INVASIVE PLANT SPECIES IMPACTS ON CARBON AND NITROGEN CYCLING IN INLAND MICHIGAN WETLANDS

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ABSTRACT

INVASIVE PLANT SPECIES IMPACTS ON CARBON AND NITROGEN CYCLING IN INLAND MICHIGAN WETLANDS

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Plant traits are often the central focus of ecological investigations into ecosystem structure and function because of the need to simplify complex plant communities to a few traits of importance. Invasions by invasive species can have major impacts on ecosystem function by altering the presence and/or dominance of plant traits that influence ecosystem energy flow and nutrient cycling. The broad goal of this dissertation was to investigate the ecosystem consequences of invasive plant species in temperate wetlands, which are important ecosystems for the cycling of carbon (C) and nitrogen (N), focusing on *Phragmites australis* (Cav) Trin. Ex Steud*, Phalaris arundinacea* L. and *Typha* ×*glauca* Godr. X. I hypothesized that within inland Michigan wetlands, the degree of invasion would be correlated with increased C and N stocks due to the high production of low quality litter from these invasive plants. I found evidence that both soil and ecosystem C stocks increased due to the presence of these invasive species. Additionally, I found significant differences for C and N mineralization among species linked to the quality of their litter (C:N ratios), with *P. australis* soil having the lowest C and N mineralization and *P. arundinacea* soil the highest.

Phragmites australis is a tall, high biomass invasive species that is a relatively recent invader into the wetlands of the Great Lakes states. To investigate the effects of living biomass and litter on C and N cycling, I manipulated *P. australis* litter and biomass within

plots at three wetland sites and then monitored abiotic conditions and performed a number of biogeochemical assays. Removing *P. australis* litter and biomass had the hypothesized effects of increasing light levels at the soil surface and increasing soil temperature, though these effects did not influence litter bag decomposition, *in situ* N mineralization, or potential denitrification rates. Biomass removal did affect porewater ion concentrations by decreasing Na^+, Cl^- and Ca^{2+} concentrations and increasing NO_3^- concentration. Though not initially hypothesized, all C and N cycling rates showed strong site effects caused by different hydrologic conditions among sites.

To separate litter quality, litter diversity, and soil origin from other controls of decomposition, I performed two laboratory incubations using litter and soil collected from monospecific stands of the three focal invasive species plus *Carex lacustris*, a native sedge. I found support for my prediction that the four species differed in litter quality and that litter C:N ratio was negatively related to C and N mineralization rates. I also found strong soil origin effects related to soil nutrient availability, which have not been found before within a similar experimental framework. The second incubation showed that while litter diversity significantly affected litter decomposition rates, the effects were more dependent of the identity of the species than just the number of species.

Taken together, these results suggest that invasive species can influence C and N cycling in inland Michigan wetlands, and that some of the biogeochemical effects, like increased C storage, could be a positive outcome of invasion. These effects can also be linked to key plant traits, such as litter quality and biomass production, and supports the conjecture that invasive species alter ecosystem function by changing the composition or dominance of plant traits within the community.

This work is dedicated to my grandmother, Helen Dombrowski.
Thank you for believing in me.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1
Introduction

Carbon and Nitrogen Cycling in Wetlands

Human-induced alteration to global biogeochemical cycles is creating considerable public and scientific interest today. Of special concern are the anthropogenic activities that have directly and indirectly increased the amount of reactive nitrogen (N) (NO_3^- and NH_4^+; Galloway et al. 2003) entering terrestrial and aquatic ecosystems, due to: (1) increased cultivation of leguminous crops that biologically fix un-reactive N (N_2) to ammonium, (2) combustion of fossil fuels resulting in the creation of NO_x species, and (3) the use of large quantities of NH_4^+-based fertilizers to increase crop yield worldwide (Vitousek et al. 1997; Driscoll et al. 2003; Galloway et al. 2003). These inputs have contributed to elevated levels of reactive N that result in major ecological and human health problems, such as tropospheric ozone formation, acid rain, high ammonium-N toxicity to fish, carcinogenic effects of increased nitrate levels in drinking water, and stimulation of pathogenic microbes which can cause public health risks (Vitousek et al. 1997; Driscoll et al. 2003; Galloway et al. 2003; Zedler 2003). Aquatic ecosystems are particularly sensitive to nutrient loading, which can result in eutrophication, algal blooms, high biological oxygen demand, and the formation of dead zones (Livingston 2000).

Carbon cycling also has been significantly impacted by human activities, such as the use of combustion engines, the processing of natural gas and crude oil, and deforestation (Hoffert et al. 1998). These actions have resulted in rising concentrations of CO_2 and CH_4

in the atmosphere. Both CO_2 and CH_4 are greenhouse gases and are hypothesized to drive most of the anthropogenic climate change now underway (in addition to N_2O), and, therefore, understanding how they cycle through the environment is of great importance. While the majority of the carbon in the biosphere is stored in the world's ocean, primarily as bicarbonate (Schlesinger 1997), there has been substantial interest in understanding the dynamics of terrestrial carbon sources and sinks because they are potentially very active and thus can have significant impacts on carbon cycling. Heimann and Reichstein (2008) suggested that on a global scale terrestrial ecosystems will provide a positive feedback to global warming, mainly by permafrost thawing, the microbial priming effect, and the interaction between carbon and nitrogen cycles, though the magnitude of effects is less well known. Soils are the largest terrestrial carbon pool, though living plants (trees, grasses, etc.) are also a significant pool of terrestrial carbon. Although forest and grassland soils can store organic matter for long periods of time, wetlands, by far, store the largest amount of carbon on an areal basis, storing a global total of approximately 300 – 700 billion tons of carbon (Bridgham et al. 2006), with the majority in northern peatlands. Wetlands are significant sinks of carbon due to their high productivity and anaerobic conditions that promote slow decomposition, thus resulting in the buildup of organic matter. Euliss et al. (2006) estimated that North American prairie wetland restoration alone has the potential to sequester 378 Tg of carbon over a 10-year time period, offsetting the annual fossil fuel carbon emissions in North America by 2.4%.

There is a strong coupling between C and N cycling (Schlesinger 2011). This strong coupling occurs for two main reasons: (1) there is a stoichiometric requirement of C and N

for most organisms, so limitation of one element will usually limit the cycling of the other (assimilatory coupling) and (2) the metabolic capabilities of many microbes allow for the catalysis of energy releasing reactions, usually by means of changes in oxidation states of C or N (dissimilatory coupling) (Burgin et al. 2011). In dissimilatory reactions, the elements are not incorporated into the biomass of the organism and are instead released directly back into the environment. The coupling of C and N cycling can have consequences to the structure and function of many ecosystems. For example, in a laboratory incubation using soil collected from an old-growth coniferous soil, Hart et al. (1994) found a strong positive relationship between CO_2 evolution and gross N mineralization. Microbial growth efficiency declined over the incubation period (456 days) suggesting the use of lower quality substrates as C availability declined. Thomas et al. (2010) studied the effects of N deposition on northeastern forests and found that N deposition increased the growth of the majority of study species, and thus increased tree C storage. In wetlands, one of the best examples of the tight coupling between C and N is the denitrification process. In low oxygen conditions, denitrifiers can convert NO_3^- to N_2O and N_2 by a series of redox reactions that use NO_3^- as an electron acceptor and organic carbon as an electron donor. This is one of several metabolic pathways responsible for the decomposition of organic matter in anoxic conditions.

Wetlands are usually found in depressional areas on the landscape and as a result they receive large amounts of water from surface and ground water inflow, making them vulnerable to flooding, nutrient loading, and other types of disturbance (Zedler and Kercher 2004). Wetlands are defined as ecosystems on the interface between terrestrial

and aquatic habitats (Mitsch and Gosselink 2000). The three main features that distinguish a wetland are: (1) a zone of saturation (at the soil surface or within the rooting zone) for at least part of the year, (2) unique soil characteristics arising from saturation (e.g., gleying) and (3) the presence of hydrophytes (plants adapted to saturated soil conditions) with the exclusion of plants intolerant to waterlogging (Mitsch and Gosselink 2000). While wetlands provide a host of ecosystem services such as wildlife habitat, flood control, and shoreline stabilization (Zedler 2003), one of the main services provided by these ecosystems is the enhancement of water quality via the retention and removal of excess N (Saunders and Kalff 2001; Zedler 2003; Verhoeven et al. 2006). Wetlands have played major roles of improving water quality in a number of climatic, ecological, and land-use settings (Zedler 2003). Wetlands retain and remove N through several physical and biological processes, including N retention via plant N uptake for metabolic processes, cation exchange in organic-rich soils, and longer-term sedimentation and burial (Reddy and Delaune 2008). Microbially-mediated transformations of N in wetlands, including mineralization, nitrification, and denitrification, are particularly important because these processes result in the conversion of reactive N to less reactive species (N_2O and N_2) that are emitted to the atmosphere.

Wetlands furnish ecosystem services disproportionately to their area, particularly in the case of water quality improvement (Zedler 2003; Verhoeven et al. 2006). Nitrogen retention and removal by wetlands has received particular attention in North America and Europe where excessive N is a concern. Wetlands can reduce the ecological and health risks posed by excess N in aquatic ecosystems, especially in areas where agriculture or other land uses create runoff of N originating from fertilizers (Verhoeven et al. 2006).

Wetland N cycling is sensitive to changes in soil temperature, redox status, available nutrients, and pH (Reddy and DeLaune 2008) (Figure 1-1; pathways 5, 6, and 8). Because of their role as landscape sinks for nutrients and organic matter, along with having periods of drying and rewetting, wetlands as known as biogeochemical "hotspots" for N (McClain et al. 2003). Wetlands are very productive ecosystems, and in most cases, rates of photosynthesis (primary production) are greater than decomposition creating organic matter rich soil that contains nutrients as well as electron donors (carbon) needed for many N transformation processes. For example, nitrification, the microbially-mediated conversion of ammonium to nitrate, needs an aerobic environment and ammonium to occur. Conversely, denitrification requires nitrate as the oxidant, labile organic carbon as the electron donor, and an anaerobic environment. These contrasting requirements limit the rate of these N cycling processes in most ecosystems, but wetlands provide aerobic and anaerobic conditions that vary spatially and/or temporally, which can greatly increase the rates of processes like denitrification. (McClain et al. 2003; Reddy and DeLaune 2008).

Despite their benefits to water quality, the majority of the wetlands in the United States have been drained or degraded, often for agricultural purposes (Zedler 2003). Over the past several decades, mitigation and conservation programs in the United States have been established to protect remaining wetlands, though they remain vulnerable to perturbations such as changes in natural hydrologic flow and nutrient loading from surrounding land-uses (Saunders and Kalff 2001; Zedler 2003; Verhoeven et al. 2006). In the Great Lakes region, an area dominated by wetlands, water, and water-based recreation, increasing invasions by exotic species are considered to be one of the most critical threats to biodiversity and ecosystem stability (Zedler and Kercher 2004).

Wetlands are especially vulnerable to biological invasions because of their landscape setting where they often receive inflows of water and propagules, and because they often are periodically disturbed by flooding and drying events (Zedler and Kercher 2004; Magee and Kentula 2005). While wetland plants can be considered stress-tolerators (Grime 2002) because they are adapted to environments that are highly variable (e.g., seasonally flooded), humans have introduced additional disturbances, such as nutrient loading, that native species are not adapted to. Nutrient loading usually favors species that respond positively to high nutrient availability (Davis et al. 2000), which is the case for most wetland invasive plants. Recent research has shown that tall, clonal species with runner behavior (i.e., species with long spacers between ramets, which includes most wetland invasive plant species) respond to nitrogen addition by increasing in abundance, while short, clumping species (many native wetland plant species) decreased in abundance (Gough et al. 2012). This may be one of the main mechanisms for the invasibility of wetlands.

Wetland plants influence wetland soils and hydrology through their structure, life history, degree of clonality, root characteristics, and quantity of litter inputs (Ehrenfeld 2003) (Figure 1-1; pathways 2 and 3). Plants and their litter influence abiotic factors (e.g., water availability and/or soil temperature), thereby indirectly affecting microbial decomposition rates and soil carbon sequestration (Eviner and Chapin 2003; Euliss et al. 2006; Lal 2008). For example, Fisk and Schmidt (1995) found that relationships between N mineralization, soil temperature, and water in alpine tundra communities varied among plant communities. It is possible that invasion by high-productivity plant species that generate low quality litter could increase C and N storage, thereby enhancing an ecosystem

service provided by wetlands. A meta-analysis of 94 experimental studies across a variety of ecosystems found that invasive species influence C and N cycling by increasing C stocks (root and shoot), plant N concentration, and inorganic N pools in the soil, and, although this pattern was not influenced by ecosystem, there was variation in the strength of effect among plant functional types (Liao et al. 2008).

As mentioned above, wetland hydrology has great influence on the structure and function of wetlands (Hamilton 2002; Trebitz et al. 2005; Sierszen et al. 2006). Directly, flooding decreases the oxygen concentration within soil or sediment, which has implications for soil respiration and decomposition (van der Valk 1991; Neckles and Neill 1994). The reduction of available oxygen due to flooding, and therefore a reduction in decomposition, is one of the main reason wetlands accumulate organic matter in their soils or sediments. Flooding not only changes ecosystem processes directly (e.g., prolonged soil anaerobiosis), but also indirectly through influence on the plant community composition. Because flooding can be a common disturbance in wetlands, many studies have focused on how flooding can affect wetland plant communities (e.g., Hudon 2004; Kercher and Zedler 2004). While most wetland plants can survive periods of inundation, plant species vary in their tolerance to the duration and depth of flooding (Cronk and Fennessy 2001). This variation in flood tolerance among species is one of the main causes for differences in plant community composition among wetlands (Sharitz and Pennings 2006). Flooding causes multiple challenges for plant species: (1) oxygen diffuses 10,000x slower in water than in air, thus roots are oxygen limited, (2) anaerobic conditions produce toxic substances such as Fe (II), H_2S, and organic acids that can harm plant roots, and (3) complete submergence of the plant will lead to a reduction of light, CO_2, and oxygen to the shoots (Cronk and

Fennessy 2001). Wetland plants have multiple adaptations to cope with flooding stress, including passively or actively transporting oxygen from the shoots to the roots for root respiration, releasing oxygen from the surface of the root creating an oxic environment that protects the root from Fe (II) and H_2S, and, in many cases, tall, emergent stature that ensures direct interface with the atmosphere in most conditions. The effect of flooding on wetland plant community composition can then influence ecosystem function by altering the predominant plant traits within the community.

Invasive Species and Plant Functional Traits

The human-caused redistribution of species has recently accelerated with the economic globalization of human society (Theoharides and Dukes 2007). Invasions by exotic species are considered to be one of the most critical threats to biodiversity and ecosystem stability (Walker and Steffen 1997). Invasive species threaten ecosystem function on multiple levels resulting in economic costs and ecological damage. The cost of damage, control, and removal of invasive species reaches approximately $137 billion annually for the United States (Pimentel et al. 2000, 2004). The resulting effects of species redistribution caused by the introduction of exotic species on ecosystem-level processes are largely unknown. While a small percent of introduced species become established, with an even smaller amount becoming invasive (Williamson and Fitter 1996), those species that invade successfully often become dominant and can alter community structure and composition (Levine et al. 2002). For example, garlic mustard (*Alliaria petiolata*) invasion into North American woodlands has resulted in the alteration of under-story plant communities (Meekins and McCarthy 2000; Stinson et al. 2007). Closer examination also

has shown that garlic mustard may change the tree community to more shade tolerant species whose seedlings can withstand the intense shading created by garlic mustard stands (Stinson et al. 2007). In addition, garlic mustard produces allelopathic chemicals that inhibit the growth other plants, potentially via inhibition of mycorrhizal fungi (Weir 2007).

While much research has investigated why certain plant species become invasive while others do not, it is not entirely clear what mechanisms are responsible for invasion success. Some of the most supported hypotheses include the enemy release hypothesis, broader tolerance hypothesis, efficient use hypothesis, hybrid vigor hypothesis, and allelopathy hypothesis (Zedler and Kercher 2004), though none can explain all invasions. There is much evidence that propagule pressure is a strong determinant of invasion success (Lockwood et al. 2005), and more recent theory suggests that invasion is a complex process with many stages including transport, introduction, establishment, and spread (Blackburn et al. 2011). The spread of invasive species have also been connected to economic activity; Taylor and Irwin (2004) were able to explain 75% of the variation in the number of exotic plants in the 50 states with a population-economic model that incorporated human population and real estate.

There are also contrasting patterns for the effect of invasive species on species diversity. On a global scale it seems clear that species diversity is decreasing as a result of many human activities, such as habitat destruction, over-hunting, and species introductions (Kerr and Currie 1995). On a local scale (study sites less than a few dozen hectares) species loss seems to follow the general global pattern; species introductions decrease species richness (Sax and Gaines 2003). The pattern for regional scales (between global

and local scale) is quite different, as there seem to be diversity increases associated with species introductions (Sax and Gaines 2003). This contrasting effect of introduced species is not easily explained, though the regional increase is likely partially caused by species redistribution by humans, increasing the total number of species on a continental scale, while at the local scale invasive species are known to competitively exclude most other species where they occur. The decrease in species diversity at a local scale is also more severe in anthropogenic environments (Sax and Gaines 2003). Another paradox related with scale is the positive association found between natives and exotic species richness at broad scales contrasted with the negative associations at fine scales (Fridley et al. 2007). These paradoxes show our incomplete understanding of species invasions and seem to suggest that more focused studies are needed to resolve these dilemmas. Regardless of the mechanism of these patterns, it is clear that invasive species negatively impact species diversity on global and local scales.

When an invasive plant becomes locally dominant, it can directly and indirectly impact the invaded community (Figure 1-1). Invasive and resident species likely differ in key plant traits such as tissue quality, productivity (above- and below-ground), and phenology. Ecosystem processes are sensitive to the identity of plant species that comprise community species pools (Eviner and Chapin 2003), thus invasions into natural communities can alter ecosystem-level processes by introducing novel plant traits that influence soil environments, soil microorganism composition, and/or nutrient cycling. In a mixed deciduous forest in New York it was shown that invasive woody species accelerated decomposition rates, which in turn resulted in increased N loss from litter (Ashton et al. 2005). What was interesting about this study was that all litter in the invaded sites (exotic

and native species) decomposed faster compared to the non-invaded sites. This showed that species-specific litter decomposition was not the only important difference between invaded and non-invaded sites and that invasive species indirectly affected ecosystem processes (Ashton et al. 2005).

Variation in plant traits within a community is known to influence many aspects of ecosystem function (Chapin 2003). Biomass accumulation and other plant traits that affect organic matter quality can directly and indirectly influence an ecosystem's ability to store carbon (C) and nitrogen (N) within litter and soil layers (Ehrenfeld 2003). Previous work has shown that plant functional group diversity had strong effects on total plant N, plant productivity, and light penetration in an experimental grassland in Minnesota (Tilman et al. 1997), while functional group composition was a more important predictor of ecosystem-level processes than functional group richness in a serpentine grassland in California (Hooper and Vitousek 1997). Plant functional traits such as leaf tissue chemistry, rooting depth, and canopy structure affect ecosystem productivity and C and N cycling (Tilman et al. 1997; Ehrenfeld 2003; Eviner and Chapin 2003).

Plant functional traits are useful to study because they allow us to simplify complex and seemingly species-specific controls on ecological/biogeochemical processes to a few plant traits of dominant influence (Eviner and Chapin 2003; McGill et al. 2006). This allows predictions to be made about the outcomes of ecological phenomena, such as invasion, succession, or competition, with some confidence given knowledge of important functional traits of the invading or competing species (Westoby and Wright 2006). For example, *Spartina alterniflora* invasion into native *Phragmites australis* and *Scirpus mariqueter* populations in China caused an increase in net primary production and C and N storage.

This was, in part, the result of *S. alterniflora* having a longer growing season, denser canopy, and greater root biomass than either native species (Liao et al. 2007). In addition, *Lythrum salicaria* invasion of *Typha latifolia* stands in New York resulted in higher soil organic matter (OM) content and greater N mineralization rates. While hydrology was a major control on N transformations, *L. salicaria* total biomass was more than double that of *T. latifolia*, which increased litter inputs and resulted in higher soil OM content (Fickbohm and Zhu 2006). If a trait is found to strongly affect ecosystem processes, e.g., decomposition, then predictions can be made about the magnitude of effect on that process for species that share that trait.

Although the benefits of investigating the influential functional traits affecting ecosystem processes have been made clear (Diaz and Cabido 2001; Lavorel and Garnier 2002; Arndt 2006; Schindler and Gessner 2009), not all variation in ecosystem function, such as C and N mineralization, can be completely explained by a few functional traits. In some cases, species identity is the best predictor of ecosystem level effects (Wardle et al 2003). Therefore, if possible, it is important to simplify complex plant community data to a few key traits of large effect, as well as to investigate if specific dominant species are strongly influencing the overall ecosystem function, perhaps by novel traits not shared by the rest of the community. Invasions by exotic species are a good example of situations where both approaches may be useful.

Phragmites australis has been shown to reduce the availability of inorganic N in wetlands and increase organic N retention (Findlay et al. 2002) compared to native *Spartina* species, likely due to differences in litter chemistry. Orwin et al. (2008) demonstrated that C substrate identity can influence soil chemistry, microbial metabolism,

and soil microbial community structure, all of which could influence ecosystem processes such as decomposition and NPP (through effects on plant available nutrients). Angeloni et al. (2006) showed that invasion by *Typha* ×*glauca* into a Michigan marsh increased soil organic matter and altered the composition of the bacterial and specifically denitrifier communities, which could have been influenced by the higher aboveground biomass of *T.* × *glauca* compared to the invaded community. While it is possible that plant invasions in temperate wetlands could stimulate the short-term uptake of N through increased biomass production, invasive species could have different longer-term impacts on N transformations in wetlands through more complex controls on soil microenvironments and substrate quality.

Phragmites australis (common reed), *Phalaris arundinacea* (reed canarygrass), and *Typha* ×*glauca* (hybrid cattail) are some of the most successful invasive species in North American wetlands (Zedler and Kercher 2004). *Phragmites australis* is invading wetlands throughout the United States, and is of particular concern in the Midwest states such as Michigan (Marks et al. 1994). *Phragmites australis* is a high-biomass invasive (Windham and Lathrop 1999; Minchinton and Bertness 2003), and can have high N use efficiency and low litter quality (Findlay et al. 2002). In Michigan wetlands, *P. australis* occurs in the same wetland zone as *Typha latifolia* and is still in the process of aggressively invading into sites where it has colonized (S. Hamilton, personal communication). *Phragmites australis* has been shown to reduce the availability of inorganic N in wetlands (Windham 2001) and increase N retention (Findlay et al. 2002) compared to *Spartina* species, likely due to differences in litter chemistry. Similar comparisons with *Typha latifolia* have yet to be made.

Phalaris arundinacea and *T.* ×*glauca* are similar to *P. australis* in that they produce higher biomass compared to native species, spread through clonal growth, and displace native wetland plant communities (Lavergne and Molofsky 2004; Wetzel and van de Valk 1998; Hudon 2004). Exotic genotypes of *P. arundinacea* was introduced to the United States ca. 1850, though native populations are known to have existed prior to that introduction (Lavergne and Molofsky 2004). Invasive genotypes have been shown to come primarily from Europe and after introduction for forage, bioremediation, and erosion control, invasive genotypes hybridized with the less aggressive native genotypes (Lavergne and Molofsky 2007). Currently, the distribution of *P. arundinacea* includes most of the northern half of the United States. *Phalaris arundinacea* is extremely phenotypically plastic and can adjust morphologically to match abiotic conditions (Martina 2006), which has been assumed to aid it when invading in to new areas. *Typha* ×*glauca*, currently distributed across the eastern United States, is a hybrid between *T. latifolia* (native) and *Typha angustifolia* (exotic) that is very aggressive and can out-compete the native cattail when they co-occur. *Typha angustifolia* is native to Europe but the date of introduction to the United States is not well known, though it was likely at the beginning of the 20th century.

For invasive species that are more productive (in terms of biomass) than natives, such as *P. australis*, *P. arundinacea,* and *T.* ×*glauca,* one of two mechanisms may be responsible: (1) invasives have higher nitrogen use efficiency (NUE, biomass per unit N) than natives or (2) they respond to nutrient loading more than natives. While it is widely hypothesized that the latter is the correct mechanism in most circumstances (Zedler and Kercher 2004; Tyler et al. 2007), invasive species can still invade wetlands at low nutrient levels (Green and Galatowitsch 2002) and there is also evidence that invasives are successful because of

greater NUE (Funk and Vitousek 2007), at least in low resource habitats. A thorough understanding of how these invasive plants are influencing C and N cycling, along with the controlling factors, will help to predict the impacts of invasion. While there has been some research on the effects of invasive species in wetlands (e.g., Findlay et al. 2002; Angeloni et al. 2006; Liao et al. 2007), few studies have directly investigated the mechanisms responsible for ecosystem process changes due to invasion and even fewer have looked at multiple invasive species simultaneously.

Decomposition: Abiotic and Biotic Determinants

Decomposition is an essential ecosystem process defined as the physical and chemical breakdown of organic matter (Chapin et al. 2002). During decomposition, organic matter is broken down into its chemical components releasing CO_2 to the atmosphere and nutrients to the soil. Decomposition consists of three basic processes: leaching, fragmentation, and chemical alteration (Chapin et al. 2002). Leaching is the mobilization of labile soluble materials into water, such as sugars and amino acids, from decomposing OM. In a litter bag field incubation investigating the controls on decomposition in two Mediterranean ecosystems, it was found that leaching was an important phase in decomposition that lasted 2-4 months (Gallardo and Merino 1993). Fragmentation is the physical breakdown of large detritus mainly by soil animals (though freeze-thaw and wet-dry cycles can also fragment organic matter) that increases surface area for microbial activity and can be a very important process in environments where soil animals thrive (Verhoef and Brussaard 1990). Soil animals can also influence the magnitude and direction of the effects of litter diversity on decomposition (Hattenschwiler and Gasser 2005). Chemical alteration mainly

occurs by the action of microbial activity though some alteration can occur spontaneously, such as when exposed to UV radiation (Austin and Ballare 2010).

Microbes (mostly fungi and bacteria) release extracellular enzymes that react with organic matter resulting in the breaking of chemical bonds. Ultimately, through microbial respiration, organic matter is used as an energy source and oxidized to CO_2 (in aerobic environments). The majority of soil respiration is due to the action of both bacteria and fungi, though aspects of how they influence decomposition can differ in important ways. Fungi are the main initial decomposers of dead plant material because they are capable of producing enzymes that can break down most plant compounds, including lignin (Chapin et al. 2002). Though they are the dominant decomposers in ecosystems like forests because of their large networks of hyphae, they are poor decomposers in low oxygen environments like wetlands. Conversely, bacteria can survive in oxygen poor soils (e.g., wetlands) and are responsible for a myriad of chemical transformations, such as nitrification, denitrification, and methanogenesis that occur in low oxygen conditions, though in soils that have drying-rewetting cycles, bacteria are not nearly as dominant, and in some cases fungi can become dominant (Strickland and Rousk 2010).

Many factors influence microbial biomass and activity and thus decomposition rates, including temperature, moisture, oxygen concentration, and organic matter quality (Wardle 1992). Generally, increasing temperature can directly enhance decomposition by promoting microbial activity with almost zero microbial activity at very low temperatures. Reichstein et al. (2000) incubated subalpine soil at three temperatures (5, 15, and 25°C) and found that increasing the temperature by 10 degrees nearly tripled C mineralization rates. While in many laboratory incubations the effect of temperature on the

mineralization of soil organic carbon is obvious, a study by Giardina and Ryan (2000) compiled data from 82 sites and five continents and determined that decomposition of organic matter found in forest mineral soils is not controlled by temperature limitations to microbial activity. This apparent lack of a temperature effect on decomposition may actually be due to multiple environmental constraints on carbon mineralization, which might also be sensitive to global warming (Davidson and Janssens 2005).

Soil moisture is needed due to the requirement of water for normal biotic activity, though as soil moisture increases to fully saturated conditions, oxygen diffusion is reduced leading to anoxic conditions. Oxygen is required for aerobic respiration, which yields more energy gain compared to anaerobic respiration. In the absence of oxygen, NO_3^- is used as an electron acceptor (denitrification) followed by Mn^{4+}, Fe^{3+}, SO_4^{2-}, CO_2, and finally H^+, with the order dictated by the energy efficiency of the various electron acceptors (more energy is gained by using NO_3^- as an electron acceptor compared to Mn^{4+}) (Reddy and Delaune 2008). Because more energy is gained during aerobic respiration versus anaerobic respiration, low oxygen concentrations usually results in low decomposition rates, though anaerobic respiration using NO_3^- can be almost as efficient. Oxygen availability can also influence humic acid formation; Ramunni et al. (1987) found that the formation of humic acids during decomposition increased in the presence of oxygen.

Finally, organic matter quality can be a major control on decomposition rates. Organic matter quality is dependent on its chemical composition, including size of molecules, type of chemical bonds, regularity of structures, toxicity, and nutrient concentration (Chapin et al. 2002). Lignin is very recalcitrant (hard to decompose) because

it is a large phenolic compound with a very irregular structure, making enzymatic degradation difficult. Consequently, plant material with high concentrations of lignin decomposes slower than material with low lignin concentrations (Melillo et al. 1982). Organic matter C:N ratios or lignin:N ratios have a negative relationship with decomposition rates and are regularly used as metrics of substrate quality (lability) in the literature. In a study of seven canopy species from a subtropical evergreen forest in Japan, Xu and Hirata (2005) found decomposition rates were strongly controlled by N and lignin content, as well as C:N and lignin:N ratios. In a different study that spanned 10 years, 21 sites, and seven biomes, Parton et al. (2007) found that N release during decomposition was mainly controlled by initial N concentrations, i.e., litter with high C:N ratios (low N content) released less N over the incubation period than litter with low C:N ratios (high N content). They also found that net N release started when litter C:N ratios fell below about 40 (range of 31-48).

Besides the four major determinants of decomposition described above, litter diversity can have profound effects on decomposition rates, though the relationship between litter diversity and decomposition can be positive, negative, or neutral (Gessner et al. 2010). Blair et al. (1990) used the leaf litter from three common woody species (*Acer rubrum*, *Cornus florida,* and *Quercus prinus*) to test the effects of single- versus mixed-species litter bags on decomposition and found that mixed-species litter bags had greater decomposition rates than single-species litter bags, but the effects of the mixed-species litter bags could not be predicted from the single-species litter bags. They hypothesized that the difference between mixed- and single-species litter decomposition was explained by changes in the decomposer community (Blair et al. 1990). In a similar experiment performed to

determine if enhanced decomposition due to increased litter diversity was caused by litter species composition or alteration to the microenvironment, it was found that there was more support for changes to the microenvironment (physical factors and decomposer community) than for litter species composition (Hector et al. 2000). Litter diversity effects on decomposition may be specifically important in plant invasions because, initially, the addition of an invasive species may increase litter diversity, but later in the invasion, if the invasive species forms a monospecific stand, litter diversity would be lower.

Wetlands are highly productive ecosystems that undergo periodic flooding events; therefore, productivity is usually greater than decomposition causing the accumulation of organic matter in wetland soils (Mitsch and Gosselink 2000). Because of the low oxygen levels in the soil, fungi and soil animals cannot survive and therefore bacteria mediate the majority of decomposition. In flooded soils, the absence of both fungi and soil animals (Anderson and Smith 2000) slows decomposition due to the positive effects of fragmentation by soil animals and lignin breakdown by fungi (Hattenschwiler et al. 2005). As discussed above, wetlands are considered biogeochemical "hotspots" because of the landscape sink nature of wetlands combined with wetting and drying events (McClain et al. 2003). These "hotspots" are created by the diversity of redox reactions that often occur in close proximity to each other due to the micro-gradients of oxygen concentrations in soil pore space (Burgin et al. 2011), which often vary spatially and temporally. This makes wetlands the site of multiple transformation pathways for many elements, including C, N, Fe, P, S, and Hg.

Wetland plants can influence all of the major determinants of decomposition discussed above, including temperature (soil shading; Eviner and Chapin 2003), soil moisture

(evapotranspiration; Goulden et al. 2007), oxygen availability (radial oxygen loss; Soukup et al. 2007) and organic matter quality (litter quality; Quested et al. 2007). Therefore, plant community composition is an important factor to consider when attempting to understand elemental cycling in wetlands. Additionally, plants can "condition" soil by influencing the soil microbial community, soil nutrient availability, priming effect (Fontaine et al. 2003), or by all of the above effects. This conditioning can then feed back to alter the decomposition of plant OM inputs, such as litter. The "home-field advantage" phenomenon, described by Gholz et al. (2000) and Ayres et al. (2009), is an example of soil conditioning where soil microbes become accustomed to OM inputs by the dominant plant species, resulting in the OM inputs from that plant species decomposing faster (Strickland et al. 2009). A more complete understanding of plant effects on C and N cycling in wetlands is required to be able to predict the effects of plant invasion on biogeochemical cycling.

Research Objectives

The broad goal of my dissertation research was to quantify the ecosystem consequences of invasive plant species in temperate wetlands, focusing on *Phragmites australis, Phalaris arundinacea*, and *Typha* ×*glauca*. The first part of my research (Chapter 2) characterizes the spatial variability in C and N storage and organic matter quality in 24 wetlands in south-central Michigan. In Chapter 3, I investigate the mechanisms by which *P. australis* influences C and N cycling in three wetlands in central Michigan. In Chapter 4, to understand the effects of litter quality, soil origin, and plant diversity on C and N mineralization, I performed two laboratory incubations using litter and soil collected from monospecific stands of invasive species. Together, these approaches allowed me to test the

following hypotheses regarding direct and indirect effects of invasive plants on plant community composition and ecosystem processes in wetlands:

Hypothesis 1 (Chapter 2): Invasion into wetlands by plant species that typically form monospecfic stands reduce species richness and evenness, and therefore there will be a negative relationship between the invasive species dominance and Shannon diversity.

Hypothesis 2 (Chapter 2): Invasive plants have higher nitrogen-use efficiency (NUE) than natives, leading to higher biomass per unit of available N as well as lower N content of biomass and litter (Figure 1-1; pathway 1, 10).

Hypothesis 3 (Chapter 2): The lower quality (lower N content, higher C:N ratio) of litter from the invasive species results in lower rates of decomposition, increasing rates of organic matter storage in invaded wetlands (Figure 1-1; pathway 8, 9).

Hypothesis 4 (Chapter 2 and 4): Variation among invasive species effects on C and N mineralization rates can be attributed to differences in plant traits among species, specifically litter quality (Figure 1-1; pathway 3, 8, 9).

Hypothesis 5 (Chapter 3): Invasive plants (specifically, *Phragmites australis*) influence C and N cycling directly by affecting plant N uptake (Figure 1-1; pathways 1, 4 and 1, 5, 9) and indirectly by affecting soil climate (Figure 1-1; pathways 2, 6, 9 and 3, 7,6, 9) and the quality and quantity of organic matter inputs (Figure 1-1; pathway 3, 8, 9).

Hypothesis 6 (Chapter 4): C and N mineralization rates depend not only on the species-specific quality of the litter (hypothesis 4), but also on the conditioning of the soil by the dominant species. The soil conditioning effect can be due to the priming effect (Figure 1-1; pathway 3,8,9), differences in soil microbial community (Figure 1-1; pathway 9), or soil nutrient availability (Figure 1-1; pathways 1,5,9 and 8,9).

Hypothesis 7 (Chapter 4): Mixtures of litter from different plant species have higher decomposition rates compared to litter from monospecific stands due to higher substrate diversity (Fig. 1-1; pathway 3,8,9). Hence, invasive species decrease decomposition rates by reducing species (and hence litter) diversity.

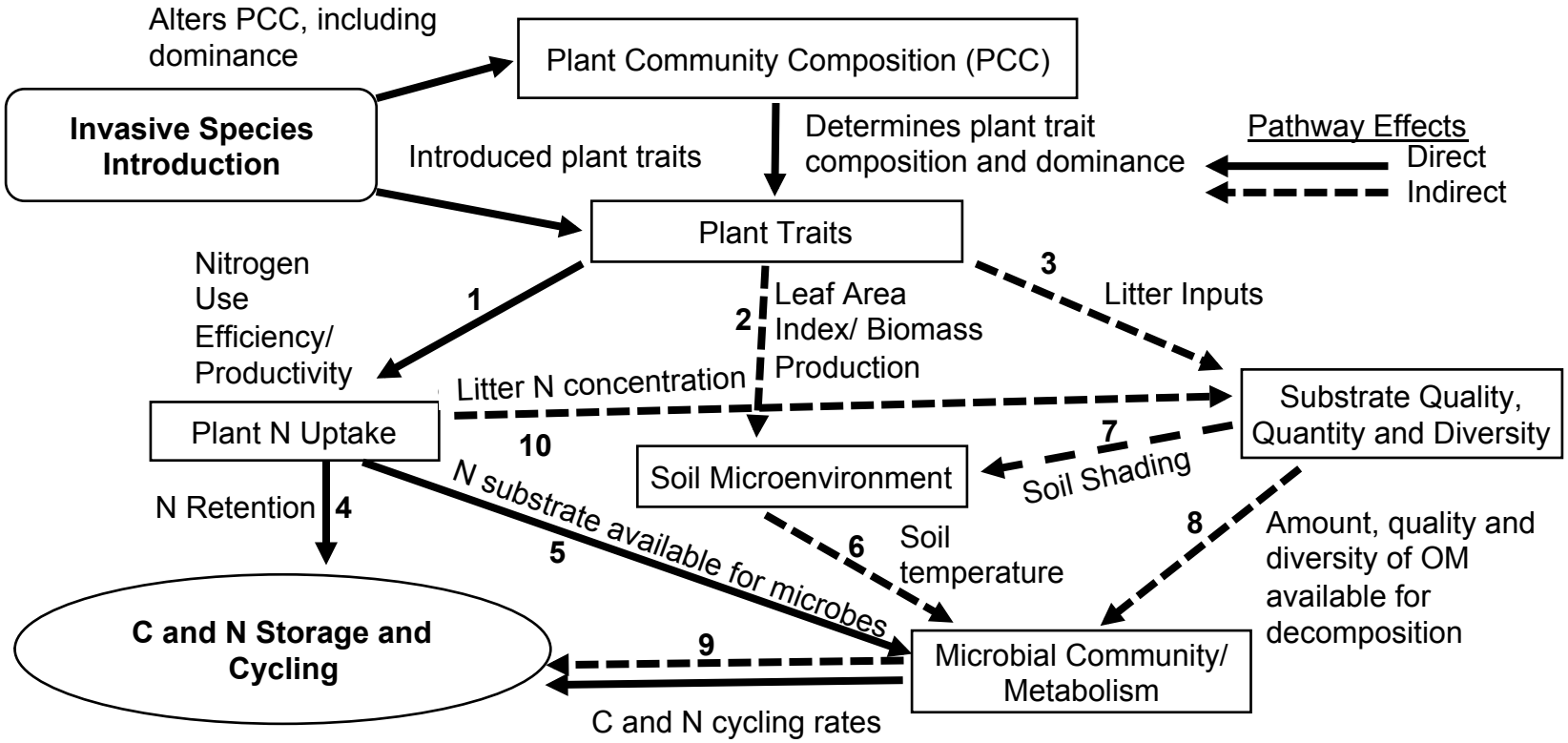

Figure 1-1. Interactions between plant community composition (PCC), plant traits and soil microenvironment influence ecosystem processes. Plant community composition effects on C and N cycling are mediated though plant traits directly (plant N uptake) and indirectly (substrate quality and soil microenvironment alteration). Invasive species introduce novel plant traits into a community and potentially can alter ecosystem function. Arrows represent pathways of influence and are addressed in the text.

Chapter 2
Organic Matter Accumulation and Quality in Michigan Wetlands: Consequences of Invasive Plants

Brief Rationale

Wetlands provide a number of valuable ecosystem services, such as wildlife habitat, flood control, shoreline stabilization, and C and N retention and storage (Zedler 2003). There has been increasing interest in understanding the spatial and temporal pattern of C dynamics in different ecosystems due to the concern of increasing greenhouse gas (CO_2 and CH_4) concentrations in the atmosphere (Smith et al. 2012). Globally, wetlands only cover 2-6% of the land area, but store 14.5% of the terrestrial C stocks (Post et al. 1982) mostly due to their high productivity and low decomposition rates caused by prominent anaerobic conditions (Reddy and DeLaune 2008). While most of the global wetland C stocks are associated with peatlands in northern latitudes (Roulet 2000), temperate wetlands can also be important landscape sinks for both C and N (Euliss et al. 2006). The role wetlands play in N retention has been known for a while, though the exact mechanisms and processes associated with N cycling in wetlands is still an active area of study (Chapman et al. 2006). Wetlands are known as biogeochemical "hotspots" because of the allochthonous inputs of carbon substrates and nutrients, along with a fluctuating hydrology causing temporal variation to the redox status of the sediment, which creates the ideal environment for N processes such as denitrification (McClain et al. 2003). Wetlands are therefore known to both store N and lose N through denitrification.

For some of the same reasons that make wetlands biogeochemical hotspots, wetlands also can be one of the most invaded ecosystems, given that they serve as sinks for invasive species propagules (carried by inflows of surface water), nutrients, and

disturbance (Zedler and Kercher 2004). Disturbance (Eschtruth and Battles 2009), propragule pressure (Lockwood et al. 2005), and nutrient enrichment (Davis et al. 2000) have all been shown to increase the colonization and establishment of invasive species, making wetlands very susceptible to invasion. Additionally, many of the species that invade wetlands are clonal species that can form monospecific stands, which reduces diversity and species interactions. Invasive species in temperate wetlands are usually high biomass producers associated with thick litter layers. Because these species usually produce more biomass than native species, they either have higher nitrogen use efficiencies (NUE: the amount of biomass produced per unit N) or are able to access a larger pool of N compared to natives. Organic matter buildup beneath monospecific stands of aggressive wetland invaders, such as *Typha* ×*glauca*, has been documented (Ehrenfeld 2003; Angeloni et al. 2006), though the pattern across different wetland types has not been evaluated. With the prevalence of invasion in temperate wetlands it is important to understand the effect of invasion on wetland processes such as C and N storage and organic matter quality. It is possible that invasion by high biomass invasive species could increase wetland C and N storage and, therefore, could be a positive outcome of invasion.

While there has been some research on the effects of invasive species in wetlands (e.g., Findlay et al. 2002; Angeloni et al. 2006; Liao et al. 2007), few studies have directly characterized the spatial variability in C and N storage and organic matter quality and even fewer have looked at multiple invasive species simultaneously. Additionally, though these species likely have cumulative effects, they can also differ in plant traits, e.g., litter quality, and therefore it is important to understand the differences among invasive species. The broad goal of this chapter is to quantify the consequences of invasive species in temperate

wetlands on C and N storage and organic matter quality. In the first part of this chapter, I quantified the effects of invasive species on plant community composition, litter and soil C and N stocks, and OM quality. In the second part of this chapter, I used two soil assays to determine differences in C and N mineralization rates from soil collected from monospecific stands of the most dominant invasive species.

Objectives, Hypotheses, and Predictions

Objective 1: To determine the cumulative effect of invasive wetland plants on species diversity, C and N storage, and organic matter quality.

Hypothesis 1: Invasion into wetlands by species that can form monospecfic stands reduces species richness and evenness.

Prediction 1.1: There will be a negative relationship between invasive species dominance and Shannon diversity.

Hypothesis 2: Invasive plant species have higher nitrogen-use efficiency (NUE) than native species, leading to lower N content of their biomass and litter.

Prediction 2.1: There will be a positive correlation between the degree of invasion (total biomass of invasive species) and the overall plant community NUE.

Prediction 2.2: Degree of invasion will be positively correlated with organic matter C:N ratios (litter and soil).

Hypothesis 3: Invasive plant species have litter with higher C:N ratios than native species' litter, leading to increased organic matter storage from slower rates of decomposition.

Prediction 3.1: There will be a positive correlation between the degree of invasion and litter C and N stocks, soil C and N stocks, and total ecosystem C and N stocks.

Prediction 3.2: Based on hypothesized higher NUE of invasives, litter, soil, and ecosystem N stocks could either decrease or increase with the degree on invasion. There is uncertainty because while the overall decrease in tissue N content should decrease N stocks, the decreased N content could also lower the quality of the OM and therefore lead to low decomposition rates and N buildup.

Objective 2: For objective 1, I tested the cumulative effects invasive species have on C and N stocks and OM quality, though there was evidence for variation among invasive species in key plant traits that could influence the species-specific magnitude of effect. This variation was examined through the use of two assays to determine differences in C and N mineralization rates from soil collected from monospecific stands of the most dominant invasive species. An additional objective was to be able to link the variation among invasive species to plant traits, such as litter quality.

Hypothesis 4: The quality (C:N ratio) of soil collected within monospecific stands of the most dominant invasive species from the wetland survey will influence C and N

mineralization rates, and the variation in soil quality will mainly be due to litter quality differences among species.

Prediction 4.1: The will be a negative relationship between soil C:N ratios and C and N mineralization rates.

Prediction 4.2: Soil collected from monospecific stands of the species with the most recalcitrant litter (high C:N ratios) will have the highest soil C:N ratios, and therefore the lowest mineralization rates.

Methods

Study sites and sampling for wetland survey

This study was conducted near the W.K. Kellogg Biological Station (KBS) on Michigan State University property in southwestern Michigan, and at Lake Lansing Park (LLP) in central Michigan during peak biomass growth (late June to early August). The region around KBS lies on a glacial landscape and has abundant wetland cover (approx. 10%), with low levels of residential development in a landscape dominated by forests, row-crop agricultural fields, and abandoned fields. The wetlands of Lake Lansing Park are embedded in a mosaic of woodlots and residential areas and are contiguous with the lake system.

In the summer of 2007, 23 wetlands near KBS and one wetland at LLP were sampled (for GPS coordinates of each site see Table 2-1). Wetland sampling sites were chosen to span the diversity of wetlands around KBS that support emergent vegetation including the

focal invasive species, along with an additional depressional wetland at LLP dominated by *Phragmites australis*. Wetlands were classified based on their hydrogeomorphic setting as well as the dominant water source, estimated using dissolved magnesium (Mg^{2+}) in the wetland surface water as an indicator of precipitation versus groundwater dominance; higher Mg^{2+} concentrations indicated higher groundwater contribution to standing water (Stauffer 1985, Whitmire and Hamilton 2008). Categories include depressional (precipitation dominated), intermediate (in between groundwater and precipitation dominance), groundwater dominated, and lakeside. Lakeside wetlands were given a unique classification due to the overriding effect an adjacent lake has on the physical and chemical wetland environment (HGM classification; Brinson 1993). These wetlands were relatively small, usually with a total surface area of less than one hectare. The majority of the study sites had relatively pure stands of *P. australis, Phalaris arundinacea,* and *Typha* spp. (hereafter *Phragmites*, *Phalaris*, *Typha*, respectively), or mixtures of relatively pure stands of these species. The most common native, non-invasive graminoid species at these sites included sedges (*Carex* spp.), rushes (*Juncus* spp.), and spike-rushes (*Eleocharis* spp.). Other common wetland species found at these sites included buttonbush (*Cephalanthus occidentalis*), pickerelweed (*Pontederia cordata*), spatterdock (*Nuphar advena*), and smartweed (*Polygonum* spp.).

