ABSTRACT

Title of Thesis: VEGETATION AND NUTRIENT DYNAMICS
OF FORESTED RIPARIAN WETLANDS IN
AGRICULTURAL SETTINGS

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Exported agricultural nutrients have been a major supply of excess nutrients into the environment. Riparian wetlands may play an important part in mitigating these nutrients and thus preventing them from migrating downstream in high concentrations. Two riparian wetland systems, one was influenced by agriculture (agricultural site) and one was not (reference site), were studied in Maryland. Both the plant community structure and abiotic factors were studied. The agricultural site had lower overall species richness and tree diversity than the reference site. Also the tree leaf litter and herbaceous leaves at the agricultural site had higher nitrogen and phosphorus concentrations and higher productivity based on fixed carbon than the reference site. Two nutrient enrichment experiments were conducted at the reference site to determine the nature of nutrient limitation. The results from these studies indicate that both plant communities are nitrogen limited. Furthermore, individual species showed a response to increased nutrients.

VEGETATION AND NUTRIENT DYNAMICS OF FORESTED RIPARIAN
WETLANDS IN AGRICULTURAL SETTINGS

By

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Thesis submitted to the Faculty of the Graduate School of the
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Advisory Committee:
Professor Andrew H. Baldwin, Chair
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Dedication

I would like to dedicate this work to my mom and dad. Without your love, support, and encouragement I would never have made it this far. Dad, it was your love of science and the outdoors that got me interested in environmental science in the first place. Your knowledge and advice helped me immensely. Mom, it was your support that kept me going all this time. I'll always remember you sitting in the living room helping me make my leaf litter traps. There are no words to express how indebted I am to both of you. Thank you.

Acknowledgements

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To the rest of EQL – especially Cathleen Hapeman, Swati Mookherji, Jennifer Klemens, Reuben Anderson, Ali Sadeghi, Emy Pfeil, and Laura McConnell– thanks for all your encouragement throughout the years!

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Chapter 1: Introduction

BACKGROUND

The release of nutrients into natural systems is a continuing problem in the United States (Carpenter *et al.* 1998). Agriculture has been a major source of this nutrient flux (Peterjohn and Correll 1984; Brenner 1995). Today, the amount of nutrients applied as fertilizer into agricultural fields is greater than that removed in the crop biomass (Carpenter *et al.* 1998). This imbalance results in the export of excess nutrients from the fields and may be transported to surface waters downstream via runoff and infiltration of rain water.

Riparian wetland systems may play an important role in retaining nutrients because of their position in the landscape (Gilliam 1994; Bischoff *et al.* 2001; Casey *et al.* 2001). Bischoff *et al.* (2001) have shown that wetland soils can provide long-term storage of nutrients while the vegetation provides short-term storage. Phosphorus sorption and denitrification are important mechanisms of nutrient retention and removal in the soil (Mitsch and Gosselink 1993). Wetlands can remove 90 to 100% of NO_3^- that enter enters the system (Todd *et al.* 1984; Cooper 1990; Bischoff *et al.* 2001).

Researchers have also found that plant uptake is an important pathway in nutrient cycling (Mitsch and Gosselink 1993). One study found that over 50 kg/ha/yr of N could be removed via plant uptake in riparian forests (Todd *et al.* 1984). Furthermore, studies have indicated that natural wetlands can act as sinks for phosphorus contained in agricultural runoff (Chambers *et al.* 1993). Todd *et al.* (1984) determined that woody vegetation in riparian forests could remove 3.8 kg/ha/yr of P while non-woody vegetation removed 3.3 kg/ha/yr.

Not only does the vegetation in these systems affect the fate of these excess nutrients, but excess nutrients can also impact the plant community structure (Verhoeven *et al.* 1994). Introduction of nutrients into a natural system can limit the productivity of some species while enhancing the productivity of others. Nutrient enrichment can change species composition and reduce the species diversity of wetlands (Morris 1991; Bedford *et al.* 1999). Nitrogen, phosphorus, or both sometimes limit the growth of plants. Thus, the dynamics of the plant community can be altered by the import of agricultural nutrients.

FORESTED RIPARIAN WETLANDS

Forested riparian wetlands, which may also be known as bottomland hardwood and floodplain forests, are important because of their location in the landscape. They occur between at the interface of terrestrial and aquatic ecosystems. These wetlands are frequently flooded and often have high water tables because of their close proximity to streams and rivers.

There are three major features of riparian wetlands: (1) they are linear in form since they are close to rivers and streams; (2) they interact with energy and materials from upstream areas more so than any other ecosystem; (3) they are linked with upstream, downstream, upslope, and downslope areas (Mitsch and Gosselink 1993). These characteristics mean that these areas may play an important role in treating pollutants carried from upstream or upslope regions. Of course, their success in attenuating or treating these pollutants is dependent on many factors, including the existing vegetation and soils and the frequency of flooding.

The vegetation is an important factor influencing the ecosystem's functions

(Silvan *et al.* 2004). The plants provide bank stability, flood control for areas downstream because they slow the flow of water, temperature control via shading, leaf litter to the system which can increase microbial activity, and wildlife habitat.

European wetlands have been largely studied and classified as either being nitrogen or phosphorus limited (Bedford *et al.* 1999). However, the classification of nutrient limitation in North American wetlands is still lacking and disputes within the scientific community about the nature of limitation in North American wetlands are still prevalent (Bedford *et al.* 1999).

SITE SELECTION AND LOCATION

I studied two riparian wetlands. One was influenced by agricultural runoff that contained high concentrations of nutrients while the other was not directly influenced by agriculture. Both sites are found in Laurel, Maryland, which is about 30 miles northeast of Washington, D.C., and are part of the Chesapeake Bay Watershed. They are both located on the grounds of Beltsville Agricultural Research Center (BARC, part of the U.S. Department of Agriculture) and are within 1.9 km of each other (Figure 1-1). The proximity of the two sites to each other increases the probability of having similar plant species, soils, and hydrology at both sites. In addition, both sites were adjacent to 1st order streams.

The agricultural site chosen for this project (known as OPE^3 – Optimizing Production Inputs for Economic and Environmental Enhancment) is part of an international research program involving many U.S. government agencies, universities, and private industries. This site is considered to be an agricultural riparian forest buffer.
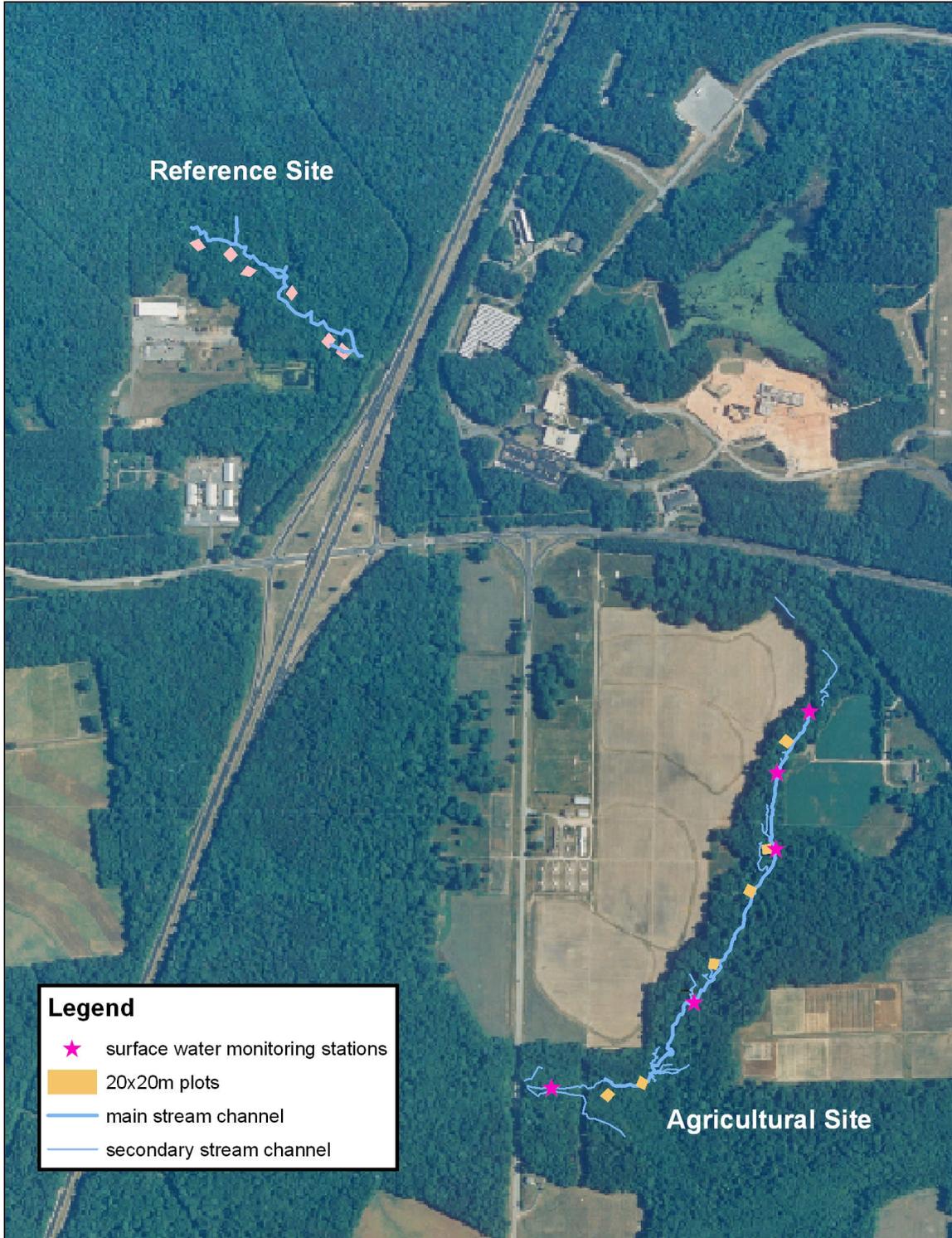
Figure 1-1. Location of research sites in Laurel, Maryland. Both sites are located on the grounds of the Beltsville Agricultural Research Center (BARC, part of the USDA).

These buffer areas consist of trees and other vegetation and are located between agricultural croplands and streams (or other waterbodies) or a groundwater recharge area (Palone and Todd 1998). Such areas are very important in retaining nutrients and other pollutants because their positions in the landscape actively slow the flow of water thereby providing the opportunity for retention and/or biodegradation of chemicals.

The overall goal of the OPE[3] project is to investigate environmental issues in both the agricultural fields and the nearby forested riparian wetland. This site was chosen for this project because it is surrounded by corn (*Zea mays* L.) fields that regularly receive chemical fertilizer and manure for increased productivity. The area has been receiving agricultural runoff for over 50 years. Like most forests surrounding agricultural fields, this area is deeply fragmented in nature. The soil is mainly Typic Haplosaprist (Johnston silt-loam series) (Angier *et al.* 2002) indicating that it is very poorly drained, highly organic, and subject to flooding with a seasonally high water table at or near the surface (Kirby *et al.* 1967).

This site had been instrumented with weirs, leaf litter traps, and sap flow sensors. The height of and nutrient concentrations in groundwater had also been studied. Moreover, surface water was collected regularly. During storm events, automated samplers situated beside each weir were collected stream water samples while during baseflow conditions, samples were collected manually and tested for nutrients. Thus the hydrology has been well studied. Elevated concentrations of nitrogen in the surface water, ground water, and the vegetation (to a limited extent) have already been documented within this system. Preliminary vegetation samples also showed that the leaves of *Symplocarpus foetidus* L. Salisb. ex Nutt. (skunk cabbage) have high

concentrations of phosphorus. However, the characteristics and role of vegetation in nutrient dynamics had not been extensively studied.

The purpose of my study was to compare the plant community structures and nutrient dynamics at OPE[3] (agricultural site) to a non-agriculturally impacted wetland (reference site). I developed several criteria for the location of the reference site. It had to be a forested riparian wetland with similar hydrology and plant species as the agricultural site. Skunk cabbage was used as an indicator species since it is commonly found in areas where upwelling of water is observed. Additionally, the site could not border any agricultural fields or other areas that may result in high nutrient loads into the system.

The reference site, which was also located in Laurel, Maryland, was chosen because it matched these criteria.   It was a contiguous wetland forest. Upon examination of the Prince Georges County Maryland soil survey maps (Kirby *et al.* 1967), the three soil series listed at the reference site were Elkton Silt Loam, Keyport Silt Loam, and Christiana Silt Loam. I recognize that looking at soil surveys is not a replacement for on-site investigations of soils, but it does allow me to estimate the types of soils present. The Elkton Silt Loam soil series consists of poorly drained silt loam over clay or clay loam with seasonally high water tables 0-1 ft below the soil surface (Kirby *et al.* 1967). The Keyport soil series contain soils that are moderately well drained, fine sandy loams, silt loams, and silty clays that overlay thick layers of clay or silty clay that have seasonal water table 1-2 ft below the soil surface (Kirby *et al.* 1967). The Christiana Silt Loam soil series, on the other hand, is typified by well-drained soils clayey throughout the soil profile with a thin surface layer of sandy or silt loam (Kirby *et al.* 1967).

To test the site's suitability, preliminary groundwater and surface water samples were collected in June 2002 at the reference site and the agricultural site to compare concentrations of ammonium, nitrate, and phosphate. One surface water sample was taken and had a nitrate concentration of 0.59 mg/L. The groundwater was collected by digging holes in the ground with a shovel until the groundwater was found. Since 2002 was a drought year, the groundwater was found at a depth between 1.5 and 2.5 feet in all reference site holes and between 1 and 1.5 ft in depth at the agricultural site. The data revealed that the ammonium concentrations were twice as high at the reference site as at the agricultural site with the P-value for a t-test was slightly above 0.05 (Table 1-1).

	Groundwater		
	AGRICULTURAL	**REFERENCE**	**P**
Ammonium	0.7 ± 0.10	1.4 ± 0.30	0.054
Nitrate	0.06 ± 0.03	0.05 ± 0.01	0.90
Phosphate	0.06 ± 0.01	0.04 ± 0.02	0.46

Table 1-1. Means ± SE for ammonium, nitrate, and phosphate concentrations in groundwater in mg/L. P-values are for t-tests of site effects.

This difference may be due to differences in water saturation. The agricultural site is generally more saturated than the reference site. This saturation would thus promote anaerobic conditions. Since mineralization rates are usually higher in aerobic rather than anaerobic conditions, the mineralization rates may be higher at the reference site which could lead to higher ammonium concentrations (Richardson and Vepraskas 2001). However, no significant difference between sites was observed for nitrate and phosphate (Table 1-1). This insignificance may have been due to the drought. Nitrate typically is dissolved in water and transported into nearby areas. Since the agricultural site receives

most of its nitrate via groundwater transport, the concentration entering the site would have been lower due to the drought. In addition, denitrification may have been high resulting in lower nitrate concentrations. Also, phosphorus is typically bound to soil particles and is largely transported through the environment by surface processes, such as runoff. During the drought there mechanism for phosphate transportation into the wetland at the agricultural site was probably limited. Moreover, there is normally very little phosphorus within the water column because living plants take up most of the available phosphorus (Richardson and Vepraskas 2001).

PROJECT JUSTIFICATION AND OBJECTIVES

Excess nutrients in agricultural runoff can have detrimental effects on downstream ecosystems. The presence of high levels of nitrogen and phosphorus in particular is a serious problem in the United States (Carpenter *et al.* 1988). These additional nutrients provide food for algae, causing algal blooms to become prevalent. The elevated algal populations prevent light from penetrating the deeper waters. This lack of light can then cause a decrease in submerged aquatic vegetation (SAV) since photosynthesis rates are decreased. When these algal populations die and begin to decompose oxygen is utilized thereby hindering the amount remaining for other biological activities. The death of fish and other organisms, including both plants and animals, often follow.

Therefore studies assessing the effectiveness of natural systems, like forested riparian wetlands, to filter these excess nutrients is crucial. Likewise, it is important to determine if the ecosystem changes in response to excess nitrogen and phosphorus to ensure the continued removal of these nutrients. Determining the long-term impact of

agricultural runoff on wetland ecosystems will lead to better management practices and a greater understanding of the role of vegetation in nutrient retention within agricultural watersheds.

For my project, two riparian wetlands were studied. One was surrounded by agricultural fields consisting of corn and the other was not in an agricultural setting. Thus, the two communities could be compared in terms of plant structure and nutrient dynamics. The objectives of my research were to:

1. determine the plant community structure of each site

2. investigate if higher nutrients at the agricultural site cause higher biomass production and nitrogen, phosphorus, and the amount of carbon fixed in tree litter than at the reference site

3. evaluate if the herbaceous vegetation in riparian forests in agricultural and non-agricultural settings is limited by nitrogen, phosphorus, both, or neither (i.e., the nature of nutrient limitation).

PROJECT OVERVIEW

Research was conducted in 2002, 2003, and 2004. In 2002, six permanently marked 20 x 20 m plots were set up at the agricultural site (Figure 1-2). Due to time constraints, only two plots were setup at the reference site. However, in 2003, four more 20 x 20 m plots were added at the reference site making a total of six (Figure 1-3). Two 1 x 2 m herbaceous monitoring plots were created within each of these larger plots. Percent cover and frequency of herbaceous vegetation by species and abiotic factors, like bare ground, coarse woody debris, etc., were determined in 2002 and 2003. Furthermore, aboveground net primary production was estimated using the biomass of tree leaf litter
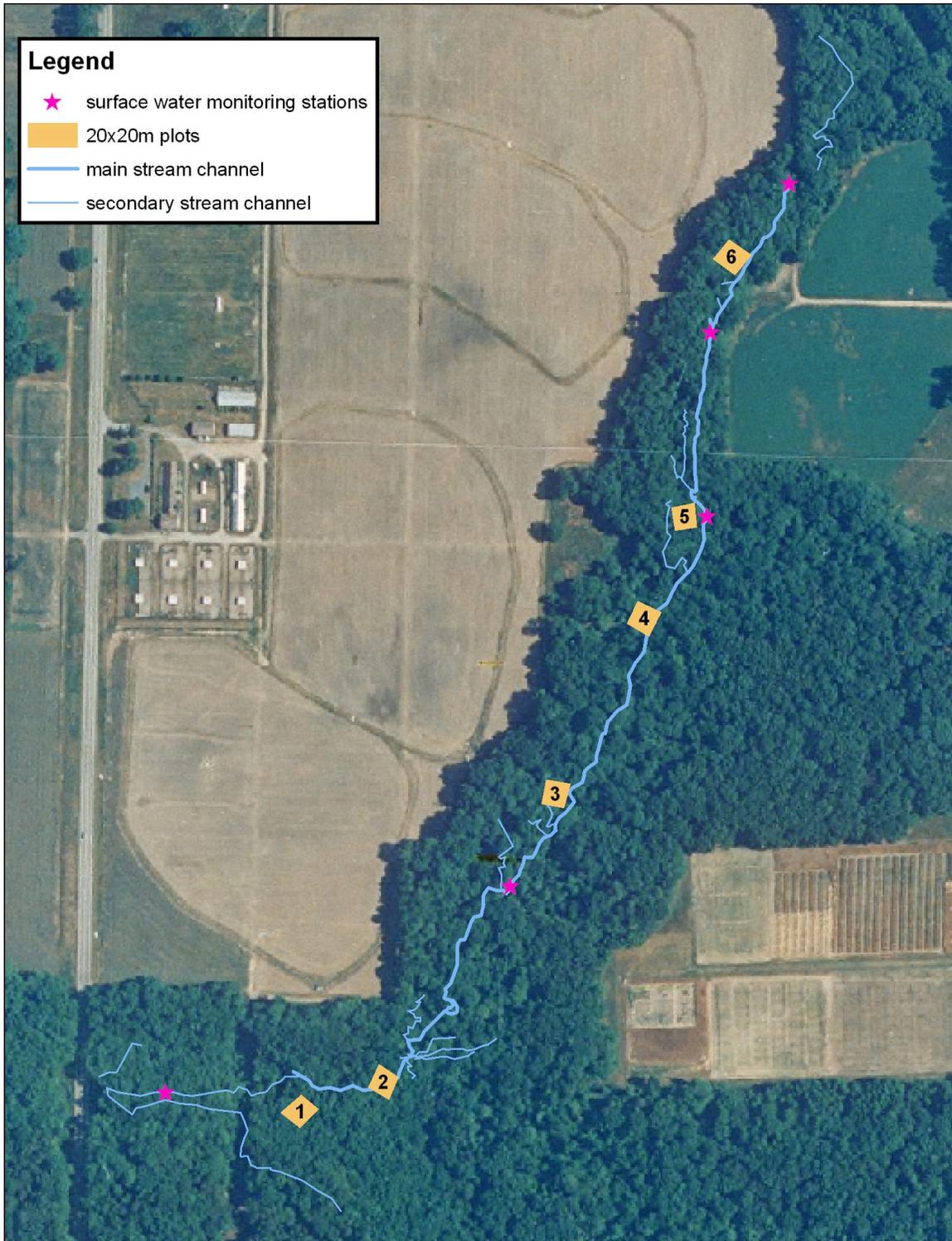
Figure 1-2. Close up view of the agricultural site. The yellow squares mark the locations of the 20 x 20 m plots within the wetland while the pink stars mark the locations of the surface water monitoring stations.
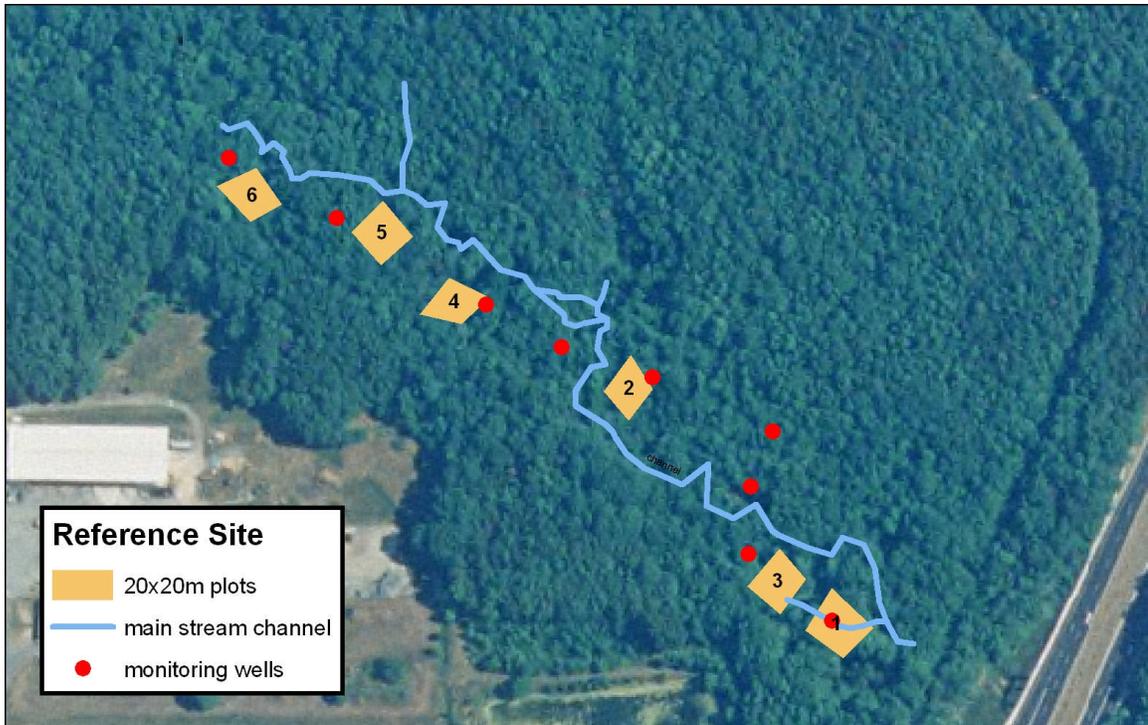
Figure 1-3. Close up view of the reference site. They yellow squares mark the locations of the 20 x 20 m plots within the wetland while the red dots mark the locations of the monitoring wells.

and peak herbaceous aboveground biomass. Two 1 x 1 m leaf litter traps were installed within each larger plot. Leaf litter was collected weekly during the fall season in 2002, 2003, and 2004. The litter was separated by species in 2002 but kept combined irregardless of species in 2003. The names and authorities of all the species in this study were obtained using the USDA Plants Database (USDA/NRCS National Plant Data Center 2004).

Nine shallow monitoring wells were also randomly installed throughout the reference site to measure the depth to the groundwater and to obtain samples for nutrient analysis (Figure 1-3). Wells were not installed at the agricultural site since the groundwater depth and nutrient concentrations had already been documented (Angier 2001; Angier *et al.* 2002).

Tree surveys were also conducted in 2002 at the agricultural site and in 2003 at the agricultural and reference sites within each 20 x 20 m plot. Each tree, shrub, and sapling was tagged and identified. The diameter at base height (DBH) of each tree was measured. For shrubs, the height and stem density of each species were determined. Sapling heights were likewise measured. Leaf area index (LAI) and photosynthetically active radiation (PAR) were also determined.

Two fertilization experiments were conducted at the reference site. The agricultural site was not included since plants at this location have been exposed to agricultural inputs of nutrients for decades. The vegetation at this site therefore may not behave in a similar manner to the addition of nutrients as non-agriculturally influenced plants may. The objective of both experiments was to determine if the herbaceous vegetation in riparian forests in agricultural and non-agricultural settings was limited by N, P, both, or neither (i.e., the nature of nutrient limitation).

