1   Biogeochemical and plant trait mechanisms drive enhanced methane emissions

2   in response to whole-ecosystem warming

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27   **Abstract**

28   Climate warming perturbs ecosystem carbon (C) cycling, causing both positive and negative

29   feedbacks on greenhouse gas emissions. In 2016, we began a tidal marsh field experiment in two

30   vegetation communities to investigate the mechanisms by which whole-ecosystem warming alters C gain,

31   via plant-driven sequestration in soils, and C loss, primarily via methane (CH_4) emissions. Here, we

32   report the results from the first four years. As expected, warming of 5.1 °C more than doubled CH_4

33   emissions in both plant communities. We propose this was caused by a combination of four mechanisms:

34   (i) a decrease in the proportion of CH_4 consumed by CH_4 oxidation, (ii) more C substrates available for

35   methanogenesis, (iii) reduced competition between methanogens and sulfate reducing bacteria, and (iv)

36   indirect effects of plant traits. Plots dominated by *Spartina patens* consistently emitted more CH_4 than

37   plots dominated by *Schoenoplectus americanus*, indicating key differences in the roles these common

38   wetland plants play in affecting anaerobic soil biogeochemistry and suggesting that plant composition can

39   modulate coastal wetland responses to climate change.

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41   **1. Introduction**

42   Methane (CH_4) is a potent greenhouse gas that contributes to 15-19 % of total greenhouse gas

43   radiative forcing (IPCC, 2013) and has a sustained-flux global warming potential that is 45 times that of

44   CO_2 on a 100-year timescale (Neubauer & Megonigal, 2015). Wetlands are the largest natural source of

45   CH_4 to the atmosphere and were recently identified as the largest source of uncertainty in the global CH_4

46   budget (Saunois et al., 2016). Recent estimates calculate that CH_4 emissions from vegetated coastal

47   wetlands offset 3.6 of the 12.2 million metric tons (MMT) of CO_2 equivalents accumulated by these

48   ecosystems each year (EPA, 2017). Despite this, there is still a substantial knowledge gap regarding how

49   global change factors, such as climate warming, will alter coastal wetland CH_4 emissions (Mcleod et al.,

50   2011) even though these feedbacks have the potential to shift coastal wetlands from being a net sink of C

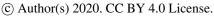51   to a net source (Al-Haj & Fulweiler, 2020; Bridgham et al., 2006).

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52   The net flux of CH_4 to the atmosphere from any ecosystem represents the balance between the

53   amount of CH_4 produced (methanogenesis), the amount of CH_4 oxidized (methanotrophy), and the rate of

54   CH_4 transport from the soil. Rates of methanogenesis are driven by low redox conditions and substrate

55   availability, while aerobic CH_4 oxidation requires both O_2 and CH_4 as substrates. Roots and rhizomes in

56   wetland ecosystems influence methane-related substrates through at least two mechanisms: (i) deposition

57   of organic compounds that support multiple pathways of heterotrophic microbial respiration, including

58   methanogenesis, and (ii) release of O_2 that simultaneously promotes CH_4 oxidation (Philippot et al., 2009;

59   Stanley & Ward, 2010) and regeneration of competing electron acceptors such as Fe(III) and SO_4. Root

60   exudates, which typically include low molecular weight compounds, may either be more readily used by

61   microbes than existing soil C (Kayranli et al., 2010; Megonigal et al., 1999) or may prime microbial use

62   of soil C (Basiliko et al., 2012; Philippot et al., 2009; Robroek et al., 2016; Waldo et al., 2019). Root

63   exudates can also decrease CH_4 oxidation by stimulating use of O_2 by other aerobic microbes (Lenzewski

64   et al., 2018; Mueller et al., 2016). Consequently, wetland CH_4 emissions are strongly linked to a wide

65   variety of plant traits that govern the supply of reductive (organic carbon) and oxidative (O_2) substrates to

66   soils (Moor et al., 2017; Mueller et al., in press).

67   Although it is understood that wetland plants are a primary control on CH_4 emissions and that much

68   of their influence is mediated through conditions in the rhizosphere (Waldo et al., 2019), there are

69   surprisingly few data, especially from coastal wetlands, that couple plant responses to the dynamics of

70   electron donors (organic C), electron acceptors (O_2, SO_4), and the rates of competing (sulfate reduction vs

71   methanogenesis) or opposing (CH_4 production vs CH_4 oxidation) microbial processes. The general lack of

72   process data on wetland CH_4 cycling makes it difficult to forecast ecosystem responses to climate change.

73   For example, the well-documented observation that warming increases wetland methane emissions can be

74   either amplified or dampened depending on changes in plant activity (e.g. primary production) or plant

75   traits (e.g. community composition) (Mueller et al., in press). Vegetation composition has been shown to

76   be a stronger control on CH_4 emissions than ~1 °C of warming in northern peatlands (Ward et al., 2013)

77   and Chen et al. (2017) proposed that warming effects on plant functional types can drive C flux responses

78   that cannot otherwise be explained by abiotic conditions. In freshwater marshes, plant species and growth

79   trends have also been linked to seasonal shifts in pools of dissolved CH_4 and DIC (Ding et al., 2005;

80   Stanley & Ward, 2010) and methanogenesis dynamics (Sorrell et al., 1997).

81   Tidal wetlands are particularly good model systems for determining the mechanisms by which

82   warming alters CH_4 emissions. Not only will the CH_4 cycle respond to the direct effects of warming, but

83   the temperature effects on the outcome of competition for electron acceptors is relatively easily observed

84   because of the abundance of SO_4. Thermodynamic theory in which terminal electron acceptors (TEAs)

85   are used in order of decreasing thermodynamic yield is commonly interpreted to mean that a system will

86   support only one form of anaerobic respiration at time, with methanogenesis occurring only when pools

87   of more energetically favorable TEAs have been depleted. However, in real systems with spatial and

88   temporal variability in the supply of electron donor substrates and TEAs, all forms of anaerobic

89   metabolism occur simultaneously (Megonigal et al. 2004, Bridgham et al., 2013). Much of this spatial and

90   temporal variation arises from the distribution and activity of roots and rhizomes as mediated by the

91   rhizosphere (Neubauer et al., 2008). Global change factors such as warming will further affect the spatial

92   distribution of key metabolic substrates. In addition, the relatively limited species diversity in saline tidal

93   wetlands allows species-level effects on CH_4 cycling to be delineated more easily than in diverse

94   freshwater wetlands.

