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MENSURATIVE AND MANIPULATIVE
EXPERIMENTS TEST EFFECTS OF GLOBAL CHANGE
DRIVERS ON GREENHOUSE GAS FLUXES IN
COASTAL MARSHES
BY
ROSE MARIE MARTIN

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
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UNIVERSITY OF RHODE ISLAND

2015

DOCTOR OF PHILOSOPHY DISSERTATION

OF

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ABSTRACT

For centuries, coastal marshes have been subjected to anthropogenic stressors. Great expanses of coastal marshes were drained and filled to make way for development, and those that remained were diked and ditched, encroached upon by upland development, and used for agricultural purposes such as livestock grazing. Today, as the values and services coastal marshes provide to human society are understood, marshes are protected from direct degradation. However, especially in developed and densely populated estuaries such as Narragansett Bay, coastal marshes are subject to impacts including nutrient pollution and introduction of invasive species. Global climate change and associated sea level rise further threaten coastal ecosystems. As marsh vegetation community structure, biogeochemistry, and microbial and faunal assemblages shift in response to anthropogenic impacts and global change, ecosystem function is likely to be altered as well. Since coastal marshes provide highly valued services such as coastline protection, wildlife habitat, nitrogen (N) transformations and carbon (C) sequestration, understanding the outcomes of these functional shifts is an important research concern. Of particular interest is the potential for impacts to coastal marshes' important ecosystem service of C sequestration, since perturbations to this function could result in climate change-exacerbating feedbacks.

Coastal marshes are such effective C sinks due to their high productivity and associated carbon dioxide (CO₂) uptake, slow decomposition, and minimal emission

of climate-altering greenhouse gases (GHGs). However, emission of GHGs may be stimulated by several of the global change drivers coastal marshes face. These potential drivers include N pollution, which can stimulate emission of the potent GHG nitrous oxide (N₂O) from coastal marshes, and invasion of the aggressive introduced grass *Phragmites australis*, which may stimulate emission of methane (CH₄). Testing how these impacts may interact to alter fluxes of GHGs in coastal marshes is important for a clear understanding of the role that coastal marshes play in global climate and whether this role is likely to be affected by a changing climate.

Very recently, development of novel technologies for measuring GHG concentrations *in situ* in real time have made simultaneous measurement of the GHGs CO₂, CH₄, and N₂O a possibility, and have opened the door to experiments that will improve understanding of coastal marsh GHG flux dynamics and their response to changes to the coastal marsh ecosystem.

The objective of the research projects presented in this dissertation was to elucidate responses of coastal marsh GHG fluxes to drivers of global change including climate change, N pollution, and invasion of *Phragmites australis*. Four research projects employing mensurative and manipulative experiments incorporating cavity ringdown spectroscopy (CRDS) technology for GHG flux measurement were conducted. First, GHG flux dynamics in native vegetation and *Phragmites*-dominated coastal marsh zones along a salinity gradient were characterized to determine whether *Phragmites* invasion may potentially affect marsh GHG fluxes. Next, the effect of vegetation presence on GHG fluxes and their diurnal variability in a coastal marsh

were tested with the aim of better understanding mechanisms underlying coastal marsh GHG fluxes. Since *Phragmites* removal as part of restoration activities is a commonly employed management activity in coastal marshes, the third experiment tested effects of coastal marsh restoration activities and *Phragmites* removal on GHG flux dynamics. Finally, to examine potential interactive impacts of climate change, N pollution and *Phragmites* presence on GHG flux dynamics, a multifactorial greenhouse experiment was conducted in chambers that simulated elevated atmospheric CO₂ and temperatures expected to occur by the end of the century.

Results of these experiments revealed a potentially complicated role of *Phragmites* in mediating GHG fluxes from coastal marshes under conditions of global change. While *Phragmites*-dominated marsh zones consistently emitted more CH₄ relative to native vegetation marsh zones, they also had substantially greater CO₂ uptake per unit area. Within *Phragmites* stands, clearing vegetation resulted in an increase in CH₄ emissions that was exacerbated by the loss of photosynthetic CO₂ uptake. Testing effects of N pollution and climate change on GHG fluxes revealed that *Phragmites*-dominated marshes might emit more CH₄ under conditions of climate change. While further research is required to determine the spatial and temporal consistency of the effects and to continue clarifying mechanisms, results presented in this dissertation make clear the potential for *Phragmites australis* invasion to alter marshes' role in a changing global climate.

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I have had the privilege of working with a remarkably accomplished, collaborative dissertation committee. First, I sincerely thank my dissertation advisor, Dr. Serena Moseman-Valtierra. When I found my way to her office to awkwardly introduce myself one morning nearly four years ago, I could not have imagined the opportunities for professional and personal growth that would lie ahead for me as a member of the “MV lab”. Through her high expectations and unwavering belief in my abilities, Serena helped me to push the boundaries of my comfort zone and grow in confidence as a researcher, leader and mentor. I will always be thankful for the creativity, insight and kindness with which she guided me through my Ph.D. I also am deeply grateful to my committee members Dr. Jennifer Bowen, Dr. Laura Meyerson, Dr. Alison Roberts and Dr. Cathleen Wigand for guiding me as well as challenging me during my time at URI. In addition to serving as dissertation committee members, these outstanding researchers have become important role models for me as I begin my career as a woman in science.

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Several facilities at URI provided necessary equipment and assistance with key components of my dissertation work. I sincerely thank the URI Genomics and

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Last, but never least, I thank my parents, Albert and Linda Cournoyer. As first generation college students who became teachers, they placed great value on education and the privileges and opportunities it provided. They were the first educators in my life, and awakened in me a love for and insatiable curiosity about the natural world that

has led me to pursue a career in science. I am profoundly grateful for that, and for their wisdom in allowing me always to chart my own course.

DEDICATION

To Albert and Linda Cournoyer, for instilling in me from my earliest memory a love of learning, a belief in the power of education, and the courage to follow my own path

PREFACE

This dissertation is prepared in manuscript format. Chapter 2, entitled “Greenhouse gas fluxes vary between *Phragmites australis* and native vegetation zones in tidal marshes along a salinity gradient”, was published in *Wetlands* in September 2015. Chapter 3, entitled “Plant manipulations and diel cycle measurements indicate distinct drivers for carbon dioxide and methane fluxes in a *Phragmites australis* coastal marsh”, has been submitted to *Aquatic Botany*. Chapter 4, entitled “*Phragmites australis* removal: Brackish marsh restoration tests invasive plant effects on greenhouse gas fluxes”, has been submitted to *Wetlands Ecology and Management*. Chapter 5, entitled “Different short-term responses of greenhouse gas fluxes to simulated global change drivers in salt marsh mesocosms”, is in review at *Journal of Experimental Marine Biology and Ecology*. Chapter 2 is presented as it was accepted for publication in September 2015, and Chapters 3-5 are presented exactly as submitted to their respective journals. Journals, submission/publication dates, and additional authors on these manuscripts are noted at the beginning of each chapter. Appendices presented at the end of the dissertation contain data and methodology conducted in support of the objectives of this dissertation that were not included in the prepared manuscripts.

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CHAPTER 1

COASTAL MARSHES OF THE ANTHROPOCENE: TESTING FUNCTIONAL RESPONSES TO DRIVERS OF GLOBAL CHANGE

On geologic timescales, coastal marshes are a brief glimmer on the face of the planet as they accrete and subside at the interface of land and sea. In the context of human civilization, however, they have seemed until recent decades to be as unchanging as the rhythm of the tides that simultaneously build and erode them. As trade and transportation encouraged human settlement by the sea, coastal marshes often stood in the way of progress and were ditched, diked and drained into oblivion without regard for their fragility and ecological significance. As we have come to understand as a society the many benefits and services provided by these ecosystems, the protection of those that remain is strictly enforced. However, in a world increasingly shaped by anthropogenic activity, coastal marshes are subject to a novel confluence of conditions that may alter community structures and functional responses, and ultimately even shift the role marshes play in regulating global climate.

Overview of global changes impacting coastal salt marshes

As a result of increased human-mediated release of GHGs to the atmosphere (Forster et al 2007; Susan 2007), average global temperatures have risen approximately 0.8°C (Susan 2007) over the past century. By the year 2100, global temperatures are projected by a consensus of modeling scenarios to increase by up to

4.5°C (Susan 2007), with extensive ecological consequences predicted to result (Walther et al 2002). While CO₂ emissions have increased most dramatically and typically are the target of management for climate change impacts (Susan 2007), methane (CH₄) and nitrous oxide (N₂O) have much higher global warming potentials per molecule over a 100 year basis (21 and 300 times that of CO₂, respectively (Forster et al 2007)) and thus the potential to cause more rapid warming (Susan 2007) and associated environmental impacts (Walther et al 2002).

Salt marshes typically are net sinks of GHGs. High levels of productivity and anaerobic conditions slow decomposition and promote significant C storage (Chmura et al 2003a; Adams et al 2012) as well as limited release of stored C as CO₂ via decomposition (Mitsch and Gosselink 2000; Chmura et al 2003a). Although plant-mediated transport has been shown to be responsible for a large proportion of CH₄ emissions from freshwater wetlands (Sorrell et al 1997; Henneberg et al 2012), emissions from salt marshes are typically smaller. Regular inundation of salt marsh soils by seawater ensures an abundance of sulfate-reducing bacteria (Madigan 2012), which outcompete methanogenic archaea that produce CH₄ as a product of their energy metabolism (Bartlett et al 1987a; Mitsch and Gosselink 2000; Poffenbarger et al 2011; Madigan 2012). Likewise, N₂O emissions from coastal marshes tend to be minimal. N entering salt marshes is either assimilated by plants and microbes (Valiela and Cole 2002; Lovell 2005) or lost as N₂ gas through denitrification (Mitsch and Gosselink 2000), a process that typically consumes N₂O (Kool et al 2010). But whereas unaltered coastal marshes have great potential to mediate climate change

through C storage, changing climate and human impacts can negatively affect marshes' abilities to store C and facilitate transformation of reactive N to inert N₂ gas, and may even shift them toward acting as net sources of GHGs (Moseman-Valtierra et al 2011).

Over the course of the next century, warming temperatures (Susan 2007; Gedan and Bertness 2009; Gedan and Bertness 2010a), elevated atmospheric CO₂ concentrations (Taiz and Zeiger 2002; Eller et al 2014), rising sea level (Donnelly and Bertness 2001a; Craft et al 2009), and shifts in species ranges associated with climate change (Walther et al 2002; Begon et al 2009) are expected to alter salt marsh structure and function. Coastal marshes, especially those in southern New England (Bricker-Urso et al 1989), have also been subjected to anthropogenic stressors including development (Bertness et al 2002), eutrophication (Turner et al 2009; Deegan et al 2012), and introduction of non-native species (Chambers et al 1999; Amsberry et al 2000; Meyerson et al 2009a) for the past several decades. The confluence of these numerous impacts is likely to result in widespread transformations to the coastal marsh ecosystem, including potential changes to ecosystem functions. Whereas the effects of each of these individual stressors on coastal marshes have been documented, less research has been conducted on potential interactions between stressors. Since coastal marshes are already heavily impacted by N loading (Turner et al 2009; Deegan et al 2012) and exotic species invasions (Chambers et al 1999) as the result of anthropogenic activities, these ecosystems may be particularly vulnerable to the effects of climate change. Furthermore, since perturbations to salt marsh

ecosystems have been shown to increase emissions of the climate-altering greenhouse gases CH₄ (Cheng et al 2007; Tong et al 2012; Mozdzer and Megonigal 2013) and N₂O (Cheng et al 2007; Moseman-Valtierra et al 2011) climate-driven stresses to salt marshes may create a feedback effect on climate warming and offset marshes' ecosystem service of C storage (Mitsch and Gosselink 2000).

Invasion of coastal marshes: Impacts of *Phragmites australis*

Exotic species invasions of salt marshes have been associated with changes to community structure (Bertness et al 2002; Ravit et al 2003), trophic function (Levin et al 2006), and biogeochemistry (Tong et al 2012). Invasive plants, as compared with native vegetation, have been associated with greater emission of CH₄ from coastal marshes which have been attributed to rhizosphere impacts and internal gas transport (Cheng et al 2007; Tong et al 2012). In North American coastal marshes, the presence of an invasive lineage of *Phragmites australis* has increased in recent decades (Chambers et al 1999; Meyerson et al 2009a). Whereas a native lineage of the species has been present in North America for tens of thousands of years (Meyerson et al 2010), the invasive lineage appears in the herbarium record beginning during the 19th century (Saltonstall 2002; Saltonstall 2003a). The relatively recent massive expansion of this robust grass has been attributed to several factors. Its requirement for abundant N is being met by anthropogenic eutrophication (Mozdzer and Zieman 2010), and when released from competition for N, invasive *Phragmites australis* (hereafter, *Phragmites*) outcompetes native species by shading (Chambers et al 1999),

competition for nutrients (Mozdzer and Zieman 2010), and rapid spread by both clonal replication (Amsberry et al 2000) and seeding (McCormick et al 2009). Genetic diversity in *Phragmites*, once thought to be minimal within the introduced, invasive lineage, is actually quite extensive as the result of multiple introductions (Lambertini et al 2012; Meyerson and Cronin 2013), hybridization (Meyerson et al 2009b), and long-distance dispersal (Lambertini et al 2012). As a result of this ability to capitalize on available N and its extensive genetic diversity, it is likely that *Phragmites* will continue to thrive and adapt to coastal wetland stressors, potentially exacerbating invasions.

Phragmites may alter marsh GHG emissions via several mechanisms: changes to its rhizosphere and associated microbial communities (Ravit et al 2003; Armstrong et al 2006), conduction of products of microbial respiration from the rhizosphere to the atmosphere (Brix 1989; Brix 1990) via a massive (Colmer 2003) and specialized (Armstrong et al 2006) internal gas transport system, and photosynthetic uptake of CO₂ (Figure 1). At rates greater than native mid-high marsh perennials which have been shown to transport minimal O₂ (Maricle and Lee 2007), *Phragmites* is known to adaptively oxygenate its rhizosphere via internal Venturi and humidity-induced pressure flows (Armstrong 2000; Colmer 2003). Due to high water requirements, *Phragmites* facilitates evapotranspirative water loss from soil, and so has been shown to locally lower water tables (Windham and Lathrop 1999) and thus potentially favor aerobic soil processes. *Phragmites* has been shown to support unique microbial communities relative to native species (Ravit et al 2003), likely as a result of its

specialized root zone aeration mechanisms (Armstrong et al 1992; Armstrong et al 1992). In addition to root zone oxygenation, *Phragmites*' massive aerenchyma and pressure-driven transport system are also responsible for conducting rhizosphere-derived gases, most notably CH₄ (Brix et al 2001), through the plants' aerenchyma and into the atmosphere (Armstrong et al 1996; Grosse et al 1996; Beckett et al 2001; Colmer 2003). Shifts in microbial communities that favor or exclude CH₄ or N₂O-producing functional groups could translate into changes in GHG flux magnitude. Such emissions may potentially be offset by *Phragmites*' photosynthetic uptake of CO₂ during the growing season (Martin and Moseman-Valtierra, 2015).

Figure 1. Potential effects of Phragmites on rhizosphere processes and GHG fluxes. Emissions are shown in green and uptake is shown in orange.

Potential interactions of eutrophication and *Phragmites* invasions in a changing climate

Climate change and associated sea level rise are predicted to alter marsh hydrology (Craft et al 2009), physical structure (Deegan et al 2012), and species distribution and abundance (Donnelly and Bertness 2001a; Gedan and Bertness 2010a; Gedan et al 2011). As anthropogenic and climatic drivers continue to alter biotic and abiotic components of coastal marshes, they may increasingly function as "novel

ecosystems” (Hobbs et al 2009; Hobbs et al 2011) that bear little resemblance to their present-day counterparts. Such marshes will require adaptive management techniques designed to preserve ecosystem function and services (Buchsbaum and Wigand 2012), which must be based upon a sound understanding of salt marsh responses to complex interacting stressors such as eutrophication, *Phragmites* effects and climate change.

Nitrogen loading and *Phragmites* spread will likely both be exacerbated by climate change. As a result of predicted increasing frequency and intensity of rainfall events (Susan 2007), inputs of land-derived N to coastal marshes are expected to rise. Further, aerial deposition of N is predicted to increase as climate change progresses (Templer et al 2012). Since *Phragmites* benefits from increased N availability (Mozdzer and Zieman 2010), eutrophication-stimulating facets of climate change could facilitate its invasion as well. Elevated atmospheric CO₂ (up to 300 ppm increase expected by the end of the 21st century (Susan 2007)) may also serve to promote *Phragmites* success. Since it utilizes the C₃ photosynthetic pathway, *Phragmites* is capable of accelerating its photosynthetic rate under conditions of elevated atmospheric CO₂, unlike native salt marsh grasses which generally use the C₄ pathway (Taiz and Zeiger 2002). This phenomenon, especially under conditions of readily available N, has the potential to allow for more rapid growth and colonization by *Phragmites* relative to its native marsh counterparts. Higher temperatures associated with climate change may enhance genetic diversity and thus adaptive ability among *Phragmites* populations, since summer temperatures predicted for the end of the century have been shown to more than double *Phragmites* germination rate

relative to rates observed under present-day temperatures (Martin and Moseman-Valtierra, *unpublished data*). While an exacerbation of *Phragmites*' spread will likely lead to decreased marsh plant biodiversity (Minchinton and Bertness 2003) and potentially to associated alterations to nutrient cycling, the plants' copious biomass may serve to sequester C and its massive root system has been suggested to promote marsh accretion (Rooth et al 2003), which could help marshes' elevation keep pace with predicted sea level rise. Additionally, the plants' reportedly high capacity for assimilation of N (Farnsworth and Meyerson 2003; Mozdzer and Zieman 2010) could help to preserve the critical marsh ecosystem function of interception of land-derived N.

In general, anthropogenic drivers of global change are likely to interact with a warming climate to impact ecologically important coastal marshes in ways that may be cumulative or synergistic. Since coastal wetlands are so vulnerable to effects of climate change and have such a significant role to play in mediating harmful climate effects, a mechanistic understanding of their response to the multiple stressors to which they will be subjected in the coming decades is of vital importance. In particular, while biological invasions of coastal wetlands have traditionally been viewed through the lens of biodiversity preservation and conservation concerns, impacts of such invasions on biogeochemistry and nutrient cycling must be studied in order to gauge their present and future roles in ecosystem functioning and climate feedbacks.

Objectives of the dissertation

The objective of this dissertation was to elucidate the effects of global change drivers, particularly invasion of *Phragmites*, on coastal marsh GHG flux dynamics by employing a series of mensurative and manipulative field and mesocosm experiments. First, edaphic conditions and GHG fluxes from marshes dominated by *Phragmites* and native marsh species were characterized along environmental gradients (Chapter 2). Next, with the goal of better understanding mechanisms driving observed patterns in GHG dynamics, effects of *Phragmites* removal on GHG fluxes and edaphic conditions was tested across diurnal cycles (Chapter 3). To discern effects of *Phragmites* removal on marsh function within a context of coastal marsh restoration, GHG fluxes, edaphic and pore water chemistry, and decomposition rates were measured under several management scenarios (Chapter 4). Finally, to investigate potential synergisms between global change drivers, a multifactorial mesocosm experiment was performed. Presence of *Phragmites*, N overenrichment, and simulated climate change were tested for independent and combined effects on mesocosm plant performance, GHG flux and edaphic responses (Chapter 5).

Quantifying greenhouse gas fluxes in coastal marshes: challenges and novel technologies

Historically, there have been constraints involved in field measurements of GHG fluxes in coastal wetland ecosystems. Given the highly spatially and temporally heterogeneous nature of coastal wetland GHG fluxes (driven by differences in

hydrology, vegetation type, season, and diel stage), real-time, continuous flux measurements are needed. However, the most commonly used method, analysis using a gas chromatograph (GC) of gas concentrations in air samples collected at intervals from within a chamber placed on the marsh surface, is time-consuming and therefore limits the amount of sampling that may reasonably be performed. Other disadvantages of the GC method for measuring GHG fluxes include artifacts such as warming associated with lengthy (several hours) chamber deployments, the need for gas sample storage, and the non-continuous nature of the data.

Recently, instruments employing novel technologies for *in situ*, continuous gas concentration measurements have become commercially available. For the work presented in this dissertation, the primary means of GHG flux measurement was a Cavity Ringdown Spectroscopy (CRDS) analyzer (model G2508, capable of measuring CO₂, CH₄, and N₂O concurrently) manufactured by Picarro Labs (Santa Clara, CA). This instrument was found to measure GHG fluxes with greater precision than a GC (Shimadzu GC-2014) (Brannon et al., *submitted*), and enabled greater sampling replication and frequency than would have been feasible using the GC method.

Figure 2. Schematic showing setup of the analyzer and static flux chamber used for GHG flux measurements. The analyzer's inlet and outlet are connected to a flux chamber using nylon tubing connected to 2 chamber ports.

CRDS allows for sensitive monitoring of multiple gases at the parts per billion (or trillion) level in seconds. The analyzer's internal cavity, along with a deployed static flux chamber and nylon tubing to connect the analyzer and chamber (Figure 2), form a closed system in which concentrations of gases are measured over the course of a chamber deployment period. The G2508 analyzer's cavity, into which air from the flux chamber is drawn, contains 3 mirrors which reflect a continuous, traveling light wave from a single-frequency diode laser. Gas concentration is determined by the decay rate of light reflected between mirrors that reflects absorption by gas molecules. When the laser is turned off, the light within the analyzer cavity continues to be reflected between mirrors. When gas molecules are introduced into the analyzer cavity and absorb light, the "ring down time", or time until light is lost from the cavity, is decreased relative to ringdown time in the absence of the gas. The laser is tuneable to different wavelengths over the target gases' spectral absorption lines, and so determines concentrations for each gas.

The G2508 analyzer reports measured GHG concentrations every few seconds. The slope of the linear change in CRDS-measured gas concentrations over time, along with the Universal Gas Law, was used to compute a GHG flux using the following formula:

$$\text{Flux} = dC/dt(PV/RAT)$$

V = chamber volume
R = Gas constant
T = Field-measured air temperature
P = Field-measured pressure
A = Chamber footprint

CHAPTER 2
GREENHOUSE GAS FLUXES VARY BETWEEN *PHRAGMITES AUSTRALIS*
AND NATIVE VEGETATION ZONES IN TIDAL MARSHES ALONG A
SALINITY GRADIENT

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Abstract

The replacement of native species by invasive *Phragmites australis* in coastal wetlands may impact ecosystem processes including fluxes of the greenhouse gases (GHGs) carbon dioxide (CO₂) and methane (CH₄). To investigate differences in daytime CH₄ and CO₂ fluxes as well as vegetation properties between *Phragmites* and native vegetation zones along a salinity gradient, fluxes were measured via cavity ringdown spectroscopy in 3 New England coastal marshes, ranging from oligohaline to polyhaline. While daytime CH₄ emissions decreased predictably with increasing soil salinity, those from *Phragmites* zones were larger (15 to 1,254 $\mu\text{mol m}^{-2} \text{h}^{-1}$) than those from native vegetation (4-484 $\mu\text{mol m}^{-2} \text{h}^{-1}$) across the salinity gradient. *Phragmites* zones displayed greater daytime CO₂ uptake than native vegetation zones (-7 to -15 $\mu\text{mol m}^{-2} \text{s}^{-1}$ vs. -2 to 0.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at mesohaline-polyhaline, but not oligohaline, sites. Results suggest that vegetation zone and salinity both impact net emission or uptake of daytime CO₂ and CH₄ (respectively). Future research is warranted to demonstrate *Phragmites*-mediated impacts on GHG fluxes, and additional measurements across seasonal and diel cycles will enable a more complete understanding of *Phragmites*' net impact on marsh radiative forcing.

Introduction

Invasive plants can alter the structure and function of coastal wetlands. Exotic species invasions of coastal wetlands are known drivers of ecosystem-level change including impacts to vegetation (Bertness et al 2002) and microbial (Ravit et al 2003) community structure, trophic function (Levin et al 2006), and biogeochemistry (Windham and Ehrenfeld 2003; Tong et al 2012). Invasive plants can alter components of nitrogen (N), carbon (C), and water cycling via differences in productivity and rhizosphere conditions including nutrient uptake, soil oxygenation, and availability of C exudates relative to native plants (Ehrenfeld 2003).

In North American coastal wetlands, the presence of the invasive grass *Phragmites australis* has increased steadily in recent decades (Chambers et al 1999; Meyerson et al 2009) with potential implications for ecosystem function. *Phragmites* has been shown to outcompete native species by shading (Chambers et al 1999), capitalizing on nutrient availability (Mozdzer and Zieman 2010), and rapidly spreading by both clonal replication (Amsberry et al 2000) and seeding (McCormick et al 2009). Although *Phragmites* invasion is known to exclude native high marsh vegetation (Minchinton et al 2006) and therefore reduce species richness in the high marsh community (Silliman and Bertness 2004), ways in which ecosystem functions may be affected by *Phragmites* invasion are less well-understood.

The replacement of native vegetation with invasive *Phragmites*-dominated communities (hereafter *Phragmites* zones) can mediate significant shifts in net CO₂ and CH₄ fluxes. Generally, coastal wetlands emit minimal carbon dioxide (CO₂) and

methane (CH₄) (Mitsch and Gosselink 2000; Poffenbarger et al 2011; Madigan 2012). *Phragmites* may increase marsh CO₂ uptake in the short term due to its greater productivity relative to smaller native species (Windham 1999). It may also contribute to decreased CH₄ emissions as a result of rhizosphere methanotrophy, since *Phragmites*' physiology often leads to a notable oxygenation of its rhizosphere (Armstrong 2000; Colmer 2003). However, invasive *Phragmites* also has the potential to exacerbate marsh CH₄ emissions relative to native vegetation (Mozdzer and Megonigal 2013). *Phragmites*' provision of labile organic C to its rhizosphere (Ravit et al 2003; Lovell 2005; Armstrong et al 2006) may result in increased methanogen presence or activity. *Phragmites* may also directly drive CH₄ emissions since its pressure-driven transport system is known to conduct rhizosphere-derived CH₄ (Brix et al 2001) through the plants' massive aerenchyma and into the atmosphere (Armstrong et al 1996; Grosse et al 1996; Beckett et al 2001; Colmer 2003).

Characterization of CO₂ and CH₄ fluxes from marshes vegetated with invasive *Phragmites* and native vegetation could provide insight into potential impacts of *Phragmites* invasion on marsh GHG flux dynamics. However, few have performed such investigations (Emery and Fulweiler 2014), and no studies to date compare greenhouse gas (GHG) fluxes from *Phragmites* and the high marsh native perennials (such as *Spartina patens*, *Distichlis spicata* and *Juncus gerardii*) that *Phragmites* is likely to displace as it invades from upland borders and along creek banks (Chambers et al 1999; Silliman and Bertness 2004).