A randomly placed linear transect was set up in the emergent vegetation zone at each site. Six 1.0 x 0.5 m (0.5 m^2) quadrats were established along each transect for vegetation, litter, and soil sampling. The quadrats were randomly placed (1-10 m distance between quadrats) along the transect using a random number table. In each quadrat, all

plant species that had developed past the seedling stage were identified and percent cover was estimated for each. Species-specific aboveground biomass was clipped at the soil surface, dried at 65°C for 48 hours, and weighed on a top-loading balance. Litter depth was measured and a known area of litter (629 cm^2) was removed from the soil surface, avoiding mostly decomposed material beneath the relatively fresh litter layer. Litter samples were collected, dried, and weighed in the same manner as biomass samples. Surface soil was sampled to 10 cm depth using a soil corer (246 cm^3) and samples were transported to the lab on ice and frozen until processed. For processing, soils were thawed, sieved (4 mm), sub-sampled for bulk density and gravimetric water content (GWC) determination, and then dried to a constant mass at 80°C.

Carbon and nitrogen analysis

Dried aboveground biomass and litter samples were ground and homogenized using a cyclone sample mill (Udy Corporation, Fort Collins, CO) and dried soil samples were ground using a mortar and pestle. Percent C and N were measured for aboveground biomass, litter, and soil using a Costech Elemental Combustion System 4010 (Costech Analytical Technologies Inc.). Samples were run in duplicate with atropine used as a standard every 10 samples. Subsamples of dry soil from all sites were tested for carbonate minerals and concentrations were found to be negligible. Aboveground biomass C and N stocks were calculated by multiplying each species' tissue %C and %N by its total dry biomass and then summing species-specific C and N stocks for each quadrat. Litter and soil C and N stocks were calculated by multiplying %C or %N by total litter mass. Soil C and N

stocks were calculated from soil %C or %N, soil bulk density, and sampling depth. Total C and N ecosystem stocks were calculated by summing soil, litter, and biomass stocks for each site. Carbon to nitrogen ratios were used as a measure of organic matter (OM) quality. Low C:N ratios indicate high quality OM and high C:N ratios indicate recalcitrant OM.

Plant aboveground tissue %N was used to determine plant- and plot-level nitrogen use efficiencies (NUE). NUE was defined as the amount of biomass produced per unit N (van Ruijuen and Berendse 2005) and was calculated on the plot level by first determining the NUE for each species in each plot (total biomass divided by the tissue N content). Though similar, NUE is not perfectly scaled with biomass C:N ratio because %C varied among species (~ 40 to 50 %C), and, therefore, both can be informative. This is an acceptable way to calculate NUE in this study because all species were collected at peak biomass and were either annual or perennial deciduous growth forms, in which all biomass was produced during a single growing season. For buttonbush (woody habit), the only abundant species with perennial aboveground biomass, leaves, and stems were separated and an NUE estimate was made using leaf tissue. A weighted NUE average was then calculated for each plot and averaged for each site (hereafter called the Site NUE).

Estimation of plant abundance

Percent cover was estimated for every plant species in each of the six 0.5 m^2 quadrats at each site. This percent cover was then assigned to a cover class from 1 to 6 (1: 0-5% cover; 2: 6-25%; 3: 26-50%; 4: 51-75%; 5: 76-95%; 6: 96-100%). Shannon's diversity index was calculated using percent cover data following Seefeldt and McCoy (2003) and was used as a measure of biodiversity (incorporating both species richness and

evenness). The percent of the total biomass from invasive species (referred to hereafter as invasive species dominance), which included the combined biomass of *Phragmites*, *Phalaris*, and *Typha*, was calculated by dividing their combined biomass by the total biomass in each quadrat. Native species dominance was calculated similarly (total native species biomass divided by total biomass) with non-native, non-invasive species excluded from both metrics. *Phalaris* %biomass was calculated by dividing *Phalaris* biomass in a quadrat by total biomass in that quadrat. In some cases (see statistical analysis section) *Phalaris* was analyzed instead of the invasive species category due to its presence at the majority of sites sampled (Table 2-1).

Soil carbon quality assay

To determine dominant species effects on organic matter quality (as aerobic C mineralization rates) in these wetlands, a laboratory incubation was done at two temperatures (7°C and 23°C) using soils collected under monospecific stands (> 90% cover) of the three most dominant species sampled during the survey study. Monospecific stands were used because species effects on soil C quality are more likely to be detected when only one species is present. The two temperatures used represent growing season average temperature (~ 23°C) and non-growing season average temperature (~ 7°C) for southern Michigan. Soils were incubated at two temperatures so the Q_{10} for each species-specific soil could be calculated. The temperature coefficient, Q_{10}, is a metric that describes the rate of change in a biological or chemical system with an increase of 10°C. This allowed a comparison to be made of temperature effects on C mineralization rates due

to dominant species. Q_{10} was calculated with the following equation: Q_{10} = $(R_2/R_1)^{10/(T_2-T_1)}$, where R_1 is the C mineralization rate at low temperature, R_2 is the C mineralization rate at high temperature, T_1 is the low temperature and T_2 is the high temperature in degrees Celsius (Solondz et al. 2008).

Eleven wetland sites were sampled for the incubations: 4 *Typha* dominated, 4 *Phalaris* dominated, 2 *Phragmites* dominated and 1 *Carex lacustris* dominated. For a site to be considered dominated by a specific species, that species had to consist of > 90% of the total biomass. Because the aggressive genotype of *Phragmites* is a recent invader to inland Michigan (Hamilton, personal communication), only two sites from the 24 that were surveyed could be classified as a monospecific stand. Additionally, due to low native species abundance, only one *C. lacustris* dominated area was included in this incubation. Because of the absence of replication at the site level, *C. lacustris* was left out of statistical analyses but is placed in some figures as a native reference. Soil samples were collected in February 2007 at the end of the growing season. Five soil cores (5 cm depth) were collected at each site using a linear transect similar to the design described above (11 sites x 5 cores = 55 cores). These soil cores were sealed in whirl-pak plastic bags (Nasco), immediately placed on ice, and transported to the laboratory where they were kept frozen (-20°C) until the start of the incubation.

Prior to the start of the incubation, soils were thawed at room temperature for 24 hours and hand sieved to remove rocks and large roots. Each soil core was then sub-sampled for bulk density and GWC determination. The dry mass of the sub-sample was measured by oven drying at 80°C for 48 hours. Dry mass values were used in bulk density

and GWC calculations. Dried soils were then ground and homogenized using a mortal and pestle and run on an elemental combustion system (Costech ECS 4010, Valencia, CA) for %C and %N analysis. Samples were run in duplicate with atropine used as a standard every 10 samples. The remaining wet soil was then split into 2 subsamples of approximately 10 g and placed into a 250 mL incubation jar (Chromatographic Specialties, Inc.; Ontario, Canada). These incubation samples were equilibrated for 7 days at 4°C to allow the C mineralization pulse from root death to pass (Weintraub and Schimel 2003). Duplicate jars were then randomly assigned a temperature treatment, flushed with ambient air (~ 400 ppmv CO_2), and loosely sealed with lids fitted with septa to allow room air to enter the jar until the start of a CO_2 extraction round (see below). The two temperature treatments consisted of high temperature (room temperature, ~ 23°C) and low temperature (cold room, ~ 7°C). All jars (110 total; 4 vegetation classes x 1, 2, or 4 replicate sites x 5 replicate cores per site x 2 temperatures) were incubated in the dark for 36 days. Similar soil moisture conditions were created across all incubation samples by bringing each to a comparable moisture content using distilled water before the beginning of the experiment (40-60% representing ideal non-limiting conditions). Moisture content was maintained weekly over the course of the incubation by adding an appropriate volume of distilled water after each jar was weighed to determine water loss.

Carbon mineralization rates were estimated for six 24-hour periods over the 36-day experiment, on days 1, 3, 8, 15, 22, and 36. During each period, a 10 mL headspace gas sample was taken from each jar immediately after jars were sealed (time = 0) using a three-way stopcock-fitted syringe, and then every 12 hours over the 24 hour period, except for

the first round, which was 48 hours. Before gas samples were extracted, each jar was shaken by hand to mix soil pore spaces and to release trapped gas bubbles. After headspace samples were collected, jars were injected with 10 mL of N_2 gas to ensure constant air pressure and volume. After each round, lids were removed, samples were gently flushed with ambient air, and jars were wrapped with plastic wrap to prevent moisture loss.

Each 10 mL headspace sample was analyzed for CO_2 partial pressure by injecting 5 mL of sample into a PP-system EGM-4 infrared gas analyzer (IRGA). The IRGA was standardized using a CO_2 standard gas after every 10 injections. Carbon dioxide concentrations were corrected for headspace dilution caused by the added N_2 gas. The slope of CO_2 concentrations over the incubation period was used to calculate overall C mineralization rates. These overall mineralization rates were divided by soil dry weight (g) to report data on a mass basis (umol CO_2 g^{-1} day^{-1}) and by total soil C (SC) to report data on a SC basis (umol CO_2 g^{-1} SC day^{-1}). Slopes were discarded if R^2 values were less than 0.75, which comprised less than 2% of the slopes.

N mineralization and nitrification assay

To determine rates of net N mineralization and nitrification in these wetlands, an aerobic laboratory incubation was conducted using soils collected under monospecific stands (> 90% cover) of *Phragmites*, *Phalaris*, and *Typha*. Eight wetland sites were used in all: 3 *Typha* dominated, 3 *Phalaris* dominated, and 2 *Phragmites* dominated, and were a

subset of the wetlands used for the C quality incubation. Soil samples were collected at the end of the 2008 growing season. Three soil cores (10 cm depth) were collected at each site from permanent plots that were established during the summer of 2008 as part of a larger research project (8 sites x 3 cores = 24 cores). These soil cores were sealed in whirl-paks (Nasco, Fort Atkinson, WI), immediately placed on ice, and transported to the laboratory where they were kept at 4°C for 2 days until the start of the incubation to reduce microbial activity.

Soil samples for assays of N mineralization and nitrification were processed similarly to the C mineralization soil samples. After sieving, 10 g sub-samples were taken from the remaining wet soil: one sub-sample was used to determine initial NO_3^- and NH_4^+ concentrations by the KCl extraction method (Robertson 1999), while the other was placed in a 100 ml plastic container. After 30 days of aerobic incubation at room temperature, the KCl extraction method was used to determine final NO_3^- and NH_4^+ concentrations. All extracts were analyzed by the microplate method for nitrate and ammonium using protocols developed by Dr. David Rothstein (Dept. of Forestry, MSU). Net rates of N mineralization and nitrification were calculated from the changes in inorganic N (NH_4^+ + NO_3^-) or NO_3^-, respectively, during the incubation period (initial – final pool sizes). Similar soil moisture conditions were created across all incubation samples by the same method used in the C quality incubation. N mineralization and nitrification rates were calculated on a soil mass and soil carbon basis similar to the C quality incubation.

Statistical analyses

Wetland survey

Because the majority of wetlands sampled were invaded by *Phalaris*, *Phragmites*, and/or *Typha* (23 out of 24; Table 2-1) simple linear regression (SLR) analysis was used to determine the relationship between site Shannon diversity index and invasive species dominance (%biomass of total community biomass) (Hypothesis 1). To address hypothesis 2, another SLR was performed to determine the relationship between vegetation NUE and invasive species dominance. For Shannon diversity and NUE SLRs, all 24 wetlands were included; for the other analyses only 20 wetlands were used due to incomplete datasets from 4 wetlands.

To address hypothesis 3, general linear models were constructed to determine the controlling factors for biomass, litter, soil, and total ecosystem C and N stocks. To explore species controls on biomass C and N stocks, I constructed general linear models using species composition data (invasive species and native species dominance or biomass) as predictor variables. Invasive and native species dominance (percent of plant community) was used instead of invasive and native biomass for biomass C stocks because there is an allometric relationship between biomass C and total biomass, therefore these variables would be auto-correlated. This allometric relationship does not necessarily exist for biomass N stock because species' NUE can influence the amount of biomass %N present. For this reason invasive species biomass was used to predict biomass N stocks. For litter C and N stocks, species composition data and aboveground biomass chemistry (%C, %N, and C:N ratio) were used as predictor variables. After no significance was found for any plant community predictor variable, *Phalaris* abundance was included as a predictor variable in

place of invasive species dominance due to its presence at most sites (Table 2-1). To determine species effects on soil C and N stocks and soil C:N ratios, general linear models were constructed that included plant community composition, litter chemistry (%N, %C, and C:N ratio), and litter quantity (mass and bulk density) as predictor variables. Finally, for total ecosystem C and N stocks, general linear models were constructed using plant community data as predictor variables. No lower level categories (soil, litter, or biomass characteristics) could be included as predictors because those data were used to calculate related stocks, and thus would be auto-correlated with total ecosystem stocks.

For all general linear models, site ID was included in the model as a random factor, treating the design as nested (quadrats nested within site). Model selection procedures based on Akaike's information criterion (AIC) values were used following Zuur et al. (2009). Briefly, the most complex biologically meaningful model (i.e., the most complex model constructed from non-autocorrelated terms that hypothetically could influence the predictor variable) was compared to models of decreasing complexity based on individual model AIC values. The best-fit model was the one with the lowest AIC value and highest Akaike weights (w_i), which describe the weight of evidence for one model compared to the other models. Once the best model was selected a tTable (R code) data frame was constructed to determine individual variable significance. Here I mainly report results for the final "best-fit" model. Full model selection results can be found in Appendix A. Wetland classification (see above) was also included as a predictor variable to the best-fit model determine if its inclusion would improve the fit of the model, therefore minimizing the possible confounding effect of wetland class on C and N stocks (for instance, if depressional wetlands have high OM accumulation independent of plant community, but

also were highly invaded). In all cases adding wetland classification improved the fit of the model (i.e., it lowered the model AIC), but in no case was wetland classification significant and is therefore left out of the results section. Model diagnostics were assessed for the best-fit model to determine if the residuals were normally distributed and displayed constant error variances. Log transformations were used to correct for any heteroscedasticity. All statistical tests were performed in R (version 2.11.1, 2010).

Assays of C quality and N transformations

The general goals for the statistical analysis of the C quality assays were twofold: (1) to determine treatment (vegetation type and temperature) effects on C mineralization rates and (2) to explore the relationships between C mineralization and organic matter quality (C:N ratio) (hypothesis 4). To investigate treatment effects on C mineralization rates, I used a repeated measures analysis of variance (ANOVA) and Tukey's HSD multiple comparison tests were used to determine vegetation and temperature effects, as well as their interaction on C mineralization rates. For the ANOVA, soil core ID was nested within site and included as a random effect. I used linear regression models to examine the effects of soil C:N ratios on C mineralization rates. Species effects on Q_{10} values, litter and soil %C, %N, and C:N ratios and N transformation rates were determined using general linear models. All statistical tests were performed in R (version 2.11.1, 2010).

Table 2-1. GPS coordinates of the 24 survey sites with their hydrological classification, which is based on magnesium concentrations (see methods for more details), and the presence or absence of the most dominant invasive species. *Phalaris* = *Phalaris arundinacea*, *Typha* = *Typha* x*glauca*, *Phragmites* = *Phragmites australis*.

Site	Classification	*Phalaris*	*Typha*	*Phragmites*	GPS Coordinates
LLP	precipitation			X	42° 46.117N, 085° 23.533W
LS	precipitation	X			42° 24.228N, 085° 23.087W
LTER	precipitation	X			42° 24.794N, 085° 22.455W
NLCL	lake	X	X		42° 28.287N, 085° 27.128W
NLCLP	lake		X	X	42° 28.254N, 085° 27.782W
P1	precipitation	X			42° 29.352N, 085° 27.100W
P11	precipitation	X			42° 28.339N, 085° 27.696W
P18	intermediate	X	X		42° 28.733N, 085° 27.553W
P26N	intermediate	X	X		42° 28.689N, 085° 27.768W
P26S	intermediate	X	X		42° 28.689N, 085° 27.768W
P3	precipitation	X			42° 29.341N, 085° 28.424W
P5	intermediate	X			42° 28.518N, 085° 27.882W
P8	intermediate	X			42° 28.274N, 085° 27.797W
P9	precipitation	X			42° 28.267N, 085° 27.726W
Parker	lake	X			42° 28.926N, 085° 27.753W
SMCL	lake		X	X	42° 28.737N, 085° 27.547W
SP7	intermediate	X	X		42° 28.396N, 085° 27.862W
SWMCL	lake			X	42° 28.965N, 085° 27.861W
SWP7	intermediate	X	X		42° 28.285N, 085° 28.005W
TM	groundwater	X			42° 24.497N, 085° 24.455W
TM2	groundwater	X	X		42° 24.497N, 085° 24.455W
WMCL	lake		X		42° 28.965N, 085° 27.861W
EF1	lake		X		42° 21.414N, 085° 21.939W
LOP	groundwater				42° 22.101N, 085° 21.656W

Results

Wetland survey

Twenty-three of the 24 wetlands surveyed were invaded by at least one of the three focal invasive species (*Phragmites*, *Phalaris*, and *Typha*). As invasive species dominance increased, Shannon Diversity decreased (Figure 2-1; adjusted $R^2 = 0.75$, $p < 0.001$). A marginally significant positive relationship was found between site nutrient use efficiency (NUE) and invasive species biomass (Figure 2-2; adjusted $R^2 = 0.10$, $p = 0.08$), though this relationship was mainly due to one site, which was a monospecific stand of *Phragmites* that produced a lot of biomass and had a high NUE. After excluding this site, there was no relationship between NUE and invasive species biomass. Additionally, there was a significant positive relationship between total biomass and invasive species biomass (Figure 2-3; adjusted $R^2 = 0.61$, $p < 0.001$).

The model that included both invasive species and native species dominance was the best for predicting biomass C stock (Table 2-2). This model had an Akaike weight (w_i) of 1.00, giving strong support for this model, while there was little support for the null model (intercept only) ($w_i < 0.001$). Both invasive and native species biomass had a positive effect on biomass C stocks, though neither was significant. For biomass N stock, the model containing only total biomass was the best fit ($w_i = 0.99$). In this model, total biomass had a significant positive relationship with biomass N stock (Table 2-2), and similar to biomass C stock, the null model had very little explanatory power ($w_i < 0.001$).

The most parsimonious model for litter C stock contained biomass %C, %N, and C:N ratio as predictor variables (Table 2-3; w_i = 0.48). The model containing *Phalaris arundinacea* biomass and biomass %C, %N, and C:N ratio had an Akaike weight of 0.29 and a ΔAIC value of less than 2, giving support for both models, though there is more support for the model that excluded *P. arundinacea* biomass (based on a higher Akaike weight). In the best-fit model, biomass %N and C:N ratio had a positive effect on litter C stock, while biomass %C had a negative effect, though no variable in the best-fit model was significant (Table 2-3). There was little support for any of the candidate models having a greater fit than the null model for litter N stock. The best-fit model contained biomass %N (Table 2-3; w_i = 0.61) but was less than 2 ΔAIC from the null model, giving little evidence for this model fitting the data in a meaningful way. In the best-fit model, biomass %N had a positive effect on litter N stocks. The most parsimonious model for predicting litter C:N ratios contained invasive species biomass and biomass %N (Table 2-3; w_i = 0.45). In the best-fit model, biomass %N had a significant negative relationship with litter C:N ratios (Figure 2-4), meaning that as biomass %N increased, litter C:N ratio decreased, showing the tight link between standing biomass nutrient concentrations and litter quality. Invasive species biomass had a positive relationship with litter C:N ratio, though this relationship was not significant.

For soil C stock, the full model, which included invasive biomass, native biomass, litter mass, litter %C and % N, and C:N ratio (Table 2-4; w_i = 0.68), was more than three times more plausible than the next best approximating model. In the full model, invasive species biomass had a significant positive relationship and with soil C stocks (Figure 2-5).

Also, litter mass had a marginally significant negative relationship with soil C stock. In the best-fit model, native species biomass, litter %C, %N, and C:N ratio all had a positive effect on soil C stocks, though they were not statistically significant. The candidate model containing native species biomass, litter mass, litter %C and %N, and C:N ratio was the best fit model for predicting soil N stocks (Table 2-4; w_i = 0.72). In this model, native species biomass and litter mass both had a significant negative effect on soil N stocks, with litter mass having a slightly larger effect. Though not significant, litter %C and C:N ratio had a negative effect on soil N stocks, while litter %N had a positive effect on soil N stock. For soil quality (C:N ratio), the model that contained litter %N as the only predictor variable was the best-fit model (Table 2-4; w_i = 0.76). In the best-fit model, litter %N had a significant positive influence on soil C:N ratios, meaning as the litter N content increased so did soil C:N ratios.

Of the four candidate models predicting ecosystem C stock, the most parsimonious contained both invasive species and native species biomass (Table 2-5; w_i = 0.74). For this model, invasive species biomass had a significant positive relationship with ecosystem C stock (Figure 2-6). For ecosystem N stock, the candidate model containing only native species biomass was the most approximating (Table 2-5; w_i = 0.51), though the null model had a ΔAIC less than 2 giving support to both models. For the best-fit model, native species biomass had a significant negative relationship with ecosystem N stock, meaning that as native species biomass increased, ecosystem N stocks decreased, which is similar to the relationship native species biomass had on soil N stocks.

Soil carbon quality assay

Carbon mineralization (C-min) rates were fairly constant over the duration of the 36-day incubation for most treatment combinations, except for the *Phalaris* soil at high temperature, which decreased over the first 15 days but then stabilized throughout the end of the incubation (Figure 2-7; significant three-way interaction, $p = 0.001$; Table 2-6). *Phalaris* soil at high temperature also had the greatest C-min rates over the duration of the experiment. At high temperature, *Typha* soil C-min rates were significantly less than *Phalaris*, but greater than *Phragmites*, suggesting that *Phalaris* soil was the most labile and *Phragmites* was the most recalcitrant. Carbon mineralization rate of *Phragmites* soil at high temperature was not statistically different from *Phalaris* soil at low temperature. The same rank order of soil lability was found at low temperature and high temperature: *Phalaris* > *Typha* > *Phragmites* (significant species main effect, $p = 0.03$; Table 2-6). As expected, carbon mineralization at high temperature was greater than low temperature among all species (significant temperature main effect, $p < 0.001$; Table 2-6).

Cumulative C-min showed a pattern similar to C-min rates: *Phalaris* soil had the greatest cumulative C-min, followed next by *Typha* soil, and then finally *Phragmites* soil, and this pattern was the same at both temperatures, though high temperatures led to higher cumulative C-min than low temperatures (Figure 2-8; significant main effects of species and temperature, $p = 0.03$ and $p < 0.001$, respectively; Table 2-7). Similar to C-min rates, cumulative C-min results suggest that *Phalaris* soil is more labile than either *Typha* or *Phragmites* soil, with the latter being the least labile.

There were significant species effects on pre-incubation soil %C, %N, and C:N ratio (Table 2-8). *Typha* and *Phragmites* soil had highest soil %C, while *Phalaris* had the lowest

(significant species effect, $p < 0.05$). Soil %N followed a similar pattern with *Typha* and *Phragmites* having the greatest soil %N and *Phalaris* the least (significant species effect, $p = 0.05$). Although not significant, *Typha* did have a greater amount of soil %N than *Phragmites*, which is reflected in their difference in soil C:N ratio. Soil C:N ratio was the highest in *Phragmites* soil and lowest in *Typha* and *Phalaris* soil (significant species effect, $p = 0.01$). *Phalaris arundinacea* soil C:N ratio showed the most variation and was not statistically different from *Typha* soil.

To determine if litter chemistry followed a similar pattern as soil chemistry, litter %C, %N, and C:N ratios were analyzed for species effects (Table 2-8). There was no difference in litter %C among species, but *Phalaris* had the highest litter %N compared to *Typha* and *Phragmites* litter (marginally significant species effect, $p = 0.08$). Litter C:N ratio followed a similar pattern as soil C:N ratio: *Phragmites* litter had the highest litter C:N ratio and *Phalaris* had the lowest litter C:N ratio (significant species effect, $p = 0.03$).

To determine possible controls on cumulative C-min, linear and nonlinear regression was used to verify the predicted influence soil C:N ratio (quality) has on decomposition rates. As predicted, there was a negative relationship between cumulative C-min at high (Figure 2-9; adjusted $R^2 = 0.53$, $p < 0.001$) and low (Figure 2-9; adjusted $R^2 = 0.40$, $p < 0.001$) temperatures and soil C:N ratio. Soil C:N ratio was a better predictor of cumulative C-min than soil %C (high temperature: adjusted $R^2 = 0.28$; low temperature: adjusted $R^2 = 0.24$) or %N (high temperature: adjusted $R^2 = 0.17$; low temperature: adjusted $R^2 = 0.12$).

As discussed in the methods section, Q_{10} is a metric that describes the rate of change in a biological or chemical system with an increase of 10°C, and using Q_{10} allows a comparison to be made of temperature effects on C-min rates due to species. Though there was a pattern to the effect species had on Q_{10}, with *Typha* soil having the lowest Q_{10} and *Phragmites* and *Phalaris* soil having almost equal Q_{10}, this was only marginally significant (Figure 2-10; $F_{2,8} = 3.46$, $p = 0.083$). These results suggest that *Typha* soil is more resistant to the effects of temperature change than the other species' soil.

N mineralization and nitrification assay

Potential N mineralization and nitrification rates were almost identical because the majority of the ammonium mineralized was nitrified to nitrate. Nitrogen mineralization rates were greatest in *Phalaris* soil and the least in *Typha* soil (Figure 2-11; significant species effect, $F_{2,5} = 20.93$, $p = 0.003$). Nitrification followed a similar pattern, though slightly less so (marginally significant species effect, $p = 0.06$). The low N mineralization and nitrification rates of *Phragmites* soil is in agreement with the C-min results that suggested *Phragmites* soil is more recalcitrant than soil collected from other invasive species monospecific stands. Though there were no significant differences in soil C:N ratios among the three soils collected for this incubation ($p = 0.46$), there were significant differences among species for soil %C and %N (significant species effect, both $p < 0.01$; Table 2-9). These differences were mainly caused by *Phragmites* soil having greater %C and %N, though *Typha* soil did have slightly greater %C than *Phalaris* soil. Soil C:N ratio

did not have a significant relationship with N mineralization rates (adjusted R^2 = 0.04, p = 0.17).

Table 2-2. Summary of the best-fit model for biomass C and N stocks. Dominance refers to the proportion of the total biomass from invasive or native species. Terms in bold indicate significant predictors. Df (Den) indicates the denominator degrees of freedom.

Biomass C Stock

Term	estimate	t-value	Df (Den)	P-value
Invasive Species Dominance	475.18	0.49	97	0.627
Native Species Dominance	444.39	0.45	97	0.650

Biomass N Stock

Term	estimate	t-value	Df (Den)	P-value
Total Biomass	0.02	11.65	98	< 0.001

Table 2-3. Summary of the best-fit model for litter C and N stocks and litter C:N ratios. Terms in bold indicate significant predictors. Df (Den) indicates the denominator degrees of freedom.

Litter C Stock

Term	estimate	t-value	Df (Den)	P-value
Biomass %N	72.29	0.55	96	0.585
Biomass %C	-2.50	-0.48	96	0.631
Biomass C:N	0.57	0.18	96	0.868

Litter N Stock

Term	estimate	t-value	Df (Den)	P-value
Biomass %N	0.92	0.43	98	0.667

Litter Quality (C:N Ratio)

Term	estimate	t-value	Df (Den)	P-value
Invasive Species Biomass	4.70	1.06	97	0.292
Biomass %N	-15.97	-3.6	97	< 0.001

Table 2-4. Summary of the best-fit model for soil C and N stocks and soil C:N ratios. Terms in bold indicate significant predictors. Df (Den) indicates the denominator degrees of freedom.

Soil C Stock

Term	estimate	t-value	Df (Den)	P-value
Invasive Species Biomass	2.43	2.02	93	0.046
Native Species Biomass	2.04	0.81	93	0.423
Litter Mass	-0.97	-1.98	93	0.051
Litter %C	39.55	0.96	93	0.338
Litter %N	1363.80	1.49	93	0.138
Litter C:N	14.63	0.54	93	0.590

Soil N Stock

Term	estimate	t-value	Df (Den)	P-value
Native Species Biomass	-0.48	-2.29	92	0.024
Litter Mass	-0.13	-2.87	92	0.005
Litter %C	-4.37	-1.16	92	0.250
Litter %N	21.80	0.27	92	0.791
Litter C:N	-0.83	-0.34	92	0.734

Soil Quality (C:N Ratio)

Term	estimate	t-value	Df (Den)	P-value
Litter N	1.21	2.27	98	0.025

Table 2-5. Summary of the best-fit model for ecosytem C and N stocks. Terms in bold indicate significant predictors. Df (Den) indicates the denominator degrees of freedom.

Ecosystem C Stock

Term	estimate	t-value	Df (Den)	P-value
Invasive Species Biomass	2.91	2.34	97	0.021
Native Species Biomass	2.74	1.04	97	0.297

Ecosystem N Stock

Term	estimate	t-value	Df (Den)	P-value
Native Species Biomass	-0.43	-2.05	98	0.043

Table 2-6. Results of repeated measures ANOVA for the effect of Species, Temperature and Time on C mineralization throughout the 36 day incubation.

Between Subjects

Source	num df	den df	F	P-value
Species	2	8	5.29	0.0340
Temperature	1	47	423.02	<0.0001
Species x Temperature	2	47	8.32	0.0008

Within Subjects

Source	num df	den df	F	P-value
Time	1	494	1.26	0.2620
Time x Species	2	494	4.89	0.0079
Time x Temperature	1	494	3.16	0.0763
Time x Species x Temp.	2	494	7.00	0.0010

Table 2-7. Summary of ANOVA examining the effect of Species and Temperature on cumulative C mineralization.

Source	df	den df	F	P>F
Species	2	8	5.82	0.0276
Temperature	1	47	1673.30	< 0.0001
S x T	2	47	2.05	0.1398

Table 2-8. Soil and litter %C, %N, and C:N ratio of the four species used in the organic matter quality incubation. Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Values are means ± SE.

Species	Soil %C	Soil %N	Soil C:N	Litter %C	Litter %N	Litter C:N
Phragmites	18.5 ±4.2[a]	1.29 ±0.31[a]	14.3 ±0.5[a]	42.7 ±1.5	1.07 ±0.20[a]	42.0 ±6.9[a]
Phalaris	10.7 ±3.1[b]	0.87 ±0.21[b]	12.0 ±0.8[b]	40.4 ±1.8	1.68 ±0.20[b]	25.5 ±3.4[b]
Typha	18.2 ±2.4[a]	1.56 ±0.22[a]	11.7 ±0.3[b]	40.6 ±3.5	1.23 ±0.15[a]	34.3 ±6.6[c]
Carex	10.5 ±3.2[b]	0.88 ±0.28[b]	12.1 ±0.6[b]	*no data*	*no data*	*no data*

Table 2-9 Soil %C, %N, and C:N ratio of the four species used in the N mineralization/nitrification incubation. Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Values are means ± SE.

Species	Soil %C	Soil %N	Soil C:N
Phragmites	31.7 ±4.9[a]	2.27 ±0.35[a]	14.0 ±0.4
Phalaris	4.3 ±0.8[b]	0.30 ±0.06[b]	14.7 ±0.7
Typha	8.5 ±2.3[c]	0.59 ±0.16[b]	14.7 ±0.7

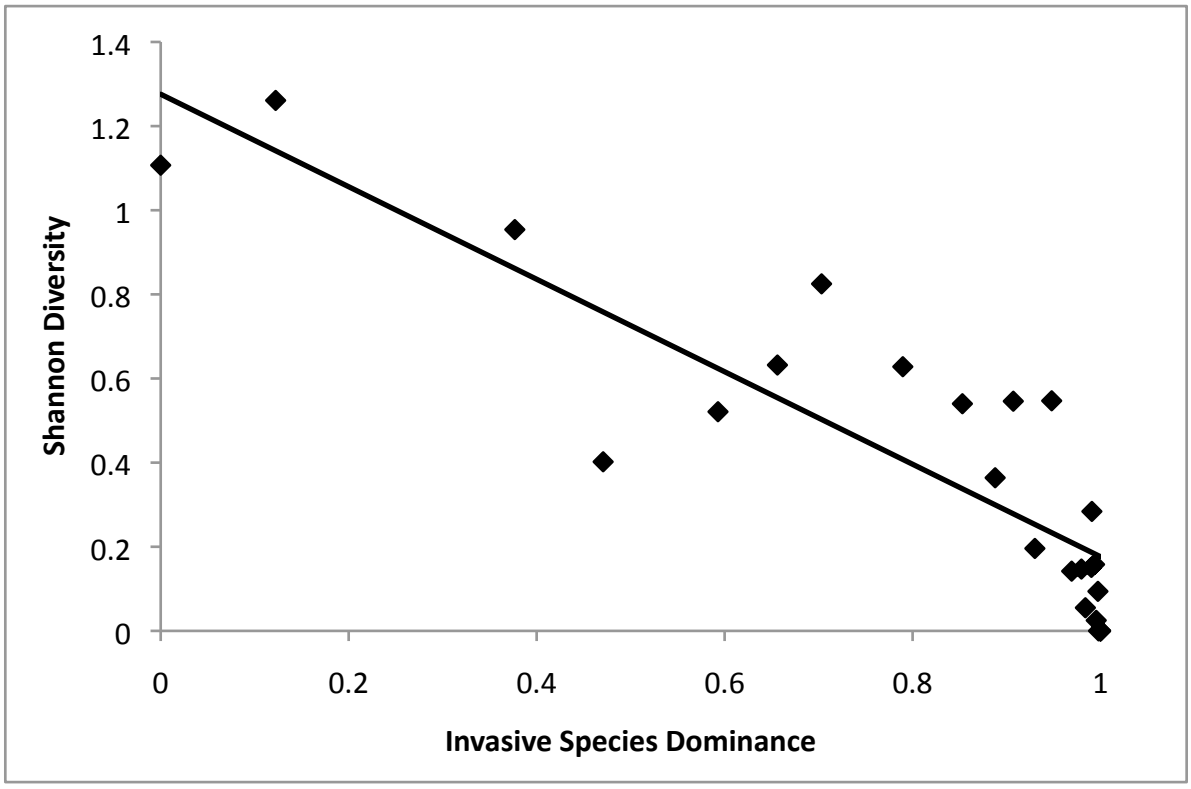

Figure 2-1. Regression between invasive species dominance (percent biomass of community) and Shannon Diversity index (adjusted $R^2 = 0.75$, $p < 0.001$).

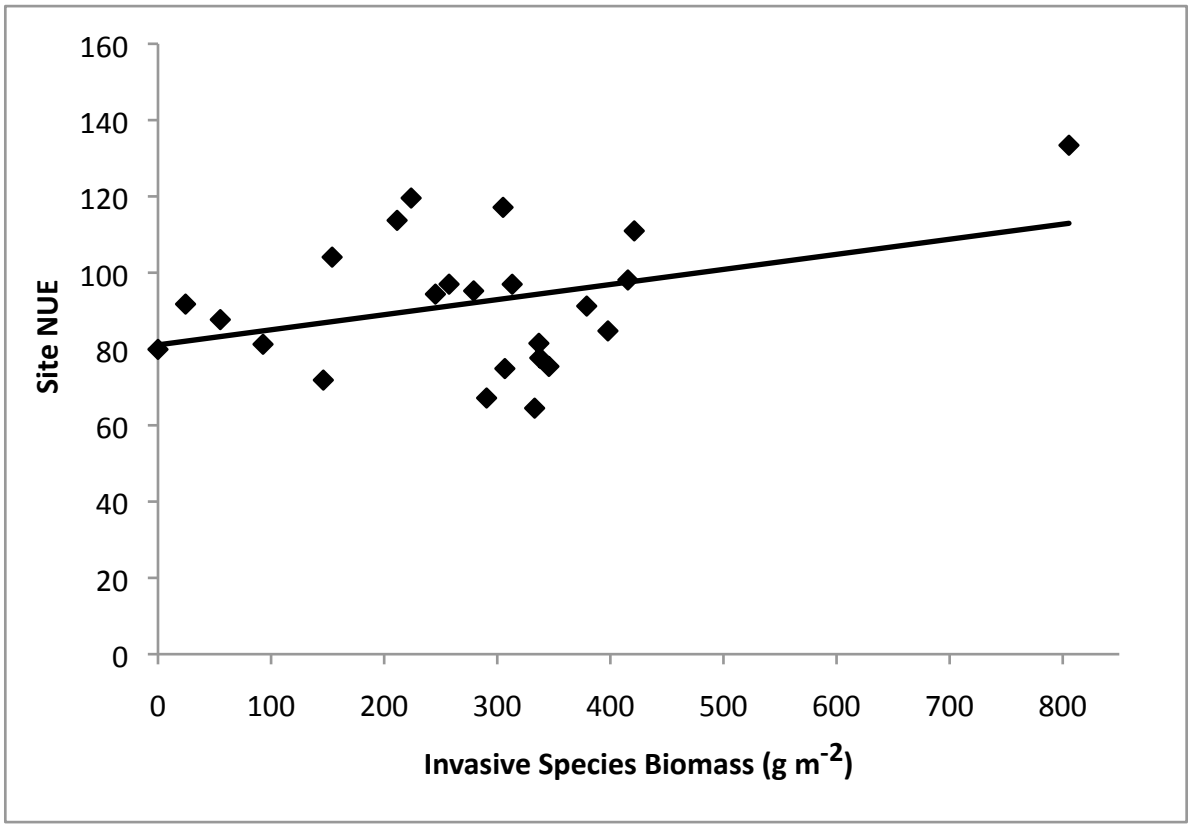

Figure 2-2. Regression between invasive species biomass (cumulative biomass of *Phragmites australis*, *Phalaris arundinacea*, and *Typha* spp.) and site nitrogen use efficiency (adjusted $R^2 = 0.10$, $p = 0.08$).

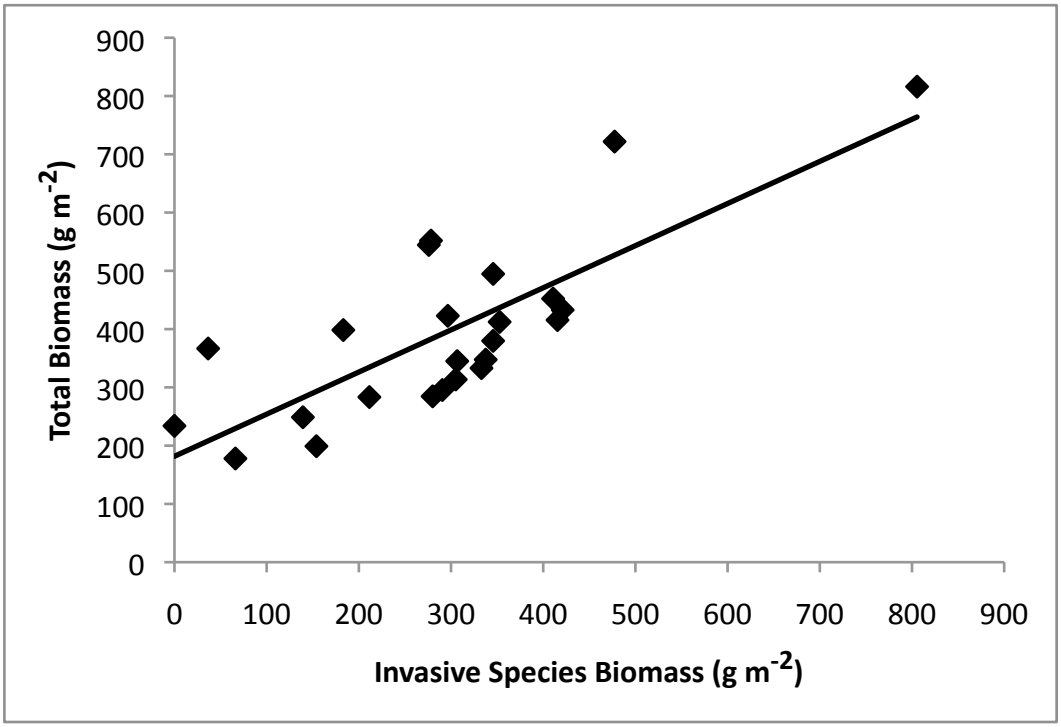

Figure 2-3. Regression between invasive species biomass (cumulative biomass of *Phragmites australis*, *Phalaris arundinacea*, and *Typha* spp.) and total biomass (adjusted $R^2 = 0.61$, $p < 0.001$).

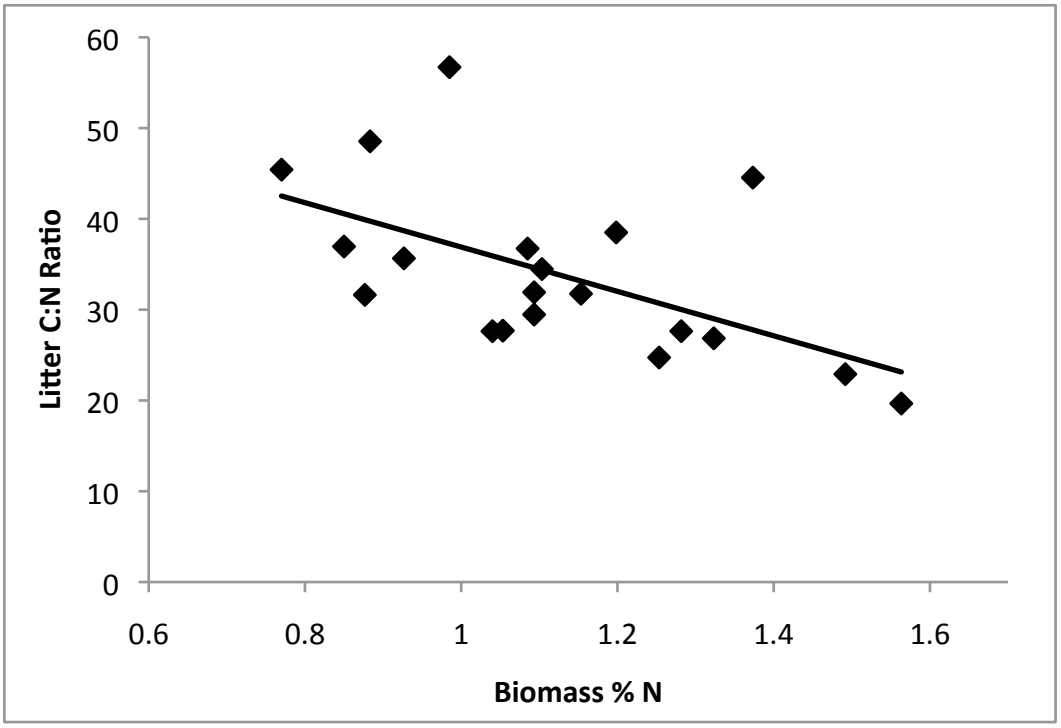

Figure 2-4. Regression between biomass % N and litter C:N ratio (adjusted $R^2 = 0.32$, $p < 0.001$). Biomass %N was a significant predictor in the best-fit model for litter C:N ratios.

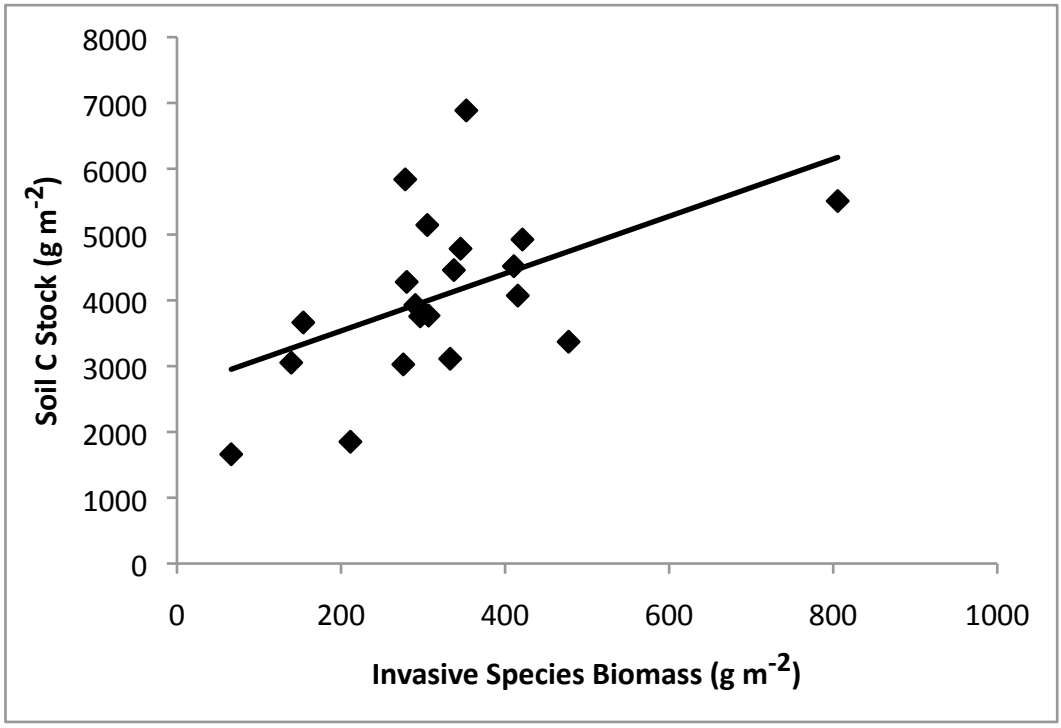

Figure 2-5. Regression between invasive species biomass (cumulative biomass of *Phragmites australis*, *Phalaris arundinacea*, and *Typha* spp.) and soil C stock (adjusted R = 0.27, $p < 0.001$). Invasive species biomass was a significant predictor in the best-fit model for soil C stock.

