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Effects of Invasion by the Common Reed (*Phragmites australis*) on Carbon Transformations in a Great Lakes Marsh

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EFFECTS OF INVASION BY THE COMMON REED (*PHRAGMITES AUSTRALIS*) ON
CARBON TRANSFORMATIONS IN A GREAT LAKES MARSH

by

Shawn Trevor Duke

Thesis

Submitted to the Department of Biology

Eastern Michigan University

In partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

in

Biology with a concentration in Ecology and Organismal Biology

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Effects of invasion by the common reed (*Phragmites australis*) on carbon transformations in

a Great Lakes marsh

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DEDICATION

To my daughter Rachel

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I would like to express my sincerest gratitude to all the family members and friends who have motivated and supported me throughout this research. Completion of this thesis would not have been possible without your patience and assistance. I would like to thank my advisor, Dr. Kristin E. Judd, whose guidance through the planning and completion of this research was invaluable. Her foresight and encouragement supported my growth as an ecological researcher. Dr. Steven N. Francoeur provided mindful and patient advising on experimental development and analysis. Dr. Gary L. Hannan provided guidance and encouragement that improved the technical and botanical integrity of this research. I would also like to thank all the field and laboratory assistants and collaborators who made this project a success, including Audrey Johnson, Jennifer Kirk, Jay Krystyniak, Jerry Tyrrell, Penelope Richardson-Bristol, and Grace Carpenter.

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ABSTRACT

The common reed (*Phragmites australis*) is a highly productive invasive grass that alters the wetland physiochemical environment and produces toxic secondary metabolites. Plant litter decomposition, effects of water level on soil microbes, and soil microbial response to leachate additions were investigated in *Phragmites* invaded and pre-invaded *Typha* sites. Less litter mass was lost from *Phragmites* than *Typha* in both sites during the initial 144 days. Annual mass loss from both species' litter was greater in the *Phragmites* site. Lower water levels resulted in greater CO₂ than CH₄ production in both *Phragmites* and *Typha* soils. Higher water levels resulted in greater CH₄ than CO₂ production in both soils and greater CH₄ production in *Typha* than *Phragmites* soils. Introducing *Phragmites* leachate to *Typha* soils resulted in less (not significant) microbial respiration than *Typha* leachate or dH₂O. Post-invasion environmental conditions enhanced gaseous carbon release, but high primary productivity resulted in net carbon storage.

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INTRODUCTION

Wetlands provide beneficial ecological services at the population, ecosystem, and global levels (Mitsch and Gosselink 2007). One important ecosystem service they provide is carbon sequestration. Marshes are highly productive wetlands that rank with the most concentrated terrestrial stores of carbon per unit area (Zedler and Kercher 2005). Plant invasions can significantly alter several wetland functional processes, including carbon transformations (Ehrenfeld 2003). An introduced genotype, haplotype M, of the common reed (*Phragmites australis* subsp. *australis* (Cav.) Trin ex Steudel), which is considered to be the ancestral lineage of populations in Europe, Asia, and Africa (Lambertini et al. 2006), has been aggressively invading Lake Erie coastal wetlands since the late 1980s to early 1990s (Saltonstall 2002, Wilcox et al. 2003). In contrast to the native subspecies (*P. australis americanus*), colonization by the introduced subspecies results in the development of monocultures that replace more diverse pre-invaded communities largely populated by hybrid cattail (*Typha x glauca* Godr.) in this area (Tulbure et al. 2007). *Phragmites*' dominance is achieved through competitive advantages derived from high rates of primary productivity (causing decreased light infiltration, air temperatures, and soil temperatures within stands), a large proportion of recalcitrant biomass, clonal growth, aerenchyma for bulk O₂ flow, and secondary metabolites that are allelopathic (phytotoxic) and antimicrobial (Anesio et al. 1999, Meyerson et al. 2000, Bains et al. 2009, Duan et al. 2009, Rudrappa et al. 2009). Other potential environmental impacts resulting from *Phragmites* invasion are modified soil structure, moisture content, and O₂ concentration (redox conditions) (Neubauer et al. 2005, Duan et al. 2009, Weidenhamer and Callaway 2010). Changes in the physiochemical environment brought about by *Phragmites* invasion could alter organic

matter turnover rates. Chemical and physical environmental modifications also affect microhabitats, which in turn could shift decomposer community structure and function (Kourtev et al. 2002, Lin et al. 2002, Reynolds et al. 2003, Ehrenfeld et al. 2005, Wolfe and Klironomos 2005).

Because wetlands are ecosystems with the potential to serve as substantial carbon sinks, quantification of carbon flow through freshwater marshes before and after invasion is necessary to accurately develop global carbon budgets (Figure 1). In addition to accumulating large quantities of plant biomass each year, *Phragmites* may impact wetland carbon fluxes through several other pathways, including plant tissue decomposition, soil microbial respiration, and soil methane production. These pathways can be influenced by environmental factors such as temperature, oxygen availability, and light availability and biological factors such as species diversity, microbial community structure, and microbial community function (Anesio et al. 1999, Meyerson et al. 2000, Rothman and Bouchard 2007, Rudrappa et al. 2009).

Increased production of recalcitrant structural molecules in *Phragmites* biomass could significantly alter nutrient availability and decomposer activity in invaded wetlands. Marshes are distinctly important wetlands because they couple high primary productivity with slow decomposition in anaerobic sediments. In inundated marshes, plant-produced organic matter (litter) falls to the soil surface and then decomposes at low rates, resulting in accretion (Figure 1). This accumulation of litter at the soil surface has been shown to decrease seedling survival of competing plant species (Vaccaro et al. 2009) and could provide invasive *Phragmites* with a mechanism to store nutrients for long term use. When standing dead plant material falls to the litter layer, soluble compounds are leached and rapidly taken up by

microbes, followed by slower decomposition that is typically not nutrient limited (Agoston-Szabo and Dinka 2008). *Phragmites* has higher lignin content than the species it replaces, and the lignin contained in *Phragmites* litter has been shown to decompose at a much lower rate than lignin in *Typha* litter (11% *Phragmites*: 71% *Typha* acid detergent lignin loss after two years) (Agoston-Szabo and Dinka 2008). This resistance to decomposition may be a major factor that benefits invasive plants by providing them with the ability to store organic material in the litter and soil layers. Accumulated carbon could be used to fuel microbial transformations, such as nitrogen fixation, making nutrients available that invasive plants strongly compete for (Rooth et al. 2003, Tuchman et al. 2009, Vaccaro et al. 2009). This shift to higher molecular weight organic material in *Phragmites* invaded wetlands could lead to selection for less energetically efficient decomposers that more completely degrade complex molecules. These factors suggest that *Phragmites* invasion may have significant effects on the organisms that mediate nutrient cycling, the structure of wetland food webs, and the global carbon cycle.

Phragmites invasion could also alter the rate of litter decomposition in wetlands by changing the composition and abundance of plant material and by changing site specific environmental characteristics. Due to the high primary productivity and high lignin content of *Phragmites*, carbon turnover rates would be expected to decrease in invaded wetlands. While evidence for *Typha* litter decomposing more rapidly than *Phragmites* litter exists (Findlay et al. 2002, Agoston-Szabo and Dinka 2008), *Phragmites* litter can decay at a higher rate than *Typha* litter (Mason and Bryant 1975). Differences in reported decomposition rates may be due to a combination of plant litter chemistry and variation in environmental factors,

but the relative importance of plant chemistry and environmental factors is currently not well understood.

Soil and litter associated bacteria and fungi are important contributors to carbon transformations in freshwater wetlands (Kominkova et al. 2000, Brix et al. 2001). Since the structure and function of microbial communities can be altered by plant invasions (Wolfe and Klironomos 2005), quantifying microbial carbon production before and after invasion is necessary when developing carbon budgets. Microbial decomposition of emergent macrophytes begins during the standing dead stage. Post-senescent *Phragmites* leaves are shed within a few months, while culms can stand for several years. When standing dead litter falls to the soil surface, microbial community composition associated with the plant materials changes and is influenced by the benthic environment (Kominkova et al. 2000). The level of microbial activity on decaying organic matter can be estimated by measuring CO₂ production (as a product of aerobic respiration), which is also a good indicator of labile carbon availability (McLauchlan and Hobbie 2004).

Another important wetland functional process that is impacted by plant invasions is methanogenesis. Wetlands are a major source of this potent greenhouse gas. In wetlands, methane is produced when redox potential is low (under anaerobic conditions) by methanogens that use CO₂ as a terminal electron acceptor. Since *Phragmites* can enrich the rhizosphere with oxygen and lowers surface water levels, CO₂ production could be enhanced and CH₄ production suppressed by a shift towards aerobic respiration in *Phragmites* dominated wetlands (Herbst and Kappen 1999, Windham and Lathrop 1999, Rothman and Bouchard 2007). However, increased rates of soil CH₄ production were observed in laboratory experiments when *Phragmites* root exudates were introduced to soils (Duan et al.

2009). The chemical composition of *Phragmites* derived root exudates may promote methanogenesis, increasing the relative amount of CH₄ produced. Additionally, when high water levels are present in *Phragmites* invaded sites, large emissions of CH₄ into the atmosphere are possible.

One major factor that could be influencing microbial processes in *Phragmites* invaded wetlands is the introduction of chemical leachates containing novel toxic molecules. Allelochemicals are secondary metabolites produced by plants that inhibit the growth of competitors. Allelopathic molecules are produced by both *Phragmites* and *Typha* (Bains et al. 2009, Jarchow and Cook 2009). Extracts of both plants also have antimicrobial properties, inhibiting bacterial, algal, and fungal growth (Aliotta et al. 1990, Anesio et al. 1999, Li and Hu 2005, Varghese et al. 2009). While *Typha*-derived compounds can inhibit some laboratory cultured microbes, gallic acid produced by *Phragmites* is antimicrobial in the aquatic environment, and its effect may be enhanced by exposure to ultraviolet radiation (King-Thom et al. 1995, Anesio et al. 1999, Rudrappa et al. 2009). Gallic acid is present in *Phragmites* root, stem, and leaf tissues. Another molecule produced by *Phragmites*, ethyl-2-methyl acetoacetate (EMA), causes algal cell death by excessive ion leakage due to an increased proportion of unsaturated fatty acids in the cell membrane (Li and Hu 2005). The time scale for microbes to gain resistance to *Phragmites*-derived toxins is not well understood. Leaching of these and other molecules from detritus could alter carbon emissions by changing wetland microbial community structure and function.

