ABSTRACT

Title of Thesis: Herbaceous Plant Tissue Nutrients as Indicators of Nutrient Limitation and Tree Establishment in Created Forested Wetlands of Virginia

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Due in part to construction techniques, soil nutrient content within created wetlands are often lower than those in naturally occurring wetlands and can lower tree growth rates. Slow growing trees are at greater ecological risk of mortality and some regulatory programs have minimum growth requirements. The purpose of this study was to explore evidence of nutrient limitations to tree growth. We examined the relationship between tissue nutrients of two colonizing herbaceous species, *Scirpus cyperinus* and *Juncus effusus,* soil nutrient content, colonizing vegetation aboveground biomass and planted tree size in three created wetlands in Loudoun County, Virginia. Dominance of *S. cyperinus* or *J. effusus* was determined in 1-m^2 plots adjacent to planted trees, and when dominant, *S. cyperinus* or *J. effusus* tissues were obtained and tissue N and P content was analyzed. Results detected indicators of nutrient limitation which suggests that soil amendments could increase tree growth in some created wetlands.

HERBACEOUS PLANT TISSUE NUTRIENTS AS INDICATORS OF NUTRIENT LIMITATION AND TREE ESTABLISHMENT IN CREATED FORESTED WETLANDS OF VIRGINIA

By

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Approved:

Robert B. Atkinson, Chair ____________________

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DEDICATION

To my family, friends, and professors

for their endless love and support

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Introduction

Less than a century ago, wetlands were not viewed as valuable ecosystems and often were converted to agricultural purposes (Tiner, 1987). Dahl (1990) reported that the state of Virginia alone has lost approximately 42% of its original wetlands because of farming and development. Regulations on human activities in and near wetlands have been established to decrease the amount of destruction since the enactment of the Clean Water Act of 1972 (EPA, 2016). Some impacts to wetlands are unavoidable and created or restored wetlands are required to compensate for the loss. Several studies have found that created wetlands, however, do not generally mimic the structure and function of naturally occurring wetlands. Construction techniques may cause nutrient levels within created wetlands to be lower than those in naturally occurring wetlands (Kangas et al., 2016). Nitrogen and phosphorus nutrient limitation can lower tree growth rates and increase the risk of mortality. If trees aren't adequately established, success criteria may not be met and forested wetland structure and function are not replaced.

Plant tissue nutrient concentrations may be related to soil nutrient availability and therefore provide an indication of limits to primary production; however, we found no studies examining herbaceous vegetation tissue nutrients as a predictor of the establishment of planted trees within created wetlands. The purpose of this study is to predict nutrient limitation and tree size within created forested wetlands using plant tissue nutrient content of *S. cyperinus* and *J. effusus*.

Literature Review

Forested Wetland Definition and Functions

Wetland ecosystems include bogs, swamps, marshes, fens, wet meadows, peatlands, pocosins, etc. (Tiner, 1998). To be defined as a wetland, an area must exhibit positive indicators of three characteristics including wetland hydrology, hydric soil, and hydrophytic vegetation (EL, 1987). To meet the wetland hydrology requirement, there must be evidence of saturated soil that has persisted at least 5% of the growing season, approximately 2 weeks in Virginia. When this soil saturation requirement is met, anoxic conditions prevail at some frequency and duration and soils typically develop indicators such as low chroma beneath the A horizon. Anoxic wetland soil conditions favor adapted plant species (i.e., hydrophytes, Table 1) and when these species are dominant, the plant community qualifies as hydrophytic vegetation (USACE, 2010). Each species in the community is typically listed in The National Wetland Plant List (Lichvar et al., 2016) and the wetland indicator status for each species and region is provided (Table 1.1). Hydrophytes include species listed as obligate wetland (OBL), facultative wetland (FACW), or facultative (FAC) species (Lichvar et al., 2012).

In 2004, the conterminous United States had approximately 39.8 million acres (~16,100,000 hectares) of wetlands. Freshwater wetlands accounted for 86% of the wetlands and 62% of the freshwater wetlands were forested. According to the Chesapeake Bay Program, there were approximately 114,000 hectares of wetlands in the Chesapeake Bay watershed in 2010, 86% of which were non-tidal (CBP, 2017).

Forested wetlands are defined as those that have woody vegetation ≥ 6 m in height (Stedman and Dahl, 2008); this wetland type is common in the eastern United States (FGDC, 2013). Primary production is high in many forested wetlands and this high productivity is associated with valuable ecosystem services such as providing habitat for plants and animals, creating riparian buffers, and limiting effects of floods.

Table 1.1. Indicator status and description for each indicator status category (USDA Plants Database, 2016).

Indicator Status	**Designation**	**Comment**
Obligate Wetland (OBL)	Hydrophyte	Almost always occur in wetlands [>99% occurrence]
Facultative Wetland (FACW)	Hydrophyte	Usually occur in wetlands, but may occur in non-wetlands [67-99% occurrence]
Facultative (FAC)	Hydrophyte	Occur in wetlands and non-wetlands [34-66% occurrence]
Facultative Upland (FACU)	Non-hydrophyte	Usually occur in non-wetlands, but may occur in wetlands [1-33% occurrence]
Obligate Upland (UPL)	Non-hydrophyte	Almost never occur in wetlands [<1% occurrence]

Wetland Mitigation, Creation and Success

In 1948, the Federal Water Pollution Control Act was enacted and served as the first law addressing water pollution. In the 1970's, water pollution and environmental concerns continued to rise within the United States and the Act was amended and renamed the Clean Water Act (EPA, 2016). Section 404 of the Clean Water Act pertains to regulations near or within wetlands and requires permits for the discharge of dredged or fill materials into wetlands (EPA, 2016).

One way to compensate for the destruction of wetlands is through the creation of wetlands for mitigation banking or compensation. A mitigation bank is an area of land (wetland) that is managed to compensate for impacts to wetlands (EPA, 2016). As of 2011, there were 563 active wetland mitigation banks accounted for within the United States, 81 of which were in Virginia (EM, 2017).

Created wetlands are monitored to determine compliance with permit conditions. Performance standards, or observable and measurable attributes, must be assessed. The majority of performance standards are measured early on, following the creation of the wetland, and some meet required performance standards in just 5 years (Spieles et al., 2006). However, wetlands develop at different rates and some may require longer term monitoring. According to findings in a study by Van den Bosch and Matthews (2017), long-term monitoring could be necessary to determine successful wetland restoration and creation.

Some performance standards that are often associated with wetland monitoring include density and cover of planted and/or colonizing vegetation (Kusler, 2006); and ecological studies of wetland compensation sites have frequently quantified vegetation biomass. A study by Dee and Ahn (2012), which used soil characteristics to predict the development of the herbaceous community in compensatory wetlands, determined that most macronutrients (except phosphorus) increased with site age, but no tissue macronutrients were significant predictors of aboveground biomass. Another way to use vegetation and soil measurements to assess the success of wetlands is by quantifying plant tissue nutrient levels.

Importance of Soil and Plant Nutrients in Wetlands

Wetland soils parameters are frequently investigated as they are so closely linked to many wetland functions (Ballantine and Schneider, 2009) and usually influence primary productivity (Wetzel and Van der Valk, 1998). It is generally assumed that an increased amount of nutrients within the soil would increase the productivity and biomass of plants growing in the area (DeBerry and Perry, 2015), but in newly created wetlands, nutrients may not be present in high levels.

Nitrogen and phosphorus are often limited within wetlands (Wassen et al., 1995). Many studies have shown that created wetlands have significantly lower nutrient levels than natural wetlands (Moser et al., 2009; Stolt et al., 2000; Fennessy et al., 2008). Soil nutrient availability may be influenced by commonly used wetland creation practices. By excavating surface soil horizons during construction, soil nutrients, particularly nitrogen which is commonly associated with organic matter, may be removed (Mitsch and Gosselink, 2000). Phosphorus may be less limiting than nitrogen in created wetland soils because it is more commonly associated with clay particles that may eluviate from surface horizons during soil development and may illuviate into horizons that persist following construction (Mitsch and Gosselink, 2000).

Some range of macro-nutrient availability is required for plant community development in created wetlands, and some studies have determined tissue nutrient concentrations to be better predictors of primary production than soil nutrient content. Atkinson et al. (2010), in a study of 20-year-old created wetlands in Virginia, found that nutrient concentrations within herbaceous plant tissues were positively associated with peak aboveground biomass of colonizing herbaceous species. Evidence of plant tissue nutrients within herbaceous species predicting primary productivity of other herbaceous species is common (Atkinson et al., 2010; Bedford et al., 1999), however after an extensive literature search, no studies have used nutrient levels within colonizing herbaceous vegetation to predict the growth of planted trees within created wetlands.

Scirpus cyperinus (L.) Kunth

Scirpus cyperinus is an invader of disturbed wetlands and tolerates a wide range of environmental conditions, including water levels and pH (Wilcox et al., 1995). Due to the extensive size and structure of its seed heads, *S. cyperinus* can colonize and become a dominant species within wetland ecosystems (Atkinson et al. 2005). *Scirpus cyperinus* has an advantage in the environment it colonizes, as a result of its tussock growth form, which benefits the plant when competing for space. This attribute is important to remember when assessing the success of created wetlands as *S. cyperinus* might compete with other herbaceous vegetation (Atkinson et al., 2005) and with small planted saplings (Wilcox et al., 1985), but no negative effects were detected by Wright (2015). When nutrient levels are high within wetlands, *S. cyperinus* is known to have higher C/N ratios than forb species (Hopfensperger, 2014). *Scirpus cyperinus* also tends to have a low decomposition rate which doesn't allow for nutrients to leach back out into the surrounding soil (Kao et al., 2003; Atkinson and Cairns, 2001).

Juncus effusus L.

Juncus effusus, also known as common rush or soft rush, is a rhizomatous perennial rush found in freshwater environments with sandy/peaty acidic soils. It is commonly associated with *S. cyperinus* because neither species is generally shade tolerant. In a study by Kuehn and Suberkropp (1998), C:P was 4300 +/- 280 and N:P was 54 +/- 4 in *J. effusus* litter. *Juncus effusus* had a 1.0-1.5% tissue N content in a study by McJannet et al. (1995).