95   Methane flux measurements are a metric of broader shifts in redox potential and biogeochemical

96   cycling, as they are sensitive to virtually all processes that regulate availability of electron donors and

97   electron acceptors. Emissions are commonly predicted to increase with future climate warming, including

98   from coastal wetlands (Al-Haj & Fulweiler, 2020), but there is minimal prior understanding of the

99   underlying mechanisms, which was the focus of this study. Our objectives were to explore the

100   mechanisms that drive enhanced CH_4 emissions under warming. To accomplish this, we measured

101   monthly CH_4 emissions from 2016 through 2019 and coupled these flux measurements with analysis of

102   porewater biogeochemistry and vegetation biomass and composition.

103

104   **2. Materials and Methods**

105   2.1 Site Description and Experimental Design

106   The Salt Marsh Accretion Response to Temperature eXperiment (SMARTX) was established in the

107   Smithsonian's Global Change Research Wetland (GCReW) in 2016. GCReW is part of Kirkpatrick

108   Marsh, a microtidal, brackish high marsh on the western shore of the Chesapeake Bay, USA (38°53' N,

109   76°33' W). Soils are organic (>80 % organic matter) to a depth of 5 m, which is typical of high marshes

110   in the Chesapeake Bay. The marsh is typically saturated to within 5-15 cm of the soil surface, but

111   inundation frequency varies across the site, from 10-20 % of high tides in high elevation areas to 30-60 %

112   of tides in low elevation areas.

113   SMARTX consists of six replicate transects, three located in each of the two dominant annual plant

114   communities (Fig. S1). In the C_3-dominated community (herein the 'C_3 community') the C_3 sedge

115   *Schoenoplectus americanus* (herein *Schoenoplectus*) composes more than 90 % of the aboveground

116   biomass (Table 1). In the C_4-dominated community (herein the 'C_4 community'), 75 % of the

117   aboveground biomass was initially composed of two C_4 grasses (*Spartina patens* and *Distichlis spicata*,

118   herein *Spartina* and *Distichlis*, respectively). However, by 2019, *Spartina* and *Distichlis* declined to 56 %

119   of the aboveground biomass (Table 1).

Table 1. Relative contribution to total aboveground biomass from C_3 sedges (*Schoenoplectus americanus*) and C_4 grasses (*Spartina patens* and *Distichlis spicata*) in each plant community. Values are means and SE (n = 12).

Year	C₃ community		C₄ community	
	% C_3	% C_4	% C_3	% C_4
2016	93 (3)	8 (3)	8 (2)	76 (6)
2017	91 (3)	9 (3)	10 (3)	64 (4)
2018	95 (1)	5 (1)	15 (4)	65 (7)
2019	93 (2)	4 (1)	23 (5)	56 (6)

120   Each transect is an active warming gradient consisting of unheated ambient plots and plots that

121   are heated to 1.7 °C, 3.4 °C, and 5.1 °C above ambient. All plots are 2 x 2 meters with a 20 cm-wide

122   buffer around the perimeter. Aboveground plant-surface temperature is elevated via infrared heaters and

123   soil temperature is elevated via vertical resistance cables (Rich et al., 2015). Soils are heated to a depth of

124   1.5 m, which is the depth most vulnerable to climate or human disturbance (Pendleton et al., 2012).

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125   Aboveground and belowground temperature variation are assessed via thermocouples embedded in

126   acrylic plates at plant canopy level and inserted into the soil, respectively, and the temperature gradient is

127   maintained by integrated microprocessor-based feedback control (Rich et al., 2015). Noyce et al. (2019)

128   provides additional details of the heating system. Warming began on 1 Jun 2016 and has continued year-

129   round.

130   2.2 Methane flux measurements

131   Methane emissions were measured monthly year-round from May 2016 to Dec 2019 using a static

132   chamber system. One permanent 160 cm^2 aluminum base was inserted 10 cm into the soil in each plot in

133   Apr 2016. On each measurement date, clear chambers (40 x 40 x 40 cm) were gently placed on top of

134   each base and secured with compression clips. Chambers consisted of an aluminum frame with

135   transparent sides made of polychlorotrifluoroethylene film (Honeywell International) and closed-cell

136   foam on the base. Depending on the height of the vegetation at the time of measurement, chambers were

137   stacked up to four high (total height of 40 to 160 cm) (Fig. S2). The advantage of this stacking method is

138   that it uses the minimal chamber volume necessary, while also allowing for plant growth. After

139   placement, the chambers were left open for at least 10 minutes, to minimize disturbance effects and allow

140   air inside the chamber to return to ambient conditions. During data collection, chambers were covered

141   with a transparent polycarbonate top equipped with sampling tubes, a fan to circulate air inside the

142   chambers, a PAR sensor, and thermocouples. The sealed chamber was covered with a foil shroud to block

143   out all light and to minimize changes in temperature and relative humidity during the measurement

144   period. An UltraPortable Greenhouse Gas Analyzer (Los Gatos Research, CA) was used to measure

145   headspace CH_4 concentrations for 5 min. Plots were accessed from permanent boardwalks elevated 15 cm

146   above the soil surface to avoid compressing the surrounding peat and altering CH_4 emissions. Fluxes were

147   calculated as the slope of the linear regression of CH_4 concentration over time. The 40 fluxes where $p >$

148   0.05 were assigned a value of ½ the limit of detection of the system (Wassmann et al., 2018; Table S1).

149   This was 1 % of fluxes during the growing season and 4.5 % of fluxes over the remaining months. For

150   2017-2019, monthly measurements were scaled to annual estimates by regressing CH_4 emissions against

151 daily mean soil temperature and day of year (as a proxy for phenological status). Annual estimates were

152 not calculated for 2016 because flux measurements did not start until May.

