

Different short-term responses of greenhouse gas fluxes from salt marsh mesocosms to simulated global change drivers

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Received: 27 October 2016/Revised: 12 May 2017/Accepted: 16 May 2017/Published online: 25 May 2017
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Abstract Salt marshes are valued as important greenhouse gas (GHG) sinks, but global changes in climate, nitrogen (N) pollution, and exotic species invasion may alter this marsh function. With the goal of better understanding the potential responses of coastal marsh GHG fluxes to interacting global changes, a multifactorial experiment was conducted. Two climate treatments (present-day and end-of-century temperatures and carbon dioxide (CO₂) concentrations) and two N treatments (non-enriched and simulated eutrophic estuary conditions) were applied to mesocosms containing either invasive *Phragmites australis* (Cav. Trin. Ex Steud.) or native *Spartina patens* (Aiton) Muhl. vegetated soil cores. Fluxes of CO₂, methane (CH₄), and nitrous oxide (N₂O) were measured in each mesocosm before and after treatment using cavity ring-down spectrometry, along with

vegetation growth, edaphic conditions, and pore water chemistry. Methane emissions increased in *P. australis* but not in *S. patens* mesocosms under climate change conditions, while CO₂ fluxes were similar between vegetation types and treatments. Nitrous oxide emissions increased with N loading from both *S. patens* and *P. australis* mesocosms, but were decreased in N-enriched *S. patens* mesocosms under climate change conditions. These findings demonstrate complex GHG flux responses to global change and suggest the potential for vegetation community-specific responses, though further research is needed to test mechanisms underlying observed GHG flux patterns.

Keywords *Spartina patens* · *Phragmites australis* · Methane · Nitrous oxide · Climate change · Eutrophication

Handling editor: Chris Joyce

Electronic supplementary material The online version of this article (doi:10.1007/s10750-017-3240-1) contains supplementary material, which is available to authorized users.

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Introduction

Climate change caused by increased anthropogenic greenhouse gas (GHG) emissions drives significant ecosystem-scale changes. Since many salt marshes already are altered by anthropogenic stressors like nitrogen (N) pollution and exotic species invasion, these ecosystems will likely experience a particularly complex interaction of global changes. As a result, salt marsh community structure and ecosystem function

may be perturbed, potentially with implications for marshes' carbon (C) and N cycling and their associated ecosystem service as important C and GHG sinks (Chmura et al., 2003; Mcleod et al., 2011).

Climate change and N enrichment can shift salt marsh vegetation community structure by altering plant productivity and competition. Plants that use the C₃ photosynthetic pathway, such as sedges and *Phragmites australis* (Cav. Trin. Ex Steud.), respond with increased C assimilation to elevated atmospheric CO₂. Meanwhile, C₄ photosynthetic plants such as *Spartina patens* (Aiton) Muhl. and *Spartina alterniflora* Loisel. are generally unaffected by increases in atmospheric CO₂ and instead display positive responses to warming (Sage & Kubien, 2007; Gedan & Bertness, 2010). Therefore, climate change (warming and increasing atmospheric CO₂) may alleviate effects of abiotic stress on some species, allowing them to colonize previously inaccessible environments (Lissner et al., 1999; Eller et al., 2014). Nitrogen enrichment may further alter species assemblages by shifting competitive balance that drives plant species zonation (Bertness et al., 2002), and may facilitate invasion of species with greater N use efficiency or demand (Mozdzer & Zieman, 2010; Kettenring et al., 2011; Mozdzer & Megonigal, 2012). Thus, N enrichment and climate change may act in combination to alter vegetation communities. For instance, elevated atmospheric CO₂ has been shown to drive deep root production in invasive *P. australis*, allowing access to deep pore water nutrient pools (Mozdzer et al., 2016). Also, while N enrichment has been shown to enhance C₃ plant response to elevated atmospheric CO₂ (Oren et al., 2001; Reich et al., 2006), it has also been shown over longer terms to stimulate spread of C₄ species in a coastal marsh setting, which may dampen ecosystem-scale response to CO₂ (Langley & Megonigal, 2010).

Salt marshes are often jointly impacted by N enrichment and invasion of *P. australis*, an aggressive, monoculture-forming reed (Chambers et al., 1999; Silliman & Bertness, 2004), and this combination of invasion-driven plant community shifts and N enrichment likely stimulates changes to marsh C and N cycling. Nitrogen in marshes is transformed via various microbial pathways that can yield the potent GHG nitrous oxide (N₂O) (Wrage et al., 2001; Kool et al., 2011), and N enrichment pulses have been shown to stimulate its emission from salt marshes

(Moseman-Valtierra et al., 2011). Since *P. australis*-dominated marshes display differences in pools and fluxes of N relative to native vegetation marshes (as reviewed in Windham & Meyerson (2003)), predominating N transformations and the degree to which N₂O emissions are stimulated by N enrichment in invaded and native vegetation zones may differ. Invasive *P. australis* may also affect marsh C cycling. Invasive *P. australis* has been associated with greater CH₄ emissions (Mozdzer & Megonigal, 2013; Mueller et al., 2016) and CO₂ uptake than native marsh vegetation zones (Martin & Moseman-Valtierra, 2015). These findings are consistent with greater photosynthetic uptake and increased labile organic C provision due to the plant's greater biomass (Windham, 2001) relative to native species, and the plant's gas transport system which accelerates soil-atmosphere exchange of GHGs (Armstrong & Armstrong, 1991; Brix et al., 1996; Colmer, 2003). However, rhizosphere oxygenation by plants with greater biomass such as *P. australis* may decrease net CH₄ emission (Sutton-Grier & Megonigal, 2011), complicating what is known about potential influence of the plant on marsh GHG flux dynamics.

