Title: Plant manipulations and diel cycle measurements test drivers of carbon dioxide and methane fluxes in a *Phragmites australis*-invaded coastal marsh

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Abstract

Invasion of coastal marshes by *Phragmites australis* may alter carbon cycling, including fluxes of the greenhouse gases (GHGs) carbon dioxide (CO₂) and methane (CH₄). Understanding patterns and drivers of these GHG fluxes in *P. australis*-invaded coastal marshes is critical to predicting how this widespread biological invasion may impact carbon (C) sequestration in coastal marshes. The objectives of this study were (1) to test effects of *P. australis* aboveground vegetation removal on GHG fluxes over short timescales (up to 4 months) and (2) to contrast diel patterns of GHG fluxes in *P. australis*-vegetated and cleared plots. First, effects of mechanical aboveground *P. australis* biomass removal on GHG fluxes and soil variables were tested over a series of short-term durations (from minutes to months). Next, on 3 dates, GHG fluxes were measured every 3 hours over complete diel cycles. Net daytime CO₂ uptake (-60 to -100 mmol m⁻² s⁻¹) was observed where *P. australis* was left intact. All durations of vegetation removal produced similar CO₂ emissions to those measured from intact *P. australis* plots during evening hours. CH₄ fluxes did not differ where *P. australis* was removed or left intact. Greater daytime CH₄ emissions (75-100 μmol m⁻² h⁻¹) were found than at night (20-40 μmol m⁻² h⁻¹) from both cleared and vegetated plots. Results of this study suggest that CO₂ fluxes in this system vary primarily due to substantial photosynthetic uptake by *P. australis*, and that CH₄ emissions are likely driven by abiotic factors, such as temperature, that vary on diel cycles. Calculation of net GHG fluxes in this *P. australis*-invaded coastal marsh indicates that it is a GHG sink during the growing season.

Keywords: Carbon dioxide, methane, *Phragmites australis*, salt marsh, cavity ringdown spectroscopy, diel patterns

1. Introduction

Characterizing carbon dioxide (CO₂) and methane (CH₄) fluxes from coastal marshes is critical to understanding the role these marshes play in global climate. In wetlands, fluxes of CO₂ and CH₄ may vary spatially with vegetation communities (Mozdzer and Megonigal, 2013) and abiotic conditions (Bartlett et al., 1987; Ma et al., 2012; Poffenbarger et al., 2011) and across a range of temporal scales from diel (Tong et al., 2013) to annual (Segarra et al., 2013).

Shifts in vegetation community structure may drive changes in GHG flux patterns as plant communities are characterized by distinct physiological traits and impacts on environmental conditions. Recent studies demonstrate the potential for *Phragmites australis*, a common invader of North American wetlands (Chambers et al., 1999), to alter coastal marsh CO₂ and CH₄ fluxes. Martin and Moseman-Valtierra (2015) measured GHG fluxes from *P. australis* and native species (*Spartina patens* and *Distichlis spicata*) zones monthly during a growing season in three New England marshes along a salinity gradient and found greater CH₄ emission (by up to 3 orders of magnitude) and substantially greater CO₂ uptake (up to 30x greater) in the *P. australis* zones, making *P. australis* zones greater net daytime GHG sinks. Recent work by Mueller et al., 2015 demonstrates increasing CH₄ emission from a coastal marsh along transects of *P. australis* invasion into native high marsh vegetation, supporting the idea that increased CH₄ emissions are exacerbated by *P. australis* invasion. Improved understanding of the balance of CO₂ and CH₄ fluxes in *P. australis*-dominated coastal marshes is needed to predict whether such wetlands will function as net GHG sinks or sources.

Drivers and patterns of CH₄ fluxes in *P. australis*-dominated freshwater wetlands have been well-characterized. *P. australis* mediates diel patterns of CH₄ emission due to light-driven patterns of convective gas transport (which carries rhizosphere gases through plant culms and

into the atmosphere) (Brix et al., 1996, Grünfeld and Brix, 1999; Van Der Nat et al., 1998). However, less is known about CH₄ flux dynamics in *P. australis*-invaded coastal brackish and salt marshes. There are several key differences between freshwater and coastal wetlands in environmental conditions that influence both plant processes and abiotic controls on GHG fluxes, with seawater inundation resulting in distinct soil biogeochemistry and microbial communities relative to freshwater wetlands (Poffenbarger et al., 2011) as well as differences in *P. australis* growth (David M Burdick et al., 2001; Chambers et al., 1998). The need to characterize patterns and drivers of CH₄ fluxes in *P. australis* invaded coastal marshes is particularly pressing in light of these marshes' potential role in sequestering C.

