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Quantifying relationships between phosphorus availability and mycorrhizal associations in wetland plants

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ABSTRACT

Increasing evidence indicates that plant community structure and therefore ecosystem function are mediated by below-ground fungal communities that form intracellular associations with plant roots called mycorrhizal associations. Arbuscular mycorrhizal fungi (AMF) are a type of mycorrhiza that colonize the plant host intracellularly but maintain hyphae outside the root cell for resource acquisition. The importance and function of AMF associations are well-documented in terrestrial ecosystems, but are less understood in aquatic or semi-aquatic systems. Phosphorus availability is the primary factor influencing mycorrhizal colonization and growth in terrestrial soils, with phosphorus-abundant soils leading to a decrease in mycorrhizal growth. However, the relationship between arbuscular mycorrhizal colonization and phosphorus supply in wetland systems is not well understood. Previous studies have examined this relationship, but have been limited by methodology and have indicated the need for studies closely mimicking natural conditions. To address this need, a field-based study was performed examining the mycorrhizal colonization of wetland plants growing in a natural wetland. Since field studies allow for only limited isolation and control of variables, a greenhouse study was also performed to isolate the impacts of phosphorus on mycorrhizal colonization in wetland plants. This study showed that phosphorus concentrations between 10 and 30 µg/L PO_4-P are sufficient to alter mycorrhizal colonization in wetland plants, but the responses are species-specific. This variable impact on mycorrhizal colonization could induce species-specific responses in wetland plants, leading to shifts in community composition of wetland vegetation and ecosystem functioning.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Arbuscular mycorrhizal fungi and their relationship to phosphorus availability in wetland environments

Ecosystem services of wetlands

Wetlands are amongst the most valuable and productive ecosystems throughout the world (Clarkson et al., 2013), providing numerous social, economic, environmental and cultural benefits, termed ecosystem services (Costanza and Folke, 1997). These services include maintaining water quality and supply, regulating atmospheric gases, sequestering carbon, preventing erosion, maintaining biodiversity, and providing cultural, recreational, and educational resources (Table 1.1). Given societies' dependence on ecosystem services, the sustainable management of these resources is necessary (Clarkson et al., 2013). Despite the numerous benefits associated with maintaining wetlands, many are being degraded due to anthropogenic activities, primarily by altering hydrology, affecting water quality and availability (Erwin, 2009; Vorosmarty and Sahagian, 2000). Wetlands cover only 1.5% of the surface of the Earth, yet they provide 40% of global ecosystem services (Zedler and Kercher, 2005). North America has lost about 50% of its original wetland cover, with the largest decline of any wetland category being forested freshwater wetlands, which mostly consist of riverine flood plains (Tockner and Stanford, 2002). Southern Ontario has lost the majority of its wetlands, with some regions experiencing as much as a 70-90% reduction in wetland area (Erwin, 2009). Without solid wetland management strategies, rapid extinctions of wetland species and loss of ecosystem services are projected within the next few decades (Tockner and Stanford, 2002). Rivers are included under the broadest definition of a wetland, and riverine wetlands are among the most impacted by human activities (Withers and Jarvie, 2008). Between 1879 and 1972 the aquatic surface area along the Missouri River decreased by 50%, with a 63% increase in cultivated land area and a 85% reduction in

commercial fishery yield (Galat et al., 1998). In addition, between 1880 and 1990 over 40 freshwater fish species went extinct in North America (Tockner and Stanford, 2002). Currently, more than 50% of the world's streams and rivers cross one or more dams, and this fraction could increase to 90% by 2030 (Cappellen and Maavara, 2016). Rivers and their associated riparian areas, reservoirs, and floodplains contribute to a plethora of essential ecosystem services, including supplying water for drinking, sanitation, irrigation, and industrial uses (Cappellen and Maavara, 2016). There is an urgent need to preserve existing, intact flood plain rivers as strategic global resources and to begin to restore hydrologic dynamics, sediment transport and riparian vegetation to those rivers that retain some level of ecological integrity (Tockner and Stanford, 2002).

Role of vegetation in wetlands

Many important benefits of wetlands are due to the presence of wetland plants. Wetland plants contribute to maintaining water quality by providing a stable surface for physical filtration (Brix, 1994), the oxygenation of the water column and sediments, and reductions in water current velocity (Petticrew and Kalff, 1992). This contribution of wetland plants to maintaining water quality is primarily achieved through physical means, with wetland plants contributing to sedimentation of fine particles and their associated nutrients, heavy metal, and organic contaminants (Petticrew and Kalff, 1992). Water current velocity was reduced by 30-40% compared to control treatments through seagrass (*Posidonia oceanica*) beds as a result of physical filtration, and particle velocity was reduced primarily through collision with seagrass leaves (Hendriks et al., 2008). In littoral zones of lakes, water current velocity was reduced by as much as 2.0 cm s^{-1} with

an increase in leaf area index of wetland plants (Petticrew and Kalff, 1992). In growth room studies using plants taken from freshwater environments, it was found that submerged roots of *Potamogeton* spp. could provide up to 68 mg O_2 m^2 h^{-1} to the water column (Sand-jensen et al., 1981). Wetland plants act as a sink for greenhouse gases by the photosynthetic assimilation of CO_2 from the atmosphere and sequestration of the organic matter produced in wetland soils (Brix et al., 2001). Restored wetlands in the Sacramento-San Joaquin Delta were net sinks for CO_2, sequestering up to 397 g C m^{-2} yr^{-1} (Knox et al., 2015). Wetland plants can also act as sources of CH_4 by favouring microbial anaerobic decomposition and internal gas transport (Brix et al., 2001), with restored wetlands emitting between 39 to 53 g C m^{-2} yr^{-1} (Knox et al., 2015). Wetland plants can provide attachment points for microbial biofilms, providing variable habitats suitable for increased biodiversity (Bianciotto and Bonfante, 2002; Brix, 1994). Wetland plants also provide increased surface area for epiphytic associations and refugia for invertebrates and grazers (Withers and Jarvie, 2008). Additionally, vegetation makes wetlands aesthetically pleasing, which can provide cultural and recreational benefits (Brix, 1994). Given the many contributions that wetland plants make to the provision of ecosystem services, it is of critical importance to understand the fundamental components that can influence plant performance and community structure, and ultimately ecosystem functioning in wetlands.

Importance of mycorrhizal colonization in wetland plants

Increasing evidence indicates that plant community structure and therefore ecosystem function are mediated by below-ground fungal communities that form

intracellular associations with plant roots called mycorrhizal associations (van der Heijden et al., 1998a). Arbuscular mycorrhizal fungi (AMF) are a type of mycorrhizal fungi that colonize the plant host intracellularly but maintain hyphae that extend outside of the root for resource acquisition (Smith, 1990). Hyphae are the main vegetative growth form of AMF, and are filamentous structures which ramify through the soil (Mcgonigle et al., 1990). The hyphae form a net-like mycelium surrounding the plant roots and penetrate the root cell walls, invaginating the cell membrane (Figure 1.1). The net-like mycelium also interacts with the environment, forming a hyphal network which can extend the plant root's nutrient and water acquisition capability (Friese and Allen, 1991), and connect to other plants, exchanging nutrients horizontally (Bonfante and Anca, 2010). During this succession of events, AMF interact with bacteria which occupy specific fungal niches such as spores and hyphae (Bianciotto and Bonfante, 2002). The rhizospheric microbiota changes occurs when mycorrhizas form, and beneficial effects are the result of additive or synergistic interactions amongst all rhizosphere microbes (Bianciotto and Bonfante, 2002). Energy is primarily stored as lipids within hyphal swellings called vesicles (Figure 1.1), which may develop thick walls in older roots and can function as propagules (Biermann and Linderman, 1983). Arbuscules are intricately branched tree-like structures found on the interior of the cortical cells of vascular plants. The arbuscule is the main defining feature of AMF and they are formed with repeated dichotomous branching and reduction of hyphae which can end with hyphae less than 1 µm in diameter (Brundrett et al., 1985). The arbuscules are theorized to be the location at which the fungus exchanges nutrients with the plant host (Smith, 1990), and they are the main morphological characteristic used for identification purposes (Figure 1.1).

The importance and function of AMF associations are well documented in terrestrial ecosystems (van der Heijden et al., 2004). These associations are widespread and occur in the majority of vascular plant families (Bever et al., 1996). AMF can act as support systems for seedling establishment in terrestrial soils by adding seedlings into existing hyphal networks and by supplying nutrients to the seedlings (van der Heijden, 2004). AMF have been shown to influence the success of seedling establishment in new soils, with plants grown in AMF inoculated soil increasing growth by up to almost 40% compared to non-mycorrhizal treatments (Klironomos, 2002). Plants have been shown to rely to different degrees on their mycorrhizal partners, with biomass of *B. erectus, B. pinnatum* and *P. vulgaris* increased respectively, by 55%, 70%, and 17% in microcosms inoculated with AMF compared to non-mycorrhizal treatments (van der Heijden et al., 2004). Plant diversity and plant community structure have also been shown to be influenced by AMF presence and community structure (van der Heijden et al., 1998b). Greenhouse experiments using single plants indicate AMF can facilitate phosphorus uptake, and that there is large functional diversity among various AMF taxa with respect to phosphorus acquisition (Koch et al., 2004). Other studies have also indicated that the presence of AMF can enhance nitrogen acquisition (Hawkins et al., 2000; Hodge et al., 2010).

The links between AMF and plant diversity and community structure are less understood in aquatic or semi-aquatic systems than in terrestrial systems (White and Charvat, 1999). Early studies indicated there were few mycorrhizal fungi occurring in aquatic or wetland plants (Khan, 1974; Powell, 1975). However, recent studies have found significant mycorrhizal colonization in *Mimulus ringens* and *Lycopus americanus*

in prairie fens (Turner et al., 2000), in *Taxodium distichum* in a Cypress swamp (Kandalepas et al., 2010), as well as in 31 of 37 plant species in a bottomland hardwood forest (Stevens et al., 2010). Benefits to maintaining AMF colonization in inundated conditions have been identified in several plant families. AMF-inoculated *Oryza sativa* plants showed up to 30% increased Zn acquisition in an upland nursery, possibly as a result of the increased absorption of nutrients due to the greater surface area of AMF hyphae extending from the roots (Purakayastha and Chhonkar, 2001). AMF inoculated *O. sativa* also showed increased shoot weight, root weight, root length, and root volume when compared to non-mycorrhizal treatments (Purakayastha and Chhonkar, 2001). AMF colonized *Casuarina equisetifolia* plants were shown to adapt to flooding, with inoculated plants increasing nodule dry weight after 4 days of flooding by 45% compared to non-mycorrhizal treatments (Osundina, 1998). This was achieved partly by more extensive development of adventitious roots and hypertrophied lenticels, which increased O_2 diffusion to the roots and to the upper zone of the flooded soil (Osundina, 1998). AMF have also shown an ability to alter plant community structure in mesocosm studies simulating calcareous fens, by increasing above-ground biomass by 21.6% and decreasing Shannon Diversity by 0.5 in AMF inoculated plant communities compared to non-AMF plant communities (Wolfe et al., 2006). This decrease in diversity in the plant community could be due to the relative dependence of plants on their mycorrhizal partners. Since the study conducted by Wolfe et al. (2006) was only conducted over a 12-week period, it is possible these negative effects may become neutral or even positive over longer time periods. These varying effects of AMF in wetlands suggest potential

parallels with terrestrial ecosystems, therefore, understanding the impacts and constraints on AMF is necessary to maintain wetland ecosystem function.

Plant performance is inextricably linked to nutrient uptake, with a reduction in nutrient uptake leading to reductions in plant performance. Interpreting changes in plant community dynamics is, therefore, reliant on the understanding of the relationships among the various pathways for resource uptake (Stevens and Peterson, 2007). The uptake of nutrients by plants occurs much faster than nutrient replenishment in the soil, the latter occurring primarily through diffusion in terrestrial systems (Marschner and Dell, 1994). This forms water and mineral depletion zones in soils immediately around the root, limiting further nutrient uptake. AMF have access beyond the zone of depletion by extending extracellular hyphae further than the plant roots, enabling the plant to acquire previously unavailable nutrient supplies (Clark and Zeto, 2000). Mycorrhizal hyphae are able to penetrate deeper into soils due to the smaller diameter relative to plant roots (Clark and Zeto, 2000). AMF have been shown to aid host plants in the acquisition of water, phosphorus, and other nutrients by forming branching hyphal networks in soil (Harner et al., 2011; Jeffries et al., 2003). Since AMF hyphae are obligate aerobes, it is unclear the extent to which hyphae can extend into wetland soils due to oxygen limitation (Carvalho et al., 2004). Improved phosphorus (P) acquisition is the most common benefit attributed to AMF associations in terrestrial host plants (Bolan, 1991). However, in addition to contributing up to 80% of plant P, AMF has been shown to contribute as much as 25% of plant N, 10% of plant K, 25% of plant Zn, and 60% of plant Cu in experimental chambers (Marschner and Dell, 1994). This improved nutrient and water acquisition capability provides a clear benefit to the host plant. This is done through both

mechanical and biochemical processes. Mycorrhizal hyphae is further able to facilitate the movement of phosphorus in soils by increasing the soil volume explored, reducing the distance phosphate ions must diffuse to come into contact with plant roots, and by increasing the surface area for absorption (Bolan, 1991). Mycorrhizal hyphae have also been shown to exude phosphatases which break down organic phosphorus in the soil, allowing previously unavailable sources of phosphorus to be accessed (Joner et al., 2000). Individual plants can uptake phosphorus from the soil phosphorus pools primarily by two main mechanisms: 1) They acquire inorganic phosphorus directly across the plant root cell wall, or 2) they acquire phosphorus through a mycorrhizal symbiosis (Schachtman et al., 1998). When plants transport phosphorus directly into the cell from the environment, they must overcome soil phosphorus concentrations which are usually very low (10 μM) while plant phosphorus need is very high (Schachtman et al., 1998). To counteract this, plants take inorganic phosphorus (Pi) across the cell membrane using specialized transporter proteins. Addition of Pi results in a depolarization of the plasma membrane and acidification of the cytoplasm of plant root cells (Ullrich and Novacky, 1990). The depolarization indicates that Pi does not enter simply as $H_2PO_4^-$ or HPO_4^{2-}, both of which would lead to membrane hyperpolarization. From these results, it is likely that Pi is co-transported with positively charged ions (Schachtman et al., 1998).

Controls of AM colonization in terrestrial systems

Species and strains of AMF differ in their ranges of tolerance of physical and chemical properties of soil (Abbott and Robson, 1991). The primary abiotic factors known to influence the abundance and distribution of AMF in terrestrial systems are

water, oxygen, salt, and nutrient availability (Read, 1991). Numerous studies have found that flooding of agricultural and non-agricultural terrestrial plants leads to reduced colonization. For example, continuous flooding in growth room studies representing natural salt marshes has been shown to lead to reduced colonization and AM fungal growth in *Aster tripolium* (Carvalho and Correia, 2003). Plant root colonization of corn (*Zea mays* L.) by AMF has also been shown to be reduced by up to 34% in flooded agricultural fields, compared with non-flooded fields (Ellis, 1998). This reduction in colonization may have been due to a decline in photosynthetic capacity, stomatal conductance and nutrient uptake by inundated host plants in response to oxygen deficient soils (Carvalho and Correia, 2003). Excess salts in terrestrial soils have been shown to decrease mycorrhizal colonization in multiple plant species. Germination of spores and subsequent hyphal growth of AMF in *Amania s*p. has been shown to be reduced by increased concentrations of salts in soils (Juniper and Abbott, 1993). Pfeiffer and Bloss (1998) showed that adding 750 mg NaCl per kg of soil decreased the root length of guayule (*Parthenium argentatum*) colonized by *Glomus intraradices* by 31%. Numerous studies have shown nutrient availability to have various impacts on AMF colonization. Increased nutrient availability has shown to decrease mycorrhizal branching density by as much as five times in vegetation dominated by mosses, grass (*Deschampsia flexuosa*), and bilberry (*Vaccinium myrtillus*) (Majdi et al., 2001). In a summary of multiple studies examining mycorrhizal responses to P and N, mycorrhizal abundance decreased by 15% under N fertilization and 32% under P fertilization (Treseder, 2004).

P availability is the primary factor influencing mycorrhizal colonization and growth in terrestrial soils, with phosphorus-abundant soils leading to a decrease in

mycorrhizal growth (Abbott et al., 1984; Treseder, 2004). Increasing soil P was shown to inhibit mycorrhizal infection and hyphal production in soybeans (*Glycine max*), with decreases in mycorrhizal infection by up to 20% in soil with added P (Asimi and Gianinazzi, 1980). Soil P enrichment has also been shown to decrease the length of plant root infected by AMF by as much as 60% in subterranean clover (*Trifolium subterraneum* L.). Increased soil P availability has been shown to decrease density of mycorrhizal chlamydospores in the soil from 95/g soil to 5.4/g soil in split root experiments with Sudangrass (*Sorghum vulgare* Pers.) (Steirle et al., 1978).

Effects of phosphorus on water quality

Increased anthropogenic activities have led to significant increases in phosphorus concentrations in agricultural and urban watersheds (Zhang, 2016). Nutrient loads can originate from storm water runoff, discharge from ditches and creeks, groundwater seepage, aquatic weed control, naturally occurring organic inputs, and atmospheric deposition (Ouyang et al., 2006). In Canada, agriculture is the primary contributor of P to watersheds, with fertilizer sales increasing from 210 000 tonnes in 1966 to 2.4 million tonnes in 2013 (Zhang, 2016). These inputs can lead to changes in species composition in aquatic plant communities within the river basin (David and Gentry, 2006). Rivers are particularly vulnerable to nutrient loading due to their proximity to urban centers, sensitivity to land use changes, and extent of exploitation (Withers and Jarvie, 2008). Current levels of P in the Grand River watershed have routinely exceeded the Provincial Water Quality Objective for rivers and streams (30 µg/L P) with recorded values higher than 200 µg/L P (Loomer and Cooke, 2011). Due to the well-studied relationship

between increased P supply and AMF, phosphorus enrichment in wetlands could disproportionately affect mycorrhizal communities and lead to changes in wetland functioning.

Controls of AMF colonization in wetlands

Wetland mycorrhizas are relatively poorly studied, although increasing evidence is indicating parallels between established relationships of AMF in terrestrial systems and those found in aquatic conditions. Mycorrhizal colonization in wetlands has been shown to be inversely correlated with water availability in *Lythrum salicaria* (L.), with hyphal and arbuscular colonization being significantly higher in drier regions (Stevens and Peterson, 1996). Increased salinity levels were shown to affect the establishment of AM colonization, AM fungal growth and activity within roots and extraradical mycelium growth (Carvalho and Correia, 2003). Triclosan (TCS) is a widely used antibacterial compound found in pharmaceuticals and personal care products, and exposure can negatively impact AMF communities (Twanabasu et al., 2013). Although there have been few studies examining the impacts of abiotic factors on mycorrhiza in wetlands, the studies that have been done have indicated parallels with terrestrial environments.

Relatively few studies have examined the relationship between AM and P in wetland plants. Of the three studies which examined this relationship, all found that P availability negatively impacted mycorrhizal colonization in wetland plants. In the emergent plant, *Lythrum salicaria* L., it was found that mycorrhizal colonization was absent at concentrations above 1000 µg PO_4/L (White and Charvat, 1999). Cattails (*Typha* spp.; Typhaceae) are an important component of many wetlands worldwide that

have generally been found to be nonmycorrhizal (Bagyaraj et al., 1979; Khan, 1974). However, more recent studies have shown that *Typha angustifolia* can be colonized in controlled conditions as a function of P availability, with increased phosphorus leading to decreases in AMF (Tang et al., 2001). Stevens et al. (2002) showed that plants inoculated with AMF can influence plant performance and that the AM association can become uncoupled at high levels of P availability. As the demand grows for preserving and re-establishing wetland plant communities for the many beneficial functions they provide, it will be increasingly necessary to understand the role AMF play in these areas (Stevens et al., 2002). Due to the increasing P enrichment in soils and aquatic systems (Bennett et al., 2001), there is a potential for an uncoupling of the relationship between wetland plants and AM communities, resulting in shifts in plant community structure and wetland ecosystem function.

Previous studies on the effects of P supply on mycorrhizal colonization in wetland plants

Despite the tremendous impacts of AM on plant communities in terrestrial systems, relatively few studies have examined how phosphorus exposure affects this relationship in wetland environments. All three studies which did examine this relationship in wetlands found that P availability negatively impacted mycorrhizal colonization. However, the authors noted that the sensitivity and extrapolation of these studies are limited by methodology.

The study performed by White and Charvat (1999) examined mycorrhizal responses in *Lythrum salicaria* L. at five phosphorus levels (0, 100, 1000, 10000, or

47500 µg PO_4/L nutrient solution) grown over 49 days. Each plant was inoculated with approximately 100 field-collected spores. Plants were grown in growth chambers and watered with Hoagland's solution with the appropriate P concentration. Mycorrhizal inoculation was found at 0, 100, and 1000 µg PO_4/L, with colonization increasing from 0 to 1000 µg PO_4/L. Almost no AMF were found at 10000 or 47500 µg PO_4/L (Figure 1.2). Due to the static non-renewal approach to P application, the authors indicated a need for studies mimicking natural conditions (White and Charvat, 1999). The static non-renewal approach allows for only a finite amount of P which is not seen in the natural environment. It is also not clear whether P concentrations were limiting throughout the study in the higher concentrations, as they were not quantified throughout the duration of the study.

The study performed by Tang et al. (2001) examined mycorrhizal responses in *Typha angustifolia* at four phosphorus levels (95, 950, 9500, and 47500 µg PO_4/L) and two inoculum densities (200 or 500 spores per pot) grown over 13 weeks. Inoculum was obtained from soil pot cultures of Sudan grass, *Sorghum sudanese*, that contained an isolate of *Glomus mossae.* Plants were grown in growth chambers and watered with 70 mL of modified half-strength Hoagland's solution with the appropriate P concentration weekly. Mycorrhizal colonization was found at 95, 950, and 9500 µg PO_4/L, with colonization increasing from 95 to 950 µg PO_4/L before a significant drop in colonization at 9500 µg PO_4/L. AMF were absent at 47500 µg PO_4/L (Figure 1.2). Because previous field studies examining the interaction between phosphorus and AMF had conflicting results, the authors indicated a need for studies which varied multiple characteristics individually and in tandem under the most natural conditions possible (Tang et al., 2001).

This study differed greatly in P application and environmental parameters from field conditions, with a non-renewal approach providing a finite amount of P to the system. In this study it was also not clear whether P concentrations were limiting in the higher concentrations, as they were not quantified throughout the duration of the study.

The study performed by Stevens et al. (2002) examined mycorrhizal responses in *Lythrum salicaria* L. in inundated conditions at five phosphorus levels (0, 1250, 5000, 10000, or 40000 µg PO_4/L) with or without AM inoculation (AM+ or AM-). Plants were grown for six weeks before AM inoculation then harvested six weeks after inoculation. Mycorrhizal inoculum was obtained from AM cultures from soils collected at several wetland sites in Southern Ontario. Plants were grown under greenhouse conditions and inundated in deionized water. Phosphorus was added as $NaH_2PO_4 \cdot 2H_2O$ and, unlike the previous studies, checked at 12 day intervals and additional P was added to maintain desired levels. Total plant height, root and shoot fresh weights and shoot dry weight were assessed and roots were digitized using a Hewlett Packard ScanJet 4C/T scanner and the total root length and total root surface area determined using WinRhizo (Regent Instruments Inc. Quebec City, Quebec). Levels of AM colonization generally decreased with increasing P supply, with arbuscules, vesicles, and internal hyphae showing significant decreases at 5000 µg PO_4/L (Figure 1.2). This study also showed that AMF have the potential to alter at least some aspects of plant morphology under inundated conditions. Total plant height was shown to significantly decrease at 10000 µg PO_4/L in AM inoculated plants. This may mean that AMF contribute little to P acquisition in aquatic or semi-aquatic systems (Stevens et al., 2002).

These studies indicate that with increasing phosphorus concentrations, there is an increase in mycorrhizal colonization until the concentration reaches between 1250 to 5000 µg PO_4/L. Between 1250 and 5000 µg PO_4/L, mycorrhizal colonization was drastically reduced. This relationship has been identified in many plant species, suggesting that phosphorus is limiting to both fungus and plant growth at the lower concentrations (Mendoza and Pagani, 1997). In addition, it shows that plants exhibit control over fungal morphogenesis within root tissues, primarily through reducing intercellular hyphal branching (Gianinazzi-Pearson, 1996). The main drawback in these studies was the phosphorus exposure system. Two of these studies used static non-renewal approaches and one used a static renewal approach with a finite amount of P available to the plant. These conditions are typically not seen in natural watersheds. Because these studies were growth room studies without continuous P application, the lowest concentrations which can impact mycorrhizal colonization in natural wetlands is currently unknown. There have also been limited field studies examining the AM status of plants along phosphorus gradients in natural wetlands. To address this shortcoming, the current field study was conducted in a natural wetland, allowing for constant P inputs from the natural environment. Since field studies do not allow for the isolation of individual variables, the mesocosm study used a continuous flow-through exposure system, allowing for constant availability of P for uptake to the plant. The previous studies also used P concentrations almost an order of magnitude higher than those seen in natural rivers (47500 µg PO_4/L in greenhouse study compared to 7000 µg PO_4/L, the highest concentration detected in Ontario). These previous studies also used one plant species as the study organism. This study examines multiple plant families and is more

representative of the plant community. Because this study is more closely simulating natural conditions and there is a continuous application of P, I predict mycorrhizal colonization to decrease at lower concentrations than those found in previous studies.

Purpose of thesis

Because wetland functioning is vital to maintaining essential ecosystem services and because plants and their mycorrhizal partners are essential in facilitating wetland ecosystem functioning, understanding the impacts that changes in mycorrhizal colonization can have on plant community and performance is crucial. If the relationship is the same as in terrestrial systems, limits to phosphorus availability could alter mycorrhizal colonization and plant community structure, ultimately impacting ecosystem functioning. Given the limitations in the previous studies examining the effects of P availability on mycorrhizal associations I ask the following question:

- **Do phosphorus concentrations found in the Grand River Watershed affect mycorrhizal colonization in wetland plants?**

This question is important because if the interactions of mycorrhiza in aquatic or semi-aquatic conditions mirror those in terrestrial systems, the dependence of wetland plants on their mycorrhizal partners would vary, meaning some plants would be impacted more than others by reduced AMF performance. If increased phosphorus availability led to species-specific reductions in mycorrhizal colonization, this could lead to altered plant community composition, diversity, and performance. To answer this question, I had two main objectives. The first objective was:

1. **To determine if water quality is correlated with mycorrhizal colonization of wetland plants in the natural environment.**

To do this, I performed a field study which characterized the mycorrhizal colonization of wetland plants along a water quality gradient. We considered naturally-occurring wetland plants receiving water continuously from natural sources. It was expected that changes in phosphorus availability would correlate to changes in arbuscular mycorrhizal colonization of wetland plants. The second objective was:

2. **To determine if phosphorus availability impacts mycorrhizal colonization in wetland plants.**

To isolate phosphorus as an explanatory variable, I performed a greenhouse study which characterized the mycorrhizal colonization of wetland plants at four phosphorus concentrations. We used a continuous flow-through application which provided stable concentrations of phosphorus throughout the experiment. We also used plant seeds collected in the field as well as an unsorted field inoculum of mycorrhizal spores. For both studies, various wetland plants were chosen as test species due to their prevalence in wetland habitats in Southern Ontario and their capacity to form mycorrhizal associations. This thesis will demonstrate the interactions between phosphorus and AMF across multiple wetland plant families.