To better understand potential interactions of N enrichment, *P. australis* invasion, and climate change (elevated CO₂ and temperatures) on salt marsh function, the goal of this study was to examine relatively short-term effects of these global changes on GHG fluxes from native *S. patens* and invasive *P. australis* mesocosms. In a multifactorial design, mesocosms containing vegetated soil cores from either *S. patens* or *P. australis* stands were subjected to increased temperatures and atmospheric CO₂ levels associated with end-of-century climate predictions (Stocker, 2013) and/or to N enrichment consistent with some of the highest estimated loads in Narragansett Bay, Rhode Island, USA. Initial and final soil variables, plant metrics and GHG (CO₂, CH₄, and N₂O) fluxes were measured in this 10-week mesocosm experiment. Increased CH₄ emission was expected under conditions of simulated climate change due to amplified methanogenesis in response to warming, and to increases in biomass and C provision. In *P. australis* mesocosm, CH₄ emissions were expected to be larger commensurate with expected greater biomass. Increased N₂O emissions were hypothesized to result from N enrichment of cores containing both plant species.

Methods

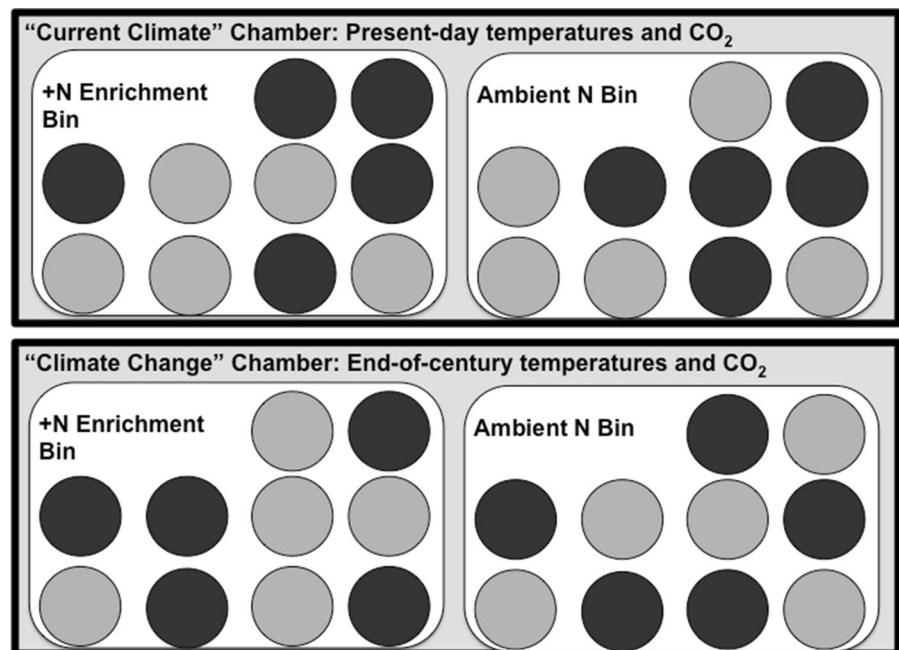
The $2 \times 2 \times 2$ factorial design (climate \times N \times plant species) consisted of two climate treatments (400 ppm carbon dioxide (CO₂) and present-day temperatures, and 700 ppm CO₂ and present-day temperatures +4.5°C); two N treatments (non-enriched; enriched with 19.7 g N m⁻² wk⁻¹); and two plant species (*P. australis* and *S. patens*). Mesocosms subject to present-day CO₂ and temperature treatments and not receiving N enrichment served as controls. Treatments ($n = 5$ per species for each of the 4 treatment combinations) were applied for 10 weeks to mesocosms containing field-collected vegetated soil cores with invasive *P. australis* or *S. patens* (Fig. 1). Logistics of limited growth chamber availability and space for N treatment bin placement necessitated an isolative segregation design for this experiment (Hurlbert, 1984). As such, growth chambers were carefully controlled and monitored throughout the study to ensure consistency of desired climate conditions. Mesocosms subjected to present-day temperatures and CO₂ levels are hereafter referred to as current climate, and those subjected to higher temperatures and elevated CO₂ are hereafter referred to as climate change. Greenhouse gas (CO₂, CH₄, and N₂O) fluxes, soil variables (salinity, pH, moisture), and vegetation

characteristics (stem height and above and below-ground biomass for both species and stem density for *P. australis*) were measured before and after the 10-week treatment period.

Experimental design

Mesocosms consisted of 20-cm diameter by 20-cm-long intact, vegetated soil cores placed in 6.3 l perforated nursery pots of the same dimensions. During mid-May, early in the growing season, 20 cores from each vegetation zone (*P. australis* and *S. patens*) were collected. Vegetation zones consisted of small (~0.1 ha), clearly defined bands of *S. patens* in the mid-high marsh and *P. australis* adjacent to the marsh-upland border, typical of New England coastal marshes (Bertness, 1991). In both vegetation zones, cores were collected within approximately 1 m of the *P. australis*/*S. patens* border in an effort to constrain between-zone differences unrelated to dominant plant species cover. To ensure that soil and plants were subjected to a low-N environment prior to experimental enrichments, cores were collected from Fox Hill marsh in Jamestown, RI (further described in Martin & Moseman-Valtierra, 2017), which is located in a minimally developed watershed at the low end of the N gradient in Narragansett Bay (Wigand et al., 2003).

