MYCORRHIZAL COLONIZATION AND OVERALL FUNGAL COMMUNITIES CHANGE IN RESPONSE TO UPLAND AND WETLAND SITE CONDITIONS IN *ACER* FORESTS OF THE VIRGINIA COASTAL PLAIN

by

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Dedication

This work is dedicated to my wife Rebecca and daughter Alexandra.

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Abstract

MYCORRHIZAL COLONIZATION AND OVERALL FUNGAL COMMUNITIES CHANGE IN RESPONSE TO UPLAND AND WETLAND SITE CONDITIONS IN ACER FORESTS OF THE VIRGINIA COASTAL PLAIN

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Mycorrhizal associations between plants and fungi involve the symbiotic transfer of soil nutrients from fungi to plant and the transfer of photosynthate from plant to fungi. Arbuscular mycorrhiza (AM) are widespread occurring in about 67% of all angiosperms and nearly all non-Pinaceae gymnosperms. One of the primary nutrients provided by AM fungi is phosphorus. Wetland soils are subjected to period of low oxygen. Two primary factors proposed in the literature as limiting the extent of mycorrhizal colonization of wetland plants are: 1) the low oxygen environment; and 2) the presence of adequate available phosphorus characteristic of many wetlands.

The present study evaluated AM colonization of *Acer* roots and the fungal community structure in paired upland/wetland locations in the Virginia Coastal Plain. The range of observed AM colonization, based on 60 samples collected on 11 dates covering 3 years,

ranged from 4% to 55%. The average colonization by site ranged from 15% to 31%. Of the two wetland-upland paired sites, the difference in percent colonization was significant at only one location. In that location, the wetland site had a higher percent colonization than the upland site. Further, the site with the greatest percent colonization in this study was a wetland site. Overall, soil redox potential and available phosphorus concentration were not significant in explaining the difference in AM colonization of *Acer* roots.

A comparison of fungal diversity between the pooled wetland site community and the pooled upland site community was conducted and the difference was significant ($p = 0.003$), but the upland sites exhibited the lower diversity. Communities from all sites were dissimilar. Proximity exerted a greater effect over community structure than did upland versus wetland status. That is, the paired upland/wetland sites at each location were more similar than any comparisons with other locations. This study concludes that forested wetland environments neither limit AM colonization nor reduce the overall fungal community.

1.0 Introduction

Arbuscular mycorrhiza (AM) occur in about 67% of all angiosperms and nearly all non-Pinaceae gymnosperms (Brundret 2002). Mycorrhizal hyphae arise from spores and other vegetative propagules which germinate in the rhizosphere. A thickening of hyphae in the epidermis, stimulated by plant exudates, facilitates the fungi-plant recognition process and hyphae are allowed to penetrate the plant root. The symbiotic transfer of nutrients between the plant and fungi occurs, primarily, at specialized fungal structures called arbuscules and potentially at intracellular hyphal coils (Smith and Smith, 1997).

The soil environment surrounding the roots, or rhizosphere, is penetrated by fine, highly branched fungal hyphae that become functional extensions of the host's root system. The fungal hyphae not only extend the area of soil from which the plant can mine nutrients - they are also more efficient than the roots at extracting nutrients from the soil solution (Bolan, 1991). The hyphae transport some of these nutrients to the root. In soils that are low in nutrients or moisture, fungal hyphae can enhance plant growth, reproduction, and survivorship (Smith and Read, 1997). Consequently, numerous studies have indicated that mycorrhizal plants are often more competitive and better able to tolerate environmental stresses than are non-mycorrhizal plants, particularly the stress of lower

nutrient availability (Mosse et. al, 1981; Harley and Smith, 1983; Faber et. al., 1991; Eckhard et. al., 1992; Smith and Read 1997; George, 2000).

Natural conditions in soils may affect the extent to which vesicular AM fungi colonize plant roots. Some of these conditions include soil composition, moisture, temperature, pH, cation exchange capacity, soil compaction, metals and pesticides (see reviews by Entry et. al., 2002, Muthukumar et. al., 2004). Soil moisture deficits were found to increase colonization percentages of roots (Simpson and Daft, 1990). In a study involving 19 wetland plant species situated on differing hydrologic gradients, Wetzel and van der Valk (1996) found that the only factors influencing mycorrhizal colonization were soil pH, host plant species, and soil phosphorus. Muthukumar et. al. (2004) found a strong positive correlation between soil pH, and soil N and colonization of sedges. The study found negative correlations between soil P and K and mycorrhizal colonization and a strong negative correlation between soil organic matter and mycorrhizal colonization.

An early study indicated that wetland plants, or plants adapted to saturated, low-oxygen soils, did not form mycorrhizal associations (Khan, 1974). However, many recent studies have indicated that many wetland plants are capable of forming mycorrhizal associations, but generally have less root infections as the substrate becomes more saturated or ponded (Liberta et al., 1983; Anderson et al., 1984; Lodge, 1989; van Duin et al., 1990; Rickerl et al., 1994; Wetzel and van der Valk, 1996). In contrast, Brown and Bledsoe (1996) found AM colonization often exceeded 30% at soil redox potentials of

less than 150 mV and demonstrated that the effect of soil redox on AM colonization was insignificant. Aziz et. al. (1995) found that wetland plants were colonized to the same extent as the non-wetland plant species when they were growing on comparable soils. Typical forested wetland species such as green ash (*Fraxinus pennsylvanica*), red maple (*Acer rubrum*), and sycamore (*Platanus occidentalis*) are capable of forming AM associations in greenhouse studies (Kormanik et al., 1982).

Two primary factors proposed in the literature as limiting the extent of mycorrhizal colonization of wetland plants are: 1) the low oxygen environment; and 2) the presence of adequate available phosphorus characteristic of many wetlands. Wetland environments are oxygen-limited for some period of time during the growing season (Mitsch and Gosselink, 2007). Because the mycorrhizal fungi are aerobic organisms, wetlands present a harsh environment. Miller and Bever (1999) suggest that the fungi may rely on the plant host to provide oxygen through its roots or that certain species of fungi may require less oxygen. In addition, wetland plants may develop adventitious roots that are located just below the soil surface where anoxic conditions do not exist or are less frequently encountered (Mitsch and Gosselink, 2007). Adventitious roots may present an environment suitable for mycorrhizal colonization.

Another potential limiting factor is the availability of soil phosphorus in wetlands. Wetland soils typically have ample nutrient reservoirs, though they are not always readily available for uptake by plants (Mitsch and Gosselink, 2007). Some studies have

suggested a relationship between high soil phosphorus (P) concentrations and the absence or scarcity of mycorrhizal colonization. This suggestion is based largely on studies of upland plants that have shown diminishing colonization with increasing P amendment (Mosse et al., 1981; Hayman, 1983; Bentivenga and Hetrick, 1992; Tresseder 2004; Covacevich 2006; and Nagy et al. 2009). Simply stated, if P were readily available to a plant, it would be less likely to invest fixed carbon in a fungal symbiont to mine additional P from the soil.

Evidence to support a relationship between available P and mycorrhizal colonization is inconclusive for wetland soils. In support of the relationship, Anderson et al. (1984) suggested that phosphorus availability played a role in decreasing AM colonization and functionality in emergent wetland plants. Søndergaard and Laegaard (1977), in a survey of aquatic macrophytes from oligotrophic lakes in Denmark, suggested that the high observed colonization by AM was at least partially due to the low phosphorus concentrations found in the lake water and sediments. Amijee et al. (1993) found that the odds of successful mycorrhizal colonization were reduced by 37% to 70% by additions of 450 mg/kg and 750 mg/kg of P. Other studies (Tanner and Clayton 1985; White and Charvat, 1999; Muthukumar et. al, 2004) also support the trend of reduced mycorrhizal colonization of wetland plants in soils with higher soil P concentrations.

In contrast, Brejda et al. (1998) found that AM colonization of the wetland grass *Panicum virgatum* was significantly correlated with the pH of the soil, but not with any other

property of the inoculant soil, including available phosphorus. Hoefnagels et al. (1993) in field studies of *Spartina patens* found no obvious relationship between percent root length colonized by AM and soil P, pH, Na, Ca, Mg, or soluble salts. Miller et al. (1999) did not find a correlation between soil P availability and AM fungal colonization of wetland plants from the genus *Carex*. Wetzel and van der Valk (1996) found no relationship between AM colonization and available phosphorus in North Dakota wetlands. Phosphorus availability was not a factor in determining AM colonization of herbs within freshwater wetlands in Indiana (Bauer et al, 2003).

Still, a review of the literature shows that the highest observed mycorrhizal colonization percentages generally occur under low soil P availability. However, the reported available P levels from these wetlands studies are relatively low compared to those investigated in upland and/or agricultural environments. Variability of the percent colonization levels at low soil P concentrations may be natural variability (i.e., dependent on the fungi and plant hosts). Data from natural environments are currently insufficient for establishing the available P threshold concentration that inhibits AM colonization.

Infrequently evaluated in past studies is the composition of the fungal community, including AM, and how it may vary with environmental conditions. Miller and Bever (1999) found a greater diversity of fungal species at the drier end of a moisture gradient and suggested that the presence of flooding may eliminate some species Mosse et al.

(1981) concluded that different fungal species and strains were adapted to particular levels of soil nutrients.

The literature contains numerous studies in which a single sampling date is used to assess the extent of mycorrhizal colonization. This "snapshot" assessment is likely not reflective of true conditions because the AM fungi colonize developing fine roots which turn over rapidly (Friese and Allen, 1991) and may be deciduous in the same fashion as leaves (Pregitzer et al., 2002). Intuitively, AM colonization would be expected to increase with additional demands for nutrients by the host plant. Studies both support (Bohrer et al., 2004; Likar et al., 2009) and refute (e.g., Miller and Sharitz, 2000) this assumption. Indeed, Lawrence et. al. (2003) found that AM colonization of sugar maple was relatively constant from April through October.

Even less frequently evaluated is the seasonal change in fungal community composition. Santos-Gonzalez et. al (2007) did not find a significant seasonal change in the patterns of the AM community composition as a whole. However, the presence of AM in *A. dioica* decreased dramatically in autumn, while an increased presence of *Ascomycetes*, presumed by the authors to be dark septate fungi, was detected.

Therefore, recent studies have challenged the assumptions about mycorrhizal associations in wetlands. The questions that remain are numerous. Specifically, does anoxia reduce mycorrhizal colonization in wetlands? Does soil phosphorus reduce mycorrhizal colonization in wetlands and if so, at what concentration does phosphorus become an

inhibitor? Does the community transition seasonally based on environmental conditions? Do different fungal communities occupy environmental niches based on site hydrology, as plants communities do?

The latter question presents problems in a field study because of the possibility of some host specificity for the fungal symbiont. There is no conclusive evidence of strict host-fungus specificity with arbuscular mycorrhizas (Smith and Read, 1997). However, Helgason et al. (2002) in trap studies found 2 fungal symbionts which colonized multiple hosts, but failed to colonize roots of *Acer pseudoplatanus* L. The study's results indicate that host specificity may occur and that it was demonstrated in the genus *Acer*.

In many wetlands the composition of the plant community transitions along the hydrologic gradient (Mitsch and Gosselink, 2007). Therefore, the present study investigated a plant host that occupies the entire gradient (i.e., one that tolerates a wide range of hydrologic conditions).

The present study targets the wetland/upland complexes containing red maple (*Acer rubrum*) and silver maple (*Acer saccharinum*) in the Virginia Coastal Plain. Virginia's Coastal Plain is a young landscape sculpted during the last few million years by the repeated rising and falling of sea level during several cycles of Pleistocene glaciation. The Coastal Plain is underlain by a wedge of sediments that increases in thickness from the Fall Line to the continental shelf. The broad flat topography of the Virginia Coastal

Plain offers subtle variations in the extent to which soils are saturated, flooded, or ponded within a relatively homogenous environmental setting.

Braun (1950) and others recognized the diversity of Virginia's plant community which includes both "northern" and "southern" species. Braun (1950) divided Virginia's Coastal Plane accordingly, with the James River (i.e., Richmond) serving as a rough boundary. The northern Coastal Plain generally contains upland and wetland vegetation with more northern affinities. That is, plant communities of the northern Virginia Coastal Plain are more closely aligned with those of the greater northeastern U.S. region.

Red maple is widely distributed in the eastern U.S. (Fowells 1965) and is found in a wide variety of settings, including both uplands and wetlands (Abrams, 1998). Based on 100,000 Forest Inventory and Analysis plots on both private and public lands in the eastern United States, monitored and administered by the US Forest Service, red maple occurs in more than 85 percent of the grid cells and in terms of importance, it is ranked first in the mesophytic forest region and in the top 10 of all regions except the oak–hickory forest (Dyer 2006).

Red maple is the dominant tree in the vast majority of broad-leaved deciduous wetland forests in the Northeast, but because of its wide range of adaptability it is classified as a facultative species, that is, one that is equally likely to occur in wetlands and uplands (Reed 1988). Red maple trees can develop numerous shallow lateral roots instead of a taproot to help avoid anaerobic stress in wetlands and create distinct red maple swamps

(Tiner 1991; Mitsch and Gosselink 1993). Will et. al. (1995) found that rhizosphere inundation of red maple seedlings from a Virginia Coastal Plain wetland induced lenticel hypertrophy (pores in the bark allowing for gas exchange with the atmosphere) and adventitious root formation. Golet (1993) estimated that more than 870,082 acres of broad-leaved deciduous wetlands occur in 6 Northeastern states, most of which are dominated by red maple. Given its significance, surprisingly few studies have evaluated the ecology, function, and associations of red maple.

Even fewer studies have evaluated silver maple. River floodplain and lake floodplain forests are locations where silver maple may be the dominant tree species (Golet 1993). Red and silver maples are closely associated and may hybridize naturally. For example, around Lake Champlain in Vermont, silver maple occupies the lakeward edge while red maple occupies the landward edge. In the middle, both species are present, and a hybrid maple, known as *Acer X freemanii*, has been identified that displays characteristics of both (Golet 1993). The so called Freeman maple has become a popular ornamental species.

Past studies have indicated that *Acer* does form mycorrhizal associations (Moizuk and Livingston, 1966; Antibus et. al., 1997; Cooke and Lefor, 1998; Lovelock and Miller, 2002; St Clair and Lynch, 2005; Wiseman, 2005) and is colonized by AM fungi (Yawney and Schultz, 1990). Further, the genus *Glomus* has been identified as the primary fungal

symbiont for *Acer* (Kormanik et. al., 1982; Yawney and Schultz, 1990; Helgason et. al., 2002).

1.1 Overview of Existing Studies

Phosphorous and Mycorrhizal Colonization of Roots

Many studies, largely based in a greenhouse or field studies with scaled P additions, have shown that AM colonization is reduced with increasing P. Phosphorus additions to the soil may reduce percent root colonization by increasing root growth (Smith and Walker, 1981), and also by reducing primary and secondary infection rates (Amijee et al., 1993; Bruce et al., 1994).

Amijee et al. (1989) found that when bicarbonate-soluble phosphorus exceeded 140 mg/kg the rate of mycorrhizal colonization decreased. Similarly, a soil P level of 133 mg/kg was found to inhibit mycorrhizae (Abbott and Robson, 1977 & 1978).

Tresseder (2004) reviewed 18 field studies involving P additions with a measured response in AM colonization. The review revealed colonization percentages between 52-90% of levels found in control plots. The mean reduction was about 32%. Covacevich (2006) found that mycorrhizal colonization decreased with increasing available soil P in fertilized plots growing tall fescue and wheatgrass. Nagy et al. 2009 found that tomato

root colonization was highest at low soil P and was decreased by 72% in plants supplied with 60 mg/kg of P and, with increasing soil P, identified a shift in the relative contribution of P to the plant via the mycorrhizal pathway towards the plant root pathway.

The host plant is active in allowing the colonization by fungi. Conceptually, if P were readily available to a plant, it would be less likely to invest fixed carbon in a fungal symbiont to mine additional P from the soil.

As previously described, the wetland environment offers codependence of some variables. As such, a single relationship between soil properties and levels of mycorrhizal colonization of wetland species are rarely reported in ecological studies (Anderson et al. 1984, Rickerl et al. 1994). Limited field studies have examined mycorrhizal relationships in the wetland environment and even less in forested wetlands.

Some examples supporting the inverse relationship between soil P and mycorrhizal colonization include Stevens et al. 2002, who found that levels of AM colonization generally decreased with increasing P supply. Wetzel and van der Valk (1995) found higher colonization rates of wetland herbs in prairie potholes with low P concentrations than in sites with higher P availability. Other studies (Tanner and Clayton 1985, White and Charvat 1999) also support the trend in mycorrhizal colonization of wetland plants. Indeed, a Spearman's rank correlation of 66 data entries from existing literature indicates

that colonization levels in sedges (wetland herbs) are negatively correlated to soil P (Muthukumar et. al, 2004).

Tang et al. (2001) grew *Typha angustifolia* in factorial combinations of four phosphorus concentrations (1, 10, 100, and 500 μM P). Hyphal root length did not differ statistically among the 1, 10, and 100 treatments but was zero at the 500 μM P treatment. The results argue for a possible threshold concentration of P that begins to inhibit mycorrhizal colonization.

Cornwell et al., 2001 found that the phosphorus additions to a wetland herb, *Solidago patula*, produced root colonization that was significantly less than the group of treatments without a P addition. However, the study also found habitats with low P that supported wetland herbs with little or no colonization, thus suggesting that soil P alone was not determining the extent of mycorrhizal colonization.

Other studies have shown no clear relationship between soil P and mycorrhizal colonization. Mosse et al. (1981) found a paradox in that many pot studies and field trials purport colonization to be sensitive to added nutrients, yet some field surveys suggest nutrient levels to be unimportant. They concluded that different fungal species and strains were adapted to particular levels of soil nutrients. Consequently, the ecological relevance of many nutrient experiments becomes questionable when the levels of added nutrient exceed the range naturally encountered by the fungal isolate.

Wetzel and van der Valk (1996) found no relationship between AM colonization and available phosphorus in North Dakota wetlands. Phosphorus availability was not a factor in determining mycorrhizal colonization of herbs within freshwater wetlands in Indiana (Bauer et al, 2003). The authors suggested that the levels of total phosphorus (ranging from 0.1 to 0.6%) were either not large enough to exclude mycorrhizal colonization completely, or mycorrhizae were transporting other limiting elements to the plants.

Hoefnagels et al. (1993) in field studies of *Spartina patens* found no obvious relationship between percent root length colonized and soil P, pH, Na, Ca, Mg, or soluble salts. Greenhouse studies using *S. patens* seedlings planted in 2 different soils revealed lower percent root length colonization in the soil with a greater P concentration. However, the P concentration in the soil was not believed to be high enough to suppress colonization.

Brejda et al. (1998) found that mycorrhizal colonization of *Panicum virgatum* was significantly correlated with the pH of the soil, but not with any other property of the inoculant soil, including available phosphorus. Ahn-Heum et al. (1999) in N-limited tallgrass prairies, found that nitrogen addition significantly increased percent mycorrhizal root colonization in N amended plots. Root colonization by AM fungi was not affected by P amendment. However, in P amended plots extraradical mycorrhizal hyphae growth was significantly decreased by phosphorus addition.

Miller (2000) found that AM colonization showed only a weak negative correlation with available soil P for *Leersia. hexandra* ($p = 0.048$), and no correlation for *Panicum hemitomon* in wetlands with a mean P concentration of 8.9 μg/g. For multiple *Carex* (sedge) species, Miller et al (1999) found that available phosphorus was not a significant predictor of the presence of colonization in wetlands with soil P concentrations between 7-22 μg/g.

Table 1 and Figure 1 summarize the data gathered from various studies involving wetland plants, mycorrhizal colonization, and available soil phosphorus. All of the studies reported here involved herbaceous plant hosts, except for Keeley (1980) who evaluated *Nyssa sylvatica* (black gum) seedlings harvested from "a sandy loam soil of relatively low fertility" and did not report the available P concentration. Quantifying inhibitions caused by flooding versus those linked to high available P concentrations is realistically not possible given the codependence of the variables and the scope of the studies currently available. Because most of the studies were not designed to determine the effect of soil P, many studies that report mycorrhizal colonization do not report soil P or report results in ranges of observed colonization values over different sites. Still, the highest reported soil P concentrations generally correspond with mycorrhizal colonization percentages at the low end and the highest observed mycorrhizal colonization percentages generally occurred under low soil P availability.

From this limited set of data points, it is clear that a lot of variability in colonization levels has been reported for soil P levels below 25 µg/g. Because field studies that identified soil P levels above 25 µg/g and also evaluated mycorrhizal colonization are uncommon, it is not possible to conclude that the same level of variability does not occur in this soil P range.

However, it is certain that some of the variability at any soil P level is attributable to the fact that different host plants were evaluated. Wetzel and Van der Valk (1996) found that the host plant species influenced fungal colonization and other studies have reinforced the observation that some species are colonized at higher rates than others (Cooke and Lefor, 1998).

Therefore, mycorrhizal colonization compared across several studies that examined different host species is complicated. This study examined a common host species in paired sets located in close proximity to each other to minimize the effects of variables other than the availability of P and soil oxygen (redox potential).