The purpose of this study was to quantify the effects of *Phragmites* invasion on three major carbon pathways in a freshwater marsh. The first objective was to investigate controls on plant litter decomposition by separating the effects of plant litter species (*Typha* or

Phragmites) and site specific environmental characteristics, such as surface water depth and soil temperature using reciprocal plant litter transplant experiments. I hypothesized that *Phragmites* litter would decompose at a lower rate than *Typha* litter regardless of site because it has a higher lignin content than *Typha* and that the litter of both plant species would decompose at a higher rate in the *Phragmites* site due to its influence on environmental conditions. The second objective was to determine how the plant community and water level influence the release of CO₂ and CH₄ from wetland soils. I hypothesized that lower water levels would result in more CO₂ and less CH₄ production and that higher water levels would result in more CH₄ and less CO₂ production in both plant communities, because O₂ is more available when soils are not saturated. I predicted that since microbial communities associated with *Phragmites* soils are adapted to lower water levels, CO₂ production in *Phragmites* soils would be higher than in *Typha* soils when water levels were low, and since *Typha* associated microbial communities are adapted to higher water levels, CH₄ production would be higher in saturated *Typha* soils than equally saturated *Phragmites* soils. To test this hypothesis, CO₂ and CH₄ production in *Phragmites* and *Typha* soils was measured for each plant community by incubating soils under aerobic (field moist) conditions and anaerobic (submersed) conditions. The third objective was to determine the impacts of plant leachates and soil type on microbial soil respiration. I hypothesized that because *Phragmites* produces molecules that are toxic to aquatic microbes and has high lignin content, *Phragmites* leachates would inhibit microbial respiration more than *Typha* leachates in both soil types. I predicted that since *Phragmites* associated microbial communities should have more adaptations to resist and degrade these molecules, the effects of inhibition would be greater when *Phragmites* leachates were added to *Typha* soils. To test this hypothesis, soils from

each community type were submersed in leachates from the stems and leaves of each plant species, and CO₂ production was measured.

MATERIALS AND METHODS

Study Site

A Lake Erie coastal marsh located in the Brancheau unit of the Detroit River International Wildlife Refuge, Monroe Co., MI. (41.984696 N, 83.248848 W) was sampled for this study (Figure 2). The marsh is largely dominated by two species of emergent macrophytes, *Phragmites australis* and *Typha x glauca*. Plant and soil samples were collected from five independent one m² plots within an area dominated by *Phragmites* and five in an adjacent area dominated by *Typha*.

Plant Litter Decomposition

Reciprocal Transplant Experiments

To quantify plant litter decomposition rates, mass loss from senesced *Phragmites* and *Typha* aboveground biomass was measured in a reciprocal transplant experiment using litter bags (two litter species: *Phragmites* and *Typha* x two sites: *Phragmites* and *Typha*). Standing dead stems and leaves were collected in October 2010 from the Brancheau wetland. Samples were air dried to a constant weight, then cut and weighed to a mass of 3.75 g stem and 1.25 g leaf to reflect a stem-to-leaf mass ratio of ~3:1 (Bellavance and Brisson 2010). A five-gram sample of plant material was transferred into each of 84 produce-style plastic bags, 42 bags containing *Phragmites* litter and 42 bags containing *Typha* litter, with a mesh size of five mm. Plant stem and leaf litter was cut to a minimum length of 20 cm to prevent fragment loss from the bags. Anchoring fixtures were used to secure litter bags at the litter layer (Figure 3). Four bags, two containing *Phragmites* litter and two containing *Typha* litter, were secured to each anchoring fixture with cable ties before placement in the wetland. In the wetland field

site, two fixtures were placed one m from a non-trampled corner of each of the 10 permanent plots.

Half of the litter bags were collected in the spring (April 25, 2011) and the remaining bags in the fall (November 11, 2011), allowing for the determination of initial mass loss during the cold season (144 d from December to April), subsequent warm season (200 d from April to November) decomposition, and annual decomposition (344 d from December to November). One set of control bags was transported to each vegetation type at time zero and then immediately brought back to the lab. The mass loss from this bag was subtracted from the initial litter mass in each bag to control for mass loss due to transport. On each sampling date one fixture with four attached bags (two each of *Phragmites* and *Typha* litter) was collected from each plot. Litter was carefully rinsed, transferred to pre-labeled paper bags of known weight, and then oven dried at 60 °C. Mass loss from time zero to collection 1 and collection 2 was determined using equation 1.

$$(1) \quad \text{mass loss (\%)} = \frac{t_x}{t_0} \times 100\%$$

Where t_0 is the initial litter mass and x indicates the sampling period (x = 1 for the spring collection and x = 2 for the fall collection).

Data Analysis

Mass loss for each of the four litter x site treatment combinations was compared for each interval by two-factor ANOVA using Systat version 13 (Systat Software, San Jose, CA). To determine mass loss during the warm season (April to November), the mean annual

mass loss was subtracted from the cold season mass loss and the error propagated using equation 2, where δx is the uncertainty in the annual mass loss and δy is the uncertainty in the cold season mass loss.

$$(2) \quad \delta q = \sqrt{[(\delta x)^2 + (\delta y)^2]}$$

Carbon Dioxide and Methane Production in Wetland Soils

I. Water Level Manipulations

Wetland soil samples collected from *Phragmites*- and *Typha*-dominated sites were treated with two water levels to determine if CO₂ and CH₄ production in *Phragmites*-associated soils differed from production in *Typha*-associated soils. The two water level treatments were chosen to simulate saturated (low O₂) and field moist (high O₂) conditions.

Soil Collection

Soil samples were collected from *Phragmites*- and *Typha*-dominated sites at the Brancheau wetland on July 28, 2010. Soil samples from the organic and mineral horizons to a depth of ~20 cm were collected from 1 m outside each permanent plot using flame-sterilized metal corers. A minimum of four core samples (> 210 g wet weight) were collected from each plot and pooled into a flame-sterilized mixing bowl. Visible plant material including stems, leaves, and roots were removed before transferring soils to sterile bags. Samples were transported on ice and then stored in a refrigerator at 5 °C upon arrival at the laboratory. Soil subsamples from each plot were oven dried at 60 °C to determine the field

moist to oven dry ratio. Dry sub-samples were combusted at 500 °C to determine the ash free dry mass after recording dry weights.

Soil Incubations

Two 30.0 g subsamples taken from the homogenized *Phragmites* soil and two from the homogenized *Typha* soil collected from each plot were incubated in sterilized chambers. Chambers were composed of a 520 mL glass container with an air tight metal lid that was fitted with a sealed rubber septum. Within 24 h of collection, one of the subsamples from each plot was sealed in a chamber with atmospheric O₂ levels, and the other subsample received an anoxic treatment following methods adapted from (Nadelhoffer 1990, Updegraff et al. 1995, Moore and Dalva 1997, Yavitt et al. 1997, Updegraff et al. 1998). To remove dissolved and headspace oxygen, 75.0 mL of distilled water was added to anoxic chambers, which was then sparged with N₂ for two minutes at a pressure of 25 kPa. The lid was then secured for a four-minute N₂ headspace flush at 12.5 kPa. To flush the headspace, two 22-gauge needles were simultaneously inserted through the septum. One needle was open to the atmosphere while the other carried pressurized N₂. This flushing procedure resulted in a concentration of < 5% total O₂ in preliminary trials. Chambers were incubated in a dark plant growth chamber at 21.7 °C and monitored with a HOBO Box Car Pro data logger (Onset, Cape Cod, MA). This temperature setting was based on the average sediment temperature from the 10 plots measured during the July 28, 2010, soil collection.

Gas Sampling

Chambers were removed from the plant growth chamber for headspace gas sampling on days one (July 30, 2010), four, eight, and eighteen of the incubations. Gas samples were collected using a 22-gauge needle attached to a 10 mL syringe with a three-way valve to puncture the septum. To purge the valve and syringe of external air, 2.0 mL of headspace gas was drawn into the syringe then exhausted through the open end of the valve to the atmosphere. A seven mL headspace gas sample was drawn into the syringe and the valve sealed. Oxic chambers were open to the atmosphere for 10 s after sampling to allow ambient air to enter the chamber. Anoxic chambers received a headspace gas injection from a syringe pre-filled with 9.0 mL N₂. Gas samples were analyzed using a Shimadzu 2014 Gas Chromatograph to determine CH₄ and CO₂ concentrations. Triplicate standards of four known gas concentrations of CO₂ and four of CH₄ encompassing the experimental range were analyzed on each sampling date. Ambient air samples were also analyzed during each interval to determine initial gas concentration in vented chambers. Gaseous carbon sampling and analysis methodology was adapted from Moore and Dalva 1997, Scanlon and Moore 2000, and Glatzel et al. 2004.