P. australis invasion may alter coastal marsh GHG fluxes either by direct mechanisms including photosynthetic uptake and gas transport or indirectly by modifications to the environment. *P. australis* is more productive than native high marsh grasses (Windham and Lathrop, 1999) and so its invasion may result in an increase in net marsh CO₂ uptake. *P. australis* has also been shown to conduct rhizosphere-derived gases, especially CH₄, into the atmosphere through its massive internal gas transport system (Armstrong et al., 1992). In mesocosm experiments, this direct influence of aboveground *P. australis* biomass on CH₄ emission has been illustrated by positive correlation of CH₄ emission with *P. australis* root mass, ramet density, and leaf area (Mozdzer and Megonigal, 2013). However, root zone oxygenation (Armstrong et al., 1992; Colmer, 2003) and locally lowered water tables (Windham and Lathrop, 1999) can promote CH₄ oxidation and thus lower net CH₄ emissions (Madigan, 2012). Provision of organic C exudates (Lovell, 2005) may sustain methanogens or CO₂ producing communities

of different abundance or structure relative to those supported by native plant communities (Ravit et al., 2003).

Measuring GHG fluxes before and immediately after removing aboveground biomass tests direct effects (i.e., convective transport) on fluxes, while vegetation removal over longer durations tests for potential indirect effects, such as rhizosphere oxygenation and exudate provision, on GHG fluxes. In this study, we performed two experiments to improve understanding of drivers of GHG fluxes in a *P. australis*-invaded coastal salt marsh. Experiment 1, conducted during the 2014 growing season, tested effects of mechanical aboveground *P. australis* biomass removal on GHG fluxes and surface soil variables over a series of short-term durations (from several minutes following the manipulation and 1-4 months). Aboveground biomass removal was hypothesized to reverse CO₂ fluxes from photosynthetic uptake (negative fluxes) to emission (positive fluxes) and to rapidly decrease CH₄ emission as plant-mediated transport was possibly curtailed. In the months following *P. australis* removal, a decline in root zone oxygenation and/or increase in organic substrates from senescing roots was expected to result in increased CH₄ emission relative to vegetated plots.

Experiment 2, conducted during June 2015, tested effects of diel period on GHG fluxes from cleared and vegetated areas in a *P. australis* marsh. Soil, pore water and plant variables were also measured and tested for relationships to GHG fluxes across diel cycles. Methane emissions were expected to be greater during daytime in both vegetated and cleared areas due to increases in methanogenesis rates as soil temperatures warmed. However, vegetated plots were expected to emit more daytime CH₄ due to plant-mediated transport. In vegetated plots, CO₂ uptake by plants during the day and emission at night (due to diel photosynthetic patterns) was

106 expected, while CO₂ emissions from cleared plots were hypothesized to vary to a lesser extent on
107 diel cycles due to removal of intact plants and loss of their photosynthetic activity.

2. Methods

2.1 Study Site

Fox Hill Marsh is located on the west coast of Conanicut Island in the town of Jamestown, Rhode Island. The approximately 0.34-acre invasive *Phragmites australis* stand used for this study is positioned at the western edge of the marsh system. Downslope of the *P. australis* stand, marsh vegetation is dominated by *S. patens*. Soil type in the *P. australis* stand consists of Ipswich Peat and Succotash Sand (Rector, 1981). Groundwater levels in the *P. australis* stand (determined with water loggers at 3 points throughout the stand during the 2015 growing season) were approximately 35-40 cm from the soil surface, and the stand rarely experienced tidal inundation. The marsh has been estimated to received small inputs of anthropogenic N (at approximately $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) relative to marshes further north in Narragansett Bay (Wigand et al., 2003), and previous measurements at the site revealed an absence of detectable nitrous oxide (N_2O) fluxes (Martin and Moseman-Valtierra, 2015) with a 30-second averaging period and minimal detection limit of approximately $1.4 \text{ } \mu\text{mol m}^{-2} \text{ hr}^{-1}$ (Brannon et al., 2016).

2.2 Experimental Designs

For experiments performed as part of this study, round PVC collars (24 cm tall x 30 cm diameter) to support static flux chambers for GHG flux measurements were used as units of replication. On each collar, six evenly spaced, approximately 2 cm diameter drainage holes were positioned beneath the soil surface to avoid pooling of tidal or rainwater.