Relationships Between Plant Tissue and Soil Nutrients

Published studies identify three relationships between plant tissue and soil nutrients. Plants could uptake nutrients passively, suggesting that there is a positive relationship between plant tissue and soil nutrient content (*sensu*, Atkinson et al., 2010; Bedford et al., 1999). Chapin (1980) reported that plants in infertile environments can act as nutrient accumulators by storing excess nutrients. There could also be no relationship between plant and soil nutrient content as reported by Willby et al. (2001) and Atkinson et al. (2010). The failure to detect the relationship could occur for both nutrient accumulators and passive nutrient uptakers and might be influenced by season and year (Whigham et al., 2002).

Purpose

In created forested wetland mitigation sites, growth and survival of planted trees is important in determining creation success. In a study by Wurst (2014), it was suggested that the facultative plant community within created wetland sites in Loudoun County could limit the growth of the planted tree species due to competition. Wright (2015), on the other hand, concluded that there was not enough evidence to say that the herbaceous vegetation surrounding the trees impacted the growth of planted trees and recommended against controlling native species around planted. If native species are not controlled around the planted trees, their tissue nutrient content would be available for predictive analysis. The relationship between tissue nutrient concentrations of two colonizing herbaceous species, *S. cyperinus* and *J. effusus*, were examined as a predictor of tree height, canopy diameter and stem diameter at groundline at the same mitigation sites in Loudoun County. The objective of this study was to compare plant tissue nutrient content from the colonizing herbaceous species *S. cyperinus* and *J. effusus* with N and P soil nutrient concentrations, the biomass of surrounding vegetation, and tree morphometric measurements from neighboring planted tree species to predict nutrient limitation and tree size in created forested wetlands of Virginia.

CHAPTER TWO:
HERBACEOUS PLANT TISSUE NUTRIENTS AS INDICATORS OF NUTRIENT LIMITATION AND TREE ESTABLISHMENT IN CREATED FORESTED WETLANDS OF VIRGINIA

Introduction

Less than a century ago, wetlands were not viewed as valuable ecosystems and often were converted for agricultural purposes (Tiner, 1987). The state of Virginia alone has lost approximately 42% of its original wetlands because of farming and development (Dahl, 1990). Regulations addressed wetland destruction as early as the Clean Water Act of 1972 (EPA, 2016). Some impacts to wetlands are unavoidable so created or restored wetlands are required to compensate for the loss. Created wetlands, however, do not generally function the same way as naturally occurring wetlands. For example, a study by Atkinson et al. (2001) reported that plant decomposition and litter accumulation functions were still developing 20 years after creation. Some divergence in functions between natural and created wetlands may result from differences in nutrient concentration which may result from construction techniques that remove surface soil layers (Kangas et al., 2016). Nutrient limitation can lower tree growth rates and slow growing trees are at greater ecological risk of mortality.

After an extensive literature search, studies examining herbaceous vegetation tissue nutrients and their relationship to aboveground biomass were found (Atkinson et al., 2010; Bedford et al., 1999; Dee and Ahn, 2012), however no studies predicting the establishment of planted trees within created wetlands using herbaceous vegetation tissue nutrients were found. The purpose of this study was to compare plant tissue nutrient content from the colonizing herbaceous species *S. cyperinus* and *J. effusus* with N and P soil nutrient concentrations, the biomass of surrounding vegetation, and tree morphometric measurements from neighboring planted tree species to assess nutrient limitation and tree size.

Methods

All field data were collected between July 31st and August 13th of 2015, which was near the peak of Virginia's growing season (June-September; Weakley et al. 2012).

Location/Site Description

Non-tidal created forested wetland mitigation sites including Phases I, II, and III of the Loudoun County Stream and Wetland Mitigation Bank (LCSWMB, Appendix A), established by Wetland Studies and Solutions, Inc. (WSSI) in 2006, were examined in this study. These wetlands were created by stripping, stockpiling topsoil, adding lime amendments and disking. A seeded mix of wetland annuals and perennials were added to the sites following construction. These wetlands were part of a seven-year study examining tree survival and growth within created non-tidal forested wetlands.

Planted Trees

In March 2009, 1,596 trees representing 7 species and 3 stocktypes (bare root, tubeling, 1-gallon pots) were planted in the LCSWMB mentioned above, following the procedures described by Wurst (2014). Sapling species planted included *Betula nigra* L. (river birch), *Liquidambar styraciflua* L. (sweetgum), *Platanus occidentalis* L. (American sycamore), *Salix nigra* Marshall (black willow), *Quercus bicolor* Willd. (swamp white oak), *Quercus palustris* Münchh. (pin oak), and *Quercus phellos* L. (willow oak).

Tree Morphometric Measurements

In summer 2015, live trees (n=206) within the second plot (plot = 3 rows, 7 trees per row) of every 3- or 4- plot array in LCSWMB Phases I, II, and III (Appendix A), were measured to quantify (1) height, (2) canopy diameter, and (3) stem diameter at groundline. Tree height was determined by measuring to the top of the highest woody stem using a meter stick or stadium rod. Tree canopy was measured from leaf tip to leaf tip at three angles laterally across each tree to calculate an average canopy width. Stem diameter at groundline was measured at the base of each planted tree using 0.1-mm graduated micro-calipers (Swiss Precision Instruments, Garden Grove, California).

Colonizing Herbaceous Vegetation Visual Analysis

In summer 2015, a subset (n=63) of the 206 living trees were selected to determine the colonizing herbaceous vegetation structure within LCSWMB Phases I, II, and III. Colonizing herbaceous vegetation was defined as any vegetation, other than planted trees, found within the plots. Coverage estimates were made within 1-m^2 subplots surrounding the selected planted trees to determine if *S. cyperinus* or *J.*

effusus was the dominant species. A modified 50/20 rule was used to determine vegetative coverage (FICWD, 1989). If the relative cover of *S. cyperinus* or *J. effusus* was > 50% of vegetation cover within the 1-m^2 subplot, it was considered the dominant species and was collected and processed to quantify tissue nutrient content.

Plant Collection and Tissue Analyses

A total of 51 planted trees, all within LCSWMB Phase III, had *S. cyperinus* as the surrounding dominant herbaceous species in the subplot. There were 14 subplots that contained *J. effusus* as a dominant species occurring with planted trees among LCSWMB Phases I, II, and III. When *S. cyperinus* or *J. effusus* was the dominant herbaceous vegetation in the 1-m^2 subplot around the planted tree, all aboveground plant tissues (seed head, stem, leaves, etc.) were collected from a randomly selected 0.25-m^2 nested quadrant. Plant tissues were sorted in the field in order to separate the dominant species, *S. cyperinus* and *J. effusus,* for easy accessibility during tissue nutrient analysis. All aboveground tissues collected from each 0.25-m^2 plot were dried at 80°C for 24 hours and weighed to determine total aboveground biomass.

Following the determination of aboveground dry weight of plant biomass, the *S. cyperinus* and *J. effusus* tissues were prepared for nutrient analyses. Seed heads were removed from the *S. cyperinus* and *J. effusus* prior to grinding as they were not found on all of the plant tissue samples. Stems and leaves from dried *S. cyperinus* and *J. effusus* plant tissues were pre-ground in a coffee grinder and approximately 5.0 grams from each 0.25-m^2 region of each subplot were stored in labeled scintillation vials. Samples were ground to pass through a sieve in a Thomas Wiley Mini Mill at the Keck Laboratory on the campus of the College of William and Mary. Determination of *S. cyperinus* and *J. effusus* tissue nitrogen content was performed using a Perkin-Elmer 2400 CHNS/O Analyzer at William and Mary's Keck Lab. Phosphorus content within *S. cyperinus* and *J. effusus* samples was determined through an ashing/acid extraction technique (Chambers and Fourqurean, 1991). A sub-sample (~20 mg) of milled tissue samples were weighed out, then ashed in a muffle furnace at 450 °C for 4 hours. After cooling, the ash was re-suspended in 10 mL of 1M HCl, capped, and heated to 80 °C for 1 hour. A 0.5-mL aliquot was removed, diluted to 10 mL with deionized water, and analyzed for dissolved inorganic phosphate using the colorimetric molybdate-ascorbate method (Parsons et al., 1984). Absorbance was read using a Thermo Spectronic Genesys 5 UV-Visible Spectrophotometer.

Soil Analysis

Soil nitrate/nitrite/ammonium and phosphate concentrations determined by Wurst (2014) were used to evaluate the relationship with herbaceous tissue nutrient concentration. In that study, soil samples were obtained between the 3rd and 4th tree of 7 in the middle row of 3 rows within each plot in summer 2011. Nitrate/nitrate (NO_3 + NO_2) and ammonium (NH_4) content were determined using KCl extraction and phosphate (PO_4) was determined through a Mehlich-3 extraction. Soil nutrient concentrations were paired with tissue nutrient concentrations within the same plot.

For the purposes of the tissue nutrient and soil nutrient comparison, it was assumed that soil nutrient content was unchanged from 2011 to 2015.

Statistical Analysis

The Rstudio data analysis software (Rstudio Team, 2015) was used to examine relationships and to predict nutrient limitation and tree establishment. Tissue P data were normally distributed for *S. cyperinus* and *J. effusus*, so a t-test was used to determine if tissue P content differed between the two dominant species. However, due to non-normally distributed *S. cyperinus* tissue N, a Mann-Whitney U test was used to determine if tissue N content differed between *S. cyperinus* and *J. effusus*.

Due to non-normality in the tissue nutrient content and tree morphometric data, even after transformation, Generalized Additive Models (GAM), which use non-parametric smoothing functions, were used to analyze the relationship between tissue nutrient content of *S. cyperinus* and *J. effusus* and tree morphometric parameters (height, canopy diameter and stem diameter at groundline). GAMs, using tissue nutrient content of *S. cyperinus* and *J. effusus* vs. total aboveground biomass of colonizing species, were also performed to aid in predicting nutrient limitation. The variable, tree species, was included in all tissue nutrient content vs. tree morphometric parameter models as a covariate. Akaike information criterion (AIC) values were used to determine the most predictive model. A P-value threshold for significance was set at 0.05.

