± 0.7	1.8 ± 0.2	2.8 ± 2.9
Tiedeman S	5.5	0.73	15.4	6.7	8.6 ± 2.0	8.8 ± 1.7	0.00	5.2 ± 0.6	1.7 ± 0.3	2.3 ± 0.8
Intertidal	1.4	0.89	14.3	7.3	10.0 ± 0.5	8476 ± 9573	5.00	3.1 ± 6.3	3375 ± 3917	6.4 ± 4.9

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Table 2. General linearized models (GLM) wherein log-transformed CH_4 production rate is the response variable and ecosystem type (freshwater or intertidal), time period (June or August), porewater acetate level, and total sulfate/nitrate availability are potential factors. Positive (↑) or negative effects (↓) of continuous factors are indicated. Models are ranked in order of the lowest Akaike information criterion corrected for low samples sizes (AIC_c) along with delta AIC_c (Δ_i) and Akaike weights (ω_i). The three models with a larger AIC_c than the null are not presented.

Model #	GLM	AIC_c	Δ_i	ω_i
1	ecosystem + time period + acetate (↑) + sulfate/nitrate (↓)	123.6	0.0	0.59
2	ecosystem + acetate (↑) + sulfate/nitrate (↓)	125.2	1.6	0.26
3	ecosystem + time period + acetate (↑)	127.2	3.6	0.10
4	ecosystem + acetate (↑)	128.3	4.8	0.06
5	ecosystem + time period + sulfate/nitrate (↓)	136.0	12.4	0.00
6	ecosystem + sulfate/nitrate (↓)	137.6	14.1	0.00
7	time period + sulfate/nitrate (↓)	138.2	14.6	0.00
8	sulfate/nitrate (↓)	139.1	15.5	0.00
9	time period + acetate (↑) + sulfate/nitrate (↓)	139.6	16.0	0.00
10	acetate (↑) + sulfate/nitrate (↓)	139.7	16.2	0.00
11	ecosystem + time period	141.9	18.3	0.00
12	ecosystem	143.3	19.7	0.00
13	null	156.8	33.2	0.00

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Table 3. General linearized models (GLM) wherein ΔCH_4 production rate (treatment minus control) is the response variable and the macrophyte species added (buckbean, horsetail, lily, or marestail), porewater acetate availability, and total sulfate and nitrate are potential factors. Positive (↑) or negative effects (↓) of continuous factors are indicated. Models are ranked in order of the lowest Akaike information criterion corrected for low samples sizes (AIC_c) along with delta AIC_c (Δ_i) and Akaike weights (ω_i). The three models with a larger AIC_c than the null are not presented.

Model #	GLM	AIC_c	Δ_i	ω_i
1	acetate (↓)	172.3	0.0	0.43
2	acetate (↓) + species	173.7	1.4	0.21
3	acetate (↓) + sulfate/nitrate (↑)	175.1	2.8	0.11
4	species	175.4	3.0	0.09
5	null	175.9	3.6	0.07

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Figure Captions

Figure 1. Conceptual diagram illustrating the potential effects of warming, sea-level rise, and increased organic matter (OM) availability on CH_4 production in coastal wetlands. These three global change mechanisms are all indirect consequences of rising CO_2 levels.

Figure 2. Aerial photo of the Copper River Delta taken by the USDA Forest Service depicting the major wetland ecosystem types extending from glaciers to ocean.

Figure 3. Mean CH_4 production rates (μmol day^{-1} per bottle) from Copper River Delta freshwater (n = 9) and intertidal (n = 5) wetlands during June (a) and August (b), 2014. Error bars represent standard errors. Bottle incubations were conducted with approximately 60 mL of sediment and 60 mL of water.

Figure 4. Mean CH_4 production rates (μmol day^{-1} per bottle) from freshwater wetland sediments incubated with freshwater (FW/FW; n = 5) and other sediments from the same freshwater wetlands incubated with brackish water from intertidal marsh (FW/INT; n = 5). Error bars represent standard errors. This sea-level rise simulation was conducted over a 14d period in June 2014. Bottle incubations were conducted with approximately 60 mL of sediment and 60 mL of water.

Figure 5. Mean CH_4 production rates (μmol day^{-1} per bottle) from organic matter treatments (CTL = control, BB = buckbean *Menanythes trifoliata*, HT = horsetail *Equisetum variegatum*, LI = lily *Nuphar polysepalum*, and MT = marestail *Hippuris vulgaris*) replicated in five freshwater wetlands during August 2014. Error bars represent standard error. Bottle incubations were conducted with approximately 60 mL of sediment and 60 mL of water.