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Tables and Figures

Table 1.1: Ecosystem services and their functions provided by wetlands. Table is modified from Costanza et al., 1997. This study estimated the current economic value of 17 ecosystem services across 16 different biomes. For the entire biosphere it was estimated that the total value ranged from US$ 16-54 trillion per year, with an average of US$ 33 trillion per year.

Ecosystem service	Ecosystem functions	Examples
Gas regulation	Regulation of atmospheric chemical composition	CO_2/O_2 balance, SO_x levels
Climate regulation	Regulation of global temperatures, precipitation, climatic processes	Greenhouse gas regulation
Disturbance regulation	Capacitance, damping and integrity of ecosystem response to environmental fluctuations	Storm and flood control
Water regulation	Regulation of hydrological flows	Water provisioning for agriculture
Water supply	Storage and retention of water	Water provisioning by watersheds, reservoirs and aquifers
Erosion control & sediment retention	Retention of soil within an ecosystem	Prevention of soil loss by wind and runoff
Soil formation	Soil formation processes	Weathering of rock and accumulation of organic material
Nutrient cycling	Storage, internal cycling, processing and acquisition of nutrients	Nitrogen fixation, N, P and other elemental or nutrient cycles
Waste treatment	Recovery of mobile nutrients and removal or breakdown of excess or xeric nutrients and compounds	Waste treatment, pollution control
Pollination	Movement of floral gametes	Provisioning of pollinators for the reproduction of plant populations
Biological control	Trophic-dynamic regulations of populations	Predator control of prey species, reduction of herbivory by predators
Refugia	Habitat for resident and transient populations	Nurseries, habitat for migratory species
Food production	Portion of gross primary production extractable as food	Production of fish and game, crops
Raw materials	Portion of gross primary production extractable as raw materials	Production of lumber, fuel or fodder
Genetic resources	Sources of unique biological materials and products	Medicine, products for materials science
Recreation	Providing opportunities for recreational activities	Eco-tourism, sport fishing
Cultural	Providing opportunities for non-commercial uses	Aesthetic, artistic, educational, spiritual, scientific values

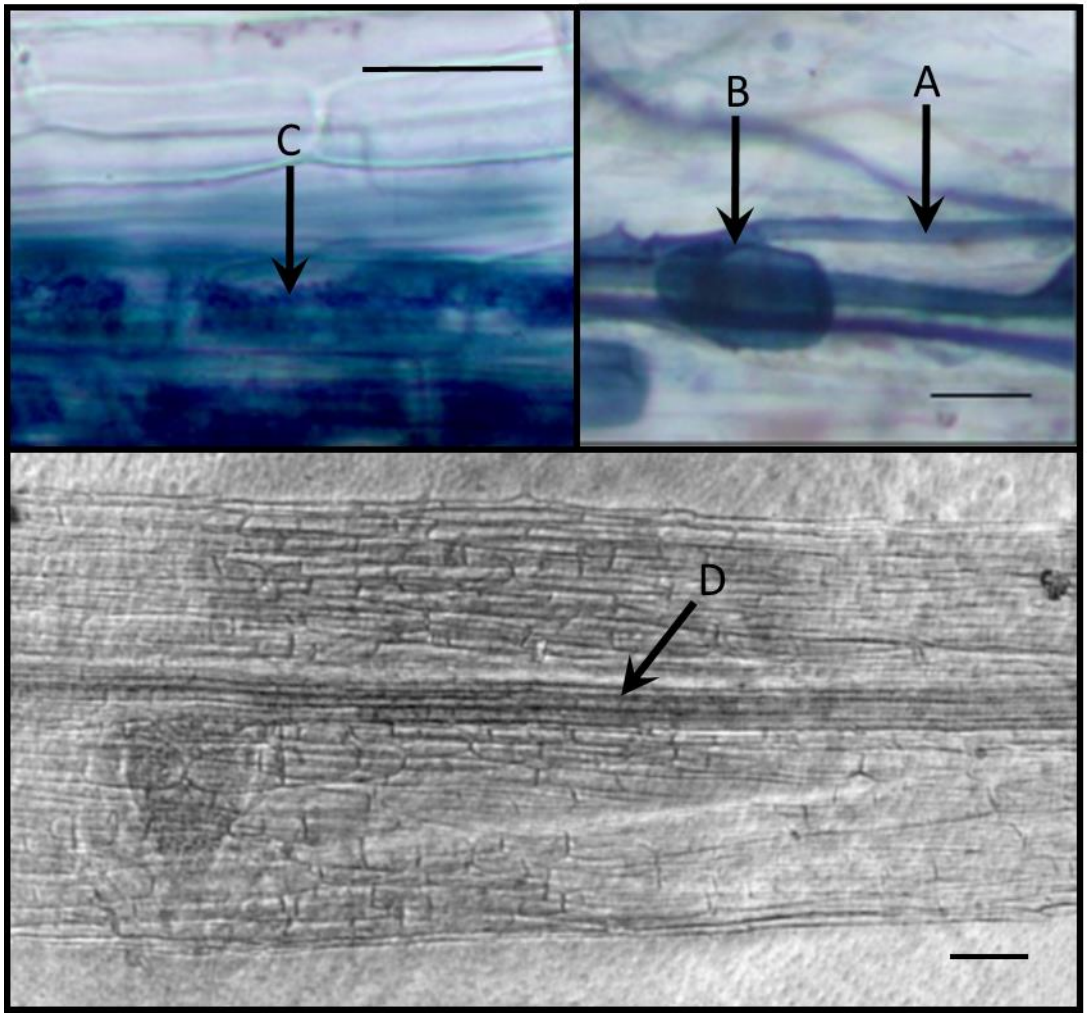

Figure 1.1: Cleared and stained roots of three different plant roots from the mesocosm experiment. Roots were cleared in a 10% KOH and stained with 5% ink-vinegar solution with household vinegar (5% acetic acid). A - C are structures of arbuscular mycorrhizal fungi (AMF); D is a non-colonized root section.
A: Hyphae in a *Phalaris arundinacea* root. Scale bar = 25 µm.
B: Vesicle in an *Phalaris arundinacea* root. Scale bar = 25 µm.
C: Arbuscule in a *Echinochloa crus-galli* root. Scale bar = 50 µm.
D: Non-colonized area of a root from *Verbena hastata*. Non-colonized cells appear translucent. The vascular cylinder (arrow) is visible as a dark central structure. Scale bar = 50 µm.

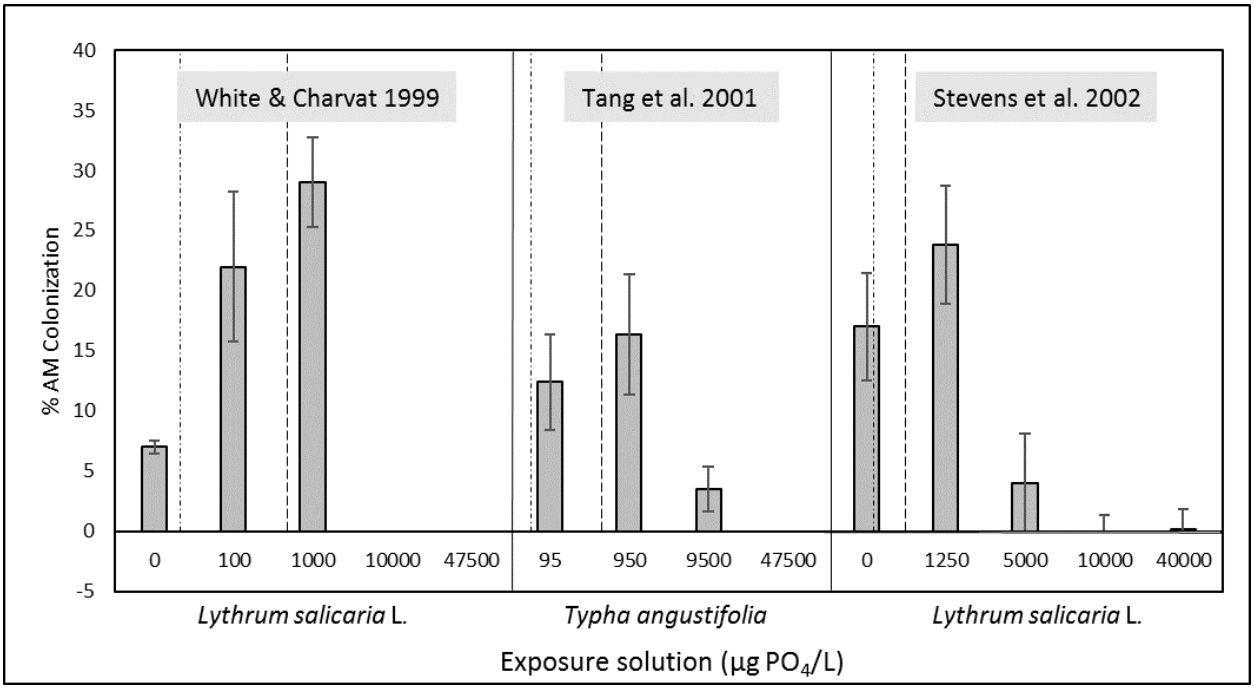

Figure 1.2: Summary of previous studies comparing mycorrhizal colonization with varying phosphorus availability in aquatic conditions. All three studies were controlled experiments with phosphorus injections (Graphs modified from Stevens et al., 2002; Tang et al., 2001; White and Charvat, 1999). Dashed line indicates uppermost concentrations found in the Grand River watershed (640 µg/L P). Dotted line indicates the Provincial Water Quality Objective for total phosphorus (30 µg/L P). Error bars +/- one standard error.

CHAPTER 2

Quantifying relationships between phosphorus availability and mycorrhizal associations in wetland plants

Abstract

The relationship between arbuscular mycorrhizal colonization and phosphorus supply in wetland systems is not well understood. Studies conducted at the field scale do not easily allow the isolation and control of independent variables, so to isolate the impacts of phosphorus supply on mycorrhizal colonization in wetlands, we performed a greenhouse-based mesocosm study. Six plant species (*Solidago canadensis*, *Eupatorium perforliatum*, *Echinochloa crus-galli*, *Verbena hastata*, *Phalaris arundinacea*, and *Epilobium parviflorum*) were grown at four different phosphorus concentrations (10, 30, 640, 7000 µg/L PO_4-P) to determine if phosphorus supply influences the mycorrhizal colonization of wetland plants. Phosphorus concentrations altered hyphal, vesicular, and arbuscular colonization at concentrations between 10 and 30 µg/L PO_4-P. In *Solidago canadensis*, *Eupatorium perforliatum* and *Echinochloa crus-galli*, an increase in phosphorus supply led to a decrease in mycorrhizal colonization. In contrast, in *Verbena hastata*, *Phalaris arundinacea*, and *Epilobium parviflorum* there was no decrease in mycorrhizal colonization with an increase in phosphorus supply. This study shows that phosphorus concentrations found in the Grand River watershed are sufficient to alter mycorrhizal colonization in wetland plants, but the responses are species-specific. This variable impact on mycorrhizal colonization could induce species-specific responses in wetland plants, leading to changes in ecosystem functioning.

Introduction

Importance of mycorrhizal colonization in wetlands

Increasing evidence indicates that plant community structure and, therefore, ecosystem function are mediated by below-ground fungal communities that form intimate associations with plant roots called mycorrhizal associations (van der Heijden et al., 1998a). Arbuscular mycorrhizal fungi (AMF) are a type of mycorrhizal fungi that colonizes the plant host intracellularly but maintain hyphae that extend outside of the root for resource acquisition and are characterized by three structures: arbuscules, vesicles, and hyphae (Smith, 1990). Hyphae are the main vegetative growth form of AMF (Figure 2.1A), and are filamentous structures which ramify through the soil, acquiring and transporting resources to the host plant (Mcgonigle et al., 1990). Vesicles are hyphal swellings which store energy as lipids, and may develop thick walls in older roots and function as propagules (Biermann and Linderman, 1983) (Figure 2.1B). The arbuscule is the main defining feature of AMF and they are formed with repeated dichotomous branching and reduction of hyphae within cortical cells which can end with hyphae less than 1 µm in diameter (Brundrett et al., 1985) (Figure 2.1C), and are theorized to be the location at which the fungus exchanges nutrients with the plant host (Smith, 1990). Understanding the impacts that biotic and abiotic factors have on these structures provides insight into the functionality of AMF.

The importance and function of AMF associations are well documented in terrestrial ecosystems (van der Heijden et al., 2004). These associations are widespread and occur in the majority of vascular plant families (Bever et al., 1996), and have been shown to improve seedling establishment (van der Heijden, 2004), increase seedling

biomass (van der Heijden et al., 2004), and influence plant diversity and plant community structure (van der Heijden et al., 1998b). Greenhouse experiments using single plants indicate AMF facilitate phosphorus (P) uptake (Koch et al., 2004), as well as enhance nitrogen acquisition (Hawkins et al., 2000; Hodge et al., 2010).

The links between AMF and plant diversity and community structure are less understood in aquatic or semi-aquatic systems than in terrestrial systems (White and Charvat, 1999). Early studies indicated there were few mycorrhizal fungi occurring in aquatic or wetland plants (Khan, 1974; Powell, 1975). However, recent studies have found significant mycorrhizal colonization in prairie fens (Turner et al., 2000), Cypress swamps (Kandalepas et al., 2010), and in bottomland hardwood forests (Stevens et al., 2010). The prevalence of AMF in wetlands is now recognized, but the dependency of wetland plants on their AMF partners and the factors that affect AMF colonization in wetland habitats are poorly understood (Cornwell et al., 2001; Stevens and Peterson, 2007; Stevens et al., 2002).

Benefits to maintaining AMF colonization in wetland vegetation have been identified in several plant families. AMF-inoculated plants showed increased Zn acquisition and biomass in inundated farm soil due to the greater surface area of the AMF hyphae extending from the roots (Purakayastha and Chhonkar, 2001), increased flood tolerance in inundated topsoil due to greater development of adventitious roots and hypertrophied lenticels (Osundina, 1998), as well as an ability to alter plant community structure in mesocosm studies (Wolfe et al., 2006). The widespread occurrence and beneficial effects of AMF noted in wetlands suggest parallels with terrestrial ecosystems.

Given the importance of AMF to plant communities and ecosystem function in terrestrial habitats, understanding the impacts and constraints on AMF in wetlands may allow for more successful wetland management, and creation of restoration efforts that maintain or enhance wetland functions.

Controls in AMF colonization

Species and strains of AMF differ in their range of tolerance of physical and chemical properties (Abbott and Robson, 1991). The primary abiotic factors known to influence the abundance and distribution of AMF in terrestrial systems are water, salt, and nutrient availability (Read, 1991). AMF colonization has been shown to be impacted by flooding (Ellis, 1998; Carvalho and Correia, 2003), excess salts (Juniper and Abbott, 1993), and nutrient availability (Majdi et al., 2001). In a summary of multiple studies examining mycorrhizal responses to P and N, mycorrhizal abundance decreased by 15% under N fertilization and 32% under P fertilization (Treseder, 2004). P availability is the primary factor influencing mycorrhizal colonization and growth in terrestrial soils, with phosphorus-abundant soils leading to a decrease in mycorrhizal growth (Abbott et al., 1984; Treseder, 2004). This is due to the lower concentrations representing phosphorus limiting conditions and the plant exhibiting control over fungal morphogenesis within plant root cells (Gianinazzi-Pearson, 1996). These environmental factors also exist in wetlands, although the extent of the impacts are less understood.

Wetland mycorrhizas are relatively poorly studied, although increasing evidence is indicating parallels between established relationships of AMF in terrestrial systems and those found in aquatic conditions. Mycorrhizal colonization in wetlands has been shown

to be reduced by increased water availability (Stevens and Peterson, 1996), excess salt availability (Carvalho and Correia, 2003), and down the drain compounds such as Triclosan (Twanabasu et al., 2013). Relatively few studies have examined the relationship between AM and P in wetland plants. Of the three studies which examined this relationship, all found that P availability impacted mycorrhizal colonization in wetland plants (White and Charvat, 1999; Tang et al., 2001; Stevens et al., 2002). Due to the increasing P enrichment in soils and aquatic systems worldwide (Bennett et al., 2001), there is a potential for impacts on mycorrhizal colonization in wetland systems. Since plant species depend to different extents on mycorrhizal partners (Stevens et al., 2011), these impacts could be particularly damaging to some species while not impacting others. This could cause shifts in plant community structure and wetland ecosystem function.

P in wetlands

Increased anthropogenic activities have led to significant increases in phosphorus concentrations in agricultural and urban watersheds (Zhang, 2016). Nutrient loads can originate from storm water runoff, discharge from ditches and creeks, groundwater seepage, aquatic weed control, naturally occurring organic inputs, and atmospheric deposition (Ouyang et al., 2006). In Canada, agriculture is the primary contributor of P to watersheds (Zhang, 2016). Rivers are particularly vulnerable to nutrient loading due to their proximity to urban centers, sensitivity to land use changes, and extent of exploitation (Withers and Jarvie, 2008). Current levels of P in the Grand River watershed have routinely exceeded the Provincial Water Quality Objective for rivers and streams (30 µg/L P) with recorded values higher than 200 µg/L P (Loomer and Cooke, 2011).

These inputs can lead to changes in species composition in aquatic plant communities within the river basin (David and Gentry, 2006). Due to the well-studied relationship between increased P supply and AMF, nutrient enrichment in wetlands could disproportionately affect mycorrhizal communities and lead to changes in wetland functioning.

Limitations in previous studies

Previous studies have examined the relationship between phosphorus availability and mycorrhizal colonization in terrestrial systems (Abbott et al., 1984; Asimi et al., 1980), but there is a lack of research into these interactions in aquatic or semi-aquatic conditions (Feng et al., 2002; White and Charvat, 1999). Previous studies which examined the effects of phosphorus availability on mycorrhizal colonization in inundated conditions have found that AMF colonization is reduced at levels as low as 5000 µg/L P (Tang et al., 2001; White and Charvat, 1999; Stevens et al. 2002). The study performed by White and Charvat (1999) examined mycorrhizal responses in *Lythrum salicaria* L. and showed reductions in vesicular and arbuscular colonization between 1000 and 10000 µg PO_4/L. The study performed by Tang et al. (2001) examined mycorrhizal responses in *Typha angustifolia* and showed reductions in hyphal and arbuscular colonization between 950 and 9500 µg PO_4/L. The study performed by Stevens et al. (2002) examined mycorrhizal responses in *Lythrum salicaria* L and showed reductions in hyphal, vesicular, and arbuscular colonization between 1250 and 5000 µg PO_4/L. While these P levels far exceed levels noted in the Grand River watershed it must be emphasized that these were greenhouse/growth room studies and the application of these results to natural

watersheds is limited. These studies were limited due to their static non-renewal (Tang et al., 2001; White and Charvat, 1999), or static renewal (Stevens et al., 2002) application of nutrient solution (Figure 2.2), as well as the reliance on a single species of wetland plant as a study organism.

Objective of current study

This study was conducted to determine the relationship between phosphorus supply and mycorrhizal colonization in wetland plants. The research question was: Do phosphorus levels found in the Grand River Watershed affect mycorrhizal colonization in wetland plants? To answer this question, we assessed six plant species representing different plant families for mycorrhizal colonization structures after exposure to varying levels of phosphorus representative of levels found in the Grand River watershed. This greenhouse study used a continuous flow-through application that provided stable concentrations of phosphorus to the plants (Figure 2.2). This exposure system is more representative of conditions found in wetlands, where the nutrient depletion zones of wetland plants are consistently being recharged through diffusion. We also used plant seeds collected in the field as well as an unsorted field inoculum of mycorrhizal spores.

Hypothesis

In consideration of previous studies that examined the effects of phosphorus availability on mycorrhizal colonization in inundated conditions, we predict that with increasing phosphorus supply there will be a decrease in mycorrhizal colonization. We also predict that due to the flow-through exposure system applying a continuous

application of P, AMF colonization will be reduced at much lower concentrations than in previous studies.

Materials and methods

Plants

Based upon a preliminary assessment of the AMF status of wetland plants in southern Ontario and their abundance in local wetlands, six wetland plant species were selected for this study: *Phalaris arundinacea* (L.) (Reed Canary Grass) and *Echinochloa crus-galli* (L.) (Cockspur Grass) in the family Poaceae, *Solidago canadensis* (L.) (Canadian Goldenrod) and *Eupatorium perforliatum* (L.) (Boneset) in the family Asteraceae, *Verbena hastata* (L.) (American Vervain) in the family Verbenaceae, and *Epilobium parviflorum* (S.) (Hoary Willowherb) in the family Onagraceae.

Flow-through exposure system

A flow-through exposure system was established in the Center for Cold Regions and Water Science, Environmental Greenhouse at Wilfrid Laurier University, Waterloo, ON. Exposure solutions (10, 30, 640, and 7000 µg/l PO_4-P) were added to obtain 1/64 strength Long Ashton nutrient levels (Hewitt, 1966) in all nutrients except phosphorus. This concentration of nutrients resulted in levels comparable to those present in North American rivers and have been found to promote mycorrhizal associations in wetland plants (Stevens et al., 2010). Solutions were mixed in 19 L high-density polyethylene (HDPE) reservoirs and replenished every 48 h to maintain flow. Exposure solutions were delivered to plastic potting trays (11 × 21.37 × 2.44 in, standard size seedling trays without drainage holes) via a 12-channel peristaltic cassette pump (Masterflex Variable-

Speed Drive, Cole-Parmer Canada, Montreal, QC) at a constant flow rate of 2.5 mL/min. Three channels on the pump were utilized for each treatment. Seedling growth inserts were placed in the trays. Each insert was filled with 115 g of soil containing unsorted mycorrhizal spores collected from the field at West Montrose (43°35‘18.7” N 80°28’14.0” W) which was used to inoculate the plants. Soil from West Montrose was used because the Grand River Conservation Authority has deemed this an area of high water quality with low phosphorus concentrations (Loomer and Cooke, 2011). To prevent algal growth, the soil surface was covered with light impenetrable fabric with a small opening in the fabric for the shoots to pass through. Opaque black microbore tubing was utilized for the lines between the buckets and the pump, and santoprene microbore tubing (Microbore two-stop tube set, Cole-Parmer Canada, Montreal, QC) was attached from each peristaltic pump cassette (Masterflex small cartridges, Cole-Parmer Canada, Montreal, QC). All reservoirs and the peristaltic pump were shielded from the light by light impenetrable fabric.

Seeds of experimental plants were germinated in Petri dishes on the surface of filter paper moistened with deionized water in experimental growth chambers (16/8 light–dark cycle and temperature 24–28 °C). Immediately after radical emergence, seedlings were transplanted to the seedling growth inserts and inoculated with the soil containing unsorted mycorrhizal spores. Seedlings were randomly assigned locations in the inserts within each tray. Each insert contained 3 plants of the same species and were maintained under greenhouse conditions (16/8 light–dark cycle and temperature 24–28 °C) for 30 days.

Exposure solution with phosphorus

Nutrient solutions were made from the same stock solutions, with phosphorus application broken down into four different concentrations (10, 30, 640, 7000 µg/L PO_4-P) in the form of NaH_2PO_4. Reverse osmosis (RO) water was utilized for the nutrient solution and tested for phosphorus. The RO water contained under 10 µg/L PO_4-P which was the method detection limit. The nutrient solution was run through the system for one week prior to seedling transplant to bring the system to equilibrium and stabilize phosphorus levels. The first concentration was identical to the lowest detectable concentration found in the Grand River Watershed (10 µg/L PO_4-P). The second concentration was equal to the Ontario Provincial Water Quality Objective for total phosphorus (30 µg/L PO_4-P, PWQO 1994). The third concentration was 640 µg/L PO_4-P, the maximum concentrations found in the Grand River Watershed during the sampling period (May to November 2015) from Chapter 3. The fourth concentration was 7000 µg/L PO_4-P, which is the highest concentration of phosphorus found in water within Ontario by the Provincial Water Quality Monitoring Network. There were three trays at each phosphorus concentration, in a 3x4 design.

Phosphorus exposure concentration analysis

All phosphorus exposure concentrations were verified by instrumental analysis of water samples collected from trays prior to seedling transplant after equilibration of the exposure system. Two water samples from the input and the outflow of each tray were collected weekly to monitor and maintain desired phosphorus levels. Orthophosphate levels were determined using Hach Method 10209 for Ultra Low Range Reactive

Phosphorus (HACH Company, 2011). 0.2 mL of Reagent B was added to 3.5 mL of water sample in a TNT 843 Reagent Vial using a micropipette. The sample vial was inverted three times then allowed to sit for 10 minutes. The sample was then poured into a 5-cm semi-micro cuvette and quantified with a spectrophotometer at 880 nm (DR3900 Hach Spectrophotometer, HACH Company, 3020 Gore Rd. London, ON N5V 4T7).

Quality assessment/quality control

Quality control samples were included with each water sampling episode. The analysis included a replicate method blank (reverse osmosis water), and a replicate of blank analyte spike (reverse osmosis water spiked with phosphorus). All quality control samples received the same extraction preparation as experimental samples.

Plant performance

All plants were harvested 30 days after treatment application and above and below-ground plant performance quantified. Pots were emptied under water and the root systems gently agitated to remove any remaining sand particles. Roots and shoots were separated, lightly blotted between paper towel, and root and shoot lengths were obtained. Fresh weights of root and shoot systems were then measured. Entire root systems were digitized using a Hewlett Packard ScanJet 4C/T scanner and the total shoot surface area determined using WinRhizo (Regent Instruments Inc. Quebec City, Quebec). Root systems were stored in 50% ethanol for AM quantification.

Root harvesting, processing, and AM quantification

The root sections harvested were selected randomly and placed in Falcon™ 50 mL Conical Centrifuge Tubes (Fisher Scientific Company, 112 Colonnade Road, Ottawa, ON, K2E 7L6). A clearing agent (10% KOH) was added, completely covering the roots. They were put in the vacuum oven (Thermo Scientific Lindberg Blue M) for 1 h 30 min at 95° Celsius at 25inHG (Vierheilig et al., 1998). The samples were retrieved from the vacuum oven and rinsed with 10% acetic acid for 3 minutes at room temperature. A solution was added composed of 5% Sheaffer Skrip Black Ink (Sheaffer Slovakia s.r.o., Priemyselnà 1, 926 01 Sered', Slovak Republic), 5% acetic acid and 90% deionized water. The samples were again put into the vacuum oven for 1 h 30 min at 95° Celsius at 25inHG. The samples were retrieved from the vacuum oven and rinsed with 5% acetic acid for 10 minutes at room temperature. The acetic acid was replaced by 50% glycerol, and the sample was allowed to sit overnight. The stained root samples were mounted on 75x25x1 mm frosted VWR Microscope Slides (VWR International). Prepared slides were viewed at 200 × magnification using a Nikon Eclipse E600 Microscope (Nikon Instruments Inc. 1300 Walt Whitman Road Melville, NY 11747-3064, U.S.A.). Colonization levels were assessed using the magnified intersections method (Mcgonigle et al., 1990). The percentage of hyphal, arbuscular, and vesicular AMF colonization was calculated after assessing a total of 100 fields of view for each sample.