The first experiment occurred in 2003. Twenty 2 x 1 m plots were also randomly set up. Five of these plots were fertilized with nitrogen; five were fertilized with phosphorus; five received both nitrogen and phosphorus; and five received no treatment as controls. All herbaceous vegetation within these plots was later harvested and separated by species to determine if fertilization had any affect on biomass production, nitrogen and phosphorus concentrations, and element ratios. All species within these plots were harvested and examined. Aboveground net primary production, plant tissue concentrations of nitrogen, phosphorus, and carbon, species cover, and species diversity were measured to assess the community response at the reference site to nutrient enhancement. The plant tissue concentrations will be measured to determine the

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relationship between nutrient limitation and vegetation N:P ratios using the methods of Koerselman and Meuleman (1996).

The second experiment, which occurred during the 2003-2004 growing season, involved the fertilization of *Symplocarpus foetidus* only. Forty plants were randomly chosen in a small area that was not previously utilized in another of the other studies at the reference site. Ten plants received nitrogen, ten received phosphorus, ten received both nitrogen and phosphorus, and ten received no treatment. The plants were harvested so that biomass and tissue concentrations of nitrogen, phosphorus, and carbon could be examined to determine if any treatment effects existed. Plant length, width, and height were also measured.

Chapter 2: Plant Community Structure and Abiotic Factors in an Agriculturally Enriched and a Non-Enriched Wetland

INTRODUCTION

Forested riparian wetlands are exposed to a variety of pollutants entering from upstream and upslope. Therefore, emphasis is often placed on their ability to treat agricultural runoff and groundwater that contains high amounts of these pollutants, particularly nutrients. However, the influence of agriculture on the plant community structure of these ecosystems is also important since it is a major factor that determines the ability of a wetland to transform, store, or remove nutrients. Some studies have found that an increase in available nutrients often causes a change in species composition, decrease in species richness, decrease in species diversity, decrease in species evenness (i.e. dominance of one or a few species), and invasion of exotic species (Bedford *et al.* 1999). Moreover, long term effects of disturbance can cause changes in species composition, the rate of stand development and competition between plant species (Roberts and Gilliam 1995).

Forested riparian wetlands bordering agricultural lands may be impacted by both excess nutrients and pesticides. These "buffer systems" are often fragmented. This characteristic may also play an important role in plant community structure. Species richness in temperate wetlands has been related to nutrient availability, hydrology, and topography (Bedford *et al.* 1999).

In this project I compared the plant community structure and abiotic factors of a forested riparian wetland in an agriculturally influenced setting with one in a non-

agricultural setting. The objectives of this study were to gain a better understanding of the influence of long term disturbance due to agricultural effluent on species richness, plant diversity, and community evenness. I anticipated that the agricultural site would have lower species richness, diversity, and evenness than the reference site since the excess nutrients entering from the agricultural fields may promote the spread of a few species that are adept at utilizing the nutrients.

METHODS

The number of trees, saplings, and shrubs were determined in each of six marked 20 x 20 m plots that were established randomly throughout the agricultural site in 2002. However, due to time constraints, only two were placed at the reference site in 2002. Four more plots were added in 2003 at the reference site to achieve a total of six plots.

The following criteria were used to distinguish trees, saplings, and shrubs. Species greater than 4 m in height with a tree growth habit at maturity were considered trees. Species less than 4 m in height with a tree growth habit at maturity were treated as saplings. Those species with a shrub growth habit at maturity were treated as shrubs.

Within the plots, each tree was taxonomically identified according to Gleason and Cronquist (1991) and the diameter at breast height (DBH) was measured at a height of 1.4 m above the ground. Heights were measured to the nearest 0.01 m for saplings. Each shrub was likewise taxonomically identified and the number of stems and height to the nearest 0.01 m were measured.

Herbaceous vegetation and abiotic factors, including bare ground, coarse woody debris, leaf litter, and water, were surveyed in two 2 x 1 m quadrats that were permanently marked within each of the larger plots. Most plants were identified in the

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field based on recognition. However, if a plant could not be positively identified in the field, a sample was taken from nearby and brought inside the laboratory and keyed out using Gleason and Cronquist (1991). A sampling frame subdivided into 32 cells was placed over each 2 x 1 m quadrat to determine the frequency of occurrence of individual species and the percent coverage of each species within these cells. The percent cover is the area of ground surface that each plant occupies if projected vertically onto the surface within the quadrat expressed as a percentage. Percent cover values can be greater than 100% since plants often overlap each other. The cover values used were: <0.1%, 0.1% increments from 0.1-1%, 1% increments from 1-10%, and 5% increments above 10%.

The leaf area index (LAI), which is a dimensionless expression of the foliage area per unit area of ground surface (e.g. m^2/m^2), is a good indicator of aboveground biomass. Woody LAI was measured approximately 2 m above the ground surface using a digital canopy meter (model LAI-2000, LI-COR, Lincoln, Nebraska). Photosynthetically active radiation (PAR), which is the light between the wavelengths of 400 and 700 nm that is absorbed by chlorophyll, was also measured in September of 2003 at both sites using a LI-COR 1 m quantum sensor with an LI-250 light meter (LI-COR, Lincoln, Nebraska). The plots were divided into two equal halves (each 10 x 20 m) and measurements were taken in the middle of each half. The wand was held approximately 2 m above the ground while turning in a circle (radius ≈ 1.5 m) over a 15 second averaging period.

Nine shallow monitoring wells, which consisted of PVC pipe with slits, were installed to a depth of about 1.1 m in topographically influenced areas (like near the base of a hill) at the reference site. These wells allowed for the determination of the water table height and nutrient concentrations. This depth was chosen because during the

drought year (2002) groundwater was obtained by digging holes in the ground and the maximum depth to the groundwater was 0.8 m. Dr. Jonathan Angier, a USDA research hydrologist, helped determine these key areas by looking at the landscape in the field. No monitoring wells were placed at the agricultural site, however, because this site had already been well instrumented with both monitoring wells and nested piezometers and an abundance of data already existed.

The depth to the water table was determined a few times using an automated tape that was lowered into the well and beeped when water was encountered. After the depth had been measured, the water was pumped out of the well and the well was allowed to replenish. It sometimes took over 24 hours for water to refill in the wells to their previous level. Once this occurred, groundwater samples were collected and placed in the refrigerator for 24 hours to allow suspended sediments to settle. They were then analyzed for ammonium, nitrate/nitrite, and phosphate using a Flow Injection Analyzer (Lachat QuikChem 8000, Milwaukee, Wisconsin).

In addition, two surface soil samples were randomly collected at a depth of 0-15 cm within each 20 x 20 m plot at both sites in 2004. These two samples were then combined together in the field and air dried using fans in the laboratory. They were then homogenized using a mortar and pestle. Basic soil characteristics were examined for each site. I will use a subsample of each soil sample to determine the soil texture using a Bouyoucos hydrometer. This method measures the density of the suspended soil, which varies with the amount and type of particles suspended. It is imperative to recognize the influence of organic matter on the hydrometer readings. Since it was anticipated that the soils would contain a high amount of organic matter, the samples were oxidized prior to

analysis using hydrogen peroxide.

Elemental analyses were also performed on the subsamples from each soil sample. Samples were analyzed for total organic carbon and nitrogen via dry combustion using a LECO TruSpec 8000 CN Analyzer (St. Louis, Missouri). The Kjeldahl digestion method was used to determine total phosphorus. Digests were then run on a Flow Injection Analyzer (Lachat QuikChem 8000, Milwaukee, Wisconsin). Nutrients in plant available forms were also analyzed. Exchangeable ammonium and nitrate were determined by using a potassium chloride extraction (Mulvaney 1996). Phosphate was determined using the dilute acid extraction procedure (also known as North Carolina or Mehlich-1 P test) (Kuo1996).

RESULTS

Trees and Shrubs

The relative abundance and richness of tree species at both sites were examined in 2003 by plotting the abundance of each species by their rank in abundance (Figure 2-1). The first thing to notice is that each site contains a total of seven tree species. Next, the curves reveal that *Acer rubrum* L. is the most prevalent species at both sites. However, while this species appears to be the most dominant at the agricultural site, it seems to be co-dominating with *Nyssa sylvatica* Marsh and *Ilex opaca* Ait. at the reference site. These graphs also reveal that there is higher species evenness at the reference site than the agricultural site since the slope of reference site curve is less steep.

Excess nutrients in agricultural effluent may be retained by nearby riparian buffer
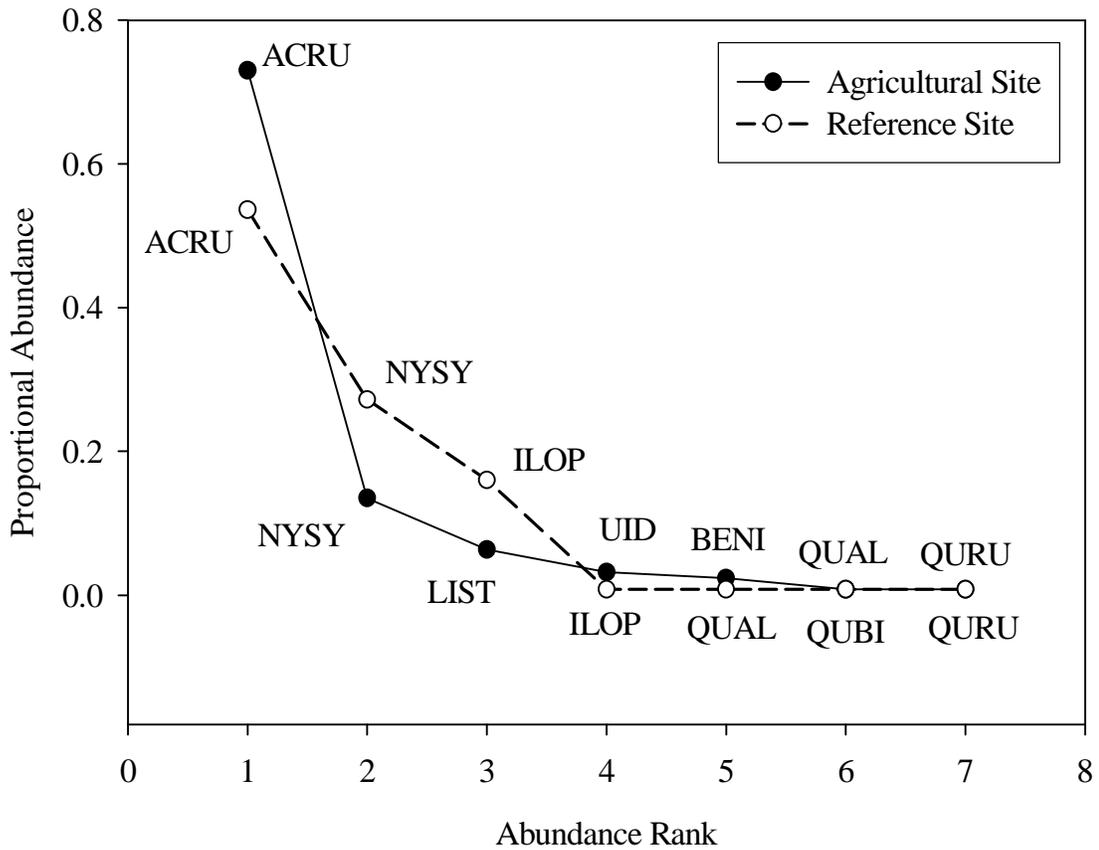
Figure 2-1. Rank-abundance curves for tree species by site. Values on the X-axis are the abundance ranks for each species. Values on the Y-axis are the proportional abundance (number of times each species occurred divided by the total number of trees). The reference site has greater species evenness than the agricultural site but both communities have the same species richness. ACRU=*Acer rubrum***; ILOP=***Ilex opaca***; LIST=***Liquidambar styraciflua***; NYSY=***Nyssa sylvatica***; UID=***Unidentified species***; BENI=***Betula nigra***; QUAL=***Quercus alba***; QUBI=***Quercus bicolor***; QURU=***Quercus rubra***.**

vegetation, but these nutrients may negatively impact the ecosystem's species diversity, which is an important measure of ecosystem health. Shannon-Wiener Diversity Indices were also calculated for each site using the following equation:

$$H' = -\sum_{i=1}^{s} p_i \log_e p_i$$

where H ' is the value of the Shannon-Wiener diversity index, p_i is the proportion of the ith species, $\log_e$ is the natural logarithm of p_i, and s is the number of species in the community. The diversity index of the trees at the agricultural site was 0.950 while the diversity index of the reference site was 1.14. Thus, the reference site has higher tree species diversity than the agricultural site. This higher value reflects a greater evenness of species composition at the reference site since both sites had the same number of species. The shrub diversity index at the agricultural site was 1.23 while that at the reference site was 0.637.

There was also a greater frequency of smaller trees at the reference site than at the agricultural site (Figure 2-2). The diameters of most trees at the reference site fall between 5 and 25 cm at the reference site while those at the agricultural site fall between 30 and 45. The mode of the DBH at the reference site is 10 cm while that at the agricultural site is 30 cm.

The density of trees on a per hectare basis was also examined for both forests (Table 2-1). *Acer rubrum* was the dominant tree found at the agricultural site. There was not a significant difference between sites or between species.
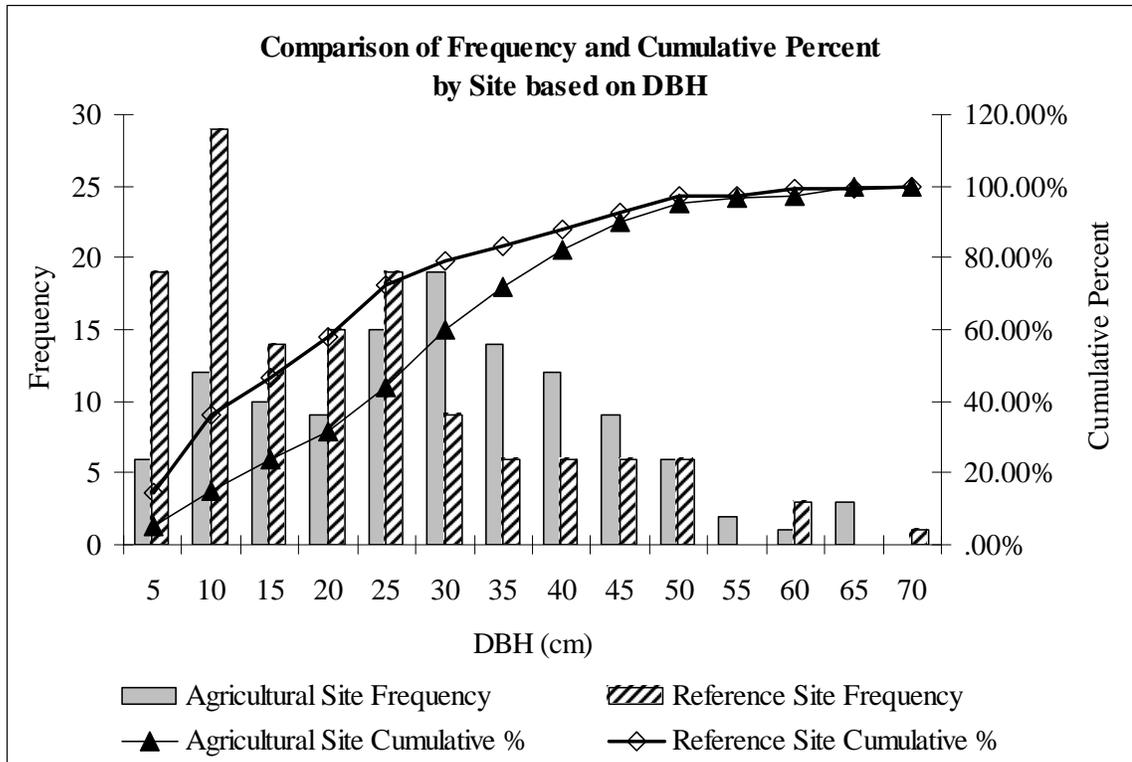
Figure 2-2. Comparison of frequency and cumulative percent of trees by site based on DBH. Values on the X-axis are DBH classes in increments of 5 cm. Values on the left Y-axis are number of occurrences of trees in each class while those on the right Y-axis are cumulative percent of the observations.

The amount of light penetrating through the canopy affects plant growth. PAR measurements were taken to assess potential light differences. Recall that PAR was taken at a height of about 2 m above the ground. Therefore this measurement would be an assessment of light penetrating the tree canopy without the influence of existing shrubs. No significant difference was found between sites (P>0.05) although the agricultural site did have a higher average PAR (190.2 ± 23.27 µmol s^{-1} m^{-2}) than the reference site (112.2 ± 51.79 µmol s^{-1} m^{-2}). LAI measurements were likewise taken. It was found that the agricultural site did have a lower average LAI (2.6 ± 0.08) than the reference site (3.1 ± 0.22), but was not significantly different.

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SPECIES	AGRICULTURAL	REFERENCE	P
	Mean ± SE	Mean ± SE	
Acer rubrum L.	31 ± 1.3	24 ± 1.5	0.0007
Betula nigra L.	28	--	--
Ilex opaca Ait.	5 ± 1.5	1.6	--
Liquidambar styraciflua L.	--	22 ± 4.2	--
Nyssa sylvatica Marsh	8 ± 1.4	9 ± 1.2	0.73
Quercus alba L.	22	57	--
Quercus bicolor Willd.	--	29	--
Quercus rubra L.	36	65	--
UID	25 ± 3.4	--	--
Overall Average	**27 ± 1.3**	**20 ± 1.3**	**0.0004**

Table 2-1. Tree density per hectare for each species present at both sites. P-values are for t-tests of site effects. A double dash (--) indicates that the species was not present at the designated site and therefore a t-test was not performed.

SPECIES	AGRICULTURAL	REFERENCE	P
	Mean ± SE	Mean ± SE	
Acer rubrum L.	383 ± 71.2	279 ± 53.0	0.27
Betula nigra L.	13 ± 12.5	--	--
Ilex opaca Ait.	33 ± 23.9	4 ± 4.2	0.28
Liquidambar styraciflua L.	--	83 ± 30.1	--
Nyssa sylvatica Marsh	71 ± 29.2	142 ± 73.8	0.39
Quercus alba L.	4 ± 4.2	4 ± 4.2	1.0
Quercus bicolor Willd.	--	4 ± 4.2	--
Quercus rubra L.	4 ± 4.2	4 ± 4.2	1.0
UID	17 ± 12.4	--	--
Total	**525 ± 80.6**	**521 ± 104.2**	**0.98**

Table 2-2. Diameter at base height (DBH) in cm for each tree species present by site. P-values are for t-tests of site effects. A double dash (--) indicates that one or no trees of that species was present at least one of the designated sites and therefore a t-test was not performed.

However, a significant difference in DBH existed overall and for *Acer rubrum* between sites (Table 2-2). It is important to note that the agricultural site has a greater number of shrubs than the reference site (Table 2-3).

SPECIES	AGRICULTURAL		REFERENCE	
	No. of Stems	Height	No. of Stems	Height
Ilex verticillata (L.) Gray	4 ± 1.2	3 ± 0.3	0.3 ± 0.3	4
Lyonia ligustrina (L.) DC.	1 ± 0.4	2 ± 0.5	0.2 ± 0.2	2
Unidentified Species	3 ± 1.0	4 ± 0.2	--	--
Viburnum recognitum Fern.	7 ± 3.5	3 ± 0.2	--	--

Table 2-3. Average number of stems ± SE and height (m) ± SE for shrubs per 0.04 ha plot at both sites.

There were more saplings at the agricultural site than at the reference site (Table 2-4), but I saw many *Acer rubrum* seedlings (some actually growing at the base of the *Symplocarpus foetidus* leaves) at the reference site which were not measured because

Site	Species	Frequency	Circumference	Height
Agricultural	*Betula nigra*	3	0.04 ± 0.002	1.6 ± 0.7
Agricultural	*Ilex opaca*	7	0.07 ± 0.02	1.0 ± 0.1
Agricultural	*Nyssa sylvatica*	3	0.5 ± 0.2	2.9 ± 0.3
Reference	*Nyssa sylvatica*	1	0.09	4

Table 2-4. Average sapling circumference (cm) ± SE and height (m) ± SE at both sites. There was only one sapling at the reference site so a standard error could not be computed here.

they we were generally less than 5 cm in height.

The basal area of the trees is another important characteristic of both systems. While a significant difference does not exist between sites on the whole, a significant difference does exist between sites for the most dominant tree at both sites – *Acer rubrum* L. (red maple) (Table 2-5). Indeed, the average basal area of the red maples found at the

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agricultural site is more than double that at the reference site.

Herbaceous Vegetation

A total of 24 herbaceous species were found within the plots at the agricultural site while a total of 35 were found at the reference site. The herbaceous plant diversity was found to be slightly lower at the reference site (H'=1.26) than the agricultural site (H'=1.31). *Symplocarpus foetidus* had the highest percent of cover and frequency at the agricultural site (Table 2-6). While *Symplocarpus foetidus* contributed the highest percent cover at the reference site, *Microstegium vimineum* (Trin.) A. Camus, an introduced species that spreads very rapidly, occurred most frequently. The plant with the second highest cover and frequency at the agricultural site was *Impatiens capensis* Meerb. while that at the reference site was *Microstegium vimineum*. It is also important to note that the agricultural site has significantly higher percentage of cover and frequency of the two most dominating species at that site – *Symplocarpus foetidus* and *Impatiens capensis* – than the reference site (Table 2-6). Both systems are dominated by these species, but the reference site is also highly dominated by *Microstegium vimineum*.

Abiotic Factors

The cover and frequency of bareground, coarse woody debris, leaf litter, and standing water were also examined (Table 2-7). It was found that bareground at the reference site had significantly higher percentage of cover and frequency of occurrence than the agricultural site. However, the agricultural site had higher percent cover of coarse woody debris, leaf litter, and water.

Surface water was collected at both sites in September 2003 and analyzed for

SPECIES	AGRICULTURAL		REFERENCE		P
	Num. of trees	Mean ± SE	Num. of trees	Mean ± SE	
Acer rubrum L.	92	13398 ± 1493.4	67	6270 ± 1190.9	0.0039
Betula nigra L.	1	106	0	--	--
Ilex opaca Ait.	8	24 ± 13.9	1	0.3 ± 0.3	0.15
Liquidambar styraciflua L.	0	--	20	2180 ± 1116.5	--
Nyssa sylvatica Marsh	17	319 ± 175.7	34	538 ± 394.5	0.62
Quercus alba L.	1	62	1	430 ± 429.7	0.43
Quercus bicolor Willd.	0	--	1	112 ± 112.2	--
Quercus rubra L.	1	172 ± 172.3	1	557 ± 557.3	0.53
UID	4	355 ± 315.7	0	--	--
Total	**124**	**14436 ± 1299.3**	**125**	**10087 ± 1706.6**	**0.070**

Table 2-5. Basal area (cm^2) in 0.04 ha plots and total number of trees for each species present at both sites. P-values are for t-tests of site effects for basal area. A double dash (--) indicates that the species was not present at the designated site and therefore t-tests were not performed.