153 2.3 Porewater sampling and analysis

154   Porewater samples were collected in May, Jul, and Sep of each year using stainless steel 'sippers'

155 permanently installed in each plot. Each sipper consisted of a length of stainless-steel tubing, crimped and

156 sealed at the end, with several slits (approximate width 0.8 mm) cut in the bottom 2 cm. The aboveground

157 portion of each sipper was connected to Tygon Masterflex® tubing capped with a 2-way stopcock. In

158 May 2016, duplicate clusters of sippers were installed in each of the 30 plots at 20, 40, 80, and 120 cm

159 below the soil surface. An additional set of 10 cm-deep sippers was installed in 2017. In this study we

160 defined samples from 10-20 cm as "rooting zone" samples and samples from 40-120 cm as "deep peat"

161 samples. On sampling dates, porewater sitting in the sippers was drawn up and discarded, after which 60

162 mL of porewater from each depth (30 mL from each sipper) was withdrawn and stored in syringes

163 equipped with 3-way stopcocks. A 10 mL-aliquot of each sample was filtered through a pre-leached 0.45

164 µm syringe-mounted filter, preserved with 5 % zinc acetate and sodium hydroxide, and frozen for future

165 SO_4 and Cl analysis. Dissolved CH_4 was extracted from 15 mL of porewater in the syringe by drawing 15

166 mL of ambient air and shaking vigorously for 2 min to allow the dissolved CH_4 to equilibrate with the

167 headspace. Headspace subsamples were then immediately analyzed on a Shimadzu GC-14A gas

168 chromatograph equipped with a flame ionization detector. The remaining porewater was used to measure

169 pH, H_2S, and NH_4; those data are not reported here.

170   SO_4 and Cl were measured on a Dionex ICS-2000 ion chromatography system (2016-2018) or a

171 Dionex Integrion (2019). On the Dionex ICS-2000 samples were separated using an AA11 column with

172 30 mM of KOH as eluent; on the Dionex Integrion samples were separated using an AA11-4µm-fast

173 column with 35 mM KOH. Sulfate depletion (Sulfate$_{Dep}$) was calculated based on measured porewater

174 concentrations of SO_4 (SO_{4pw}) and Cl (Cl_{pw}) and the constant molar ratio of Cl to SO_4 in surface seawater

175 (R_{sw} = 19.33; Bianchi 2006) using the following equation: Sulfate$_{Dep}$ = Cl_{pw} / R_{sw} - SO_{4pw}. Based only on

176 seawater inputs, the ratio of Cl to SO_4 would remain constant, but under anaerobic conditions SO_4 can be

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177   reduced by sulfate reducing bacteria, altering this ratio. As a result, SO_4 depletion can be used as a proxy

178   for SO_4 reduction rates.

179   2.4 Plant biomass measurements

180   Measurements of *Schoenoplectus, Spartina*, and *Distichlis* biomass were conducted during peak

181   biomass of each year (29 Jul – 2 Aug) as described by Noyce et al. (2019). *Schoenoplectus* biomass was

182   estimated using non-destructive allometric techniques (Lu et al., 2016) in 900-cm^2 quadrats, and *Spartina*

183   and *Distichlis* biomass were estimated through destructive harvest of 25-cm^2 subplots.

184   2.5 Data analysis

185   Statistics were conducted in R (version 3.6.3). ANOVA tests were used to compared means between

186   warming treatments and plant communities, with Tukey's HSD test for *post hoc* analyses. The 'growing

187   season' was defined as May through Sep, based on *Schoenoplectus* growth trends (see Fig. S3). Due to

188   the non-normal distribution of the CH_4 flux (Fig. S4) and porewater data, values were log transformed

189   prior to calculating analyses.

190

191   **3. Results**

192   3.1 Environmental conditions and experiment performance

193   The growing season of 2016 was the hottest of the four years, with growing season temperatures

194   averaging >1 °C above the other three years (Table 2). While 2017 through 2019 had similar summer

195   temperatures, they had very different precipitation regimes; 2018 was much wetter on average and 2019

196   was slightly drier (Table 2). During all years, temperatures in the experimental plots were successfully

197   shifted by the target differentials of +1.7, +3.4, and +5.1 °C above the ambient plots (Fig. 1; Noyce et al.,

198   2019).

Table 2. Growing season (May-Sep) temperature and precipitation. Temperature data are means (SE) of daily averages from ambient plots and precipitation is total from May through September.

Year	Mean aboveground temperature (°C)	Mean soil temperature (°C)	Total precipitation (cm)
2016	24.7 (0.3)	22.4 (0.2)	51.0
2017	22.0 (0.3)	20.7 (0.2)	51.1
2018	23.7 (0.3)	20.8 (0.2)	86.4
2019	23.6 (0.3)	20.9 (0.2)	43.7

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199   3.2 Methane fluxes

200   Methane emissions increased with soil temperature ($R^2 = 0.41$, $p < 0.001$) (Fig. 1). Emissions from

201   all treatments had strong seasonal trends; fluxes were highest in the C_3 community in Jun through Aug

202   and peak fluxes in the C_4 community were shifted about a month later to Jul through Sep (Fig. S4).

203   Whole-ecosystem warming increased CH_4 emissions throughout the growing season ($F_{3,439} = 3.7$, $p =$

204   0.012; Fig. 2). Across all four years, 5.1 °C of warming more than doubled growing season emissions,

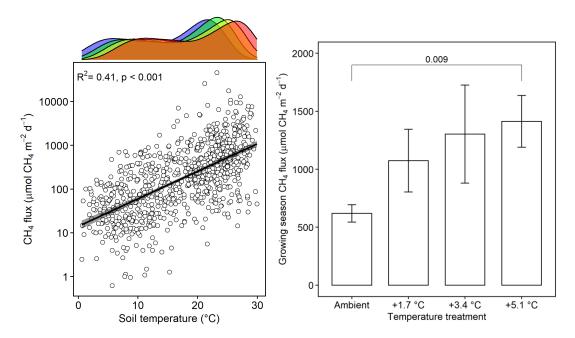205   from 624 to 1413 µmol CH_4 m^{-2} d ($p_{adj} = 0.01$; Fig. 2).

