ABSTRACT

Title of Document: **EFFECTS OF *PHRAGMTES AUSTRALIS* (COMMON REED) INVASION ON NITROGEN CYCLING, POREWATER CHEMISTRY AND VEGETATION STRUCTURE IN A BRACKISH TIDAL MARSH OF THE RHODE RIVER, MARYLAND.**

Justin Eric Meschter, Master of Science, 2015

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Phragmites australis is one of the most widespread invasive species in wetland habitats of North America. Conversion of existing wetland ecosystems to *Phragmites*-dominated communities decreases overall plant diversity and alters biogeochemical cycles, which can negatively affect ecosystem processes. Previous studies demonstrated that *Phragmites* has a significantly greater above-ground nitrogen demand than native plants, likely due to its greater biomass. To evaluate how invasion by *Phragmites* alters standing stock nitrogen, I measured above- and below-ground biomass and nitrogen stocks in both the invasive and native plant communities to examine how *Phragmites* is meeting its documented increased nitrogen demand in the Rhode River, a sub-estuary of the Chesapeake Bay in Edgewater, Maryland. I also quantified deep N uptake using a ^{15}N tracer study. I

found that *Phragmites* roots significantly deeper than native marsh grass communities and has the ability to utilize deeper nitrogen pools and take up nitrogen from deeper depths. This enhanced rooting structure gives the invasive *Phragmites* the ability to potentially access lower salinity water, as well as tap nutrients unavailable to native marsh plant communities.

EFFECTS OF *PHRAGMTES AUSTRALIS* (COMMON REED) INVASION ON NITROGEN CYCLING, POREWATER CHEMISTRY AND VEGETATION STRUCTURE IN A BRACKISH TIDAL MARSH OF THE RHODE RIVER, MARYLAND.

By

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Thesis submitted to the Faculty of the Graduate School of the
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Dedication

This thesis is dedicated to my family, for all of their love and support during my studies. The encouragement and support was never ending, and a vital part of my enthusiasm throughout. In particular, I would like to dedicate this work to my grandmother, Joan Meschter “Nana”. She passed on her love of nature and the outdoors to me at a young age, which played a large part in my interests and career path. She is always up to speed on my studies and always interested, so I would like to dedicate this thesis to her, along with the rest of my family.

Acknowledgements

I would like to thank several groups of people for the tremendous support, guidance and help during my studies at The University of Maryland. I want to thank the members of my committee: Andrew Baldwin, Thomas Mozdzer, Patrick Megonigal and Martin Rabenhorst for their continued mentoring, and contributions they made to my work. I would also like to thank the entire Biogeochemistry Laboratory at the Smithsonian Environmental Research Center for help in both the lab and field over the last three years. Most importantly I would like to thank my family and friends for the continued support and patients. My research was supported by the Maryland Sea Grant Fellowship Program and the Smithsonian Fellowship Program.

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Chapter 1: Introduction to Thesis

Invasive species are a major threat to native plant biodiversity and ecosystem services worldwide (Mack et al., 2000). Wetlands are particularly susceptible to invasion because they are dynamic ecosystems characterized by frequent changes in flooding, sedimentation rates, debris deposition, and salinity (Zedler and Kercher, 2004). These changes and disruptions affect resource availability or space, allowing new species to successfully establish. The novel traits that often accompany plant invasion directly affect a wide range of ecosystem properties such as light availability, litter quality and primary production, which in turn cause indirect changes in microbial community structure and processes such as decomposition rates. The ecosystem effects of novel plant species can equal or exceed those of perturbations caused by eutrophication or climate (Ehrenfeld and Scott, 2001). Although studies have tended to consider such perturbations separately, interactions among global change factors are expected. It is presently unclear how invasive plant species will react to elevated CO_2, accelerated sea level rise, or increased nutrient loading in coastal wetland systems. The specific goals of my research were inspired by the fact that understanding the interactions among global change factors requires significant insights into how various species acquire and compete for resources.

The introduced invasive common reed, *Phragmites australis* (Cav.) Trin. ex Steud. (hereafter *Phragmites*) is a densely growing, large perennial grass that has been shown to alter biogeochemical processes and nutrient cycling in marsh ecosystems (Chambers, 1997). The native lineage of *Phragmites* has been in a slow

decline and has disappeared from much of its historical range (Meyerson et al., 2010), while the non-native (invasive) lineage is by far the most common lineage presently in North America (Saltonstall and Stevenson, 2007, Lambert et al., 2010). In comparison to native herbaceous plant communities in tidal wetlands, introduced *Phragmites* stands are typically larger in stature, more dense and more productive (Meyerson et al., 2000, Mozdzer and Megonigal, 2012, Mozdzer et al., 2013), often growing in monoculture. As a result, conversion from a native marsh community to a *Phragmites*-dominated system tends to raise reduction-oxidation potentials, deepen water tables (Windham and Lathrop, 1999), increase soil surface elevation (Chambers, 1997), alter porewater chemistry (Meyerson et al., 1999) and increase methane emissions (Mozdzer and Megonigal 2013). Conversion of native wetland plant communities to *Phragmites*-dominated communities also decreases overall plant diversity, which has a direct impact on several ecosystem services such as a reduction in avian and animal habitat, leading to a reduction in animal assemblages (Chambers et al., 1999).

Nitrogen (N) cycling within marsh systems is largely driven by internal plant and microbial transformations, with lower rates of N inputs and exports into and out of the system (Bowden, 1987, White and Howes, 1994). Because tidal marshes tend to be nitrogen-limited systems, increased nitrogen inputs from anthropogenic sources is expected to impact existing pools and M cycles. *Phragmites* has an unusually high N demand (Chambers, 1997, Meyerson et al., 2000, Mozdzer et al., 2010, Mozdzer et al., 2013), making it likely that increased anthropogenic N pollution alone cannot explain the proliferation of the invasive *Phragmites* in an otherwise closed system,

and that other changes within the system must take place to meet the increased N demand. The increased demand for N must be met by either an increase in N from an import process (N fixation, atmospheric deposition, uptake from the water column), from an increase in N transformations within the system, or access to deeper nutrient pools and N-rich porewater that is unavailable or inaccessible to native communities. Without such perturbations of the N cycle, N-limited systems would not be able to support the higher N demand of boveground biomass caused by a species shift from native plant species to invasive *Phragmites australis* monocultures.

Currently, tidal marsh systems are viewed as sinks for excess nutrients, such as N, due to the large biomass stocks and slow decomposition rates. With the invasion of *Phragmites,* I observed a shift in the cycling of N and a potential release of carbon, facilitated by changes to soil and porewater characteristics. Previous studies indicate that *Phragmites* invasion draws down porewater N concentrations and increase soil N mineralization rates (Chambers, 1997, Windham and Ehrenfeld, 2003), while producing litter that has a slower decomposition rate than native vegetation (Findlay et al., 2002). In this study, I aim to examine several potential biogeochemical changes that may result from the invasion of *Phragmites* in to tidal marsh systems in a sub-estuary of the Chesapeake Bay, Maryland. The objectives of my thesis project were:

1. Quantify the porewater N, soil-extractable N, resin-extractable N, and biomass standing stock nitrogen pools in order to compare the availability of N in invasive versus native plant communities.

2. Compare the rooting depth distributions of *Phragmites* and native plant communities. Rooting depth profiles may explain how the invasive species is circumventing competition and accessing N pools that are not available to native communities.
3. Examine N uptake by the native and introduced plant species. Does a deeper rooting profile lead to N uptake at deeper depths by the introduced species?
4. Compare porewater chemistry (sulfide, sulfate, chloride, pH, and methane) between the native and invasive plant communities in order to determine how a species alters soil biogeochemical cycles.
5. Quantify potential changes in methane production and emissions from native and invasive plant communities in order to determine whether a species shift has increased greenhouse gas emissions from a system that has traditionally been categorized as a carbon sink.

These objectives support my goals to understand: 1) how species shifts alter tidal marsh ecosystem N pools, 2) where invasive *Phragmites* is gaining access to N that supports greater N demand, and 3) how *Phragmites* affects soil biogeochemical cycles. Collectively, this work and previous studies will provide insights on the mechanisms by which *Phragmites* invasions alter tidal marshes, which provide important ecosystem services.

Chapter 2: Nitrogen uptake by deeply rooting *Phragmites australis* to meet greater aboveground nitrogen demand compared to native plant communities

Abstract

Invasive species threaten plant biodiversity and native ecosystems worldwide. Common reed, *Phragmites australis*, is one of the most widespread invasive species in wetland habitats of North America. Conversion of existing wetland ecosystems to *Phragmites*-dominated communities decreases overall plant diversity and alters biogeochemical cycles, which can negatively affect ecosystem processes. When compared to native plant communities, *Phragmites* has a well-documented greater above and belowground productivity, which results in a significantly increased nitrogen demand. However, it is unclear how *Phragmites* persists in nutrient-limited systems when faced with limited N-availability. I hypothesized that deep rooting is a mechanism by which *Phragmites* can access deep N pools circumventing nutrient competition and that deep N access may be the "missing N" needed to satisfy the well-documented N demand of this invasive plant. To evaluate the importance of deep rooting, I first examined N pools and rooting depth profiles to 3 meters in depth in both *Phragmites*-dominated and native plant communities in two brackish marsh systems in the Rhode River sub-estuary of the Chesapeake Bay, in Maryland. I also conducted a ^{15}N uptake study at three depths, up to 80 cm below the soil surface, to evaluate the contribution of deep N uptake. I report that introduced *Phragmites* has 6 to 8 times the above-ground standing stock N, 2 to 3 times the total belowground

biomass, and roots up to 3 times deeper than native plant communities at depths exceeding 3 meters. Our ^{15}N tracer demonstrated that *Phragmites* is capable of utilizing N pools from 20, 40 and 80cm depths, whereas native N uptake in the plant communities was constrained to the upper 20cm. Our data suggest that deeper rooting is a mechanism by which *Phragmites* can access previously buried N pools needed to satisfy its high N demand and fuel invasion in low nutrient ecosystems.

Introduction

Invasive species are a major threat to native plant diversity and ecosystem services worldwide (Mack et al., 2000). Plants must be able to compete successfully for N in order to persist in communities, and have evolved a wide variety of traits and strategies for doing so (Grace, 2012). Plant demand for nitrogen (N) typically far exceeds supply, making N the foremost nutrient that limits productivity in most terrestrial wetland ecosystems (Valiela et al., 1973, Bedford et al., 1999). The physiological, morphological and life history strategies implemented by plants to compete for N, P, water and other resources drive biogeochemical cycles, and is ultimately expressed as a variety of ecosystem services such as N burial or denitrification (Neubauer et al., 2005).

This ecological relationship between plant traits and element cycles means that changes in plant community composition will likely alter soil biogeochemical cycles. Invasive species provide one of the more dramatic examples of this linkage because they often introduce both novel plant traits and simultaneously come to dominate the ecosystem. The introduced lineage of the common reed, *Phragmites australis* (Cav.) Trin. ex Steud. (hereafter *Phragmites*) is a densely growing, large,

invasive perennial grass that can significantly alter biogeochemical processes and nutrient cycling in marsh ecosystems. In comparison to native herbaceous plant communities in tidal wetlands, introduced *Phragmites* stands are typically larger in stature, more dense and more productive (Meyerson et al., 2000, Mozdzer and Megonigal, 2012, Mozdzer et al., 2013), often growing in monoculture. The present study sought to investigate the influence of non-native lineages of *Phragmites australis* on soil N distribution in a tidal wetland ecosystem.

Species composition shifts can alter net primary production with direct consequences for nutrient pools and nutrient cycling. Nitrogen is a macro nutrient that regulates wetland plant growth (Odum, 1988). Wetland N cycling is largely regulated by biomass uptake and microbial recycling (Hopkinson and Schubauer, 1984). Nitrogen can be retained within the system through plant uptake, microbial immobilization and deep sediment burial (Neubauer et al., 2005). Early studies have demonstrated that *Phragmites* has a higher standing stock biomass and nitrogen content than many marsh plant species including *Typha angustifolia* (Findlay et al., 2002), *Spartina pectinata* (Rickey and Anderson, 2004), *Phragmites australis ssp. americanus* (Mozdzer et al., 2013), *Spartina patens (Meyerson et al., 2000)*, and *Schenoplectus americanus* (unpublished data), and therefore a higher demand for N than these native plant species (Windham and Ehrenfeld, 2003, Mozdzer et al., 2010). To the extent that *Phragmites* can meet this higher demand, it may reduce the availability of nitrogen to native species. The greater nitrogen demand must be met by either an increase in nitrogen from an import process (N fixation, atmospheric deposition, uptake from the water column), from an increase in N transformations

within the system, or accessing nutrient pools previously unavailable or accessible to native communities. Introduction of an invasive species into a system can place stresses on nutrient acquisition in native plants. Species that are able to uptake nutrients and avoid competition, can utilize energy for other purposes such as production of aboveground biomass.

Changes in plant community composition can alter root depth distributions in brackish tidal wetlands. Native plant species in brackish tidal wetland (*Spartina patens, Schenoplectus americanus* and *Distichlis spicata*) are typically shallow-rooted with a majority of the belowground biomass concentrated in the upper 30 cm of the soil profile, sharply decreasing with depth (Saunders et al., 2006). Due to the shallow rooting profiles in native wetland plant communities, few studies have examined rooting profiles or the influences of plant roots below a depth of 30 cm. However, changes in plant community composition by novel invaders may change root depth distributions and associated biogeochemical processes. For example, *Phragmites* belowground biomass has been shown to be significantly greater than several native plants species in the upper 50 cm of the soil (Windham, 2001), including *Spartina patens* (0.40 m), *Schenoplectus americanus* (0.65 m) (Saunders et al., 2006), *Spartina alterniflora* (0.50 m)(Blum, 1993) and *Typha angustifolia* (0.50 m)(Templer et al., 1998). Deeper rooting profiles may allow communities to access new nutrient-rich soil pools, circumvent nutrient competition and access deeper water sources of lower salinty (Lissner and Schierup, 1997). Deep rooting may also provide a source of carbon for microbial communities (Raich and Nadelhoffer, 1989), increase redox

potentials at deeper depths (Armstrong et al., 1996) and increase overall plant biomass.

With the invasion of *Phragmites*, it is unclear as to how the changes caused by this novel plant species will affect the capacity of wetland ecosystems to continue to be sinks for excess nutrients. If the nitrogen obtained by *Phragmites* is from an anthropogenic or external source, it may be bound up in the vegetation or soils and the system will be a nitrogen sink.

In this study I aimed to compare plant community rooting depth, nitrogen pools, and plant uptake of nitrogen between the invasive *Phragmites* and native plant communities. My goal was to better understand the mechanisms used by *Phragmites* to meet its high demand for nitrogen. I hypothesized that deeper rooting profiles are providing access to deep, nutrient rich porewater, allowing *Phragmites* to access nitrogen not available to native species, allowing them to meet a higher nitrogen demand. These deeper pools of nitrogen are inaccessible to native plant species, further promoting *Phragmites* invasion in some wetland ecosystems.

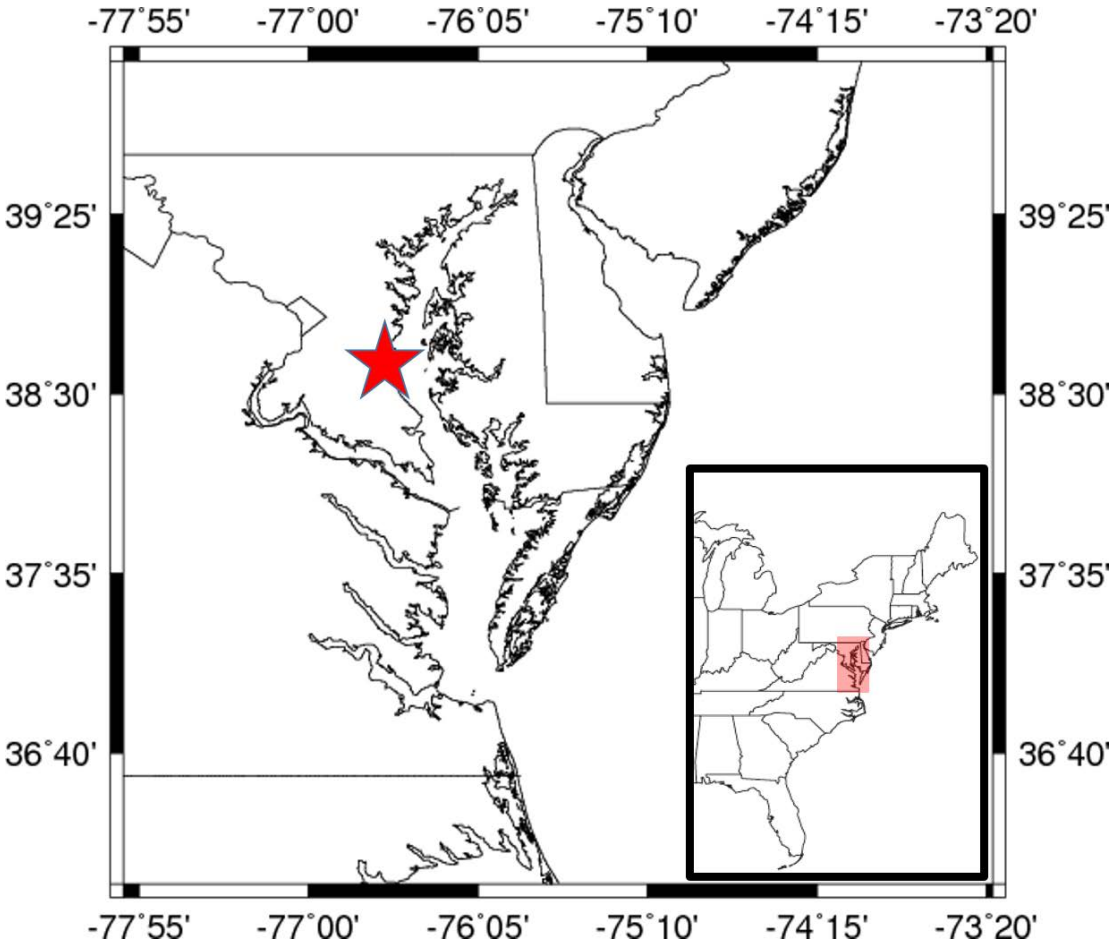
Methods

Study Site

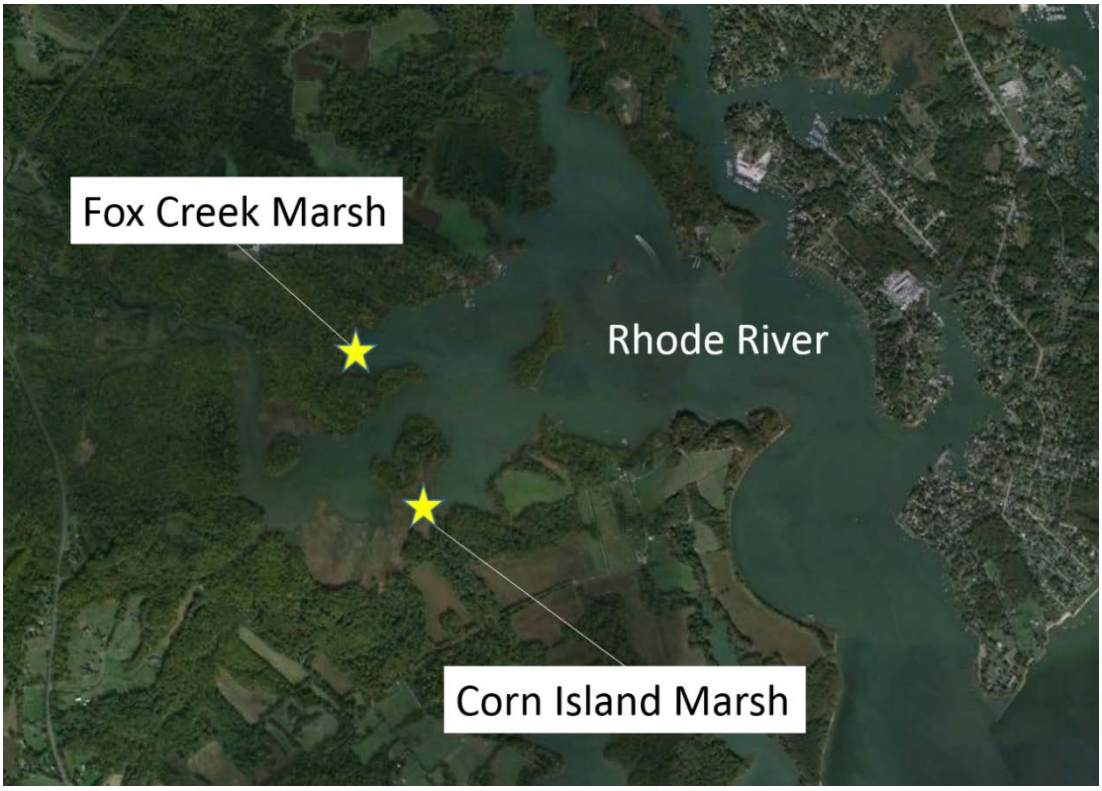
Two field sites were selected in brackish tidal marshes of the Smithsonian Environmental Research Center to investigate belowground difference between native marsh communities and areas that have been invaded by the invasive lineage of the common reed *Phragmites australis*. Fox Creek and Corn Island marshes are within the Rhode River sub-estuary the Chesapeake Bay, in Edgewater, MD, USA (Figure 1,