Data Analysis

Gas samples collected during a four-day interval (days four through eight) of the experiment were analyzed to determine CO₂ and CH₄ production. This interval was selected because it allowed for sustained microbial activity to occur, while minimizing potential long-term chamber effects. Standard curves were created using peak areas for standards of known CO₂ and CH₄ concentration. Using the regression equation from the standard curve and the

ideal gas law, sample gas concentrations were determined. The ambient gas concentrations at the beginning of an interval were subtracted from the final concentration by analyzing air samples from time zero or the previous sampling date. Atmospheric pressure was calculated using elevation and the growth chamber temperature. Headspace volume was calculated using equation 3, where 520 mL is the volume of the sealed chamber and x is the total volume occupied by soil and/or liquid.

$$(3) \quad V \text{ (mL)} = 520 \text{ mL} - x$$

The ambient concentration at the beginning of the sampling interval (measured on the previous sampling date) was subtracted from the final concentration and the total moles (n) of CO₂ and CH₄ produced during the interval calculated (equation 4). The calculated number of moles was then multiplied by the molar mass of each gas to determine production. Daily

$$(4) \quad \text{Total moles (n)} = \frac{(\text{sample ppm} \times P \text{ (atm)} \times 10^{-6} \times V(L))}{R \text{ (L atm mol}^{-1} \text{K}^{-1}) \times K^{\circ}(K)}$$

production rates of CO₂ and CH₄ were determined by dividing by the total number of days (4) in the interval. To standardize CO₂ and CH₄ production per gram of wetland soil, dry weights of soils in each chamber were estimated by determining the proportion of dry to wet soil in three 30.0 g samples of homogenized *Phragmites*-associated and *Typha*-associated soils by oven drying. The recorded wet mass of each sample was multiplied by this proportion to estimate the dry weight. The calculated gas production was then divided by the estimated soil dry mass (equation 5).

$$(5) \quad C \text{ Production } \left(\frac{\mu\text{g}}{\text{Soil (g)d}} \right) \\ = \left(\frac{\text{total moles (mol)} \times \text{gas molar mass } \left(\frac{\text{g}}{\text{mol}} \right) \times 10^6 \left(\frac{\mu\text{g}}{\text{g}} \right)}{\text{days in sampling interval (d)}} \right) \times (\text{soil dry mass (g)})^{-1}$$

The production rates of CO₂ and CH₄ under oxic and anoxic conditions were calculated for each dominant vegetation type using calculated values from the five chambers (plots) for each vegetation x water level treatment. Log transformations were performed due to unequal variance, and gas production rates were compared using two-factor (*Phragmites*, *Typha* x oxic, anoxic) ANOVA.

II. Plant Litter Leachate Additions

To test whether *Phragmites* leachates have a stronger antimicrobial effect than *Typha* leachates, wetland soil samples collected from sites dominated by each vegetation type were exposed to whole plant leachates from each plant species and a control solution of dH₂O. Leachates were extracted from standing dead above ground biomass because it represents the stage at which the plant material would fall into the litter layer. The dH₂O control was used to compare the leachate additions to a baseline microbial activity level under conditions of equal water availability.

Plant Litter Collection and Leachate Extraction

Above ground biomass from standing dead *Typha* and *Phragmites* was collected from the Brancheau wetland in November 2010. Plants were air dried for ~6 months. Stems and leaves were cut to a length of 10 cm and submerged in a flask filled with 1.0 L dH₂O. To represent the average proportion of stem to leaf tissue found a plant, 30.0 g of litter was

added in the ratio of 3 g stem:1 g leaf (Bellavance and Brisson 2010). A third control flask contained dH₂O only. Leachate and dH₂O flasks were stored at a constant temperature of 5 °C in the dark for 10 d. On day 11, stems and leaves were removed from the leachate flasks and the remaining solutions were vacuum filtered through coarse Whatman paper then a Whatman 0.7 µm pore size GF/F filter into sterile flasks.

Leachate Additions and Soil Incubations

Soil samples were collected on July 19, 2011, from the Brancheau wetland following the same protocol as described for water level treatments. Soils were homogenized, and 30 g samples of *Phragmites*- and *Typha*-associated soils were individually transferred to each of 24 sterilized incubation chambers (48 total). Each of the three leachate types (30.0 mL) was distributed to eight chambers containing *Phragmites* soils and eight chambers containing *Typha* soils. Chambers were incubated in a dark plant growth chamber at a constant temperature of 21.7 °C for 16 d.

Gas Sampling

Soil microbial CO₂ and CH₄ production in response to leachate treatments was measured by sampling the headspace of experimental incubation chambers on days one (July 21, 2011), eight, and 16, using the same procedure described in the water level soil incubation experiments. This experimental period was selected to allow for sustained microbial activity to occur, while also minimizing long-term chamber effects, and was based on results of the water level incubations. An ambient air sample was collected when the

chambers were initially sealed and during each gas sampling date (while venting) to be used for subtraction of initial gas concentrations in the chambers during data analysis.

Data Analysis

The production rate of CO₂ and CH₄ from each soil x leachate combination was determined using the calculated values from the eight replicates by equations three through five. The seven day interval from days one through eight was selected for analysis. The three leachate treatments were compared using one factor ANOVA and a Tukey HSD test (SYSTAT). The data set did not require transformation.

Plant, Soil, Water, and Environmental Characteristics

Plant Biomass

Above ground primary production of both *Phragmites* and *Typha* was determined to estimate the amount of carbon stored in this pool and maximum potential litterfall. Live above-ground biomass (LAGB) was measured by collecting all of the living above-ground shoots within a 25 cm x 25 cm satellite plot, drying and weighing. Productivity was then calculated per m² by multiplying the satellite plot production by 16. To estimate the annual accumulation of litter, total LAGB was multiplied by annual percent litter mass loss of each plant species in the dominant site (*Phragmites* litter in *Phragmites* site and *Typha* litter in *Typha* site).

Water Level and Soil Temperature

Surface water levels and soil temperatures were measured to determine if either factor could be influencing litter decomposition rates. Surface water depths were analyzed using four measurements of the distance from the soil surface to the water surface within each of the five permanent plots in both the *Phragmites* and *Typha* sites on nine sampling days. Soil temperatures were analyzed by collecting a soil pore water sample from each permanent plot at a depth of ~10 cm and recording the temperature on eight sampling days. The surface water levels and soil temperatures were determined for each site during the cold (two dates during April) and warm (seven dates during May through November) seasons. While surface water levels and soil temperatures were measured during the study period, litter collections did not occur during particular water levels or temperatures. Repeated measures ANOVA was used to test for significant differences in water depths and soil temperatures between sites during both the cold and warm seasons.

Dissolved Organic Carbon

To standardize CO₂ and CH₄ production per dissolved organic carbon (DOC) molecule and estimate a DOC production rate from litter, two replicate subsamples (20 mL) of each filtered leachate (*Phragmites*, *Typha*, and dH₂O) were collected and then acidified with HCl and analyzed for DOC concentration. Duplicate samples were transferred into 20 mL sterile glass bottles from the one L leachate samples, refrigerated, and then analyzed using a Shimadzu TOC following EPA method 415.1. The DOC concentration was converted into units of $\frac{mg}{L}$ and carbon production calculated by equation 6.

$$(6) \quad C \text{ Production} \left(\frac{\mu\text{g}}{\text{Soil (g)}d \text{ DOC} \left(\frac{\text{mg}}{\text{L}} \right)} \right) = \left(\frac{C(\mu\text{g})}{\text{Soil (g)}d} \right) \times \left(\text{DOC} \frac{\text{mg}}{\text{L}} \right)^{-1}$$

To determine the relative aromaticity of the organic material extracted from each litter type, the specific ultraviolet absorbance at a wavelength of 254 nm (SUVA₂₅₄, a measurement that corresponds to the recalcitrance of DOC) was calculated (Weishaar et al. 2003). A 20 mL subsample of each filtered leachate solution (*Phragmites*, *Typha*, and dH₂O) was collected then acidified with HCl and transferred to a quartz cuvette for analysis with a spectrophotometer following EPA method 415.3. Absorbance and DOC concentrations were used to calculate SUVA₂₅₄ by equations 7 and 8 from EPA method 415.3 (sec. 12.2), where UVA is the ultraviolet absorbance in absorbance units, A is the measured ultraviolet absorbance at 254 nm, d is the quartz cuvette path length, and DOC is the dissolved organic carbon concentration.

$$(7) \quad UVA (cm^{-1}) = \frac{A}{d(cm)}$$

$$(8) \quad SUVA_{254} \left(\frac{L}{mg-M} \right) = \frac{UVA(cm^{-1})}{\left(\text{DOC} \frac{\text{mg}}{\text{L}} \right) \left(100 \frac{\text{cm}}{M} \right)}$$

Soil Bulk Density

Bulk density was determined by collecting two replicate soil core samples of 375 mL, oven drying the soil, and then determining the oven dry mass to field moist volume. The mean dry weight was divided by whole core volume using equation 9.

$$(9) \quad \text{Bulk Density} \left(\frac{g}{L} \right) = \frac{\text{soil dry weight}(g)}{\text{soil volume}(L)}$$

Samples used to determine bulk density were then combusted at 500 °C to a constant mass to determine AFDM. Percent carbon was calculated by equation 10. The bulk density for each dominant vegetation type was used to determine CO₂ and CH₄ production per unit area by equation 11.

$$(10) \quad \text{Carbon (\%)} = \frac{\text{Organic Matter (\%)}}{2} = \frac{(\text{AFDM})(\text{Soil Dry Mass}^{-1}) \times 100}{2}$$

$$(11) \quad \text{C Production} \left(\frac{\mu\text{g}}{\text{m}^2 \text{ d}} \right) = \left(\frac{\mu\text{g}}{\text{gSoil d}} \right) \times \text{bulk density} \left(\frac{\text{g}}{\text{L}} \right) \times \frac{1 \text{ L}}{10^{-3} \text{ m}^3} \times \text{sampling depth (m)}$$

RESULTS

Plant Litter Decomposition

During the cold season (3 December 2010 to 25 April 2011), the litter mass loss from *Typha* bags was significantly greater than mass loss from *Phragmites* bags, regardless of whether bags were in *Phragmites* or *Typha* sites ($p < 0.01$) (Figure 4). During the warm season, there was no significant difference in litter mass loss from the two plant species or between sites ($p > 0.05$) (Figure 4). On an annual basis (344 d) litter at the *Phragmites* site, regardless of whether it was *Phragmites* or *Typha* litter, had greater mass loss ($p < 0.05$) (Figure 4).

Water Level Manipulations

During the four-day soil incubation, there was no significant difference in the rate of CO₂ production between soils from *Phragmites* and *Typha* sites under oxic (field moist) or anoxic (submersed) conditions ($p > 0.05$) (Figure 5). Significantly more CO₂ was produced in oxic soils than anoxic soils ($p < 0.01$) regardless of associated vegetation (site) type, and significantly more methane was produced in *Typha* soils than *Phragmites* soils under anoxic conditions ($p < 0.05$) (Figure 6). Methane production was also significantly greater in submersed (anoxic) soils than field moist (oxic) soils ($p < 0.01$) regardless of site.