Figure 1. CH_4 emissions from each plot versus the soil temperature at the time of measurement. Density plot shows the range of soil temperatures in each treatment.

Figure 2. Comparison of CH_4 emissions from each warming treatment during the growing season (May-Sep). Means include both the C_3 and C_4 community and all years of measurement. Error bars indicate SE. Horizonal bars indicate means that are significantly different and the corresponding p_{adj}.

206   Mean CH_4 emissions were higher from the C_4 community than from the C_3 community both during

207   the growing season ($F_{1,70} = 22.0$ $p < 0.001$; Fig. 3a) and on an annual basis ($F_{1,70} = 10.2$, $p = 0.002$; Fig.

208   3b). Mean annual CH_4 emissions ranged from 58 mmol CH_4 m^{-2} yr^{-1} (ambient) to 343 mmol CH_4 m^{-2} yr^{-1}

209   (+5.1 °C) in the C_3 community and from 55 mmol CH_4 m^{-2} yr^{-1} (ambient) to 879 mmol CH_4 m^{-2} yr^{-1} (+5.1

210   °C) in the C_4 community (Table S2). Under ambient conditions, growing season CH_4 fluxes were almost

211   twice as large from C_4 plots, whereas under low warming (1.7-3.4 °C) this difference increased to more

212   than three times as large (Fig. 3a). From 2017-2019, CH_4 emissions were positively related to *Spartina*

213   and *Distichlis* aboveground biomass across all warming treatments and negatively related to

214   *Schoenoplectus* biomass (Fig. 4a,b). In 2016, however, the direction of those relationships in both plant

215   communities were the exact opposite, with *Spartina* and *Distichlis* biomass negatively related, and

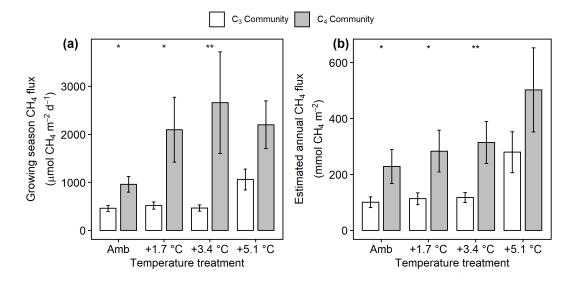216   *Schoenoplectus* biomass positively related, to CH_4 emissions (Fig. 4a,b).

Figure 3. Comparison of CH_4 emissions from the C_3 community dominated by *Schoenoplectus* (open bars) and the C_4 community dominated by *Spartina* and *Distichlis* (grey bars). (a) During the growing season (May-Sep) and (b) scaled to a year. Means are averaged across all sampling dates for 2017 – 2019. Error bars indicate SE. Asterisks indicate significant differences between C_3 and C_4 means at a given temperature (* $p_{adj} <0.05$, ** $p_{adj} < 0.01$).
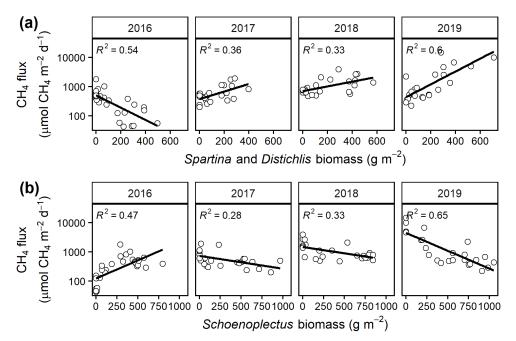
Figure 4. Mean growing season (May-Sep) CH$_4$ emissions from each plot versus the biomass of (a) C$_3$ (*Schoenoplectus*) and (b) C$_4$ (*Spartina* and *Distichlis*) plants. All regressions are significant at $p = 0.05$.

217   3.3 Porewater chemistry

218   Under ambient conditions, porewater collected from the C$_4$ community had lower salinity ($F_{1,315}$ =

219   15.5, $p < 0.001$), more dissolved CH$_4$ ($F_{1,315}$ = 45.0, $p < 0.001$; Fig. 5a,b), and less SO$_4$ ($F_{1,315}$ = 61.5, $p <$

220   0.001; Fig 6a) than the C$_3$ community. In the C$_3$ community, warming increased dissolved CH$_4$ in both

221   the rooting zone porewater (i.e. 10-20 cm) ($F_{3,225}$ = 5.45, $p = 0.001$; Fig. 5a) and in the deep peat (40-120

222   cm) ($F_{3,368}$ = 13.6, $p < 0.001$; Fig. 5b). Dissolved CH$_4$ concentrations were relatively similar in the

223   ambient, +1.7 °C, and +3.4 °C treatments, but more than doubled with +5.1 °C of warming in both the

224   rooting zone (59 to 125 µmol CH$_4$ L^{-1}, p_{adj} = 0.001) and the deeper porewater (43 to 1254 µmol CH$_4$ L^{-1},

225     $p_{adj} < 0.001$). In the C$_4$ community there was minimal effect of warming treatment on porewater in the

226   rooting zone ($F_{3,230}$ = 5.45, $p = 0.67$; Fig. 5a), but all levels of warming decreased dissolved CH$_4$ below 40

227   cm ($F_{3,379}$ = 39.3, $p < 0.001$), with concentrations in the +3.4 and +5.1 plots less than a third of the