Statistical analysis of plant performance in response to phosphorus availability

Plant performance variables (root length, root weight, shoot height, shoot weight, root to shoot height ratio, root to shoot weight ratio, and shoot surface area) were

examined for their relationship with phosphorus supply using multivariate analysis of variance (MANOVA) using IBM SPSS Statistics 23 (IBM). Since the plant performance variables were not normally distributed, they were log transformed. Fixed factors were plant species and phosphorus concentration.

Statistical analysis of mycorrhizal colonization in response to phosphorus availability

Since there were problems normalizing the mycorrhizal colonization data, a generalized linear mixed model (GLMM) using IBM SPSS Statistics 23 (IBM) was used to fit the data. To determine if phosphorus levels were affecting mycorrhizal colonization, logistic regression was performed on each response variable (hyphae, vesicles, and arbuscules). The design was binary logistic, and the response variables were modelled as presence/absence in each view counted to reflect the AMF quantification method (100 views/plant). There were three channels utilized for each exposure concentration which fed into three separate trays. There were four levels of phosphorus concentration (10, 30, 640, and 7000 µg/l PO_4-P), six plant species and four replicate plants obtained from each tray for each species at the single harvest after 30 days. The predictive factors were phosphorus concentration and plant species. Main effects as well as interaction of phosphorus concentration and plant species were modelled and multiple comparisons were conducted.

Results

Measured phosphorus concentrations exceeded targeted levels (10, 30, 640, 7000 µg/L PO_4-P) on the day the plants were planted, but the concentration of phosphorus was reduced to desired levels throughout the experiment. Averaged across all sampling days,

concentrations of P for each tray over the course of the entire experiment were +/- 15% of the desired concentrations except for one tray at 10 µg/L PO_4-P (40% higher) and one tray at 30 µg/L PO_4-P (30% lower) (Table 2.1).

Effects of plant species and phosphorus concentration on plant performance

There was a statistically significant effect of plant species ($F_{30,288} = 38.373$, $p < 0.001$; Wilks' $\Lambda = .051$) and phosphorus concentration ($F_{18,288} = 3.015$, $p < 0.001$; Wilks' $\Lambda = .817$), but no interaction between plant species and phosphorus concentration ($F_{90,288} = 1.159$, $p = 0.152$; Wilks' $\Lambda = .679$) on plant performance variables (Table 2.2). Phosphorus concentration had significant effects on shoot height ($F_{3,288} = 2.63$; $p < .05$; partial $\eta^2 = .030$), root weight ($F_{3,288} = 2.676$; $p < .05$; partial $\eta^2 = .030$), root-shoot height ratio ($F_{3,288} = 3.339$; $p < .05$; partial $\eta^2 = .037$), root-shoot weight ratio ($F_{3,288} = 6.426$; $p < .001$; partial $\eta^2 = .070$), and shoot surface area ($F_{3,288} = 6.836$; $p < .001$; partial $\eta^2 = .074$) (Table 2.3). Generally, with an increase in phosphorus availability, there were increases in plant shoot height and weight and decreases in plant root weight and height. This led to a reduction of the root:shoot weight ratio (Figure 2.3 & 2.4).

Effects of plant species and phosphorus concentration on mycorrhizal colonization

Under controlled conditions in an inundated flow-through system, AMF were able to colonize all six plant species as a function of P availability. Mycorrhizal colonization in plant roots across all six species was very high. There was detectable colonization in 94% of plants in the experiment, and most colonized plants had both observable vesicles (76% average) and arbuscules (83% average) (Table 2.4).

Mean hyphal colonization of plant root cells varied between 20% (*Epilobium parviflorum*) and 51% (*Eupatorium perforliatum*). The tests of model effects showed that species ($X^2_5 = 1697.830$, $p < 0.001$), phosphorus concentration ($X^2_3 = 98.137$, $p < 0.001$) and the interaction between plant species and phosphorus concentration ($X^2_{15} = 104.269$, $p < 0.001$) had significant effects on hyphal colonization (Table 2.5). In *S. canadensis*, *E. perforliatum* and *E. crus-galli*, an increase in phosphorus concentration led to a decrease in hyphal colonization. In *V. hastata* and *P. arundinacea*, P concentration led to inconsistent effects, but generally hyphal colonization decreased with increasing P. In *E. parviflorum* there was no significant change in hyphal colonization with an increase in phosphorus supply (Figure 2.5).

Mean vesicular colonization of plant root cells varied between 8% (*Phalaris arundinacea*) and 32% (*Verbena hastata*). The tests of model effects showed that plant species ($X^2_5 = 140.749$, $p < 0.001$), phosphorus concentration ($X^2_3 = 12.435$, $p < 0.05$) and the interaction between plant species and phosphorus concentration ($X^2_{15} = 254.317$, $p < 0.001$) had significant effects on vesicular colonization (Table 2.5). In *S. canadensis* and *E. perforliatum*, an increase in phosphorus concentration led to a decrease in vesicular colonization. In *E. crus-galli*, *E. parviflorum*, *P. arundinacea*, and *V. hastata* there was no significant change in vesicular colonization with increasing P supply (Figure 2.6).

Mean arbuscular colonization of plant root cells varied between 7% (*Epilobium parviflorum*) and 35% (*Eupatorium perforliatum*). The tests of model effects showed that plant species ($X^2_5 = 1750.721$, $p < 0.001$), phosphorus concentration ($X^2_3 = 13.719$, $p < 0.05$) and the interaction between plant species and phosphorus concentration ($X^2_{15} =$

194.250, $p < 0.001$) had significant effects on arbuscular colonization (Table 2.5). In *V. hastata*, an increase in phosphorus concentration led to a decrease in arbuscular colonization. In *S. canadensis*, *E. perforliatum*, and *E. crus-galli* there was no significant change in arbuscular colonization with increasing P supply. In *E. parviflorum* and *P. arundinacea*, an increase in P supply led to an increase in arbuscular colonization (Figure 2.7).

Discussion

Plant performance response to phosphorus availability

The P concentrations achieved in this study corresponded to those found in southern Ontario watersheds (Loomer and Cooke, 2011) and represented a range of P concentrations detectable by the equipment employed. While the two highest levels of P applied far exceeded the Provincial Water Quality Objective of 30 µg/L P, they were considerably lower than those used in Long Ashton nutrient solution (approximately 200 mg/l; Stevens et al., 2002). In this study, we showed that wetland plants have species-specific responses to increased phosphorus availability in their performance. Generally, with decreased phosphorus availability, plant shoot height, weight, and surface area decreased and root weight and length increased, leading to higher root to shoot height and weight ratios. Since plant performance was reduced at the lowest levels of P supply, 10 and 30 µg/L PO_4-P, and peaked at 7000 µg/L PO_4-P, it is likely that the lower concentrations represented P limiting conditions. This result was consistent with the nitrogen-carbon balance concept, which is when there is an increase in minerals closely associated with the synthesis of new tissues (N, P, S) there is a decrease in the root:shoot

ratio (Ericsson, 1995). The decrease of aboveground structures and increase in below-ground structures in low phosphorus is indicative of nutrient-limiting conditions (Drew and Saker, 1978).

Since there was no control treatment without mycorrhizal fungi, it is impossible to determine if AMF colonization had any effects on plant performance in this experiment. Early studies showed either low levels of mycorrhizal colonization in wetland habitats (Khan, 1974; Powell, 1975), or a negative relationship between soil moisture and mycorrhizal colonization, particularly a decrease in hyphal and arbuscular structures in inundated soils (Stevens and Peterson, 1996). However, more recent studies have discovered arbuscular mycorrhizal fungi in wetland environments primarily in dicotyledons (Cornwell et al., 2001). Dicotyledons are one of two groups of flowering plants. They have two embryonic leaves or cotyledons. They have vascular bundles in concentric circles in the stem. Monocotyledons are the other group whose seed only contains one cotyledon. The vascular bundles are scattered throughout the stems. The morphological explanation for the prevalence of mycorrhiza in dicots is that monocotyledons had more extensive aerenchymous tissue, giving them an increased ability to transport oxygen to their roots (Cornwell et al., 2001). In my study, I discovered AMF colonized roots in both monocots and dicots, and AMF were present in roots of all plant species examined in this study (*Phalaris arundinacea*, *Solidago canadensis*, *Eupatorium parviflorum*, *Epilobium perforliatum*, *Echinochloa crus-galli*, and *Verbena hastata*). The presence of plants heavily colonized by mycorrhiza in this study indicates that the mycorrhizal inoculum retrieved from the field were compatible with the host plants. Since the mycorrhiza were able to colonize the plants so successfully, it could

indicate that there is still a functional role of the fungus in the mycorrhizal symbiosis in wetlands, though that role is not well understood (Muthukumar et al., 2004). It could also mean that the plants' control over the mediation of the mycorrhizal relationship is reduced with increased soil moisture. It is hypothesized that plants emit signal molecules which precedes successful root colonization (Akiyama et al., 2005). These molecules could be diluted when soil moisture is too high, disrupting signal recognition.

Trends in the response of mycorrhizal structures to phosphorus availability

I hypothesized that the inverse relationship between mycorrhiza and phosphorus availability found in terrestrial systems would persist in inundated conditions (Read, 1991), but that was only the case in three of the six plant species examined in this study. In *S. canadensis*, *E. perforliatum* and *E. crus-galli*, an increase in phosphorus concentrations led to a decrease in hyphal colonization. This could indicate that at high phosphorus concentrations, the plant can depress the amount of carbohydrates it allocates to the fungi as it does not require the phosphorus and water AMF can provide. This could also indicate that at high levels of phosphorus and water there is an uncoupling of the mycorrhizal symbiosis due to interference with the signaling compounds exuded by the plant roots (Stevens et al., 2002), leading to a reduction in the ability of mycorrhizal spores to identify host plants. In previous studies, mycorrhizal colonization was depressed at P concentrations between 1250 and 5000 µg/L P (Stevens et al., 2002; Tang et al., 2001; White and Charvat, 1999). These studies had static systems which were either injected or pulsed, which could have varied the availability of the P as well as the concentrations of P directly available to the plant. In my study, I showed phosphorus

supply can have species-specific impacts on mycorrhizal colonization in concentrations as low as 10 to 30 µg/L P in a flow through system. This system closely simulated natural conditions by supplying phosphorus continuously using a peristaltic pump. This depression of mycorrhizal colonization at such low levels could be because my study had a continuous application of P, so there was a constant supply of P for the plant to uptake.

The precise functioning of P uptake by mycorrhizas are not well understood. The first phase in the development of an AMF symbiosis is spore germination, where the spore responds to external factors in the environment and uses triacylglycerol and glycogen stores to extend a singular hypha into the environment. Plant root exudates and volatiles, including CO_2 can also stimulate germination (Harrison, 2005). Should the hypha fail to encounter a host, the fungus will stop hyphal growth while still maintaining enough energy to spare for another attempt. Once the hypha reaches the vicinity of a plant root, respiration and growth of the fungus increases substantially, indicating that it perceives something exuded by the root (Harrison, 2005). It is possible that at this step, the presence of excess water and phosphorus interferes or dilutes the exudate, reducing the signal and, thereby, reducing the ability of the spore to detect compatible hosts.

In *S. canadensis* and *E. perforliatum*, there was also a decrease in vesicles found in colonized plants with increasing phosphorus. This could be due to their reduced intake of photosynthates received from the host plant, reducing both the need for storage structures as well as the resources needed to grow them. In *S. canadensis* there was an increase in arbuscules, perhaps indicating the need of the fungi for more carbohydrates,

and, therefore, more arbuscules to increase surface area to allow transfer from the plant to the fungus.

In *V. hastata* and *P. arundinacea*, hyphal colonization decreased with increasing P, but not linearly. This showed the various phosphorus levels were having an effect on mycorrhizal structures, but there was no specific cut-off point as hyphae were depressed at different levels of phosphorus concentrations. In *V. hastata*, vesicular colonization increased from 10 to 640 µg/L PO_4-P and then dropped to its' original level at 7000 µg/L PO_4-P but arbuscular colonization decreased significantly between 10 and 30 µg/L PO_4-P. *P. arundinacea* had no increase in vesicular or arbuscules, indicating a possible resiliency to excess phosphorus exposure. In *E. parviflorum* there was no change in hyphal colonization with an increase in phosphorus supply. Vesicular colonization also did not increase, but arbuscular colonization increased, at 30 and 7000 µg/L PO_4-P. This depressed response could again indicate resiliency to increased phosphorus exposure.

In this study, I showed that phosphorus concentration at levels found in the Grand River watershed could alter the mycorrhizal colonization of six plant species, although responses were species-specific. Since plant performance is inextricably linked to resource uptake, and the mycorrhizal symbiosis is one of the pathways of acquiring P for a plant (Stevens and Peterson, 2007), any impacts on AMF could induce species-specific responses in host plants. Due to plants having differential responses to AMF colonization, the alteration of these below-ground fungal communities has the potential to impact plant performance and impact wetland ecosystem functioning. Future studies should investigate these relationships by applying treatments with and without mycorrhizal

inoculum to closely examine if P supply and mycorrhizal colonization have impacts on plant performance. It is also important to perform this study across multiple plant species, as phosphorus concentrations have variable impacts on plant and rhizospheric fungal communities. Future studies should also examine a greater range of P concentrations to determine the range of AMF sensitivity.

Conclusion

Phosphorus concentrations found in the Grand River watershed are sufficient to impact mycorrhizal colonization in wetland plants. This impact is species-specific and could result in altered plant community structure, leading to changes in ecosystem functioning.

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Tables & Figures

Table 2.1: Measured phosphorus levels in each tray as PO_4-P. Trays were given 7 days to acclimate to the exposure solution before seedlings were planted. This study was performed to indicate the effects of phosphorus on mycorrhizal colonization in a wetland system. Six different plants were harvested and assessed for mycorrhizal colonization after exposure to four levels of phosphorus (10, 30, 640, and 7000 µg/L PO_4-P) for 30 days.

Desired Concentration	Average Concentration	Stabilized	Planted				Harvest
	14	9	29	13	12	7	13
10	10	12	9	9	9	12	8
	12	22	1	5	17	12	12
	30	31	41	32	26	21	28
30	23	30	7	31	21	16	30
	31	28	45	34	32	19	26
	652	640	731	612	684	591	652
640	658	640	749	607	672	664	614
	654	651	748	641	605	619	662
	7084	7104	7798	6021	7831	6329	7422
7000	7139	7013	8115	5899	8010	6082	7712
	7196	6996	8023	6104	8140	6700	7213
Units	µg/L PO_4-P	Week	0	1	2	3	4

Table 2.2: Results of multivariate analysis of variance (MANOVA) on phosphorus concentration and plant performance variables. Variables tested were root length, shoot height, root weight, shoot weight, root:shoot weight ratio, and leaf surface area. Trays were given 7 days to acclimate to the exposure solution before seedlings were planted. This study was performed to indicate the effects of phosphorus on mycorrhizal colonization in a wetland system. Plants were harvested and assessed after 30 days in the exposure solution.

Effect	Wilks' Lambda	F	Hypothesis df	Error df	P value	Partial Eta Squared
Intercept	0.003	12332.743	6	259.000	0.000	0.997
Plant Species	0.051	38.373	30	1038.000	0.000	0.524
P concentration	0.817	3.015	18	733.048	0.000	0.074
Plant Species * P concentration	0.679	1.159	90	1463.128	0.424	0.057

Table 2.3: Summary of two-way ANOVAs assessing the effects of phosphorus concentration and plant species on the plant performance of six wetland plants. Variables tested were root length, shoot height, root weight, shoot weight, root:shoot weight ratio, and leaf surface area. Trays were given 7 days to acclimate to the exposure solution before seedlings were planted. This study was performed to indicate the effects of phosphorus on mycorrhizal colonization in a wetland system. Plants were harvested and assessed after 30 days in the exposure solution. Significant effects (p=<0.05) are in bold.

Response Variable	Plant Species		P Concentration		Plant Species x P Concentration	
	F	P-Value	F	P-Value	F	P-Value
Root Length	93.533	**0.000**	1.513	0.212	0.805	0.672
Root Weight	139.054	**0.000**	2.833	**0.039**	0.865	0.604
Shoot Height	321.630	**0.000**	2.821	**0.039**	0.843	0.629
Shoot Weight	126.099	**0.000**	0.877	0.454	0.928	0.533
R:S Weight Ratio	15.293	**0.000**	6.395	**0.000**	1.405	0.145
Shoot Surface Area	123.025	**0.000**	7.017	**0.000**	0.824	0.650

Table 2.4: Overall colonization of mycorrhizal structures in all plants in the mesocosm. Trays were given one week to acclimate to the exposure solution for a week before seedlings were planted. This study was performed to indicate the effects of phosphorus on mycorrhizal colonization in a wetland system. Six different plants were harvested and assessed for mycorrhizal colonization after exposure to four levels of phosphorus (10, 30, 640, and 7000 µg/L PO_4-P) for 30 days.

	Hyphae	Vesicles	Arbuscules
Overall Colonization (%)	93	76	83

Table 2.5: Effects of phosphorus supply on mycorrhizal colonization of the three mycorrhizal structures assessed across six plant species. Summary of logistic regressions performed on mycorrhizal structures. This study was performed to indicate the effects of phosphorus on mycorrhizal colonization in a wetland system. Six different plants were harvested and assessed for mycorrhizal colonization after exposure to four levels of phosphorus (10, 30, 640, and 7000 µg/L PO_4-P) for a month. Trays were given one week to acclimate to the exposure solution for a week before seedlings were planted.

Structure		Plant species	P concentration	Plant species x P concentration
Hyphae	Square	1697.83	98.137	104.269
	Df	5	3	15
	P value	0.000	0.000	0.000
Vesicles	Square	140.749	12.435	254.317
	Df	5	3	15
	P value	0.000	0.006	0.000
Arbuscules	Square	1750.721	13.719	194.25
	Df	5	3	15
	P value	0.000	0.003	0.000

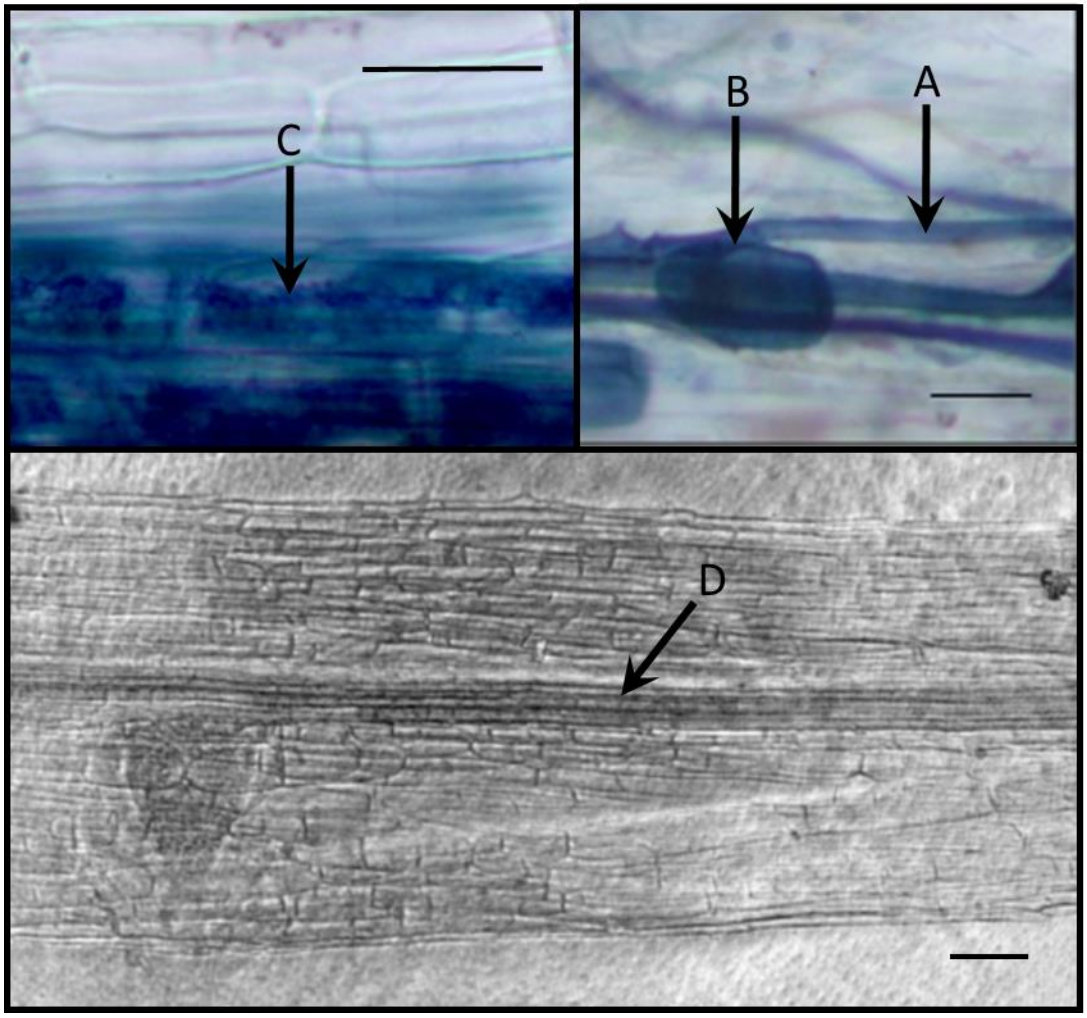

Figure 2.1: Cleared and stained roots of three different plant roots from the mesocosm experiment. Roots were cleared in a 10% KOH and stained with 5% ink-vinegar solution with household vinegar (5% acetic acid). A - C are structures of arbuscular mycorrhizal fungi (AMF); D is a non-colonized root section.
A: Hyphae in a *Phalaris arundinacea* root. Scale bar = 25 µm.
B: Vesicle in an *Phalaris arundinacea* root. Scale bar = 25 µm.
C: Arbuscule in a *Echinochloa crus-galli* root. Scale bar = 50 µm.
D: Non-colonized area of a root from *Verbena hastata*. Non-colonized cells appear translucent. The vascular cylinder (arrow) is visible as a dark central structure. Scale bar = 50 µm.

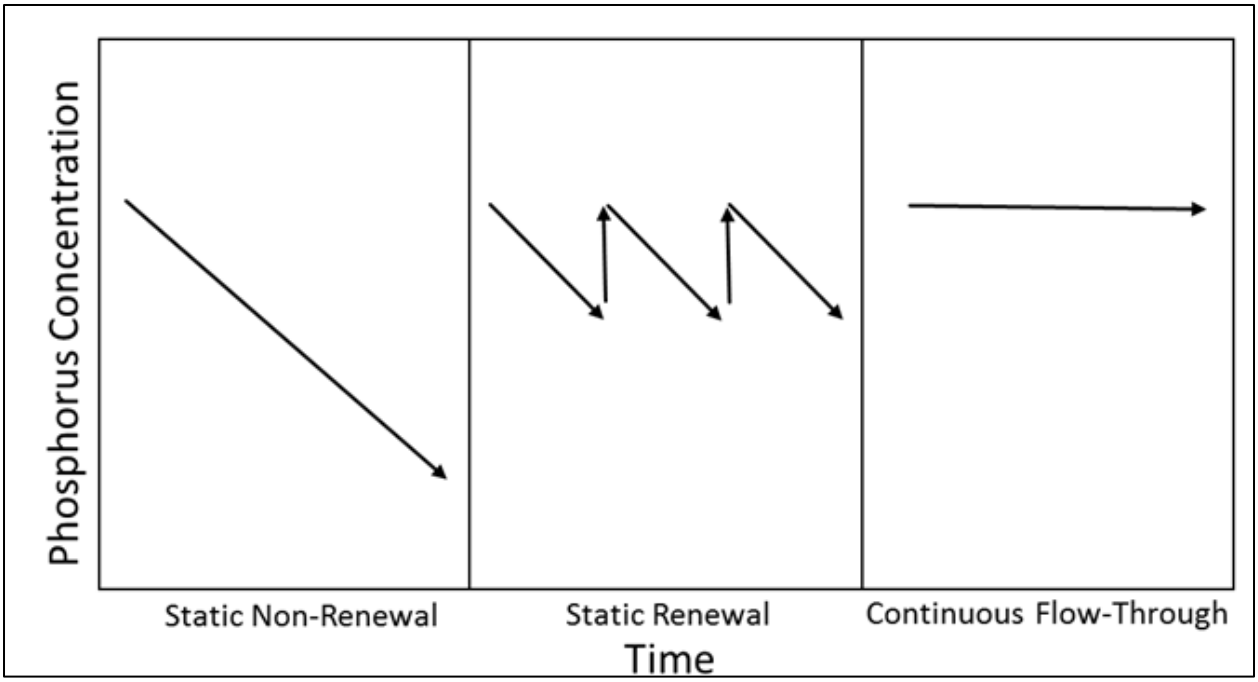

Figure 2.2: The different phosphorus exposure methods used in various greenhouse experiments. The static non-renewal approach is a single injection of phosphorus into the exposure solution, and the concentration decreases as the plant uptakes the phosphorus. The static renewal approach is an injection that is assessed periodically and topped up to maintain a desired concentration. The continuous flow-through system was used in my experiment, where a concentration is maintained throughout the experiment, which closely mimics natural conditions.