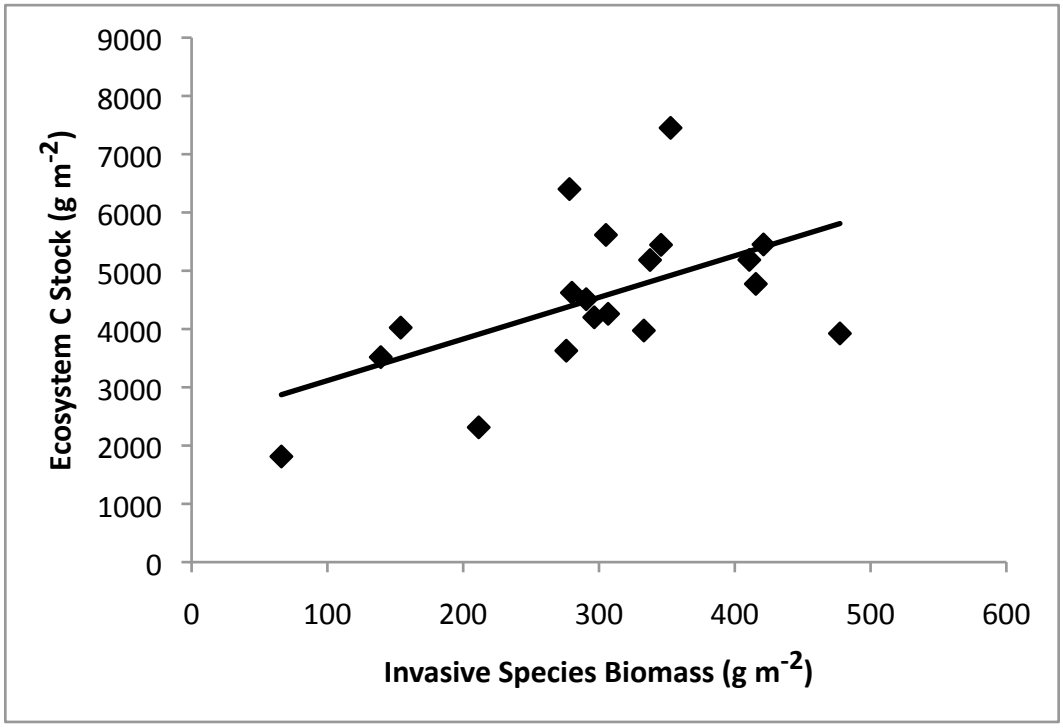

Figure 2-6. Regression between invasive species biomass (cumulative biomass of *Phragmites australis*, *Phalaris arundinacea* and *Typha* spp.) and ecosystem C stock (adjusted $R^2 = 0.35$, $p < 0.001$). Invasive species biomass was a significant predictor in the best-fit model for ecosystem C stock.

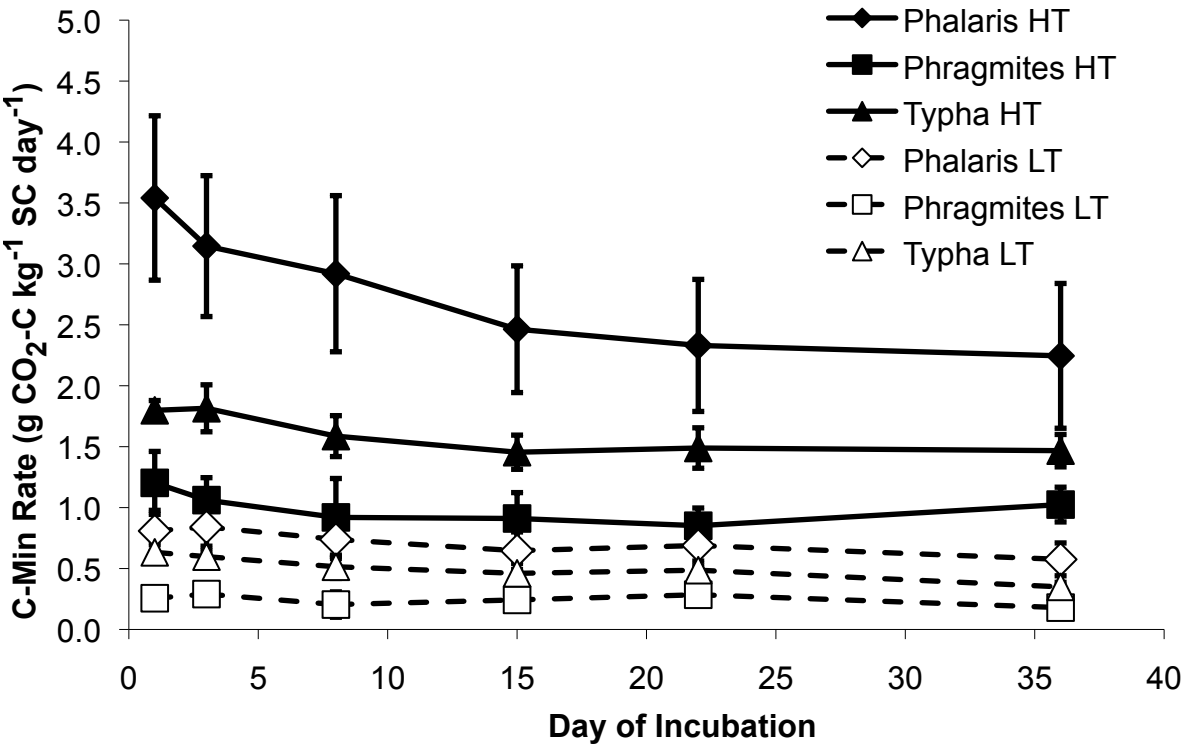

Figure 2-7. Carbon mineralization rates over the 36-day laboratory incubation. Repeated measures ANOVA showed that there was a significant 3-way interaction between species, temperature and time ($F_{2,494} = 7.00$, $p = 0.001$). Error bars represent 1 SE.

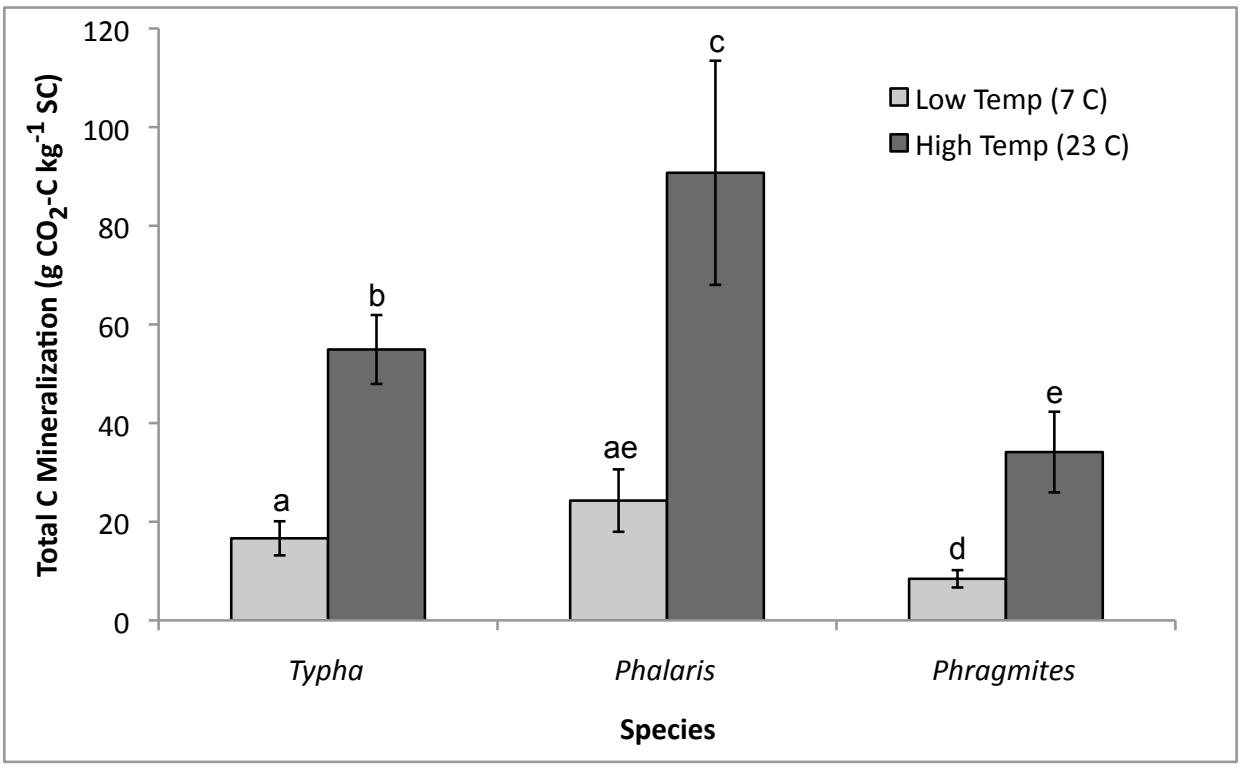

Figure 2-8. Mean cumulative C mineralization over the 36-day laboratory incubation. The main effects of species ($F_{2,8} = 5.82$, $p = 0.028$) and temperature ($F_{2,47} = 1673.30$, $p < 0.001$) were significant, but not their interaction. Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

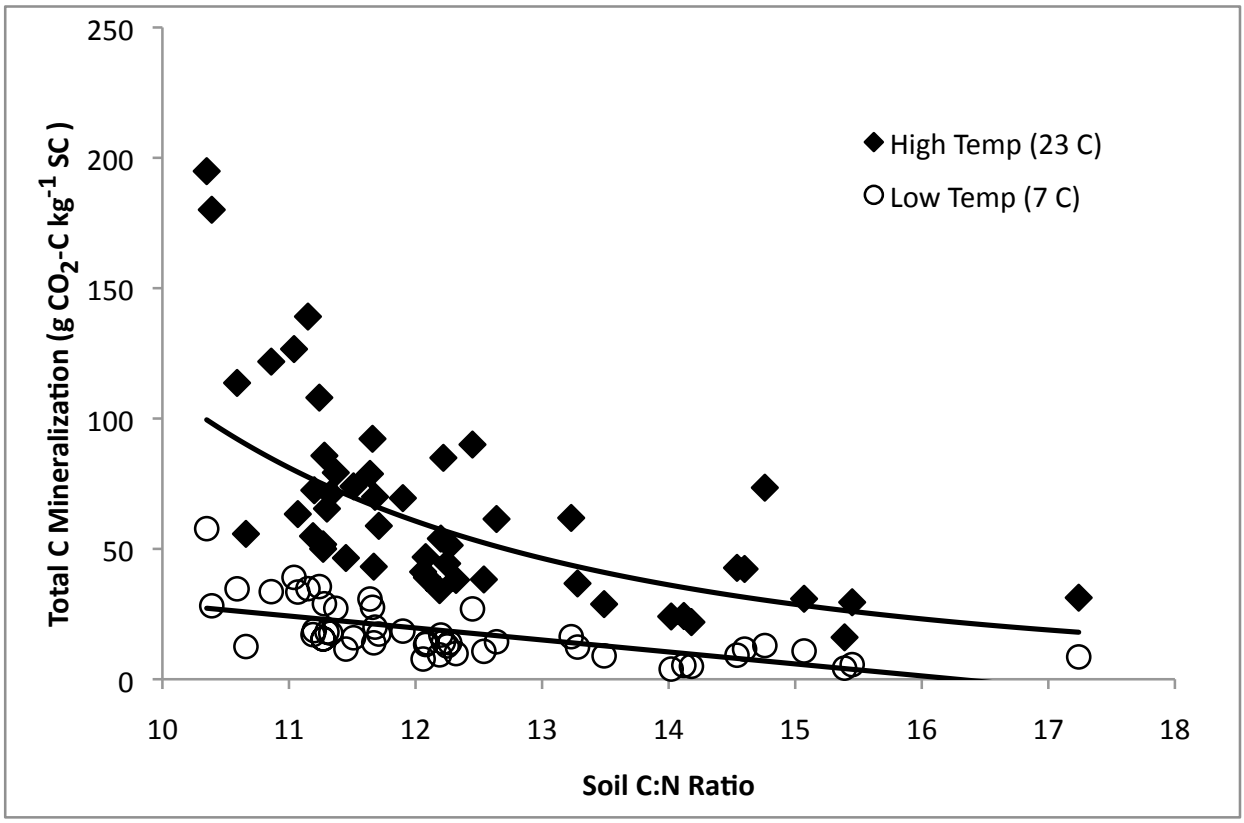

Figure 2-9. Regression between soil C:N ratio and cumulative C mineralization at high (21°C; adjusted $R^2 = 0.53$, $p < 0.001$) and low (7°C; adjusted $R^2 = 0.40$, $p < 0.001$) temperatures.

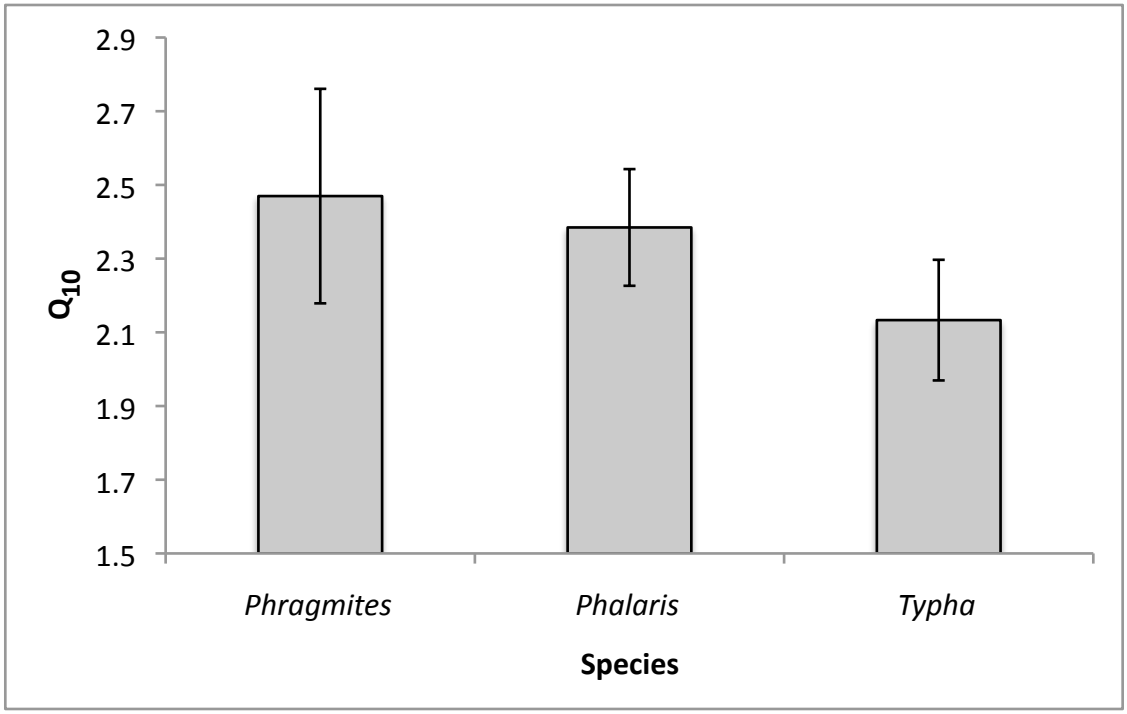

Figure 2-10. Comparison of mean Q_{10} values among soil collected from monospecific stands of *Phragmites australis*, *Phalaris arundinacea*, and *Typha* spp. The effect of species was marginally significant ($F_{2,8} = 3.46$, $p = 0.082$). Error bars represent 1 SE.

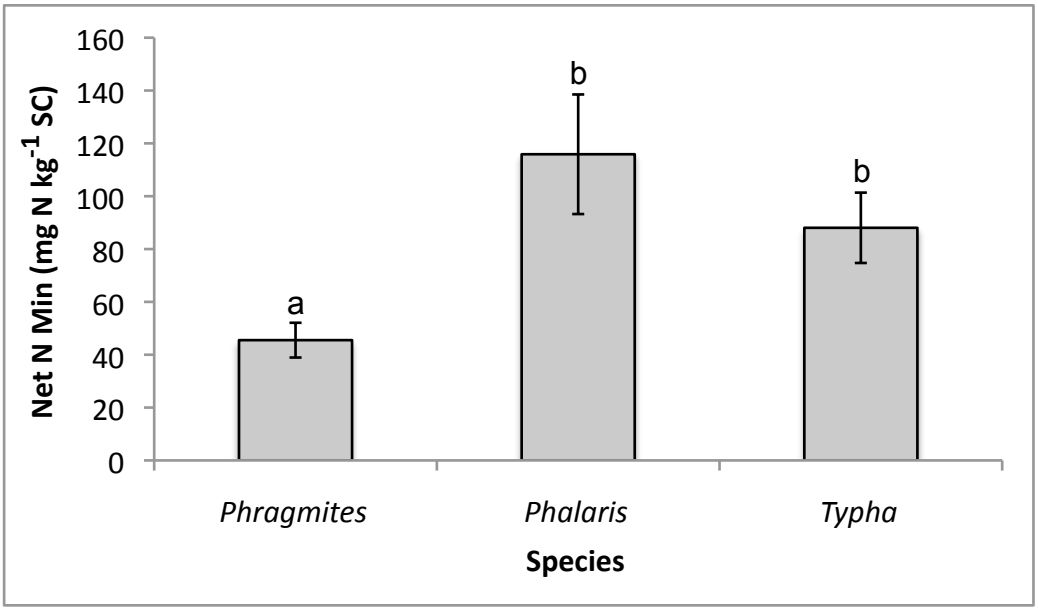

Figure 2-11. Comparison of mean net N mineralization rates among soil collected from monospecific stands of *Phragmites australis*, *Phalaris arundinacea*, and *Typha* spp. The effect of species was significant ($F_{2,5} = 22.32$, $p = 0.003$). Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

Discussion

The negative relationship between invasive species dominance and Shannon diversity is a pattern that has been found in investigations at similar spatial scales (Sakai et al. 2001; Hejda et al. 2009), though the relationship isn't usually as strong as the one found in this wetland survey (Figure 2-1). Conversely, Houlahan and Findley (2004) found little evidence for invasive species having a larger effect than natives on plant diversity in 58 Ontario inland wetlands, though community dominants (native or exotic) did decrease diversity. When invasive plants successfully invade they usually out-compete other plants and either reduce their population and/or cause them to go locally extinct (Sakai et al. 2001; Allendorf 2003). This consequence of invasion usually causes species diversity to decline on a local scale (though on larger spatial scales species diversity can increase because at larger spatial scales those species that are threatened at smaller spatial scales still persist). The strong pattern seen in this study likely is partly due to wetlands being particularly susceptible to invasion due to their hydrogeomorphic placement on the landscape that results in high levels of disturbance, nutrient enrichment, and propagule pressure (Zedler and Kercher 2004). Additionally, wetland invasive species are known to form monospecific stands, which reduce local diversity to an even greater extent.

While I found some support for my prediction that there will be a positive correlation between the degree of invasion and the overall plant community NUE (Figure 2-2), this pattern was driven entirely by LLP (site dominated by *Phragmites*). The lack of a relationship is somewhat surprising because wetland invasive species are known to usually produce greater amounts of biomass than natives (Ehrenfeld 2003; Liao et al. 2008) and, therefore, either have higher NUE or are using a larger N pool than natives. My results suggest the latter and could be connected to the their ability to respond to larger N pools

than natives (Davis et al. 2000) that are more adapted to low N conditions (Daehler 2003), though not always (Funk and Vitousek 2007). While there have been similar studies investigating NUE of invasive species (Funk and Vitousek 2007), this is the first, that I am aware of, that has looked at the NUE of the entire community. Future studies should investigate the N pools used by wetland invasive species compared to native plants to determine if invasive species do use a larger N pool than natives.

The most parsimonious model for biomass C stock included both invasive and native species dominance, though neither had a significant relationship with biomass C stock. The relationship between biomass C stock and invasive species dominance is reasonable because invasive species are usually high biomass producing species (Ehrenfeld 2003; Liao et al. 2008), so the more dominant they are the more C should be in aboveground biomass. The positive influence of native species dominance is harder to explain, but could indicate that species identity (invasive or native) didn't truly matter. Total biomass was the best predictor of biomass N stock suggesting that regardless of community composition (native or invasive), wetlands with more biomass also had more N stored in the aboveground biomass. This is further evidence for invasive species not having a higher NUE than native species because otherwise there would likely not be a relationship between total biomass and biomass N stocks as invasive species constitute the majority of the biomass at most of these sites.

The best-fit models for litter C and N stocks contained tissue chemistry predictors with biomass %N and C:N ratios having a positive effect and biomass %C a negative effect. These individual variables were not significant, however, and for litter N stock there was not a statistical significance between the best-fit model and the null model, so while tissue

chemistry traits likely do influence litter C and N stocks, in this data set it is hard to have confidence in their importance. The most parsimonious model for litter C:N ratio had both native species and biomass %N as predictor variables, though only biomass %N was significant. As mentioned in the results section, the negative relationship found between litter C:N ratio and biomass %N (as biomass %N increases, litter C:N ratio decreases) shows the link between living biomass nutrient concentrations and the litter quality; the living biomass N content influenced the litter by increasing the quality (lowering the C:N ratios) as N content increased. The link between living tissue chemistry and litter quality is a logical relationship but has not been shown before at the community level as in this study, though it has been inferred (Wardle et al. 2004). The lack of invasive species influence on litter C and N stocks and quality refuted predictions 2.2 and 3.1, and showed that, as a whole, invasive species don't differ from natives in their ability to alter litter stocks and quality. This conclusion is supported by Herr-Turoff and Zedler (2005) that found that *Phalaris* invasion did not alter a wetland's ability to retain N. It should be stated, though, that the litter collected in the wetland survey was litter that had accumulated on the soil surface over multiple growing seasons and therefore might not be an indicator of fresh litter quality.

Though the full model was the best-fit model for soil C stocks, only invasive species biomass and litter mass were significant. The positive influence invasive species had on soil C stocks supports hypothesis 3 that as the dominance of invasive species increases so will the organic matter storage of the wetlands they invade (Figure 2-5). This relationship was also seen in a meta-analysis by Liao et al. (2008) that found invasive species increased root C stock by 5% and shoot C stock by 133%. Additionally, He et al. (2011) found that

Phalaris had a greater seasonal C gain compared to the native species *Carex stricta*. The negative influence of litter mass seems counterintuitive as more litter mass should mean more organic matter being incorporated into the soil, but, as mentioned above, the litter collected in the wetland survey was litter that had accumulated on the soil surface over multiple growing seasons. The accumulation of the litter may be due to the recalcitrance of the litter, i.e., lower quality litter resisted decomposition and thus was not added to the soil C stocks. The negative relationship found between litter mass and soil N stocks follows the same logic used for its same relationship found with soil C stocks. As for the negative relationship between native species biomass and soil and ecosystem N stocks, this could be caused by native species having lower detrital N concentrations or could be a function of native species producing less biomass than invasives. Therefore, wetlands that are more dominated by natives produce less biomass, and since biomass N stocks was a function of total biomass in this study, those wetlands might have less organic N inputs (Ehrenfeld 2003).

The positive relationship between litter %N and soil C:N ratios seems counterintuitive if litter N was directly incorporated into soil OM as in Melillo et al. (1989) because one would expect soil C:N ratio to decrease as litter with higher N content was incorporated into the soil. Similar to the effects of litter mass on soil C and N stocks, this relationship could be caused by the litter collected in this study having accumulated over multiple years, so it is possible that having higher litter %N might be indicative of N being locked up in litter and not being incorporated into the soil or N immobilization in the litter (Melillo et al. 1989).

For ecosystem C stocks, invasive species had a positive influence on the C storage within the wetlands surveyed in this study (Figure 2-6). This is in support of hypothesis 3 and suggests that invasive plant species play an important role in the ecosystem functioning of temperate wetlands. Due to the nature of this survey study, the exact mechanisms by which invasive species were affecting wetland C storage is unknown, but there is evidence from the controlling factors of the other C and N stocks in this study that plant traits such as biomass N concentration and litter quality could be important. Since there wasn't an effect of invasive species on litter C and N stocks or quality, invasive species may be influencing soil and ecosystem C stocks by greater belowground biomass production than natives (Liao et al. 2008). Many studies have found that *Phragmites, Phalaris*, and *Typha* spp. produce more belowground biomass (and overall biomass) than natives (Green and Galatowitsch 2001 and 2002; Vymazal and Kropfelova 2005). Understanding how wetlands function changes following invasions is critical if we are to properly manage these nearly ubiquitous invasions, and if there is a positive aspect to invasion, such as increase C storage, then this needs to be considered when determining management for invasive wetland species. More detailed research is needed to determine the degree and mechanism of this apparent relationship.

While the cumulative effect of invasive wetland plant species (*Phragmites*, *Phalaris*, and *Typha*) were shown in this study, their differences in key plant traits, such as litter quality, is important to elucidate as these species likely have unique individual effects on C and N cycling (Eviner 2004). The results of the carbon quality incubation clearly showed that there are differences in C mineralization rates from soil collected within monospecific stands of each of these species (Figure 2-8), which is in support of hypothesis 4. Soil

collected under populations of *Phalaris* had the greatest C mineralization rates, followed by *Typha* and then *Phragmites*. This suggests that the OM in *Phalaris* soil was the most labile of the three species and this lability can be connected to *Phalaris* having the highest quality soil and litter, while *Phragmites* had the lowest quality soil and litter. The difference among these invasive species in litter quality could also explain why there was not a clear relationship between invasive species biomass and litter quality in the wetland survey. The importance of soil quality on decomposition was further illustrated by the strong relationship between soil C:N ratio and total C mineralization, which was stronger at high temperatures than at low temperatures. The relationship between OM quality (C:N ratios) and C mineralization has been shown in other studies (Melillo et al. 1982; Taylor et al. 1989; Xu and Hirata 2005; Parton et al. 2007), and further supports the role soil quality on decomposition. The difference between temperatures is likely caused by greater microbial activity at higher temperatures that probably enhances the controlling effect of the quality of the organic matter.

Though the difference among species in the Q_{10} response was only marginally significantly different (Figure 2-10), because *Typha* soil had the lowest Q_{10} value (2.13) and thus was the most resistant to the effects of temperature change, *Typha* monospecific stands may be less altered by a warming climate, though the difference among species was minimal. The range of Q_{10} values among studies species (2.13 to 2.47) fell within the range of 1.6 to 4.8 found for wetland soils (Nieveen et al. 1998; Bubier et al. 2003; Lafleur et al. 2005) and the Q_{10} for *Phragmites*, 2.47, was very close to what Zhou et al. (2009) found (2.38) for soil collected within monospecific stands of *Phragmites* in Northeast China. In

comparison to other ecosystems, the range I found in the study species was similar to what Bekku et al. (2003) found for temperate (2.9), tropic (2.1) and arctic (3.4) soils, though arctic soils were about 1 Q_{10} greater than *Phragmites* monospecific stands.

Phalaris soil had the highest N mineralization and nitrification rates, which corresponded with *Phalaris* soil having the highest C mineralization rates (Figure 2-11). This showed that *Phalaris* soil was more active in these laboratory incubations compared to *Typha* and *Phragmites*, giving further support to hypothesis 4. N mineralization rates were not correlated with soil C:N ratio and, additionally, soil %C and %N did not match those found for the C quality incubation for two reasons: (1) soil used in the C quality incubation was collected from a 5 cm depth and soil used in the N mineralization incubation was collected from a 10 cm depth and (2) a different monospecific stand of *Phragmites* was used in place of NLCLP that had much greater soil %C. Specifically, using a deeper soil core could have incorporated more mineral material and thus decrease soil %C and %N as seen in comparing the soil from the two incubations (Tables 2-8 and 2-10). Similar to these results, Bridgham et al. (1998) found that N mineralization varied greatly across an ombrotrophic-minerotrophic gradient in northern Minnesota and that the dominant wetland community had strong direct and indirect effects.

The C and N mineralization rates differences among species appear to be a consequence of—or at least correlated with—differences in plant traits, such as litter quality. Though it was useful to look at the cumulative effects of these invasive species, it is just as important to be aware of their species-specific differences to better understand their individual effects. Species differences are known to influence many aspects of ecosystem functioning (Eviner and Chapin 2003; Wardle et al. 2004) and these differences

can be especially important when determining the effects of species, like invasive species, that can dominate large spatial areas, like wetlands, that are “hot spots” for biogeochemical cycling.

Chapter 3
Effects of Aboveground Biomass and Litter on Biogeochemical Cycling in *Phragmites australis* Stands

Brief Rationale

The results from Chapter 2 showed that invasive species have an effect on C and N stocks and organic matter quality, and that these effects likely differ among the three invasive species studied (*Phragmites australis*, *Phalaris arundinacea*, and *Typha* spp.). While only two sites were dominated by *P. australis* (hereafter *Phragmites*) in the wetland survey, those sites had the most biomass and soil organic matter of all sites surveyed. Additionally, species-specific investigation into soil and litter quality showed that soil beneath monospecific stands of *Phragmites* had high soil C:N ratios (low quality) and decomposed more slowly compared to those dominated by *P. arundinacea* and *Typha* spp.. It is therefore critical to investigate, in more detail, the mechanisms by which *Phragmites* alters the wetlands it invades due to its large and expanding distribution in the United States.

Phragmites is a cosmopolitan wetland species found on all continents except Antarctica (Cronk and Fennessy 2001). In some areas of Europe, *Phragmites* is in decline and wetland restoration and management have focused on increasing its presence and spread. In North America, *Phragmites* was only a minor component of wetland vegetation for thousands of years, but is now one of the most aggressive wetland invaders in the United States (Chambers et al. 1999). The spread of the aggressive genotype is attributed to multiple European introductions along the northern Atlantic coast in the 19th century (Plut et al. 2011). Though the exact origin of the invasive genotype is not known, recent evidence suggests the United Kingdom as the most likely source (Plut et al. 2011). This

genotype is salt tolerant and can maintain horizontal clonal growth (up to 4.5 meters per year) allowing it to establish and spread in coastal wetlands displacing native plants, such as *Spartina* spp. Once established on the east coast, the European genotypes quickly spread and are now found in all 48 conterminous states (Chambers et al. 1999). The native genotype is much less aggressive than the exotic, which is now outcompeting native *Phragmites*, as well as most other wetland plants in sites where it becomes established (Plut et al. 2011).

Its capacity for horizontal growth and salt tolerance makes roadside ditches in northern climates, where road deicing salts are used, ideal environments for invasion and spread. *Phragmites* can grow to heights of 4-5 meters allowing it to outcompete most wetland plants for light. Additionally, the standing dead stems of *Phragmites* usually resist decomposition and persist in wetlands for years (Graneli 1990; Schieferstein 1997; Gessner 2001). The large amount of litter produced every year shades the soil surface further restricting competitors' access to light. In addition to the effects *Phragmites* can have to the native plant community (and other trophic levels), it can also impact nutrient cycling. Otto et al. (1999) showed that inorganic N availability was higher in *Phragmites* stands compared to *Lythrum salicaria* and *Typha angustifolia* stands, while Findlay et al. (2002) found detrital N standing stocks were greater in populations of *Phragmites* versus *T. angustifolia*. The presence of *Phragmites* usually leads to organic matter buildup, which can change not only the C storage of the wetland, but the hydrology as well (Able et al. 2003). While the above studies have been able to show effects of *Phragmites* invasion on biogeochemical cycling, few studies have tried to elucidate the mechanisms of change. It has been shown that radial oxygen loss (ROL), the loss of oxygen through the rooting

system of emergent wetland plants, is important for the health and survival of *Phragmites* (Armstrong and Armstrong 2001; Colmer 2003; Soukup et al. 2007), and it is possible that this oxygen release in the rooting zone could have impacts on biogeochemical cycling by creating oxidizing conditions in an otherwise anaerobic environment.

It is important to understand the mechanisms by which *Phragmites* alters the biotic and abiotic environment because then we may be able to predict the consequences of *Phragmites* invasion and possibly invasions by other species with similar traits, as well as to better manage their impact. For example, if it is found that the litter of *Phragmites* significantly reduces light levels at the soil surface, then the practice of killing stands with glyphosate and then leaving the litter standing might not be advantageous because of the negative effects low light levels could have on native recruitment. One way to determine these mechanisms is to manipulate both the litter and biomass in a field experiment and then monitor both the abiotic environment and C and N cycling within manipulated plots.

Objectives, Hypotheses, and Predictions

Objective: To determine the mechanisms by which *Phragmites* influences ecosystem function in the field. *Phragmites* invasion is likely to cause changes to the soil microenvironment (e.g., temperature, redox potential, etc) due to the high production of biomass, with ramifications for soil microbial activity and biogeochemical processes, such as N and C cycling, but also plant available cations and anions. Such changes are important to understand the full impact of *Phragmites* invasion, as well as attempted eradication.

Hypothesis*: Phragmites* exerts both direct and indirect influences on biogeochemistry;

direct effects include plant nutrient uptake into live biomass and indirect effects include changes in the soil microenvironment and in OM matter input quality and quantity. As a result, I predict the following:

- Removal of *Phragmites* litter will increase light levels to the soil surface, which will result in increased diurnal maximum soil temperature, though living biomass should still shade soil. Increased temperature will enhance *in situ* N mineralization and decomposition rates compared to reference plots. Cessation of litter inputs also will decrease OM quality of the soil as the labile OM pool is mineralized and not replenished to the same degree as in the reference plots, though effects on OM quality likely would take more than a few years to become detectable.
- Removal of living biomass will increase light levels to the soil surface, which will result in increased diurnal maximum soil temperature, though the litter layer should still provide partial shading of the soil. Increased temperature should enhance *in situ* N mineralization and decomposition rates compared to reference plots. Reduced N and other plant nutrient uptake by vegetation should increase their soil pore concentration. Additionally, the sediment anoxic zone should increase due to a reduction in radial oxygen loss from rooting system.
- Because the total removal treatment was initiated in 2010, a soil organic matter effect would probably take years to become detectable. This treatment was mainly established to determine light levels and temperature in the absence of both litter and vegetation. The total removal of both the living biomass and litter of *Phragmites* should result in the greatest increase in light levels to the soil surface, which will

result in increased diurnal maximum soil temperature. This increased temperature should enhance *in situ* N mineralization and decomposition rates compared to all other treatments, but the greatest difference should be when compared to reference plots.

Methods

Site description and experimental treatment design

I conducted a field experiment that manipulated aboveground biomass and litter levels in three temperate wetlands. The experiment was designed to quantify the direct and indirect effects of *Phragmites* on the soil microenvironment and biogeochemical cycling. Three sites were selected in 2008 that were monospecific stands of *Phragmites* of similar size (see below); two sites were located within Lake Lansing Park in central lower Michigan (Figure 3-1; 42°46'07"N 85°23'32"W); a third site was located within a wetland environment surrounding Glasby Lake in southwestern Michigan (Figure 3-2; 42°28'52"N 85°25'02"W).

Lake Lansing Park is embedded in a mosaic of lakes, woodlots, and residential areas. The total area of the park is 166 hectares and consists mainly of deciduous forest, coniferous forest, and a large interconnected wetland complex of approximately 80 hectares. The park has been extensively invaded by a variety of invasive plant species, including *Phragmites, P. arundinacea*, and *Typha* × *glauca*. The wetland complex contains a mosaic of monospecific stands of these three species, though some areas are mixtures of these along with some native species. Some of the more conspicuous native species include *Carex* spp., *Juncus* spp., *Sambucus* spp., *Leersia oryzoides*, *Eutrochium maculatum, Impatiens* spp., and *Asclepias incarnata*. Both LLP sites consisted of pure stands of *Phragmites* of a

total area less than 1 hectare. LLP1 was located within an inlet of the major wetland complex that dominates the park, while LLP2 was separated from the major wetland complex by areas of higher elevation and a gravel road. Due to this spatial separation and different hydrology (see results) these two sites were considered independent. Subsequent to the completion of this research, beginning in 2011, the LLP sites were subjected to control measures intended to eradicate *Phragmites* from within the park.

The experimental wetland site adjacent to Glasby Lake (referred to hereafter as Glasby) was similar in size to LLP1 and LLP2 with other invasive species such as *P. arundinacea* and *T.* ×*glauca* surrounding the *Phragmites* stand, comparable to the LLP sites. The region around Glasby Lake lies on a glacial landscape and has abundant wetland cover (~ 10%) with low levels of residential development in a landscape dominated by forests, row-crop agricultural fields, and abandoned fields. Glasby Lake itself is approximately 10 hectares of open water with more extensive wetlands surrounding the lake. The amounts of live and dead *Phragmites* biomass, as well as soil and water characteristics, at these three sites are compared in Table 3-1. The data for Table 3-1 were collected over the growing seasons of 2008-2010 and the methodology used to collect the field data can be found in Appendix B.

To separate the effects of living vegetation versus litter on the soil microenvironment and biogeochemical cycling, experimental manipulation of litter and aboveground living biomass (AGB) was initiated in the summer of 2008. At each of the three sites described above, I created ten 2 x 2 m (4 m^2) plots randomly distributed throughout the site, and assigned each plot to either a reference, an AGB removal, a litter removal, or a total removal treatment (n=3 for all treatments except n=1 for total removal treatment). I

marked the corner of each with PVC that stood 1 m from the soil surface for three corners of the plot and 3 m for the fourth corner. These ten plots were randomly established within each site and then each plot was randomly assigned a treatment. Vegetation was cleared around each plot (0.5 m) to establish a buffer zone and no experimental samples (except for AGB) were collected within 20 cm from the outer edge of the plot to reduce edge effects. The height of these stands exceeded 3 m and thus the buffer zone had little effect on light penetration to the ground surface. Reference and biomass removal plots were established at the beginning of the growing season in 2008, litter removal plots at the beginning of growing season in 2009, and total removal plots at the beginning of the growing season in 2010.

For AGB removal treatment plots, all individual plants were cut at the soil surface and removed from the plot (standing and fallen litter remained). For litter removal treatment plots, all standing and fallen litter was collected by hand and removed from the plot (AGB remained). For the total removal plots, all AGB and litter (standing and fallen litter) was removed by hand. Total removal plots were established to determine light levels and temperature in the absence of both litter and vegetation. Litter and AGB were not altered in the reference plots. Manipulations were maintained every three weeks during the growing season (May to October) by removing any vegetation regrowth from the AGB removal plots and any newly fallen litter in the litter removal plots. In the fall of 2009, all of the senesced AGB in the litter removal plots was cut at the soil surface and moved to the AGB removal plots to prevent any litter deposition in litter removal plots, as well as to ensure the AGB removal plots continued to receive litter inputs. Monitoring of the reference and manipulation plots started in growing season of 2008 (May) and continued

through the growing season of 2009 and ended past the growing season of 2010 November). While root decay may have occurred in the AGB removal plots, because regrowth from rhizomes persisted throughout the growing season, it is unlikely that substantial root decomposition occurred. Regardless, roots and rhizomes were removed from soil cores collected for assays that might be influenced by root decay, e.g., for the *in situ* N mineralization assay.

Abiotic and biotic measurements

To monitor water table levels, Solinst pressure transducers were installed at each site along with Solinst barometric pressure loggers to correct for atmospheric pressure. Pressure transducers were suspended within a ~1.5-m long PVC pipe with holes drilled through the sides to allow water to enter the pipe. PVC pipes where surrounded with a small mesh size material to keep soil and debris from entering. Pressure transducers within the PVC structure were installed in a randomly selected plot 1 m below the soil/sediment surface. Pressure transducers were installed in early summer of 2008, 2009, and 2010 and removed in late October each year to avoid winter damage and to download data. Water table measurements in 2008 were taken only at LLP1 and Glasby. Manual water level measurements were also taken at each plot every third week of the growing season. All plots experienced relatively similar hydrologic conditions within a site, therefore it is possible to compare site hydrologic conditions based on pressure transducer measurements at a single point.

To determine treatment effects on soil characteristics, soil samples were collected at each plot at the end of the growing season and analyzed for bulk density, % moisture, and

%C and N to determine C and N storage. Surface soil was sampled to a 10 cm depth using a soil corer (246 cm^3) and samples were transported to the lab on ice and kept at 4°C until processed (~ 3 days). For processing, soils were sieved (4 mm), sub-sampled for bulk density and gravimetric water content (GWC) determination, and then dried to a constant mass at 80°C. Dried soils were then ground and homogenized using a mortal and pestle and run on an elemental combustion system (Costech ECS 4010, Valencia, CA) for %C and %N analysis. Samples were run in duplicate with atropine used as a standard every 10 samples. For the soil samples collected in 2010, the 10 cm soil cores were split into two depths, 0-5 cm and 5-10 cm, and analyzed separately, including for NH_4^+ + NO_3^- concentration (see N mineralization and nitrification methods below) to determine possible depth effects.

Surface water samples were taken on a monthly basis during the 2009 and 2010 growing seasons as baseline data to compare among sites and to determine treatment effects on water chemistry (Table 3-1). These three sites varied seasonally and among years in the presence and depth of water above the soil surface. Water samples were collected when standing water was greater than 5 cm using a 250 ml polyethylene bottle submerged below the water surface. Samples were analyzed for soluble reactive phosphorus (SRP), ammonium, total alkalinity, dissolved organic carbon (DOC), and other major cations and anions (NO_3^-, Cl^-, Na^+, Mg^{2+}, Ca^{2+}, K^+). Dissolved oxygen, specific conductivity (SpC), water temperature, pH, and oxidation-reduction potential (ORP) were monitored using an YSI 556 Multi-Probe System.

After all experimental manipulations were established at the three *Phragmites* sites,

HOBO logging thermistors were installed (4 vertical positions: ambient [60 cm above soil surface], litter layer, and at 2- and 10-cm soil depths) at one random plot per treatment per site to determine if the experimental treatments affected litter and soil temperatures. From 8 July to 29 October 2010 soil temperatures at six depths (soil surface, 2, 10, 20, 30, 40 cm) were manually measured with a Multilogger Thermometer (OMEGA Engineering, Inc.) at each treatment plot at each site. Manual temperature measurements were made to check the accuracy of HOBO temperature probe measurements, as well as for analysis of depth profiles. In summer 2010, light levels (photosynthetically active radiation) at the ground level within each plot were estimated monthly using an AccuPAR LP-80 Ceptometer (ICT International).

Litter bag decomposition assay

To quantify *in situ* decomposition rates a litter bag assay was performed using both *Phragmites* stem tissue and Whatman cellulose filters as a standard substrate. Litter bags (10 cm x 10 cm) were created using Nylon mesh (mesh size = 0.25 mm) and thread. Approximately 1 g of air-dried *Phragmites* stem litter was placed in the litter bag, along with one air-dried Whatman cellulose filter paper (90 mm diameter, ~ 1.12 g). Samples of the air-dried stem litter and filter paper were dried to a constant mass at 80°C and weighed, and the percent difference was used to correct the air-dried weights. The litter bags were then sealed using a heated hand impulse sealer with plastic as the sealant. Litter bags were connected with braided Nylon fishing line to a marker flag and then either placed on the soil surface or 10 cm below the soil surface. Five litter bags were placed in each plot, three on the soil surface and two at 10 cm below the soil surface (total = 150

litter bags: 90 at soil surface, 60 at 10 cm soil depth). Litter bags were installed in each plot on 29 July (Glasby) and 30 July (LLP1 and LLP2) in 2010. After 18 days in the field, one randomly selected soil surface litter bag was removed from each plot, returned to the lab, and kept at 4°C until processed. After a total of 42 and 90 days in the field, one surface and one buried litter bag were removed (randomly selected), returned to the lab, and kept at 4°C until processed. During the time the litter bags were in the field, water levels were ~ 19 cm above the soil surface at Glasby, 55 cm below the soil surface at LLP1 and fell from 5 cm above the soil surface to below the soil surface at LLP2. For processing, litter bags were gently washed with distilled water to remove the majority of the soil particles. The cellulose filter was separated from the stem litter, placed in aluminum foil, dried to a constant mass at 80°C and weighed.

Porewater equilibrators

To investigate possible treatment effects on the chemistry of water in the root zone, porewater equilibrators were installed at each site for approximately 20 days, from 11 August (Glasby), 12 August (LLP2), and 16 August (LLP1) 2011 to 31 August, 1 September, and 2 September 2011, respectively. At Glasby and LLP2, a porewater equilibrator was installed at one randomly selected reference, AGB removal, and litter removal treatment plot. At LLP1, due to low water table levels, porewater equilibrators were only installed at a reference and AGB removal plot. The porewater equilibrators were constructed out of clear acrylic plastic with a total length of 60 cm and width of 10 cm. Fourteen paired wells ran from top to bottom of the porewater equilibrator (3.5 cm distance between well pairs) with each well holding 12 ml of water. Before installation, each porewater equilibrator was

submerged in filtered water (Pall Supor 450 membrane) and one Biotrans^{TM} nylon membrane (0.2 μm pore size) filter membrane sheet was laid on top of the porewater equilibrator, covering the wells. The faceplate was then securely fastened to seal the membrane over the well openings. Each porewater equilibrator was then placed in a vertical acrylic tank filled with filtered water and deoxygenated overnight by pumping N_2 gas through a bubbler into the bottom of the tank for approximately 12 hours. Tanks containing the porewater equilibrators were then transported in deoxygenated water to each site. For installation, porewater equilibrators were driven into the soil until only the top two well pairs were above the soil surface in the surface water (LLP2 and Glasby). Due to low water table levels, installation at LLP1 included digging a pilot hole to determine the location of the water table and then installing the porewater equilibrator to that depth. After approximately 20 days in the field, porewater equilibrators were removed and analyzed for total dissolved P, NO_3^-, Cl^-, Na^+, Mg^{2+}, Ca^{2+}, K^+ and silicate.

N mineralization and nitrification assays

In the summer of 2010, *in situ* net N mineralization and nitrification rates were assayed in each plot using the buried polyethylene bag technique (Eno 1960; Binkley and Hart 1989). Conducting the N transformation assay in the field is useful because possible treatment effects, such as temperature differences, can be included in the incubation. Three pairs of soil cores (10 cm depth, 246 cm^3) were removed from each plot (6 cores x 10 treatment plots x 3 sites = 180 cores). Soil core pairs were taken from a random location within each plot, with a distance of 5 cm between paired soil cores. One core from

each pair was placed in a polyethylene bag and buried at the same depth it was taken, while the other soil core was transported on ice to the laboratory for initial N pool size determination. Soil cores were incubated in the field from 15 July to 15 August (30 days), after which they were transported to the lab for final N pool size determination. From the time of initial *in situ* setup to the end of the incubation, water levels were above the soil surface at Glasby and LLP2, while at LLP1 the water table was ~ 20 cm below the soil. For processing, soils were sieved (4 mm), sub-sampled for determination of bulk density, gravimetric water content (GWC) and concentrations of NH_4^+ and NO_3^-, and then dried to a constant mass at 80°C. Dried soils were ground using a mortal and pestle and analyzed on an elemental combustion system (Costech ECS 4010, Valencia, CA) for %C and %N. Concentrations of inorganic N for both initial and final soil cores were determined on subsamples of all soils via extraction with 2 M KCl and analyzed by the microplate method for nitrate and ammonium using protocols developed by Dr. David Rothstein (Dept. of Forestry, MSU). Net rates of N mineralization and nitrification were calculated from the changes in inorganic N ($NH_4^+ + NO_3^-$) or NO_3^--N, respectively, during the incubation period (initial – final pool sizes).