Figure 6. Actual response of ΔCH_4 production (treatment–control) plotted against the predicted response from model-averaged parameter estimates of the macrophyte species added (BB= buckbean *Menanythes trifoliata*, HT = horsetail *Equisetum variegatum*, LI = lily *Nuphar polysepalum*, and MT = marestail *Hippuris vulgaris*), porewater acetate availability, and total sulfate and nitrate. The dotted black line depicts the 1:1 line.

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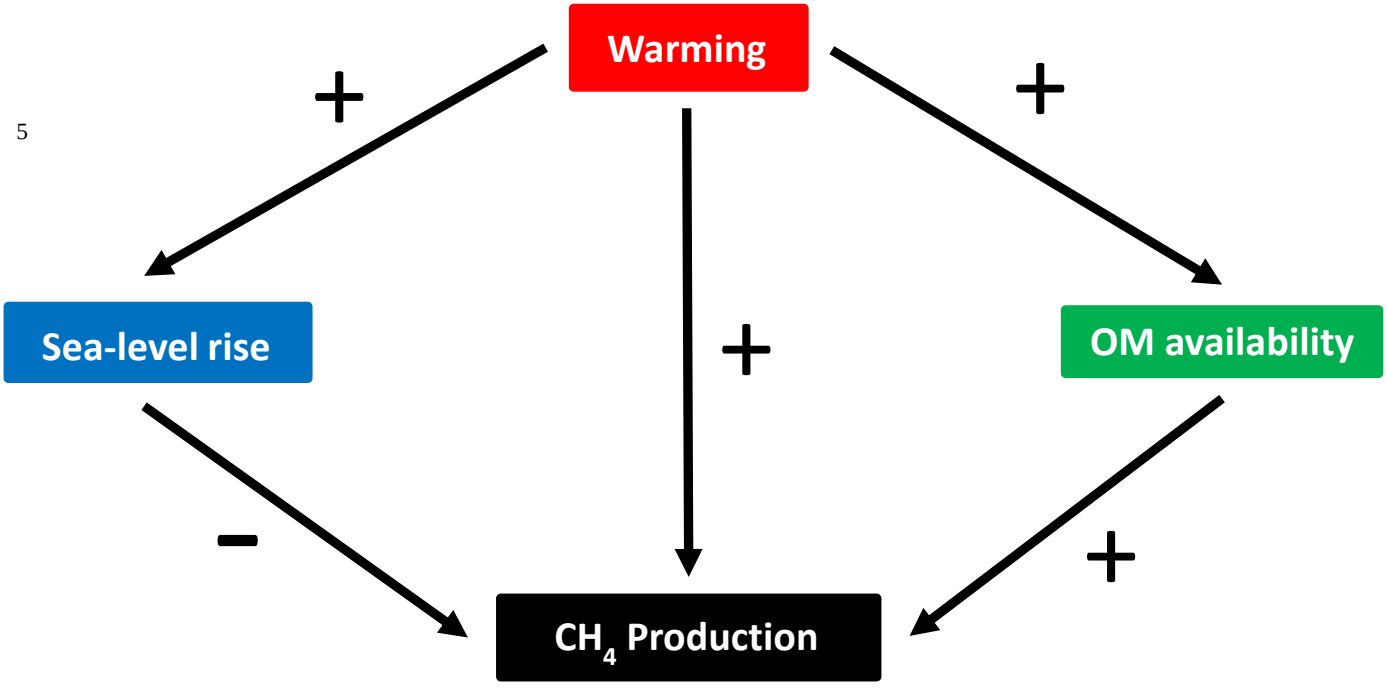