Factors other than vegetation type also affect marsh GHG fluxes, and must be taken into account when attempting to discern impacts of changing plant communities. Salinity is known to be a major control on CH₄ fluxes in coastal wetlands (Poffenbarger et al 2011) as frequent inundation with seawater replenishes sulfate to soil bacterial communities that outcompete methanogenic archaea (Mitsch and Gosselink 2000; Madigan 2012). Salinity also is understood to constrain *Phragmites* distribution (Burdick et al 2001), and while *Phragmites* is capable of growing at marine strength salinities (Chambers et al 2003), it often displays reduced vigor and success when exposed to increased seawater inundation (Hanganu et al 1999). A comparison of GHG fluxes from *Phragmites* and native vegetation stands along a natural salinity gradient could begin to distinguish between biotic and abiotic controls on flux differences.

The objective of this research was to compare CH₄ and CO₂ fluxes from *Phragmites* and native high marsh vegetation zones during a growing season in three Southern New England coastal marshes that span a natural salinity gradient. Surface soil properties, plant variables and pore water sulfide also were measured and tested for their relationship to observed GHG fluxes. *Phragmites* zones were hypothesized to have higher CH₄ emissions but also higher rates of CO₂ uptake than native high marsh zones given the plant's greater gas transport and productivity rates. CH₄ fluxes were expected to decrease with increasing marsh salinity in both *Phragmites* and native high marsh zones.

Materials and Methods

Study Sites

Study sites were chosen to allow for comparison of GHG fluxes between *Phragmites* and native high marsh vegetation zones along a salinity gradient. Three *Phragmites*-invaded southern New England coastal wetlands were selected: two in lower Narragansett Bay, Rhode Island (Round Marsh and Fox Hill) and one in Waquoit Bay, Massachusetts (Sage Lot) (Table 1, Figure 1). Sage Lot is located in a watershed with minimal development and minimal N loads (Valiela and Cole 2002), and Round Marsh and Fox Hill are located in lower Narragansett Bay where N concentrations from anthropogenic activities are estimated to be low (Wigand et al 2003). Native vegetation consists primarily of *Spartina patens* and *Distichlis spicata* (see Table 1 for details), and *Phragmites* invasion encroaches from the upland edge of all marsh sites. Sites increase in growing season surface soil salinity from oligohaline levels in Round Marsh to polyhaline in Sage Lot, with Fox Hill being intermediate (Table 1). All sites experienced flooding with 32 ppt seawater in both vegetation zones during spring tides.

Experimental Design

At each site, GHG fluxes were compared between the *Phragmites* and native high marsh vegetation zones using 3 replicate plots per zone. At Round Marsh, 3 plots were selected in each vegetation zone with approximately 30 m of spacing between plots. At Fox Hill and Sage Lot, three plots were selected in the native high marsh

zone, but in the *Phragmites* zone where pilot trials indicated high gas flux variability, 3 pairs of plots (with 0.3 m spacing between paired plots) were established (data from the paired plots were averaged prior to statistical analyses, as detailed below). In *Phragmites* zones, plots were established approximately 1m from the leading, seaward edge of the stand, and in native vegetation zones plots were placed at random. *Phragmites*-mediated changes to edaphic conditions and microbial communities at the leading edge of the invasion have persisted for shorter duration than in older, interior portions of the stand. Therefore, comparisons between native and *Phragmites* zones represent conservative estimates of potential changes in GHG flux dynamics due to invasion.

For GHG flux measurements, bases were installed in each selected plot to support static flux chambers. Bases were installed in the early spring (at least 2 weeks prior to first measurements) so as to permit recovery of vegetation, and were left in place for the duration of the growing season to minimize soil disturbance. *Phragmites* zone chamber bases consisted of PVC rings (24 cm tall x 30 cm diameter), and native vegetation chamber bases consisted of stainless steel rings (9 cm tall x 30 cm diameter). *Phragmites* and native vegetation bases were inserted 8 cm into the soil and both types featured drainage holes positioned just beneath the soil surface to allow for lateral water movement after tidal inundation or rainfall.

Edaphic Variable and Plant Metrics

Surface soil (top 3 cm) salinities were measured at each plot within chamber

bases once per month from April-August 2014 at the time of GHG flux measurements. Soil was pressed against paper filters using small syringes to extract water, which was analyzed for salinity using a handheld refractometer. Soil pore water was collected at each site in May, July and late August from each plot during GHG flux measurements using 15 cm Rhizon Soil Moisture Samplers (Ben Meadows, Janesville, WI), preserved using 1 M zinc acetate, and frozen until analysis. Pore water sulfide concentrations were analyzed using standard colorimetric techniques (Cline 1969). Mid-growing season soil pH, oxidation-reduction potential, temperature and moisture point measurements were performed just outside each chamber base once each during June and July GHG flux measurements for a total of 2 measurements per plot. Soil oxidation-reduction (redox) potential (ORP) and pH were measured using an ORP probe and pH/ORP meter (Mettler Toledo, Greifensee, Switzerland) and pH meter (ExStick[®] Instruments, Nashua, NH). Soil temperature was measured with a digital thermometer inserted into soil at a depth of 15 cm, and soil moisture content was measured using a volumetric water content sensor (Decagon Devices, Pullman, WA) inserted 5 cm into soil. Soil for organic content analysis was collected from each plot in August. Using a cutoff syringe, approximately 10 mL of soil was collected. Soil was dried, weighed, and placed in a muffle furnace at 500 °C overnight. Change in weight was defined as loss on ignition (LOI), a proxy for soil organic content.

To characterize *Phragmites* vegetation at each site, densities of live and dead stems and average stem height within chamber bases were recorded during the growing season once plants were mature (July). For stem height, 10 stems per plot

were selected randomly and their heights were averaged.

GHG Flux Measurements

Daytime GHG flux measurements were performed during the early (April-May), mid- (June-July), and late (late August) growing season during 2014. Gas measurements were conducted for 6-10 minutes per plot, based on observed periods for linear rates of change. All GHG flux measurements were performed between 9:00 AM and 3:00 PM and within 3 h of low tide.

A cavity ring down spectroscopy (CRDS) analyzer (Picarro G2508) was used to measure CO₂, CH₄ and N₂O concentrations in real-time. The analyzer cavity, together with a flux chamber and connective tubing, creates a closed system within which gas concentration changes over time are measured with a flow rate of ~230 sccm and frequency of approximately 1 measurement/sec. The gas analyzer simultaneously measures H₂O vapor concentrations and reports the dry mole fraction of the other target gases, and this corrected value was used for all flux calculations. H₂O vapor saturations never exceeded 2.5% over the course of any measurements.

The analyzer was connected via nylon tubing to transparent polycarbonate chambers, which were placed into the previously-installed bases. Vegetation was left intact inside the chambers. A 0.02 m³ polycarbonate chamber was used for native species zone measurements following previously-described methods (Moseman-Valtierra et al 2011). In order to accommodate tall (up to 2 m) *Phragmites* plants, a 2 m tall, 0.3 m diameter transparent polycarbonate tube (Rideout Plastics[®]) was modified

to extend the shorter polycarbonate chamber which was sealed to the extender using a polyethylene closed-cell foam collar with its channel filled with water (for a total chamber volume of 0.15 m³). Extender support bases were designed to create a gas-tight fit between base and extender. Two small fans attached to the inside of the polycarbonate chamber (10-cm fans) and extender (20-cm fans) ensured air mixing during measurements. A stainless steel 55 cm long, 0.8 mm diameter pigtail was used for pressure equilibration. Hobo[®] data loggers (Onset, Bourne, MA) were suspended within chambers during all flux measurements to monitor air temperature at 30 s intervals.

GHG fluxes were calculated using chamber size and footprint. The Ideal Gas Law ($PV = nRT$) was used to calculate changes in gas concentrations over time using field-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 0 (3.8% of CO₂ and 7.6% of CH₄ measurements). When slopes had an R² value of less than 0.85, data were not included in the analysis (5.7% of CO₂ and 1.9% of CH₄ measurements). The relatively short time period of these greenhouse gas flux measurements are not designed to capture ebullitive fluxes, and thus may represent underestimates of total gas emissions, particularly for CH₄ (Tokida et al 2005). However, our high resolution measurement of gas concentrations does enable detection of the rapid (often step-shaped) changes in gas concentrations that occur during ebullition to be very well resolved and distinguished from diffusive flux during the periods of chamber deployment

(Middelburg et al 1996). We did not detect ebullition from this dataset.

Statistical Analyses

When two sets of measurements were taken during a portion of the growing season (n=3 seasonal stages: early, mid or late), averages for the two sampling dates were computed by plot. At Fox Hill and Sage Lot where *Phragmites* zone measurements were conducted in duplicate, averages of measurements from pairs of plots were used for statistical analyses. Therefore, for GHG flux, soil salinity and porewater sulfide data, each site had 3 data points per vegetation zone per growing season period for a total of 18 data points per site. CO₂ and CH₄ fluxes, soil salinity and porewater sulfide were compared between vegetation zones at each site using a 2-factor ANOVA with vegetation type and growing season period (early, mid, late) as the two factors, and comparisons were drawn between sites using a 1-factor ANOVA.

For edaphic variables, June and July data were averaged for the two sampling dates by plot. Edaphic and plant variable data collected from pairs of *Phragmites* plots were averaged and the means of the two values were used for statistical analysis. Therefore, for pH, redox potential, temperature, moisture, and soil organic C, each site had n=3 data points per vegetation zone for the mid growing season only (for a total of 6 data points per site). Edaphic variables were compared using two-factor ANOVA with site and vegetation type as main effects. *Phragmites* vegetation characteristics (stem height, live and dead stem counts) were compared between sites using a one-factor ANOVA. Data were aligned then rank-transformed prior to ANOVA analyses

(Salter and Fawcett 1993; Wobbrock et al 2011) to account for deviations in normality while allowing for tests of effect interaction (Seaman Jr et al 1994). Tukey's HSD test was used for post-hoc pairwise comparisons when appropriate.

Potential relationships between edaphic and vegetation variables and GHG fluxes were investigated using Spearman's R Correlation Test.

All statistics were performed in R (R Core Team, 2012) and interpreted at a significance level of 0.05.

Results

Edaphic Variables & Vegetation Characteristics

Confirming the expected salinity gradient, soil salinity differed significantly between all 3 sites and was highest at Sage Lot and lowest at Round Marsh, with Fox Hill intermediate (Table 2). Significant differences in salinity between vegetation zones (*Phragmites* and native) were present only at Fox Hill (Table 2), with salinity higher by several ppt in the native vegetation zone during the mid and late growing season stages.

Porewater sulfide concentrations ranged from 0 to approximately 250 μM , although one sample (from native vegetation at Sage Lot) had a sulfide concentration of over 1,000 μM (Table 2). Concentrations did not differ significantly between vegetation zones at any site, but did display between-site differences when averaged across all dates, with Sage Lot sulfide concentrations (139.00 ± 17.77) significantly greater than those at Round Marsh (31.57 ± 16.31) (Table 2).

Surface soil pH averaged across vegetation zones at Sage Lot was significantly greater than at Round Marsh and Fox Hill (Table 3). Surface soil oxidation-reduction potential averaged across vegetation zones was significantly lower at Sage Lot than Round Marsh. Soil temperature (at 15 cm depth) averaged across vegetation zones was higher by approximately 3°C at Sage Lot and Fox Hill than at Round Marsh (Table 3). Soil moisture (at 5 cm depth) averaged across vegetation zone differed between the three sites, decreasing from Sage Lot to Round Marsh with Fox Hill intermediate. Significant site x vegetation zone interaction indicated that *Phragmites*

zone soil moisture at Fox Hill was similar to Round Marsh, while native vegetation soil moisture at Fox Hill was similar to Sage Lot (Table 3). Soil organic content averaged between zones was significantly greater at Fox Hill than Round Marsh (Table 3).

Phragmites stand structure varied along the salinity gradient. Although not significant, trends in *Phragmites* stem height and live and dead stem counts were observed between sites (Table 3). Average *Phragmites* stem height displayed a trend of decrease with increasing site salinity. Live and dead *Phragmites* stem densities were generally greater at higher-salinity sites (Fox Hill and Sage Lot) than at Round Marsh.

GHG fluxes

Daytime CH₄ fluxes were significantly greater (by up to several orders of magnitude) in *Phragmites* zones than in native vegetation zones at all sites (Figure 2) and were orders of magnitude larger for both vegetation zones at oligo-mesohaline Round Marsh than polyhaline Sage Lot (Figure 3). CH₄ emissions were highly variable and ranged from 0-4,206 $\mu\text{mol m}^{-2} \text{h}^{-1}$. They increased after the early growing season at meso-polyhaline Fox Hill (trend) and polyhaline Sage Lot (significantly); by contrast, however, oligo-mesohaline Round Marsh displayed a trend of larger CH₄ emissions during the early growing season, which declined later in the growing season (Figure 2).

Daytime CO₂ fluxes ranged from -37 to +7 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with significantly greater uptake (by 5-15 times during the mid growing season) in the *Phragmites* zone than in native vegetation at Fox Hill and Sage Lot, the higher-salinity sites (Figure 2). The greatest *Phragmites* zone CO₂ uptake (approximately 2x as much as at Round Marsh and Sage Lot), as well as the greatest native vegetation zone CO₂ emission (positive fluxes), occurred at Fox Hill (intermediate salinity). CO₂ fluxes varied across the growing season at Round Marsh and Fox Hill, with the least CO₂ uptake occurring during the early growing season. For CO₂ fluxes at Fox Hill, interaction of vegetation type and seasonal stage were significant, indicating that greatest CO₂ uptake occurred in *Phragmites* zones during the mid growing season.

No detectable N₂O fluxes were observed (with a 30-second averaging period and minimal detection limit of approximately 1.4 $\mu\text{mol m}^{-2} \text{hr}^{-1}$) (Brannon et al. *in prep*).

Across all sites, CH₄ emissions in *Phragmites* zones (but not native vegetation) were negatively correlated with salinity (Spearman's $r = -0.43$, $p = 0.04$). Soil redox potential was negatively correlated with CO₂ flux magnitude in native vegetation (Spearman's $r = -0.88$, $p < 0.01$), while soil temperature (Spearman's $r = 0.78$, $p = 0.01$), and soil moisture (Spearman's $r = 0.72$, $p = 0.04$) were positively correlated with CO₂ flux magnitude in that zone. No other significant relationships were found between GHG fluxes and edaphic and plant variables.

Discussion

Phragmites zones were consistently associated with larger CH_4 emissions across the salinity gradient

Site histories prior to *Phragmites* invasions may vary. Therefore, experimental manipulations would be required in order to determine whether *Phragmites* invasion drove the observed consistent greater emission of CH_4 compared to native vegetation zones. However, the clear association between *Phragmites* presence and increased daytime CH_4 emissions during the growing season across sites suggests a role of this invasive species in driving GHG dynamics. These findings are consistent with *Phragmites*' known promotion of advective and diffusive fluxes of gases from soils to atmosphere (Armstrong et al 1996; Brix et al 1996; Colmer 2003), as well as potential greater C substrate provision (as a result of greater *Phragmites* biomass relative to native species) to methanogens in the form of rhizodeposition or litter (as reviewed in Lovell 2005).

In salt marshes, plant zonation follows strong gradients in multiple environmental conditions. Significantly larger emissions from *Phragmites* than from native vegetation zones at all sites along the salinity gradient may thus reflect a combination of edaphic and plant-driven factors. CH_4 emissions differed between zones despite similarity in mid-growing season surface soil variables (pH, redox, temperature, moisture, and organic content), a finding that may suggest direct CH_4 emission enhancement by *Phragmites*. However, the similarity of edaphic variables in surface soils (0-15 cm) does not rule out potential for significant differences in these

and other factors between vegetation zones at depths greater than we sampled.

Phragmites' ability to alter conditions in its rhizosphere environment is well-documented, with reported effects including decreased surface soil salinity (Windham and Lathrop 1999), oxygenation of the rhizosphere (Colmer 2003) and enhanced sediment accretion (Rooth et al 2003). Given the plant's characteristic deep (up to 1 m) root system (Brix 1987; Moore et al 2012), it is reasonable to suspect that *Phragmites* rhizosphere conditions may have contributed to the observed pattern of CH₄ emissions.

While this study found differences in CH₄ emissions between *Phragmites* and native high marsh vegetation zones, an investigation comparing GHG fluxes between *Phragmites* and low marsh native *Spartina alterniflora* did not. Emery and Fulweiler (2014) measured GHG fluxes between January and September from *Phragmites* and *S. alterniflora* zones at Plum Island Estuary (mesohaline) and found that GHG fluxes (CO₂, CH₄ and N₂O) at this site did not differ between the two vegetation zones. The authors' reported growing season CH₄ fluxes were highly variable, but generally fall within the ranges of those we observed at higher-salinity sites. Their single exceptionally high *S. alterniflora* CH₄ flux of over 18,000 $\mu\text{mol m}^{-2} \text{h}^{-1}$, however, exceeds our greatest measured fluxes at any site by an order of magnitude. The discrepancy between our findings and those of Emery and Fulweiler are likely due a combination of methods differences, key ecophysiological differences in *S. alterniflora* and the high marsh species *S. patens* and *D. spicata*, and effects of the season during which measurements were conducted. In addition, the ability of CRDS

technology to detect fluxes over much shorter periods than gas chromatograph-based methods (6-10 minutes vs. 60 minutes) may have allowed for better differentiation between vegetation zones.

In a mesocosm experiment, greater CH₄ emissions were attributed to more abundant biomass of invasive relative to native *Phragmites* (Mozdzer and Megonigal 2013). In our study, however, metrics indicative of *Phragmites* biomass (stem density and height) did not correlate with CH₄ emissions, suggesting that differences in subsurface soil conditions or belowground biomass may instead be responsible for observed patterns of CH₄ fluxes.

Greater CO₂ uptake by Phragmites zones may suggest potential for enhanced C sequestration

Greater CO₂ uptake by *Phragmites* relative to native vegetation zones over the course of the growing season at Fox Hill and Sage Lot is reasonable given *Phragmites*' substantially greater biomass (Windham 2001) (and therefore more photosynthetic uptake) relative to smaller native high marsh species. At oligohaline Round Marsh, mid-growing season uptake by native vegetation greater than that measured at other sites could be due to greater aboveground biomass (which was not measured in this study) or to reduced salinity stress.

At Round Marsh and Sage Lot, mid-season *Phragmites* zone CO₂ uptake is similar to that reported by Emery and Fulweiler (2014) (approximately 11 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Mid-growing season uptake at intermediate-salinity Fox Hill, however, averaged

more than twofold greater at about $30 \mu\text{mol m}^{-2} \text{s}^{-1}$. Such difference in uptake magnitude between Fox Hill and Sage Lot is surprising since *Phragmites* live stem densities and heights were similar between these sites (Table 3), and may suggest an influence of soil-driven CO_2 emission that counters plant-mediated uptake at Sage Lot.

Although CH_4 fluxes in *Phragmites* zones were larger than in native vegetation zones, they were small compared to measured *Phragmites* zone CO_2 uptake rates on a gram-to-gram C basis. Therefore, based on daytime, low tide fluxes measured during this growing season study, net CH_4 emissions were not sufficient to offset net CO_2 uptake. However, CO_2 uptake is diminished (and emissions therefore increased) during the evening, and studies over annual or tidal cycles will likely exhibit reduced overall uptake of CO_2 .

Phragmites' substantial increase in daytime CO_2 uptake relative to native vegetation, coupled with its known slow rates of decomposition and high productivity rates relative to *S. patens* (Windham 2001) and its promotion of marsh accretion (Rooth et al 2003), may suggest that its presence could ultimately enhance marsh C sequestration. However, such a conclusion must be based on more detailed temporal GHG flux measurements (seasonal and diel), including longer term gas ebullition studies, and coupled with measurements of long-term C sequestration rates.

Comparing GHG emissions across seasonal stages and complex environmental gradients

Since *Phragmites* commonly invades marshes from the landward edge (Amsberry et al 2000) and therefore displaces native high marsh species, GHG fluxes need to be characterized in order to assess ecosystem-scale response to a changing vegetation community. This study broadens understanding of growing season patterns of daytime CO₂ and CH₄ fluxes across the complex marsh landscape and over a growing season period.

Phragmites zones exhibited distinct temporal CH₄ flux trends along the salinity gradient, with fluxes increasing over the course of the growing season in the meso-polyhaline sites and decreasing at the oligohaline site. The observed increase in net CH₄ emissions from early to late seasonal stages in the more saline sites may imply a role of plant-mediated transport and/or an increase as the growing season progresses in microbial CH₄ production that is not being offset by increased microbial CH₄ oxidation. At the oligohaline site, observed temporal patterns of CH₄ fluxes may imply that vegetation presence decreases CH₄ emissions (potentially by soil oxygenation) and/or that microbial CH₄ production decreases as the growing season progresses.

The difference in CH₄ emissions between sites is consistent with the known control of salinity on marsh CH₄ emission (Bartlett et al 1987; Mitsch and Gosselink 2000; Poffenbarger et al 2011; Madigan 2012), but other variables (soil moisture, redox potential, and pore water sulfide concentration) also vary along the salinity

gradient. CH₄ emissions were greatest at Round Marsh, the site of lowest soil salinity, moisture and porewater sulfide and least reduced conditions, and smallest at Sage Lot, which was characterized by greatest soil salinity, moisture and sulfide concentrations and most reduced conditions. Fox Hill's soil conditions were intermediate. These findings support known roles of salinity and sulfate availability as strong predictors of marsh CH₄ emission magnitude, but contradict known positive relationships between methanogenesis and anaerobic, reduced soil conditions. Given the difficulty in determining relative contributions of soil variable and plant-mediated effects on GHG fluxes, future research should be directed toward experimentation to discern biotic and abiotic feedbacks along these environmental gradients.

Conclusions

Phragmites-dominated zones were characterized by significantly larger daytime CH₄ emissions than native high marsh vegetation zones along the natural salinity gradient, and larger daytime CO₂ uptake rates were observed in *Phragmites* zones in meso-polyhaline marshes. Although this study is not able to discern relative impacts of physical and biological controls on observed CO₂ and CH₄ fluxes, it reveals differences between two marsh zones for which GHG fluxes had not previously been compared and therefore confirms a need for future manipulative experiments to test mechanisms driving flux differences. In order to determine whether *Phragmites* may affect marsh net GHG uptake and C sequestration in the long term, future studies

should monitor GHG fluxes over annual and diel cycles and investigate how rates of *Phragmites*-zone C sequestration compare with rates in native vegetation zones.

Figure 1. Map of study sites in Narragansett Bay (Round Marsh and Fox Hill) and Waquoit Bay (Sage Lot Pond).

Table 1. Study site characteristics and GHG flux measurement replication details

Site	Salinity Class	Native high marsh species	Replication	Months Measured
Round Marsh <i>Jamestown, RI</i>	oligohaline- mesohaline	<i>Spartina patens</i> <i>Distichlis spicata</i>	<i>Phragmites</i> : n= 3 Native: n = 3	May, June, July, August
Fox Hill <i>Jamestown, RI</i>	mesohaline- polyhaline	<i>Spartina patens</i> <i>Distichlis spicata</i>	<i>Phragmites</i> : n= 3 (3 pairs) Native: n = 3	April, May, June, July, August
Sage Lot <i>Falmouth, MA</i>	polyhaline	<i>Distichlis spicata</i>	<i>Phragmites</i> : n= 3 (3 pairs) Native: n = 3	May, June, July, August

Table 2. Mean salinity (ppt) and pore water sulfide concentration $\pm$ SE measured during the early, mid and late growing seasonal stages and results of ANOVA tests

	Average Surface Salinity (ppt) ± SE			Veg. Type	Results of ANOVA tests			Site
	Early (Apr – May)	Mid (June – July)	Late (Aug)		Season	Veg Type x Season Stage		
<i>Round Marsh</i>								
<i>Phragmites</i>	4.00 ± 1.26	5.15 ± 1.36	-- **	$F_{1,8}=1.42$,	$F_{1,8}=0.73$,	$F_{1,8}=0.50$,	$F_{2,44}=88.89$, p < 0.01*	
Native Veg.	8.00 ± 4.31	13.33 ± 4.70	-- **	p=0.26	p=0.41	p=0.50		
<i>Fox Hill</i>								
<i>Phragmites</i>	7.13 ± 1.04	28.00 ± 3.30	24.33 ± 3.93	$F_{1,12}=16.68$,	$F_{1,12}=12.46$,	$F_{1,12}=1.17$,	Tukey HSD: RM ^c FH ^b SL ^a	
Native Veg.	10.67 ± 0.44	34.17 ± 1.59	33.6 ± 1.83	p<0.01*	p<0.01*	p=0.34		
<i>Sage Lot</i>								
<i>Phragmites</i>	26.75 ± 0.75	28.33 ± 0.11	33.67 ± 0.49	$F_{1,11}=1.48$,	$F_{1,11}=31.60$,	$F_{1,11}=0.17$,		
Native Veg.	25.67 ± 1.33	28.33 ± 0.44	32.67 ± 1.45	p=0.25	p<0.01*	p=0.85		
	Average Pore water sulfide (µM) ± SE			Veg. Type	Results of ANOVA tests			Site
	Early (Apr – May)	Mid (June – July)	Late (Aug)		Season	Veg Type x Season Stage		
<i>Round Marsh</i>								
<i>Phragmites</i>	106.97 ± 71.04	0.00 ± 0.00	17.51 ± 15.21	$F_{1,9}=0.62$, p	$F_{1,9}=1.96$ p	$F_{1,9}=1.14$	$F_{2,44}=6.96$, p < 0.01*	
Native Veg.	20.95 ± 13.98	0.00 ± 0.00	50.83	=0.45	=0.20	p=0.36		
<i>Fox Hill</i>								
<i>Phragmites</i>	72.80 ± 70.13	56.41 ± 56.41	27.07 ± 26.06	$F_{1,6}=0.01$ p	$F_{1,6}=0.74$ p	$F_{1,6}=0.62$	Tukey HSD: RM ^b FH ^{ab} SL ^a	
Native Veg.	180.10 ± 1.74	88.61 ± 87.31	148.21 ± 79.36	=0.90	=0.50	p=0.57		
<i>Sage Lot</i>								
<i>Phragmites</i>	0.00 ± 0.00	14.16 ± 7.70	103.53 ± 74.55	$F_{1,12}=1.73$ p	$F_{1,12}=1.53$ p	$F_{1,12}=0.62$		
Native Veg.	73.13 ± 66.20	91.58 ± 59.12	514.07 ± 410.39	=0.21	=0.25	p=0.56		

F statistics, degrees of freedom, and significance values are reported for 2-factor (Veg. Type x Seasonal Stage) and 1-factor (Site) ANOVA tests