Denitrification assay

A laboratory denitrification assay was performed to determine treatment effects on denitrification potential. On 5 November 2011, a 10 cm soil core (246 cm^3) was collected from each treatment plot at each site, transported to the lab on ice, and kept at 4°C until processed. At the time of soil harvest, water levels were above the soil surface (~ 30 cm) at

Glasby, at the soil surface at LLP2, and below the soil surface at LLP1. On 6 November 2011, soil cores were sieved (4mm) and then two subsamples (25 ml) were kept on ice and transported to Dr. Jennifer Tank's lab at the University of Notre Dame where the assay was performed (10 treatment plots x 3 sites x 2 replicate subsamples = 60 assay samples). On 7 November 2011, denitrification rates were determined using the chloramphenicol-amended acetylene (C_2H_2) inhibition technique (Groffman et al. 2006) modified by the Tank Lab. Briefly, each 25 ml subsample was added to a 125 ml media bottle and 50 ml unfiltered water from the Glasby site was added and mixed with the soil to make a slurry. The media bottle was sealed with an *n*-butyl rubber septum in the lid to allow gas samples to be collected with a 5 ml syringe. Chloramphenicol was added to the slurry to inhibit *de novo* enzyme production. Before gas samples were taken, the headspace of the media jars was purged with helium for 10 minutes and mechanically agitated to achieve anoxic conditions throughout the slurry. Acetylene gas (15 ml) was then added to the media bottle to prevent the conversion of N_2O to N_2, allowing the concentration of N_2O to be used for denitrification rate determination. Five 5 ml gas samples were collected starting at time 0 and then hourly for 4 hours (total of 5 samples per replicate). Samples were injected into a 3 ml scintillation jar and then analyzed for N_2O on a gas chromatograph with an electron capture detector. Denitrification rates were calculated for each replicate from the linear increase in N_2O concentration over the course of the 4-hour incubation. Concentrations were corrected for dilution by the addition of N_2 after each sample was collected and rates were expressed on a volumetric and gravimetric basis.

Statistical analyses

Site differences in key soil characteristics were analyzed by general linear models (GLM) to help explain the pattern in variation among sites for the ecosystem process data collected in this study. Four separate multivariate analysis of variance (MANOVA) tests were performed for two sets of response variables: 1) litter bag decomposition rate, denitrification rate, net N mineralization rate, and nitrification rate (hereafter rate response variables), and 2) soil characteristics collected in 2010. For each set of response variables the effects of treatment and site were tested individually for a total of four MANOVA tests. The multivariate analysis was used to protect follow-up ANOVA analysis from inflated alpha, which can happen when multiple ANOVA tests are performed (Scheiner 2001). The Pillai-Bartlett trace (hereafter Pillai) was used as the test statistic for each MANOVA. Separate GLMs (ANOVAs) were then run to assess which factors significantly influenced individual response variables.

For the analysis of light levels, soil temperature, soil C and N storage, soil NH_4^+ and NO_3^- concentrations, *in situ* net N mineralization/nitrification rates, potential denitrification rates, and litter bag decomposition rates, GLMs were used to determine treatment effects. For these analyses, "site" was considered a random factor and "plot" was nested within "site". Porewater equilibrator data were analyzed differently because there was no replication at the treatment plot level, instead there was only one equilibrator per treatment per site, and therefore plot was not nested within site. Additionally, depth was included as a fixed factor for those analyses that included it. Tukey's HSD multiple comparison tests were performed to determine significant differences among factor levels.

For the biogeochemical rate assays (N mineralization, nitrification, denitrification, and decomposition), both simple linear regression and multiple regression analyses were performed to determine significant controlling soil factors on rates. Many of the measurements and assays described above were additionally analyzed with "site" as a fixed effect (without "treatment" as a fixed effect) because of the strong site effects that were present, which can be linked to differences in hydrology. Model diagnostics were assessed for each analysis to determine if the residuals were normally distributed and displayed constant error variances. Log transformations were used to correct for any heteroscedasticity. All variances are presented as standard errors. All statistical tests were performed in R 2.13.2 (R Development Core Team 2011).

Table 3-1. Variation among sites in plant characteristics, soil characteristics, and water chemistry. Variance expressed as standard error. AGB = aboveground biomass, DO = dissolved oxygen, SpC = specific conductivity, ORP = oxidation reduction potential, OM = organic matter. * indicates data collected in 2010.

	Plant Characteristics (2009)						
Site	Plant Height (cm)	AGB (g)	AGB per tiller (g)	Standing litter ($g\ m^{-2}$)	Ground Litter ($g\ m^{-2}$)	Total Litter ($g\ m^{-2}$)	Rhizome ($g\ m^{-2}$)
LLP1	361 ±9.4	1742 ±489	47.8 ±6.0	580 ±335	1545 ±1426	2126 ±1315	584 ±186
LLP2	383 ±29.0	1668 ±422	55.2 ±8.3	1157 ±344	1184 ±397	2341 ±145	813 ±596
Glasby	391 ±15.0	1868 ±263	69.5 ±10.6	724 ±291	1225 ±549	1950 ±548	1422 ±576
	Soil Characteristics (2009)						
Site	OM Depth (cm)	Bulk density ($g\ cm^{-3}$)	Ammonium* ($mg\ N\ kg^{-1}$)	Nitrate* ($mg\ N\ kg^{-1}$)	Soil %C	Soil %N	Soil C:N
LLP1	66.7 ±10.2	0.255 ±0.030	4.16 ±1.55	747.64 ±329.77	25.07 ±1.76	0.25 ±0.03	13.45 ±0.48
LLP2	55.2 ±6.2	0.161 ±0.033	14.36 ±4.78	69.68 ±97.42	40.06 ±1.96	0.16 ±0.03	13.07 ±0.68
Glasby	53.9 ±9.2	0.157 ±0.460	19.79 ±7.66	77.55 ±84.72	37.32 ±5.36	0.16 ±0.05	14.18 ±0.34
	Water Chemistry (2010)						
Site	SpC ($\mu S\ cm^{-1}$)	DO ($mg\ L^{-1}$)	%DO	pH	Salinity (ppt)	ORP (mV)	
LLP1	0.737 ±0.75	0.6 ±0.16	5.9 ±1.5	7.46 ±0.19	0.36 ±0.04	-179 ±56	
LLP2	0.744 ±0.12	0.64 ±0.15	6.5 ±1.4	6.71 ±0.09	0.36 ±0.06	-5 ±11	
Glasby	0.442 ±0.10	0.54 ±0.14	5.1 ±1.2	6.48 ±0.08	0.21 ±0.04	-18.2 ±14	
	Major Ions (ppm; 2009 and 2010)						
Site	Cl^- (ppm)	NO_3^- (ppm)	SO_4^{2-} (ppm)	Ca^{2+} (ppm)	Mg^{2+} (ppm)	Na^+ (ppm)	K^+ (ppm)
LLP1	30.69 ±7.43	0.017 ±0.028	6.64 ±12.10	86.73 ±26.03	18.08 ±6.12	16.51 ±4.63	2.86 ±0.64
LLP2	72.54 ±23.10	0.005 ±0.001	1.00 ±0.16	90.46 ±27.13	23.12 ±5.12	27.17 ±13.18	0.52 ±0.77
Glasby	56.88 ±13.75	0.005 ±0.006	0.48 ±0.21	67.90 ±28.86	16.51 ±5.20	36.29 ±13.60	0.36 ±0.13

Table 3-1 (continued)

Roots ($g\ m^{-2}$)	Total BGB ($g\ m^{-2}$)
149 ±112	734 ±278
224 ±94	1038 ±642
1350 ±445	2773 ±812

Soil C Stock ($g\ m^{-2}$)	Soil N Stock ($g\ m^{-2}$)
6368 ±523	473 ±38
6425 ±1136	491 ±84
5678 ±994	401 ±74

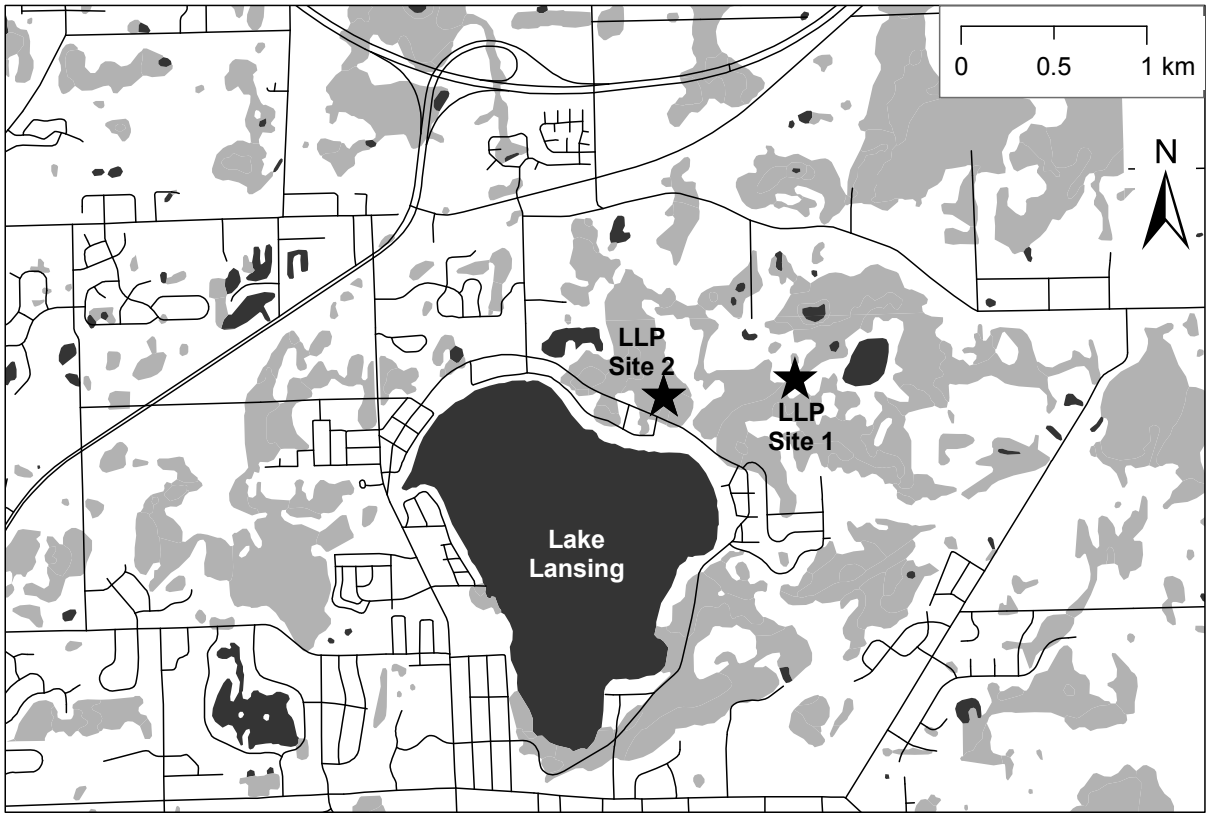

Figure 3-1. Map showing the location of the two monospecific stands of *Phragmites australis* at Lake Lansing Park (LLP1 and LLP2), Haslett, MI.

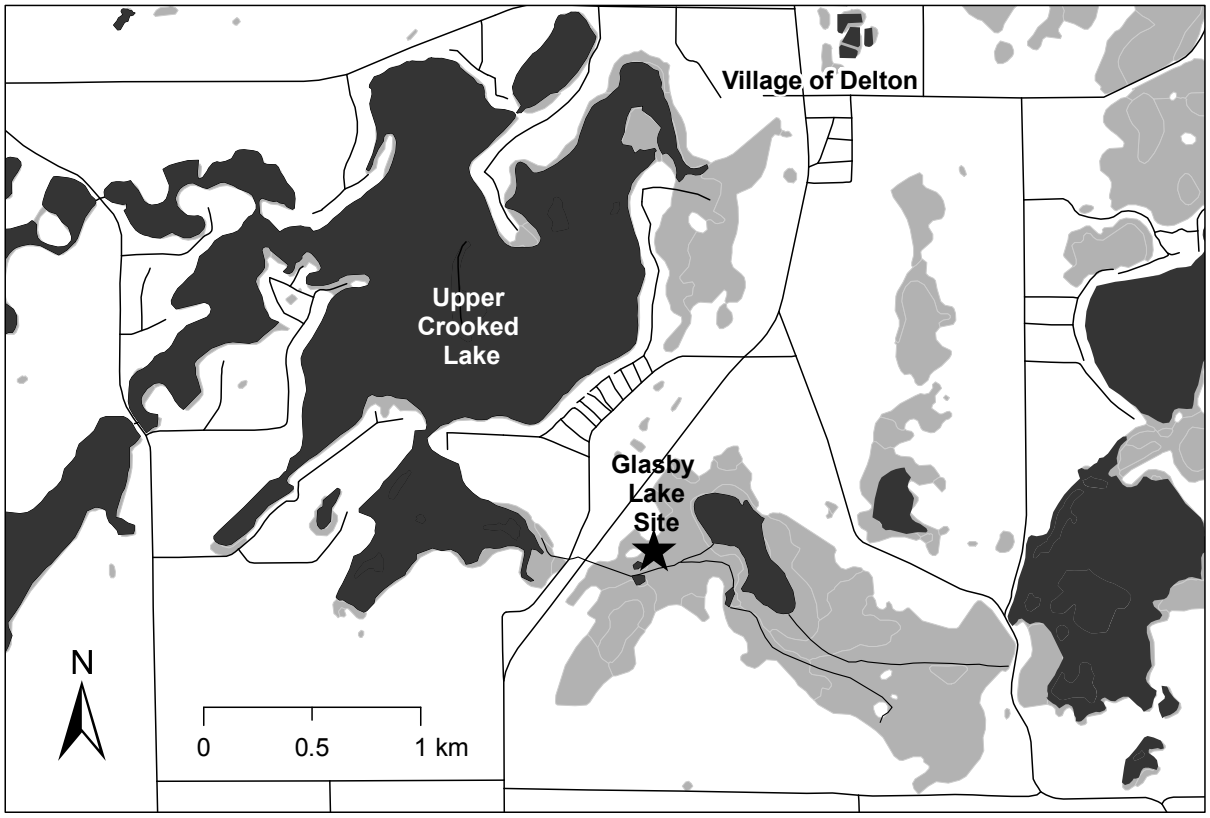

Figure 3-2. Map showing the location of the monospecific stand of *Phragmites australis* in the wetland area surrounding Glasby Lake (Glasby), Delton, MI.

Results

Site Characteristics

For soil characteristics, multivariate analysis showed significant effects of site and depth (Pillai = 1.51, approx. $F_{16,48} = 16.06$, $p < 0.001$, Pillai = 0.57, approx. $F_{8,41} = 6.84$, $p < 0.001$, respectively). There was also a significant site x depth interaction (Pillai = 0.51, approx. $F_{16,84} = 1.80$, $p = 0.045$). Protected univariate analysis showed there were significant site effects for nitrate, ammonium, and soil C:N ratio (Table 3-2). LLP1 soil had greater nitrate availability and lower soil moisture and ammonium availability than either LLP2 or Glasby, while Glasby had a higher soil C:N ratio than LLP1 and LLP2. Soil C and N stocks and soil % C and N were also significantly different among sites (Table 3-2). There was a significant site x depth interaction for both soil % C ($p = 0.040$) and % N ($p = 0.085$): LLP1 and Glasby had greater soil % C and N at 0-5 cm depth compared to at 5-10 cm depth, but there were no differences between depths at LLP2. Soil C and N stocks followed a similar pattern of higher stocks at 5-10 cm depth compared to at 0-5 cm depth (significant depth effect; $p < 0.001$ for both soil C and N stock), along with LLP1 having greater stocks than LLP2 and Glasby (significant site effect; Table 3-2).

Water table position measured in 2008-2010 (Figures 3-3 to 3-5) by pressure transducers installed at each site are informative because they help to explain the variation among sites for many of the variables discussed above. There are a number of points to be made about the water table position data:

(1) Glasby had a higher water table position throughout all recorded time periods compared to LLP1, while LLP2 had the highest water table position throughout 2009 and the second highest position in 2010;

(2) Wet and cool spring and summer conditions in 2009 lead to higher than average water levels across central Michigan, leading to high water table position at LLP1 and LLP2;

(3) In September of 2008 and 2010, there was a dramatic increase in water table position for most sites following heavy rainfall;

(4) In 2010, when most of the data analyzed above were collected, the Glasby water level was always at least 10 cm above the soil surface, while LLP1's water level was always below the soil surface. LLP2's water level was closer to the soil surface and shifted from above to below the soil surface multiple times over the recorded period in 2010.

A summary of the average water table position can be found in Figure 3-6. These site differences in hydrology likely explain the variation in soil moisture differences among sites: LLP2 had the greatest soil moisture, followed by Glasby, and then LLP1 (significant site effect; $p < 0.001$).

While there was little difference in total aboveground biomass production and plant height among sites, there was a significant site effect on rhizome, root, and total belowground biomass (BGB) (total BGB: $F_{2,12} = 8.35$, $p = 0.005$). Glasby, the most consistently saturated site, had the highest BGB, while LLP2 and LLP1, which had either fluctuating water levels or water levels consistently below the soil surface, had significantly lower BGB.

Litter and AGB effects on soil and porewater chemistry, light levels, and temperature

For soil characteristics, results from the MANOVA indicated that when analyzed for the effects of treatment and depth, treatment was not significant (Pillai = 0.23, approx. $F_{16,84} = 0.69$, $p = 0.798$) but depth was (Pillai = 0.51, approx. $F_{8,41} = 5.41$, $p < 0.001$). Protected ANOVAs indicated bulk density, stocks of soil C and N, and soil C:N ratio were all significantly greater at 5-10 cm depth compared to at 0-5 cm depth (Table 3-3). Conversely, ammonium, nitrate, and soil % C and N were all significantly greater at 0-5 depth compared to at 5-10 cm depth (Table 3-3).

Photosynthetically active radiation (PAR) below the canopy varied across treatments with the highest values recorded in the total removal plots (781 ± 191 μmol m^{-2} sec^{-1}), nearly 18 fold greater than in the reference plots (44 ± 15 μmol m^{-2} sec^{-1}) (Figure 3-7; significant treatment effect, $p < 0.001$). Levels of PAR in the biomass removal and litter removal plots were intermediate, and did not statistically differ from one another.

Averaged across sites, soil temperature measurements made during midday visits ranged from 17.1 ± 0.54 ^{o}C at a depth of 40 cm for the reference treatment to 24.77 ± 2.67 ^{o}C at the soil surface for the total removal treatment (Figure 3-8). In all treatments besides total removal, there was a gradual decrease in temperature from ambient to 40 cm soil depth (significant depth effect; Table 3-4). For the biomass removal treatment, there was a significant decrease in temperature from ambient air to the soil surface followed by a decrease from a soil depth of 2 cm to 40 cm similar to other treatments (significant treatment x depth interaction; Table 3-4). The total removal treatment showed an increase

in temperature from ambient to soil surface, though they were not significantly different. Reference plots had the lowest recorded soil temperature for each soil depth, as well as ambient temperature (significant treatment effect; Table 3-4).

For HOBO datalogger temperature measurements recorded in 2010, which included all four treatment plots, there was an interaction between depth and date: temperature variation among days was greater for ambient air readings compared to soil readings (Figures 3-9 to 3-12; significant depth x date interaction; Table 3-5). From 12 July to the beginning of September, total removal plots had the highest temperatures followed by litter removal plots, biomass removal plots, and reference plots. Starting at the beginning of September and lasting until the end of the recording period (28 October), the rank order of decreasing temperature changed to reference, biomass removal, total removal, and finally litter removal (significant treatment x date interaction; Table 3-5). The significant treatment effect ($p = 0.003$) was caused by total removal plots having higher temperatures compared the other treatment levels, from the start of the recording period to approximately the beginning of September.

Analysis of porewater equilibrator wells after field incubation revealed distinct changes in major solute chemistry with depth below the water table. There was a significant treatment effect on Na^+ and Cl^- concentrations, as the biomass removal treatment plots had lower concentrations of these relatively conservative ions compared to the reference and litter removal plots (Figures 3-13 and 3-14; significant treatment effect; Table 3-6). Concentrations of Mg^{2+} were highest in the litter removal plots compared to reference and biomass removal plots (Figure 3-15; significant treatment effect; Table 3-6)

and this pattern was stronger at greater depth, though the interaction was not significant. Concentrations of Ca^{2+} were highest in reference plots compared to biomass and litter removal plots (Figure 3-16; significant treatment effect; Table 3-6). Across treatments, Mg^{2+} and Ca^{2+} concentrations increased with depth (significant depth effect; Table 3-6). Sulfate and nitrate decreased with well depth (Figure 3-17 and 3-18; significant depth effect; $p < 0.001$), and though there were no significant effects of treatment for sulfate, nitrate was significantly lower in reference plots than litter removal plots (significant treatment effect; $p = 0.049$, Table 3-6). Total dissolved phosphate (TDP) and potassium did not vary with depth or among treatments.

Carbon and nitrogen cycling

For the carbon and nitrogen cycling rate response variables (litter bag decomposition rate, denitrification rate, net N mineralization rate, and nitrification rate), there were no significant effects of litter and/or biomass removal treatment (MANOVA; Pillai = 0.30, approx. $F_{15,72} = 0.52$, $p = 0.919$), but that there were significant site effects (Pillai = 1.29, approx. $F_{10,48} = 8.74$, $p < 0.001$).

For univariate analysis, there were no significant treatment effects on *in situ* N mineralization or nitrification rates, though there were strong site effects. Both Glasby and LLP2 had positive N mineralization rates (3.99 and 4.96 mg N kg^{-1} soil, respectively) while negative N mineralization (i.e., net immobilization and/or denitrification of dissolved inorganic N) occurred at LLP1 (-0.88 mg N kg^{-1} soil) (Figure 3-19; significant site effect,

$F_{2,87} = 65.57$; $p < 0.001$). Net nitrification rates were negative for LLP1 and LLP2 (-0.39 and -0.15 mg N kg soil^{-1}, respectively) and zero for Glasby (Figure 3-20; significant site effect, $F_{2,87} = 1.29$; $p < 0.001$). Because LLP1 and LLP2 had positive soil nitrate concentrations at the beginning of the incubation, the low net nitrification rates were likely caused by denitrification (nitrate loss) occurring throughout the 30-day incubation. Soil moisture and soil % N were positively related to net N mineralization (Figure 3-21 and 3-22; adjusted $R^2 = 0.297$, $p < 0.001$, adjusted $R^2 = 0.406$, $p < 0.001$, respectively). Ammonium was positively related to soil moisture (Figure 3-23; adjusted $R^2 = 0.125$, $p < 0.001$), while nitrate was negatively related to soil moisture (adjusted $R^2 = 0.045$, $p < 0.025$), though the relationship was weak.

There were no treatment effects on litter bag decomposition rates, though there were significant depth effects. Trends in decomposition were similar for both *Phragmites* stems and filter paper: filter paper (Figure 3-24; $p < 0.001$; Table 8) and stem (Figure 3-25; $p = 0.015$; Table 3-7) material incubated within soil had a lower decomposition rate compared to when incubated on the soil surface, though there were no differences within total removal plots. When litter bag assay data were analyzed to determine site x depth effects, their interaction was found to be significant. For stem decomposition, rates at both depths at LLP1 and Glasby were not different from one another, but at LLP2 stem tissue decomposed faster on the soil surface and slower in soil compared to LLP1 and Glasby (Figure 3-26; significant site x depth interaction; Table 3-7). For filter paper decomposition, there was no statistical difference among sites for filter paper incubated in

soil, while filter paper incubated on the soil surface decomposed faster. This faster decomposition on the soil surface was not observed at LLP1 (Figure 3-26; significant site x depth interaction; Table 3-7). Overall, filter paper (k constant = 0.0114 d^{-1}) decomposed at a faster rate than *Phragmites* stem tissue (k constant = 0.0022 d^{-1}) ($p < 0.001$).

Similar to the *in situ* N mineralization/nitrification incubation and litter bag assay, no significant treatment effects were found for potential denitrification rates. There were strong site effects, and expressing denitrification rates on a volumetric (cm^{-3}) or gravimetric (g^{-1} soil and g^{-1} soil C) basis yielded similar results: both LLP1 and LLP2 had significantly greater N_2O production rates than Glasby (Figure 3-28; $p < 0.001$ for all cases; for cm^{-3} $F_{2,27} = 11.41$), which had almost no detectable amount of N_2O production. Because mode of expression did not affect statistical conclusions, only denitrification rates expressed on a volumetric basis (cm^{-3}) will be discussed further. The best fit multiple regression model (based on AIC values and overall model significance) showed denitrification rates were positively related to soil nitrate (Figure 3-29; $p < 0.001$) and negatively related to soil C:N ratio (Figure 3-30; $p < 0.001$), with the overall model explaining 41% of the variation in denitrification rates.

Table 3-2. Mixed model F-values for the effect of Site and Depth on Bulk Density, Ammonium, Nitrate, Soil %C, Soil %N, Soil C:N ratio, and Soil C and N Stock. df indicates degrees of freedom. Significance is denoted with asterisk(s).

Source	df	Bulk Density	Ammonium	Nitrate	Soil %C	Soil %N	Soil C:N	Soil C Stock	Soil N Stock
Site	3	29.56**	23.10**	18.44**	53.50**	65.52**	23.25**	4.76*	7.88**
Depth	1	32.99**	8.21*	6.07*	7.12*	16.80**	17.11**	33.80**	19.79**
T x D	3	4.13*	1.39	0.51	3.41*	2.58*	3.72*	0.32	0.27

*p < 0.05
**p < 0.001

Table 3-3. Mixed model F-values for the effect of Treatment and Depth on Bulk Density, Ammonium, Nitrate, Soil %C, Soil %N, Soil C:N ratio, and Soil C and N Stock. df indicates degrees of freedom. Significance is denoted with asterisk(s).

Source	df	Bulk Density	Ammonium	Nitrate	Soil %C	Soil %N	Soil C:N	Soil C Stock	Soil N Stock
Treatment	3	0.22	1.23	1.87	0.55	0.34	0.65	0.89	0.94
Depth	1	27.34**	8.09*	6.15*	6.34*	15.16**	14.57**	33.08**	19.46**
T x D	3	0.08	0.75	0.03	0.10	0.15	0.12	0.26	0.28

*p < 0.05
**p < 0.001

Table 3-4. Summary of two-factor ANOVA for the effect of Treatment and Depth on soil temperature. den df indicates the denominator degrees of freedom and num df indicates numerator degrees of freedom.

Source	num df	den df	F	P-value
Treatment	3	174	9.33	<0.0001
Depth	6	174	62.41	<0.0001
T x D	18	174	2.07	0.0087

Table 3-5. Summary of Repeated Measures ANOVA for the effect of Treatment, Depth, and Time on soil temperature during the 2010 growing season. den df indicates denominator degrees of freedom and num df indicates numerator degrees of freedom.

Between Subjects

Source	num df	den df	F	P-value
Treatment	3	28	5.82	0.0032
Depth	3	28	3.83	0.0203
Treatment x Depth	9	28	0.13	0.9982

Within Subjects

Source	num df	den df	F	P-value
Time	108	3181	1518.51	<0.0001
Time x Treatment	324	3181	2.46	<0.0001
Time x Depth	324	3181	31.84	<0.0001
Time x Treat. x Depth	972	3181	0.64	1.0000

Table 3-6. Mixed model F-values for the effect of Treatment and Depth on TDP (total dissolved phosphate), Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, SO_4^{2-}, Cl^{-}, and NO_3^{-} concentrations from porewater equilibrators. Significance is denoted with asterisk(s). df indicates degrees of freedom.

Source	df	TDP	Mg^{2+}	Ca^{2+}	Na^{+}	K^{+}	SO_4^{2-}	Cl^{-}	NO_3^{-}
Treatment	2	1.82	3.29*	7.54*	4.93*	2.03	1.55	17.34**	3.16*
Depth	13	1.30	9.20**	5.44**	0.30	0.39	4.48**	0.54	2.91*
Treatment x Depth	26	0.66	0.99	0.61	0.78	1.23	0.21	0.21	0.51

*$p < 0.05$
**$p < 0.001$

Table 3-7. Summary of two-factor ANOVAs for the effect of Treatment and Depth on stem and filter decomposition and Site and Depth on stem and filter decomposition (expressed as first-order rate constants, k). den df indicates the denominator degrees of freedom and num df indicates numerator degrees of freedom.

Source	num df	den df	F	P-value
Stem k constant				
Treatment	3	44	0.60	0.6178
Depth	1	44	6.43	0.0148
T x D	3	44	1.06	0.3745
Filter k constant				
Treatment	3	44	0.67	0.5717
Depth	1	44	27.21	<0.0001
T x D	3	44	1.62	0.1976
Stem k constant				
Site	2	54	1.69	0.1933
Depth	1	54	6.95	0.0110
S x D	2	54	5.12	0.0092
Filter k constant				
Site	2	54	21.50	<0.0001
Depth	1	54	35.71	<0.0001
S x D	2	54	10.33	0.0002

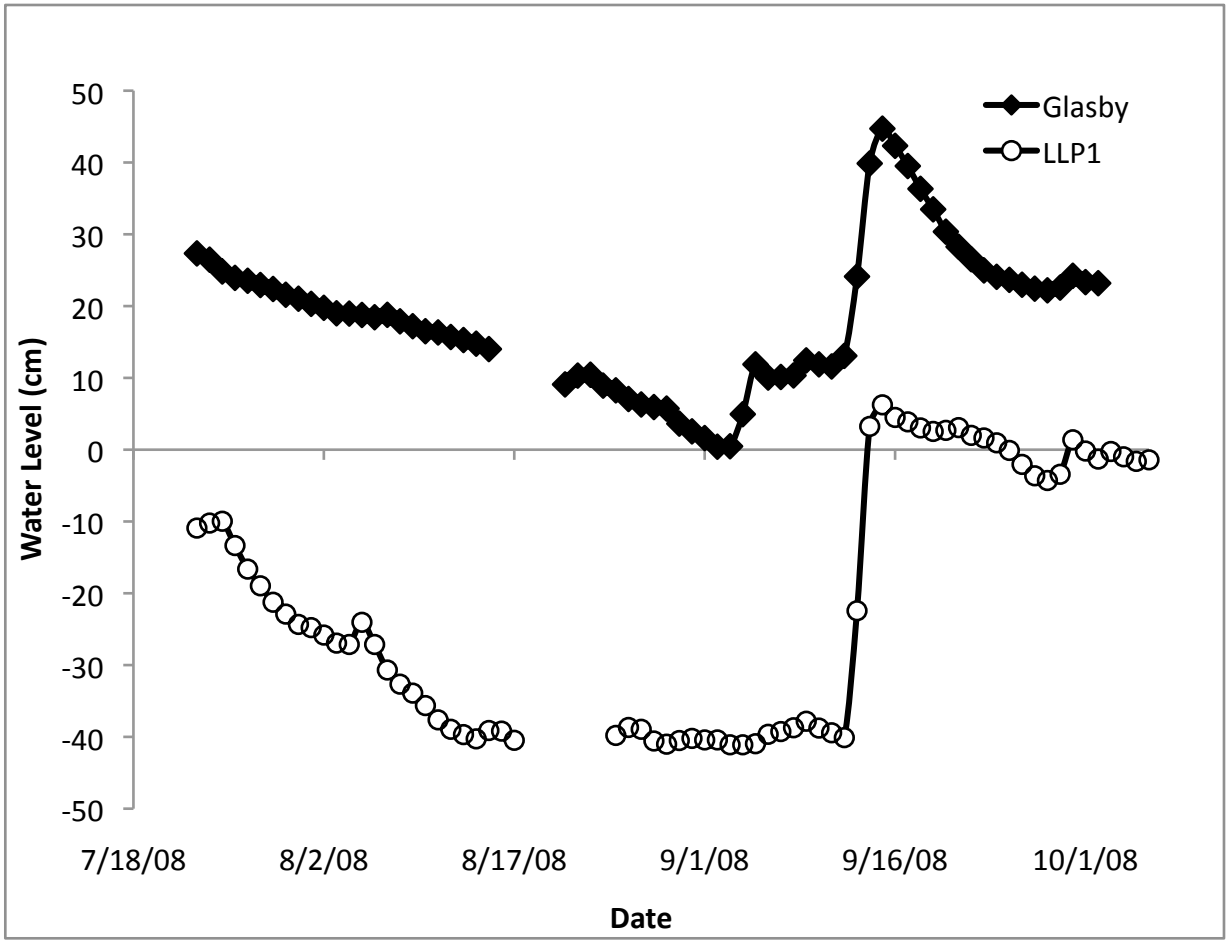

Figure 3-3. Water table position at Glasby and LLP1 from July to October of 2008. Zero water level represents the soil surface with positive water levels indicating flooding (standing water) and negative values indicating a water table below the soil surface.

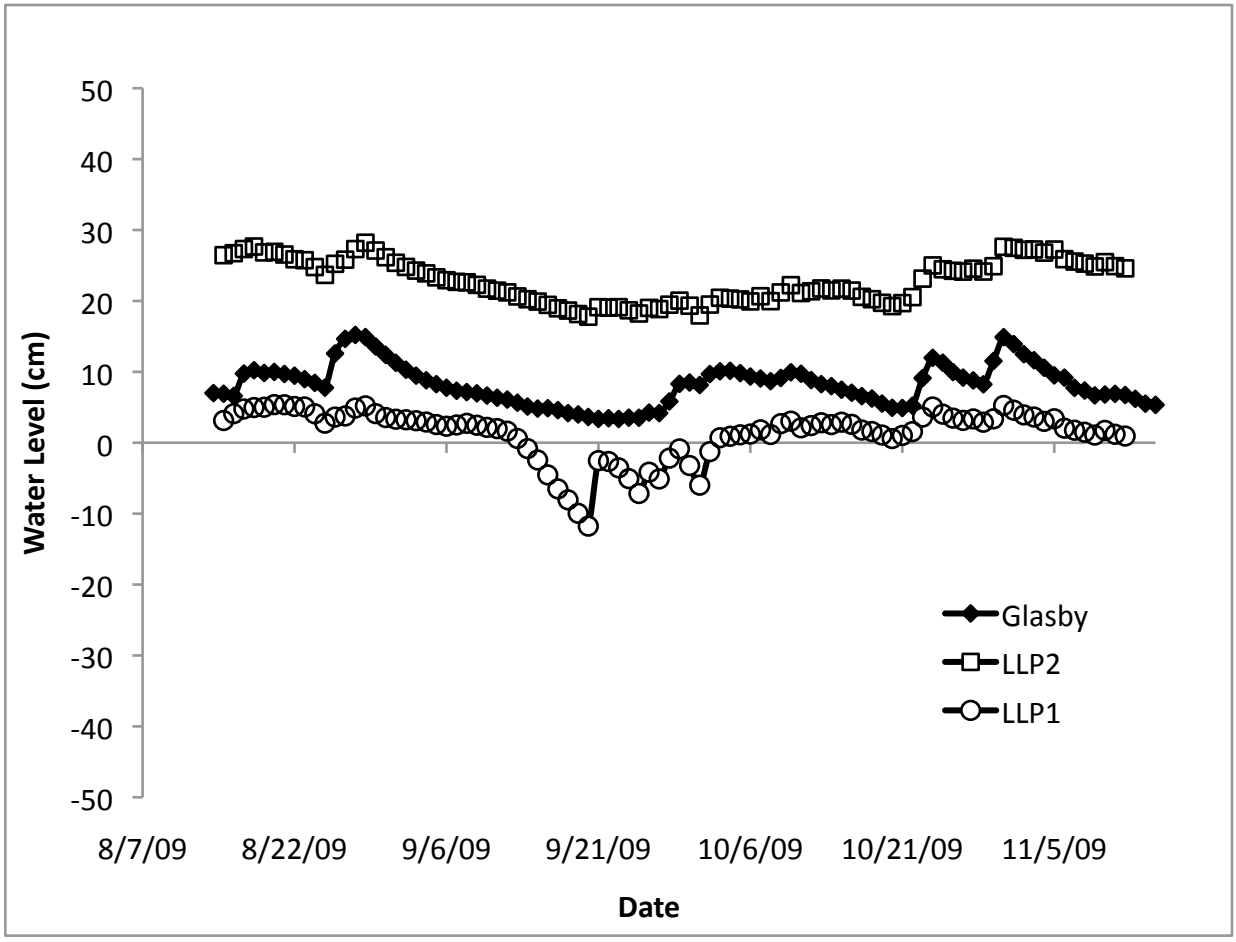

Figure 3-4. Water table position at Glasby, LLP1, and LLP2 from August to November of 2009. Zero water level represents the soil surface with positive water levels indicating flooding (standing water) and negative values indicating a water table below the soil surface.

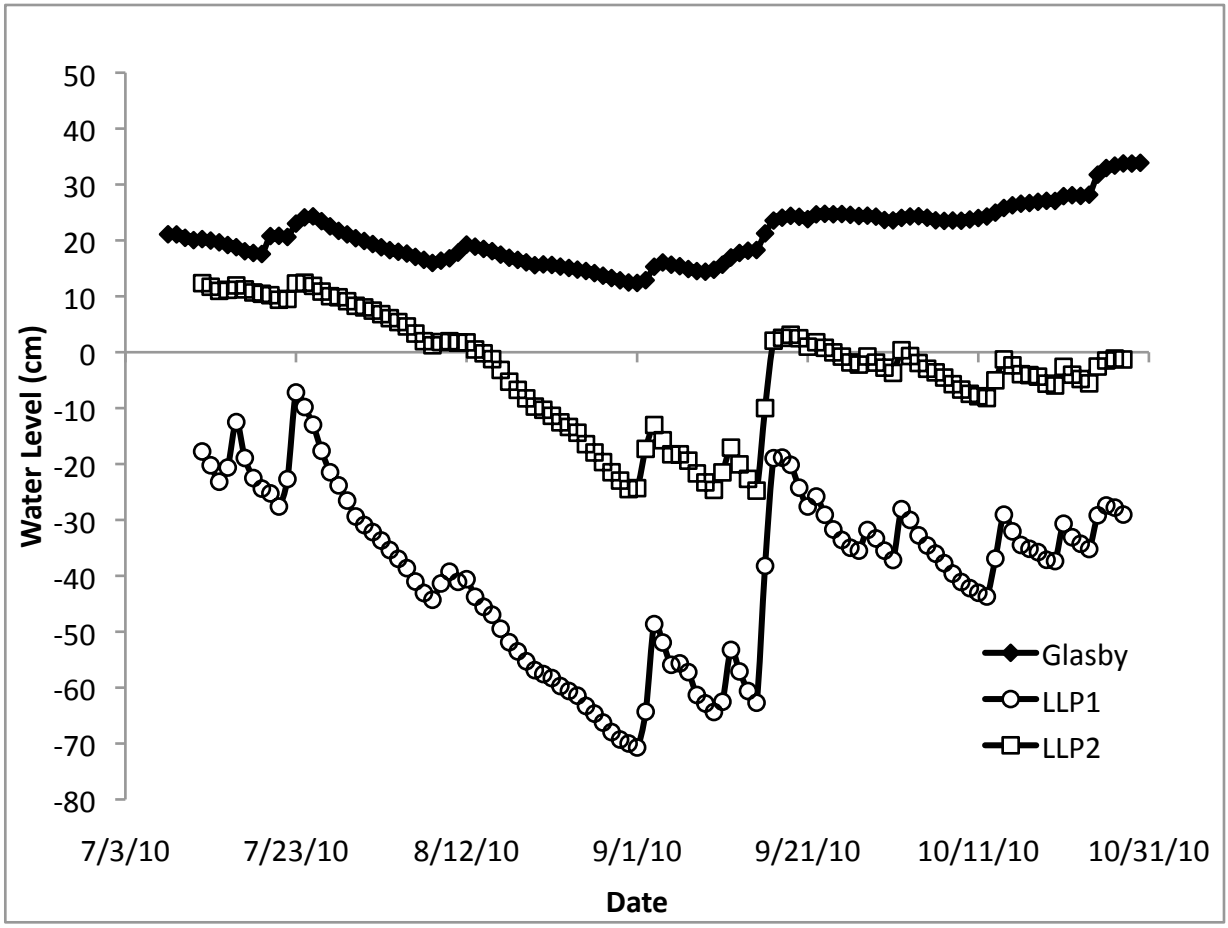

Figure 3-5. Water table position at Glasby, LLP1, and LLP2 from July to October of 2010. Zero water level represents the soil surface with positive water levels indicating flooding (standing water) and negative values indicating a water table below the soil surface.

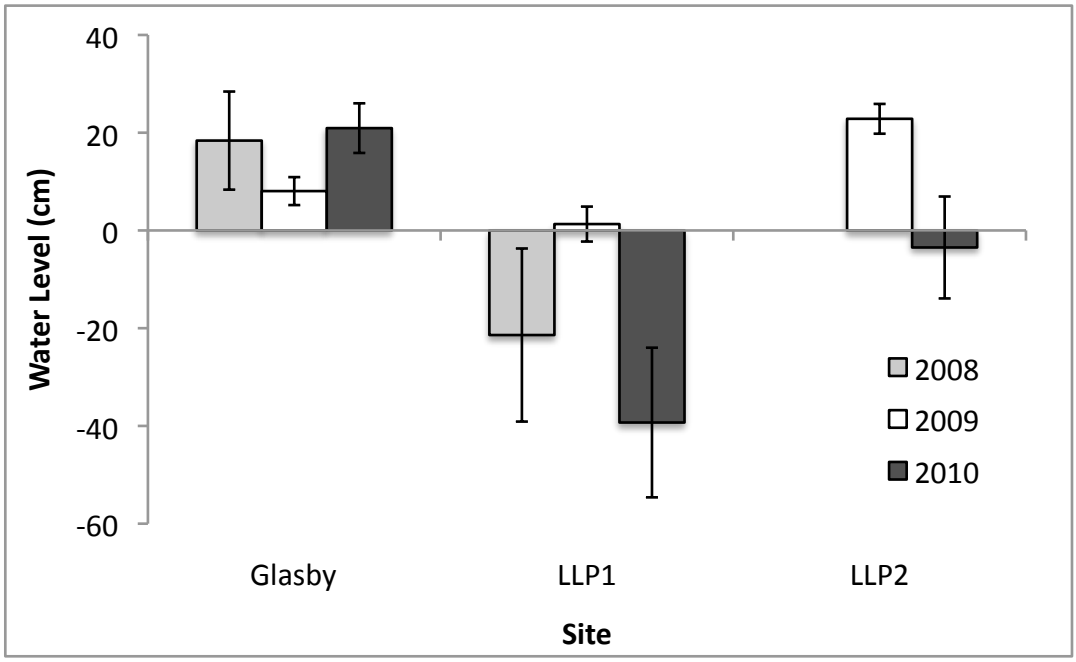

Figure 3-6. Water table position at Glasby, LLP1, and LLP2 for 2008 to 2010 (see methods for exact time periods). Zero water level represents the soil surface with positive water levels indicating flooding (standing water) and negative values indicating a water table below the soil surface. Error bars represent 1 standard deviation.