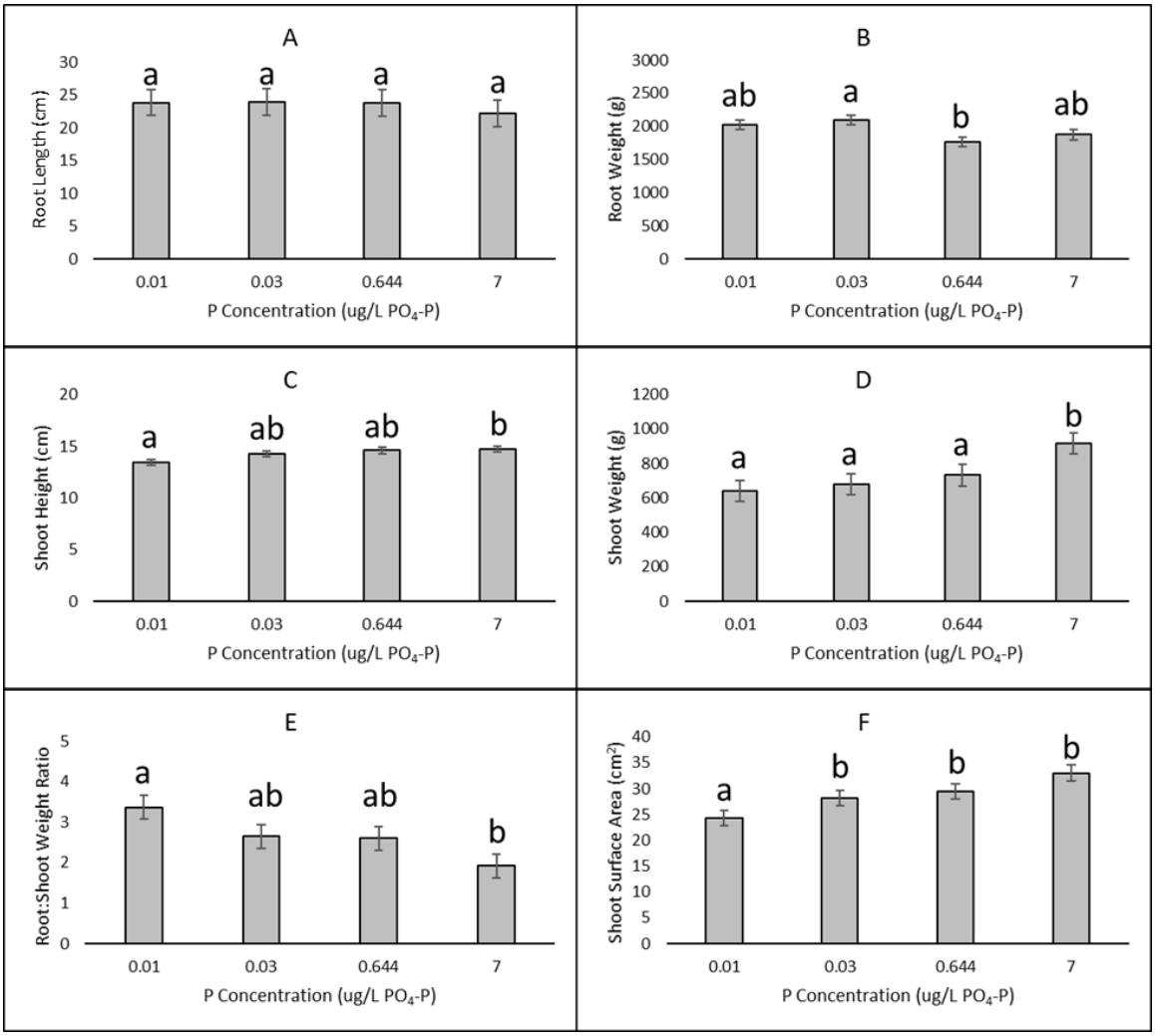

Figure 2.3: Plant performance of all six plant species at the four phosphorus concentrations. Effects of phosphorus supply on plant performance across all six plant species were determined using two-way analysis of variance (ANOVA). Means were compared using Tukey HSD post-hoc tests. This study was performed to indicate the effects of phosphorus on mycorrhizal colonization in a wetland system. Six different plants were harvested and assessed for mycorrhizal colonization after exposure to four levels of phosphorus (10, 30, 640, and 7000 µg/L PO_4-P) for 30 days. Trays were given 7 days to acclimate to the exposure solution for a week before seedlings were planted. Bars with the same letter are not significantly different. A – Root length vs. P availability. B – Root length vs. P availability. C – Shoot height vs. P availability. D – Shoot weight vs P availability. E – Root:shoot weight ratio vs. P availability. F – Shoot surface area vs. P availability. Error bars indicate +/- one standard error.

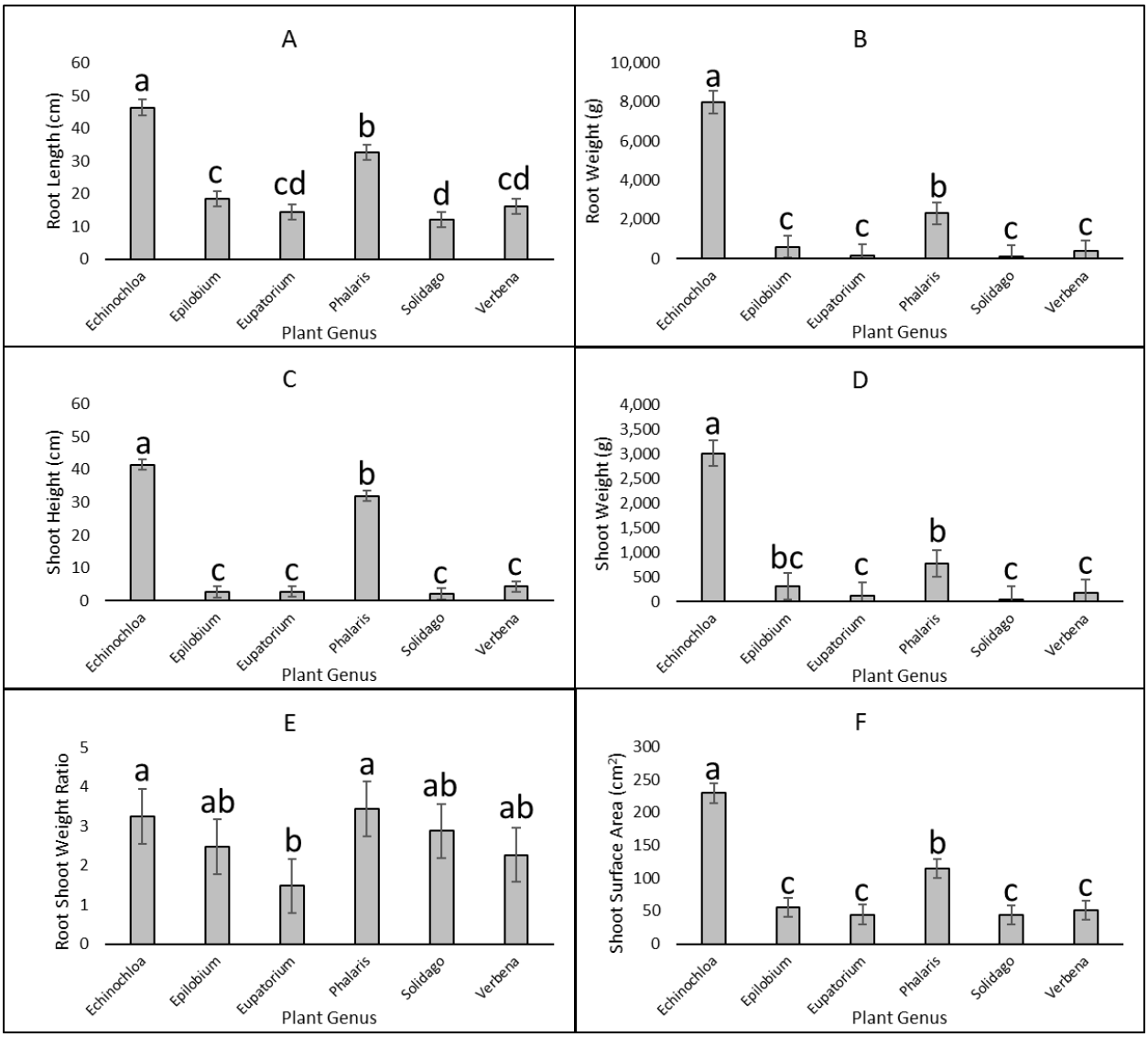

Figure 2.4: Plant performance of each plant at all four phosphorus concentrations. Effects of phosphorus supply on plant performance across all six plant species were determined using multivariate analysis of variance (MANOVA). Means were compared using Tukey HSD post-hoc tests. This study was performed to indicate the effects of phosphorus on mycorrhizal colonization in a wetland system. Six different plants were harvested and assessed for mycorrhizal colonization after exposure to four levels of phosphorus (10, 30, 640, and 7000 µg/L PO_4-P) for 30 days. Trays were given 7 days to acclimate to the exposure solution for a week before seedlings were planted. Bars with the same letter are not significantly different. A – Root length vs. P availability. B – Root weight vs. P availability. C – Shoot height vs. P availability. D – Shoot weight vs P availability. E – Root:shoot weight ratio vs. P availability. F – Shoot surface area vs. P availability. Error bars indicate +/- one standard error.

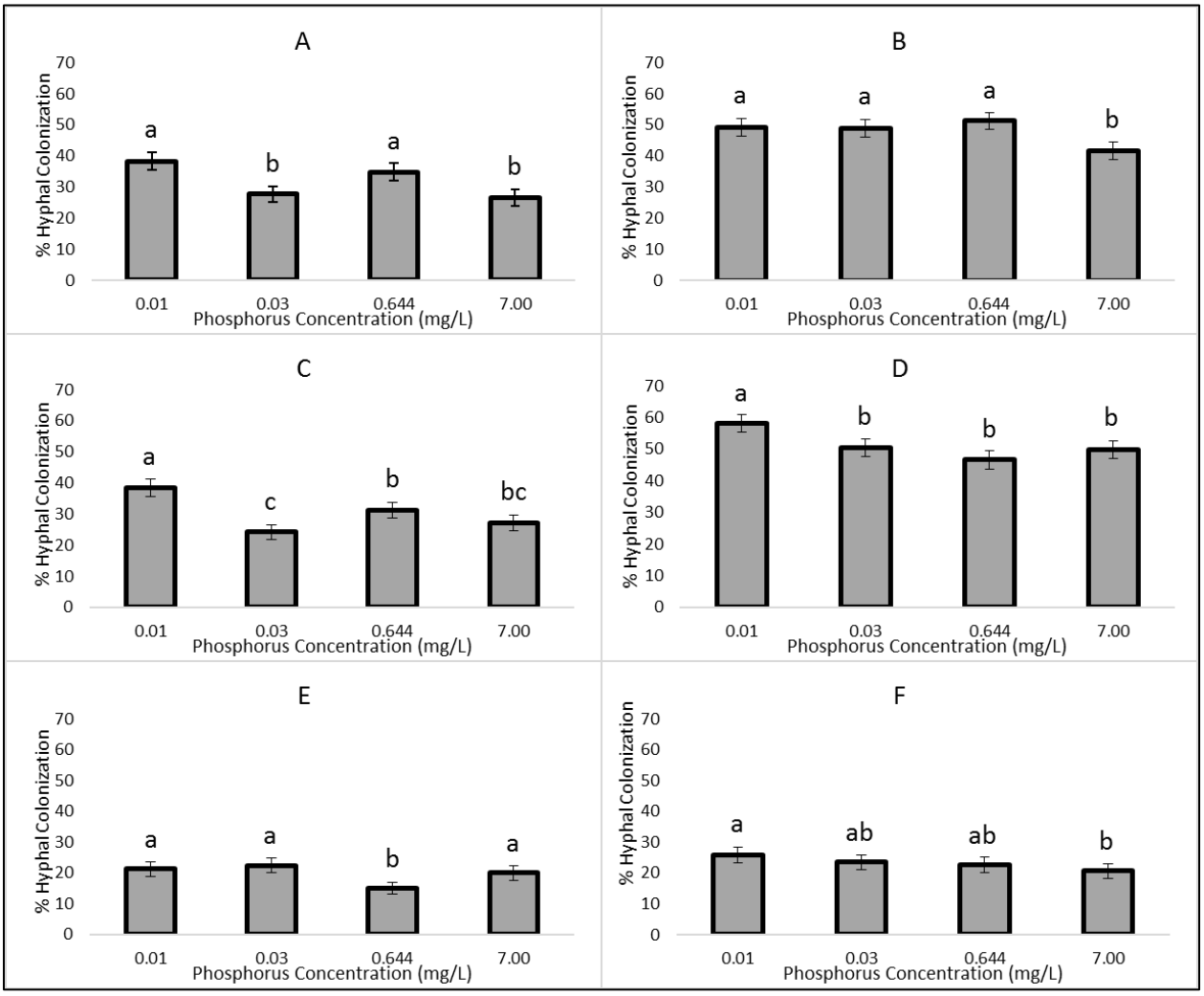

Figure 2.5: Mean hyphal colonization of all six plant species at the four phosphorus concentrations. Effects of phosphorus supply on hyphal colonization across all six plant species were determined by logistic regression. Parameters were compared using the Wald test. This study was performed to indicate the effects of phosphorus on mycorrhizal colonization in a wetland system. Six different plants were harvested and assessed for mycorrhizal colonization after exposure to four levels of phosphorus (10, 30, 640, and 7000 µg/L PO_4-P) for 30 days. Trays were given 7 days to acclimate to the exposure solution for a week before seedlings were planted. Bars with the same letter are not significantly different. A – Mean hyphal colonization of *Verbena hastata*. B – Mean hyphal colonization of *Solidago canadensis*. C – Mean hyphal colonization of *Phalaris arundinacea*. D – Mean hyphal colonization of *Eupatorium perforliatum*. E – Mean hyphal colonization of *Epilobium parviflorum*. F – Mean hyphal colonization of *Echinochloa crus-galli*. Error bars indicate +/- one standard error.

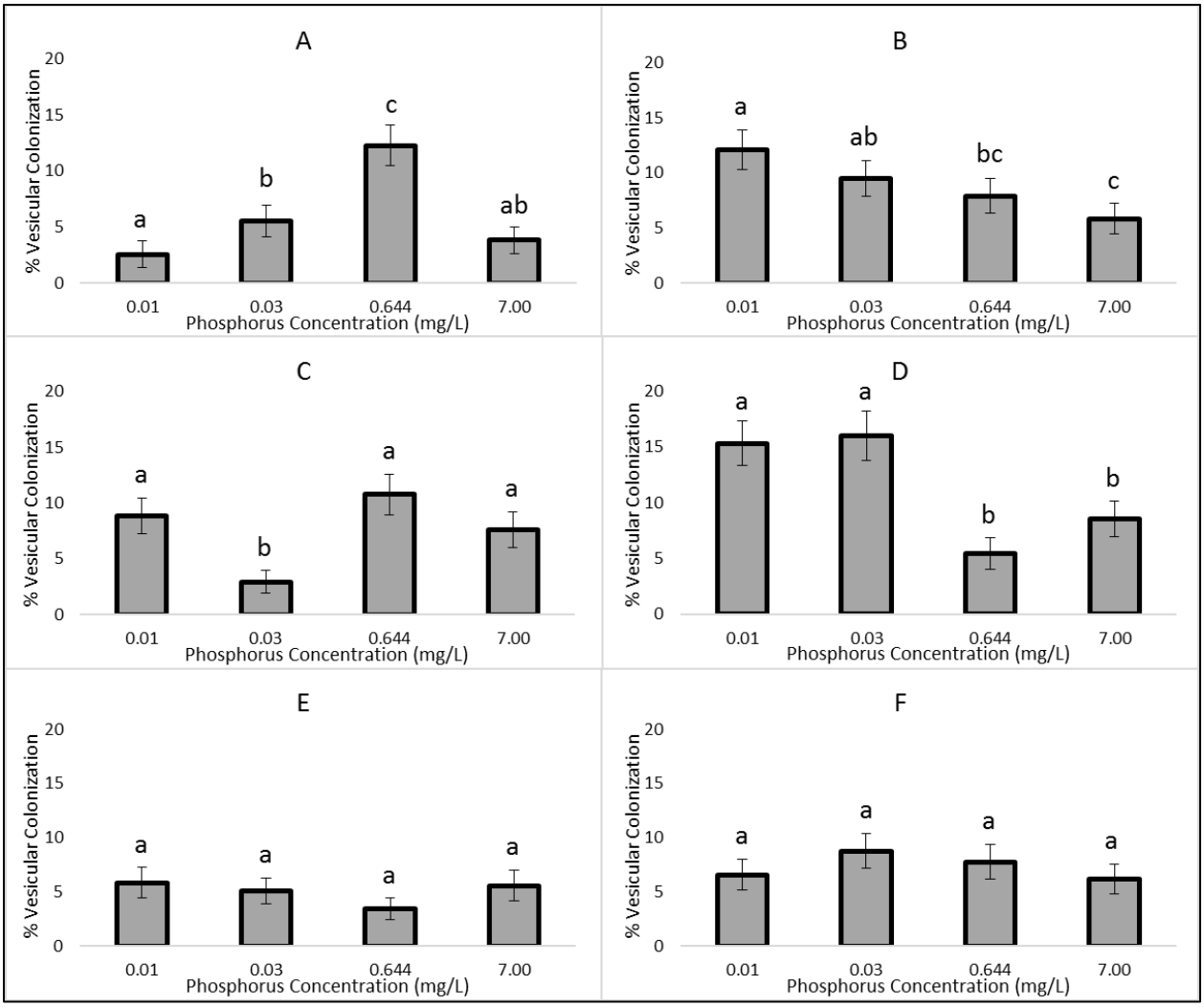

Figure 2.6: Mean vesicular colonization of all six plant species at the four phosphorus concentrations. Effects of phosphorus supply on vesicular colonization across all six plant species determined by logistic regression. Parameters were compared using the Wald test. This study was performed to indicate the effects of phosphorus on mycorrhizal colonization in a wetland system. Six different plants were harvested and assessed for mycorrhizal colonization after exposure to four levels of phosphorus (10, 30, 640, and 7000 µg/L PO_4-P) for 30 days. Trays were given 7 days to acclimate to the exposure solution for a week before seedlings were planted. Bars with the same letter are not significantly different. A – Mean vesicular colonization of *Verbena hastata*. B – Mean vesicular colonization of *Solidago canadensis*. C – Mean vesicular colonization of *Phalaris arundinacea*. D – Mean vesicular colonization of *Eupatorium perforliatum*. E – Mean vesicular colonization of *Epilobium parviflorum*. F – Mean vesicular colonization of *Echinochloa crus-galli*. Error bars indicate +/- one standard error.

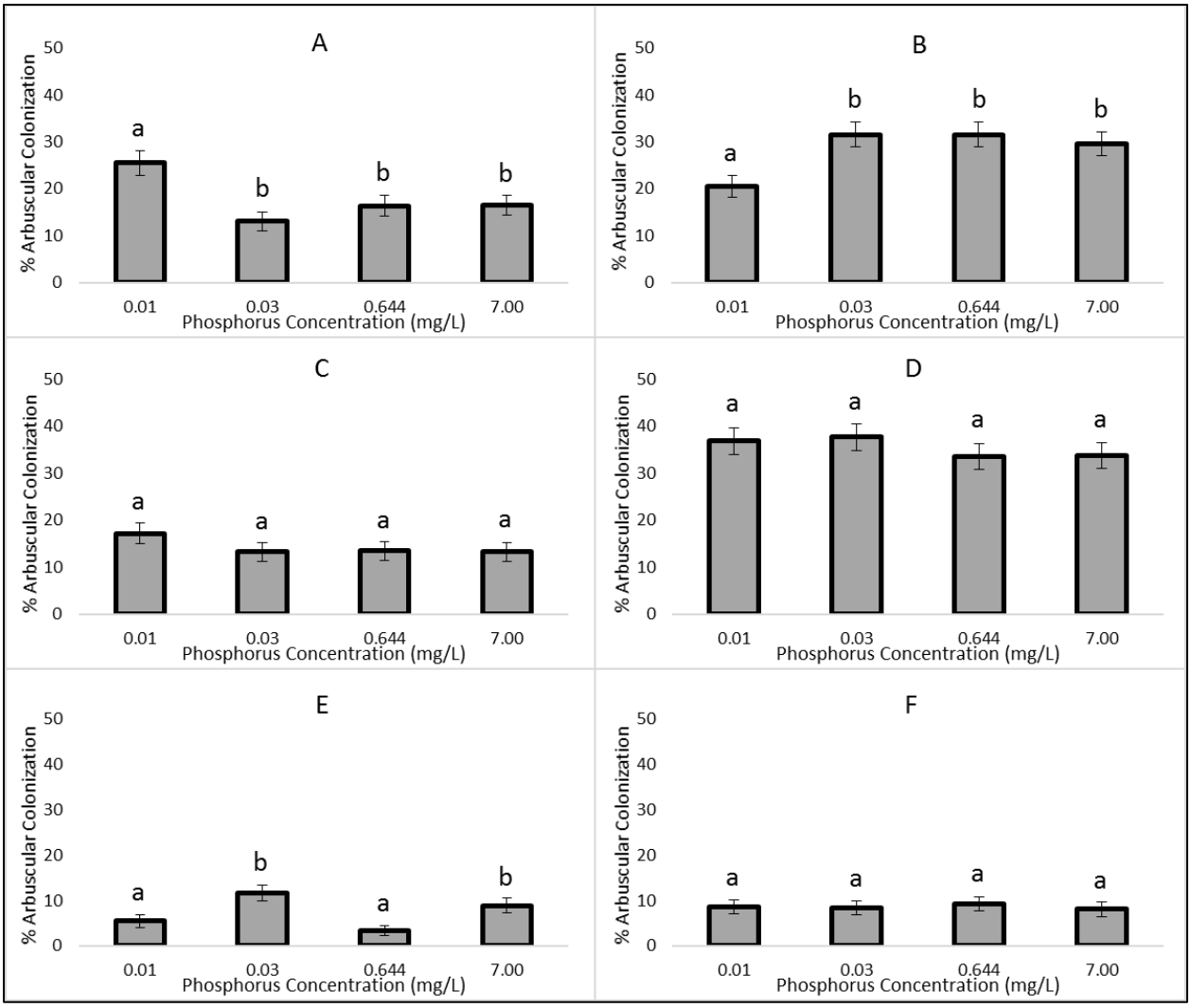

Figure 2.7: Mean arbuscular colonization of all six plant species at the four phosphorus concentrations. Effects of phosphorus supply on arbuscular colonization across all six plant species determined by logistic regression. Parameters were compared using the Wald test. This study was performed to indicate the effects of phosphorus on mycorrhizal colonization in a wetland system. Six different plants were harvested and assessed for mycorrhizal colonization after exposure to four levels of phosphorus (10, 30, 640, and 7000 µg/L PO_4-P) for 30 days. Trays were given 7 days to acclimate to the exposure solution for a week before seedlings were planted. Bars with the same letter are not significantly different. A – Mean arbuscular colonization of *Verbena hastata*. B – Mean arbuscular colonization of *Solidago canadensis*. C – Mean arbuscular colonization of *Phalaris arundinacea*. D – Mean arbuscular colonization of *Eupatorium perforliatum*. E – Mean arbuscular colonization of *Epilobium parviflorum*. F – Mean arbuscular colonization of *Echinochloa crus-galli*. Error bars indicate +/- one standard error.

CHAPTER 3

Quantifying relationships between phosphorus availability and mycorrhizal associations in a natural wetland

Abstract

The relationship between phosphorus availability and mycorrhizal colonization has been well studied in terrestrial plant species, but has been insufficiently examined in aquatic and semi-aquatic plant species. To determine the relationship between phosphorus availability and arbuscular mycorrhizal fungi in a natural wetland, two field studies were conducted. In the first study, *Phalaris arundinacea* plants were harvested and assessed for mycorrhizal structures at six locations along a water quality gradient on the Grand River Watershed. Phosphorus availability was shown to have little effect on the mycorrhizal status of *P. arundinacea* with weak Pearson's correlations ($r^2 < 0.4$, *p*-value > 0.05). In the second study, three plant species were assessed at two sites of varying water quality on the Grand River comparing the mycorrhizal response. No arbuscules were found in *Potamogeton* sp., but they were found in *Iris* sp. and *Veronica* sp. Mycorrhizal colonization were compared using logistic regression, showing mean hyphal colonization in *Iris* was higher in West Montrose (mean = 0.66, 95% CI: 0.60 - 0.71) than at Rare (mean = 0.45, 95% CI: 0.39 - 0.51). Mean hyphal colonization in *Veronica* was higher at Rare (mean = 0.59, 95% CI: 0.54 - 0.65) than in West Montrose (mean = 0.49, 95% CI: 0.44 - 0.55) indicating that mycorrhizal colonization varied depending on site, but this response is species-specific.

Introduction

Importance of mycorrhizal colonization in wetlands

Increasing evidence indicates that plant community structure and, therefore, ecosystem function are mediated by below-ground fungal communities called mycorrhizal associations (van der Heijden et al., 1998a). Arbuscular mycorrhizal fungi (AMF) are a type of fungus that colonizes plant host root cells but also maintain hyphae that extend outside of the root for resource acquisition. AMF are characterized by three structures: arbuscules, vesicles, and hyphae (Smith, 1990). Hyphae are the main vegetative growth form of AMF (Figure 3.1A), and are filamentous structures which ramify through the soil, acquiring and transporting resources to the host plant (Mcgonigle et al., 1990). Vesicles are hyphal swellings which store energy as lipids, and may develop thick walls in older roots and function as propagules (Biermann and Linderman, 1983) (Figure 3.1B). The arbuscule is the main defining feature of AMF and they are formed with repeated dichotomous branching and reduction of hyphae within cortical cells which can end with hyphae less than 1 µm in diameter (Brundrett et al., 1985) (Figure 3.1C). The arbuscules are theorized to be the location at which the fungus exchanges nutrients with the plant host (Smith, 1990). Understanding the impacts that biotic and abiotic factors have on these structures provides insight into the functionality of AMF.

The biotic and abiotic factors that contribute to AMF associations are well-documented in terrestrial ecosystems (van der Heijden et al., 2004). These associations occur in the majority of vascular plant families (Bever et al., 1996), and have been shown to improve seedling establishment (van der Heijden, 2004), increase seedling biomass

(van der Heijden et al., 2004), and influence plant diversity and plant community structure (van der Heijden et al., 1998b). Greenhouse experiments indicate AMF have been shown to facilitate phosphorus (P) uptake (Koch et al., 2004), and enhance nitrogen acquisition (Hawkins et al., 2000; Hodge et al., 2010).

The links between AMF and plant diversity and community structure are not well understood in aquatic or semi-aquatic systems (White and Charvat, 1999). Early studies discovered few mycorrhizal fungi occurring in wetland plants (Khan, 1974; Powell, 1975). However, more recent studies have found mycorrhizal colonization in prairie fens (Turner et al., 2000), Cypress swamps (Kandalepas et al., 2010), and in bottomland hardwood forests (Stevens et al., 2010). The presence of AMF in wetlands is now recognized, but the dependency of wetland plants on their AMF partners and the factors that affect AMF colonization in wetland habitats are poorly understood (Cornwell et al., 2001; Stevens and Peterson, 2007; Stevens et al., 2002).

Benefits to maintaining AMF colonization in wetland vegetation has been identified in several plant families. AMF-inoculated wetland plants have shown increased Zn acquisition in inundated farm soil (Purakayastha and Chhonkar, 2001), increased biomass in an upland nursery (Purakayastha and Chhonkar, 2001), increased flood tolerance in inundated topsoil (Osundina, 1998), as well as the capability of altering plant community structure in mesocosm studies (Wolfe et al., 2006). The widespread occurrence, and the beneficial effects of AMF found in wetlands suggest parallels with terrestrial ecosystems. Given the importance of AMF to plant communities and ecosystem function in terrestrial habitats, understanding the impacts and constraints on

AMF in wetlands may allow for more successful wetland management, and restoration efforts that maintain or enhance wetland functions.