Fig. 1 Schematic showing the experimental design. Two growth chambers were used to simulate present-day (top) and end-of-century (bottom) temperature and CO₂ conditions. In each chamber, two bins bathed experimental mesocosms (pots containing vegetated cores) in the N enrichment (left) and ambient N (right) treatments. *P. australis* and *S. patens* mesocosms (represented by light and dark circles, respectively) were treated as experimental units



Collecting 20-cm-deep cores excluded a portion of deeper belowground biomass, particularly for deep-rooting *P. australis* (Mozdzer et al., 2016).

Mesocosms were randomly placed in one of two vented chambers (Conviro® Model PGR15, Controlled Environments Ltd., 590 Berry St, Winnipeg, MB R3H 0R9, Canada) that were used to control climate (temperatures and atmospheric CO₂ levels). Mesocosms were treated as experimental units since each contained an independent vegetated soil core from a distinct point in the field, and since measurements were performed on individual potted cores. One chamber was set to mimic present-day CO₂ levels and temperatures, and temperature and atmospheric CO₂ concentrations were elevated in the second chamber consistent with increases predicted to occur by the year 2100 (Susan, 2007) (Fig. 1; Table 1). Day and evening temperatures for the current climate chamber were determined using average high and low July temperatures for Rhode Island (NOAA). These temperatures were raised by 4.5°C for the climate change treatment chamber based on the median of the upper bounds of model-predicted temperature increases for the year 2100 (Susan, 2007). Humidity was held at ambient levels in the two chambers. Light was provided in each chamber by 8 fluorescent and 6 incandescent bulbs for 15 h with 9 h of darkness. Resulting irradiance averaged 393.7 μmol m⁻² s⁻¹ (photosynthetic photon flux density, PPFD), comparable to that reported in previous, similar experimental setups (Eller et al., 2014).

Mesocosms were submerged at constant depths of approximately 10–15 cm in 20-cm-deep plastic bins (1 bin per N treatment and 2 bins per climate chamber, Fig. 1) containing unfiltered seawater collected from Narragansett Bay at the University of Rhode Island Graduate School of Oceanography, directly across the bay from the core collection site. Seawater was diluted to match previously measured site pore water salinities of 10–15 ppt. Mesocosms were distributed randomly in treatment bins and were moved to new locations and

rotated within the bin on a biweekly basis to minimize positional effects. To maintain soil flushing and ameliorate toxic salt buildup, 2 l of fresh water was applied per bin biweekly using a watering can to simulate rainfall.

Mesocosms receiving N enrichment treatments were supplemented with 19.7 g N m⁻² wk⁻¹ for a final loading rate of 177.3 g N after 10 weeks of treatment, scaled down from estimated anthropogenic N loading to Apponaug Cove Marsh in upper Narragansett Bay (10,253 kg N ha⁻¹ year⁻¹) (Wigand et al., 2003). Enrichments were performed weekly using ammonium nitrate dissolved in 0.5 l field-collected seawater per bin diluted to a salinity of 12 ppt, and non-enriched mesocosms received an identical volume of unenriched diluted seawater. Ammonium nitrate solution or plain diluted seawater was applied by spraying directly onto the soil surface of each mesocosm. Ammonium nitrate was employed with the intent of testing effects of two forms of N together, since both are present in Narragansett Bay from sewage and groundwater inputs and the forms are readily interconverted. The aim of this experiment was to test impacts of N loading (by either form) and not to discern mechanisms for N₂O production.

Edaphic variable measurements

Pore water salinity and sulfide, and soil pH and moisture were measured on the first (week 1, the day after collection) and last (week 10) days of the experiment for each mesocosm pot. Soil surface pH was measured using a pH meter (ExStick® Instruments, Nashua, NH). Soil moisture content was monitored using a volumetric water content sensor (Decagon Devices, Pullman, WA) inserted 5 cm into soil. Pore water from mesocosm depths of approximately 10 cm was sampled using Rhizon Soil Moisture Samplers (Ben Meadows, Janesville, WI) from two replicates per vegetation type per treatment. Pore water was analyzed for salinity using a handheld

Table 1 Temperatures and atmospheric CO₂ settings in growth chambers used to simulate present-day and year 2100 conditions

	Daytime temperature (°C)	Nighttime temperature (°C)	CO ₂ level (ppm)
Current climate	28	18	390
Climate Change (year 2100)	33	23	700

refractometer and for sulfide concentration using standard colorimetric techniques (Cline, 1969).

Plant characteristics measurements

Average stem height (based on ten measurements of randomly selected stems from the stem base to the tallest leaf tip) for both species and live stem density for *P. australis* was recorded for each mesocosm pot on the first and last days of the experiment. Stem density counts were not performed for *S. patens* mesocosms due to logistical constraints given the species' exceptionally high stem density (up to >300 stems per mesocosm pot). Above and belowground biomass (live and dead combined) from all mesocosms was harvested at the conclusion of the 10-week experiment. Aboveground biomass was clipped at the soil surface and rinsed, and belowground biomass was washed to remove soil debris. Biomass was oven-dried to constant mass at 90°C prior to weighing.