Scirpus cyperinus tissue N content and soil nutrient content (NO_3+NO_2 and NH_4) data used to test for associations between *S. cyperinus* tissue N and soil nutrient content was normally distributed so Pearson Correlation tests were conducted. Soil PO_4 content, however, was not normally distributed so a Spearman Correlation was conducted to test for associations between *S. cyperinus* tissue P and soil PO_4 content. *Juncus effusus* tissue N and P and soil nutrient content (NO_3 + NO_2, NH_4, and PO_4) data were used to test for associations between *J. effusus* tissue and soil nutrient content were normally distributed so Pearson Correlation tests were conducted.

Soil nutrient content (NH_4 and NO_3+NO_2) data near *S. cyperinus* and *J. effusus* subplots were normally distributed, however, soil PO_4 content near *S. cyperinus* subplots was not normally distributed. T-tests were used to determine if soil NH_4 and NO_3 +NO_2 differed between *S. cyperinus* and *J. effusus*-dominated subplots and a Mann-Whitney U test was used to determine if soil PO_4 differed between *S. cyperinus* and *J. effusus*-dominated subplots.

Results

Tissue nutrient content of *S. cyperinus* and *J. effusus* were examined to determine whether nutrient limitation was occurring within LCSWMB Phases I, II, and III, as well as to predict tree establishment. Concentration of N in *S. cyperinus* tissue N ranged from 1.65% to 2.93% and average N was 2.05% (Table 2.1). *Juncus effusus* tissue N ranged from 1.51% to 2.03% and average N was 1.72% (Table 2.1).

Scirpus cyperinus had significantly higher tissue N (W = 596.5, $P < 0.05$) than *J. effusus* (Figure 2.1).

Scirpus cyperinus tissue P ranged from 0.15%-0.27% and average P was 0.20% (Table 2.1). *Juncus effusus* tissue P ranged from 0.10%-0.16% and average P was 0.14% (Table 2.1). *Scirpus cyperinus* had significantly higher tissue P ($t = 5.81$, $df = 35.12$, $P < 0.05$) than *J. effusus* (Figure 2.2).

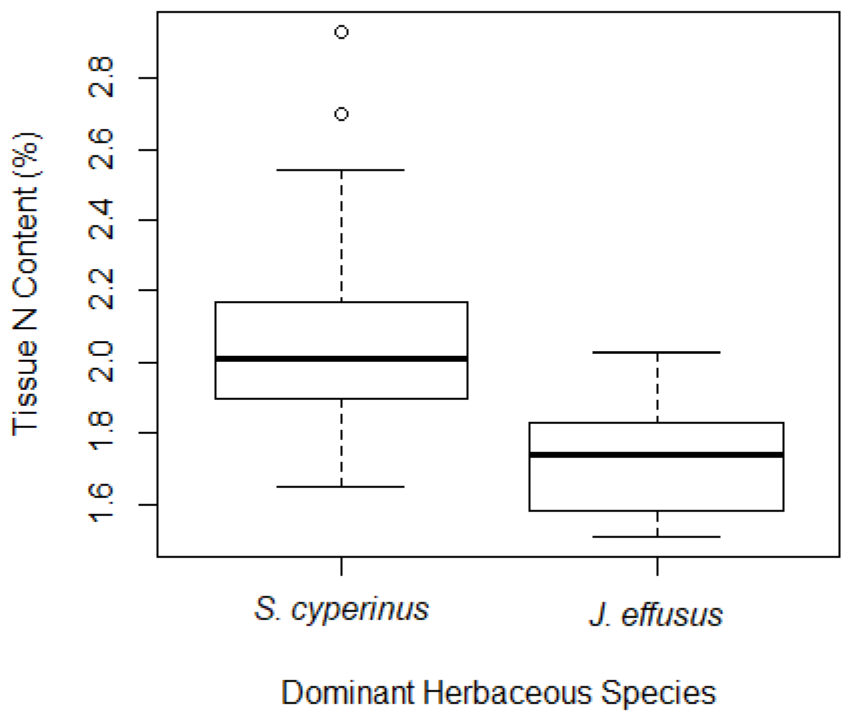


Figure 2.1. Plot of *S. cyperinus* (n=49) and *J. effusus* (n=14) tissue N content.

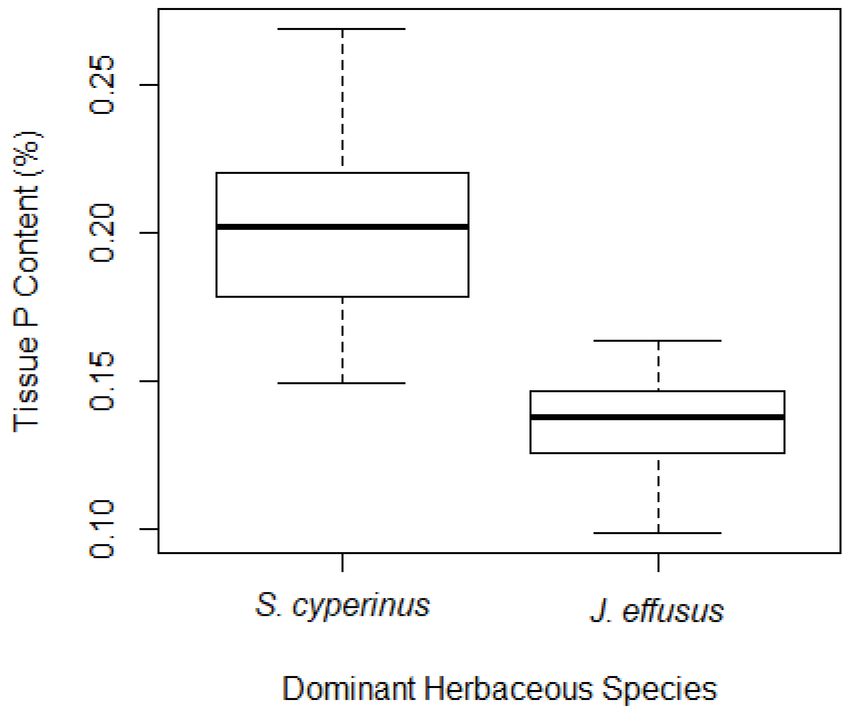


Figure 2.2. Plot of *S. cyperinus* and *J. effusus* tissue P content.

Scirpus cyperinus tissue N:P ranged from 7.65%-17.97% and average N:P was 10.30% (Table 2.1). *Juncus effusus* tissue N:P ranged from 9.52-18.40 and average N:P was 13.02 (Table 2.1).

Trees within *S. cyperinus*-dominated subplots had a mean height of 149.82 ± 79.32 cm, canopy diameter of 92.80 ± 56.42 cm, and stem diameter at groundline of 3.59 ± 2.23 cm. Trees within *J. effusus*-dominated subplots had a mean height of 157.36 ± 92.83 cm, canopy diameter of 94.26 ± 76.83 cm, and stem diameter at groundline of 4.03 ± 2.74 cm. Mean tree height, canopy diameter and stem diameter at groundline for each planted tree species in *S. cyperinus* and *J. effusus*-dominated subplots can be found in Table 2.2.

Table 2.1. Tissue N, P, and N:P within the two dominant herbaceous species *S. cyperinus* (n=49) and *J. effusus* (n=14).

	N (%)		**P (%)**		**N:P**	
Species	**Range**	**$\bar{x}$ ± stdev**	**Range**	**$\bar{x}$ ± stdev**	**Range**	**$\bar{x}$ ± stdev**
S. cyperinus	1.65-2.93	2.04 ± 0.26	0.15-0.27	0.20 ± 0.03	7.65-17.97	10.30 ± 1.84
J. effusus	1.51-2.03	1.72 ± 0.16	0.10-0.16	0.14 ± 0.02	9.52-18.40	13.02 ± 2.85

Table 2.2. Planted tree mean (± standard deviation) height, canopy diameter, and stem diameter at groundline in 2015 for *S. cyperinus* and *J. effusus*-dominated subplots.

Tree Species	**Common Name**		***S. cyperinus***				***J. effusus***		
			Height (cm)	**Canopy Diameter (cm)**	**Stem Diameter at Groundline (cm)**		**Height (cm)**	**Canopy Diameter (cm)**	**Stem Diameter at Groundline (cm)**
Betula nigra	River birch	n=10	180.80 ± 94.71	99.90 ± 64.45	3.69 ± 2.35	n=2	292.00 ± 107.48	144.17 ± 46.43	4.35 ± 0.49
Liquidambar styraciflua	Sweet gum	n=5	216.80 ± 75.96	95.27 ± 23.22	4.82 ± 1.63	--	--	--	--
Platanus occidentalis	American sycamore	n=1	93	37.33	2.2	--	--	--	--
Quercus bicolor	Swamp white oak	n=11	115.09 ± 44.18	77.70 ± 27.18	3.13 ± 1.79	n=2	92.50 ± 74.25	55.50 ± 49.26	2.95 ± 2.62

Table 2.2. Continued

Tree Species	**Common Name**	***S. cyperinus***				***J. effusus***			
			Height (cm)	**Canopy Diameter (cm)**	**Stem Diameter at Groundline (cm)**		**Height (cm)**	**Canopy Diameter (cm)**	**Stem Diameter at Groundline (cm)**
Quercus palustris	Pin oak	n=13	103.54 ± 47.55	60.85 ± 29.43	2.15 ± 1.14	n=5	109.20 ± 24.51	59.67 ± 33.28	3.14 ± 1.57
Quercus phellos	Willow oak	n=2	129.50 ± 150.61	117.33 ± 37.71	4.1 ± 1.13	n=3	124.00 ± 75.29	54.22 ± 54.42	2.43 ± 1.00
Salix nigra	Black willow	n=7	212.14 ± 63.90	164.90 ± 78.58	6.03 ± 2.87	n=2	258 ± 36.77	229.67 ± 79.67	9.40 ± 2.69

Scirpus cyperinus (woolgrass) Models

Planted trees (n=49) and the associated dominant species *S. cyperinus* were analyzed to determine potential relationships between tissue nutrient content and tree morphometric parameters (planted tree height, canopy diameter, stem diameter at groundline). Results from Generalized Additive Models examining the relationships between *S. cyperinus* tissue nutrient content and height, canopy diameter, and stem diameter at groundline of planted trees found no significant model (Table 2.3). However, a Generalized Additive Model detected a significant positive relationship between *S. cyperinus* tissue P and total aboveground biomass of colonizing species (($P = 0.009$), Table 2.3 and Figure 2.3).