Table 1. Literature Review of Mycorrhizal Colonization and Available Soil P in Wetlands

Table 1. Literature Review of Mycorrhizal Colonization and Available Soil P in Wetlands						
Available Soil P	**Host Species**	**Percent Colonization**	**Study Location**	**Type**	**Comments**	**Source**
[1]40 µg/g	Phalaris arundinacea	0	SD	AM	Prairie potholes	Rickerl et al., 1994
[1]40 µg/g	Scirpus fluviatilis	0	SD	AM	Prairie potholes	Rickerl et al., 1994
7-22 µg/g	Carex stricta	0	IL	AM	Emergent wetland	Miller et al, 1999
[1]40 µg/g	Typha X glauca	2	SD	AM	Prairie potholes	Rickerl et al., 1994
7-22 µg/g	Carex blanda	5	IL	AM	Emergent wetland	Miller et al, 1999
[1]29 µg/g	Typha X glauca	8	SD	AM	Prairie potholes	Rickerl et al., 1994
6 ± 1 mg/dm	Spartina patens	8	NC	AM	Coastal salt marsh	Hoefnagels et al., 1993
[1]29 µg/g	Scirpus fluviatilis	9	SD	AM	Prairie potholes	Rickerl et al., 1994
0.1-5.0 µg/g	Scirpus maritimus	9.6-15.2	ND	AM	Shallow emergent	Wetzel and van der Valk, 1996
6.0-6.8 µg/g	Various emergent	18.3 (mean)	IA	AM	Low prairie	Wetzel and van der Valk, 1996
7-22 µg/g	Carex gravida	20	IL	AM	Emergent wetland	Miller et al, 1999
1.2-1.7 µg/g	Hordeum jubatum	20.2-34.2	ND	AM	Wet meadow	Wetzel and van der Valk, 1996
[1]29 µg/g	Phalaris arundinacea	24	SD	AM	Prairie potholes	Rickerl et al., 1994
2 ± 1 mg/dm	Spartina patens	26	NC	AM	Coastal salt marsh	Hoefnagels et al., 1993
7-22 µg/g	Carex vulpinoidea	28	IL	AM	Emergent wetland	Miller et al, 1999
2.0-4.4 µg/g	Various emergent	28.1(mean)	ND	AM	Low prairie	Wetzel and van der Valk, 1996
1.2-1.7 µg/g	Carex atheroides	28-56.4	ND	AM	Wet meadow	Wetzel and van der Valk, 1996
[1]29 µg/g	Apocynum Cannabinum	29	SD	AM	Prairie potholes	Rickerl et al., 1994
7-22 µg/g	Carex stipata	32	IL	AM	Emergent wetland	Miller et al, 1999
"low"	Nyssa sylvatica	32-45	GA	AM	Greenhouse (flooded)	Keeley, 1980
23 ± 3.2 µg/g	Jaumea carnosa	33	CA	AM	Marine	Brown and Bledsoe, 1996
1.2-1.7 µg/g	Distichlis stricta	33.3-71.8	ND	AM	Wet meadow	Wetzel and van der Valk, 1996
106 ± 4 mg/dm	Spartina patens	34	NC	AM	Coastal salt marsh	Hoefnagels et al., 1993
8.3-36.4 µg/g	Spartina pectinata	39.5	IA	AM	Wet meadow	Wetzel and van der Valk, 1996
11 ± 2 mg/dm	Spartina patens	50	NC	AM	Coastal salt marsh	Hoefnagels et al., 1993
13 ± 0.9 µg/g	Jaumea carnosa	51	CA	AM	Tidal channel	Brown and Bledsoe, 1996
4.4 µg/g	Dasiphora floribunda	66.7	NY	AM	Calcareous fen	Van Hoewyk et al, 2001

Table 1. Literature Review of Mycorrhizal Colonization and Available Soil P in Wetlands

Available Soil P	Host Species	Percent Colonization	Study Location	Type	Comments	Source
1.2-1.7 µg/g	Spartina pectinata	68.6	ND	AM	Wet meadow	Wetzel and van der Valk, 1996
3.6 µg/g	Dasiphora floribunda	70.3	NY	AM	Calcareous fen	Van Hoewyk et al, 2001
7.8 µg/g	Dasiphora floribunda	74.1	NY	AM	Calcareous fen	Van Hoewyk et al, 2001
15.7 µg/g	Dasiphora floribunda	76.7	NY	AM	Calcareous fen	Van Hoewyk et al, 2001
3.1 µg/g	Dasiphora floribunda	91.7	NY	AM	Calcareous fen	Van Hoewyk et al, 2001

[1] Authors reported total P and organic P. Inorganic P was estimated by total P – organic P.

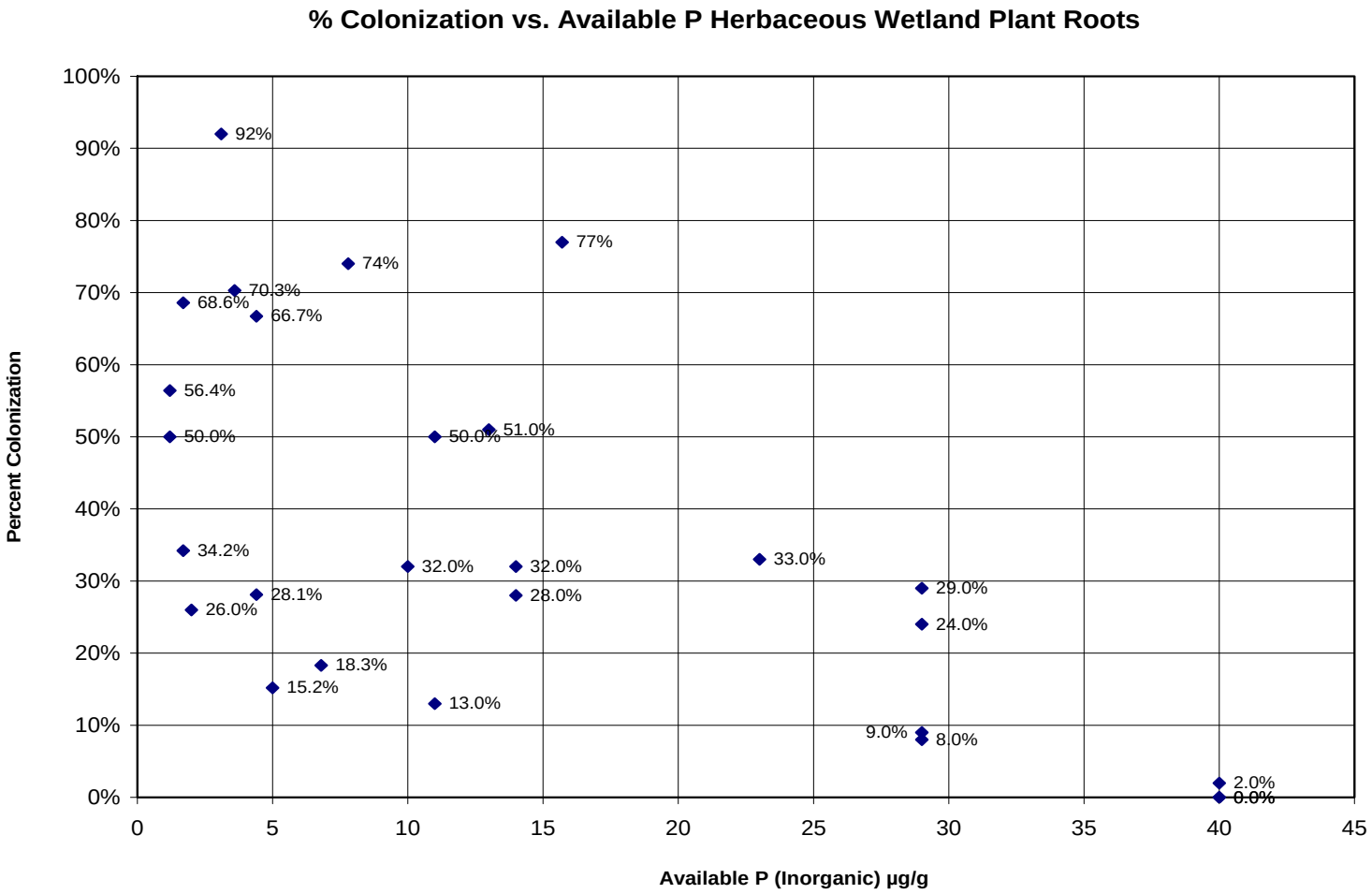

Figure 1. Literature review of Reported Percent Colonization of Herbaceous Wetland Plant Species

Mycorrhiza in Wetland Habitats

As described previously, an early study indicated that wetland plants did not form mycorrhizal associations (Khan, 1974) or that AM fungi were poorly suited to living in wet environments (Mosse et al., 1981). Subsequent studies have changed the discussion from *if* there are wetland plant-AM fungi associations to *how* they are affected by water stress. Specifically, these studies confirm that wetland plants are capable of forming mycorrhizal associations, but generally are colonized at a lower rate as the substrate becomes more saturated or ponded (Liberta et al., 1983; Anderson et al., 1984; Lodge, 1989; van Duin et al., 1990; Rickerl et al., 1994; Wetzel and van der Valk, 1996; Miller, 2000).

Cooke and Lefor (1998) suggested that AM fungi may be able to tolerate flooding by concentrating in oxygen rich portions of the root or rhizosphere. Miller and Bever (1999) also suggested that the fungi may rely on the plant host to provide oxygen through its roots, but as an alternative explanation raised the possibility that certain species of fungi may require less oxygen. The study identified differences in the mycorrhizal fungal community based on differing hydrologic regimes. However, their identification of the communities was based on spores, rather than colonizing fungi. Spore numbers bear little relation to the amount of root colonized (Robinson-Boyer et al., 2009) and the community composition determined from spores can be different than those colonizing roots (Wu et al. 2007). As sporulation may be a response to unfavorable conditions, the community analysis may not specifically identify those species that are flood tolerant.

Observed decreases in percent colonization in areas subjected to water stress could be an expression of colonization strategies by specific AM fungi that are either tolerant or intolerant of flooding (Ray and Inouye, 2006; Miller and Bever, 1999). Alternatively, water stress reduces net photosynthesis, growth, and biomass production (Will et. al., 1999), thereby reducing both the available substrate and photosynthate for mycorrhizal fungi. This drop in photosynthetic activity may reduce new colonization and promote senescence of existing associations. Certainly, both phenomena may occur in wetland environments.

Among grasses and sedges, Miller et. al. (1999) in a review of AM colonization of sedges (*Carex* spp.), found that the probability of infection was lower in areas with high soil moisture. The probability of infection was particularly low in areas with low soil pH and high moisture. Miller and Sharitz (2000) found that flooding roots of semi-aquatic grasses reduced the initiation of colonization, but once the fungi were established in the roots they were able to maintain and expand with the growing root system. Anderson et al. (1984) found AM colonization in herbaceous plants growing in the wettest habitats along a soil moisture gradient, but these associations did not include arbuscules. Stevens and Peterson (1996) conducted field studies along an existing water gradient using roots from *Lythrum salicaria* (purple loosestrife) and found that hyphal and arbuscular colonization levels were significantly higher in the dry and intermediate regions of the gradient than in the wet regions.

Less commonly evaluated are wetland trees. Moizuk and Livingston (1966) observed that all red maple seedlings on a Massachusetts bog mat possessed mycorrhizae, but did not quantify the colonization or the hydrology. Lodge (1989) found that mycorrhizal formation within the roots of willow and cottonwood trees was greater in moist soil than in very dry or flooded soils. The study suggested the existence of an optimal moisture range for mycorrhizal colonization. In examination of sweetgum tree roots in a Mississippi impoundment, Filer (1975) found mycorrhizae in roots from flooded areas, but the proportion of cells occupied was lower than in roots from non-flooded trees. The author concluded that flooding not only reduced existing AM populations but also prevented the formation of new ones.

Jurgensen et. al. (1997) reported finding studies of mycorrhizal colonization of only 14 different bottomland hardwood tree species. Of the 14, 5 supported AM colonization only 2 supported ectomycorrhizae (EM), and 7 supported both AM and EM. In general, AM are thought to be more tolerant of flooded conditions than are EM. Kormanik et. al. (1982) investigated 8 tree species, many of which are common in wetlands, and found strong AM colonization in each.

Khan (1993) examined wetland trees of South Wales and found that AM were absent or less frequent in roots growing in swamps, water or sediments with low redox values (i.e., 150 mV) than in terrestrial soils with higher redox values (i.e., 300 mV). The study also

found that mycorrhizal spore densities were positively correlated to redox potential values.

Cantelmo and Ehrenfeld (1999) found that Atlantic white cedar (*Chamaecyparis thyoides*) roots from the tops of hummocks had the greatest amounts of intraradical AM hyphae, while the roots from the bottom positions had very little. The authors also suggested the possibility of a zone of maximal arbuscular production that would vary in response to water table fluctuations. Specifically, they theorized that colonization during the "wetter" period may have shown higher colonization in hummock tops when these microsites were moist but aerobic.

Mycorrhizal Colonization of *Acer*

Studies evaluating AM in temperate forest tree species are uncommon. The various species of *Acer* develop only AM associations (Bainard et. al. 2011; van Diepen et. al. 2007; Jurgensen et. al. 1997). Wiseman and Wells (2005) reported a mean AM colonization level of 22% for red maple forest trees in the upper South Carolina Piedmont. Red maple seedlings in a forested Maryland Coastal Plain site exhibited AM colonization levels of 19% to 37% (Lovelock and Miller 2002).

Bainard et. al. 2011 is the only study found that reported AM colonization in *Acer saccharinum*. Levels were found to be 38.2 ± 6.40 in rural trees and 16.6 ± 3.88 in urban trees.

Brundrett et al. (1990) noted that red maple typically possessed lower levels of AM colonization than did sugar maples. Reported AM colonization intensities for sugar maple seedlings in southern Quebec were between 12-32% (Coughlan et al. 2000). Zahka et al. (1995) examined mature sugar maples in northern Vermont and found AM colonization levels between 9-33%.

Fungal Communities

Studies examining the fungal community composition have yielded differing conclusions regarding whether or not hydrology was significant and have identified other environmental variables that correlated with fungal community composition. Previous studies have: found a greater diversity in the fungal community at the drier end of a moisture gradient (Miller and Bever, 1999); suggested that different fungal species and strains were adapted to particular levels of soil nutrients (Mosse et al., 1981); found greater species richness in rural versus urban environments (Lawrynowicz 1982); and found greater diversity in AM communities in lakes with low dissolved P concentrations than in the lakes with relatively high dissolved P concentrations (Baar et. al., 2011)..

Further, Yu et. al. (2009) in examining forested wetlands in the New Jersey Pinelands found similar microbial communities present in mineral soils, regardless of the hydrologic conditions, and significantly different microbial communities in organic soils. Burke et. al. (2009) found community correlations with environmental variables differed seasonally. June fungal communities significantly correlated with soil pH, soil moisture,

and soil C and N at fine spatial scales, while in September, fungal communities were significantly correlated with labile P, soil C, and C/N ratio. .

Gottel et. al. (2011) examined the bacterial and fungal communities from the rhizosphere or endosphere environment of *Populus deltoides* trees growing in upland and bottomland soil types. The study found that both bacterial and fungal communities did not differ significantly in higher-order composition in either the rhizosphere or endosphere environment. The communities also did not vary even though the upland and bottomland soils supported statistically different tree sizes and age classes.

1.2 Study Design

The present study was designed to examine both the extent of upland and wetland AM communities in the Virginia Coastal Plain. To limit the previously identified variables (e.g., various soil constituents and the host plant), paired sites with differing hydrologic conditions were selected. The root source and host plant selected was *Acer*, due to its wide adaptability. The following sections will describe the sites that were sampled, the methods employed, and the results organized generally by the variable. Specifically, the available soil phosphorus and measured levels of soil redox are described and discussed as potential contributors to the observed levels of mycorrhizal colonization of *Acer* roots and as factors affecting observed variability to the overall fungal community. Sampling was conducted over multiple seasons to capture any seasonal variability.

Based on my review of the literature, I hypothesize that:

1. mycorrhizal associations will be present at both upland and wetland sampling locations;
2. the percentage of colonization will be lower at the wetland sites due to the constraints of anoxia;
3. the percentage of colonization will be inversely proportional to soil phosphorus levels;
4. both the number and diversity of mycorrhizal fungal species will decrease in the wetter sampling locations; and
5. different fungal communities will occupy the upland and wetland niches.

2.0 Site Descriptions

Six sites were chosen for sampling. Sites were paired at three locations. The three locations are relatively close to each other in northern Virginia (see Figure 2). The northernmost site, Franconia Bog (FB), is located in a residential/townhome development located east of Springfield Mall in the Franconia/Springfield area. Franconia Bog is a seep wetland associated with gravel terraces. The paired sites at FB are FB1 and FB2.

Approximately 3.5 miles southeast of the FB location is the Huntley Meadows (HM) location. The sample sites are located along a trail within the Fairfax County Park Authority's Huntley Meadows Park. Huntley Meadows Park is a 1,424-acre wetland preserve. Approximately 800 acres of Huntley Meadows Park are classified as wetland. This represents the largest non-tidal freshwater wetland area in northern Virginia. The paired sites at HM are HM1 and HM2.

Approximately 9.5 miles south of the FB location and 10 miles southwest of the HM location lies the final location, the Occoquan Wildlife Refuge (OC). The refuge is a 579-acre property that is administered by the U.S. Fish and Wildlife Service. Approximately 270 acres of the Woodbridge Refuge are classified as wetland. The sample sites are located on the eastern shore of the refuge along Belmont Bay of the Potomac River. The paired sites at OC are OC1 and OC2.

The site coordinates are:

OC1 (38°38'49.02"N, 77°13'27.82"W), OC2 (38°38'41.45"N, 77°13'27.54"W), HM1 (38°45'23.57"N, 77° 6'1.20"W), HM2 (38°45'24.18"N, 77° 6'5.20"W), FB1 (38°46'30.36"N, 77° 9'45.61"W), and FB2 (38°46'28.01"N, 77° 9'37.89"W).

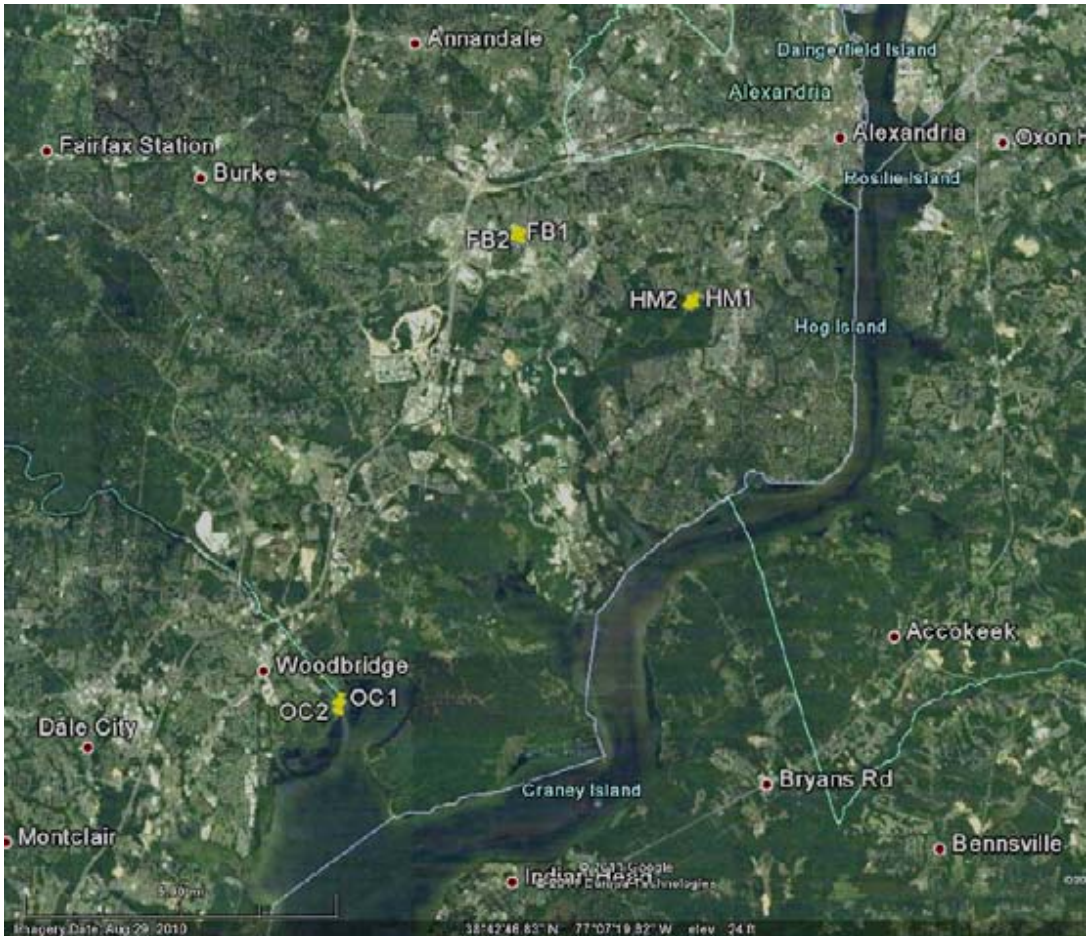

Figure 2. Regional Location of Sampling Sites.

2.1 Geology and Soils

All six sample sites are located within Virginia's Coastal Plain. The topography of the Coastal Plain is a terraced landscape that stair-steps down to the coast and to the major rivers. The risers (scarps) are former shorelines and the treads are emergent bay and river bottoms. This landscape was formed over the last few million years as sea level rose and fell in response to the repeated melting and growth of large continental glaciers and as the Coastal Plain slowly uplifted.

The Virginia Coastal Plain sediments rest on an eroded surface of Precambrian to early Mesozoic rock. Two-thirds of the sediments are comprised of late Jurassic and Cretaceous clay, sand, and gravel. These sediments were stripped from the Appalachian mountains, carried eastward by rivers and deposited in deltas in the newly formed Atlantic Ocean basin. A sequence of thin, fossiliferous marine sands of Tertiary age overlies the older strata. They were deposited during the repeated marine transgressions across the Coastal Plain.

Quaternary (Miocene) deposits occur on low-lying terraces near the Potomac River. The lowest and youngest consists of silt, clay, and fine-grained sand and gravel, of (possibly) estuarine origin, formed when sea level was about 50 feet higher than present.

Soil parent material in Virginia's coastal plain consists of sedimentary rocks overlain with typically thick soils (2–8 m deep) (Markewich et al., 1990). Soils of the four wetland sites (OC2, HM2, FB1, and FB2) are subjected, at least seasonally, to saturation.

Under prolonged submergence, mineral soils develop redoximorphic features associated with anaerobic soil metabolism. As oxygen is excluded by submergence, soil organisms must use other soil constituents as their oxidizing agents in deriving energy from organic matter. This typically occurs in the sequence: nitrate ions to nitrogen, manganic manganese to manganous, ferric iron to ferrous, and then sulfate ions to sulfide (Mitsch and Gosselink, 2007).