* = Significant at $\alpha = 0.05$

** Salinity was measured on this date at an exceptionally high, late tide resulting in plot inundation and so is not included

Table 3. Edaphic and plant variable averages $\pm$ SE measured during the mid growing season (June – July) and results of ANOVA tests

	pH	Redox potential (mV)	Soil Temp. (°C)	Soil Moisture (%)	Soil organic content (%)	Live stem height (cm)	Live stems	Dead stems
<i>Round Marsh</i>								
<i>Phragmites</i>	6.18 $\pm$ 0.42	258.33 $\pm$ 181.86	17.82 $\pm$ 0.57	55.30 $\pm$ 2.15	23.07 $\pm$ 1.24	176.80 $\pm$ 8.74	4.00 $\pm$ 2.00	6.50 $\pm$ 1.50
Native Veg.	6.47 $\pm$ 0.40	217.22 $\pm$ 156.16	17.88 $\pm$ 0.62	55.52 $\pm$ 3.18	26.09 $\pm$ 2.66	--	--	--
<i>Fox Hill</i>								
<i>Phragmites</i>	6.95 $\pm$ 0.16	39.00 $\pm$ 53.76	20.15 $\pm$ 0.50	56.71 $\pm$ 2.28	52.28 $\pm$ 13.60	134.09 $\pm$ 19.18	8.67 $\pm$ 1.15	16.00 $\pm$ 2.16
Native Veg.	6.36 $\pm$ 1.41	-29.50 $\pm$ 98.17	20.68 $\pm$ 0.48	63.71 $\pm$ 0.84	47.61 $\pm$ 3.13	--	--	--
<i>Sage Lot</i>								
<i>Phragmites</i>	7.46 $\pm$ 0.14	0.08 $\pm$ 101.98	21.15 $\pm$ 0.77	66.25 $\pm$ 0.81	45.77 $\pm$ 4.18	123.13 $\pm$ 7.62	9.67 $\pm$ 0.33	15.67 $\pm$ 3.86
Native Veg.	7.13 $\pm$ 0.15	-83.83 $\pm$ 24.06	20.82 $\pm$ 0.83	64.55 $\pm$ 0.85	25.74 $\pm$ 6.00	--	--	--
Results of 2-factor ANOVA								
Veg. zone	$F_{1,11} = 0.19$, p = 0.67	$F_{1,12} = 0.94$, p = 0.35	$F_{1,12} = 3.00$, p = 0.91	$F_{1,12} = 2.89$, p = 0.11	$F_{1,11} = 2.68$, p = 0.13	--	--	--
Site	$F_{2,11} = 6.84$, p = 0.01*	$F_{2,12} = 5.27$, p = 0.02*	$F_{2,12} = 20.76$, p < 0.01*	$F_{2,12} = 34.76$, p < 0.01*	$F_{1,11} = 5.87$, p = 0.02*	$F_{2,6} = 4.80$, p = 0.06	$F_{2,6} = 5.10$, p = 0.05*	$F_{2,6} = 3.68$, p = 0.09
Veg. zone x Site	$F_{2,11} = 0.65$, p = 0.54	$F_{2,12} = 0.06$, p = 0.94	$F_{2,12} = 0.80$, p = 0.47	$F_{2,12} = 56.54$, p = 0.01*	$F_{1,11} = 1.32$, p = 0.30	--	--	--
Tukey HSD for Site	Round Marsh ^b Fox Hill ^b Sage Lot ^a	Round Marsh ^b Fox Hill ^{ab} Sage Lot ^a	Round Marsh ^b Fox Hill ^a Sage Lot ^a	Round Marsh ^c Fox Hill ^b Sage Lot ^a	Round Marsh ^b Fox Hill ^a Sage Lot ^{ab}	--	Round Marsh ^a Fox Hill ^a Sage Lot ^a	--

F statistics, degrees of freedom, and significance values are reported for 2-factor (Veg. Type x Site) ANOVA tests

In the Tukey HSD row, sites not connected by the same letter are significantly different

* = Significant at $\alpha = 0.05$

Figure 2. Average CH_4 (top row) and CO_2 (bottom row) fluxes during the early (April-May), mid (June-July) and late (August) growing season stages at the 3 study sites. Standard error bars are shown. Lowercase letters indicate significant differences between growing season periods; season flux measurements not connected by the same letter are significantly different.

F statistics, degrees of freedom, and significance values are reported for 2-way (Veg. Type x Seasonal Stage) ANOVA tests

*Significant at $\alpha = 0.05$

Figure 3. Average CH₄ (top) and CO₂ (bottom) fluxes from Phragmites and native vegetation zones averaged over the growing season (Early, Mid and Late seasonal stages) for each site.

F statistics, degrees of freedom, and significance values are reported for 1-factor ANOVA tests for site differences.

Lowercase letters represent the results of a Tukey HSD test. Bars within vegetation type not connected by the same letter are significantly different.

* = Significant at $\alpha = 0.05$

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CHAPTER 3

PLANT MANIPULATIONS AND DIEL CYCLE MEASUREMENTS INDICATE DISTINCT DRIVERS FOR CARBON DIOXIDE AND METHANE FLUXES IN A *PHRAGMITES AUSTRALIS* COASTAL MARSH

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Keywords: Salt marsh, diurnal cycles, cavity ringdown spectroscopy, *Phragmites australis*

Highlights

- Greenhouse gas fluxes varied in a *Phragmites australis* coastal marsh on diel cycles, with greatest carbon dioxide (CO₂) uptake ($-18 \mu\text{mol m}^{-2} \text{s}^{-1}$) and methane (CH₄) emission ($120 \mu\text{mol m}^{-2} \text{h}^{-1}$) during daylight hours. At night, average CO₂ and CH₄ emission of $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $20 \mu\text{mol m}^{-2} \text{h}^{-1}$, respectively, were observed.
- Aboveground vegetation removal resulted in a shift within minutes from CO₂ uptake to emission of similar magnitudes, but did not significantly affect CH₄ fluxes even after 4 months.
- CO₂ fluxes appear to be driven primarily direct plant-mediated processes, while CH₄ flux drivers appear to consist of abiotic and/or indirect plant effects.
- Daytime greenhouse gas flux measurements (alone) may overestimate net GHG uptake in coastal *Phragmites australis* marshes. In this experiment, the weighted average of net GHG uptake (in CO₂ equivalent units) across diel stages was about 20% of those calculated from daytime-only measurements.

Abstract

Substantial greenhouse gas (GHG) fluxes in *Phragmites*-invaded coastal marshes may be driven by plant-mediated and/or abiotic processes. The aim of this study was to elucidate carbon dioxide (CO₂) and methane (CH₄) patterns and drivers in a *Phragmites*-invaded coastal marsh during the active growing season. Specific objectives of this study were (1) to test effects of *Phragmites* aboveground vegetation removal on GHG fluxes over timescales ranging from instantaneous to 4 months and (2) to discern diel patterns of GHG fluxes in *Phragmites*-vegetated and cleared plots from measurements performed every 3 hours over complete diel cycles on 3 measurement dates. At all durations of vegetation removal, CO₂ uptake in vegetated plots consistently shifted to CO₂ emission when plots were cleared. CO₂ fluxes from vegetated plots were significantly different during the day (with uptake of around -15 $\mu\text{mol m}^{-2} \text{s}^{-1}$) than at night (when emission around 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was observed), while those from cleared plots did not vary over diel cycles. Nighttime CH₄ fluxes (20 $\mu\text{mol m}^{-2} \text{h}^{-1}$) from vegetated and cleared plots were on average only half the size of daytime fluxes (120 $\mu\text{mol m}^{-2} \text{h}^{-1}$) and were similar to each other. These results suggest that diel patterns of CO₂ are driven primarily by plants while patterns of CH₄ are driven by abiotic and/or indirectly plant-mediated effects, and that investigations that rely exclusively on daytime measurements from vegetated plots may overestimate net GHG uptake in CO₂ equivalent units in *Phragmites* marshes during the growing season.

1. Introduction

Characterizing net greenhouse gas (GHG) fluxes from coastal marshes is critical to understanding the role these marshes play in global climate. However, the high spatial and temporal variability of coastal marsh GHG fluxes complicates research efforts to quantify them and to understand their drivers. Fluxes of carbon dioxide (CO₂) and methane (CH₄) may vary with vegetation communities (Mozdzer and Megonigal 2013) and abiotic conditions (Bartlett et al 1987b; Poffenbarger et al 2011; Ma et al 2012) 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. Different plant communities influence GHG flux dynamics due to distinct physiological traits and associated impacts on soil biogeochemistry. Plants may “directly” affect GHG fluxes via rates of photosynthetic uptake of CO₂ (Taiz and Zeiger 2002) and direct gas transport between rhizosphere and atmosphere (Colmer 2003). “Indirect” plant affects on GHG fluxes may occur due to rhizosphere oxygenation (Maricle and Lee 2007), carbon (C) source provision (Lovell 2005), nutrient uptake (Windham and Meyerson 2003), and microbial community influences (Bodelier et al 2006) that can either enhance or reduce net CO₂ and CH₄ production.

Phragmites australis invasion dramatically alters vegetation community structure in coastal wetlands. As it makes its way into marshes from upland edges and along creek banks, the invasive grass *Phragmites australis* replaces native high marsh species (Silliman and Bertness 2004a) and has been shown to substantially increase marsh productivity (Windham 2001), alter soil chemistry (Windham and Lathrop

1999) and to support different soil microbial communities than native species (Ravit et al 2003). *Phragmites* invasion may affect marsh GHG flux dynamics via rhizosphere effects such as root zone oxygenation (Armstrong et al 1992; Colmer 2003) and locally lowered water tables (Windham and Lathrop 1999) that may promote CH₄ oxidation (Madigan 2012), or by provision of organic C exudates (Lovell 2005) that sustain methanogens or CO₂ producing communities. The plant 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 *Phragmites* biomass on CH₄ emission has been illustrated by positive correlation of CH₄ emission with *Phragmites* root mass, ramet density, and leaf area (Mozdzer and Megonigal 2013).

To determine how *Phragmites* invasion may affect GHG fluxes in coastal marshes, recent studies have compared fluxes from invasive *Phragmites* marsh zones to those vegetated with native species. Martin and Moseman-Valtierra (*in press*) measured GHG fluxes from *Phragmites* and native species (*Spartina patens* and *Distichlis spicata*) zones monthly during a growing season in three New England marshes and found greater CH₄ emission (by up to 3 orders of magnitude) but substantially greater CO₂ uptake (up to 30x greater) in the *Phragmites* zones. However, in another New England marsh, (Emery and Fulweiler 2014) found no difference in daytime GHG fluxes from *Phragmites* and the lower marsh zone dominated by *Spartina alterniflora* measured monthly over 1 year.

While these comparisons suggest potential for *Phragmites* invasion to affect marsh GHG budgets under some environmental conditions, experimental evidence is needed to confirm whether differences between vegetation-defined zones are driven by *Phragmites* invasion. Experimental manipulation of *Phragmites* plants could help begin to distinguish the role of *Phragmites* presence on GHG fluxes from that of pre-existing differences in soil conditions. Measuring GHG fluxes before and immediately after removing aboveground biomass would test direct effects (i.e., convective transport) on fluxes, while vegetation removal over longer durations could test for indirect effects, such as rhizosphere oxygenation and exudate provision, on GHG fluxes.

GHG fluxes vary over diel cycles. In combination with vegetation manipulations, measuring GHG fluxes over diel cycles may be used to test biotic and abiotic drivers of GHG fluxes. Light availability, air and soil temperatures, and relative humidity, as well as differences in plant-associated processes such as photosynthesis and root zone oxygenation (Brix 1988), vary on diel cycles. These differences affect biogeochemistry and microbial communities, as well as interactions between plants and microbes, which drive marsh GHG flux dynamics. In freshwater systems, *Phragmites* has been shown to mediate 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), (Van Der Nat et al 1998; Grünfeld and Brix 1999). Testing GHG flux patterns in cleared and vegetated plots across diel cycles could determine whether plant-mediated drivers also control

GHG fluxes in saline, coastal *Phragmites*-dominated systems, or whether drivers are primarily abiotic.

Studies of GHG flux measurements in coastal marshes typically restrict measurements to daytime. Relying exclusively on daytime fluxes could fail to account for potentially significant diel effects on marsh GHG dynamics. Although some studies simulate night conditions for GHG flux measurements using darkened chambers, this technique likely obscures day-night contrasts in GHG fluxes since rhizosphere connectivity allows continuation of *Phragmites*-mediated gas transport (Armstrong et al 1992; Colmer 2003). In support of this idea, a recent study demonstrated that in freshwater *Phragmites* stands, measurements with dark and light chambers showed similar CH₄ fluxes, despite previous investigations (Van Der Nat et al 1998; Grünfeld and Brix 1999) showing greater daytime than nighttime fluxes in *Phragmites* stands. Measurements of coastal marsh GHG fluxes over diel cycles will better inform estimates of GHG flux dynamics in *Phragmites*-invaded systems, and coupled with C sequestration rates, will allow for more accurate assessment of *Phragmites*'s potential to affect the role coastal marshes play in global climate.

The objectives of this investigation were to (1) test effects of mechanical aboveground *Phragmites* biomass removal on GHG fluxes and surface soil variables over durations of several minutes, one week, and 1-4 months (2) to test effects of diel period on GHG fluxes from cleared and vegetated areas in a *Phragmites* marsh. Soil, pore water and plant variables were also measured and tested for relationships to GHG fluxes across diel cycles. Aboveground biomass removal was hypothesized to reverse

CO₂ fluxes from photosynthetic uptake (negative fluxes) to emission (positive fluxes) at all durations of vegetation clearing. Aboveground biomass removal was hypothesized to result in a rapid decrease in CH₄ emission as plant-mediated transport (Armstrong et al 1992) was curtailed. At longer durations (months) following removal, a lack of root zone oxygenation (Colmer 2003) was expected to result in increased CH₄ emission relative to vegetated plots (Mitsch and Gosselink 2000), and as senescing root zone material provided a labile organic substrate (Madigan 2012). Emission of CH₄ and uptake of CO₂ in vegetated plots were expected to be greater during daylight hours due to convective transport and photosynthesis, respectively. In unvegetated plots, emission of CH₄ and CO₂ were expected to be greater during daylight hours, when warmer soil temperatures could stimulate more rapid microbial metabolic rates (Madigan 2012).

2. Methods

2.1 Study Site

Fox Hill Marsh is located on the west coast of Conanicut Island in the town of Jamestown, RI and 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). The approximately 0.34-acre invasive *Phragmites* stand (Figure 1) used for these investigations is positioned at the western edge of the marsh system and abuts an access road to a local RV campsite. Soil type in the stand consists of Ipswich Peat and Succotash Sand (Rector 1981), and loss on ignition analysis indicates that it contains approximately 27.13-46.80 % organic matter. Average *Phragmites* height at this site is approximately 2 m, and decreases to 0.5 m toward the seaward, *Spartina patens* dominated high marsh. Groundwater levels in the *Phragmites* stand (determined with water loggers during the 2015 growing season) are approximately 35-40 cm from the soil surface, and the stand rarely experiences tidal inundation, even during spring tides.

2.2 Testing effects of vegetation clearing on GHG fluxes

Effects of vegetation clearing on GHG fluxes and soil variables were determined monthly for up to 4 months in 3 cleared and 3 pairs of intact plots within the invasive *Phragmites* stand between May-August 2014. For GHG flux measurements, PVC bases to support static flux chambers were installed prior to the start of the growing season in the *Phragmites* stand. Three clusters of 3 flux chamber bases (clusters 9 m apart, bases within clusters 0.3 m apart) were established in April

(for a total of 9 bases) and used for monthly measurements. One base per cluster ($n=3$ total) was cleared of aboveground vegetation (by clipping at the soil surface) in April and maintained on a weekly basis for the duration of the experiment. Vegetation was allowed to persist in the other two bases per cluster, with one of these bases used for the short-term clipping experiment in August.

To test for instantaneous effects (within minutes) of vegetation removal on GHG fluxes, fluxes were measured before and immediately after clipping aboveground vegetation during August. During August, one vegetated base from each cluster was used to test for immediate effects of vegetation clearing on GHG fluxes. To capture potentially rapid GHG flux changes accompanying vegetation clipping, GHG flux measurements were performed 2 times on each of the bases in sequence: with vegetation intact and within several minutes of vegetation being clipped at the soil surface. A third measurement, with clipped stem bases plugged with petroleum jelly, was performed on each base to test for gas transport through stem bases that remained after clipping.

GHG flux comparisons for cleared and intact plots at all durations of vegetation removal were based on day-time measurements (detailed in section 2.4) that were conducted between 9:00 AM and 3:00 PM and within 3 h of low tide. Bases were left in place for the duration of the growing season to allow for observation of effects of season-long vegetation clearing. Drainage holes were positioned beneath the soil surface to avoid pooling of tidal or rainwater within bases.

Within each base, soil surface salinity, pH, moisture, temperature, and oxidation-reduction potential (redox) data were collected at the time of GHG flux measurements (at least monthly). For surface salinity measurements, soil was pressed against paper filters using small syringes to extract water, which was analyzed for salinity using a handheld refractometer. Soil surface pH was measured using a pH meter (ExStick[®] Instruments, Nashua, NH). Soil moisture content was measured using a volumetric water content sensor (Decagon Devices, Pullman, WA) inserted 5 cm into soil. Soil temperature was measured with a digital thermometer inserted into soil at a depth of 10 cm. Soil surface redox was measured using an ORP probe (Mettler Toledo, Greifensee, Switzerland).

2.3 Testing Effects of Diel Cycles on GHG fluxes under cleared and vegetated conditions

GHG flux (detailed in Section 2.4) and edaphic variable measurements were performed approximately every 3 hours over a series of 3 complete diel cycles (24-h periods) during June 2015 for a total of 23 sets of measurements. Three plots (A, B and C), approximately 3m by 3m, were chosen randomly within the same portion of the *Phragmites* stand used for the 2014 clearing experiment. Within each plot, paired (vegetation intact and cleared) flux chamber bases were installed (and vegetation was clipped at the soil surface) 1 week prior to measurements. Destructive sampling harvest for testing relationships of GHG fluxes to plant biomass necessitated moving bases after each measurement date; bases (both for intact and cleared vegetation) were

moved approximately 0.3 m within plots. Within each plot was installed a pore water sampling well (~60 cm deep) and a deployment well containing a Hobo® U20L-04 water level logger (Onset, Bourne, MA). Pore water sampling wells and water level loggers were left intact for the duration of the experiment.

Air temperature and relative humidity were recorded during each set of diel stage measurements with a Pocket Weather Meter (Kestrel Instruments) and soil temperature was measured with a digital thermometer inserted 10 cm into the soil. Air and soil temperature and humidity were averaged for each diel measurement set. Light intensity during the time of each GHG flux measurement was recorded using Hobo® Pendant loggers (Onset, Bourne, MA) and was averaged for the duration of chamber deployments. Surface soil redox was measured every other measurement set (for a total of 4 sets of measurements per sampling date). During June 2015, soil organic content was determined from 6 soil samples within the *Phragmites* stand (2 each from plots A, B and C).

On each of the 3 sampling dates, during each diel GHG flux measurement set, pore water from a depth of 60 cm was collected using wells constructed of 1.5” diameter PVC pipe cut to lengths of 60 cm based on observed *Phragmites* active root depths at the site. PVC wells were capped at the bottom with mesh screen and at the top with a vinyl cap fitted with ports to accommodate a handheld pump and to support a length of Tygon® tubing positioned with its bottom end just above the base of the well. 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 about 10 minutes water was collected for analysis using a syringe. Pore water (60 cm) was analyzed for redox potential, pH, salinity and sulfide concentration, and pore water from the 1st of the 3 sampling dates was analyzed for ammonium concentration.

On the 2nd and 3rd sampling dates, during every other measurement set, pore water was collected from each plot at a depth of 10 cm using Rhizon Soil Moisture Samplers (Ben Meadows, Janesville, WI). Pore water (10 cm) from the 2nd and 3rd measurement dates was analyzed for redox potential, pH, salinity and sulfide concentration.

Soil surface and pore water oxidation-reduction potential (ORP) was measured using an ORP probe (Mettler Toledo, Greifensee, Switzerland). Pore water from 10 cm and 60 cm was analyzed for salinity using a handheld refractometer. Pore water pH was measured using an OrionTM Star A326 Multiparameter Meter (ThermoScientific). Pore water (from 60 and 10 cm depths) for sulfide concentration analysis was preserved immediately using 1 M zinc acetate. Pore water was transported back to the lab on ice and frozen until analysis, and sulfide and ammonium concentrations were quantified using standard colorimetric techniques (Cline 1969; Solorzano 1969).

At the conclusion of GHG flux and edaphic sampling on each of the 3 measurement dates, stem density and average height were recorded and aboveground biomass was harvested from within all vegetated bases. Average stem height was determined from measurement of 10 randomly chosen stems per base. Biomass was

rinsed, dried in a vented oven for several days, separated into leaves and stems, and weighed.

2.4 GHG Flux Measurements

A cavity ring down spectroscopy (CRDS) analyzer (Picarro G2508) was used to measure CO₂, CH₄ and N₂O concentrations in real-time (as described in Martin and Moseman-Valtierra, *in press*). Gas concentration changes within a closed system comprised of the analyzer cavity, flux chamber and connective tubing were recorded at a frequency of approximately every second. Dry mole fractions for all gases (corrected by the analyzer software for water vapor concentrations) were used for all flux calculations.

The analyzer was connected using nylon tubing to a transparent polycarbonate chamber, which was placed into the previously-installed bases. A 2 m tall, 0.3 m diameter transparent polycarbonate tube (Rideout Plastics®) was capped with a shorter 0.02 m³ polycarbonate chamber which was sealed to the tube using a polyethylene closed-cell foam collar (for a total chamber volume of 0.15 m³). Chamber support bases were designed to create a gas-tight fit between base and chamber. Small fans (2 20-cm diameter within the chamber and 2 10-cm diameter within the cap) ensured air mixing during measurements. A stainless steel 55 cm long, 0.8 mm diameter pigtail was used for pressure equilibration, and Hobo® Pendant data loggers (Onset, Bourne, MA) were suspended within the chamber during all flux measurements to monitor air temperature and light intensity at 30 s intervals during flux measurements. Gas

measurements were conducted for 5-10 minutes per plot, based on observed periods for linear rates of change.

GHG fluxes were calculated using chamber size and footprint area. The Ideal Gas Law ($PV = nRT$) was used to calculate changes in gas concentrations over time using chamber air temperatures and atmospheric pressure. When slopes had an R^2 value of less than 0.85, data were not included in the analysis (1 instance each for CO_2 and CH_4 out of 24 measurements in the 2014 experiment, and 1 instance for CO_2 and 2 instances for CH_4 out of 138 measurements for the 2015 experiment). No detectable N_2O fluxes were observed (with a 30-second averaging period and minimal detection limit of approximately $1.4 \mu\text{mol m}^{-2} \text{hr}^{-1}$) (Brannon et al. *in prep*) for either experiment.

2.6 Statistical Analyses

2.6.1 Testing immediate to season-long effects of vegetation clearing on GHG fluxes and soil conditions (2014)

GHG flux and edaphic variable data points from the 2 intact vegetation bases per cluster were averaged, and that value was used for analysis. To test for effects of vegetation presence and duration of vegetation removal on GHG fluxes and soil variables, 2-factor ANOVAs (vegetation clearing x measurement month) were performed. CH_4 data were log-transformed prior to ANOVAs due to non-normality. Tukey HSD tests were used for post-hoc analysis when ANOVA tests indicated

significant differences between months or an interaction of month and vegetation clearing.

For the 2014 clipping experiment, GHG fluxes from immediately before and after clipping aboveground biomass and after plugging clipped stems were compared using a nonparametric Friedman rank sum test due to non-normality and heteroscedasticity.

2.6.2 Testing Effects of Diel Cycles on Edaphic Conditions and Diel Cycles and Vegetation Removal on GHG fluxes (2015)

For the 2015 experiment, 8 diel stages of approximately 3 hrs each spanning a 24-hr period were defined (Table 2) for the 3 dates in the study. Kruskal-Wallis nonparametric tests were used, due to non-normality of data, to compare light intensity, soil and air temperature, and relative humidity between these 8 diel stages. Post-hoc comparisons of means were performed where appropriate using the R package *pgirmess* for Kruskal-Wallis tests (Giraudoux, 2014).

To test effects of diel stage and vegetation presence on GHG fluxes, linear mixed effects analysis of the relationship between vegetation presence and/or diel stage and response variables was performed. Vegetation presence and diel stage (1-8) were treated as fixed effects, and plot and measurement date were treated as random effects. An interaction of plot and measurement date was specified to account for the repeated measures nature of the experimental design. Residual plots were used to confirm that assumptions of homoscedasticity and normality were met. To obtain p-

values to assess significance of the effect of vegetation presence and diel stage on response variables, likelihood ratio tests of full models against models with the fixed vegetation or diel stage effect removed were performed. To test for interactive effects of vegetation presence and diel stage on GHG fluxes, models with and without an interaction term were compared using likelihood ratio tests. Water level loggers did not indicate any tidal effect on groundwater level, and so tidal stage was not included as an effect in the analysis.

Due to non-normality of data, Spearman's Correlation Analysis was used to test for relationships between edaphic variables (separately from vegetated and cleared plots, across the entire diel cycle) and vegetation variables (from vegetated plots, for each diel stage) and CO₂ and CH₄ fluxes. Correlations between vegetation variables and GHG fluxes were tested for each diel period since, due to the nature of the experimental design, vegetation data was collected once per measurement date.

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

3. Results

3.1 Effects of 1-4 month vegetation clearing on GHG fluxes

During the 2014 vegetation clearing experiment, some soil variables differed between months, but none were affected by vegetation clearing (Table 1). Soil temperature was significantly lower during June than in July or August. Soil salinity was lower during May and highest during June, with July and August salinities intermediate. Soil moisture was greater in July than in May. There was an interactive effect of month and vegetation presence for soil moisture (Table 1), which indicated significantly moister soil in July in vegetated and cleared bases than in cleared bases in May. There was a trend of lower pH during July than May or June ($p=0.05$), but Tukey HSD tests did not indicate significant differences between monthly means.

CO₂ was consistently emitted within cleared bases (with fluxes ranging from 0 to 10.94 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and taken up within bases containing intact vegetation (with fluxes ranging from 0 to -37 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (Figure 2A). CO₂ fluxes were significantly affected by vegetation clearing ($F_{1,20}=24.54$, $p<0.005$). CO₂ 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 ($F_{3,20}=3.34$, $p=0.04$) that indicated a significant difference in fluxes from cleared and vegetated plots only during the month of June (Figure 2A).