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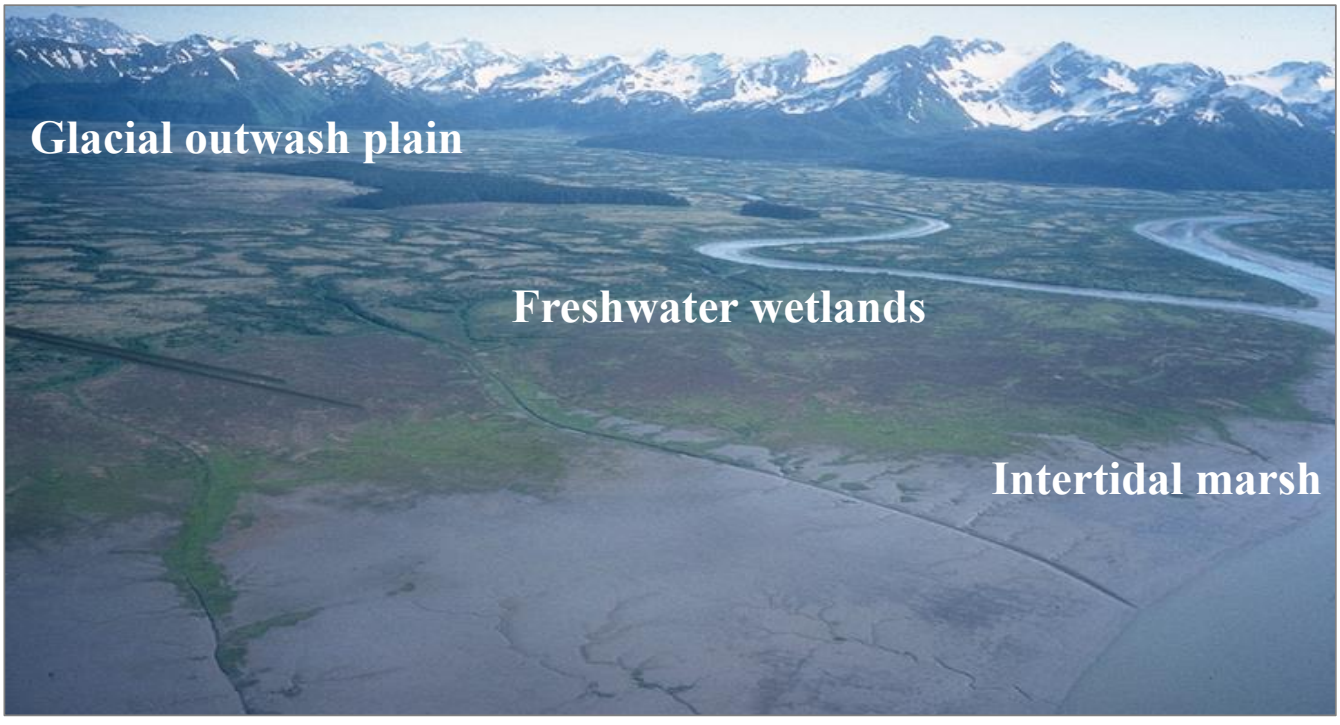

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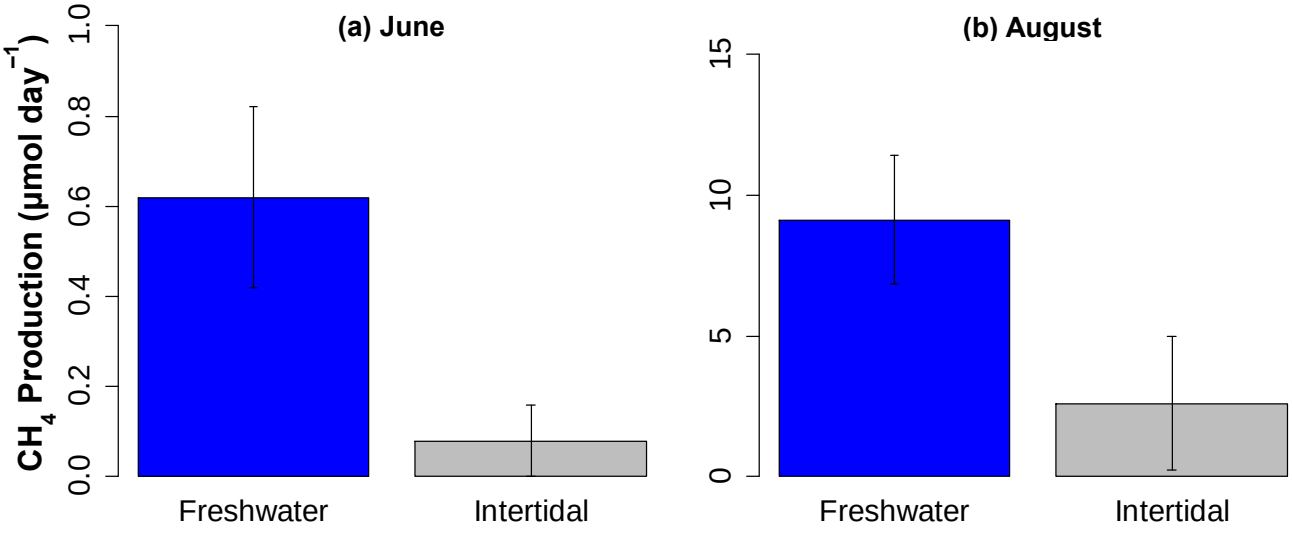