Effects of Litter Leachates on Soil Respiration and Methanogenesis

In *Phragmites* soils, *Typha* leachate addition resulted in significantly greater CO₂ production than dH₂O addition ($p < 0.05$) (Figure 7). Carbon dioxide production in *Phragmites* soils treated with either leachate type was significantly greater than production in

all *Typha* soil treatments ($p < 0.05$). In *Typha* soils, leachate additions had no significant effect on CO_2 production ($p > 0.05$). While not significant, less CO_2 production occurred when *Phragmites* leachate was added than when *Typha* leachate or dH_2O was added.

Plant, Soil, Water, and Environmental Characteristics

Aboveground plant biomass (primary productivity) was higher for *Phragmites* than *Typha* in the Brancheau marsh (Table 1). The DOC concentrations for the three leachate treatments were: 0.0 ppm (dH_2O), 83.07 ppm (*Phragmites*), and 183.0 ppm (*Typha*). These concentrations were slightly higher than soil water DOC concentrations measured in the marsh during a concurrent study (K. Judd, unpublished data, 2012). The specific ultraviolet absorbance of *Typha* leachates was 1.15 times greater than it was for *Phragmites* leachates (Table 3). Carbon dioxide and methane production were both higher in *Phragmites*-associated soils than *Typha*-associated soils when standardized for DOC concentration (Table 3). Water levels in the *Phragmites* site were significantly lower than levels in the *Typha* site during both the cold and warm seasons ($p < 0.05$) (Figure 8). There was no significant difference between soil temperatures in *Phragmites* and *Typha* sites in either season ($p > 0.05$) (Figure 9). Bulk density and percent soil carbon were higher in the *Phragmites*-invaded site (Table 2). Using the bulk density data, areal CO_2 and CH_4 production rates were estimated using the two treatments that most closely matched natural conditions (*Phragmites* leachate on *Phragmites* soil and *Typha* leachate on *Typha* soil). It was found that CO_2 production per m^2 of wetland was higher in the *Phragmites* site, while CH_4 production was similar in both community types (Table 3).

DISCUSSION

Post-invasion environmental conditions in the Brancheau marsh were stronger controls on carbon transformations than the chemical composition of plant litter. The prediction that less decomposition of *Phragmites* litter than *Typha* litter would occur in both sites was not supported, suggesting that differences in chemical composition were not controlling decomposition rates. *Phragmites* and *Typha* litter lost more mass in the invaded site, suggesting that *Phragmites* may be influencing the physiochemical environment to enhance decomposition. Significantly more CH₄ was produced in *Typha*-associated soils than *Phragmites*-associated soils under anoxic conditions as predicted, suggesting that higher levels of anaerobic microbial activity occurred in the *Typha* site. There was no significant difference in CO₂ production between sites under oxic conditions; therefore, the prediction that *Phragmites*-associated soil microbial communities would be more active than *Typha*-associated communities under aerobic conditions was not supported. While both *Typha* and *Phragmites* leachates were readily metabolized by *Phragmites*-associated soil microbes, significantly less consumption occurred when leachates were introduced to *Typha* associated soil microbes during aerobic incubations. Furthermore, slightly less CO₂ was produced in *Typha* soils treated with *Phragmites* leachates than when treated with dH₂O or *Typha* leachates, indicating the potential for microbial inhibition by *Phragmites* leachate. An alternative explanation is that the *Typha*-associated microbes were not adapted to produce enzymes necessary to degrade *Phragmites*-derived humic compounds.

Significantly higher annual plant litter mass loss in the *Phragmites* site than the *Typha* site, regardless of litter species, suggests that *Phragmites* invasion can increase the rate of carbon turnover and emissions to the atmosphere from the wetland detrital carbon

pool. During the 2010 growing season, *Phragmites* fixed more than two times the amount of carbon fixed by *Typha* and carbon composed 42% of *Phragmites* biomass and 43% of *Typha* biomass (K. Judd, unpublished data, 2012); therefore, the *Phragmites* invaded site served as a net carbon sink. Similar mass loss from *Phragmites* and *Typha* litter in both community types follows the trend of similar decomposition rates from both plant species reported in previous studies, suggesting that annual productivity is a major factor in wetland carbon accumulation. Higher rates of *Typha* decomposition during the cold season may have been due to a larger proportion of labile (non-humic) organic material in the *Typha* litter compared to *Phragmites* litter. An alternative explanation is that antimicrobial compounds leached from *Phragmites* litter upon falling to the wetland surface suppressed microbial decomposition. During the warm season, decomposition rates were similar between sites and for *Phragmites* and *Typha* litter. It is possible that differences were not detected during the warm season because measurements were indirect (subtraction of the mean cold season loss from the total loss), which increased the error in measurement. This error could be reduced by introducing a separate set of litter bags into the wetland at the end of the cold season for fall collection. While the characteristics of invasive *Phragmites* that increase decomposition rates (high primary productivity, dense growth, high evapotranspiration rates, etc.) were expected to have a maximal impact on litter mass loss during the growing season, comparatively more labile carbon in *Typha* litter is biologically available in the spring, and the warm season is the peak period for decomposer activity. These factors may have contributed to an equalization of decomposition rates.

Significantly lower surface water levels in the *Phragmites*-invaded site may influence higher rates of microbial respiration (Figure 8). A difference in water levels may have

resulted from increased evapotranspiration in the invaded site. While higher rates of evapotranspiration are associated with *Phragmites* invasion (Herbst and Kappen 1999, Goulden et al. 2007, Borin et al. 2011), these rates are strongly influenced by local conditions and require further investigation within the study site. Proximity to Lake Erie may have been a factor that influenced site specific water level; however, it is unlikely that higher lake surface water levels would have caused comparatively lower water levels in the *Phragmites* site because it is nearer to the pelagic zone. Low water levels (field moist vs. saturated) increased the rate of soil microbial CO₂ production during incubations. Greater CO₂ production in invaded sites with lower water levels may also result from increased methane oxidation (Roslev and King 1996). Lower water levels could also explain the significantly higher rates of litter decomposition in the *Phragmites* site.

The predicted decrease of soil temperatures resulting from reduced light infiltration into the invaded site was not supported (Figure 9). This suggests that soil temperature was not a major post-invasion driver of litter decomposition rates. Increased soil temperatures during the growing season have been shown to significantly increase the wetland function of nutrient storage (Picard et al. 2005). While decreased water levels and increased temperature can increase microbial activity, no significant difference between soil temperatures in the *Phragmites*-invaded site and the pre-invaded *Typha* site was observed during this study, suggesting that lower water levels after *Phragmites* invasion were likely the main driver of increased rates of litter decomposition compared to *Typha* sites.

The results of this study did not indicate that *Phragmites*-associated microbial communities were more active than *Typha*-associated communities under oxic conditions because CO₂ production was not significantly different in the two soil types. The similar

response of soil microbes may be attributable to the high energy yield of aerobic respiration, which occurs at a rate that is several times faster than anaerobic carbon mineralization (D'Angelo and Reddy 1999). Another possible explanation is that since both plant species transport O₂ to the root zone, aerobic microbes are constantly active in both soil types. Significantly higher rates of CH₄ production in saturated *Typha* soils than saturated *Phragmites* soils suggest that *Typha* associated microbial communities may be better adapted to saturated conditions. A change in the anaerobic microbial community resulting from *Phragmites* invasion would be functionally significant because the beneficial processes of denitrification and sulfate reduction require specific anoxic conditions (Faulwetter et al. 2009). Evidence for inhibited microbial community function, measured as decreased removal rates of N and P, was observed when another grass (*Phalaris arundinacea*) invaded *Typha* sites (Fraser et al. 2004). Other explanations for greater methane production in *Typha*-associated soils, such as substrate availability, less methane oxidation and physiochemical characteristics, cannot be eliminated.

The results suggest that *Phragmites* stem and leaf litter leachates may inhibit microbial activity during wetland invasion. I predicted that exposure of *Phragmites* and *Typha* soils to *Phragmites* leachates would result in less gaseous carbon production than exposure to *Typha* leachates. While not statistically significant, microbial respiration rates in *Typha* soils were lower in response to *Phragmites* leachate addition than *Typha* leachate or dH₂O addition. This contrasted the trend towards greater respiration rates in *Phragmites* soils in response to *Phragmites* or *Typha* leachate addition versus dH₂O addition. This trend occurred despite greater aromaticity of *Typha* leachates, as measured by specific ultraviolet absorbance. This suggests that soil microbial communities associated with *Phragmites*

monocultures may be more adapted to toxic molecules, such as the gallotanin or EMA, known to be produced in *Phragmites* leaves and stems (Anesio et al. 1999, Rudrappa et al. 2009). Another possible explanation is that these antimicrobial molecules are also allelopathic and microbial inhibition is secondary to phytotoxicity.

The results of this study indicated that *Phragmites*-associated soil microbes were more active than *Typha*-associated soil microbes and that CO₂ production from invaded wetlands was greater on an areal and DOC corrected basis. While areal estimates considered gaseous carbon production only to the sampled soil depth of 20 cm and are not estimates of total emissions, both the aerobic and anaerobic zones were represented in the analysis. Higher respiration rates in *Phragmites*-associated soils occurred despite less DOC availability in *Phragmites* litter leachates. One possible explanation is that *Phragmites*-associated soil microbes were not nutrient limited at the lower DOC concentration, and these microbes were adapted to readily metabolize the *Phragmites*-derived leachate, which was not found to be more recalcitrant in SUVA analysis. Greater decomposition and increased respiration in the *Phragmites*-invaded site supports the hypothesis that invasive plants benefit from litter accumulation, providing an energy source to microbes that mediate nutrient transformations, such as nitrogen fixing bacteria (Tuchman et al. 2009). An increase in sample size for each soil x leachate treatment could result in significant differences becoming detectable in the trend towards inhibition of *Typha*-associated microbes by *Phragmites*-derived leachates. A future experiment that could be conducted to determine antimicrobial properties of *Phragmites* leachates would be to isolate phenolic compounds found in *Phragmites* leachates and apply them to soil microbial communities under pre-invaded and post-invaded conditions.