2.2.1 Experiment 1: Testing effects of *P. australis* clearing on GHG fluxes

Collars for GHG flux measurements were installed during spring 2014 in the *P. australis* stand and left in place for the duration of the growing season (May-August 2014). There were 3 plots containing 3 collars each. In early spring, one collar per plot was cleared of aboveground vegetation (by clipping at the soil surface) and roots and rhizomes around the plot were severed to depths of 0.2 m. Vegetation was allowed to persist in the other two collars.

To test for effects of vegetation removal on GHG fluxes over the course of the growing season, fluxes were measured on a monthly basis from May to August from each of the 9 collars. Within each collar, soil surface salinity, pH, moisture, temperature, and oxidation-reduction potential (redox) data were also collected at the time of GHG flux measurements.

In August 2014, an additional experiment tested near-immediate effects of vegetation removal on GHG fluxes by measuring them before and within 2 minutes of clipping aboveground vegetation from one intact vegetation collar per plot. To ensure that clipped stems (< 2 cm tall) were not facilitating gas transport in the cleared collars, clipped stem bases were plugged with petroleum jelly and a third gas flux measurement was performed within 12 minutes of vegetation clipping.

All GHG flux measurements for Experiment 1 were conducted between 9:00 AM and 3:00 PM and within 3 h of low tide.

2.2.2 Experiment 2: Testing Effects of Diel Cycles on GHG fluxes under cleared and vegetated conditions

Three plots were established within the same portion of the *P. australis* stand used for the 2014 clearing experiment, but plot locations were changed to avoid effects of the previous year's vegetation clearing. Within each of three plots, two paired (vegetation intact and cleared) collars were installed 1 week prior to measurements. Vegetation was cleared following the same methods as Experiment 1. GHG fluxes were measured approximately every three hours over three 24-h periods during June 2015. Diel cycles (24-h) were broken down into eight "diel periods" defined as the following: (1) 8:00-11:00, (2) 11:00-14:00, (3) 14:00-17:00, (4) 17:00-20:00, (5) 20:00-23:00, (6) 23:00-3:00, (7) 3:00-5:00, and (8) 5:00-8:00. Destructive sampling harvest for testing relationships of GHG fluxes to plant biomass necessitated moving collars (approximately 0.3 m) within plots after each measurement date.

During each diel period, air temperature, relative humidity, soil temperature, light intensity, and rooting-depth pore water (60 cm depth) sulfide, salinity, pH and redox potential were measured. On each sampling date, stem height, density and aboveground biomass were measured from the vegetated collars. Groundwater depth in the *P. australis* stand was monitored over the duration of the experiment.

2.3 Measurement of environmental and soil variables and pore water chemistry

For Experiment 1, pore water salinity, soil surface pH, moisture content, redox, and temperature were measured as described previously in Martin and Moseman-Valtierra, 2015.

For Experiment 2, air temperature and relative humidity were measured using a Pocket Weather Meter (Kestrel Instruments). Light intensity was recorded using Hobo[®] Pendant loggers (Onset, Bourne, MA). Pore water was collected using 3 wells (one per plot) constructed of 1.5" diameter PVC pipe cut to lengths of 60 cm based on observed *P. australis* active root depths at the site. To extract pore water, wells were evacuated of water using a 50 mL syringe, a handheld siphon pump was used to create a vacuum to draw pore water into the well, and after 10 minutes water was collected for analysis. Pore water pH was measured using an Orion[™] Star A326 Multiparameter Meter (ThermoScientific). Pore water redox was measured using an ORP probe (Mettler Toledo, Greifensee, Switzerland). Pore water sulfide concentrations were quantified using standard colorimetric techniques (Cline, 1969; Solorzano, 1969). Average stem height was determined from measurement of 10 randomly chosen stems per collar. Biomass was rinsed, dried to constant mass in a vented oven for several days, separated into leaves and stems, and weighed. To monitor groundwater levels, three Hobo[®] U20L-04 water level loggers (Onset, Bourne, MA) were deployed in the *P. australis* stand in the vicinity of the experimental plots for the duration of the experiment.

For Experiments 1 and 2, a cavity ring down spectroscopy (CRDS) analyzer (Picarro G2508) and a 2 m tall, 0.3 m diameter transparent flux chamber were used to measure CO₂ and CH₄ concentrations in real-time (as described in Martin and Moseman-Valtierra, 2015). GHG fluxes were calculated using the slope of the linear change in gas concentration over time (Martin and Moseman-Valtierra, 2015). Since fluxes were measured using transparent chambers, CO₂ fluxes represent the net contributions of respirative emissions and photosynthetic uptake. Ebullitive CH₄ fluxes were defined as those where gas concentration change over time

195 followed a “stepped” pattern resulting from episodic increase of CH₄ emission (Middelburg et
196 al., 1996).