CH₄ fluxes were variable and ranged from 4-537 $\mu\text{mol m}^{-2} \text{h}^{-1}$ in vegetated bases and 15-435 $\mu\text{mol m}^{-2} \text{h}^{-1}$ in unvegetated bases (Figure 2B). During August, fluxes of nearly 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (during one pair of pre- and post-clipping measurements) were measured from one vegetated base. CH₄ fluxes differed between

months, with significantly smaller emissions during May than any other month ($F_{3,20}=5.67$, $p=0.006$). However, CH_4 fluxes were not affected by vegetation clearing ($F_{1,20}=0.07$, $p=0.80$), and there was no interactive effect of month and vegetation clearing ($F_{3,20}=0.36$, $p=0.78$).

3.2 Immediate effects of vegetation removal (clipping) and stem base plugging on GHG fluxes

Clipping aboveground vegetation resulted in immediate reversal of CO_2 fluxes from uptake ($-15.00 \pm 3.90 \mu\text{mol m}^{-2} \text{s}^{-1}$) to emission ($5.84 \pm 1.35 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Figure 3A). Plugging stem bases did not affect CO_2 fluxes (relative to clipping alone) ($X^2_2=6$, $p=0.05$). However, neither clipping nor plugging stems significantly affected CH_4 fluxes (Figure 3B) ($X^2_2=0.67$, $p=0.72$).

3.3 Effect of diel cycle stage on edaphic conditions

As expected, diel stages were characterized by differences in environmental variables (Table 2). Light intensity was strongest during midday and weaker during earlier and later daylight hours. Air temperature was significantly higher during the day than at night by about 18°C and relative humidity displayed the opposite pattern with highest humidity recorded at night. Soil temperature was warmer late in the day and coolest at night and in the morning by about 2°C . Pore water salinity at 10 cm was significantly higher at night than in the morning by about 5 psu. Pore water redox potential at 60 cm was significantly greater during morning than later diel stages. Pore

water sulfide concentrations at 60 cm were generally higher at night than in the morning. No other pore water variables or surface soil redox potential varied significantly with diel stage, and groundwater levels remained approximately 35-40 cm from the soil surface in all 3 plots.

3.4 Effect of diel stage on GHG fluxes from cleared and vegetated areas

CO₂ fluxes varied with diel stage ($X^2_{7}=44.52$, $p<0.005$) and vegetation presence ($X^2_{1}=45.93$, $p<0.005$) (Figure 4A). Fluxes at night (when emission occurred in the absence of photosynthesis) were significantly different than daytime fluxes (when photosynthetic uptake occurred). Vegetation removal resulted in an absence of daytime CO₂ uptake relative to intact vegetation. Vegetation removal significantly affected CO₂ fluxes, with greater uptake from vegetated bases (where fluxes ranged from -37.17 to 11.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$) than unvegetated bases (where fluxes ranged from 0.84 to 10.61 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Although not found to be significant with post-hoc pairwise comparisons, vegetated bases at night (between 20:00 and 5:00) displayed a trend of greater CO₂ emission (at least 1.5 times as much) as unvegetated bases (Figure 4A). There was an interactive effect of vegetation presence and diel stage on CO₂ fluxes ($X^2_{7}=179.00$, $p<0.005$), with significantly greater uptake occurring in vegetated bases during daylight hours than from cleared or vegetated bases at night.

CH₄ fluxes varied with diel stage ($X^2_{7}=48.49$, $p<0.005$) but not with vegetation presence ($X^2_{7}=2.94$, $p<0.09$). There was no interactive effect of vegetation presence and diel stage ($X^2_{7}=1.33$, $p=0.98$) on CH₄ fluxes. CH₄ fluxes were variable and

ranged from 0.55-266.75 $\mu\text{mol m}^{-2} \text{h}^{-1}$ in vegetated bases and from 3.04 to 527.84 $\mu\text{mol m}^{-2} \text{h}^{-1}$ in cleared bases. Daytime CH_4 emissions were twice as large as night emissions (Figure 4B). A single very large ebullative CH_4 flux was measured (2,851.77 $\mu\text{mol m}^{-2} \text{h}^{-1}$) during the second visit from a cleared base in 1 plot (out of 3 total) at night, and was excluded from analysis (and figures) due to its outlier status.

3.5 Relationships between edaphic variables and GHG fluxes across diel cycles

CO_2 fluxes from bases with vegetation cleared were positively correlated with 10 cm pore water pH ($P=0.57$, $p=0.006$).

CH_4 fluxes from vegetated bases were positively correlated with 60 cm pore water pH ($P=0.65$, $p<0.005$) and negatively correlated with 10 cm and 60 cm pore water salinity ($P=-0.64$, $p=0.002$, $P=-0.50$, $p<0.005$). CH_4 fluxes from bases with vegetation cleared were negatively correlated with 10 cm pore water redox ($P=-0.63$, $p=0.004$).

3.6 Relationships between vegetation characteristics and GHG fluxes at each diel stage

Relationships of *Phragmites* biomass and density characteristics (summarized in Table 4) to CO_2 and CH_4 fluxes varied by diel stage.

CO_2 fluxes were negatively correlated with stem biomass during diel stages 8:00-11:00 ($P=-0.93$, $p<0.005$), 11:00-14:00 ($P=-0.88$, $p=0.007$), 14:00-17:00 ($P=-0.77$, $p=0.02$) and 17:00-20:00 ($P=-0.82$, $p=0.01$) and positively correlated with stem

biomass during night diel stages 20:00-23:00 ($P=0.66$, $p=0.02$) and 3:00-5:00 ($P=0.73$, $p=0.03$). CO_2 fluxes were negatively correlated with leaf mass during diel stage 8:00-11:00 (8:00-11:00) ($P=-0.68$, $p=0.05$) and positively correlated with leaf mass during diel stage 3:00-5:00 ($P=0.78$, $p=0.02$). CO_2 fluxes were negatively correlated with average stem height during diel stage 17:00-20:00 ($P=-0.77$, $p=0.02$) and positively correlated with average stem height during diel stages 20:00-23:00 ($P=0.87$, $p=0.005$), 6 ($P=0.75$, $p=0.03$) and 3:00-5:00 ($P=0.90$, $p=0.002$).

CH_4 fluxes were positively correlated with live stem density during diel stages 3:00-5:00 ($P=0.69$, $p=0.04$) and 5:00-8:00 ($P=0.94$, $p=0.005$) and negatively correlated with average stem height during diel stage 5:00-8:00 ($P=-0.94$, $p=0.02$).

4. Discussion

4.1 Plant-driven controls on CO₂ fluxes

Consistent reversal from CO₂ uptake to emission with aboveground *Phragmites* removal was observed across the range of instantaneous (clipped aboveground biomass), week-long (for the diel experiments), and season-long time scales. In cleared (clipped) plots, CO₂ emissions measured June-August from all experiments were remarkably consistent (averaging about 4-6 $\mu\text{mol m}^{-2} \text{s}^{-1}$) despite differences in *Phragmites* removal durations. These results suggest that CO₂ flux dynamics are primarily driven by magnitude of photosynthetic uptake and plant-mediated gas transport rather than by longer-term impacts of *Phragmites* on soil conditions or microbiota. Photosynthetic uptake as the primary driver of CO₂ flux dynamics in this study is further underscored by strong correlations between CO₂ uptake and *Phragmites* stem and leaf biomass during daylight hours (Section 3.6) and minimal correlation with soil variables.

The notable period of minimal CO₂ uptake and emission from vegetated bases (May) reflects minimal photosynthetic activity by *Phragmites* plants of (< 0.3 m tall) in the early growing season. Cleared plots produced similar, small emissions (Figure 2), and so smaller fluxes measured after 1 month of *Phragmites* clearing (relative to fluxes later in the growing season) likely reflect seasonal abiotic or microbial drivers rather than effects of vegetation removal duration.

While CO₂ fluxes from vegetated plots clearly were driven by magnitude of photosynthetic uptake, evening emissions and emissions from cleared plots may have been driven by soil microbial and/or plant root respiration. Aboveground vegetation

removal in cleared plots did not completely destroy belowground structures, even over the course of months, as indicated by the need for biweekly clipping of a few stems per plot. Diel experiments indicate a trend of slightly greater CO₂ emission from vegetated relative to cleared (for 1 week) plots at night (Figure 4), likely due to respirative emission of CO₂ from aboveground structures (Taiz and Zeiger 2002). As nighttime CO₂ emissions from vegetated plots were only about 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ greater than those from cleared plots, it can be concluded that the bulk of nighttime CO₂ emission is due to soil microbial or belowground plant structure respiration.

Global changes, such as changes to vegetation communities as a result of exotic species invasions, could affect net marsh GHG fluxes (Ehrenfeld 2003; Lovell 2005), either by enhancing net GHG uptake or exacerbating emissions. Since results of this investigation with invasive *Phragmites* indicate that CO₂ fluxes are driven primarily by vascular plant photosynthetic activities, it may be hypothesized that plant community shifts that alter aboveground biomass will affect CO₂ uptake magnitude. Contrasts thus far of GHG fluxes from native marsh grass and *Phragmites* zones in coastal marshes indicate that differences can be substantial (Martin and Moseman-Valtierra *in press*), but mechanisms driving GHG fluxes may also differ due to species-specific productivity, photosynthetic and gas conductance rates between *Phragmites* and shorter statured native high marsh grasses. Therefore, future investigations that manipulate native marsh vegetation to contrast controls on GHG drivers with those of *Phragmites* would improve mechanistic understanding of how *Phragmites* invasion

4.2 Vegetation manipulations and diel cycle experiments suggest primarily abiotic CH₄ flux drivers

In contrast to the CO₂ fluxes, results of this study suggest that direct plant-mediated processes are not the primary drivers of CH₄ fluxes. CH₄ fluxes did not differ significantly between intact and cleared plots after any duration of vegetation clearing, displayed similar patterns over diel cycles, and clipped stems were demonstrated not to conduct gases, indicating a lack of direct (i.e., convective gas transport) vegetation effects. These findings are in agreement with those of another study that compared GHG emissions from vegetated and unvegetated (vegetation naturally absent) plots in a *Phragmites*-invaded coastal marsh (Emery and Fulweiler 2014); the authors found no difference in CH₄ emissions or biogeochemical conditions between the 2 plot types.

The observed diel pattern of greater daytime than nighttime CH₄ emissions from *Phragmites* marshes is well supported in the literature, and has been attributed to mainly plant-mediated transport, but also to higher daytime soil temperature. Experiments conducted in freshwater mesocosms (Grünfeld and Brix 1999), freshwater wetlands (Van Der Nat et al 1998; Brix et al 2001), 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 greater (by about a factor of about 6) daytime emissions. These freshwater studies attribute the observed diel pattern (from vegetated marshes) to

Phragmites convective gas transport (Brix et al., 2001) and (for unvegetated areas) to increased ebullition of CH₄ under conditions of higher daytime temperatures (Van Der Nat et al., 1998). In a brackish marsh, Tong et al (2013) measured GHG fluxes over diurnal cycles from intertidal portions of a *Phragmites* marsh in China (where the plant is native). They also report greater CH₄ emission by a factor of 4 during the day than at night, and attributed these findings to either soil temperature (which was found to be correlated with CH₄ flux magnitude) or to known mechanisms of *Phragmites* gas transport.

Observed diel CH₄ emission patterns in this experiment do not appear to agree with previous studies' findings that *Phragmites*-mediated transport is the primary driver of diel CH₄ flux patterns, given the lack of significant difference between vegetated and cleared *Phragmites* plots. Rather, the diel pattern observed in this study could be attributed mainly to abiotic drivers. Of the abiotic conditions that varied on diel cycles (Table 1), soil temperature seems the most probable driver of CH₄ flux diel patterns. Soil temperature, which was generally greater during the daytime (Table 1), drives rates of methanogenesis (Madigan 2012), which would increase daytime CH₄ emissions when soil was warmer.

Differences between findings of aforementioned freshwater studies in which CH₄ emissions in vegetated plots in *Phragmites* stands are primarily driven by plant-mediated transport and this experiment may be due to physiological constraints affecting *Phragmites* in saline coastal systems. *Phragmites*, while sufficiently salt-tolerant to invade coastal wetlands, does not grow optimally under saline conditions

(Lissner and Schierup 1997). High pore water sulfide levels in seawater-influenced relative to freshwater wetlands represent another *Phragmites* stressor (Chambers et al 1998). Therefore, *Phragmites* convective processes that drive gas transport may be more pronounced in freshwater wetlands, while abiotic conditions are main drivers under saline conditions.

4.3 The need to consider diel variation driving CO_2 and CH_4 fluxes

Accurate estimates of net marsh GHG fluxes must account for diel variability. The limited daytime window into coastal marsh GHG flux dynamics presented by most studies, by excluding nighttime fluxes, may bias estimates of net system GHG dynamics. Using daytime GHG flux measurements, *Phragmites* marsh zones have been shown to emit more CH_4 but take up more CO_2 relative to native high marsh vegetation zones, making them larger daytime GHG sinks (Martin and Moseman-Valtierra, *in press*). Based on results of this experiment, reliance on daytime measurements only results in overestimation of both CH_4 emission (which is smaller at night) and CO_2 uptake (which reverses to emission). If daytime only fluxes from vegetated bases in this experiment were used to calculate net GHG emissions (in CO_2 equivalent units, using a global warming potential factor of 21 for CH_4), estimated net uptake is substantially greater ($-2,570 \text{ mg } CO_{2 \text{ eq.}} \text{ m}^{-1} \text{ h}^{-1}$) than if a weighted average of fluxes from all diel stages ($-531 \text{ mg } CO_{2 \text{ eq.}} \text{ m}^{-1} \text{ h}^{-1}$) is used. Notably, the full diel cycle weighted average net GHG fluxes from *Phragmites* during the diel experiments still indicates that the zone is a net GHG sink. However, diel cycle GHG flux

measurements from the native vegetation zones would help to more accurately determine the extent to which *Phragmites* invasion affects net marsh GHG exchange.

4.4 Conclusions

In conclusion, results of this study indicate that *Phragmites* coastal marsh CO₂ and CH₄ fluxes have distinct drivers and patterns of variability over diel cycles that may differ from those in freshwater systems. CO₂ flux variability was driven primarily by plant photosynthetic effects rather than abiotic or microbial conditions that affect soil respiration. CH₄ diel patterns, in contrast, appear mainly due to abiotic conditions that vary on diel cycles, and potentially may be attenuated in vegetated plots by the indirect plant effect of root zone oxygenation. Results also have implications for understanding net GHG balance of a system, as they demonstrate that GHG fluxes vary significantly on diel cycles, and so daytime-only measurements overestimate both CO₂ uptake and CH₄ emission. Future studies of diel variation and mechanisms by which different plant communities affect GHG fluxes will improve predictions of how changing vegetation communities, such as invasion of native high marsh vegetation by *Phragmites*, could affect coastal marsh GHG budgets.

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Figure 1. The *Phragmites* stand at Fox Hill salt marsh in Jamestown, RI used for experiments presented in this paper. The extent of the stand is indicated in yellow outline.

Table 1. Average monthly edaphic variables from intact and cleared plots and results of ANOVA tests

	Average Monthly Edaphic Variables $\pm$ se		Results of ANOVA and Tukey Tests			
	Intact	Cleared	Month	Vegetation Clearing	Month x Veg. Clearing	Tukey HSD Test Results
<i>Soil redox (mV)</i>	May: -- June: -79.00 $\pm$ 68.63 July: -154.17 $\pm$ 83.95 August: -260.33 $\pm$ 27.46	May: -- June: -214.33 $\pm$ 80.58 July: -132.67 $\pm$ 81.28 August: -268.00 $\pm$ 72.34	$F_{2,16}=0.82$, p=0.45	$F_{1,16}=1.48$, p=0.24	$F_{2,16}=3.25$, p=0.07	--
<i>Soil pH</i>	May: 7.00 $\pm$ 0.13 June: 7.20 $\pm$ 0.09 July: 6.70 $\pm$ 0.27 August: --	May: 7.15 $\pm$ 0.13 June: 7.21 $\pm$ 0.11 July: 6.62 $\pm$ 0.32 August: --	$F_{2,16}=3.62$, p=0.05*	$F_{1,16}=0.00$, p=0.99	$F_{2,16}=0.22$, p=0.81	--
<i>Soil moisture (%)</i>	May: 52.71 $\pm$ 1.83 June: 52.86 $\pm$ 2.41 July: 60.56 $\pm$ 1.00 August: 64.09 $\pm$ 0.26	May: 50.79 $\pm$ 2.11 June: 58.93 $\pm$ 0.38 July: 58.88 $\pm$ 1.74 August: 63.63 $\pm$ 0.83	$F_{2,16}=11.35$, p<0.005*	$F_{1,16}=0.01$, p=0.95	$F_{2,16}=3.71$, p=0.05*	May-Cleared ^c , May-Intact ^{bc} , June-Cleared ^{abc} , June-Intact ^{bc} , July-Cleared ^{ab} , July-Intact ^a
<i>Soil temp. (°C)</i>	May: -- June: 19.18 $\pm$ 0.2 July: 21.08 $\pm$ 0.37 August: 20.62 $\pm$ 0.17	May: -- June: 18.97 $\pm$ 0.19 July: 21.37 $\pm$ 0.26 August: 20.67 $\pm$ 0.67	$F_{2,16}=19.26$, p<0.005*	$F_{1,16}=0.05$, p=0.83	$F_{2,16}=0.15$, p=0.86	June ^b , July ^a , Aug. ^a
<i>Soil surface salinity (psu)</i>	May: 4.50 $\pm$ 0.5 June: 33.0 $\pm$ 3.70 July: 18.00 $\pm$ 2.24 August: 24.33 $\pm$ 2.03	May: 4.67 $\pm$ 0.33 June: 28.33 $\pm$ 6.01 July: 17.33 $\pm$ 4.48 August: 22.33 $\pm$ 3.93	$F_{3,16}=17.0$, p<0.005*	$F_{1,16}=0.48$, p=0.50	$F_{3,16}=0.15$, p=0.92	May ^c , June ^a , July ^b , Aug. ^{ab}

F statistics, degrees of freedom, and p values are shown for 2-factor (Month x Vegetation presence) ANOVAs.

Tukey HSD test results are shown for Month or Month x Vegetation interaction. Items not connected by the same letters are significantly different.

* Significant at $\alpha = 0.05$

Figure 2. Average CO₂ (A) and CH₄ (B) fluxes for each month for intact and cleared plots during 2014. 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.

Figure 3. Average CO₂ (A) and CH₄ (B) fluxes from intact and clipped vegetation during August 2014. Standard error bars are shown.

Table 2. 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 post- hoc comparison
<i>Soil Temperature (°C)</i>	18.52 $\pm$ 0.27	19.34 $\pm$ 0.23	21.74 $\pm$ 0.44	21.44 $\pm$ 0.28	20.32 $\pm$ 0.29	19.74 $\pm$ 0.28	19.22 $\pm$ 0.26	18.46 $\pm$ 0.14	X ² ₇ =71.16, p<0.005*	Diel Stage: 1 ^d , 2 ^{cd} , 3 ^{ab} , 4 ^a , 5 ^{abc} , 6 ^{bcd} , 7 ^{cd} , 8 ^d
<i>Air Temperature (°C)</i>	77.81 $\pm$ 1.10	82.79 $\pm$ 0.70	85.56 $\pm$ 0.27	71.99 $\pm$ 0.50	69.14 $\pm$ 0.47	65.67 $\pm$ 0.81	66.20 $\pm$ 0.77	64.75 $\pm$ 0.77	X ² ₇ =116.58, p<0.005*	Diel Stage: 1 ^{ab} , 2 ^a , 3 ^a , 4 ^b , 5 ^{bc} , 6 ^c , 7 ^c , 8 ^c
<i>Relative Humidity (%)</i>	60.51 $\pm$ 0.22	55.49 $\pm$ 0.74	57.55 $\pm$ 1.04	62.81 $\pm$ 0.96	75.98 $\pm$ 1.5	85.13 $\pm$ 1.79	90.63 $\pm$ 1.17	89.4 $\pm$ 0.28	X ² ₇ =116.58, p<0.005*	Diel Stage: 1 ^{bc} , 2 ^b , 3 ^b , 4 ^{bc} , 5 ^a , 6 ^a , 7 ^a , 8 ^{ab}
<i>Light Intensity (lum/ft)</i>	3,237 $\pm$ 298.14	4,250 $\pm$ 378.32	4,188.72 $\pm$ 393.02	1,243.79 $\pm$ 235.07	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.29 $\pm$ 0.29	484.27 $\pm$ 123.57	X ² ₇ =124.76, p<0.005*	Diel Stage: 1 ^{ab} , 2 ^a , 3 ^a , 4 ^{ab} , 5 ^c , 6 ^c , 7 ^c , 8 ^{ab}

X² statistics, degrees of freedom, and significance values are reported for Kruskal-Wallis tests

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 $\alpha = 0.05$

Table 3. Surface soil and pore water variables $\pm$ standard error for the each diel stage averaged across measurement dates

Diel Stage:		1	2	3	4	5	6	7	8
10 cm depth	Redox (mV)	256.5 ± 32.18	--	214.5 ± 22.72	--	243.00 ± 13.36	287.00 ± 60.85	204.5 ± 19.09	--
	pH	6.73 ± 0.18	--	6.72 ± 0.09	--	6.13 ± 0.08	6.23 ± 0.53	6.50 ± 0.03	--
	Salinity (psu)	16.67 ± 2.17	--	18.83 ± 2.12	--	22.67 ± 1.87	18.33 ± 2.12	22.80 ± 2.11	--
	Sulfide (uM)	0.00 ± 0.00	--	0.00 ± 0.00	--	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	--
60 cm depth	Redox (mV)	140.67 ± 26.46	42.22 ± 17.09	67.11 ± 13.75	61.22 ± 11.70	45.11 ± 14.49	36.11 ± 16.49	27.88 ± 10.07	47.17 ± 18.33
	pH	6.72 ± 0.08	6.62 ± 0.08	6.67 ± 0.07	6.81 ± 0.06	6.69 ± 0.08	6.67 ± 0.07	6.69 ± 0.07	6.60 ± 0.08
	Salinity (psu)	9.5 ± 1.32	10.11 ± 1.76	10.56 ± 1.61	9.78 ± 1.13	11.11 ± 1.50	12.56 ± 1.27	10.76 ± 1.40	11.17 ± 1.78
	Sulfide concentration (uM)	22.57 ± 6.25	52.24 ± 11.42	51.47 ± 18.52	86.02 ± 23.73	62.25 ± 16.92	96.87 ± 24.84	95.30 ± 25.57	87.61 ± 22.54
	Ammonium concentration (mM)	7.31 ± 4.51	8.39 ± 1.93	6.90 ± 4.52	11.04 ± 3.05	10.28 ± 4.72	10.72 ± 1.95	10.35 ± 4.64	16.74 ± 6.07
Surface soil	Redox (mV)	267.42 ± 38.92	199.17 ± 43.72	285.75 ± 47.64	197.67 ± 28.37	270.00 ± 41.52	245.00 ± 35.28	281.18 ± 55.26	--

Table 4. Results of likelihood ratio and Tukey HSD tests for effects of diel stage on pore water and soil variables at soil surface and 10 cm and 60 cm depths

		Results of likelihood ratio tests	Results of Tukey HSD tests
<i>10 cm depth</i>	Redox (mV)	$X^2_4=3.66$, $p=0.45$	--
	pH	$X^2_4=6.74$, $p=0.15$	--
	Salinity (psu)	$X^2_2=12.07$, $p=0.02^*$	Diel Stage: 1 ^b , 3 ^{ab} , 5 ^{ab} , 6 ^a , 7 ^{ab} ,
<i>60 cm depth</i>	Redox (mV)	$X^2_7=22.24$, $p=0.002^*$	Diel Stage: 1 ^a , 2 ^b , 3 ^{ab} , 4 ^a , 5 ^b , 6 ^b , 7 ^b , 8 ^{ab}
	pH	$X^2_7=11.51$, $p=0.12$	--
	Salinity (psu)	$X^2_7=7.86$, $p=0.34$	--
	Sulfide concentration (uM)	$X^2_1=14.86$, $p<0.005$	Diel Stage: 1 ^b , 2 ^{ab} , 3 ^{ab} , 4 ^a , 5 ^{ab} , 6 ^a , 7 ^a , 8 ^a
	Ammonium concentration (mM)	$X^2_7=4.21$, $p=0.76$	--
<i>Surface soil</i>	Redox (mV)	$X^2_6=4.42$, $p=0.62$	--

X^2 statistics, degrees of freedom, and p values are shown for likelihood ratio tests comparing null and full models for effects of diel stage on soil and GHG flux variables.

In Tukey comparison column, diel stages not connected by the same letter are significantly different. Letters a and b represent higher and lower values, respectively.

* Significant at $\alpha = 0.05$

Table 5. *Phragmites* 2015 vegetation characteristics averaged over 3 measurement dates

<i>Average Height (cm)</i>	107.80 ± 5.71
<i>Stem density (stems m⁻²)</i>	91.47 ± 6.77
<i>Biomass (leaves) (g m⁻²)</i>	177.21 ± 40.88
<i>Biomass (stems) (g m⁻²)</i>	244.85 ± 60.88

Figure 4. CO₂ (A) and CH₄ (B) fluxes averaged over 3 measurement dates for vegetated and cleared plots at 8 diel stages (1-4 daytime, 5-8 nighttime). 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.

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CHAPTER 4

PHRAGMITES AUSTRALIS REMOVAL: BRACKISH MARSH RESTORATION TESTS INVASIVE PLANT EFFECTS ON GREENHOUSE GAS FLUXES

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Abstract

Coastal marsh restoration techniques, including hydrologic reconnection and invasive species control, aim to improve function of marshes impacted by anthropogenic activities. While much research has tested effects of restoration on species assemblages and habitat value, less is known about impacts to coastal marsh biogeochemistry. Since coastal marshes are valued for their ability to sequester greenhouse gases (GHGs), it is important to understand the effect of restoration on these services. The objective of this research was to test effects of *Phragmites australis* aboveground biomass -employed as part of a larger tidal reconnection project-on carbon dioxide (CO₂) and methane (CH₄) fluxes from a brackish New England coastal marsh. First, GHG fluxes were compared over the course of a growing season between a *Phragmites* stand cleared mechanically (but being recolonized within months of initial removal) and an uncleared stand in the same marsh system. CO₂ uptake increased dramatically in the cleared stand as *Phragmites* regrew, but CH₄ emissions stayed constant and were small relative to those in the uncleared stand, consistent with increased drainage in the cleared stand. The following year, to test mechanisms of *Phragmites* impact on GHG fluxes, GHG fluxes were compared between intact *Phragmites* plots, cleared *Phragmites* plots with litter, and cleared *Phragmites* plots without litter. *Phragmites* clearing (independent of litter removal) resulted in increased CO₂ and CH₄ emissions. Results suggest that *Phragmites* removal, in the absence of native vegetation recolonization, may diminish

GHG sequestration of coastal marshes in the short term, and that longer-term impacts warrant investigation.