Table 2.3. Results from Generalized Additive Models examining the relationship between *S. cyperinus* tissue nutrient content and height, canopy diameter, and stem diameter at groundline of planted trees and aboveground biomass of colonizing species. Tree species was included in the nutrient content and tree morphometric parameter models as a covariate (* signifies the best fit model, ** indicates significant P-value).

Dependent Variable	Model Syntax	Degrees of Freedom	AIC	P-value
Height (cm)	Height vs. N%	9.00	562.50	0.286
	Height vs. P%	9.00	562.36	0.264
	Height vs. N% & P%	10.00	563.32	--
Canopy Diameter (cm)	Canopy Diameter vs. N%	9.00	528.39	0.997
	Canopy Diameter vs. P%	9.00	528.01	0.579
	Canopy Diameter vs. N & P%	10.00	530.01	--
Stem Diameter at Groundline (cm)	Stem Diameter at Groundline vs. N%	9.00	214.41	0.669
	Stem Diameter at Groundline vs. P%	9.00	214.27	0.587
	Stem Diameter at Groundline vs. N% & P%	10.00	216.12	--
Total Aboveground Biomass of Colonizing Species (g/m^2)	Aboveground Biomass vs. N%	3.00	555.47	0.977
	Aboveground Biomass vs. P%	3.0	548.35*	0.009**
	Aboveground Biomass vs. N% & P%	4.00	550.16	--

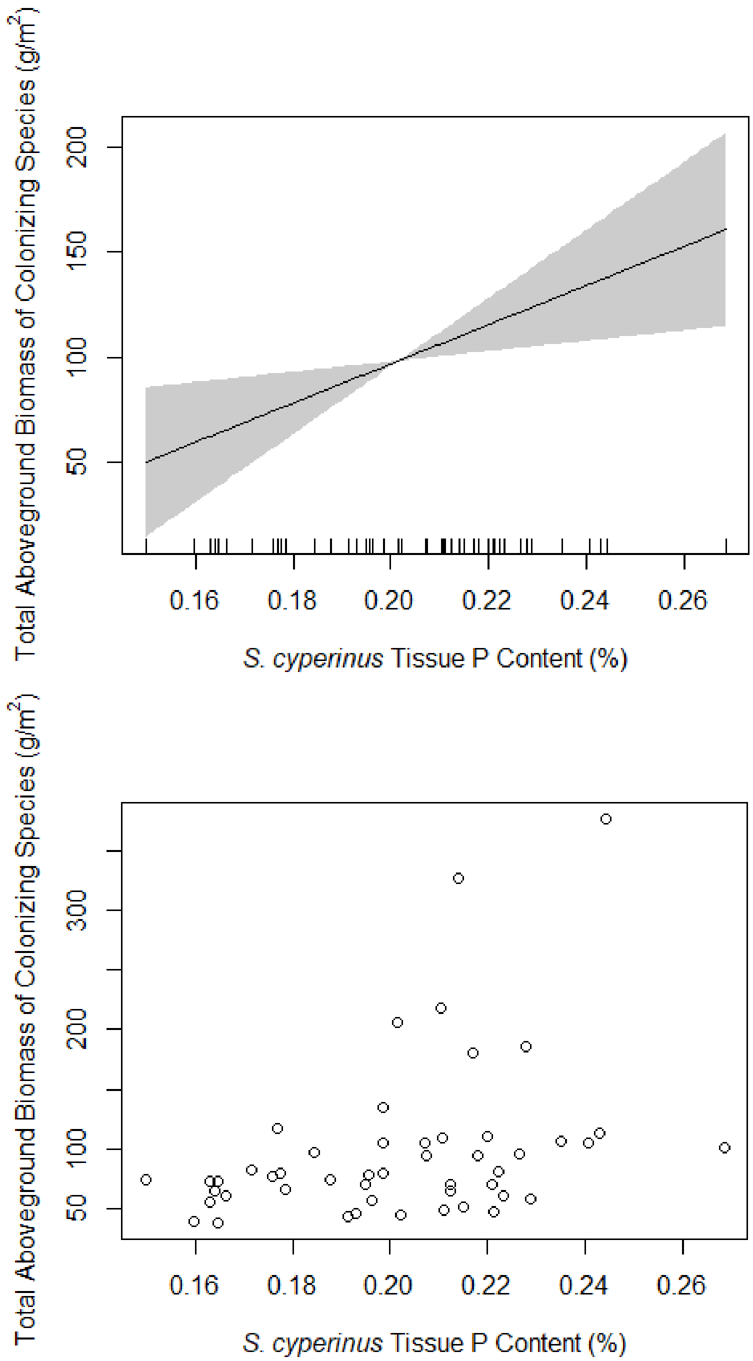


Figure 2.3 [A, B]. Plots of *S. cyperinus* tissue P content (%) in relation to total aboveground biomass of colonizing species (g/m^2) (P = 0.009). [A] The line represents a best fit of the data and the shaded region represents a 95% confidence interval. [B] The scatter plot for the same data.

Juncus effusus (soft rush) Models

Planted trees (n=14) and the associated dominant species *J. effusus* were analyzed to determine potential relationships between tissue nutrient content and tree morphometric parameters. Results from Generalized Additive Models examining relationships between *J. effusus* tissue nutrients and height and canopy diameter suggested that the best fit models include P, however there were no significant relationships in the models at the alpha = 0.05 level (Table 2.4). There were marginally significant relationships between canopy diameter and tissue P content as well as between stem diameter at groundline and tissue N content. A Generalized Additive Model examining relationships between *J. effusus* tissue nutrients and aboveground biomass suggested that the best fit model include N as a predictor. There was a positive relationship between *J. effusus* tissue N and total aboveground biomass of colonizing species ((P = 0.017), Table 2.4, Figure 2.4).

Table 2.4. Results from Generalized Additive Models examining the relationship between *J. effusus* tissue nutrient content and height, canopy diameter, and stem diameter at groundline of planted trees and aboveground biomass of colonizing species. Tree species was included in the nutrient content and tree morphometric parameter models as a covariate (* signifies the best fit model, ** indicates significant P-value).

	Model Syntax	**Degrees of Freedom**	**AIC**	**P-value**
Height (cm)	Height vs. N%	5.31	161.94	0.783
	Height vs. P%	4.72	140.05*	0.101
	Intercept only model	2.00	160.08	--
Canopy Diameter (cm)	Canopy Diameter vs. N%	7.16	155.61	0.542
	Canopy Diameter vs. P%	3.00	128.46*	0.075
	Intercept only model	2.00	154.30	--
Stem Diameter at Groundline (cm)	Stem diameter at groundline vs. N%	8.35	56.92	0.085
	Stem diameter at groundline. P%	3.00	57.05	0.606
	Intercept only model	2.00	60.42	--
Total Aboveground Biomass of Colonizing Species (g/m^2)	Biomass vs. N%	7.69	139.13*	0.017**
	Biomass vs. P%	3.00	154.10	0.289
	Intercept only model	2.00	153.47	--

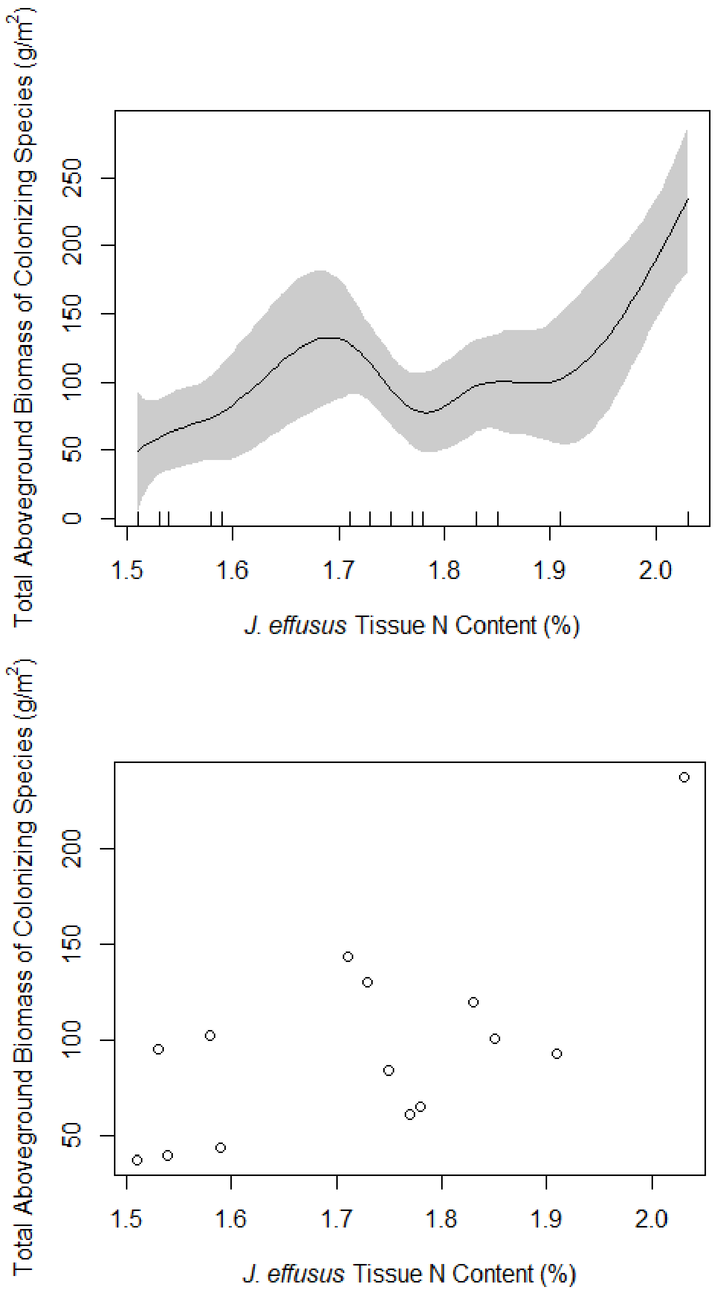


Figure 2.4 [A, B]. Plot of *J. effusus* N content (%) in relation to total aboveground biomass of colonizing species (g/m^2) (P = 0.017). [A] The line represents a best fit of the data and the shaded region represents 95% confidence intervals. [B] Scatter plot for the same data.