CONCLUSIONS

Post-invasion environmental factors had a strong influence on wetland carbon transformations. While the invaded site functioned as a net carbon sink, high rates of litter decomposition and CO₂ production (with site specific leachate additions) in the invaded marsh suggest that the organic matter entering invaded wetlands moves rapidly from both plant biomass and soil to the atmosphere through aerobic respiration. These aerobic processes may have been enhanced by both lower water levels and potentially increased bulk O₂ transport to the rhizosphere in the invaded site; however, microbial respiration rates were similar in both *Phragmites*- and *Typha*-associated soil types when not submerged, suggesting that increased oxygen availability may equalize microbial activity. *Phragmites* invasion may result in lower methane emissions from wetland soils. Since methanogenesis was significantly lower in saturated *Phragmites*-invaded soils than saturated pre-invaded soils and invasion is associated with lower water levels (likely resulting from higher *Phragmites* transpiration rates and a limiting factor in obligate anaerobe activity), post-invasion CH₄ emission is expected to decrease. Since other environmental factors could influence this trend, multiple carbon pathways in a variety of wetlands should be quantified with concurrent environmental monitoring. While increased microbial respiration occurred in soils of the invaded site, the possibility of microbial inhibition resulting from *Phragmites*-derived leachate additions was observed. This supports the hypothesis that litter chemical composition provides a mechanism for invasive plants to store energy in biomass, which is a nutrient pathway that is not currently well understood. Microbial inhibition may be particularly important at the invasion front, where competition for nutrients is high and suppression of decomposition would benefit a rhizomatous invader. While this study focused

on short-term carbon fluxes, which vary with annual primary productivity, the results suggest that long-term carbon emission potential from invaded wetlands may be enhanced by greater carbon storage in invaded soils and greater post invasion O₂ availability due to lower water levels.

REFERENCES

- Agoston-Szabo, E. and M. Dinka. 2008. Decomposition of *Typha angustifolia* and *Phragmites australis* in the littoral zone of a shallow lake. *Biologia* **63**:1104-1110.
- Aliotta, G., M. D. Greca, P. Monaco, G. Pinto, A. Pollio, and L. Previtera. 1990. In vitro algal growth inhibition by phytotoxins of *Typha latifolia* L. *Journal of Chemical Ecology* **16**:2637-2646.
- Anesio, A. M., C. M. T. Denward, L. J. Tranvik, and W. Graneli. 1999. Decreased bacterial growth on vascular plant detritus due to photochemical modification. *Aquatic Microbial Ecology* **17**:159-165.
- Bains, G., A. S. Kumar, T. Rudrappa, E. Alff, T. E. Hanson, and H. P. Bais. 2009. Native plant and microbial contributions to a negative plant-plant interaction. *Plant Physiology* **151**:2145-2151.
- Bellavance, M.-E. and J. Brisson. 2010. Spatial dynamics and morphological plasticity of common reed (*Phragmites australis*) and cattails (*Typha* sp.) in freshwater marshes and roadside ditches. *Aquatic Botany* **93**:129-134.
- Borin, M., M. Milani, M. Salvato, and A. Toscano. 2011. Evaluation of *Phragmites australis* (Cav.) Trin. evapotranspiration in Northern and Southern Italy. *Ecological Engineering* **37**:721-728.
- Brix, H., B. K. Sorrell, and B. Lorenzen. 2001. Are *Phragmites*-dominated wetlands a net source or net sink of greenhouse gases? *Aquatic Botany* **69**:313-324.
- D'Angelo, E. M. and K. R. Reddy. 1999. Regulators of heterotrophic microbial potentials in wetland soils. *Soil Biology and Biochemistry* **31**:815-830.
- Duan, X. N., X. K. Wang, and Z. Y. Ouyang. 2009. Influence of Common Reed (*Phragmites australis*) on CH₄ production and transport in wetlands: Results from single-plant laboratory experiments. *Water Air and Soil Pollution* **197**:185-191.
- Ehrenfeld, J. G. 2003. Effects of Exotic Plant Invasions on Soil Nutrient Cycling Processes. *Ecosystems* **6**:503-523.
- Ehrenfeld, J. G., B. Ravit, and K. Elgersma. 2005. Feedback in the plant-soil system. *Annual Review of Environment and Resources* **30**:75-115.
- Faulwetter, J. L., V. Gagnon, C. Sundberg, F. Chazarenc, M. D. Burr, J. Brisson, A. K. Camper, and O. R. Stein. 2009. Microbial processes influencing performance of treatment wetlands: A review. *Ecological Engineering* **35**:987-1004.

- Findlay, S. E. G., S. Dye, and K. A. Kuehn. 2002. Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. *Wetlands* **22**:616-625.
- Fraser, L. H., S. M. Carty, and D. Steer. 2004. A test of four plant species to reduce total nitrogen and total phosphorus from soil leachate in subsurface wetland microcosms. *Bioresource Technology* **94**:185-192.
- Glatzel, S., N. Basiliko, and T. Moore. 2004. Carbon dioxide and methane production potentials of peats from natural, harvested and restored sites, eastern Québec, Canada. *Wetlands* **24**:261-267.
- Goulden, M. L., M. Litvak, and S. D. Miller. 2007. Factors that control *Typha* marsh evapotranspiration. *Aquatic Botany* **86**:97-106.
- Herbst, M. and L. Kappen. 1999. The ratio of transpiration versus evaporation in a reed belt as influenced by weather conditions. *Aquatic Botany* **63**:113-125.
- Jarchow, M. E. and B. J. Cook. 2009. Allelopathy as a mechanism for the invasion of *Typha angustifolia*. *Plant Ecology* **204**:113-124.
- King-Thom, C., G. Zhaoa, E. Stevens, B. A. Simcoa, and C. I. Weib. 1995. Growth inhibition of selected aquatic bacteria by tannic acid and related compounds. *Journal of Aquatic Animal Health* **7**:46-49.
- Kominkova, D., K. A. Kuehn, N. Busing, D. Steiner, and M. O. Gessner. 2000. Microbial biomass, growth, and respiration associated with submerged litter of *Phragmites australis* decomposing in a littoral reed stand of a large lake. *Aquatic Microbial Ecology* **22**:271-282.
- Kourtev, P. S., J. G. Ehrenfeld, and M. Häggblom. 2002. Exotic Plant Species Alter the Microbial Community Structure and Function in the Soil. *Ecology* **83**:3152-3166.
- Lambertini, C., M. Gustafsson, J. Frydenberg, J. Lissner, M. Speranza, and H. Brix. 2006. A phylogeographic study of the cosmopolitan genus *Phragmites* (Poaceae) based on AFLPs. *Plant Systematics and Evolution* **258**:161-182.
- Li, F. M. and H. Y. Hu. 2005. Isolation and characterization of a novel antialgal allelochemical from *Phragmites communis*. *Applied and Environmental Microbiology* **71**:6545-6553.
- Lin, Y.-F., S.-R. Jing, T.-W. Wang, and D.-Y. Lee. 2002. Effects of macrophytes and external carbon sources on nitrate removal from groundwater in constructed wetlands. *Environmental Pollution* **119**:413-420.
- Mason, C. F. and R. J. Bryant. 1975. Production, Nutrient Content and Decomposition of *Phragmites communis* Trin. and *Typha angustifolia* L. *Journal of Ecology* **63**:71-95.

- McLauchlan, K. K. and S. E. Hobbie. 2004. Comparison of labile soil organic matter fractionation techniques. *Soil Science Society of America Journal* **68**:1616-1625.
- Meyerson, L. A., K. Saltonstall, L. Windham, E. Kiviat, and S. Findlay. 2000. A comparison of *Phragmites australis* in freshwater and brackish marsh environments in North America. *Wetlands Ecology and Management* **8**:89-103.
- Mitsch, W. J. and J. G. Gosselink. 2007. *Wetlands*, 4th ed. John Wiley & Sons, Inc., New York.
- Moore, T. R. and M. Dalva. 1997. Methane and carbon dioxide exchange potentials of peat soils in aerobic and anaerobic laboratory incubations. *Soil Biology & Biochemistry* **29**:1157-1164.
- Nadelhoffer, K. J. 1990. Microlysimeter for measuring nitrogen mineralization and microbial respiration in aerobic soil incubations. *Soil Science Society of America Journal* **54**:411-415.
- Neubauer, S. C., K. Givler, S. K. Valentine, and J. P. Megonigal. 2005. Seasonal patterns and plant-mediated controls of subsurface wetland biogeochemistry. *Ecology* **86**:3334-3344.
- Picard, C. R., L. H. Fraser, and D. Steer. 2005. The interacting effects of temperature and plant community type on nutrient removal in wetland microcosms. *Bioresource Technology* **96**:1039-1047.
- Reynolds, H. L., A. Packer, J. D. Bever, and K. Clay. 2003. Grassroots Ecology: Plant-Microbe-Soil Interactions as Drivers of Plant Community Structure and Dynamics. *Ecology* **84**:2281-2291.
- Rooth, J. E., J. C. Stevenson, and J. C. Cornwall. 2003. Increased sediment accretion rates following invasion by *Phragmites australis*: The role of litter. *Estuaries* **26**:475-483.
- Roslev, P. and G. M. King. 1996. Regulation of methane oxidation in a freshwater wetland by water table changes and anoxia. *FEMS Microbiology Ecology* **19**:105-115.
- Rothman, E. and V. Bouchard. 2007. Regulation of carbon processes by macrophyte species in a Great Lakes coastal wetland. *Wetlands* **27**:1134-1143.
- Rudrappa, T., Y. S. Choi, D. F. Levia, D. R. Legates, K. H. Lee, and H. P. Bais. 2009. *Phragmites australis* root secreted phytotoxin undergoes photo-degradation to execute severe phytotoxicity. *Plant Signaling & Behavior* **4**:506-513.
- Saltonstall, K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences of the United States of America* **99**:2445-2449.