1. Introduction

Coastal marshes have been subject to human impacts since at least the Middle Ages (Gedan et al. 2009). Marshes were diked and drained to accommodate agriculture and development, used as pasture for livestock grazing, and ditched to control biting insect populations. Today, the value of coastal wetlands for shoreline protection (Costanza et al. 2008), nutrient pollution interception (Valiela and Cole 2002) and carbon (C) sequestration (Chmura et al. 2003; Mcleod et al. 2011) is understood by scientists and managers, and legislation protects remaining marshes from direct damage. However, vestiges of past exploitation remain in the form of impoundments, diking and drainage ditches that alter tidal flow (Roman and Burdick 2012). In addition, marshes are increasingly affected by anthropogenic impacts including nutrient over-enrichment (Turner et al. 2009; Deegan et al. 2012), climate change (IPCC 2007) and associated sea level rise (Donnelly and Bertness 2001; Craft et al. 2009), and the invasion of non-native species (Chambers et al. 2003; Silliman and Bertness 2004; Meyerson et al. 2009). The historical and present-day stressors which impact coastal marshes may greatly influence ecosystem function and provision of important ecosystem services.

Coastal marshes play an important role in global C cycling. Due to their high productivity (Mcleod et al. 2011), low decomposition rates (Mitsch and Gosselink 2000), and minimal emission of greenhouse gases (GHGs), they store C at rates greater even than terrestrial forests (Mcleod et al. 2011). Carbon dioxide (CO₂) uptake is substantial during the growing season due to plant productivity and

photosynthetic uptake. Emissions of methane (CH₄), a GHG with 21 times the radiative forcing potential of CO₂ over a 100 year basis (Forster et al., 2007), are minimal due to the known control of seawater presence on microbial processes that drive CH₄ production (Poffenbarger et al. 2011). Improved understanding of this ecosystem service has prompted assessment of the merits of making coastal marsh restoration activities eligible for credits in C markets (Emmett-Mattox et al. 2010). Within such a framework, restoration or conservation of coastal wetlands could be used to offset C credits. As climate change progresses and efforts to align monetary and environmental protection goals continue, improved understanding of the response of coastal wetland C cycling to restoration activities could ensure increased funding for coastal conservation work.

With the aim of returning degraded coastal marshes to a state of functionality (or pre-degradation conditions), many marsh ecosystem restoration projects employ techniques for tidal reconnection. Tidal flow restriction can alter coastal marsh C cycling through impacts to both abiotic and biotic marsh function. Disruption to natural tidal flow can result less frequent flushing with seawater and/or poor drainage that allows freshwater to collect on the marsh surface. These phenomena have implications for biogeochemistry, with tidally restricted marshes often having lower salinity and less sulfidic and reduced soil than unimpacted marshes (Anisfeld 2012), which can lead to increased emission of methane (CH₄).

Another impact of lowered soil salinity and sulfide concentrations associated with restricted tidal flow is invasion of *Phragmites australis* (Chambers et al. 2012),

since the plant is more sensitive than native species to high soil salinity and sulfide concentrations but may establish itself under conditions of minimal seawater influence (Bart and Hartman 2003). As a result, tidally restricted marshes often become dominated by *Phragmites* as it outcompetes native species, and are transformed into potentially highly productive (Windham 2001) but low-diversity (Silliman and Bertness 2004) systems.

Independently or together with changes in tidal flow, *Phragmites* invasion may affect marsh GHG fluxes. *Phragmites* may alter root zone conditions in ways that enhance (provision of C substrates) or inhibit (oxygenation of rhizosphere) methanogenesis (Colmer 2003; Lovell 2005; Armstrong et al. 2006). Since *Phragmites* is highly productive (Windham 2001) and may take up more CO₂ relative to native vegetation (Martin and Moseman-Valtierra, 2015), a reversal from CO₂ uptake to emission in the short term, and potentially decreases in CO₂ uptake in the longer term, will result. Due to its large internal ventilation system, the plant is known to conduct products of root zone microbial metabolism (CO₂, CH₄) into the atmosphere via humidity- and Venturi-induced convection (Armstrong et al. 1996; Colmer 2003). Therefore, *Phragmites* removal (especially in conjunction with tidal flow restoration) is likely to reduce CH₄ emission (Burden et al. 2013; Drexler et al. 2013).

A variety of control measures are used to reduce *Phragmites* cover as part of restoration, and some may affect coastal marsh GHG fluxes. In some instances, restoration of tidal flow is used to alter soil conditions to be unfavorable for

Phragmites and therefore shift species assemblages to native marsh perennials (Chambers et al. 2012). Tidal flow restoration is likely to have the secondary effect of decreasing CH₄ emissions from wetlands, but may substantially diminish CO₂ uptake if *Phragmites* cover is reduced and native vegetation fails to recolonize. Methods (sometimes used in combination with tidal flow restoration) for *Phragmites* removal commonly include herbicide application and less effective mechanical methods such as mowing, which actually stimulates an increase in *Phragmites* shoot density (Hazelton et al. 2014), a phenomenon which could ultimately result in intensified plant-mediated impacts to GHG fluxes. *Phragmites* cutting results in the presence of abundant litter on the marsh surface. Detrital leaf litter plays an important role in coastal marsh food webs as a substrate for decomposers (Teal 1962). Along with edaphic conditions including soil moisture, pH, and redox potential, organic C availability is a control on soil microbial processes that drive CO₂ and CH₄ production (Madigan 2012). Organic C inputs such as plant root exudates and litter may provide C sources that are more labile than organic matter available in soil, or may “prime” soil microbial communities for enhanced decomposition rates (Kuzyakov 2002). Demonstrating the stimulative effect of organic C inputs on GHG fluxes, a study of effects of organic amendments (leaf litter and compost) on CO₂ and CH₄ production potential found significant increases in both gases with both amendments (Morrissey et al. 2014). Litter may also alter abiotic conditions, such as lowering soil temperature by shading and increasing soil moisture by attenuating evaporation effects.

Research investigating effects of restoration activities on coastal marsh ecosystem functions includes monitoring of vegetation communities (Buchsbaum et al. 2006; Chambers et al. 2012; Smith and Warren 2012) and faunal habitat (Raposa and Roman 2001; Roman et al. 2002; Raposa and Roman 2003; Gratton and Denno 2005; Dibble et al. 2013). However, there have been fewer studies of biogeochemical responses of marshes to restoration (Portnoy and Giblin 1997; Portnoy and Valiela 1997; Portnoy 1999), and fewer still that test effects of restoration on the critical function of C cycling (Burden et al. 2013; Drexler et al. 2013). Only one known study, conducted in China where *Phragmites* is native and *Spartina alterniflora* is an aggressive invasive, has tested effects of an invasive species eradication program on GHG fluxes (Sheng et al. 2014).

The goal of this study was to investigate effects of marsh-scale *Phragmites* removal conducted as part of restoration activities on GHG fluxes in a brackish marsh, and to test impacts of plot-scale manipulations of aboveground biomass and/or plant litter in affecting plant regrowth and GHG fluxes. Specifically, objectives were as follows: (1) to compare GHG fluxes over course of a growing season between a *Phragmites* stand recently subjected to mechanical clearing and an intact stand within the same marsh system and (2) to test effects of *Phragmites* removal and litter clearing on GHG fluxes (measured in light and dark). It was hypothesized that *Phragmites* regrowth would increase and removal would decrease CH₄ emission, given known gas transport via plant tissues from soils to the atmosphere. It was expected that *Phragmites* removal would cause a shift from photosynthetic CO₂ uptake to emission

(due to net respiration). Clearing leaf litter was hypothesized to decrease emission of CH_4 and CO_2 due to removal of a C substrate for soil microbial respiration and methanogenesis.

2. Methods

2.1 Study Site

Round Marsh (Figure 1), a 27.1 ha marsh complex owned by the town of Jamestown, Rhode Island and the Rhode Island Audubon Society, was chosen for these experiments. Vegetation at the site is characteristic of New England salt marshes; the high marsh is dominated by *Spartina patens* and *Distichlis spicata*, and low marsh vegetation consists primarily of *Spartina alterniflora*. Tidal flow is restricted to the eastern part of Round Marsh by a road with a small culvert bisecting the marsh, and artificial channels connecting the marsh's eastern reaches to the main creek have become clogged. These conditions have impeded tidal inundation and drainage of freshwater from the marsh surface. Increased surface water pooling and vegetation dieback in the high marsh zone, and progressive invasion of *Phragmites* in recent years, have been attributed to these hydrologic conditions (Anne Kuhn and Wenley Ferguson, personal communication). Based on surveys performed by Save The Bay in their summer 2012 and 2013 statewide salt marsh assessment, the eastern portion of Round Marsh was targeted for restoration. Restoration work at the site included dredging the clogged channel that runs through the *Phragmites* stand, digging a runnel network to promote drainage and tidal exchange, and mulching *Phragmites* using low-impact equipment with the aim of decreasing its extent and coverage and allowing native vegetation to recolonize. No herbicides were used as part of *Phragmites* management.

Two *Phragmites* stands, the stand on the far eastern side of the marsh where mulching is being performed (hereafter “restored stand”) and a smaller, intact stand on the northern edge of the marsh (hereafter “reference stand”), were examined (Figure 1). The restored stand is approximately 1.63 acres in extent, and the reference stand is about 0.13 acres. The restored stand abuts a forested freshwater swamp to the southeast and the reference stand is situated on the north edge of the marsh south of agricultural fields and a band of woody shrubs. Efforts to promote hydrologic reconnection, including channel excavation (near the restored stand) and runnel construction (near both stands) was performed with the aim of improving freshwater drainage and tidal flow. Soils in both stands consist of mucky peat, with soil organic content greater than 75% in both stands (Martin, unpublished data). Over the course of the experiment, the two stands were observed to flood only during one exceptionally high tide in August 2014 (Martin, personal observation).

2.2 Experimental Setup

A mensurative comparison of GHG fluxes and edaphic conditions between the restored and reference stands (Experiment 1) was conducted during summer 2014. The first cutting of the restored *Phragmites* stand, as well as excavation of the main channel running through it (Figure 1) and construction of runnels, took place in late March and early April 2014. Within a week of mulching, the reference stand was selected based on similarity in soil temperature, moisture, surface salinity (Table 1) and hydrology to the restored stand. In each stand, PVC bases to support chambers for

GHG flux measurements were installed at least 2 weeks prior to the first set of measurements and were left intact for the duration of each experiment to avoid soil disturbance. Six bases were installed haphazardly at least 15 m apart within each of the reference and restored stands. Bases were treated as experimental units and were used as locations for edaphic conditions, pore water and vegetation measurements. On a monthly basis beginning in May and ending in late August, GHG fluxes (CO₂ and CH₄) and soil conditions (moisture, temperature, oxidation-reduction (redox) potential and surface salinity) were measured. Pore water sulfide concentration was determined for May, July and August. Vegetation characteristics (stem density and average height) were measured during June and July.

During November 2014, the restored stand was cut for a second time, and more extensive runnels were added to the existing network based on observed surface water pooling in the high marsh adjacent to the *Phragmites* stand. A second experiment, performed during summer 2015 in the restored stand, tested responses of GHG fluxes to 2 treatments: *Phragmites* cutting alone and *Phragmites* cutting with litter cleared (Experiment 2). In May 2015, 3 plots (each 20 m²) were established in the restored stand. *Phragmites* was left intact in one plot and cut by hand at the soil surface in the two others. In one of the cut plots, *Phragmites* litter was removed by thorough raking, and the other had litter left in place after cutting. New shoots were clipped if needed in cut and cleared plots on a biweekly basis for the duration of the experiment. Treatment plots will be referred to hereafter as “control”, “cut”, and “cleared”. Four PVC bases to support chambers for GHG flux measurements were installed

haphazardly in each plot at least 2 weeks prior to May measurements and were left intact for the duration of the experiment. Bases were treated as experimental units and were used as locations for edaphic conditions, pore water and vegetation measurements. Measurements of GHG fluxes (CO_2 and CH_4), edaphic conditions (soil temperature, moisture, redox potential, surface salinity, pore water salinity), pore water chemistry (sulfide and ammonium concentrations), and vegetation characteristics were performed on 4 occasions: once in May as a baseline to test for uniform conditions between the plots prior to application of treatments, and on 3 occasions beginning about 1 month after treatment application (1 in June, 2 in July). On the final measurement date, light and dark GHG flux measurements were performed on the control and cut plots to separate plant (photosynthetic) and soil microbial respiration effects on CO_2 fluxes.

Litterbags containing *Phragmites* litter (one per flux chamber base) were deployed for 1 month to test for differences in decomposition rates between the 3 treatments.

2.3 Soil and vegetation characteristics and pore water chemistry measurements

Collection of soil variable data and pore water was conducted just outside chamber bases at the time of GHG flux measurements. Soil redox potential (ORP) was measured using an ORP probe and pH/ORP meter (Mettler Toledo, Greifensee, Switzerland). Soil temperature was measured with a digital thermometer inserted into 10 cm into soil, and soil moisture content was measured using a volumetric water

content sensor (Decagon Devices, Pullman, WA) inserted 5 cm into soil. Surface soil salinity was measured by pressing soil against paper filters using plastic syringe to extract water and measuring its salinity with a handheld refractometer.

Litterbags to estimate decomposition rates in the 3 treatments contained weighed, oven-dried *Phragmites* leaf and stem litter collected from the site. Litterbags were buried 15 cm beneath the soil surface and were deployed for approximately 1.5 months. Post-deployment litter was dried and weighed. Mass lost during deployment was used as a proxy for decomposition.

Phragmites stem densities and average heights from within chamber bases were recorded. For average stem height, 10 stems per plot were selected for measurement at random and their heights were averaged.

Pore water for sulfide and ammonium concentration analysis was collected during baseline measurements and on 2 post-treatment dates using 15 cm Rhizon Soil Moisture Samplers (Ben Meadows, Janesville, WI), preserved using 1 M zinc acetate, and frozen at -20 C until analysis. Pore water salinity was measured using a handheld refractometer. Pore water sulfide and ammonium concentrations were analyzed using standard colorimetric techniques (Cline 1969; Solorzano 1969).

2.4 GHG Flux Measurements

All GHG flux measurements were performed between 9:00 and 16:00 to avoid confounding effects of diurnal variability and were conducted for 5-10 minutes per plot, based on observed periods for linear rates of change. A cavity ring down

spectroscopy (CRDS) analyzer (Picarro G2508) and transparent static flux chamber were used to measure CO₂, CH₄ and N₂O concentrations in real-time (as described in Martin and Moseman-Valtierra, 2015)). Hobo[®] data loggers (Onset, Bourne, MA) were suspended within chambers during all flux measurements to record air temperature and light intensity at 30 s intervals.

On the last measurement date for the 2015 experiment, both light and dark measurements were performed. Dark measurements were conducted by placing a light-blocking, light-colored cloth sheath over the capped chamber for the duration of measurements.

GHG fluxes were calculated using chamber size and footprint area. The Ideal Gas Law ($PV = nRT$) was used to calculate changes in gas concentrations over time using within-chamber air temperatures and ambient atmospheric pressure. When slopes had an R^2 value of less than 0.85, data were not included in the analysis (4 instances for CO₂ and 3 for CH₄ during 2014 and 1 instance for each GHG during the 2015 experiments).

2.5 Statistical Analysis

2.5.1 2014 Stand Comparison (Experiment 1)

For the 2014 stand comparison experiment, early-season (April) edaphic conditions were compared between restored and reference stands using Wilcoxon Signed-Rank Tests to account for non-normality and heteroscedasticity.

To compare GHG fluxes and soil conditions between the 2 stands and among the 4 months of measurement, 2-factor repeated measures ANOVA tests were used. Stand and month were treated as fixed effects, and plot was treated as a random effect. CH₄ fluxes were log-transformed prior to analysis due to extreme skew. Pairwise t-tests were used for post-hoc pairwise comparisons when appropriate.

Plant characteristics (density and average stem height) were compared between restored and reference stands using 1-factor ANOVAs.

Relationships between GHG fluxes and soil, pore water, and plant variables were tested for in each stand using Spearman's correlation analysis.

2.5.2 2015 Vegetation and Litter Removal Experiment (Experiment 2)

To test for uniform plot conditions prior to treatments, GHG fluxes, edaphic conditions, pore water sulfide and ammonium concentrations and vegetation characteristics were compared between the 3 plots (control, cut and cleared) using Kruskal-Wallis tests to account for non-normality and heteroscedasticity.

To compare GHG fluxes (excluding dark chamber fluxes) and soil and pore water conditions between the 3 treatments (control, cut and cleared) from 3 measurement dates, repeated-measures ANOVA tests were performed as described previously. Treatment was set as a fixed effect, and measurement date was treated as a random effect. CH₄ fluxes were log-transformed prior to analysis due to extreme skew. Pairwise t-tests were used for post-hoc pairwise comparisons when appropriate.

Light vs. dark GHG fluxes from the control and cut treatments were compared within each treatment using paired Student's t-tests. To test for differences in CO₂ emission not driven by photosynthetic effects, dark CO₂ fluxes from control plots were compared with light CO₂ fluxes from cleared and cut plots (from the same measurement date) using a 1 factor ANOVA.

Effects of treatment on litterbag decomposition rates were tested using a 1-factor ANOVA.

All statistical analyses were performed in R (R Core Team, 2012). Statistics were interpreted at a significance level of 0.05.

3. Results

3.1 Comparison of restored and reference *Phragmites* stands (2014)

Since *Phragmites* grew back in the area where it had been cleared during summer 2014, this experiment tested GHG fluxes and edaphic conditions between the 2 stands over a period of *Phragmites* re-colonization of the restored stand.

Over the course of the growing season, *Phragmites* quickly re-vegetated the mulched (restored) stand and (averaged over June and July measurements) was taller ($F_{1,16}=221.50$, $p<0.001$) and denser ($F_{1,22}=4.28$, $p=0.05$) than in the reference stand. In the reference stand, *Phragmites* sprouts around 10 cm in height were observed in May, and sprouts were observed in the restored stand 1 month later (about 2 months after mulching). In June, *Phragmites* plants in the reference stand were taller (at 157.82 ± 14.23 cm) than plants in the restored stand (at 43.77 ± 8.31 cm). Also, in June, restored stand *Phragmites* plants were twice as dense as those in the reference stand (6.67 ± 1.45 stems per chamber base vs. 3.67 ± 1.38 stems per chamber base). By July, *Phragmites* in the restored stand was 3 times as dense (with 11.50 ± 1.73 stems per chamber base relative to just 4.17 ± 1.54 stems per chamber base in the reference stand), but reference plants were still taller (173.23 ± 4.83 vs. 65.81 ± 4.41).

In the earliest comparison (April 2014) of soil conditions in the restored and reference *Phragmites* stands (during the week following first mulching), soil salinity, moisture, and temperature were similar between stands ($W=16.5$, $p=0.87$; $W=13$, $p=0.48$; $W=7$, $p=0.89$, respectively). These similarities supported our choice of reference stand.

Beginning in May, several soil variables differed between reference and restored *Phragmites* stands (Tables 1 and 2). For the duration of the growing season, soil moisture was consistently slightly higher in the reference stand. Redox potential (Eh) (measured only in July and August) was substantially greater in the restored stand (310.67 ± 39.56 mV in July and 128.33 ± 66.51 mV in August) than in the reference stand (20.33 ± 72.12 mV in July and 17.5 ± 17.43 mV in August). Soil temperature was greater in the restored stand during May, but did not differ between stands during later months. Neither surface soil salinity nor pore water sulfide concentrations differed between stands.

Most soil variables varied over the course of the growing season (Tables 1 and 2). Soil temperature increased as the growing season progressed in both stands. Salinity measured during August was significantly higher in both stands than salinity measured during other months due to unusually high tides on that date causing seawater inundation prior to measurements. In both stands, pore water sulfide concentrations were significantly higher in May (reference: 106.97 ± 71.03 μmol , restored: 95.52 ± 38.52 μmol) than in July (when concentrations were 0 μmol in both stands) or August (reference: 17.51 ± 15.21 μmol , restored: 5.33 ± 6.53 μmol).

CO₂ fluxes, which did not differ significantly between stands (Figure 2), ranged from slight emission in May (around 3 $\mu\text{mol m}^{-1} \text{s}^{-1}$ from both stands) to uptake of greater than -15 $\mu\text{mol m}^{-1} \text{s}^{-1}$ in July from both stands. CO₂ fluxes in May (when emission occurred) differed significantly from fluxes measured in July (when the most uptake occurred).

CH₄ fluxes ranged from just over 0 $\mu\text{mol m}^{-1} \text{h}^{-1}$ in the restored stand in May to nearly 4,000 $\mu\text{mol m}^{-1} \text{h}^{-1}$ in the reference stand during May. CH₄ fluxes decreased substantially in the reference stand but increased slightly in the restored stand as the growing season progressed (Figure 2). CH₄ fluxes were significantly greater from the reference stand only during May, and were similar between the 2 stands for the remainder of the growing season.

GHG fluxes did not significantly correlate with soil, pore water or plant variables in either reference or restored stands.

3.2 Effect of Phragmites and litter removal on GHG fluxes (2015)

Baseline measurements indicated that plots did not differ significantly in any of the variables measured prior to initiation of the experimental treatments (Table 4), except for a 1°C higher average soil temperature in the control plots. This trend did not persist in subsequent measurements, and so was not thought to bias results.

Soil conditions varied among sampling visits, but none differed among treatments (Table 3, Table 5). Litterbag mass loss (a proxy for decomposition) generally was greater in cut and cleared than in control plots (Table 3), although not statistically significant ($F_{2,8}=2.06$, $p=0.19$).

CO₂ and CH₄ fluxes were both significantly affected by treatment (Table 4). CO₂ fluxes during baseline measurements were small (between -3 and 3 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (Figure 3A). After treatment, fluxes increased in magnitude and averaged between -18 and 8 $\mu\text{mol m}^{-2} \text{s}^{-1}$. CO₂ fluxes differed significantly between cut and cleared plots

and the control plot ($p < 0.001$ in both cases). Emission of similar magnitude was observed from cut and cleared plots and net uptake due to photosynthesis was observed in the control plot. Although CH_4 fluxes displayed great spatial variability, treatment effects were discerned. While CH_4 fluxes from the control plot (less than $200 \text{ umol m}^{-2} \text{ h}^{-1}$) did not increase on average from baseline to post-baseline measurements, fluxes from the cut and cleared plots more than doubled (from less than $150 \text{ umol m}^{-2} \text{ h}^{-1}$ to over $300 \text{ umol m}^{-2} \text{ h}^{-1}$) (Figure 3B). Post-hoc pairwise t-tests indicate that CH_4 emissions were significantly greater from the cleared than intact plot ($p = 0.05$), and that there was a trend of greater emission from the cut relative to intact plot ($p = 0.09$). Emissions did not differ between cut and cleared plots ($p = 0.65$).

Light and dark CO_2 fluxes from control plots differed significantly ($t_2 = 2.85$, $p = 0.04$), with uptake observed during light measurements and emission observed during dark measurements. When dark CO_2 fluxes from the control plot were compared to fluxes from cut and cleared plots on the last sampling date, there was a strong trend ($F_{2,9} = 3.78$, $p = 0.06$) of smaller emissions from cleared than from cut or control plots (Figure 4). Neither light/dark CO_2 emissions in the cut treatment ($t_2 = 2.85$, $p = 0.10$) nor light/dark CH_4 emissions within the control ($t_3 = 1.71$, $p = 0.18$) and cut ($t_2 = -0.10$, $p = 0.93$) plots differed.

4. Discussion

4.1 Restoration effects on soil conditions and *Phragmites*

Results of this restoration endeavor over the short terms of this study indicate increased drainage (but not tidal inundation) and rapid revegetation by *Phragmites* within a few months.

Although excavation of small runnels (< 0.3 m in diameter) to promote hydrological connectivity was performed throughout the eastern portion of Round Marsh, the bulk of excavation (dredging of an existing manmade channel > 1m in diameter) took place immediately north of the restored stand. Resulting increased drainage in the restored stand is reflected in measured soil characteristics, which indicated decreased moisture and increased redox potential both relative to the reference stand (Table 1) and between 2014 and 2015 (Table 3). While channel excavation and runnels promoted increased aeration of restored stand peat by allowing freshwater drainage, they do not appear to have promoted increased tidal inundation. Pore water salinity and sulfide concentrations did not differ between reference and restored stands in 2014, and in the restored stand salinities remained low and pore water sulfide concentrations were nearly 0 μM by 2015 (Table 3).

The rapid regrowth of the restored *Phragmites* stand during the 2014 growing season was likely stimulated by drying soil conditions, since oxic stress and high sulfide concentrations have been shown to restrict *Phragmites* spread (Chambers et al., 2012; Bart and Hartman, 2003). Observed greater stem densities in the restored than reference *Phragmites* stand may support previous findings that cutting, in the absence

of treatment with herbicides or other control measures, stimulates increases *Phragmites* stem density (Hazelton et al., 2014).

4.2 Peat drainage and *Phragmites* presence may enhance marsh net GHG uptake

Restoration-mediated changes (increased drainage and *Phragmites* recolonization) may have driven increases in net GHG uptake. Small restored stand CH₄ emissions (relative to the reference stand) reflected more aerated soil conditions, and CO₂ uptake increased substantially with *Phragmites* regrowth (without increases in CH₄ emission).

Methanogenesis takes place under anoxic, highly reduced conditions. Therefore, soil aeration due to improved drainage likely diminished restored stand CH₄ fluxes (relative to the reduced, wetter reference stand) (Figure 2). Restored stand CH₄ emissions were minor relative to emissions of up to over 800 $\mu\text{mol m}^{-1}\text{h}^{-2}$ measured during the 2014 growing season in an irregularly flooded *Phragmites* stand in a marsh of mesohaline soil salinity located in Jamestown, RI (Martin and Moseman-Valtierra, 2015, Martin and Moseman-Valtierra, *in review*). However, they were several times greater than CH₄ emissions measured in a *Phragmites* stand in Falmouth, MA (less than 25 $\mu\text{mol m}^{-1}\text{h}^{-2}$) that was inundated daily and had correspondingly high soil salinities (mesohaline) (Martin and Moseman-Valtierra, 2015). These comparisons suggest that in terms of CH₄ emission attenuation, restoration-mediated increases in marsh drainage could produce results comparable to

increasing tidal inundation, a known strong control on CH₄ production (Poffenbarger et al., 2011).