Tests of Tissue Nutrient and Soil Nutrient Content

Average soil NH_4 (0.08 ± 0.04 μmol/cm^3) in *S. cyperinus*-dominated subplots was not significantly greater than in *J. effusus*-dominated subplots (0.10 ± 0.08 μmol/cm^3) (t = 0.54, df = 4.75, P = 0.611). Average soil NO_3 + NO_2 content (0.06 ± 0.04 μmol/cm^3) in *S. cyperinus*-dominated subplots was also not significantly greater than in *J. effusus*-dominated subplots (0.04 ± 0.03 μmol/cm^3) (t = -1.47, df = 9.74, P = 0.172). Average soil PO_4 content in *S. cyperinus*-dominated subplots (1.75 ± 1.11 μmol/cm^3) was greater than in *J. effusus*-dominated subplots (0.64 ± 0.56 μmol/cm^3) (W = 9, P = 0.023; Figure 2.5).

Table 2.5. Average soil nutrient content in *S. cyperinus* and *J. effusus*-dominated plots.

		Soil NH_4 (μmol/cm^3)		**Soil NO_3 + NO_2 (μmol/cm^3)**		**Soil PO_4 (μmol/cm^3)**
Species		**$\bar{x}$ ± stdev**		**$\bar{x}$ ± stdev**		**$\bar{x}$ ± stdev**
S. cyperinus	n=13	0.08 ± 0.04	n=11	0.06 ± 0.04	n=13	1.75 ± 1.11
J. effusus	n=5	0.10 ± 0.08	n=3	0.04 ± 0.03	n=5	0.64 ± 0.56

There was no relationship between *S. cyperinus* tissue N and soil NH_4 (r_p = -0.33, df= 11, P = 0.272) and no significant relationship between tissue N and soil NO_3 + NO_2 (r_p = 0.35, df = 9, P = 0.292). There was a marginally significant positive relationship between *S. cyperinus* tissue P and soil PO_4 (r_s = 0.48, S = 188, P = 0.097; Figure 2.6).

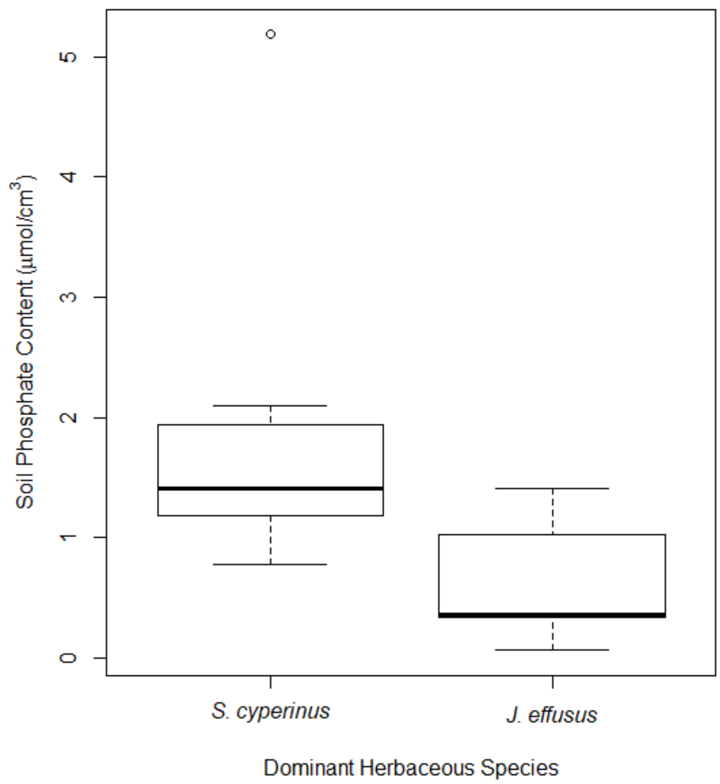


Figure 2.5. Plot of soil phosphate content (µmol/cm^3) in *S. cyperinus* and *J. effusus*-dominated subplots.

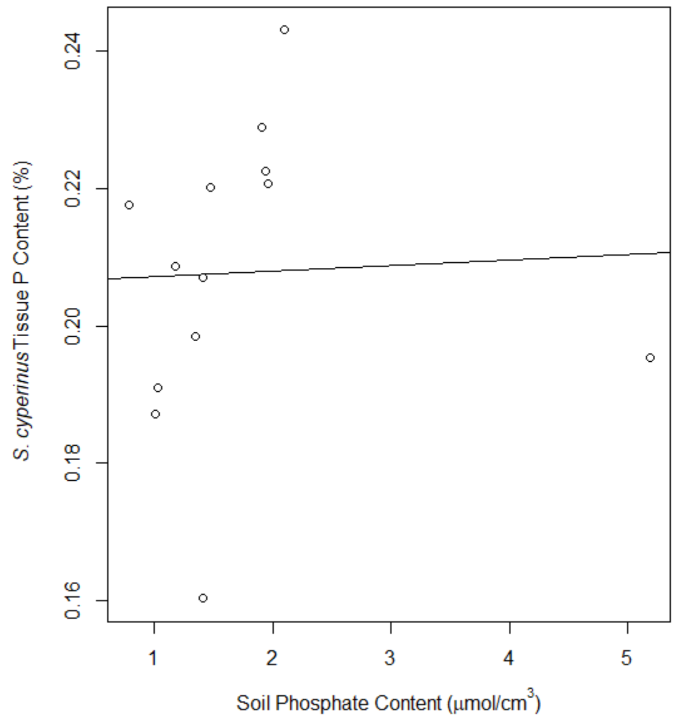


Figure 2.6. Plot of *S. cyperinus* tissue P content (%) and soil phosphate content (µmol/cm^3).

There were no significant relationships between *J. effusus* tissue N and soil NH_4 (r_p = 0.44, df = 3, P = 0.447), N and soil NO_3 + NO_2 content (r_p = 0.13, df = 1, P = 0.918) or tissue P and soil PO_4 content (r_p = 0.39, df = 3, P = 0.519).

Discussion

There was evidence of nutrient limitation to tree growth in this study. While Wurst (2014) failed to detect a relationship between soil nutrients and tree growth in our sites, in the current study tissue P content for *S. cyperinus* and tissue N content for *J. effusus* were positively related to aboveground biomass of colonizing species which suggests nutrient limitations to plant growth in general. However, we were unable to find any significant models between tissue nutrient contents and tree morphometric parameters.

<u>Tissue Nutrient Content Status</u>

In the current study, *S. cyperinus* tissue N (2.04%) was greater than that reported (0.95%) in a study of *S. cyperinus* N by Atkinson et al. (2010) in herb-dominated 20-year-old created wetlands in southwest Virginia. Our *S. cyperinus* tissue N fell within the range (0.83-4.20%) reported in a study by Bedford et al. (1999), which examined nutrient availability in wetlands, tissue N from an assemblage of herbaceous species. In *J. effusus*, tissue N (1.72%) was lower than that reported (2.49%) in a study by Shen et al. (2003), which examined J. effuses nutrient uptake.

A study by McJannet et al. (1995) examined wetland plants of different functional groups and habitats by loading N and P into wetlands to determine the effect on tissue nutrient content of plant species. Tissue N content in *S. cyperinus* (2.04%) and *J. effusus* (1.72%) in the current study were both greater than that in the McJannet et al. (1995) study, which reported tissue N content of 1.5% in *S. cyperinus* and 1.4% in *J. effusus*. McJannet et al. (1995) assumed that the plants used in their study were not nutrient limited, however, their tissue N contents fell within the range (0.83-4.20%) for nutrient limited plants reported in the Bedford et al. (1999) paper.

In the current study, *S. cyperinus* tissue P (0.20%) was similar to that reported (0.19%) in the Atkinson et al. (2010) study. Our *S. cyperinus* tissue P fell within the range (0.10-0.64%) reported in the Bedford et al. (1999) study. In *J. effusus*, tissue P (0.14%) was lower than the maximum reported (0.36%) in the Shen et al. (2003) study and is likely due to the fact that nutrients in our study weren't loaded into the wetland.

Nutrient conditions have been found to change during the first several years of created wetland soil development and may account for differences in *S. cyperinus* tissue N content. The Atkinson et al. (2010) study examined nutrient content from live tissues collected in 20-year-old created wetlands while the current study sampled from 7-year-old created wetlands. Based on a study by Dee and Ahn (2012), which was examining development of the plant community and soil properties in created wetlands ranging from 3-10 years in age, the 10-year-old site had significantly higher soil organic matter content than younger sites. Nitrogen is generally associated with organic matter content (Mitsch and Gosselink, 2000), and older sites showed higher organic matter and soil nitrogen concentrations. A study by Wurst (2014), which occurred within the same created wetlands as the current study, determined that organic matter was higher than those reported by Dee and Ahn (2012) for wetlands of similar age. This suggests that the current study's soils might be more developed than typical created wetlands of the same age and could result in higher nitrogen content in the soil and plant tissues.

<u>Relationships Between Plant Tissue and Soil Nutrients</u>

A study by Willby et al. (2001) examining the responses of herbaceous plant tissue nutrient content to nutrient availability among vegetation types in European marshes found that, as in the current study, tissue N and P were independent of respective soil nutrient concentrations. In that study, early colonizing species in disturbed sites, a category that describes *S. cyperinus* or *J. effusus* in the current study, were found to rapidly deploy nutrients into tissue growth rather than accumulating nutrients in plant tissues. The failure to detect a relationship could also occur in the case of nutrient accumulators or plants that passively uptake nutrients (positive relationship between plant tissue nutrients and soil nutrients).

However, because this was a field study and some variables were unable to be controlled, we evaluated P-values under an alpha = 0.1 level. There was a marginally significant positive association between *S. cyperinus* tissue P and soil PO_4 ($P = 0.097$). This suggests that passive (positive relationship between plant tissue nutrients and soil nutrients) uptake could be occurring.