Map 2). At Fox Creek, the predominant vegetation type is a mixture of C_4 grasses *Spartina patens* and *Distichlis spicata*, and the invasive lineage of *Phragmites australis*. In contrasts, at Corn Island, there is a mixed plant community containing *Spartina patens* and the C_3 sedge *Schoenoplectus americanus*, which is likewise being invaded by *Phragmites australis*. These sites were selected based on the inclusion of a well-developed stand of *Phragmites australis* and a well-established native community typical of brackish wetland systems. The *Phragmites* communities at both sites were established sometime between 1970 and 2007, based on a regional survey of the Rhode River (McCormick et al., 2010). The mean tidal range is 44 cm and the average salinity is 10 part per thousand (ppt) but ranges from 4 to 15 ppt seasonally(Langley et al., 2009).

Soils at both sites were highly organic (%OM > 30%), but bulk density and organic matter content was more uniform with depth at the Corn Island site than the Fox Creek site. At Corn Island, both plant communities had peaty soils with a histic epipedon and were organic matter content ranging from 42% at the soil surface to >70% from 50-100 cm depth (Appendix 1). Soils in the Native community at Fox Creek were similar to those at Corn Island, but soils in the Invasive community at Fox Creek had comparatively high bulk density and low organic matter content (<16%) from 20-60 cm depth (Appendix 1) and would likely be classified as a thapohistic sulfaquent (M. Rabenhorst, Per. Comm.). Below the 1 m depth both sites were highly organic, with organic matter contents >50% (Appendix 2).



Map 1. Study site along the Chesapeake Bay, on the Rhode River in Edgewater Maryland.



Map 2. Fox Creek Marsh and Corn Island Marsh study sites located on the Rhode River, a sub-estuary of the Chesapeake Bay in Edgewater Maryland. Photo curtesy of Google Earth.

Aboveground Biomass and Standing Stock Nitrogen

Aboveground biomass was sampled in August 2012 and 2013 (Appendix 3), corresponding to peak biomass period for both *Phragmites* and native communities (Windham and Lathrop, 1999). Aboveground biomass in both communities was destructively harvested using clip plots (0.25m x 0.25m) that were subsequently dried (60^{o}C) to constant mass and weighed for total aboveground biomass by plant community. Additionally, twenty *Phragmites* stems of various sizes were randomly collected throughout the stand and separated by plant tissue type (leaf, stem, and flower), dried and weighed to calculate the proportion of the total biomass each tissue type comprises to more accurately assess total standing nitrogen.

N content was determined by plant species and tissue type on dried samples that were first homogenized using an (SPEX SamplePrep 8000D Mixer Mill, NJ, USA) ball grinder and analyzed for C and N content on an EAI CE-440 Elemental Analyzer (Exeter Analytical, INC, North Chelmsford, MA). Plant tissue nitrogen content and total plot-wise biomass was used to calculate standing stock plant nitrogen above and belowground (belowground biomass collection method described below "root depth distributions) in both communities (Windham and Ehrenfeld, 2003).

Porewater Nitrogen

Five cm diameter soil cores (n=2 for each community) were taken to a depth of 3 meters, or deeper, depending on depth of refusal using a barrel corer (appendix table 2) with added handle extensions. Cores were sectioned in 5cm sections in the first 30cm, 10cm increments from 30-100cm depth, 20cm increments from 1m to 2m depth and 25cm increments at depths beyond 2m. Core sections were immediately wrapped in plastic to limit exposure to ambient air, and placed in a COY laboratories anaerobic chamber (vinyl anaerobic chamber with automatic airlock (AALC), COY Lab Products, Michigan, USA) upon return to the lab (within 1 hour of sample collection). Inside the chamber, porewater immediately was pressed from each core section through a (Whatman no. 1 filter paper) filter (0.45 μm). Porewater samples were frozen until ammonium (NH_4^+) was determined using an indophenol blue method (Solorzano, 1969).

Soil Extractable Nitrogen

In July 2013, a new set of replicate cores (n=5) were taken to a depth of 1m in both the native and invasive communities (appendix table 2). At three depths, 20, 40 and 80cm (± 2.5cm), 5 cm segments were removed and immediately wrapped in plastic to limit interaction with oxygen. Samples were returned to lab and placed in a COY laboratories anaerobic chamber. From each core, 5g field moist soil was removed and extracted with 50 mL 2M KCl. Samples were shaken on an orbital platform shaker for 2 hours at 150 r.p.m and subsequently filtered (0.45μm). Inorganic N (NH_4^+-N) was determined colorimetrically with an Astoria-Pacific autosampler analyzer (API 300, Astoria-Pacific Inc, Oregon, USA)

N availability via Ion-exchange Resin

To provide a measure of inorganic nitrogen availability over time between the native and invasive plant species I used mixed-bed cation-anion exchange resin. Mixed bed resins were used to provide a measure of N availability overtime, in conjunction with instantaneous measures of soil extractable N and porewater N measures (McKinley et al., 2009). Although not a direct measure of N mineralization, it can be used as a proxy estimate of potential N mineralization in both vegetation communities at different depths. Each nylon resin bag contained 10.0 ± 0.5g of mixed-bed cation-anion exchange resin (Resin Dowex Marathon MR-3 (H/OH) MB, part #109005036, Siemens Industry, Inc.) (Binkley and Matson, 1983, Theodose and Martin, 2003) that was fitted into the end of a 1inch PVC pipe. The resins were inserted at 20, 40 and 80cm depths, the same depths at which porewater wells (described below) were inserted. These depths were used at both sites and in both

vegetation communities. Replicate clusters (n=5) were installed in both the native and invasive communities with each cluster including a resin bag at each target depth. The resin bags were inserted into the soil at a 60% angle from the horizontal, after soil removal using an auger. An outer 1.25in PVC pipe was placed in the hole to provide easier replacement of the resin bag. The resin bag, situated at the end of a 1.00in PVC pipe tube, was securely inserted into the outer tube and enclosed to ensure the resin bag was in full contact with the soil. The exchange resins were deployed 01 August 2013 and collected 30 October 2013 (appendix table 2). After removal the resins were oven dried (60^{o}C) and sieved (500 μm and 100 μm sieves) to remove loose sediment and fine roots. The resins were then extracted in 80 ml of 1M KCl, and shaken for 4 hours at 150 RPM. Ammonium concentrations of the extracts were determined colorimetrically (indophenol blue method (Solorzano, 1969)).

Porewater analysis

To estimate porewater N availability, porewater wells were installed in 5 replicate clusters at depths of 20, 40 and 80 cm in both the native and invasive plant communities at both sites. Porewater wells were constructed of Teflon tubing with holes drilled in the bottom 5 cm of the tube sealed at the bottom with silicone, with a 3 way stopcock at the top used for sampling. The wells were randomly distributed within both sites. Porewater was collected once a month for 6 months during the growing season in both 2013 and 2014 (appendix table 2). Samples were collected from all three depths in both communities with the exception of the 40cm and 80cm depth in the invasive community at Fox Creek. I attributed the inability to extract porewater to a mineral horizon found between 30 and 90 cm in the *Phragmites*

(described below), which restricted the removal of porewater in the method used for this study.

Root Depth Distributions

Belowground root biomass was estimated with soil cores (n=2 for each community) to a depth of 3 meters. Five cm diameter soil cores were taken (appendix table 2). Cores were sectioned as described above for porewater nitrogen, and roots were collected from each core section after porewater was squeezed from the soil. Core sections were washed through a 2mm and 1mm sieve to remove sediment and loosen the root mass. Samples were sorted by hand, separating live from dead roots based on visual interpretation of coloration of the roots and root turgidity (Windham, 2001), and live biomass was sorted and separated into fine roots, C_3 rhizome, C_4 rhizome, C_3 red roots (Saunders et al., 2006) and *Phragmites* rhizomes. Sorted samples were dried at 60^{o}C to constant mass and weighed. Belowground biomass was converted to grams per meter^2.

A model for the vertical distribution of roots was presented by Gale and Grigal (Gale and Grigal, 1987). The asymptotic equation:

$$Y = 1 - \beta^d$$

where Y is a cumulative root fraction (between 0 and 1) between the surface to a depth d (cm), and β is the fitted coefficient is used to model root depth distributions. In this model, β is the only parameter that is estimated and can provide an index of root distribution based on depth. Low β values indicate a higher proportion of roots near the surface, while a high β values indicates more roots at depth. β values were fit to both communities to compare root depth distribution.

Plant Nitrogen Uptake

To estimate how plant N uptake differs between both plant communities at multiple depths, I initiated a ^{15}N tracer experiment. One meter square plots were randomly selected in both the invasive (n=4) and native (n=5) plant communities, with one replicate per depth and plant community. In each community one plot was designated for 20cm, 40cm, 80cm depth addition, and a reference plot. In the native community, a 10cm plot was also added to increase the chances of detecting a signal in a shallow rooting community. On July 24th, 2013 (appendix table 2), the newest full, non-budding *Phragmites* leaf was collected from every stem within each invasive plot. In the native plot, 10 full *S. patens* stems were collected haphazardly throughout the plot (spaced to cover entire plot), to serve as time zero ^{15}N vegetation concentrations. On July 26th 2013, highly enriched $^{15}NH_4Cl$ (99% atom percent ^{15}N, Cambridge Isotope Laboratory, Andover MA, USA), was injected into each depth plot using a 3/16 inch stainless steel rod with, 20, 1/16 inch holes at the bottom 5cm of the rod. To increase the probability of measuring ^{15}N uptake, a higher concentration of $^{15}NH_4Cl$ was used at deeper depths. In each plot, ^{15}N was added to 8.1 L of DI water. At the 10cm and 20cm depth plots, I added 1g $^{15}NH_4Cl$, in the 40cm plot, I added 2g $^{15}NH_4Cl$, and in the 80cm plot, I added 6g $^{15}NH_4Cl$. Each plot was injected 81 times in a 9x9 grid (injections 10cm apart), with each injection being 100ml $^{15}NH_4Cl$ solution. After each injection 50ml of DI water was used to flush the injection rod.

Two weeks after injection, newly budded *Phragmites* leaves were collected from each stem within each plot in the invasive community. In the native community,

again 10 full *Spartina patens* stems were collected haphazardly from each plot. Plant samples were dried (60 ^{o}C) to constant mass, and homogenized using a ball grinder (SPEX SamplePrep 8000D Mixer Mill, NJ, USA), and subsamples were sent to UC Davis Stable Isotope Facility for [^{15}N].

The percent of ^{15}N label taken up in each plot was calculated as follows:

$$\%Uptake = \left(\frac{(\Delta_{at-\%}) - (T_{0\ at-\%})\ x\ Tot_{Leaf\ N}}{g^{15}N\ Injected}\right) x\ 100$$

Where $\Delta_{at-\%}$ is the ^{15}N atom percent in the sample, $T_{0\ at-\%}$ is the background ^{15}N concentration in the vegetation, and $Tot_{Leaf\ N}$ is the total leaf based nitrogen in each plot. %Uptake is a measure of how much of the total label was utilized by the vegetation in each plot.

Statistical Analysis

The effects of vegetation community composition on soil and vegetation nitrogen pools were analyzed using fixed effect model ANOVA (Proc ANOVA, SAS Enterprise, SAS Institute Inc., Cary, North Carolina), based on the variable being tested. Analyses were made between communities within each site only. Differences in soil profile and porewater characteristics prevented between site analyses of some belowground variables. Depth variables for soil extractable and resin N (20, 40 and 80 cm) were analyzed using a repeated measures ANOVA, for any depth-related differences between communities. Least square mean comparisons were done with a Tukey post-hoc adjustment. The experiment was not set up with blocks, as no know blocking factors were present in the system. In this study, $P < 0.05$ was considered to be significant for these analyses.

Results

Biomass and Standing Stock Nitrogen

At both sites, Fox Creek and Corn Island, invasive *Phragmites* has a greater aboveground biomass ($p<0.0001$, df=8, and $p=0.0023$, df=8 respectively) than the native plant communities (figure 2.1). At Fox Creek, invasive *Phragmites* had over 6 times more aboveground biomass than the native community (3391 ± 989 g m^{-2} vs 490 ± 72 g m^{-2}). Corn Island also demonstrated similar patterns, but lower overall biomass with *Phragmites* having only 3.7 times more biomass (2499 ± 838 g m^{-2} vs. 660 ± 246 g m^{-2}). There was no observed difference in aboveground biomass between sites in the native community ($p=0.6892$, df=16), or the invasive community ($p=0.1848$, df=16). Belowground biomass at both sites was consistently higher, but not significantly greater, in the invasive community at both Fox Creek (3056 ± 2751 g m^{-2} vs 769 ± 138 g m^{-2}, $p=0.2330$, df=2) and Corn Island (2418 ± 1637 g m^{-2} vs 1817 ± 587 g $m^{-}2$, $p=0.7310$, df=2) compared to the native vegetation (figure 2.1). There is no significant site difference in belowground biomass within communities, although the native community at Corn Island has over twice the average belowground biomass than the native community at Fox Creek.

Aboveground standing stock plant nitrogen content was significantly greater in the invasive species at both sites (Fox Creek $p<0.0001$, df=8, Corn Island $p<0.0009$, df=8). At Fox Creek, invasive *Phragmites* had over 10 times more aboveground standing stock N than the native community (42 ± 12 g N m^{-2} vs 3 ± 0.5

g N m^{-2}). Corn Island also demonstrated similar patterns, but lower overall N with *Phragmites* having only 5 times more standing stock N (31 ± 10 g N m^{-2} vs. 6 ± 1 g N m^{-2}). Nitrogen content belowground (figure 2) was not significantly different between communities at either site (p=0.6203, df=2 at Fox Creek and p=0.9617, df=2 at Corn Island) or between sites (figure 2.2). In the invasive community, leaf biomass consisted of 30% of the total plot biomass, while the stems consisted of the other 70% of the biomass (Table 1). Conversely, the leaf tissue in the *P. australis* consisted of nearly 66% of the total plot N, while the stems accounted for the other 44%. In the native communities, *Spartina patens* accounted for 87% of the aboveground N at Fox Creek and 44% at Corn Island. *Schenoplectus americanus* accounted for 52% of the aboveground N at Corn Island (Table 1).

Table 1. Community composition and biomass partitioning at Fox Creek and Corn Island marshes, based on m^2 plot measurements (n=5) ± 1 standard deviation. Coarse roots were combined with fine roots in this table. Red roots have been previously identified by coloration methods and isotopic ($\delta^{13}C$) signature to be C_3 S*choenoplectus americanus* (Saunders et al., 2006).

Invasive Community		% N	Fox Creek Marsh		Corn Island Marsh	
			% Total Biomass	% Total N	% Total Biomass	% Total N
Phragmites australis						
Aboveground	Leaf	2.796	29.5 ±1.1	65.9 ±2.9	29.2 ±1.3	65.9 ±3.5
	Stem	0.605	70.2 ±1.0	34.1 ±0.6	70.5 ±1.1	34.1 ±0.6
Belowground	Fine Roots	1.195	27.1	47.1	29.1	49.5
	Rhizome	0.490	72.9	52.9	70.9	50.5
Native Community						
Aboveground	*Spartina patens*	0.714	86.7 ±1.2	86.3 ±1.1	53.9 ±17	44.0 ±17.8
	Schenoplectus americanus	1.233	0.0	0.0	43.3 ±17	53.8 ±17.9
	Distichlis spicata	0.711	13.2 ±1.2	13.7 ±1.1	2.80 ±1.8	2.10 ±1.3
Belowground	Fine Roots	1.155	26.4	27.7	12.0	11.6
	Red/Dark Roots	1.4	0.0	0.0	14.6	17.2
	C_3 Rhizome	1.2	0.0	0.0	45.0	45.4
	C_4 Rhizome	1.080	73.6	72.3	28.4	25.7

Soil Properties and Nutrient Availability

Soil bulk density at both sites showed differences between the two communities at different depths (figure 2.3). In general, at both sites there were zones

of higher bulk density in the introduced community, composed of a mineral soil (7% OM) (sandy clay loam, 10 YR 4/2 and 10 YR 3/3 at Fox Creek and Corn Island respectively). In contrast, the native community at both sites maintained a constant bulk density down the soil profile, composed of a thick root mat in the upper portion with peat below. At Fox Creek there was an increase in bulk density in the invasive species between 20 and 90cm, which reached a max bulk density of 1g cm^{-3} at a depth of 50cm. In the native community there is a slight increase in bulk density from 0.10 g cm^{-3} to 0.20 g cm^{-3} at a depth of 40cm (figure 2.3), before returning to a consistent 0.10 g cm^{-3} to a depth of 2m. At Corn Island the top 40cm of the invasive community has an increase in bulk density 0.30 g cm^{-3} before exhibiting a consistent, lower bulk density to a depth of 2m.