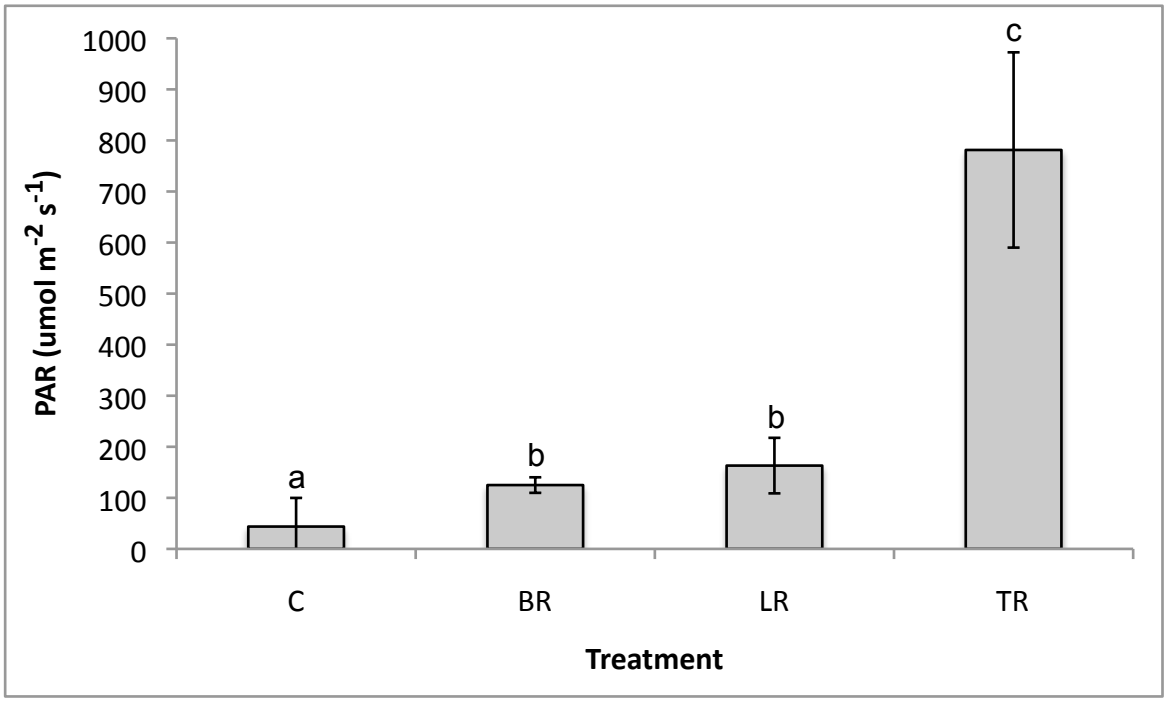

Figure 3-7. Mean light levels (PAR) measured directly above the soil surface within control (C), biomass removal (BR), litter removal (LR), and total removal (TR) treatment plots averaged across sites. There was a significant treatment effect ($F_{3,81} = 109.95$, $p < 0.001$). Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

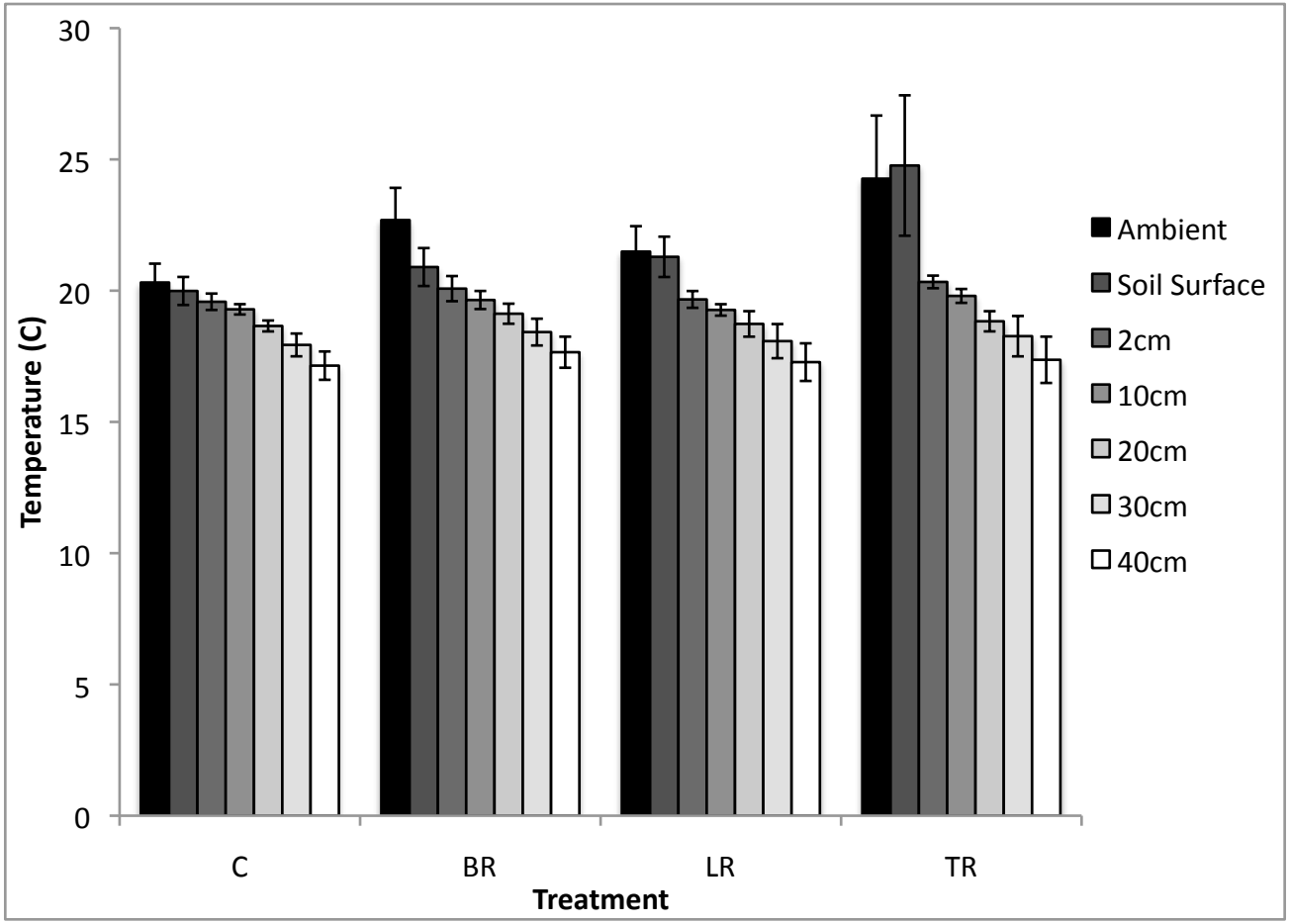

Figure 3-8. Mean manual temperature measurements taken at 6 soil depths (and ambient) within control (C), biomass removal (BR), litter removal (LR), and total removal (TR) treatment plots averaged across sites. There was a significant interaction between treatment and depth ($F_{18,174} = 2.07$, $p < 0.009$). Error bars represent 1 SE.

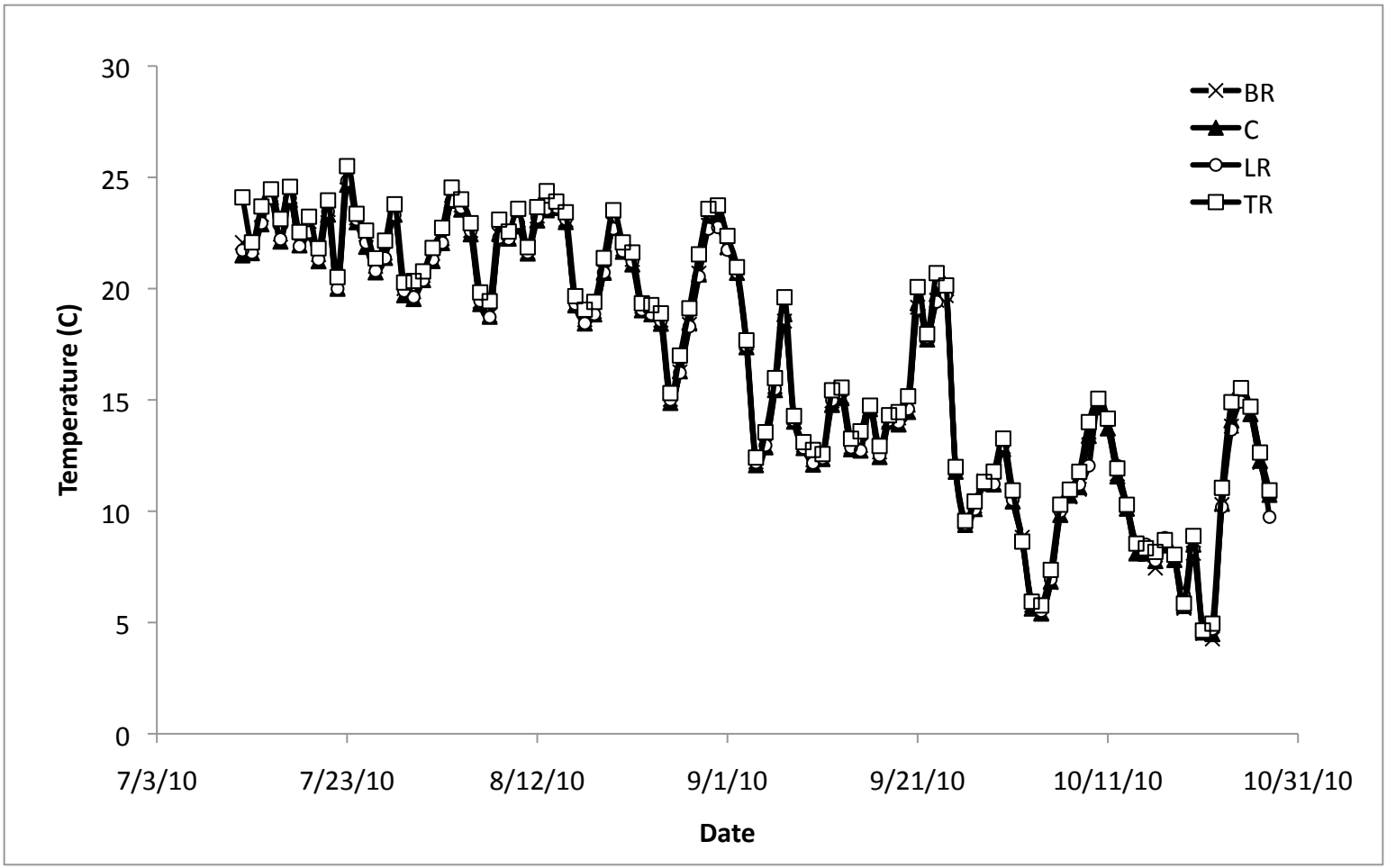

Figure 3-9. Daily ambient mean temperature from July to October 2010 within control (C), biomass removal (BR), litter removal (LR), and total removal (TR) treatment plots.

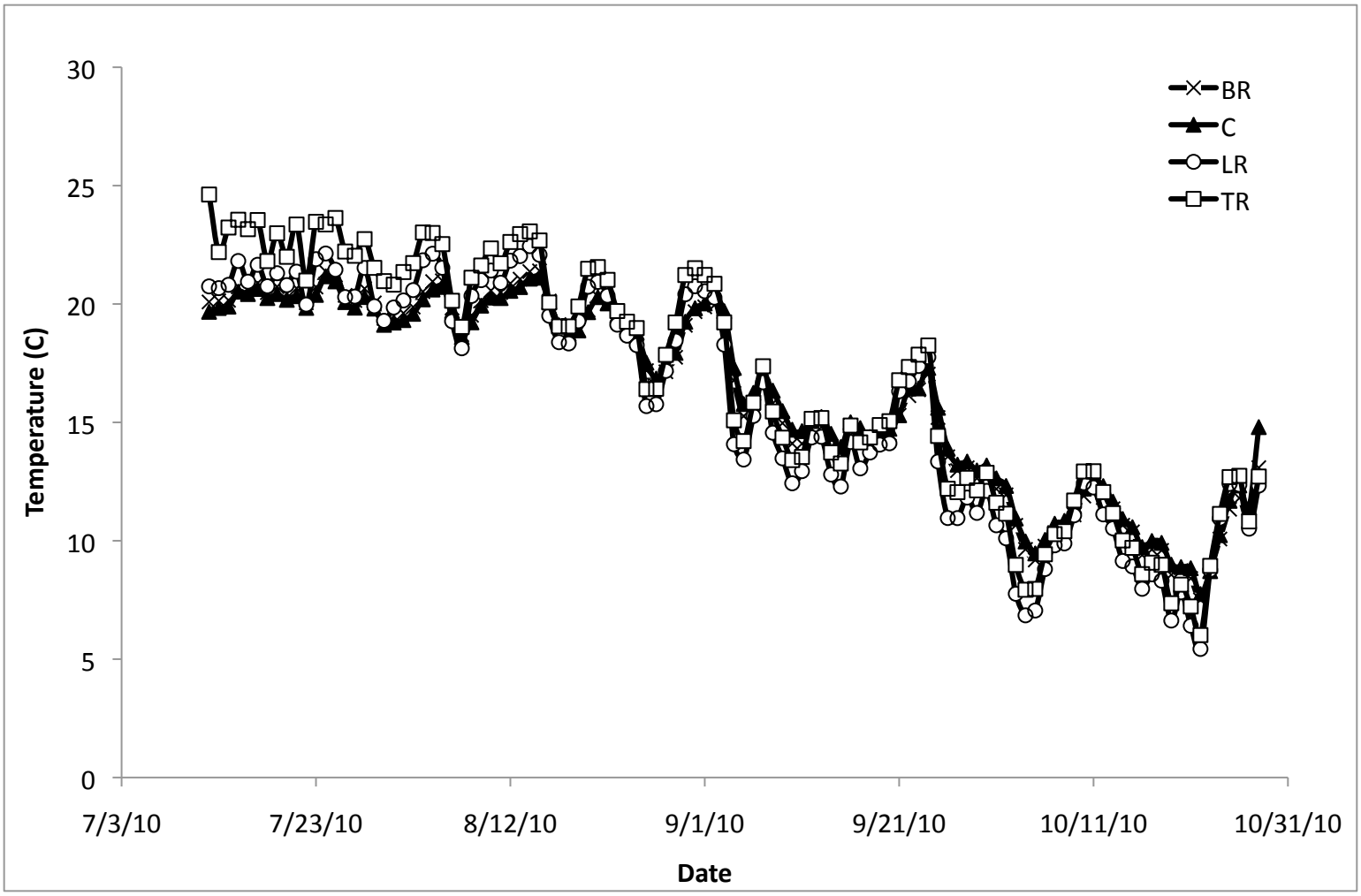

Figure 3-10. Daily soil surface mean temperature from July to October 2010 within control (C), biomass removal (BR), litter removal (LR), and total removal (TR) treatment plots.

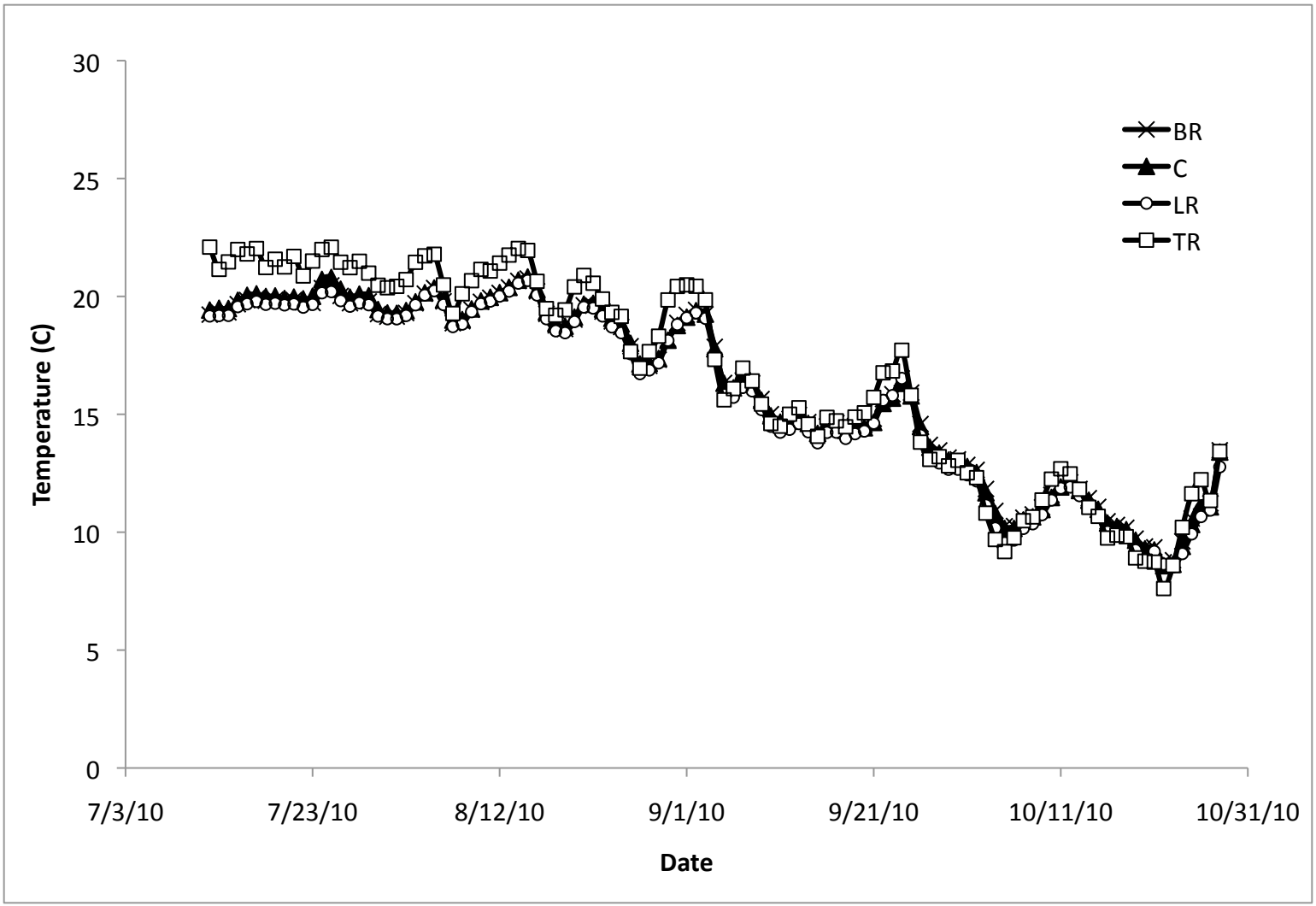

Figure 3-11. Daily mean temperature at 2 cm soil depth from July to October 2010 within control (C), biomass removal (BR), litter removal (LR), and total removal (TR) treatment plots.

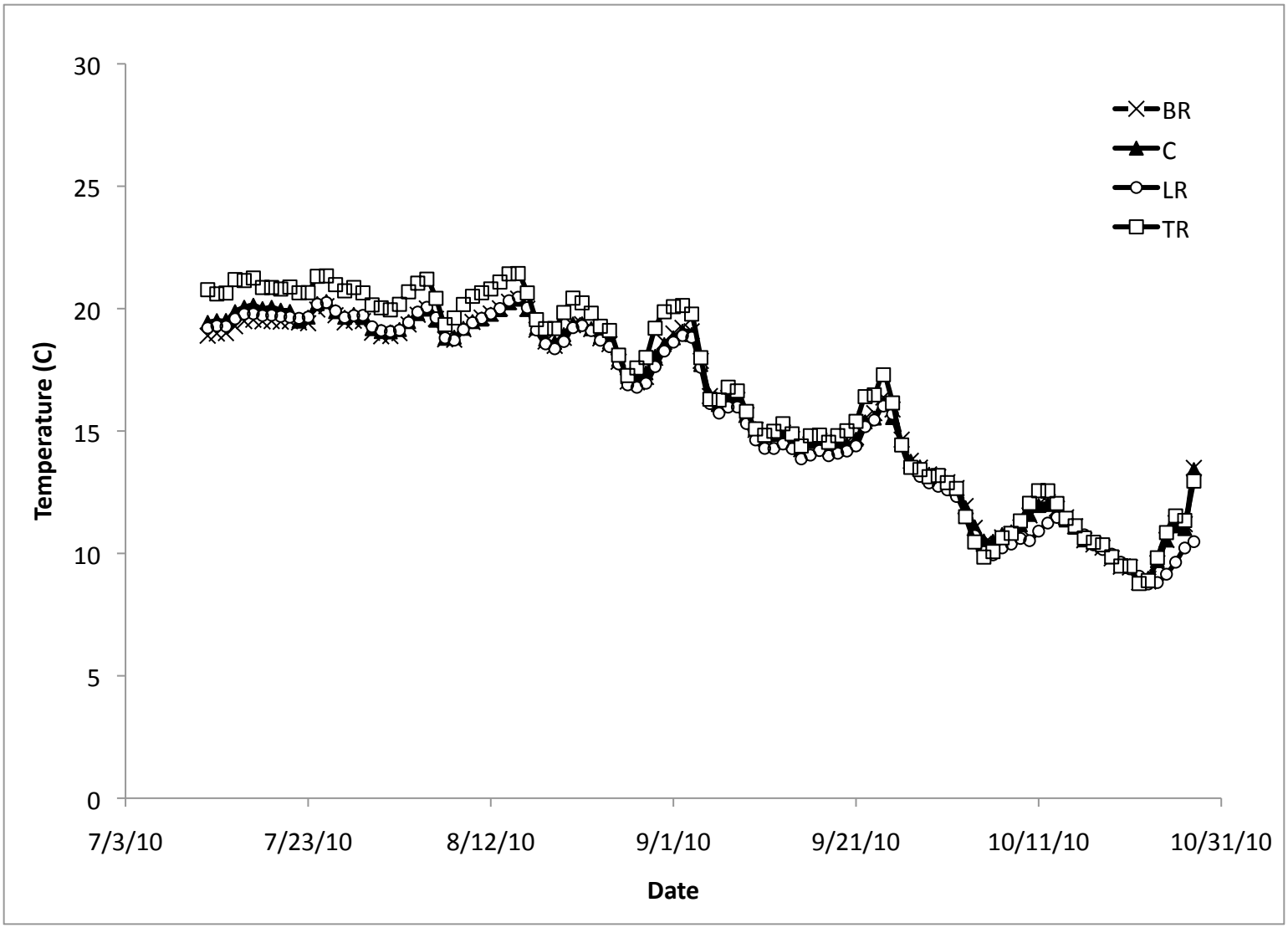

Figure 3-12 Daily mean temperature at 10 cm soil depth from July to October 2010 within control (C), biomass removal (BR), litter removal (LR), and total removal (TR) treatment plots.

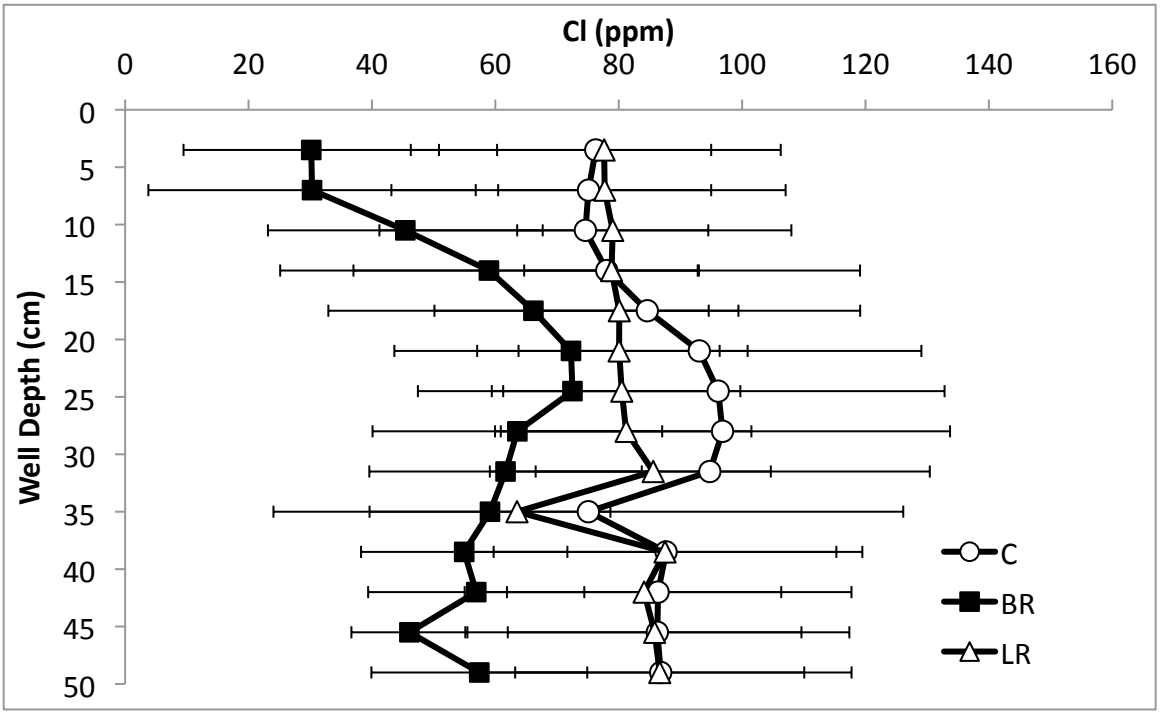

Figure 3-13. Mean Cl^- concentrations from porewater equilibrators within control (C), biomass removal (BR), and litter removal (LR) treatment plots averaged across sites. There was a significant effect of treatment ($F_{2,63} = 17.34$, $p < 0.001$) on Cl^- concentrations, but not depth or their interaction. Error bars represent 1 SE.

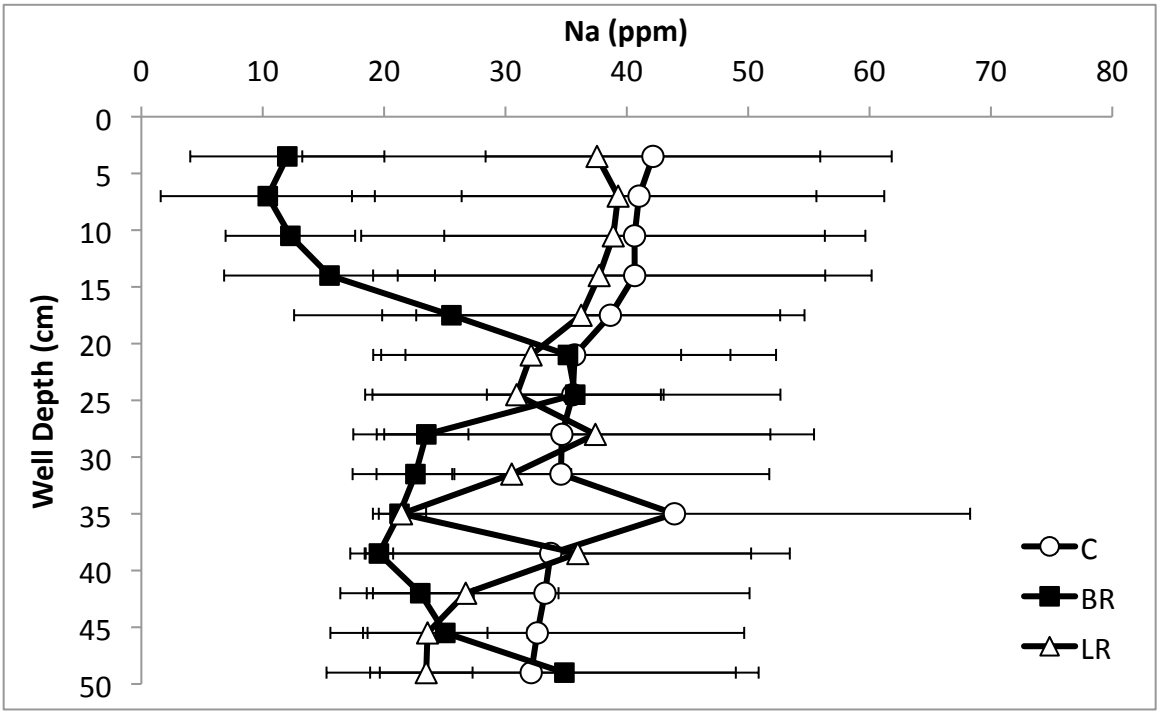

Figure 3-14. Mean Na^+ concentrations from porewater equilibrators within control (C), biomass removal (BR), and litter removal (LR) treatment plots averaged across sites. There was a significant effect of treatment ($F_{2,63} = 4.93$, $p = 0.01$) on Na^+ concentrations, but not depth or their interaction. Error bars represent 1 SE.

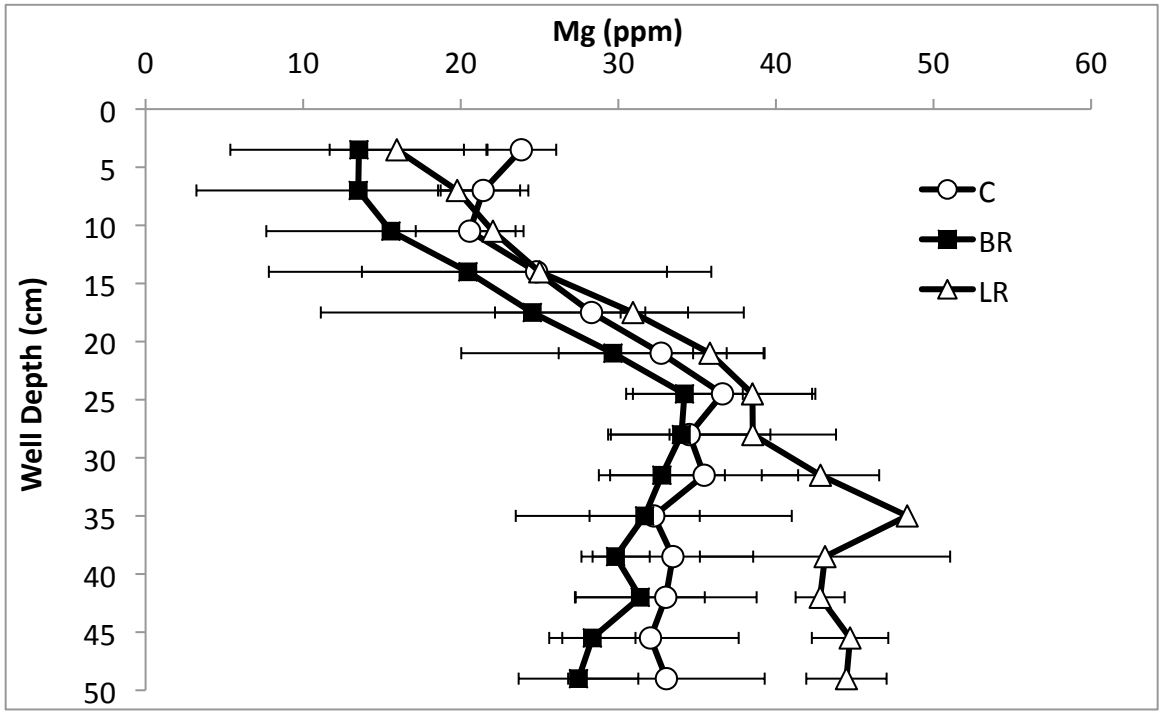

Figure 3-15. Mean Mg^{2+} concentrations from porewater equilibrators within control (C), biomass removal (BR), and litter removal (LR) treatment plots averaged across sites. There was a significant effect of treatment ($F_{2,63} = 3.29$, $p = 0.043$) and depth ($F_{13,63} = 9.20$, $p < 0.001$) on Mg^{2+} concentrations, but not their interaction. Error bars represent 1 SE.

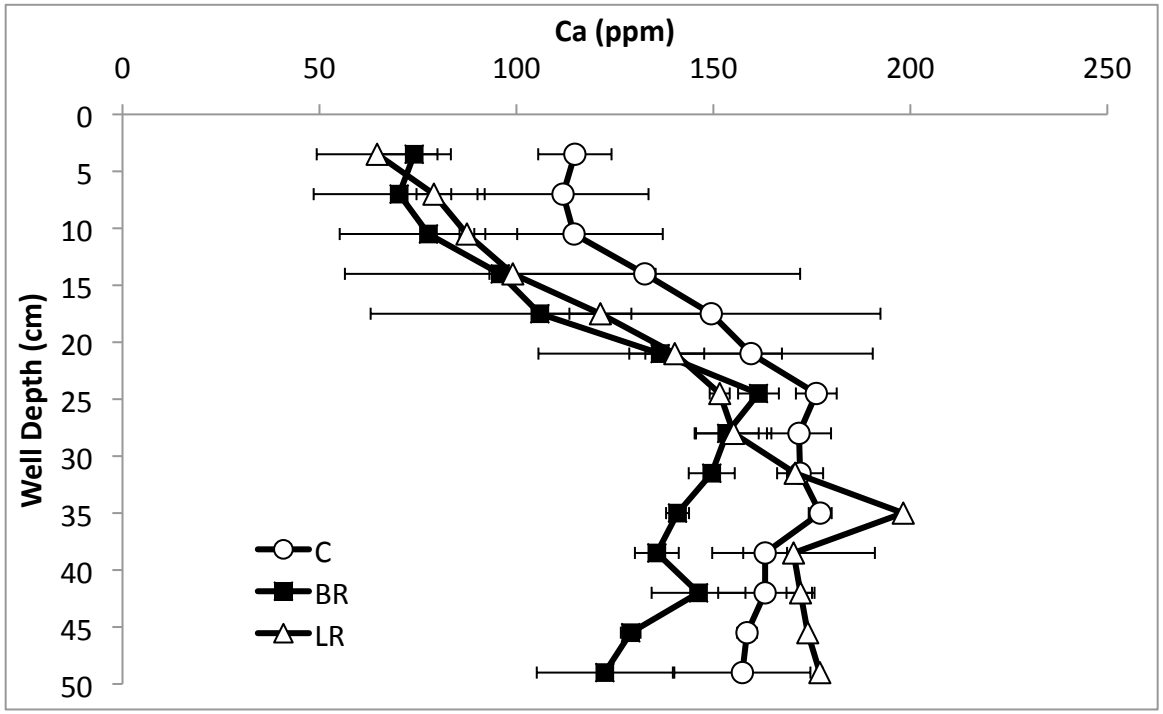

Figure 3-16. Mean Ca^{2+} concentrations from porewater equilibrators within control (C), biomass removal (BR), and litter removal (LR) treatment plots averaged across sites. There was a significant effect of treatment ($F_{2,63} = 7.54$, $p = 0.001$) and depth ($F_{13,63} = 5.44$, $p < 0.001$) on Ca^{2+} concentrations, but not their interaction. Error bars represent 1 SE.

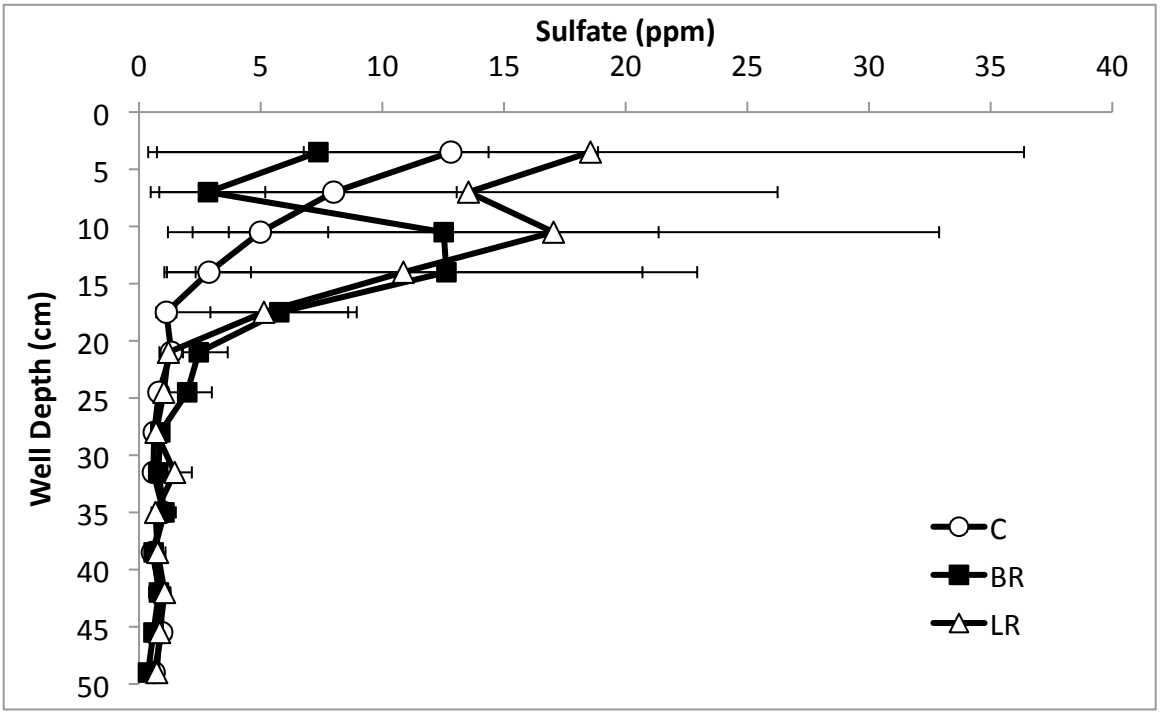

Figure 3-17. Mean SO_4^{2-} concentrations from porewater equilibrators within control (C), biomass removal (BR), and litter removal (LR) treatment plots averaged across sites. There was a significant effect of depth ($F_{13,63} = 4.48$, $p < 0.001$) on SO_4^{2-} concentrations, but not treatment or their interaction. Error bars represent 1 SE.

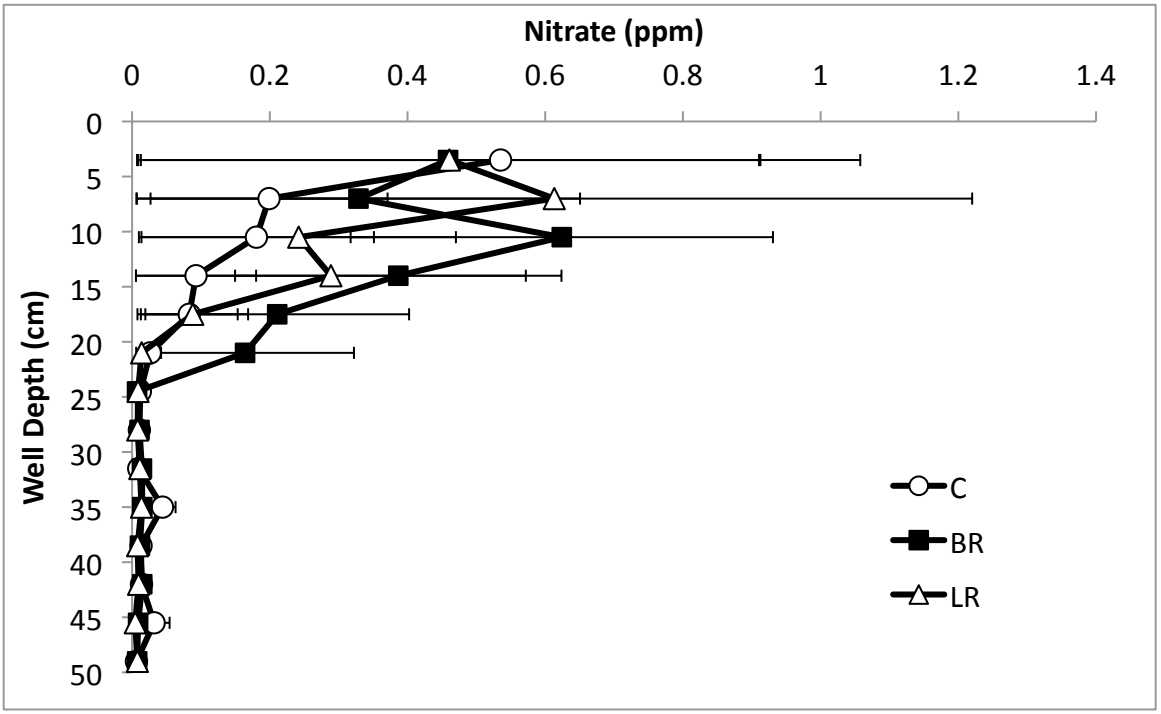

Figure 3-18. Mean NO_3^--N concentrations from porewater equilibrators within control (C), biomass removal (BR), and litter removal (LR) treatment plots averaged across sites. There was a significant effect of depth ($F_{13,63} = 3.16$, $p = 0.049$) and treatment on NO_3^- ($F_{13,63} = 2.91$, $p = 0.002$) concentrations, but not their interaction. Error bars represent 1 SE.

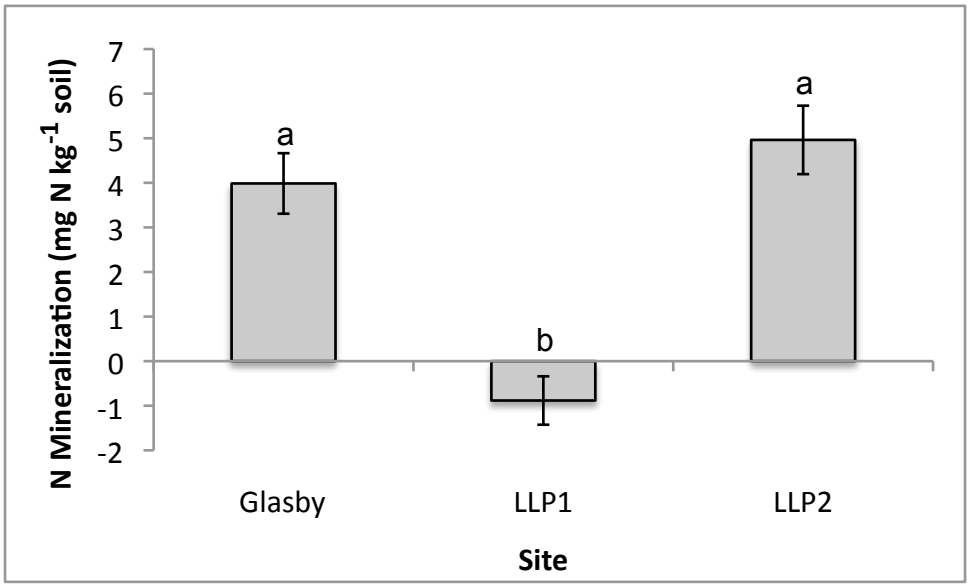

Figure 3-19. Mean net N mineralization rates among soil collected within monospecific stands of *Phragmites australis* at Glasby, LLP1, and LLP2. The effect of site was significant ($F_{2,87} = 65.57$; $p < 0.001$). Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

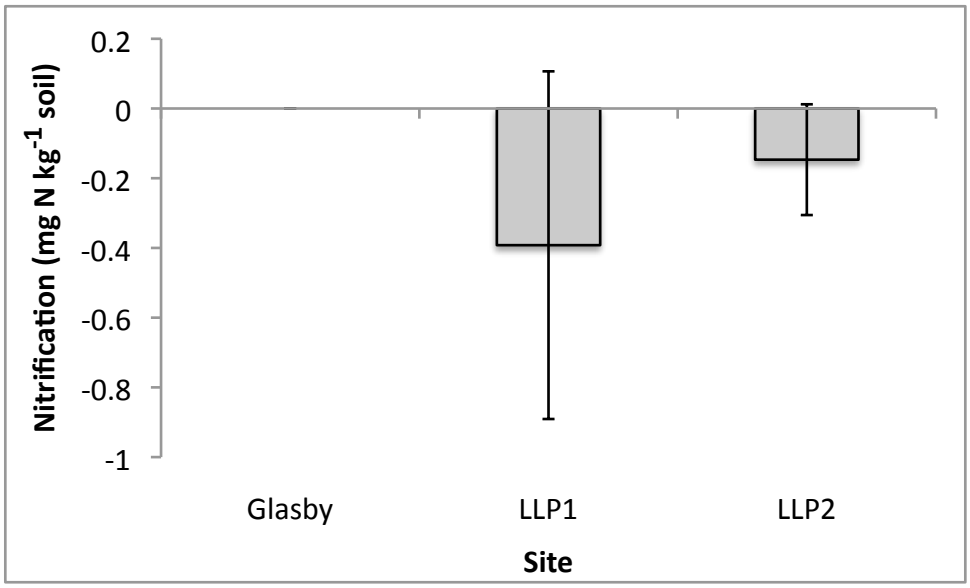

Figure 3-20. Mean net nitrification rates among soil collected within monospecific stands of *Phragmites australis* at Glasby, LLP1, and LLP2. The effect of site was significant ($F_{2,87} = 1.29$; $p < 0.001$). Error bars represent 1 SE.

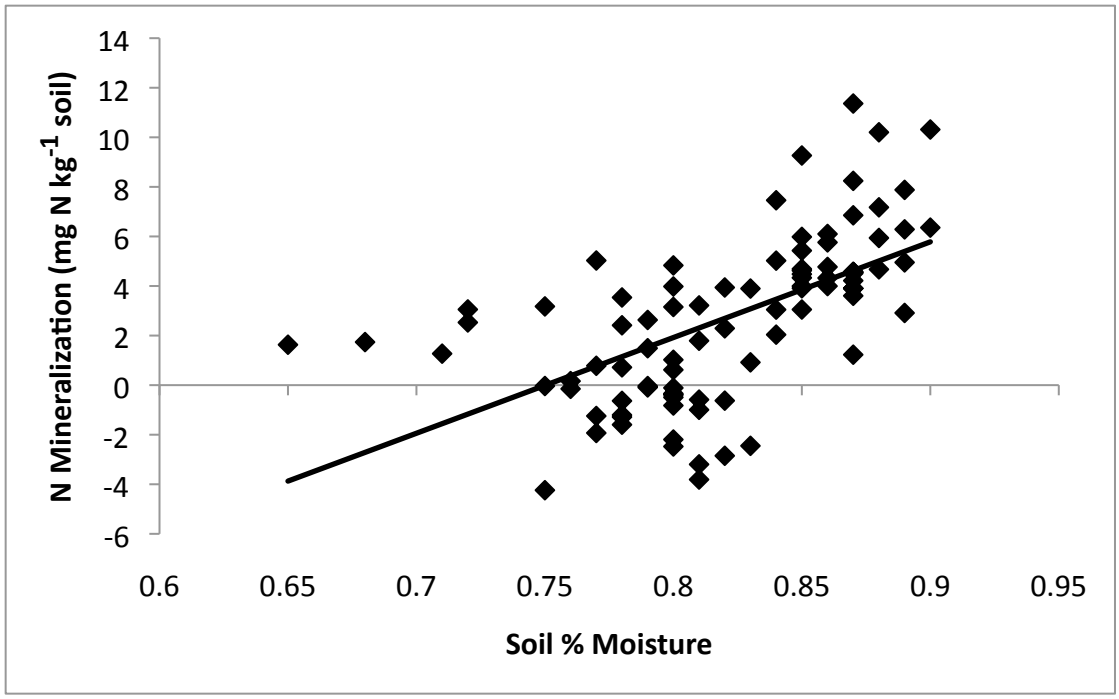

Figure 3-21. Regression between soil % moisture and net N mineralization rates (adjusted $R^2 = 0.297$, $p < 0.001$).

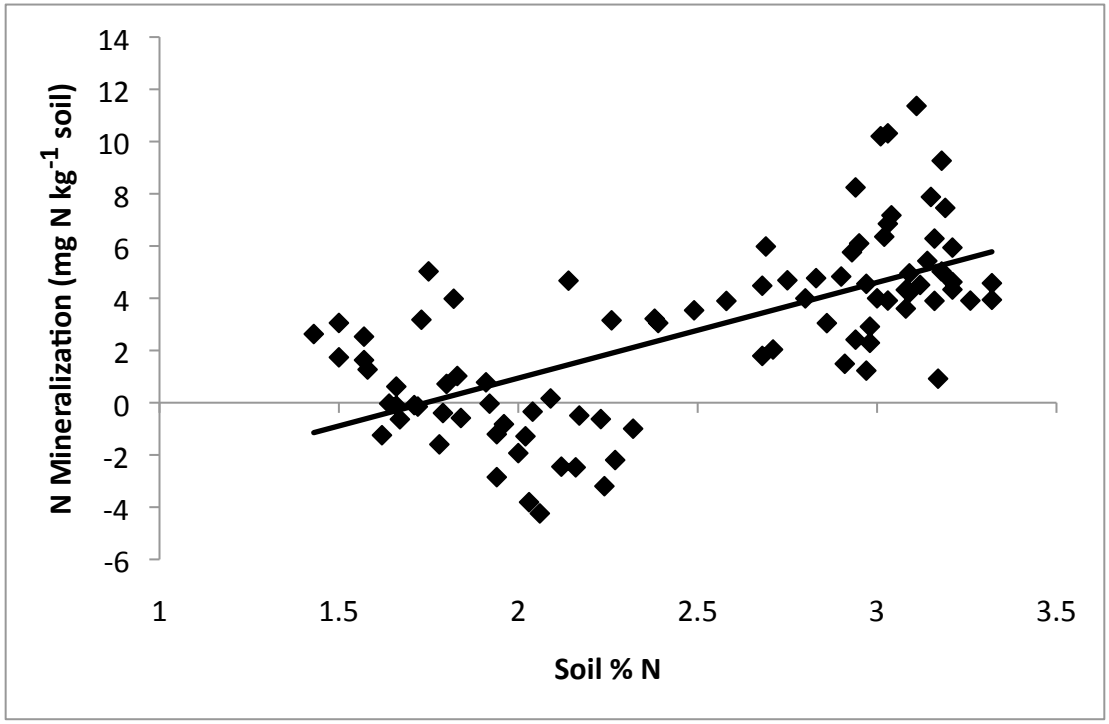

Figure 3-22. Regression between soil % moisture and net N mineralization rates (adjusted $R^2 = 0.406$, $p < 0.001$).