GHG flux measurements

Greenhouse gas (CO₂, CH₄, and N₂O) flux measurements were performed for each mesocosm on the first (week 1) and last (week 10) days of the experiment. Mesocosms were removed from chambers immediately prior to GHG flux measurements, which were conducted under low-light, indoor conditions (light intensity less than 27.9 lum/m² compared with 464.5–929.0 lum/m² measured during daylight hours at the site of core collection). Light levels were constant over the course of measurements. Week 1 measurements were performed the day after core collection. A cavity ring-down spectroscopy analyzer (Picarro G2508) was used to measure GHG concentrations in real-time (as described in Martin & Moseman-Valtierra, 2015). The analyzer was connected by nylon tubing to a 0.025-m³ transparent polycarbonate chamber (93 cm tall and 20 cm in diameter) (Rideout Plastics^R), which was sealed to the edge of the mesocosm pot using a polyethylene closed-cell foam collar with its channel filled with water. Small electric fans attached to the inside of the chamber mixed air during measurements. A stainless steel of 55-cm-long and 0.8-mm-diameter pigtail was used for pressure equilibration. Gas measurements were conducted for 3 min per mesocosm, based on observed periods for linear rates of change.

Greenhouse gas fluxes were calculated using chamber volume and footprint. The Ideal Gas Law ($PV = nRT$) was used to calculate changes in gas concentrations over time using measured air temperatures and atmospheric pressure. Cases in which no change in gas concentration over time was detectable for the duration of the measurement period were classified as having a flux of zero. When slopes had an R^2 value of less than 0.85 due to potential measurement error (two occurrences for CH₄, no occurrences for CO₂ or N₂O), data were not included in the analysis.

Statistical analysis

To check for uniformity of initial edaphic, plant, and GHG flux conditions prior to experimentation, differences between mesocosms randomly assigned to different treatment groups were tested in week 1 using two-factor ANOVAs (N treatment × climate conditions) for each vegetation type. By chance, significant differences in pH were present between mesocosms assigned to current climate plus N and climate change treatment without N treatment groups in *P. australis* mesocosms ($F_{1,18} = 6.41, p = 0.02$). *S. patens* mesocosms assigned to treatments of current climate and unenriched and climate change treatment with N enrichment had significantly larger initial CH₄ emissions ($F_{1,18} = 12.86, p < 0.01$) by a factor of 2. Initial CO₂ fluxes from *S. patens* mesocosms were significantly greater under the unenriched current climate than all other treatments ($F_{1,18} = 22.6, p < 0.01$) by a factor of 2. However, by week 10 none of the chance initial statistically significant differences persisted. Therefore, initial differences were not considered to have biased results, and statistical analyses were performed on final measurements (wk 10).

Two-factor ANOVAs (climate treatment × N) were used to compare the effect of treatments and their potential interactions on soil and plant variables for each vegetation species. Three-factor ANOVAs were used to test for main effects and interactions of vegetation species, N, and climate on CO₂ and CH₄ fluxes. Tukey's HSD test was used for post hoc pairwise comparisons when appropriate. Mesocosms were treated as units of replication. All data (with the exception of CO₂ fluxes which were normally distributed) were aligned then rank-transformed prior to ANOVA analyses (Salter & Fawcett, 1993; Wobbrock et al., 2011) to account for deviations in normality

while allowing for tests of effect interaction (Seaman et al., 1994). A Kruskal–Wallis Rank Sum test was used, due to non-normality of data, to test the effect of climate conditions on N₂O fluxes observed only from N-enriched mesocosms. Due to zero inflation and exceptionally high variability, pore water sulfide concentrations were assessed only qualitatively. Spearman's Correlation Analysis was used to test for correlations between measured soil and plant parameters and GHG fluxes for each vegetation zone. All statistics were performed in JMP 10.0 and interpreted at a significance level of 0.05.

Results

Mesocosm conditions: climate, soil, and pore water variables

Approximately, daily measurements indicated that the desired climate parameters (Table 1) were maintained throughout the experiment. Temperatures were held within 0.5°C, and CO₂ levels (mean ± SE) were maintained close to intended levels for current (396 ± 4.5 ppm) and climate change treatment (667 ± 12.7 ppm) chambers. Between initial and final measurements, soil pH generally decreased in all mesocosms; increases in pH (by about 0.5 units) were observed only in climate change treatment and N-enriched *P. australis* mesocosms (Appendix 1, Supplementary Material; Table 2). In *S. patens* mesocosms, N enrichment resulted in significantly lower final pH ($p < 0.01$) in treatment vs. control mesocosms, while climate change treatment had no effect. In *P. australis* mesocosms, final soil pH was affected interactively by climate conditions and N enrichment ($p < 0.01$), with the 2 treatments stimulating an increase of about two units relative to control and climate change plus N treatments (Table 2). Soil moisture generally increased slightly between initial and final measurements in all mesocosms (Appendix 1, Supplementary Material; Table 2). In *S. patens* mesocosms receiving N enrichment, final soil moisture was significantly lower than in non-enriched mesocosms ($p = 0.04$) (Table 2). Final *P. australis* mesocosm moisture was not affected by either treatment. Despite efforts to control soil salinity, pore water salinity in all mesocosms increased between initial and final measurements by about 15 ppt to

approximately that of seawater (~32 ppt) (Appendix 1, Supplementary Material; Table 2). Final pore water salinity was not significantly affected by treatments.

Spartina patens pore water sulfide concentrations were exceptionally variable when measured during wk 1 of the experiment and ranged from 0 to nearly 300 μM, while *P. australis* pore water sulfide concentrations were 0 μM (Appendix 1, Supplementary Material). By the end of the experiment, *S. patens* pore water sulfide concentrations remained highly variable but did not generally change in magnitude, and *P. australis* concentrations remained close to 0 μM, with one mesocosm having a concentration of 11 μM (Table 2).