SPECIES	COVER (%)			FREQUENCY (%)		
	AG.	REF.	P	AG.	REF.	P
Apios americana Medik.	0	0.1 ± 0.09	0.19	0	2 ± 1.6	0.27
Arisaema triphyllum (L.) Schott	0	4 ± 1.9	0.060	0	11 ± 4.4	0.033
Boehmeria cylindrica (L.) Sw.	0.4	1 ± 0.8	0.57	3 ± 3.1	2 ± 1.6	0.77
Carex folliculata L.	0	0.2	0.34	0	1 ± 0.5	0.34
Carex intumescens Rudge	0.8 ± 0.54	0.5 ± 0.42	0.68	2 ± 1.6	2 ± 1.6	1.0
Carex lurida Wahlenb.	0	0.4	0.34	0	2 ± 1.6	0.34
Carex sp. 1	0	0.4	0.34	0	1 ± 1.0	0.34
Carex sp. 2	0	0.4	0.34	0	1 ± 1.0	0.34
Carex sp. 3	0	0.3	0.34	0	1 ± 0.8	0.34
Cinna arundinacea L.	2.2 ± 2.08	5.3 ± 3.9	036	13.3 ± 2.3	25 ± 10.9	0.34
Claytonia virginica L.	0.9 ± 0.3	2.7 ± 2.1	0.40	8.9 ± 2.4	12.5 ± 6.4	0.60
Cyperaceae	0.3 ± 0.3	0	0.22	1 ± 1.1	0	0.24
Dioscorea villosa L.	0	0.7 ± 0.50	0.14	0	2 ± 1.3	0.11
Duchesnea indica (Andr.) Focke	0.4	0	0.34	4 ± 3.4	0	0.34
Galium tinctorium L.	0.7 ± 0.43	0	0.15	4 ± 2.1	0	0.087

Table 2-6. Percent cover and frequency for herbaceous species by site. P-values are for t-tests of site effects.
(Page 1 of 3)

SPECIES	COVER (%)			FREQUENCY (%)		
	AG.	REF.	P	AG.	REF.	P
Grass1	0	1 ± 0.47	0.047	0	10 ± 4.4	0.045
Impatiens capensis Meerb.	**6 ± 1.5**	**2 ± 0.87**	**0.015**	**39 ± 9.8**	**9 ± 3.4**	**0.014**
Juncus effusus L.	0	0.7 ± 0.47	0.18	0	2 ± 1.1	0.13
Leersia oryzoides (L.) Sw.	0.2 ± 0.1	0.1	0.54	1 ± 0.6	0.3 ± 0.3	0.24
Microstegium vimineum (Trin.) A. Capus	**0**	**17 ± 4.2**	**0.0020**	**0**	**61 ± 10.1**	**<0.0001**
Moss	2 ± 0.7	0.8 ± 0.56	0.17	13 ± 4.5	3 ± 2.1	0.059
Onoclea sensibilis L.	0	1	0.34	0	0.3 ± 0.3	0.34
Osmunda cinnamomea L.	**4 ± 2.3**	**10 ± 6.4**	**0.41**	**15 ± 8.1**	**15 ± 8.3**	**0.97**
Panicum clandestinum (L.)	0	0.4 ± 0.26	0.14	0	2 ± 1.1	0.17
Parthenocissus quinquefolia (L.)	0.1	0.1	1.0	1 ± 1.0	0.3 ± 0.3	0.48
Peltandra virginica (L.) Schott	0.4 ± 0.41	0	0.31	1 ± 0.6	0	0.19
Pilea pumilia (L.) Gray	0.2 ± 0.11	0.3 ± 0.26	0.66	2 ± 1.1	1 ± 0.8	0.58
Polygonum arifolium L.	4 ± 3.3	2 ± 1.7	0.64	9 ± 7.5	3 ± 1.7	0.47
Polygonum punctatum Ell.	1 ± 0.5	0.7 ± 0.43	0.63	15 ± 6.4	6 ± 3.3	0.25
Polygonum sagittatum L.	0	0.1	0.34	0	0.3 ± 0.3	0.34

Table 2-6 (Page 2 of 3)
(Continued)

SPECIES	COVER (%)			FREQUENCY (%)		
	AG.	REF.	P	AG.	REF.	P
Rubus L.	0.7 ± 0.47	0	0.18	3 ± 2.0	0	0.18
Sagittaria latifolia Willd.	0	0.04	0.34	0	1 ± 0.5	0.34
Smilax rotundifolia L.	2 ± 0.6	0.4 ± 0.42	0.17	8 ± 2.9	2 ± 1.3	0.058
Spp. 1	0	0.1	0.34	0	1 ± 1.0	0.34
Spp. 2	0.1 ± 0.09	0	0.19	6 ± 5.2	0	0.26
Spp. 3	0	0.1	0.34	0	0.3 ± 0.3	0.34
Spp. 4	0.5 ± 0.29	0	0.14	6 ± 3.9	0	0.14
***Symplocarpus foetidus* (L.) Salisb. Ex Nutt.**	**71 ± 6.7**	**46 ± 8.5**	**0.031**	**94 ± 6.0**	**58 ± 11.6**	**0.015**
Thalictrum pubescens Pursh	0	0.3 ± 0.26	0.22	0	2 ± 1.3	0.26
Toxicodendron radicans (L.) Kuntze	0.3	0.3 ± 0.13	1.0	3 ± 2.6	2 ± 1.1	0.79
Unidentified Species	**2.1 ± 0.48**	**0**	**0.061**	**59 ± 8.8**	**0**	**<0.0001**
Violet	0	0.3	0.34	0	1 ± 0.5	0.34

Table 2-6 (Page 2 of 3)

(Continued)

	COVER (%)			FREQUENCY (%)		
	AG.	REF.	P	AG.	REF.	P
Bareground	0.8 ± 0.56	19 ± 4.7	0.0022	4 ± 2.5	25 ± 4.4	0.0003
Coarse Woody Debris	4 ± 1.2	2	0.4955	22 ± 5.7	2 ± 2.1	0.0061
Leaf Litter	10 ± 2.7	7 ± 4.2	0.3949	63 ± 12.3	14 ± 8.4	0.0030
Water	8 ± 5.8	2 ± 1.4	0.3119	9 ± 6.5	2 ± 1.4	0.28

Table 2-7. Percent cover and frequency of abiotic factors by site. P-values are for t-tests of site effects. Bareground is ground devoid of vegetation; coarse woody debris includes fallen trees and branches; leaf litter is litter cover the ground; water includes standing and flowing water.

nitrate. Although no significant difference was detected between sites (P=0.13), the agricultural site (1.3 ± 0.30) had almost twice the amount of nitrate as the reference site (0.7 ± 0.03).

Results from the monitoring wells revealed that in July of 2003, the groundwater was found at an average depth of 18 cm while in September it was at a depth of 38 cm. These shallow depths (compared to 2002 when I had to dig more than 1 m in depth to reach the groundwater) were expected since the area is a wetland and Maryland experienced its wettest year on record in 2003 (NOAA/USHCN). These water levels are also within the range for the soil series present there (i.e. soil with water tables between 0-5 ft) (Kirby *et al.* 1967).

A loamy soil texture was present at the agricultural site while a silt loam soil texture was present at the reference site. Nutrient analyses revealed that both nitrogen and carbon concentrations were significantly higher in the agricultural soil than the reference soil (Table 2-8). However, no differences in nitrate and ammonium concentrations or the C:N ratio were detected (Table 2-8). The C:N ratios were both about 15.6.

Parameter	Agricultural Site	Reference Site	P
Nitrate (mg/L)	1.83 ± 0.5	1.42 ± 0.6	0.59
Ammonium (mg/L)	6.23 ± 1.4	4.21 ± 0.6	0.20
N (%)	0.57 ± 0.6	0.31 ± 0.1	0.050
C (%)	8.97 ± 1.7	4.81 ± 0.96	0.060
C:N	15.59 ± 0.6	15.69 ± 0.7	0.92

Table 2-8. Soil concentrations of nitrate, ammonium, nitrate, and carbon and the C:N ratio at both sites. There was a significant difference between sites in total nitrogen and total carbon concentrations.

DISCUSSION

Trees and Shrubs

The prevalence of certain tree species can often predict the nature of flooding in an area (Kozlowski 2002). Both systems are largely dominated by *Acer rubrum*. Kozlowski found that *Acer rubrum* usually dominates riparian forests with short periods of flooding (2002). *Nyssa sylvatica* was also prevalent at both sites along with *Quercus alba*. These species often occur on high ridges that have very short flooding regimes (Kozlowski 2002). *Lyquidambar styraciflua* likewise occurs at the reference site. This species usually occurs on ridges that experience short flooding (Kozlowski 2002).

The DBH of trees found at the agricultural site was significantly greater than at the reference site. This site effect is due to the significantly higher DBH of *Acer rubrum* L. (red maple) at the agricultural site. Most trees at the agricultural site had DBH values of 30 cm while most at the reference site had values of 10 cm. The nutrients entering the agricultural system may be causing the trees to grow at a faster rate than those at the reference site. The productivity of this system may indeed be increasing due to these elevated nutrients. These results have also been found by Tessier and Raynal (2003). They found that *Acer rubrum* trees may be limited by nitrogen and therefore may experience higher growth rates in environments where nitrogen is prevalent.

It was somewhat surprising that the sites were not significantly different in terms of tree density. It was anticipated that the reference site, which is a large expanse of contiguous forest, would have a greater density than the agricultural site, which is deeply fragmented in nature.

Both light and nutrient availability influence plant growth and survival. To

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determine the influence of light on each ecosystem, PAR and LAI measurements were collected. Neither PAR nor LAI were significantly different between sites. Recall that the agricultural site was found to have lower tree diversity but higher shrub diversity. I took both measurements at a height that included the shrub canopy. The higher number of shrubs at the agricultural site may be influencing both of these measurements causing an insignificant difference between sites. Thus, it may be inferred that most differences between sites are due more to discrepancies in nutrient regimes than light regimes. However, since Hurricane Isabel had impacted the area just a few days prior to my measurement dates, some leaves and trees had fallen. In fact, two trees at each site had fallen near plots. My PAR values (112.2 ± 51.79 µmol s^{-1} m^{-2} for the agricultural site and 190.2 ± 23.27 µmol s^{-1} m^{-2} for the reference site) are consistent with other forest-field systems. Cadenasso *et al.* (1997) studied PAR in two well-developed upland forests and compared measurements along the forest-field edge boundaries. They found PAR values ranging between 50 and 150 µmol s^{-1} m^{-2} within the forest (Cadenasso *et al.* 1997).

No difference in the density of the systems was found. It is beneficial to have similar values between sites to reduce the error that this may cause in other studied parameters like herbaceous plant growth. However, these results were not anticipated. As stated previously, the agricultural site is fragmented and has a high potential for disturbance. Wind can penetrate this ecosystem more easily than at the reference site and could potentially cause more trees to fall. Therefore it was thought that the trees at the agricultural site would have a higher turnover rate resulting in less density. However, it is important to note that the agricultural site has a greater number of shrubs than the reference site. These shrubs may be blocking light to the lower canopy, which includes

seedlings. This may be one reason for the lower regeneration of trees at the agricultural site. Furthermore, since the reference site has a larger population of smaller trees, the chances of these trees surviving environmental and climatic changes to reach maturity is also greater than at the agricultural site.

Furthermore, recall that there was a significantly greater amount of leaf litter present on the ground of the agricultural site than the reference site. This litter can further block sunlight from seedlings. Also, it has been found that litter can actually compete with plants for nutrients (Xiong, Nilsson, and Johansson 2001). The litter may be one reason for the lower regeneration of trees at the agricultural site. Furthermore since the reference site has a larger population of smaller trees, the chances of these trees surviving environmental and climatic changes to reach maturity is also greater than at the agricultural site.

Although the overall basal area of the trees was not significantly different between sites, it was significantly different for *Acer rubrum*. This result further confirms that the *Acer rubrum* trees at the agricultural site are larger than those at the reference site. Tessier and Raynal (2003) studied the N:P ratios in plant foliage. They concluded that *Acer rubrum* was nitrogen limited and in the presence of unlimited quantities of nitrogen, *Acer rubrum* trees would experience higher growth rates (Tessier and Raynal 2003). Since nitrogen is readily available at the agricultural site, these trees are probably growing at a faster rate than those found at the reference site.

Herbaceous Vegetation

The herbaceous vegetation at the reference site had a slightly lower diversity index than that at the agricultural site. This result was the opposite of what I had

anticipated. It is probably caused by a larger number of minor herbaceous species at the reference site. The reference site actually had higher herbaceous species richness than the agricultural site. This result was anticipated since the nutrients entering the agricultural site from surrounding fields probably have promoted the growth of species capable of adjusting to and thriving in communities with high nutrient levels. Indeed, nutrient enrichment in several wetland types has resulted in a decrease in species richness (Vermeer and Berendse 1983; Bedford *et al.* 1999).

Both systems are dominated by a few herbaceous species. *Microstegium vimineum* occurred most frequently at the reference site. This species has many qualities that make it invasive and able to adapt to disturbance quite easily. These characteristics include easily adapting to changing environmental conditions, an annual life history, the production of large quantities of seeds, a lack of predators or pathogens, and clonal growth to allow for the of necessary resources.

Abiotic Factors

The higher concentrations of nitrate found in the surface water at the agricultural site were expected since these levels are often increased in waterways bordering agricultural fields (Todd *et al.* 1984; Stone *et al.* 1998; Angier *et al.* 2002). I also expected the total nitrogen and carbon in the soils to be higher at the agricultural site than at the reference site because I thought productivity and nutrient concentrations in the plant litter would be higher at the agricultural site resulting in more litter with elevated concentrations being returned to the ecosystem.

The higher concentrations of nitrogen in the soil at the agricultural site may be due to the elevated concentrations of nitrogen entering from the agricultural fields. The

higher carbon concentrations may be related to the higher nitrogen concentrations.

Perhaps the additional nitrogen stimulates soil microorganisms involved in the

breakdown of organic matter. Carbon would therefore be released. Fenn *et al.* (1998)

found that soils with C:N ratios greater than 20 were more effective in retaining nitrogen

than those less than or equal to 10. This indicates that the soils at both sites may

potentially be effective at retaining nitrogen.

CONCLUSIONS

Although the two ecosystems are similar are many respects, there are some key

differences. The agricultural site has lower species richness and is less diverse than the

reference site. Although both systems are dominated by *Acer rubrum*, the reference site

appears to be dominated with two additional species. The increased nutrients entering the

agricultural system could be causing these results by selecting for species that are better

able to cope with disturbance. *Acer rubrum* may be one of these species since its

diameter and basal area are so significantly higher at the agricultural site than at the

reference site. It is also interesting that *Microstegium vimineum*, an introduced species, is

so prevalent at the reference site.

Chapter 3: Biomass and Nutrient Concentrations of Woody Plant Litter and Herbaceous Vegetation in Agricultural Settings

INTRODUCTION

The nutrient enrichment of natural waters has been due in part to agricultural practices (Peterjohn and Correll 1984; Schröder et al. 2004). Pollution due to elevated nitrogen is of particular concern since it can cause eutrophication problems downstream. While best management practices (BMPs), have been developed to reduce the export of agricultural effluent, it is unrealistic to presume that nutrients are not entering adjacent areas. One BMP that has received much attention is the use of buffer strips. These vegetated areas can be composed of herbaceous vegetation and/or woody vegetation (trees and shrubs). The vegetation in these areas acts to slow the flow of water thereby trapping suspended solids and allowing for plant uptake of nutrients to occur. The soils in these areas are also important. Bischoff *et al.* (2001) found that wetlands stored 99% of their nitrogen in peat, organic soil, and mineral soil and <0.1% in foliage and herbaceous vegetation and woody tissues.

While it is important to determine the effectiveness of these buffer systems to mitigate nutrients, it is equally imperative to study the impacts of the nutrients on these ecosystems. Nutrient cycling is an important function of ecosystems that may be affected by elevated nutrients from agriculture.

Plant uptake is an important pathway in the cycling of nutrients (Mitsch and Gosselink 1993). The concentrations of nitrogen, phosphorus, and carbon in herbaceous

vegetation and tree leaf litter are important since they are ultimately returned to the ecosystem at the end of the growing season, incorporated into the soil via decomposition, and made available for use by soil microbes.

The concentrations of nutrients in herbaceous biomass and tree litter are an important factor in nutrient cycling. The resorption of nutrients is an important conservation mechanism. It reflects the degree to which nutrients are retained by the plant. The concentrations of nutrients resorbed by vegetation influences competition, nutrient uptake, and productivity (Killingbeck 1996).

Calculating nutrient use efficiencies (NUE), which are the litter biomass per unit mass of nutrient, provide an insight into the role of nutrients in ecosystems. There has been some disagreement within the scientific community about whether areas of high nutrient availability result in lower or higher nutrient use efficiencies (Killingbeck 1996). Several studies have found that these efficiencies are lower in nutrient rich areas (Vitousek 1982; Boerner 1984; Anderson and Eickmeier 2000; Koutroubas *et al.* 2000). Chapin and Kedrowski (1983) found that four Alaskan tree species growing in low nutrient environments did not resorb a greater amount of nitrogen and phosphorus than those in high nutrient areas.

Determining how the import of agricultural nutrients impact the vegetation component of nutrient cycling in buffer systems is important since plants play an essential role in pollution mitigation. In my research, I studied the vegetation component of nutrient cycling in two forested riparian wetlands: one wetland was influenced by agriculture (the agricultural site) while the other was not (the reference site).

I developed two objectives for this project. The first was to determine if nutrients entering from surrounding agricultural fields were impacting the concentrations of nitrogen, phosphorus, and carbon in tree litter and herbaceous vegetation collected at the agricultural site by comparing their concentrations to those found at the reference site. Once these concentrations were determined, nutrient loads, NUEs, and element ratios (N:P, C:N, and C:P) were calculated and statistical tests were performed to determine if any differences existed between sites and species. The second objective was to determine if nitrogen, phosphorus, and carbon concentrations in the litter changed during the period of senescence. If the nutrient concentrations at the beginning of the season were more elevated at the agricultural site than the reference site I would presume that the nutrient concentrations in green tree leaves were also higher. A change in the nutrient concentrations during the period of senescence would imply that the trees are effectively resorbing the nutrients from their leaves.

Four main hypotheses were developed to address these objectives. First, higher nutrient availability at the agricultural site would result in elevated nutrient concentrations and higher biomass production in both leaf litter and herbaceous vegetation. Second, the trees found at the reference site would have higher NUEs because the nutrients are presumably more limited in this environment and these plants would probably need to conserve nutrients more than those in the agricultural site. Third, the nutrient concentrations would differ by species within the woody and herbaceous communities since some species may be more adept at resorbing nutrients than others. Fourth, I anticipated that the nutrient concentrations would change over time since the leaves that abscise late in the season are attached to the trees for a longer period of time

thereby providing a greater opportunity for nutrient retention.

METHODS

Field and Laboratory Techniques. In 2002, six 20 x 20 m (400 m^2) plots were randomly established at the agricultural site and two at the reference site. In 2003 four more plots were added at the reference site. Two 1 m^2 leaf litter traps were randomly placed within each of the larger plots. The traps were constructed from PVC pipe and nylon screening and positioned approximately 0.5 m above the ground. In 2002 litter was collected four times (November 6, 15, 20 and December 23) and was kept separated by date. Litter was likewise collected weekly in 2003 from October 10 through November 18 and on January 13 in 2004. The litter was dried at 60°C until constant weight was achieved. In 2002 the litter was separated by species, but combined in 2003 because I did not expect intraspecific variation to change between years. The litter from both years was weighed and homogenized using a Wiley Mill.

A 2.5 m^2 ring was randomly placed at seven locations within each 400 m^2 plot so that herbaceous vegetation could be harvested in the middle of July in 2002 and at the beginning of August in 2003. All herbaceous vegetation was separated by species *in situ* at the time of collection, dried at 60°C until constant weight was achieved, weighed, and homogenized using a Wiley Mill.

Total nitrogen and carbon concentrations in the litter and herbaceous vegetation were determined via dry combustion using a LECO Tru-Spec CN Analyzer (St. Louis, Missouri). Total phosphorus concentrations were determined using a modified (instead of adding 3.5 mL of sulfuric acid to each sample, I added 5 mL) Kjeldahl digestion method. Digests were then run on a Flow Injection Analyzer (Lachat QuikChem 8000,

Milwaukee, Wisconsin). In 2003 only the litter obtained between October 10 and November 14 was analyzed for nutrient concentrations since litter collected later in the season did not have enough biomass for analysis.

It has been suggested that looking simply at nutrient concentrations in litter may lead to biased results since specific leaf mass (SLM) changes during senescence (Aerts 1996). Nonetheless changes in SLM are generally small (Chapin and Moilanen 1991), so I assumed that comparisons based on concentration were reasonable. However, I also converted nutrient concentrations for each sample into units of mass by multiplying the concentration by the sample biomass and I then used these values to calculate nutrient loads, NUEs, and element ratios.

Statistical Analyses. The statistical program SAS (v. 8 for Windows, SAS Institute, Cary, NC, USA) was used in all statistical analyses. Comparisons of biomass, nutrient concentrations, nutrient loads, NUEs, and element ratios were compared between sites for both years using t-tests. During 2002 differences between species both within and between sites were also examined using tests of fixed effects. Changes in nutrient concentrations during the period of senescence were also examined separately for 2002 and 2003 by performing repeated measures analyses. I did not average these two years together because in 2002 I had insufficient plot replication at the reference site. Moreover, 2002 was a drought year while 2003 was a "normal" year. Comparisons were also made for each year within sites between dates using repeated measure analyses and between sites on specific dates using the Tukey-Kramer procedure.

RESULTS

Results varied depending on the year examined. In 2003, the nitrogen

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concentration in tree leaf litter was significantly higher at the agricultural site than at the reference site (Table 3-1). The concentration of phosphorus was not significantly different between sites in 2002; however, it was marginally significantly higher at the agricultural site for 2003 (Table 3-1). Unlike nitrogen and phosphorus, the concentration of carbon was statistically higher at the agricultural site than at the reference site during both years (Table 3-1).

In 2003, the nutrient use efficiency of nitrogen, phosphorus, and carbon were all significantly higher in the tree litter from the reference site than at the agricultural site in 2003 (Table 3-1). Although neither the N:P nor the C:P ratios was significantly different between sites in 2003 (Table 3-1). The C:N ratio was significantly higher at the reference site than at the agricultural site (Table 3-1).

In 2003, the leaf litter biomass was not significantly different between sites at the 0.05 significance level using t-tests (Table 3-1). However, it was slightly higher at the reference site than at the agricultural site during both years. Although significant differences were observed in nutrient concentrations, none were detected for the nutrient loads of nitrogen, phosphorus, and carbon in 2003 (Table 3-1).

However, in 2002, there were no observed differences between sites for tree litter biomass, nitrogen and carbon concentrations, nitrogen and phosphorus NUEs, nitrogen, phosphorus, and carbon loads, and N:P, C:P, and C:N ratios (Table 3-1). In fact, only two significant differences were found. The concentration of carbon was significantly higher at the agricultural site than at the reference site (Table 3-1).

Significant differences within sites between species existed for nitrogen, phosphorus, and carbon concentrations as well as C:N ratios in tree leaf litter (Figure 3-1;

Figure 3-2; Figure 3-3; Figure 3-4). C:P ratios differed by species at the agricultural site but not at the reference site (Figure 3-5). A significant species effect and an insignificant site effect were found for N:P ratios so the species effect was averaged across sites (Figure 3-6). Only a few differences in the studied parameters were found when comparing species between sites. Both of these differences involved carbon. The concentration of carbon in *Liquidambar styraciflua* and *Quercus rubra* were significantly higher at the agricultural site than at the reference site (Figure 3-3). Likewise, the C:N ratio in *Quercus falcata* was significantly higher at the agricultural site than at the reference site (Figure 3-4).

Differences between species both within and between sites were examined in 2002 only. Statistical tests of fixed effects revealed that a significant site*species interaction existed for nitrogen so interaction effects are presented.

Changes in nutrient concentrations during the senescence (i.e. fall) season were examined for both years. In 2002, no changes in nitrogen (Figure 3-7a), phosphorus (Figure 3-7b), or carbon (Figure 3-7c) concentrations over time at either site and between sites by date were detected. In 2003, the concentration of nitrogen at the agricultural site did change significantly over time whereas it did not at the reference site (Figure 3-8a). From October 10 through November 5 in 2003 a decreasing trend was observed at the agricultural site (Figure 3-8a). However, surprisingly on the last date a significant increase occurred. Significant differences were also observed between sites in 2003 throughout the entire season except for on October 29 and November 5.