198 2.5 Statistical Analyses

199 2.5.1 Experiment 1

200 Two-factor ANOVAs were used to test for main effects and interactions of vegetation
201 presence and duration of vegetation removal (month) on GHG fluxes and soil variables. CH₄
202 data were log-transformed due to non-normality. Significant differences were interpreted at
203 $\alpha=0.05$. Tukey HSD tests were used for post-hoc analysis when appropriate.

204 For the August 2014 clipping experiment, GHG fluxes from before and after clipping
205 aboveground biomass and stem plugging were compared using a nonparametric Friedman rank
206 sum test due to non-normality and heteroscedasticity. Significant differences were interpreted at
207 $\alpha=0.05$.

209 2.5.2 Experiment 2

210 Linear mixed effects analysis of the relationship between vegetation presence and/or diel
211 period on CO₂ and CH₄ fluxes, pore water pH, redox, salinity and sulfide concentrations, and soil
212 surface redox were performed. Vegetation presence and diel period were treated as fixed effects,
213 and plot and measurement date as random effects. Interaction of plot and measurement date was
214 specified to account for the repeated measures nature of the experimental design. Residual plots
215 were used to confirm that assumptions of homoscedasticity and normality were met. To obtain
216 p-values to assess significance of the effect of vegetation presence and diel stage on GHG fluxes,

217 likelihood ratio tests of full models against models with the fixed vegetation or diel period effect
218 removed were performed. To test for interactive effects of vegetation presence and diel period
219 on GHG fluxes, models with and without an interaction term were compared using likelihood
220 ratio tests. Significant differences were interpreted at $\alpha=0.05$.

221 Kruskal-Wallis nonparametric tests were used, due to non-normality of data, to compare
222 light intensity, soil and air temperature, and relative humidity between the eight diel stages.
223 Significant differences were interpreted at $\alpha=0.05$. Post-hoc comparisons of means were
224 performed where appropriate using the R package *pgirmess* (Giraudoux, 2014).

225 Due to non-normality of data, Spearman's Correlation Analysis was used to test for
226 relationships between vegetation variables from vegetated plots for each diel stage and CO₂ and
227 CH₄ fluxes. Significant differences were interpreted at $\alpha=0.10$.

228 All statistical analyses were performed in R (R Core Team, 2012). The *lme4* package
229 (Bates, Maechler & Bolker, 2012) was used for linear mixed effects analyses.

3. Results

3.1 Experiment 1: Testing effects of *P. australis* clearing on GHG fluxes

During the 2014 vegetation clearing experiment, none of the soil variables were affected by vegetation clearing although some differed between months (Supplemental Table 1).

CO₂ was generally emitted within cleared collars and during May before plants had grown (0 to 20 mmol CO₂ m⁻² h⁻¹) and then CO₂ was taken up within collars containing intact vegetation from June-August (-50 to -110 mmol CO₂ m⁻² h⁻¹) (Figure 1A). Clearing vegetation significantly increased CO₂ fluxes ($F_{1,20}=24.54$, $p<0.005$), but fluxes did not differ significantly between months ($F_{3,20}=1.85$, $p=0.17$). There was an interactive effect of month and vegetation clearing ($F_{3,20}=3.34$, $p=0.04$).

CH₄ fluxes were not affected by vegetation clearing ($F_{1,20}=0.07$, $p=0.80$) and were variable (Figure 1B). During August, fluxes of nearly 2,000 μmol CH₄ m⁻² s⁻¹ (during both pre- and post-clipping measurements) were measured from one vegetated collar. CH₄ fluxes differed between months, with significantly smaller emissions during May than any other month ($F_{3,20}=5.67$, $p=0.006$). There was no interactive effect of month and vegetation clearing.

In the August experiment testing near-immediate effects of vegetation removal on GHG fluxes, aboveground vegetation clipping resulted in rapid reversal of CO₂ uptake (-55 mmol m⁻² s⁻¹) to emission (8 mmol m⁻² s⁻¹) and did not affect CH₄ fluxes ($X^2_2=0.67$, $p=0.72$) (Supplemental Figure 1). Plugging clipped stems did not affect CO₂ or CH₄ fluxes ($X^2_2=6$, $p=0.05$), demonstrating that clipped culms in the vegetation clearing treatment were not directly conducting rhizosphere gases.