Soil extractable N (NH_4-N) (figure 2.4) varied significantly by site and depth. At Fox Creek, the invasive community has significantly lower soil N availability ($p<0.001$, df=46). I observed a significant increase in total soil bound nitrogen, from KCl extracts, as depth increased in the native ($p<0.0064$, df=46) but not in the invasive community. In contrast, no community differences in soil extractable N were found at Corn Island, but extractable N appears to increases in the native community with depth.

Porewater N (NH_4-N) availability also exhibited consistent patterns among vegetation type, both with depth and time (Appendix 4). Similar to extractable N, porewater N concentration was higher in the native community than invasive community at Fox Creek (figure 2.5). Porewater N concentration in the native community increased with depth from around 40 μmol N-NH_4 L^{-1} at the surface, to

over 1200 μmol N-NH_4 L^{-1} at a depth of 3 meters. Conversely, porewater N in the invasive community at Fox Creek maintains a consistent, lower, concentration of N-NH_4 L^{-1} to a depth of 3 meters. Unlike the large differences between communities observed at Fox Creek, porewater N concentrations did not differ significantly between communities at Corn Island. Both communities had a small increase in N-NH_4 concentration around the 50 cm depth, with the invasive community being slightly lower than the native between 50 and 200 cm (figure 2.5).

Resin N was highly variable and does not reflect any community ($p=0.7625$, $df=45$) or site differences ($p=0.5382$, $df=45$) (figure 2.6). In contrast to porewater, resin extracted N shows a trend of decreasing in the native community as depth increases at both sites ($p=0.0033$), while the invasive community does not exhibit the same trend.

Table 2. β values and their associated r^2 values based on the collected rooting data at Fox Creek Marsh and Corn Island Marsh, fit to the 1987 Gale and Grigal model (eq. 1), root biomass percent in the upper 40cm of soil, and the total root biomass (kg m^{-2}). Larger β values correspond to deeper rooting profiles, and are represented graphically with the data in figure 5.

Site	Community	β	r^2	% Root biomass upper 30 cm	Total root biomass (kg m^{-2})
Fox Creek Marsh	Invasive	0.98	0.94	35	3.05
	Native	0.79	0.91	99	0.77
Corn Island Marsh	Invasive	0.96	0.95	68	2.45
	Native C_3	0.92	0.97	96	1.08
	Native C_4	0.90	0.95	97	0.73
Total	Invasive	0.97	0.86	52	2.74
	Native	0.88	0.91	97	1.29

Root Depth Distributions

Introduced *Phragmites* rooted deeper than native vegetation communities at all sampled locations. The deepest rooting profile was the invasive community at Fox Creek marsh, followed by the invasive community at Corn Island marsh (β=0.98 and 0.96 respectively) (figure 2.7). *Phragmites* consistently rooted to nearly 3 meters in depth at both sites (*e.g.* 320 cm at Fox Creek and 280cm at Corn Island). In contrast, the native community at Fox Creek showed the shallowest rooting profile (β=0.79), with just over 99% of the root biomass found within the upper 30cm of the soil, and the deepest live root within 75 cm of the soil surface. At Corn Island 97% of the root mass was observed in the upper 30cm in the native community (Table 2). The native C_3 community (β=0.92) has a deeper rooting profile than the C_4 native community (β=0.90) at Corn Island with the deepest observed live root observed around 110cm depth. The invasive community overall averaged 52% of the root biomass in the upper 30cm of the soil, while the invasive species was 97%. On average, the invasive community has a total root biomass of 2.74 kg m^{-2} while the native is 1.29 kg m^{-2}.

Nitrogen Uptake

I observed ^{15}N uptake in both plant communities, with the greatest uptake observed in the *Phragmites* community at all depths. The highest uptake of the ^{15}N (as $^{15}NH_4Cl$, 99% atom percent ^{15}N) was found the invasive community at the 40cm depth plot, which corresponded to the greatest abundance in roots (figure 2.8). ^{15}N uptake was lower in the 20cm and 80cm depth plots in the invasive community (Figure 8). In contrast, ^{15}N uptake rates were there greatest at the surface and

decreased significantly with depth, with 80cm showing very little, but detectable uptake at 80cm in the native plant community. These patterns in the native community correlate well with the reported rooting profiles, suggesting that our estimates of live roots match uptake patterns in the field.

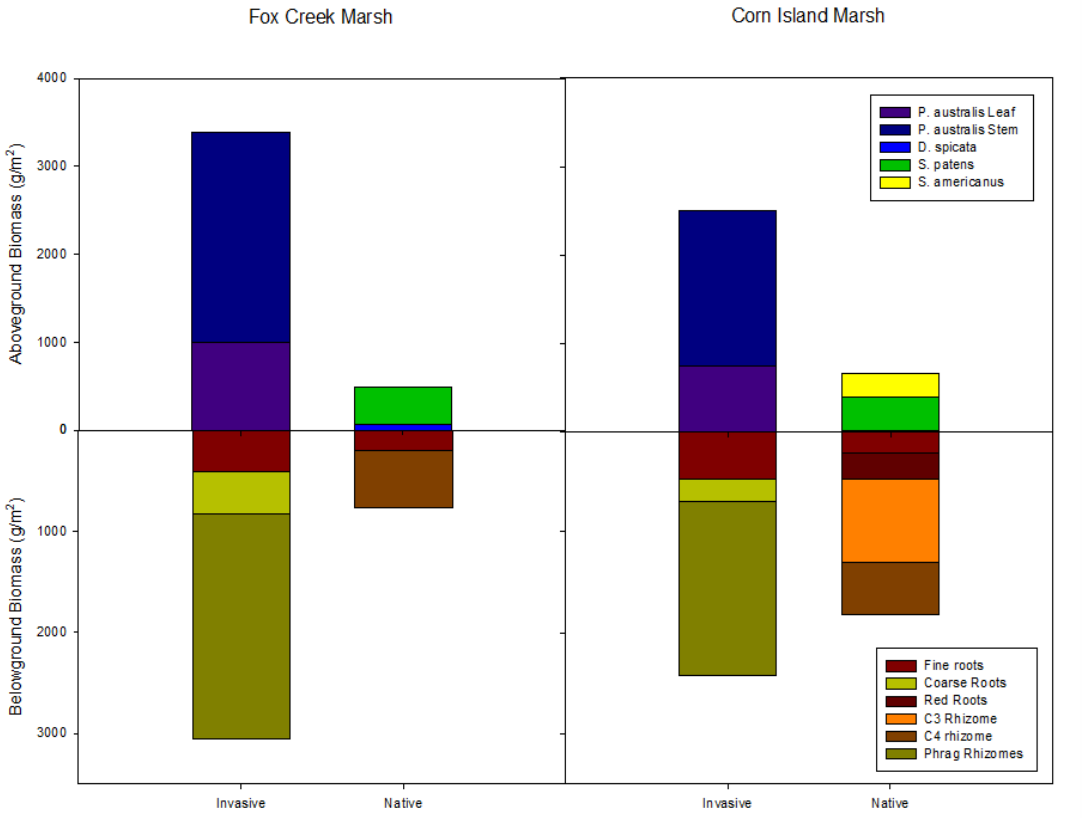


Figure 1. Above- and below-ground biomass (g m^{-2}) in the invasive and native plant communities broken into plant species (native community) and plant component (invasive community). Belowground composition is broken into root and rhizome type on both communities. Plots (n=5) measured at both Fox Creek and Corn Island marshes. Error bars represent ±1 S.E. Letters represent significant differences in total biomass (above- and below-ground separated) between communities and sites ($p<0.05$).

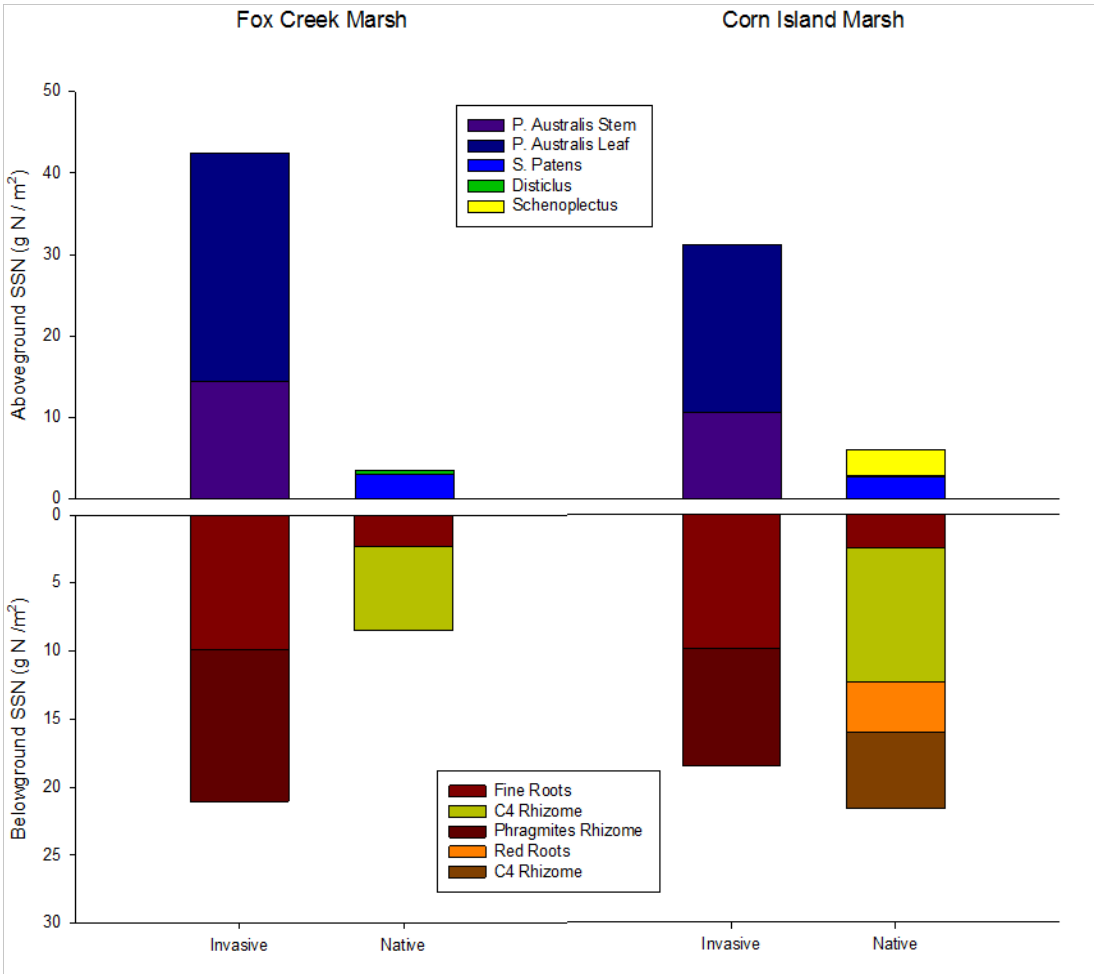


Figure 2. Above- and below-ground nitrogen content (g m^{-2}) in the invasive and native plant communities broken into plant species (native community) and plant component (invasive community). Belowground composition is broken into root and rhizome type on both communities. Plots (n=5) measured at both Fox Creek and Corn Island marshes. Error bars represent ±1 S.E. Letters represent significant differences in total N content (above- and below-ground separated) between communities and sites ($p<0.05$).

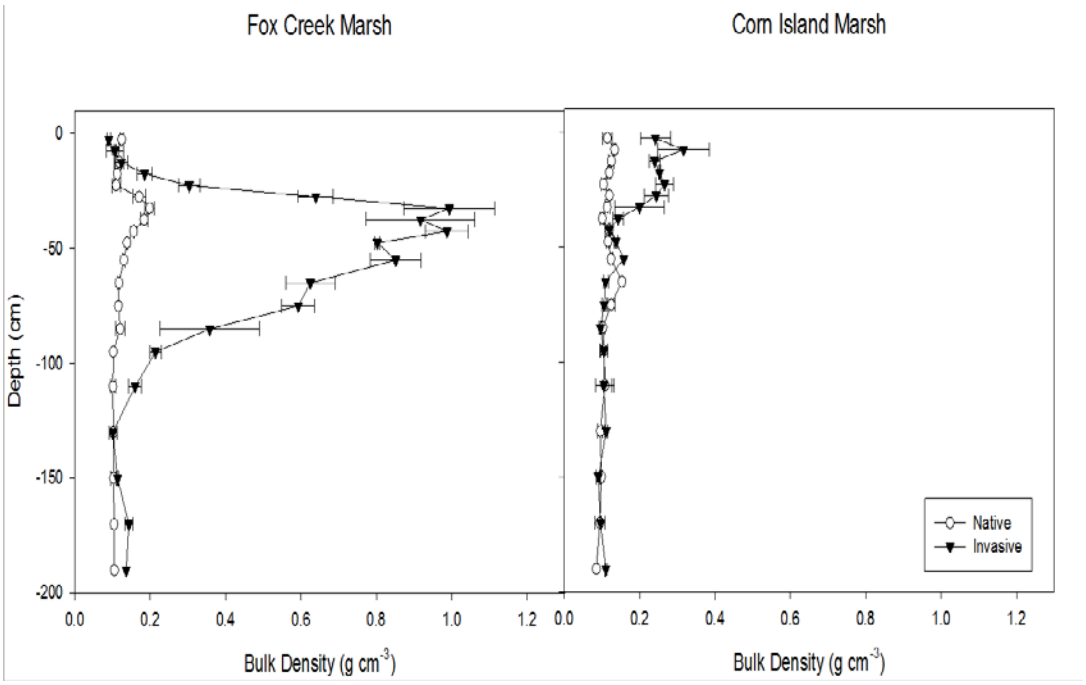


Figure 3. Bulk density (g cm^{-3}) measurements to a depth of 2m (n=2) at Fox Creek and Corn Island Marshes comparing native and invasive communities. Values are the averages at each depth with error bars denoting ±1.0 standard error. The invasive community has a greater bulk density at Fox Creek (between 20 and 100cm depth) and at Corn Island (between 0 and 50cm depth) than the native community (α=0.05).

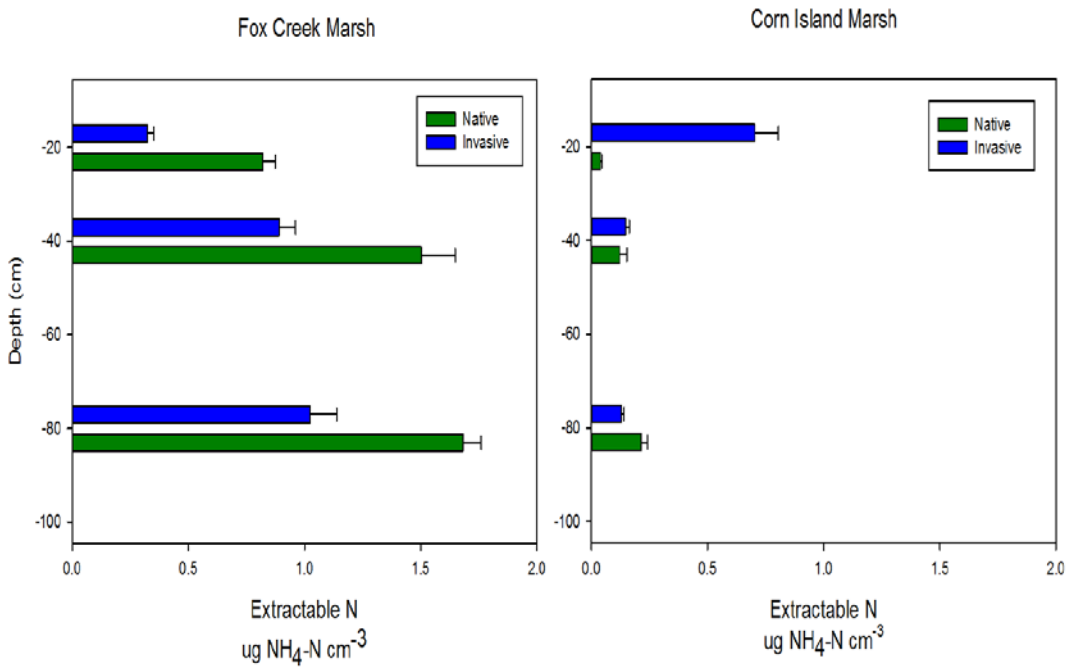


Figure 4. Soil extractable N (NH_4-N) extracted via 2M KCL extraction at 20, 40 and 80cm depths in the invasive and native communities at Fox Creek and Corn Island Marshes. Displayed as µg NH_4-N per cm^{-3} of soil averages (n=5) with error bars denoting ± 1 S.E.

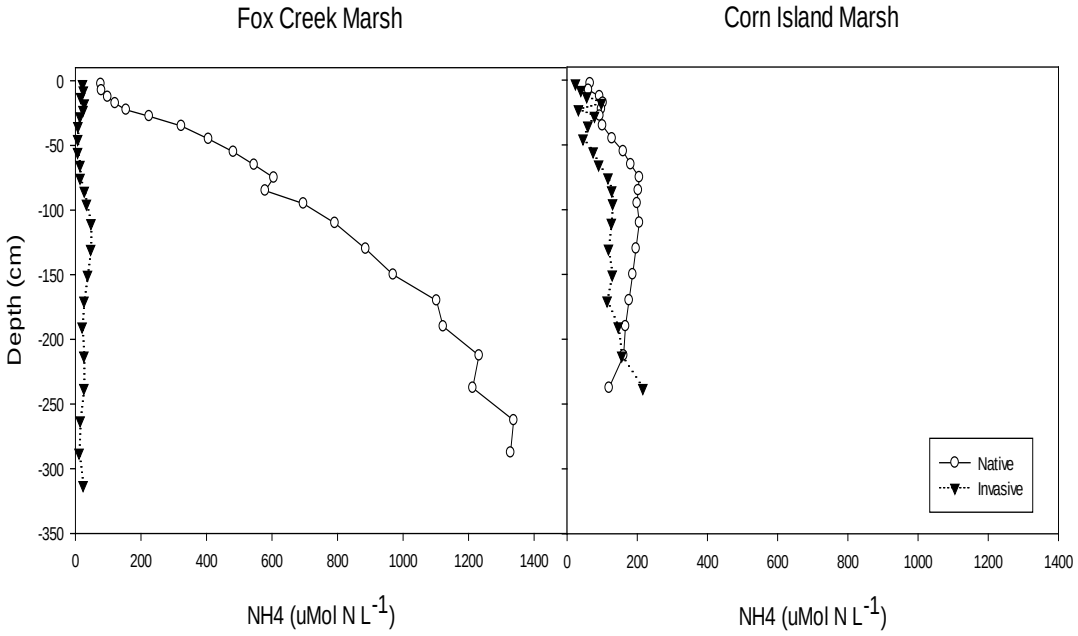


Figure 5. Porewater N (NH_4-N) to a depth of 250+ cm in the native and invasive vegetation communities at Fox Creek and Corn Island marshes. For each sampling, n=2 cores in each community at both sites for a total of n=8 cores.