Parameter	Agricultural Site 2002	Reference Site 2002	P	Agricultural Site 2003	Reference Site 2003	P
Biomass (kg/ha)	3282.63 ± 383.6	4645.58 ± 1192.7	0.1799	1981.75 ± 216.9	2450.1 ± 197.9	0.14
N (%)	0.80 ± 0.03	0.74 ± 0.04	0.3707	1.16 ± 0.03	0.99 ± 0.03	0.0023
P (%)	0.05 ± 0.01	0.06 ± 0.004	0.7761	0.08 ± 0.005	0.07 ± 0.002	0.055
C (%)	51.50 ± 0.1	50.35 ± 0.1	0.0009	51.03 ± 0.4	49.88 ± 0.2	0.025
N (kg/ha)	26.17 ± 3.3	34.84 ± 10.7	0.3100	22.54 ± 2.2	24.4 ± 2.3	0.57
P (kg/ha)	1.71 ± 0.1	2.77 ± 0.9	0.4461	1.58 ± 0.2	1.68 ± 0.1	0.70
C (kg/ha)	1689.68 ± 195.4	2338.42 ± 604.6	0.2052	1010.61 ± 108.2	1222.1 ± 98.5	0.18
N – NUE	127.60 ± 4.7	135.78 ± 6.9	0.4059	87.28 ± 2.5	101.2 ± 2.9	0.0048
P – NUE	1908.21 ± 148.9	1767.19 ± 134.5	0.6315	1270.92 ± 75.5	1468.1 ± 46.2	0.050
N:P Ratio	15.28 ± 1.6	12.99 ± 0.4	0.4490	14.68 ± 1.1	14.53 ± 0.4	0.90
C:N Ratio	65.72 ± 2.4	68.36 ± 3.3	0.5890	44.5 ± 1.1	50.47 ± 1.4	0.0082
C:P Ratio	982.80 ± 76.5	890.17 ± 66.0	0.5412	647.36 ± 36.8	732.46 ± 22.5	0.077

Table 3-2. Biomass, nutrient concentrations, nutrient loads, nutrient use efficiencies, and element ratios for tree litter at both sites in 2002 and 2003. Significance values were determined using t-test analyses.
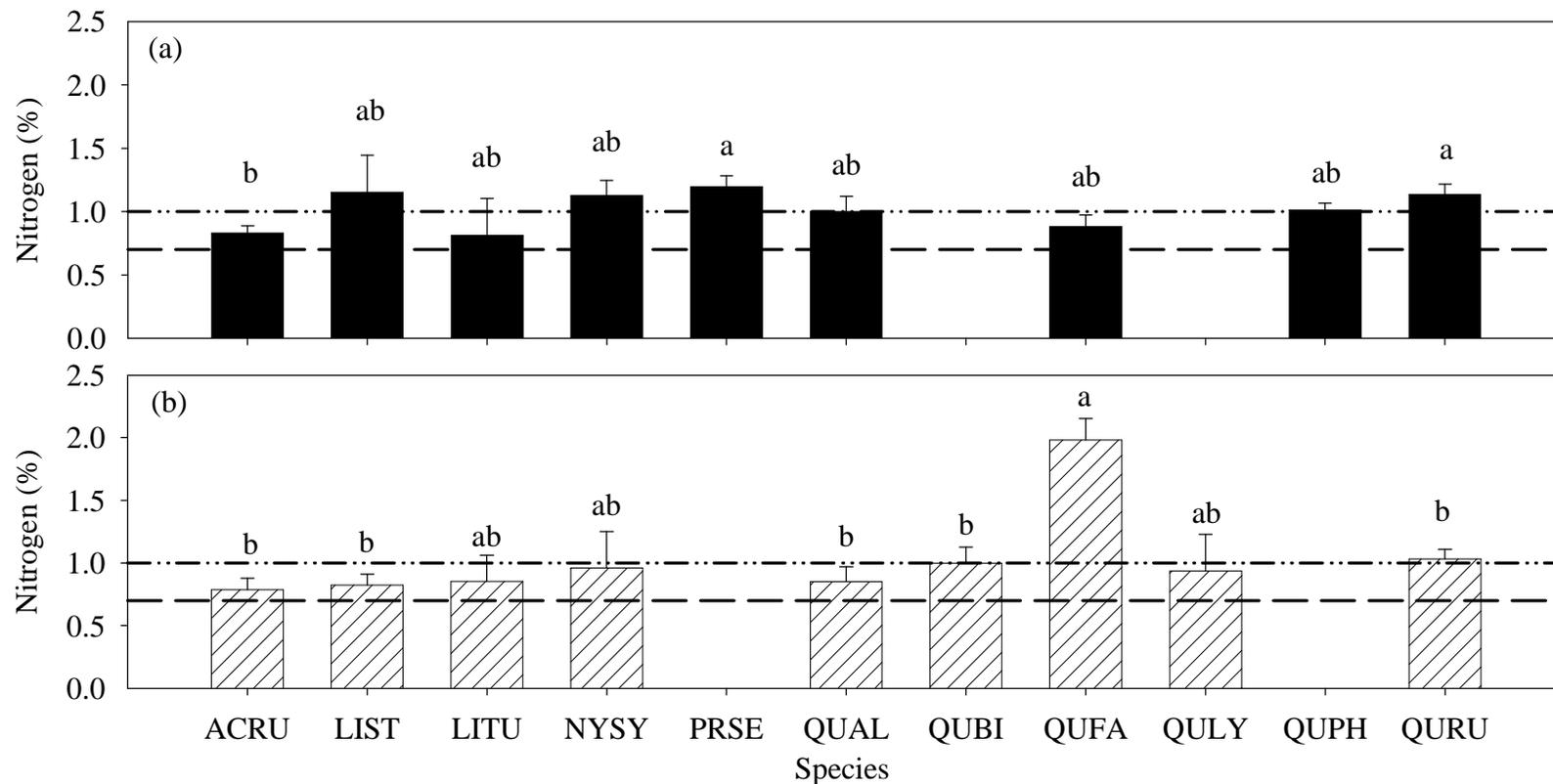
Figure 3-1. Differences in nitrogen concentration in tree litter by species at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different at the 0.05 level by the Tukey-Kramer procedure. There were no significant differences between sites at the 0.05 level by the Tukey-Kramer procedure within species. The horizontal lines represent resorption levels for deciduous tree species proposed by Killingbeck (1996). ACRU=Acer rubrum; LIST=Liquidambar styraciflua; LITU=Liriodendron tulipifera; NYSY=Nyssa sylvatica; PRSE=Prunus serotina; QUAL=Quercus alba; QUBI=Quercus bicolor; QUFA=Quercus falcata; QULY=Quercus lyrata; QUPH=Quercus phellos; QURU=Quercus rubra.
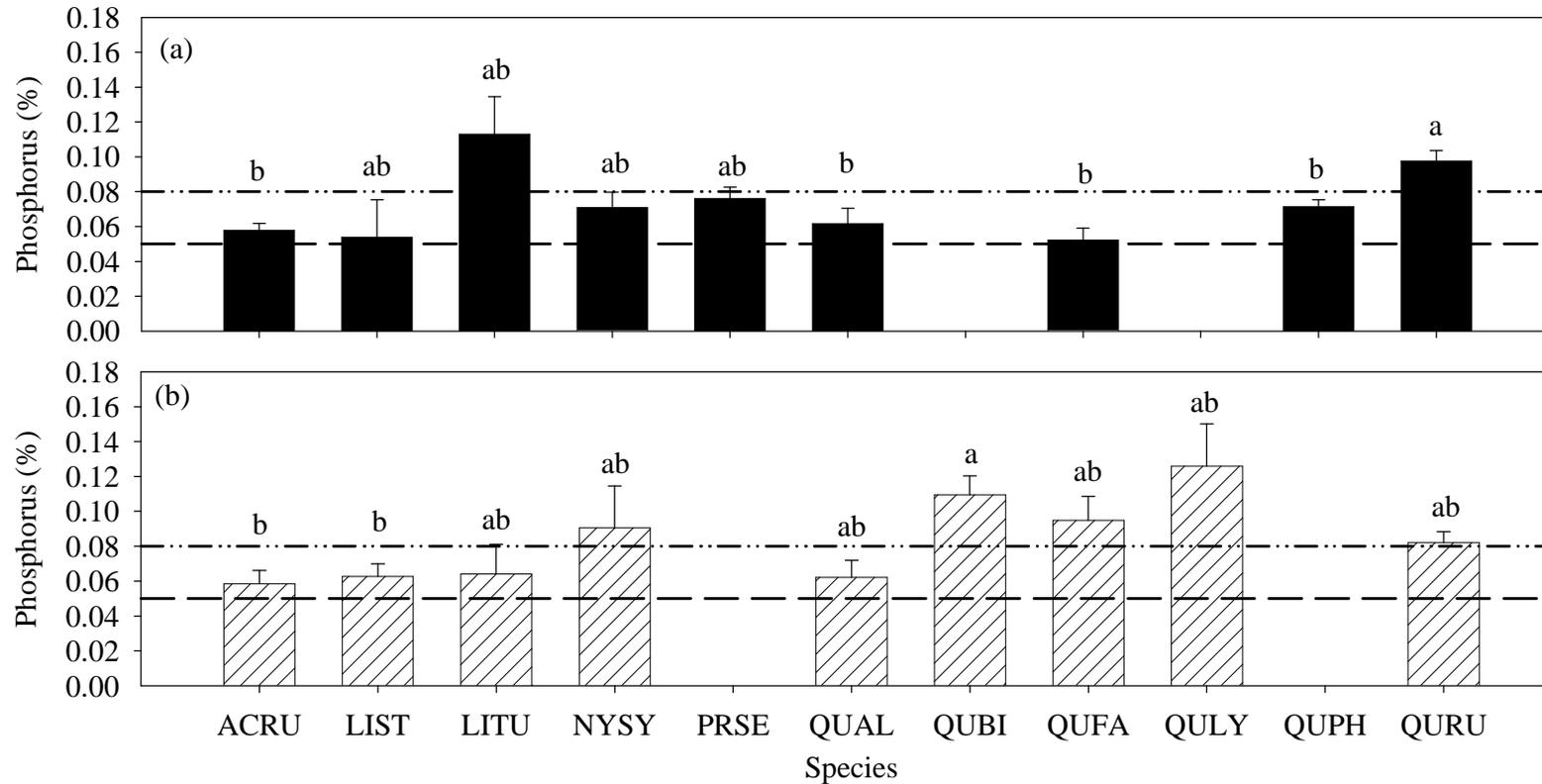
Figure 3-2. Differences in phosphorus concentration in tree litter by species at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different at the 0.05 level by the Tukey-Kramer procedure. There were no significant differences between sites at the 0.05 level by the Tukey-Kramer procedure within species. The horizontal lines represent resorption levels for deciduous tree species proposed by Killingbeck (1996). Values less than 0.05 % represent complete resorption while those above 0.08 % represent incomplete resorption. Values between these concentrations represent intermediate resorption. ACRU=Acer rubrum; LIST=Liquidambar styraciflua; LITU=Liriodendron tulipifera; NYSY=Nyssa sylvatica; PRSE=Prunus serotina; QUAL=Quercus alba; QUBI=Quercus bicolor; QUFA=Quercus falcata; QULY=Quercus lyrata; QUPH=Quercus phellos; QURU=Quercus ruba.
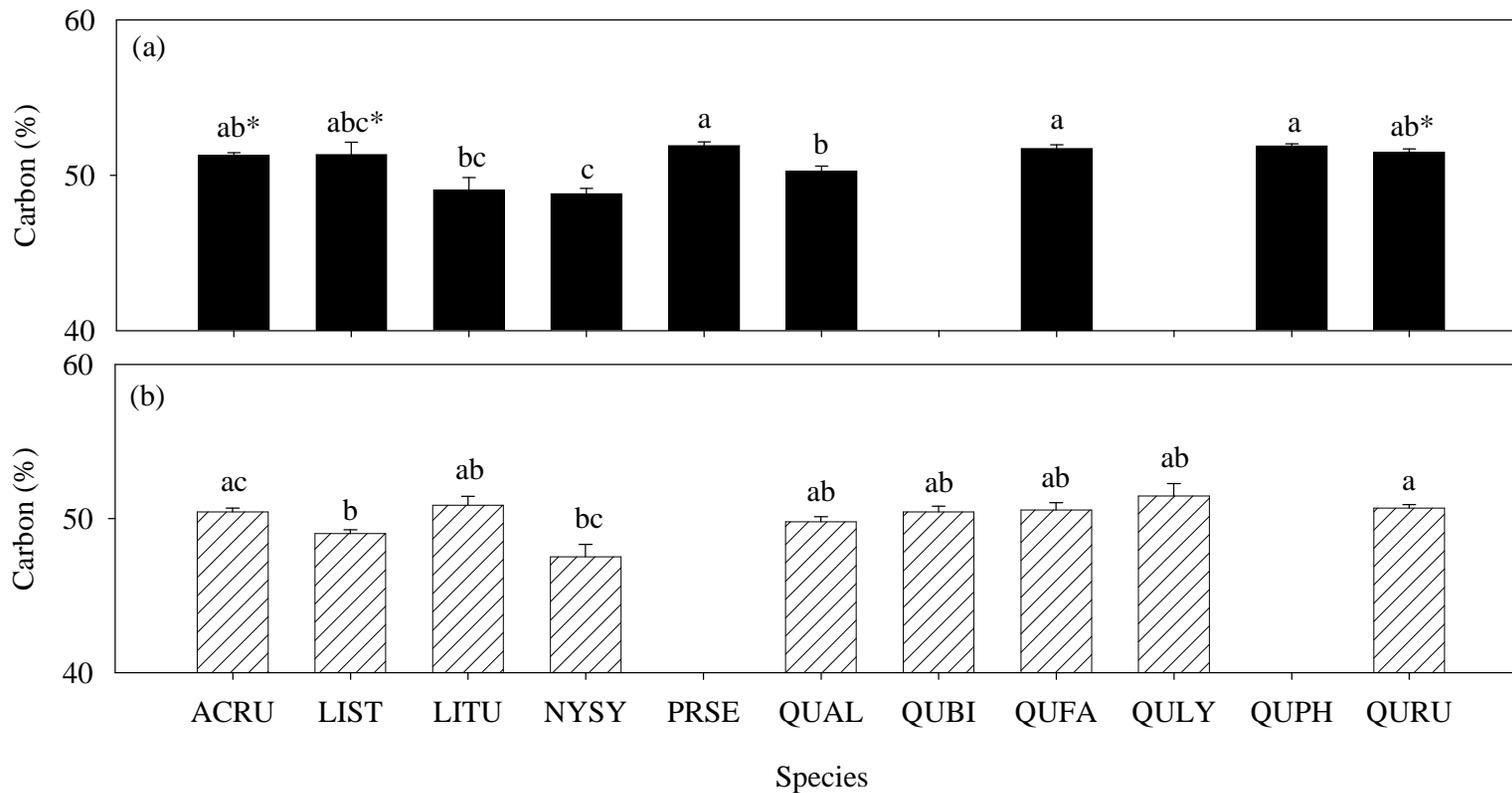
Figure 3-3. Differences in carbon concentration in tree litter by species at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites at the 0.05 level by the Tukey-Kramer procedure within species. ACRU=Acer rubrum; LIST=Liquidambar styraciflua; LITU=Liriodendron tulipifera; NYSY=Nyssa sylvatica; PRSE=Prunus serotina; QUAL=Quercus alba; QUBI=Quercus bicolor; QUFA=Quercus falcata; QULY=Quercus lyrata; QUPH=Quercus phellos; QURU=Quercus rubra.
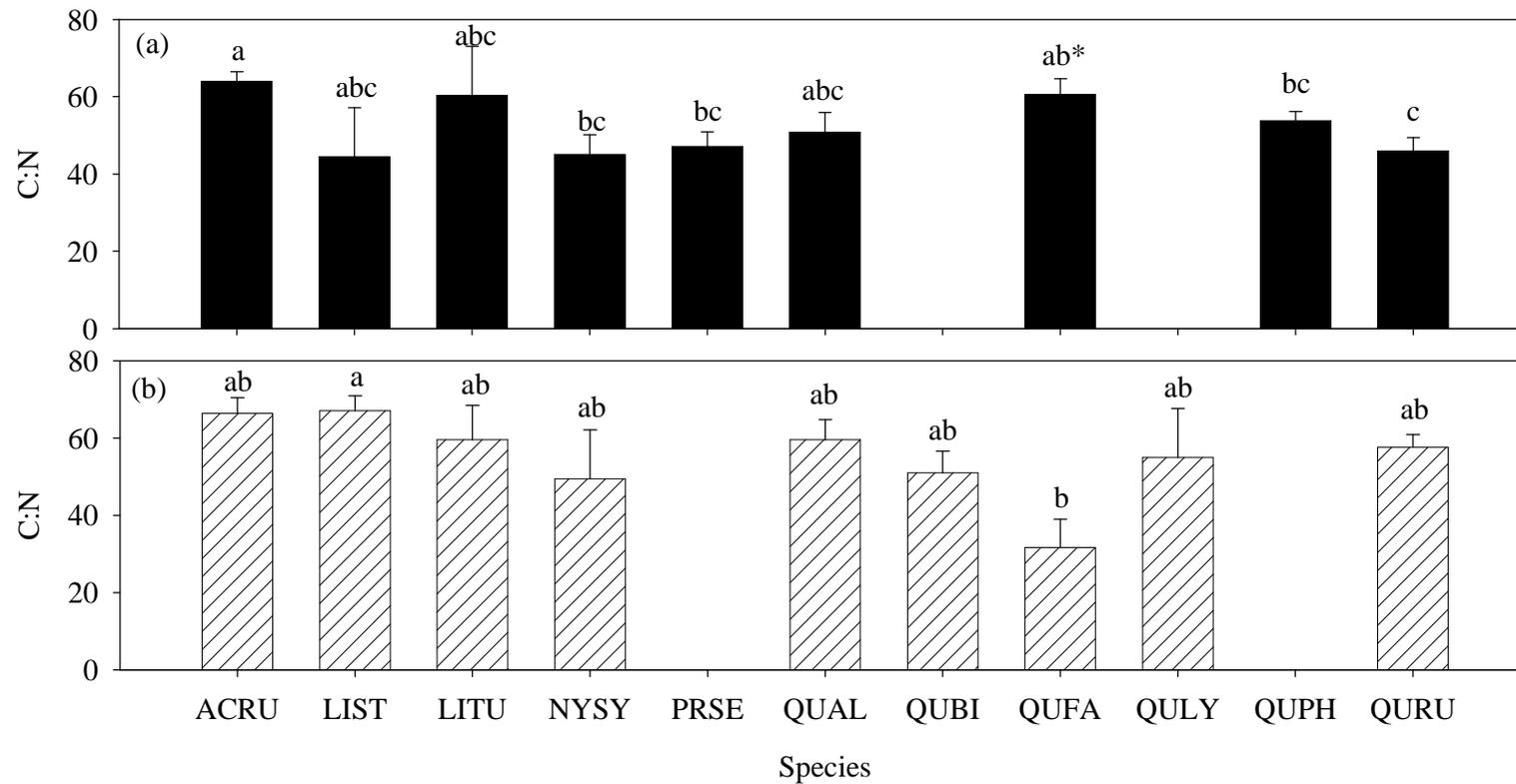
Figure 3-4. Differences in C:N ratios in tree litter by species at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. ACRU= Acer rubrum; LIST=Liquidambar styraciflua; LITU=Liriodendron tulipifera; NYSY=Nyssa sylvatica; PRSE=Prunus serotina; QUAL=Quercus alba; QUBI=Quercus bicolor; QUFA=Quercus falcata; QULY=Quercus lyrata; QUPH=Quercus phellos; QURU=Quercus rubra.

The concentration of phosphorus on the first sampling date in 2003 at the agricultural site was significantly greater than that on subsequent sampling dates (Figure 3-8b). At the agricultural site, a decreasing trend was observed for this year in general except for the last sampling date. There were also significant differences between sites at the beginning and end of the season. In addition, the carbon concentrations in 2003 showed that a significant increase occurred at the beginning of the season at the agricultural site (Figure 3-8c). Nevertheless, no difference was observed at the reference site throughout the season (Figure 3-8c). There was a difference between sites in the middle of the season at the agricultural site but no difference at the reference site.

Overall comparisons by site for herbaceous vegetation during both years revealed little difference between the two sites. Indeed, in 2002, the only significant difference that existed was in the N:P ratio, which was found to be significantly higher at the reference site than at the agricultural site (Table 3-2). In 2003, the concentration of carbon was marginally significantly higher at the agricultural site than at the reference site (Table 3-2).