The most visible change associated with this process is the reduction of the red and brown compounds of ferric iron to blue-grey compounds of ferrous iron. Subsequent translocation of soluble ferrous iron to zones where oxygen enters the soil–such as at the soil surface or near plant roots–produces reddish-brown mottles of insoluble ferric iron. Likewise there may be movement and re-oxidation of manganous manganese forming black manganic compounds. These changes produce the characteristic redoximorphic features of submerged mineral soils (Kirk, 2004). The average lag period between the onset of soil saturation and onset of reduction at depths of 15-cm depths in soils of the Atlantic Coastal Plain in North Carolina was found to be 21 days (He et al., 2003).

Occoquan Wildlife Refuge

Since the Refuge was formerly a federal military installation, the Natural Resources Conservation Service (NRCS) has not mapped soil types. However, in 2007 a Soils Resource Specialist from the NRCS conducted a general soil survey of the Refuge and constructed a generalized soils map. According to that mapping, the OC1 site is categorized as Comus Loam (0-2% slope). Comus loam is a nearly level to gently sloping, well drained soil with a seasonal high water table depth of greater than 6 feet (USFWS, 2009).

The OC2 site was mapped as Marumsco loam (0-4% slopes). Marumsco loam is a nearly level to moderately sloping, very deep, moderately well drained soil with a seasonal high water table as high as 15 inches below the soil surface.

Soil sieve analyses (Bouyoucos, 1962) of the OC1 and OC2 sample sites classified both soils as sandy loam. Both soils were 56% sand. OC1 was 34% silt, versus 32% for OC2. OC1 was 10% clay, while OC2 was 12% clay. Based on 4 samples subjected to loss on ignition, the OC1 soil is 5.7% organic matter by weight while the OC2 soil is 9.1% organic matter by weight. The average soil pH at both sites was 5.5.

Soil color at OC1 was consistent with an upland soil. The soil color at OC2 would not grade out as a wetland soil using the Munsell soil chart. However, the plant community was comprised almost entirely of hydrophytic vegetation and indicators of wetland

hydrology were present. As will be noted later in the discussion of site hydrology, OC2 was flooded, ponded, or saturated during extended parts of each growing season.

Huntley Meadows

The soils at the HM1 and HM2 sites are mapped as Glenelg silt loam. Soil sieve analyses (Bouyoucos, 1962) of the HM1 sample site classified the soil as loam (52% sand, 34% silt, and 14% clay). Based on 4 samples subjected to loss on ignition, the HM1 soil is 5.2% organic matter by weight. The average soil pH was 5.2. Soil color was consistent with a upland soil.

Soils at the HM2 sample site were classified as sandy loam (58% sand, 24% silt, and 18% clay). Based on 4 samples subjected to loss on ignition, the HM2 soil is 4.6% organic matter by weight. The average soil pH was 4.5. Soil color was consistent with a wetland soil. Soils were reduced and contained oxidized rhizospheres (National Research Council, 1995).

Franconia Bog

The soils in the general area are mapped as Fairfax loam, Glenelg silt loam, and Huntington silt loam. Because of the level of development, the specific soil type at the site was not mapped. Soil sieve analyses (Bouyoucos, 1962) of the FB1 and FB2 sample sites classified the soils as loamy sand (80% sand) and sandy loam (74% sand), respectively. FB1 was 6% clay, while FB2 was 12% clay. However, a large fraction of the soil at FB1 was comprised of organic material. In general, a 4-5 inch layer of organic material overlay gravel and sand. Based on 4 samples subjected to loss on ignition, the FB1 soil is 18.3% organic matter by weight. The average soil pH was 4.4. Soil color was consistent with a wetland soil.

The FB2 site lies contiguous with a small intermittent drainage from a culvert that is at least partially augmented by stormwater runoff from parking areas and lawns associated with the townhomes that surround the site. At the site, a thick layer of mineral soil (sandy loam) overlay gravel. It is likely that the sandy loam is partially or mostly depositional material carried by the intermittent stream. Based on 4 samples subjected to loss on ignition, the FB2 soil is 3.8% organic matter by weight. The average soil pH was 5.2. Soil color was consistent with a wetland soil (Environmental Laboratory. 1987).

The USDA (1999) defines organic wetland soils as having an organic carbon content between 12–18% if the soil contains less than 60% clay. Based on this definition, the FB1 site would be categorized as an organic wetland soil.

Soils from the 6 sites were analyzed in August 2007 and again, 6 months later, in February 2008. The August sample date was chosen to reflect the biotic depletion of plant nutrients, which occurs throughout the growing season and would be close to completion in late August. In addition, August represents the third consecutive month of dry conditions at the wetland sites. The February sample represents the end of a period of higher soil moisture and minimal nutrient depletion by plants. A summary of the soils analysis from the 6 sites is provided in Table 2. The soil samples were not drained prior to packaging for shipment to the laboratory. Therefore, constituents found in the soil water, which are readily available for plant uptake, were analyzed as well. The results for soil phosphorus are discussed in Section 4.1.

Table 2. Soil Chemistry for the Sample Sites

Table 2. Soil Chemistry													
Site	**OM**	**pH**		**CEC (%)**		**K (mg/Kg)**		**Mn (mg/Kg)**		**Fe (mg/Kg)**		**Ca (mg/Kg)**	
	(%)	**Aug.**	**Feb.**	**Aug.**	**Feb.**	**Aug.**	**Feb.**	**Aug.**	**Feb.**	**Aug.**	**Feb.**	**Aug.**	**Feb.**
FB1	18.3	4.4	4.5	4.0	3.6	72	78	6	4	444	831.5	170	177
FB2	3.8	5	5.5	5.5	7.1	75	96.5	10	14.5	633	567.5	555	825.5
HM1	5.2	5.3	5.1	6.7	10.9	113.5	103.5	204.5	120.5	209	226.5	1105	1025
HM2	4.6	4.5	4.6	8.0	13.7	94.5	93	35.5	62.5	469.5	496	720	733.5
OC1	5.7	5.5	5.6	6.0	8.1	115	100	90	75.5	271.5	253	1035	957
OC2	9.1	5.5	5.6	6.3	8.1	88	89	47	83.5	308	402.5	1060	903.5

2.2 Hydrology

Occoquan

Situated on a neck between Belmont and Occoquan Bays, topographic relief at the Occoquan Wildlife Refuge is slight. Stream erosion is the primary cause of existing topographic relief in the region. Approximately two-thirds (387 acres) of the refuge lies within the 100-year tidal floodplain.

The Refuge is located at the mouth of the Occoquan River. Occoquan Bay borders the facility to the south. Belmont Bay, which is located on the facility's northeast side, is mainly fed by the Occoquan River. The paired sampling sites (OC1 and OC2) are located on the northeast section of the refuge, and are separated from Belmont Bay by Deep Hole Point Road, a gravel road that is a perimeter barrier to normal tidal flow over the shoreline (see Figure 3). Tide data for High Point in Occoquan Bay shows a tidal range of about 1.6-1.8 feet (http://tidesandcurrents.noaa.gov/tides07/tab2ec2c.html).

Based on field observations, debris left by normal high tides generally did not carry over Deep Hole Point Road. However, under storm conditions a wrack line was observed west (up gradient) of both OC1 and OC2. During approximately two years of observations, water was ponded at the OC1 site (see Figure 4) on only two occasions.

The OC2 site is bounded to the east by Deep Hole Point Road and Belmont Bay and to the west by a tidal marsh that is fed through a break in the road south of the site. Ponding at OC2 was observed following large storm events during the summer months. Extended ponding and/or saturation were observed starting in September and ending after complete leaf-out in the spring (see Figure 5). Although not monitored, the wet conditions observed in fall may be related to the senescence of tidal marsh vegetation and the corresponding reduction in water lost to the atmosphere via evapotranspiration. Under these conditions, shallow groundwater at OC2 could be augmented by expansion of the tidal freshwater marsh to the west of the site. Hussey and Odum (1992) showed that vegetated tidal freshwater marshes in Virginia may lose more water to the atmosphere through plant transpiration than through evaporation.

Figure 3. The Occoquan Wildlife Refuge and Sampling Locations.

Figure 4. The OC1 Upland Site.

Figure 5. The OC2 Wetland Site

Huntley Meadows

In 1975, Huntley Meadows was acquired by the Fairfax County Park Authority (FCPA). Three years later, beavers dammed Barnyard Run, forming a large emergent wetland area. In 1981, the FCPA built a boardwalk across the marsh for public access. From the parking and visitors center, located just off Lockheed Boulevard, a trail leads down to the boardwalk (Figure 6).

The HM1 site is located about 150 feet down the trail and on the north side. The area was consistently dry, except for brief periods during rainfall events (Figure 7). The HM2 site is located about 320 feet west of the HM1 site and 540 feet northeast of an extensive emergent marsh. The HM2 site contains little herbaceous understory (Figure 8). The

HM2 site was ponded and/or saturated with the hydroperiod of a classical seasonally inundated forested wetland. Generally, the site was wet over the winter and into early May. Evapotranspiration by the forest community draws down groundwater levels following full leaf-out. During sampling, leaf drop occurred in late October into early November and leaf-out began in mid April. Based on observations, the hydrology of the site was driven by groundwater and precipitation.

Figure 6. Sample Sites at Huntley Meadows Park

Figure 7. The HM1 Upland Site.

Figure 8. The HM2 Wetland Site.

Franconia Bog

Simmons and Strong (2002) refer to the Franconia Bog as a remnant of a series of Magnolia Bogs, which they define as fen-like seeps associated with high elevation gravel terraces of the inner Coastal Plain near the fall line between the Coastal Plain and Piedmont physiographic provinces in the mid-Atlantic region. The site is located in a townhome development near Springfield, VA (Figure 9). These areas are typically fed by spring or seep flow from a gravel and sand aquifer over a thick, impervious layer of underlying clay which prevents the downward infiltration of water.

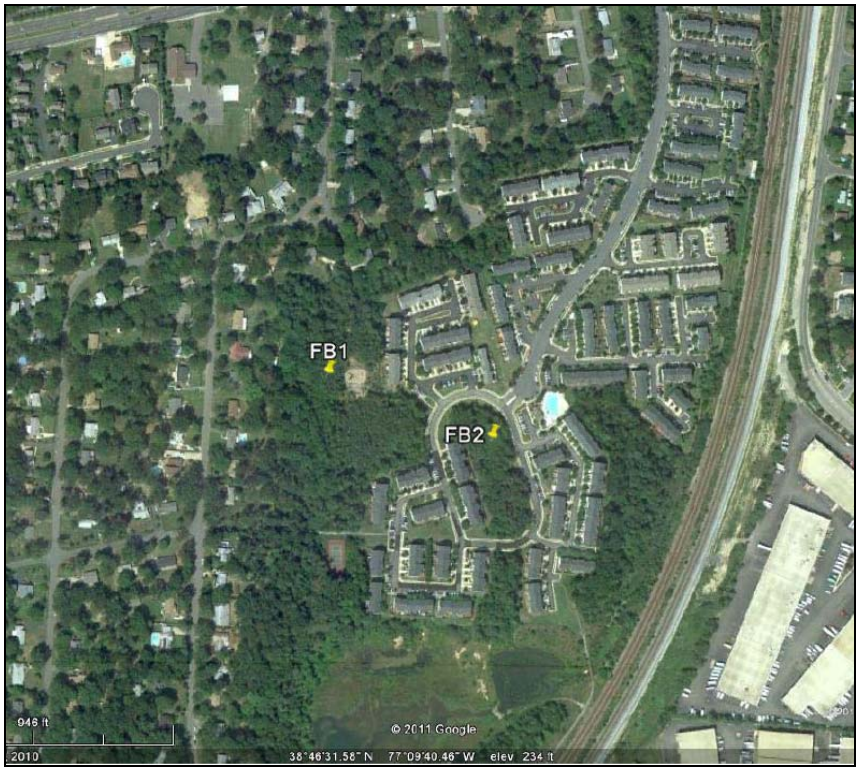

Figure 9. Sample Sites at the Franconia Bog

The FB1 site is in a slight depression, but the organic material in the soil and the shallow depth to gravel would not be conducive to retaining water derived from precipitation or from surface flow (Figure 10). The major hydrologic input to FB1 is almost certainly groundwater. Drawdowns observed during the summer months were also likely the result of evapotranspiration by the forested habitat.

The FB2 site also receives groundwater inputs, but some percentage is likely conveyed to the site via the small, intermittent stream that is located within three feet of the study tree (Figure 11). The stream also delivers stormwater runoff from impervious surfaces within the townhouse development (see Figure 12).

Figure 10. The FB1 Wetland Site.

Figure 11. The FB2 Wetland Site.

Figure 12. Stormwater culvert to FB2 site.

As described above, precipitation plays a significant role in the hydrology of the four wetland sites. In general, the period in which hydrologic monitoring was performed (July 2006 – May 2008) was a dry period (see Table 3). In fact, 2007 was the driest year in the 46-year period compiled. While 2006 was a fairly wet year, the spring (March-May) was dry. The total precipitation over the three months was only 6.8 inches, which was the fourth driest spring over the same period. Spring precipitation in 2008 was the highest for the period totaling 20.09 inches. The summer period (June-August) offered little relief during 2007. Total precipitation for summer 2007 was 6.4 inches, which was the driest summer in the 46-year period compiled.

Table 3. Precipitation During the Sampling Period.

Precipitation (Inches)					
Year	**spring**	**summer**	**fall**	**winter**	**Annual**
2005	13.91	12.32	12.17	7.63	45.97
2006	6.80	15.72	17.40	7.51	46.21
2007	6.72	6.40	7.45	6.43	28.24
2008	20.09	9.80	10.57	6.98	47.11
Data generated from National Climactic Data Center, Reagan National Airport via http://www.ncdc.noaa.gov/oa/climate/research/cag3/z0.html					

2.3 Vegetation

Red maple (*Acer rubrum*) is one of the most abundant species in eastern forests. It is abundant on bottom lands and is tolerant of water-logged soils and flooding. It is a "supergeneralist", growing on the widest variety of sites and in the greatest range of

conditions (sunny or shady, high or low nutrients, dry or moist) of any North American species. It is found from sea level to about 900 m (3,000 ft) and grows over a wide range of microhabitat sites (Walters and Yawnery, 1990). Red maple seedlings in wet areas produce short taproots with long, well-developed laterals. On dry sites, they develop long taproots with much shorter laterals (Hutnick and Yawney, 1961). Under flood conditions, many adventitious roots develop (Hosner and Boyce, 1962).

Red maple roots are primarily horizontal and form in the upper 25 cm (10 in) of soil (Walters and Yawney, 1990). Horizontal, woody roots grow outward from the tree. Non-woody feeder roots fan upward, mostly within the upper 8 cm (3 in) of mineral soil. These non-woody feeder roots are the ones colonized by AM.

Red maple was chosen as the sample tree for the present study because of its ability to occupy such as wide range of hydrologic and soil conditions. However, at the Occoquan Refuge, very few red maple trees were observed occupying a wetland habitat. In general, sweet gum (*Liquidambar styraciflua*) occupied the dry/upland edge of habitats and then transitioned into black gum (*Nyssa sylvatica*) and green ash (*Fraxinus pennsylvatica*) as the habitat grew wetter. The northeast corner of the refuge, however, contains a mixed stand of silver maple (*Acer saccharinum*) that is common in the moist upland soils down to the wet forest found along the tidal freshwater marsh.

Silver maple is similar to red maple and the two species are known to hybridize naturally (Little, 1979). Silver maple has a shallow, fibrous root system. Silver maple does not

compete well in upland habitats and appears as a dominant species only in streamside communities or on the fringes of lakes or backwaters of streams (Walters and Yawney, 1990).

Red maple is one of the first trees to flower in the spring, appearing from March to May depending upon elevation and latitude (Walters and Yawney, 1990). Fruit and leaf development follow and occur between April and June. Silver maple is the first of the maples to bloom in North America, beginning as early as February and extending into May (Schopmeyer, 1974). Ripening and dispersal of fruits begins in April and ends in June. Consequently, resource needs for both species would be expected to be highest from April through June, when both leaf and fruit production is underway.

At all sample sites, study trees were selected, at least in part, based on a relatively open understory and a lack of other woody vegetation in proximity to the study tree. A circular plot with an area of 10 square meters (1.78-meter radius) was evaluated and a visual assessment of percent cover was determined within the plot. Observations were recorded on August 13, 2006.

Occoquan

At OC1, the study tree was a mature *A. saccharinum* with a diameter-at-breast-height (dbh) of just over 14.2 inches (36.1 cm). No other trees occupied the 10-square-meter plot. The understory consisted of *Microstegium vimenium* (70% cover), *Comelina*

virginica (8% cover), and *Toxicodendron radicans* (2% cover). The remaining area was open with no vegetation. Other nearby woody vegetation included *A. saccharinum, L. styraciflua, Smilax rotundifolia,* and *Hamamelis virginiana*.

At OC2, the study tree was an *A. saccharinum* with a dbh of just over 7.7 inches (19.6 cm). About 50% of the circular plot was unvegetated and blackened. The dominant herb was *Carex crinata* (25% cover), *M. vimenium* (6%), *S. rotunfifolia* (5%), and *T. radicans* (2%). Although the closest woody vegetation was a single *F. pennsylvanica*, numerous *A. saccharinum* occupy the stand.

Huntley Meadows

At HM1, the study tree was a mature *A. rubrum* with a dbh of 12.2 inches (31 cm). About 20% of the circular plot was unvegetated. As observed throughout Huntley Meadows, *M. vimenium* was a dominant understory herb and covered about 70% of the plot. *Bohmeria cylindrica* covered about 5% of the plot area. The canopy was closed by four *A. rubrum*, one *L. styraciflua*, and one *N. sylvatica*.

At HM2, the study tree was a multiple trunk *A. rubrum* with dbh's of 14.6 inches (37.1 cm) and 14.8 inches (37.6 cm). About 90% of the circular plot was unvegetated. *M. vimenium* covered about 5% of the plot while *Lycopus virginicus* covered about 3%. The canopy was closed by five *A. rubrum* and one *L. styraciflua*.

Franconia Bog

Approaching the FB1 site, you traverse a narrow forested habitat bounded on one side by a small stream and on the other by a playground and townhouse development. The most distinguishing characteristic of the habitat is the patches of sphagnum moss (*Sphagnum spp*.) intermixed with *Lycopodium obscurum*. Shrubs include *Magnolia virginiana*, *Rhododendron viscosum*, and *Vaccinium corymbosum*.

The canopy near the study tree contains six *A. rubrum*, two *L. styraciflua*, and one *Pinus virginiana*. The understory is 90% open with *Carex sp* (4%), *N. sylvatica* (2%), *R. viscosum* (2%), *V. corymbosum* (1%), and *Osmunda cinnamomea* (1%). The study tree is a multiple-trunk (3) *A. rubrum*. The dbh for the three trunks is 7.4 inches (18.8 cm). 7.6 inches (19.3 cm), and 7.8 inches (19.8 cm).

FB2 site is also forested, but lacks the sphagnum moss community of FB1. The study tree, located adjacent to an intermittent drainage fed by a culvert, was also a multiple-trunk (2) *A. rubrum* with dbh's of 13.5 inches (34.3 cm) and 5.8 inches (14.7 cm). About 95% of the circular plot was unvegetated while *L. virginicus* covered about 4%. The canopy was closed entirely by six *A. rubrum*.

3.0 Materials and Methods

Manipulation of the environment during in situ studies is often problematic. However, the control derived from a greenhouse study is even more problematic because of the uncertainties related to extrapolating the results to field conditions. For example, Stevens and Peterson (1996) found higher colonization by mycorrhizal fungi in wetter areas during the field studies, but the opposite relationship was observed from their greenhouse studies. Accordingly, the present study relies solely on field investigations of three sites with paired trees sampled over several seasons.

The collection of tree roots began in April 2005 and was conducted quarterly through May 2008. Roots were collected on eleven dates. Soil and roots were excavated along lateral roots generally within 1.5 m of the sample tress. Roots and soil were secured in Ziploc bags and stored on ice for transfer to the laboratory. Root samples were collected by removing the fine attached roots with flame-sterilized forceps and gloved hands from the root masses after thorough cleaning with deionized water (Hawkes et al, 2006). Between 2-4 g of fine roots were stored at -20 C until staining. An additional 1-2.4 g of roots per sample was set-aside for DNA extraction and stored at –80ºC (Hawkes et al., 2006). Soils were frozen and stored separately.

3.1 Root Staining and Microscopy

Various methods have been used to clear and stain roots. Tree roots are difficult to clear because they contain high levels of phenolic materials (Sylvia et al., 1998). A review of methods used is summarized in Table 4. Thawed samples were analyzed by taking subsamples of about 2 g of fine root (<1 mm diameter). Root samples were cleared by autoclaving in 10% KOH for 0.75 h and rinsed with deionized distilled water. Two additional protocols subsequent to KOH treatment were evaluated: 1) alkaline peroxide (2 ml of NH_4OH, 15 ml of 10% H_2O_2 , and 83 ml of deionized H_2O, sit for 0.5 h at room temperature, as generally described in Kormanik & McGraw, 1982; and 2) acidic bleach (5% NaOCI, acidified with several drops of 5 M HCI, sit for 1 h at room temperature). Using either approach, root pieces were still highly pigmented. So, both clearing techniques were used in sequence, with the acidic bleach step used first, followed by rinsing with deionized distilled water.

Table 4. Clearing and Staining Methods for Tree Roots.