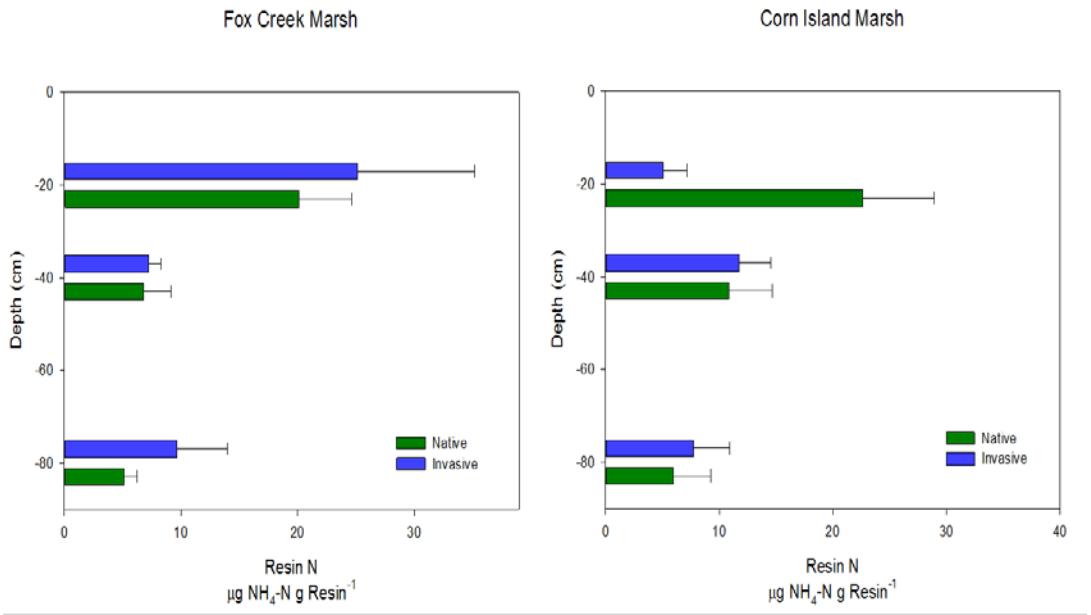


Figure 6. Resin extracted N (NH_4-N) extracted via 1M KCL extraction at 20, 40 and 80cm depths in the invasive and native communities at Fox Creek and Corn Island Marshes. Displayed as µg NH_4-N per gram of dry resin averages (n=5) with error bars denoting ± 1 S.E. Resins were deployed for 8 weeks during the summer of 2013.

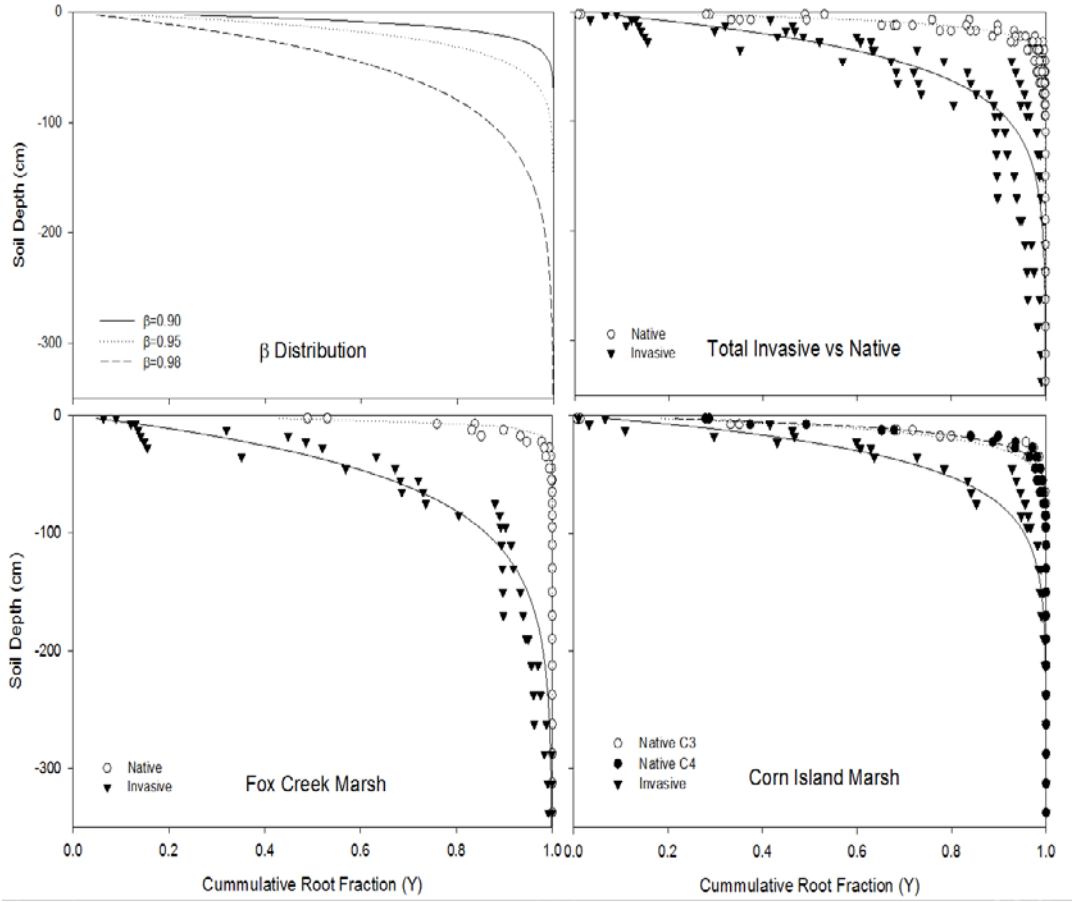


Figure 7. Cumulative root distribution (fraction, Y) as a function of soil depth. Site and community specific βvalues can be found in table 2. β values are the least squares fit to the root distribution model (eq. 1) by Gale and Grigal (1987). Based on the model ($Y=1-\beta^d$), βis a proportion between 0 and 1, based on the soil depth (d) and cumulative root fraction (Y). As shown in the top left panel, higher βvalues correspond to deeper rooting profiles.

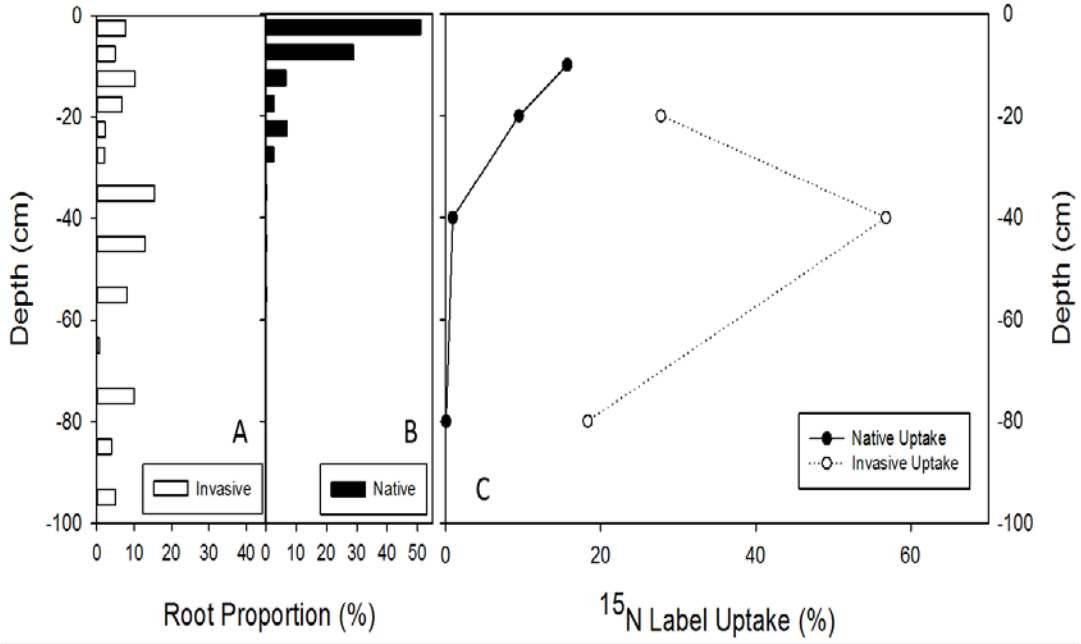


Figure 8. Rooting proportion of total rooting biomass at each depth increment in the invasive *Phragmites* (A) and native (B) vegetation communities. ^{15}N (labeled as $^{15}NH_4Cl$, 99% atom percent ^{15}N) uptake (C) from 10 (native only) 20, 40 and 80 cm depth plots in both the native and invasive plant communities at Fox Creek marsh.

Discussion

Biomass and Rooting Depth

A community shift to *Phragmites* changes the root depth distribution, biomass, and SSN availability that together fundamentally alter ecosystem function in invaded wetlands. With the invasion of *Phragmites* I see a significant increase in above and belowground biomass, aboveground SSN, increased rooting depth, and differences in belowground N pools. The ability of *Phragmites* to uptake N from deeper depths than native communities gives it another competitive edge, and allows it to tap into nutrient rich, deep N pools. The depth to which *Phragmites* can root, and access N, is a novel trait that is not seen in the native wetland plant species in this study. This study had limited root measurement replication due to the depth of rooting work, but other studies have shown significantly greater belowground productivity in *Phragmites* in comparison to similar native vegetation communities (Moore et al., 2012) suggesting that my study has broader implications.

Assessment of vegetation rooting depth and distribution, and belowground differences observed in *Phragmites* are important to understand ecosystem functioning, and the cycling of nutrients. It has been widely reported that *S. patens*, *S. americanus* and *D. spatica* have relatively shallow rooting systems which are concentrated in the upper portion of the soil profile (Windham and Lathrop, 1999, Windham, 2001, Saunders et al., 2006). However, I found that the extensive rhizosphere of *Phragmites* is located at a deeper depth than that of the native communities presented in this study, and other reported studies. As a consequence, I hypothesize that this deeper rooting system may directly influence biogeochemical

processes by potentially oxidizing the soil and providing labile carbon for microbial respiration at deeper depths.

Replacement of native communities by introduced *Phragmites* profoundly alters both below and aboveground N stocks. Specifically, invasion increases nitrogen retention within the vegetation and also a shift to an increased aboveground nitrogen standing stock by six to eight times (Figure 2). Such profound changes have also been observed by Windham and Meyerson (2003) and Templer et. al. (Templer et al., 1998) among others, who report a significant increase in N retention and N demand in wetland ecosystems that are shifting towards *Phragmites* as the primary vegetation type. In contrast, native communities have greater belowground nitrogen pools in the root systems compared to aboveground tissue pools. A community shift to a *Phragmites*-dominated community maintains similar belowground N pools, due to low N concentrations in *Phragmites* rhizomes, but vastly increases aboveground standing stock nitrogen.

Changes in plant community composition and resulting changes in tissue N content may influence decomposition processes at the ecosystem level. In contrast to the native *Spartina* grasses, which are primarily leaves, aboveground biomass in introduced *Phragmites* is predominantly composed of stems (70%), while the nitrogen-rich leaves comprise less of the aboveground biomass (30%) (table 1). Previous studies have shown slower rates of decomposition of *Phragmites* compared to several native species, with *Phragmites* having 30% slower decomposition than native vegetation due to higher lignin concentrations (Windham, 2001). The greater demand for N in *Phragmites*, paired with slower rates of decomposition points

towards *Phragmites* invasion increasing the ability of wetland systems to retain excess nitrogen and become a larger N pool.

Deep rooting may be a strategy to provide a competitive advantage over native, shallow rooting plant communities. With a deeper rooting profile in the introduced species, *Phragmites* can now access nutrient pools below 40 cm, allowing it to bypass competition with the native species, which is utilizing nutrients in the upper 30 cm of the soil profile (figure 6). High salinity environments are stressful for plants, so a deeper, more extensive rooting system may be a method of accessing freshwater in otherwise saline habitats (Lissner and Schierup, 1997). This could cause a shift in nutrient allocation away from regulating osmotic pressure due to higher salinity, toward biomass production aboveground. At the Fox Creek site, lower salinity porewater was observed in the *Phragmites*, which was not found in the native vegetation. This source of freshwater is not fully known, but upland freshwater inputs are thought to be the source.

Soil Properties and Nutrient Availability

The mineral lens observed at the Fox Creek site at of depth of 20 to 80 cm (figure 7) is likely to change patterns of nutrient retention and uptake compared to soils with higher organic matter content. The increase in clay content (sandy clay loam) will increase the cation exchange capacity (Helling et al., 1964), possibly lower root production and growth (Unger and Kaspar, 1994), and may slow down water movement (Nye and Tinker, 1977). Each of these factors has the potential to alter nutrient uptake by plants. The presence of the mineral layer could be due to weather

events that deposited mineral-rich sediment onto the soil surface from the Rhode River, erosion of mineral soils from the bluffs that surround this marsh, are it could represent a period of time when the marsh surface was lower in elevation relative to sea level, causing longer periods of inundation and therefore sediment deposition. More certain is that these soils supported native plants at the time the mineral-rich sediments were deposited, most likely communities dominated by a C_3-C_4 mix of S. *americanus* and S. *patens*. *Phragmites* introduction and growth has been shown to be facilitated by disturbances to tidal wetland systems (Bart and Hartman, 2000, Minchinton, 2002, Minchinton and Bertness, 2003), which would explain the initial introduction in to the Fox Creek system as an increase in bulk density restricted root growth. At Corn Island, a smaller increase in bulk density was observed in the upper 30 cm of the soil profile in the introduced community, which is likely due to the fact *Phragmites* slows tidal waters, allowing smaller sediment particles to settle out and become part of the soil profile.

Phragmites depletions in both porewater and extractable N pools is site specific in this study, pointing towards other factors potentially contributing to meeting higher N demands. These same differences are not as profound at Corn Island, which could be due to the inclusion of an additional species (S. *americanus*) found in the native community. The two invasive communities have similar pools of N but have greater N pools than the two native communities. Changes in plant communities are associated with differences in nutrient availability at the two sites. It is clear that *Phragmites* alters porewater N availability at our sites, consistent with previous findings in brackish and salt marshes (Chambers, 1997, Templer et al., 1998,

Meyerson et al., 2000, Windham, 2001, Windham and Meyerson, 2003, Mozdzer et al., 2010). These changes are likely attributed to greater N demand (Figure 2), which is a generalizable phenomenon in *Phragmites*-invaded wetlands (Meyerson and Cronin, 2013). One explanation for the lower porewater and soil-extractable N at Corn Island in the native community compared to Fox Creek could be the presence of S. *americanus*, which may increase the ability of the native community to draw down available N pools. With *S. americanus* rooting slightly deeper than S. *patens* and *D. spatica*, it may also access deeper N pools. With the addition of *S. americanus* at Corn Island, there is a slight increase in aboveground and belowground standing stock N, which may reflect a higher demand for N.

The influence of invasive *Phragmites* on N cycling is difficult to assess in the Fox Creek Marsh because differences in soil characteristics suggest that soil element cycles were different even before the invasive species became established. However, at the Corn Island site, our data suggests higher rates of N mineralization in the invasive community than the native community at the soil surface (20 cm). Lower-soil extractable N and porewater N in the *Phragmites* community at Corn Island suggests that decomposition, and thus N mineralization, of recently deposited organic matter has declined in the introduced community (Appendix 5). One interpretation of this pattern is that *Phragmites* tissue is more recalcitrant to decomposition than native tissue. Previous studies have reported greater rates of nitrogen mineralization in *Phragmites* than native plant communities (Windham and Ehrenfeld, 2003, Windham and Meyerson, 2003). In comparison to an upland forested community, I observed 8-10 times greater resin extractable NH_4 at similar depths (McKinley et al., 2009).

Plant Nitrogen Uptake

Our data suggest that the uptake of N by *Phragmites* at depth may account for some of the additional N needed to satisfy the high N demand of the invasive *Phragmites.* Introduced *Phragmites* showed greater N uptake compared to the native community at all depths. N uptake rates mirror root depth distribution in both plant communities, with uptake occurring in the *Phragmites* at every depth I measured. Given this observation and my data showing live roots present at depths >3 m, I suggest that *Phragmites* is capable of extracting N at deeper depths than native plant species. By focusing root mass at depths that contain more N (*e.g.* 40 cm) *Phragmites* may have a competitive advantage by avoiding direct nutrient competition with native species that have allowed it to establish in pristine, low-N systems. McKinley et al., 2009 reported similar deep N uptake in a terrestrial forested system where a tracer was put in to the water table (~250cm depth), with uptake observed several days later. *Phragmites* exhibited deep rooting profiles similar to temperate forests (Jackson et al., 1996), suggesting that I should expect to see similar uptake from such depths. The introduced *Phragmites* communities generally showed higher rates of nitrogen uptake and the ability to draw down available N pools more than the native community providing nitrogen to meet a greater N demand in the introduced *Phragmites* community. With site difference in available N between communities it leads up to believe there is potentially other complicating processes involved in N cycling and N pools.

Although my data provide convincing evidence of deep N uptake, I acknowledge several limitations. When calculating the deep N uptake I made the

assumption that ^{15}N concentrations were homogenous in leaves, which does not account for potential remobilization of nitrogen from older leaves to newly produced leaves. With such low N concentrations in *Phragmites* stems, I did not account for any ^{15}N that may have been associated with the stems or inflorescence, which can range from 25% to 30% of total plant N (Table 1) (Windham and Meyerson, 2003). The calculated values are also not accounting for any N uptake that was used in root production.

Conclusions

Phragmites produces 3-5 times more aboveground biomass and nearly double belowground root biomass than the native plant communities it displaces. This increase in aboveground biomass represents a shift nitrogen allocation in wetland vegetation from a belowground-dominated to aboveground-dominated sink (Appendix 5). With an increase in production, and a decrease in decomposition, these changes may lead to an increased ability of the system to act as a nitrogen sink. However, paired with potentially enhanced N mineralization (not directly measured in this study), it is unclear if a species shift to *Phragmites* alters the overall N sink function of wetland ecosystems, as this would require a better understand of the fate of N during plant decomposition as organic matter accumulates in the soil.