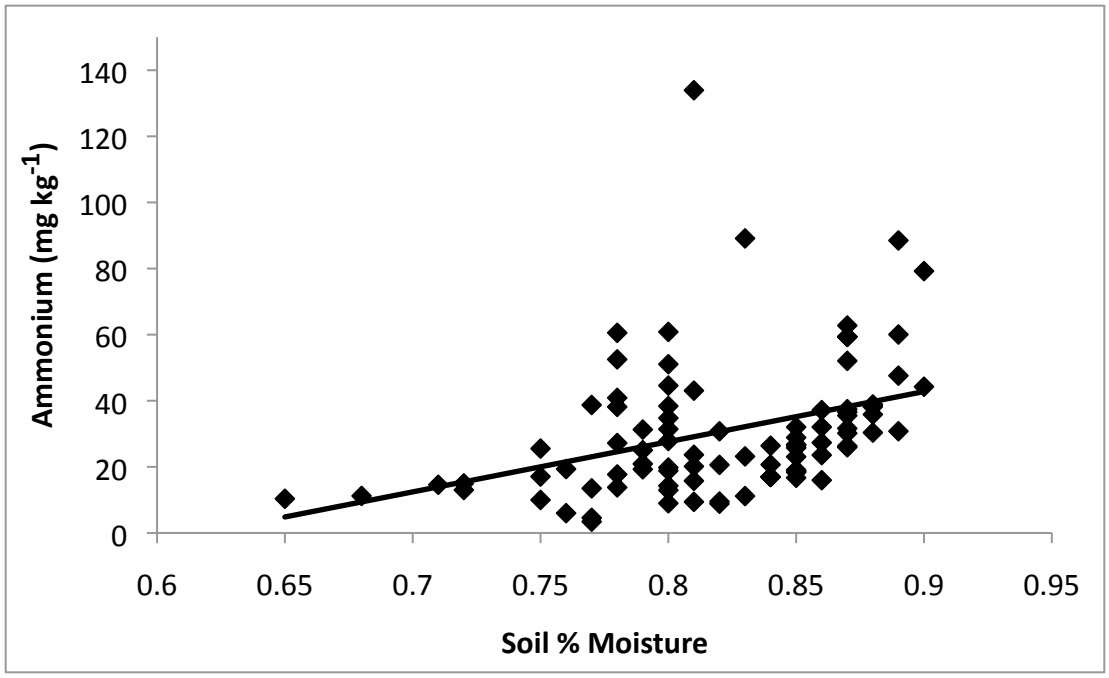

Figure 3-23. Regression between soil % moisture and net N mineralization rates (adjusted $R^2 = 0.125$, $p < 0.001$).

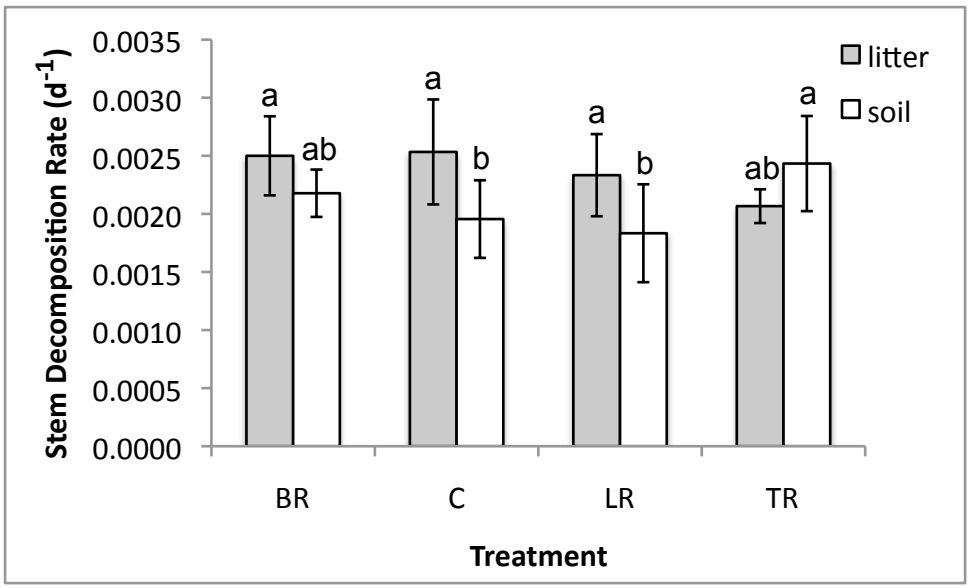

Figure 3-24. Mean first order rate constants (k; day^{-1}) for stems incubated within control (C), biomass removal (BR), litter removal (LR), and total removal (TR) treatment plots over a 90 day time period averaged across sites. There was a significant effect of depth ($F_{1,44} = 6.43$; $p = 0.014$), but not treatment or their interaction. Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

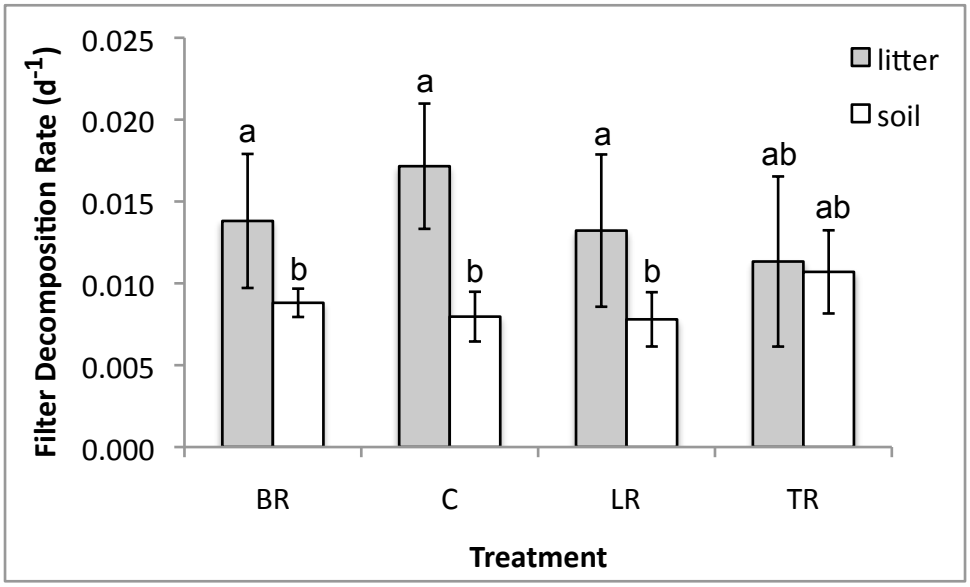

Figure 3-25. Mean first order rate constants (k; day^{-1}) for filters incubated within control (C), biomass removal (BR), litter removal (LR), and total removal (TR) treatment plots over a 90 day time period averaged across sites. There was a significant effect of depth ($F_{1,44}$ = 6.43; $p < 0.001$), but not treatment or their interaction. Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

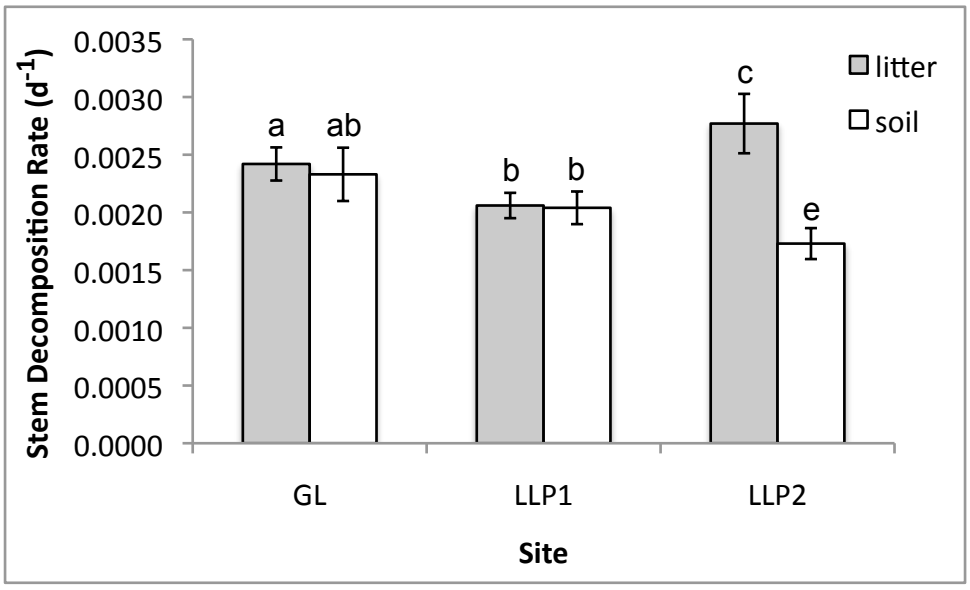

Figure 3-26. Mean first order rate constants (k; day^{-1}) for stems incubated at Glasby (GL), LLP1, and LLP2 sites over a 90 day time period. There was a significant interaction between site and depth ($F_{2,54}$ = 5.12; $p = 0.009$). Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

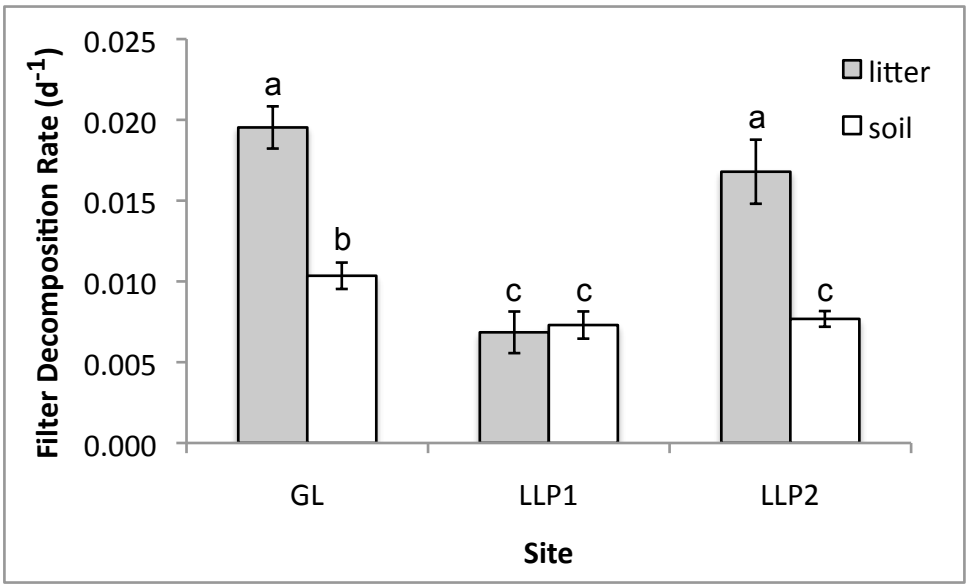

Figure 3-27. Mean first order rate constants (k; day^{-1}) for filters incubated at Glasby (GL), LLP1, and LLP2 sites over a 90 day time period. There was a significant interaction between site and depth ($F_{2,54} = 10.33$; $p < 0.001$). Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

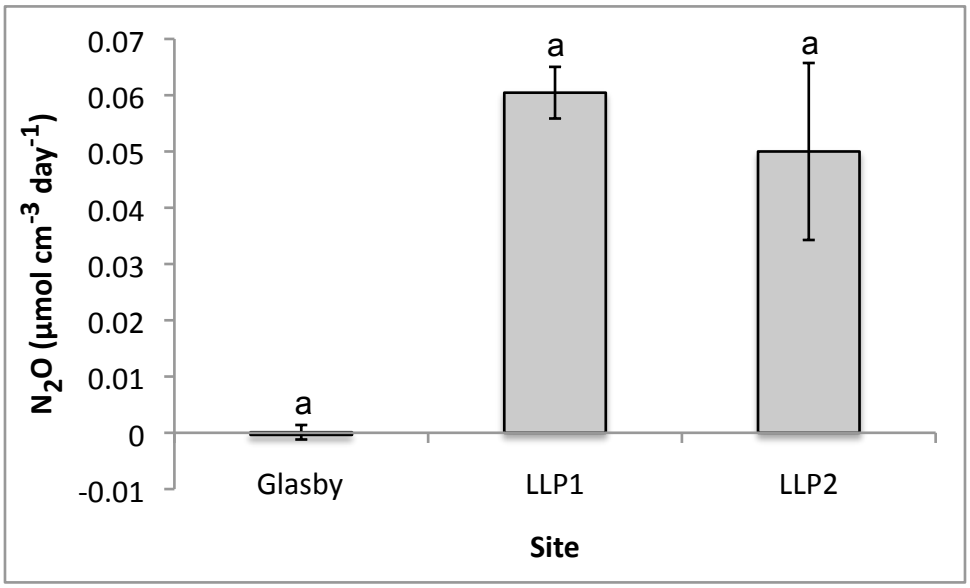

Figure 3-28. Mean denitrification rates (N_2O production) among soil collected within monospecific stands of *Phragmites australis* at Glasby, LLP1, and LLP2. The effect of site was significant ($F_{2,27} = 11.41$; $p < 0.001$). Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

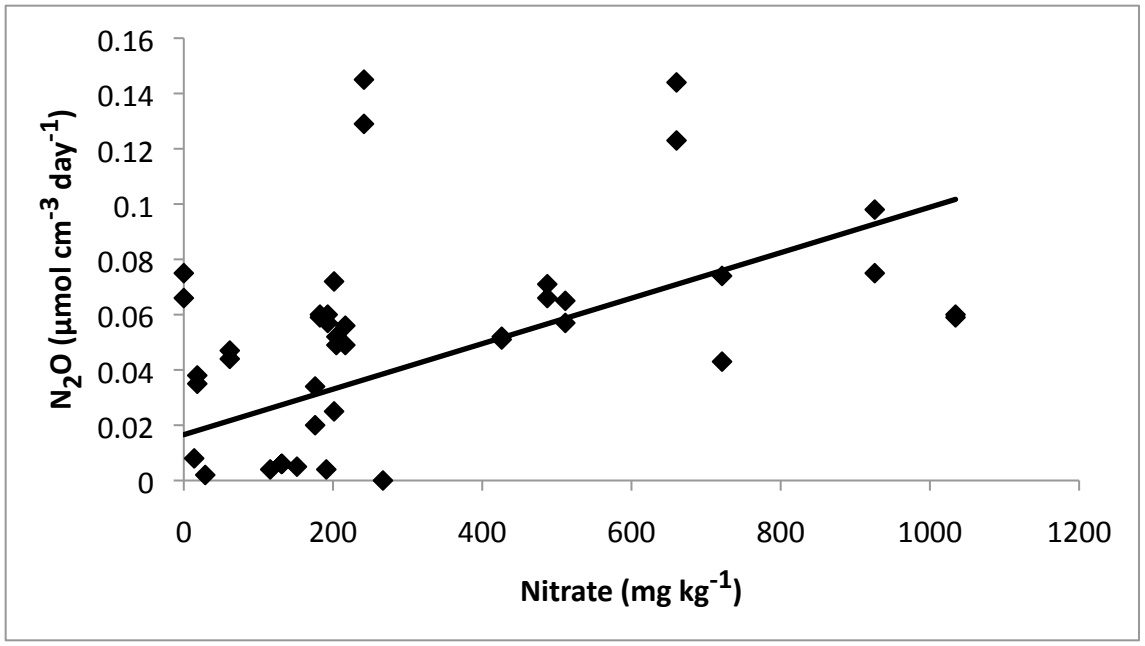

Figure 3-29. Regression between nitrate and denitrification rates (N_2O production)(adjusted $R^2 = 0.310$, $p < 0.001$).

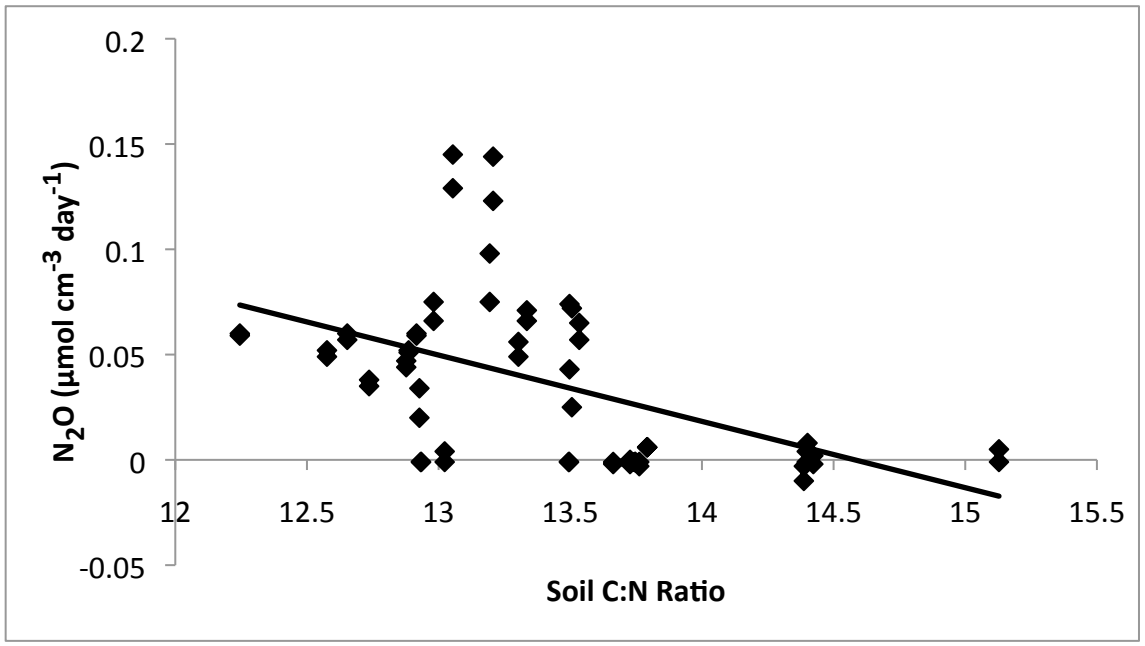

Figure 3-30. Regression between soil C:N ratio and denitrification rates (N_2O production) (adjusted $R^2 = 0.252$, $p < 0.001$).

Discussion

Water table position varied among study sites seasonally and among years, and when above the soil surface, the depth of standing water varied as well. These hydrologic differences created a gradient in water table position among sites, especially in 2010, that likely influenced several of the results discussed above. The more pronounced gradient in 2010 is notable because that was the year most of the investigations occurred, including all of the process rate assays. Throughout the study period in 2010, the water table was above the soil surface (standing water) at Glasby, fluctuated above and below the soil surface at LLP2, and was below the soil surface at LLP1 (Figure 3-5). Differences in water table position likely influenced site soil moisture, availability of nutrients due to changes in redox potential caused by drying and rewetting, and, if the differences in hydrology occurred on a long enough time scale, site organic matter buildup. Data support these predictions; LLP1 (lowest water table) had the lowest soil % moisture, the lowest ammonium concentrations, the highest nitrate concentrations, and the lowest soil % C (an estimate of organic matter) (Table 3-1). Ju et al. (2006) modeled hydrologic effects on C cycling in forests and wetlands in Canada and found that drainage class greatly influenced a wetlands ability to accumulate C, with poorly drained wetlands accumulating the most and well-drained wetlands accumulating the least. This supports my findings that LLP1 (well-drained) had the lowest soil %C and Glasby (pooly drained) the highest. The remainder of this section will focus on treatment and site effects on the ecosystem parameters measured in this study, with hydrology as the main mechanism for variation among sites.

The treatment effects on both light levels and temperatures during the growing season followed my predictions: control plots had the lowest light levels and the lowest

temperatures (for manual measurements), total removal plots had the highest light levels and temperatures, and both biomass removal and litter removal plots had intermediate levels. These data suggest a number of effects of biomass and litter removal. First, light levels were dramatically reduced (18 fold difference) in *Phragmites* stands compared to when *Phragmites* was absent (Figure 3-7). Second, litter and biomass removal both increased light levels to the same degree, which was significantly less of an increase compared to when it was completely removed. This also supports my predictions that both litter and biomass removal treatments would increase light levels, but the remaining litter (biomass removal) or biomass (litter removal) would reduce light levels compared to when completely removed (total removal). Similarly, Farrer and Goldberg (2009) found, in a litter transplant experiment, that *Typha* ×*glauca* litter significantly reduces soil surface light levels, but, interestingly, live *T.* ×*glauca* did not greatly affect light levels. One explanation for this difference between the effects of live biomass of *Phragmites* and *T.* × *glauca* on light levels is that *Phragmites* stems have a branching structure to them, while *T.* ×*glauca* has a much more linear structure. Third, even though there were differences in light levels and temperatures among treatment plots, the differences in light level among plots were of a much greater magnitude than differences in temperature. One possible reason for this magnitude discrepancy is that light level determination requires the ceptometer user to record light levels around noon on a cloudless sky. These conditions produce the most extreme difference in light levels among treatments but are likely not to be the most common circumstance each site experienced. Conversely, while manual temperature measurements were taken within a similar time range as light levels (11:00 am to 3:00 pm) on rainless days, cloudless skies were not requisite of temperature data

collection. These differences likely mean that manual temperature measurements likely were closer to actual soil conditions. Another possibility is the low temperature of the surrounding *Phragmites* stands reduced the temperature within the 2 x 2 m plots.

The tall stature and ability of living biomass and litter of *Phragmites* to shade the soil surface likely facilitates its invasion into native communities by creating light limitation below its canopy. Because *Phragmites* is a clonal species, and, therefore, new ramets can receive resources from parent plants, its own success will not be greatly affected by the low light conditions. One consequence for wetland restoration of the significant treatment effects on light levels and temperature is it is common practice to manage monospecific stands of *Phragmites* by the use of glyphosate, which usually successfully kills off the majority of individuals. If this sudden die-off isn't followed by a removal of all of the standing litter, light levels might still be low enough to impede the colonization of more desirable wetland plants until standing dead biomass decomposes, which can take several years. Farrer and Goldberg (2009) also found that shading effects from *T.* ×*glauca* litter decreased the abundance and diversity of native species. Gordon (1998) found that the ability to shade native competitors was a commonality among invasive species in Florida.

The temperature dataloggers allowed me to capture seasonal variation in temperature. Data from the HOBO dataloggers showed that the largest temperature difference among treatments was at the soil surface where total removal and litter removal plots had the largest temperature difference compared to reference plots (Figure 3-10). This temperature difference among treatments changed at the beginning of September to litter and total removal plots having the lowest temperatures, though before the beginning

of September total and litter removal plots periodically had the lowest soil surface on relatively cool days (less than ~ 19°C). This change that started in September was not predicted and could be due a lower capacity for total and litter removal plots to hold heat close to the soil surface during periods of cooling weather and, thus, were more dependent on incoming solar radiation to maintain high soil surface temperatures. MacKinney (1929) found similar effects of forest litter on temperature fluctuations over multiple seasons; litter acted like an insulator by decreasing daily high temperatures and increasing daily lows, and this effect diminished with soil depth. Soil temperatures at 2 and 10 cm depths showed that total removal plots were the only manipulated plots that had temperatures different from reference plots, though this difference was much reduced after September (Figures 3-10 and 3-11). These data suggest that litter plays a substantial role in the temperature regulation of the soil and these effects are likely to be greater with high litter producing species such as *Phragmites*.

The significant treatment effects on porewater chemistry likely are from the direct effect of *Phragmites*. Concentrations of Na^+ and Cl^- were lower in the biomass removal plots compared to the reference plots, which could be indicative of reduced evaporative concentration by evapotranspiration. A similar pattern of increased salinity due to plant transpiration was found in mangrove populations in French Guiana (Marchand et al. 2004), and *Spartina alterniflora* is known to alter the concentration of Na^+, Cl^-, and other salts due to ion exclusion and secretion when growing in brackish waters (Bradley and Morris 1991). The same mechanism could also cause the lower concentrations of Ca^{2+} in the biomass removal plots, though why there were also decreased Ca^{2+} concentrations in the

litter removal plots is less apparent. Similarly, the significant, albeit weak, pattern of lower nitrate concentrations in the reference plots could be explained by direct plant uptake, though nitrate concentrations were not lower in the litter removal plot where live biomass remained. Findlay et al. (2003) showed that nitrate in porewater wasn't affected by the presence of living *Phragmites*, though ammonium did increase with the removal of *Phragmites*.

The lack of any treatment effects on litter bag decomposition, N mineralization, nitrification, and denitrification was surprising, especially because there were significant treatment effects on light levels and soil temperatures. With an increase in light levels, and subsequent soil temperatures, *in situ* litter bag decomposition and net N mineralization rates should have increased due to the temperature sensitivity of these ecosystem processes. For *in situ* N mineralization/nitrification rates, one possible cause for the lack of treatment effects could have been that the soil cores were 10 cm in depth and treatment effects on soil temperature were greater closer to the soil surface, so using a 10 cm core could have obscured any treatment effect present near the soil surface. For the litter bag assay, the majority of the time (~ 60 of the total 90 days) the litter bags were in the field total and litter removal plots had lower temperatures than reference plots, which was opposite of what was predicted and likely led to the lack of treatment effects on decomposition rates. Potential denitrification was conducted in the laboratory free from temperature variation experienced in the field, so differences in denitrification potential would have been indicative of either organic matter quality changes or nitrate availability, though no treatment effects were detected. Contrary to what I found, Findlay et al. (2003) found that one year after *Phragmites* removal by herbicide, potential denitrification

dropped by 50% (compared to living plots) and this decrease was possibly due to the cessation of oxygen release by the roots which allowed for nitrification and therefore nitrate needed for denitrification. The buildup of ammonium after *Phragmites* removal gave further evidence for this mechanism (Findlay et al. 2003).

While no significant treatment effects were detected for any ecosystem process rate investigated, there were significant site effects. Net N mineralization rates were the lowest at LLP1 where rates were negative (indicative of net N immobilization and/or denitrification), while rates at Glasby and LLP2 were positive (Figure 3-19). The biggest difference between LLP1 and the other two sites was the low position of the water table causing drier conditions. Likely due to the more aerobic soil conditions, nitrate levels were higher at LLP1 than the other two sites, and ammonium concentrations were low (Table 3-1). The negative net N mineralization and nitrification rates therefore could have been a consequence of denitrification occurring within the sealed soil cores in the field, because while LLP1 was the driest site, it was likely still moist enough to have anaerobic soil microsites, which have been shown to be sites of denitrification (Burgin et al. 2011). Because LLP1 had the greatest starting nitrate concentrations and ended with the lowest, nitrification followed by denitrification is a likely fate of remineralized N. While microbial N immobilization is another possibility for the negative net rates at LLP1, it is unlikely based on the results from Chapters 2 and 4 that showed when incubated in the lab, soil incubated alone rarely shows signs of N immobilization. The positive relationship between soil %N and net N mineralization rates indicates that soils with more N (likely in organic forms) have a greater potential for mineralization (Figure 3-22) and has been seen in similar *in situ* N mineralization assays (Finzi et al. 1998).

There were no depth effects on stem decomposition at Glasby or LLP1 and rates were comparable between sites. At LLP2 there were strong depth effects with stem decomposition being the greatest at the soil surface (Figure 3-26). One possible explanation for this pattern is LLP1 and Glasby were relatively constant in water table position over the course of the incubation, though the actual water table position varied considerably between sites; the water table at LLP1 was below the soil surface and Glasby had constant flooded conditions. Conversely, LLP2's water table fluctuated from above to below the soil surface multiple times throughout the assay periods, which could have created ideal conditions for decomposition as the wetting, drying, and rewetting likely added moisture, nutrients, and aerobic conditions. Jarvis et al. (2007) showed that drying and wetting conditions increased decomposition of Mediterranean soils, but there is also evidence that in some cases a wetting-drying cycle doesn't have much effect (Day 1983) and the magnitude of effect may be greater for arid and semiarid soils (Borken and Matzner 2009). Filter paper decomposition was higher on the soil surface at Glasby and LLP2 compared to LLP1 (Figure 3-27), which could be due to those two sites having periods of complete inundation that drove the breakdown of the filter paper by both physical and microbial means (increased microbial colonization when submerged in water [Glasby and LLP2] compared to when on a relatively dry soil surface [LLP1]) (Day 1983). The higher decomposition rates for filter paper compared to stem tissue shows the recalcitrant nature of the stem tissue and the high organic matter buildup seen in *Phragmites* stands attests to this: the more labile leaves decompose quickly while the recalcitrant stems buildup in the soil (Kominkova et al. 2000; Agoston-Szabo and Dinka 2008).

The absence of potential denitrification at Glasby is probably due to the low nitrate levels in the soil (Figure 3-28; Findlay et al. 2003; Reddy and DeLaune 2008), which is likely caused by previous denitrification that already removed nitrate from the soil/water column. Also, the soil C:N ratio was the highest at Glasby compared to LLP1 and LLP2 (Table 3-1) and soil organic matter quality has been shown to influence denitrification rates (Burford and Bremner 1975; Reddy and DeLaune 2008). The most consistently high rates of denitrification were from LLP2 and can, as for the N mineralization rates, be attributed to the fluctuating position of the water table that created an ideal environment for the alternation between nitrification and denitrification (McClain et al. 2003; Jacinthe et al. 2012). The multiple regression analysis showing nitrate concentration and soil C:N ratio as significant predictors of denitrification further supports this explanation for the low denitrification rates at Glasby, as Glasby had both low nitrate concentrations and low soil quality.

Taken together, the significant effects of site on litter bag decomposition, N mineralization/nitrification, and denitrification, along with the differences in soil characteristics among sites, suggests the strong influence site differences can have on wetland biogeochemical cycling, even within monospecific stands of the same plant species. The most conspicuous difference among sites was their hydrology. The differences in hydrology among sites likely influenced the differences seen in biogeochemical process rates, nutrient availability (particularly nitrate), soil moisture, soil organic matter, and possibly the depth of the belowground biomass placement of *Phragmites* (Table 3-1). Changes in hydrology have been shown to alter almost every aspect of the physical and chemical nature of wetlands (LaBaugh 1986; van der Valk 2000).

The BGB differences seen among sites (highest at Glasby and LLP2, compared to LLP1) unlikely demonstrates a true difference in BGB production because AGB was comparable among sites, and probably suggests *Phragmites* places most of its BGB close to the soil surface in consistently waterlogged systems to avoid anaerobic soil conditions. At LLP1, where the water table was consistently below the soil surface, *Phragmites* was likely to extend its BGB well below the top 30 cm of soil to scavenge for more nutrients without having the negative effects of anaerobiosis. This behavior has been seen in other plants; for example, Schwintzer and Lancelle (1983) showed the rooting depth and root morphology of *Myrica gale*, a shrub found in peatlands, was dependent on the depth of the water table with roots growing more horizontally the closer the water table was to the soil surface.

Future studies should further examine the effects of flooding regime on biogeochemical cycling within *Phragmites* stands, along with the more direct effects of flooding regime on the plasticity of *Phragmites*. Additionally, this study only looked at biogeochemistry within *Phragmites* stands, but should be expanded to include other dominant wetland species to determine species identity matters on the hydrology effects seen in this investigation. Some of the expected effects of living biomass and litter of *Phragmites* on C and N cycling were not seen in this study, which was likely a consequence of the relatively short time span of the study. Replicating this study over a longer time scale, e.g., 5-15 years, would allow for more gradual effects, such as changes in soil OM quality and quantity, to occur. It is likely that longer studies would find that living *Phragmites* is integral to maintaining high soil OM matter content usually seen under monospecific stands by continual inputs of litter and significant soil shading that decreases OM decomposition.

Chapter 4
Effects of Litter Quality, Soil Origin, and Plant Species Diversity on Decomposition in Temperate Wetlands

Brief Rationale

The chemical and physical characteristics of plant litter have been shown to influence ecosystem processes in many environments (Melillo et al. 1982; McClaugherty et al. 1985; D'Antonio and Vitousek 1992; Cornwell et al. 2008), and this becomes especially apparent when an invasive plant becomes abundant in a new environment. For example, invasion of *Bromus tectorum* (cheatgrass) into the arid grasslands of the Colorado Plateau (USA) decreased N mineralization rates due to the significantly greater C:N ratio of litter inputs from *B. tectorum* compared to native species (Evans et al. 2001). Species-specific litter quality (C:N, lignin:N, etc.) can influence C and N cycling by controlling decomposition rates (Ehrenfeld 2003; Eviner and Chapin 2003; Eviner 2004; Orwin et al. 2008). *Myrica faya*, a nitrogen-fixer that invaded young volcanic sites on Hawaii, was shown to enhance N cycling by producing N-rich litter that decomposed quickly releasing plant available N (Vitousek et al. 1987). Species-specific differences in litter quality is a potentially important aspect of invasions by exotic plants in wetlands because often the invasive species becomes dominant over all other species. Invasive species can also invade into monospecific stands of other invasive species, though the effects of this type of invasion on biogeochemical cycling is largely unknown

To separate species-specific litter quality from other controls on decomposition and N transformation rates, I performed a laboratory incubation using both litter and surficial soil from wetlands with nearly monospecific stands of three widespread invasive wetland plants, *Phalaris arundinacea*, *Phragmites australis*, and *Typha* ×*glauca* (hereafter *Phalaris*,

Phragmites, and *Typha*, respectively), as well as from an additional wetland dominated by *Carex lacustris* (a native sedge; hereafter *Carex*). The sedge-dominated wetland represents a community that has not been invaded and hence served as a reference relative to invaded plots. All four study species are in the same functional group (clonal, emergent wetland dominants; Boutin and Keddy 1993), so if significant differences are found among species this might suggest that invasions can alter carbon cycling even if the plants are similar in growth form.

Additionally, few studies have tried to determine if dominant plant species invoke strong soil legacy effects (termed "soil origin effects" in this study) that can influence the decomposition of organic matter inputs. Soil origin effects can have significant influences on C and N cycling independent of the characteristics of the litter inputs or climate. The mechanisms of soil origin effects can range from differences in soil microbial community to nutrient availability to organic matter content. For example, soil microbial communities can be adapted to the particular physical and chemical characteristics of the litter they are accustomed to decomposing (Gholz et al. 2000; Strickland et al. 2009). This has been referred to as "home-field advantage" (Ayres et al. 2009) and usually results in higher decomposition rates of resident litter. If dominant plant species from the same functional group can influence C and N cycling by species-specific litter and soil origin effects, then there might be less justification for categorizing wetland species, especially invasive species, into generalized functional groups.

While the incubation briefly explained above explored the effects of litter identity and soil source on C and N mineralization, I also was interested in the effects of litter diversity on C and N mineralization. For this, I conducted a second incubation experiment in which I

mixed different combinations of litter from the four species described above into a common soil. Litter diversity has been shown to enhance decomposition in some experiments, but the outcomes of mixing are not always predictable from single species litter treatments (Blair et al. 1990; Hector et al. 2000). Understanding the consequence of litter diversity is important because invasive species often become dominant but may or may not form monospecific stands. In most cases, plant invasion decreases the diversity of litter inputs to soil systems due to a decrease in plant species diversity. If litter diversity were correlated with decomposition rates, then one outcome of monospecific stand formation (and hence a single species litter layer) would be a reduction in overall decomposition and an increase in organic matter buildup. In other words, invasive species would decrease decomposition rates when they reduce species (litter) diversity.

Objectives, Hypotheses, and Predictions

***Objective 1*:** To separate species-specific litter quality from other controls on C and N mineralization rates and to determine if dominant wetland species "condition" soil substrate.

***Hypothesis 1*:** C and N mineralization rates will depend not only on the species-specific quality of the litter (high quality = high mineralization rates), but also on the conditioning of the soil by the dominant species (significant soil effect). As a result, I predict:

1. The four study species will vary in litter quality (C:N ratio) with *Phragmites* litter being the most recalcitrant (higher C:N ratio) and *Carex* the least (lower C:N ratio)

2. There will be a negative relationship between litter C:N ratios and C and N mineralization rates
3. After background soil C mineralization rates (based on soil emissions of CO_2 without added litter) are factored out of total C mineralization (soil + litter C mineralization), there will still be a significant soil effect, indicating a soil origin effect.

Objective 2: To determine if wetland species litter diversity influences C and N mineralization. This is an important component of the biogeochemical implications of invasions because the initial stages of invasion should result in a diverse litter layer, with C and N cycling feedbacks potentially affecting the ability of the invader to become dominant.

Hypothesis 2: Mixtures of litter from different dominant wetland species will have higher decomposition rates compared to single litter type additions due to higher substrate diversity. As a result, I predict:

1. There will be a positive relationship between litter diversity and cumulative C mineralization.
2. Species-specific litter decomposition rates in single litter treatments will predict which litter diversity treatments will decompose the fastest, e.g., the two species with highest decomposition rates in single litter treatments will result in the highest decomposition rates when incubated together.

Methods

Litter quality and soil origin incubation

The laboratory incubation was setup as a 4 x 5 factorial treatment design and was conducted from 23 April to 29 June 2010. Four soil origin factor levels were crossed with five factor levels of species-specific litter for a total of 20 treatment combinations. The four soil origin levels were soil collected within monospecific stands of *Phalaris*, *Phragmites*, *Typha*, and *Carex*. The species-specific litter levels were the same four species as the soil origin factor with the addition of a no litter addition factor level. Each treatment combination was replicated six times for a total 120 experimental subjects (120 incubation jars = 4 soil origin levels x 5 litter levels x 6 replicates). The factorial design enabled the detection of interactions between soil origin and species-specific litter, as well as the influence of soil origin identity on litter decomposition.

Site description and sample processing

Soil and litter samples used for this laboratory incubation were collected from Lake Lansing Park North (LLP)(42°46'07"N 85°23'32"W), Haslett, MI. Lake Lansing Park North is embedded in a mosaic of lakes, woodlots, and residential areas. The total area of the park is 166 hectares and consists mainly of deciduous forest, coniferous forest, and a large interconnected wetland complex of approximately 70 hectares. The park has been extensively invaded by a variety of invasive plant species, including *Phalaris*, *Phragmites*, and *Typha*. The wetland complex has become a mosaic of monospecific stands of these three species, though some areas are mixtures of these three invasive species along with some native species. Some of the more conspicuous native species include *Carex* spp.,

Juncus spp., *Sambucus canadensis*, *Leersia oryzoides*, *Eutrochium* spp., *Asclepias incarnata*, *Echinocystis lobata*, and *Impatiens capensis*. Litter was collected from monospecific stands of the four study species, *Phalaris*, *Typha*, *Phragmites*, and *Carex* (Figure 4-1), during the fall of 2009 after all plants had senesced, but before the first snowfall to avoid leaching. *Carex*, a native sedge, was used in this experiment because it has a tendency to form monospecific stands similar to the invasive plant species in this study and thus allowed for the comparison between native and invasive species with similar growth habits. Each monospecific stand was greater than 200 m^2 and at least 90% of the species cover was of the study species. Each monospecific stand had similar hydrology: early spring rain would raise water levels to a maximum (< 1 m above ground), and by the end of the growing season the water level was below the soil surface.

Within each monospecific stand, fresh species-specific litter (recently senesced and fallen) was collected within six 1-m^2 plots using linear transect sampling with 2.5 meters between each plot. In Spring 2010, six 10-cm deep cores (166 cm^3) were collected directly adjacent from each litter sampling plot and sealed in whirl-pak plastic bags (Nasco), immediately placed on ice, and transported to the laboratory where they were kept at 4°C for 2 days until the start of the incubation.

After being stored at 4°C for 2 days, I sieved (4 mm) each core to remove large roots and rocks. Each soil core was then sub-sampled for bulk density and % moisture determination (60 g), as well as for initial nitrate and ammonium concentration determination (15 g, see below for N mineralization/nitrification methods). The dry mass of the 60 g sub-sample was measured after oven drying at 80°C for 48 hours. Dried soils

were then ground and homogenized using a mortal and pestle and run on an elemental combustion system (Costech ECS 4010, Valencia, CA) for %C and %N analysis. Samples were run in duplicate with atropine used as a standard every 10 samples. The remaining wet soil was then split into five subsamples of 20 g and each subsample was placed into a 250 mL incubation jar (Chromatographic Specialties, Inc.; Ontario, Canada). These incubation soils were equilibrated for 7 days at 4°C prior to litter treatment addition to allow the C mineralization pulse from root death to pass. Soil C:N ratios are expressed on a mass basis.

Litter collected at each monospecific stand was air dried at room temperature and stored for 6 months before processing. All litter samples taken from a species-specific monospecific stand were combined and mixed to homogenize litter characteristics. Litter was then separated by organ (stems, leaves, and inflorescences). Species-specific organ subsamples were then cut by hand and sieved through a 4- and 2-mm mesh. The 2 to 4 mm litter pieces were used in the experiment to ensure contact with the soil within each incubation jar. For each species' litter, multiple subsamples were taken from the homogenized mixture for chemical analysis. Litter %C and %N were determined on an elemental combustion system (see above) and C:N ratios are expressed on a mass basis.

For each replicate of each treatment combination, one gram of litter (1:1 ratio of stems to leaves) was hand mixed into the soil to maximize physical contact between soil and litter. Jars were then allowed to equilibrate for 3 days and were sealed with lids fitted with septa to allow room air to enter the jar until the start of a CO_2 sampling round (see below). All jars (120 total) were incubated in the dark for 68 days. Similar soil moisture conditions were created across all incubation samples by bringing each to comparable

moisture contents before the beginning of the experiment (40-60% representing ideal non-limiting conditions). Moisture content was maintained weekly over the course of the incubation by adding an appropriate volume of distilled water after each jar was weighed to determine water loss.

Carbon mineralization assay

Carbon mineralization rates were estimated during seven 24-hour periods over the 68-day incubation (days 1, 4, 8, 13, 26, 41, and 68). During each period, a 10 mL headspace gas sample was taken from each jar immediately after jars were sealed (time = 0) using a three-way stopcock-fitted syringe, and then after 6, 12, and 24 hours. Before gas samples were extracted, each jar was shaken by hand to mix soil pore spaces and to release trapped gas bubbles. After headspace samples were collected, jars were backfilled with 10 mL of N_2 gas to ensure constant air pressure and volume. After each round, lids were removed, samples were gently flushed with ambient air, and jars were wrapped with plastic wrap to prevent moisture loss.

The headspace gas samples were analyzed for CO_2 concentrations by injecting the 10 mL sample into a PP-system EGM-4 infrared gas analyzer (IRGA). The IRGA was standardized using a CO_2 standard gas after every 20 injections. Carbon dioxide concentrations were corrected for headspace dilution caused by backfilling with N_2 gas. The slope of CO_2 concentrations over the incubation period was used to calculate C mineralization rates. No slope had an R^2 value of less than 0.85 and the majority (>95%)

were greater than 0.95. CO_2 production was assumed to be due to C mineralization (decomposition).

The C mineralization rates for litter addition treatments were background-corrected based on CO_2 production in No Litter treatment counterparts (assumed to represent ambient soil C mineralization) for the particular day of measurement, and then divided by litter dry weight to report data on a mass basis (g CO_2-C kg^{-1} litter C day^{-1}). First order rate constants (k, day^{-1}) were calculated by taking the natural log (In) of the C mineralization rates and then determining the slope of the In transformed rates over the time course of the incubation. Total CO_2 production was calculated by fitting a nonlinear curve (log) to the time course of rates described above, then interpolating daily CO_2 production and summing these values.

N mineralization/nitrification assay

Initial NO_3^- and NH_4^+ concentrations were determined from the 15 g subsample collected at the time of initial soil processing using the KCl extraction method (Robertson 1999). After the 68-day incubation period, the contents of each jar (litter and soil) were extracted for final NO_3^- and NH_4^+ concentrations using the same method. Concentrations of NO_3^- and NH_4^+ in the extracts were analyzed by the microplate method using protocols developed by Dr. David Rothstein (Dept. of Forestry, MSU). Net rates of N mineralization and nitrification were calculated from the changes in inorganic N ($NH_4^+ + NO_3^-$) or NO_3^-,

respectively, during the incubation period (initial – final pool sizes/incubation period). N mineralization and nitrification rates were expressed on a soil mass basis similar to the C mineralization component of the experiment.

Litter diversity incubation

The litter diversity incubation was designed as a single factor treatment structure and was conducted from 1 December 2010 to 24 January 2011. The single factor of species-specific litter diversity consisted of 16 factor levels of different combinations of litter collected from monospecific stands of *Phalaris*, *Phragmites*, *Typha*, and *Carex*. The 16 treatment levels consisted of all possible species combinations within a litter diversity level and were as follows: single species (4), two species (6), three species (4), all species (1) and no litter (1). A replacement design (as opposed to an additive design) was used for litter mixtures (see below). All litter treatment levels were incubated in a "common soil", meaning the soil was not collected from a monospecific stand of any of the four species. Instead, the soil was collected from a shallow, unvegetated ephemeral pond to minimize the possible conditioning effects plant species could have on the soil. Each treatment level was replicated seven times for a total 112 experimental subjects (incubation jars). This experimental design was developed to test the effects of wetland species litter diversity on C and N transformation rates.

Site description and sample processing

Soil and litter samples used for this laboratory incubation were collected from Lake Lansing Park North, within the same wetland complex used for the soil origin incubation

described above (Figure 4-1). Litter was collected using the same protocol and from the same monospecific stands of the four study species as in the soil origin incubation. During the fall of 2010, litter was collected after all plants had senesced, but before the first snowfall to avoid leaching. Within a unvegetated ephemeral wetland, twenty 10-cm deep soil cores (166 cm^3) were collected during fall 2010 using linear transect sampling with one meter between each core. These soil cores were sealed in whirl-pak bags (Nasco), immediately placed on ice, and transported to the laboratory where they were kept at 4°C for 2 days until the start of the incubation.

After being stored at 4°C for 2 days, each core was sieved (4 mm) to remove large roots and rocks. Each sieved soil core was then combined and homogenized in a large plastic container. Eighteen sub-samples (30 g) were taken for bulk density and % moisture determination, and eighteen more subsamples were taken for initial nitrate and ammonium concentration determination (15 g, see below for N mineralization/nitrification methods). The dry mass of the eighteen 30 g sub-samples was measured by oven drying at 80°C for 48 hours. Dried soils were then ground and homogenized using a mortal and pestle and run on an elemental combustion system (Costech ECS 4010, Valencia, CA) for %C and %N analysis. Samples were run in duplicate with atropine used as a standard every 10 samples. From the homogenized wet soil, 112 30-g subsamples were placed into separate 250 ml incubation jars (Chromatographic Specialties, Inc.; Ontario, Canada). These incubation soils were equilibrated for 7 days at 4°C prior to litter treatment addition to allow the C mineralization pulse from root death to pass. Litter collected at each monospecific stand was air dried at room temperature for two weeks before processing. Litter %C and %N were determined on an elemental combustion system, while %lignin

was determined by near infrared reflectance spectrophotometry (samples analyzed by Litchfield Analytical Services, MI; McLellan et al 1991).