Controls in AMF colonization

Species and strains of AMF differ in their range of tolerance of physical and chemical properties (Abbott and Robson, 1991). The primary abiotic factors known to influence AMF abundance and distribution in terrestrial systems are water, salt, and nutrient availability (Read, 1991). AMF colonization has been shown to be decreased by increased soil moisture likely due to the decline in oxygen availability and photosynthetic capacity, stomatal conductance and nutrient uptake (Ellis, 1998; Carvalho and Correia, 2003), excess salts through reductions in arbuscular and vesicular colonization (Juniper and Abbott, 1993), and nutrient availability by reducing the amount of nutrients necessary for synthesis of new tissues (Majdi et al., 2001). In a summary of multiple studies examining mycorrhizal responses to P and N, mycorrhizal abundance was reduced by 15% under N fertilization and 32% under P fertilization (Treseder, 2004). P availability is the primary factor influencing mycorrhizal colonization and growth in terrestrial soils, with phosphorus-abundant soils leading to reductions in mycorrhizal growth (Abbott et al., 1984; Treseder, 2004). These environmental factors also exist in wetlands, although the extent of the impacts is less understood.

Wetland mycorrhizas are relatively poorly studied, although increasing evidence is indicating parallels between established relationships of AMF in terrestrial systems and those found in aquatic conditions. Mycorrhizal colonization in wetlands has been shown to be reduced by increased water availability (Stevens and Peterson, 1996), excess salt

availability (Carvalho and Correia, 2003), and down the drain compounds such as Triclosan, a widely used antibacterial found in pharmaceuticals and personal care products (Twanabasu et al., 2013). Relatively few studies have examined the relationship between AM and P in wetland plants. Of the three studies which examined this relationship, all found that P availability impacted mycorrhizal colonization in wetland plants (White and Charvat, 1999; Tang et al., 2001; Stevens et al., 2002). Due to the increasing P enrichment in soils and aquatic systems worldwide (Bennett et al., 2001), there is a potential for impacts on mycorrhizal colonization in wetland systems. Since plant species depend to different extents on mycorrhizal partners (Stevens et al., 2011), these impacts could be particularly damaging to some species while not impacting others. This could cause shifts in plant community structure and wetland ecosystem function.

P in wetlands

Increased anthropogenic activities have led to significant increases in P loading in agricultural and urban watersheds (Zhang, 2016). Nutrient loads can originate from various sources, including storm water runoff, discharge from ditches and creeks, groundwater seepage, aquatic weed control, naturally occurring organic inputs, and atmospheric deposition (Ouyang et al., 2006). In Canada, agriculture is the primary contributor of P to watersheds (Zhang, 2016), and rivers are particularly vulnerable to nutrient loading due to their proximity to urban centers, sensitivity to land use changes, and extent of exploitation (Withers and Jarvie, 2008). Current levels of P in the Grand River watershed have routinely exceeded the Provincial Water Quality Objective for rivers and streams (30 µg/L P), with recorded values as high as 200 µg/L P (Loomer and

Cooke, 2011). These increased P inputs could lead to changes in species composition in aquatic plant communities (David and Gentry, 2006). Due to the well-studied relationship between increased P supply and AMF in terrestrial systems, if that relationship is maintained in wetland systems nutrient enrichment could disproportionately affect mycorrhizal communities and lead to changes in wetland functioning.

Limitations in previous studies

Previous studies have examined the relationship between phosphorus availability and mycorrhizal colonization in terrestrial systems (Abbott et al., 1984; Asimi et al., 1980), although there is a lack of research into these interactions in aquatic conditions (Feng et al., 2002; White and Charvat, 1999). Previous studies which examined the effects of phosphorus availability on mycorrhizal colonization in inundated conditions have found that AMF colonization is reduced at levels as low as 5000 µg/L P (Tang et al., 2001; White and Charvat, 1999; Stevens et al. 2002). The study performed by White and Charvat (1999) examined mycorrhizal responses in *Lythrum salicaria* L. and showed reductions in vesicular and arbuscular colonization between 1000 and 10000 µg PO_4/L. The study performed by Tang et al. (2001) examined mycorrhizal responses in *Typha angustifolia* and showed reductions in hyphal and arbuscular colonization between 0.950 and 9500 µg PO_4/L. The study performed by Stevens et al. (2002) examined mycorrhizal responses in *Lythrum salicaria* L and showed reductions in hyphal, vesicular, and arbuscular colonization between 1250 and 5000 µg PO_4/L. While these P levels far exceed levels noted in the Grand River watershed it must be emphasized that studies were greenhouse/growth room studies and the application of these results to natural watersheds

is limited. These studies were limited due to their static non-renewal (Tang et al., 2001; White and Charvat, 1999), or static renewal (Stevens et al., 2002) application of nutrient solution (Figure 3.2), as well as the reliance on a single species of wetland plant as a study organism.

Objective of current study

The primary objective of this study was developed to determine the relationship between phosphorus availability and arbuscular mycorrhizal fungi in a natural wetland. The secondary objective was to determine if other water quality parameters were also affecting mycorrhizal colonization. To address these objectives, I assessed various wetland plants for mycorrhizal structures at locations of varying water quality in the Grand River Watershed. This experiment was a completely randomized design assessing plants in the natural environment. Two studies were performed: the first field study assessed the mycorrhizal colonization of a single plant species across a water quality gradient over the course of a year to capture seasonal variation. The second field study assessed the mycorrhizal colonization of three wetland plants at two sites with different water quality to examine species-specific responses of wetland plants.

Hypothesis

In consideration of previous studies that examined the effects of phosphorus availability on mycorrhizal colonization in inundated conditions, I predict that with increasing phosphorus availability, there will be a decrease in mycorrhizal colonization. I also predict that due to the flow-through exposure system applying a continuous application of P, AMF colonization will be reduced at much lower concentrations than in

previous studies. In addition, since there is a plethora of environmental variables not accounted for such as down the drain compounds, heavy metals, etc., it is anticipated that there will also be variation in mycorrhizal colonization due to confounding factors.

Materials and methods

Field Study 1

Study organism

Reed Canary Grass (*Phalaris arundinacea* L.) was selected as a study organism because it was present at all sites within 1 m of the Grand River bank. This places *Phalaris* in the riparian wetland zone, which makes it heavily impacted by river water quality. It grows very rapidly, removes nutrients from the water, and is used in constructed wetlands for wastewater removal (Vymazal and Kropfelova, 2005). It is also known to be colonized by mycorrhizal fungi (Lorimer and Bauer, 1983).

Study sites

Six sampling sites were chosen based on the GRCA Nutrient Quality Index and the 2011 Water Quality Report based on water quality data from 2003-2008 in the Grand River Watershed (Loomer and Cooke, 2011). Water quality data provided by the Provincial Water Quality Monitoring Network (PWQMN) was consulted to determine that past trends are consistent with data collected during field sampling. Six sites were chosen, each classified as either good (Shand Dam and West Montrose), intermediate (Bridge & Lancaster and Conestogo), or poor (Doon and Glen Morris) water quality sites (Figure 3.3). Each site was within 100 meters of a provincial water quality monitoring site which is sampled every month for common water quality parameters.

Shand Dam (43°43'28.5"N 80°20'37.2"W, Figure 3.3A) is the northernmost site. Agricultural land surrounds it, with a weeping bed on the northwestern shore slightly upstream of the study site. The site is approximately 1 km downstream from the Belwood Lake reservoir dam. The West Montrose site (43°35'18.0"N 80°28'14.1"W, Figure 3.3B) is approximately 20 km downstream from Shand Dam. Agricultural land surrounds it, with a stream carrying agricultural effluent 50 m upstream of the study area and a trailer park 100 m downstream of the study area. The Conestogo site (43°31'30.7"N 80°30'50.7"W, Figure 3.3C) is the only site which is not on the Grand River proper. It is on the Conestogo River approximately 4.5 km downstream from St. Jacobs and 3.5 km upstream of the junction with the Grand River. Agricultural land surrounds it, and it receives effluent from the St. Jacobs wastewater treatment plant. The Bridge and Lancaster Site (43°28'55.0"N 80°28'51.3"W, Figure 3.3D) is about 10 km south of the junction between Conestogo River and the Grand River in a heavily urban area, and has a stream 100 m north of the sampling locations. The Doon site (43°23'10.3"N 80°23'14.5"W, Figure 3.3E) is at the southeast of Kitchener near Cambridge in a heavily urban area. Glen Morris (43°16'41.6"N 80°20'33.4"W, Figure 3.3F) is the southernmost site sampled in the watershed and is under the bridge at Glen Morris. It is approximately 10 km downstream of Cambridge and is surrounded by agricultural land.

Sampling plan

Sampling took place every six weeks throughout the year with a total of five sampling events from May 20, 2015 – November 11, 2015 to capture growing season variation. During each sampling event, all six sites were sampled within 10 days of each

other, with the sampling split into two separate days of 3 sites each (1 from good, 1 from intermediate, 1 from poor water quality) which was determined with a random number generator before sampling began. Rectangular quadrats of 50 cm × 120 cm were placed in four locations at each site on populations of *Phalaris arundinacea* growing within 1 m of the river bank. These quadrats were chosen by areas populated by the test species and within 100 m of provincial water quality monitoring stations. Piezometers 30 cm in length were installed in the center of each quadrat for pore water extraction two weeks before the start of the sampling season. The quadrats were divided into eight sub-quadrats to allow for destructive sampling. The sampled sub-quadrats were 50 cm x 15 cm and were chosen using a random number generator from undisturbed sub-quadrats. Once at a site, all four quadrats were sampled. At each quadrat, pictures of the sampled sub-quadrats were taken prior to sampling with cards showing the site, the quadrat and sub-quadrat numbers (Figure 3.4). Two *Phalaris arundinacea* plants were taken in labelled plastic bags for assessing AMF colonization. Soil moisture was measured from the sub-quadrat prior to sampling using a soil moisture probe (HH2 W.E.T. Sensor, Delta-T Devices, Cambridge, UK) and a 200 mL pore water sample was then taken from the quadrat piezometer and stored in a 250 mL Nalgene sample container for further nutrient analysis. At each sub-quadrat, common water quality parameters (Table 3.1) were quantified at the time of sampling using a YSI Professional Plus Multiparameter Instrument (YSI 1700/1725 Brannum Lane, Yellow Springs, OH 45387 USA) with an attached Professional Plus Quatro Field Cable using Temperature/Conductivity, DO, ORP, and pH probes. River water was tested for the same parameters and stored in the 250 mL Nalgene sample containers. Pore and river water samples were then retrieved

from the field and tested for NH_3-N, NO_3-N, NO_2-N, and PO_4-P in the lab within 12 hours.

Nutrient analysis

The nutrient tests used were those which were most likely to capture the concentration range of the water within the site based on historic water quality data retrieved form the Provincial Water Quality Monitoring Network (PWQMN). Water was collected from the quadrat piezometer using a 250 mL Nalgene sample bottle which had been cleaned and rinsed three times using deionized water prior to sampling events. Orthophosphate levels were determined using Method 10209 Ultra Low Range Reactive Phosphorus (0.010 – 0.500 mg/L PO_4-P) and quantified at 880 nm with a DR3900 Benchtop Spectrophotometer (HACH Sales & Service Canada Ltd. 3020 Gore Road, London, ON, N5V 4V7). Nitrite levels were determined using Method 8507 Nitrite, Low Range (0.005 to 0.350 mg/L NO_2-N) for water, wastewater and seawater (HACH Company, 2012) and quantified at 507 nm with a DR/890 Hach Spectrophotometer (HACH Sales & Service Canada Ltd. 3020 Gore Road, London, ON, N5V 4V7). Nitrate levels were determined using Method 8171, Mid Range (0.2 to 5.0 mg/L NO_3-N) for water, wastewater and seawater (HACH Company, 2012) and quantified at 400 nm with a DR/890 Hach Spectrophotometer (HACH Sales & Service Canada Ltd. 3020 Gore Road, London, ON, N5V 4V7). Ammonia levels were determined using Method 8155 (0.02 to 0.50 mg/L NH_3-N) for water, wastewater and seawater (HACH Company, 2012) and quantified at 655 nm with a DR/890 Hach Spectrophotometer (HACH Sales & Service Canada Ltd. 3020 Gore Road, London, ON, N5V 4V7).

Field Study 2

Study organisms

Based upon a preliminary assessment of the presence of wetland plants in the two study sites, three plant species were selected for this study: *Veronica* sp. (Speedwell) in the family Plantaginaceae, *Iris sp.* in the family Iridaceae, and *Potamogeton* sp. (Pondweed) in the family Potamogetonaceae. These species were selected because they are prevalent within the Grand River watershed at both sites that were sampled and are present throughout the watershed. *Veronica* sp., Lamiales: Plantaginaceae is a flowering dicot originating from the northern hemisphere. It is either an herbaceous annual or a perennial plant and are commonly used as a ground cover plant. *Veronica* as a weed is known to outcompete lawn grasses (Figure 3.5). *Iris* sp., Asparagales: Iridaceae are perennial flowering plant which spread from creeping rhizomes. They have erect flowering stems which can be branched or simple. They have fan-shaped inflorescences and contain six lobed flowers. There are 300 species in the genus (Figure 3.6). *Potamogeton* sp., Alismatales: Potamogetonaceae is a genus of freshwater aquatic plant known by the common name Pondweed, although it is unrelated to Canadian pondweed. Stems range from 10 cm to 6 m in length. All species are perennial, but some are significantly reduced in winter. The plant is wholly submerged with thin, translucent leaves. The flowers are green to brown in colour and are composed of four rounded segments borne in a spike. The fruits are spherical and are green to brown and usually 1-3 mm in diameter. *Potamogeton* are found in freshwater rivers throughout the world, with there being 94 species identified worldwide (Figure 3.7).

Study sites

The two sampling sites were chosen based on water quality data obtained in the first field study. The Rare site was selected as a "poor water quality" site as it was near the Doon sampling site in the previous field study. The West Montrose site was selected as a "good water quality" site as it was the same as the West Montrose site used in the previous field study.

Sampling plan

Three different plant species were collected from the field with three replicates each over two days from both sites. Three root samples of each plant were harvested at each site and placed in Nasco Whirl-Paks™ (Fisher Scientific Company, 112 Colonnade Road, Ottawa, ON, K2E 7L6) for mycorrhizal assessment. Harvested roots were rinsed in tap water then fixed and stored in 50% ethanol. On the first day, two samples of *Potamogeton* sp. and *Iris* sp. were taken and one sample of *Veronica* sp. was taken from the West Montrose site. One sample of *Potamogeton* sp. and *Iris* sp. was taken and two samples of *Veronica* sp. were taken from the Rare site. On the second day, one sample of *Potamogeton* sp. and *Iris* sp. were taken and two samples of *Veronica* sp. were taken at the West Montrose site. Two samples of *Potamogeton* sp. and *Iris* sp. were taken and one sample of *Veronica* sp. was taken from the Rare site. Water quality for the two sampling sites were displayed as an average of two sampling periods which bracketed the study date (Table 3.2).

Root harvesting, processing, and AM quantification

The root sections harvested were selected randomly and placed in Falcon™ 50 mL Conical Centrifuge Tubes (Fisher Scientific Company, 112 Colonnade Road, Ottawa, ON, K2E 7L6). A clearing agent (10% KOH) was added, completely covering the roots. They were put in the vacuum oven (Thermo Scientific Lindberg Blue M) for 1 h 30 min at 95° Celsius at 25inHG. The samples were then retrieved from the vacuum oven and rinsed with 10% acetic acid for 3 minutes at room temperature. A solution was then added composing of 5% Sheaffer Skrip Black Ink (Sheaffer Slovakia s.r.o., Priemyselnà 1, 926 01 Sered', Slovak Republic), 5% acetic acid and 90% deionized water. The samples were again put into the vacuum oven for 1 h 30 min at 95° Celsius at 25inHG. The samples were then retrieved from the vacuum oven and rinsed with 5% acetic acid for 10 minutes at room temperature. The acetic acid was then replaced by 50% glycerol, and the sample was allowed to sit overnight. The stained root samples were then mounted on 75x25x1 mm frosted VWR Microscope Slides (VWR International). Prepared slides were viewed at 200 × magnification using a Nikon Eclipse E600 Microscope (Nikon Instruments Inc. 1300 Walt Whitman Road Melville, NY 11747-3064, U.S.A.). Colonization levels were assessed using the magnified intersections method (Mcgonigle et al., 1990). The percentage of hyphal, arbuscular, and vesicular AM colonization was calculated after assessing a total of 100 fields of view for each sample.

Data analysis

Field Study 1

Water quality among sites

The sites were classified into good (Shand Dam & West Montrose), intermediate (Conestogo and Bridge & Lancaster), and poor (Doon & Glen Morris) water quality groups using the Grand River Conservation Authority (GRCA) 2011 Water Quality report and the Nutrient Quality Index. It was essential to determine if phosphorus availability and mycorrhizal colonization correlated with these classifications. As part of data exploration, we ran individual analysis of variances (ANOVAs) on phosphorus, mycorrhizal structures, and other water quality variables both within and between water quality groupings at each sampling event. Since the results of the ANOVA determined there were both within and between site differences at each time point for the explanatory variables, multiple comparisons were also performed on mycorrhizal structure data assessed from plants sampled from the field.

Phosphorus availability correlated with mycorrhizal colonization

To determine if phosphorus availability was correlated with mycorrhizal colonization, a Pearson correlation was performed. Phosphorus concentrations were compared with mycorrhizal structures (hyphae, vesicles, and arbuscules) to determine linear relationships at each time point and over the entire sampling period.

Determination of trends in water quality

To determine relationships among water quality parameters in addition to phosphorus, a comparison was made with a PCA using IBM SPSS Statistics 23 (IBM) to

determine prevalent trends in water quality. Normalized variables used within the PCA were DO, water depth, water temperature, PO_4-P, NO_2-N, NO_3-N, NH_3-N, TDS, conductivity, pH, and ORP. Univariate descriptions were displayed as well as a coefficient correlation matrix, with KMO and Bartlett's test of Sphericity to test if the data were acceptable to use in a PCA. The method used for extraction was principal components based on the fixed number of factors determined using the Monte Carlo Simulation.

Prevalent trends in water quality and nutrient supply

Since the contrasts from the multiple comparisons showed inconsistencies both within and between water quality groups, a comparison was made between water quality variables using a principal components analysis (PCA) in IBM SPSS Statistics 23 (IBM) to determine prevalent trends in water quality and nutrient supply. Prior to completing the PCA, a Monte Carlo simulation was performed to determine the number of principal components to use in the model. Syntax was obtained from Brian O'Connor SPSS Stats (O'Connor, 2000). The number of simulated datasets was set to 1000 and the desired statistical percentile was set at 95%. This allows percentiles associated with the eigenvalues that will be estimated on 1000 datasets generated from the Monte Carlo Simulation. The program also displays the 50^{th} percentile, or the mean eigenvalue as well as the 95^{th} percentile. To meet the assumptions required for this test, the explanatory variables were all normalized using various accepted methods. Dissolved oxygen (DO), PO_4-P, NO_2-N, and NO_3-N were all Ln transformed. Conductivity, total dissolved solids (TDS), and oxidation-reduction potential (ORP) were all square root transformed. Water

depth and NH_3-N were log transformed. Water temperature and pH were normally distributed. Four component scores were saved as variables using the regression method and displayed on a coefficient matrix for use in further analyses and labelled as "PC Score 1, 2, 3, and 4" (representing 70.94% of the variation of the dataset).

Assessment of site differences using PC scores from principal component analysis

To determine if the water quality parameters were different at each site, multivariate analysis of variance (MANOVA) was performed on the PC scores extracted from the principal component analysis. Site and month were set as fixed factors and the four PC scores extracted from the PCA were set as dependent variables.

Assessment of mycorrhizal colonization by site and time

To determine if mycorrhizal colonization was different at each site and over time, logistic regression was performed. Mycorrhizal structures (hyphae, vesicles, and arbuscules) were examined for a relationship with site and time using multiple regression.

Water quality changes correlated with mycorrhizal colonization

To determine if water quality was correlated with mycorrhizal colonization, PC scores saved from the PCA on water quality from previous sampling events were used as predictors for current mycorrhizal colonization trends. In addition to assessing direct correlation between water quality parameters and mycorrhizal colonization, the previous months' water quality was also used as explanatory variables. This is because there aren't usually immediate morphological and physiological responses in organisms so this could account for a delayed effect of water quality on mycorrhizal structures. To determine if

mycorrhizal colonization was correlated with water quality parameters, a repeated measures analysis of variance on lagged and non-lagged PC scores was performed using R Version 3.3.2. An error term was supplied to reflect that month is nested within quadrats within sites. Month, quadrat, and site were also sources of variability.

Field Study 2

Assessment of mycorrhizal colonization between sites

To determine whether mycorrhizal colonization varied between sites, logistic regression was performed using IBM SPSS Statistics 23 (IBM) on each response variable (hyphae, vesicles, and arbuscules) modelled as presence/absence in each cell counted (100 cells/plant). The predictive factors were the species of plant. Main effects of plant species were modelled.

Results

Field Study 1

Water quality among sites

Oxidation-reduction potential, dissolved oxygen, water depth, water temperature, and NH_3-N didn't vary among sites. PO_4-P, NO_2-N, NO_3-N, pH, total dissolved solids, and conductivity were significantly different between GRCA groupings, indicating that these variables changed between groups and required further study (Table 3.3). From the contrast statements, it was determined that there were significant differences of means within water quality groups, indicating that the groupings were not properly captured by the parameters that were tested. Contrast statements and ANOVA results for water quality variables at each sampling time are provided in the Appendix. Mycorrhizal

structures were also shown to vary depending on the site and time, but were not consistently different among water quality groups. Contrast statements and ANOVA results for mycorrhizal structures at each sampling time are provided in the Appendix.

The results of the analysis of variance showed that the predicted groupings based off historical data didn't persist in the conditions assessed during the study. Phosphorus concentrations did not vary within the good ($F_{5,120} = 1.266$, $p = 0.207$), intermediate ($F_{5,120} = -0.517$, $p = 0.606$), or poor ($F_{5,120} = 1.908$, p = 0.059) water quality groups. P concentrations were statistically different between good and intermediate sites ($F_{5,120} = -3.846$, $p < 0.001$), and good and poor sites ($F_{5,120} = -3.685$, $p < 0.001$). However, P concentrations did not vary between intermediate and poor quality sites ($F_{5,120} = 0.136$, $p = 0.892$) (Table 3.4). This indicates that the intermediate and poor quality sites had phosphorus levels that were statistically the same.

Hyphal colonization did not vary within the intermediate ($F_{5,120} = 1.143$, $p = 0.255$) water quality group, but it did vary within the good ($F_{5,120} = 2.178$, $p = 0.031$), and poor ($F_{5,120} = 2.309$, $p = 0.023$) water quality groups (Table 3.4). This indicates that the good and poor water quality sites had statistically different mycorrhizal colonization within water quality groups, so using them as distinct groupings could not be done using mycorrhizal colonization as a predictor.

Phosphorus availability correlated with mycorrhizal colonization

There were no correlations between phosphorus and hyphae (r = 0.133, n = 120, p = 0.148), vesicles (r = 0.110, n = 120, p = 0.232), or arbuscules (r = 0.065, n = 120, p =

0.483) over the entire sampling period. Phosphorus concentrations also did not correlate to any mycorrhizal structures over any sampling time periods (Appendix).

Determination of trends in water quality

Since the water quality didn't correspond to the GRCA groupings, and phosphorus was not directly correlated with mycorrhizal colonization, the use of PCA to determine trends in water quality was required. Bartlett's test of sphericity was significant ($df = 55$, $p < .001$) for the PCA, indicating that the null hypothesis can be rejected and that the data is appropriate for a PCA (Table 3.5). The values found in the communalities table (Table 3.6) indicated that four principal components should be used for the PCA. A total cumulative variance of 70.94% was explained by the model (Table 3.7). In this table, there are no variables with low values, indicating that each variable is well represented by the PC Scores. The component matrix shows the component loadings, or the correlations between component and variable. Correlations under 0.2 were suppressed and do not appear on the table (Table 3.8).

Four main components were extracted from the principal components analysis for the first study. PC Score 1 accounted for 24.127% of the variation and had pH, ORP, NO_3-N, NO_2-N, and dissolved oxygen strongly positively loaded. PC Score 2 accounted for 21.103% of the variation and had water depth and temperature loaded strongly. PC Score 3 was the most applicable to the overall research goal, as it was positively loaded with total dissolved solids, conductivity, NO_3-N, NO_2-N, and PO_4-P. It only accounted for 14.113% of the overall variance, however. PC Score 4 accounted for 11.513% of the variance and had ORP and NH_3-N positively loaded, with NO_2-N negatively loaded.

Assessment of site differences using PC scores from principal component analysis

To determine if there was a site and time effect on water quality parameters, a MANOVA using PC Scores from the PCA was performed. A statistically significant multivariate analysis of variance interaction effect was obtained between site and month, $F_{80,120} = 2.303$, $p < .001$; Wilk's $\lambda = .185$ (Table 3.9) indicating significant differences between sites in terms of water quality. PC scores are loaded onto box plots to display differences between sites at each sampling period (Figures 3.8-3.11).

Assessment of mycorrhizal colonization by site and time

To determine if there was a site and time effect on mycorrhizal structures, logistic regression analysis was performed. Logistic regression analysis on mycorrhizal structures showed significant differences in the interaction between site and month for hyphae ($X^2_5 = 790.496$, $p < 0.001$), vesicles ($X^2_5 = 243.413$, $p < 0.001$), and arbuscules ($X^2_5 = 540.395$, $p < 0.001$) (Table 3.10). Mean hyphal, vesicular, and arbuscular colonization are shown in Figures 3.12-3.14.

Water quality changes correlated with mycorrhizal colonization

The repeated measures ANOVA results indicate that hyphal colonization significantly differed depending on lagged PC Score 2 ($F_{1,83} = 4.701$; $p < 0.05$) and PC Score 3 ($F_{1,83} = 4.161$; $p < 0.05$) and non-lagged PC Score 1 ($F_{1,83} = 4.431$; $p < 0.05$) (Table 3.11). There were no significant differences with vesicular colonization (Table 3.12). Arbuscular colonization significantly differed depending on Lagged PC Score 2 ($F_{1,83} = 5.121$; $p < 0.05$) and PC Score 1 ($F_{1,83} = 6.485$; $p < 0.05$) (Table 3.13).