3.2 Experiment 2: Testing Effects of Diel Cycles on GHG fluxes under cleared and vegetated conditions

Several environmental variables significantly changed over diel cycles ($P < 0.005$) including light intensity, air and soil temperature, and relative humidity (Table 1). As expected, light intensity was strongest during midday, air temperature was about 18°C higher during the day than at night, and relative humidity was lower (about 30% relative to daytime) at night. Several soil and pore water variables differed between diel periods (Supplemental Tables 2 and 3). Soil temperature was warmer late in the day and coolest at night and in the morning by about 2°C. Pore water redox potential and sulfide concentrations varied slightly between some diel stages, though diel patterns were not apparent. No other pore water variables or surface soil redox potential varied significantly with diel stage. Groundwater levels remained approximately 35-40 cm from the soil surface in all 3 plots during the study period.

CO₂ was taken up by vegetated plots during the day and emitted from all plots at night and from unvegetated plots during the day (Diel stage: $X^2_{\gamma}=44.52$, $p<0.005$; Vegetation presence: $X^2_{\gamma}=45.93$, $p<0.005$) (Figure 2A). There was an interactive effect of vegetation presence and diel stage on CO₂ fluxes ($X^2_{\gamma}=179.00$, $p<0.005$), with significantly greater uptake occurring in vegetated collars during daylight hours than from cleared or vegetated collars at night.

Daytime CH₄ emissions were significantly greater than night emissions and did not differ between cleared and unvegetated plots (Diel stage: $X^2_{\gamma}=48.49$, $p<0.005$; Vegetation presence: $X^2_{\gamma}=2.94$, $p<0.09$) (Figure 2B). There was no interactive effect of vegetation presence and diel stage ($X^2_{\gamma}=1.33$, $p=0.98$) on CH₄ fluxes. A single (out of 138 total flux measurements) very

large ebullitive CH₄ flux was measured (2,851 $\mu\text{mol m}^{-2} \text{h}^{-1}$) from a cleared collar at night, and was excluded from analysis and figures due to its outlier status.

P. australis at the study site averaged 108 ± 6 cm in height. Stem density was 92 ± 7 stems m^{-2} , and stem and leaf biomass were 177 ± 41 and 245 ± 61 g, respectively. CO₂ fluxes were negatively correlated with stem biomass during morning (diel stages 1 (*Spearman's* $r = -0.93$, $p < 0.005$) and 2 (*Spearman's* $r = -0.88$, $p = 0.007$)). CO₂ fluxes were positively correlated with average stem height during the evening (diel stages 5 (*Spearman's* $r = 0.87$, $p = 0.005$) and 7 (*Spearman's* $r = 0.90$, $p = 0.002$)). CH₄ fluxes were positively correlated with live stem density during early morning (diel stage 8 (*Spearman's* $r = 0.94$, $p = 0.005$)). No other correlations were significant.

4. Discussion

4.1 Carbon Dioxide fluxes

CO₂ uptake (-60 to -100 mmol m⁻² h⁻¹) in intact *Phragmites australis* was comparable to coastal marsh *P. australis* fluxes measured using similar methods (Emery and Fulweiler, 2014; Martin and Moseman-Valtierra, 2015) and was an order of magnitude greater than CO₂ uptake values in native high marsh salt marsh grasses *Spartina patens* and *Distichlis spicata* (Martin and Moseman-Valtierra, 2015). Evening and unvegetated plot daytime CO₂ emissions were similar to reported soil respiration rates measured between *S. patens* plants in coastal marshes of southern New England (Wigand et al., 2009), suggesting that *P. australis* marsh respiration rates are similar to those in native vegetation. Collectively, these results further support that *P. australis* may play a strong role in C sequestration as it expands along the upland borders of coastal marshes.

These experiments allowed for further determination of the mechanisms driving the large CO₂ uptake in *P. australis*-invaded coastal marshes. Results of Experiment 1 demonstrate photosynthetic uptake by *P. australis* as a strong control on CO₂ fluxes in vegetated plots. CO₂ uptake generally tracked plant development, increasing between May (as plants were just beginning to sprout) and June (when plants were well-developed). As hypothesized, aboveground *P. australis* vegetation clearing reversed daytime CO₂ fluxes from net uptake to emission, and this reversal was both rapid and sustained through a growing season. Consistency in respirative emission from cleared plots immediately after clipping and throughout the growing season suggests minimal influence of rhizosphere effects on CO₂ fluxes. Monthly measurements demonstrate smaller net CO₂ emission occurring during the month of May, reflecting lack of

plant influence on C exchange in the marsh and/or differences in microbial or abiotic drivers relative to other months in the growing season and are consistent with previous observations of greater CO₂ emissions from salt marshes during summer months (Morris and Whiting, 1986).