- Scanlon, D. and T. Moore. 2000. Carbon dioxide production from peatland soil profiles: The influence of temperature, oxic/anoxic conditions and substrate. *Soil Science* **165**:153-160.
- Tuchman, N. C., D. J. Larkin, P. Geddes, R. Wildova, K. Jankowski, and D. E. Goldberg. 2009. Patterns of environmental change associated with *Typha x glauca* invasion in a Great Lakes coastal wetland. *Wetlands* **29**:964-975.
- Tulbure, M. G., C. A. Johnston, and D. L. Auger. 2007. Rapid invasion of a Great Lakes coastal wetland by non-native *Phragmites australis* and *Typha*. *Journal of Great Lakes Research* **33**:269-279.
- Updegraff, K., S. D. Bridgham, J. Pastor, and P. Weishampel. 1998. Hysteresis in the temperature response of carbon dioxide and methane production in peat soils. *Biogeochemistry* **43**:253-272.
- Updegraff, K., J. Pastor, S. D. Bridgham, and C. A. Johnston. 1995. Environmental and substrate controls over carbon and nitrogen mineralization in northern wetlands. *Ecological Applications* **5**:151-163.
- Vaccaro, L. E., B. L. Bedford, and C. A. Johnston. 2009. Litter accumulation promotes dominance of invasive species of cattails (*Typha* Spp.) in Lake Ontario wetlands. *Wetlands* **29**:1036-1048.
- Varghese, A., U. Gavani, S. Abraham, B. G. T. Parambi, and A. J. Sathianarayanan. 2009. Phytochemical screening and antimicrobial investigation of *Typha angustifolia* Linn. *International Journal of Chemical Sciences* **7**:1905-1910
- Weidenhamer, J. D. and R. M. Callaway. 2010. Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. *Journal of Chemical Ecology* **36**:59-69.
- Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of Specific Ultraviolet Absorbance as an Indicator of the Chemical Composition and Reactivity of Dissolved Organic Carbon. *Environmental Science & Technology* **37**:4702-4708.
- Wilcox, K. L., S. A. Petrie, L. A. Maynard, and S. W. Meyer. 2003. Historical distribution and abundance of *Phragmites australis* at Long Point, Lake Erie, Ontario. *Journal of Great Lakes Research* **29**:664-680.
- Windham, L. and R. G. Lathrop, Jr. 1999. Effects of *Phragmites australis* (Common Reed) invasion on aboveground biomass and soil properties in brackish tidal marsh of the Mullica river, New Jersey. *Estuaries* **22**:927-935.
- Wolfe, B. E. and J. N. Klironomos. 2005. Breaking new ground: Soil communities and exotic plant invasion. *Bioscience* **55**:477-487.

Yavitt, J. B., C. J. Williams, and R. K. Wieder. 1997. Production of methane and carbon dioxide in peatland ecosystems across north America: Effects of temperature, aeration, and organic chemistry of peat. *Geomicrobiology Journal* **14**:299-316.

Zedler, J. B. and S. Kercher. 2005. Wetland resources: Status, trends, ecosystem services, and restorability. Pages 39-74 *Annual Review of Environment and Resources*. Annual Reviews, Palo Alto.

Table 1. Annual above ground biomass production and litter mass loss from: *Phragmites* in the *Phragmites* site and *Typha* in the *Typha* site during a 344 d period from Dec 2010 to Nov 2011, and estimated annual litter accumulation. Live above ground plant biomass (n=5) and litter mass loss (n=10) reported as mean (\pm 1 SD).

	<i>Phragmites</i>	<i>Typha</i>
Live Above-ground Plant Biomass (g m ⁻²)	3343.7 \pm 1510.52	1510.5 \pm 632.9
Litter Mass Loss (%)	49.1 \pm 5.1	39.4 \pm 17.9
Annual Litter Accumulation (g m ⁻²)	1702	915

Table 2. Percent soil carbon, bulk density and estimated total mass of carbon in one m³ of soil in the *Phragmites* and *Typha* dominated sites. Soil C (n=5) and bulk density (n=2) are reported as the mean (\pm 1 SD).

	<i>Phragmites</i>	<i>Typha</i>
Soil Carbon (%)	44.8 \pm 1.3	44.2 \pm 1.4
Bulk Density (kg/m ³)	405.0 \pm 58.6	342.8 \pm 80.4
Total soil carbon (kg/m ³)	181.4	151.5

Table 3. Rates of DOC leaching, specific ultraviolet absorbance and gaseous carbon production (standardized for DOC concentration and per m² wetland). Areal estimates were based on production when *Phragmites* leachates were added to *Phragmites* soil and *Typha* leachates were added to *Typha* soil treatments in aerobic incubations to approximate normal wetland conditions. Values are the mean (\pm 1 SD) (n=8).

	<i>Phragmites</i>	<i>Typha</i>
DOC leaching Rate		
$\left(\frac{DOC(\frac{mg}{L})}{g\text{Litter } d\text{Leached}}\right)$	2.77×10^{-1}	6.10×10^{-1}
SUVA		
$\left(\frac{L}{mg-M}\right)$	8.40×10^{-1}	9.64×10^{-1}
CO ₂ Production		
$\left(\frac{\mu g}{Soil (g) d DOC(\frac{mg}{L})}\right)$	1.86 ± 0.26	0.49 ± 0.27
CH ₄ Production		
$\left(\frac{\mu g}{Soil (g) d DOC(\frac{mg}{L})}\right)$	$7.71 \times 10^{-4} \pm 9.49 \times 10^{-4}$	$4.83 \times 10^{-4} \pm 4.37 \times 10^{-4}$
CO ₂ Production		
$\left(\frac{\mu g}{m^2 d}\right)$	$1.56 \times 10^7 \pm 2.19 \times 10^6$	$8.16 \times 10^6 \pm 4.47 \times 10^6$
CH ₄ Production		
$\left(\frac{\mu g}{m^2 d}\right)$	6474.8 ± 7968.6	8042.9 ± 7287.3

Table 4. Comparison of mass loss from *Typha* and *Phragmites* litter in this study to three previous studies that reported decomposition rates of litter originating from both plant species within a wetland.

Author(s)	Site Type	Mass Loss (%)		Length (days)
		<i>Phragmites</i>	<i>Typha</i>	
This study (Phragmites Site)	Great Lakes coastal marsh	49.1	49.0	344
This study (Typha Site)	Great Lakes coastal marsh	40.7	39.4	344
(Agoston-Szabo and Dinka 2008) leaf and culm	Shoreline of small shallow freshwater lake	NA	50	292
(Agoston-Szabo and Dinka 2008) culm only	Shoreline of small shallow freshwater lake	50	NA	574
(Agoston-Szabo and Dinka 2008) leaf only	Shoreline of small shallow freshwater lake	50	NA	242
(Findlay et al. 2002)	Tidal freshwater marsh	~5	~30	400
(Mason and Bryant 1975)	Shoreline of small shallow freshwater lake	NA	50	368
(Mason and Bryant 1975)	Shoreline of small shallow freshwater lake	50	NA	200

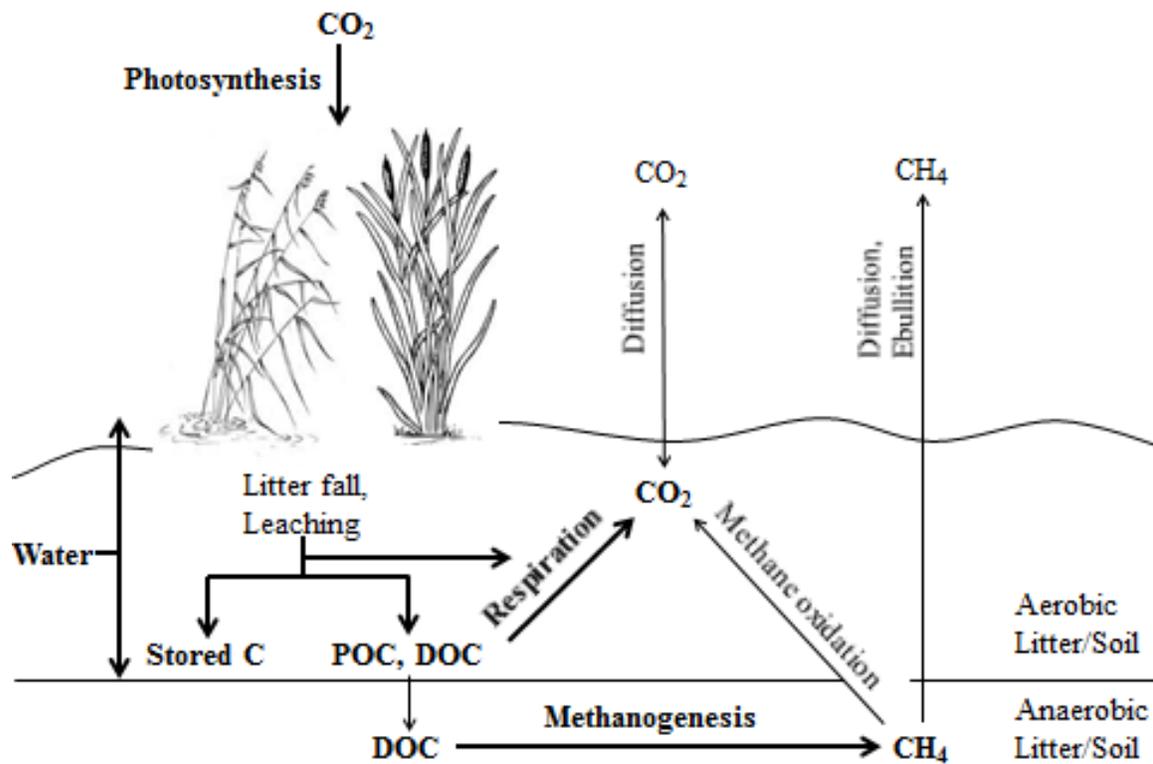


Figure 1. Conceptual diagram showing the major carbon transformations occurring in a Great Lakes coastal marsh. Bold arrows show the pathways investigated during this study. Diagram was modified from Brix et al. 2001 and Mitsch and Gosselink 2007. *Phragmites* illustration by Miranda Waugh.