Field Study 2

Assessment of mycorrhizal colonization among sites

Hyphae

The tests of model effects showed that hyphal colonization varied among species ($X^2_2 = 260.184$, $p < .001$) and the interaction between site and species ($X^2_2 = 30.747$, $p < .001$) (Table 3.14). Mean hyphal colonization in *Iris* was higher in West Montrose (mean = 0.66, 95% CI: 0.60 - 0.71) than at Rare (mean = 0.45, 95% CI: 0.39 - 0.51). Mean hyphal colonization in *Veronica* was higher at Rare (mean = 0.59, 95% CI: 0.54 - 0.65) than in West Montrose (mean = 0.49, 95% CI: 0.44 - 0.55). Mean hyphal colonization in *Potamogeton* was higher at Rare (mean = 0.07, 95% CI: 0.05 - 0.10) than in West Montrose (mean = 0.05, 95% CI: 0.03 – 0.08) (Table 3.15, Figures 3.15 & 3.16).

Vesicles

The tests of model effects showed that vesicular colonization varied among species ($X^2_2 = 50.025$, $p < .001$), and the interaction between site and species ($X^2_2 = 39.772$, $p < .001$) (Table 3.16). Mean vesicular colonization in *Iris* was higher in West Montrose (mean = 0.19, 95% CI: 0.15 - 0.24) than in Rare (mean = 0.15, 95% CI: 0.11 - 0.20). Mean vesicular colonization in *Veronica* was similar between Rare (mean = 0.14, 95% CI: 0.11 - 0.19) and West Montrose (mean = 0.14, 95% CI: 0.10 - 0.18). Mean vesicular colonization in *Potamogeton* was higher at West Montrose (mean = 0.04, 95% CI: 0.02 - 0.07) than at Rare (mean = 0.03, 95% CI: 0.01 – 0.05) (Table 3.17, Figures 3.15 & 3.16).

Arbuscules

The tests of model effects showed that arbuscular colonization varied among species (X^2_2 = 44623.784, p < .001), site (X^2_2 = 30.582, p < .001), and the interaction of site and species (X^2_2 = 39.772, p < .001) (Table 3.18). Mean arbuscular colonization in *Iris* was higher in West Montrose (mean = 0.32, 95% CI: 0.27 - 0.37) than in Rare (mean = 0.29, 95% CI: 0.24 - 0.34). Mean arbuscular colonization in *Veronica* was higher at Rare (mean = 0.24, 95% CI: 0.19 - 0.29) than in West Montrose (mean = 0.02, 95% CI: 0.01 - 0.04). Arbuscules were absent in *Potamogeton* at both sites (Table 3.19, Figures 3.15 & 3.16).

Discussion

Water quality in the Grand River watershed

Water quality trends in the Grand River watershed did not follow the groupings which were determined based on the GRCA Nutrient Quality Index and the 2011 Water Quality Report, meaning water quality was different than predicted. The multiple comparisons performed showed that the intermediate and poor water quality groups were not statistically different in terms of phosphorus. Mycorrhizal colonization also did not correspond to the water quality groupings, with hyphae and arbuscules showing within group variation in the good and poor water quality groups. This could be because the water quality has changed since the watershed was assessed in 2008, either due to increases in urban activities, changes in wastewater systems, or seasonal variations. Waterloo Region has increased in population from 477160 in 2006 (Statistics Canada, 2016) to a population of 575000 in 2015 (Parkin, 2016), an increase of 20% over 9 years.

This could account for decreases in water quality in heavily populated areas and a trend towards more people populating rural towns north of Waterloo. Another factor which may have affected the results of the study was that during the growing season, major upgrades to the Kitchener wastewater treatment plant were under way. This would mainly affect the poor water quality sites (Doon and Glen Morris), which were downstream to the newly upgraded wastewater treatment plant. Within the year that upgrades were implemented, there was a 70% reduction in the occurrence of intersex fish, with an expected full recovery of the fish population (Hicks et al., 2017). It could be expected that with increases in wastewater treatment capabilities, that water quality no longer follows the water quality groups determined from historic data, and could be an indication why the Glen Morris site had better water quality than previous measurements indicated. Since the water quality groupings did not reflect the water quality of the study, a closer look at water quality parameters was required.

The PCA showed that there were four main components to water quality in the Grand River. PC Score 3 was the most applicable to the overall research goal of finding interactions with phosphorus, as it was strongly loaded with total dissolved solids, conductivity, NO_3-N, NO_2-N, and PO_4-P. It only accounted for 14.113% of the overall variance, however. PC Score 3 could be used to explain fertilizer and phosphorus inputs from agricultural and urban runoff.

The PC showed that PC Score 1 accounted for 24.127% of the variation and had pH, ORP, NO_3-N, NO_2-N, and dissolved oxygen strongly positively loaded. This is unsurprising, given that dissolved oxygen, ORP, and pH are well known to be strongly

positively correlated in nature (Zang et al., 2011). PC Score 2 accounted for 21.103% of the variation and had water depth and temperature loaded strongly. These two parameters are closely tied together, as water depth correlates strongly with colder and more consistent temperatures in nature (Schmidt et al., 2006). PC Score 4 accounted for 11.513% of the variance and had ORP and NH_3-N positively loaded, with NO_2-N negatively loaded. This component could be representing the nitrogen cycle, with NO_2-N being reduced to NH_3-N (Nayyar, 2009).

Correlations between water quality and mycorrhizal colonization in Phalaris arundinacea

The variation in mycorrhizal colonization were both seasonal and site specific, with colonization increasing over the summer. Since PC Score 1 was shown to be significantly correlated with the presence of mycorrhizal structures, it is important to show the break down of the different positively loaded parameters. It is well known that mycorrhizal colonization in terrestrial systems is heavily impacted by soil pH, with a desired pH between 5.5 and 6 (Koptur and Volin, 2015). Mycorrhizal colonization is also negatively correlated with soil moisture, as it is an obligate aerobe and increases water uptake for the plant, conveying additional benefits as a symbiont (Purakayastha and Chhonkar, 2001). Both PC Scores 2 and 3 were shown to be correlated with decreases in mycorrhizal colonization when lagged. This could be due to delayed effects associated with the increased presence of total dissolved solids and nutrients such as phosphorus. Since the main benefit of AMF for plants is their improved nutrient uptake, particularly

phosphorus, it makes sense that with more available nutrients in the water column, there would be a decreased need for the mycorrhizal symbiosis for the plant (Bolan, 1991).

Water quality effects on mycorrhizal colonization in Potamogeton sp., Iris sp., and Veronica sp.

The results of the logistic regression show that mycorrhizal colonization varies with site water quality, although it primarily affects the presence of arbuscules (Figures 3.15 & 3.16). Mycorrhizal colonization impacts also depend on the host plant species. Colonization of AMF in *Potamogeton* sp. was very low at both sites, so determining trends was difficult. There also were no arbuscules present, so it is possible that a different type of mycorrhiza colonized it. In *Iris* sp., all three mycorrhizal structures were more prevalent at the good quality site (West Montrose) than the poor quality site (Rare). This follows the well-known relationship in terrestrial systems where mycorrhizal colonization is inversely related to nutrient availability (Treseder, 2004), as well as previous studies which have found a similar relationship in wetland environments (Stevens et al., 2002; Tang et al., 2001; White and Charvat, 1999). In *Veronica* sp., colonization of hyphae and arbuscules were higher in the poor quality site (Rare) than in the good quality site (West Montrose). This could indicate that the water quality parameters we examined weren't properly capturing important environmental variables, or that the plants assessed had a mycorrhizal response that reacted differently amongst plant species.

Conclusion

Because wetland functioning is vital to maintaining essential ecosystem services, and because plants and their mycorrhizal partners are essential in facilitating wetland ecosystem functioning, understanding the impacts that changes in mycorrhizal colonization can have on plant community and performance is crucial. If the relationship is the same as in terrestrial systems, limits to phosphorus availability could alter mycorrhizal colonization, causing impacts to ecosystem functioning. About half of the global wetland area has been lost (Zedler and Kercher, 2005), so understanding the factors which affect wetland plant community composition are essential for wetland restoration and management (Zedler, 2000).

In the first field study, phosphorus availability is shown to be correlated with mycorrhizal colonization of *Phalaris arundinacea* in a natural wetland. However, the mycorrhizal responses vary depending on the site and time and have significant interactions, which makes drawing conclusions particularly difficult. It is also shown that phosphorus availability is only a small portion of the environmental factors which are correlated with AMF. In the second field study, water quality is shown to vary with mycorrhizal colonization of three wetland plants in a natural wetland. These effects are not consistent across all plant species, and they elicit species-specific responses. This is particularly alarming because if water quality changes impact species differently, shifts in community structure could occur which could lead to changes in ecosystem functioning. Since field studies do not allow for the isolation of individual variables, future studies should examine the effects of nutrients, particularly phosphorus, on mycorrhizal colonization in controlled greenhouse studies. Future studies should also examine the

effects of various water quality and nutrient parameters on mycorrhizal colonization in controlled studies across multiple plant species.

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Tables & Figures

Table 3.1: Environmental parameters tested using the YSI Professional Plus meter. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters were measured in pore water in each quadrat as well as in the river water. A principal components analysis was used to determine prevailing trends in water quality, and logistic regression was used on the extracted component loadings to determine if water quality influenced mycorrhizal structures.

Material	Equipment Used
pH	YSI Professional Plus
Temperature	
Oxidation-reduction potential (ORP)	
Dissolved oxygen (DO)	
Total dissolved solids (TDS)	
Conductivity	

Table 3.2: Water Quality of the two sampling sites from Field Study 2 (Rare and West Montrose). Data displayed is an average of two sampling periods (October 6, 2015 and November 10, 2015) which bracketed the study date. Water samples were taken directly from the river at the displayed water depth in the center of the river and nutrient tests were done at the lab within 24 hours.

Site	West Montrose	Rare	Units
Flow	0.2	0.3	m/s
Water depth	22.5	25	cm
Monitoring depth	15	15	cm
Water temperature	8.9	11.35	°C
pH	8.15	8.355	-
Dissolved oxygen	10.745	13.44	mg/L
Total dissolved solids	292.5	402.725	mg/L
Oxidation-reduction potential	61.8	53.125	mV
Conductivity	307.4	477.8	Cus/cm
Total suspended solids	5	7.5	mg/L
Nitrite	0.007	0.049	mg/L NO_3-N
Nitrate	0.65	0.9	mg/L NO_2N
Ammonium	0.03	0.02	mg/L NH_3-N
Phosphorus	69.5	92	µg/L PO_4-P

Table 3.3: Results of analysis of variance on site differences on water quality parameters. Six sites along a water quality gradient were assessed for 11 water quality parameters as shown in this table five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters were measured in pore water in each quadrat as well as in the river water.

Water Quality Parameter	Sum of Squares	df	Mean Square	F	P - value
pH	7.702	5	1.54	16.577	0.000
ORP	65.968	5	13.194	2.249	0.054
Dissolved oxygen	4.389	5	0.878	2.236	0.056
Water Depth	0.178	5	0.036	1.115	0.356
Water Temperature	250.546	5	50.109	1.656	0.151
PO_4-P	9.498	5	1.9	4.865	0.000
NO_2-N	14.35	5	2.87	3.327	0.008
NO_3-N	17.782	5	3.556	9.425	0.000
NH_3-N	2.697	5	0.539	2.268	0.052
Total dissolved solids	868.311	5	173.662	9.285	0.000
Conductivity	949.535	5	189.907	10.025	0.000

Table 3.4: Contrast tables of the multiple comparisons conducted examining site differences within and between water quality groups. Shand Dam and West Montrose represented the "Good Water Quality" group, Conestogo and Bridge & Lancaster represented the "Intermediate Water Quality" group, and Doon and Glen Morris indicated the "Poor Water Quality" group. Six sites were assessed for water quality parameters and mycorrhizal colonization along a water quality gradient five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters and mycorrhizal structures were compared.

Contrast Coefficients

	Site					
Contrast	Shand Dam	West Montrose	Conestogo	Bridge & Lancaster	Doon	Glen Morris
1	1	-1	0	0	0	0
2	0	0	1	-1	0	0
3	0	0	0	0	1	-1
4	1	1	-1	-1	0	0
5	1	1	0	0	-1	-1
6	0	0	1	1	-1	-1

	Contrast	Value of Contrast	Std. Error	t	df	P - value
PO_4-P	1	0.2506	0.19759	1.268	113	0.207
	2	-0.1022	0.19759	-0.517	113	0.606
	3	0.382	0.20017	1.908	113	0.059
	4	-1.0747	0.27944	-3.846	113	**0.000**
	5	-1.0364	0.28127	-3.685	113	**0.000**
	6	0.0383	0.28127	0.136	113	0.892
Hyphae	1	16.15	7.415	2.178	114	**0.031**
	2	8.475	7.415	1.143	114	0.255
	3	17.125	7.415	2.309	114	**0.023**
	4	-26.075	10.487	-2.486	114	**0.014**
	5	-2.475	10.487	-0.236	114	0.814
	6	23.6	10.487	2.25	114	**0.026**
Vesicles	1	4.9	2.656	1.845	114	0.068
	2	1.65	2.656	0.621	114	0.536
	3	3.5	2.656	1.318	114	0.190
	4	-6.45	3.755	-1.717	114	0.089
	5	-0.4	3.755	-0.107	114	0.915
	6	6.05	3.755	1.611	114	0.110
Arbuscule	1	11.725	5.292	2.216	114	**0.029**
	2	7.1	5.292	1.342	114	0.182
	3	4.85	5.292	0.917	114	0.361
	4	-9.975	7.484	-1.333	114	0.185
	5	-0.275	7.484	-0.037	114	0.971
	6	9.7	7.484	1.296	114	0.198

Table 3.5: KMO and Bartlett's Test for principal components analysis of water quality parameters. Six sites were assessed for 11 water quality parameters along a water quality gradient as shown in Table 3.3 five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters were measured in pore water in each quadrat as well as in the river water.

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.527
Bartlett's Test of Sphericity		
	Approx. Chi-Square	749.177
	df	55
	P - value	.000

Table 3.6: Raw data eigenvalues, mean & percentile random data eigenvalues for Monte Carlo simulation. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters were measured in pore water in each quadrat as well as in the river water. A principal components analysis was used to determine prevailing trends in water quality, and logistic regression was used on the extracted component loadings to determine if water quality influenced mycorrhizal structures.

Root	Raw Data	Means	Prcntyle
1.000000	**2.658725**	**1.531139**	**1.678636**
2.000000	**2.321567**	**1.370353**	**1.475881**
3.000000	**1.554374**	**1.250371**	**1.336809**
4.000000	**1.268750**	**1.150083**	**1.220127**
5.000000	0.873251	1.059413	1.122733
6.000000	0.791226	0.973392	1.033002
7.000000	0.614572	0.895402	0.951476
8.000000	0.459579	0.817420	0.877810
9.000000	0.342019	0.736443	0.798755
10.000000	0.100395	0.655925	0.724071
11.000000	0.015541	0.560058	0.642606

Table 3.7: Total variance explained for principal components analysis of water quality parameters. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters were measured in pore water in each quadrat as well as in the river water. A principal components analysis was used to determine prevailing trends in water quality, and logistic regression was used on the extracted component loadings to determine if water quality influenced mycorrhizal structures.

	Initial Eigenvalues			Extraction Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.654	24.127	24.127	2.654	24.127	24.127
2	2.321	21.103	45.230	2.321	21.103	45.230
3	1.553	14.123	59.352	1.553	14.123	59.352
4	1.266	11.513	70.866	1.266	11.513	70.944
5	.877	7.976	78.842			
6	.790	7.186	86.027			
7	.613	5.573	91.601			
8	.466	4.237	95.838			
9	.342	3.108	98.946			
10	.100	.913	99.859			
11	.016	.141	100.000			

Table 3.8: Component Matrix[a] for principal components analysis of water quality parameters. Four components were extracted. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters were measured in pore water in each quadrat as well as in the river water. A principal components analysis was used to determine prevailing trends in water quality, and logistic regression was used on the extracted component loadings to determine if water quality influenced mycorrhizal structures.

	Component			
	1	2	3	4
Dissolved oxygen	0.700			-0.254
Water depth	-0.527	0.751		0.219
Water temperature	-0.481	0.780		0.252
PO_4-P		0.389	0.444	-0.279
NO_2-N		0.288	0.514	-0.546
NO_3-N	0.573		0.387	0.202
NH_3-N	0.514			0.452
Total dissolved solids	-0.338	-0.676	0.583	
Conductivity	-0.417	-0.516	0.647	0.288
pH	0.742	0.318	0.247	
Oxidation-reduction potential (ORP)	0.438	0.247		0.613

Table 3.9: ANOVA obtained from principal component analysis results examining interactions between site and month on PC scores. Four PC scores were assessed. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters were measured in pore water in each quadrat as well as in the river water. A principal components analysis was used to determine prevailing trends in water quality, and logistic regression was used on the extracted component loadings to determine if water quality influenced mycorrhizal structures.

Effect	Test	Value	F	Hypothesis df	Error df	P - value
Intercept	Wilks' Lambda	1	.000[b]	4	87	1
Site	Wilks' Lambda	0.144	11.516	20	289.496	0
Month	Wilks' Lambda	0.04	31.286	16	266.427	0
Site * Month	Wilks' Lambda	0.185	2.303	80	345.625	0

Table 3.10: Results of logistic regression on hyphae, vesicles, and arbuscules. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters were measured in pore water in each quadrat as well as in the river water. A principal components analysis was used to determine prevailing trends in water quality, and logistic regression was used on the extracted component loadings to determine if water quality influenced mycorrhizal structures.

Structure	Source	Wald Chi- Square	Df	P - value
Hyphae	Site	417.792	5	0.000
	Month	92.35	4	0.000
	Site(Month)	790.469	20	0.000
Vesicles	Site	54.134	5	0.000
	Month	62.019	4	0.000
	Site(Month)	243.413	20	0.000
Arbuscules	Site	123.364	5	0.000
	Month	30.19	4	0.000
	Site(Month)	540.395	20	0.000

Table 3.11: Repeated measures analysis of variance (ANOVA) between lagged and non-lagged PC Scores and hyphal colonization. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters were measured in pore water in each quadrat as well as in the river water. A principal components analysis was used to determine prevailing trends in water quality, and logistic regression was used on the extracted component loadings to determine if water quality influenced mycorrhizal structures.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
PC Score 1 Lagged	1	1126	1126	2.130	0.14820
PC Score 2 Lagged	1	2485	2485	4.701	**0.03301** *
PC Score 3 Lagged	1	2199	2199	4.161	**0.04455** *
PC Score 4 Lagged	1	33	33	0.062	0.80401
PC Score 1	1	2342	2342	4.431	**0.03831** *
PC Score 2	1	380	380	0.718	0.39908
PC Score 3	1	411	411	0.777	0.38046
PC Score 4	1	305	305	0.577	0.44952
site	1	5247	5247	9.927	**0.00227** **
Residuals	83	43870	529		
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

Table 3.12: Repeated measures analysis of variance (ANOVA) between lagged and non-lagged PC Scores and vesicular colonization. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters were measured in pore water in each quadrat as well as in the river water. A principal components analysis was used to determine prevailing trends in water quality, and logistic regression was used on the extracted component loadings to determine if water quality influenced mycorrhizal structures.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
PC Score 1 Lagged	1	1.24	1.236	0.761	0.386
PC Score 2 Lagged	1	3.13	3.131	1.928	0.169
PC Score 3 Lagged	1	0.15	0.145	0.089	0.766
PC Score 4 Lagged	1	0.09	0.091	0.056	0.814
PC Score 1	1	6.31	6.311	3.886	0.052 .
PC Score 2	1	2.32	2.321	1.429	0.235
PC Score 3	1	1.84	1.844	1.135	0.290
PC Score 4	1	1.31	1.307	0.805	0.372
site	1	3.72	3.716	2.288	0.134
Residuals	83	134.8	1.624		
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					

Table 3.13: Repeated measures analysis of variance (ANOVA) between lagged and non-lagged PC Scores and arbuscular colonization. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. Water quality parameters were measured in pore water in each quadrat as well as in the river water. A principal components analysis was used to determine prevailing trends in water quality, and logistic regression was used on the extracted component loadings to determine if water quality influenced mycorrhizal structures.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
PC Score 1 Lagged	1	0.89	0.888	0.282	0.5969	
PC Score 2 Lagged	1	16.13	16.131	5.121	0.0262	*
PC Score 3 Lagged	1	2.62	2.617	0.831	0.3646	
PC Score 4 Lagged	1	0.00	0.001	0.000	0.9895	
PC Score 1	1	20.43	20.427	6.485	0.0127	*
PC Score 2	1	0.92	0.922	0.293	0.5900	
PC Score 3	1	0.51	0.509	0.162	0.6887	
PC Score 4	1	0.01	0.013	0.004	0.9482	
site	1	13.00	12.997	4.126	0.0454	*
Residuals	83	261.43	3.150			
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

Table 3.14: Logistic regression showing effects of site on hyphal colonization in three plant species (*Iris* sp., *Veronica* sp., and *Potamogeton* sp.). Three different wetland plant species (*Iris* sp., *Veronica* sp., and *Potamogeton* sp.) were assessed at two field sites for presence of mycorrhizal structures (hyphae, vesicles, and arbuscules). One site was a good water quality site (West Montrose) and the other was a poor water quality site (Rare Charitable Research Reserve). This study was performed on plants in the natural environment.

	Type III		
Source	Wald Chi-Square	df	P - value
(Intercept)	126.676	1	0.000
Site * Species	30.747	2	0.000
Site	0.043	1	0.836
Species	260.184	2	0.000

Table 3.15: Estimated marginal means of hyphal colonization showing interactions between plant species and site. Effects of site on hyphal colonization across all three plant species determined by a logistic regression. This study was performed to indicate the effects of site on mycorrhizal colonization in a wetland system. Three different plants were harvested and assessed for mycorrhizal colonization at two different field sites, one with good water quality (West Montrose) and a site with poor water quality (Rare Charitable Research Reserve). This study was performed on plants in the natural environment.

				95% Wald Confidence Interval	
Site	Species	Mean	Std. Error	Lower	Upper
Rare	Iris	.45	.029	.39	.51
	Potamogeton	.07	.015	.05	.10
	Veronica	.59	.028	.54	.65
West Montrose	Iris	.66	.027	.60	.71
	Potamogeton	.05	.013	.03	.08
	Veronica	.49	.029	.44	.55

Table 3.16: Logistic regression showing effects of site on vesicular colonization in three plant species (*Iris* sp., *Veronica* sp., and *Potamogeton* sp.). Three different wetland plant species (*Iris* sp., *Veronica* sp., and *Potamogeton* sp.) were assessed at two field sites for presence of mycorrhizal structures (hyphae, vesicles, and arbuscules). One site was a good water quality site (West Montrose) and the other was a poor water quality site (Rare Charitable Research Reserve). This study was performed on plants in the natural environment.

	Wald Chi-Square	df	P - value
(Intercept)	3591.909	1	.000
Site	30.582	1	.000
Species	44623.784	2	.000
Site * Species	39.772	2	.000

Table 3.17: Estimated marginal means of vesicular colonization showing interactions between plant species and site. Effects of site on vesicular colonization across all three plant species determined by a logistic regression. This study was performed to indicate the effects of site on mycorrhizal colonization in a wetland system. Three different plants were harvested and assessed for mycorrhizal colonization at two different field sites, one with good water quality (West Montrose) and a site with poor water quality (Rare Charitable Research Reserve). This study was performed on plants in the natural environment.

			95% Wald Confidence Interval	
Species	Mean	Std. Error	Lower	Upper
Iris	.17	.015	.14	.20
Potamogeton	.03	.007	.02	.05
Veronica	.14	.014	.11	.17

Table 3.18: Logistic regression showing effects of site on arbuscular colonization in three plant species (*Iris* sp., *Veronica* sp., and *Potamogeton* sp.). Three different wetland plant species (*Iris* sp., *Veronica* sp., and *Potamogeton* sp.) were assessed at two field sites for presence of mycorrhizal structures (hyphae, vesicles, and arbuscules). One site was a good water quality site (West Montrose) and the other was a poor water quality site (Rare Charitable Research Reserve). This study was performed on plants in the natural environment.

	Wald Chi-Square	df	P - value
(Intercept)	3591.909	1	.000
Site	30.582	1	.000
Species	44623.784	2	.000
Site * Species	39.772	2	.000

Table 3.19: Estimated marginal means of arbuscular colonization showing interactions between plant species and site. Effects of site on arbuscular colonization across all three plant species determined by a logistic regression. This study was performed to indicate the effects of site on mycorrhizal colonization in a wetland system. Three different plants were harvested and assessed for mycorrhizal colonization at two different field sites, one with good water quality (West Montrose) and a site with poor water quality (Rare Charitable Research Reserve). This study was performed on plants in the natural environment.

				95% Wald Confidence Interval	
Site	Species	Mean	Std. Error	Lower	Upper
Rare	Iris	.29	.026	.24	.34
	Potamogeton	.00	.000	.00	.00
	Veronica	.24	.025	.19	.29
West Montrose	Iris	.32	.027	.27	.37
	Potamogeton	.00	.000	.00	.00
	Veronica	.02	.008	.01	.04

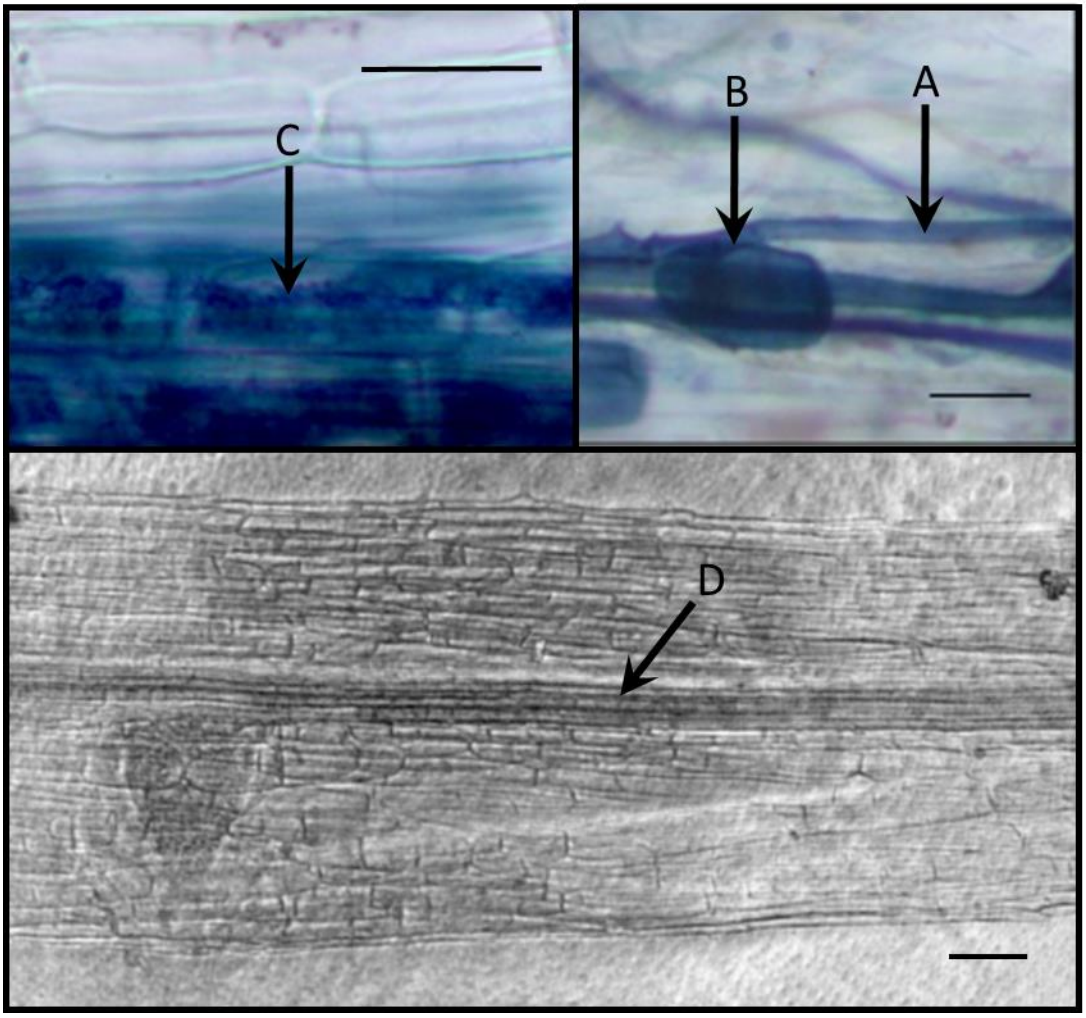

Figure 3.1: Cleared and stained roots of three different plant roots from the mesocosm experiment. Roots were cleared in a 10% KOH and stained with 5% ink-vinegar solution with household vinegar (5% acetic acid). A - C are structures of arbuscular mycorrhizal fungi (AMF); D is a non-colonized root section.
A: Hyphae in a *Phalaris arundinacea* root. Scale bar = 25 µm.
B: Vesicle in an *Phalaris arundinacea* root. Scale bar = 25 µm.
C: Arbuscule in a *Echinochloa crus-galli* root. Scale bar = 50 µm.
D: Non-colonized area of a root from *Verbena hastata*. Non-colonized cells appear translucent. The vascular cylinder (arrow) is visible as a dark central structure. Scale bar = 50 µm.