Our study offers another independent line of support for the strong role of *P. australis* in driving CO₂ fluxes in coastal marshes. If plant photosynthesis exerts strong control on CO₂ fluxes, then one expects diel patterns to mirror changes in periods of photosynthetic activity. This idea was largely confirmed in Experiment 2. CO₂ fluxes from vegetated plots displayed the expected pattern of net uptake during daylight hours as plants photosynthesized and net emission in the evening when photosynthetic uptake did not outpace plant and soil respiration. In contrast, CO₂ emissions from unvegetated plots were, as hypothesized, consistent (approximately 20 mmol m⁻² h⁻¹) across diel cycles. This stability is surprising given observed shifts in temperature, light, and humidity on this time scale (Table 1), which should influence microbial respiration in soils, particularly in these plots without aboveground plant structure to buffer the benthos (Whitcraft and Levin, 2007). The observation of diel shifts in CO₂ fluxes from vegetated plots alone thus highlights the stronger role of plant activity over microbial respiration in influencing net carbon exchange.

Differences in CO₂ fluxes despite a similarity of soil variables between cleared and vegetated plots in Experiments 1 and 2 suggest that CO₂ fluxes are primarily driven by the magnitude of *P. australis* photosynthetic uptake during the growing season. Evening CO₂ emissions from vegetated collars were comparable to cleared collar measurements (Figure 2a), which suggests that respiration was similar between our cleared and uncleared treatments, potentially due to respiration by belowground structures at greater depths than those severed.

Notably, some abiotic conditions (soil moisture, temperature and salinity) did vary during the growing season in our study, and they likely moderated the magnitude of CO₂ uptake (for example, leading to declines between June and August, Figure 1A). We acknowledge that CO₂ flux variations over annual cycles must be considered in the estimation of marsh GHG budgets and abiotic drivers are quite likely to be more important outside of active growing seasons.

4.2 Methane fluxes

The stability of CH₄ fluxes in response to *P. australis* removal was surprising. A decrease in CH₄ emission was hypothesized due to anticipated loss of plant-mediated transport after *P. australis* removal. However, vegetation clearing had no significant effect on CH₄ fluxes. Abiotic controls such as salinity and temperature (Poffenbarger et al., 2011; Van Hulzen et al., 1999) are known to strongly influence CH₄ fluxes. Combined with the finding (tested by plugging clipped stems in Experiment 1) that cut stems were not transporting rhizosphere-derived gases, results of this study demonstrate that plant-mediated transport was not driving CH₄ fluxes in this system. These findings contradict results of experiments in freshwater systems that demonstrate *P. australis*-mediated CH₄ transport (Armstrong and Armstrong, 1991; Beckett et al., 2001; Brix et al., 1996; Duan et al., 2006) as a major driver of CH₄ emission. However, our finding is in agreement with results of an experiment that found no difference in coastal marsh CH₄ fluxes between *P. australis* vegetated and naturally unvegetated plots (Emery and Fulweiler, 2014).

The hypothesis of greater CH₄ emission during daylight hours than in the evening was supported and was surprisingly consistent in both vegetated and unvegetated collars (Experiment

2). This observed diel pattern of greater daytime than nighttime CH₄ emissions from *P. australis* marshes is well supported in the literature and has been attributed primarily to light-driven patterns of plant-mediated transport. Experiments conducted in freshwater mesocosms (Grünfeld and Brix, 1999), freshwater wetlands (Brix et al., 2001; Van Der Nat et al., 1998; Whiting and Chanton, 1996) and brackish marshes (Tong et al., 2013) all demonstrated significantly greater daytime than nighttime CH₄ emissions. In freshwater systems, daytime emissions were 1.6 (Grünfeld and Brix, 1999) to 4 (Van Der Nat et al., 1998) times greater than nighttime emissions, consistent with this study's over twofold greater daytime emissions. In our study, a similar light-dependent plant transport mechanism for large daytime methane emissions cannot be invoked because cleared collars displayed the same diel CH₄ flux pattern as vegetated collars (Figure 2b). This finding suggests either that 1) CH₄ flux drivers differed between the cleared and vegetated collars (yet resulted in similar diel CH₄ emissions patterns) or 2) that CH₄ fluxes in this system are driven by abiotic processes and/or 3) indirect plant-mediated processes persist as an artifact of the experimental design in the clipped plots and they influence the CH₄ emissions. The possibility that different drivers are mediating similar CH₄ flux patterns in vegetated and cleared plots is consistent with findings from freshwater constructed wetlands and mesocosms, where CH₄ emissions were attributed to diffusive plant transport when vegetation was present and to ebullition in the absence of plants (Van Der Nat et al., 1998). Such a scenario seems unlikely to drive observed CH₄ flux patterns in our study, however, because of similar edaphic properties in clipped and vegetated plots (Supplemental Table 1) as well as close concordance of the CH₄ fluxes with variation in abiotic parameters on diel scales. Further, our vegetation clipping experiments indicated that plant-mediated transport is not driving observed