Vegetation characteristics

P. australis average stem height remained approximately the same between initial and final measurements, with plants averaging only a third as tall as those from the core collection site (Martin & Moseman-Valtierra, 2015). However, *S. patens* stem height increased approximately 4-fold between initial and final measurements (Appendix 1, Supplementary Material; Table 2), with final plant heights matching those from the sampling site. Average final stem heights were not affected by N addition or climate change simulation for either species.

Final *P. australis* stem density was interactively affected by treatments ($p = 0.04$), with N enrichment and climate change treatment combined resulting in decreased stem density relative to climate change treatment and N treatments applied independently.

In *P. australis* mesocosms, N enrichment stimulated a minor (1.5%) increase in final belowground biomass ($p = 0.02$). There were no significant main effects or interactions of N enrichment or climate change treatment on *S. patens* above or belowground biomass or on *P. australis* aboveground biomass (Table 2).

GHG fluxes

All mesocosms emitted CH₄ and CO₂ during time zero and time final measurements, while N₂O emissions were observed only from mesocosms receiving N enrichment during time final measurements.

Phragmites australis mesocosms subjected to climate change treatment displayed some exceptionally

Table 2 Final edaphic and plant variables \pm SE after 10 weeks of N and/or “climate change” treatments and results of 2-factor ANOVA for all treatment combinations

Average edaphic and plant variables and GHG fluxes \pm SE		Results of 2-factor ANOVA				
Control	Nitrogen	Climate change	Nitrogen + climate change	N	Climate	N \times climate
Soil pH						
<i>P. australis</i>	6.20 \pm 0.15	8.06 \pm 0.04	8.06 \pm 0.23	6.61 \pm 0.08	$F_{1,19} = 0.52, p < 0.48$	$F_{1,18} = 2.51, p = 0.13$ $F_{1,18} = 16.40, p < 0.01^*$
<i>S. patens</i>	3.98 \pm 0.05	5.68 \pm 1.24	3.91 \pm 0.36	5.81 \pm 0.01	$F_{1,19} = 42.07, p < 0.01^*$	$F_{1,18} = 0.00, p = 1.00$ $F_{1,18} = 0.73, p = 0.41$
Pore water salinity (ppt)						
<i>P. australis</i>	33.0 \pm 5.66	30.00 \pm 7.07	21.50 \pm 19.09	30.00 \pm 8.49	$F_{1,7} = 0.21, p = 0.67$	$F_{1,7} = 0.21, p = 0.67$ $F_{1,7} = 0.21, p = 0.67$
<i>S. patens</i>	32.50 \pm 3.54	32.00 \pm 1.73	26.00 \pm 0.00	27.50 \pm 4.95	$F_{1,7} = 0.22, p = 0.67$	$F_{1,7} = 6.12, p = 0.07$ $F_{1,7} = 0.00, p = 1.00$
Pore water sulfide (μM)						
<i>P. australis</i>	0.00 \pm 0.00	0.00 \pm 0.00	5.52 \pm 7.8	0.00 \pm 0.00	–	–
<i>S. patens</i>	246.32 \pm 70.00	0.00 \pm 0.00	145.67 \pm 9.99	0.00 \pm 0.00	–	–
Soil moisture (%)						
<i>P. australis</i>	64.32 \pm 1.13	65.14 \pm 0.42	65.40 \pm 0.79	61.65 \pm 2.50	$F_{1,19} = 0.46, p = 0.51$	$F_{1,19} = 0.04, p = 0.83$ $F_{1,19} = 2.06, p = 0.17$
<i>S. patens</i>	63.60 \pm 1.13	63.46 \pm 0.74	64.92 \pm 0.52	61.19 \pm 0.46	$F_{1,19} = 5.19, p = 0.04^*$	$F_{1,19} = 0.32, p = 0.58$ $F_{1,19} = 4.54, p = 0.05^*$
Stem density per mesocosm pot						
<i>P. australis</i>	3.80 \pm 1.16	7.80 \pm 1.02	8.20 \pm 2.22	6.40 \pm 1.57	$F_{1,19} = 0.41, p = 0.53$	$F_{1,19} = 1.75, p = 0.20$ $F_{1,19} = 4.96, p = 0.04^*$
Stem height (cm)						
<i>P. australis</i>	39.27 \pm 1.14	53.27 \pm 5.30	54.88 \pm 6.00	44.03 \pm 9.42	$F_{1,17} = 0.11, p = 0.75$	$F_{1,17} = 0.61, p = 0.45$ $F_{1,17} = 2.49, p = 0.14$
<i>S. patens</i>	30.23 \pm 1.84	36.57 \pm 5.16	36.40 \pm 1.11	31.30 \pm 3.06	$F_{1,19} = 0.00, p = 1.00$	$F_{1,19} = 0.02, p = 0.89$ $F_{1,19} = 3.75, p = 0.07$
Aboveground biomass (g)						
<i>P. australis</i>	13.20 \pm 1.72	29.60 \pm 4.03	34.45 \pm 6.42	26.04 \pm 5.24	$F_{1,19} = 0.86, p = 0.37$	$F_{1,19} = 0.50, p = 0.49$ $F_{1,19} = 2.45, p = 0.14$
<i>S. patens</i>	16.74 \pm 1.60	21.98 \pm 5.57	16.72 \pm 4.62	23.38 \pm 4.49	$F_{1,19} = 2.55, p = 0.13$	$F_{1,19} = 0.08, p = 0.78$ $F_{1,19} = 0.41, p = 0.53$
Belowground biomass (g)						
<i>P. australis</i>	36.25 \pm 6.00	76.56 \pm 26.51	70.38 \pm 13.10	86.44 \pm 14.80	$F_{1,19} = 0.81, p = 0.02^*$	$F_{1,19} = 2.73, p = 0.12$ $F_{1,19} = 0.04, p = 0.84$
<i>S. patens</i>	151.38 \pm 23.69	156.24 \pm 36.41	149.30 \pm 17.17	122.12 \pm 10.56	$F_{1,18} < 0.01, p = 0.95$	$F_{1,18} = 0.88, p = 0.36$ $F_{1,18} = 0.52, p = 0.48$