Table 4. Clearing and Staining Methods for Tree Roots			
Reference	**Tree Species**	**Stain**	**Clearing Method**
Cheng and Widden, et al. (2005)	Sugar maple	CBE	10% KOH (1h-15 PSI)-H_20 rinse,35% H_2O_2 (1h)-H_20 rinse, 15% HCL (0.25h)
Reich and Barnard, 1984	Apple	TB	10% KOH (0.5h @ 90c)- 10 % Chlorox (5 min)- 1% HCL (3 min)
Trowbridge and Jumpponen (2004)	Shrub willows	TB	10% KOH (0.5h @ 90c)-1% HCL (1 min)
Sylvia et al. (1998)	23 tree species	TB	1.8 M KOH (1h-80°C)-rinseed (3x)- 3% NaOCI, acidified with several drops of 5 M HCI (50 seconds 70F)-rinsed (5x)-HCL
Bohrer (2001)	*Vangueria infausta*	TB	2.5% KOH (0.5h-96c)- 30% H_2O_2 (0.5h)-1% HCL (10h)-
Onguene and Kuyper (2001)	97 tree species	AF	10% KOH (24h-72F)- (3 ml of NH_4OH and 30 ml of 10% H_2O_2 to 567 ml of tap water- 1h) –rinsed H_2O (5x) - 1% HCl (3 min)- destained for 2 to 3 days in a lactic acid
St. Clair and Lynch (2005)	Sugar maple and red maple	TB	10% KOH (15 min.) – H_20 rinse, 5% H_2O_2 (2 min.)-H_20 rinse - 5% HCl (1 min.)
AF = Acid-Fuschin, CBE = Chlorazol Black E, TB = Trypan Blue			

After incubating in acidic bleach, as described above, roots were rinsed 5X with deionized distilled water, incubated in alkaline H_2O_2, as described above, rinsed 5X with deionized distilled water, and acidified in 1N HCl for 5 minutes. The cleared roots were then stained with trypan blue in lactic acid solution (875 ml lactic acid, 63 ml glycerin, and 62 ml of deionized water). The staining was fixed by autoclaving the roots and stain mixture for 0.25 h. Roots were left in the staining solution overnight. Stained roots were then rinsed with ethanol and destained for 2-3 days, as necessary, in a 90% lactic acid solution and stored in glycerol/lactic acid before microscopic examination.

Colonization was measured on 1-cm length pieces of fine roots (5-10 per slide), following the protocol of McGonigle et al. (1990). Stained roots were randomly selected, placed on a glass slide, and gently squashed under a cover glass to observe the anatomy of AM fungi under a compound microscope at 100-400x magnification and to estimate colonization. One hundred fields of view per sample were tallied. The following structures were counted from samples: hyphae without septa, vesicles, arbuscules, hyphal coils, and spores.

3.2 DNA Extraction

Root samples designated for DNA extraction were thawed at room temperature. Fine roots were ground in clean, autoclaved plant blenders (Iberbach Corp.) powered by commercial blender (Waring) and passed through a series of three clean, autoclaved USA Standard Testing Sieves (Fisher Scientific): 500 μm, 212 μm, and 106 μm in descending order (Torzilli et al., 2006). The 106 μm fraction was rinsed 10X with autoclaved deionized water and stored at –20ºC (Ritchie et al., 2000) until the extraction step.

DNA was extracted from roots using a BIO101-FastDNA Spin Kit for Tissues following a modified protocol in which an extra ceramic bead was added to Tissue Matrix tubes to maximize lysing of fungi and plant cells during bead-beating (Torzilli et al., 2006). Additionally, 800 μl CLS-VF solution and 200 μl PPS were added to Tissue Matrix tubes in place of CLS-Y solution in the root DNA extraction procedure to improve the

extraction of DNA from recalcitrant plant tissues (personal communications with Dr. Torzilli).

3.3 ARISA Fingerprinting

Automated Ribosomal Intergenic Spacer Analysis (ARISA)[1] was used to characterize mixtures of fungal community amplicons from environmental DNA extracts. A select set of extracts was evaluated using various primer pair sets developed by Redecker (2000) to amplify the Internal Transcribed Spacer 1 (ITS1) region of 18S ribosomal RNA genes from the total fungal community DNA while not amplifying the co-occurring DNA of bacteria, plants, and other organisms. Specific primers included ACAU1660 for the genus *Acaulospora*, LETC1670 and GLOM5.8R for genus *Glomus*, and GIGA5.8R for genus *Gigaspora*. Of these, only the primer pair of ITS1F-GLOM5.8R yielded visible PCR product (data not shown). This would indicate that only the genus *Glomus* was present in the samples. However, the results were not consistently reproducible. Therefore, the primer pair ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (TGTGTTCTTCATCGATG) was used to amplify the overall fungal community, rather than targeting a specific genus of arbuscular mycorrhizae. As the present study evaluated only fine roots that were thoroughly rinsed, blended, and rinsed again, most if not all fungal DNA associated with the environment external (i.e., fungi associated with the soil

[1] Alternatively known as Amplicon Length Heterogeneity PCR (ALH-PCR) or Length-Heterogeneity PCR (LH-PCR) based on the variation in amplicon length associated with different taxa (Ritchie et al., 2000; Suzuki et al., 1998; Yang et al., 2006).

environment rather than the tree roots) to the fine roots was eliminated from the amplification step.

DNA extracts were generally diluted 1:5 for PCR based on the results of preliminary reaction optimization experiments (data not shown). DNA extracts from the 7/23/06 sample date were not diluted because sufficient PCR product was not produced using a 1:5 dilution. Immediately prior to each PCR run a "master mix" was prepared containing the following components per sample or reaction: 8 µl DEPC water, 2 µl 10X Rx. Buffer, 2 µl of 25 mM MgCl mix, 1.9 µl '2.0mM each' dNTPs, 2 µl BSA 0.1%, 1 µl forward primer 10 µM, 1 µl reverse primer 10 µM, and 0.1 µl Taq polymerase 5 units/µl. After delivering 18 µl aliquots of master mix per sample to separate reaction tubes, 2 µl of diluted DNA extract was added to each tube, with 2 µl of diluted lichen DNA serving as positive controls and master mix minus DNA representing a negative control. The complete reaction conditions were as follows: 35 cycles at 30 seconds denaturation [95ºC], 30 seconds annealing [Tm = 55ºC], 2 min. + 5 sec/cycle extension [72ºC], followed by 45 min. extra extension step (Torzilli et al., 2006). All PCR reaction mixtures included ITS1F primers fluorescently labeled by FAM marker to allow analysis by high throughput capillary electrophoresis. Duplicate or triplicate PCRs, where possible, were run for each fingerprinted sample to check for reproducibility and to validate individual peaks and their locations (Torzilli et al., 2006).

All PCR products were subjected to ARISA generated electropherograms in which amplicons of different sizes on the X-axis and fluorescence intensities on the Y-axis (from the fluorescent-labeled ITS1F primer integrated during PCR) represented fungal OTUs (operational taxonomic units). Peak heights represented relative OTU abundances. Since each OTU represented a presumptive fungal species, the fingerprint or community profile gave a general snapshot of a particular fungal community's composition (Martin and Rygiewicz, 2005; Suzuki et al, 1998). Peak-calling was performed using SpectruMedix software with peaks below 1% relative abundance threshold not included in the analysis. Data derived from the community DNA fingerprints were exported to Microsoft Excel after processing by a Perl script program written by Dr. Patrick Gillevet (personal communications; Torzilli et al., 2006). Once in Excel, errors in the automated assignment, or binning, of peaks to basepair numbers were corrected by comparing adjacent columns of relative abundances by OTU for instances where rare or singleton values likely corresponded to unusual gaps in the adjacent column of a more-abundant fungus, the misplaced value being moved to fill the gap where appropriate.

3.4 Hydrologic Measurements

Hydrologic monitoring of the 6 sites began in July 2006 and was performed at approximately 3-week intervals between February and November until May 2008. Monitoring was continued over the winter but less frequently. In total, hydrologic data was collected on 28 dates.

An unlined soil hole was bored using a 2-inch-diameter soil auger. The holes were bored to a depth of between 44 and 55 cm. The water elevation in these holes was measured concurrent with soil redox measurements. Readings were recorded as negative centimeters below the soil surface elevation. If the hole was dry, a measurement of -44 cm was recorded so that a consistent definition of "dry" could be applied across all locations.

In June of 2006, two to three platinum electrodes were inserted at each site in nests to measure the redox potential of the soils (see Figure 13). Two electrodes were inserted in upland sites and three electrodes were inserted in wetland sites. The electrodes were left in place during the study, except for occasional cleaning. The electrodes were planted to a depth of 8-10 cm below the surface, based on the depths where roots were harvested. The electrodes were planted somewhat deeper (18 cm) at the HM1 site because of the depth of roots at that location.

A voltmeter was attached to the platinum wire and also to an Ag/AgCl reference electrode. Measurements were recorded in millivolts (mV). Readings were corrected for a standard hydrogen electrode by adding +244 mV to the field reading. Readings were averaged to produce a single value for each site on each date.

Figure 13. Nested Redox Probes at the HM2 site (July 2006).

3.5 Soil Phosphorus Evaluation

Soil chemistry, including soil P, was evaluated using 2 sampling dates. While some temporal variability in soil P would be expected to occur throughout the period of field collections, these changes would be the result of seasonal fluxes (e.g., changes in the level of soil reduction in wetland soils, mineralization, and biotic utilization).

Seasonal variability in labile P has not been consistently reported for forested wetlands and floodplains. In a study of coastal floodplain forests in Georgia, Wright et. al. showed insignificant variation in available P within mesocosm and nonmesocosm control soils between July and December. Stoekel et. al. 2001 found no seasonal differences in the inorganic pool of P within forested floodplains in the Coastal Plain of South Carolina. However, Fabre et. al. (2006) found winter (January-February) peaks in inorganic P followed by a decline in March with relatively stable values through August. Increasing concentrations of inorganic P were observed starting in September. The authors concluded that peaks during winter were a consequence of P leaching from fresh litter (leaf fall) accumulated from November to January and to the limitation of mineralization processes during winter low temperatures. They ascribed the reduction in labile P during the spring to plant and microbial uptake as well as release to the water column during flood events in the adjacent river.

For the present study, the August sample date was chosen to reflect the biotic depletion of P by plants, which occurs throughout the growing season and would be close to completion in late August. In addition, August represents the third consecutive month of elevated redox levels at the wetland sites. Under higher redox conditions, iron phosphate complexes are stable and soil P is not mobilized in the soil column. Soil P sorbed by Fe minerals may become mobile when Fe (III) is reduced to Fe (II) during periods of low redox potential. The potential release and movement of P in the soil column is captured by the February sampling date. February represents the end of a period of lower redox and minimal depletion by plants (see Results).

Soils containing roots were collected in 2-gallon Ziploc bags. Roots were removed and the soils for each site were composited. From each composite, duplicate samples were prepared for analysis. The February 2006 and August 2007 samples were sent to the Virginia Cooperative Extension Soil Testing Laboratory at Virginia Polytechnic University (VT), Blacksburg, VA. Duplicates of the August 2007 soils and separate February 2008 samples were sent to A&L Eastern Agricultural Laboratories, Inc. (A&L), Richmond, VA A&L conducted a soil sieve analysis (Bouyoucos, 1962) of the August 2007 samples. A&L used the Mehlich-3 protocol to extract available P, K, Ca, Mg, Mn, Fe, Cu, Zn (Wolf & Beegle, 1995) while the VT lab used the Mehlich-1 protocol. In general, Mehlich-1 is less efficient at removing P from soils than Mehlich-3. As a result, the P concentration yielded using Mehlich-1 underestimates the soil P concentration.

Mehlich-1 also underestimates most extractable micronutrients (Gartley et al., 2002). Therefore, the A&L results were used to report soil chemistry concentrations for the sites.

3.6 Statistical Analysis

The diversity of fungal communities was measured by Shannon's log e diversity (H') on Multi-Variate Statistical Package (MVSP) Version 3.2 software by Kovach Computing Services. Species-saturation was measured by the generation of Mao Tau rarefaction curves using EstimateS Version 8.2.0 software. The rarefaction analyses of the ARISA fingerprints utilized the OTU abundances of each sample to calculate species accumulation with increasing collection effort.

As data was collected during the study, it was entered into Microsoft Office Excel 2003 spreadsheets for future analysis and the generation of summary figures. Tests of normalcy were performed with XLSTAT Version 2011.4.03. An arcsine transformation was performed on percentages (i.e., percent colonization and organic matter). Analysis of Variance (ANOVA) was conducted using the MySTAT (version 12.02.00) a student version of SYSTAT 32-bit Unicode software. All significance estimates (p-values) for comparisons between sites and/or dates and hydrologic, soil chemistry (phosphorus), percent AM colonization, and Shannon's Index values were determined using the ANOVA function of MySTAT. Box plots for data were also generated using MySTAT.

Principal Coordinate Analysis (PCO) with Bray Curtis distances was used to examine differences between samples in overall fungal and plant community structure. PCOs were carried out for all fungal communities derived from ARISA fingerprinting. Canonical Correspondence Analysis (CCA) with detrending was applied to the fungal community data to evaluate correlations between fungal community structures by sample and the corresponding environmental variables. CCA ordinated samples in terms of Bray Curtis distances, which is suitable for representing species-abundance data (Legendre and Legendre, 1998). MVSP was used to generate the analysis of fungal community graphics and significance estimates (p-values).

Mean Bray Curtis distance values for fungal communities were compared using R software version 2.14.0 with the ANOSIM (Analysis of similarity) vegan package. ANOSIM provides a way to test statistically whether there is a significant difference between two or more groups of sampling units and operates directly on a dissimilarity matrix. If two groups of sampling units are really different in their species composition, then compositional dissimilarities between the groups ought to be greater than those within the groups. The anosim statistic R is based on the difference of mean ranks between groups. Distance values can be interpreted as the average proportion of dissimilarity between two compared groups of communities (Legendre and Legendre, 1998).

4.0 Results

4.1 Phosphorus

Phosphorus is an important plant macronutrient that is a component of key molecules such as nucleic acids, phospholipids, and ATP. Inorganic phosphorus (P) moves by active transport (against a gradient) into mycorrhizal hyphae, passively to the fungus-root interface, and then actively into the root. The hyphae establishes the concentration gradient that favors movement of P to the root interface by the hydrolysis of polyphosphate (Bolan, 1991). The fungal symbiont provides nutrients and minerals and in return accepts photosynthate from the plant. Typically, about 5–10% the total amount of carbon fixed by the plant is contributed to the fungi (Johnson et al., 2005).

The soil available-P concentration is the amount of P which can be extracted or mined by plant roots and utilized for growth and development. Soil P can be described in terms of recently adsorbed labile phosphate, which is readily taken up by biota, and non-labile phosphate which may be occluded, precipitated, or bound to organic matter. Plants use mostly labile P that reaches roots by diffusion. Phosphate transport across the root is usually faster than diffusive transport in soil. Consequently, the concentration of

phosphate in the soil solution immediately surrounding the root is depleted over time (Marschner and Dell 1994).

The small diameter (down to < 2 μm) of mycorrhizal hyphae increases the surface area of absorption and enables hyphal penetration into pores in soils and organic matter that cannot be entered by root hairs. Mycorrhizal hyphae on a unit-weight basis absorb higher amounts of P than non-mycorrhizal plants in soils and the hyphae have a higher efficiency for phosphorus uptake than plant roots (Smith and Read 2008). The hyphae extend as much as much as 12 cm from the root surface (Habte and Osorio, 2001) and exploit pools of available P.

Mycorrhizal hyphae can store larger amounts of absorbed P than plant roots. The bulk of the phosphate is stored as polyphosphates, mainly in granular form. Phosphate stored in solid form in hyphal vacuoles is osmotically neutral and does not increase the differential in the concentration gradient between the hyphae and the soil (Bolan, 1991). The concentration of inorganic P inside the hyphae is approximately 1,000 times higher than that in soil solution (Gianinazzi-Pearson and Gianinazzi, 1986).

Karandashov and Bucher (2005) provides a nice synthesis of the phosphate transport process performed in the mycorrhizal relationship. Essentially, phosphate transporters located in extradical hyphae absorb phosphate and condense it into polyphosphate or incorporate it into nucleic acids, phospholipids and other phosphorylated molecules.

These phosphate-containing compounds are transported into the intracellular mycelium where transfers with the host plant occur through specialized fungal structures (e.g., arbuscules and hyphal coils).

Wetland Soils

Wetland soils typically have ample nutrient reservoirs, though they are not always readily available for uptake by plants (Mitsch and Gosselink, 2007). In fact, P may be a limiting nutrient in many types of wetland systems including northern bogs, freshwater marshes, and deepwater swamps. A substantial proportion of P may be inaccessible to plants because it complexes with organic litter and peat or with inorganic sediments. Under aerobic conditions, insoluble phosphates may be precipitated with ferric iron, calcium, and aluminum.

Under reduced conditions, the soil may release phosphate from ferric phosphate as iron is reduced from Fe^{+3} to Fe^{+2}. The availability of P in wetlands, then, may be determined more by the soil environment and the frequency and duration of flooding than by the magnitude of external inputs. The impact of a reduced soil environment on primary production and decomposition may drive the availability of P in the wetland (Mitsch and Gosselink 2007).

In addition, P may be released from geochemical pools through the hydrolysis and dissolution of Fe and Al phosphates or through the release of clay-associated phosphates through anionic exchange. Under anaerobic conditions, the demand for P may decrease, thus increasing the P available (Mitsch and Gosselink, 2007).

Still, available P in forested wetlands may be limiting to plants. In Georgia Coastal Plain forested wetlands, Wright et. al. (2001) found that resin-extractable P was only 9.5 mg/kg. Jones et. al. 2006 found available P concentrations of between 6.4-12.8 ppm in forested wetlands of southern Alabama. In groundwater-fed wetlands, incomplete decomposition forms peat which may leave P tied up in partially decayed materials. Groundwater flow may periodically flush the small concentration of available P that does accumulate.

The soil P concentrations for February and August are shown in Figure 14. The figure shows the range of observed concentrations and includes the values for the duplicates analyzed. Soil P at the OC1, OC2, and HM2 sites were similar. The HM1 site had the highest levels of available P while the FB2 site had the lowest. Both FB sites were low in available P. The available P concentrations reported by the VT lab using the Mehlich-1 protocol were significantly lower but also indicated that the FB2 site had the lowest P and the HM1 site had the highest P (results not shown).

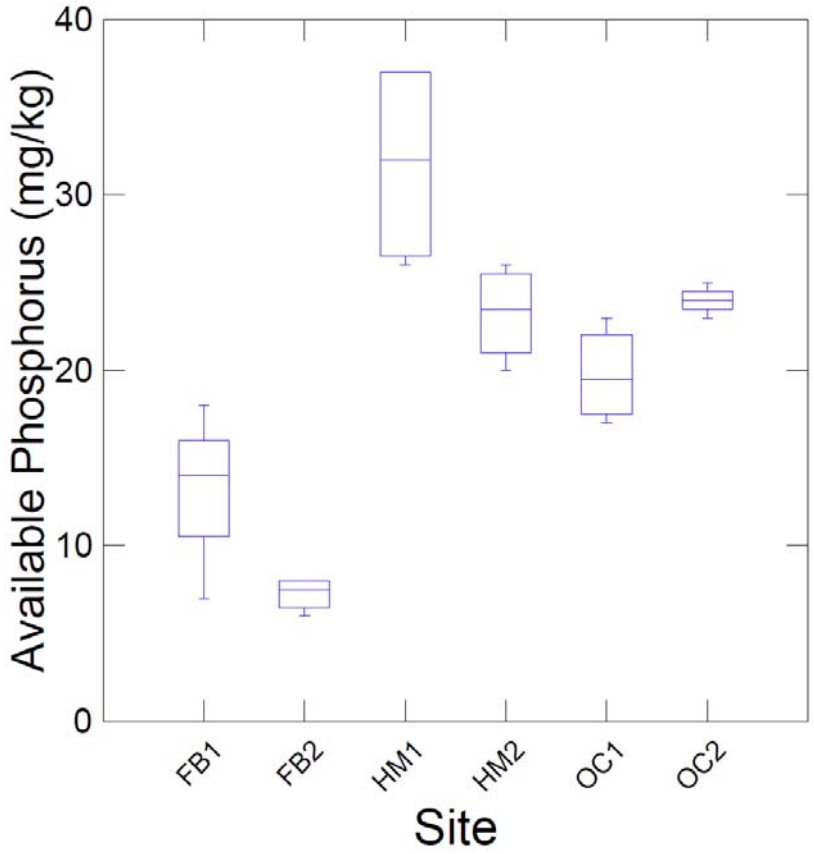

Figure 14. Available Soil P – Combined Summer (August 2007) and Winter (February 2008).
Note: In each box plot, the center horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall. The lines extend out to high and low observed values.

It was expected that the FB1 site would have low available P because the site is groundwater fed and the soil is organic. However, the FB2 site was expected to have higher P concentrations because it received discharge from the storm culvert and ditch and because it had much lower OM content (3.8% vs. 18.3%) and a higher clay content (12% vs. 6%) than the FB1 site. The P adsorption potential of soils has been found to strongly correlate with the percentage of clay (Mozaffari and Sims, 1994; Axt and Walbridge, 1999). Although the ditch water was not analyzed, it is likely that it

contained low levels of P. The drainage area for the culvert was mostly paved and contained only a small grassed area which may not have been fertilized. The mineral soil layer at the FB2 site may have been deposited by a limited number of larger storms. It is possible that the flow conveyed through the ditch was predominantly groundwater that flushed the mineral soils periodically and kept the available P levels low.

The available soil P in August was higher than in February (Table 5 and Figure 15), though not significantly ($p = 0.218$). The FB2 and OC2 sites showed almost no decrease from summer 2007 to winter 2008. Both sites are wetland sites.

Table 5. Available P Concentrations at the 6 Sites.

Table 5. Available P				
Site	**Aug (mean)**	**Std. Dev.**	**Feb. (mean)**	**Std. Dev.**
FB1	16	2.8	10.5	5.0
FB2	7.5	0.7	7	1.4
HM1	37	0	26.5	0.7
HM2	25.5	0.7	21	1.4
OC1	22	1.4	17.5	0.7
OC2	24.5	0.7	23.5	0.7

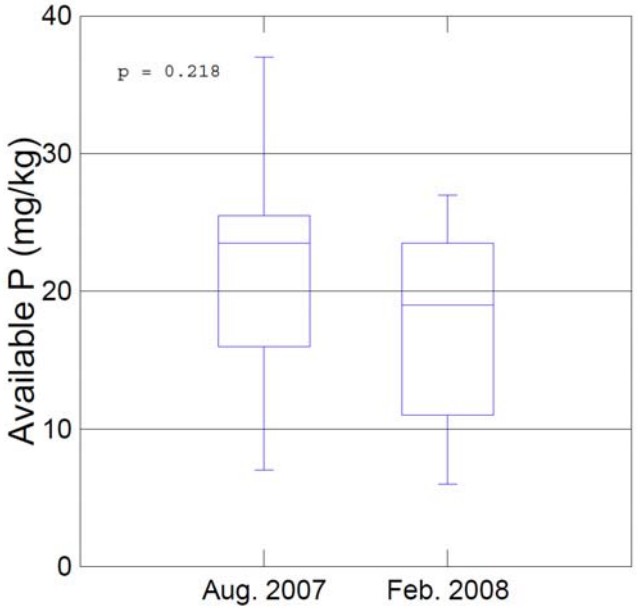

Figure 15. Available P for August 2007 and February 2008 at all sites.