The remaining litter processing was carried out as in soil origin incubation. For each replicate of each treatment level, 2 g of litter was hand mixed into the common soil to maximize physical contact between soil and litter. As mentioned above, litter was added within a replacement design, as opposed to an additive design. The same total amount of litter was added to each incubation jar regardless of litter diversity treatment, e.g., 2 g of *Phragmites* litter was added for the single species additions, but for the two species litter addition of *Phragmites* and *Phalaris*, 1 g of each was added. A replacement design was used to keep the total amount of litter the same because changing the amount of total litter added would alter the decomposition rates and would confound any diversity effects. The stem:leaf ratio of added litter differed among species (3:1 ratio for *Phalaris* and *Phragmites* and 1:1 ratio for *Typha* and *Carex*) in this experiment to approximate stem:leaf ratios that occur in the field. Jars were then allowed to equilibrate for 3 days after which they were loosely sealed with lids (fitted with septa) to allow room air to enter the jar until the start of a CO_2 extraction round (see below). All jars (112 total) were incubated in the dark for 55 days. Non-limiting soil moisture conditions were created similar to the soil origin incubation.

Carbon mineralization assay

Carbon mineralization rates were estimated during six 24-hour periods over the 55-day incubation (days 1, 3, 7, 15, 29, and 55). I estimated CO_2 production and C

mineralization rates following the same methods as outlined in incubation 1 (litter quality and soil origin incubation). No slope had an R^2 value of less than 0.85 and the majority (>95%) were greater than 0.90. The C mineralization rates for litter addition treatments were background-corrected based on the No Litter treatment for a particular day of measurement and then divided by litter dry weight to report data on a mass basis (g CO_2-C kg^{-1} litter C day^{-1}). First order rate constants (k, day^{-1}) and total CO_2 production were calculated as in the soil origin incubation.

N mineralization/nitrification assay

Initial NO_3^- and NH_4^+ concentrations were determined from the 15 g subsample collected at the time of initial soil processing using the KCl extraction method (Robertson 1999). After the 55-day incubation period, the contents of each jar (litter and soil) were extracted for final NO_3^- and NH_4^+ concentrations using the same method. Concentrations of NO_3^- and NH_4^+ in the extracts were analyzed by the microplate method using protocols developed by Dr. David Rothstein (Dept. of Forestry, MSU). Net rates of N mineralization and nitrification were calculated from the changes in inorganic N ($NH_4^+ + NO_3^-$) or NO_3^-, respectively, during the incubation period (initial – final pool sizes/incubation period). N mineralization and nitrification rates were calculated on a soil mass and soil carbon basis similar to the C mineralization component of the experiment.

Statistical analysis

General linear models (GLMs) were used to test for treatment effects on first order rate constants, total C mineralization, N mineralization, and nitrification. Species effects on soil %C, %N, and C:N ratio and litter chemistry (%C, %N, C:N ratio, %lignin, lignin:N ratio) were determined using GLMs. Tukey's HSD multiple comparison tests were performed to determine significant differences between factor levels. To determine the relationship between litter diversity and total C mineralization, a regression analyses was performed with litter diversity treatments 1 though 4 as the independent factor. Simple linear regression was used to investigate both soil and litter quality controls on C and N mineralization rates. Model diagnostics were assessed for each analysis to determine if the residuals were normally distributed and displayed constant error variances. Log transformations were used to correct for any heteroscedasticity. All statistical tests were performed in R 2.13.2 (R Development Core Team 2011).

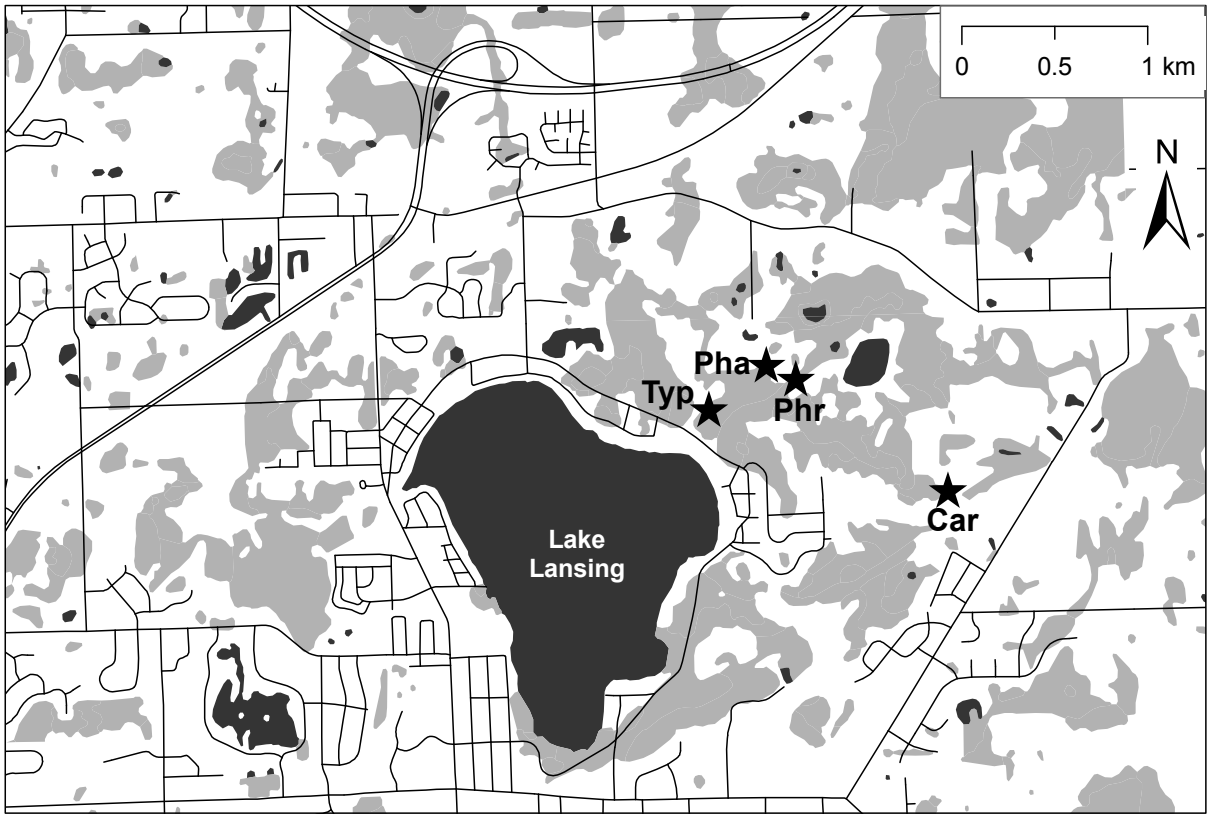

Figure 4-1. Map showing the location of the monospecific stands of *Phragmites australis* (Phr)*, Phalaris arundinacea* (Pha)*, Typha* ×*glauca* (Typ), and *Carex lacustris* (Car) in the wetland area within Lake Lansing Park North, Haslett, MI.

Results

Litter quality and soil origin incubation

First order rate constants (k, decomposition rate) varied among treatment combinations, with the highest rate constant occurring when *Phragmites* litter was incubated in *Phalaris* soil (0.0282 ± 0.012 day^{-1}) and the lowest when *Typha* litter was incubated in *Carex* soil (0.0085 ± 0.003 day^{-1}) (Table 4-1). For a species-specific litter type, there was a general pattern of the highest decomposition rate occurring when incubated in *Phragmites* soil, followed by *Phalaris*, *Typha*, and *Carex*, except for *Phragmites* litter which showed no pattern among the soil it was incubated in (marginal litter x soil interaction; $p = 0.068$). Averaged over soil origin, *Phragmites* litter had the highest decomposition rate and *Typha* litter the lowest (main effect of litter; $p < 0.001$; Table 4-2). The only treatment combination that had a comparable decomposition rate compared to *Phragmites* litter was *Phalaris* litter when incubated in *Phragmites* soil. When decomposition rates were averaged over species-specific litter type, *Carex* soil had the lowest and *Phragmites* soil the highest (main effect of soil origin; $p < 0.001$; Table 4-2)

Phragmites and *Phalaris* litter had the highest total carbon mineralization rates (C-min), followed by *Carex* and then *Typha* when averaged across soil origin (Figure 4-2; significant main effect of litter; $p < 0.001$; Table 4-2). When averaged across species-specific litter, soil collected under monospecific stands of *Typha* and *Carex* had the greatest total C-min, followed by *Phalaris* and then *Phragmites* (Figure 4-2; significant main effect of soil origin; $p < 0.001$; Table 4-2). Overall, the treatment combination that yielded the highest C-min was *Phalaris* litter incubated with *Typha* soil, whereas *Typha* litter incubated

with *Phragmites* soil yielded the lowest C-min. The interaction between species-specific litter and soil origin was not significant. The significant effect of soil origin on total C-min after background C-min attributed to soil alone was factored out suggests the importance of soil origin.

There were significant species effects on soil %C, %N, and C:N ratio (Table 4-3). *Phragmites* soil had the highest soil C:N ratio (significant main effect of species; $p < 0.001$; Table 4-3), indicating that the quality of its soil organic matter was poorer compared to soils of different species origin, and this is consistent with total C-min results suggesting *Phragmites* soil organic matter was the least labile. *Phalaris* and *Carex* soil had intermediate soil C:N ratios with *Carex* soil C:N ratios significantly greater than that of *Typha*. Soils collected from monospecific stands of *Phalaris* and *Typha* had the highest soil %C and %N, while soils collected from *Phragmites* and *Carex* had the lowest C:N ratios, and for soil %N, *Phragmites* had significantly less than *Carex* (significant species effect; $p < 0.001$; Table 4-3).

Litter N content significantly differed among species. *Typha* and *Carex* litter had the highest litter C:N ratios at ~ 65, followed by *Phalaris* at ~ 40 and *Phragmites* at ~28 (significant species effect; $p < 0.001$; Table 4-3). For litter %N, *Phragmites* litter contained the greatest amount, followed by *Phalaris* and then *Typha* and *Carex*, which were not significantly different from each other (significant species effect; $p < 0.001$; Table 4-3). A different pattern arose for litter %C; *Phalaris* and *Typha* litter had the highest %C, followed by *Carex* litter and finally *Phragmites* (significant species effect; $p < 0.001$; Table 4-3). *Phragmites* litter had the lowest C:N ratios due both to the highest litter %N and the lowest litter %C among all species.

Past studies have shown that soil and litter C:N ratio (indicators of quality) can have significant controlling effects on decomposition rates (Melillo et al. 1982; Taylor et al. 1989, Berg 2000). To test this, simple linear regression was used to determine if a relationship between total C-min and either litter or soil C:N ratio existed. There was no relationship between total C-min and soil C:N ratio when all treatment combinations were included in the analysis (Figure 4-3; adjusted $R^2 = 0.003$, $p = 0.423$), nor was this relationship significant when all litter addition treatment combinations were removed and only No Litter addition (soil only) treatments were analyzed (Figure 4-4; adjusted $R^2 = 0.077$, $p = 0.105$). There was a significant positive relationship between total C-min (No Litter treatments only) and soil %N (Figure 4-5; adjusted $R^2 = 0.192$, $p = 0.018$). For litter addition treatments, litter C:N ratio had a negative relationship with total C-min (Figure 4-6; total C-min; adjusted $R^2 = 0.524$, $p < 0.001$), though the relationship was much stronger at the beginning of the incubation (Figure 4-7; Day 1 C-min; adjusted $R^2 = 0.861$, $p < 0.001$). These data indicate that the influence of litter quality on C-min decreased throughout the incubation.

Net N mineralization and nitrification rates were almost identical to each other because the majority of the ammonium mineralized was nitrified to nitrate; therefore only net N mineralization (N-min) rates will be discussed. Similar to total C-min, only the main effects of soil origin and species-specific litter were significant, not their interaction (Figure 4-8; $p < 0.001$ for both; Table 2). Both positive and negative N-min rates were found, indicating N immobilization occurred in some treatment combinations (negative N-min rates). For the No Litter addition treatments, all soil-origin treatment levels had positive N-

min rates, with *Phragmites* and *Typha* soil having the highest and *Phalaris* and *Carex* having the lowest rates. *Phragmites* litter was the only litter addition treatment level that resulted in positive N-min rates for all soil origins (it also had the lowest litter C:N ratio: Figure 4-8). Among soils that *Phragmites* litter was incubated in, *Phragmites* and *Typha* soil resulted in the highest N-min rates, while *Phalaris* and *Carex* soil resulted in the lowest. For other litter types (*Phalaris*, *Typha*, and *Carex*) most of the N-min rates were negative, except for when *Phalaris* litter was incubated in *Typha* soil, which resulted in positive values that were comparable to low values for *Phragmites* litter. Regression analysis was used to determine the relationship between N-min rates and litter C:N ratio and a significant negative curvilinear relationship was found (Figure 4-9; $R^2 = 0.407$, $p < 0.001$). No significant relationship was found for N-min rates and soil C:N ratio. These results suggest, as for C-min, litter C:N ratio partially controlled N-min rates.

Litter diversity incubation

Among single species litter addition treatments, *Phragmites* litter had the lowest decomposition rate constant and *Phalaris* litter the highest (treatment effect; $p < 0.001$; Tables 4-4 and 4-5). For the double species litter addition treatments, all litter mixtures that included *Phragmites* litter had the lowest decomposition rate compared to all other litter combinations. There were no differences among triple litter addition species mixtures, which also were not different from the non-*Phragmites* double species litter mixtures and the all species litter mixture.

Total C-min varied among litter addition treatments (overall mean: 271 g CO_2-C kg^{-1} litter C) with the greatest difference being between *Typha* litter addition and *Phragmites* + *Phalaris* litter addition (Figure 4-10; significant treatment main effect; $p < 0.001$; Table 4-5). Single or multiple species additions that included *Typha* litter had the lowest total C-min, while litter mixtures that included *Phalaris* usually had the highest total C-min. For the single litter addition treatments, *Carex* litter had the greatest total C-min and *Typha* litter the least. The treatment with the greatest total C-min was the two species litter addition of *Phragmites* and *Phalaris*, which was significantly greater than when either of these two species were incubated alone, indicating the possibility of a non-additive relationship between these two types of litter. Though there was not a strong effect of litter diversity on total C-min, there was an observable decrease in the variance of total C-min as litter diversity increased.

Soil used in this incubation, which was collected from the ephemeral pond at LLP, had a %C of 23.29 (± 0.36), a %N of 1.72 (± 0.03), and a C:N ratio of 13.51 (± 0.09). Litter C:N ratios were higher and differed among species; *Phalaris* and *Typha* had the highest C:N ratio at ~ 62.5 and *Phragmites* had the lowest C:N ratio at 47.2 (significant species effect; $p < 0.001$; Table 4-6). Percent lignin and lignin:N ratios followed a similar pattern among species. The highest % lignin was observed in *Typha* litter, with *Carex* and *Phalaris* having the lowest % lignin and *Phragmites*, *Phalaris*, and *Carex* having lower lignin:N ratio than *Typha* (significant species effect; $p < 0.001$; Table 4-6).

Simple linear regression analysis for total C-min on litter C:N ratios showed a much weaker negative relationship than for the litter quality and soil origin incubation, though the relationship was still significant (Figure 4-11; adjusted $R^2 = 0.131$, $p < 0.001$). The

relationship between total C-min and % lignin was somewhat stronger (Figure 4-12; adjusted $R^2 = 0.228$, $p < 0.001$) and the relationship between total C-min and lignin:N ratio was the best fit among all the litter quality predictors (Figure 4-13; $R^2 = 0.313$, $p < 0.001$). Litter diversity did not have a large impact on total C-min, though the relationship was significant (Figure 4-14; adjusted $R^2 = 0.075$, $p = 0.002$), and as mentioned before, there was a reduction in the variance of total C-min as litter diversity increased.

As with the litter quality and soil origin incubation, net N mineralization rates mirrored net nitrification rates, therefore, only N-min rates will be discussed. The only treatment that had positive N-min rates was the No Litter addition treatment, the remaining litter addition treatment levels all had negative N-min rates, indicating net N immobilization (Figure 4-15; significant litter treatment effect; $p < 0.001$; Table 4-5). Though the No Litter addition treatment was significantly different from all litter addition treatment levels, none of the litter addition treatments were statistically distinct from the rest. The consistent trend for N immobilization for each litter addition treatment level can be explained in the same way as the soil origin incubation, even though the same species-specific litter was used in both incubations.

Table 4-1. Average decomposition rates expressed as first-order rate constants (k; day^{-1}) for each litter x soil origin treatment combination.

Soil Addition	Litter Addition							
	Phragmites	Std. Error	*Phalaris*	Std. Error	*Typha*	Std. Error	*Carex*	Std. Error
Phragmites	0.0264	0.011	0.0267	0.011	0.0160	0.007	0.0218	0.009
Phalaris	0.0282	0.012	0.0221	0.009	0.0143	0.006	0.0166	0.007
Typha	0.0266	0.011	0.0175	0.007	0.0138	0.006	0.0170	0.007
Carex	0.0253	0.010	0.0159	0.006	0.0085	0.003	0.0147	0.006

Table 4-2. Summary of the two-factor ANOVAs for the effect of species-specific litter and soil on decomposition constant (K), cumulative C mineralization, and N mineralization.

Source	num df	den df	F	P>F
K constants				
Soil	3	80	12.35	<0.0001
Litter	3	80	51.12	<0.0001
Soil x Litter	9	80	1.87	0.0683
Cumulative C mineralization				
Soil	3	80	11.29	<0.0001
Litter	3	80	69.43	<0.0001
Soil x Litter	9	80	1.60	0.1283
N Mineralization				
Soil	3	100	14.07	<0.0001
Litter	4	100	92.30	<0.0001
Soil x Litter	12	100	0.97	0.4871

Table 4-3. Soil and litter %C, %N, and C:N mass ratios of the four species used in the litter quality and soil origin incubation. Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Values are means ± SE.

Species	Soil %C	Soil %N	Soil C:N	Litter %C	Litter %N	Litter C:N
Phragmites	24.8 ±1.07^{a}	1.78 ±0.10^{a}	14.0 ±0.32^{a}	41.4 ±0.15^{a}	1.48 ±0.01^{a}	28.0 ±0.32^{a}
Phalaris	40.22 ±0.39^{b}	3.10 ±0.03^{b}	13.0 ±0.08^{b}	45.6 ±0.14^{b}	1.14 ±0.03^{b}	40.0 ±0.94^{b}
Typha	39.10 ±3.62^{b}	3.13 ±0.28^{b}	12.4 ±0.17^{c}	45.5 ±0.18^{c}	0.62 ±0.01^{b}	74.3 ±1.37^{c}
Carex	31.13 ±1.09^{c}	2.37 ±0.09^{c}	13.1 ±0.07^{b}	43.1 ±0.16^{c}	0.62 ±0.01^{c}	69.8 ±1.51^{c}

Table 4-4. Average decomposition rates expressed as first-order rate constants (k; day^{-1}) for each litter diversity treatment.

Litter Diversity	Species	k constant (day^{-1})	Standard Error
1	*Phalaris*	0.0222	0.0084
1	*Typha*	0.0191	0.0072
1	*Phragmites*	0.0173	0.0066
1	*Carex*	0.0206	0.0078
2	*Phragmites + Phalaris*	0.0192	0.0072
2	*Phragmites +Typha*	0.0212	0.0080
2	*Phragmites + Carex*	0.0211	0.0080
2	*Phalaris + Typha*	0.0239	0.0090
2	*Phalaris + Carex*	0.0249	0.0094
2	*Typha + Carex*	0.0240	0.0091
3	*Phragmites + Phalaris + Typha*	0.0227	0.0086
3	*Phragmites + Phalaris + Carex*	0.0232	0.0088
3	*Phragmites + Typha + Carex*	0.0223	0.0084
3	*Phalaris + Typha + Carex*	0.0241	0.0091
4	All Species Litter	0.0233	0.0088

Table 4-5. Summary of ANOVAs for the effect of Treatment on decomposition (K) constant, cumulative C mineralization, and N mineralization.

Response	num df	den df	F	P>F
K Constant	14	90	2.50	0.0047
Cumulative C mineralization	14	90	8.08	<0.0001
N mineralization	15	96	845.21	<0.0001

Table 4-6. Litter C:N mass ratios, % lignin, and lignin:N mass ratios of the four species used in the litter diversity incubation. Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Values are means ± SE.

Species	C:N	% Lignin	Lignin:N
Phragmites	47.2 ±0.03^{a}	11.1 ±0.40^{a}	12.4 ±0.45^{a}
Phalaris	62.0 ±1.10^{b}	8.9 ±0.24^{b}	12.6 ±0.47^{a}
Typha	63.0 ±0.81^{b}	13.7 ±0.02^{c}	19.1 ±0.20^{b}
Carex	53.7 ±2.0^{c}	10.0 ±0.30^{b}	12.1 ±0.10^{a}

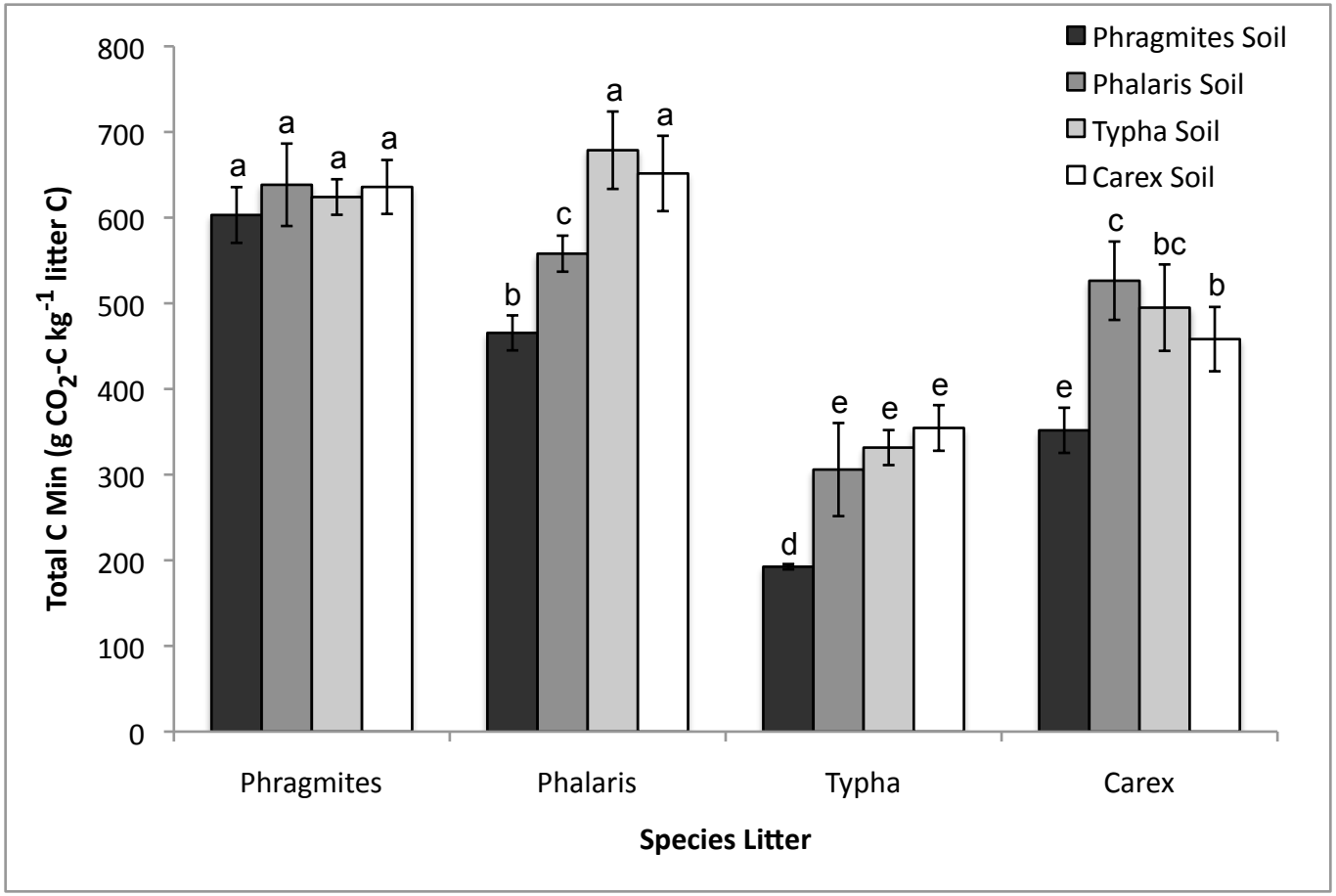

Figure 4-2. Mean cumulative C mineralization over the 68-day litter quality and soil origin laboratory incubation. The main effects of species-specific litter ($F_{3,80} = 69.43$, $p < 0.001$) and soil origin ($F_{3,80} = 11.29$, $p < 0.001$) were significant, but not their interaction. Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

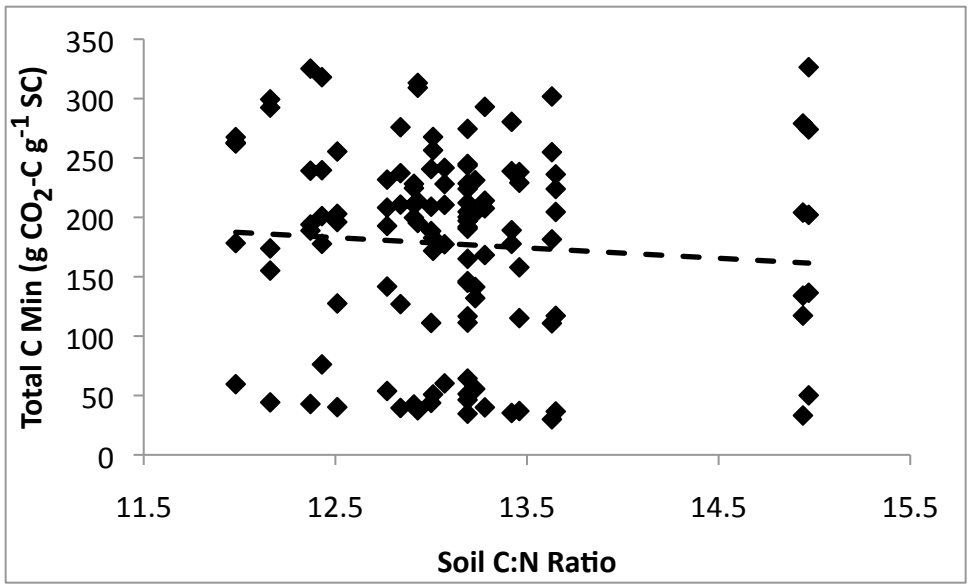

Figure 4-3. Regression between soil C:N ratio and cumulative C mineralization (all treatments) (adjusted R^2 = 0.003, p = 0.423).

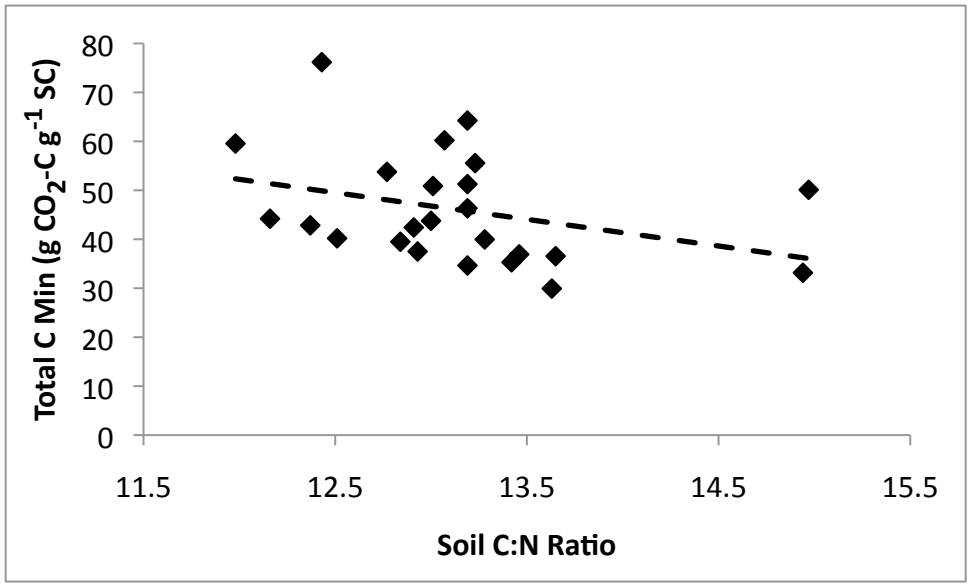

Figure 4-4. Regression between soil C:N ratio and cumulative C mineralization (no litter treatments only) (adjusted R^2 = 0.077, p = 0.105).

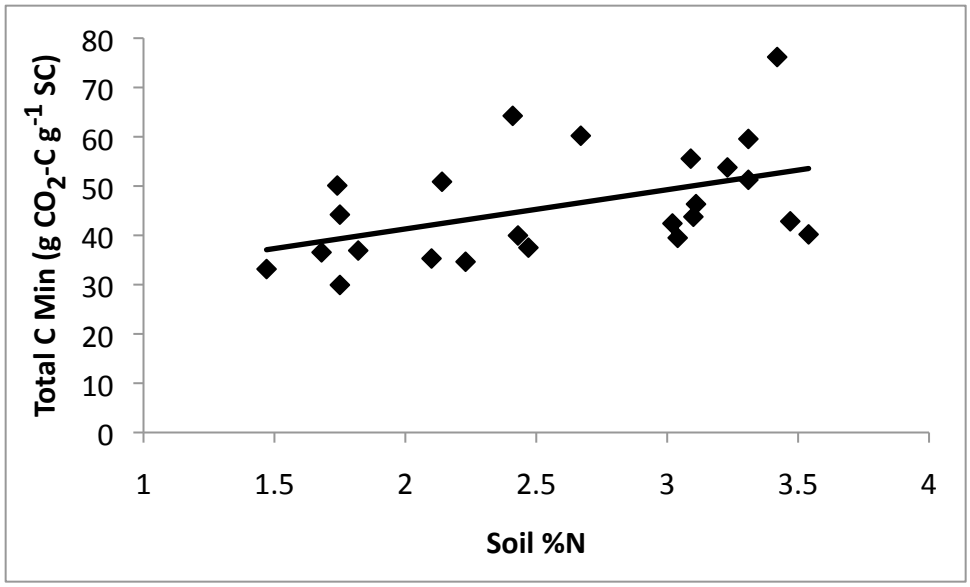

Figure 4-5. Regression between soil %N ratio and cumulative C mineralization (no litter treatments only) (adjusted $R^2 = 0.192$, $p = 0.018$).

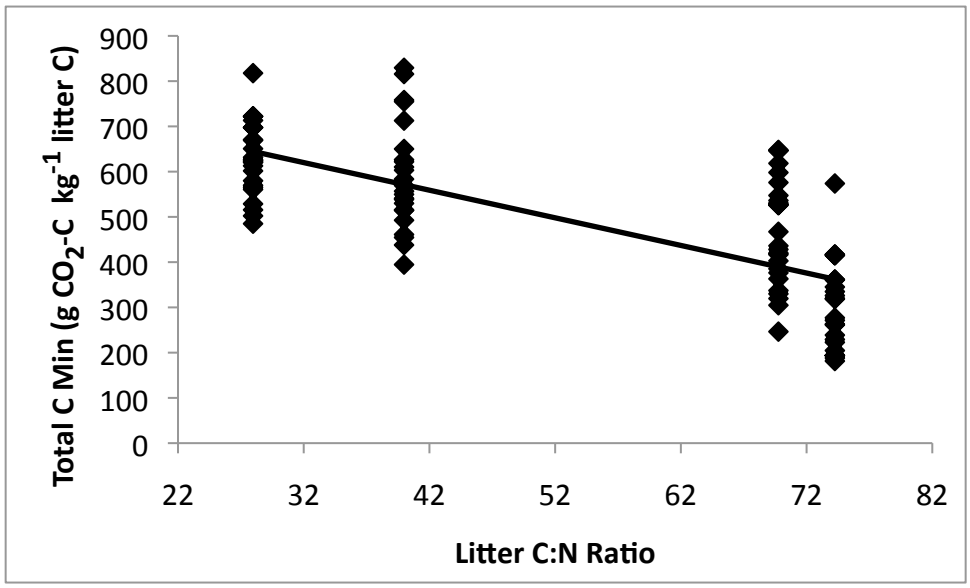

Figure 4-6. Regression between litter C:N ratio and cumulative C mineralization (litter addition treatments only) (adjusted $R^2 = 0.524$, $p < 0.001$).

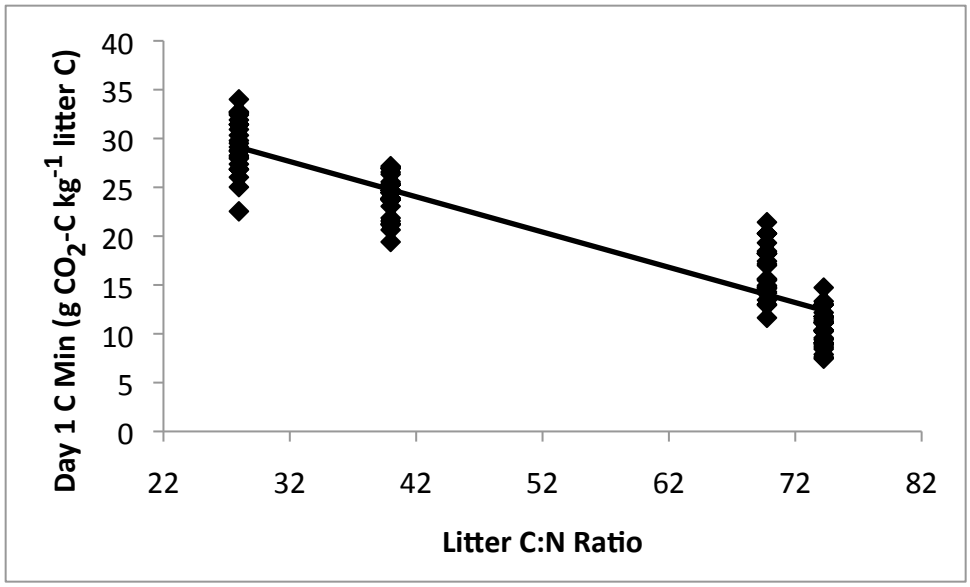

Figure 4-7. Regression between litter C:N ratio and day 1 C mineralization (Litter addition treatments only) (adjusted $R^2 = 0.861$, $p < 0.001$).

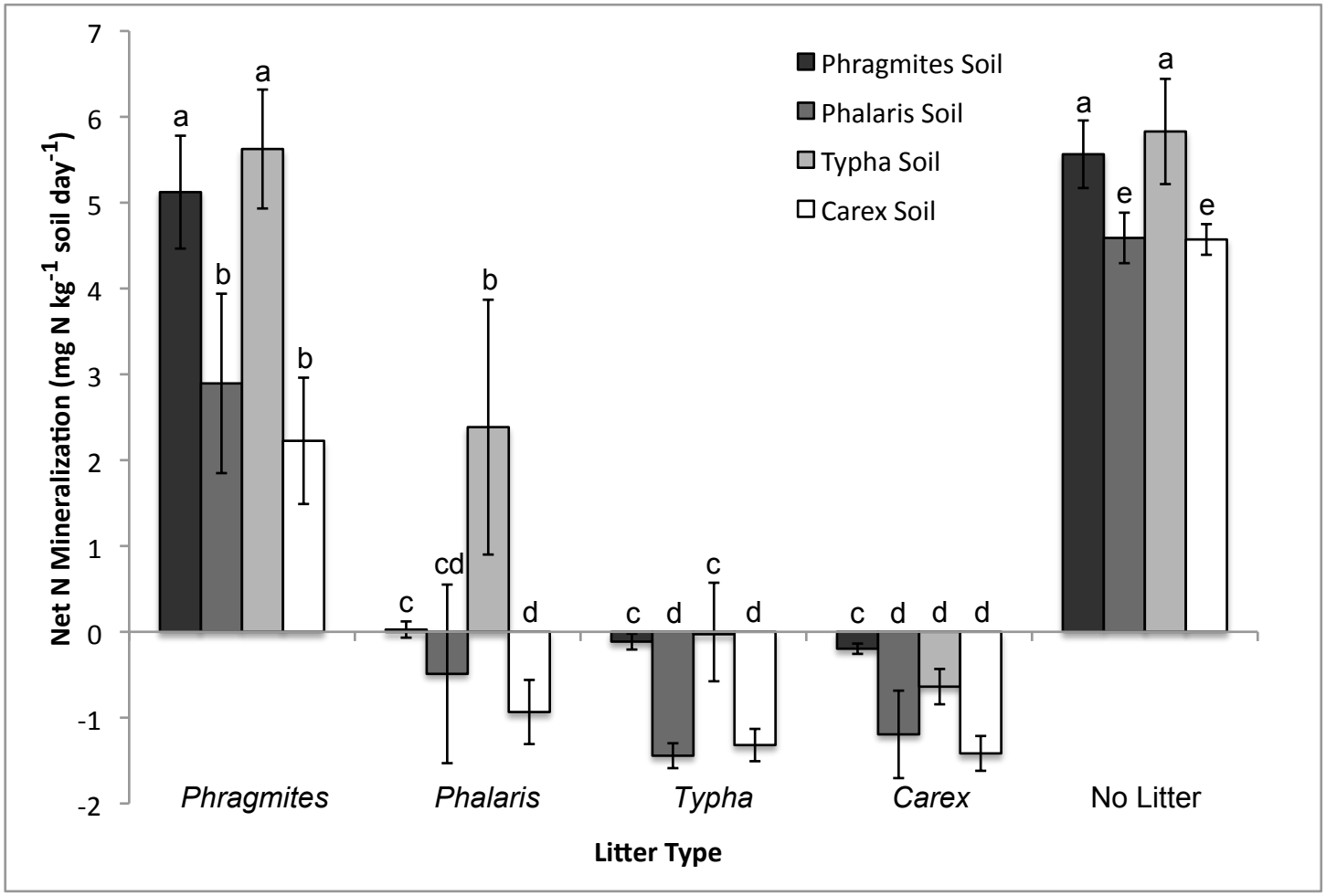

Figure 4-8. Mean net N mineralization over the 68-day litter quality and soil origin laboratory incubation. The main effects of species-specific litter ($F_{3,100} = 92.30$, $p < 0.001$) and soil origin ($F_{12,100} = 14.07$, $p < 0.001$) were significant, but not their interaction. Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

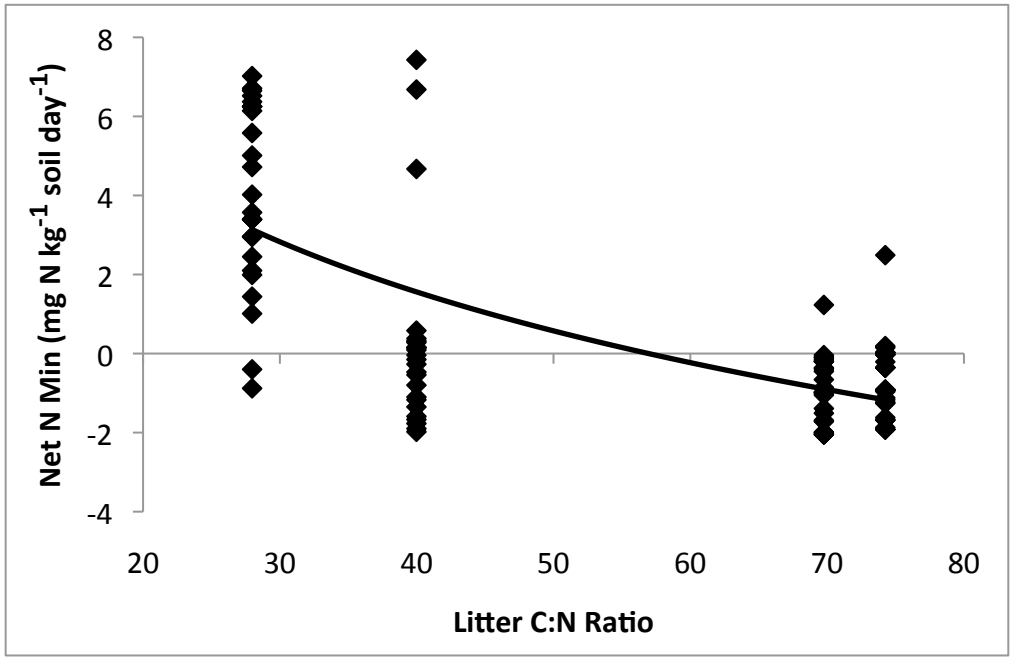

Figure 4-9. Regression between litter C:N ratio and net N mineralization (adjusted R^2 = 0.452, $p < 0.001$).

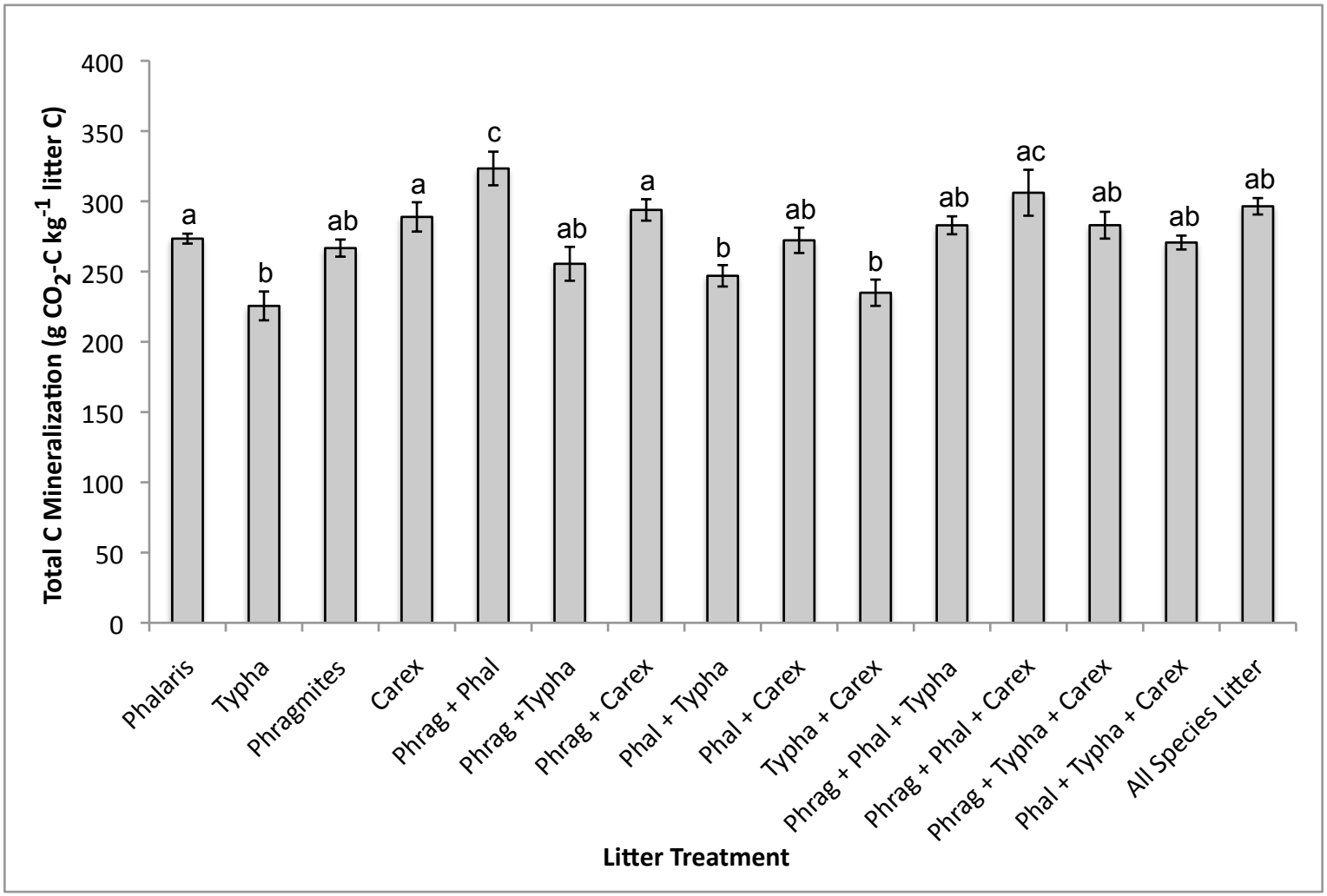

Figure 4-10. Mean cumulative C mineralization over the 55-day litter diversity laboratory incubation. The main effect of litter treatment was significant ($F_{14,90} = 8.08$, $p < 0.001$). Same letter superscript denotes nonsignificant differences according to Tukey post hoc tests. Error bars represent 1 SE.

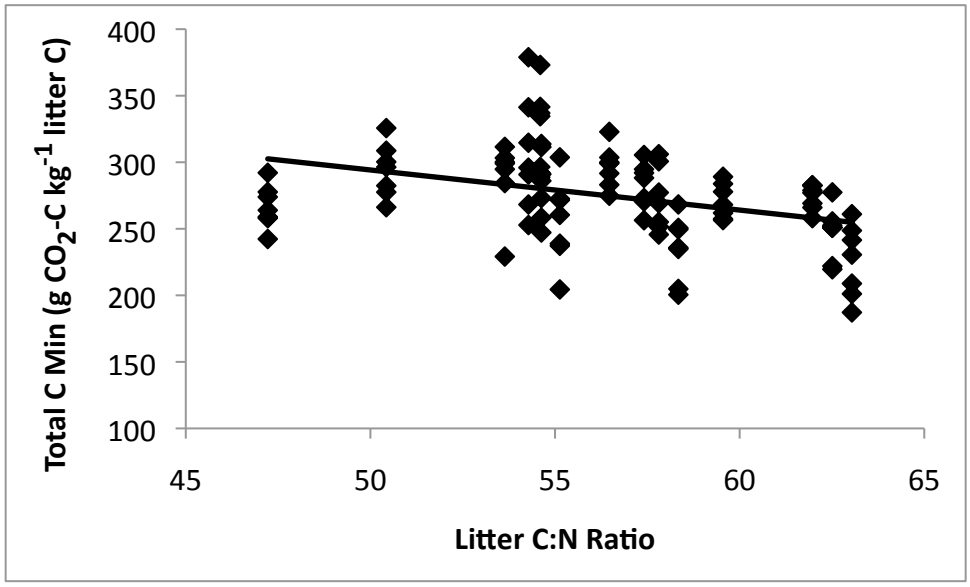

Figure 4-11. Regression between litter C:N ratio and cumulative C mineralization (adjusted $R^2 = 0.131$, $p < 0.001$).