In 2002, differences existed at both sites between herbaceous species. The nitrogen concentrations differed by species within both the agricultural and reference sites (Figure 3-9). The phosphorus concentrations differed by species within the agricultural site but not within the reference site (Figure 3-10). Moreover, the phosphorus concentrations were significantly higher in *Impatiens capensis* Meerb., *Polygonum arifoliam* L, and *Symplocarpus foetidus* (L.) Salisb. ex Nutt at the agricultural site than at the reference site (Figure 3-10). The carbon concentrations also differed between species within the agricultural site but not within the reference site (Figure 3-11). In addition, differences in
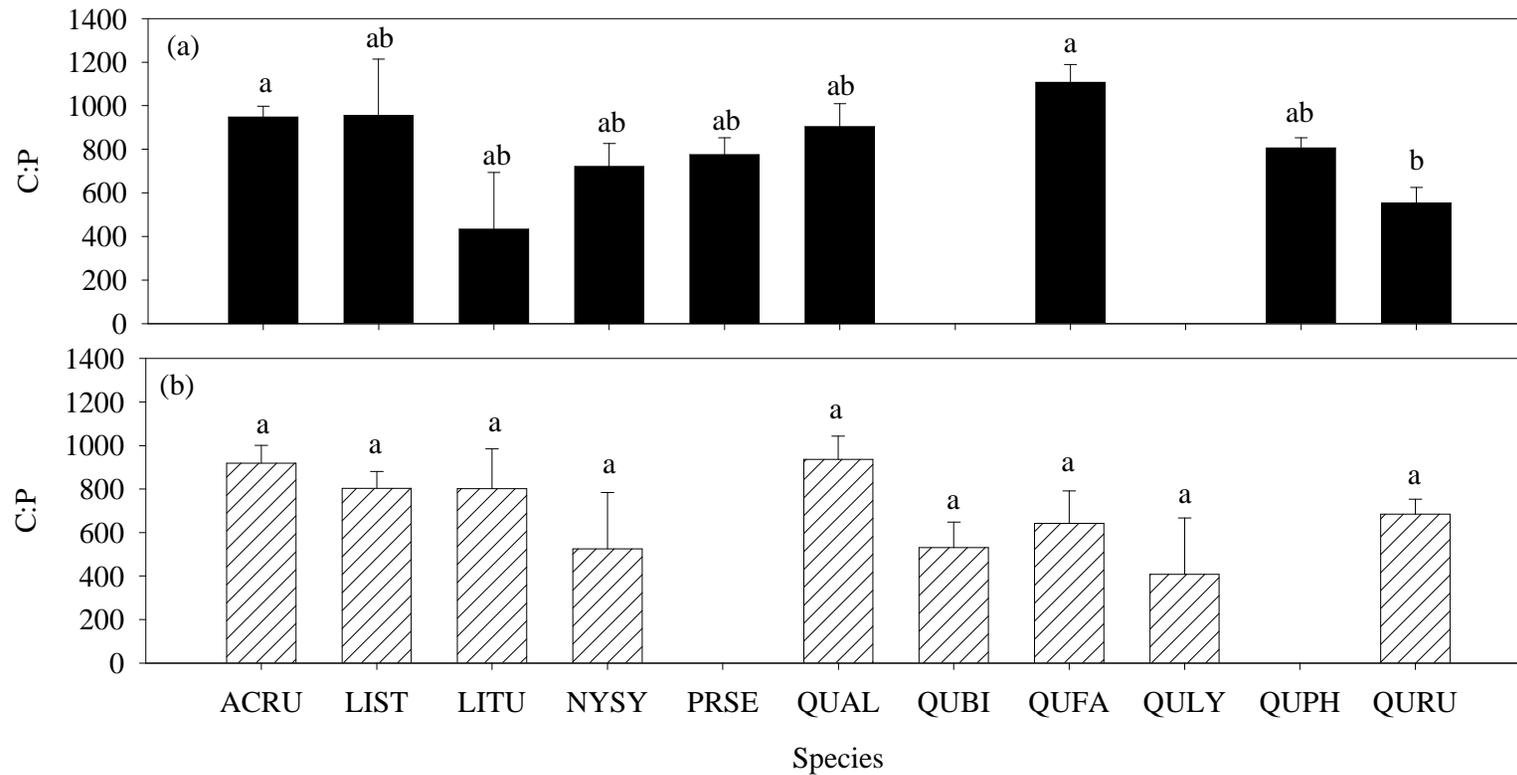
**Figure 3-5.** Differences in C:P ratios in tree litter by species at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different at the 0.05 level by the Tukey-Kramer procedure. There were no significant differences between sites within species at the 0.05 level by the Tukey-Kramer procedure. ACRU= Acer rubrum; LIST=Liquidambar styraciflua; LITU=Liriodendron tulipifera; NYSY=Nyssa sylvatica; PRSE=Prunus serotina; QUAL=Quercus alba; QUBI=Quercus bicolor; QUFA=Quercus falcata; QULY=Quercus lyrata; QUPH=Quercus phellos; QURU=Quercus rubra.
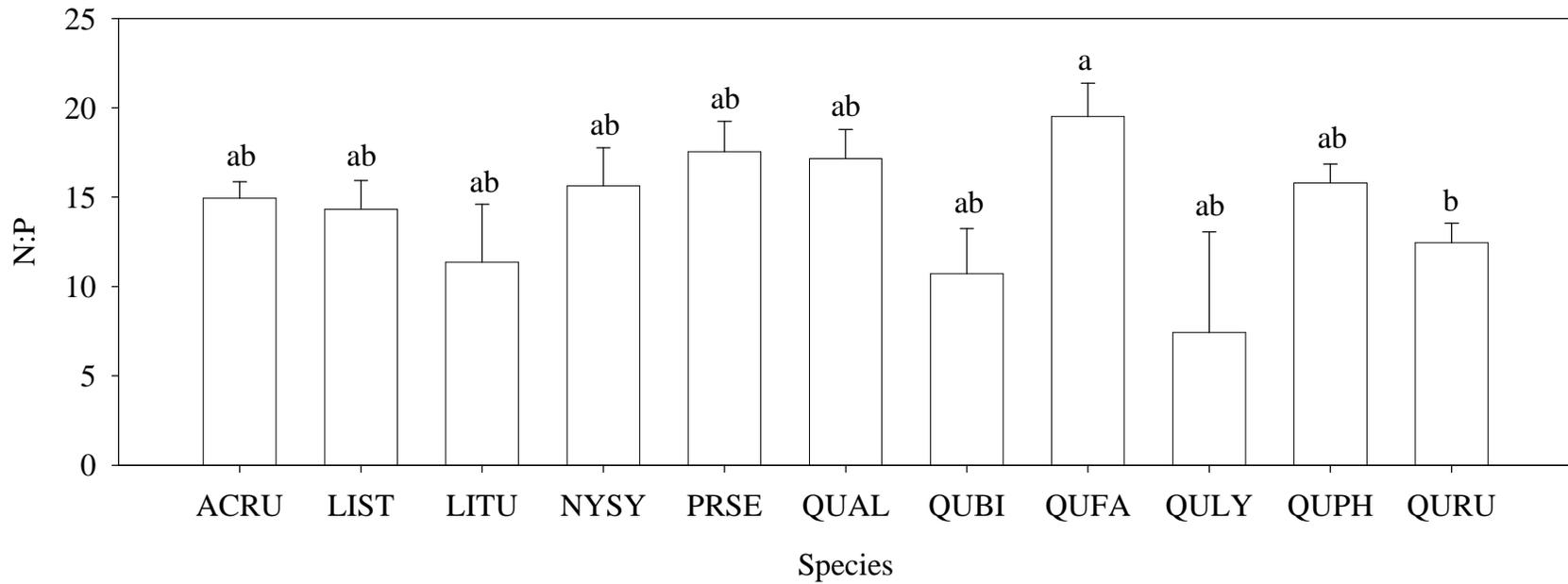
Figure 3-6. Differences in N:P ratios in tree litter by species. Since the site*species interaction was insignificant (P=0.6147),the species effect is   shown averaged across site. A significant species effect (P=0.0412) and an insignificant site effect (P=0.5365) were found. Means with identical letters are not significantly different at the 0.05 level by the Tukey-Kramer procedure. Significant differences exist between species. ACRU=Acer rubrum; LIST=Liquidambar styraciflua; LITU=Liriodendron tulipifera; NYSY=Nyssa sylvatica; PRSE=Prunus serotina; QUAL=Quercus alba; QUBI=Quercus bicolor; QUFA=Quercus falcata; QULY=Quercus lyrata; QUPH=Quercus phellos; QURU=Quercus rubra.
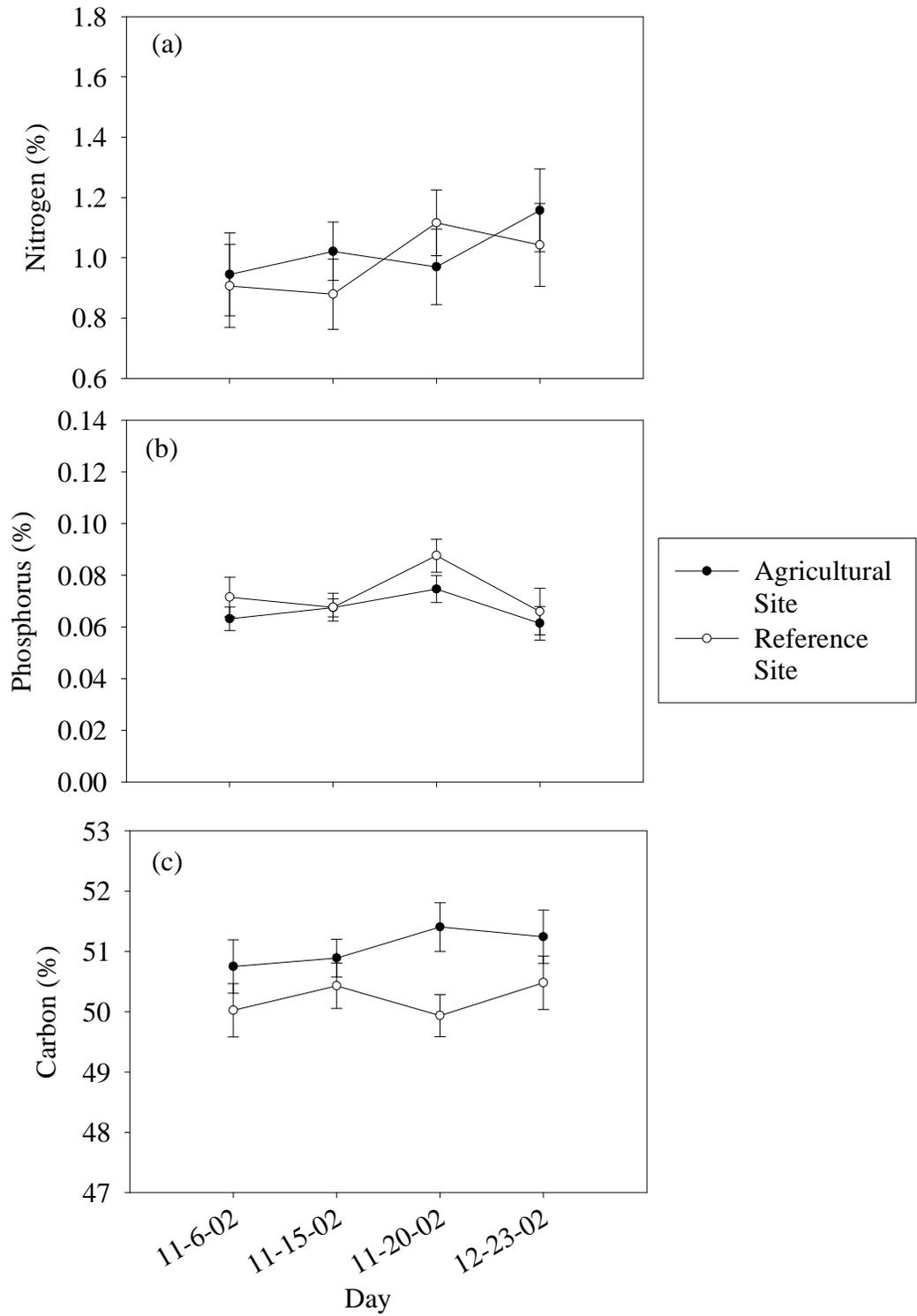
Figure 3-7. Change in nutrient concentrations in tree litter over time in 2002 for (a) nitrogen, (b) phosphorus, and (c) carbon. No significant differences exist within sites between dates or between sites within dates at the 0.05 level.
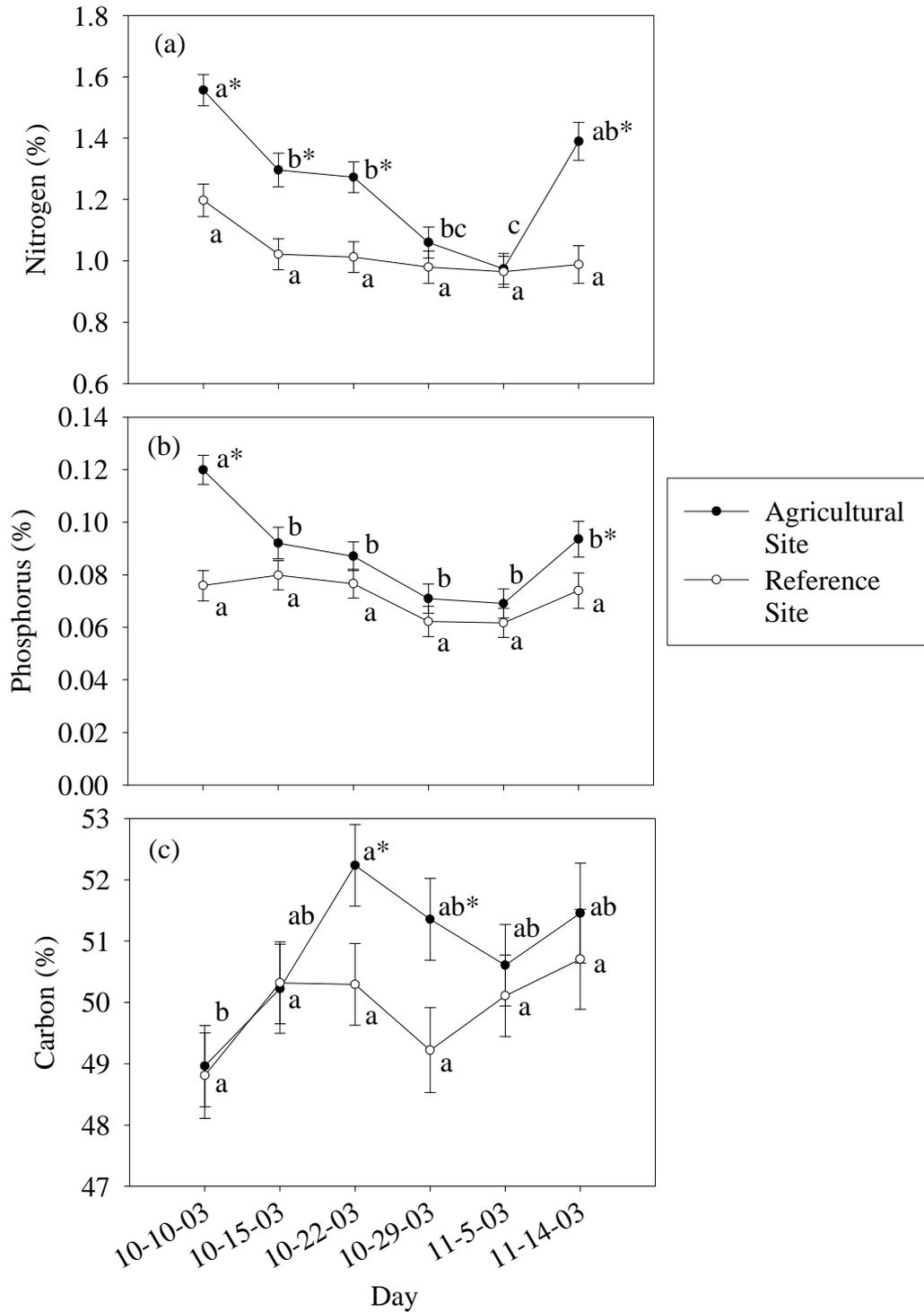
Figure 3-8. Change in nutrient concentrations in tree litter over time in 2003 for (a) nitrogen, (b) phosphorus, and (c) carbon. Means with identical letters are not significantly different between dates within site at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists within dates between sites at the 0.05 level. Significant differences were observed over time at the agricultural site but not at the reference site and between sites on some days.

N:P ratios between species at both sites and between sites for *Symplocarpus foetidus* existed (Figure 3-12). Also, C:P (Figure 3-13) and C:N (Figure 3-14) ratios differed by species at the agricultural and reference sites.

In 2003, the concentration of nitrogen differed by species and site (Figure 3-15) while carbon concentrations (Figure 3-16) and the C:N ratios (Figure 3-17) varied by species only. The C:P ratios differed by species at the agricultural site, but not at the reference site (Figure 3-18). The phosphorus concentrations did not differ by species within sites but *Leersia oryzoides* and *Symplocarpus foetidus* contained significantly higher concentrations at the agricultural site than at the reference site (Figure 3-19). However, the N:P ratios showed no differences between species or site (Figure 3-20).

DISCUSSION

A higher concentration of nitrogen was found in the litter from the agricultural site than the litter from the reference site during the 2003 season. This may be due to the elevated amount of nitrate entering the agricultural site from the surrounding fields. These results agree with other studies. Boerner (1984) studied fertile and infertile forests and determined that the nitrogen concentrations in litter were higher at the fertile site than at the infertile site. This elevation in nitrogen may help promote nitrogen mineralization at the agricultural site by stimulating soil microbes involved in the breakdown the nitrogen. However, phosphorus concentrations were significantly higher at the agricultural site than at the reference site in 2003. As stated earlier, the concentration of phosphate in shallow groundwater samples was higher at the agricultural site than at the reference site. Thus, more phosphorus is probably available for plant uptake at the enriched site. Moreover, more carbon was present in the litter from the agricultural site

Parameter	Agricultural Site 2002	Reference Site 2002	P	Agricultural Site 2003	Reference Site 2003	P
Biomass (kg/ha)	1006.0 ± 393.60	2111.9 ± 603.93	0.2024	2322.5 ± 932.61	2573.0 ± 413.40	0.81
N (%)	2.4 ± 0.16	2.3 ± 0.07	0.7857	3.1 ± 0.19	2.8 ± 0.17	0.16
P (%)	0.3 ± 0.03	0.2 ± 0.02	0.1098	0.3 ± 0.03	0.3 ± 0.02	0.11
C (%)	40.3 ± 0.33	40.6 ± 0.38	0.6615	41.7 ± 0.92	36.6 ± 2.20	0.057
N (kg/ha)	26.2 ± 11.10	49.7 ± 15.58	0.3181	80.0 ± 36.84	72.0 ± 14.04	0.84
P (kg/ha)	6.1 ± 1.13	14.2 ± 0.79	0.6510	6.5 ± 2.34	5.8 ± 1.38	0.81
C (kg/ha)	406.0 ± 160.60	859.1 ± 252.98	0.2022	990.8 ± 413.46	946.2 ± 171.30	0.92
N – NUE	42.5 ± 3.50	42.9 ± 1.29	0.9561	32.4 ± 2.18	36.9 ± 2.57	0.21
P – NUE	338.4 ± 37.19	492.3 ± 51.13	0.0767	377.7 ± 38.43	494.9 ± 58.64	0.13
N:P Ratio	8.0 ± 0.53	11.5 ± 31.08	0.0249	11.7 ± 1.06	13.3 ± 0.99	0.31
C:N Ratio	17.1 ± 1.42	17.4 ± 0.36	0.9211	13.5 ± 0.73	13.2 ± 0.24	0.78
C:P Ratio	136.6 ± 15.34	199.9 ± 22.60	0.0789	156.8 ± 14.63	175.6 ± 12.72	0.35

Table 3-2. Biomass, nutrient concentrations, nutrient loads, nutrient use efficiencies, and element ratios for herbaceous vegetation at both sites in 2002 and 2003. Significance values were determined using t-test analyses.
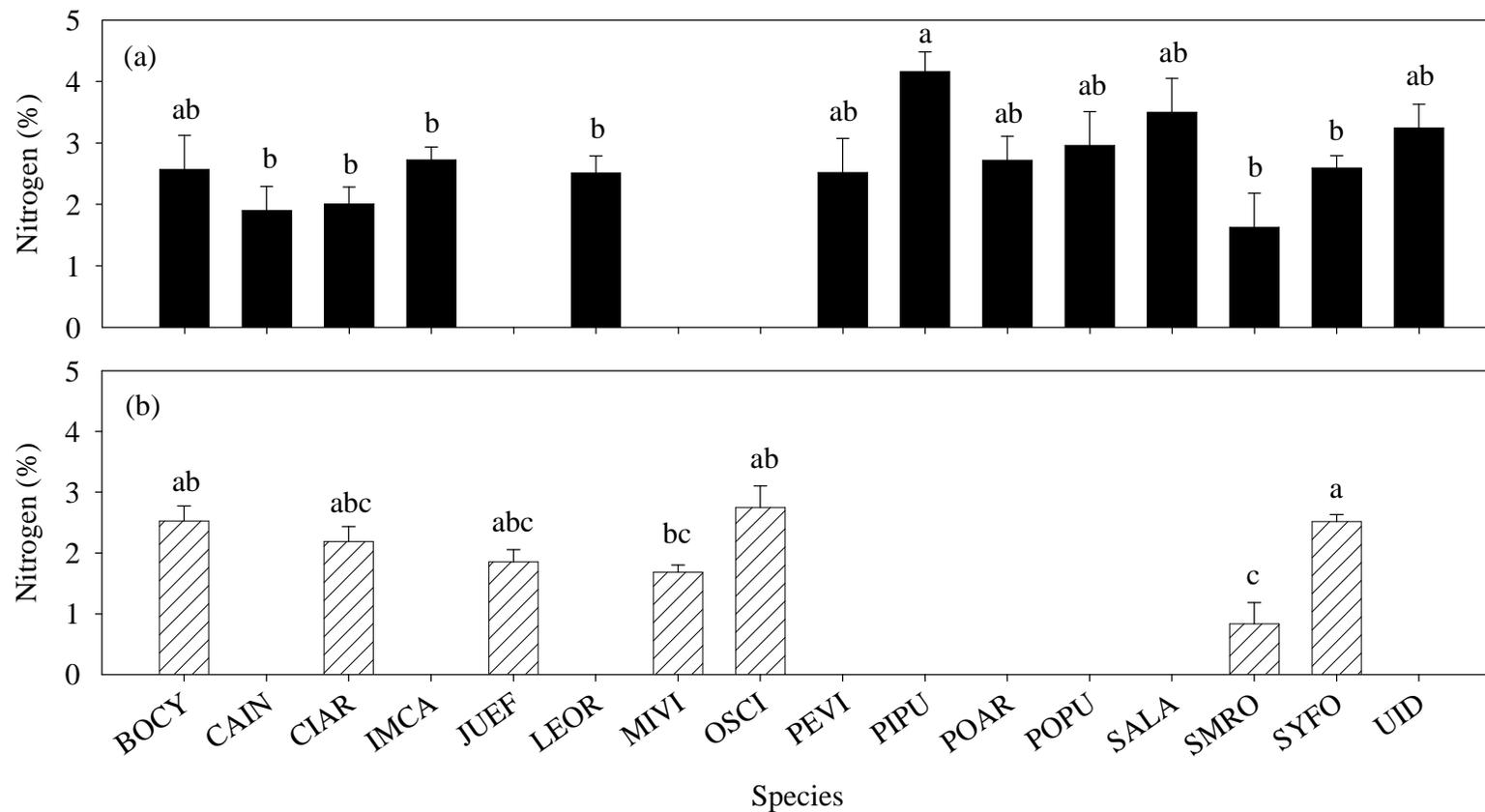
Figure 3-9. Differences in nitrogen concentrations in herbaceous vegetation by species in 2002 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. There were no significant differences between sites within species at the 0.05 level using the Tukey-Kramer procedure. BOCY= *Boehemeria cylindrica***; CAIN=***Carex intumescens***; CIAR=***Cinna arifolium***; IMCA=***Impatiens capensis***; JUEF=***Juncus effusus***; LEOR=***Leersia oryzoides***; MIVI=***Microstegium vimineum***; OSCI=***Osmunda cinnamomea***; PEVI=***Peltandra virginica***; PIPU=***Pilea pumila***; POAR=***Polygonum arifolium***; POPU=***Polygonum punctatum***; SALA=***Sagittaria latifolia***; SMRO=***Smilax rotundifolium***; SYFO=***Symplocarpus foetidus***; UID=unidentified species.**
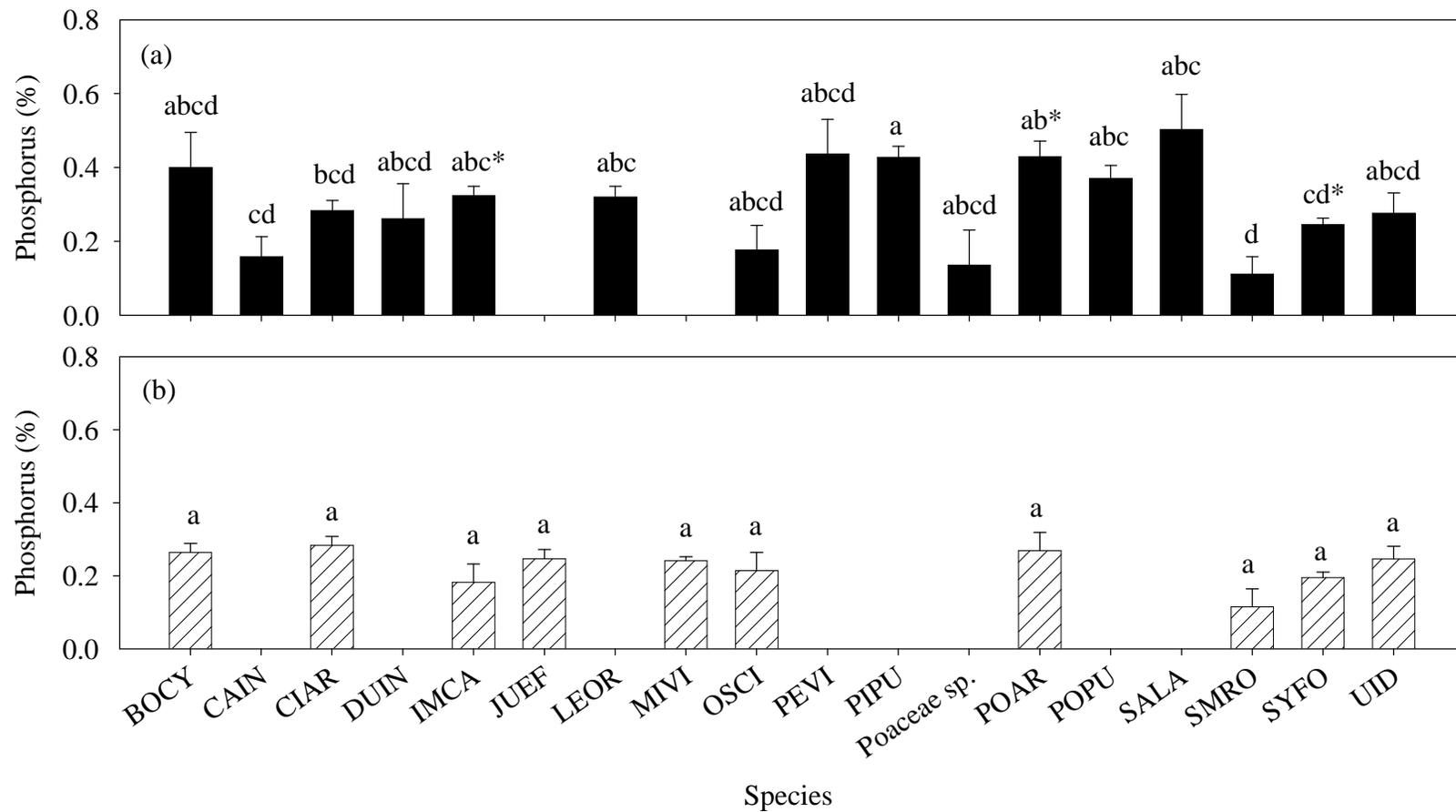
Figure 3-10. Differences in phosphorus concentrations in herbaceous vegetation by species in 2002 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. **BOCY=** *Boehemeria cylindrica*; **CAIN=***Carex intumescens*; **CIAR=***Cinna arifolium*; **IMCA=***Impatiens capensis*; **JUEF=***Juncus effusus*; **LEOR=***Leersia oryzoides*; **MIVI=***Microstegium vimineum*; **OSCI=***Osmunda cinnamomea*; **PEVI=***Peltandra virginica*; **PIPU=***Pilea pumila*; **POAR=***Polygonum arifolium*; **POPU=***Polygonum punctatum*; **SALA=***Sagittaria latifolia*; **SMRO=***Smilax rotundifolium*; **SYFO=***Symplocarpus foetidus*; **UID=unidentified species.**

Figure 3-11. Differences in carbon concentrations in herbaceous vegetation by species in 2002 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. BOCY= *Boehemeria cylindrica*; CAIN=*Carex intumescens*; CIAR=*Cinna arifolium*; IMCA=*Impatiens capensis*; JUEF=*Juncus effusus*; LEOR=*Leersia oryzoides*; MIVI=*Microstegium vimineum*; OSCI=*Osmunda cinnamomea*; PEVI=*Peltandra virginica*; PIPU=*Pilea pumila*; POAR=*Polygonum arifolium*; POPU=*Polygonum punctatum*; SALA=*Sagittaria latifolia*; SMRO=*Smilax rotundifolium*; SYFO=*Symplocarpus foetidus*; UID=unidentified species.

Figure 3-12. Differences in N:P ratios in herbaceous vegetation by species in 2002 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. BOCY= *Boehemeria cylindrica*; **CAIN=***Carex intumescens*; **CIAR=***Cinna arifolium*; **IMCA=***Impatiens capensis*; **JUEF=***Juncus effusus*; **LEOR=***Leersia oryzoides*; **MIVI=***Microstegium vimineum*; **OSCI=***Osmunda cinnamomea*; **PEVI=***Peltandra virginica*; **PIPU=***Pilea pumila*; **POAR=***Polygonum arifolium*; **POPU=***Polygonum punctatum*; **SALA=***Sagittaria latifolia*; **SMRO=***Smilax rotundifolium*; **SYFO=***Symplocarpus foetidus*; **UID=unidentified species.**

Figure 3-13. Differences in C:P ratios in herbaceous vegetation by species in 2002 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. BOCY= *Boehemeria cylindrica*; CAIN=*Carex intumescens*; CIAR=*Cinna arifolium*; IMCA=*Impatiens capensis*; JUEF=*Juncus effusus*; LEOR=*Leersia oryzoides*; MIVI=*Microstegium vimineum*; OSCI=*Osmunda cinnamomea*; PEVI=*Peltandra virginica*; PIPU=*Pilea pumila*; POAR=*Polygonum arifolium*; POPU=*Polygonum punctatum*; SALA=*Sagittaria latifolia*; SMRO=*Smilax rotundifolium*; SYFO=*Symplocarpus foetidus*; UID=unidentified species.

**Figure 3-14.** Differences in C:P ratios in herbaceous vegetation by species in 2002 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. BOCY= *Boehemeria cylindrica*; CAIN=*Carex intumescens*; CIAR=*Cinna arifolium*; IMCA=*Impatiens capensis*; JUEF=*Juncus effusus*; LEOR=*Leersia oryzoides*; MIVI=*Microstegium vimineum*; OSCI=*Osmunda cinnamomea*; PEVI=*Peltandra virginica*; PIPU=*Pilea pumila*; POAR=*Polygonum arifolium*; POPU=*Polygonum punctatum*; SALA=*Sagittaria latifolia*; SMRO=*Smilax rotundifolium*; SYFO=*Symplocarpus foetidus*; UID=unidentified species.