228   concentrations in the ambient plots (155 vs 56 and 40 µmol CH$_4$ L^{-1}, $p_{adj} < 0.001$; Fig. 5b).
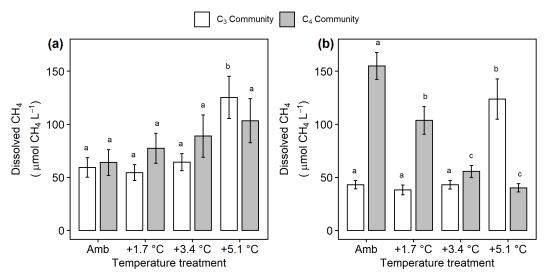
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Figure 5. Comparison of dissolved CH_4 from the C_3 community dominated by *Schoenoplectus* (open bars) and the C_4 community dominated by *Spartina* and *Distichlis* (grey bars). (a) In the dominant rooting zone (10-20 cm) and (b) below the rooting zone (40-120 cm). Means are averaged across all sampling dates for 2016 – 2019. Error bars indicate SE. Letters indicate temperature treatments that are significant different from each other (p_{adj} <0.05) within the same plant community.
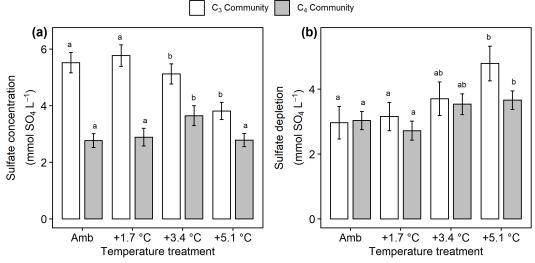

Figure 6. Comparison of sulfate concentrations and estimated sulfate depletion from the C_3 community dominated by *Schoenoplectus* (open bars) and the C_4 community dominated by *Spartina* and *Distichlis* (grey bars). (a) Sulfate in the entire soil profile and (b) sulfate depletion in the rooting zone. Means are averaged across all sampling dates for 2016 – 2019. Error bars indicate SE. Letters indicate temperature treatments that are significant different from each other (p_{adj} <0.05) within the same plant community.

229   In the C_3 community, warming of +3.4 and +5.1 °C reduced SO_4 concentrations ($F_{3,597}$ = 11.7, *p* <

230   0.001), but warming effects on SO_4 cycling in the C_4 community were more mixed with +3.4 °C

231   increasing SO_4 (p_{adj} = 0.013) but no other treatments having large effects (Fig. 6a). In all plots, the

232   measured concentrations of rooting zone SO_4 were lower than expected based on salinity (Fig. 6b),

233   indicating that SO_4 reduction occurred. In both plant communities, the +5.1 °C treatments increased this

234   SO_4 depletion effect compared to ambient (C_3: $F_{3,349}$ = 3.96, p = 0.008; C_3: $F_{3,358}$ = 3.04, p = 0.029) (Fig.

235   6b). In both plant communities, dissolved CH_4 was highest when SO_4 concentrations were below 5 mmol

236   SO_4 L^{-1} (Fig. S5).

237

238   **4. Discussion**

239   Soil temperature (both seasonal and experimental) and plant traits were both strong drivers of CH_4

240   emissions from this site. This follows prior field, mesocosm, and incubation studies across a variety of

241   wetlands, in which temperature has been shown to be a strong predictor of CH_4 emissions (e.g. Al-Haj

242   and Fulweiler, 2020; van Bodegom and Stams, 1999; Christensen et al., 2003; Dise et al., 1993; Fey and

243   Conrad, 2000; Liu et al., 2019; Ward et al., 2013; Yang et al., 2019; Yvon-Durocher et al., 2014) and in

244   which plant functional type has an interacting effect (Chen et al., 2017; Duval & Radu, 2018; L. Liu et al.,

245   2019; Mueller et al., in press; Ward et al., 2013). Methane emissions are a function of the balance

246   between methanogenesis, CH_4 oxidation, and CH_4 transport, so explaining these results requires some

247   combination of stimulation of methanogenesis, reduction of CH_4 oxidation, or increase in CH_4 transport.

248   Prior data from brackish wetlands are limited, but incubation studies on freshwater wetland soils

249   typically show large increases in CH_4 fluxes with warming (Duval & Radu, 2018; Hopple et al., 2020;

250   Inglett et al., 2012; Sihi et al., 2017; van Bodegom & Stams, 1999; Wilson et al., 2016), indicating that

251   warming alters belowground processes. Though there is some evidence that rhizosphere temperature

252   alters CH_4 transport through rice aerenchyma (Hosono & Nouchi, 1997), any transport-driven effects in

253   this ecosystem would be transient unless there was a simultaneous increase in net CH_4 production (i.e. an

254   increase in methanogenesis that was not completely offset by methanotrophy). Instead, we observed a

255   sustained increase in CH_4 emissions, suggesting large shifts in anaerobic metabolism, especially with +5.1

256   °C of warming. We propose four potential and non-exclusive mechanisms to explain the temperature-

257   driven increase in CH$_4$ emissions: (1) shifted ratios of CH$_4$ production to oxidation, (2) increased substrate

258   availability, (3) reduced competition with sulfate reducers, and (4) indirect plant trait effects (Fig. 7).
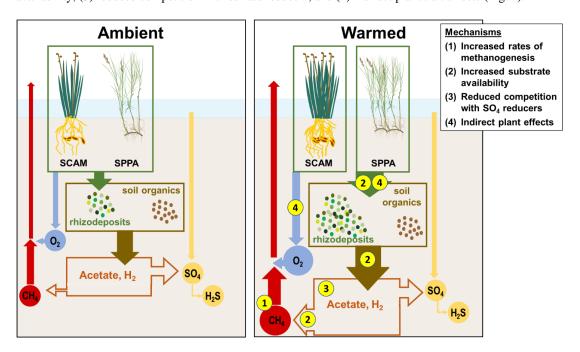

Figure 7. Schematic of mechanisms driving enhanced CH$_4$ emissions in response to warming. SCAM = *Schoenoplectus americanus*. SPPA = *Spartina patens*. Left: Processes under ambient conditions. Plants add organic compounds to the soil, which are transformed into other low molecular weight organic compounds. This pool, and processed soil organic matter, support terminal respiration processes dominated by SO$_4$ reduction over CH$_4$ production in organic-rich brackish marsh soils. Plants also transport O$_2$ which supports oxidation of a fraction of the CH$_4$ before it can be transported out of the soil. Right: Processes under warmed conditions. 1) Rates of CH$_4$ production increase more than rates of CH$_4$ oxidation. 2) Substrate availability increases as plants add more rhizodeposits and organic matter is more rapidly fermented to low molecular weight organic compounds and H$_2$. 3) The pool of electron donors available to methanogens increases as SO$_4$ reducers become SO$_4$ limited. 4) The dominant plant species have different effects on these process with *S. americanus* driving a net increase in O$_2$ transport and *S. patens* driving a net increase in rhizodeposits.