In contrast to the hypothesized increase in CH₄ emission with *Phragmites* presence, results of Experiment 1 indicated that the plant did not exacerbate and possibly even decreased CH₄ emissions. Emissions in the restored stand were very low relative to the reference stand in the spring, and remained constant throughout the growing season as *Phragmites* regrew. The pattern in the reference stand (with its anoxic, reduced soil) of decreasing emissions as vegetation matured (Figure 2) may indicate plant-driven promotion of CH₄ oxidation (and therefore decreased CH₄ emission). *Phragmites* can ameliorate anoxic soil conditions that support methanogenesis by oxygenating and drying its root zone. When compared to adjacent marsh vegetated with native high marsh species, redox potentials in a *Phragmites* stand in a brackish New Jersey tidal marsh were found to be higher (Windham and Lathrop 1999), with increases of +400 mV (Armstrong et al. 2006). *Phragmites* takes up abundant water via evapotranspiration, and can locally lower water tables (Windham and Lathrop 1999), and resulting aerobic soil conditions could impede methanogenesis.

While *Phragmites* recolonization of the restored stand was not associated with an increase in CH₄ emission, it was accompanied by a dramatic increase in CO₂ uptake that trended toward outpacing uptake in the reference stand by August (Figure 2). Greater restored stand CO₂ uptake can likely be attributed to more substantial

Phragmites biomass and reflects greater stem density in the restored stand (although biomass itself was not measured).

4.3 Potential inhibition and stimulation of GHG emissions by Phragmites and associated litter

In the 2015 *Phragmites* clearing experiment, negative CO₂ fluxes in the control (intact *Phragmites*) plot demonstrate photosynthetic uptake greater in magnitude than CO₂ emission from plant and heterotrophic respiration. The trend of smaller CO₂ emissions from cleared than from cut or reference plots (Figure 4) indicates a stimulative effect of *Phragmites* litter on soil microbial respiration. This modest increase in CO₂ emission (about 4 $\mu\text{mol m}^{-2}\text{s}^{-1}$) is small relative to net CO₂ uptake, as demonstrated by fluxes in control plots where litter was left intact. However, results of this experiment suggest that mechanically clearing *Phragmites* and resultant increase in litter could exacerbate CO₂ emission, particularly if the area is not either recolonized by *Phragmites* or another species with CO₂ uptake rates that would outpace respirative emission.

Results of the 2015 experiment suggest that rather than exacerbating CH₄ emissions, *Phragmites* presence may in fact attenuate emissions (as in Grünfeld and Brix, 1999) relative to unvegetated brackish marsh soil. Greater CH₄ emissions from cut and cleared treatment plots than in the control plot support the notion that for intact plants, oxygenation of its root zone (Colmer 2003) limits anaerobic methanogenesis, and may perhaps couple methanogenesis and methanotrophy (Lovell

2005), decreasing net emissions where plants remain intact. An alternative explanation for greater CH₄ emission in the cut and cleared plots is greater provision of organic C substrate for methanogenesis from decaying root or rhizome material if removal of aboveground structures resulted in senescence of belowground structures. Unlike for CO₂, leaf litter decomposition does not seem a likely driver of CH₄ emissions, given the comparable emissions between cut and cleared plots on the short timescale of this study. This finding is potentially explained by the comparatively smaller suite of microbes and more limited metabolic strategies involved in CH₄ production relative to CO₂. While microbial groups likely to capitalize on labile organic C provided by litter availability are numerous and so likely present in soil, there are few taxa of methanogenic archaea that metabolize a small group organic substrates produced by bacterial fermentation (Madigan 2012).

4.4 Implications for management: Decreased GHG emissions as a restoration goal

Results of these experiments suggest that effects of the restoration activity (increased drainage and *Phragmites* recolonization) likely enhanced the marsh function of GHG uptake, at least over the short duration of this experiment. However, potential longer-term impacts of the restoration conditions achieved, as well as ecosystem services other than GHG sequestration, must be considered.

Although marsh drainage associated with this restoration project likely resulted in decreased CH₄ emissions, drainage may ultimately promote a loss of C storage. Marsh peat aeration accelerates decomposition as oxygen becomes available to

microbial communities, resulting in loss of buried C pools (Portnoy, 1999).

Restoration of tidal inundation is known to diminish CH₄ production (Poffenbarger et al., 2011) while maintaining reduced conditions conducive to C storage. Therefore, restoration projects that restore tidal inundation rather than exclusively promote drainage of standing water are desirable in terms of maximizing sequestration of C and GHGs.

The findings of these experiments suggest a potential role for *Phragmites* in maintaining or enhancing a coastal marsh ecosystem service, and therefore raise questions about *Phragmites* management. Coupled with the observed substantial uptake of CO₂ in reference and revegetating (restored) *Phragmites* stands and the 2015 control *Phragmites* plot, increases in CH₄ from plots cleared of *Phragmites* suggest that its removal could potentially be of detriment to marsh ecosystem GHG sequestration. Using a global warming potential of 21 (IPCC 2007) to compute net daytime, growing season GHG fluxes in CO₂ equivalent units from post-treatment averages, cleared and cut plots were net emitters of GHGs (956 and 1,230 mg m⁻² h⁻¹, respectively) over the short time period of this study, while the control plot had a net CO₂ equivalent uptake rate twice as large (-2,707 mg m⁻² h⁻¹) due to substantial CO₂ uptake and smaller CH₄ emissions. These findings indicate that further tests of effects of *Phragmites* removal on GHG flux dynamics in coastal marshes are warranted. Introduced *Phragmites* is exceptionally genetically diverse (Saltonstall 2003), and regional and environment-specific differences in ecology and physiology could affect its responses to removal as well as its impacts on C cycling and GHG flux dynamics.

Therefore, future investigations should test applicability of these findings over varying spatial scales and across gradients of environmental conditions.

While the 2 experiments presented here respectively tested effects of *Phragmites* recolonization and compared *Phragmites*-vegetated and bare marsh, it must be considered that the aim of *Phragmites* removal is to facilitate re-colonization of native marsh vegetation. Therefore, in theory, the marsh from which *Phragmites* was eradicated would be colonized by native species, which has been shown to typically occur within three or more years following restoration activities (Konisky et al. 2006). Nevertheless, as reviewed in Chambers et al. 2012, restoration of tidal flow may not achieve a return to the desired native species assemblage. Rather, the invasive plant may recolonize, or native species may fail to establish in the cleared area. In the case of the mechanically-cleared *Phragmites* stand in this experiment, revegetation was rapid and, in keeping with results of previous investigations (see Hazelton et al., 2014), *Phragmites* in the stand that had been cleared grew back at densities substantially greater than in the reference stand. Other, reportedly more effective methods of *Phragmites* removal such herbicide use and burning (Hazelton, 2014), though, are likely to sufficiently damage plants such that regrowth is minimized and native plants may colonize if conditions are favorable. Depending on methods and frequency of *Phragmites* removal, unvegetated swathes of marsh may persist for months or years, potentially resulting in increased emission of CH₄ with no compensatory CO₂ uptake.

The goal of restoration projects may be to return ecosystems to supposed former functional states or conditions, or they may target optimization of a single ecosystem service. In the case of the latter, prioritizing one ecosystem service may occur at the expense of others. While a *Phragmites*-dominated marsh may provide the ecosystem service of C storage as a result of abundant biomass production (Windham 2001), sediment accretion (Rooth et al. 2003), and net GHG uptake, effects of *Phragmites* on aspects of ecosystem function including wildlife habitat provision and species diversity must undoubtedly also be accounted for in management context.

Figure 1. Round Marsh with *Phragmites* reference and restored stands indicated in red.

Table 1. Edaphic characteristics $\pm$ se for reference and restored *Phragmites* stands

	April	May	June	July	August
Soil Temperature (C)					
<i>Reference Stand</i>	9.27 $\pm$ 0.16	13.42 $\pm$ 0.41	16.30 $\pm$ 0.25	19.08 $\pm$ 0.18	20.42 $\pm$ 0.24
<i>Restored Stand</i>	8.92 $\pm$ 0.11	11.95 $\pm$ 0.26	16.63 $\pm$ 0.20	19.00 $\pm$ 0.09	19.85 $\pm$ 0.03
Soil Moisture (%)					
<i>Reference Stand</i>	58.60 $\pm$ 1.49	54.39 $\pm$ 0.91	51.42 $\pm$ 1.93	57.47 $\pm$ 1.58	--
<i>Restored Stand</i>	56.57 $\pm$ 1.21	49.35 $\pm$ 1.29	46.43 $\pm$ 1.39	50.27 $\pm$ 1.26	--
Soil Salinity (ppt)					
<i>Reference Stand</i>	4.58 $\pm$ 1.45	1.33 $\pm$ 0.49	4.33 $\pm$ 0.71	4.50 $\pm$ 1.48	28.00 $\pm$ 2.08
<i>Restored Stand</i>	4.42 $\pm$ 0.74	3.67 $\pm$ 0.33	2.33 $\pm$ 0.21	4.20 $\pm$ 1.24	18.00 $\pm$ 1.79

Figure 2. Daytime CO₂ (A) and CH₄ (B) fluxes from reference and restored *Phragmites* stands measured monthly from May-August. Standard error bars are shown.

Table 2. Results of statistical tests of effects of stand and month on GHG fluxes and abiotic variables from comparisons of reference and restored *Phragmites* stands

	Results 2-factor (Stand x Month) ANOVAs			Results of post-hoc pairwise t-tests for month
	Stand	Month	Stand x Month	
Soil redox (mV)	$F_{1,18}=5.26$, $p=0.03^*$	$F_{2,18}=0.04$, $p=0.96$	--**	--
Soil moisture (%)	$F_{1,33}=5.00$, $p=0.03^*$	$F_{3,33}=14.18$, $p=0.001^*$	$F_{2,33}=0.45$, $p=0.64$	May ^{ab} , June ^a , July ^b , Aug ^{bc}
Soil temp.(°C)	$F_{1,36}=0.52$, $p=0.48$	$F_{3,36}=411.64$, $p<0.001^*$	$F_{3,36}=5.82$, $p=0.002^*$	May ^a , June ^b , July ^c , Aug ^d
Surface salinity (psu)	$F_{1,32}=0.35$, $p=0.56$	$F_{3,32}=135.74$, $p<0.001^*$	$F_{1,33}=10.54$, $p<0.001^*$	May ^a , June ^a , July ^a , Aug ^b
Pore water sulfide (umol)	$F_{1,10}=1.39$, $p=0.27$	$F_{2,10}=5.52$, $p=0.02^*$	$F_{2,10}=0.02$, $p=0.98$	May ^a , July ^b , Aug ^b
CH ₄ flux (umol m ⁻² h ⁻¹)	$F_{1,32}=1.19$, $p=0.28$	$F_{3,32}=0.92$, $p=0.44$	$F_{3,32}=4.30$, $p=0.01^*$	--
CO ₂ flux (umol m ⁻² s ⁻¹)	$F_{1,31}=0.00$, $p=0.99$	$F_{3,31}=4.66$, $p=0.008^*$	$F_{3,31}=1.81$, $p=0.17$	May ^a , June ^{ab} , July ^b , Aug ^{ab}

F statistics, degrees of freedom, and p-values are reported for 2-factor (*Phragmites* stand x Month) ANOVAs. In post-hoc comparison column, months not connected by the same letter are significantly different

* = Significant at $\alpha = 0.05$

**Insufficient month data for test of interaction (only July and August measured)

Table 3. Pre- and post-treatment edaphic and vegetation characteristics from the summer 2015 vegetation clearing experiment

	<i>Moisture (%)</i>	<i>Temp.(°C)</i>	<i>Redox (Eh)</i>	<i>Salinity (psu)</i>	<i>Sulfide (uM)</i>	<i>Ammonium (uM)</i>	<i>Stem Density</i>	<i>Avg. Height (cm)</i>	<i>%Litterbag mass lost</i>
Baseline (Pre-Treatment)									
<i>Control</i>	25.03±6.45	14.98±0.18	511.75±15.16	2.67±1.63	4.14±4.78	9.27±4.92	6.00±2.83	58.85±7.45	--
<i>Cut</i>	29.73±1.59	13.98±0.20	507.00±24.72	16.75±10.97	0.00±0.00	13.37±4.26	5.25±1.66	51.30±3.25	--
<i>Cleared</i>	23.25±4.17	13.88±0.32	460.00±23.41	20.25±3.69	0.00±0.00	33.35±5.74	6.25±2.23	59.44±2.44	--
Post-Treatment*									
<i>Control</i>	36.58±1.84	17.95±0.74	444.64±16.42	3.33±0.93	2.29±1.96	11.95±2.78	10.38±0.94	75.09±6.85	14.67±3.71
<i>Cut</i>	30.13±2.46	17.52±0.81	443.83±15.89	3.00±1.08	0.00±0.00	7.33±1.85	--	--	21.10±4.87
<i>Cleared</i>	35.83±3.57	17.73±0.75	470.00±13.96	3.92±1.11	0.00±0.00	7.38±1.64	--	--	24.47±4.75

*Averaged over 3 post-treatment measurement dates in June and July, with the exception of litterbag mass lost (quantified between early June and late July)

Table 4. Results of statistical comparisons of baseline variables prior to vegetation and litter removal

Variable	Results of Kruskal-Wallis Test
Moisture	$X^2_2=6.86$, $p=0.39$
Temperature	$X^2_2=7.04$, $p=0.03^*$ <i>Control warmer by about 1°C</i>
Redox	$X^2_2=4.16$, $p=0.13$
Salinity	$X^2_2=6.10$, $p=0.05$
Sulfide	$X^2_2=2.00$, $p=0.37$
Ammonium	$X^2_2=3.80$, $p=0.15$
Stem Density	$X^2_2=0.51$, $p=0.78$
Avg. Height	$X^2_2=2.91$, $p=0.23$

X^2 statistics, degrees of freedom, and significance values are reported for Kruskal-Wallis tests of differences between pre-treatment plots

* = Significant at $\alpha = 0.05$

Table 5. Results of statistical tests of effect of treatment (Intact, Cut, and Cleared) and site visit on GHG fluxes and edaphic variables from the summer 2015 experiment

	Results 2-factor (Treatment x Date) ANOVAs			Results of post-hoc pairwise t-tests for visits 1-3
	<i>Treatment</i>	<i>Visit</i>	<i>Treatment x Visit</i>	
<i>Soil redox</i>	$F_{2,23}=1.08$, p=0.36	$F_{2,23}=4.53$, p=0.02*	$F_{4,23}=0.64$, p=0.64*	1 ^a 2 ^b 3 ^{ab}
<i>Soil moisture</i>	$F_{2,24}=0.45$, p=0.64	$F_{2,24}=21.72$, p<0.001*	$F_{4,24}=1.58$, p=0.21	1 ^a 2 ^a 3 ^c
<i>Soil temp</i>	$F_{1,36}=0.57$, p=0.57	$F_{3,36}=528.07$ p<0.001*	$F_{3,36}=1.05$, p=0.40	1 ^a 2 ^b 3 ^c
<i>Surface salinity</i>	$F_{2,24}=0.75$, p=0.48	$F_{3,24}=137.58$, p<0.001*	$F_{1,24}=4.47$, p=0.008*	1 ^a 2 ^b 3 ^c
<i>Pore water ammonium</i>	$F_{2,15}=1.33$, p=0.29	$F_{1,15}=8.00$, p=0.01*	$F_{2,15}=1.10$, p=0.36	1 ^a 2 ^b 3 ^c
<i>CH₄ flux</i>	$F_{2,24}=3.27$, p=0.05*	$F_{2,24}=1.24$, p=0.31	$F_{2,24}=0.56$, p=0.69	--
<i>CO₂ flux</i>	$F_{2,24}=5.50$, p=0.01*	$F_{2,24}=0.41$, p=0.67	$F_{2,24}=0.95$, p=0.45	--

Figure 3. CO₂ (A) and CH₄ (B) fluxes measured in control, cut, and cleared plots on baseline (May) and each of the 3 post-treatment measurement dates in 2015. Standard error bars are shown. The dashed lines indicate average CO₂ uptake (-10 umol m⁻² s⁻¹) and CH₄ emission (125 umol m⁻² h⁻¹) from the restored plot during 2014 when vegetation was intact (June-August).

Figure 4. CO₂ fluxes measured on one date from cut and treatment plots and from control plots in the dark.

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CHAPTER 5

DIFFERENT SHORT-TERM RESPONSES OF GREENHOUSE GAS FLUXES TO SIMULATED GLOBAL CHANGE DRIVERS IN SALT MARSH MESOCOSMS

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Abstract

Coastal marshes are valued for ecosystem services such as carbon (C) storage, nitrogen (N) transformation and coastline protection, but they are facing numerous global changes including climate change, eutrophication and exotic species invasion. Such perturbations to marsh ecosystems will likely alter plant and microbial community structure and biogeochemistry, including that of greenhouse gas (GHG) fluxes. With the goal of better understanding potential responses of coastal marsh function to interacting global changes, a multifactorial experiment was conducted. Two climate treatments (simulated future or present day carbon dioxide (CO₂) and temperature conditions) and two N treatments (non-enriched and enriched with 19.7 g N m⁻² wk⁻¹) were applied independently and in combination for 10 weeks to mesocosms containing field-collected vegetated soil cores with invasive *Phragmites australis* or native *Spartina patens*. Edaphic properties (soil pH, salinity and moisture), plant growth (stem height and density and above and belowground biomass), and fluxes of CO₂, methane (CH₄) and nitrous oxide (N₂O) fluxes were measured before and after 10 weeks of treatment. GHG fluxes were measured using cavity-ring down spectrometry. When “climate change” and N enrichment were applied independently, *P. australis* stem density nearly doubled. N enrichment significantly increased belowground biomass for *P. australis* but not *S. patens* and did not affect aboveground biomass production of either species. CO₂ fluxes did not differ between vegetation types or treatments. “Climate change” significantly increased CH₄ emissions relative to emissions from “current climate” treatments in *P.*

australis mesocosms, but did not affect CH₄ emissions from *S. patens* mesocosms. N₂O emissions increased from both *S. patens* and *P. australis* mesocosms, although emissions were lower from the *S. patens* mesocosms under “climate change” than “current climate” conditions. These results suggest that while global change impacts on CH₄ fluxes are likely to vary across marsh vegetation zones, N loading may consistently increase N₂O emissions in both native *S. patens* and invasive *P. australis* zones, although responses may be climate-dependent.

Highlights

- *Phragmites australis* stem density increased with nitrogen (N) enrichment and simulated climate change applied independently, but did not significantly change in response to the combination of both N loading and climate change.
- N addition stimulated shallow belowground biomass production (0-20 cm) in mesocosms containing *P. australis* and did not affect *Spartina patens* or *P. australis* aboveground biomass.
- Simulated climate change increased methane (CH₄) emissions from coastal marsh mesocosms containing invasive *P. australis* but did not affect emissions from those with native *S. patens*.
- N loading (19.7 g N m⁻² wk⁻¹) dramatically increased N₂O emissions from both *P. australis* and *S. patens* mesocosms under “climate change” and “current climate” conditions. However, for *S. patens*, “climate change” mesocosms emitted half as much N₂O as “current climate” mesocosms. *P. australis* mesocosm N₂O emissions were similar under the two climate treatments.

1. Introduction

Significant increases in global air and ocean temperatures and atmospheric CO₂ concentrations in coming years (IPCC, 2007) may impact coastal ecosystems in many ways. In coastal marshes, global climate change may cause warming along with increased runoff from more frequent and intense precipitation events (IPCC, 2007) and exacerbated exotic species invasions (Hellmann et al 2008). Coastal marshes are already heavily impacted by anthropogenic nitrogen (N) loading (Nixon 1995) and exotic species invasions (Minchinton and Bertness 2003; Silliman and Bertness 2004a), and increased run-off and precipitation as well as warming may exacerbate coastal eutrophication (Rabalais et al 2009). Thus, coastal marshes may experience particularly complex synergistic impacts of anthropogenic and climate-driven stressors.

1.1 Potential impacts of climate change on coastal marshes

Warmer temperatures and elevated atmospheric CO₂ concentrations may affect coastal marsh vegetation by causing shifts in plant productivity and community composition and exacerbating exotic species invasion. Experimental simulated warming in salt marshes has been associated with increases in *S. patens* aboveground productivity (Gedan and Bertness 2010b) and also with shifts in marsh species prevalence (Gedan and Bertness 2009). Elevated atmospheric CO₂ (up to a 300 ppm increase expected by the end of the 21st century (IPCC, 2007)) may favor C₃ plants capable of accelerating photosynthetic rates to capitalize on available CO₂ (Taiz and

Zeiger 2002). Unlike native salt marsh grasses, which generally use the C₄ pathway, the invasive grass *Phragmites australis* (Gleason and Cronquist 1991) uses the C₃ pathway and so its spread may be exacerbated by rising atmospheric CO₂ concentrations. In support of this idea, elevated temperature and CO₂ (at levels expected by the year 2100) have been shown to alleviate salinity stress on two *P. australis* haplotypes, with implications for the plant's invasion of coastal marshes (Eller et al 2014).

Climate change impacts to marsh biogeochemical cycles (such as those of N and C) may reflect direct responses by microbes and plants to warming temperatures or may be indirectly related to changes in communities of plants and microbial associates of their rhizospheres. Warmer temperatures stimulate an increase in rates of microbial enzymatic reactions (Madigan 2012) as well as differences in plant growth and functioning (Taiz and Zeiger 2002). At higher temperatures and low humidity, convective flow within *P. australis* takes place at higher rates (Grünfeld and Brix 1999), and so warmer temperatures could lead to the emission of more rhizosphere-derived GHGs into the atmosphere from *P. australis*-dominated marshes. Over longer terms, changes in plant communities may alter marsh microbial community diversity (Ravit et al 2003) as plants either compete with microbes for nutrients or create favorable microenvironments for them as they oxygenate sediments (Armstrong 2000) and provide C as exudates or senesced material (Lovell 2005). Plant species-specific rhizosphere processes such as water uptake, organic substance uptake and release, competition for mineralized N, and preferred substrate utilization affect intensity of

soil organic matter decomposition (reviewed in Kuzyakov, 2002), and so changes to plant communities may drive changes to C and N cycling.

1.2 Potential impacts of nitrogen loading on coastal marshes

Anthropogenic N loading is another major driver of global change in coastal ecosystems. In salt marshes, N loading can drive increases in microbial activities and soil respiration, as well as in aboveground vegetation biomass allocation at the expense of marsh-stabilizing belowground biomass (Deegan et al 2012). An abundance of N also may alter plant community structure (Bertness et al 2002) including exacerbation of invasion by species such as *P. australis* (Mozdzer and Zieman 2010; Kettenring et al 2011). In addition, N enrichment may significantly affect GHG fluxes from marshes. N in marshes is transformed via various microbial pathways (denitrification, nitrification, dissimilatory nitrate reduction to ammonia), many of which can yield the potent greenhouse gas N₂O (Wrage et al 2001; Kool et al 2011). Since N₂O has a radiative forcing potential 300 times that of CO₂ (Forster et al 2007), increased emissions may have a greatly detrimental impact on climate. Pulses of N have been demonstrated to significantly increase emission of N₂O (Moseman-Valtierra et al 2011), indicating the susceptibility of coastal marsh N cycling to perturbation by external inputs. N overenrichment can also decrease C sequestration by promoting increased rates of organic matter decomposition (Deegan et al 2012) and by inhibiting CH₄ oxidation, both of which could further increase net GHG emissions from marshes (Liu and Greaver 2010). Impacts of N on GHG fluxes from marshes

may be mediated by plant uptake of nutrients, which is influenced by plant productivity and community composition, and therefore depends on climatic conditions. Thus, impacts of N and climate change must be investigated together in order to accurately predict marsh ecosystem response to future environmental shifts.

1.3 Objectives of the study

As anthropogenic and climatic drivers alter biotic and abiotic components of coastal marsh systems, these marshes may increasingly function as "novel ecosystems" (Hobbs et al 2009; Hobbs et al 2011) that bear little resemblance to their present-day counterparts. The objective of this study was to investigate short-term effects of potentially interacting drivers of global change (N and climate change) on plant growth and GHG fluxes from native *Spartina patens* (Gleason and Cronquist 1991) and invasive *P. australis* mesocosms. Since plant community composition changes over long time scales and co-varies with edaphic conditions in marsh ecosystems, a space-for-time approach that employs vegetation and associated soil collected from the two contrasting marsh zones (*S.patens* and *P. australis*) was used.

In a factorial design, mesocosms containing vegetated cores from the two vegetations zones were subjected to increased temperatures and atmospheric CO₂ levels associated with end-of-century climate predictions and/or to N enrichment consistent with some of the highest estimated loads in Narragansett Bay, Rhode Island. This allowed for comparison of how two different communities of plants and soil microbial communities, in *S. patens* or *P. australis* zones, respond to simulated

environmental changes. Initial and final soil variables, plant metrics and GHG (CO₂, CH₄ and N₂O) fluxes were measured in this 10-week experiment. Growth of both plant species was hypothesized to increase in response to N enrichment, and *P. australis*, a C₃ plant, was hypothesized to respond favorably to increased atmospheric CO₂ availability. Increased N₂O emissions were expected to result from N enrichment of cores from both vegetation zones, and emission of all GHGs was expected to increase under conditions of simulated climate change as warmer temperatures accelerated microbial metabolism. *P. australis* mesocosms were expected to emit more GHGs relative to *S. patens* mesocosms as a result of *P. australis*'s active internal gas ventilation systems.

2. Methods

Responses of salt marsh mesocosms containing invasive *P. australis* and native *S. patens* were tested under four climate change and N enrichment scenario combinations in a factorial design with 5 replicates of each plant species per scenario (Fig. 1). GHG (CO₂, CH₄ and N₂O) fluxes, soil variables (salinity, pH, moisture), and vegetation characteristics (stem height and above and belowground biomass for both species and stem density for *P. australis*) were measured before and after the 10-week treatment period. 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”.

2.1 Mesocosm Design & Experimental Setup

Mesocosms were designed to mimic conditions in a New England salt marsh. Vegetated cores (approximately 20 cm by 20 cm) were collected during mid-May, early in the growing season. To ensure that soil and plants were subjected to a low-N environment prior to experimental enrichments, cores were collected from single stands of *P. australis* and *S. patens* in Fox Hill marsh in Jamestown, RI, which is located in a minimally developed watershed at the low end of the N gradient in Narragansett Bay (Wigand et al 2003). It is recognized that collecting 20 cm deep cores excluded deeper belowground biomass, particularly for *P. australis*, since the species' roots can reach depths of up to 3 m (Justin Meschter, unpublished data) but given setup size restrictions it was not logistically feasible to collect deeper samples.

Despite this shallow depth, active growth was documented throughout the experiment for both plant species.

Field-collected cores of marsh soil and associated *S. patens* or *P. australis* plants were placed into perforated 2-gallon nursery pots. Pots and cores were placed in 8” deep plastic bins containing diluted field-collected seawater that matched field porewater conditions (salinities of 10-15). Bins were allowed to drain and water was replaced approximately every four weeks, in accordance with tidal flooding frequency at the site from which soil cores were collected. To maintain soil flushing and ameliorate toxic salt buildup, 2L of fresh water was applied per bin bi-weekly using a watering can to simulate rainfall, which maintained water salinity in bins between approximately 12 and 15. However, surface and subsurface soil salinity still increased by the end of the experiment (Appendix 1).