Significantly deeper rooting depths, and the ability to obtain nutrients from these depths, shows a potential method to meet higher demands for nitrogen. Accessing nutrients unavailable to native plant communities may help *Phragmites* to avoid direct competition. A second method not directly measured in this study is the increase in N transformations (*i.e.* mineralization), with deeper rooting *Phragmites*,

providing a source of carbon to fuel the transformation. Historically, wetland ecosystems have been viewed as a sink for excess nutrients, but if the introduction of *Phragmites* increases rates or mineralization at deeper depths, where old organic nitrogen has been buried and brings it to the surface it could start to decrease the effectiveness of the system as a sink. Long term studies on the effects of *Phragmites* invasion could help to further answer these questions.

Chapter 3: Effects of *Phragmites australis* introduction on soil porewater chemistry and methane emissions.

Abstract

Tidal marshes are highly productive ecosystems with the potential to sequester large amounts of carbon dioxide and emit methane (CH4). The carbon sink function is relatively well quantified in salt and brackish wetlands, but there is relatively little known about CH_4 emissions from these ecosystems. In particular, it is unclear how species shifts may change the radiative forcing of coastal wetland ecosystems where high sulfate availability is expected to suppress methane production (citation). An introduced lineage of the common reed, *Phragmites australis*, is rapidly invading North American tidal wetlands. As plant communities shift it is important to understand how these species shifts may change the ecosystem carbon balance. I quantified rates of CH_4 emissions between introduced *Phragmites australis* and native plant communities (*Spartina patens, Distichlis spicata* and *Schoenoplectus americanus*) in a brackish Chesapeake Bay tidal marsh using static flux chambers along four transects between the two communities. Both clear and opaque chambers were used to control light versus dark conditions. Methane emissions were measured in the native community, *P. australis* community and a transition zone containing both native and introduced plant communities over a period of two hours. Diffusive CH_4 emissions were significantly greater in the *Phragmites*-dominated communities than native communities. Assuming that CH_4 emissions via ebullition are negligible or unchanged by *Phragmites*, the result suggests that this invasive species can

increase methane emissions and has the potential to change the radiative forcing, or the change in energy balance contributing to climate change, of a tidal wetland.

Introduction

Wetland ecosystems occupy a small area of the planet but have a large role in the sequestration of carbon from the atmosphere as a long term CO_2 sink (Weston et al., 2014). Water logged conditions prohibit aerobic respiration, so slower, anaerobic respiration pathways are utilized to break down organic matter, which in turn slows rates of decomposition in wetland ecosystems allowing carbon to be sequestered (Chmura et al., 2003). Coastal wetlands have the capacity to sequester carbon at a greater rate per unit area, than many major terrestrial carbon sinks and, thus, are a large part in the global carbon cycle (McLeod et al., 2011). Salt and brackish marsh ecosystems are more commonly known as C sinks in comparison to freshwater marshes, as sequestration can be lower due to higher rates of methane (CH_4) emissions (Chmura et al., 2003). Methane emissions offset the reduction in radiative forcing of carbon sequestration (Bridgham et al., 2006). Although CH_4 fluxes are much smaller than CO_2 fluxes, CH_4 is a more potent greenhouse gas (GHG) than CO_2 and is responsible for nearly 30% of total anthropogenic radiative forcing (IPCC 2013).

Methane emissions are highly variable from wetland ecosystems, both spatially and temporally. Additionally, vascular plants have been pointed out as a key factor in controlling methane emissions (Joabsson et al., 1999). Different plant communities have the ability to affect several key processes associated with methane emissions from wetland ecosystems. Vegetation can provide not only a conduit for

methane release from the soil, but can also provide organic compounds through root exudation that can increase methane production. Conversely, root oxygen loss can increase methane consumption via methanotrophy (Ding et al., 2005).

Phragmites australis invasion of wetland ecosystems alters biogeochemical cycling, including a variety of processes that directly influence the rate of methane emissions by altering the production, consumption and transport of CH_4. *Phragmites* has a relatively deep rooting zone (Chapter 2) that may allow for increased root oxygen loss, a process that would simultaneously inhibit CH_4 production and stimulate rhizosphere CH_4 oxidation. Finally, *Phragmites* is able to ventilate CH_4 to the atmosphere via a pressurized oxygen flow (Brix et al., 1996), bypassing the aerobic zone at the soil surface that supports CH_4 oxidation. A previous study reported that 98% of the CH_4 emitted from *Phragmites*-dominated wetlands passes through the stems (Brix et al., 2001). Certain native plant communities can provide the same conduit for gas release through the aerenchyma, but *Phragmites* has been shown to actively ventilate the rhizosphere.

The goal of this study was to quantify CH_4 emissions from plant communities dominated by *Phragmites*, native species, or a mix of *Phragmites* and native species, and to identify processes by which *Phragmites* invasion may influence CH_4 cycling through differences in porewater chemistry and laboratory soil incubations. I hypothesized that introduced *Phragmites australis* causes a net increase in CH_4 emissions, and that the mechanism is primarily through plant release, as increased oxidation rates could negate some of the belowground production.

Methods

Site Description

Two sites were used to investigate differences in porewater chemistry between plant communities dominated by introduced *Phragmites australis* versus native plant species. Fox Creek marsh and the Corn Island marsh are located at the Smithsonian Environmental Research Center, part of the brackish Rhode River sub-estuary of the Chesapeake Bay (Fig x, Map 2). Dominant plants at Fox Creek Marsh are a mixture of the native species *Spartina patens* and *Distichlis spaicata*, and the invasive lineage of *Phragmites australis*; predominant plant species at Corn Island are *Spartina patens*, *Schoenoplectus americanus* and *Phragmites australis*. These sites were selected because they have both a well-developed stand of *Phragmites australis* and a well-established native community typical of brackish wetland systems.

Porewater Analysis

Porewater sippers were used to monitor monthly changes in dissolved nutrients and redox-active elements during the 2013 growing season. Wells were constructed of Teflon tubing with holes drilled in the bottom 5 cm of the tube. Silicone was used to seal the bottom end of the tube, and the top was sealed with a 3 way stopcock for sampling. Wells were installed in five replicate clusters at depths of 20, 40 and 80 cm, with full sets in both the native and invasive plant communities. The wells were haphazardly distributed within both sites. Porewater was collected once a month for the six months (June thru October 2013, and May 2014) that correspond to the growing season in these marsh systems. At Fox Creek the samples

were collected from the 20 cm-deep wells in both communities each sampled month, and at 40 and 80 cm in the native community. Samples of porewater from 40 and 80 cm depths at the Fox Creek *Phragmites* site were only collected in June and July because a mineral horizon at 30- 90 cm depth restricted porewater extraction using this method.

On each sampling date, a 60 ml syringe was used to collect porewater from each well and processed in aliquots for several different solutes (Table 3.1). The first 15 ml were discarded to flush any water remaining in the well from the prior sampling event. From the 60 ml syringe, 10 ml of sample was filtered through a 0.45 μm syringe tip filter into a 20 ml scintillation vial. Dissolved NH_4^+ was measured spectrophotometrically by the indophenol blue method, using a dark incubation time of two hours, and a wavelength of 640 nm (Solorzano, 1969).

From the 60 ml syringe, 3 ml of unfiltered porewater was mixed with 3 ml of sulfide anti-oxidant buffer (modified from Eaton et al, 1995), and the solution was measured with a millivolt meter (ORION Research, Beverly MA. USA.). pH was measured using a 3 ml aliquot of unfiltered sample from the original 60ml sample using a bench top pH meter (ORION research).

Dissolved CH_4 was measured using a headspace equilibration technique (Keller et al., 2009). The remaining sample from the original 60 ml syringe was expelled, leaving a 15 ml porewater sample. Next, 15 ml of ambient air was drawn into the syringe for a 1:1 porewater to ambient air ratio, then shaken vigorously for one minute to strip dissolved CH_4 into the headspace. The porewater was expelled,

and the headspace analyzed for CH_4 with a Shimadzu GC-14A gas chromatograph equipped with an FID (Shimadzu Corporation, Kyoto, Japan).

Table 3.1. Treatment of porewater samples collected by either the sipper well or soil press technique.

Porewater Analysis	Sample treatment	Volume (ml)	Preservation
Sulfide (S^{2-}), pH	Unfiltered, fixed	3	Run same day
Ammonium (NH_4^+)	0.45 μm filter	10	Frozen
Sulfate (SO_4^{2-}),	0.45 μm filter	10	1 ml 5% Zinc
Chloride (Cl^-)			Acetate, Frozen
Methane (CH_4)	Unfiltered	15	Run same day

Soil cores (n=2 for each community) were taken to a depth of 3 meters (5cm diameter) or deeper depending on depth of refusal in May of 2014. Cores were sectioned in 5cm sections in the first 30cm, 10cm increments from 30-100cm depth, 20cm increments from 1m to 2m depth and 25cm increments at depths beyond 2m. Core sections were immediately wrapped and plastic to limit exposure to ambient air, and placed in a COY laboratories anaerobic chamber (vinyl anaerobic chamber with automatic airlock (AALC), COY Lab Products, Michigan, USA) upon return to the lab (within 1 hour of sample collection). Inside the chamber, porewater immediately was pressed from each core section through a (Whatman no. 1 filter paper), before being syringe tip filtered (0.45 μm). Porewater samples were analyzed for pH, sulfide, sulfate and chloride, as previously described.

Samples for chloride and sulfate analysis were fixed with 5% zinc acetate at a 10 to 1 ratio of sample to zinc acetate. The filtered and fixed samples were diluted at a 50:1 deionized (DI) water to sample ratio, pipetted into 1.5 ml polycarbonate vials, and measured for SO_4^{2-} and Cl^- on a Dionex ICS-2000 RFIC (Dionex Corporation, Sunnyvale CA) with an auto sampler. Porewater chloride and sulfate were used to determine the depletion of SO_4^{2-} in the inventories. Sulfate depletion was calculated as:

$$(SO_4^{2-})_{Dep} = [(Cl_{pw}^-) \times (R_{sw})^{-1}] - SO_4^{2-}{}_{pw}$$

where $(SO_4^{2-})_{dep}$ is the depletion the of SO_4^{2-} inventory in mM S L^{-1}, (Cl^-_{pw}) and $(SO_4^{2-}{}_{pw})$ are the porewater concentrations of Cl^- and SO_4^{2-} measured in mM, and R_{sw} is the constant molar ratio of Cl^- to SO_4^{2-} in surface seawater (R_{sw}=19.33, Weston et al., 2006). The difference between the 'expected' SO_4^{2-} concentration and the 'measured' concentration can provide an estimate of SO_4^{2-} depletion via sulfate reduction in the soil column.

Gas Flux

Four replicate transects were established across the border between native- and *Phragmites*-dominated plant communities. On each transect there was one plot in the native plant community, one in a transitional community with recently established *Phragmites* and native species, and one in near monoculture of *Phragmites*. Methane flux measurements were conducted from August 2012 to September 2013. At each flux plot, a 50 x 50-cm aluminum flux collar was permanently inserted to a soil depth of 30cm. The top of the collar had a flat lip to accommodate a flux chamber that was removed after sampling. During each flux measurement, a clear static flux chamber

with a closed-cell neoprene rubber on the base was placed on an aluminum collar and sealed using clamps. Chambers of different heights were used to accommodate either short native stems (70 cm-tall chambers) or tall *Phragmites* stems (120 cm-tall chambers). Each chamber was fitted with Teflon tubing for collecting headspace gas samples. During each two-hour sampling period, five 20 ml gas samples were drawn at 25 minute intervals, with the first sample drawn at time 0 when the chambers were initially sealed. Gas samples were injected into evacuated Exetainer® glass vials, and CH_4 concentrations determined on a Varian 450-GC CO/CO_2 (Agilent Technologies, Santa Clara CA, USA).

Methane flux rates were calculated using a linear regression of concentration versus time. Rates with an r^2 less than 0.85 were rejected, with high values ascribed to ebullition during the sampling period and low values to leaky vials. Rates were corrected for chamber volume and temperature during the sampling period. Final rates were calculated as CH_4 flux in μmol m^{-2} min^{-1}.

Potential Soil CH_4 and CO_2 Production

Soil cores to a depth of one meter were collected along a transect in both the introduced and native community (n=5 cores in each community). From each core, soil was collected from the 20, 40 and 80 cm depths. A subsample of 10 g was slurried with 20 ml DI water (2:1 ratio of DI to soil) in 100 ml crimp-sealed glass serum vial in an anaerobic chamber, then each jar was further purged with N_2 gas. An initial sample (100 μl) was taken from each vial at time 0 and measured for CO_2 and CH_4 concentration. Serum vials were placed on a shaker table at a medium speed, and stopped to take gas samples once daily for a seven-day period CH_4 was analyzed via

direct inject port on a GC-14A gas chromatograph equipped with an FID detector (Shimadzu Corporation, Kyoto, Japan), while CO_2 concentrations were measured on LiCor LI-7000 CO_2/H_2O Analyzer (LiCor, Lincoln, NB, USA). Rates of production were calculated using a linear regression of production versus time. Production rates with an r^2 less than 0.85 were rejected. Rates were adjusted using headspace volume, temperature and soil oven-dry mass. Final rates were calculated as CH_4 or CO_2 production in units of μmol g-soil^{-1} hr^{-1}.

Potential Soil CH_4 Oxidation

Methane oxidation potential was determined on the same samples described above for rates of production. After production rates were determined, samples were opened and flushed with atmospheric oxygen while on a shaker table to ensure full penetration of oxygen into the sample. Serum vials were crimp-sealed and injected with 2000 μl CH_4 L^{-1}. A time 0 sample was collected, and serum bottles were placed on the shaker table at medium speed and sampled periodically for four days for CH_4 concentrations (previously described) to determine potential rates of methane oxidation. Rates of potential oxidation were calculated as a change in concentrations of CH_4 vs a change in time, with final rates of methane consumption in units of μmol CH_4 g-soil^{-1} hr^{-1}.

Results

Porewater analysis

Depth profiles of sulfate (figure 3.1 and chloride (figure 3.2) and sulfate depletion (figure 3.3) show differences between the invasive community at Fox Creek and the other 3 communities that were sampled in the upper 1 meter of the soil profile. At Corn Island, there were no differences in sulfate or chloride concentrations at depth between communities. Sulfate, Chloride and $Sulfate_{Dep}$ all peak within the upper meter of the soil profile. Fox Creek has lower chloride and salinity in the surface of *Phragmites* community than is observed in the native community. This difference in not seen at Corn Island, as both communities reflect similar salinity (figure 3.4). There was no community difference observed in $sulfate_{Dep}$ at Corn Island, but with depth I did not see as much variation as was seen at Fox Creek. Fox Creek shows a decrease in pH in the *Phragmites* community in the top meter of the soil (figure 3.5)

At Corn Island, there were only small differences between communities in porewater sulfide concentration at all depths during the warmest months, and slightly higher concentrations in the native community in April and October (Figure 3.6). Fox Creek followed a similar pattern at 20 cm depth except that concentrations were high in the native community in June rather than April. Depth profiles of sulfide to 3 m at both sites supports the trend in the well data for higher dissolved sulfide in native community (Figure 3.7), but the pattern was much stronger at Fox Creek than Corn Island.

Dissolved CH_4 in porewater increased with depth from 20, 40 to 80 cm in the native community at Fox Creek (Figure 3.8). I am unable to compare the native and *Phragmites* communities across depths at Fox Creek due to my limited ability to collect porewater below 30 cm in the *Phragmites*. Methane concentrations at Corn Island did not increase with depth in either community. Methane concentrations increased during the growing season at 20 and 40 cm depths in all communities and at both sites, while the seasonal pattern at 80 cm tended to be the opposite. The invasive community at Corn Island showed an increased in methane concentration during the growing season at a greater rate than the native species.

Field Methane Emissions and Soil Incubations

The *Phragmites* community at Fox Creek (Figure 3.9) had significantly higher CH_4 emissions than the native community during each sampling event ($p=0.002$). There was no statistical difference between CH_4 emissions from the native and mixed communities, but there was a general pattern of higher emissions in study plots with a mixed community than a native community. Peak emissions in all three communities occurred during the July and September sampling events.

Soil methane production potentials were greater, but not significantly, in the *Phragmites* community than native community at 40 cm ($p=0.127$) and 80 cm ($p=0.178$) depths (figure 3.10). There was no difference at the 20 cm depth between communities, but the *Phragmites* plots on average had a higher potential rate of potential production. The peak average rate of potential production in both communities was observed at the 40 cm depth. Carbon dioxide production potentials were greater in the native community than the introduced *Phragmites* community at

all 3 depths (p=0.0214) (figure 3.11). The highest potential rate of production in both communities occurred at the 80 cm depth. Potential rates of methanotrophy were higher in the *Phragmites* community at the 40 cm and 80 cm depths, with no differences observed at the 20cm depth (figure 3.12).

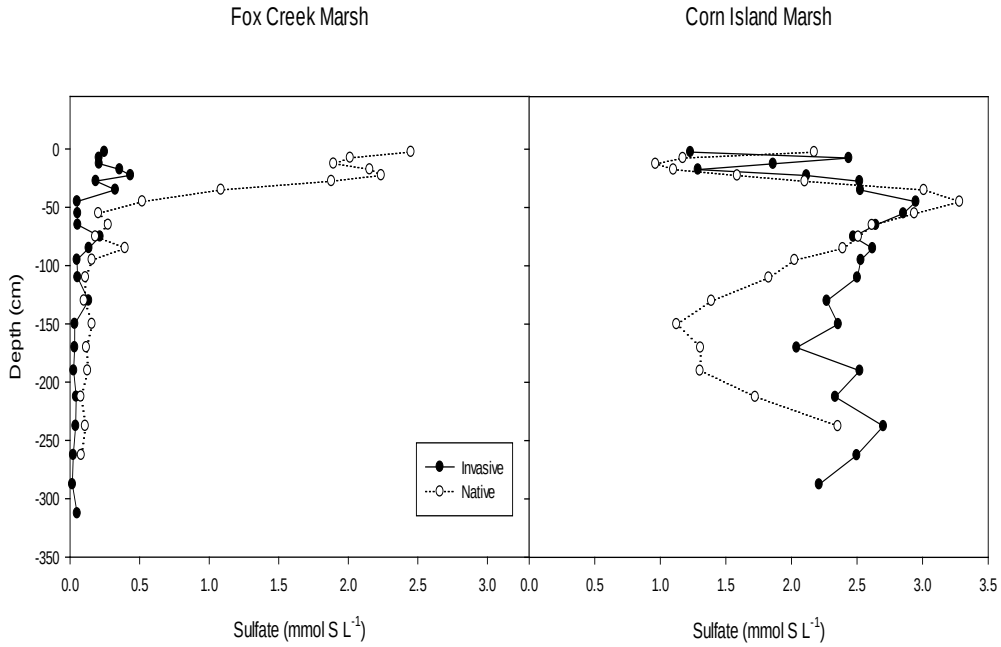


Figure 3.1. Porewater sulfate (SO_4^{2-}) to a depth of 3 meters in the native and invasive communities at Fox Creek (left) and Corn Island (right) marshes. Porewater was obtained by squeezing the soil in increments from deep rooting cores that were collected.