259   4.1 Whole-ecosystem warming promotes methanogenesis over CH$_4$ oxidation

260   Holding the supply of substrates and transport properties of the system constant, warming is expected

261   to increase rates of CH$_4$ production relative to CH$_4$ oxidation due solely to differences in the temperature

262   dependence of each process (Megonigal et al., 2016). In wetland soils, the average Q$_{10}$ of methanogenesis

263   is 4.1 compared to 1.9 for aerobic CH$_4$ oxidation (Segers, 1998), which means that a system starting with

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264   a given initial ratio between the two processes will become increasingly dominated by methanogenesis as

265   soils warm. A corollary to this expected pattern is that the ratio of the two processes should be constant if

266   the Q_{10} responses are similar, an outcome that was supported with *in situ* measurements of the two

267   processes in a tidal freshwater forested wetland (Megonigal & Schlesinger, 2002). We did not quantify

268   the temperature dependence of CH_4 production and oxidation in the present study, but based on the

269   literature (Segers, 1998) it is likely that methanogenic activity increased more than aerobic

270   methanotrophic activity in direct response to warming (Fig. 7, mechanism 1). Evidence for this is that

271   rhizosphere pools of porewater CH_4 were highest in the warmest treatment (Fig. 5a); because this

272   occurred despite either no change or an increase in aboveground biomass (Noyce et al., 2019) which

273   would by itself have lowered porewater CH_4 due to venting (plant transport), it indicates that CH_4

274   production increased relative to the sum of aerobic and anaerobic methane oxidation.

275   4.2 Whole-ecosystem warming increases substrate availability for methanogens

276   Methanogenesis can be the terminal step of anaerobic decomposition, but a consortium of microbes is

277   required to break down soil organic matter to electron donor substrates that methanogens can metabolize.

278   The final step in any decomposition pathway involves the flow of electrons from organic matter (electron

279   donors) to a TEA. Under anaerobic conditions, this is accomplished by microbes that tend to specialize in

280   one TEA and compete for organic C as an electron donor (Megonigal et al., 2004). Consequently, the

281   supply of both electron donors and TEAs regulate the multi-step process of anaerobic decomposition and

282   thus ultimately control CH_4 emissions. Methanogenic activity is typically limited by the supply of

283   electron donors, including low molecular weight organic compounds (e.g. acetate, Neubauer & Craft,

284   2009) and H_2, a product of organic matter fermentation. We propose that whole-ecosystem warming

285   increases the availability of previously limited C substrates through two pathways (Fig. 7, mechanism 2).

286   First, warming may directly influence C availability through biochemical kinetics. Even if organic

287   inputs remained constant, warming likely accelerates fermentation of soil organic matter, presumably

288   increasing substrate availability for methanogens. Second, the warmed plots had longer growing seasons

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289   than the unheated controls (Noyce et al., 2019). This increases inputs of root exudates and fresh detritus,

290   accelerating all forms of heterotrophic microbial respiration by providing organic material that can be

291   broken down into acetate, H_2, and other electron donors (Philippot et al., 2009) and stimulating CH_4

292   emissions from warmed plots, particularly during the growing season as seen here. In 2017, we measured

293   GPP at the same time as CH_4 emissions and observed that CH_4 emissions were positively correlated with

294   GPP and that this effect increased with warming (Fig. S6). Prior studies have also linked CH_4 production

295   or emissions to rates of photosynthesis (Vann & Megonigal, 2003), periods of active growth (Chen et al.,

296   2017; Ward et al., 2013), and plant senescence which coincides with a pulse of labile C from plants to

297   soils (Bardgett et al., 2005).

298   <u>4.4 Whole-ecosystem warming reduces competition with sulfate reducers</u>

299   While acetate and other low molecular weight organic compounds are important electron donors for

300   methanogenic respiration, they are also a key substrate for other microbial groups including SO_4 reducers

301   (Megonigal et al., 2004; Ye et al., 2014). As a result, consumption of the limited acetate supply by SO_4

302   reducers should (and often does) limit methanogenic activity, such that terminal microbial respiration is

303   typically dominated by SO_4 reduction in brackish marshes (Sutton-Grier et al., 2011).

304   We did not measure rates of SO_4 reduction in this study, but can use SO_4 depletion as a proxy; more

305   SO_4 depletion indicates that more SO_4 reduction has occurred. Warming generally increased SO_4

306   depletion, especially in the plots dominated by *Schoenoplectus* (Fig. 6b). Differences in SO_4 depletion

307   between plots is not driven by SO_4 inputs because the only supply of SO_4 is the tidal flow, which is the

308   same for all plots in each community of the experiment. Instead, higher rates of SO_4 reduction are most

309   likely driven by some combination of electron donor supply and kinetics. While SO_4 reducers likely

310   benefited from the increased availability of electron donors, as described above, the kinetics of SO_4

311   reduction also respond strongly to temperature (Weston & Joye, 2005).

312   When SO_4 concentrations drop below a threshold concentration, SO_4 reduction becomes SO_4-limited,

313   rather than electron donor-limited (Megonigal et al., 2004). A review of the coastal wetland CH_4 literature

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314   estimated this threshold at 4 mmol SO_4 (Poffenbarger et al., 2011), a value that is consistent with patterns

315   of porewater $[CH_4]$ and $[SO_4]$ at the GCReW site (Keller et al. 2009). As SO_4 and O_2 are the dominant

316   electron-accepting compounds that suppress methanogenesis in this organic soil, this drawdown then

317   releases the methanogens from substrate competition (Fig. 7, mechanism 3). Here, we show that $[SO_4]$ is

318   typically below 4 mmol in the +5.1 plots in the C_3 community and in all plots in the C_4 community (Fig.