Bold value indicates statistical significance

F statistics, degrees of freedom, and significance values are reported for 2-factor (climate change simulation \times N treatment) ANOVA tests

n = 5 mesocosms per treatment

“Control” refers to mesocosms kept at ambient temperature and atmospheric CO₂ with no N enrichment

“Nitrogen” refers to mesocosms enriched with ammonium nitrate

“Climate change” refers to mesocosms kept under elevated temperature and atmospheric CO₂ treatments

* Significant at $\alpha = 0.05$

large CH_4 emissions ($>5,000 \mu\text{mol m}^{-2} \text{h}^{-1}$) (Fig. 2A), and produced significantly larger CH_4 emissions than any other vegetation type/treatment combinations ($p = 0.01$) (Table 3).

N enrichment dramatically increased N_2O production from all mesocosms within that treatment group

by the end of the experiment (Fig. 2B). For *S. patens*, climate change-treated mesocosms emitted approximately half as much N_2O as current climate mesocosms ($X^2 = 0.10$, $p < 0.01$). In contrast, climate change treatment did not affect N_2O emissions from *P. australis* mesocosms ($X^2 = 6.81$, $p = 0.75$).

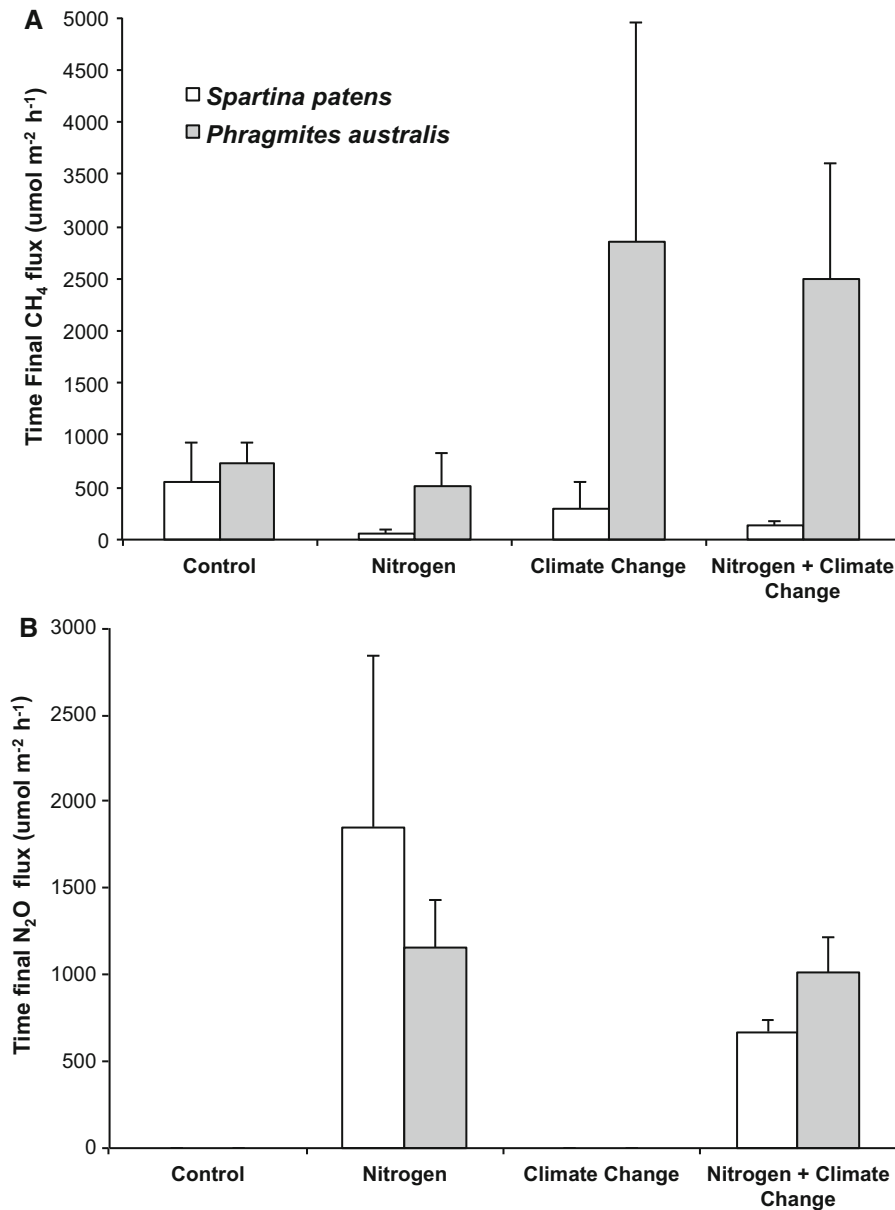


Fig. 2 Time final average CH_4 (A) and N_2O (B) fluxes \pm SE from all treatment groups. $N = 5$ mesocosms per treatment. “Control” refers to mesocosms in kept at ambient temperature and atmospheric CO_2 with no N enrichment. “Nitrogen” refers

to mesocosms enriched with ammonium nitrate. “Climate change” refers to mesocosms kept under elevated temperature and atmospheric CO_2 treatments