Figure 3-15. Differences in nitrogen concentrations in herbaceous vegetation by species in 2003 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. BOCY= *Boehemeria cylindrica***; CAIN=***Carex intumescens***; CIAR=***Cinna arifolium***; IMCA=***Impatiens capensis***; JUEF=***Juncus effusus***; LEOR=***Leersia oryzoides***; MIVI=***Microstegium vimineum***; OSCI=***Osmunda cinnamomea***; PEVI=***Peltandra virginica***; PIPU=***Pilea pumila***; POAR=***Polygonum arifolium***; POPU=***Polygonum punctatum***; SALA=***Sagittaria latifolia***; SMRO=***Smilax rotundifolium***; SYFO=***Symplocarpus foetidus***; UID=unidentified species.**

Figure 3-16. Differences in carbon concentrations in herbaceous vegetation by species in 2003 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. BOCY= *Boehemeria cylindrica*; **CAIN=***Carex intumescens*; **CIAR=***Cinna arifolium*; **IMCA=***Impatiens capensis*; **JUEF=***Juncus effusus*; **LEOR=***Leersia oryzoides*; **MIVI=***Microstegium vimineum*; **OSCI=***Osmunda cinnamomea*; **PEVI=***Peltandra virginica*; **PIPU=***Pilea pumila*; **POAR=***Polygonum arifolium*; **POPU=***Polygonum punctatum*; **SALA=***Sagittaria latifolia*; **SMRO=***Smilax rotundifolium*; **SYFO=***Symplocarpus foetidus*; **UID=unidentified species.**

Figure 3-17. Differences in C:N ratios in herbaceous vegetation by species in 2003 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. BOCY= *Boehemeria cylindrica*; **CAIN=***Carex intumescens***; CIAR=***Cinna arifolium***; IMCA=***Impatiens capensis***; JUEF=***Juncus effusus***; LEOR=***Leersia oryzoides***; MIVI=***Microstegium vimineum***; OSCI=***Osmunda cinnamomea***; PEVI=***Peltandra virginica***; PIPU=***Pilea pumila***; POAR=***Polygonum arifolium***; POPU=***Polygonum punctatum***; SALA=***Sagittaria latifolia***; SMRO=***Smilax rotundifolium***; SYFO=***Symplocarpus foetidus***; UID=unidentified species.**

Figure 3-18. Differences in C:P ratios in herbaceous vegetation by species in 2003 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. BOCY= *Boehemeria cylindrica*; CAIN=*Carex intumescens*; CIAR=*Cinna arifolium*; IMCA=*Impatiens capensis*; JUEF=*Juncus effusus*; LEOR=*Leersia oryzoides*; MIVI=*Microstegium vimineum*; OSCI=*Osmunda cinnamomea*; PEVI=*Peltandra virginica*; PIPU=*Pilea pumila*; POAR=*Polygonum arifolium*; POPU=*Polygonum punctatum*; SALA=*Sagittaria latifolia*; SMRO=*Smilax rotundifolium*; SYFO=*Symplocarpus foetidus*; UID=unidentified species.

Figure 3-19. Differences in phosphorus concentrations in herbaceous vegetation by species in 2003 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. BOCY= *Boehemeria cylindrica*; **CAIN=***Carex intumescens*; **CIAR=***Cinna arifolium*; **IMCA=***Impatiens capensis*; **JUEF=***Juncus effusus*; **LEOR=***Leersia oryzoides*; **MIVI=***Microstegium vimineum*; **OSCI=***Osmunda cinnamomea*; **PEVI=***Peltandra virginica*; **PIPU=***Pilea pumila*; **POAR=***Polygonum arifolium*; **POPU=***Polygonum punctatum*; **SALA=***Sagittaria latifolia*; **SMRO=***Smilax rotundifolium*; **SYFO=***Symplocarpus foetidus*; **UID=unidentified species.**

Figure 3-20. Differences in N:P ratios in herbaceous vegetation by species in 2003 at the (a) agricultural site and (b) reference site. Means with identical letters are not significantly different within sites at the 0.05 level by the Tukey-Kramer procedure. Letters with asterisks (*) beside them indicate a significant difference exists between sites within species at the 0.05 level by the Tukey-Kramer procedure. BOCY= *Boehemeria cylindrica***; CAIN=***Carex intumescens***; CIAR=***Cinna arifolium***; IMCA=***Impatiens capensis***; JUEF=***Juncus effusus***; LEOR=***Leersia oryzoides***; MIVI=***Microstegium vimineum***; OSCI=***Osmunda cinnamomea***; PEVI=***Peltandra virginica***; PIPU=***Pilea pumila***; POAR=***Polygonum arifolium***; POPU=***Polygonum punctatum***; SALA=***Sagittaria latifolia***; SMRO=***Smilax rotundifolium***; SYFO=***Symplocarpus foetidus***; UID=unidentified species.**

than the litter from the reference site in both years. This difference may be due to the fragmented structure of the agricultural site. Since this site is narrower, more light can potentially penetrate the canopy. This is most likely causing photosynthesis rates to increase ultimately increasing carbon production.

The nitrogen NUE was significantly higher at the reference site than at the agricultural site in 2003. Once again this difference between years could be due to the drought or lack of monitoring plot replication in 2002. It has been found that higher NUEs exist in forests with lower aboveground nitrogen circulation (Vitousek 1982). Our results appear to support their findings. Nutrient rich sites may have higher concentrations in leaf litter than non-enriched sites do. However, since nitrogen is more readily available in these enriched sites, the trees may not have to resorb higher concentrations of nitrogen from the leaves prior to abscission. Another possible reason for this discrepancy in NUEs between sites is that the nitrogen resorption rate may not increase in response to higher nitrogen concentrations. This is extremely important in the cycling of nutrients through ecosystems because if resorption rates do not increase in response to higher nutrient concentrations, then the potential for loss of nutrients via throughfall is greater. These results suggest that enriched sites may have a higher amount of nutrients returned to the system via litterfall than non-enriched sites.

N:P ratios are important because they serve as indicators of microbial processes. In 2002, the N:P ratio was higher at the agricultural site than the reference site due to the elevated nitrogen in the litter at the agricultural site. Both sites had N:P ratios greater than 12 in both years. Also, N:P ratios greater than 10 may actually cause phosphorus to be immobilized (Lockaby 1999). The C:N ratios were not significantly different in 2002.

Nevertheless in 2003 the C:N ratio was significantly higher at the reference site than at the agricultural site. This result occurs because the agricultural site has higher concentrations of both nitrogen and carbon. The C:N ratios at both sites were greater than 40 in both years. These C:N ratios are higher than those found by Bischoff *et al.* (2001). C:N ratios greater than 20 to 30:1 will cause the immobilization of some nitrogen since the microorganisms will preferentially use more easily attainable nitrogen in the soils (Barbour *et al.* 1999). This is relevant since areas with high C:N ratios may release nitrogen slowly during decomposition causing the net nitrogen mineralization to be small. C:P ratios were not significantly different between sites in either year.

Not finding a significant difference in litter biomass was definitely an unexpected result that is probably due to the characteristic differences observed at each site. Unlike the reference site, the agricultural site is fragmented in nature and winds may actually cause the export of litter from the system. Thus wind could actually limit the amount of biomass available for collection at the agricultural site. In addition, no differences in the nutrient loads were detected. This result seems counterintuitive at first. However, it is probably a reflection of the higher biomass at the reference site. The lower litter input at the agricultural site may be offsetting the nutrient loading rates due to elevated concentrations of nitrogen, phosphorus, and carbon. Bischoff *et al.* (2001) found that the average nitrogen load in a deciduous forested wetland was 20 kg/ha while the average carbon load was 661 kg/ha. When simply considering the year with normal climatic patterns (i.e. 2003), my nitrogen values (22.54 ± 2.2 kg/ha at the agricultural site and 24.4 ± 2.3 kg/ha at the reference site) are consistent with Bischoff's estimate. However, my carbon loads (1010.61 ± 108.2 kg/ha at the agricultural site and 1222.10 ± 98.5

kg/ha) are higher than those found by Bischoff (2001). Yet, the average litter biomass of their site was also lower (1302 kg/ha) than mine (1981.75 ± 216.9 at the agricultural site and 2450.1 ± 197.9 at the reference site) (Bischoff *et al.* 2001).

The 2002 data was typified by an overall lack of differentiation between the two sites for both tree leaf litter and herbaceous vegetation. The carbon concentrations and NUE in leaf litter and N:P ratios in herbaceous vegetation were the only overall differences found between sites. This may be due to several factors. A severe drought occurred from 2001-2002. By March of 2002 the Maryland Department of the Environment (MDE) had declared central Maryland, which includes both study sites, to be in an imminent emergency (MDE 2005). By August of that same year Maryland was experiencing an "exceptional drought," which means that crops would most likely be adversely impacted, fire risks were high, and water shortages were likely imminent. Since the agricultural site is strongly influenced by the contribution of groundwater, a decrease in groundwater (and surface water) levels may have limited the flow of nitrogen into the system and consequently its availability for plant uptake. Furthermore, I may not have detected a difference in 2002 because I only had two monitoring plots at the reference site compared to six at the agricultural site. This lack of replication may have limited my ability to detect any existed differences between sites.

There were significant differences between species within both sites but insignificant differences between sites by species for tree litter. The concentrations of nitrogen within the species are similar to those found in other studies. It appears that in general the *Quercus* species (oaks) had higher nutrient concentrations than the other species. These results coincide with those of Killingbeck and Tainsh (2002). They found

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that *Acer rubrum* had more proficient resorption of nitrogen than *Quercus alba* did (Killingbeck and Tainsh 2002). This result suggests that oaks may return higher concentrations of nitrogen to the system than the other species. Oak leaves are generally larger and thicker than the leaves of the other species studied here. It is easy to cite this discrepancy as a possible cause for these results. However, others have found that leaf size and thickness does not affect the resorption of nitrogen and phosphorus in *Acer rubrum* and *Quercus alba* leaves (Killingbeck and Tainsh 2002). The discrepancy may be due to differences inherent in the species. The concentrations in *Quercus alba* and *Quercus rubra* are comparative to those found in other studies (Killingbeck and Tainsh 2002; Killingbeck 1996; Boerner 1984). However, the concentrations in *Acer rubrum* and *Nyssa sylvatica* are higher at both sites in this study than in others (Killingbeck and Tainsh 2002; Killingbeck 1996; Boerner 1984).

Killingbeck (1996) proposed nitrogen resorption levels for deciduous tree species. He suggested that values below 0.7 % reflected complete resorption, those above 1.0 % reflected incomplete resorption, and those in between these values reflected intermediate resorption. Comparisons based on this classification revealed tha no species at either site resorbed nitrogen completely. There were more species at the agricultural site that would be classified as having incomplete resorption than there were at the reference site. In fact, over 77% of the species at the agricultural site resorbed nitrogen incompletely whereas over 77% of the species at the reference site resorbed nitrogen at an intermediate level.

The concentrations significantly differed depending on the species but did not differ within species between sites. I expected to find differences within species. The

Quercus rubra leaves at the agricultural site contained significantly higher concentrations of phosphorus than did *Acer rubrum, Quercus alba, Quercus falcata, and Quercus phellos*. Killingbeck (1996) found phosphorus concentrations in *Quercus rubra* leaves were 0.07 % and those in *Nyssa sylvatica* were 0.04 %. In this study, the phosphorus concentrations were slightly higher for both species– 0.098 at the agricultural site and 0.082 at the reference site for *Quercus rubra* and 0.071 at the agricultural site and 0.091 at the reference site for *Nyssa sylvatica*. *Acer rubrum* and *Quercus alba* concentrations were comparable to those found in other studies (Killingbeck and Tainsh 2002; Killingbeck 1996).

Killingbeck (1996) also proposed phosphorus resorption levels for deciduous trees. He asserted that complete resorption occurred below 0.05% while incomplete resorption was above 0.08%. Intermediate resorption occurred in between these two values. Once again no species completely resorbed the nutrient. Moreover, almost 77% of the species at the agricultural site resorbed phosphorus incompletely while slightly over 55% of the species at the reference site resorbed phosphorus at an intermediate level.

This time some differences in carbon were observed between species within site and between sites by species. Inconsistencies between species within sites could be due to differences in the ability of the trees to harvest sunlight for photosynthesis. Overall, the oak species have higher carbon concentrations in their leaves than do the other species. The oak leaves are larger than the other species thereby allowing for a greater surface area to capture light.

The carbon concentrations were also significantly greater in leaves of *Acer rubrum, Liquidambar styraciflua,* and *Quercus rubra* at the agricultural site than at the reference site. Carbon is closely linked to nitrogen (Barbour *et al.* 1999). Acquiring nitrogen requires energy supplied by carbon metabolism. In return, biomass production is often limited by nitrogen availability (Barbour *et al.* 1999). Since nitrogen is more readily available to the trees at the agricultural site, more biomass may be produced causing higher carbon concentration in the litter.

There was only one difference found between species for the N:P ratios. *Quercus falcata* leaves had significantly higher N:P ratios than did *Quercus rubra*. Boerner (1984) determined nitrogen and phosphorus concentrations on a weight basis in *Acer rubrum* and *Quercus alba* leaves in a fertile and infertile site. Because he did not present N:P ratios *per se*, I took the nitrogen and phosphorus concentrations that he presented and calculated N:P ratios for each of his study sites. I then averaged them together so that I could determine if the values obtained in my study were comparable to others. Based on data presented by Boerner (1984), the average N:P ratio for *Acer rubrum* was 11.5 and for *Quercus alba* was 12.1. These values are lower than my values of 14.9 and 17.5 for *Acer rubrum* and *Quercus alba,* respectively.

Although differences between species existed at both sites, there were more differences at the agricultural site than at the reference site. The nitrogen enrichment at the agricultural site may be causing some trees to take up more nitrogen and increase carbon production (i.e. biomass) thereby altering the natural C:N ratios. This is relevant since high C:N ratios often cause decomposers to be limited by nitrogen (Vitousek 1982). Only one difference was found between sites by species. *Quercus falcata* leaves at the

agricultural site had significantly higher C:N ratios than those at the reference site.

The leaves of *Acer rubrum* and *Quercus falcata* had significantly higher C:P ratios than did *Quercus rubra* at the agricultural site. Despite this difference at the agricultural site, there were no differences between species at the reference site. Similarly no significant differences existed between sites within species. However, the C:P ratios were higher in all but two species at the agricultural site than at the reference site. This result is attributable to differences in carbon more so than differences in phosphorus.

In 2003 the leaves fell within a six week period. A decreasing trend in nitrogen concentrations was observed in 2003 from October 10 through November 5 at the agricultural site was expected since the nutrients were being resorbed by the trees. Woodwell (1974) studied tree litter of *Quercus alba, Quercus coccinea,* and *Pinus rigida* in a New York forest. His results were consistent with mine, for he found that nitrogen concentrations in tree leaf litter decreased throughout the period of senescence (Woodwell 1974). This suggests that leaves remaining attached to trees for an extended period of time had a greater concentration of nitrogen resorbed by the woody tissues. The increase in concentration that occurred on the last date was apparently caused by a frost with a low temperature of 24°F lasting 3 days that happened just prior to this collection date (NOAA). The leaves froze, essentially trapping the nitrogen in the leaf and preventing the tree from withdrawing it. An increase was not observed on the same date at the reference site possibly because, unlike the fragmented agricultural site, the contiguous span of forest provides a buffer against low temperatures and high winds. Significant differences existed between sites for nitrogen on all dates except on October

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29 and November 5, 2003. Boerner (1984) also found that trees growing in fertile environments had higher concentrations of nitrogen in tree litter than those in infertile environments. As just previously mentioned, the frost may explain the discrepancy at the end of the year. The difference at the beginning of the year is probably a reflection of the excess nitrogen at the agricultural site.

In addition, the concentration of phosphorus did decrease over time in 2003 except for on the last collection date. Woodwell (1974) found similar results throughout the period of senesce in New York. As previously mentioned, a frost occurred just prior to that date and provides a possible explanation for the observed, yet insignificant, difference. Significant differences in phosphorus between sites were detected at the start and end of the season only. The difference at the beginning of the season is attributed to variations in phosphorus availability between the sites. The discrepancy at the end of the season is probably due to the frost.

In 2003 carbon varied significantly within and between sites by date. The observed increase in carbon at the agricultural site is expected. A significant difference between sites in the middle of the season was also seen. This result could be due to an abundance of structural carbon that remains in the leaf later in the season. As nitrogen and phosphorus are resorbed the proportion of carbon left in the leaf increases. This is important since the amount of carbon being resorbed by senescing leaves has been found to influence the efficiency of nutrient resorption (Chapin and Moilanen 1991).

Recall that in 2002 only two plots had litter traps place within them at the reference site. There may have been insufficient replication at this site to detect differences over time. There was also no difference between sites in 2002. As stated

earlier, this result may be due to climatic factors. A significant drought occurred for much of 2002. The lack of precipitation may have caused the trees to drop their leaves earlier in the season and the period of senescence may have been shortened thereby altering the normal resorption rates. The majority of the litter was collected within a three week period during 2002. Del Arco *et al.* (1991) found that when the annual rainfall influenced retranslocation efficiency. He concluded that the higher the annual rainfall, the higher the retranslocation efficiency (Del Arco *et al.* 1991). Although I did not measure retranslocation efficiency, the influence of drought may help to explain the lack of decrease in nutrients during the period of senescence.

Furthermore, overall comparisons of the herbaceous vegetation revealed only two significant differences between sites: in 2002, the N:P ratio was significantly higher at the reference site than at the agricultural site and in 2003 the concentration of carbon was marginally significantly higher at the agricultural site than at the reference site. The difference in N:P ratios can be explained by the drought of 2002. The agricultural site is largely influenced by groundwater inputs (Angier *et al.* 2002) so the drought limited the ability of the groundwater to transport nitrogen into the agricultural site. The plants found at the reference site were probably already adapted to low nitrogen availability but those at the agricultural site probably were not. The higher carbon concentrations at the agricultural site in 2003 may be attributed to the higher light levels at the agricultural causing increasing photosynthesis rates. There were some differences in nutrient concentrations and element ratios within site by herbaceous species during both years and at both sites. This difference between species has been found in other studies (Kao *et al.* 2003). Any differences that occurred between sites by species were partly due to

Symplocarpus foetidus. Thus, this species may be able to take up excess nutrients in enriched sites.

CONCLUSIONS

A lower number of significant differences were found in 2002 than in 2003. This could be due to a lower number of repetitions at the reference site. The two plots found there may not have been sufficient to detect differences. Also, since 2002 was a drought year, the behavior of nutrient cycling may have been different than in a year when normal climatic patterns occur like in 2003 possibly due to plant stress caused by a lack of water. Moreover, the influence of groundwater appears to be especially important for the vegetation at the agricultural site. Because the plants in this system appear to rely heavily on the import of nutrients via groundwater, when the levels dropped, the availability of nutrients probably did too. In addition, there were more differences detected between sites for the tree litter than for the herbaceous vegetation. This may also be attributed to the role of groundwater. The levels of nitrate in the groundwater are lowest in the shallow groundwater and increase with depth. Thus, the trees, which roots that extend deep into the soil profile, may potentially take up higher concentrations of nutrients. The herbaceous vegetation, on the other hand, has shallow roots that are exposed to lower concentrations of nutrients. Therefore, they probably are not taking up concentrations of nutrients like the trees.

During the 2003 season, the nitrogen, phosphorus, and carbon concentrations in tree litter were all significantly greater at the agricultural site than at the reference site. Moreover, the NUEs of nitrogen and phosphorus were both significantly lower at the agricultural site than at the reference site. Based on these results the trees at the

agricultural site return higher concentrations of nutrients to the system via litterfall. The

observed decrease in nutrient concentrations during the 2003 senescent period at the

agricultural site reveals that the nutrient cycles at the site may be changing in response to

the excess nitrogen and phosphorus. Despite these decreasing concentrations, nitrogen

and phosphorus significantly increased in the agricultural litter when a frost occurred.

The fragmented nature of these agricultural buffers may make more susceptible to frost

damage and could temporarily change nutrient patterns within these systems.

Chapter 4: The Effects of Nutrient Enrichment on the Herbaceous Vegetation of a Forested Riparian Wetland

INTRODUCTION

Surface and ground water exiting agricultural fields often contain high levels of nitrogen and phosphorus. These nutrients then enter nearby ecosystems where they interact with the vegetation. These additional nutrients can cause changes in the plant community to occur. Community level changes, such as a decrease in species diversity, species richness and species evenness have been documented in communities enhanced by nutrients (Roberts and Gilliam 1995; Bedford *et al.* 1999; Joyce 2001). Furthermore, species level changes, including changes in species competition and plant uptake of nutrients, can also occur due to elevated concentrations of nutrients (Roberts and Gilliam 1995). The addition of the limiting nutrient(s) (often nitrogen, phosphorus, or a combination of the two) by definition results in an increase in biomass production while the addition of non-limiting nutrients produces little if any effect (Silvan *et al.* 2004).

It is important to study the influence of nutrients on the vegetation found in natural settings so that we can understand how excess nutrients from human activities alter plant vegetation and biodiversity. Only by fertilizing these plants can we gain insight into the direct effects of the agricultural nutrients on the individual plant species and the ecosystem as a whole. Thus, we need to identify those species that are likely to be negatively impacted by increased nutrients.

It has been argued that the N:P ratios of plants can reveal whether plants are limited by nitrogen, phosphorus, or a co-limited by both nutrients. There are at least two

main schools of thought on the relationship of N:P ratios and nutrient limitation. Koerselman and Meuleman (1996) reviewed the results of forty experiments involving the fertilization of herbaceous vegetation was fertilized in natural wetland settings in Europe. They suggested that N:P ratios less than 14 reflect nitrogen limitation while those greater than 16 reflect phosphorus limitation. N:P values in between 14 and 16 represent co-limitation by nitrogen and phosphorus.

The resource ratio model, on the other hand, provides a different view. It predicts that if a species is co-limited by nitrogen and phosphorus, the addition of nitrogen will cause that species to compete for phosphorus since it would be in more limited in quantity (Tilman 1985). The species that can increase phosphorus uptake could then outcompete those species that could not. The opposite outcome would occur if phosphorus was suddenly added to the system and nitrogen became limited. This model also predicts that the concentrations of nutrients in plant biomass are a reflection of the competitiveness of the plant. Those plants that are strong competitors would contain low concentrations of the nutrient while those that are weak competitors would contain high concentrations.

I examined responses of herbaceous vegetation to excess nitrogen and phosphorus in a fertilization experiment in a forested riparian wetland. Percent cover and frequency were determined and biomass and nitrogen, phosphorus, and carbon concentrations in plant tissue were measured. Nutrient loads, nutrient use efficiencies (the plant biomass produced for every unit of nutrient mass), and element ratios were also calculated. Comparisons of these parameters between treatments were made first by averaging across species to determine community level trends and then by inspecting by species to

determine trends at the species level. Four species that were present in every plot were examined – *Impatiens capensis* (Meerb.)*, Microstegium vimineum* (Trin.) A. Capus*, Polygonum punctatum* Ell.*,* and *Symplocarpus foetidus* L. Salisb. ex. Nutt. *Microstegium vimineum* was the only non-native species present. This C_4 grass originated in Japan and has become naturalized throughout the eastern and southeastern United States (Claridge and Franklin 2002).

I hypothesized that the community as a whole would be limited by nitrogen since many freshwater wetlands have been shown to be nitrogen limited (Bedford *et al.* 1999). I also expected that resource limitation would vary by species. Furthermore, I anticipated that species would differ in nutrient concentrations, NUEs, and element ratios since there is often variability in nutrient concentrations between species.

METHODS

The fertilization experiment was conducted at the reference site only. No experiments were conducted at the agricultural site. The direct addition of nutrients into the agricultural system may have influenced studies that other researchers were performing simultaneously (like the monitoring of nitrogen concentrations in groundwater and surface water). Moreover, it was unknown whether any fertilization effects would be pronounced enough to detect since the plants at this site have been exposed to elevated concentrations of nutrients for decades.

Twenty 2 x 1 m plots were randomly distributed throughout the reference site and marked with PVC poles. Nitrogen and phosphorus were applied in a 2 x 2 factorial arrangement, resulting in four treatment combinations: (1) the addition of nitrogen, (2) the addition of phosphorus, (3) the addition of nitrogen and phosphorus, and (4) no

additions. Each treatment combination was randomly applied to five of the 2 x 1 m plots.

Fertilizer in the form of urea (N-P-K 46-0-0) for nitrogen and triple superphosphate (N-P-K 0-46-0) for phosphorus were applied. Since the study area is frequently flooded, I decided that the fertilizer should be applied below the ground surface in the rooting zone instead of simply being applied on the ground surface. By digging holes I hoped to ensure that the fertilizer would not be exported out of the system by flood waters.

I cut eight holes at equal intervals (two rows of four) into a piece of plastic that was 1 x 2 m in area. The plastic was then used as a guide and laid on the soil surface within each plot. Eight holes were then dug within each plot using a soil corer. Plots that did not receive additional nitrogen or phosphorus likewise had holes dug to maintain consistency and to ensure that possible effects due to the holes would be present in all plots. Within each of the holes 150 g of the assigned treatment was applied. Thus, nitrogen treated plots received a total of 1200 grams of nitrogen; likewise, phosphorus treated plots received a total of 1200 grams of phosphorus. Plots fertilized with both nitrogen and phosphorus received 150 g of each nutrient within each hole resulting in a total of 2400 g of fertilizer (1200 g of nitrogen and 1200 g of phosphorus). The holes were then resealed with soil.