319   6, Fig. S5). The drawdown of SO_4 may also reduce rates of anaerobic CH_4 oxidation (Hinrichs & Boetius,

320   2003). Van Hulzen et al. (1999) proposed a multi-phase system in a warming incubation experiment,

321   observing that first methanogens are out-competed for substrates by other microbes, next CH_4 production

322   increases as the supply of inhibiting TEA decreases, and finally [TEA] decreases to the point that

323   methanogenesis is controlled only by the supply of electron donors. Warming in this study decreased time

324   required for the system to pass through the first two phases (van Hulzen et al., 1999). In our study, this

325   final phase of increased methanogenic activity occurs when SO_4 concentrations dip below 4 mmol SO_4 L$^-$

326   ^{1}, which occurs most often in the +5.1 °C plots, especially in the C_3 community. This interpretation is also

327   supported by the long-term record of porewater chemistry from an allied experiment at the site,

328   demonstrating that porewater CH_4 concentrations increase as SO_4 concentrations decrease (Keller et al.,

329   2009).

330   Methanogens may also have a competitive advantage over SO_4 reducers for electron donor

331   consumption at warmer temperatures (van Hulzen et al., 1999). Sulfate reducers and methanogens have

332   very similar K_M values for acetate, but the K_M for acetoclastic methanogenesis may decrease with

333   temperature whereas K_M values for SO_4 reducers increase with temperature (van Bodegom & Stams,

334   1999). If this is the case in our system then warming would allow methanogens to use a greater proportion

335   of the available acetate and other organic compounds.

336   4.5 Plant traits modify warming effects on CH_4 cycling

337   The three biogeochemical mechanisms we propose to explain a warming-induced increase in CH_4

338   emissions should interact strongly with plant responses to warming. Relationships between plant

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339   functional groups and CH_4 emissions have been demonstrated through field studies in other wetland
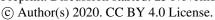
340   ecosystems such as peatlands (Bubier et al., 1995; Ward et al., 2013) (Bubier et al., 1995, Ward et al.,

341   2013) and in tidal wetland mesocosms (L. Liu et al., 2019; Martin & Moseman-Valtierra, 2017; Mueller

342   et al., in press). We provide field evidence that two species with distinct plant traits -- *Schoenoplectus* and

343   *Spartina* -- have strikingly different effects on CH_4 emissions from brackish wetlands. *Spartina*-

344   dominated communities had consistently higher CH_4 emissions under both ambient and warmed

345   conditions (Fig. 3). In most years, *Schoenoplectus* biomass was negatively correlated with CH_4 emissions,

346   while *Spartina*/*Distichlis* biomass was positively correlated. Vegetation effects are typically strongest

347   during the growing season, when the plants are actively altering rhizosphere biogeochemistry (F.-J. W. A.

348   van der Nat & Middelburg, 1998; Ward et al., 2013), which is consistent with our observations in this

349   study.

350   As with warming effects, plant-driven shifts in CH_4 emissions are the result of differing rates of CH_4

351   production, oxidation, transport, or a combination of these processes, but sustained differences in

352   emissions cannot be attributed only to transport, as discussed previously. Instead, the stimulation of CH_4

353   emissions is likely due to changes in the plant-mediated supply of electron acceptors and electron donors.

354   In a field environment, differentiating between species-specific effects and underlying environmental

355   conditions can be difficult, but mesocosm studies that control all environmental factors have also found

356   species-specific effects on CH_4 cycling (e.g. (D. Liu et al., 2014). Plants can alter CH_4 cycling by adding

357   O_2 (electron acceptor) or C substrates (electron donors) to the rhizosphere, altering the redox state. We

358   propose that *Schoenoplectus* is a net oxidizer of the rhizosphere and that *Spartina* is a net reducer, and

359   thus their presence and productivity have opposing effects on CH_4 emissions (Fig. 7, mechanism 4).

360   *4.5.1 Schoenoplectus oxidizes the rhizosphere, increasing CH_4 oxidation*

361   Species vary in their capacity to support aerobic CH_4 oxidation (van der Nat & Middelburg, 1998)

362   and *Schoenoplectus* appears to support higher rates of aerobic CH_4 oxidation than *Spartina* (Mueller et

363   al., in press). *Scirpus lacustris* is morphologically similar to *Schoenoplectus americanus* studied here and

364   has been demonstrated to have substantial rhizospheric oxidation capacity, especially during the growing

365   season (van der Nat & Middelburg, 1998). Consequently, these plants likely exert stronger control on

366   rates of CH_4 oxidation than rates of methanogenesis (van der Nat & Middelburg, 1998). We hypothesize

367   that the relatively high capacity of *Schoenoplectus* to transport O_2 held the CH_4 emissions stimulation

368   caused by modest levels of warming (+1.7 to +3.4 °C) to rates similar to under ambient conditions (Fig.

369   3). At high warming (+5.1 °C), however, *Schoenoplectus* community CH_4 emissions drastically increase

370   (Fig. 3). We suggest that this is due to the combined effects of the three mechanisms discussed

371   previously, namely the differences in the Q_{10}-values of CH_4 production and CH_4 oxidation, the increased

372   supply of organic substrate through plant productivity, and the decrease in competition for electron

373   donors due to SO_4 depletion. Collectively, when the ecosystem is warmed above current ambient

374   conditions by 5 °C or more, enhanced stimulation of CH_4 production starts to offset some of the

375   *Schoenoplectus* oxidation effect. This also offers an explanation for the positive correlation between

376   *Schoenoplectus* biomass and CH_4 emissions observed in 2016 as that was the hottest of the four years in

377   this study.