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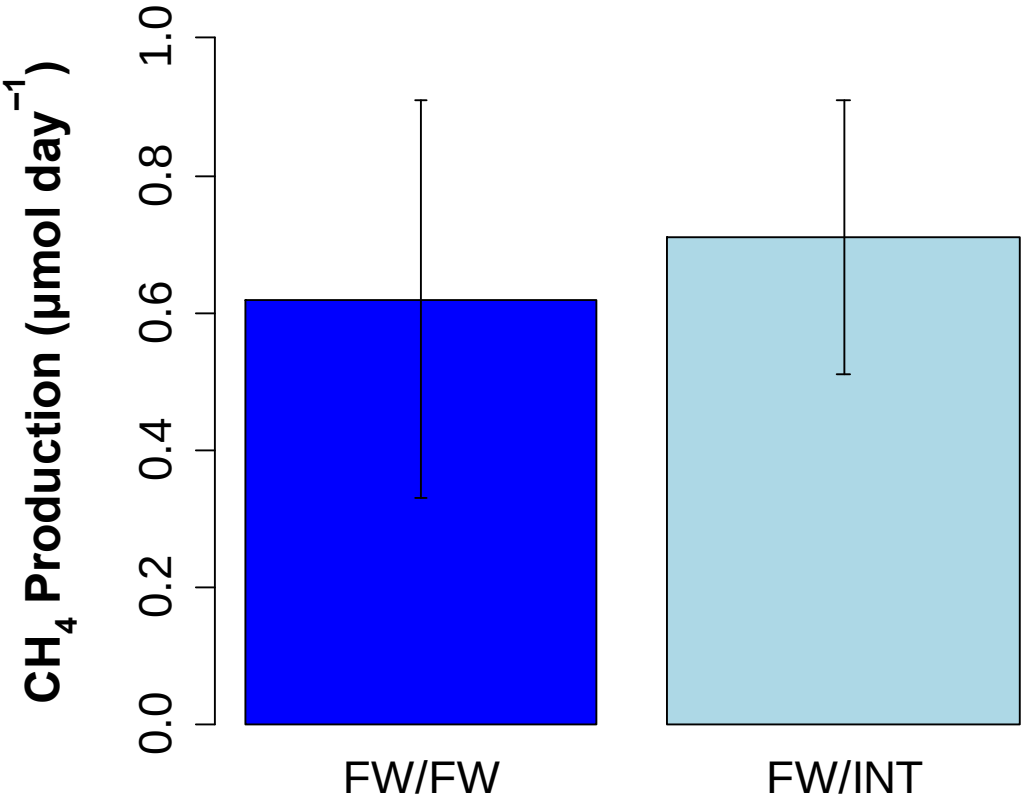

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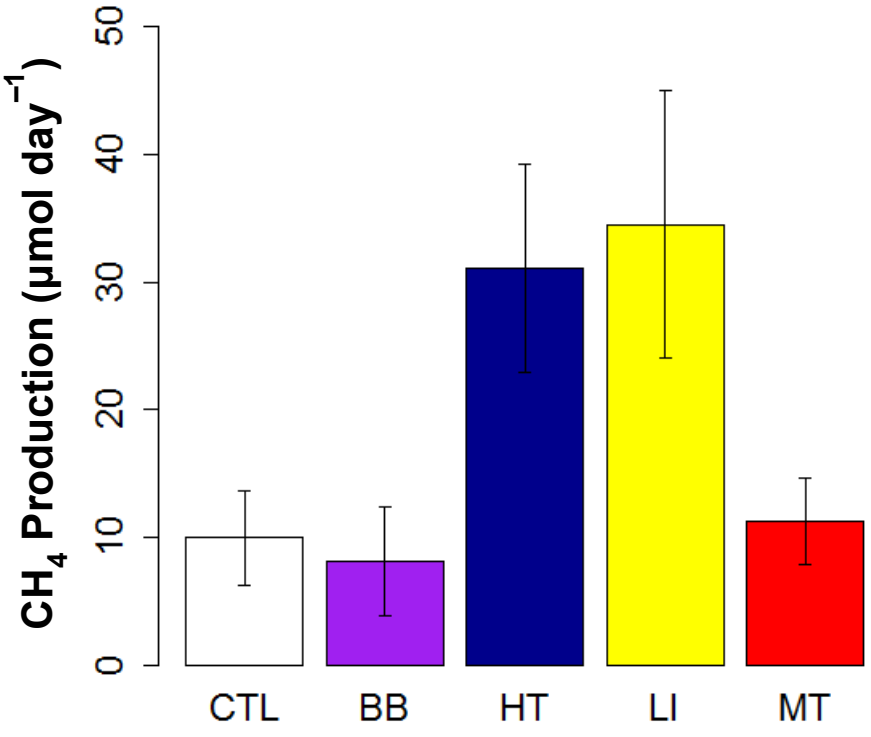

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