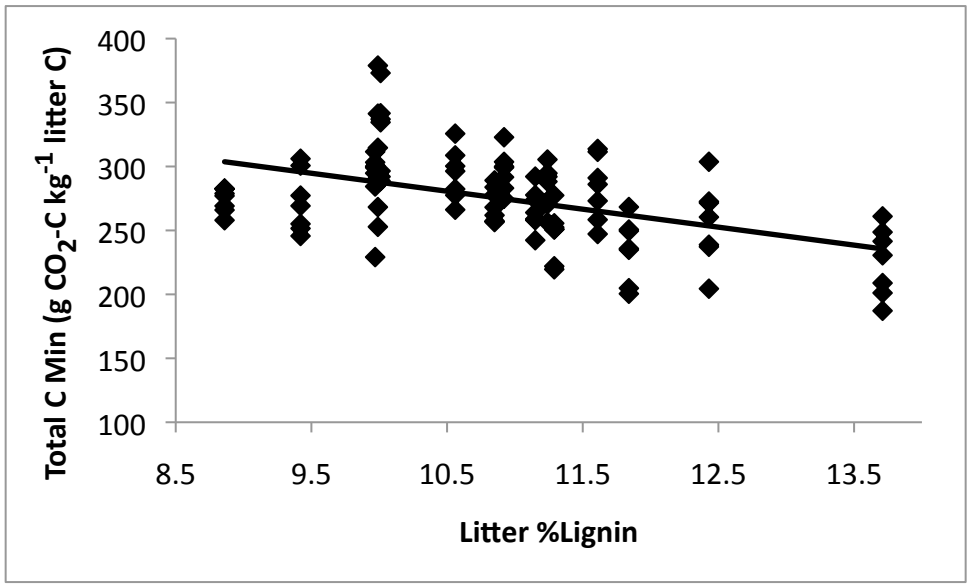

Figure 4-12. Regression between litter %lignin and cumulative C mineralization (adjusted $R^2 = 0.228$, $p < 0.001$).

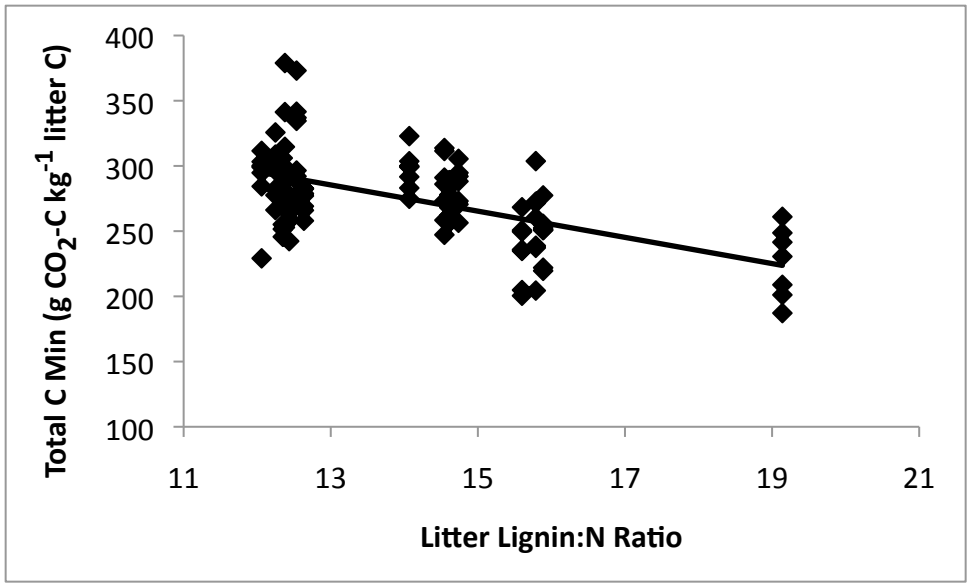

Figure 4-13. Regression between litter lignin/N ratio and cumulative C mineralization (adjusted $R^2 = 0.313$, $p < 0.001$).

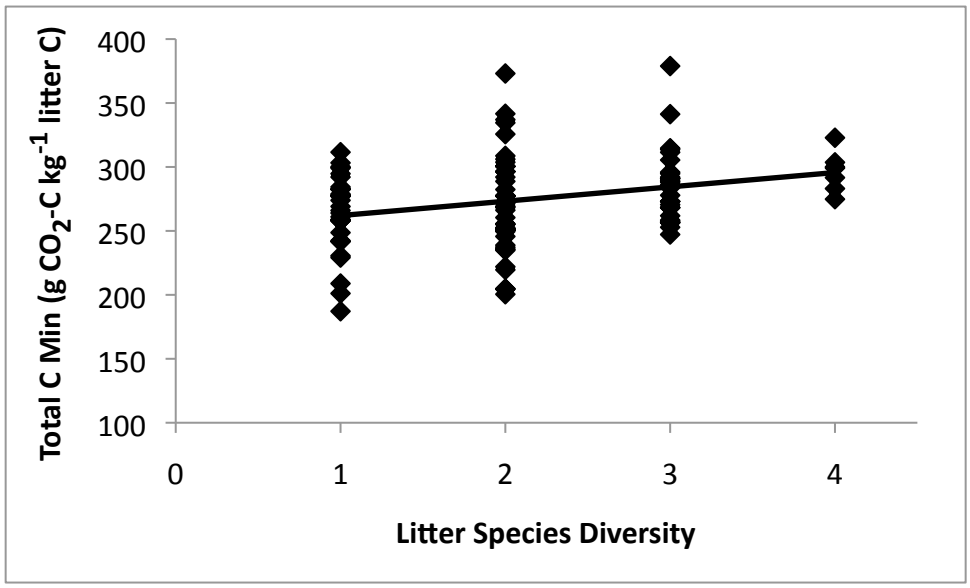

Figure 4-14. Regression between litter species diversity and cumulative C mineralization (adjusted $R^2 = 0.075$, $p = 0.002$).

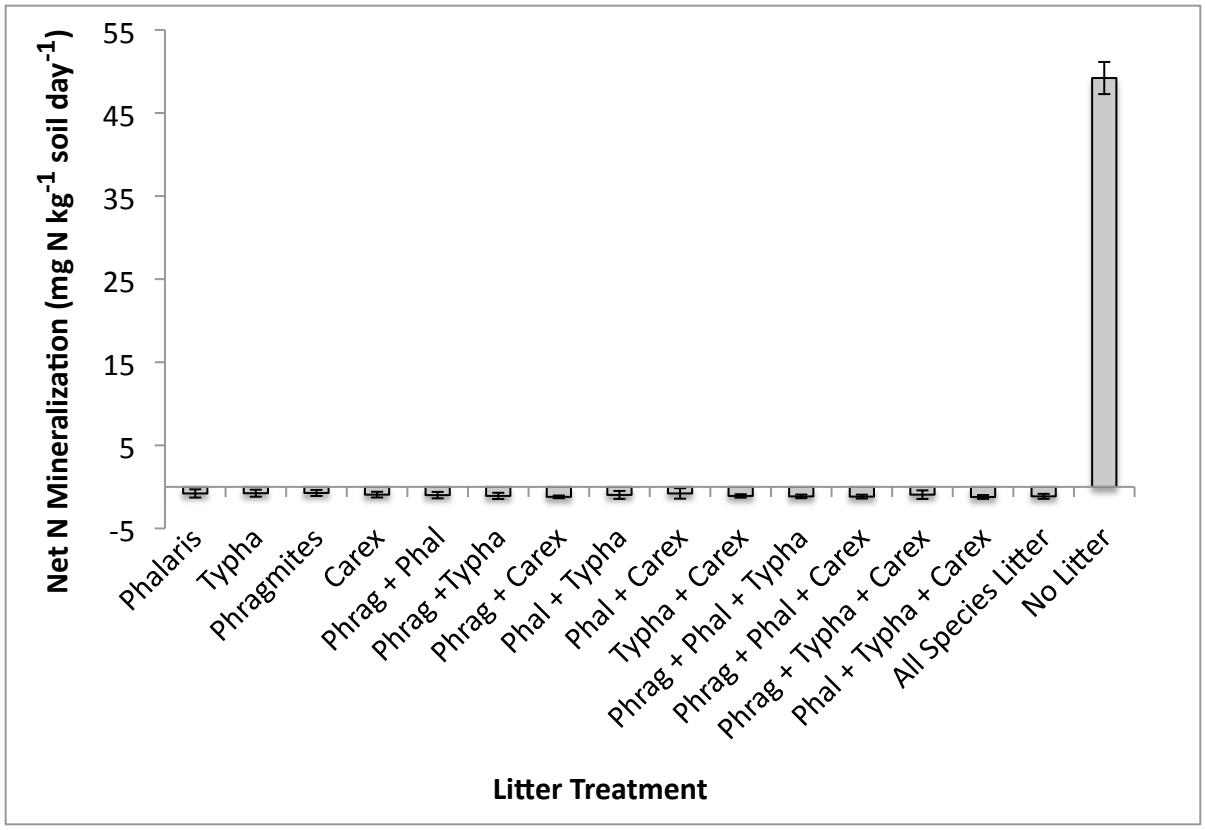

Figure 4-15. Mean net N mineralization over the 55-day litter diversity laboratory incubation. The main effect of litter treatment was significant ($F_{15,96} = 845.21$, $p < 0.001$). Only the no litter treatment was significantly different from the other treatment combinations. Error bars represent 1 SE.

Discussion

Litter quality and soil origin incubation

The high first-order decomposition rate constants and total C-min consistently observed when *Phragmites* litter was incubated in the different soil types (Table 4-1; Figure 4-2) are likely explained by the lower C:N ratio of *Phragmites* litter compared to the other three species (Table 4-3). In contrast, *Typha* litter had the lowest rate constants and total C-min among different soil types, especially when incubated in *Carex* soil. Decomposition rates were much greater in this laboratory incubation (k ranged from 0.0085 d^{-1} to 0.0282 d^{-1}) than in field incubations of *Phragmites* stem and leaf tissue whose decomposition (k) ranged from 0.0015 d^{-1} to 0.0051 d^{-1} (Gessner 2000), likely due to constant laboratory temperatures and the mechanical breakup of litter as part of the methodology. First order rate constants found for *Phragmites* stems in Chapter 3 (0.0018 to 0.0025 d^{-1}) were also lower than in this incubation, perhaps in part for the same reasons that caused the lower rates in Gessner (2000); in addition, only stems were incubated in the Chapter 3 litter bag assay, and stems are known to be more recalcitrant than leaves. However, my decomposition rates were closer to Chimney and Pietro (2006) that found a range of 0.0008 d^{-1} to 0.0568 d^{-1} for four wetlands plants incubated in a constructed wetland.

The relatively slow decomposition of *Typha* litter is probably due to its high C:N ratio and physical structure. *Typha* litter, as with its living biomass, is dominated by aerenchyma tissue (Sale and Wetzel 1983) that likely lowers physical contact with the soil, thus potentially reducing decomposition rates. It would be interesting to test if the

physical structure of the litter affects decomposition because this is an attribute of litter not usually considered to be an important control on decomposition rate. This could be tested experimentally by incubating *Typha* litter, along with other species litter that varied in aerenchyma tissue, in wetland water in addition to in soil because physical contact with water would not be restricted as it is with soil.

The effect of litter quality on the decomposition of litter has been shown previously in a wide range of plant types and ecosystems (Eviner 2004; Xu and Hirata 2005; Orwin et al. 2008; Parton et al. 2007). The influence of litter C:N ratio (quality) on total C-min rates was apparent in the regression analysis (Figure 4-6) and further supports my hypothesis that species-specific litter quality influences decomposition, though the hypothesis that *Phragmites* had the most recalcitrant litter was not supported. This relationship was stronger in the beginning of the incubation suggesting that litter quality had a stronger influence early in its decomposition, probably due to more labile litter components being mineralized first (Figures 4-6 and 4-7). Rovira and Vallejo (2002) found that when soils decompose the quality usually decreases throughout the time of the incubation, and litter in my incubation likely corresponds to what they termed "Labile Pool I", which was the most labile.

Though litter quality had large impacts on decomposition rates in the incubation experiment, the significant soil effects after rates where corrected for background soil C-min shows the influence of soil origin on decomposition rates and supports the hypothesis postulated in the introduction (hypothesis 1). This significant soil origin effect was most important for *Phalaris* litter, which showed significant differences in decomposition rate when incubated in its own soil, *Phragmites* soil, and *Carex* or *Typha* soil (Figure 4-2). Soil

origin seemed to have the least effect on *Phragmites* litter, which did not significantly vary across soil origins. Conversely, *Phragmites* soil usually had the lowest total C-min compared to other specific-species soil types (Figure 4-2). Because total C-min was corrected for background soil C-min, this effect is from an aspect of the soil other than its direct decomposition. In contrast to *Phragmites* litter, *Phragmites* soil had the lowest soil C:N ratio of all the other species-specific soil (Table 4-3). One reason for the discrepancy between the quality of the soil versus litter could be that the litter used in this incubation was a mixture of leaf and stem tissue, and *Phragmites* has extremely labile leaf tissue which likely decomposes fast in the field leaving the recalcitrant stem tissue to develop into the soil substrate (previous year leaf fall is not recognizable at the beginning of the growing season, personal observation). This low soil organic matter quality may result in a more K-selected soil microbial community, which may not be able to respond to the litter inputs as quickly compared to the other species-specific soils (Fontaine et al. 2003).

To the best of my knowledge, the soil origin effect as described in this study has not been shown before within a similar experimental framework (though see Gholz et al. 2000 and Ayres et al. 2009, for the "home-field advantage" effect), especially with soil collected under different species in the same wetland complex, though soil characteristics have been shown to influence decomposition. For example, Giardina et al. (2001) found that soil C mineralization in forests dominated by either quaking aspen or lodgepole pine were significantly different, and the difference wasn't due to soil texture, but could have been caused by the quality of the organic matter inputs. Scott et al. (1994) found that while soil texture (% sand content) alone had little effect on litter C mineralization, a metric that incorporated both soil texture and soil water pressure explained a significant amount of

the variation in native soil C mineralization. Possible mechanisms for the soil origin effect found in this incubation include interactions between the soil organic matter and litter (priming effect, Fontaine et al. 2003), differences in soil microbial community (Waldrop et al. 2000), and soil nutrient availability (Liu et al. 2006).

In this incubation, the most likely mechanism of the soil origin effect was soil nutrient availability as it explains the majority of the patterns seen in the data. First, *Phragmites* litter had the highest quality among litter types and its decomposition was not statistically significantly different among soil origins (Figure 4-2; not dependent on the N content or C:N ratio of the soil). Second, *Phragmites* soil had the lowest quality among soil types and, besides *Phragmites* litter, all other litter types incubated in *Phragmites* soil had the least C-min compared to other soil types. Third, *Typha* soil was the highest quality soil among soil types and consistently had the highest C-min for each species-specific litter types. A study by Liu et al. (2006) investigated the important soil chemical and physical factors influencing the decomposition of two dominant plant species in China's grasslands (*Allium bidentatum* and *Stipa krylovii*) and found that available soil nutrients (N and P) increased decomposition and that this effect was greater for lower quality litter. Though the data support soil nutrient availability as the mechanism of the soil origin effect, it is possible that other factors, such as the soil microbial community, played a role. While microbial "home-field advantage" has been shown in the literature (Gholz et al. 2000, Ayres et al. 2009), it is unlikely to have influenced my results because when a specific-species litter was incubated in its "home soil" it never resulted in the highest C mineralization (Figure 4-2). Future research should isolate the causal factor for the soil origin effect seen in this incubation.

The most consistently negative N-min rates (i.e., net N immobilization) occurred when *Phalaris*, *Typha*, or *Carex* litter was incubated in *Phalaris* or *Carex* soil. This pattern of N immobilization can be partly explained by the C:N ratio of the species-specific litter. It has been found that organic matter with a C:N ratio greater than approximately 30:1 to 35:1 does not supply the nutrient demand of the microbial decomposer community, forcing the microbes to scavenge available N from the soil and thus causing N immobilization (Brady and Weil 2001), though Parton et al. (2007) showed that N immobilization associated with litter quality occurred when the litter C:N ratio was less than 40. In this incubation, the C:N ratio of litter from *Phragmites* was ~ 28, which is below the critical C:N ratio of 35, and all treatment combinations that included *Phragmites* litter had positive N-min rates. Similarly, *Phalaris* litter had a C:N ratio of ~ 40 and since this is close to the critical C:N ratio of 35, immobilization was less than *Carex* and *Typha* litter (both with a litter C:N ratio >> than 35:1), especially when *Phalaris* litter was incubated with *Typha* soil, which resulted in positive N-min rates. For all litter types besides *Carex*, litter incubated in *Typha* soil had the highest N-min rates, which is likely due to *Typha* soil having the lowest C:N ratio of all soils resulting in the highest N-min rates when no litter was added.

The results from this experiment suggest that dominant plant species from the same functional group can influence C and N cycling by species-specific litter effects, and that these influences can have lasting effects in soils (soil origin effects). These results may give less justification for categorizing wetland species, especially invasive species, into generalized functional groups (Boutin and Keddy 1993). Further evidence for the lack of usefulness of functional groups was found by Bremer et al. (2007), which found that plant functional group did not affect *nirK*-type denitrifier communities, though plant species

identity did. However, plant function group classification has been found to be useful in other studies that have shown that the diversity of plant functional groups helped to resisted invasion by *Centaurea maculosa* (Pokorny et al. 2005). All four wetland species used in this incubation had different litter and soil quality properties, although they are all highly clonal, emergent wetland species that can be found in similar wetland habitats and could be classified as clonal dominants by Boutin and Keddy (1993). They do vary in some key wetland plant characteristics, such as total height, biomass production (though they are all high biomass producing species), flowering phenology, and water depth tolerance. For invasive species that have become ubiquitous wetland plants, it might be more advantageous to consider species as unique components of the plant community and then study their species-specific plant traits, instead of considering them members of a specific functional category.

Litter diversity incubation

Similar to the soil origin incubation, in the litter diversity incubation *Typha* litter had the lowest total C-min rates and decomposition rates (k constants) compared to the other single species litter addition (Figure 4-10), which, as before, is likely influenced by its high litter C:N ratio and physical structure. *Phalaris* litter had a similarly high C:N ratio but a higher total C-min and decomposition rate than *Typha* litter. The general pattern of an increase in decomposition as diversity increased was not as apparent as hypothesized. Total C-min showed a more idiosyncratic pattern in which the species added had a greater impact that just the number of species. For example, when *Phragmites* and *Phalaris* litter were incubated together, their combined effect was greater than when either was

incubated alone or any of the other litter diversity treatments. Despite this idiosyncratic pattern, litter diversity still had a significant effect on total C-min, albeit a weak one (Figure 4-14). The relationship between diversity and decomposition has been shown to be positive, neutral, or negative (Gessner et al. 2010) so the lack of a strong impact of litter diversity on decomposition is not too surprising.

Litter quality had a greater effect on decomposition than diversity, specifically litter lignin:N ratio (Figure 4-22). The importance of lignin content on decomposition, due to its recalcitrant nature, has been shown in multiple litter decomposition studies (Melillo et al. 1982; Taylor et al. 1989; Berg 2000). Litter C:N ratio had less of an effect compared to the litter quality and soil origin incubation probably because of the higher similarity between species-specific litter quality. The difference between species-specific litter C:N ratios used in this incubation compared to the litter C:N ratios for the litter quality and soil origin incubation is explained by the different stem:leaf ratios used in these incubations. For the soil origin incubation, a 1:1 stem:leaf ratio was used for each species for standardization purposes, but for this incubation (litter diversity) a 3:1 stem:leaf ratio was used for *Phragmites* and *Phalaris* and a 1:1 stem:leaf ratio was used for *Carex* and *Typha;* these ratios are closer to what is seen in the field. The higher proportion of stems to leaves decreased the lability of the overall mixture (i.e., increased the C:N ratio).

Net N mineralization rates were less variable for this incubation than the soil origin incubation as all litter addition treatments, regardless of species identity or diversity, resulted in negative N-min rates (Figure 4-15). As explained above, the litter C:N ratio of less than 30 for *Phragmites*, and near that demarcation line for *Phalaris*, was able to explain the immobilization pattern seen for the different treatments in the litter quality and soil

origin incubation. In this incubation, all of the litter C:N ratios were greater than 30 due to the different stem:leaf ratios used for *Phragmites* and *Phalaris* (3:1 versus 1:1). Therefore, since all of the added litter mixtures were above the critical C:N ratio of 35:1 (at which point microbes need to scavenge the soil for available N to support growth) all litter addition treatment levels resulted in N immobilization within the soil. These results demonstrate that recalcitrant litter, when added to the soil, reduces the available N concentration in the soil which could feedback to plant fitness. This ability of low quality C (high C:N ratio) to reduce the availability of N is well known and has been successfully used in restoration to decrease high nutrient conditions that invasive species respond positively to, to allow native species to establish (Averett et al. 2004).

Though the relationship between litter diversity and decomposition was weaker than hypothesized, the effect of litter diversity was still significant. Because of this result, one outcome of the formation of monospecific stands (and hence a single species litter layer) could be a reduction in overall decomposition and an increase in organic matter buildup. In other words, invasive species would decrease decomposition rates when they reduce species (litter) diversity. According to the results of this experiment, the identity of the plant species that forms a monospecific stand could play an important role in determining plant effects to C and N cycling. For example, in this experiment, *Typha* litter resulted in the lowest decomposition rates and could explain why *Typha* monospecific stand sites used in these incubations had the highest soil %C.

Chapter 5
Conclusions

Humans have removed the physical and biological barriers restricting the population ranges of many species around the world resulting in a global exchange of exotic species (Richardson et al. 2000). While many species are transported to new areas on freight, in ballast water, or for cultivation, only a few become established and expand into surrounding areas (Williamson and Fitter 1996). Though relatively few in number, invasive species can have numerous negative impacts on the ecology of invaded areas. On a global scale, invasive species are considered to be the one of the greatest causes of biodiversity loss, second only to habitat destruction (Walker and Steffen 1997).

Many studies have shown that invasive species dramatically alter species composition on a local scale (Zedler and Kercher 2004). And while there is still some debate about the impacts of invasive species on biodiversity at multiple spatial scales (Sax and Gaines 2003), their impact on C and N cycling seems to be more direct. For example, a meta-analysis of 94 experimental studies showed that invasive plant species significantly increased root and shoot C stocks, primary production, litter decomposition, and N availability compared to native plant communities (Liao et al. 2007).

It is important to understand the impacts of invasive plants in wetlands for numerous reasons. Because of their placement on the landscape, wetlands are one of the most highly invaded ecosystems with the resulting invasion ranging from the addition of a new co-dominant to the complete replacement of the local flora by a monospecific stand of the introduced species (Zedler and Kercher 2004). Wetlands are often important ecosystems for the biogeochemical cycling due to their high productivity, low decomposition, and spatiotemporally variable redox status due to fluctuating water table

position (McClain et al 2003; Reddy and DeLaune 2008). Therefore, the introduction of new species can affect wetland function by introducing new traits, or increasing the abundance of an already existing trait, that influences decomposition rates or redox status of the soil, such as litter quality and radial oxygen loss, respectively (Figure 1-1). Though the negative aspects of plant invasions are hard to ignore, e.g., loss of biodiversity, it is possible that plant invasions in wetlands could be partially beneficial, such as increasing C and N storage or increasing denitrification rates.

The broad goal of this dissertation was to investigate the ecosystem consequences of invasive plant species in temperate wetlands, focusing on *Phragmites australis, Phalaris arundinacea* and *Typha* ×*glauca* (hereafter, *Phragmites*, *Phalaris*, and *Typha*, respectively). These are among the most aggressive wetland invaders in the Great Lakes area and understanding their impact on biogeochemical cycling in wetlands is of great importance. In Chapter 2, I characterized the spatial variability in C and N storage and organic matter quality in 24 wetlands in south-central Michigan that support monospecific stands of these invasive species. Additionally, I used laboratory assays to determine potential differences in C and N mineralization within monospecific stands of *Phragmites, Phalaris*, and *Typha.* In Chapter 3, I investigated the mechanisms by which *Phragmites* influences C and N cycling in three wetlands in central Michigan. This was accomplished by manipulating *Phragmites* litter and living biomass within plots at each site and then monitoring abiotic conditions and performing a number of assays, including litter bag decomposition, an *in situ* N mineralization incubation, and a laboratory denitrification incubation. In Chapter 4, to understand the effects of litter quality, soil origin, and plant diversity on C and N mineralization, I performed two laboratory incubations using litter and soil collected from

monospecific stands of the three invasive species, as well as *Carex lacustris* (hereafter *Carex*), a native sedge. Together, these approaches allowed me to test multiple hypotheses regarding direct and indirect effects of invasive plants on ecosystem processes in wetlands.

I hypothesized that invasive species would have higher NUE than native species, and therefore wetlands that were more invaded would have a higher site NUE compared to those that were less invaded (Figure 1-1: pathway 1). While there was some support for this hypothesis (Figure 2-2), the exclusion of just one site removed the significant relationship, thus little support was found for this hypothesis. This lack of a convincing relationship likely indicates that instead of invasive wetland species being able to attain high biomass production through the efficient use of N, they instead are able to use different N pools than natives or respond more positively to nutrient enrichment (Davis et al. 2000).

My subsequent hypothesis that wetland C stocks would be positively influenced by invasive species was supported, although the hypothesized mechanism behind the relationship was not (invasive species have higher NUE than natives; Figure 1-1: pathway 1,10). I found evidence that both soil and ecosystem C stocks increased due to presence of high biomass producing invasive species (Figures 2-5 and 2-6) and even though invasive species did not have an influence on wetland N stocks (Figure 1-1: pathway 1,4), there was evidence that native species had a negative effect on both soil and ecosystem N stocks. This could be considered an indirect effect of invasive species because of the negative effects invasive species had on the presence and diversity of native species (Figure 2-1). As for the effect invasive species had on wetland C stocks, because there was only weak evidence for invasive species decreasing litter quality (Table 2-3) and no evidence for any effect on soil

C:N ratios, it is possible that wetland invasive species affect C stocks by some belowground mechanism, such as greater belowground biomass (BGB) production or lower quality BGB, which would be in agreement with what Liao et al. (2007) found in a meta-analysis showing that invasive species increase root C stocks.

Besides the importance of considering the cumulative effects of invasive species, I also hypothesized there would be differences among the three most dominant invasive species in their effects on soil C and N mineralization. In my wetland survey, I found considerable support for this hypothesis (Figure 2-8) and was able to connect this to the quality of their litter (Figure 1-1: pathway 3,8,9), a plant trait known to affect ecosystem functioning (Eviner and Chapin 2003; Eviner 2004). Considering the results from Chapter 1 (wetland survey and laboratory incubations) it seems that invasive species are influencing the C (and to some extent N) stocks in inland Michigan wetlands, but that the most dominant invasive species in the study region differ in important plant traits, such as litter quality, and hence the identity of the invasive species is important to understand the full effect of an invasion.

In Chapter 3, I showed how removal of *Phragmites* litter and living biomass had the hypothesized effects of increasing light levels at the soil surface and increasing soil temperature (Figure 1-1: pathways 2,6 and 3,7; Figures 3-7 and 3-8). The effect on soil temperature, though, seemed to be dependent on the time of year, as warmer months with more direct sunlight increased soil temperature when biomass and litter was removed, but when there was less direct sunlight and daytime temperatures were lower, biomass and litter removal decreased soil temperature (Figure 3-10). This contrasting effect is likely due to the litter layer's ability to insulate the soil surface. Interestingly, this increased soil

temperature did not affect rates of litter bag decomposition or *in situ* N mineralization rates as I had hypothesized (Figure 1-1: pathway 2,6,9 and 3,7,6,9). As mentioned in Chapter 3, the lack of significant treatment effect could have been due to the depth of the soil core used in the N mineralization incubation and the cooler treatment conditions found in the treatment plots when the litter bag assay was performed. Additionally, the absence of any treatment effect on potential denitrification rates was probably caused by nonsignificant treatment effects on OM quality (a potential control on denitrification rates) since the assay was done in the laboratory, free from temperature variation among treatments found in the field. Litter and biomass removal did affect porewater ion concentrations, specifically Na^+, Cl^-, Ca^{2+}, and NO_3^-, due to either direct uptake or reduced evaporative concentration by evapotranspiration (Figure 1-1: pathway 5).

Though not initially hypothesized, all process rate data (litter bag decomposition, *in situ* N mineralization, and potential denitrification) showed strong site effects. These site effects, which included high potential denitrification rates at LLP1 and LLP2 compared to Glasby (Figure 3-28), negative N mineralization and nitrification rates at LLP1 (Figures 3-19 and 3-20), and a significant interaction between site and depth for filter paper decomposition rates (Figure 3-27), were likely influenced by the very different hydrologic conditions among the three sites. Glasby had constantly flooded conditions, LLP2 had a water table that fluctuated above to below the soil surface, and LLP1 had a water table usually below the soil surface. These differences in hydrology created variation in moisture conditions and soil redox status among sites. The effects of hydrology seen in this study demonstrate the overriding importance that water table position can have within wetland sites that all support the same species, *Phragmites*, and this concords with many

studies that show the importance of hydrology to wetland function (e.g., Hamilton 2002; Trebitz et al. 2005; Sierszen et al. 2006).

In Chapter 4, I showed the effects litter quality has on decomposition rates among species within the same functional group (emergent, clonal wetland dominants; Boutin and Keddy 1993), as well as the effects of litter quality on N mineralization rates (Figure 1-1: pathway 3,8,9). I found support for my hypothesis that the four species used in this assay, *Phragmites*, *Phalaris*, *Typha*, and *Carex*, would differ in litter quality and that litter C:N ratios would be negatively related to C and N mineralization rates. Though litter C:N ratios had a clear linear relationship with decomposition (Figures 4-6 and 4-7), there seemed to be a threshold for litter quality effects on N immobilization, in which litter with a C:N mass ratio greater than ~ 35 to 40 resulted in N immobilization, while C:N ratios less than the threshold resulted in net N mineralization (Figures 4-8 and 4-9).

Besides litter quality effects on decomposition, I found strong soil origin effects (significant soil effects after litter decomposition rates where corrected for background soil C mineralization). The soil origin effect is likely due to soil nutrient availability, but it is possible that other factors, such as the soil microbial community, influenced litter decomposition rates. The different litter quality and soil origin effects on C and N mineralization for these four plant species is notable because these species are in the same functional group and, therefore, their effects on ecosystem processes can sometimes be classified together (Diaz and Cabido 1997; Craine et al. 2002; McLaren and Turkington 2010). In addition, their unique effects on decomposition and N availability could feed back to increase their fitness and offer a mechanism of individual invasion success. For example, because the decomposition of *Phragmites* leaf litter was shown to result in net N

mineralization (as opposed to N immobilization), especially when incubated in its own soil (Figure 4-14), and since *Phragmites* has been shown to have a greater response to N availability than some native and exotic plant species (Minchinton and Bertness 2003; Saltonstall and Stevenson 2007), this might result in a positive feedback that could explain its success.

There is an apparent discrepancy across experiments (Chapters 2 and 4) in regard to the quality of *Phragmites* litter. In Chapter 2, *Phragmites* litter had the highest C:N ratio compared to the other study species (indicating low quality), while in chapter 4, *Phragmites* litter added in the litter quality and soil origin incubation had the lowest C:N ratio among study species. Also, in Chapter 4, for the litter diversity incubation, *Phragmites* litter was intermediate between Chapter 2 and the other incubation in Chapter 4. As discussed in the discussion section for Chapter 4, the discrepancy between the C:N ratios of *Phragmites* litter additions in the two incubations originated from different leaf:stem ratios used. Because leaf tissue was much more labile, the difference in leaf:stem ratio significantly changed the overall litter quality. The same general explanation can be used to explain the very low quality of *Phragmites* litter observed in Chapter 2. Litter collected in Chapter 2 was from the established litter layer at each site, and therefore most of the labile tissue, such as leaf material, had likely already decomposed, leaving the recalcitrant stem tissue. The stark difference in lability between stem and leaf tissue of *Phragmites* could have consequences to biogeochemical cycling. *Phragmites* is a late season grass that doesn't flower until late August or September, after which aboveground tissue senesces. At this time leaves detach and fall to the soil surface. The large input of labile tissue to the soil likely causes a pulse of C and N mineralization and N availability. Because of this behavior

(late senescence and leaf shed), *Phragmites* likely alters C and N cycling temporally compared to earlier flowering native species that do not have as much labile leaf material.

As I hypothesized, there was a significant effect of litter diversity (Figure 1-1: pathway 3,8,9) on litter decomposition rates (Figure 4-15), which could indicate that an outcome of the formation of monospecific stands (and hence a single species litter layer) would be a reduction in overall decomposition rate and an increase in organic matter buildup, i.e., invasive species decrease decomposition rates when they reduce species (litter) diversity. However, the effects of plant species diversity—and the corresponding diversity of litter inputs—on decomposition rates were minimal and more dependent of the identity of the species than just the number of species incubated. For example, the greatest C mineralization occurred when *Phalaris* litter was incubated with *Phragmites* litter, exceeding rates when either of the two species was incubated alone, suggesting a multiplicative response (Figure 4-10).

While all of the pathways shown in Figure 1-1 were investigated to some degree for the research described above, a more thorough investigation is needed for some pathways. In particular, for pathways 2,6,9 and 3,7,6,9, significant effects of both living biomass and litter were found for light levels and soil temperatures, but these effects did not translate to significant effects on C and N cycling. A longer, multi-season litter bag assay should be able to determine if the temperature effects are large enough to influence decomposition rates.

Additionally, the field study investigating litter and living biomass effects on C and N cycling (Chapter 4) was only done in monospecific *Phragmites* stands. Other dominant invasive species should be included in such investigations to determine species differences. There was evidence from the porewater equilibrators used in Chapter 4 that *Phragmites*

affected nitrate concentrations in the soil (Figure 1-1: pathway 5), but the assay was only conducted late in the growing season. Nitrate concentrations should be measured throughout the growing season to determine the full extent of *Phragmites* impact on nitrate availability. Lastly, significant effects of litter quality, litter diversity, and soil origin (Figure 1-1: pathway 3,8,9) were found for *Phragmites*, *Phalaris*, *Typha*, and *Carex* during laboratory incubations. To understand the strength of pathway 3,8,9 in a natural setting, a field experiment needs to be conducted with the same basic experimental design as the incubations in Chapter 4, except instead of CO_2 production in jars, litter mass loss could be monitored in a reciprocal transplant field experiment.

Taken together, the results from this dissertation research show how invasive species influence C and N cycling in inland Michigan wetlands through plant traits, such as litter quality and quantity, biomass production, and direct N uptake. Most wetlands of the southern Great Lakes region are either invaded or under the threat of invasion by aggressive wetland invaders like *Phragmites*, *Phalaris*, and/or *Typha*, therefore studies like those described in this dissertation are important if the ecosystem consequences of wetland invasions are to by fully understood. If invasive species are increasing C stocks in wetlands, this could be a positive outcome of wetland invasion. The research I described here also has value for wetland managers facing challenges from invasive plants. Large sums of money, often from public funds, are spent to control the invasive plants I studied and restore native plant communities in wetlands. We need a better comprehension of their impacts on wetlands, which may not all prove to be negative, in order to focus control efforts where they are most needed.

My results also indicate some future research opportunities. First, while inland wetlands are an important feature to interior Michigan, many invaded wetlands occur throughout the extensive Great Lakes coastal zones, thus the results of Chapter 2 showing the positive effects of invasive species on inland wetland C stocks should be compared to a similar study of these coastal wetlands. Second, the major shortcoming of the biomass and litter manipulation study was the short-term nature of the experiment. A five to ten year manipulation study may be able to identify living biomass and litter effects on soil organic matter quality and quantity that could not be elucidated in the study described in this dissertation. Finally, the mechanism behind the soil origin effect found in Chapter 4 should be investigated, along with expanding the litter quality incubation experiment to the field to determine if the same patterns found in the laboratory incubation can be extended to a natural setting.

APPENDICES

Appendix A

Table A-1. Models of Biomass C Stock. Model in bold indicates the best-fit model based on lowest AIC value.

Model	AIC	Δ AIC	wi
Null	1493.53	21.91	0.00
Invasive Dominance + Native Dominance	**1471.62**	**0.00**	**1.00**
Invasive Dominance	1485.43	13.81	0.00
Native Dominance	1485.46	13.84	0.00

Table A-2. Models of Biomass N Stock. Model in bold indicates the best-fit model based on lowest AIC value.

Model	AIC	Δ AIC	wi
Null	615.13	77.74	0.00
Invasive Biomass + Native Biomass	547.48	10.09	0.01
Invasive Biomass	572.09	34.70	0.00
Native Biomass	625.19	87.80	0.00
Total Biomass	**537.39**	**0.00**	**0.99**

Table A-3. Models of Litter C Stock. Model in bold indicates the best-fit model based on lowest AIC value.

Model	AIC	Δ AIC	wi
Null	1500.44	13.68	0.00
Invasive Biomass + Native Biomass	1507.27	20.51	0.00
Phalaris Biomass	1501.86	15.10	0.00
Total Biomass	1503.87	17.11	0.00
Phalaris Biomass + Biomass N + Biomass C + Biomass C:N	1487.77	1.01	0.29
Phalaris Biomass + Biomass N + Biomass C	1490.15	3.39	0.09
Phalaris Biomass + Biomass N	1493.43	6.67	0.02
Biomass N + Biomass C + Biomass C:N	**1486.76**	**0.00**	**0.48**
Biomass N + Biomass C:N	1490.11	3.35	0.09
Biomass C:N	1499.51	12.75	0.00
Biomass N	1491.94	5.18	0.04

Table A-4. Models of Litter N Stocks. Model in bold indicates the best-fit model based on lowest AIC value.

Model	AIC	Δ AIC	wi
Null	737.54	1.52	0.28
Invasive Biomass + Native Biomass	757.66	21.64	0.00
Phalaris Biomass	745.76	9.74	0.00
Total Biomass	747.63	11.61	0.00
Phalaris Biomass + Biomass N + Biomass C + Biomass C:N	751.40	15.38	0.00
Phalaris Biomass + Biomass N + Biomass C	747.22	11.20	0.00
Phalaris Biomass + Biomass N	744.30	8.28	0.01
Biomass N + Biomass C + Biomass C:N	743.52	7.50	0.01
Biomass N + Biomass C:N	740.53	4.51	0.06
Biomass C:N	743.40	7.38	0.02
Biomass N	**736.02**	**0.00**	**0.61**

Table A-5. Models of Litter C:N ratio. Model in bold indicates the best-fit model based on lowest AIC value.

Model	AIC	Δ AIC	wi
Null	926.87	18.28	0.00
Invasive Dominance + Native Dominance	909.33	0.74	0.31
Invasive Dominance	923.25	14.66	0.00
Invasive Dominance + Biomass C + Biomass N + Biomass C:N	912.72	4.13	0.06
Invasive Dominance + Biomass N + Biomass C:N	911.54	2.95	0.10
Invasive Dominance + Biomass N	**908.59**	**0.00**	0.45
Biomass C + Biomass N + Biomass C:N	916.61	8.02	0.01
Biomass N + Biomass C:N	915.53	6.94	0.01
Biomass N	912.55	3.96	0.06
Biomass C:N	921.67	13.08	0.00

Table A-6. Models of Soil C Stock. Model in bold indicates the best-fit model based on lowest AIC value.

Model	AIC	Δ AIC	wi
Null	2105.64	38.88	0.00
Invasive Biomass + Native Biomass + Litter Mass + Litter C + Litter N +Litter C:N	**2066.76**	**0.00**	**0.68**
Invasive Biomass + Litter Mass + Litter C + Litter N	2075.76	9.00	0.01
Invasive Biomass + Litter Mass	2103.37	36.61	0.00
Invasive Biomass + Litter Mass + Litter C + Litter N + Litter C:N	2069.09	2.33	0.21
Invasive Biomass + Litter Mass + Litter N + Litter C:N	2077.23	10.47	0.00
Invasive Biomass + Litter Mass + Litter N	2084.96	18.20	0.00
Native Biomass + Litter Mass + Litter C + Litter N + Litter C:N	2070.80	4.04	0.09
Invasive Biomass	2103.62	36.86	0.00
Native Biomass	2104.12	37.36	0.00
Litter C + Litter N	2080.10	13.34	0.00
Litter N + Litter C:N	2082.79	16.03	0.00
Litter Mass	2105.62	38.86	0.00
Litter N	2090.03	23.27	0.00

Table A-7. Models of Soil N Stocks. Model in bold indicates the best-fit model based on lowest AIC value.

Model	AIC	Δ AIC	wi
Null	1548.56	16.12	0.00
Invasive Biomass + Native Biomass + Litter Mass + Litter C + Litter N +Litter C:N	1536.78	4.34	0.08
Invasive Biomass + Litter Mass + Litter C + Litter N	1539.19	6.75	0.02
Invasive Biomass + Litter Mass	1551.27	18.83	0.00
Invasive Biomass + Litter Mass + Litter C + Litter N + Litter C:N	1537.44	5.00	0.06
Invasive Biomass + Litter Mass + Litter N + Litter C:N	1540.97	8.53	0.01
Invasive Biomass + Litter Mass + Litter N	1543.43	10.99	0.00
Native Biomass + Litter Mass + Litter C + Litter N + Litter C:N	**1532.44**	**0.00**	**0.72**
Invasive Biomass	1552.70	20.26	0.00
Native Biomass	1547.90	15.46	0.00
Litter C + Litter N	1537.69	5.25	0.05
Litter N + Litter C:N	1538.09	5.65	0.04
Litter Mass	1547.61	15.17	0.00
Litter N	1541.31	8.87	0.01

Table A-8. Models of Soil C:N. Model in bold indicates the best-fit model based on lowest AIC value.

Model	AIC	Δ AIC	wi
Null	530.87	3.60	0.13
Invasive Biomass + Native Biomass + Litter Mass + Litter C + Litter N +Litter C:N	573.96	46.69	0.00
Invasive Biomass + Litter Mass + Litter C + Litter N	558.55	31.28	0.00
Invasive Biomass + Litter Mass	556.95	29.68	0.00
Invasive Biomass + Litter Mass + Litter C + Litter N + Litter C:N	565.50	38.23	0.00
Invasive Biomass + Litter Mass + Litter N + Litter C:N	560.75	33.48	0.00
Invasive Biomass + Litter Mass + Litter N	554.21	26.94	0.00
Native Biomass + Litter Mass + Litter C + Litter N + Litter C:N	561.15	33.88	0.00
Invasive Biomass	543.46	16.19	0.00
Native Biomass	539.83	12.56	0.00
Litter C + Litter N	531.79	4.52	0.08
Litter N + Litter C:N	533.65	6.38	0.03
Litter Mass	544.51	17.24	0.00
Litter N	**527.27**	**0.00**	**0.76**

Table A-9. Models of Ecosystem C Stock. Model in bold indicates the best-fit model based on lowest AIC value.

Model	AIC	Δ AIC	wi
Null	2108.37	6.79	0.02
Invasive Biomass + Native Biomass	**2101.58**	**0.00**	**0.74**
Invasive Biomass	2104.43	2.85	0.18
Native Biomass	2106.85	5.27	0.05

Table A-10. Models of Ecosystem N Stock. Model in bold indicates the best-fit model based on lowest AIC value.

Model	AIC	Δ AIC	wi
Null	1547.04	0.60	0.38
Invasive Biomass + Native Biomass	1550.75	4.31	0.06
Invasive Biomass	1550.91	4.47	0.05
Native Biomass	**1546.44**	**0.00**	**0.51**

Table A-11. Best-fit model summary table for each dependent variable.

Dependent	Model Structure	wi
Biomass C Stock	Invasive Dominance + Native Dominance	1.00
Biomass N Stock	**Total Biomass**	0.99
Litter C Stock	Biomass N + Biomass C + Biomass C:N	0.48
Litter N Stock	Biomass N	0.61
Litter C:N	Invasive Dominance + **Biomass N**	0.45
Soil C Stock	**Invasive Biomass** + Native Biomass + **Litter Mass** + Litter C + Litter N +Litter C:N	0.68
Soil N Stock	**Native Biomass** + **Litter Mass** + Litter C + Litter N + Litter C:N	0.72
Soil C:N	**Litter N**	0.76
Ecosystem C Stock	**Invasive Biomass** + Native Biomass	0.74
Ecosystem N Stock	**Native Biomass**	0.51

Appendix B

In each reference plot, the following plant traits were estimated in the 2008 and 2009 growing season: aboveground biomass (AGB) production, nitrogen use efficiency (NUE), organ specific tissue chemistry, litter mass, litter depth, litter chemistry, and plant height (plant height data were also collected in 2010). These measurements were taken for both baseline data and for the ability to compare the three *P. australis* sites. AGB production was estimated in a randomly selected 1 m^2 subplot within each permanent plot by harvesting standing biomass at the peak biomass period. Biomass samples were separated into leaves, stems, and inflorescences, weighed, and analyzed for %C and %N on a Costech Elemental Analyzer. Maximum plant height was measured once a month during the growing season. NUE was calculated as plot and plant level N productivity (g dry weight g^{-1} N) (Berendse and Aerts 1987; van Ruijuen and Berendse 2005). Litter depth was recorded and litter was collected from within the 1 m^2 subplot (629 cm^2), dried at 65°C for 48 hours, weighed and analyzed for %C and %N.

Along with these measurements, belowground biomass (BGB) was measured in the summer of 2009. BGB was estimated by coring to a depth of 30 cm using a PVC pipe (10 cm diameter). Biomass was sampled to a 30 cm depth because this is within the depth range of the majority of BGB production for *P. australis*, though it can grow deeper in drier conditions. Five BGB cores were collected for each site, one in each reference plot and two additional cores collected outside the treatment plots but within the *P. australis* stand. Soil was removed, washed, and sieved (2 mm) to separate roots and rhizomes from soil. Roots and rhizomes were then dried to constant mass at 80°C, weighed, and analyzed for %C and

%N. Seasonal variation in tissue chemistry was determined by sampling AGB three times during the growing season.

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