378   *4.5.2 Spartina reduces the rhizosphere, increasing CH_4 production*

379   The variability in quality and quantity of root exudates between plant functional types is well known

380   to affect microbial community composition and activity (Deyn et al., 2008). Methanogenesis responses to

381   warming in incubation studies are related to the lignin and cellulose content of the peat, which in turn

382   depends on the plant functional type from which the peat developed (Duval & Radu, 2018). Although

383   warming is likely increasing substrate availability across the whole experiment, the production rate of

384   labile, low molecular weight C substrates through fermentation does not increase as rapidly above 25 °C

385   (Scott C Neubauer & Craft, 2009; Weston & Joye, 2005). Microbes may also preferentially use root

386   exudates as their C sources (Delarue et al., 2014) and consequently warming effects on CH_4 production

387   should be strongest in a system where the plants are directly releasing key C substrates. We propose this

388   explains the patterns that we observed in the C_4 community. Multiple years of porewater chemistry at this

389   site show that *Spartina*-dominated communities have higher DOC and dissolved CH_4 than adjacent

390   *Schoenoplectus* communities (Keller et al., 2009; Marsh et al., 2005). Though we did not directly measure

391   root exudation, porewater DOC is partially derived from root exudation and has been used as a proxy to

392   understand the responses of root exudates to global change factors (Dieleman et al., 2016; Fenner et al.,

393   2007; Jones et al., 2009).

394   In most years, *Spartina* biomass was positively correlated with CH_4 emissions, supporting our

395   hypothesis that *Spartina* favors net CH_4 production. However, in 2016 *Spartina* biomass and CH_4

396   emissions were negatively correlated. Prior work at this site has indicated that *Spartina*/*Distichlis* biomass

397   is more negatively affected by hot and dry growing conditions than *Schoenoplectus* (Noyce et al., 2019)

398   due in part because the *Spartina*/*Distichlis* (C_4) communities are less frequently inundated. The 2016

399   growing season was substantially warmer than average (Table 2) and the heating treatments were

400   initialized on 1 Jun of that year, after the annual plants had already established and may have developed

401   adaptations to ambient, rather than elevated, temperature conditions. The combination of these two effects

402   likely led to heat stress, reducing the root exudates supplied to the rhizosphere microbial community

403   (Heckathorn et al., 2013) and thus minimizing the *Spartina* stimulation effect.

404   4.6 Comparisons with prior data

405   Methane emissions have been measured at the GCReW site previously, but this study represents the most

406   comprehensive dataset collected to date, and is thus particularly useful for advancing the process-based

407   understanding needed to improve prognostic models. Overall, our flux estimates are lower than those

408   reported previously. The earliest CH_4 fluxes were measured in a single month (July) in *Schoenoplectus*-

409   dominated plots and reported to be 331 to 6883 μmol m^{-2} d^{-1} (Dacey et al., 1994), much higher than our

410   range of 359 to 1651 μmol m^{-2} d^{-1} for ambient temperature *Schoenoplectus* plots in July. Similarly, Marsh

411   et al. (2005) reported mean growing season (May-Oct) CH_4 emissions from this site of 846 $\pm$ 111 μmol

412   CH_4 m^{-2} d^{-1}, whereas we measured 656 $\pm$ 79 μmol CH_4 m^{-2} d^{-1} over the same months. Finally, Pastore et

413   al. (2017) estimated average annual fluxes in their *Schoenoplectus*-dominated ambient CO_2 plots as 3.1 $\pm$

414   1.7 g CH_4 m^{-2} yr^{-1}, compared to our estimates of 1.6 $\pm$ 0.3 g CH_4 m^{-2} yr^{-1} for *Schoenoplectus* plots. The

415   different estimates by these studies may be partly due to interannual variability as demonstrated in our

416   data where 2018 had substantially higher fluxes than any of the surrounding years (Table S2).

417   The annual estimates reported here for ambient temperature plots trended lower than published

418   mean CH_4 emissions for mesohaline tidal marshes. Our plots ranged from 0.7 to 9.3 g CH_4 m^{-2} yr^{-1} (mean

419   = 9.3), compared to the range of 3.3 to 16.4 g CH_4 m^{-2} yr^{-1} (mean = 16.4) reported by Poffenbarger et al.

420   (2011). This difference may be explained by the fact that there was significant within-class variation in

421   the oligohaline and mesohaline salinity classes that was unexplained and their assessment was based on

422   too few data points to fully capture the variation that is expected to exist in the mesohaline class. Indeed,

423   subsequent studies have documented fluxes well below 3 g CH_4 m^{-2} yr^{-1} (Krauss & Whitbeck, 2012), and

424   even negative fluxes (Al-Haj & Fulweiler, 2020). We hypothesize that the low fluxes measured at our site

425   reflect *Schoenoplectus americanus* traits that favor CH_4 oxidation more than CH_4 production, and that the

426   high end of our range was limited by the high soil elevation (i.e. deep water table) of areas dominated by

427   *Spartina patens*, off-setting the influence of *S. patens* traits that favor CH_4 production.

428   4.7 Implications for tidal wetland carbon cycling

429   Warming accelerates rates of CH_4 emissions from brackish marshes, especially during the growing

430   season. This is driven by both direct and indirect warming effects and mediated by soil biogeochemistry,

431   but the magnitude of the warming effect is also dependent on traits of the plant species that dominate the

432   plant community. Communities dominated by *Spartina patens* increase net CH_4 emissions in response to

433   smaller increments of warming than communities dominated by *Schoenoplectus americanus*. *Spartina*-

434   dominated sites may thus have a higher likelihood of shifting from a net C sink to a net C source under

435   future warming conditions, due to this increased loss of C as CH_4. However, this effect could be mitigated

436   if these high-elevation *Spartina* marshes become dominated by *Schoenoplectus* in response to predicted

437   accelerated sea-level rise (Kirwan & Guntenspergen, 2012). In addition, *Spartina* traits are plastic and

438   influenced by factors such as soil redox conditions (Kludze & DeLaune, 1994), salinity (Crozier &

439   DeLaune, 1996), and water level (Liu et al., 2019), all of which can be expected to change plant-mediated

440   effects on CH_4 biogeochemistry. Further studies are needed to thoroughly assess the range of

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441 environmental conditions under which *Spartina* is a net reducer and *Schoenoplectus* is a net oxidizer as

442 proposed by the present study.

443

444 **Data availability**

445 All data is available from the corresponding author upon request.

446

447 **Author contributions**

448 GLN and JPM designed the study, GLN collected and analyzed the data, and GLN and JPM wrote the

449 paper.

450

451 **Competing interests**

452 The authors declare that they have no conflict of interest.

453

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461

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