CH₄ fluxes in this system. Since belowground structures persisted in the cleared plots, the contribution of root-associated methanogen communities to CH₄ fluxes in this system cannot be precluded. However, observed diel patterns in cleared and vegetated plots seem likely to be driven by environmental conditions that vary on diel cycles, which in this experiment included soil and air temperature, light and relative humidity (Table 1). Methanogenesis is strongly temperature dependent (Van Hulzen et al., 1999), so CH₄ flux patterns may be driven by warmer soil temperatures during daylight hours, as was suggested by results of a study that characterized diel patterns of CH₄ emissions from a *P. australis* vegetated salt marsh in China (where *P. australis* is native) Tong et al (2013). Soil temperature as a control on CH₄ emissions is also suggested by lower CH₄ emission rates during cooler weather in early May during 2014 (Figure 1B).

Our finding that CH₄ emissions do not appear to be driven by plant-mediated transport may be due to relative strength in abiotic and biotic CH₄ flux drivers in fresh and saltwater wetlands. While methanogenesis dominates in freshwater wetland systems, in coastal marshes abundant sulfate from seawater inundation instead supports sulfate reduction (Poffenbarger et al., 2011). Less rhizosphere CH₄ is therefore available to be transported from soil to atmosphere. In addition, coastal marsh *P. australis* plants are frequently smaller in stature than their freshwater counterparts, stressed by soil salinity and/or sulfide (Burdick et al., 2001), possibly making them less effective CH₄ conduits from soil to atmosphere.

CH₄ emissions were highly variable in this experiment, as observed recently in similar *P. australis* salt marshes (Emery and Fulweiler, 2014; Martin and Moseman-Valtierra, 2015). CH₄ emission rates of over 1,000 $\mu\text{mol m}^{-2} \text{h}^{-1}$ (on par with emissions from freshwater wetlands

(Whiting and Chanton, 2001)) were measured in a few instances, including in August 2014 from a vegetated plot (Figure 2) and June 2015 from a cleared plot (described in results and excluded from analysis). These observed fluxes represent ebullition events, which are detectable by our methods. The presence of the few ebullition events we monitored suggests that ebullition does play a role in CH₄ emission from this system. Longer incubations may allow for more accurate quantification of ebullition events in *P. australis*-invaded coastal wetlands, and lend insight to whether these events significantly exacerbate CH₄ emissions from these systems.

4.3 Greenhouse gas sequestration

To our knowledge, this is the first study to quantify net CO₂ and CH₄ fluxes from a coastal marsh invaded by *P. australis* across diel cycles. This is also the first known manipulative test of *P. australis* roles in driving coastal marsh GHG fluxes. In addition to providing insight into mechanisms driving GHG fluxes in *P. australis*-invaded coastal marshes, these data allow for calculation of the balance of GHGs emitted and taken up by this system, and therefore into the role invasion of coastal marshes by *P. australis* may play in either supporting or limiting marsh GHG sequestration. Using a time-weighted average of net GHG fluxes in terms of CO₂ equivalent units across diel cycles, this system is a net GHG sink with uptake rates of about -531 mg CO₂ eq. m⁻¹ h⁻¹. This demonstrates that high daytime CO₂ uptake by *P. australis* reported in this and a previous study (Martin and Moseman-Valtierra, 2015) in this system is more than sufficient to offset CH₄ and respirative CO₂ evening emissions during growing seasons. Annual studies of GHG exchange are warranted to accurately estimate net ecosystem exchange and understand potential shifts in long-term C sequestration in coastal marshes invaded

417 by *P. australis*. Given the highly variable nature of wetland GHG fluxes and the genetic
418 diversity and phenotypic plasticity by which *P. australis* is characterized, future research is
419 needed to investigate GHG flux patterns and drivers in *P. australis* invaded systems along
420 broader spatial gradients of abiotic conditions and *P. australis* populations. Nonetheless, results
421 of this study suggest that in some coastal marsh systems substantial CO₂ uptake driven by *P.*
422 *australis* invasion may support or enhance GHG sequestration.