The percent cover and frequency of each species in all plots were determined in the end of May. A sampling frame quadrat subdivided into 32 cells was placed over each 2 x 1 m plot to determine the frequency of occurrence of individual species (i.e., the number of cells it occurred in divided by 32 multiplied by 100%). Percent cover was determined for the entire 2 x 1 m quadrat. The percent cover is the area of ground surface that each plant occupies if projected vertically onto the surface within the quadrat

expressed as a percentage. Percent cover values can be greater than 100% since plants often overlap each other. The cover values used were: <0.1%, 0.1% increments from 0.1-1%, 1% increments from 1-10%, and 5% increments above 10%.

The aboveground biomass within each 2 x 1 m plot was harvested in June, separated by species while harvesting in the field, dried at 60°C until constant weight was achieved, weighed, homogenized using a Wiley Mill, and analyzed for nitrogen, phosphorus, and carbon. Total nitrogen and carbon were determined using a LECO Tru-Spec CN Analyzer (St. Louis, Missouri). Total phosphorus concentrations were determined using a modified Kjeldahl digestion method (instead of using 3.5 mL of sulfuric acid as called for in this method, I used 5 mL). Digests were then run on a Flow Injection Analyzer (Lachat QuikChem 8000, Milwaukee, Wisconsin).

Phosphorus concentrations were originally given in units of parts per million (ppm=μg/mL) while nitrogen and carbon were given in units of percent. The phosphorus concentrations were therefore converted to units of percent so that all data would be in the same units. Nutrient loads were calculated by dividing the nutrient mass (in units of kilograms) by the total area of each plot in hectares). Nutrient use efficiencies were calculated by dividing the biomass in units of grams by the nutrient mass (which were converted into units of grams). Element ratios were also calculated using nutrient contents in units of mass. All statistical analyses on the data were then performed using SAS (v. 8 for Windows, SAS Institute, Cary, NC, USA). The data were examined by averaging across species to investigate general plant community level trends due to nitrogen and phosphorus fertilization and by averaging within species by treatment to determine possible differences between species.

When considering overall trends due to fertilization, the addition of nitrogen and phosphorus did increase the concentration of nitrogen slightly but did not alter it significantly (Figure 4-1a; Table 4-1). Although the concentration of phosphorus was not affected by the addition of nitrogen, it was significantly increased when phosphorus was added (Figure 4-1b; Table 4-1). Carbon concentrations were unaffected by the addition of nitrogen or phosphorus (Figure 4-1c; Table 4-1).

In addition, the application of phosphorus decreased the N:P ratio significantly while that of nitrogen did not (Figure 4-2a; Table 4-1). However, neither the C:N (Figure 4-2b; Table 4-1) nor the C:P (Figure 4-2c; Table 4-1) ratios were influenced significantly by the addition of nitrogen or phosphorus.

Despite the lack of significant differences of nutrient loads due to fertilization treatment, the phosphorus load was slightly increased when nitrogen was added but decreased when phosphorus was added (Figure 4-3a; Table 4-1). The application of nitrogen did increase the nitrogen load but not significantly while the addition of phosphorus, actually decreased the nitrogen load (Figure 4-3b; Table 4-1). Moreover, carbon followed a similar trend as the other two nutrients studied. The addition of nitrogen increased the carbon load while the addition of phosphorus decreased it (Figure 4-3c; Table 4-1).

The addition of nitrogen had no significant effect on the nitrogen NUE; likewise phosphorus additions did not alter the NUE of nitrogen (Figure 4-4a; Table 4-1). Also, the NUE of phosphorus was not affected when nitrogen was added to the system, but it was significantly decreased when phosphorus was added (Figure 4-4b; Table 4-1).

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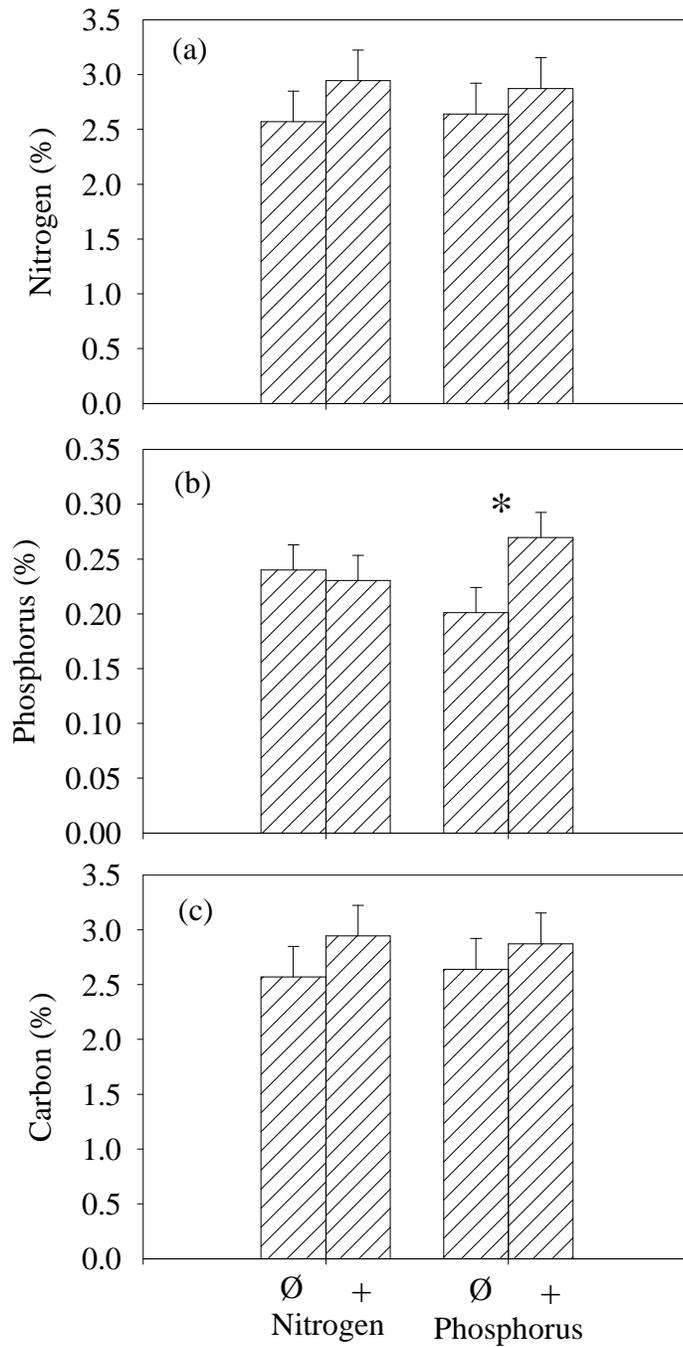
Figure 4-1. Nitrogen and phosphorus effects on the concentrations of (a) nitrogen, (b) phosphorus, and (c) carbon of all herbaceous species. Values with asterisks (*) between them indicate a significant treatment effect for that nutrient at the 0.05 level. Ø = no nutrient added; + = nutrient added.

Parameter	Nitrogen	Phosphorus	Nitrogen*Phosphorus
Phosphorus	$0.09_{(1,16)}$	$4.40_{(1,16)}$	$0.03_{(1,16)}$
Carbon	$0.90_{(1,16)}$	$0.34_{(1,16)}$	$0.10_{(1,16)}$
Nitrogen	$0.90_{(1,16)}$	$0.34_{(1,16)}$	$0.10_{(1,16)}$
C:N	$2.77_{(1,16)}$	$1.92_{(1,16)}$	$0.27_{(1,16)}$
C:P	$0.03_{(1,16)}$	$1.28_{(1,16)}$	$1.40_{(1,16)}$
N:P	$2.02_{(1,16)}$	$5.51_{(1,16)}*$	$0.28_{(1,16)}$
P load	$1.59_{(1,16)}$	$0.97_{(1,16)}$	$1.20_{(1,16)}$
N load	$2.61_{(1,16)}$	$3.00_{(1,16)}$	$1.10_{(1,16)}$
C load	$1.92_{(1,16)}$	$2.63_{(1,16)}$	$0.83_{(1,16)}$
NUE-P	$0.30_{(1,16)}$	$4.16_{(1,16)}$	$0.08_{(1,16)}$
NUE - N	$0.17_{(1,16)}$	$0.09_{(1,16)}$	$0.11_{(1,16)}$
Biomass	$1.59_{(1,16)}$	$1.81_{(1,16)}$	$0.29_{(1,16)}$
Cover	$0.30_{(1,756)}$	$0.47_{(1,756)}$	$0.13_{(1,756)}$
Frequency	$0.03_{(1,756)}$	$0.22_{(1,756)}$	$0.03_{(1,756)}$

Table 4-1. Tests of fixed effects results for all species. Values are the F values and the numbers in parentheses are the numerator and denominator degrees of freedom, respectively. N is the nitrogen treatment effect; P is the phosphorus treatment effect; N*P is the nitrogen*phosphorus interaction effect. An asterisks (*) indicates significance at the 0.05 level. There was a significant phosphorus treatment effect for the N:P ratio.
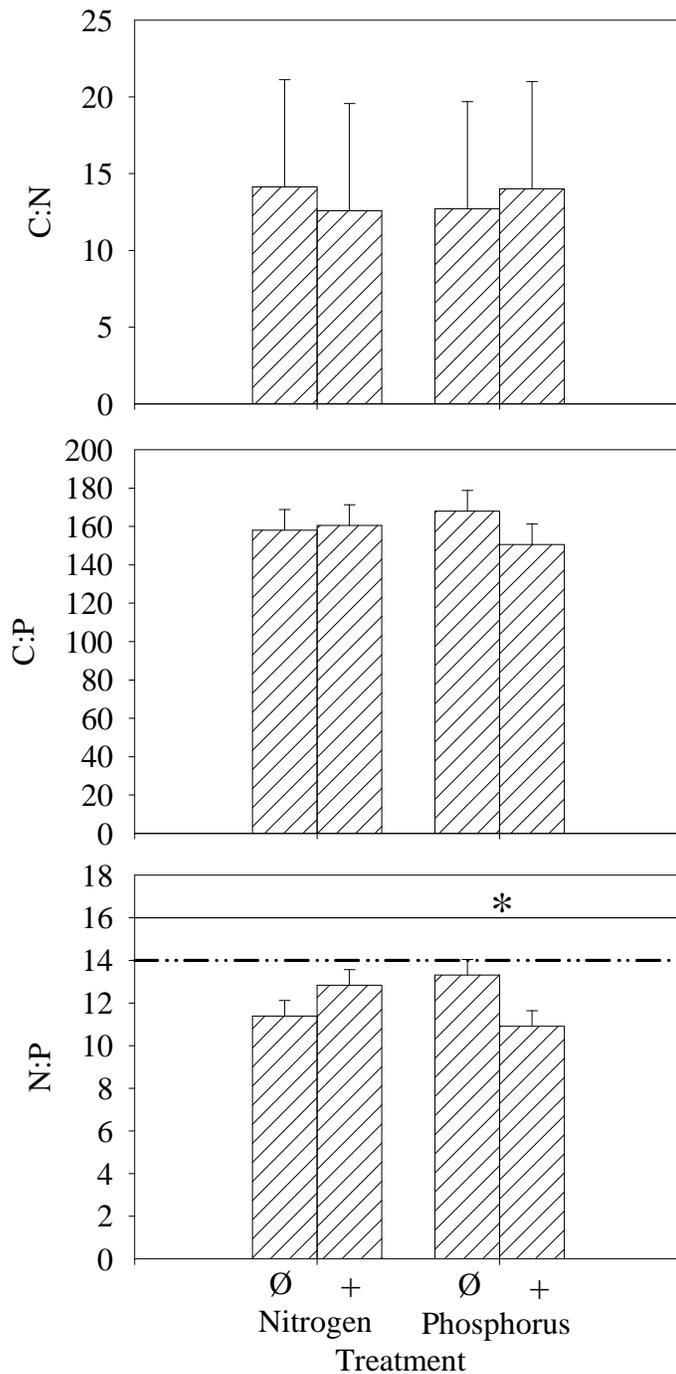
Figure 4-2. Nitrogen and phosphorus effects on element ratios of all herbaceous species. Values with asterisks (*) between them indicate a significant treatment effect for that nutrient at the 0.05 level. The horizontal lines in the N:P graph represent the cutoff values for nutrient limitation suggested by Koerselman and Mueleman (1996). Values above 16 represent phosphorus limitation while those below 14 represent nitrogen limitation. Values between 14 and 16 represent co-limitation by both nitrogen and phosphorus. The N:P ratio was significantly decreased when phosphorus was added. Ø = no nutrient added; + = nutrient added.
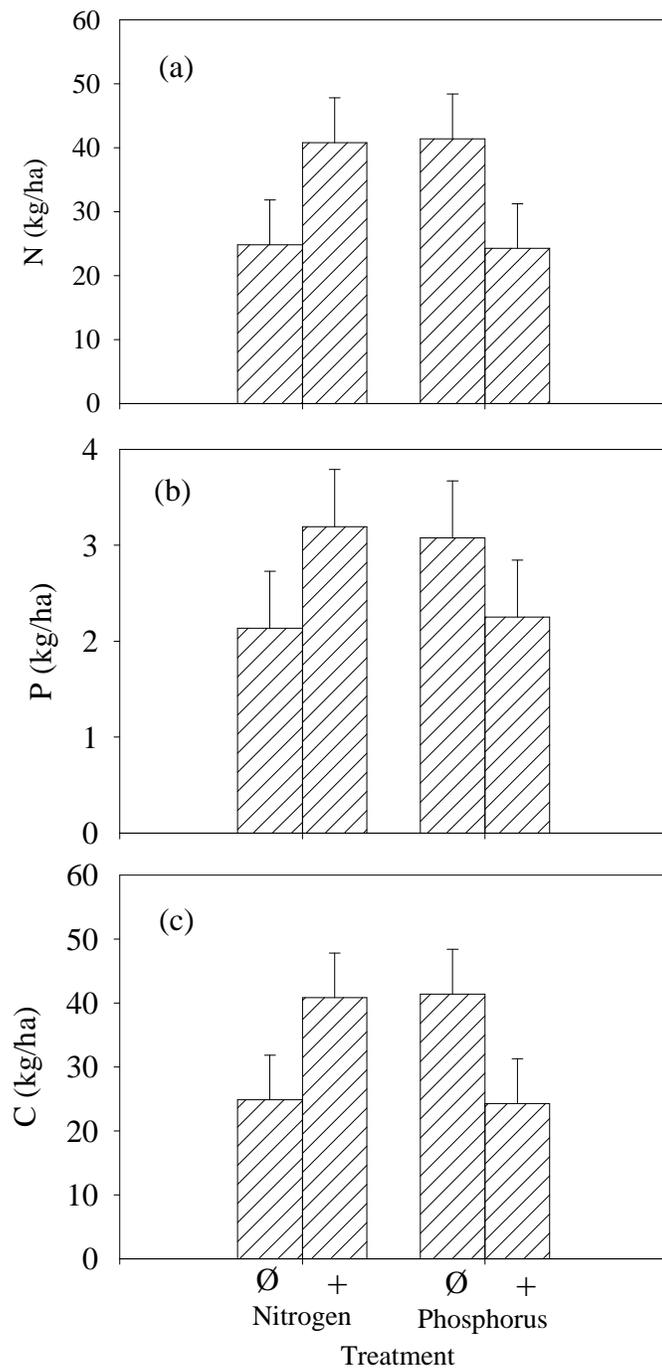
Figure 4-3. Nitrogen and phosphorus effects on the nutrient loads of (a) nitrogen, (b) phosphorus, and(c) carbon for all herbaceous species. There were no significant treatment effects at the 0.05 level. Ø = no nutrient added; + = nutrient added.
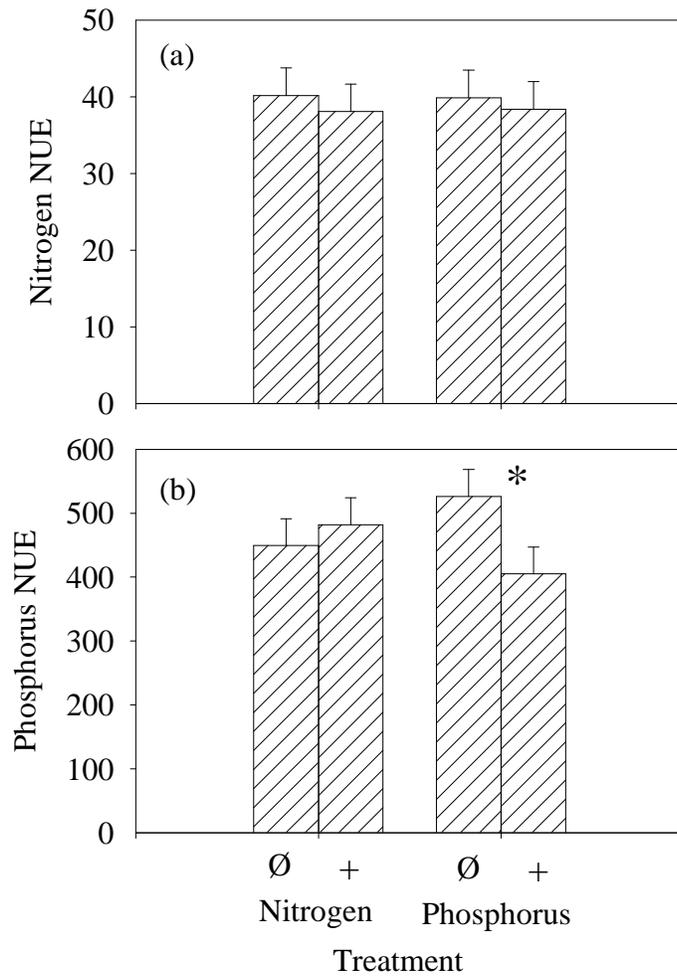
Figure 4-4. Nitrogen and phosphorus effects on the nutrient use efficiencies (NUE is the leaf litter biomass per unit nutrient content) of (a) nitrogen and (b) phosphorus of all herbaceous species. Values with asterisks (*) between them indicate a significant treatment effect for that nutrient at the 0.05 level. Ø = no nutrient added; + = nutrient added.

Neither nitrogen nor phosphorus application affected the overall cover or

frequency of the plants (Table 4-1; Table 4-2). Likewise, the addition of nitrogen or

phosphorus produced no significant differences in biomass (Figure 4-5; Table 4-1).

However, when nitrogen was added, the biomass did increase slightly while the addition

of phosphorus decreased it.

Effect	Cover (%)		Frequency (%)	
	Nitrogen	**Phosphorus**	**Nitrogen**	**Phosphorus**
Ø	2.8 ± 0.6	3.3 ± 0.6	6.2 ± 0.9	5.8 ± 0.9
+	3.2 ± 0.6	2.7 ± 0.6	5.9 ± 0.9	6.4 ± 0.9

Table 4-2. Nitrogen and phosphorus effects on percent cover and frequency of all herbaceous species. There were no differences in cover or frequency due to nitrogen or phosphorus. Ø = no nutrient added; + = nutrient added.
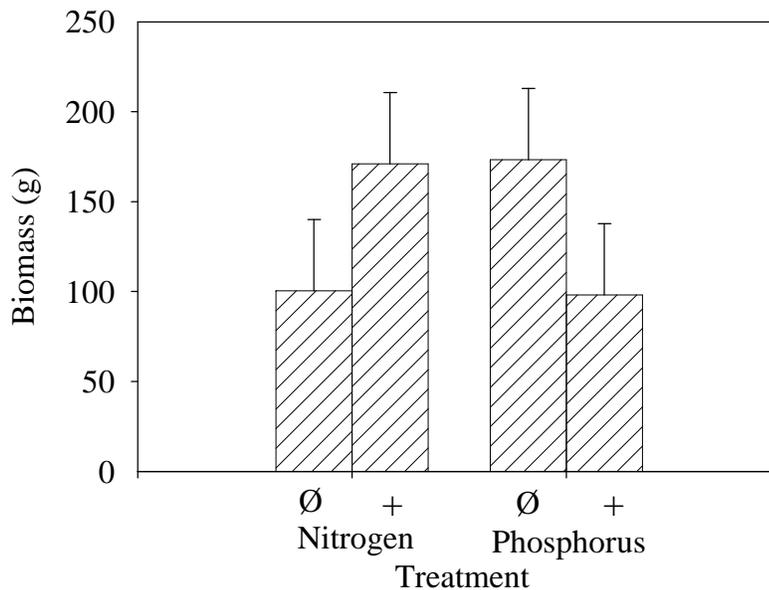
Figure 4-5. Nitrogen and phosphorus effects on the biomass of all herbaceous species. Values with asterisks (*) above them indicate a significant difference exists between treatments at the 0.05 level using tests of fixed effects. No differences in biomass were observed due to nitrogen or phosphorus additions. Ø = no nutrient added; + = nutrient added.

Differences between the most commonly occurring species were also

investigated. While there were no significant differences in cover due to nitrogen or

phosphorus additions in *Impatiens capensis* (Figure 4-6a; Table 4-3)*, Microstegium vimineum* (Figure 4-6b; Table 4-4)*,* or *Polygonum punctatum* (Figure 4-6c; Table 4-5), there was a significant interaction of nitrogen and phosphorus for *Symplocarpus foetidus* (Figure 4-6d; Table 4-6). When phosphorus was added, the percent cover was actually lower than when no phosphorus was added. The addition of nitrogen alone had the highest percent cover and the lowest occurred when both nitrogen and phosphorus were added. There were no significant differences in frequency due to the addition of nitrogen or phosphorus for any of the species (Figure 4-7 a-d; Tables 4-3, 4-4, 4-5, and 4-6).

Although the phosphorus concentration in *Impatiens capensis* was not affected significantly by the addition of nitrogen or phosphorus, it did increase slightly with the addition of phosphorus (Figure 4-8a; Table 4-3). There was an interaction effect involving nitrogen and phosphorus, but the concentration of phosphorus in *Microstegium vimineum* was always higher when phosphorus was added than when no phosphorus was added (Figure 4-8b; Table 4-4). The application of nitrogen did not affect the concentration of phosphorus in *Polygonum punctatum*, but the application of phosphorus significantly enhanced the concentration of phosphorus (Figure 4-8c; Table 4-5). Furthermore, nitrogen and phosphorus additions neither increased nor decreased the concentration of phosphorus in *Symplocarpus foetidus* (Figure 4-8d; Table 4-6). There were no significant differences in nitrogen concentration observed for any of the species at the 0.05 level using the tests of fixed effects due to the application of nitrogen or phosphorus (Figure 4-9a-d; Tables 4-3, 4-4, 4-5, and 4-6). However, the application of nitrogen did slightly increase the concentrations of nitrogen in all species (Figure 4-9a-d; Tables 4-3, 4-4, 4-5, and 4-6). Carbon concentrations were also examined. There were
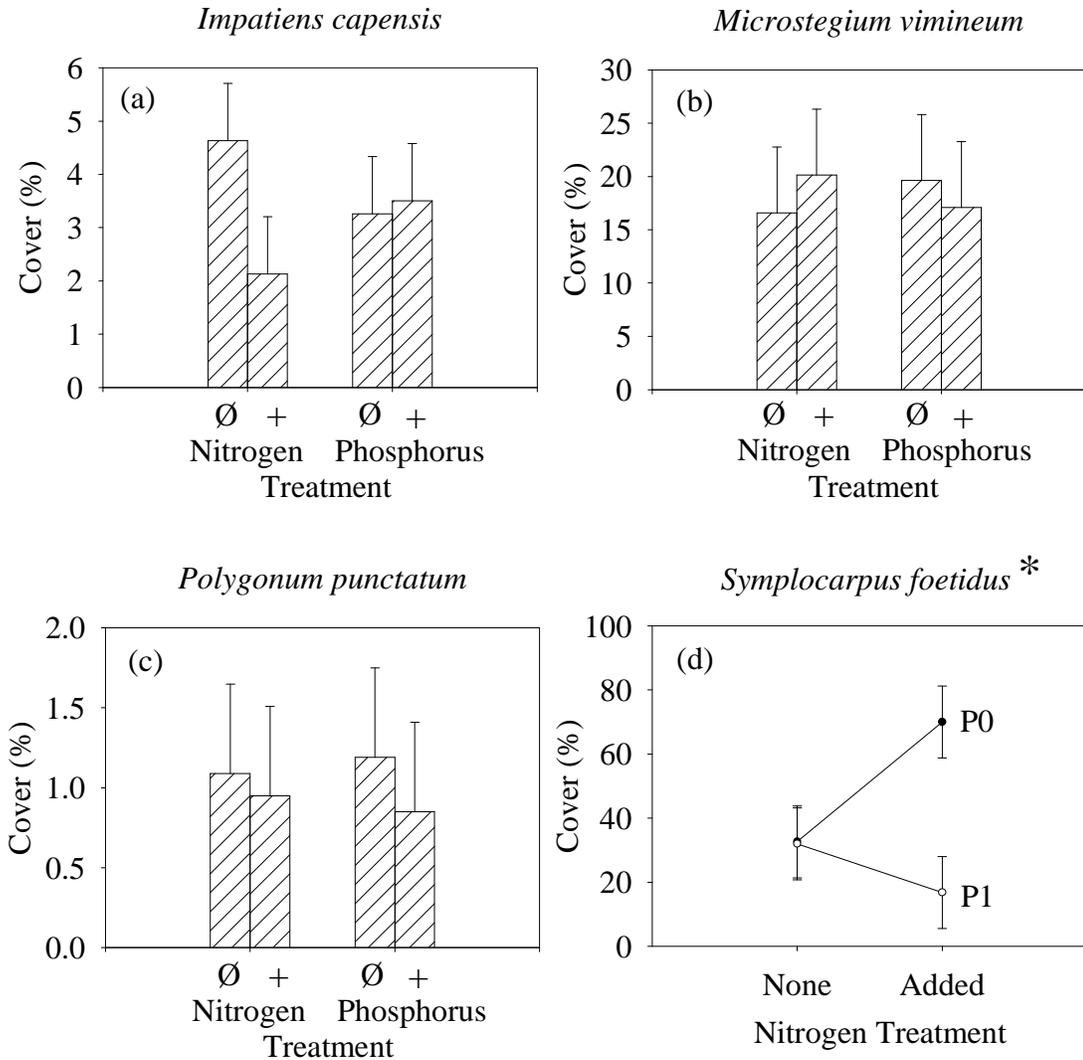
Figure 4-6. Nitrogen and phosphorus treatment effects on the percent cover of (a) Impatiens capensis, (b) Microstegium vimineum, (c) Polygonum punctatum, and (d) Symplocarpus foetidus. Values with asterisks (*) above them indicate a significant treatment effect for that nutrient at the 0.05 level. Main effects are shown for Impatiens capensis, Microstegium vimineum, and Polygonum punctatum since the nitrogen*phosphorus interactions were insignificant; nitrogen*phosphorus interactions are presented for Symplocarpus foetidus since they were significant (P=0.0326). No significant effects on nitrogen concentration were found when nitrogen or phosphorus was added. Ø = no nutrient added; + = nutrient added.