Table 3 Results of 3-factor ANOVA tests for effects of N, climate treatments, and vegetation type on CH₄ and CO₂ fluxes

Results of 3-factor ANOVA (N × CC × veg. type)

	N	CC	N × CC	Veg. type	Veg. type × N	Veg. type × CC	Veg. type × CC × N
CH ₄	$F_{1,37} = 2.57,$ $p = 0.12$	$F_{1,37} = 4.82,$ $p = \mathbf{0.04}^*$	$F_{1,37} = 0.37,$ $p = 0.55$	$F_{1,37} = 9.27,$ $p < \mathbf{0.01}^*$	$F_{1,37} = 1.18,$ $p = 0.29$	$F_{1,37} = 7.27,$ $p = \mathbf{0.01}^*$	$F_{1,37} = 0.02,$ $p = 0.90$
CO ₂	$F_{1,39} = 3.08,$ $p = 0.09$	$F_{1,39} = 0.01,$ $p = 0.91$	$F_{1,39} = 0.01,$ $p = 0.91$	$F_{1,39} = 3.15,$ $p = 0.09$	$F_{1,39} = 0.03,$ $p = 0.87$	$F_{1,39} = 0.05,$ $p = 0.83$	$F_{1,39} = 0.61,$ $p = 0.44$

Bold value indicates statistical significance

F statistics, degrees of freedom, and significance values are reported for ANOVA tests

“CC” refers to “climate conditions”

n = 5 mesocosms per treatment* Significant at $\alpha = 0.05$

Relationships of soil and plant variables to GHG flux magnitude

In *P. australis* mesocosms, CH₄ emissions were positively correlated with pH ($S = 0.52$, $p = 0.02$) and negatively correlated with stem height ($S = -0.57$, $p = 0.02$) and aboveground biomass ($S = -0.56$, $p = 0.01$). Nitrous oxide fluxes were negatively correlated with pH ($S = -0.71$, $p < 0.01$) and positively correlated with belowground biomass ($S = 0.56$, $p = 0.01$).

GHG fluxes from *S. patens* mesocosms did not correlate with any soil or plant variables.

Discussion

Distinct responses of CH₄ and N₂O emissions from *S. patens* and *P. australis* mesocosms to simulated climate change conditions suggest the potential for GHG flux dynamics in these vegetation zones to respond differently to global change drivers. However, mesocosm conditions differed from field conditions in terms of light availability, hydrology, salinity, and patterns of plant growth. Due to limitations of two climate chambers, the possibility that factors other than simulated climate differed between chambers is acknowledged. Despite these experimental artifacts, we were able to confirm that targeted temperatures and CO₂ concentrations were maintained in each chamber for the duration of this study. We use results of this mesocosm experiment to present hypotheses that may help guide future field and greenhouse studies.

Vegetation zone-specific CH₄ and N₂O flux responses to simulated climate change

Methanogenesis is highly temperature dependent (Dunfield et al., 1993), and so increased CH₄ emissions under warmer conditions were expected. The response in *P. australis* mesocosms, but not *S. patens* mesocosms, of dramatically increasing CH₄ emissions with climate change treatment suggests that *P. australis* soil was primed for increased methane production under favorable, warmer conditions. Different vegetation communities, including *S. alterniflora* and *P. australis* (Ravit et al., 2003), have been shown to support distinct microbial assemblages due to their impacts to rhizosphere conditions (Burke et al., 2002; Lovell, 2005). Greater *P. australis* mesocosm CH₄ emissions in response to the climate change treatment may have been due to *P. australis* soils sustaining more abundant or active methanogen communities over the course of the experiment due to abundance or quality of rhizosphere C exudates. Methanogenesis in the *S. patens* mesocosms may have been limited at the beginning of the experiment by prevalence of the sulfate reduction pathway, which is indicated by the presence of pore water sulfide in some mesocosms at the time of initial measurements (Poffenbarger et al., 2011). As *P. australis* growth is inhibited by high sulfide concentrations (Chambers et al., 1998), it stands to reason that *P. australis* mesocosm pore water showed minimal evidence of sulfate reduction. Although final measurements indicate reduced sulfate reduction, *S. patens* soil methanogen assemblages may have remained small.

Differences in soil pH may also have driven observed patterns of methane emission if methanogen communities in these systems are intolerant of acidic conditions. Finally, the difference in CH₄ emissions response between vegetation zones in the climate change treatment may have been due in part to enhanced CH₄ oxidation in the rhizosphere in *S. patens* mesocosms, where belowground biomass was greater (Table 2). Previous investigations that demonstrated a stimulatory effect of elevated atmospheric CO₂ on CH₄ emissions or pore water CH₄ concentrations in C₃ plant communities (*P. australis* and *Scirpus olneyi*, syn. *Scirpus americanus*) linked this response to increases in biomass (Keller et al., 2009; Mozdzer & Megonigal, 2013). In Mozdzer & Megonigal (2013), plants were grown in larger containers (15 l vs. our 6.3 l) and under freshwater conditions. These factors were more conducive to *P. australis* growth and so measurable growth responses were stimulated by treatments, while plants in our relatively short experiment displayed minimal responses to elevated CO₂ or N (Table 2).