Divergent tissue nutrient trends have been reported in other studies. McJannet et al. (1995) investigated the hypothesis that higher soil nutrient availability, which they simulated by adding fertilizer, might be associated with lower tissue nutrient concentrations; however, no trends in availability and tissue nutrient concentrations were reported in that study. Conversely, findings reported in a study by Chapin (1980), which examined the mineral nutrition of plants, suggested that species from infertile habitats accumulate and retain nutrients when available. *Scirpus cyperinus* and *J. effusus* could be storing excess nutrients in their tissues because the availability of nutrients is temporary.

Scirpus cyperinus had higher tissue N ($\bar{x}$ =2.04%) and P ($\bar{x}$ = 0.20%) content than *J. effusus* ($\bar{x}$ =1.72%, $\bar{x}$ = 0.14%, respectively). These results are similar to those found in the McJannet et al. (1995) study, which reported higher tissue N and P in *S. cyperinus* than *J. effusus*. Differences in tissue nutrient concentrations between the two herbaceous species could be due to differences in plant structure and their nutrient uptake potential. Although both species are known to be cespitose (grow in tussocks), *S. cyperinus* is strongly cespitose which could allow it to trap sediments and increase nutrient uptake. It could also be that *S. cyperinus* functions as a nutrient accumulator more than *J. effusus*.

Nutrient Limitation

The main purpose of this study was to determine whether the LCSWMB wetlands were nutrient limited based on *S. cyperinus* and *J. effusus* tissue nutrient content. In the Bedford et al. (1999) study, tissue N content ranged from 0.83-4.20% in N-limited wetlands and P content ranged from 0.10-0.64% in P-limited wetlands. Scirpus cyperinus (2.04%) and *J. effusus* (1.72%) tissue N content in the current study were within the N-limited range reported in the Bedford et al. (1999) study. *Scirpus cyperinus* (0.20%) and *J. effusus* (0.14%) tissue P in the current study also fell in the range for P-limited wetlands.

A study by Koerselman and Meuleman (1996) reviewed a total of 40 studies with reported nutrient content and developed a tool using N:P ratios to detect nutrient limitation. Koerselman and Meuleman (1996) suggested that N-limitation occurs at N:P < 14 and P-limitation at N:P > 16. In the current study, *S. cyperinus* tissues had a mean N:P of 10.30 and *J. effusus* had a mean N:P of 13.02. N:P for both *S. cyperinus* and *J. effusus* were less than 14, suggesting that nitrogen is limiting within the created forested wetlands.

In a study by Atkinson et al. (2010), which examined primary production in created wetlands, peak aboveground biomass was positively associated with *S. cyperinus* tissue P content. Similarly, our results showed a positive association between *S. cyperinus* tissue P content and total aboveground biomass of colonizing species (Figure 2.3), which suggests that P is limiting the productivity of the plants in the LCSWMB. There was also a positive association between *J. effusus* tissue N and total aboveground biomass of colonizing species (Figure 2.4), which indicates that N is also limiting the productivity of plants (P = 0.017) in these subplots.

<u>Tree Establishment</u>

Our generalized additive model results do not show any significant relationships between *S. cyperinus* tissue nutrient content and any of three tree morphometric parameters. However, two marginally significant models using *J. effusus* tissue nutrient content were detected. *Juncus effusus* tissue N content was somewhat positively related to stem diameter at groundline (P = 0.085). In a study by Hudson (2016), stem diameter at groundline was an adequate predictor of total tree biomass. Out of all the models, we would suggest that the one with stem diameter at groundline be examined further. *Juncus effusus* tissue P content was somewhat positively related to tree canopy diameter (P = 0.075). According to a study by Wright (2015) conducted at the same LCSWMB sites, canopy diameter was correlated with height and ground diameter and was omitted in an analysis of tree growth responses to herbaceous vegetation. Because of this high correlation, using canopy diameter to assess tree establishment in young created wetlands might not be necessary.

Differential initial sizes of species and planting types could obscure the growth response to tissue nutrient concentrations and planted tree stocktypes (Roquemore et al., 2014) included tubeling, bare root, or 1-gallon pots. Based on an annual report by Hudson et al. (2014) of woody vegetation in the same created wetlands as the current study, it was suggested that larger initial sizes of 1-gallon potted plants could impact survival and size of the tree. The 1-gallon potted plants had greater amounts of soil surrounding the root mass and required greater disturbance of the wetland soil for planting. Variations in planted tree survival and growth could be obscuring our Generalized Additive Model predictive capability, which potentially indicates that how stocks are grown and planted matters more than subsequent nutrient availability. One suggestion for future study would be to examine change in height, canopy diameter, and stem diameter at groundline over several years (actually model growth of planted trees) in relation to herbaceous tissue nutrient content.

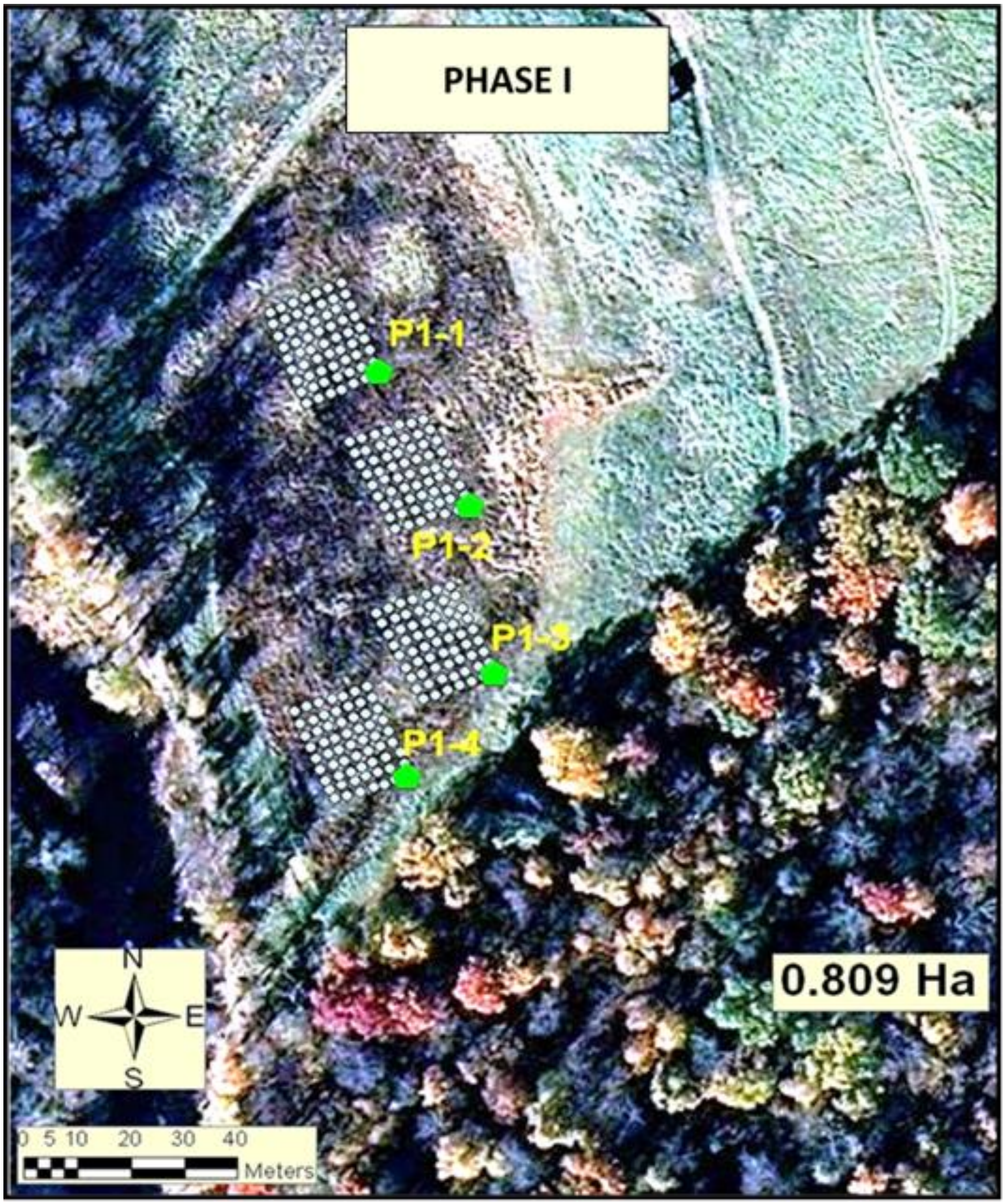
Conclusion

The first null hypothesis in this study was there is no relationship between herbaceous plant tissue nutrients and nutrient limitation in created forested wetlands of Virginia. Indicators of nutrient limitation within the created forested wetlands were found based on tissue N, P and N:P of *S. cyperinus* and *J. effusus*. The second null hypothesis for this study was there is no relationship between herbaceous plant tissue nutrients and tree establishment in created forested wetlands of Virginia. We have to accept the second null hypothesis as we were unable to find significant results in the modeling of tree establishment based on tissue nutrient content. However, we were able to predict aboveground colonizing biomass using tissue nutrient content and confirm the results of Atkinson et al. (2010).

While we were unable to find significant relationships based on models between tissue nutrient content and tree morphometric parameters, we recommend that herbaceous vegetation surrounding planted trees should not be removed as it is able to provide information on important wetland functions.