Note: In each box plot, the center horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall. The lines extend out to high and low observed values.

The largest decrease in available P was at the HM1 site, which is a forested upland. Over the August 2007 to February 2008 period, the OC2 site was the only one that exhibited redox levels low enough to reduce iron and potentially release P (see Table 9). As the OC2 site showed only a minimal decrease in soil P concentrations and low redox levels were not observed in August, it is reasonable to conclude that redox potential did not influence the soil P concentrations.

Seasonal trends in available P concentrations in floodplain soils were also observed by Fabre et. al. (2006). The study showed fluctuation points at the beginning of spring (February) and at the end of summer (August-September). However, the study found the

opposite configuration of peaks and dips. The unexpected decrease in available P at the sites reviewed in the present study during a period of low plant utilization suggests utilization by another group of organisms, the microbial community. Zou et al. (1992) determined P immobilization by the microbial community of up to 4.3 mg/kg per day in soils from a red alder stand. Evidence of microbial activity in winter soils is also suggested by gradual decreases in the soil redox potential of the HM2 and FB2 soils between October 20, 2006 and February 23, 2007.

The soil P levels for August were assigned to all spring and summer sampling dates (April 15-August 24), while the February soil P levels were assigned to all fall and winter sampling dates (October 9-February 24) to determine if Soil P explained the variability in percent AM colonization values.. The results show that soil P was not significant in explaining the variability within the 60 colonization counts ($p = 0.221$). The average AM colonization values for the growing season and non-growing season are graphed versus available P in Figure 16 for comparison with the literature values presented in Figure 1. The general trend direction is reversed for colonization as the higher soil P values do not correspond to lower mycorrhizal colonization at my sites.

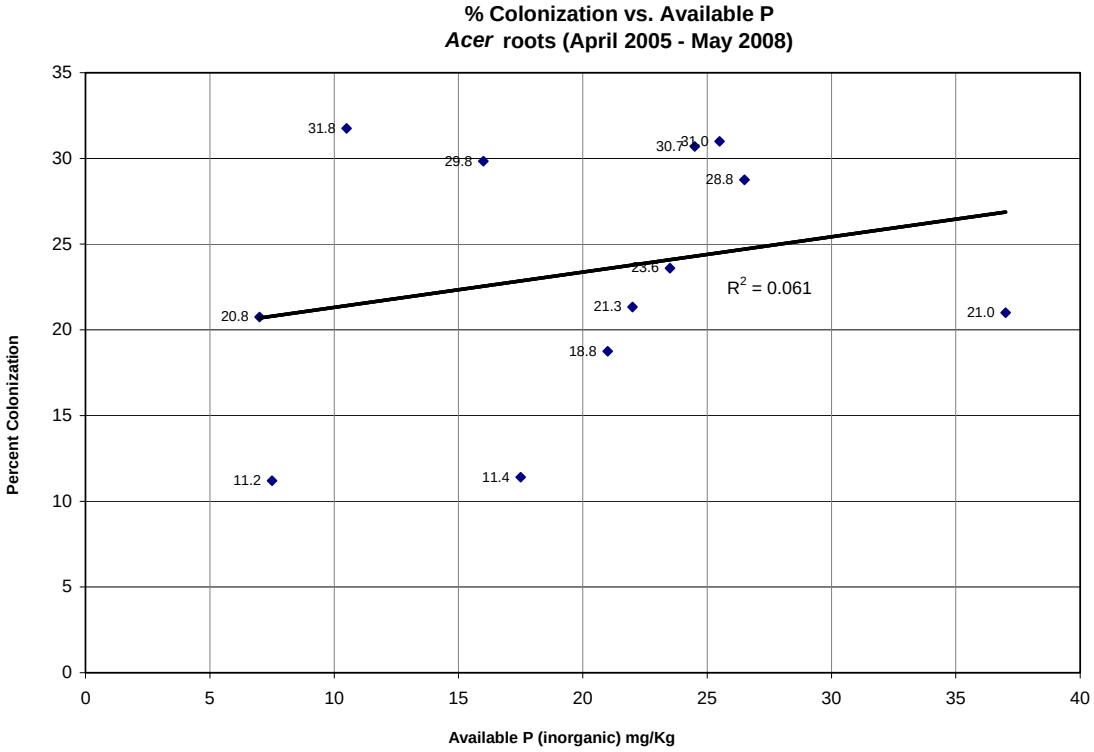

Figure 16. Available Soil P vs. Percent Mycorrhizal Colonization of *Acer* in Coastal Plain Virginia

4.2 Hydrology

When a soil is flooded or sufficiently saturated, water fills the pore spaces between soil particles and decreases the movement of oxygen into the soil from the atmosphere. Once soils are flooded, oxygen is depleted by the metabolic activities of obligate aerobes and facultative anaerobes and the soils show a decline in oxidation-reduction (redox) potential (Eh). When the roots lack oxygen, the plant's ability to transport water decreases, leading to a decrease in water uptake. The stomata close to decrease water loss and, subsequently, photosynthetic activity decreases.

Plants with adaptations allowing them to survive the challenges of water stress are termed hydrophytes, or commonly, wetland plants. Wetland plants are capable of growth in substrates that are at least periodically deficient in oxygen as a result of high water content and can possess various adaptations including physiologic adaptations, anatomic and morphologic adaptations, and life history adaptations that permit them to tolerate temporary, seasonal, or long-term soil anoxia (National Research Council, 1995).

Similar to plants, AM fungi require oxygen and must find ways of adapting when it becomes depleted in wetland habitats.

In general, the period in which hydrologic monitoring was performed (July 2006 – May 2008) was a dry period (Table 6). In 2007, the region experienced the driest year in the

46-year period compiled (1963-2008). However, spring precipitation in 2008 was the highest for the same period totaling 20.09 inches. Consequently, hydrologic monitoring captured both extremely dry and extremely wet conditions.

Table 6. Precipitation During the Sampling Period.

Table 6. Precipitation During the Sampling Period for May-August				
Year	**May Precipitation (Inches) (Historical rank)[1]**	**June Precipitation (Inches) (Historical rank) [1]**	**July Precipitation (Inches) (Historical rank) [1]**	**August Precipitation (Inches) (Historical rank) [1]**
2006	1.84 (8th driest)	12.0 (wettest)	2.46 (17th driest)	1.26 (5th driest)
2007	0.36 (driest)	2.94 (21st driest)	1.77 (6th driest)	1.69 (9th driest)
2008	11.33 (wettest)	Period not sampled	Period not sampled	Period not sampled
[1]Historical rank is for the period of 1963-2008				

Figure 17 present the monitoring results for soil redox potential at the six sites. Figure 18 presents the groundwater level elevations observed in the unlined soil holes located next to the redox probes. The adjusted redox readings for the sites were significantly different (see Figure 19). Differences between the paired sites (locations) were significant for the two upland-wetland pairs (OC1 and OC2 and HM1 and HM2) and not significant for the wetland-wetland pair at the FB site (see figure 20).

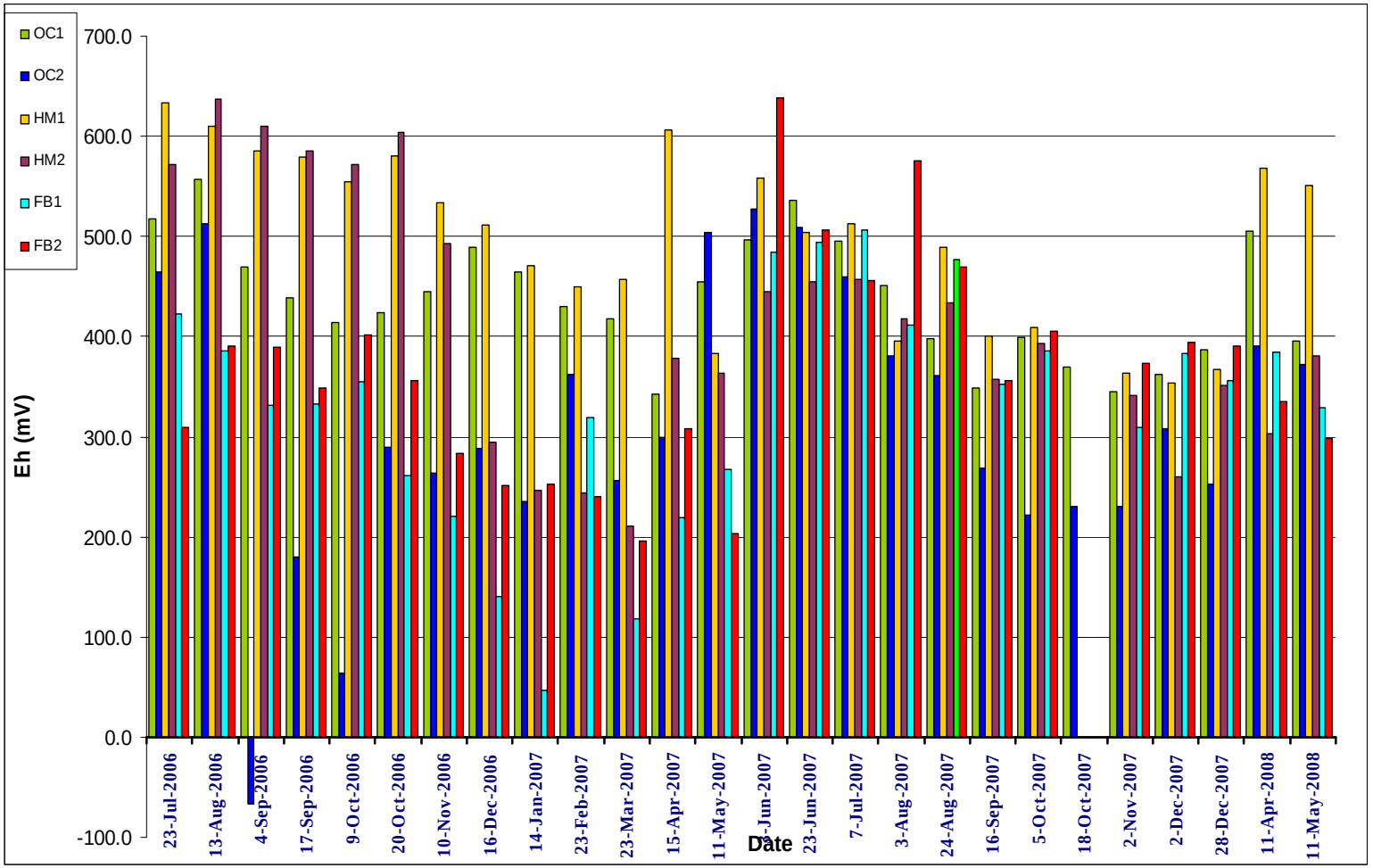

Figure 17. Adjusted Eh Values by Site and Date (July 2006 - May 2008).

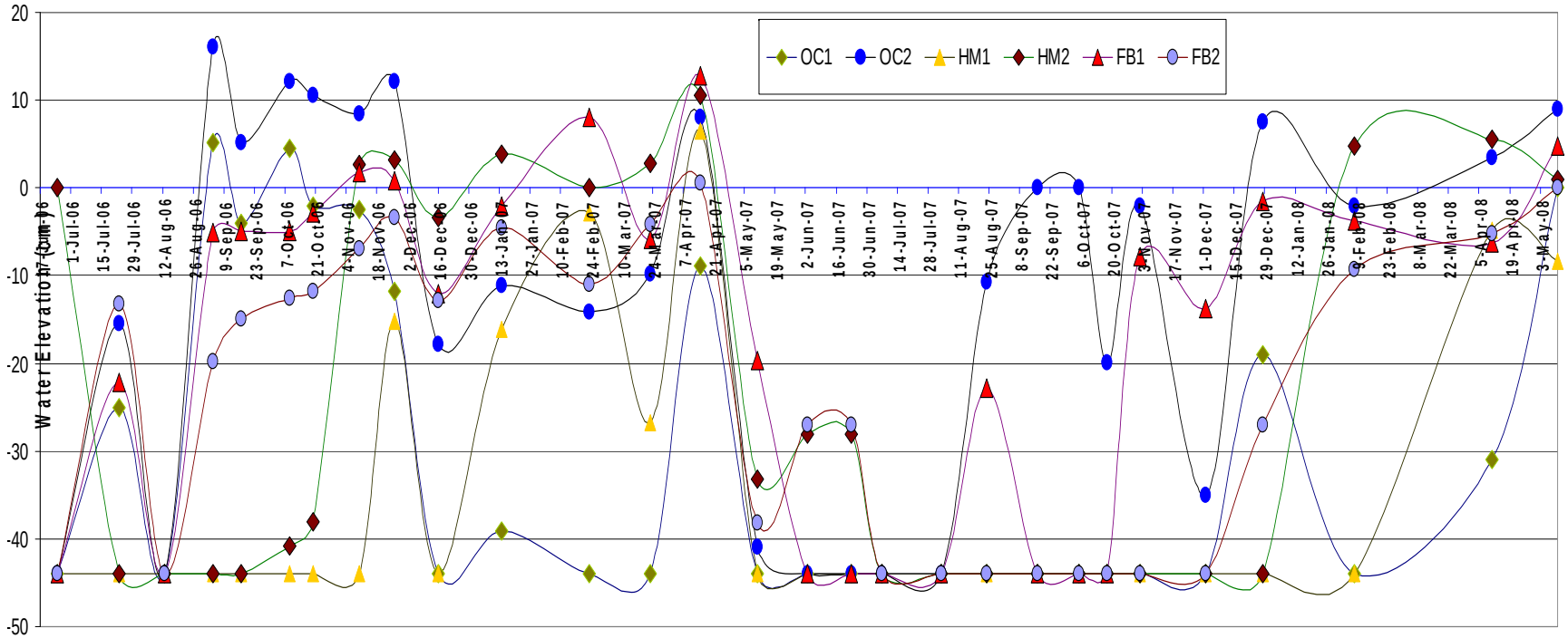

Figure 18. Observed Water Elevations in Unlined Holes (July 2006 - May 2008). *Note: Dry holes are uniformly designated as -44 cm.*

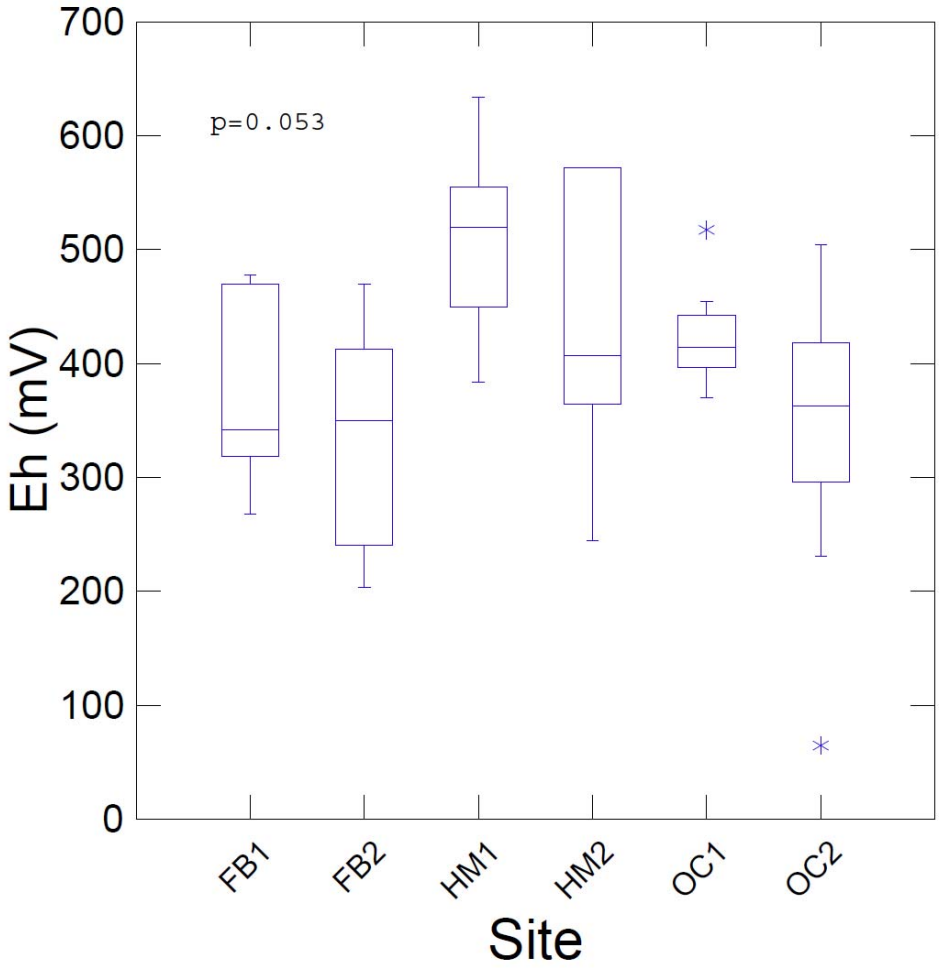

Figure 19. Eh Values for the Sampling Sites (July 2006 through May 2008).

Note: In each box plot, the center horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall. The lines extend out to high and low observed values. Observations marked "*" fall between 1.5 and 3 times the interquartile range shown by the box.

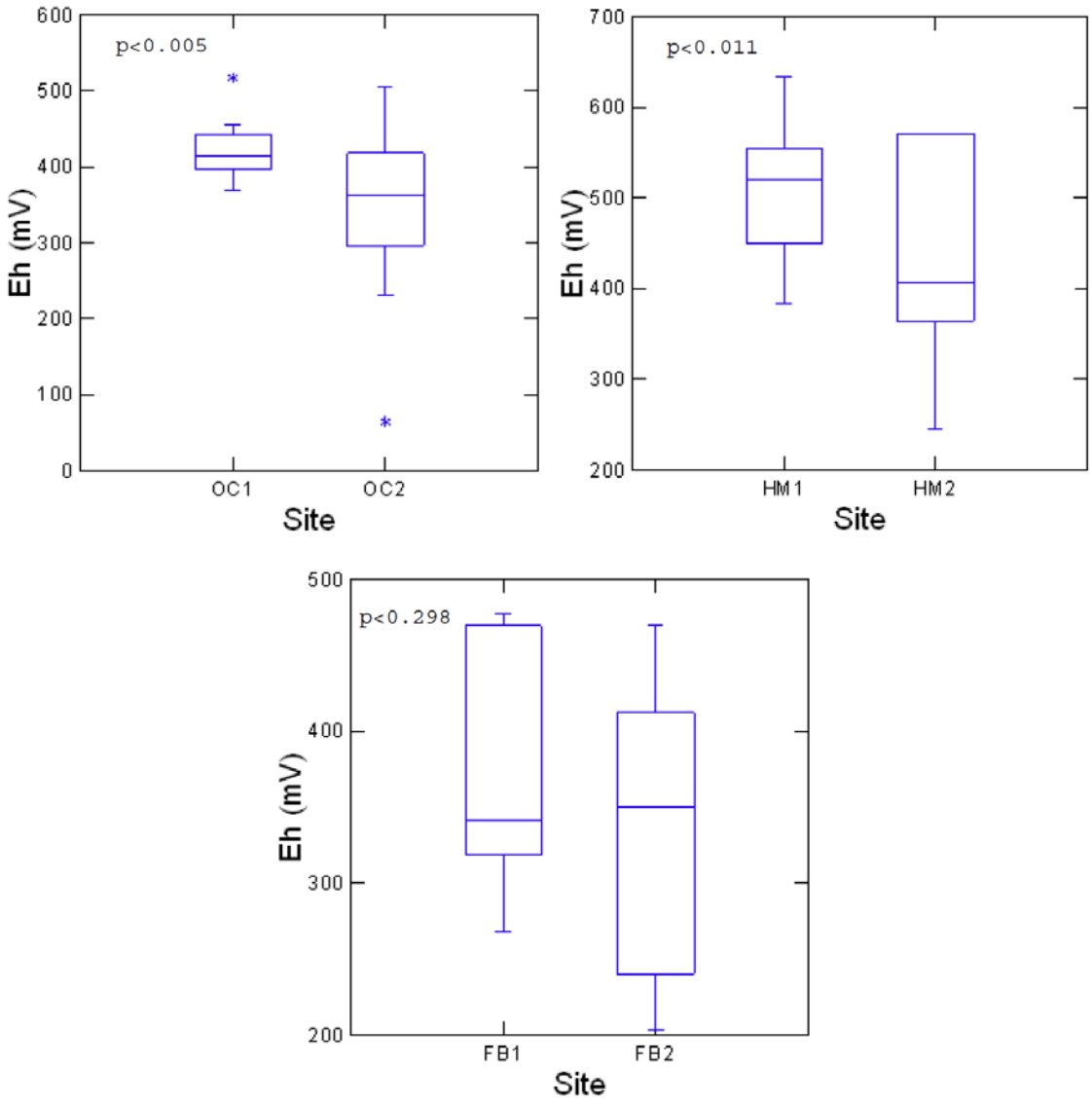

Figure 20. Comparison of the Eh Values Observed at the Paired Sites.

Note: In each box plot, the center horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall. The lines extend out to high and low observed values.

Eh values below the anaerobic threshold were infrequently observed during the sampling period, at least partially due to the low precipitation totals. For a soil to be considered

anaerobic, the Eh must be below the threshold value which varies with soil pH as follows (NTCHS, 2003):

$$\text{Anaerobic Threshold Eh (mV)} = 595 - (60 * \text{pH})$$

Using this equation, the threshold values were derived for the six sites are shown in Table 7.

Table 7. Anaerobic Threshold Values.

Table 7		
Site	**pH**	**Anaerobic Threshold (mV)**
OC1	5.5	265
OC2	5.6	259
HM1	5.2	283
HM2	4.5	325
FB1	4.4	331
FB2	5.2	283

Of the 152 Eh values recorded for the sites, 32 were below the anaerobic threshold (Table 8). The overall average Eh at the FB1 site was essentially equal to the anaerobic threshold. All others averaged Eh values above the anaerobic threshold.