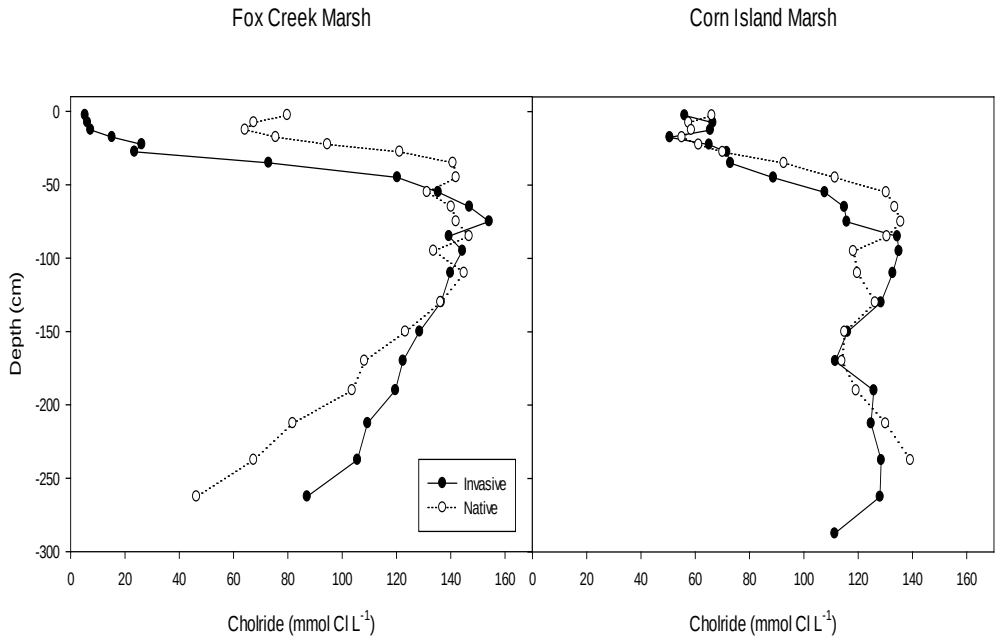


Figure 3.2. Porewater chloride (Cl^-) to a depth of 3 meters in the native and invasive communities at Fox Creek (left) and Corn Island (right) marshes. Porewater was obtained by squeezing the soil in increments from deep rooting cores that were collected.

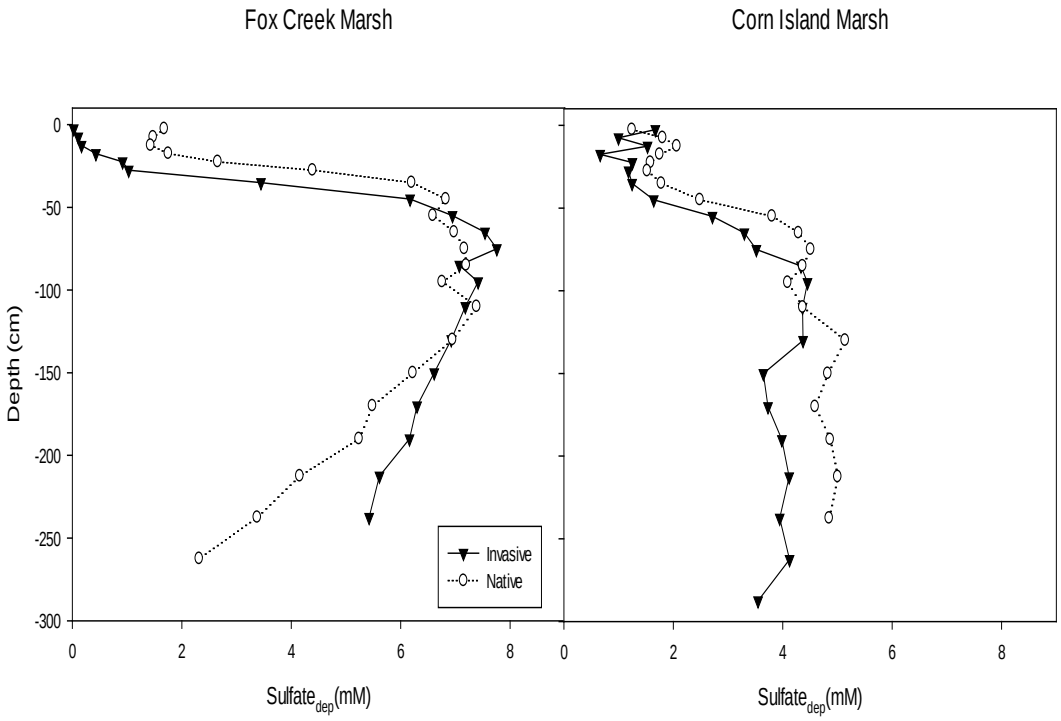


Figure 3.3. Depletion of sulfate inventories to a depth of 3 meters in the native and invasive communities at Fox Creek (left) and Corn Island (right) marshes. Porewater was obtained by squeezing the soil in increments from deep rooting cores that were collected.

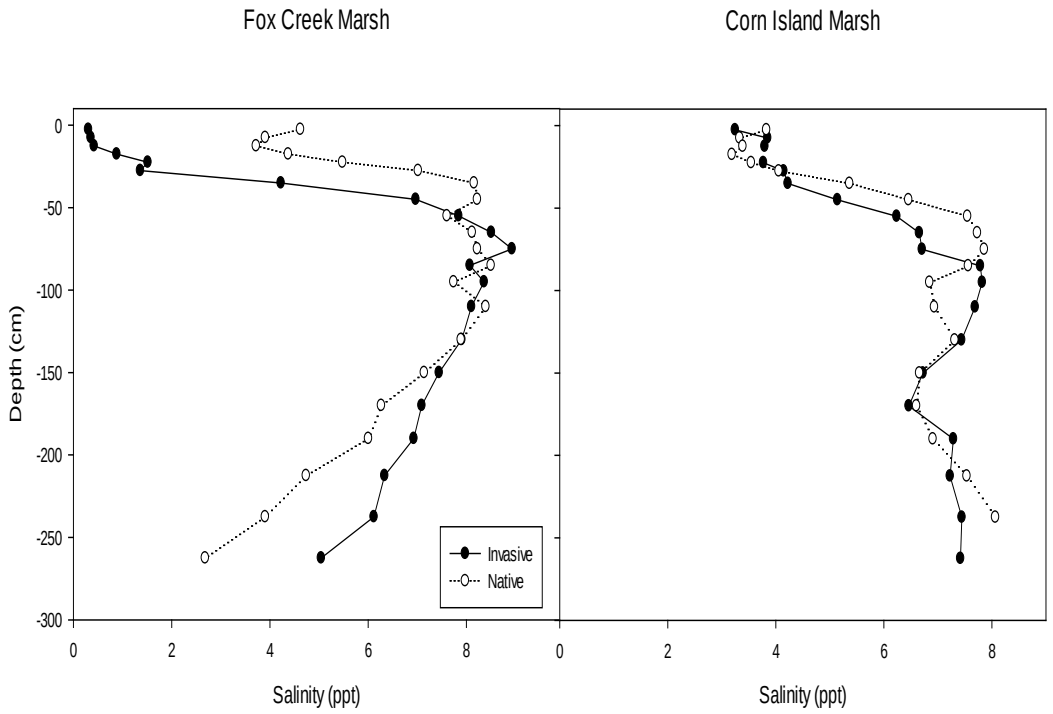


Figure 3.4. Salinity to a depth of 3 meters in the native and invasive communities at Fox Creek (left) and Corn Island (right) marshes. Salinity was calculated using Knudsen equation where: Salinity = 0.030+ 1.8050 * chlorinity.

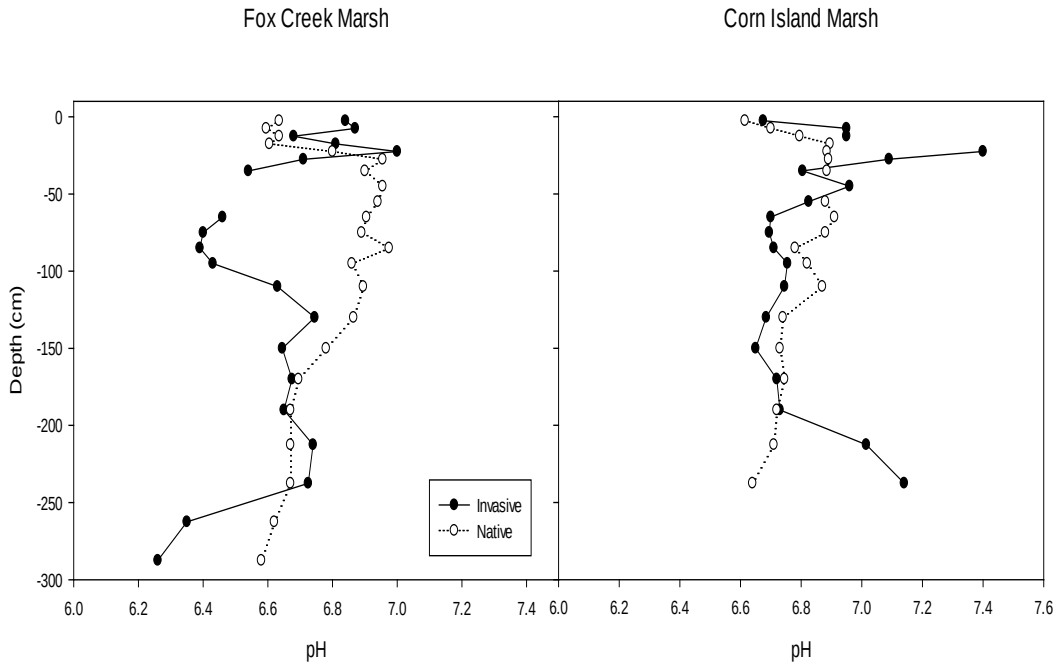


Figure 3.5. Porewater pH to a depth of 3 meters in the native and invasive communities at Fox Creek (left) and Corn Island (right) marshes. Porewater was obtained by squeezing the soil in increments from deep rooting cores that were collected.

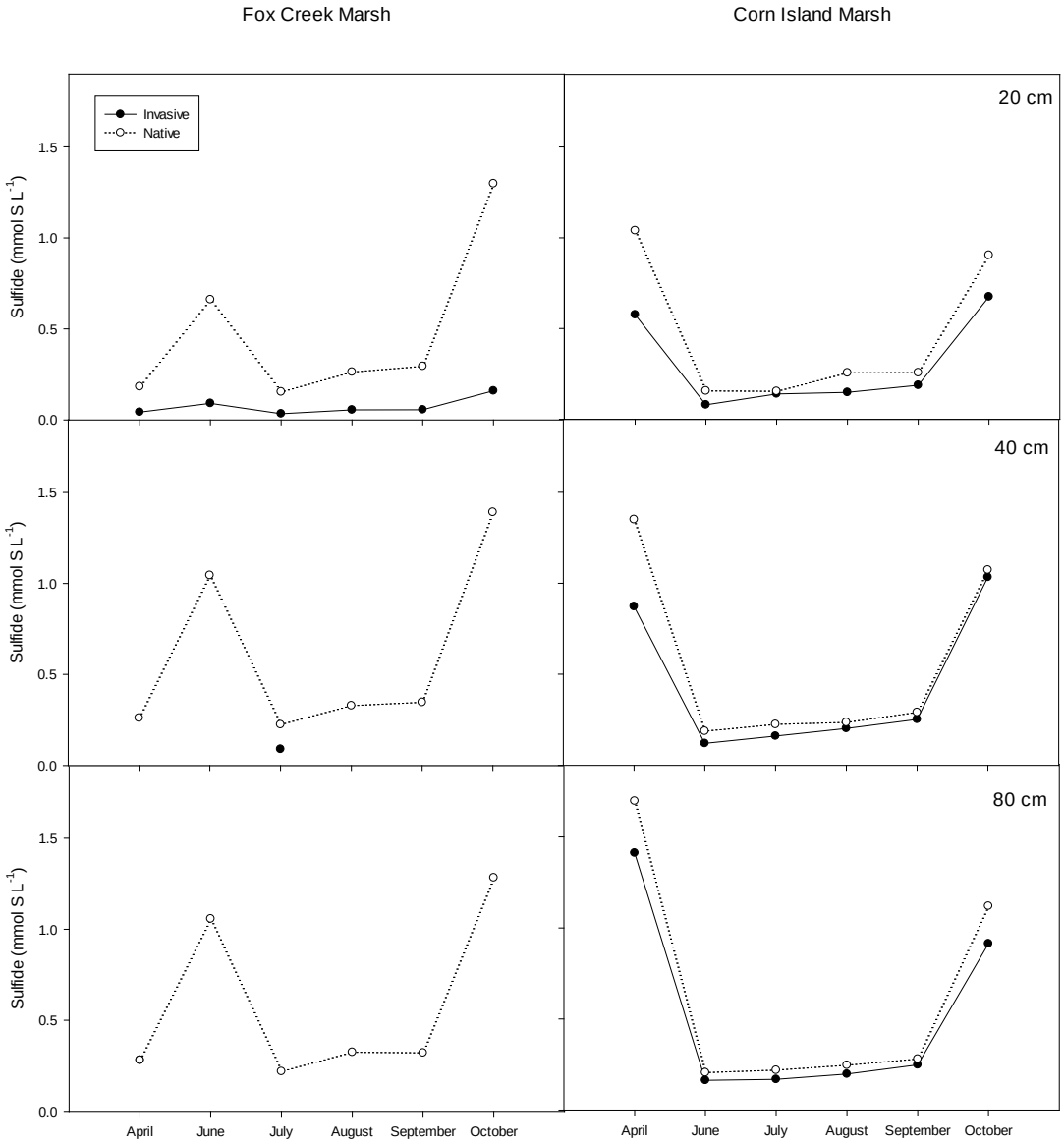


Figure 3.6. Porewater sulfide (S^{2-}) at 20, 40 and 80cm in the native and invasive communities at Fox Creek (left) and Corn Island (right) marshes during the 2013 growing season. Missing values at the 40 and 80cm depth in the invasive community at Fox Creek is due to a lack of sampling do to soil constraints. Porewater was collected using porewater sippers (described above) that were left in the systems during the duration of the growing season.

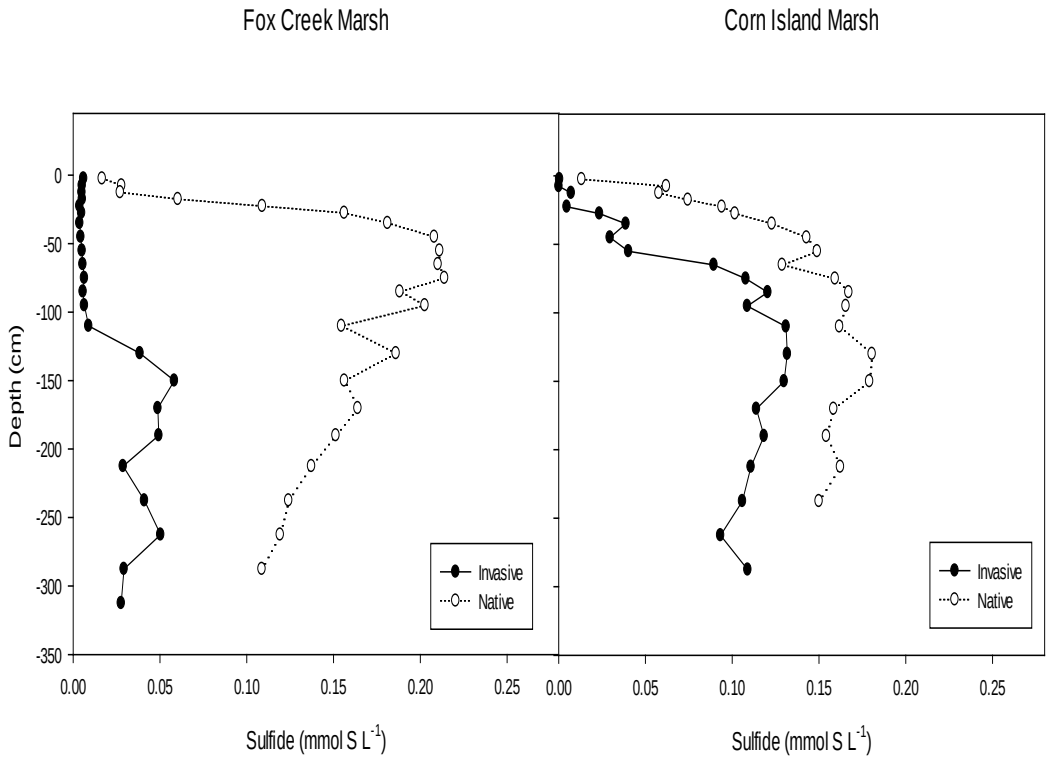


Figure 3.7. Porewater Sulfide (S^{2-}) to a depth of 3 meters in the native and invasive communities at Fox Creek (left) and Corn Island (right) marshes. Porewater was obtained by squeezing the soil in increments from deep rooting cores that were collected.