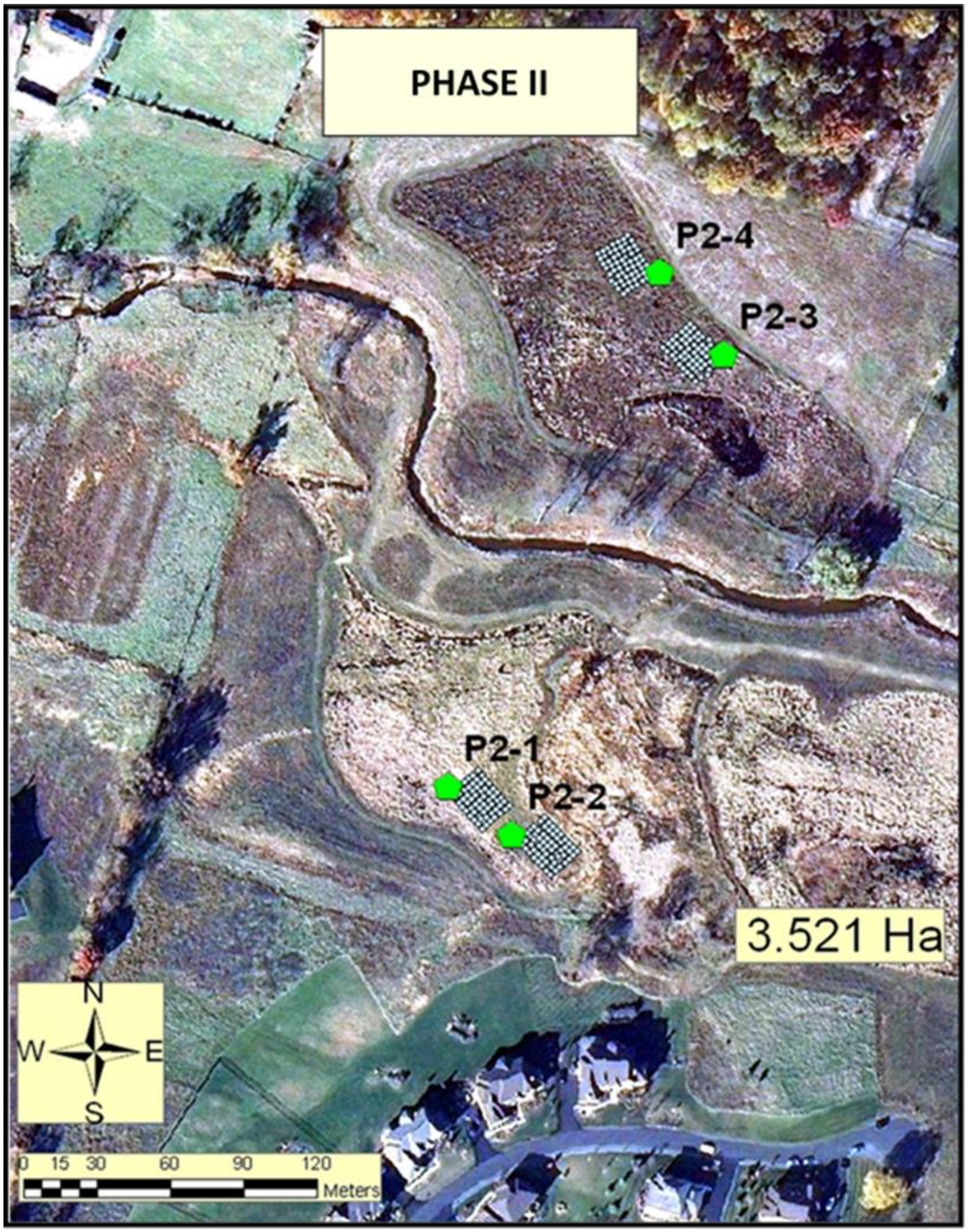
APPENDIX A: MAPS OF FIELD SITE LOCATIONS, PLOTS, AND TREES

Figure A.1. LCSWMB Phase I: Arrays are designated by green markers and individual trees (subplots) are shown in light blue markers (Hudson et al., 2013; Wurst, 2014; Wright, 2015).



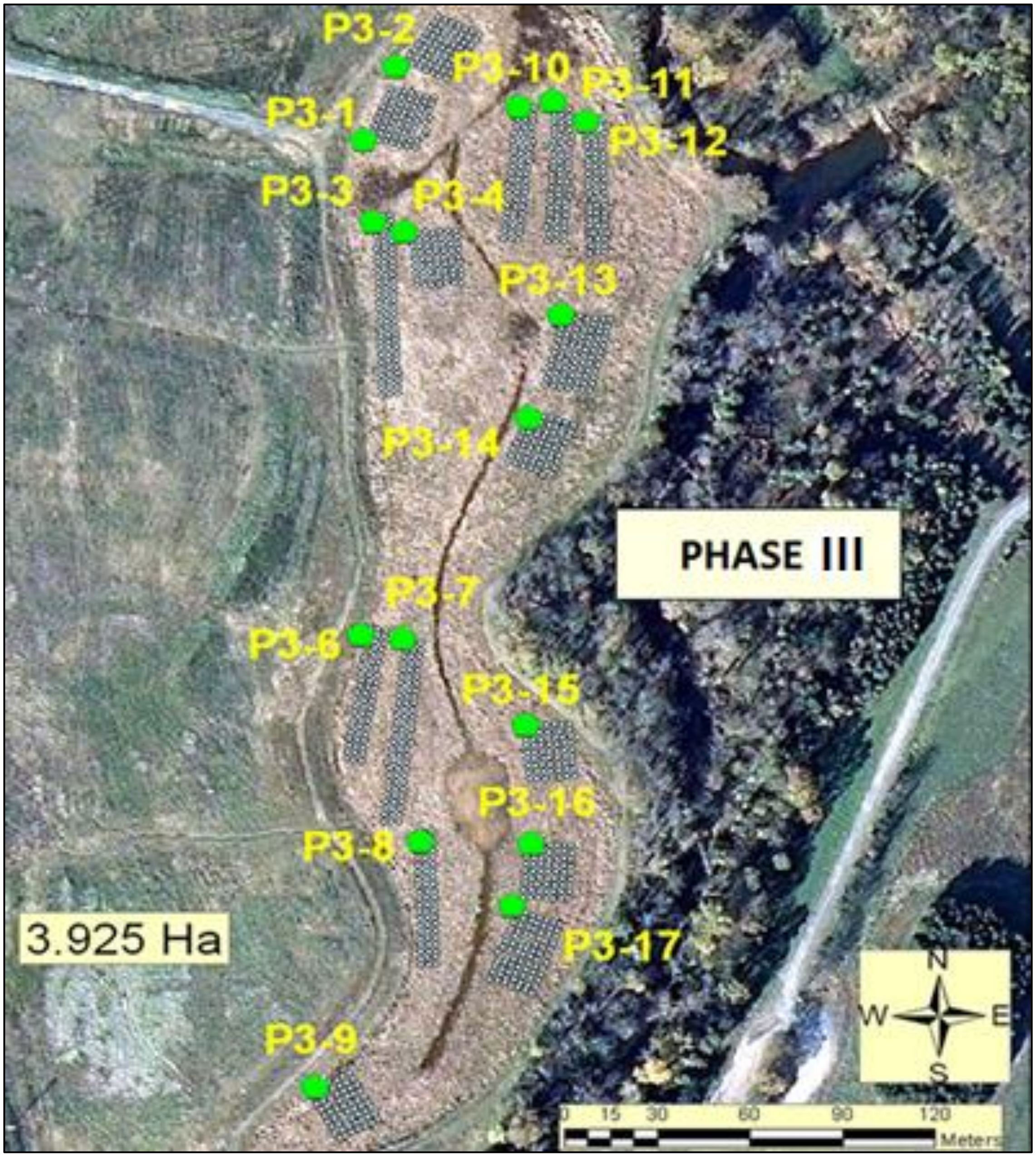
APPENDIX A: MAPS OF FIELD SITE LOCATIONS, PLOTS, AND TREES (cont.)

Figure A.2. LCSWMB Phase II: Arrays are designated by green markers and individual trees (subplots) are shown in light blue markers (Hudson et al., 2013; Wurst, 2014; Wright, 2015).



APPENDIX A: MAPS OF FIELD SITE LOCATIONS, PLOTS, AND TREES (cont.)

Figure A.3. LCSWMB Phase III: Arrays are designated by green markers and individual trees (subplots) are shown in light blue markers (Hudson et al., 2013; Wurst, 2014; Wright, 2015).



APPENDIX B: SOIL NUTRIENT RAW DATA

Table B.1. Soil nutrient content in *S. cyperinus* and *J. effusus*-dominated plots (0 values were eliminated from analysis).

S. cyperinus			
Plot	**Soil $NO_3 + NO_2$**	**Soil NH_4**	**Soil PO_4**
3.1.2	0.078	0.073	1.028
3.2.5	0.053	0.153	1.341
3.6.20	0.034	0.085	1.902
3.7.23	0.159	0.05	1.405
3.8.27	0.037	0.053	1.414
3.9.30	~~0~~	0.127	5.182
3.11.36	0.018	0.043	1.472
3.12.39	0.03	0.048	1.006
3.13.43	0.091	0.025	1.179
3.14.47	0.084	0.046	0.783
3.15.50	~~0~~	0.07	2.092
3.16.14	0.055	0.087	1.939
3.17.17	0.066	0.125	1.954

J. effusus			
Plot	**Soil $NO_3 + NO_2$**	**Soil NH_4**	Soil PO_4
1.2.5	~~0~~	0.221	0.359
2.1.2	~~0~~	0.116	0.063
2.3.9	0.017	0.016	0.336
3.1.2	0.078	0.073	1.028
3.8.27	0.037	0.053	1.414

LITERATURE CITED

Atkinson, R. B. and J. Cairns Jr. 2001. Plant decomposition and litter accumulation in depressional wetlands: functional performance of two wetland age classes that were created via excavation. *Wetlands* 21 (3): 354-362.

Atkinson, R. B., J. E. Perry, and J. Cairns Jr. 2005. Vegetative communities in 20-year-old created depressional wetlands. *Wetlands Ecology and Management* 13: 469–478.

Atkinson, R. B., J. E. Perry, G. B. Noe, W. L. Daniels, and J. Cairns Jr. 2010. Primary productivity in 20-year-old created wetlands in southwestern Virginia. *Wetlands* 30: 200-210.

Ballantine, K. and R. Schneider. 2009. Fifty-five years of soil development in restored freshwater depressional wetlands. *Ecological Applications* 19 (6): 1467-80.

Bedford, B. L., M. R. Walbridge, and A. Aldous. 1999. Patterns in nutrient availability and plant diversity of temperate North American wetlands. *Ecology* 80: 2151-2169.

Bills, J. S. 2008. Invasive reed canary grass (*Phalaris arundinacea*) and carbon sequestration in a wetland complex. Master's thesis, Indiana University, Bloomington, Indiana, USA.