Brancheau Unit Detroit River International Wildlife Refuge Newport, MI 2012



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Figure 2. Map of the Brancheau marsh study site in Newport, MI showing locations of *Phragmites* and *Typha* plots. Base layer imported from Bing Maps (Microsoft Corporation, 2012).



Angle iron used to maintain the position of four bags containing stem and leaf litter

Spike used to secure the angle iron in the wetland litter layer

Figure 3. Photograph of a 5 mm mesh litter bag secured to an angle iron that was anchored to the wetland soil with an iron spike.

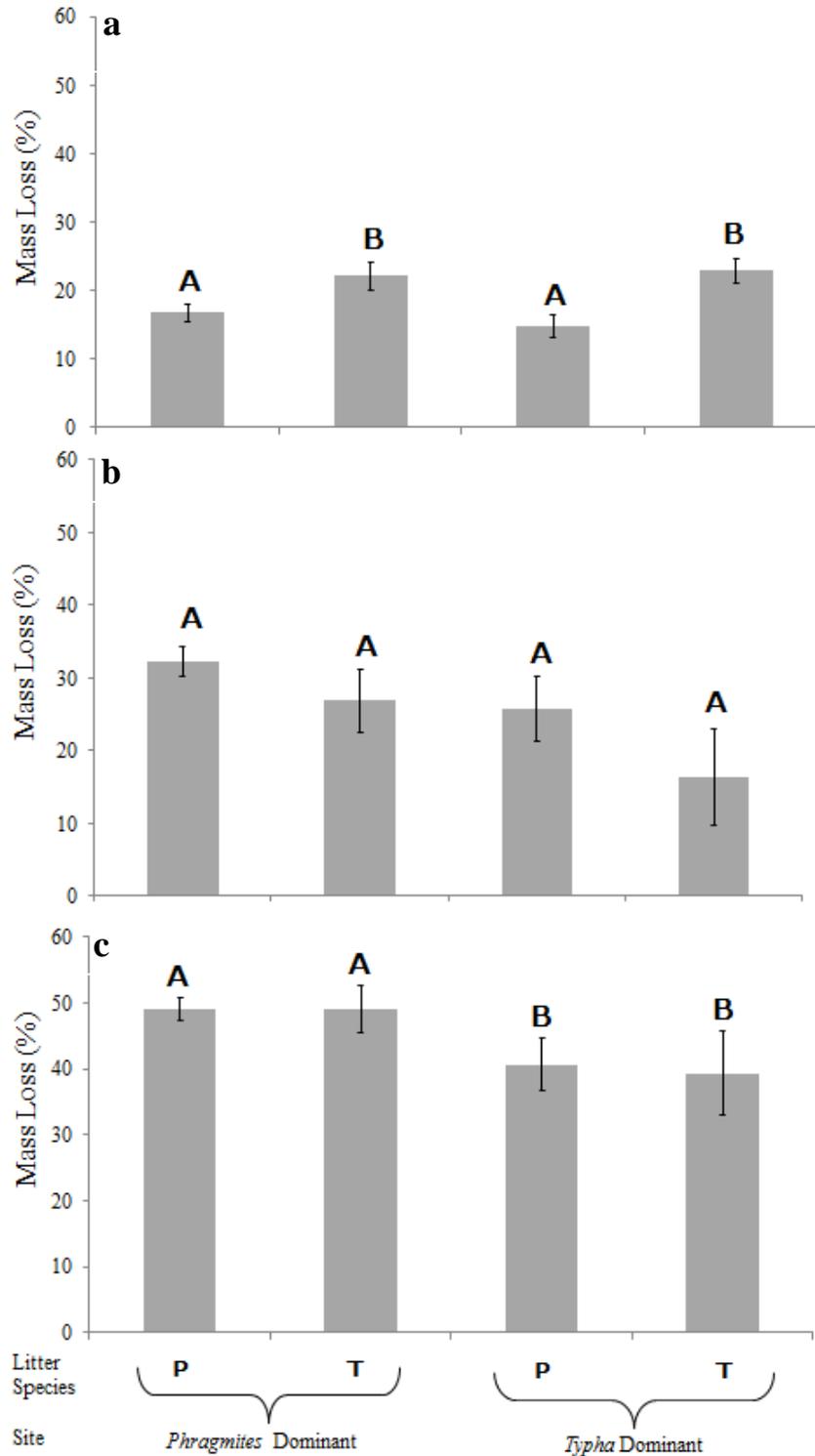


Figure 4. Mass loss (%) from *Phragmites* and *Typha* litter in sites dominated by each plant species during a) cold season (144 d from Dec 2010 to Apr 2011) b) warm season (200 d from Apr 2011 to Nov 2011) and c) annual (344 d from Dec 2010 to Nov 2011). Different capital letters above bars indicate significant differences ($p < 0.05$). Bars show mean (± 1 SD) ($n=10$).

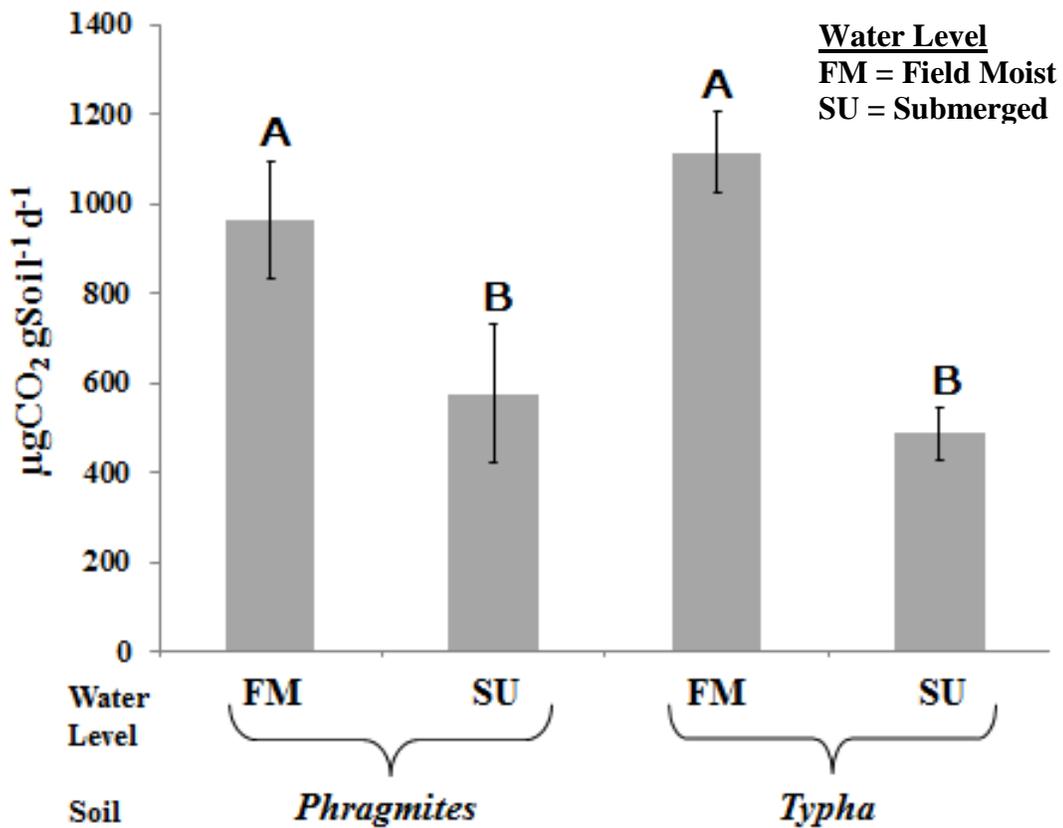


Figure 5. Carbon dioxide production during a four day incubation of field moist (FM) oxic and submerged (SU) anoxic *Phragmites* and *Typha* soils in the dark at 21.7 °C. Different capital letters above bars indicate significant differences ($p < 0.05$). Bars show mean (± 1 SE) ($n=5$).

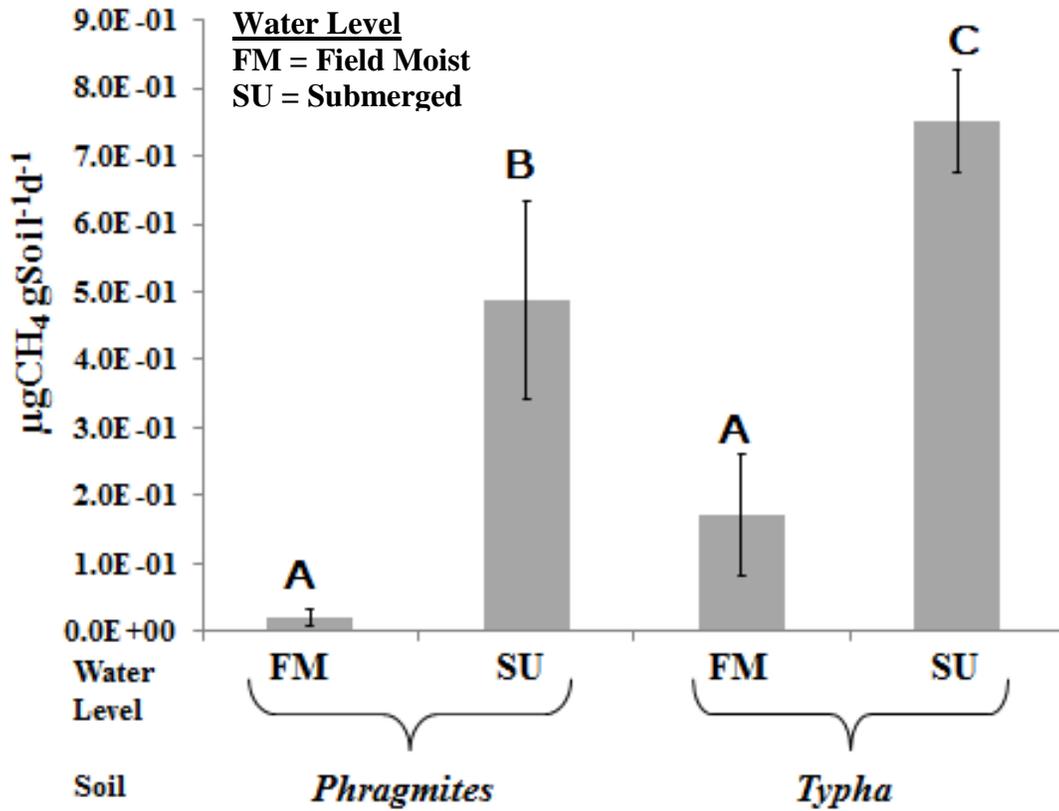


Figure 6. Methane production during a four day incubation of field moist (FM) oxic and submerged (SU) anoxic *Phragmites* and *Typha* soils in the dark at 21.7 °C. Different capital letters above bars indicate significant differences ($p < 0.05$). Bars show mean (± 1 SE) ($n=5$).