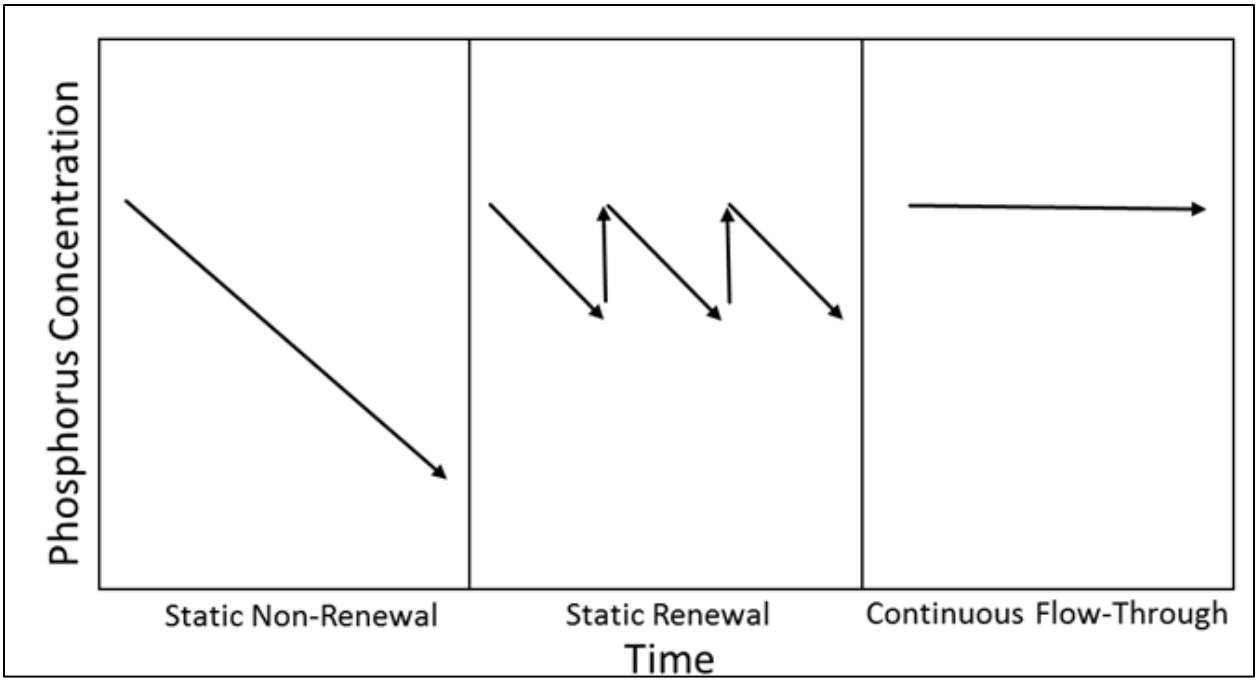

Figure 3.2: This figure displays the different phosphorus exposure methods used in various greenhouse experiments. The static non-renewal approach is a single injection of phosphorus into the exposure solution, and the concentration decreases as the plant uptakes the phosphorus. The static renewal approach is an injection that is assessed periodically and topped up to maintain a desired concentration. The continuous flow-through system was used in my experiment, where a concentration is maintained throughout the experiment, which closely simulates natural conditions.

Figure 3.3: Field Study Sites located on the Grand River Watershed. **A** - Shand Dam, and **B** - West Montrose are considered "good" water quality sites, **C** – Conestogo, and **D** - Bridge & Lancaster are considered "intermediate" water quality sites, and **E** – Doon, and **F** - Glen Morris are considered "poor" water quality sites. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. A principal components analysis was used to determine prevailing trends in water quality, and logistic regression was used on the extracted component loadings to determine if water quality influenced mycorrhizal structures. Images were retrieved from https://www.google.ca/maps/

Figure 3.4: Photograph taken at the Doon field site on the Grand River. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. A principal components analysis was used to determine prevailing trends in water quality, and logistic regression was used on the extracted component loadings to determine if water quality influenced mycorrhizal structures.

Figure 3.5: *Veronica* sp. plants at the Rare Charitable Research Reserve field study site. Image captured by Daniel Marshall, 2016. Three different plants were harvested and assessed for mycorrhizal colonization at two different field sites, one with good water quality (West Montrose) and a site with poor water quality (Rare Charitable Research Reserve). This study was performed on plants in the natural environment.

Figure 3.6: *Iris* sp. plants at the Rare Charitable Research Reserve field study site. Image captured by Daniel Marshall, 2016. Three different plants were harvested and assessed for mycorrhizal colonization at two different field sites, one with good water quality (West Montrose) and a site with poor water quality (Rare Charitable Research Reserve). This study was performed on plants in the natural environment.

Figure 3.7: *Potamogeton* sp. plants at the Rare Charitable Research Reserve field study site. Image captured by Daniel Marshall, 2016. Three different plants were harvested and assessed for mycorrhizal colonization at two different field sites, one with good water quality (West Montrose) and a site with poor water quality (Rare Charitable Research Reserve). This study was performed on plants in the natural environment.

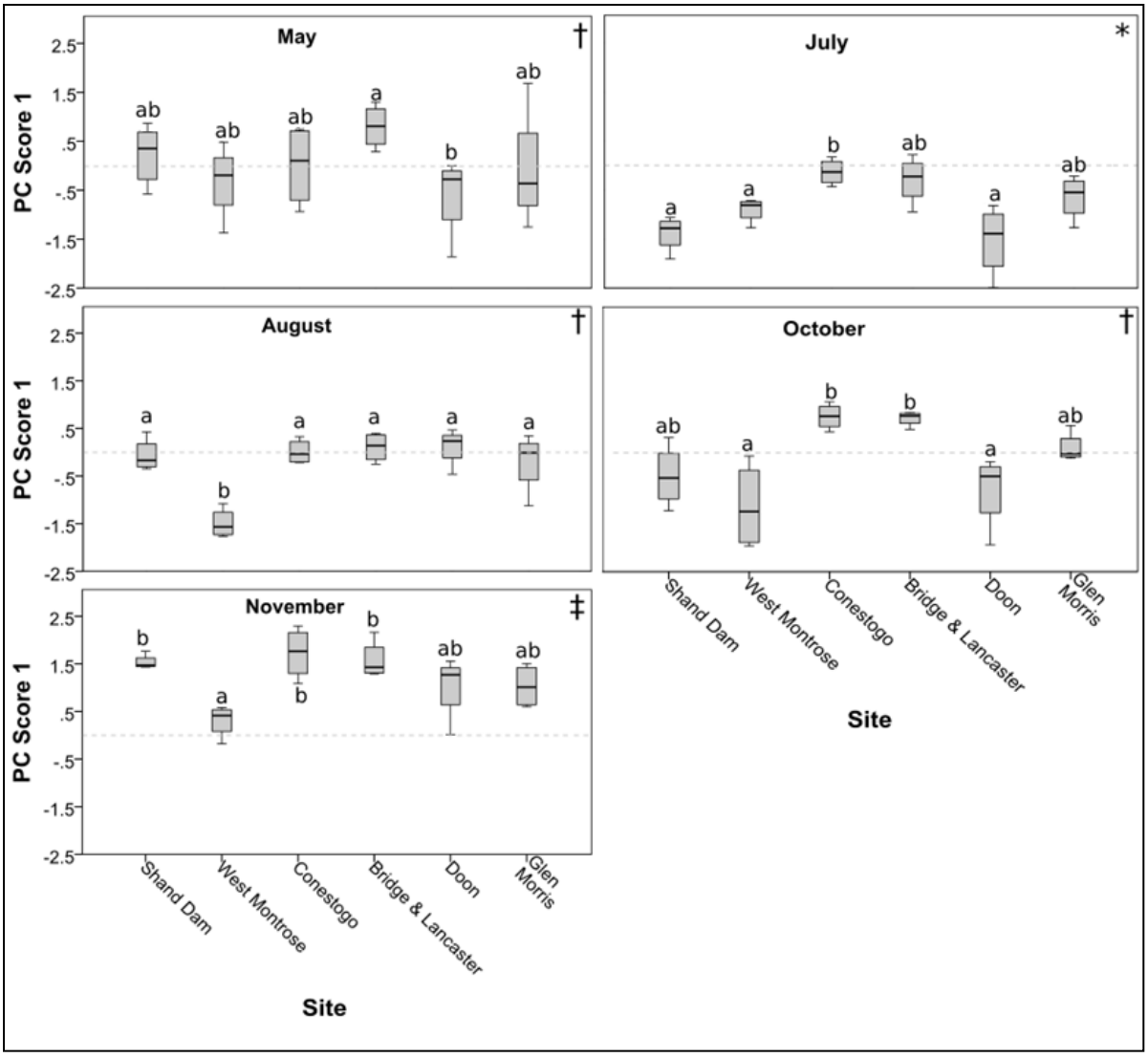

Figure 3.8: PC Score 1 extracted from a principal component analysis displayed as a function of site and time in a multivariate analysis of variance (MANOVA). Sampling took place between May and November to capture seasonal variation. There was a significant interaction between site and time, and differences between sites are shown with different letters indicating statistically different means. Symbols in the top right of the plots show differences between months.

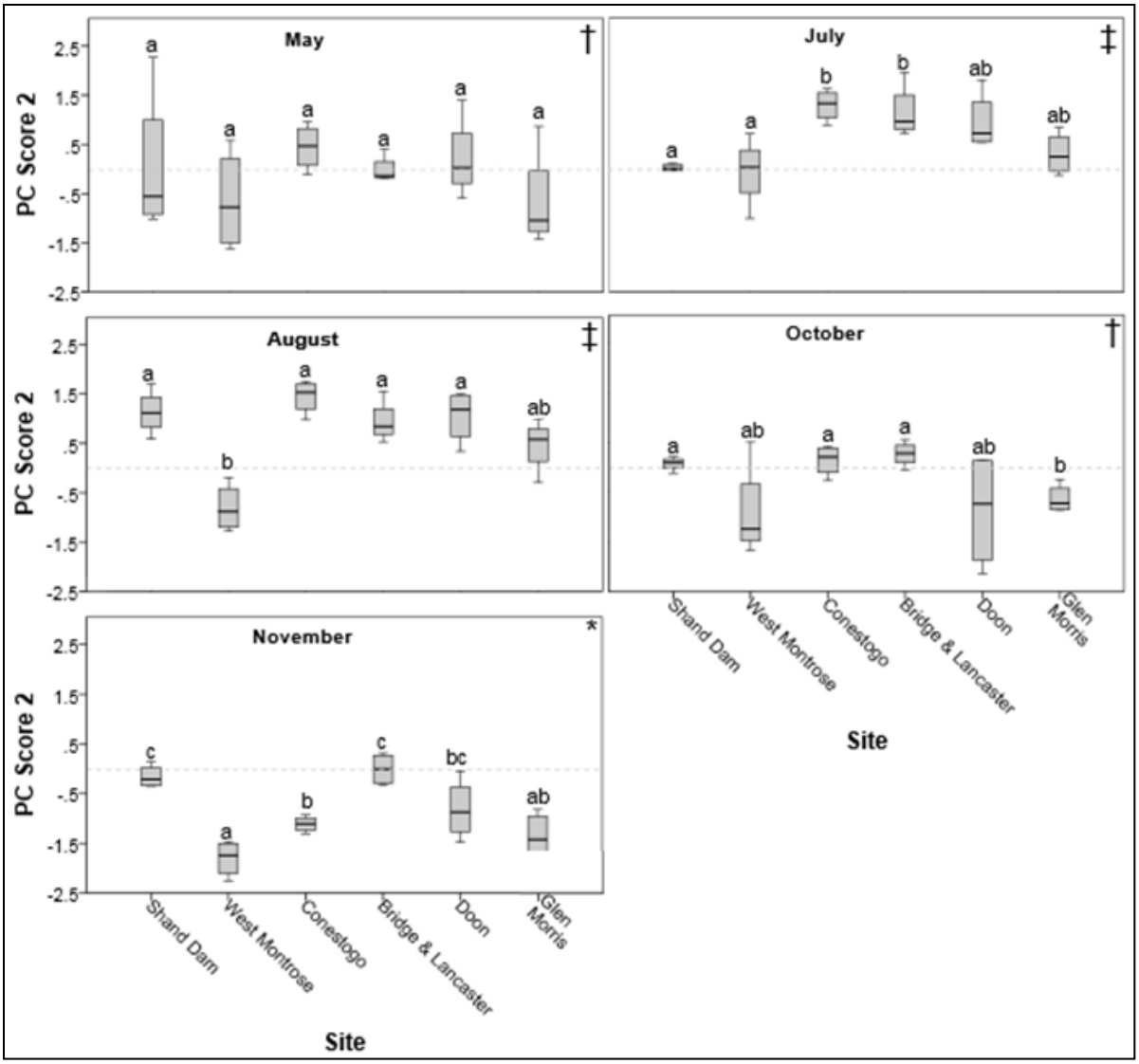

Figure 3.9: PC Score 2 extracted from a principal component analysis displayed as a function of site and time in a multivariate analysis of variance (MANOVA). Sampling took place between May and November to capture seasonal variation. There was a significant interaction between site and time, and differences between sites are shown with different letters indicating statistically different means. Symbols in the top right of the plots show differences between months.

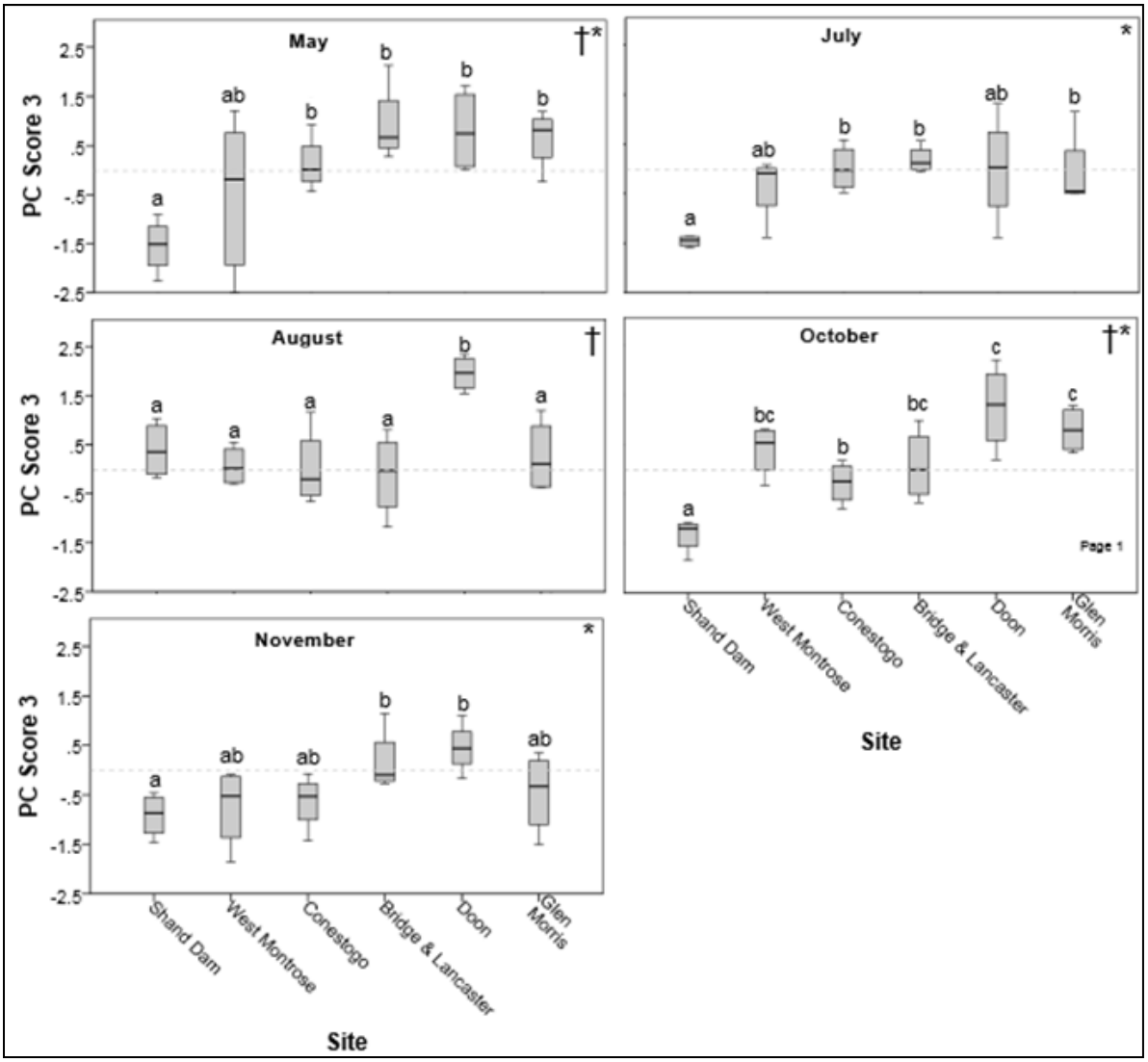

Figure 3.10: PC Score 3 extracted from a principal component analysis displayed as a function of site and time in a multivariate analysis of variance (MANOVA). Sampling took place between May and November to capture seasonal variation. There was a significant interaction between site and time, and differences between sites are shown with different letters indicating statistically different means. Symbols in the top right of the plots show differences between months.

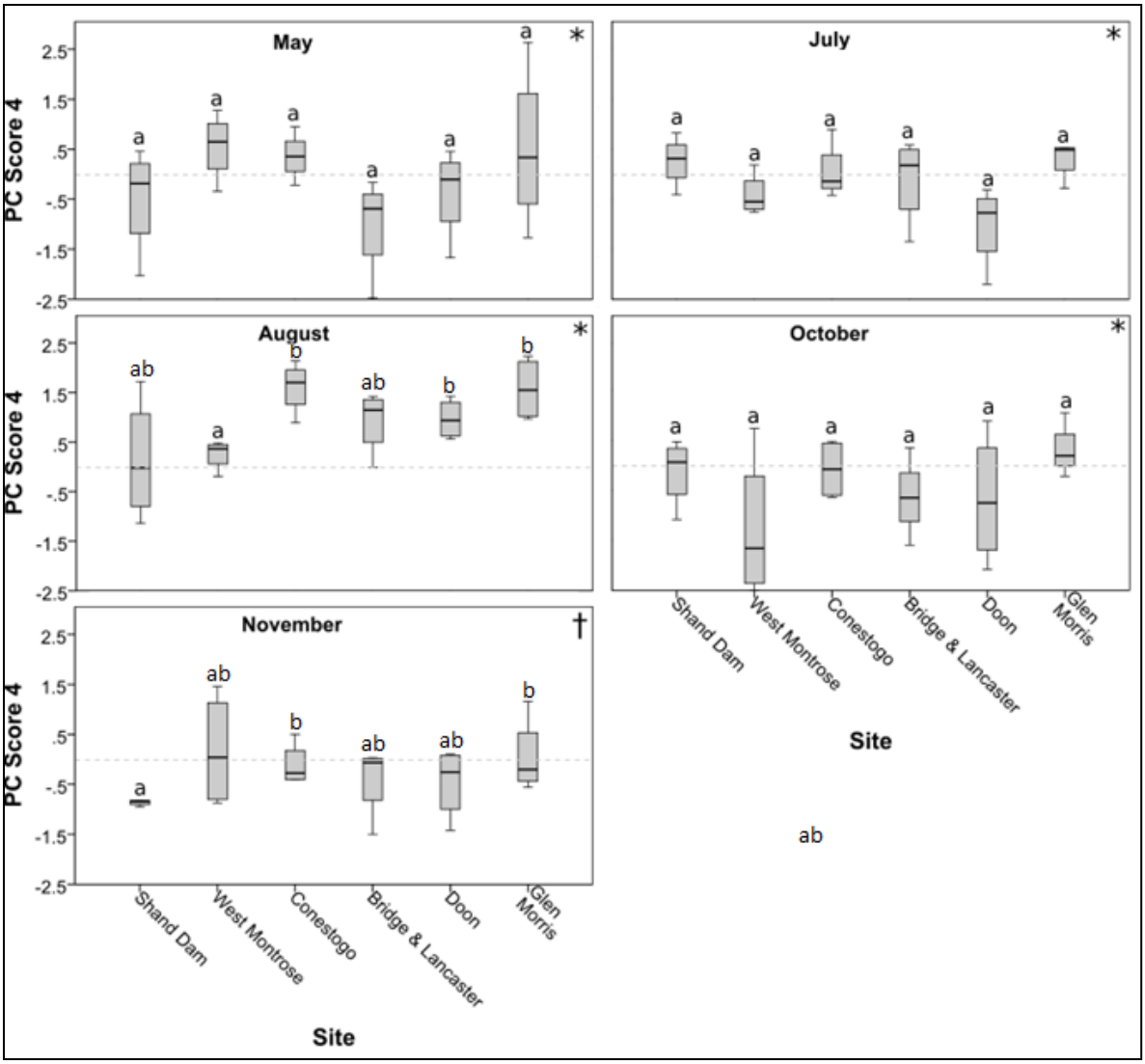

Figure 3.11: PC Score 4 extracted from a principal component analysis displayed as a function of site and time in a multivariate analysis of variance (MANOVA). Sampling took place between May and November to capture seasonal variation. There was a significant interaction between site and time, and differences between sites are shown with different letters indicating statistically different means. Symbols in the top right of the plots show differences between months.

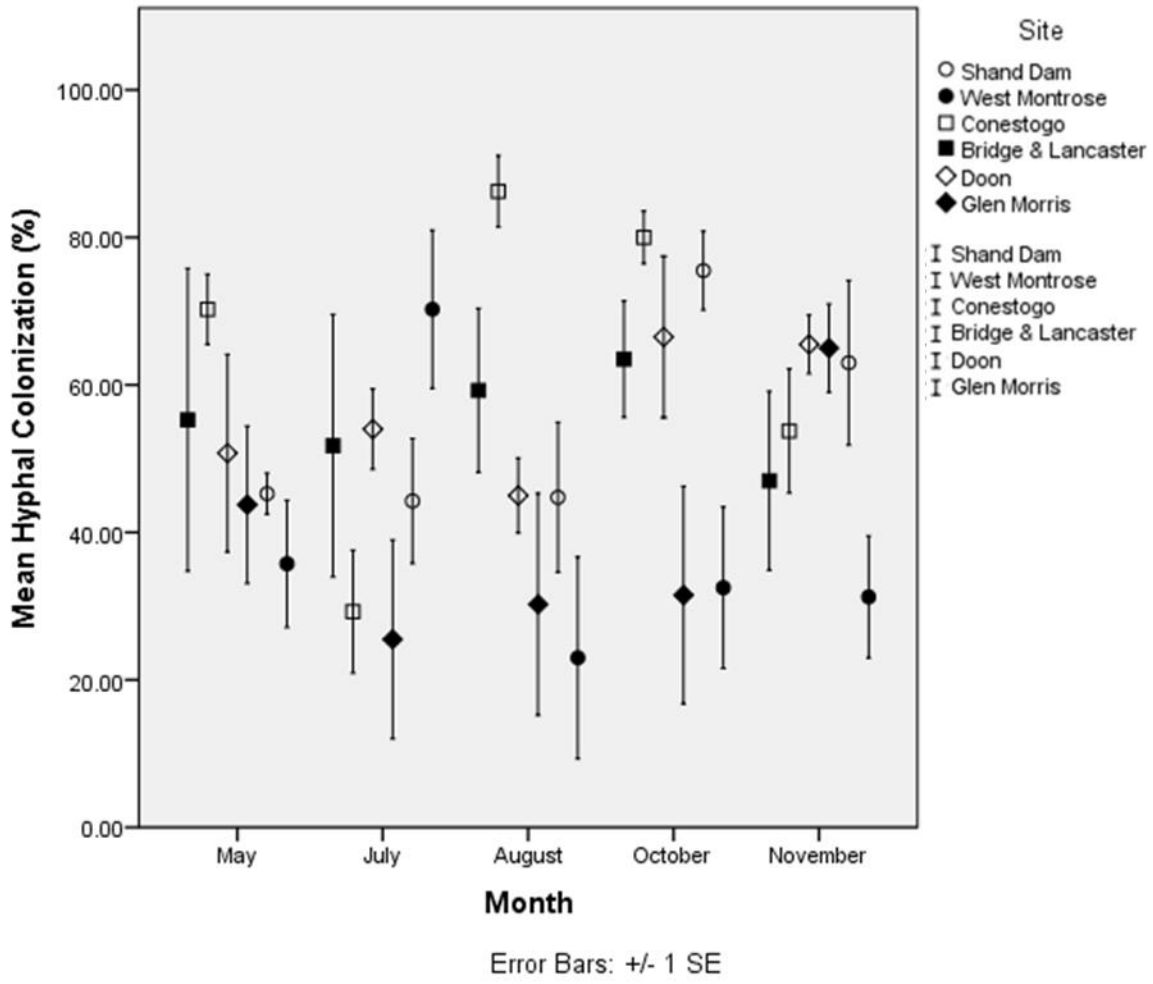

Figure 3.12: Mean hyphal colonization of all sampled sites at each month. Different symbols denote different site groupings, while symbol filling denotes different sites within groupings. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. A regression analysis was used to determine if site or time varied with mycorrhizal colonization. Confidence intervals were calculated but not included to improve clarity. Error bars denote +/- 1 standard error.