Chambers, R. M. and J. W. Fourqurean. 1991. Alternative criteria for assessing nutrient limitation of a wetland macrophyte (*Peltandra virginica* (L.) Kunth). *Aquatic Botany* 40: 305-320.

Chapin, III, F. S. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11: 233-260.

[CBP] Chesapeake Bay Program. 2017. Learn the Issues: Wetlands [online]. Website https://www.chesapeakebay.net/issues/wetlands [accessed 11 November 2017].

Dahl, T. E. 1990. Wetlands losses in the United States 1780's to 1980's. U.S. Department of the Interior, Fish and Wildlife Service, Washington, D.C. 13pp.

DeBerry, D. A., and J. E. Perry. 2015. Using the floristic quality concept to assess created and natural wetlands: Ecological and management implications. *Ecological Indicators* 53: 247-257.

Dee, S. M. and C. Ahn. 2012. Soil properties predict plant community development of mitigation wetlands created in the Virginia Piedmont, USA. *Environmental Management* 49: 1022-1036.

Dee, S. M and C. Ahn. 2014. Plant tissue nutrients as a descriptor of plant productivity of created mitigation wetlands. *Ecological Indicators* 45: 68-74.

[EL] Environmental Laboratory. 1987. Corps of Engineers Wetlands Delineation Manual. Vicksburg (MS): US Army Corps of Engineers, Waterways Experiment Station. Technical Report Y-87-1.

[EM] Ecosystem Marketplace. 2017. US wetland and stream bank dataset and analysis [online]. Website www.speciesbanking.com [accessed 01 May 2017].

EPA. 2016. Mitigation banking factsheet [online]. Website https://www.epa.gov/ [accessed 04 April 2016].

EPA. 2016. Section 404 permit program [online]. Website https://www.epa.gov/ [accessed 04 April 2016].

Fennesy, M. S., A. Rokosch, and J. J. Mack. 2008. Patterns of plant decomposition and nutrient cycling in natural and created wetlands. *Wetlands* 28: 300-310.

Federal Geographic Data Committee (FGDC). 2013. Classification of wetlands and deepwater habitats of the United States. FGDC-STD-004-2013. Second Edition. Wetlands Subcommittee, Federal Geographic Data Committee and U.S. Fish and Wildlife Service, Washington, DC.

[FICWD] Federal Interagency Committee for Wetland Delineation. 1989. Federal manual for identifying and delineating jurisdictional wetlands. Washington (DC): U.S. Army Corps of Engineers, U.S. Environmental Protection Agency, U.S. Fish and Wildlife Service, and U.S.D.A. Soil Conservation Service. Cooperative Technical Publication.

Hopfensperger, K. N., A. J. Burgin, V. A. Schoepfer, and A. M. Helton. 2014. Impacts of saltwater incursion on plant communities, anaerobic microbial metabolism, and resulting relationships in a restored freshwater wetland. *Ecosystems* 17: 792-807.

Hudson, III, H. W. 2016. Development of forested wetlands ecological functions in a hydrologically controlled field experiment in Virginia, USA. Ph.D. dissertation, Virginia Institute of Marine Science, Gloucester Point, Virginia, USA.

Hudson, III, H. W., E. Wright, R. B. Atkinson, and J. E. Perry 2014. Assessment of woody vegetation for replacement of ecological functions in created forested wetlands of the Piedmont Province of Virginia. Virginia Institute of Marine Science and Christopher Newport University, Virginia, USA.

Kao, J. T., J. E. Titus, and W. Zhu. 2003. Differential nitrogen and phosphorus retention by five wetland plant species. *Wetlands* 23: 979-987.

Koerselman, W. and A. F. M. Meuleman. 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* 33: 1441-1450.

Kuehn, K. A. and K. Suberkropp. 1998. Decomposition of standing litter of the freshwater emergent macrophyte *Juncus effusus*. *Freshwater Biology* 40: 717-727.

Kusler, J. 2006. Developing performance standards for the mitigation and restoration of northern forested wetlands. Discussion Paper, Association of State Wetland Managers, Inc. Travers City, Michigan, USA.

Langis, R., M. Zalejko, and J. B. Zedler. 1991. Nitrogen assessments in a constructed and a natural salt marsh of San Diego Bay. *Ecological Applications* 1: 40-51.

Lichvar, R. W., D. L. Banks, W. N. Kirchner, and N.C. Melvin. 2016. *The National Wetland Plant List*: 2016 wetland ratings. *Phytoneuron* 30: 1-17.

Lichvar, R. W., N. C. Melvin, M. L. Butterwick, and W. N. Kirchner. 2012. National Wetland Plant List Indicator Rating Definitions. ERDC/CRREL TR-12-1. U.S. Army Engineer Research and Development Center, Cold Regions Research and Engineering Laboratory, Hanover, New Hampshire, USA.

Livesley, S. J., A. Ossola, C. G. Threlfall, A. K. Hahs, and N. S. G. Williams. Soil carbon and carbon/nitrogen ratio change under tree canopy, tall grass, and turf grass areas of urban green space. *Journal of Environmental Quality* 45: 215-223.

McJannet, C. L., P. A. Keddy, and F. R. Pick. 1995. Nitrogen and phosphorus tissue concentrations in 41 wetlands plants: a comparison across habitats and functional groups. *Functional Ecology* 9: 231-238.

Mitsch, W. J. and J. G. Gosselink. 2000. Wetlands 3rd ed. Wiley, New York, NY.

Morgan, J. A. and P. Hough. 2015. Compensatory mitigation performance: the state of the science. *National Wetlands Newsletter* 37 (6): 1-9.

Moser, K. F., C. Ahn, and G. B. Noe. 2009. The influence of microtopography on soil nutrients in created mitigation wetlands. *Restoration Ecology* 17: 641-651.

Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. *Pergamon Press*, New York, 173pp.

Roquemore, J. D., H. W. Hudson, III, R. B. Atkinson, and J. E. Perry. 2014. Survival and growth of seven tree species from three stocktypes planted in created wetlands in Loudoun County, Virginia. *Ecological Engineering* 64: 408-414.

RStudio Team. 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. Website http://www.rstudio.com/.

Shen, W., G. Zhang, L. Ma, W. Gui, and R. Szmidt. 2003. Uptake of nitrogen, phosphorus, and potassium by mat rush and effects of nitrogen and potassium fertilizers on plant yield and quality in paddy field soil. *Journal of Plant Nutrition* 26: 757-768.

Spieles, D. J. 2005. Vegetation development in created, restored, and enhanced mitigation wetland banks of the United States. *Wetlands* 25: 51-63.

Spieles, D. J., M. Coneybeer, and J. Horn. 2006. Community structure after 10 years in two central Ohio mitigation bank wetlands. *Environmental Management* 38: 837-852.

Stedman, S. and T. E. Dahl. 2008. Status and trends of wetlands in the coastal watersheds of the Eastern United States 1998 to 2004. National Oceanic and Atmospheric Administration, National Marine Fisheries Service and U.S. Department of the Interior, Fish and Wildlife Service. 32 pp.

Stolt, M. H., M. H. Genthner, W. L. Daniels, V. A. Groover, S. Nagle, and K. C. Haering. 2000. Comparison of soil and other environmental conditions in constructed and adjacent palustrine reference wetlands. *Wetlands* 20 (4): 671-683.

Streever, B. 1999. Examples of performance standards for wetland created and restoration in Section 404 permits and an approach to developing performance standards. WRP Technical Notes Collection (TN WRP WG-RS-3.3). U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Tiner, R. W. 1987. Mid-Atlantic wetlands: a disappearing natural treasure. U.S. Fish and Wildlife Service National Wetlands Inventory Project, One Gateway Center, Newton Corner, Massachusetts, USA.

Tiner, R. W. 1998. In search of swampland: a wetland sourcebook and field guide. Rutgers University Press, New Brunswick, New Jersey, USA.

U.S. Army Corps of Engineers (USACE). 2010. Regional supplement to the Corps of Engineers wetland delineation manual: Atlantic and Gulf Coastal Plain Region (Version 2.0). U.S. Army Engineer Research and Development Center, Vicksburg, Mississippi, USA.

Van den Bosch, K. and J. W. Matthews. 2017. An assessment of long-term compliance with performance standards in compensatory mitigation wetlands. *Environmental Management* 59: 546-556.

Wassen, M. J., H. G. M. Olde Venterink, and E. O. A. M de Swart. 1995. Nutrient concentrations in mire vegetation as a measure of nutrient limitation in mire ecosystems. *Journal of Vegetation Science* 6: 5-16.

Weakley, A. S., J. C. Ludwig, and J. F. Townsend. 2012. Flora of Virginia. Bland Crowder, ed. Foundation of the Flora of Virginia Project Inc., Richmond. Fort Worth: Botanical Research Institute of Texas Press.

Wetzel, P. R. and A. G. van der Valk. 1998. Effects of nutrient and soil moisture on competition between *Carex stricta*, *Phalaris arundinacea*, and *Typha latifolia*. *Plant Ecology* 138: 179-190.

Wilcox, D. A., N. B. Pavlovic, and M. L. Mueggler. 1985. Selected ecological characteristics of *Scirpus cyperinus* and its role as an invader of disturbed wetlands. *Wetlands* 5: 87-97.

Willby, N. J., I. D. Pulford, and T. H. Flowers. 2001. Tissue nutrient signatures predict herbaceous-wetland community responses to nutrient availability. *New Phytologist* 152: 463-481.

Whigham, D., M. Pittek, K. H., Hofmockel, T. Jordan, and A. L. Pepin. 2002. Biomass and nutrient dynamics in restored wetlands on the outer Coastal Plain of Maryland, USA. *Wetlands* 22: 562-574.

Wright, D. J. D. 2015. The characterization and influence of vegetation structure surrounding planted trees in created forested wetlands in the Piedmont Province of Virginia. Master’s thesis, Christopher Newport University, Newport News, Virginia, USA.

Wurst, S. J. 2014. Modeling tree growth for piedmont created wetlands with biological and physiochemical parameters. Master’s thesis, Christopher Newport University, Newport News, Virginia, USA.