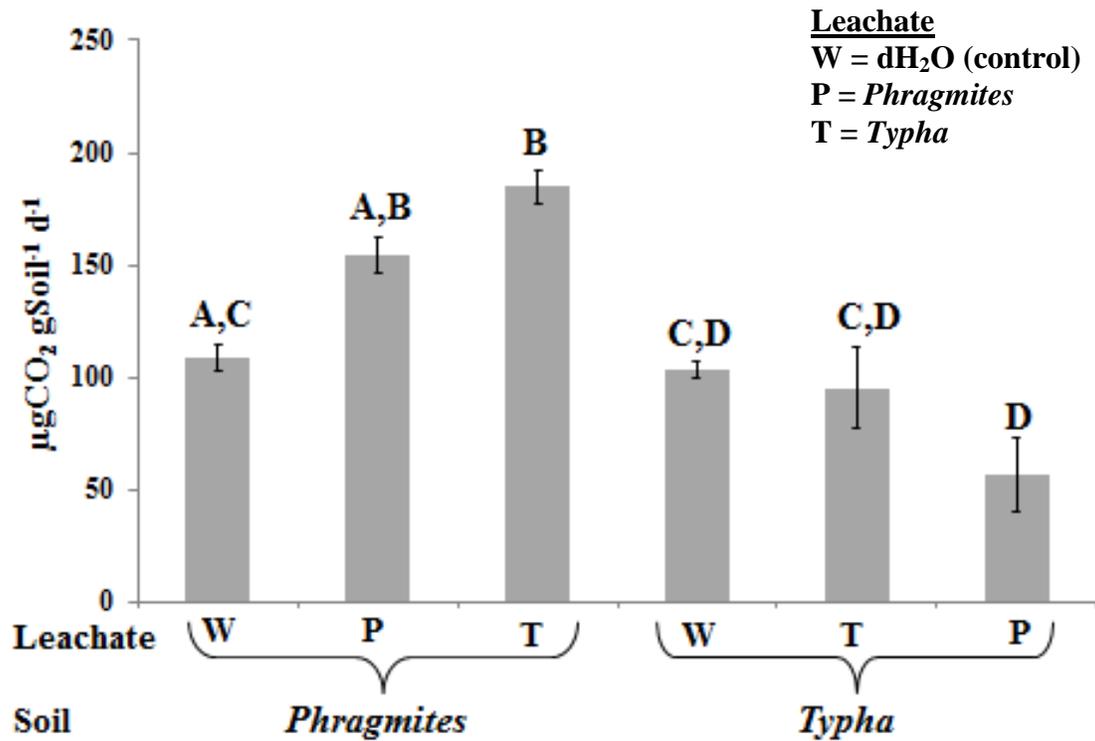


Figure 7. Carbon dioxide produced during a seven day incubation of *Phragmites* and *Typha* soils treated with dH₂O (W), *Phragmites* leachates (P) or *Typha* leachates (T) in the dark at 21.7 °C for 7 d. Different capital letters above bars indicate significant differences ($p < 0.05$). Bars show mean (± 1 SE) ($n=8$).

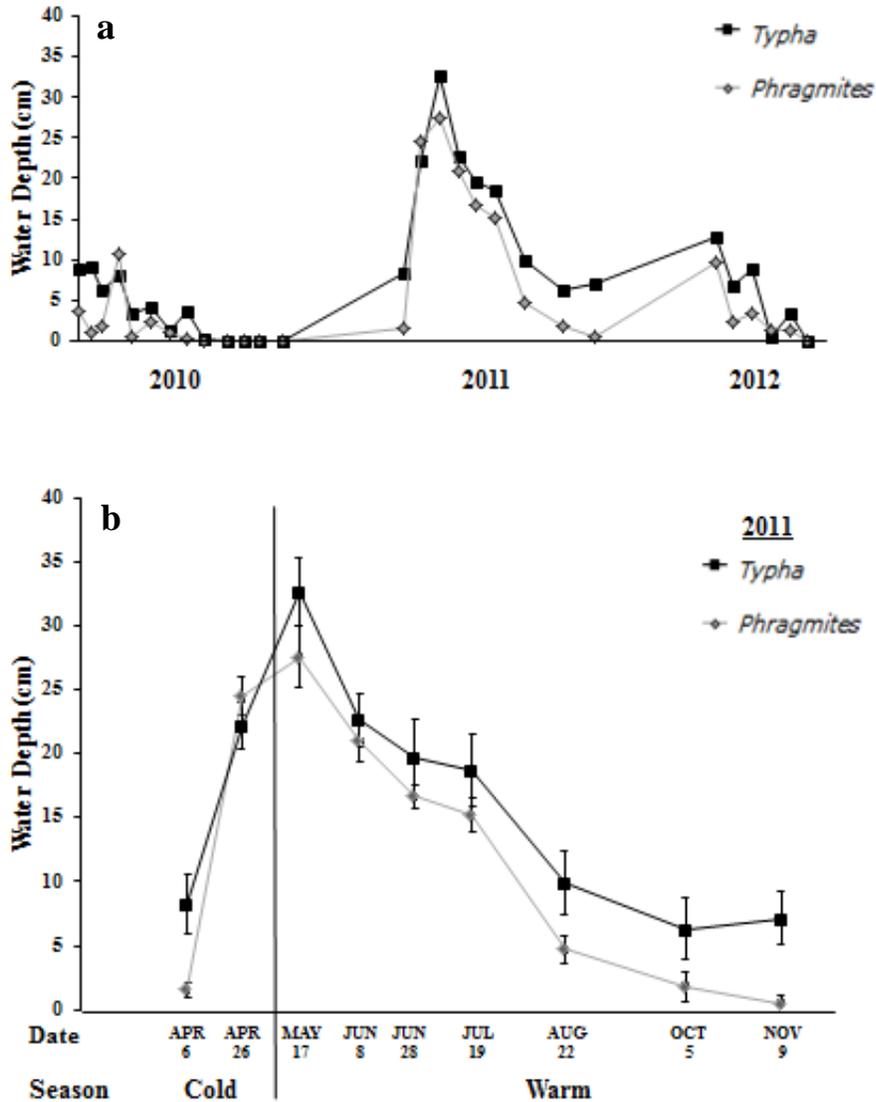


Figure 8. a) Surface water depths in the *Phragmites* and *Typha* dominated sites at the Brancheau marsh during 2010 through 2012. Points show mean (n=5) b) Inset of surface water depths within *Phragmites* and *Typha* sites on nine sampling dates during the cold (Dec 2010 to Apr 2011) and warm seasons (Apr 2011 to Nov 2011) of the litter decomposition experiment. Water levels were significantly lower in *Phragmites* sites during both the cold and warm seasons ($p < 0.05$). Points show mean (± 1 SE) (n=5).

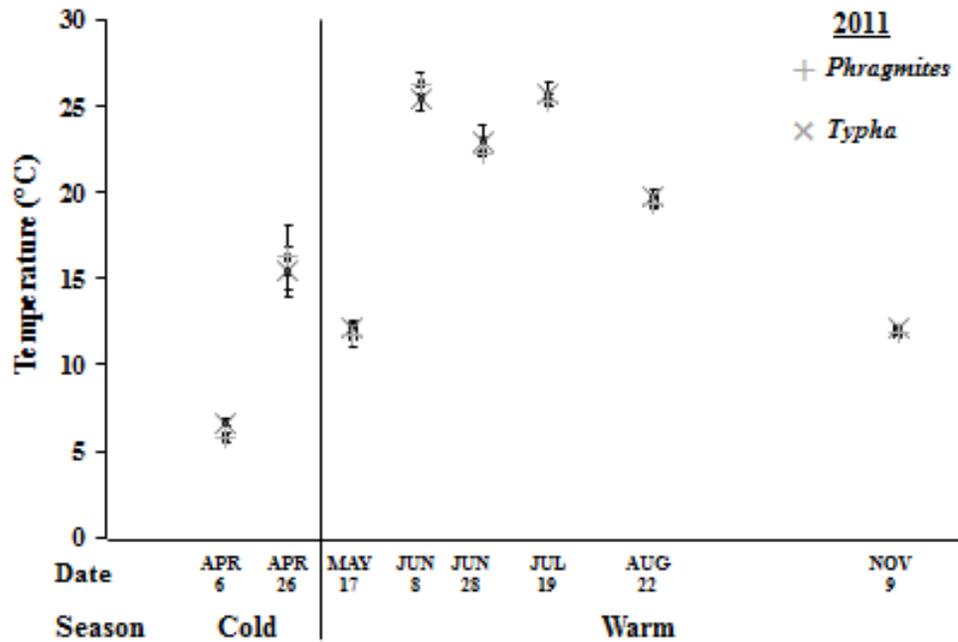


Figure 9. Soil water temperatures within *Phragmites* and *Typha* sites on eight sampling dates during the cold (Dec 2010 to Apr 2011) and warm (Apr 2011 to Nov 2011) seasons of the litter decomposition study. Water temperatures were not significantly different in *Phragmites* and *Typha* sites, during the cold or warm seasons ($p > 0.05$). Points show mean (± 1 SD) ($n=5$).