Two vented growth chambers (Conviron® Model PGR15) were used to simulate elevated temperatures and atmospheric CO₂ levels. One chamber was set to mimic present-day summer conditions in Rhode Island, and temperature and atmospheric CO₂ concentrations were elevated in the second consistent with increases predicted to occur by the year 2100 (IPCC 2007) (Table 1). Day and evening temperatures for the “current climate” chamber were determined using the high and low July temperatures for Rhode Island (NOAA). These temperatures were raised by 4.5°C for the “climate change” chamber based on the median of the upper bounds of model-predicted temperature increases for the year 2100 (IPCC 2007). Humidity was

held at ambient levels in the two chambers, and light imitated daytime in mid-summer with 15 h of light and 9 h of darkness.

Mesocosms receiving N enrichment treatments were supplemented with 19.7 g N m⁻² wk⁻¹, rates scaled down from estimated anthropogenic N loading to Apponaug Cove Marsh in upper Narragansett Bay (10,253 kg N ha⁻¹ yr⁻¹) (Wigand et al 2003). Enrichments were performed using ammonium nitrate dissolved in field-collected seawater diluted to a salinity of 12, and mesocosms outside the N enrichment treatment each received an identical volume of unenriched diluted seawater. In this experiment, ammonium nitrate was employed with the intent of providing both ammonium and nitrate to test 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.

2.2 Edaphic Variable Measurements

Surface soil (top 5 cm) and deeper soil (10-15 cm) salinity, pH and moisture were measured on the first (week 1) 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 of the top 5 cm was monitored using a volumetric water content sensor (Decagon Devices, Pullman, WA) inserted 5 cm into soil. A random subsample of surface soil (approximately 2-4 ml) was pressed against paper filters using small syringes to extract porewater for surface salinity from

each mesocosm pot. Subsurface soil (10-15 cm) pore water 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 refractometer.

2.3 Plant Characteristics Measurements

Average stem height (based on 10 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 thoroughly oven-dried for several days prior to weighing.

2.4 GHG Flux Measurements

CO₂, CH₄ and N₂O flux measurements were performed for each mesocosm on the first (week 1) and last (week 10) days of the experiment. 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 and Moseman-Valtierra, *in revision*). 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[®]), which was sealed to the edge of the mesocosm pot using a polyethylene closed-cell foam collar with its channel filled with water (Fig. 2). Small electric fans attached to the inside of the chamber mixed air during measurements. A stainless steel 55 cm long, 0.8 mm diameter pigtail was used for pressure equilibration. Gas measurements were conducted for 3 minutes per mesocosm, based on observed periods for linear rates of change. GHG 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 0. 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.

2.5 Statistical analysis

To check for uniformity of initial edaphic, plant and GHG flux conditions prior to experimentation, differences between mesocosms assigned to different treatment groups were tested in week 1 using two-factor ANOVAs (N treatment x climate conditions) for each vegetation type. Small chance differences were present in pH between mesocosms assigned to “current climate” plus N and “climate change”

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 no N and “climate change” plus N had 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 greater under the “current climate” without N treatment 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 differences persisted, patterns were significantly different from those observed by chance at week 1, and in the case of *S. patens* CO₂ final fluxes were consistent across groups despite initial differences. Therefore, initial differences were not considered to have biased results.

Two-factor ANOVAs (climate treatment x N treatment) were used to compare the effect of treatments on soil and plant variables and GHG fluxes and to test for potential interactions of climate and N effects on mesocosms from each vegetation zone. Three-factor ANOVAs (climate treatment x N treatment x vegetation type) were then used to include effects of vegetation zone and test for interactions with climate and N treatments on GHG fluxes. Tukey’s HSD test was used for post-hoc pairwise comparisons when appropriate. All data were aligned then rank-transformed prior to ANOVA analyses (Salter and Fawcett 1993; Wobbrock et al 2011) to account for deviations in normality while allowing for tests of effect interaction (Seaman Jr et al 1994). A Kruskal-Wallis Rank Sum test was used to test the effect of climate conditions on N₂O fluxes observed only from N-enriched mesocosms.

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.

3. Results

Approximately daily checks indicated that the desired temperatures were maintained and actual in-chamber CO₂ levels (396 ppm and 667 ppm for “current climate” and “climate change” chambers, respectively) were held close to intended levels (Table 1).

3.1 Soil variables

Over the course of the experiment, soil pH generally decreased; increases in pH (by about 0.5 units) were observed only in “climate change” and N enriched *P. australis* mesocosms (Appendix 1, Table 2). In *P. australis* mesocosms, N enrichment resulted in significantly lower pH, while “climate change” had no effect. In *S. patens* mesocosms, soil pH was affected interactively by climate conditions and N enrichment; within the “climate change” treatment, N enriched mesocosms had significantly higher pH (by about 2 units) (Table 2).

Soil moisture generally increased slightly over the course of the experiment in all mesocosms (Appendix 1, Table 2). *S. patens* mesocosms receiving N enrichment had significantly drier soil than those that did not (Table 2). *P. australis* mesocosm moisture was not affected by either treatment.

Despite efforts to control soil salinity, surface soil salinity increased by 30-80 in all mesocosms, although subsurface soil salinity increases were more modest (about 15) (Appendix 1, Table 2). *P. australis* mesocosms in the “climate change” treatment that received N enrichment had significantly higher surface soil salinity than those

receiving no N (Table 2). *S. patens* mesocosm surface soil salinity and subsurface soil salinity were not affected by either treatment.

3.2 Vegetation Characteristics

P. australis average stem height remained approximately the same throughout the experiment, while *S. patens* stem height increased approximately 4-fold (Appendix 1, Table 2). Average stem heights were not affected by N addition or climate change simulation for either species. By the end of the experiment, *P. australis* plants remained taller than *S. patens* plants, but by less than 20 cm on average (Table 2).

P. australis stem density remained unchanged in both control mesocosms and in mesocosms with the combination of N enrichment and “climate change.” In contrast, it nearly doubled in mesocosms with N or “climate change” applied independently (Appendix 1, Table 2).

In *P. australis* mesocosms, N enrichment significantly increased belowground biomass by approximately 1.5%. There were no significant effects of N enrichment or climate change simulation on *S. patens* above or belowground biomass or on *P. australis* aboveground biomass (Table 2).

3.3 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. CH₄ emissions were variable and ranged

from 0 – 386 $\mu\text{mol m}^{-2} \text{h}^{-1}$ during week 1 measurements (Appendix 2), and from 5 to 5402 $\mu\text{mol m}^{-2} \text{h}^{-1}$ during week 10 measurements (Table 2). Week 10 N_2O measurements from N-enriched mesocosms ranged from 515 – 3,466 $\mu\text{mol m}^{-2} \text{h}^{-1}$ (Fig. 3). While chance differences in CO_2 emission (which averaged 18-54 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were present between treatment groups at Week 1 (Appendix 2), there was no difference in emissions between groups by the end of the experiment (when emissions averaged 14-20 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

P. australis mesocosms subjected to “climate change” had some exceptionally large CH_4 emissions ($> 5,000 \mu\text{mol m}^{-2} \text{h}^{-1}$) (Figure 3A). When mesocosms with both vegetation types were compared, significantly larger CH_4 emissions were found from *P. australis* mesocosms, with an interactive effect that indicated larger CH_4 fluxes from *P. australis* mesocosms subjected to climate change than for all other vegetation type/treatment combinations (Table 2).

N enrichment dramatically increased N_2O production from all mesocosms within that treatment group by the end of the experiment (Figure 3B). For *S. patens*, “climate change” mesocosms emitted approximately half as much N_2O as “current climate” mesocosms ($X^2=0.10$, $p<0.01$). In contrast, “climate change” did not affect N_2O emissions from *P. australis* mesocosms ($X^2=6.81$, $p=0.75$).

3.4 Relationships of soil and plant variables to GHG flux magnitude

In *P. australis* mesocosms, CH_4 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 ($S=-0.56$, $p=0.01$). N_2O 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.

4. Discussion

4.1 Experimental design: Limitations of mesocosms

A mesocosm approach was chosen for this study to ensure precise simulation of predicted future climate conditions and N loading representative of heavy anthropogenic impacts. Use of growth chambers allowed for accurate simulation of desired experimental temperature and atmospheric CO₂ conditions (Table 1). However, replicating field conditions while precisely manipulating experimental factors is a common challenge inherent in mesocosm studies, and experimental artifacts including surface soil salinities greater than those measured at the field site and truncated root systems resulted in our experiment.

It was not possible with the design employed to adequately simulate tidal hydrology and associated flushing or freshwater influences of the field site from which cores were collected. As a result, soil salinities (surface and subsurface) increased over the course of the experiment. Subsurface salinities approximately doubled over the course of the experiment but remained within the range of midsummer salinity measured at the site from which vegetated cores were collected (~40) (Martin and Moseman-Valtierra, *in revision*). Surface soil salinities, however, increased to over twice that of those observed at the core collection site, placing them within range of salinities observed in more arid United States west coast salt marshes (Moseman 2007).

Long-term studies are needed to fully understand community response to global change. Due to the increasing soil surface salinity, we restricted experimental

duration to 10 wks, which potentially did not allow for observation of longer-term plant effects, including senescence and decomposition, on GHG-flux mediating soil microbial communities. While the observed increase in soil salinity was an unintended artifact of this mesocosm study, studies have reported elevated soil salinities likely attributable to drought (Hughes et al 2012) that may be linked with marsh dieback events.

Disturbance of root systems during mesocosm construction using both species was inevitable. *P. australis* roots, which in the field can reach depths of 3 m (Justin Meschter, unpublished data), were particularly truncated to fit in mesocosm pots, and much of *P. australis*'s belowground biomass was excluded, likely contributing to observed stunting in mesocosm *P. australis*.

Despite the limitations of the experimental design, we use results of this experiment to infer potential responses of coastal marsh communities to extreme conditions of disturbance, and to present hypotheses that further experiments, including field manipulations, should aim to address.

4.2 Impacts of environmental change on S. patens and P. australis

S. patens and *P. australis* displayed varying responses to “climate change” and N enrichment treatments, with *P. australis* responses likely influenced greatly by truncated root systems necessitated by this setup. Rooting depth (subsurface) salinity approaching that of seawater may also have contributed to stress in *P. australis*. While *S. patens* is a salt-tolerant halophyte, *P. australis* is well known to be stressed

by saline conditions, with one experiment demonstrating 100% mortality rates among *P. australis* grown at salinities of 35-50 (Lissner and Schierup 1997). Stem height data from this experiment illustrate *P. australis*'s potential susceptibility to salinity stress or other impacts of mesocosm growth, with plants averaging heights only around a third as tall as those at the field collection site during the 2014 growing season (Martin and Moseman-Valtierra, *in revision*).

Invasion of non-native *P. australis* is thought to be partially attributable to increases in nutrient availability that the plant is able to capitalize on and outcompete native counterparts (Rickey and Anderson 2004; Holdredge et al 2010; Mozdzer and Megonigal 2012). Increases in stem density and belowground biomass under conditions of N enrichment are not surprising, although the lack of N enrichment effect on aboveground biomass under either current or future conditions is inconsistent with known responses of *P. australis* to nutrient availability. Longer-term experiments under more natural conditions could better discern differences between responses of the two vegetation types to N enrichment.

4.3 GHG flux responses to simulated climate change vary by vegetation zone

Under “climate change” conditions, *P. australis* mesocosm CH₄ emission increased while *S. patens* mesocosm emissions were unaffected. These distinct responses may be due to differences in soil abiotic conditions, vegetation type and condition, and/or microbial communities (that may be the result of plant influence or have existed prior to plant colonization).

Soil salinity and pH effects may have driven observed fluxes through direct effects or through their impacts to vegetation. While soil salinity in mesocosms containing the two vegetation types did not generally differ, differences in this stressor's effect on the two plant species may have resulted in distinct CH₄ responses. Differences in surface soil pH may have driven observed patterns of CH₄ emission because the *S. patens* mesocosm soil was generally more acidic than *P. australis* mesocosm soil under all treatments by the end of the experiment. Low soil pH levels have been associated with decreased CH₄ production in wetland systems due to both inhibition of methanogenesis pathways and impacts to fermentation (Ye et al 2012). Manipulative experiments that alter soil pH from the two vegetation zones and measure CH₄ responses could lend support to this idea.

Since *Phragmites* oxygenates its root zone via radial oxygen loss (ROL) (Colmer 2003) when it actively grows, the taller plants with greater aboveground biomass may have resulted in a more oxygenated rhizosphere that promoted methanotrophy (CH₄ oxidation) and so decreased net CH₄ emission. However, belowground biomass and overall *P. australis* growth in our mesocosms was limited due to shallow collection depths and likely salinity stress. An alternative explanation for greater CH₄ emission from mesocosms with less aboveground biomass is that stressed and dying *P. australis* released more C in the form of senescing material to support methanogenesis than healthier *P. australis* did. The potential for this mechanism to explain observed results is further supported by the negative correlation of CH₄ fluxes in *Phragmites* mesocosms with stem height and aboveground biomass

across treatments. This possibility is also supported by the smaller increases in CH₄ production over the course of the experiment from *S. patens* mesocosms, where plants achieved heights observed in the field and appeared generally healthier. Further, CH₄ fluxes from *S. patens* mesocosms generally were more similar in magnitude to field-measured CH₄ emissions from both *S. patens* ($6 \pm 2 \mu\text{mol m}^{-2} \text{h}^{-1}$) and *P. australis* ($298 \pm 203 \mu\text{mol m}^{-2} \text{h}^{-1}$) zones of Fox Hill marsh, where plants were uninhibited by mesocosm artifacts (Martin and Moseman-Valtierra *in review*).

The observed pattern of CH₄ emission increase from *P. australis* mesocosms under “climate change” conditions may be explained by microbial community responses. The simplest potential explanation is that warming increased decomposition rates of soil organic matter (Zogg et al 1997; Kirwan and Blum 2011; Thiessen et al 2013), and rates of methanogenesis in particular have been observed to accelerate under warmer conditions (Van Hulzen et al 1999). Other potential mechanisms may be tied to differences in soil microbial community composition between *P. australis* and *S. patens* zones (as in Ravit et al., 2003), a phenomenon which may be due to plant impacts or to soil conditions existing independent of vegetation type. If plant differences alone cannot explain the larger CH₄ emissions observed in response to climate change simulations, then this study suggests that microbial communities in *P. australis* - and *S. patens* - associated soil may be differently “primed” (Kuzyakov 2002; Cheng 2009) for accelerated soil organic matter decomposition in response to warmer temperatures. *P. australis*-associated soil may support more abundant methanogenic archaea or increased expression of genes

regulating the methanogenic metabolic pathway, whether due to plant-mediated effects on the rhizosphere or independent of *P. australis* colonization. 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.

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. *P. australis* mesocosms produced CH₄ emissions that were generally within the wide range of fluxes measured during midsummer sampling at Fox Hill Marsh in the *P. australis* zone (Martin and Moseman-Valtierra *in review*), although exceptionally high wk 10 fluxes of greater than 5,000 $\mu\text{mol m}^{-2} \text{h}^{-1}$ from *P. australis* “climate change” mesocosms were several times larger than the largest field-measured fluxes (which were about 1,500 $\mu\text{mol m}^{-2} \text{h}^{-1}$). That these very large CH₄ emissions were observed at the elevated salinities present in these mesocosms contradicts known controls of salinity on CH₄ established in the field (Bartlett et al 1987b; Poffenbarger et al 2011) and may support the potential for temperature and/or plant stress to strongly influence CH₄ fluxes. However, this observation may be due to salinities that under the simulated conditions do not represent sulfate concentrations or sulfate reducing bacteria (SRB) abundances (as use of electron acceptors by SRB is the mechanism by which methanogenesis is suppressed at higher salinities).

Additional experiments have investigated impacts of invasive *P. australis* on CH₄ fluxes under futuristic atmospheric CO₂ (but no simulated warming) and N

enrichment conditions. In their mesocosm experiment, Mozdzer and Megonigal (2013) compared CH₄ production from mesocosms containing native and invasive lineages of *Phragmites* subjected to elevated atmospheric CO₂ and simulated N loading of 25 g N m⁻² y⁻¹ (40x lower than our enrichment). Emissions were positively correlated with N-stimulated increases in root mass, ramet density and leaf area, suggesting enhancement of CH₄ emissions by plants, the opposite of the pattern observed in our study. Mozdzer and Megonigal's use of larger (15L) mesocosm pots and the lack of elevated soil salinity likely allowed for less inhibition to plant growth, and healthier plants are likely been responsible for more diffusive CH₄ transport relative to the stunted plants in our experiment.

4.3 N enrichment stimulated N₂O emission in both vegetation zones

N₂O emissions are likely a function of the magnitude, form, and duration of N loading in coastal ecosystems. N enrichment stimulated N₂O fluxes from all treated mesocosms as expected, and flux magnitude was comparable for *P. australis* and *S. patens*. The absence of initial (week 1) N₂O emissions was expected based on previous measurements at the site (Martin and Moseman-Valtierra, *in revision*) and since Fox Hill marsh is located in a minimally developed watershed and receives some of the lowest estimated N loadings in Narragansett Bay, at about 10 kg N h⁻¹ yr⁻¹ (Wigand et al 2003).

A previous field study reports increased N₂O emission from coastal marshes in response to N enrichment (Moseman-Valtierra et al 2011). N₂O fluxes in that study

averaged around $1.75 \mu\text{mol m}^{-2} \text{h}^{-1}$, significantly lower than the up to nearly 3,500 $\mu\text{mol m}^{-2} \text{h}^{-1}$ fluxes 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.

While N loading rates in this experiment are high compared with those applied in other N enrichment experiments (such as Great Sippewisset Marsh at $1,500 \text{ kg N h}^{-1} \text{yr}^{-1}$ (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 and Cole 2002). The estimated N loads at Apponaug Cove ($10,253 \text{ kg N ha}^{-1} \text{yr}^{-1}$) (Wigand et al 2003) from which additions in this experiment were calculated reflect a anthropogenically impacted coastal marsh, with the surrounding watershed highly developed (>40% residential) and 74% of its N inputs derived from wastewater. Demonstrating the longer-term effects of eutrophication than those demonstrated in this experiment, Apponaug Cove marsh has experienced fragmentation and dramatic loss of high marsh vegetation. Although the N enrichment in this experiment allowed for inferences of the impacts of urban N loading on coastal marsh GHG fluxes, future experiments should employ a more subtle 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 N_2O fluxes under varying environmental conditions.

One known study has measured N₂O fluxes from a *P. australis* marsh (Emery and Fulweiler 2014), but under the low-N loads to that site minimal N₂O emission was detected. While N₂O emission in this experiment did not differ between mesocosms containing cores from the two vegetation zones, mechanisms behind observed patterns of N₂O fluxes may differ by zone. For *P. australis* but not *S. patens* mesocosms, belowground biomass was positively correlated with N₂O emission. This suggests a possible role of the *P. australis* root zones in supporting denitrifier communities responsible for N₂O production, perhaps by provision of labile organic carbon from stressed and dying plants, although nitrification and nitrifier-denitrification are also known sources of N₂O and so the observed pattern may be due to inhibition by root zone oxygenation.

N₂O fluxes from mesocosms containing cores from the two vegetation zones differed in responses to “climate change” which suggests that emissions may have been influenced by either plant, soil abiotic or microbial responses by *S. patens* mesocosms to increased temperatures and/or atmospheric CO₂. While measured soil and plant variables do not appear to explain this vegetation zone-specific N₂O response, it may be due to differences in microbial communities active in N₂O-producing pathways between the two vegetation zones that were not discerned in this study.

4.4 Future Implications for Coastal Marshes & Conclusions

Although this mesocosm design resulted in elevated salinities and vegetation responses uncharacteristic of present-day conditions at the site of vegetation core collection, plant stunting and increased soil salinities (as in Zhong et al., 2014) may represent a potential future state of coastal marshes under severe conditions of disturbance and global change. Extreme climactic events such as drought are predicted to increase with a warming climate over the next century (IPCC, 2007), as is marsh vegetation loss due to interactions of herbivory and climatic factors (Silliman et al 2005). The results of this experiment therefore provide a window into likely drivers of plant decline and GHG fluxes from marshes subjected to warming climates and anthropogenic eutrophication.

This study indicates the possibility for climate change and N enrichment to differentially affect *P. australis* and *S. patens* dominated marsh zones. Notably, under the conditions in this mesocosm experiment, “climate change” and N enrichment applied together were not observed to synergistically stimulate emission of the GHGs measured. In fact, emission of N₂O was moderated under “climate change” conditions in *S. patens* mesocosms. These results underscore the complexity of potential GHG flux responses to global change drivers. Future research should be directed to improve simulation of natural conditions and conduct field tests of global change drivers on GHG fluxes, and to better understand mechanisms (plant, microbial or abiotic soil) that influence GHG flux responses to climate change and N pollution.

Figure 1. Schematic showing the experimental design. *Phragmites* and *Spartina* mesocosms were subjected to treatments of preset-day (top) vs. year 2100 (bottom) predicted temperatures and atmospheric CO₂ concentration and ambient N(left) vs. N enrichment (right).

Figure 2. A polycarbonate chamber sealed to mesocosm pots with closed-cell foam was connected via tubing to a Picarro CRDS analyzer for measurement of real time CO₂, CH₄ and N₂O concentrations

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

	Daytime Temperature	Nighttime Temperature	CO₂ Level
Present Day	28°C	18°C	390 ppm
Year 2100	33°C	23°C	700 ppm

Figure 3. Time final CH₄ (A) and N₂O (B) fluxes from all treatment groups. “Climate change” refers to elevated temperature and atmospheric CO₂ treatments.

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	CC	N x CC
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}=42.07, p<0.01*	F _{1,18} =0.00, p=1.00	F _{1,18} =0.73, p=0.41
	<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} =0.52, p<0.48	F _{1,18} =2.51, p=0.13	F_{1,18}=16.40, p<0.01*
Salinity								
	<i>P. australis</i>	73.20 $\pm$ 10.08	58.60 $\pm$ 2.29	68.80 $\pm$ 6.76	97.00 $\pm$ 3.00	F _{1,20} =1.75, p=0.20	F_{1,20}=5.13, p<0.04*	F_{1,20}=12.74, p<0.01*
	<i>S. patens</i>	75.00 $\pm$ 9.68	67.20 $\pm$ 12.83	77.40 $\pm$ 11.23	53.60 $\pm$ 8.44	F _{1,19} =2.62, p=0.12	F _{1,20} =0.28, p=0.61	F _{1,20} =0.18, p=0.68
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								
	<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

F statistics, degrees of freedom, and significance values are reported for 2-factor (climate change simulation x N treatment) ANOVA tests “CC” refers to “climate conditions”.

* Significant at $\alpha = 0.05$

Table 3. Results of 2- and 3-factor ANOVA tests of N and climate treatments and vegetation type on CH₄ emissions

Results of 2-factor ANOVA (N x CC) for each veg. type						
		<i>N</i>		<i>CC</i>		<i>N x CC</i>
CH₄	<i>S. patens</i>	F _{1,17} =0.07, p=0.80		F _{1,17} =1.78, p=0.20		F _{1,17} =0.13, p=0.72
	<i>P. australis</i>	F_{1,19}=4.71, p=0.05*		F _{1,19} =0.57, p=0.46		F _{1,19} =2.09, p=0.17
Results of 3-factor ANOVA (N x CC x veg. type)						
	<i>N</i>	<i>CC</i>	<i>N x CC</i>	<i>Veg. Type</i>	<i>Veg. Type x N</i>	<i>Veg. Type x CC</i>
CH₄	F _{1,37} =2.57, p=0.12	F _{1,37} =4.82, p=0.04*	F _{1,37} =0.37, p=0.55	F _{1,37} =9.27, p<0.01*	F _{1,37} =1.18, p=0.29	F _{1,37} =7.27, p=0.01*
						F _{1,37} =0.02, p=0.90

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

“CC” refers to “climate conditions”.

* Significant at $\alpha = 0.05$

Appendix 1. Average soil salinity $\pm$ se from all treatments measured bi-weekly throughout the experiment

	Week 1	Week 3	Week 5	Week 7	Week 10
Control					
<i>S. patens</i>	34.40 $\pm$ 3.78	69.00 $\pm$ 10.36	60.60 $\pm$ 10.49	63.40 $\pm$ 11.45	75.00 $\pm$ 9.68
<i>P. australis</i>	16.60 $\pm$ 2.14	94.40 $\pm$ 10.85	66.00 $\pm$ 12.08	80.80 $\pm$ 9.39	73.20 $\pm$ 10.05
Nitrogen					
<i>S. patens</i>	26.20 $\pm$ 2.44	42.40 $\pm$ 5.91	53.40 $\pm$ 9.06	58.00 $\pm$ 11.37	67.20 $\pm$ 12.83
<i>P. australis</i>	15.00 $\pm$ 2.72	29.20 $\pm$ 3.90	50.40 $\pm$ 10.55	56.40 $\pm$ 10.67	58.60 $\pm$ 2.29
Climate Change					
<i>S. patens</i>	24.60 $\pm$ 4.97	50.40 $\pm$ 11.26	40.80 $\pm$ 6.00	69.40 $\pm$ 14.91	77.40 $\pm$ 11.23
<i>P. australis</i>	19.00 $\pm$ 5.61	32.80 $\pm$ 7.66	39.20 $\pm$ 5.35	68.20 $\pm$ 11.61	68.80 $\pm$ 6.76
Nitrogen + Climate Change					
<i>S. patens</i>	22.20 $\pm$ 2.08	42.00 $\pm$ 1.69	72.60 $\pm$ 7.05	68.20 $\pm$ 13.76	53.60 $\pm$ 8.44
<i>P. australis</i>	20.60 $\pm$ 4.49	33.00 $\pm$ 1.92	64.00 $\pm$ 6.55	83.80 $\pm$ 10.20	97.00 $\pm$ 3.00

Appendix 3

Appendix 3. *Spartina* and *Phragmites* mesocosm CO₂ (A) and CH₄ (B) fluxes measured on weeks 1 and 10 of the experiment.

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CHAPTER 6

CONCLUDING REMARKS: A POTENTIAL ROLE FOR *PHRAGMITES*

AUSTRALIS IN NOVEL ECOSYSTEM FUNCTION

The research presented in this dissertation sheds light on potential impacts of *Phragmites* on coastal marsh function under present-day and future conditions. In the context of the large body of literature describing responses of coastal systems to *Phragmites* invasion, these results have potential implications for management of this infamous invasive, and raise questions about the role non-native species play in maintaining ecosystem function.