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Figure 1. Average CO₂ (A) and CH₄ (B) fluxes for each month for intact (n=3) and cleared (n=3) plots. Standard error bars are shown. Letters represent results of Tukey HSD tests. Bars (CO₂) or pairs of bars (CH₄) not connected by the same letter are significantly different. Note the difference in units between CO₂ (mmol m⁻²h⁻¹) and CH₄ (umol m⁻²h⁻¹).

Figure 2. CO₂ (A) and CH₄ (B) fluxes averaged over 3 measurement dates for vegetated and cleared plots at the following 8 diel stages: (1) 8:00-11:00, (2) 11:00-14:00, (3) 14:00-17:00, (4) 17:00-20:00, (5) 20:00-23:00, (6) 23:00-3:00, (7) 3:00-5:00, and (8) 5:00-8:00. Standard error bars are shown. Letters represent results of Tukey HSD tests, and bars not connected by the same letter are significantly different. Asterisks indicate diel stages when fluxes from vegetated and cleared plots differed significantly. Note the difference in units between CO₂ (mmol m⁻²h⁻¹) and CH₄ (μmol m⁻²h⁻¹).

Table 1. Average environmental conditions $\pm$ standard error and results of Kruskal-Wallis and post-hoc means comparisons tests for 8 defined diel stages

Diel Stage:	8:00 – 11:00 (1)	11:00 – 14:00 (2)	14:00 – 17:00 (3)	17:00 – 20:00 (4)	20:00 – 23:00 (5)	23:00 – 3:00 (6)	3:00 – 5:00 (7)	5:00 – 8:00 (8)	Results of Kruskal- Wallis test	Results of diel stage post-hoc comparison
<i>Soil Temperature (°C)</i>	18.5 $\pm$ 0.2	19.3 $\pm$ 0.2	21.7 $\pm$ 0.4	21.4 $\pm$ 0.2	20.3 $\pm$ 0.3	19.7 $\pm$ 0.3	19.2 $\pm$ 0.3	18.5 $\pm$ 0.1	$X^2_7=71.2$, $p<0.005^*$	1 ^d , 2 ^{cd} , 3 ^{ab} , 4 ^a , 5 ^{abc} , 6 ^{bcd} , 7 ^{cd} , 8 ^d
<i>Air Temperature (°C)</i>	77.8 $\pm$ 1.1	82.8 $\pm$ 0.7	85.6 $\pm$ 0.3	72.0 $\pm$ 0.5	69.1 $\pm$ 0.5	65.7 $\pm$ 0.8	66.2 $\pm$ 0.8	64.8 $\pm$ 0.8	$X^2_7=116.58$, $p<0.005^*$	1 ^{ab} , 2 ^a , 3 ^a , 4 ^b , 5 ^{bc} , 6 ^c , 7 ^c , 8 ^c
<i>Relative Humidity (%)</i>	60.5 $\pm$ 0.2	55.5 $\pm$ 0.7	57.6 $\pm$ 1.0	62.8 $\pm$ 1.0	76.0 $\pm$ 1.5	85.1 $\pm$ 1.8	90.6 $\pm$ 1.2	89.4 $\pm$ 0.3	$X^2_7=116.58$, $p<0.005^*$	1 ^{bc} , 2 ^b , 3 ^b , 4 ^{bc} , 5 ^a , 6 ^a , 7 ^a , 8 ^{ab}
<i>Light Intensity (lum/ft)</i>	3,237.0 $\pm$ 298.1	4,250.0 $\pm$ 378.3	4,188.7 $\pm$ 393.0	1,243.8 $\pm$ 235.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.3 $\pm$ 0.3	484.3 $\pm$ 123.6	$X^2_7=124.76$, $p<0.005^*$	1 ^{ab} , 2 ^a , 3 ^a , 4 ^{ab} , 5 ^c , 6 ^c , 7 ^c , 8 ^{ab}

X^2 statistics, degrees of freedom, and significance values are reported for Kruskal-Wallis tests for diel stage differences in environmental variables. For each diel stage, $n=9$.

In post-hoc comparison column, diel stages not connected by the same letter are significantly different. Letters a-d represent highest-lowest values, respectively.

* = Significant at $\alpha = 0.05$