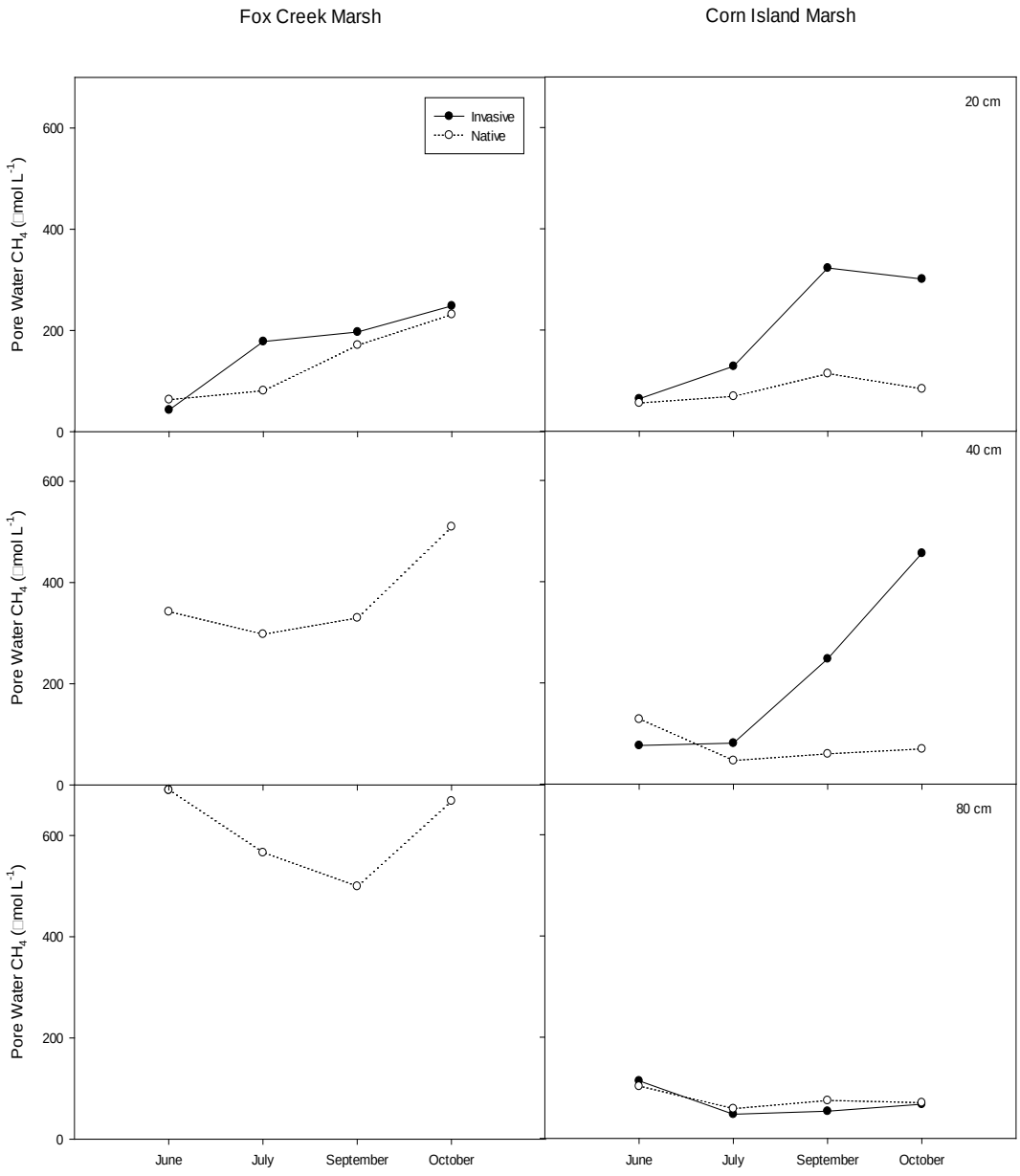


Figure 3.8. Porewater methane (CH_4) at 20, 40 and 80cm in the native and invasive communities at Fox Creek (left) and Corn Island (right) marshes during the 2013 growing season. Missing values at the 40 and 80cm depth in the invasive community at Fox Creek is due to a lack of sampling do to soil constraints. Porewater was collected using porewater sippers (described above) that were left in the systems during the duration of the growing season.

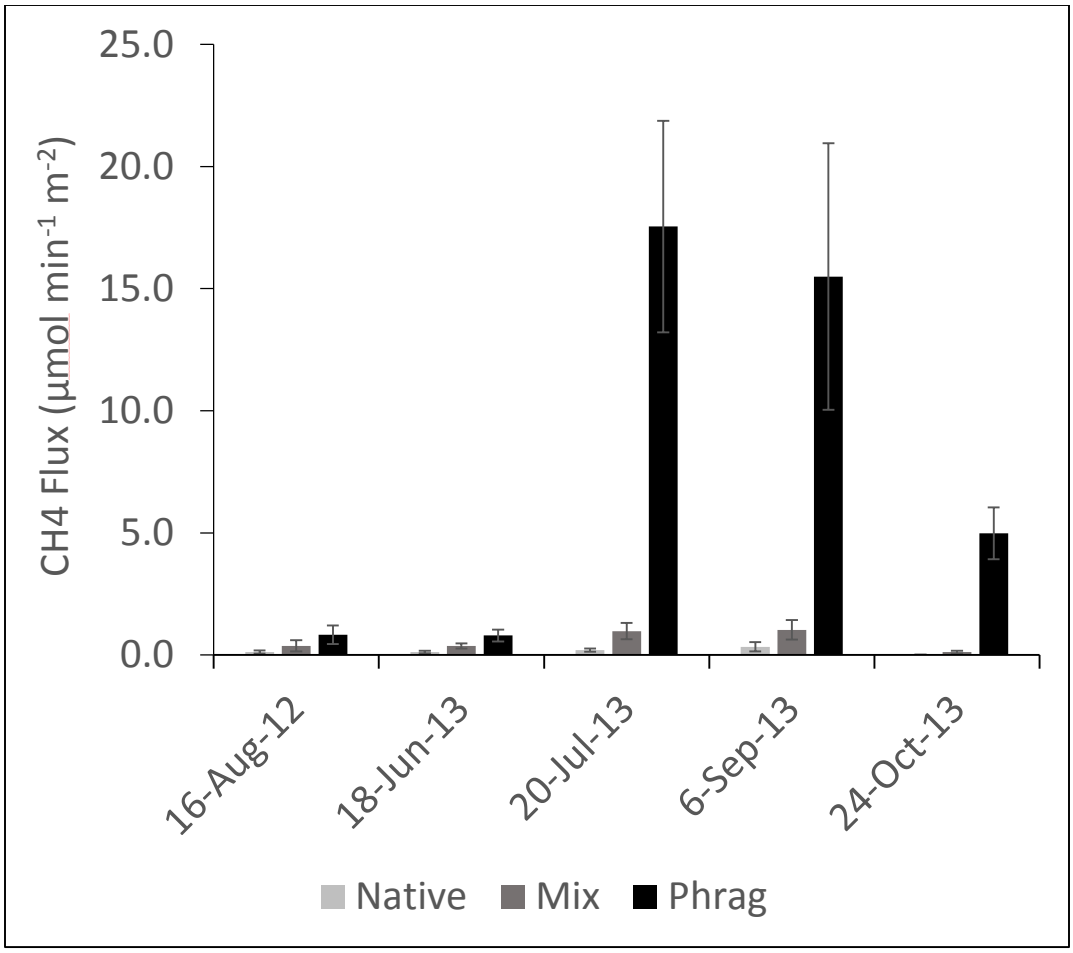


Figure 3.9. Methane emissions from native, invasive and mixed community plots during the growing season in 2013 at Fox Creek Marsh. Error bars denote standard error.

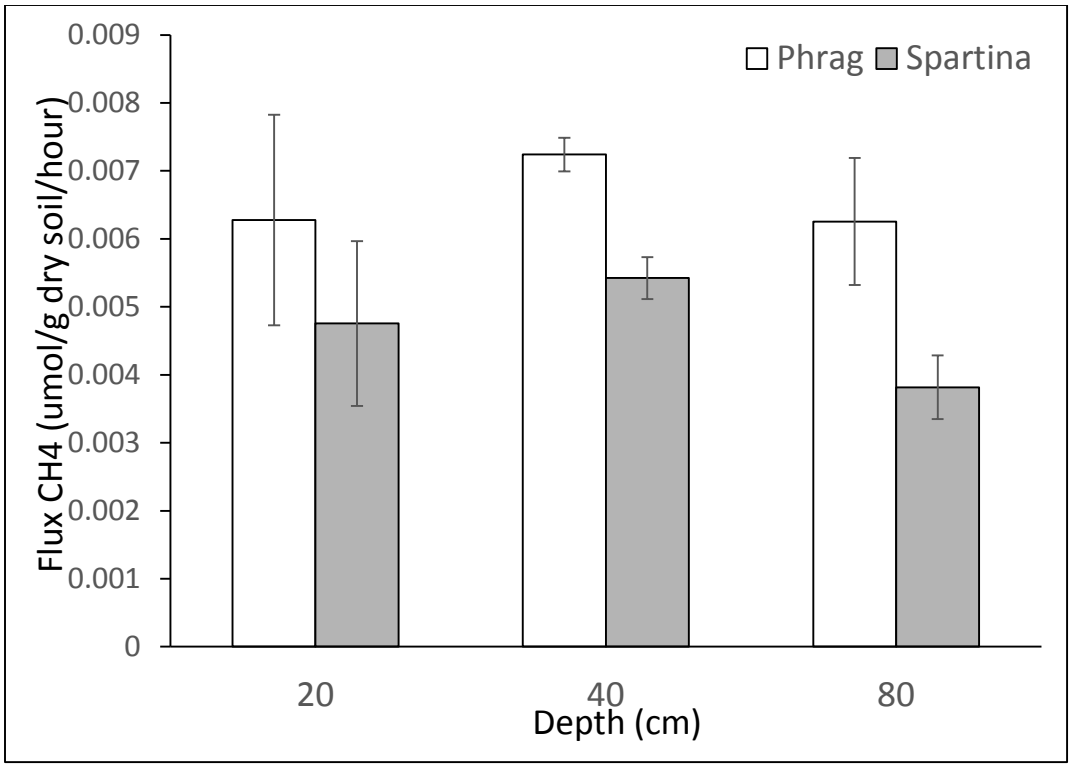


Figure 3.10. Methane flux means under anaerobic incubation conditions in umol/g dry soil hour^{-1} at 20, 40 and 80 cm depth, with error bars denoting standard error. Samples were taken from Fox Creek Marsh during the growing season in 2012.

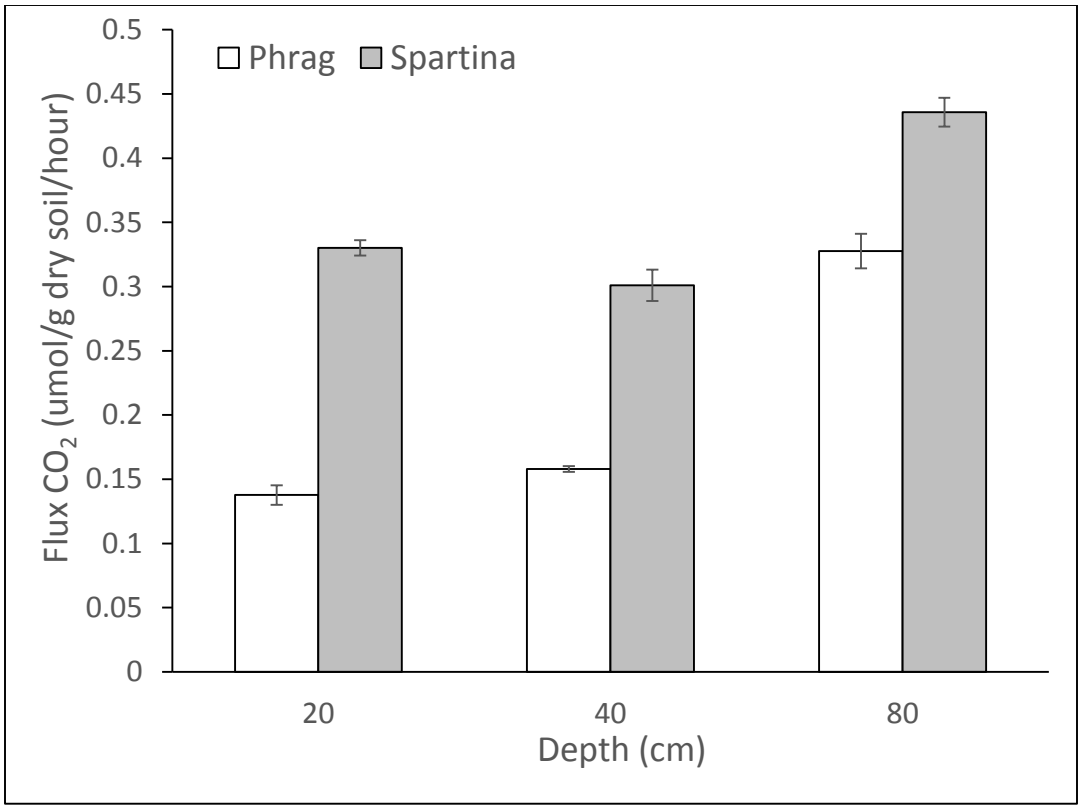


Figure 3.11. Carbon Dioxide (CO_2) flux means under anaerobic incubation conditions in umol/g dry soil hour^{-1} at 20, 40 and 80 cm depth, with error bars denoting standard error. Samples were taken from Fox Creek Marsh during the growing season in 2012.

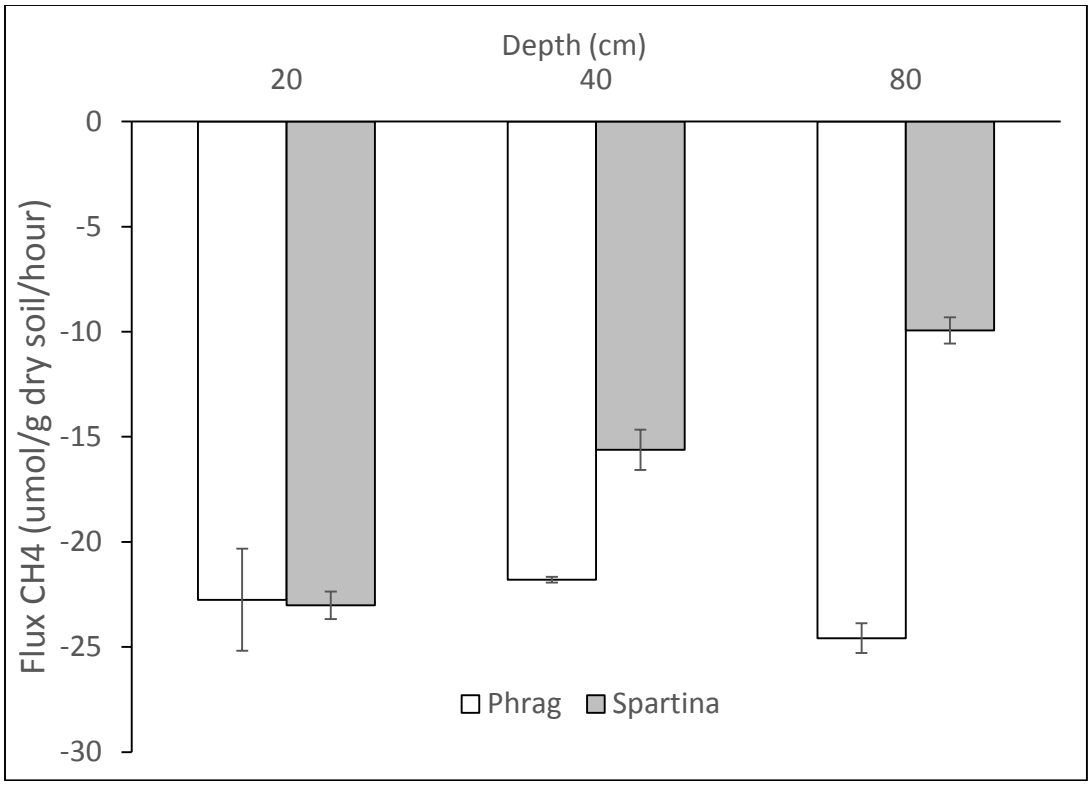


Figure 3.12. Methane flux means under aerobic incubation conditions in umol/g dry soil hour^{-1} at 20, 40 and 80 cm depth, with error bars denoting standard error. Samples were taken from Fox Creek Marsh during the growing season in 2012.

Discussion

Differences in porewater and redox chemistry between the two study sites were largely independent of plant community composition, and appear to be driven by soil composition and hydrology. In particular, the soil and porewater chemistry profiles at the Fox Creek site in the *Phragmites* community are vastly different from the other three communities in this study. The upper meter of the Fox Creek *Phragmites* community had lower porewater concentrations of sulfate (figure 3.4), chloride (figure 3.5) and salinity (3.7) than the native community at Fox Creek, the native community at Corn Island or the *Phragmites* community at Corn Island. These data suggest that there is a groundwater source in the *Phragmites*-dominated area of Fox Creek marsh, which could be a product of the geomorphology of the site, which is located at the base of a sloped upland.

Relatively low salinity conditions in the Fox Creek *Phragmites* community coincide with soil horizons characterized by relatively high bulk density and mineral content. This feature was not observed in soil cores taken in from the other sites in this study (figure 2.7), and has not been previously observed in extensive coring of nearby Global Change Research Wetland (J.P. Megonigal, pers. comm.). Other soils in the area are more uniformly 80% organic to depths of up to 5 m. Based on the depth distribution of this mineral-rich horizon (approximately 40-90 cm), it was deposited well before the invasive *Phragmites* genotype was a dominant species at the site, and it may help explain the presence of *Phragmites* near the upland edge of this marsh, and the observation that these soils are relatively less saline than other soils at the site. Many *Phragmites* stands at nearby sites, and the nearby Global

Change Research Wetland, have started on creek edges, and worked into native communities. This *Phragmites* stand colonized on the upland side of the wetland, in an area similar to that of a bluff or drainage area. This geographic difference from the Corn Island site, could suggest potential freshwater outwells through the previous discussed mineral horizon.

The mineral-rich horizon may also explain a number of biogeochemical features of the Fox Creek *Phragmites* site that contrast with the other three sites in the study. A source of freshwater can explain the relatively low sulfate concentrations, which are expected to support relatively low rates of sulfate reduction and generation of sulfide. Relatively low porewater sulfide may be explained by a combination of sulfate-limited microbial respiration and the relative abundance of iron-bearing soil minerals capable of reacting with free sulfides to form iron sulfides. Porewater pH is lower in the mineral horizon (figure 3.8), which could be due to oxidation of the iron sulfides in the presence of root oxygen loss (Armstrong et al., 1996, Brix et al., 1996). With the production and availability or iron sulfide paired with a high proportion of *Phragmites* root biomass within the mineral horizon, this oxidation process could generate small amount of acid (Sullivan et al., 1988, Schippers and Jørgensen, 2002), which would lower pH.

Perhaps the most important implication of the unique soil and redox features I found in the *Phragmites* community at Fox Creek is the potential for increased methane emissions. Low rates of CH_4 emissions from brackish systems compared to freshwater wetland systems has been attributed to high sulfate availability driving the more energy efficient microbial process of sulfate reduction (Megonigal et al., 2003,

Weston et al., 2006). Indeed, methane emissions rates from the *Phragmites* community at Fox Creek were much higher than the three other communities in this study. There are several processes that alone or in combination could increase methane emissions. I found a trend for relatively high potential rates of methane production in the Fox Creek *Phragmites* community, which may be explained by either low sulfate concentrations, high carbon inputs through deep *Phragmites* root production, or most likely a combination of these factors. The fact that potential rates of methane oxidation were also relatively high in these soils is strong evidence that deep *Phragmites* roots are influencing methane biogeochemistry at the site. High potential rates reflect a relatively large community of methanotrophs, which can occur at these depths if there root oxygen loss. Methane oxidation has been recognized as an important factor in regulation CH_4 flux (King, 1992). *In situ*, actual rates of methanotrophy are less than actual rates of methane production because methane accumulates in the porewater. The fact that porewater methane concentrations were similar in the native and *Phragmites*-dominated sites most likely reflects the that *Phragmites* is more efficient conduit for methane to escape from the soil to the atmosphere than the native species at this site because of very high plant biomass (Grünfeld and Brix, 1999).