Table 8. Average Eh Values (July 2006-May 2008) with Anaerobic Threshold.

Table 8						
Sample Date	**OC1**	**OC2**	**HM1**	**HM2**	**FB1**	**FB2**
7/23/2006	517.3	464.4	633.5	571.4	422.8	309.3
8/13/2006	557.2	512.1	610.1	636.5	385.1	391.0
9/4/2006	469.0	-67.0	585.6	609.9	330.9	389.9
9/17/2006	439.0	179.4	579.6	585.6	332.5	348.3
10/9/2006	414.1	64.5	555.2	571.6	354.5	401.9
10/20/2006	424.4	289.5	580.6	603.8	261.2	355.6
11/10/2006	444.4	264.0	533.9	492.6	221.1	283.6
12/16/2006	489.5	287.7	510.9	294.3	141.0	250.8
1/14/2007	464.2	235.5	470.3	246.9	46.4	253.0
2/23/2007	430.1	362.3	449.3	244.2	318.6	240.1
3/23/2007	417.5	255.8	456.9	210.1	118.7	195.7
4/15/2007	342.6	299.5	606.1	378.4	219.3	307.7
5/11/2007	454.8	504.3	383.3	363.9	267.8	203.2
6/3/2007	496.5	527.9	557.8	445.0	483.9	638.3
6/23/2007	535.9	509.4	504.5	455.3	494.6	506.5
7/7/2007	495.9	460.0	512.1	457.5	506.6	455.4
8/3/2007	451.1	380.8	395.9	418.0	411.4	575.6
8/24/2007	398.0	361.2	488.7	433.4	477.4	469.5
9/16/2007	348.5	268.9	400.4	357.2	352.3	355.9
10/5/2007	399.1	221.5	409.1	393.6	385.7	405.1
10/18/2007	369.7	230.9				
11/2/2007	344.6	230.3	363.8	341.1	308.7	372.8
12/2/2007	362.4	308.1	353.5	260.1	382.9	394.7
12/28/2007	386.3	252.2	367.2	350.7	355.9	390.9
4/11/2008	505.3	390.4	567.8	302.5	385.0	335.5
5/11/2008	395.6	372.4	550.8	380.6	328.7	297.7
Average	436.6	314.1	497.0	416.2	331.7	365.1
	= Eh at or below the anaerobic threshold value.					

Soil redox potential was not significant in determining the percentage of AM colonization of *Acer* roots ($p < 0.939$). In fact, there was no observed correlation (Figure 21).

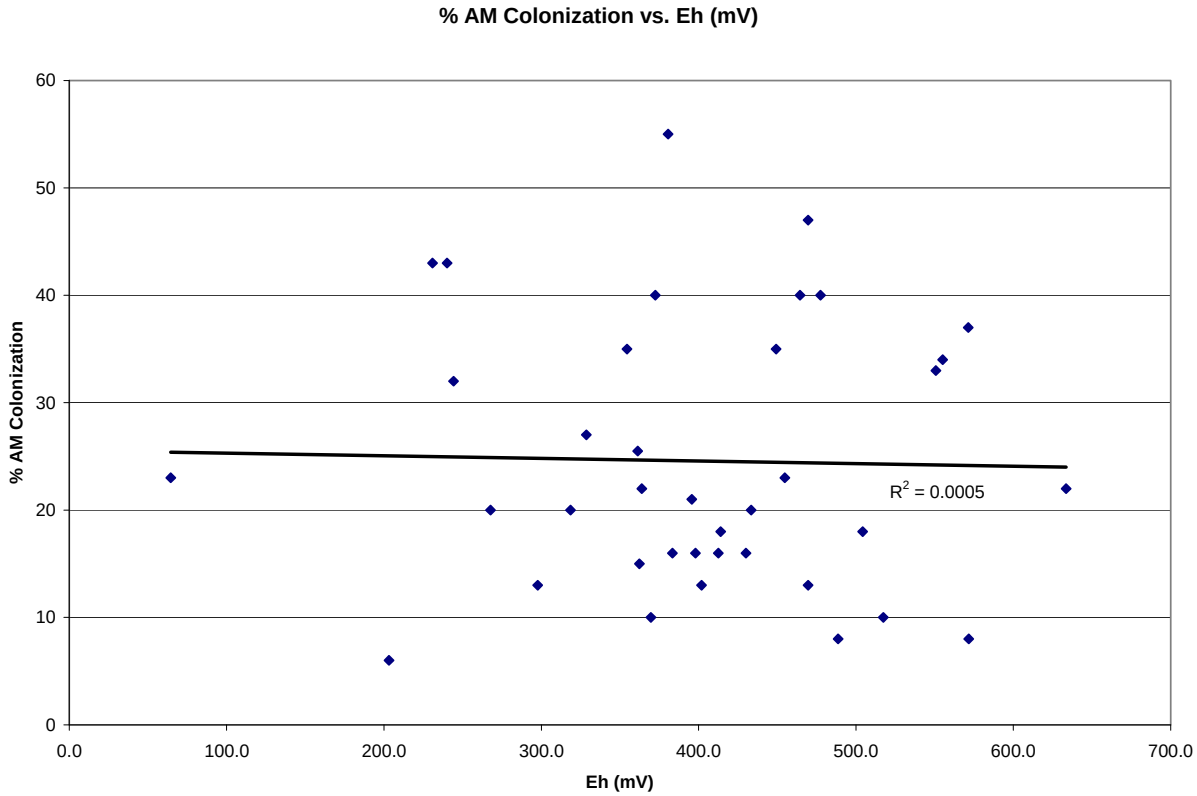

Figure 21. . Relationship between Eh Values and % AM Colonization (July 2006-May 2008).

4.3 AM Colonization

AM colonization of *Acer* roots was evaluated microscopically from field harvested roots collected between April 2005 and may 2008.

The observed percent colonization by AM of *Acer* roots, based on 60 samples collected on 11 dates covering 3 years, ranged from 4% to 55% (Figure 22). The average colonization by site was lowest at FB2 (15%) and highest at FB1 (31%). Both sites are wetlands and are separated by only about 660 feet. All 6 sites sustained colonization levels of at least 38% on at least one date. All except FB1 had minimum colonization levels of 8% or less on at least one date (Figure 23). The differences in colonization by site were significant (p=0.014). AM structures observed were almost entirely hyphae (Figure 22). Antibus et. al. (1997) found red maple roots to be heavily colonized (no percentage reported) with hyphae but arbuscules were infrequently observed.

The difference in percent AM colonization between the paired sites were significant at the FB ($p = 0.003$) and the OC ($p = 0.044$) sites. The difference was not significant at the HM location ($p = 0.774$) (see Figure 24). Season (i.e., winter, spring, summer, or fall) was not significant ($p = 0.849$) in determining percent AM colonization (Figure 25); although generally higher colonization was observed in roots collected during the summer.

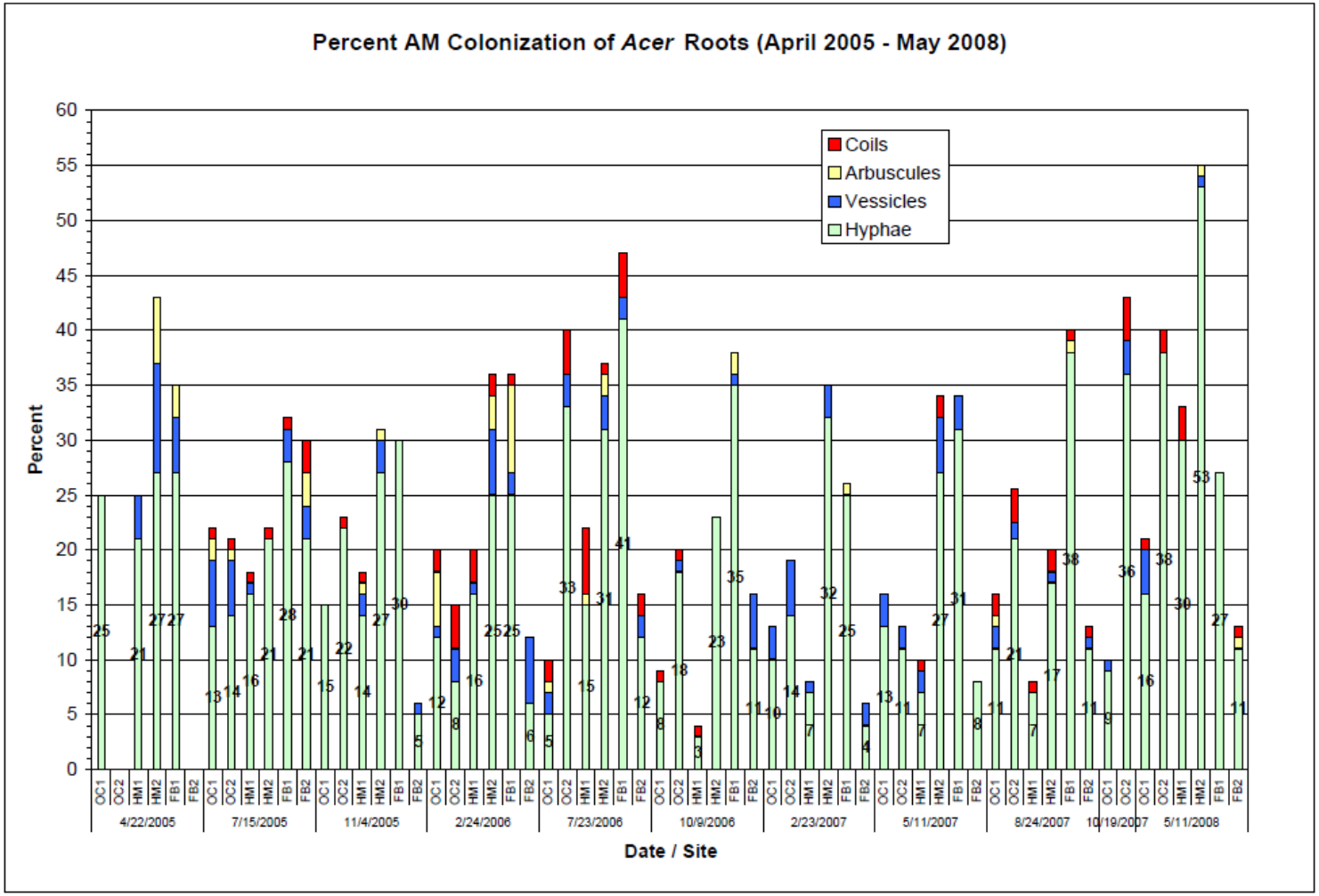

Figure 22. Percent AM Colonization by site and date (April 2005-May 2008).

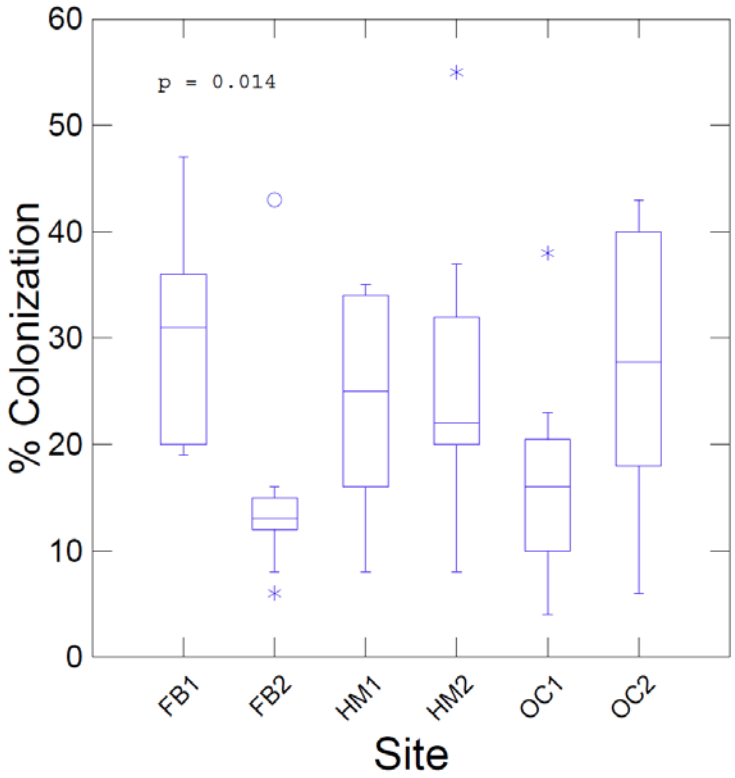

Figure 23. Percent AM Colonization of Acer roots at the sample sites (April 2005-May 2008).

Note: In each box plot, the center horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall. The lines extend out to high and low observed values. Observations marked "*" fall between 1.5 and 3 times the interquartile range shown by the box.

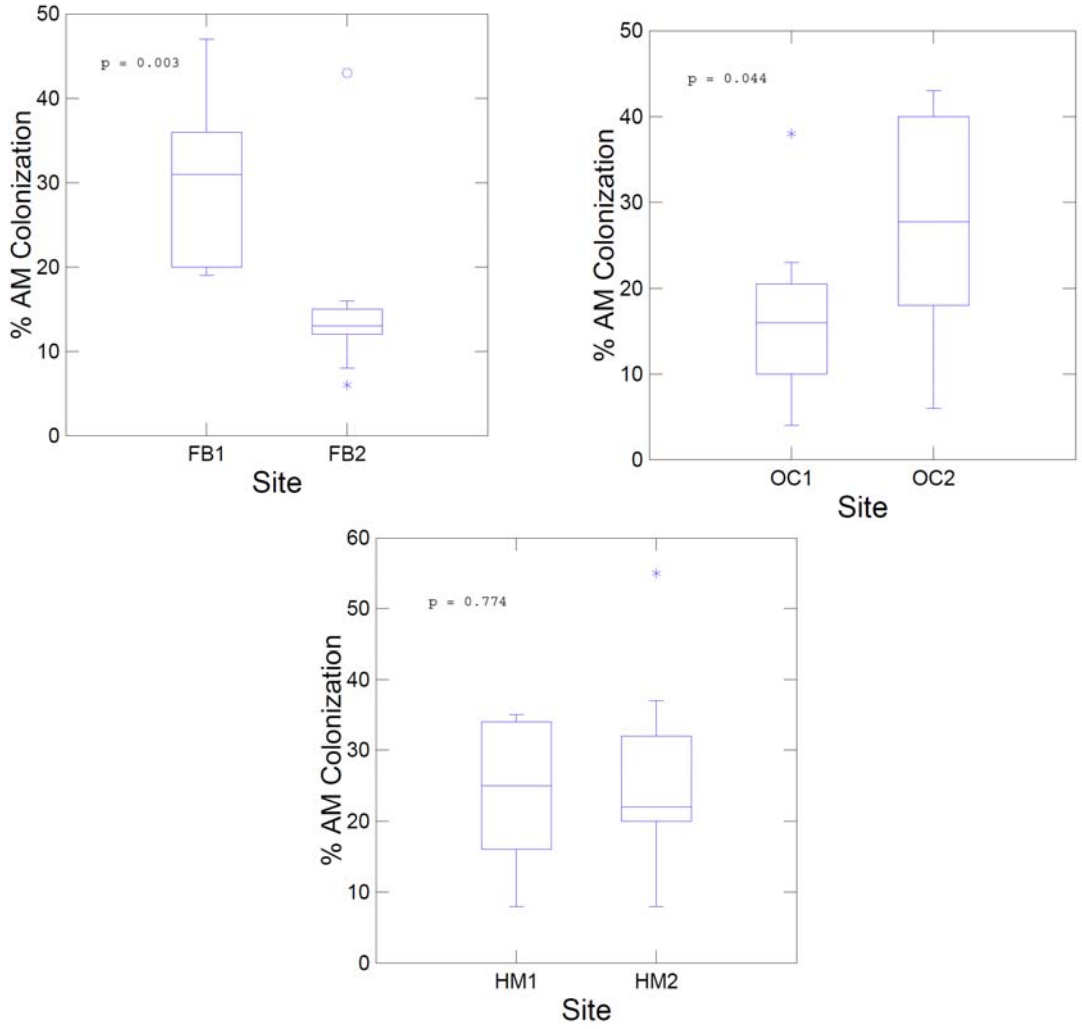

Figure 24. Comparison of percent AM Colonization between paired sites.

Note: In each box plot, the center horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall. The lines extend out to high and low observed values. Observations marked "*" fall between 1.5 and 3 times the interquartile range shown by the box.

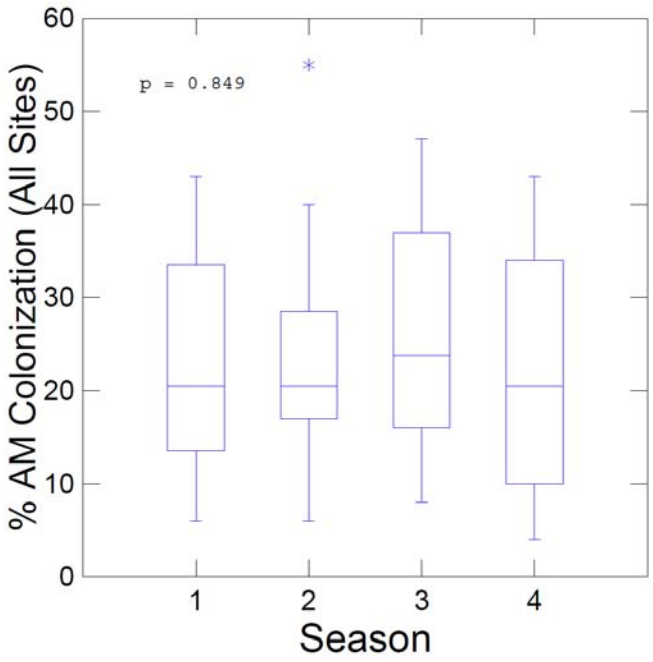

Figure 25. Percent AM Colonization of Acer roots at the sample sites by season.

1=winter, 2=spring, 3=summer, and 4=fall. Note: In each box plot, the center horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall. The lines extend out to high and low observed values. Observations marked "*" fall between 1.5 and 3 times the interquartile range shown by the box.

4.4 Fungal Community

A total of 114 replicable fluorescent peaks representing fungal OTUs were detected through the ARISA community fingerprinting of 155 PCR replicates derived from 60 root DNA extracts. The total number of OTUs observed at each for all dates ranged from 29 (OC1) to 64 (HM2) (Table 9); although the number of individual fungal OTUs observed at each site on a single date was much lower and ranged from 1 to 29. In fact, all 6 sites had individual sampling dates in which 4 or less OTUs were identified. This indicates that the number and composition of OTUs present on each date varied within each site. Only 13 OTUs occurred in 20% or more of the samples and only three were found in over a third of all samples. One OTU, 278.72, occurred in at least one sample from all 6 sites; although, overall, it occurred in only 65% of the samples. Fungal species-saturation was not met for the pooled data, but the majority of fungal OTUs were captured (about 88% of the incidence-based species estimation).

The total fungal community structure for each site is summarized by Figures 26-28 in terms of percent mean relative abundances of fungal OTUs. In order to interpret these results, ARISA data was subjected to Shannon diversity metrics (see Table 10 and Figure 29), the multivariate ordination methods of Principal Coordinate Analysis (PCO) and Canonical Correspondence Analysis (CCA), as well as similarity analysis based on mean Bray Curtis distances.

Table 9. Fungal Diversity.

Table 9 Fungal Diversity						
	FB1	**FB2**	**HM1**	**HM2**	**OC1**	**OC2**
Total No. of OTUs	41	47	50	64	29	44
Mean Shannon's Index	1.904 (0.33)	1.633 (0.55)	1.896 (0.045)	2.394 (0.37)	0.843 (0.48)	1.663 (0.52)
Mean Evenness	0.860 (0.06)	0.811 (0.08)	0.823 (0.14)	0.837 (0.11)	0.512 (0.26)	0.808 (0.08)

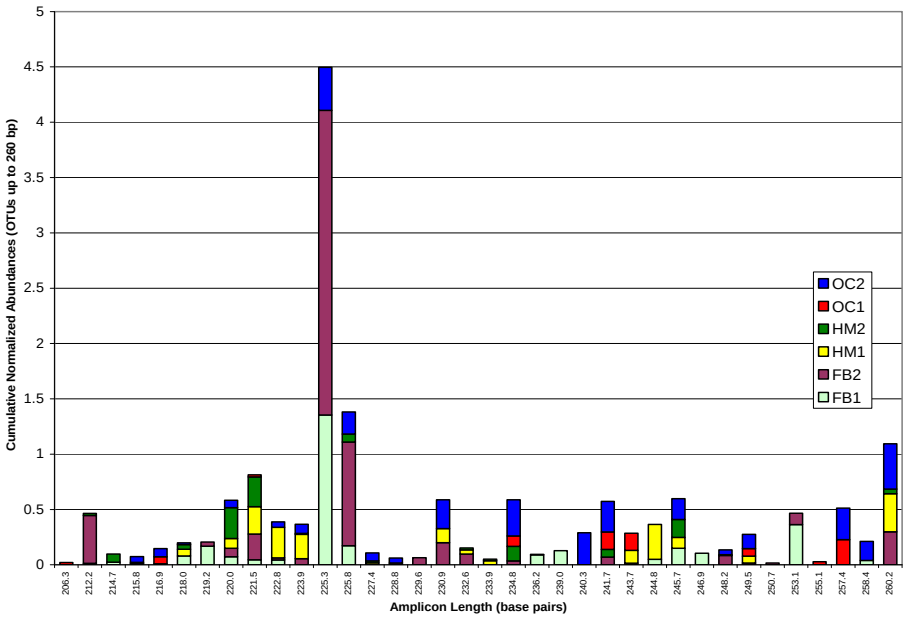

Figure 26. Cumulative abundance by OTU for each site (bp size 206-260).