CH₄ fluxes from *P. australis* and *S. patens* zones at Fox Hill marsh, the site from which mesocosm cores were collected, allow for comparison of mesocosm and field fluxes and interpretation of potential experimental artifacts. CH₄ emissions from *P. australis* and *S. patens* mesocosms were generally within the wide range of fluxes measured during midsummer sampling at Fox Hill Marsh (Martin & Moseman-Valtierra, 2015), although exceptionally high fluxes of greater than 5,000 μmol m⁻² h⁻¹ from *P. australis* climate change-treated mesocosms, which were several times larger than the largest field-measured fluxes (which were about 1,500 μmol m⁻² h⁻¹). In addition to increased temperatures and atmospheric CO₂, mesocosm artifacts such as differences in hydrology and increased plant stress due to increases in pore water salinity may have contributed to very high CH₄ emissions by decreasing oxygen availability and increasing the amount of senescent plant material relative to field conditions. In addition, lower light conditions in chambers than during midday under outdoor conditions likely altered plant function, and may have differentially affected responses of the C4 and C3 species studied in this experiment to temperature and CO₂ treatments (Ehleringer & Björkman, 1977). Climate change manipulations under field conditions are therefore warranted.

N₂O fluxes from N-enriched mesocosms of the two vegetation types also displayed distinct responses to simulated climate change, with climate change treatment ameliorating N₂O response to fertilization only among *S. patens* mesocosms. This finding may point to different predominating pathways of N transformations between soils or to differences in *P. australis* and *S. patens* responses to treatments. Differences in pH between mesocosms containing the 2 species and the negative relationship between pH and N₂O emissions for *P. australis* mesocosms may provide clues about the predominating pathway, since some pathways such as dissimilatory nitrate reduction to ammonium (DNRA) dominate at higher pH (Stevens et al., 1998). For *P. australis* but not *S. patens* mesocosms, belowground biomass was positively correlated with N₂O emission, potentially suggesting *P. australis* root zone support N₂O-generating processes such as denitrification (Findlay et al., 2003) or nitrifier-denitrification (Wrage et al., 2001; Kool et al., 2011). Further research is needed to discern predominating N transformation pathways in the two vegetation zones, and future mesocosm studies should incorporate unvegetated soil controls from each vegetation zone to separate plant-mediated and soil impacts during the course of the experiment.

N₂O emission stimulation by high rates of N loading

While N loading rates in this experiment are high compared with those applied in other N enrichment experiments [such as Great Sippewissett Marsh at 1,500 kg N h⁻¹ year⁻¹ (Fox et al., 2012)], they are designed to represent N loads in highly urban, impacted marshes and may represent conditions of increasingly degraded marshes as population growth expands (Valiela & Cole, 2002). The estimated N loads at Apponaug Cove (10,253 kg N ha⁻¹ year⁻¹) (Wigand et al., 2003) from which additions in this experiment were calculated reflect an anthropogenically impacted coastal marsh, with the surrounding watershed highly developed (>40% residential) and 74% of its N inputs derived from wastewater. The N enrichment in this experiment therefore allowed for inferences of the impacts of urban N loading on coastal marsh GHG fluxes. Future experiments might employ a subtler N loading gradient to better test thresholds of plant and GHG responses, and different N forms

(nitrate, ammonium) should be tested to better discern mechanisms of N₂O fluxes under varying environmental conditions.

Previous field studies report increased N₂O emission from a marsh dominated by native grasses in response to N enrichment (Moseman-Valtierra et al., 2011). Nitrous oxide fluxes in that study averaged around 1.75 μmol m⁻² h⁻¹, significantly lower than the fluxes, of up to nearly 3,500 μmol m⁻² h⁻¹, measured from N-enriched mesocosms by the end of this experiment. Greater emissions from the mesocosms are consistent with much larger weekly additions than the single pulses of nitrate at 1.4 g N m⁻² employed in the field experiment, and with mesocosm artifacts including diminished flushing and potentially suppressed plant-mediated N uptake.

Conclusions

Although sampling from a single salt marsh system, small sample size, and experimental artifacts preclude generalization of these results to other systems, findings from this experimental mesocosm study suggest the potential for coastal marsh vegetation zone CH₄ and N₂O flux dynamics to respond differently to climate change and N enrichment. Notably, climate change and N enrichment were not observed to interactively stimulate GHG emissions. Findings of this experiment underscore the potential complexity of GHG flux responses to global change drivers, and demonstrate that investigation of multiple stressors in combination is warranted since responses of net GHG emissions may not be additive. Future experiments, including field investigations, are necessary to elucidate drivers of differing responses of GHG fluxes to global change among marsh vegetation communities.

Acknowledgements This work was supported by the United States Department of Agriculture National Institute of Food and Agriculture (Hatch Project #229286, grant to Moseman-Valtierra) and the National Science Foundation Experimental Program to Stimulate Competitive Research Cooperative Agreement (#EPS-1004057, graduate fellowship to Martin). Sincere thanks go to C. Wigand and two anonymous reviewers for manuscript advice, and to J. Bowen, L. Meyerson, A. Roberts, and C. Wigand for their design advice. We thank I. Armitstead, I. Burns, L. Brannon, S. Doman, S. Kelley, T. Moebus, and K. Sperry for their assistance with mesocosm preparation and data collection, and C. Martin for assistance with R code for a data analysis automation script.

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