As the movement of introduced *Phragmites* into North American coastal wetlands prompted research into its impacts to habitat quality and ecosystem function as well as costly (Martin and Blossey 2013) control measures, some have questioned the wisdom of attempts to eradicate it. These authors cite the plant's numerous ecosystem services (Weis and Weis 2003; Hershner and Havens 2008; Kiviat 2013), including N uptake (Mozdzer and Zieman 2010), potential for C sequestration (Windham 2001), soil stabilization (Windham and Lathrop 1999; Rooth and Stevenson 2000), and wildlife habitat provision (Parsons 2003). Some findings presented in this dissertation, that *Phragmites*-dominated marsh takes up more carbon dioxide (CO₂) per unit area than native high marsh vegetation and that its removal results in increased methane (CH₄) emission along with the loss of a CO₂ sink, join the

growing body of literature suggesting potential positive impacts of the plant on coastal marsh carbon sequestration. Studies have demonstrated the plant's substantial biomass, slow decomposition (Windham 2001), and promotion of marsh surface accretion (Rooth et al 2003), which along with abundant CO₂ uptake and possible mediation of CH₄ emissions, could enhance carbon storage.

Others, however, point to the clear negative impacts *Phragmites* has on native plant biodiversity and, as many studies suggest, on habitat value (Benoit and Askins 1999; Chambers et al 1999; Meyerson et al 2000). Martin and Blossey (2009) point out that without considerations of timescale and a clear definition of "ecosystem services", accepting the presence of invasive *Phragmites* in wetlands may be a premature decision and an ecological mistake. This call for caution in embracing invasive *Phragmites*' presence in coastal marshes is underscored by the finding presented in this dissertation that *Phragmites* stands may be associated with increased CH₄ emission relative to native vegetation, particularly under future climatic states.

Our understanding of *Phragmites*' role in coastal marsh function remains unclear, and the context in which we understand this role is changing. Under conditions of climate change, *Phragmites*' ability to thrive in coastal marshes, and therefore its ecosystem impacts, will likely be altered. *Phragmites* is a C₃ plant, and so may be better able to capitalize on increasing atmospheric CO₂ levels than its native C₄ counterparts (a phenomenon not well-represented in the mesocosm experiment presented in Chapter 5 due to artifacts limiting *Phragmites* growth). Salinity is a main constraint on *Phragmites*' spread in coastal wetlands, and recent work has

demonstrated that elevated atmospheric CO₂ concentrations and temperatures predicted by the year 2100 were associated with better salinity tolerance (Eller et al 2014). Warming temperatures, as demonstrated in Appendix 4 of this dissertation, may enhance *Phragmites* germination rate (allowing for introduction of genetic diversity via sexual reproduction) while inhibiting germination rates of native high marsh *Spartina patens*. Since recent research makes clear the important role of genetic diversity in *Phragmites*' spread (McCormick et al 2009; Belzile et al 2010), this finding may have implications for its ability to colonize coastal marshes in a changing climate. Factors co-occurring with climate change, such as increased nitrogen (N) pollution through aerial deposition (Templer et al 2012) and increasing intensity and frequency of storm events that will exacerbate pollutant-carrying runoff (IPCC 2007) will result in greater N over-enrichment of marshes. N pollution has the potential to stimulate *Phragmites* spread as well as alter coastal marsh biogeochemistry to stimulate nitrous oxide (N₂O) emission (Moseman-Valtierra et al 2011, Chapter 5 of this dissertation). Disentangling the potential detriments and benefits of *Phragmites* in coastal marshes under future states is a likely to be among the great challenges faced by coastal marsh ecologists in the coming decades.

Hobbs et al (2006) define “novel ecosystems” as ecosystems “hav[ing] species compositions and relative abundances that have not occurred previously within a given biome”. Coastal marshes in estuaries extensively impacted by human activities may be seen as such systems; these marshes support assemblages of species that did not exist prior to anthropogenic intervention, but which will likely persist far into the

future. Under conditions of climate change, the departure of these systems from their previous state is likely to be sustained and exacerbated. As a dominant species in many of these altered coastal marshes, *Phragmites* clearly plays, and will continue to play, a leading role in the functioning of these novel systems. As is underscored by the findings of this dissertation, whether *Phragmites* will serve to resurrect our drowning coastal marshes or deplete their value to wildlife is far from being resolved. What is clear, however, is the potential for an important role for *Phragmites* in mediating fluxes of greenhouse gases from coastal systems and therefore affecting coastal marshes' effects on global climate. Future research must be directed to continue elucidating the mechanisms underlying *Phragmites*' impact on coastal marsh greenhouse gas flux dynamics, as well as determining these findings' applicability over broader spatial and temporal scales.

APPENDICES

Appendices presented in this dissertation represent results of pilot tests and method development conducted as part of this work that support the objectives of the dissertation, but that were not included in the 4 main chapters prepared for publication.

APPENDIX 1

GREENHOUSE GAS FLUXES FROM *PHRAGMITES AUSTRALIS* AND NATIVE VEGETATION MARSH ZONES AT A SITE CHARACTERIZED BY SANDY SOIL

Greenhouse gas (GHG) fluxes were measured during summer 2014 from a fringing marsh vegetated by *Spartina patens* and an adjacent *Phragmites australis* stand at Passeonkquis Cove in Warwick, RI. These results were not included in Chapter 2 of this dissertation due to the dramatically different soil type at this site. Conditions at this site differ primarily from those measured at the sites referenced in Chapter 2 in the percentage of organic content of soil. At Passeonkquis Cove, soil organic concentrations (as determined by loss on ignition) did not exceed 10%, whereas organic content at other sites where GHG flux measurements were taken generally were greater than 50% (Table 1). Soil moisture was also substantially lower at Passeonkquis Cove due to the poor moisture-holding capacity of sandy soils.

Arrangements of bases for GHG flux measurement chambers at Passeonkquis Cove were similar to those at sites described in Chapter 2, with 3 bases placed at random in the *S. patens* zone and 3 pairs of bases situated toward the seaward edge of the *Phragmites* stand. *Phragmites* and *S. patens* GHG flux measurements were performed 4 and 2 times, respectively, during summer 2014 (Figure 1). Edaphic variables were measured during June. All measurements were performed within 3 hours of low tide.

Measured CO₂ fluxes at Passeonkquis Marsh (Figure 1) were within range of those measured from Fox Hill Marsh, Round Marsh, and Sage Lot Marsh as part of other experiments presented in this dissertation. CH₄ fluxes, however, were much

smaller. These findings suggest that soil organic content as an important control on CH₄ emissions, in agreement with previously published findings (Grünfeld and Brix 1999).

Table 1. Passeonkquis Cove marsh edaphic and plant variable averages $\pm$ SE measured during June

	pH	Redox potential (mV)	Soil Temp. (°C)	Soil Moisture (%)	Soil organic content (%)	Soil salinity	Live stem height (cm)	Live stems	Dead stems
<i>Passeonkquis</i>									
<i>Phragmites</i>	7.25 $\pm$ 0.27	420.00 $\pm$ 43.13	17.90 $\pm$ 0.19	38.69 $\pm$ 14.03	6.19 $\pm$ 3.34	26.00 $\pm$ 0.00	111.62 $\pm$ 9.77	15.50 $\pm$ 3.37	7.50 $\pm$ 0.35
Native Veg.	7.25 $\pm$ 0.50	213.33 $\pm$ 94.20	19.43 $\pm$ 0.04	39.89 $\pm$ 5.78	7.56 $\pm$ 1.71	26.67 $\pm$ 2.94	--	--	--

Figure 1. CO₂ (A) and CH₄ (B) fluxes measured from *P. australis* and native vegetation zones during spring and summer 2014. CH₄ emissions from the native vegetation zone were negligible during May 2014.

APPENDIX 2

EXTRACTION OF SALT MARSH SOIL DNA AND PCR AMPLIFICATION OF THE *MCRA* GENE TO TARGET METHANOGENIC ARCHAEA

Methanogens are archaea of the phylum Euryarchaeota that produce methane (CH₄) as a byproduct of energy metabolism under anaerobic conditions. Methanogenesis is a dominant process only at very low redox potentials, when more energetically efficient terminal electron acceptors than CO₂ (such as O₂, NO₃, and SO₄) have been reduced. For this reason, it is an important terminal step in organic matter breakdown in freshwater wetlands with their anoxic, reduced sediments. Typically, however, high SO₄ concentrations in salt marshes as a result of seawater presence lead to much lower levels of methanogenesis. However, in the studies presented in this dissertation, CH₄ emission was consistently observed from *Phragmites australis* stands along varying hydrological and soil salinity gradients. It was hypothesized that root-zone dynamics of *Phragmites* may support different methanogen relative to native marsh species.

The objective of this research was to use qPCR quantify copy numbers of the gene *mcrA* (Methy-coenzyme M Reductase, a proxy for methanogenic archaea) in *Phragmites australis* and native vegetation marsh zones. Since CH₄ fluxes were consistently larger in *Phragmites* zones (Chapter 2), it was thought that this phenomenon may be due to greater methanogen abundance.

Soil Core Collection, processing, and DNA extraction

Soil cores (0-4 cm) from salt marshes of varying edaphic characteristics (Round Marsh, Fox Hill, and Sage Lot) were collected using 10 mL cutoff syringes

pounded gently into soil (samples are summarized in Table 1). One sample from a depth of 30 cm was collected using a MacAulay Peat Sampler. Cores were capped with foil and placed on dry ice for transport back to the laboratory, where they were stored at -80 °C until processing. Soil cores were then sectioned at 1-cm intervals, homogenized, and stored at -20 °C. Soil DNA was extracted using a PowerSoil[®] DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's protocol.

Amplification of mcrA to target methanogenic archaea

The primer pair ME1 and ME2 (Hales et al 1996) was used for attempted amplification of *mcrA* from collected, sectioned samples. For a positive control, frozen methanogen culture was used. PCR reactions of 25 µL contained 8 µL 5Prime Master Mix (5Prime, Inc., Gaithersburg, MD), 0.4 µL each forward and reverse primer, 1 µL DNA, and 10.2 µL PCR water. DNA concentrations ranged from approximately 2-20 ng/µL. Positive controls contained 11.2 µL water and were spiked with methanogen culture using a sterile pipette tip. Negative controls contained water in place of DNA. Reaction conditions were as follows: initial denaturation at 94 °C for 5 min, 30 cycles of 94 °C for 40 s, annealing at 50 °C for 1.5 min, and extension at 72 °C for 3 min, followed by a final extension at 7 °C for 10 min. PCR products were tested for amplification success by electrophoresis on a 1% agarose gel at 140V for 40 minutes.

Troubleshooting

Amplification of *mcrA* was consistently achieved with the positive control, but never for extracted DNA from soil samples. To test samples for lack of viability, PCR was performed on a subset of samples' extracted DNA using universal primers B27F and U1492R. Reaction conditions were: initial denaturation at 95 °C for 10 min, 30 cycles of 95 °C for 1 min, annealing at 53 °C for 1 min, and extension at 72 °C for 1 min, followed by a final extension at 7 °C for 7 min. Gel electrophoresis indicated successful amplification.

To test for inhibitors to *mcrA* amplification in extracted soil DNA, soil samples were spiked with a few µL of the methanogen culture used as a positive control and homogenized. DNA was extracted as described previously and PCR using ME1 and ME2 was performed. Electrophoresis indicated amplification of *mcrA* from samples enriched with positive controls but not from unenriched samples.

It was concluded that *mcrA* DNA was likely present in low quantities in our samples and so could not be amplified for further analysis.

Table 1. Summary of samples for which *mcrA* amplification was attempted

Site	Plot ID	Depth (cm)
Fox Hill	1	0-1
Fox Hill	1	3-4
Fox Hill	2	0-1
Fox Hill	2	3-4
Fox Hill	3	0-1
Fox Hill	3	3-4
Sage Lot	1	0-1
Sage Lot	1	3-4
Sage Lot	2	0-1
Sage Lot	2	3-4
Sage Lot	3	0-1
Sage Lot	3	3-4
Round Marsh (Ref.*)	3	0-1
Round Marsh (Ref.)	3	3-4
Round Marsh (Ref.)	4	0-1
Round Marsh (Ref.)	4	3-4
Round Marsh (Ref.)	6	0-1
Round Marsh (Ref.)	6	3-4
Round Marsh (Rem.**)	1	0-1
Round Marsh (Rem.)	1	3-4
Round Marsh (Rem.)	2	0-1
Round Marsh (Rem.)	2	3-4
Round Marsh (Rem.)	3	0-1
Round Marsh (Rem.)	3	3-4
Fox Hill	"Deep" Core	30

* *Phragmites* stand left intact ("Reference")

***Phragmites* stand cleared by mulching ("Removed")

APPENDIX 3

COMPARISON OF GREENHOUSE GAS FLUXES FROM *PHRAGMITES AUSTRALIS* AND *SPARTINA PATENS* PLANTS GROWN FROM SEED IN POTTING MIX AND TESTS OF ABOVEGROUND VEGETATION CLIPPING

In field investigations, testing direct effects of vegetation on greenhouse gas (GHG) fluxes is challenging due to confounding environmental variables. Likewise, testing direct effects of vegetation on GHG fluxes is complicated by dynamic field environments. With the aim of (1) comparing GHG fluxes from *Phragmites australis* and native high marsh *Spartina patens* vegetated soil in a controlled setting and (2) testing effects of aboveground vegetation removal on GHG fluxes, the two species were grown from seed in containers of MetroMix 510[®] potting mix (Sun Gro Horticulture, Agawam, MA). Approximately 45 seeds per species were sown in 20 cm diameter containers (n=5 containers per species). Placement in a growth chamber (set at the ambient conditions described in Chapter 5) kept temperature, light, and humidity constant for all replicates.

After about 7 weeks of growth, GHG flux measurements were performed before and immediately after clipping aboveground vegetation. This design was employed with the intent of determining whether the presence of aboveground vegetation affected GHG fluxes. Fluxes were measured as described in Chapter 5. Temperatures during measurements were consistent and ranged from 22-24 °C. Above and belowground biomass were harvested, washed, dried in a vented oven and weighed.

Biomass at 7 weeks was substantially greater for *S. patens* than for *Phragmites* (Table 1). Both CO₂ emission ($t_{16}=3.48$, $p=0.003$) and CH₄ uptake ($t_{16} = -2.97$, $p=0.009$) were significantly greater in *S. patens* relative to *Phragmites* pots. Removing aboveground vegetation did not affect fluxes of either GHG for either plant species. Both above- ($t_{5.8}=4.39$, $p=0.005$) and belowground ($t_{5.92}=3.94$, $p=0.008$) biomass were greater in *S. patens* pots.

GHG fluxes from these vegetated mesocosms displayed the opposite pattern typically observed during growing season field measurements; CO₂ was emitted (indicating more respiration than photosynthetic uptake) and CH₄ was taken up (indicating methanotrophy) (Figure 1). This finding could be due to greater biomass of *S. patens* in this experiment. In the field, *Phragmites* is generally more massive than high marsh native grasses. Greater belowground biomass of *S. patens*, in the case of this experiment, could support greater densities of microorganisms active in CO₂ production and/or CH₄ oxidation.

The lack of an immediate effect of aboveground vegetation removal on GHG fluxes is consistent with results of the field clipping experiment described in Chapter 3 of this dissertation.

These results indicate a strong role of plant species as a control on CO₂ and CH₄ fluxes. Further, they suggest that these effects are due either to belowground biomass processes or to indirect mechanisms (plant impacts to soil).

Table 1. Above- and belowground biomass dry weights for *Phragmites* and *S. patens*

	Aboveground biomass (g)	Belowground biomass (g)
<i>Phragmites</i>	0.45 ± 0.42	0.19 ± 0.18
<i>S. patens</i>	4.42 ± 0.75	2.05 ± 0.43

Figure 1. CO₂ and CH₄ fluxes from *Phragmites* and *S. patens* grown in potting mix, before and after clipping aboveground vegetation.

APPENDIX 4

EXPERIMENTAL WARMING INCREASED GERMINATION RATES FOR *PHRAGMITES AUSTRALIS* WHILE DECREASING GERMINATION RATES FOR *SPARTINA PATENS*

Introduction

Phragmites' success in coastal marshes has been attributed to its competitive ecophysiological traits (Lambert et al., 2010) as well as anthropogenic impacts including increased nitrogen (N) availability (Mozdzer and Zieman, 2010) and disturbance (Minchinton and Bertness, 2003; Silliman and Bertness, 2004). Although clonal spread has been the primary mechanism by which this C3 grass has been thought to enter coastal wetlands (Amsberry et al., 2000; Saltonstall, 2002), recent research in brackish Chesapeake Bay marshes indicates that *Phragmites* populations may expand predominantly by seed (McCormick et al., 2009). *Phragmites*' ability to reproduce sexually when favorable conditions are available and the resulting introduction of genetic diversity into their populations may ultimately affect their ability to adapt to environmental stressors, with implications for the species' invasiveness.

Factors known to affect *Phragmites* germination include soil salinity (Wijte and Gallagher, 1996) and magnitude and number of diurnal temperature fluctuations (Ekstam et al., 1999), with the most germination occurring under conditions of low salinity and large, frequent diurnal temperature fluctuations. While fewer studies of tested effects of overall temperature increases on *Phragmites* germination, a

greenhouse experiment in Australia found that *Phragmites* germination was decreased and delayed by an increase of 5°C.

As *Phragmites* enters coastal marshes, it displaces high marsh native species assemblages commonly dominated in northeastern North America by the C4 grass *Spartina patens*. Less research has focused on germination dynamics of *S. patens*, a species described as reproducing primarily by vegetative means (Hester et al., 1994). However, the plants' life history does include sexual reproduction (Lonard et al., 2010). No known studies have tested effects of temperature increases on *S. patens* germination.

Impacts of rising temperatures on germination rates of *Phragmites* and *S. patens* could impact the plants' life histories in ways that could either facilitate or inhibit the spread of *Phragmites* into *S. patens* dominated high marsh ecosystems. Therefore, the objective of this research was to test the effect of increased temperatures within the range predicted to result from climate change by the year 2100 (IPCC, 2013) on germination rates in invasive *Phragmites australis* and the native high marsh perennial grass *S. patens*.

Methods

Vented growth chambers (as used in Chapter 5) were used to test the effect of elevated temperatures predicted to be associated with climate change on germination rates of *Phragmites* and *Spartina patens*. A temperature increase of 5 °C was chosen to simulate climate warming during this century, as this increase represents the median

of the upper bounds of the lowest and highest model-predicted temperature increase ranges summarized in the IPCC 2007 Fourth Assessment (IPCC, 2007). For the present-day temperature (control) treatment, the daytime temperature in the growth chamber was set to 27.8°C, the average high July temperature in Rhode Island (NOAA). The nighttime temperature was set to 17.8°C, the average low July temperature in Rhode Island (NOAA). For end-of-century temperature (warmed) mesocosms, the daytime temperature was set to 32.8°C and nighttime temperature was set to 22.8°C. Atmospheric CO₂ and humidity were kept at ambient levels in the two chambers, and daylight simulation imitated daytime in mid-summer with 15 hours of light and 9 hours of darkness. Seeds of each species were sown on the surface of clean, sieved Metro Mix 510 commercial potting mix in 8” diameter nursery pots. Exactly 30 seeds were placed in each pot (n = 10 pots for each species for controlled and warmed treatments). *Phragmites* seeds were harvested by the University of Rhode Island Meyerson Lab from a population of invasive *Phragmites* in NY, and *S. patens* seeds were purchased from a nursery (Environmental Concern, Inc.). Seeds had been refrigerated for at least 3 months prior to the beginning of the experiment. Seedlings were counted for two weeks after first germination and were misted twice daily with tap water as necessary to keep moist. Two-factor ANOVA (species x temperature simulation) and post-hoc Tukey HSD was used to test treatment effects on germination rate. Statistical analyses were performed in JMP 10.0 and interpreted at a significance level of $\alpha = 0.05$.

Results & Conclusions

S. patens and *Phragmites* germination rates were about 25%-35% and 5-20% respectively (Figure 1). While reported germination rates for *S. patens* could not be found in the literature, germination rates for *Phragmites* were generally comparable to those reported from stands in the Chesapeake Bay (Kettenring and Whigham, 2009).

There was significant interaction between species and temperature simulation ($F_{1,39} = 26.54$, $p < 0.01$) (Figure 1), with *Phragmites* and *S. patens* germination rates responding very differently to increased temperature. *Phragmites* germination rate more than doubled under increased temperature conditions, while *S. patens*'s germination rate was significantly reduced. While control temperature conditions *S. patens*'s germination rate outpaced *Phragmites*' by a factor of 6, under warmed conditions, germination rate did not differ significantly between the two species.

Differences in the 2 species' responses to temperature increases could be due either to differences in physiological response to air and soil temperature or to secondary effects such as drier soils (although soil moisture was not measured) under elevated temperatures. Therefore, future experiments should account for soil moisture differences under varying temperature conditions. Differences in source population locations and seed storage conditions also may confound experimental outcomes. In the case of this experiment, seeds from the 2 species were acquired from different geographic regions and were not stored for the same duration. Future work should test responses of seeds collected from source populations inhabiting a common region, and should harvest and store seeds under the same conditions prior to experimentation.

In conclusion, temperature increases predicted to occur by the year 2100 were observed to significantly increase germination rates in *Phragmites* but to decrease germination rates in *S. patens*. Different germination rate responses to rising temperatures by these two species may have impacts on the species' abilities to adapt to environmental changes, and ultimately plant community composition in coastal marshes. While these findings do not account for the effect of climate change-associated impacts aside from rising temperatures or for potential population genotype-level differences within the species studied, they suggest that the responses of invasive *Phragmites* and native *S. patens* germination to climate change may vary, and that this phenomenon merits further investigation.

Figure 1. *S. spatens* and *Phragmites* germination rates (out of 30 seeds) under present day and year 2100 predicted day and night temperatures. Letters represent results of post-hoc Tukey's HSD tests. Bars not connected by the same letter are significantly different.

APPENDIX 5

DNA EXTRACTION, PCR, AND SEQUENCING PROTOCOLS FOR HAPLOTYPING *PHRAGMITES AUSTRALIS* FROM FOX HILL AND ROUND MARSH SITES

North American *Phragmites australis* populations are highly diverse.

Phragmites diversity in North America has recently been shown to be the result of multiple invasions (Meyerson et al 2009b), long-distance dispersal (Lambertini et al 2012), and hybridization (Meyerson et al 2009b; Lambertini et al 2012) between various *Phragmites* genotypes. In addition, while *Phragmites* was thought to spread predominantly via clonal reproduction, outcrossing and reproduction by seed are now understood to be important reproductive mechanisms (McCormick et al 2009).

Soil microbe communities are known to be plant species-specific (Bertin et al 2003; Ravit et al 2003). Even different genotypes of the same plant species (in this case, *Phragmites australis*) have been shown to exhibit distinct rhizosphere microbial communities when grown in a greenhouse environment (Meyerson and Bowen, *unpublished data*).

Soil microbial communities are responsible for critical nutrient cycling processes within wetland systems including nitrogen fixation, denitrification, and methanogenesis (Lovell 2005), and invasive species-induced changes in microbial community composition may impact these functions (Fu and Cheng 2002). Plant rhizospheres act as important microhabitats for microbes through provision of organic substrates (Lovell 2005) and oxygenated zones (Armstrong et al 1992). Therefore,

differences in soil microbial assemblages between vegetation communities dominated by different species, lineages or genotypes could putatively scale up to differences in ecosystem-level biogeochemical function.

At the Round Marsh study site (where research for Chapters 2 and 4 was conducted), 2 *Phragmites* stands, phenotypically resembling the common introduced haplotype M lineage (described in Chapter 1 of this dissertation, pp. 4-5), are present within the marsh system. Since greenhouse gas fluxes and edaphic conditions were compared between the 2 stands in Chapter 4, plants from the 2 stands, as well as from a nearby stand (Fox Hill) were haplotyped in accordance with previously established protocols (Saltonstall 2002) to ensure that plants were members of a common haplotype.

Sample collection

One leaf was collected from each *Phragmites* plant over 1m tall in each of 3 plots (the area within GHG sampling bases) per stand. Plots were located approximately 15-30 m apart. Tissue samples were placed in ziplock bags with desiccant packets.

DNA Extraction and Cleanup

One dry leaf tissue sample was chosen at random for each plot. Samples were ground in UV-sterilized mortars and pestles with liquid nitrogen and sterile fine sand. DNA was extracted using an E.Z.N.A.[®] SP Plant DNA Kit (Omega Bio-Tek,

Norcross, GA). Extracted DNA was purified according to the manufacturer's protocol using an UltraClean[®] PCR Clean-Up Kit (MoBio Labs, Carlsbad, CA) prior to PCR amplification.

PCR Amplification and gel electrophoresis

Two non-coding regions of chloroplast DNA were amplified based on protocols presented in (Saltonstall 2002). The primer pair trnT-trnL was used to amplify a 1200 bp region, and the primer pair rbcL-psaI were used to amplify an 80 bp region. PCR reactions (20 μ L) contained 8 μ L 5Prime Master Mix (5Prime, Inc., Gaithersburg, MD), 1 μ L of each primer (10 μ M), and 1 μ L DNA (concentrations ranged from approximately 25-220 ng/ μ L).

PCR products were purified according to the manufacturer's protocol using an UltraClean[®] PCR Clean-Up Kit (MoBio Labs, Carlsbad, CA) prior to PCR amplification.

PCR products were run for 40 minutes on a 1% agarose gel prepared with Tris-Acetate-EDTS buffer with ethidium bromide to check for amplification. For most reactions using the rbcL-psaI primer set, strong bands indicated successful amplification. For the trnT-trnL primer set, however, only very faint bands were present.

Sequencing Preparation

DNA concentrations of all PCR products were determined using a NanoDrop 8000 Spectrophotometer (Thermo Scientific, Wilmington, DE).

Each 12 μ L sequencing reaction contained 2.5 ng template DNA (calculated based on NanoDrop-indicated DNA concentrations) and 2 μ L of 2.5 μ M stock primer in PCR grade water. Reaction conditions were as follows: an initial denaturation step of 94°C for 3 min; 40 cycles of initial denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, and extension at 72°C; and a final extension step of 72°C for 60 min.

Sequencing was performed in the URI Genomics and Sequencing Center with an Applied Biosystems® 3130xL Genetic Analyzer (Thermo Scientific, Wilmington, DE).

Sequence Analysis

Sequences were inspected and edited using FinchTV software (Geospiza, Inc., Seattle, WA). The *rbcL-psaI* primer pair yielded usable sequences, while the *trnT-trnL* pair did not. Sequences were trimmed (to a length of 733 bp, based on the shortest sequence) and aligned in CLC Main Workbench (CLC Bio, Boston, MA). Alignment of sequences revealed a 100% match between sequences, suggesting that all plants were members of a common haplotype.

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