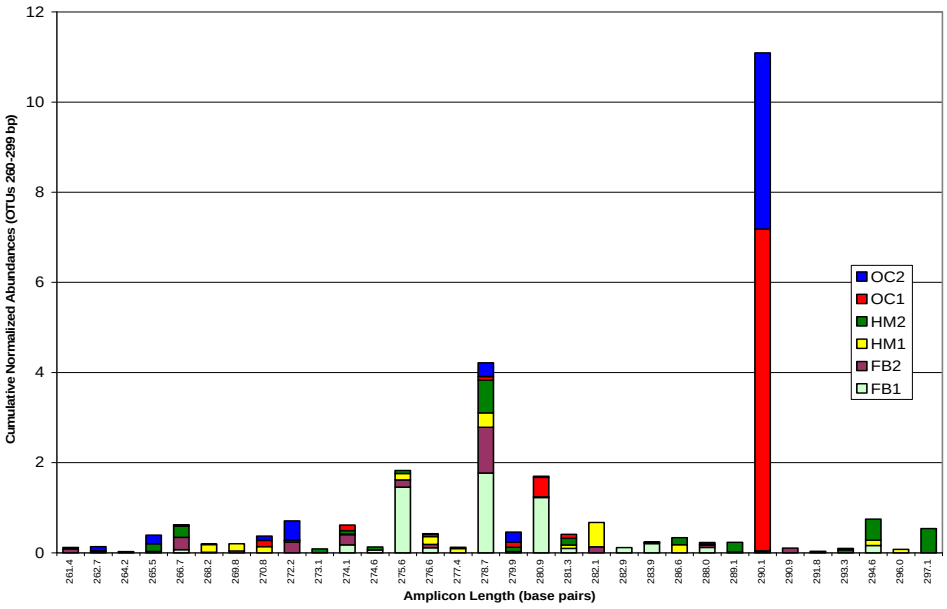

Figure 27. Cumulative abundance by OTU for each site (bp size 261-300).

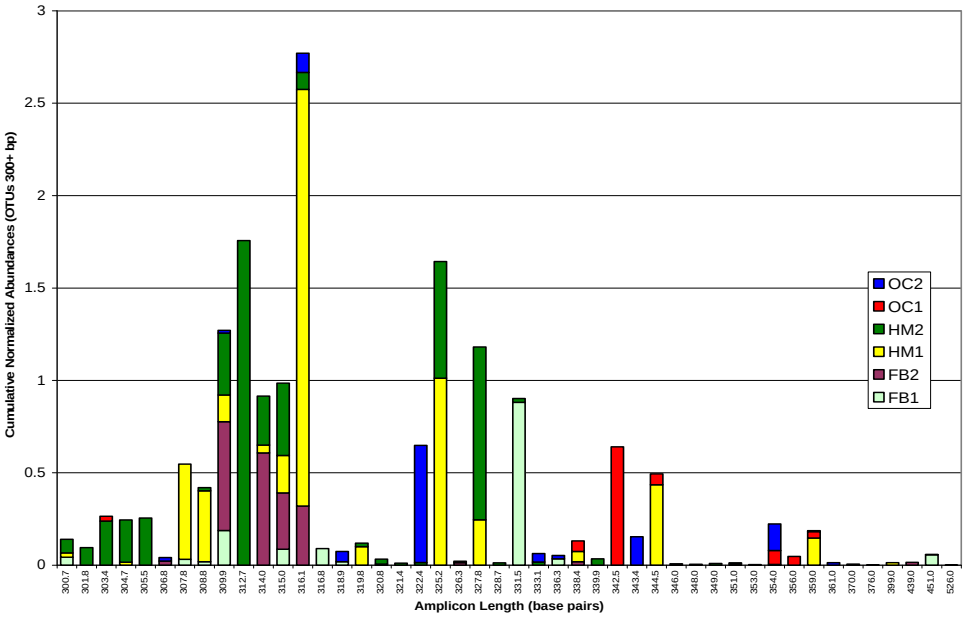

Figure 28. Cumulative abundance by OTU for each site (bp size >300).

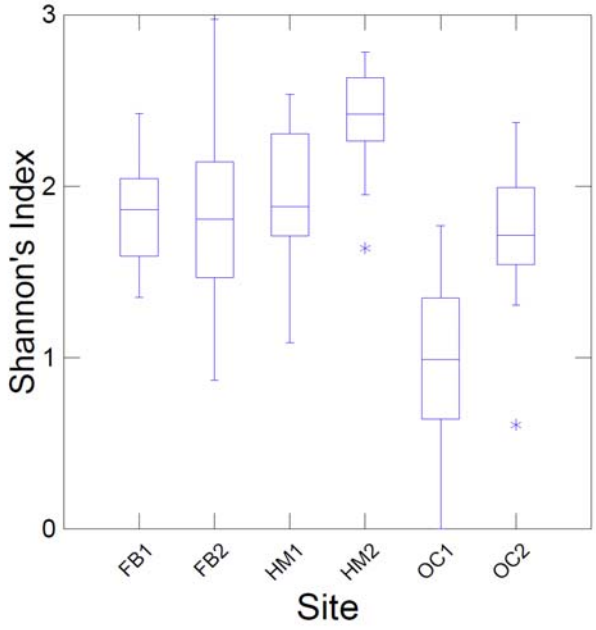

Figure 29. Shannon's Index for all samples.

Note: In each box plot, the center horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall. The lines extend out to high and low observed values. Observations marked "*" fall between 1.5 and 3 times the interquartile range

Diversity of fungal OTUs differed significantly by site ($p < 0.005$). However, based on the median Shannon's Index values, FB1, FB2, HM1, and OC2 exhibited similar levels of diversity; although the range of observed values for FB2 was notable (Figure 29). The extremes were anchored by the HM2 site (2.394 ±0.37) and the OC1 site (0.843 ±0.48). Fungal diversity between sites differed significantly for both the OC ($p = 0.004$) and the HM ($p = 0.018$) site pairs. However, the FB site pair did not differ significantly ($p = 0.720$). Because the FB site pair are both wetland sites, a comparison of fungal diversity between the pooled wetland site community and the pooled upland site community was conducted and the difference was significant ($p = 0.003$). However, because there are only 2 upland sites, the difference could be attributed to the particularly low diversity of the OC1 upland site.

Season has been identified as a variable affecting fungal community composition (Burke et. al., 2009). However, the pooled Shannon's Index values did not vary significantly by season ($p = 0.407$).

The Principal Coordinate Analysis of all fungal communities separated the communities into 3 clusters based on the 3 paired sampling locations (FB, HM, and OC). As displayed on Figure 30, Axis 1, which explained over 26.9% of the total variance, mostly separated the OC fungal communities. Axis 2, which covered an additional 13.6% of the variance between samples, largely differentiated the FB communities and the HM communities from the others. Axis 3, which explained 8.23% more of the variance,

separated the HM1 and HM2 communities. It took 16 axes to define 90% of the variance. However, the first 8 principle axes explained a cumulative 72% of the variance.

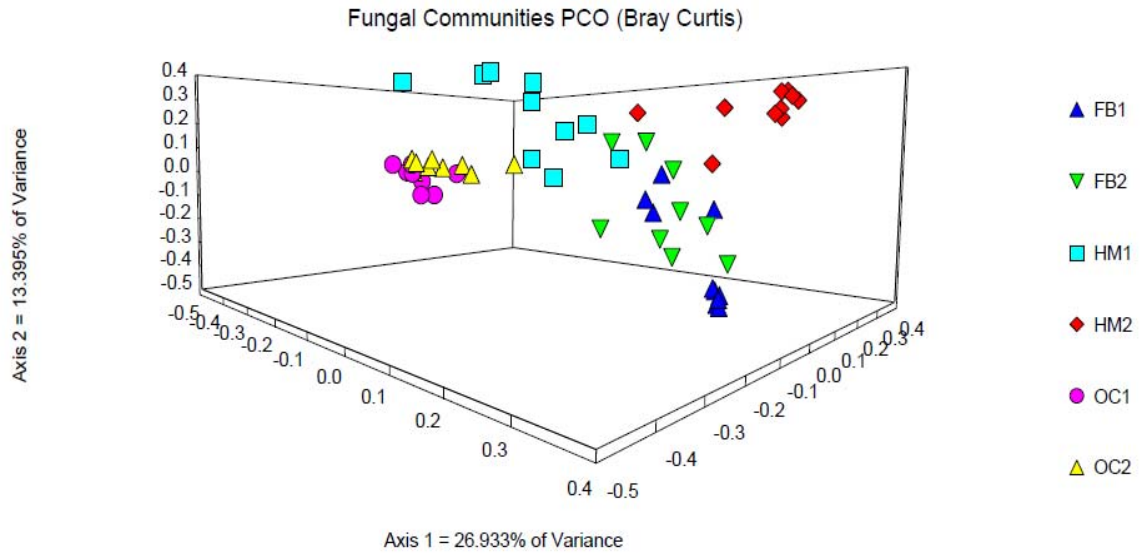

Figure 30. Principal Coordinate Analysis of Fungal Communities.

Canonical Correspondence Analysis

CCA is practically an ordination of community data followed by a multiple regression analysis between the ordination and a corresponding environmental dataset. It provides the benefits of PCO along with biplot vectors for the relationships between the environmental variables and the species variable-based ordination and graphically displays the statistical relationships between the community structures, the composing

species, and the surrounding environment. Figure 31 projects soil chemistry variables (pH, P, OM, Fe, Mn, K, Ca, and CEC). The overall test for differences between sites was significant at $p = 0.005$. The first 5 canonical axes were significant at ($p < 0.05$). Four of the environmental variables P, CEC, K, and Mn, were statistically significant at ($p < 0.05$) using the margin test. Calcium (Ca) was not significant and the remaining variables were borderline significant ($p < 0.1$).

Soil redox is excluded as a variable in the overall CCA output for two reasons. First, preliminary ordinations indicated that it was not significant. Second, root samples were collected in 2005 and early 2006 prior to installation of the platinum probes. Consequently, correlations with the ARISA output could only be evaluated for 37 samples. A separate evaluation of environmental variables that included soil redox was necessary. The CCA for the 37 samples and the redox biplot vector are shown in Figure 32. As can be seen in the figure, redox was not significant in determining the fungal community using the smaller set of data. Using the margin test, redox was not significant at $p < 0.05$.

Season is also not included in the overall CCA output. The effect of season on the fungal community was evaluated using ANOSIM (analysis of similarity) which revealed that season was insignificance ($p = 0.258$).

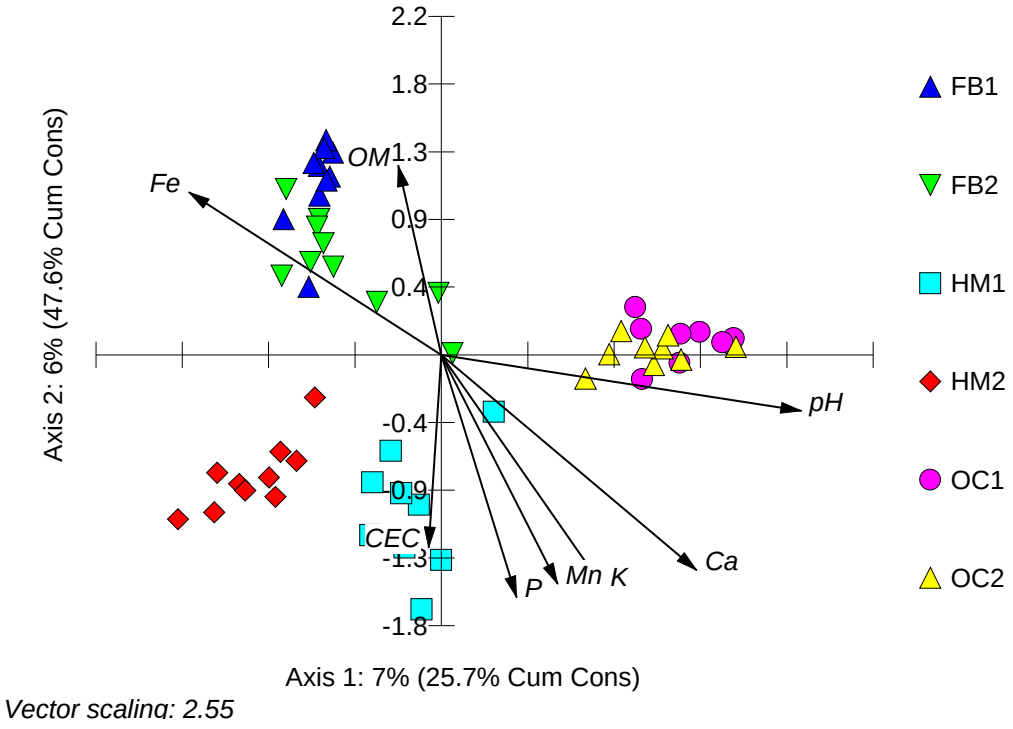

Figure 31. Canonical Correspondence Analysis of all fungal communities with soil chemistry variables.

As presented in Figure 31, the CCA of root fungi also separated fungal community structures by the 3 paired sampling locations while displaying (as biplot vectors) the direction and relative magnitudes of greatest change of the environmental variables. The first two axes were environmentally constrained and explained 13% of all variance and 47.6% of the cumulative constrained percentage of variance. Soil pH dominated Axis 1 with a biplot score of 0.836. Iron (Fe) and Calcium (Ca) also had high biplot scores of -0.468 and 0.453, respectively. All variables exhibited high biplot scores on Axis 2, as all 8 had magnitudes above 0.400. Ca (-0.859), K (-0.836), Mn (-0.835), and P (-0.831) were the highest.

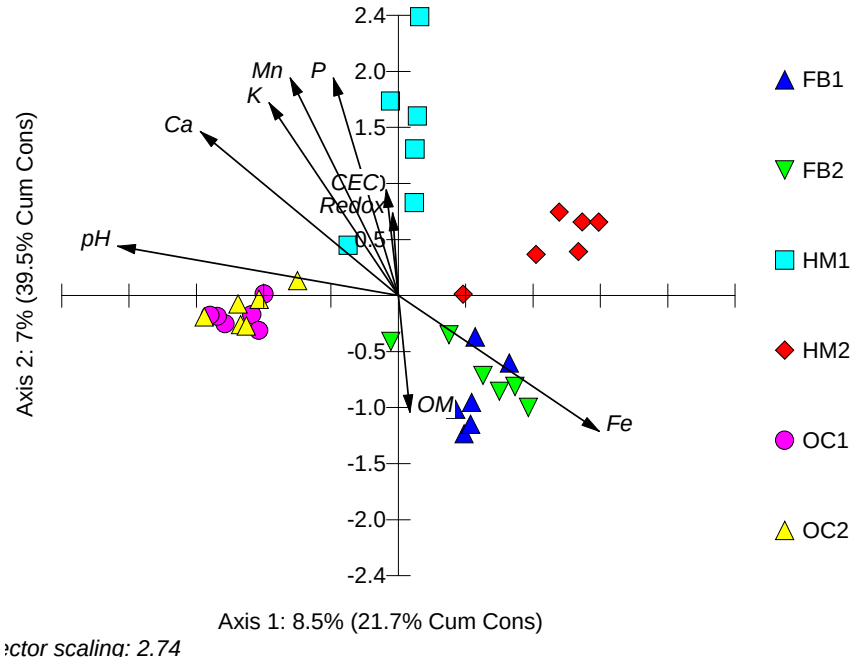

Figure 32. Canonical Correspondence Analysis of 37 fungal communities with soil chemistry variables, including redox.

Thus it appears that overall fungal community structures differed by site, but the recorded environmental variables explained a relatively small percentage of the fungal community structure. However, many of the environmental variables did correlate with the occurrence of some fungal OTUs. The OTUs with species-environment correlations of 0.6 magnitude and above for the constrained axes were evaluated with subsequent CCAs to determine their importance to the ordination results relative to the environmental variables (biplot vectors). Based on this screening, a total of 17 OTUs were found to

underlie most differences between fungal communities. Figure 33 shows the improved explanation of community variation when only the 17 primary OTUs are modeled. The explanation of total variance nearly doubles from 13% (Figure 31) to 25.8%.

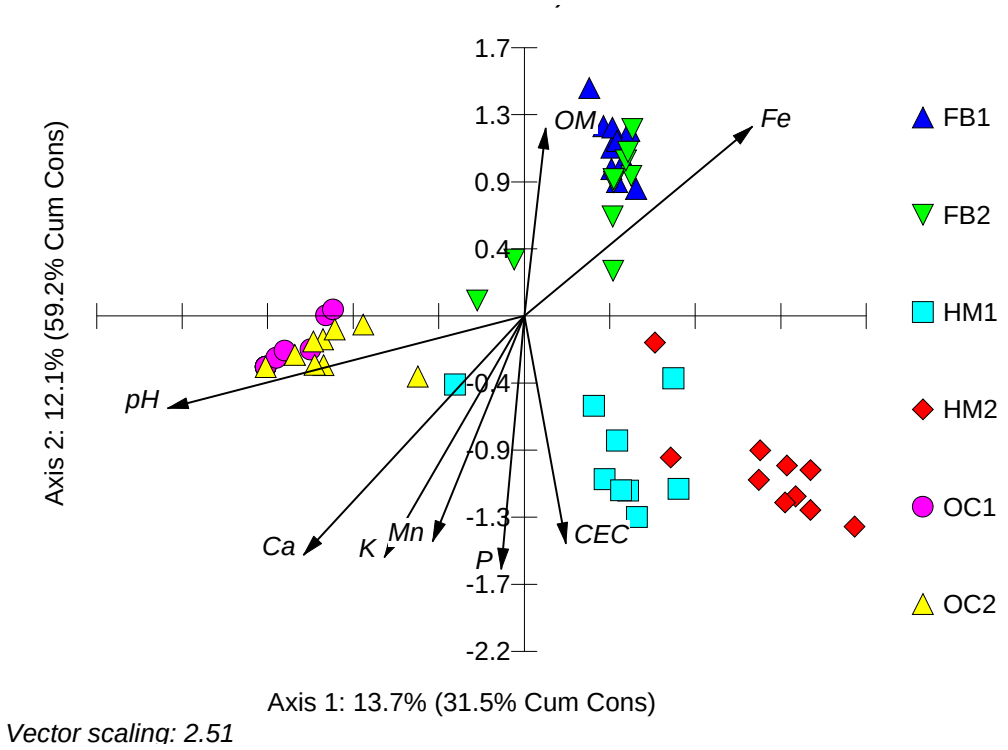

Figure 33. Canonical Correspondence Analysis of all fungal communities, 17 major OTUs only, with soil chemistry variables.

Figure 34 shows a CCA with only the 17 major OTUs and correlations with the soil chemistry vectors. Of course, the soil chemistry variables observed at each site are distinctive as well. For example, the overall CEC and P values are highest at the HM sites. Table 10 indicates the OTU/soil variable/site pair relationships.

Table 10. Relationships between 17 major fungal OTUs, soil chemistry variables, and site pairs.

Table 10		
Fungal OTUs	**Soil Chemistry Variables**	**Site Pair**
260, 263, 272, 290	pH and Ca	OC
225, 226, 276, 279, 281, 314, 331	OM and Fe	FB
246, 297, 313, 316, 325, 328	CEC and P	HM

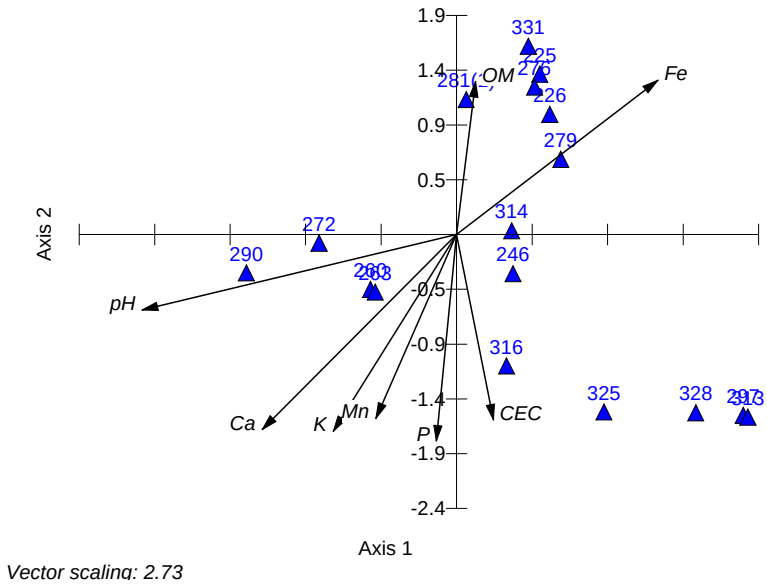

Figure 34. Canonical Correspondence Analysis of 17 major OTUs with soil chemistry variables.

The CCAs suggest that there are species-environmental correlations by site and that the 17 most abundant fungal OTUs are those most correlated/most responsible for the distinctions in community structure. OTU 290.1 clearly distinguishes the OC sites from the others. This OTU occurs in all of the OC samples, none of the FB samples, and at low levels in 4 of the HM samples (all winter samples). Figure 27 graphically displays the dominance of this OTU.

All sites significantly differed with all others in terms of OTU composition and, with the exception of OC1 (due to strong dominance by OTU 290), the norm was for samples within a site to share 37% (63% dissimilar) or less relative abundances in fungi (total fungal community structure). Sites compared between the 3 paired locations generally had 10% or less overlap in fungal OTUs. Examined collectively, the sites pool together by location (site) because the fungal communities are less dissimilar within locations than they are dissimilar between locations. The environmental variables explain the clustering by location (or dissimilarity between locations).

To quantify the distances between locations with those within the sites, Table 11 provides a ranking of comparisons by average Bray distances at 95% Confidence (Tukey's 'Honest Significant Difference' method following ANOVA for Between Group Differences).

The OCI site was dominated by a single OTU, 290.1, and had the lowest diversity of the 6 sites. Consequently, OC1 had the lowest internal variation in community structure (average Bray distance). The OC2, HM2, and FB1 sites exhibited roughly equivalent internal dissimilarity, but the HM2 site had a lower standard deviation, indicating more stability. The HM2 site also had the highest Shannon’s Index diversity score. The FB2 and HM1 sites had greater internal dissimilarity, with the HM1 communities showing the least similarity between them.

Table 11. Dissimilarity Measures (average Bray Distances).