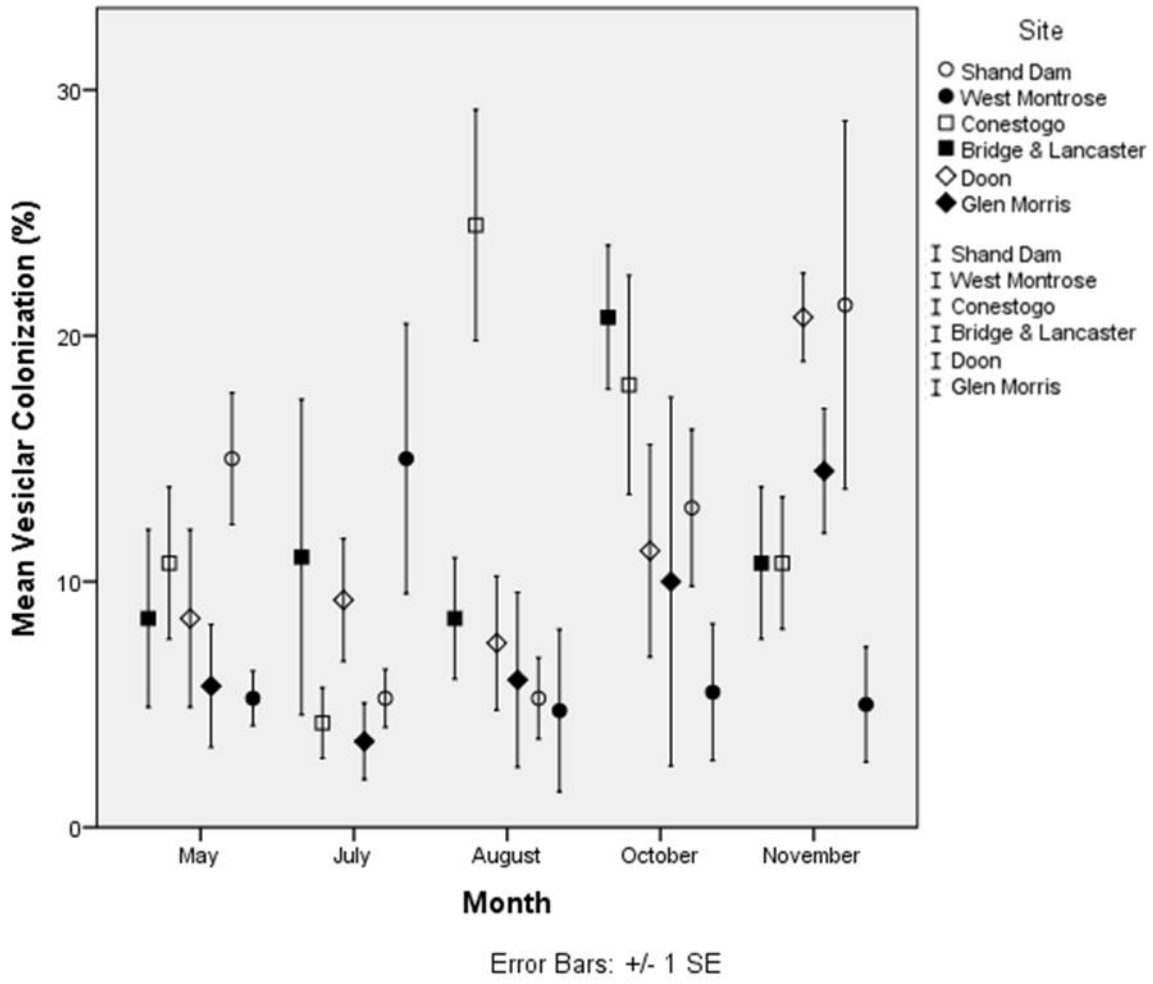

Figure 3.13: Mean vesicular colonization of all sampled sites at each month. Different symbols denote different site groupings, while symbol filling denotes different sites within groupings. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. A regression analysis was used to determine if site or time varied with mycorrhizal colonization. Confidence intervals were calculated but not included to improve clarity. Error bars denote +/- 1 standard error.

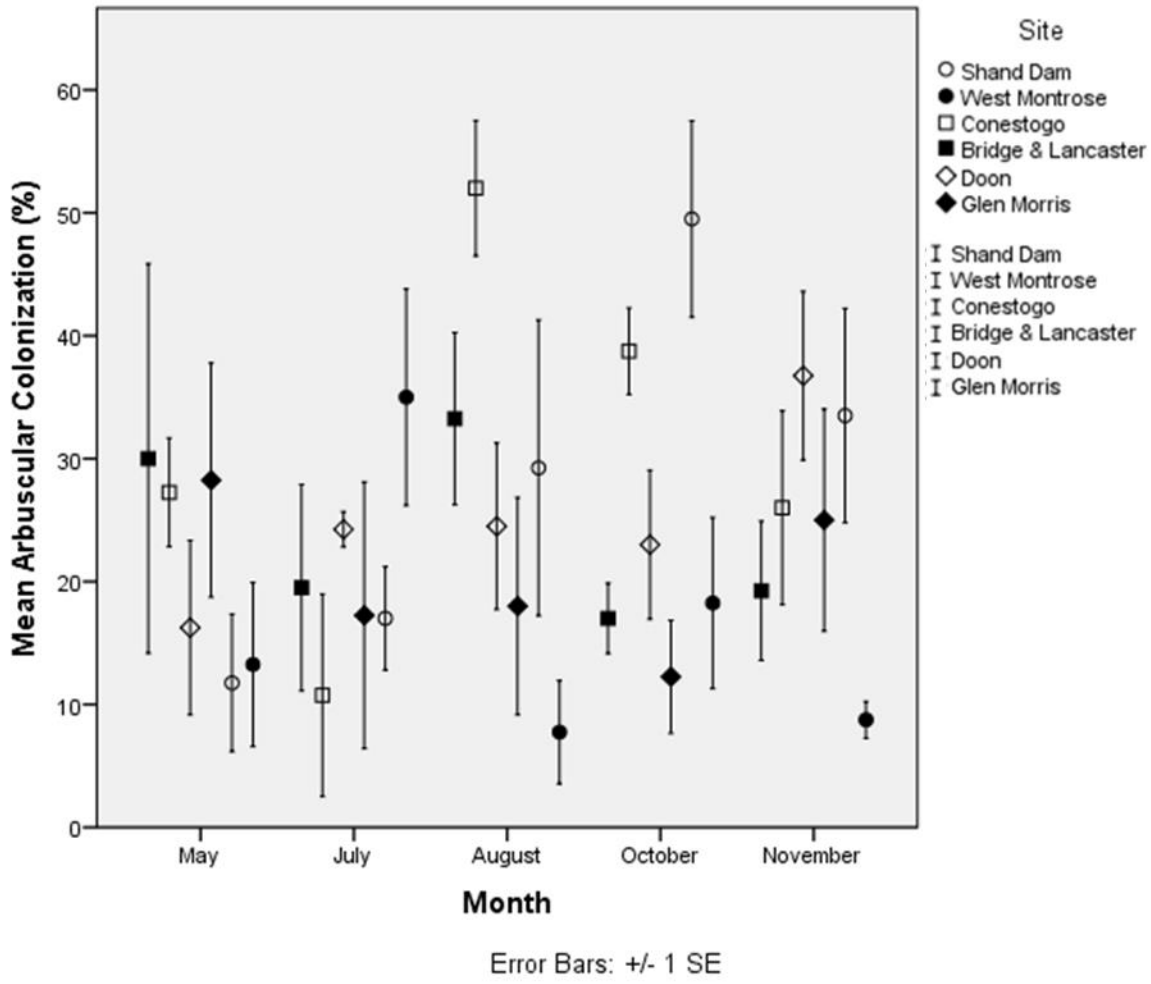

Figure 3.14: Mean arbuscular colonization of all sampled sites at each month. Different symbols denote different site groupings, while symbol filling denotes different sites within groupings. *Phalaris arundinacea* plants were harvested and assessed for colonization of mycorrhizal structures at each site five times from May 2015 – November 2015 to account for seasonal variation. A regression analysis was used to determine if site or time varied with mycorrhizal colonization. Confidence intervals were calculated but not included to improve clarity. Error bars denote +/- 1 standard error.

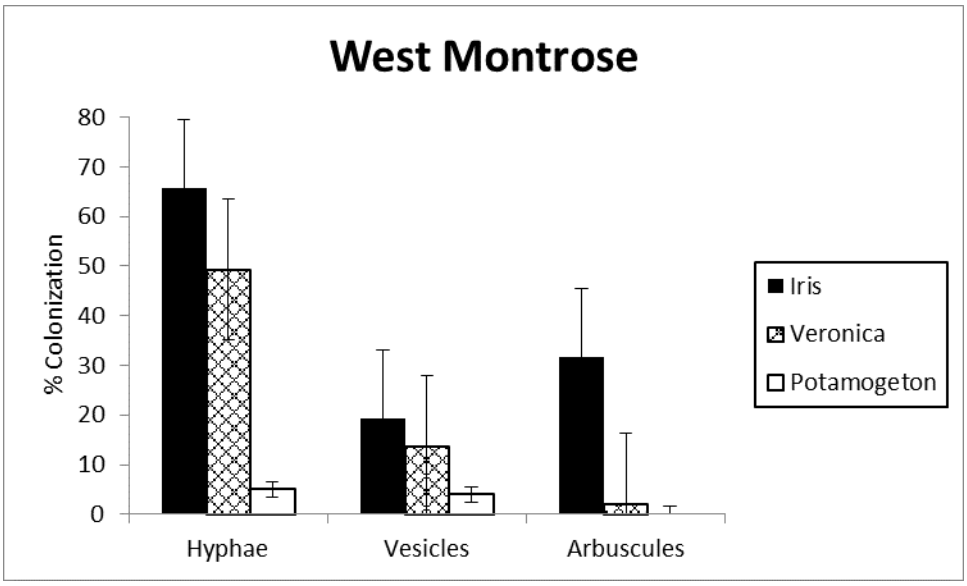

Figure 3.15: Mycorrhizal colonization at the West Montrose sampling site. Three different wetland plant species (*Iris* sp., *Veronica* sp., and *Potamogeton* sp.) were assessed at two field sites for presence of mycorrhizal structures (hyphae, vesicles, and arbuscules). One site was a good water quality site (West Montrose) and the other was a poor water quality site (Rare Charitable Research Reserve). Error bars denote +/- 1 standard error.

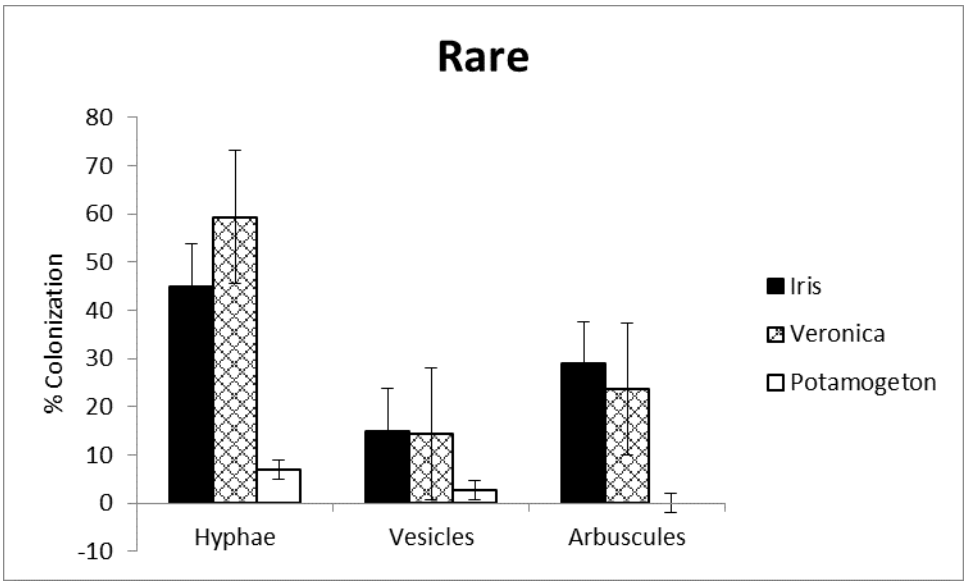

Figure 3.16: Mycorrhizal colonization at the Rare Charitable Research Reserve sampling site. Three different wetland plant species (*Iris* sp., *Veronica* sp., and *Potamogeton* sp.) were assessed at two field sites for presence of mycorrhizal structures (hyphae, vesicles, and arbuscules). One site was a good water quality site (West Montrose) and the other was a poor water quality site (Rare Charitable Research Reserve). Error bars denote +/- 1 standard error.

CHAPTER 4 – Summary Chapter

The impacts of varying phosphorus availability on the mycorrhizal colonization of wetland plants

The primary purpose of this thesis was to determine the impact of varying phosphorus availability on the mycorrhizal colonization of wetland plants. In the field studies, I investigated the effects of water quality on mycorrhizal colonization of multiple plant species in the natural environment. To do this, I performed two different field studies. The first consisted of six sampling sites along a water quality gradient, where I assessed the mycorrhizal colonization of *Phalaris arundinacea*, a wetland plant common in the Grand River watershed. The second study consisted of two sites where three wetland plant species were assessed for mycorrhizal colonization to investigate species-specific responses. The shortcoming of these two studies was that they were performed under natural conditions where phosphorus could not be isolated as the only variable, and many other environmental variables were not considered. The results from the field studies indicate that potential impacts of phosphorus on AMF were not conclusive, other than to point out the difficulties of determining correlations in ecological studies. To address this shortcoming, I performed the greenhouse study where I determined that phosphorus supply was impacting mycorrhizal colonization at levels between 10 and 30 µg/L PO_4-P. The results from the experimental chapter sheds light on the understudied relationships between mycorrhiza and phosphorus supply in wetland systems and how they differ from the same relationship in terrestrial systems. In this chapter, I discuss the implications of these results as well as conclude with a discussion of the integrative techniques utilized in my approach throughout my research.

Quantifying relationships between phosphorus availability and mycorrhizal associations in a natural wetland

Although it is well understood that phosphorus availability is negatively correlated with mycorrhizal colonization in terrestrial systems (Thomson et al., 1986), this relationship is poorly understood in wetland environments (White and Charvat, 1999). Of the studies which have examined the impacts of phosphorus availability on arbuscular mycorrhizal fungi (AMF) in wetland plants, many have indicated a need to assess plants in the natural environment (Tang et al., 2001).

In the first field study, there was a slight positive correlation between phosphorus availability and mycorrhizal colonization in *Phalaris arundinacea* plants. However, these interactions were different depending on site and seasonal variation. Previous field studies have also reported mixed results with respect to the relationship between phosphorus and mycorrhizal associations. These earlier studies have shown high levels of mycorrhizal colonization have corresponded with low phosphorus availability in wetlands (Turner et al., 2000), but other studies have shown no relationship between AM colonization and phosphorus availability in natural wetland environments (Miller and Sharitz, 2000; Wetzel and Valk, 1998).

Potential difficulties in quantifying the impacts of phosphorus on mycorrhizas in aquatic conditions could be due to the diverse forms in which phosphorus presents itself in the natural environment. It is introduced into aquatic environments though numerous chemical forms and fractions. In terrestrial systems, phosphorus is a large fraction of soil mass, but in aquatic systems it is much smaller. These fractions contain many components, which can change between dissolved or particulate states. Soluble

phosphorus is phosphorus which is smaller than 0.45 µm in diameter and is useable by plants and AMF. Particulate phosphorus fractions are those which are larger than 0.45 µm in diameter (Spivakov et al., 1999). Under pH conditions normally associated with lakes and rivers (pH 5-6), soluble phosphorus is usually found in the orthophosphate form ($H_2PO_4^-$ and HPO_4^{2-}) (Schachtman et al., 1998). Dissolved and particulate organic phosphorus fractions can undergo mineralization through bacterial decomposition which transfers the phosphorus into the soluble orthophosphate pool, which make the phosphate accessible to plants. Assessing plants in the natural environment is further complicated by various abiotic factors. Not all parameters can be assessed continuously, so there is always the possibility of the presence of confounding variables. These previous studies also only assessed a few wetland plants. Since the dependency on mycorrhizal partners by wetland plants is variable (Stevens et al., 2011), it is possible that these studies assessed plants which were not dependent on mycorrhizas. In addition, the greenhouse study concluded that phosphorus impacts mycorrhizal colonization in a species-specific manner in host plants. Mycorrhizal colonization of *Phalaris arundinacea,* the plant used in the field study, was shown not to follow the predicted pattern of an inverse relationship between mycorrhiza and phosphorus availability. Since *Phalaris* was the plant species which was used as the sample organism in the field study, this could further complicate the findings.

In the second field study, three wetland plants were assessed for mycorrhizal colonization. In each of these plant species, there were different mycorrhizal responses. In *Potamogeton* sp., there were no arbuscules found in plant roots despite the identification of root hyphae, indicating either a different type of mycorrhiza or a lack of

the functional mycorrhiza-plant relationship. *Potamogeton* sp. has been shown to have dark septate endophyte (DSE) symbionts (Kai and Zhiwei, 2006), so it is likely that the fungal hyphae found were DSE and not AMF. In *Iris* sp., hyphal colonization was lower at the low quality site, following the relationships found in terrestrial systems (Thomson et al., 1986). Interestingly, *Veronica* showed increased mycorrhizal colonization with increasing nutrient availability. Hyphal colonization in *Veronica* was higher at the low quality site, which is opposite of the relationship found in terrestrial systems. Since the role of AMF in terrestrial systems is to uptake nutrients and water, the lack of an inverse relationship with P in this study could indicate that this role is not maintained in wetland environments. At the high quality site, arbuscular colonization was significantly reduced when compared to the low quality site. This is odd as the high quality site has fewer nutrients available for uptake and arbuscules are theorized to be the site of nutrient exchange (Smith, 1990). This lack of arbuscules could indicate a breakdown of the functional relationship between mycorrhiza and plant in wetland systems.

Both field studies indicate that the relationship between phosphorus, and mycorrhizal colonization in wetland systems is complicated and requires further study. This is especially true given that there were species-specific responses to water quality and phosphorus supply. The context of these two studies must be considered. These were field based studies in the natural environment, meaning the isolation of single variables was impractical. Although other variables were quantified, the potential impacts from untested environmental variables outside the scope of this study is high. Available phosphorus has been reported as being positively correlated with soil moisture in natural wetlands (Wetzel and Valk, 1998). This indicates that it may be the high availability of

phosphorus under inundated conditions which discourage the colonization of aquatic species by mycorrhiza, rather than a lack of oxygen for the mycorrhiza (Stevens et al., 2002). Although mycorrhiza are considered to be effective for *in situ* bioremediation (Robertson et al., 2007), they may be sensitive to other contaminants and the reductions seen in this study may reflect that. Other man-made compounds, such as Triclosan (5-chloro-2-[2,4-dichlorophenoxy]phenol) are widespread contaminants present in local watersheds and are known to inhibit mycorrhiza in wetlands (Twanabasu et al., 2013). Temperature has been shown to have varying effects on spore germination, with some spores germinating only at 34°C and others germinating primarily at lower temperatures (Schenck et al., 1975). Any of these abiotic factors other than phosphorus may be the primary drivers of mycorrhizal communities in field conditions. In addition, the PCA in the first field study showed that P was only strongly correlated with two of the components, accounting for just a fraction of 35% of the variance. There are many other variables which were more strongly correlated with mycorrhizal colonization than phosphorus in the field study, so it is impossible to determine what most affects mycorrhizal colonization. Due to these complications and limitations of previous studies, the greenhouse study in chapter 2 was performed to isolate phosphorus impacts on mycorrhizal colonization.

Quantifying relationships between phosphorus availability and mycorrhizal associations in wetland plants in a controlled greenhouse-based study

There have been several previous greenhouse studies assessing mycorrhizal colonization in wetland plants in response to variation in phosphorus supply (Stevens et al., 2002; Tang et al., 2001; White and Charvat, 1999). These studies have shown a need

for a controlled greenhouse study closely mimicking natural conditions (Tang et al., 2001; White and Charvat, 1999). These studies were limited by their methodologies in two main ways. They had static renewal and static non-renewal phosphorus applications not representative of natural environments. Static renewal phosphorus application is when a single injection of phosphorus is used at the start of a study. Static non-renewal is the same, except the concentration of phosphorus is periodically quantified, then topped up as needed to maintain the desired concentration. My study was different because it had a continuous application of phosphorus in nutrient solution using a peristaltic pump. This allowed the phosphorus supply to be constant as it is in nature rather than pulsed or injected (Stevens et al., 2002; Tang et al., 2001; White and Charvat, 1999), as it may be the lack of availability rather than concentration of phosphorus which impacts colonization. The phosphorus levels were also at levels more ecologically relevant. The concentrations ranged from the benchmark for soluble reactive phosphorus (10 µg/L P) to the highest concentration found in Ontario (7000 µg/L P), as opposed to 47500 µg/L P utilized in previous studies. In my study, the substrate was soil taken from the field, allowing for more natural soil conditions and unsorted mycorrhizal inoculum. These studies also only investigated a single plant species. My study assessed multiple plant species, which was essential to determine species-specific effects.

The six plants assessed showed three different mycorrhizal responses, with three plants (*Solidago canadensis*, *Eupatorium perforliatum, Echinochloa crus-galli*) showing decreased mycorrhizal colonization with increases in phosphorus availability. Two plants showed a decrease in mycorrhizal colonization (*Phalaris arundinacea*, *Verbena hastata*) overall, but not linearly, and one plant (*Epilobium parviflorum*) showed no decrease in

mycorrhizal colonization. These varied responses indicate the potential for species-specific responses to phosphorus availability in wetland plants. This may have widespread ramifications, because previous studies have shown mycorrhizal colonization can impact plant performance in wetland systems (Stevens et al., 2002). The mechanisms behind this are unknown, although the mycorrhiza could, in this case, have draining effects on the host plants, taking carbon without providing nutrients and water in response. This does not necessarily mean that in natural conditions there is a negative effect of AMF colonization, but that the benefit could be related to factors not assessed in the study. In addition, there may be other potential parallels with terrestrial systems not yet examined.

In the greenhouse study, AMF were found at all phosphorus levels in every plant species, though overall, they decreased slightly with increasing P availability. This negative correlation is well known in terrestrial systems and may well be prevalent in aquatic systems. In terrestrial soils, nutrient and P availability are highly dependent on soil water content. The observed reductions in AM colonization in aquatic conditions may be more attributable to increased availability of P rather than direct result of oxygen deficiency or other matters related to increased water level. This is further backed up in my study because the plants maintained the AM relationship under inundated conditions regardless of soil water content. The functioning and implications of AMF in wetland habitats are still not fully understood. This is partly due to conflicting studies, resulting from differences in methodology and limitations. This study helps to show that different plant species respond uniquely to phosphorus in wetland conditions and make different demands on the AM association.

Future studies should look further into the range of phosphorus concentrations where the AMF symbiosis becomes uncoupled across multiple species. Past studies have indicated responses to phosphorus levels between 1250 and 5000 µg/L PO_4-P. This study has shown that responses in mycorrhizal colonization of some plant species can be at much lower P levels, some as low as 10 to 30 µg/L PO_4-P. It has also shown that responses in mycorrhizal colonization to phosphorus are dependent on the species of host plant. This species-specific response has the potential to impact plant morphology, and may alter plant community structure. Plants vary in their reliance on the AMF symbiosis, so conditions which lead to impacts in mycorrhizal colonization would disproportionally impact the highly dependent plants. This could lead to losses in biodiversity, and ultimately loss of ecosystem function (Figure 4.1). As the demand for preserving, re-establishing, and constructing wetland plant communities increases, it will become increasingly vital to identify and understand the role that AM fungi can play in these systems.

An integrative approach

Throughout my project, it became increasingly apparent that it was a project which included more than just the study of biology. It was important to understand the physiological responses of wetland plants and mycorrhizas, soil science, biogeochemistry, ecology, and water chemistry. Specifically, I needed to study the physiology of two different phyla and their symbiotic interactions. I also needed to understand aspects of biogeochemistry, soil science, and water chemistry to determine phosphorus loads, their origins, and pathways through a watershed, and how that impacted the two organisms I studied. The interactions between phosphorus, wetland

plants, and mycorrhizal fungi was at the core of my research and investigating these interactions required an integrated approach. Looking at a single response to a single environmental variable is important, but putting that in the context of the entire natural environment shows the importance of the research and how it could impact the world directly.

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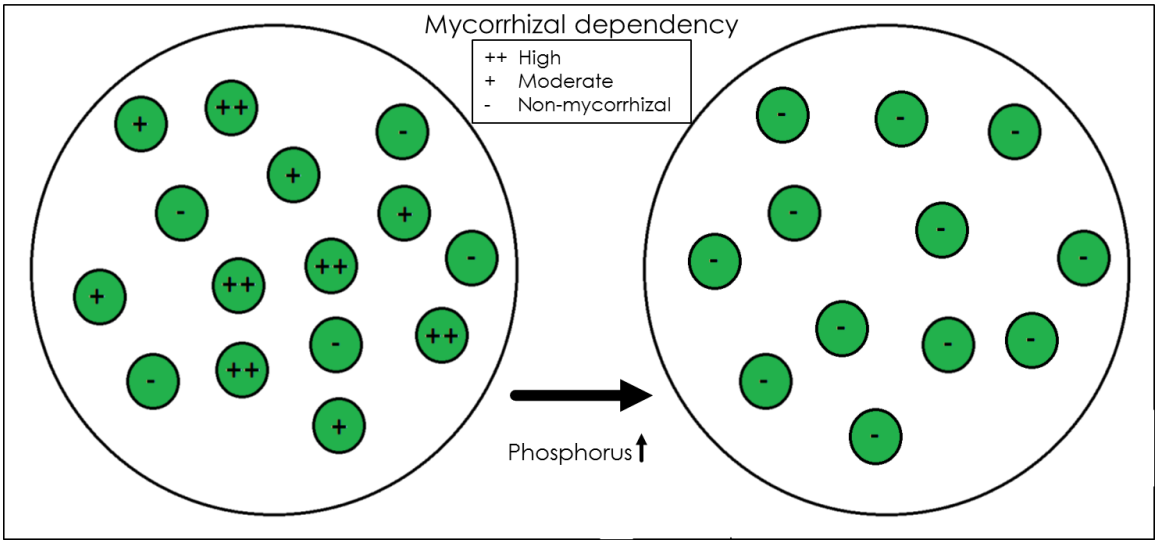

Figure 4.1: Two wetland plant communities showing shifts in plant species following phosphorus inputs. Green circles represent individual plants. With an increase in phosphorus, there could be species-specific impacts on AMF colonization in wetland plants. This could lead to decreases in biodiversity and loss of ecosystem function.