A novel aspect of this study is that porewater chemistry, soil characteristics and root biomass measured to depths much deeper than the many other soil ecology studies. With invasive *Phragmites* rooting significantly deeper than native plant species, it is apparent that future studies need to extend deeper into the soil profile. At Corn Island, in both communities, the porewater attributes measured remained

relatively constant. At Fox Creek, below 1 meter, I observed a decrease in salinity and chloride concentrations. With a deeper rooting system (2.5+ meters), the invasive species has access to water lower in salts, which may reduce salinity stress. This could provide the plant the ability to shift nutrient allocation away from osmotic regulation towards root growth and aboveground biomass growth (Lissner and Schierup, 1997). Below 1 meter, sulfate depletion increased with depth as a result of lower sulfate availability compared the potential for sulfate reduction respiration. Such conditions also favor methane production.

Collectively, my data suggest that unique hydrological features found associated with the *Phragmites* community at Fox Creek account for the high rates of methane emissions. Low salinity at this site promote relatively high *Phragmites* biomass, which in turn promotes soil microbial respiration. Because iron and sulfate availability is limited, a relatively large fraction of total microbial respiration terminates in methane production, which is efficiently vented to the atmosphere through *Phragmites* stems. Thus, I conclude that a combination of pre-existing soil conditions and *Phragmites* invasion explain the very high methane emissions at this site.

Chapter 4: Conclusion

In this study *Phragmites* produced around 4 times more aboveground biomass and nearly twice the belowground root biomass than the native communities. This greater aboveground biomass, complemented by higher tissue nitrogen concentrations, represents a fundamental shift in nitrogen storage and allocation patterns within these marsh systems. In addition to higher rates of net primary production, previous studies have shown slower rates of decomposition of *Phragmites* tissue. This coupling of processes could lead to greater pools of surface N, which in-turn is likely to alter chemical and biological properties of the marsh. With the introduction of *Phragmites* monocultures, there is a shift from native communities in which nitrogen storage was predominantly belowground, to a more above-ground dominated vegetation N sink.

Significantly deeper rooting systems in the *Phragmites* provides increased access to nutrients and potentially lower salinity porewater, both of which provide a competitive advantage. Although competition was not directly measured in this study, it can be thought that accessing larger pools of nutrients allows *Phragmites* an advantage and can aid in its invasion of native communities in similar marsh systems. Since short term studies cannot capture all aspects of invasion, such as I have studied here a space-for-time study allowed us to examine *Phragmites* monocultures as a potential "future" marsh ecosystem, and examine different above and belowground properties as changes to current native ecosystems. Although it is uncertain how individual *Phragmites* monocultures become introduced in a system, this study design allowed us to forecast changes in nitrogen pools and nitrogen cycling.

Due to limitations in site replication and continuity, it is difficult to draw clear cut conclusions in this study. Mainly, differences in soil profiles between sites, and also within sites, restrict our boundaries when looking at causality linked to *Phragmites* invasion. The mineral rich horizon in the Fox Creek *Phragmites* that is not present in the other site and communities, can help to explain differences observed in porewater pH, sulfide, chloride and methane flux, but restricts our assumptions made in regards to *Phragmites* invasion on N pools and cycling. Many of the differences in porewater and redox chemistry between sites and communities seem to be largely independent of plant community composition and more driven by soil composition and hydrology. This study illustrates that measuring multiple processes to examine the impact of plant invasion on a marsh system is important. It is unclear at this point as to the full affect differences in soil composition has on *Phragmites* invasion at these two sites, but what is clear is nitrogen pools and cycling is tightly connected to other systems properties.

A novel aspect of this study in comparison to many other soil and below-ground ecology studies, is the depth to which porewater chemistry, soil characteristics and root biomass was measured. Many studies involving invasive species examine the upper 50cm of soil, but with *Phragmites* rooting significantly deeper (2.5+ meters), it is apparent that relevant studies need to extend deeper into the soil profile to fully capture associated changes. With a deeper rooting system *Phragmites* could provide organic matter to fuel biogeochemical process not observed in native communities below 50cm depths.

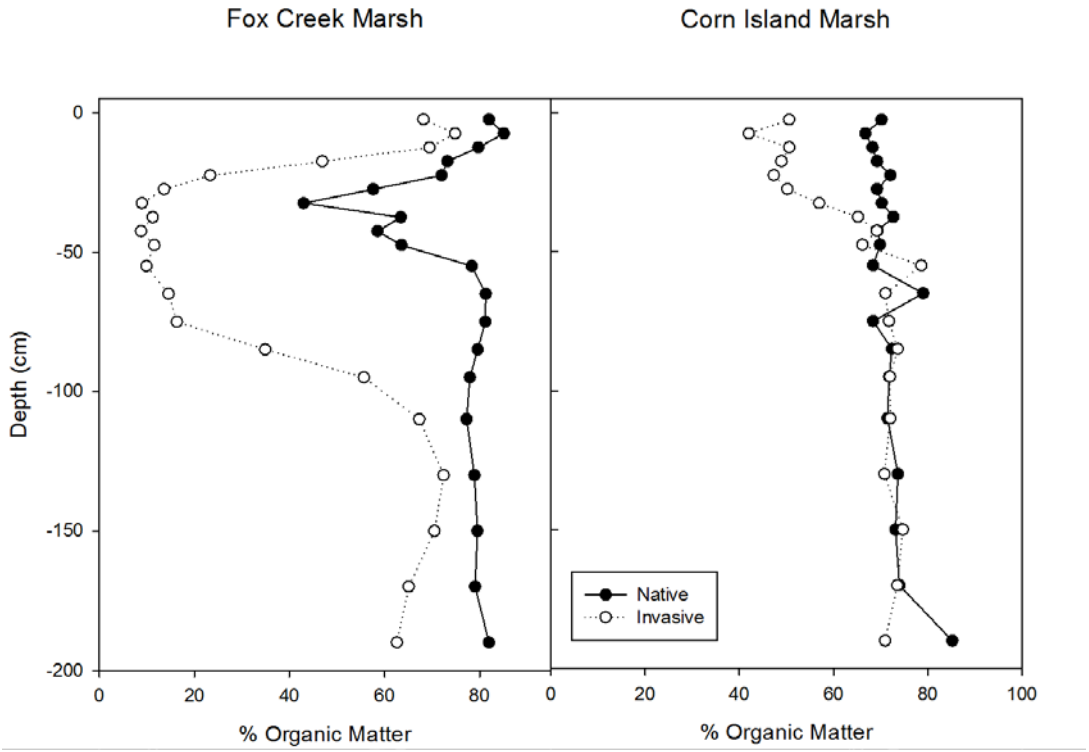
Overall, in this study *Phragmites* demonstrates the ability to access deeper depths and nutrient pools. This trait allows for greater nitrogen uptake, and paired with higher biomass production and tissue nitrogen concentrations, increases the marsh systems ability to store nitrogen. Although, this does not account for changes in nitrogen mineralization at deeper depths, which could offset some of the N-sink ability after *Phragmites* invasion. In the face of changing climate conditions, it remains unclear how different marsh processes will respond to alterations over several years and decades. Increases in anthropogenic nitrogen pollution could be offset by the increased N demand and uptake in an invasive community such as *Phragmites*. Any assumption or predictions of how plant invasion will affect N availability within an ecosystem is complicated by other chemical or physical differences within the studied systems. The fate of N in *Phragmites* dominated systems could better be understood in long term studies that include measuring other N transformations such as mineralization, denitrification and decomposition. This would provide a much clearer nitrogen budget picture, and what could be expected to be seen in marsh systems currently being invaded by *Phragmites australis*.

Appendices

Appendix 1. Fox Creek Marsh and Corn Island Marsh bulk density (g cm^{-3}) and % organic matter measured using a loss on ignition method.

	Fox Creek Marsh				Corn Island Marsh			
	P. australis		**Native**		***P. australis***		**Native**	
Depth (cm)	Bulk Density (g cm^{-3})	% OM	Bulk Density (g cm^{-3})	% OM	Bulk Density (g cm^{-3})	% OM	Bulk Density (g cm^{-3})	% OM
0-10	0.10	74.8	0.10	85.1	0.31	42.0	0.13	66.8
10-20	0.18	46.9	0.11	73.2	0.25	48.9	0.12	69.3
20-30	0.63	13.6	0.17	57.6	0.24	50.2	0.12	69.2
30-40	0.91	9.0	0.18	63.4	0.14	65.2	0.10	72.7
40-50	0.80	11.6	0.13	63.6	0.13	66.1	0.11	69.9
50-60	0.85	9.9	0.13	78.3	0.15	78.6	0.12	68.4
60-70	0.62	14.6	0.11	81.3	0.11	71.0	0.15	79.0
70-80	0.59	16.3	0.11	81.2	0.10	71.8	0.12	68.4
80-90	0.35	34.9	0.12	79.6	0.09	73.6	0.10	72.4
90-100	0.21	55.7	0.10	78.0	0.10	72.0	0.10	71.8

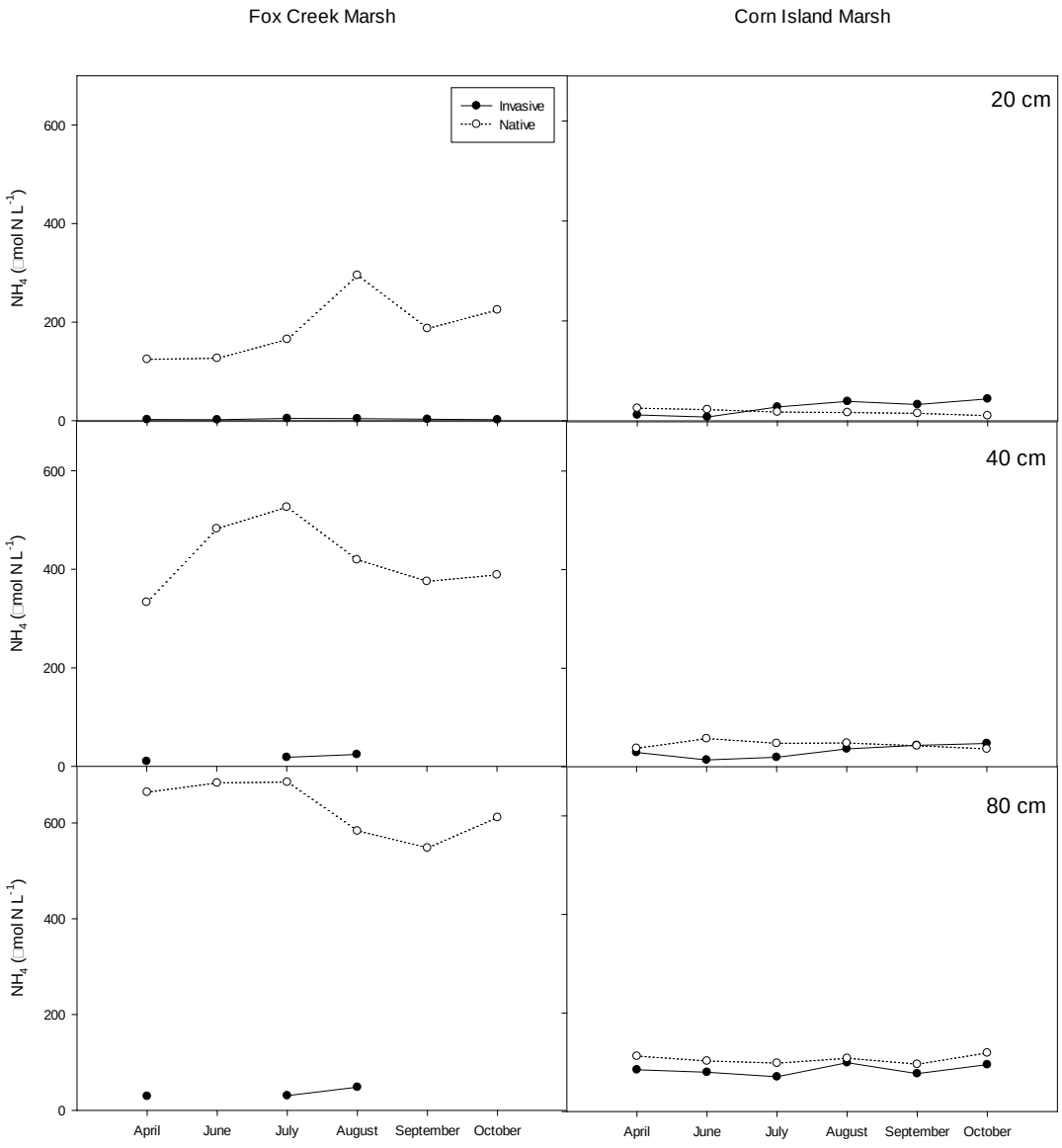
Appendix 2. Organic matter (%) at Fox Creek Marsh and Corn Island Marsh to a depth of 2 meters, determined using a loss on ignition method.



Appendix 3. Dates and coring techniques for the different procedures and analysis discussed in the methods sections.

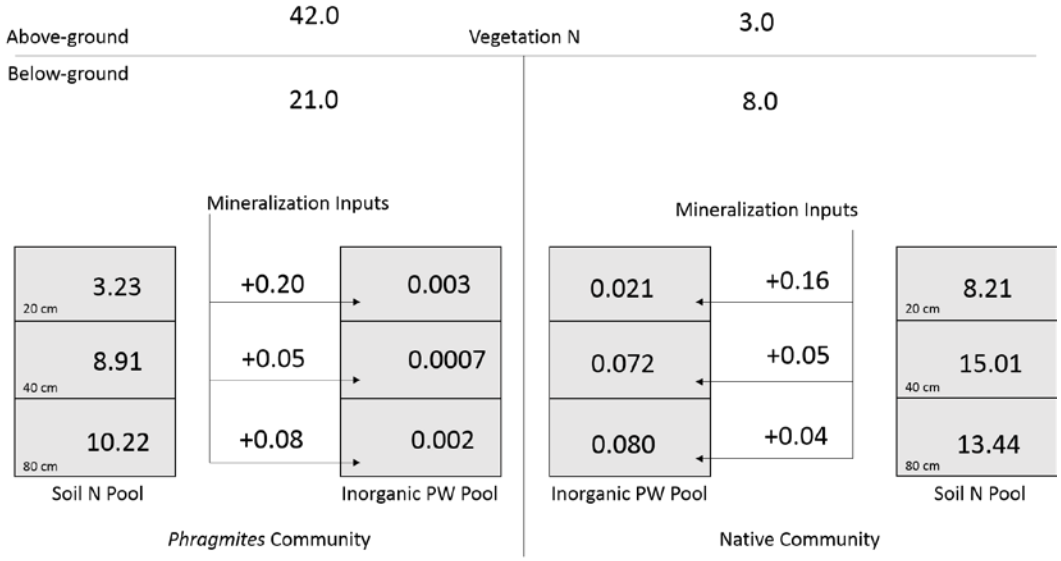
Date	Analysis/Procedure	Notes
01 May 2013	Porewater wells (20, 40, 80 cm)	Measured monthly during the growing season (May-September)
10 Jul 2013	Soil Extractable	1m punch Auger Corer (5 cm diameter)
10 Aug 2013	Aboveground Biomass Collection	0.25 x 0.25 m plots
01 Aug-30 Oct 2013	Ion Exchange Resin field measure of N availability	Mixed-bed cation-anion exchange resin
24 Jul-10 Aug 2013	^{15}N uptake injection (24 July 2013) and vegetation collection (10 August 2013)	Initial samples collected before injection. 2 week samples collected for determination
May 2014	Root Depth distributions (belowground biomass collection)	1m punch Auger Corer with extensions (5 cm diameter)
May 2014	Deep N porewater Analysis	1m punch Auger Corer with extensions (5 cm diameter)

Appendix 4. Porewater ammonium concentrations (NH_4^+) at 20, 40 and 80cm in the native and invasive communities at Fox Creek (left) and Corn Island (right) marshes during the 2013 growing season. Missing values at the 40 and 80cm depth in the invasive community at Fox Creek is due to a lack of sampling do to soil constraints.

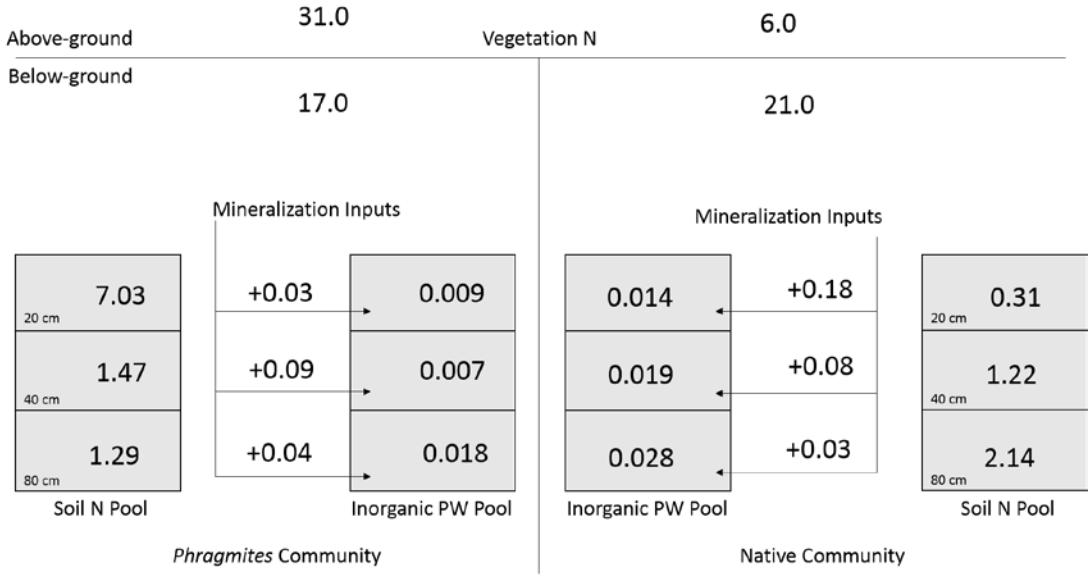


Appendix 5. Nitrogen budgets for Fox Creek Marsh (panel A) and Corn Island Marsh (panel B). Stocks (g N m^{-2}) were measured at the three soil depths (20, 40 and 80 cm), and in above- and below-ground vegetation (g N m^{-2}). Nitrogen mineralization inputs are estimated by ion-exchange resins and are displayed as grams of N per growing season (June-September) and is the rate expressed as absorption by 1 gram of ion-exchange resin.

Fox Creek Marsh



Corn Island Marsh



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