Table 11. Dissimilarity Measures		
Group Comparisons	**Average Distance Between**	**Standard Deviation**
OC1 vs. OC2	0.592^{A}	0.189
FB1 vs. FB2	0.808^{B}	0.128
FB2 vs. HM2	0.881^{C}	0.060
HM1 vs. HM2	0.888^{CD}	0.080
FB1 vs. HM2	0.901^{CD}	0.052
FB2 vs. OC2	0.918^{CD}	0.108
FB2 vs. HM1	0.920^{CD}	0.074
FB1 vs. HM1	0.943^{D}	0.058
HM2 vs. OC2	0.948^{D}	0.046
FB1 vs. OC2	0.950^{D}	0.086
HM1 vs. OC2	0.958^{DE}	0.053
FB1 vs. OC1	0.961^{DE}	0.054
HM1 vs. OC1	0.980^{DE}	0.032
HM2 vs. OC1	0.982^{DE}	0.033
FB2 vs. OC1	0.988^{E}	0.026
Site Comparisons	**Average Distance Within**	**Standard Deviation**
OC1	0.349^{A}	0.096
OC2	0.630^{B}	0.222
HM2	0.630^{B}	0.162
FB1	0.638^{B}	0.237
FB2	0.740^{BC}	0.208
HM1	0.796^{C}	0.197
^{ABCDE} - Group comparisons with the same assigned letter(s) were not significantly different whereas those not sharing the same letter were significantly different at 95% confidence according to Tukey's HSD.		

Comparing dissimilarity between sites, the OC1 site is the least similar to all of the other sites, except the OC2 site. The least observed average Bray distance was between OC1 and OC2 and the greatest average Bray distances were observed between OC1 and FB2, OC1 and HM2, OC1 and HM1, and OC1 and FB1 (in order of decreasing dissimilarity).

In terms of relative distinctiveness of the fungal communities, OC sites were significantly more similar than FB sites which were, in turn, significantly more similar than HM sites. The OC and FB sites were noticeably coherent within their locations whereas HM sites differed considerably both with each other and with the other two locations.

Site location exerted a greater effect over community structure than did upland versus wetland status. The two upland sites (HM1 and OC1) were equally to significantly less similar than all upland/wetland comparisons, which is consistent with the PCO and CCA (see Figures 30 and 31) .

5.0 Discussion

5.1 Phosphorus

Contrary to my hypothesis, increasing soil P concentrations, within the observed range of 7-37 µg/g, did not reduce mycorrhizal colonization in *Acer* roots. For example the spring/summer colonization of the FB2 site averaged 11.2% while the soil P concentration was only 7.5 µg/g. Conversely, the spring/summer colonization of the HM1 site averaged 21% with the highest observed soil P concentration (37 µg/g).

Schachtman et. al. (1998) suggested that high available soil P may not directly regulate the activity of the fungus. Rather, specific signals from the plant may cause lower mycorrhizal colonization. Signals from the plant host that would promote colonization would be based on whether or not the plant was experiencing a deficiency of P. The opposite signal, colonization inhibition, would result from the plant not experiencing any deficiency. In this case, a plant host-specific threshold soil P concentration would drive the eventual inhibition of mycorrhizal colonization. Based on the literature review, this threshold may be above 25 µg/g. Only 1 of my sites had soil P concentrations appreciably above this threshold. Further, this threshold value would vary seasonally with the differing demands of the plant host. I have suggested that the P demands for

Acer would be highest in the timeframe of early spring, coinciding with the appearance of blooms and then the production of leaves. However, my results did not identify the month or season as significant in determining mycorrhizal colonization. Overall, results from July were the highest while results from August were the lowest, but the differences were small.

Based on the lack of a spring "peak" in colonization, the study trees were apparently able to acquire the necessary P reserves to sustain growth and development without increased mycorrhizal colonization. Consequently, the observed range of soil P concentrations were apparently within the range needed by the trees using their root systems augmented by a minimal level of acquisition by mycorrhizae. It has been shown that under most conditions, plants obtain at least some P via the mycorrhizal pathway (Smith et al. 2004), if one is established. It is possible that rather than reduce overall colonization, the development of arbuscules and other transfer structures is inhibited. Arbuscules are thought to be the primary intracellular transfer location between host plant and fungi. Arbuscules were uncommon in *Acer* roots observed in this study and Antibus et. al. (1997) also reported infrequent occurrence of arbuscules in red maple roots. However, arbuscules are short-lived structures and are often absent or hard to see (due to root age and pigments) in field-collected roots (Brundrett et al., 1996). Consequently, assumptions about a lack of AM functionality based on minimal arbuscular presence may be incorrect. Although little is known about whether fungal structures are signaled by the host plant or are just reoccurring functions of the fungus, Maeda et al. (2006) found that

the physiological state of plants (sufficient P uptake or not) affects the full development of fungal structures.

5.2 Hydrology

Observed Eh values (between -67.0 mV and 638.3 mV, mean = 394.5 mV ± 125.0 mV) were generally within the range reported by Vasilas et al. 2004 for forested upland and wetland sites located within the same region as this study (Maryland and Delaware portions of the Coastal Plain). Although the FB1 site was the only one that averaged Eh values below the anaerobic threshold, it was saturated on dates that it did not meet the anaerobic threshold (see Figure 18). Turner et. al. (2000) found low but significant levels of dissolved oxygen in the root zone of fens, presumably transported by flowing groundwater.

Contrary to my hypothesis, soil redox potential was not significant in determining the percentage of AM colonization of *Acer* roots ($p < 0.939$). In fact, there was no observed correlation (Figure 21). Comparing the specific AM colonization on dates where the soils were at or below the anaerobic threshold versus dates that the soils were above the threshold also showed no significant difference ($p < 0.590$). Christensen and Wigand (1998) found no significant difference in AM colonization of *Lobelia dortmanna* (Water Lobelia) and water depth and Eh (observed values between 89-496 mV). The results in the present study can be explained by two factors: first, only moderate Eh values were

observed at the wetland sites; and second, studies indicate that AM likely use oxygen from roots to sustain themselves. The *Acer* develop hypertrophied lenticels and the HM2 soils exhibited oxidized rhizospheres, an indication of fine roots leaking oxygen into a reduced environment.

Both the OC2 and HM2 Eh values were significantly lower than their upland paired sites (Figure 20). Comparing AM colonization observed in the two upland-wetland paired sites, the percent colonization was significantly different between OC1 and OC2 ($p < 0.045$) and the OC2 (wetland) site had the higher AM colonization. There was not a significant difference in the AM colonization of the HM1 and HM2 sites ($p < 0.873$). The results are consistent with the optimal soil moisture findings of Lodge (1989) and Cantelmo and Ehrenfeld (1999). That is, the Eh values observed in the present study did not reach or sustain levels necessary to inhibit the presence of normal AM colonization levels observed throughout the period of study.

At the study sites and at forested wetlands in general, soils are subjected to seasonal or intermittent saturation, flooding, or ponding. Certainly, the sustained periods of aerobic conditions at the sites would allow colonization without inhibition. Under these conditions, the observations of Miller and Sharitz (2000) that AM fungi could colonize roots under dry conditions and then sustain themselves through wet periods would seem to apply.

Although the mechanism for supplying oxygen to AM fungi during periods of anaerobisis has been suggested, more research is needed to demonstrate the process and quantify the amount of oxygen required by the fungal symbiont. Questions remain about how the AM fungi move the borrowed oxygen to distal hyphae in an anaerobic environment and if the AM fungi continue to function as harvesters of nutrients for the plant, or if the fungi shut down under these conditions.

5.3 AM Colonization

The observed colonization levels were consistent with past studies. In particular Bainard et. al. (2011) reviewed AM colonization of seven species of *Acer*. The study found the following colonization levels for rural trees: *Acer negundo* (29.6 ± 4.79), *Acer nigrum* (34.0 ± 4.43), *Acer pensylvanicum* (50.2 ± 5.21), *Acer platanoides* (43.6 ± 4.83), *Acer rubrum* (38.2 ± 4.83), *Acer saccharinum* (38.2 ± 6.40), and *Acer saccharum* (49.4 ± 5.89).

As previously discussed, soil redox potential ($p = 0.939$) and phosphorus ($p = 0.221$) were not significant in determining the percentage of AM colonization of *Acer* roots. These results are discussed in the section 3 and 4. In addition, AM colonization did not correlate with redox (Figure 21) or season (Figure 25). Many studies have identified correlations between percent AM colonization and environmental variables. Correlations found between phosphorus and colonization and soil moisture and colonization are

discussed in Section 1.1. Zahka et al. (1995) found colonization of sugar maple roots by AM fungi at four Vermont forest sites was positively correlated with the concentration of soil K and OM. Wiseman and Wells (2005) found maple AM colonization was negatively correlated with soil pH and positively correlated with CEC.

However, Coughlan et. al. (2005) found colonization of sugar maple was positively correlated with pH. Muthukumar et. al. (2004) examined data on 65 studies where soil pH was reported and found a strong positive correlation ($r = 0.630$; $P < 0.000$) between soil pH and mycorrhizal colonization level in sedges. A negative correlation was identified between both soil K and OM and mycorrhizal colonization levels in sedges.

The conflicting results of which environmental variable affects mycorrhizal colonization are at least partially due to differences in the host species, the sampling sites, the season(s) of sampling, and methods and procedures for staining and quantifying the percent AM colonization. As previously described, the present study sought to eliminate many of these variables by using a common host species, using a common physiographic province (the Coastal Plain), sampling over multiple seasons, and using the most common staining and quantifying techniques described in the literature. However, in the present study the "site" is the only variable of significance. If "site" is significant, then one or more of the site attributes would be expected to be significant. As the environmental variables are not individually significant, then the two likely explanations are: the variable of significance was not measured; or the specific combination of

variables for each site is significant rather than any individual variable. Because virtually every environmental variable identified in a previous study was measured, the latter explanation is the most likely one. The two explanations will be discussed further in the next section (Fungal Communities).

However, more intensive sampling within the paired sites could expose a significant environmental variable.

5.4 Fungal Community

The molecular identification of root fungal OTUs focused on the entire fungal community, rather than just AM fungi. The total number of OTUs observed for all dates for a given site ranged from 29 (OC1) to 64 (HM2). However, overall a total of 114 OTUs were identified and the Shannon's Index values varied within sites (especially the FB2 site). The mean fungal diversity was similar at FB1, FB2, HM1, and OC2. The OC1 site had the lowest diversity. Burke et. al. (2009) suggested that that root-associated fungal diversity of a beech-maple forest declines as soil P levels increase. However, in this study the greatest diversity was observed at the site with the second highest available P levels (HM2). Overall, fungal community diversity was significantly different between the sites. The variability in diversity across sampling dates and between sites was unexpected given the commonality of the host species and the overall proximity of the 6 sites geographically.

Only 13 OTUs occurred in 20% or more of the samples and only three were found in over a third of all samples. One OTU, 278.72, occurred in at least one sample from all 6 sites; although, overall, it occurred in only 65% of the samples. These observations were borne out statistically with the results of a Bray distances comparison that showed that all 6 communities were different than each other and were only slightly less dissimilar with their site pairs (within locations) than with sites from other locations.

Correlations with environmental variables using PCO and CCA showed that soil redox and time of season, two variables that can affect fungal communities, were not significant at the 6 sites. The findings of the present study are consistent with those of Wolfe et. al. (2007) that found that the community composition of AM in a calcareous fen was variable over small spatial scales but was not significantly associated with soil saturation. The study suggested that that the molecular diversity of AM from saturated soils in wetlands might be comparable to that of upland systems. In a study of California tidal marshes and adjacent upland sites, AM fungal species diversity was found to be similar across soil moisture regimes (Brown and Bledsoe, 1996).

Four of the environmental variables P, CEC, K, and Mn, were statistically significant at ($p < 0.05$); however, all but Ca were moderately correlated. Burke et. al. (2009) found that root-associated fungi in a mature beech-maple forest correlated with different environmental variables in different seasons. In June, fungal communities were significantly correlated with soil pH, soil moisture, and soil C and N at fine spatial scales, while in September, fungal communities were significantly correlated with P, soil C, and C/N ratio.

About half of the variation in ecological communities can be explained by environmental and spatial variables (Cottenie, 2005). The similarities observed within locations but not between the other two locations may be a simple function of geography. Spatial diversification of fungal communities has been previously demonstrated. Lekberg et. al.

(2007) found that distance between sampling sites was positively correlated with AM fungal community dissimilarity. Öpik et al. (2006) conducted a meta-analysis showing a significant between-habitat variation in the number of AM fungal taxa colonizing single plant species and that the composition of AM fungal communities may differ between regions and habitat types. About half of the surveyed AM fungal taxa were detected at no more than one site. The communities evaluated in the present study include the entire fungal community, of which AM fungi are a subset. However, the observations from previous studies on the AM community would apply to other fungi as well.

Environmental variables were evaluated in this study and found to explain a relatively small percentage of the community variation. As mentioned earlier, physical differences exist between the sites beyond those measured in this study. For example, soil moisture has been shown to affect fungal communities and was likely different between sites. Tobermam et. al. (2008) found that summer drought negatively affected both fungal band richness and community composition. In addition, other studies have found that differences in soil moisture levels result in changes in the composition of soil fungal communities (Trudell and Edmonds, 2004; McLean and Huhta, 2000). The present study, focused on potential inhibition of AM fungi by anaerobesis, measured soil redox (oxygen content) rather than soil moisture (moisture content). The measurement of soil redox in this study identified periods of anoxia but did not detect less overt gradations in soil moisture that may have promoted or inhibited the fungal community.

One distinguishing factor about the HM1 site was the soil depth at which fine roots proliferated. As previously mentioned, *Acer rubrum* differentially deploys roots at different soil depths based on external stimuli, such as flooding. The HM1 roots were harvested at 18 cm., as opposed to < 10 cm. at the HM2 site (and all the other sites as well). Soil depth has been found to either diminish or change the structure of AM communities. Oehl et. al. (2005) found that the AM community composition changed towards deeper soil layers. Zajicek et. al. (1986) found similar AM spore affiliations but in different proportions between 20 cm. and 40 cm. of soil depth. Kabir et. al. (1998) found that AM fungus populations diminished markedly below 15 cm.

Both OC sites were observed flooded by river water at least once (data not shown). The OC1 site showed a distinct litter rack line upslope from the sampling location. Following a flooding event, the OC1 site typically dried out within a day or two, but the OC2 site stayed ponded for a week or longer (see Figure 18). Tidal or river waters have long been known as a source of fungal propagules dispersal. Chrzanowski et. al. (1982) reported 7.3×10^6 propagules per m^3 of microfungi in tides from a stream in South Carolina. The distinctive fungal community observed at the OC sites could be influenced by these flooding events and the potential for the introduction of different species of fungi.

Although no evidence for host-specificity within closely related members of a single genus of tree has been demonstrated, it is possible that *Acer saccharinum* (OC sites) supports a different fungal community than *Acer rubrum* (HM and FB sites). No studies

have indicated that the AM community would differentiate between the two *Acer* species, but it is possible that other fungal endophytes may discriminate. Further research is needed to support this conclusion.

The FB wetlands are unique due to their hydrology source; groundwater discharge through a subsurface gravel layer. The FB1 site, as previously described, retains the fen-like characteristics of a groundwater-fed wetland. The FB2 site, while significantly modified, retains the characteristic low-P of a fen, indicating that the surface water intermittently flowing through the site is probably also groundwater-derived. The disturbance at FB2 has primarily changed the soil profile and chemistry. The soil redox levels at the sites were not significantly different. However, disturbance alone has been shown to alter fungal species richness (Lawrynowicz 1982). Wiseman and Wells (2005) found that *Acer rubrum* L. trees in urban sites had significantly lower arbuscular mycorrhizal colonization compared to forested sites.

Although these physical differences may not effect the AM species composition, the overall community of endophytic fungi could be affected. Further research is needed to determine which, if any, of the OTUs identified in this study belong to endophytic fungi that may have more strictly defined environmental niches than do the AM fungi.

6.0 Conclusions

With regard to the hypotheses put forth in the introduction, the following summarizes the findings of this study.

1. **Mycorrhizal associations will be present at both upland and wetland sampling locations**. This study found mycorrhizal associations through microscopic examination in roots harvested from both upland and wetland habitats. The range of observed colonization, based on 60 samples collected on 11 dates covering 3 years, ranged from 4% to 55%. The average colonization by site was lowest at FB2 (15%) and highest at FB1 (31%). Both sites are wetland sites.

2. **The percentage of colonization will be lower at the wetland sites due to the constraints of anoxia.** This hypothesis was not validated. The difference in percent AM colonization between the paired sites was significant at the FB ($p = 0.003$) and the OC ($p = 0.044$) locations. The difference was not significant at the HM location ($p = 0.774$). Soil redox potential ($p = 0.939$) was not significant in determining the percentage of AM colonization of *Acer* roots. Of the two wetland-upland paired sites, the difference in percent colonization was significant at only the OC location. In that location, the wetland site had a higher percent colonization than the upland site.

Further, the site with the greatest percent colonization in this study, FB1, was a wetland and it had the lowest observed redox potential.

3. **The percentage of colonization will be inversely proportional to soil phosphorus levels**. This hypothesis was not validated. In this study, increasing soil P concentrations, within the observed range of 7-37 μg/g, did not reduce mycorrhizal colonization in *Acer* roots. For example the spring/summer colonization of the FB2 site averaged 11.2% while the soil P concentration was only 7.5 μg/g. Conversely, the spring/summer colonization of the HM1 site averaged 21% with the highest observed soil P concentration (37 μg/g). Based on the lack of a spring "peak" in colonization, when the nutritional demands of *Acer* are the highest, the study trees were able to acquire the necessary P reserves to sustain growth and development without needing to signal for increased mycorrhizal colonization. Consequently, the observed range of soil P concentrations were within the range needed by the trees using their root systems augmented by a minimal level of acquisition by mycorrhizae.

4. **Both the number and diversity of mycorrhizal fungal species will decrease in the wetter sampling locations.** This hypothesis was not validated. A comparison of fungal diversity between the pooled wetland site community and the pooled upland site community was conducted and the difference was significant ($p = 0.003$), but the upland sites exhibited the lower diversity. However, because there are only 2 upland sites, the difference could be attributed to the particularly low diversity of the OC1

upland site. The HM2 wetland site, a classical forested wetland with seasonal saturation, exhibited the greatest fungal community diversity and supported the greatest number of OTUs.

5. **Different fungal communities will occupy the upland and wetland niches**. Communities from all sites were dissimilar. Proximity exerted a greater effect over community structure than did upland versus wetland status. That is, the paired upland/wetland sites at each location were more similar than any other combination of sites except for the FB2-HM2 combination that was only marginally more similar than the HM1-HM2 combination. The two upland sites (HM1 and OC1) were more dissimilar than all upland/wetland comparisons. The OC sites were most similar, based largely on the predominance of one OTU (290.1).

This study is noteworthy because, if for no other reason, its findings are contrary to many previous studies. The study design in terms of variables considered and duration (3 years) exceeds most of the published studies addressing the hypotheses evaluated here. Root samples were gathered from the same trees over multiple seasons under the full suite of environmental conditions, including periodic soil anoxia. The stressor of anoxia, thought to be a likely inhibitor of both AM colonization and fungal community diversity, was not significant. Available P levels ranged from very low to moderate but were not significant in affecting AM colonization. Available P was significant in fungal community structure but did not inhibit the number or diversity of OTUs. Further, in this

study, season was not significant for determining AM colonization, fungal community diversity, or fungal community structure.

As mentioned above, the location had the greatest effect on the fungal community structure. Understanding that a location is a combination of environmental features that either optimize for or inhibit certain species, the discriminate environmental variables at each location stood out as significant for the 17 major OTUs that influenced fungal community structure. As was the case with the AM colonization results, it appears that to the extent that environmental variables determined the community, it may be the combination of those measured as part of this study and possibly others that were not included that distinguished each site's community from the others.

Previous studies (Lekberg et. al., 2007; Öpik et al., 2006) indicate that fungal community structure is variable spatially with an individual species occupying a limited number of sites. This study is consistent with those findings. The overlap between sites of individual OTUs was limited despite the fact that a common host plant was evaluated and the sites were all located within a small region of the Virginia Coastal Plain.

Considerations for Future Studies

As originally conceived, this study would have established a hydrologic gradient to further refine the differences in AM inhibition by anoxia. As this study examined the two extremes of the gradient and did not reveal a trend based on soil redox, a hydrologic

gradient would not have aided the evaluation. However, a gradient-based evaluation that included soil moisture as a variable may have provided additional information. It would also have been useful for determining if a spatial scale could be established that was small enough to reduce the level of dissimilarity observed.

My inability to find a primer set that would consistently amplify AM fungi reduced some of the connectivity between the microscopic observations of AM fungi and molecular investigations. However, based on previous studies, *Glomus* is the predominant genus found colonizing *Acer* roots (Kormanik et. al., 1982; Zahka et. al., 1995; Yawney and Schultz, 1990; Coughlan et. al., 2000: Helgason et. al., 2002). Consequently, it is likely that AM-specific primers would not have revealed the overall community structure differences between sites that the whole community did because the amplification would likely yield a relatively low diversity of AM fungal species. As an alternative to the approach used here, a nested-PCR using the general fungal primer set with a second amplification using a more-specific primer set (Martin and Rygiewicz, 2005), would allow the investigator to fingerprint both levels. This approach would retain the overall community results while also adding information on the specific changes in the AM community.

Finally, the addition of a more permanently flooded wetland site would aid the investigation by increasing the differences in soil redox. The study period included sampling dates with low redox, but the relatively dry conditions prevalent during parts of

the study period reduced the potential stressor of anoxia. Other studies that have included more extreme soil redox conditions, such as Wetzel and van der Valk, 1996, have needed to introduce another confounding variable, multiple plant hosts. Most species of trees, including *Acer*, are limited in wetlands that experience more consistent soil anoxia. The few tree species that are adapted to these conditions (e.g., *Taxodium distichum*) are not found in dry habitats. Consequently, finding a host species/habitat that would allow more extreme hydrologic conditions would be challenging.

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Literature Cited

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Curriculum Vitae

James Martin attended Indiana University, where he received his Bachelor of Science in Biology in 1987. He went on to receive his Master of Science in Environmental Science and Master of Public Affairs from the School of Public and Environmental Affairs at Indiana University in 1990. This Dissertation completes the requirements for his Doctor of Philosophy from the Department of Environmental Science and Public Policy at George Mason University. Mr. Martin is employed as an environmental scientist and serves as a Branch Chief in the Division of Gas – Environment and Engineering within the Office of Energy Projects at the Federal Energy Regulatory Commission in Washington, DC.