Parameter	Nitrogen	Phosphorus	Nitrogen*Phosphorus
Biomass	$0.24_{(1,8)}$	$0.05_{(1,8)}$	$1.23_{(1,8)}$
Phosphorus	$0.01_{(1,7)}$	$1.81_{(1,7)}$	$1.35_{(1,7)}$
Carbon	$1.14_{(1,7)}$	$0.68_{(1,7)}$	$0.03_{(1,7)}$
Nitrogen	$1.78_{(1,7)}$	$0.02_{(1,7)}$	$0.13_{(1,7)}$
C:N	$1.30_{(1,7)}$	$0.10_{(1,7)}$	$0.05_{(1,7)}$
C:P	$0.000_{(1,6)}$	$1.51_{(1,6)}$	$1.60_{(1,6)}$
N:P	$1.09_{(1,6)}$	$0.26_{(1,6)}$	$0.10_{(1,6)}$
Cover	$2.71_{(1,16)}$	$0.02_{(1,16)}$	$0.73_{(1,16)}$
Frequency	$1.40_{(1,16)}$	$1.96_{(1,16)}$	$0.93_{(1,16)}$

Table 4-3. Tests of fixed effects results for *Impatiens capensis*. Values are the F values and the numbers in parentheses are the numerator and denominator degrees of freedom, respectively. N is the nitrogen treatment effect; P is the phosphorus treatment effect; N*P is the nitrogen*phosphorus interaction effect. Neither the interactions nor the main treatment effects were significant.

Parameter	Nitrogen	Phosphorus	Nitrogen*Phosphorus
Biomass (kg/ha)	$0.69_{(1,10)}$	$0.31_{(1,10)}$	$2.31_{(1,10)}$
Phosphorus (%)	$6.64_{(1,9)}$*	$38.81_{(1,9)}$**	$9.66_{(1,9)}$*
Carbon (%)	$0.05_{(1,9)}$	$0.43_{(1,9)}$	$1.73_{(1,9)}$
Nitrogen (%)	$0.02_{(1,9)}$	$1.21_{(1,9)}$	$0.33_{(1,9)}$
C:N	$0.10_{(1,9)}$	$0.39_{(1,9)}$	$0.04_{(1,9)}$
C:P	$6.65_{(1,8)}$*	$24.15_{(1,8)}$**	$3.68_{(1,8)}$
N:P	$8.68_{(1,8)}$*	$21.82_{(1,8)}$**	$1.93_{(1,8)}$
Cover	$0.16_{(1,16)}$	$0.08_{(1,16)}$	$0.09_{(1,16)}$
Frequency	$0.09_{(1,16)}$	$0.54_{(1,16)}$	$0.06_{(1,16)}$

Table 4-4. Tests of fixed effects for *Microstegium vimineum*. Values are the F values and the numbers in parentheses are the numerator and denominator degrees of freedom, respectively. N is the nitrogen treatment effect; P is the phosphorus treatment effect; N*P is the nitrogen*phosphorus interaction effect. An asterisks (*) indicates the significance level as follows: * = <0.05; **= <0.01.

Parameter	Nitrogen	Phosphorus	Nitrogen*Phosphorus
Biomass (kg/ha)	$2.35_{(1,2)}$	$0.90_{(1,2)}$	$47.13_{(1,2)}$*
Phosphorus (%)	$1.38_{(1,2)}$	$19.26_{(1,2)}$*	$0.28_{(1,2)}$
Carbon (%)	$0.03_{(1,2)}$	$0.19_{(1,2)}$	$0.44_{(1,2)}$
Nitrogen (%)	$1.79_{(1,2)}$	$0.14_{(1,2)}$	$1.25_{(1,2)}$
C:N	$1.49_{(1,2)}$	$0.25_{(1,2)}$	$0.03_{(1,2)}$
C:P	$0.69_{(1,2)}$	$6.89_{(1,2)}$	$0.48_{(1,2)}$
N:P	$23.74_{(1,2)}$*	$81.45_{(1,2)}$*	$3.25_{(1,2)}$
Cover	$0.03_{(1,16)}$	$0.19_{(1,16)}$	$0.66_{(1,16)}$
Frequency	$0.24_{(1,16)}$	$1.07_{(1,16)}$	$0.15_{(1,16)}$

Table 4-5. Tests of fixed effects for *Polygonum punctatum*. Values are the F values and the numbers in parentheses are the numerator and denominator degrees of freedom, respectively. N is the nitrogen treatment effect; P is the phosphorus treatment effect; N*P is the nitrogen*phosphorus interaction effect. An asterisks (*) indicates significance at the 0.05 level.

Parameter	Nitrogen	Phosphorus	Nitrogen*Phosphorus
Biomass (kg/ha)	$2.30_{(1,15)}$	$0.14_{(1,15)}$	$0.04_{(1,15)}$
Phosphorus (%)	$0.11_{(1,13)}$	$1.89_{(1,13)}$	$1.81_{(1,13)}$
Carbon (%)	$1.01_{(1,15)}$	$0.41_{(1,15)}$	$0.22_{(1,15)}$
Nitrogen (%)	$0.07_{(1,15)}$	$0.63_{(1,15)}$	$0.65_{(1,15)}$
C:N	$6.16_{(1,15)}*$	$0.44_{(1,15)}$	$0.32_{(1,15)}$
C:P	$0.31_{(1,13)}$	$0.03_{(1,13)}$	$0.00_{(1,13)}$
N:P	$0.18_{(1,13)}$	$0.15_{(1,13)}$	$0.04_{(1,13)}$
Cover	$0.98_{(1,16)}$	$5.73_{(1,16)}*$	$5.48_{(1,16)}*$
Frequency	$0.00_{(1,16)}$	$0.60_{(1,16)}$	$1.01_{(1,16)}$

Table 4-6. Tests of fixed effects for *Symplocarpus foetidus*. Values are the F values and the numbers in parentheses are the numerator and denominator degrees of freedom, respectively. N is the nitrogen treatment effect; P is the phosphorus treatment effect; N*P is the nitrogen*phosphorus interaction effect. An asterisks (*) indicates significance at the 0.05 level.
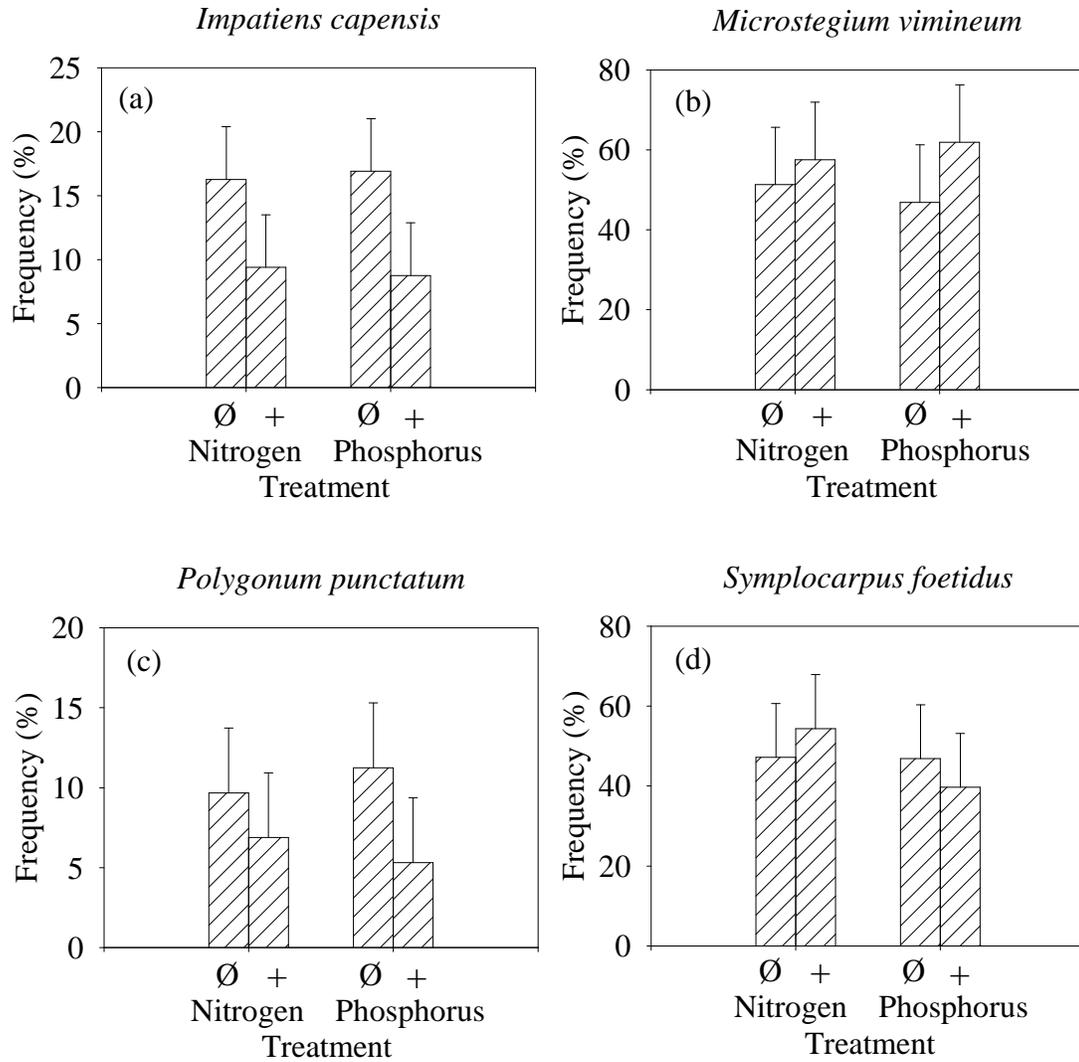
Figure 4-7. Nitrogen and phosphorus treatment effects on the frequency of (a) Impatiens capensis, (b) Microstegium vimineum, (c) Polygonum punctatum, and (d) Symplocarpus foetidus. There were no significant treatment effects at the 0.05 level. Ø = no nutrient added; + = nutrient added.
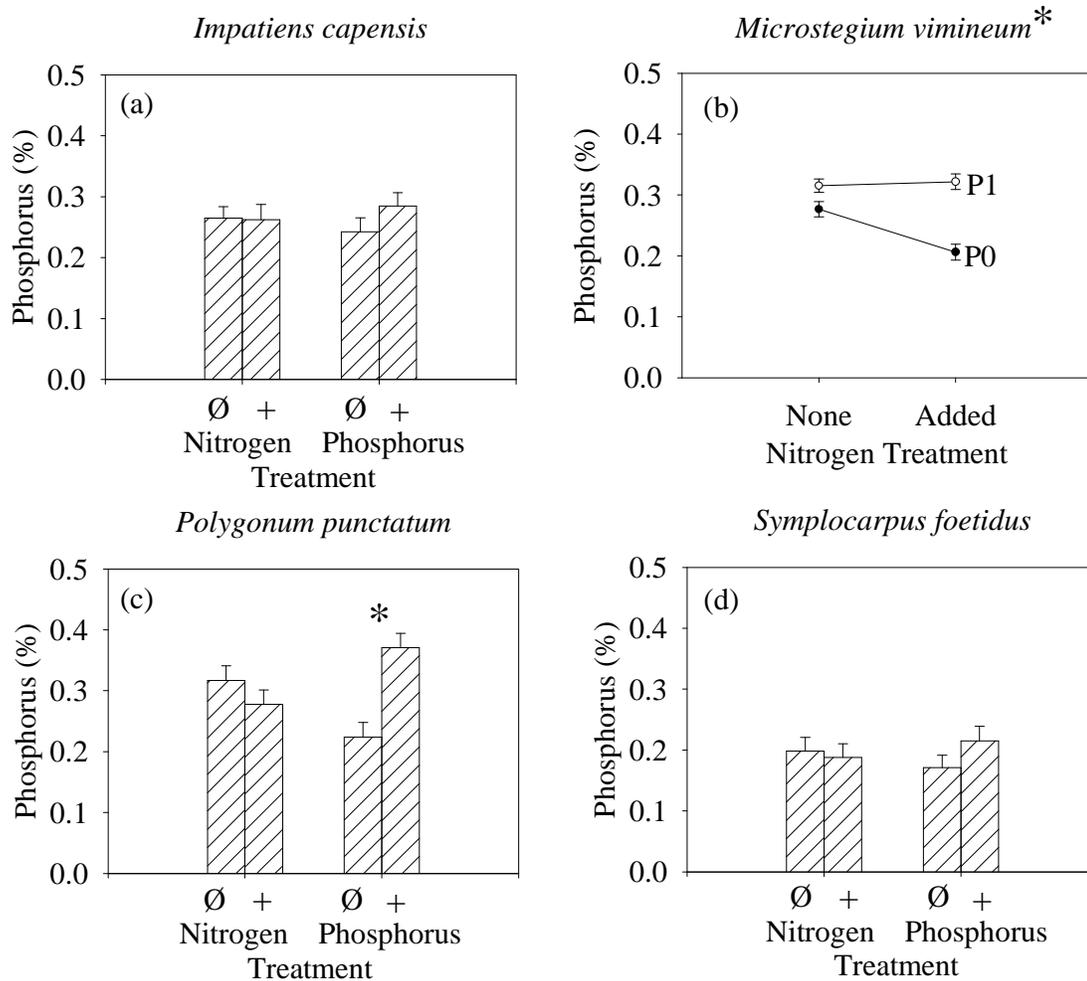
Figure 4-8. Nitrogen and phosphorus treatment effects on the phosphorus concentration in (a) Impatiens capensis, (b) Microstegium vimineum, (c) Polygonum punctatum, and (d) Symplocarpus foetidus. Values with asterisks (*) between them indicates a significant treatment effect for that nutrient at the 0.05 level. The nitrogen*phosphorus interaction is shown for Microstegium vimineum since it was found to be significant (P=0.0126). The addition of phosphorus significantly increased the concentration of phosphorus in Polygonum punctatum. Ø = no nutrient added; + = nutrient added.
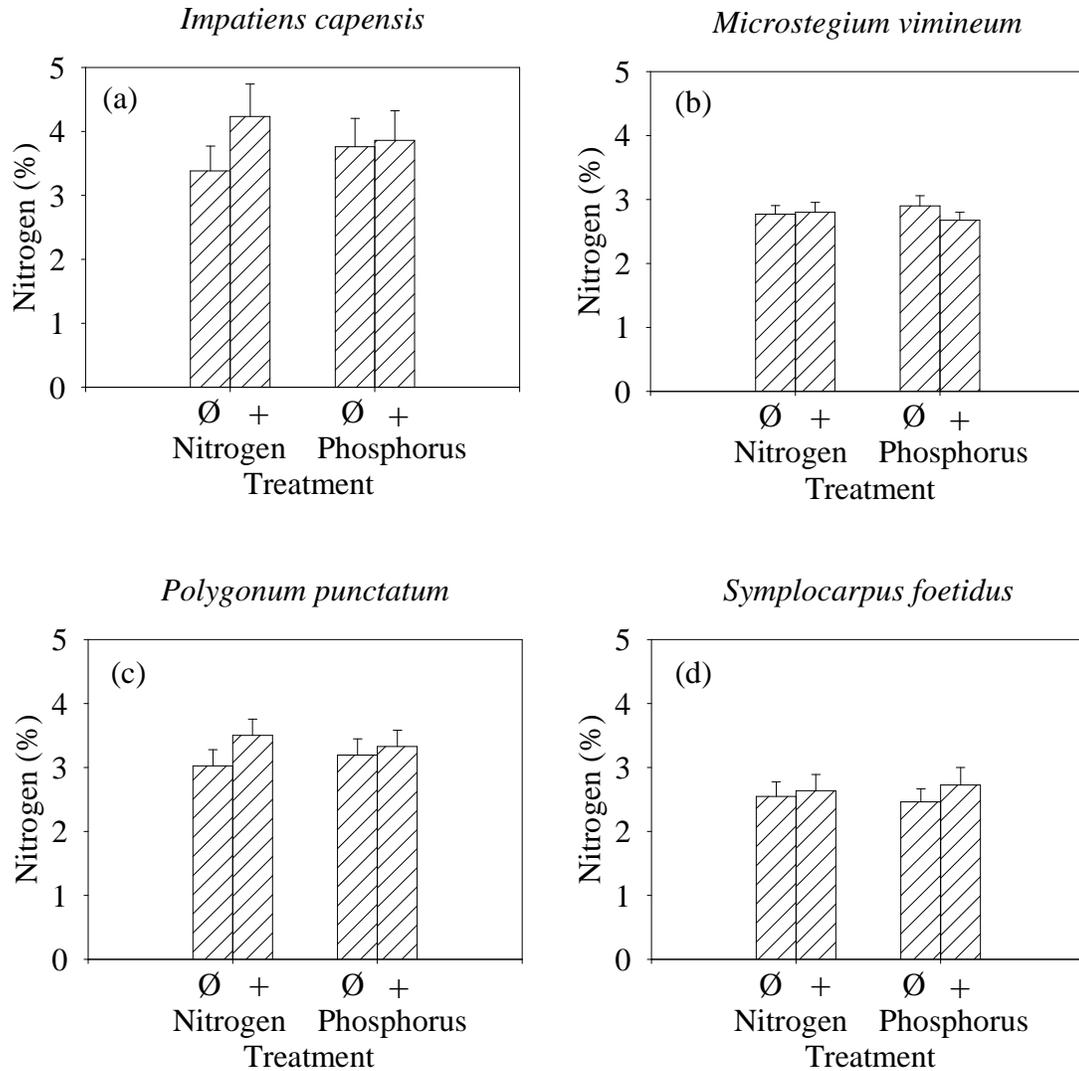
Figure 4-9. Nitrogen and phosphorus treatment effects on the nitrogen concentration in (a) Impatiens capensis, (b) Microstegium vimineum, (c) Polygonum punctatum, and (d) Symplocarpus foetidus. There were no significant treatment effects at the 0.05 level. Ø = no nutrient added; + = nutrient added.

no significant changes in carbon concentrations caused by the addition of nitrogen or phosphorus for any of the species studied (Figure 4-10a-d; Tables 4-3, 4-4, 4-5, and 4-6).

The N:P ratios increased for all species when nitrogen was added, but was significantly altered only for *Microstegium vimineum* and *Polygonum punctatum* (Figure 4-11a-d; Tables 4-3, 4-4, 4-5, and 4-6). On the other hand, the N:P ratios decreased for all species when phosphorus was added, but was significant in only the *Microstegium vimineum* and *Polygonum punctatum* biomass (Figure 4-11a-d; Tables 4-3, 4-4, 4-5, and 4-6). There were no significant trends due to the addition of nitrogen or phosphorus in the C:N ratios (Figure 4-12a-d; Tables 4-3, 4-4, 4-5, and 4-6). However, the C:N ratios of *Impatiens capensis, Polygonum punctatum,* and *Symplocarpus foetidus* did decrease somewhat when nitrogen was added (Tables 4-3, 4-5, and 4-6). The C:P ratio of all species except *Microstegium vimineum* was unaffected when nitrogen was added (Figure 4-13; Tables 4-3, 4-4, 4-5, and 4-6). When nitrogen was added to *Microstegium vimineum* the C:P ratio significantly increased (Figure 4-13b; Table 4-4). The C:P ratio of this species also significantly decreased when phosphorus was added (Figure 4-13b; Table 4-4). Although the other species did not have their C:P ratios significantly reduced by the addition of phosphorus, their ratios did decrease somewhat (Figure 4-13a,c,d; Tables 4-3, 4-5, and 4-6).

The biomass of *Impatiens capensis, Microstegium vimineum,* and *Symplocarpus foetidus* were not affected by the addition of nitrogen or phosphorus (Figure 4-14a,b,d; Tables 4-3, 4-5, and 4-6). However, the interaction of nitrogen and phosphorus did significantly affect the biomass of *Polygonum punctatum* (Figure 4-14c; Table 4-5). Since it is a rank-order interaction, meaning that the plot of the two treatment lines
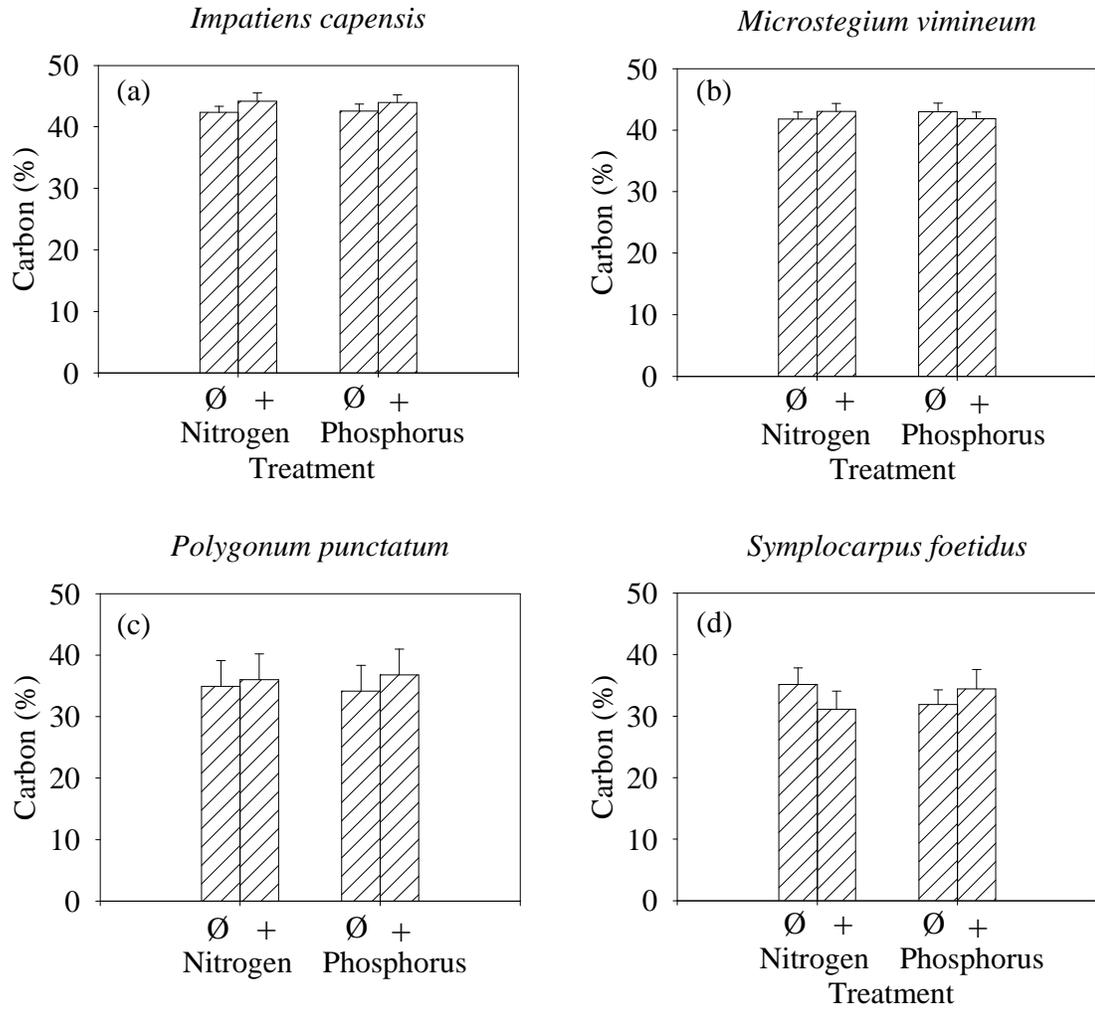
Figure 4-10. Nitrogen and phosphorus treatment effects on the carbon concentration in (a) Impatiens capensis, (b) Microstegium vimineum, (c) Polygonum punctatum, and (d) Symplocarpus foetidus. There were no significant treatment effects at the 0.05 level. Ø = no nutrient added; + = nutrient added.
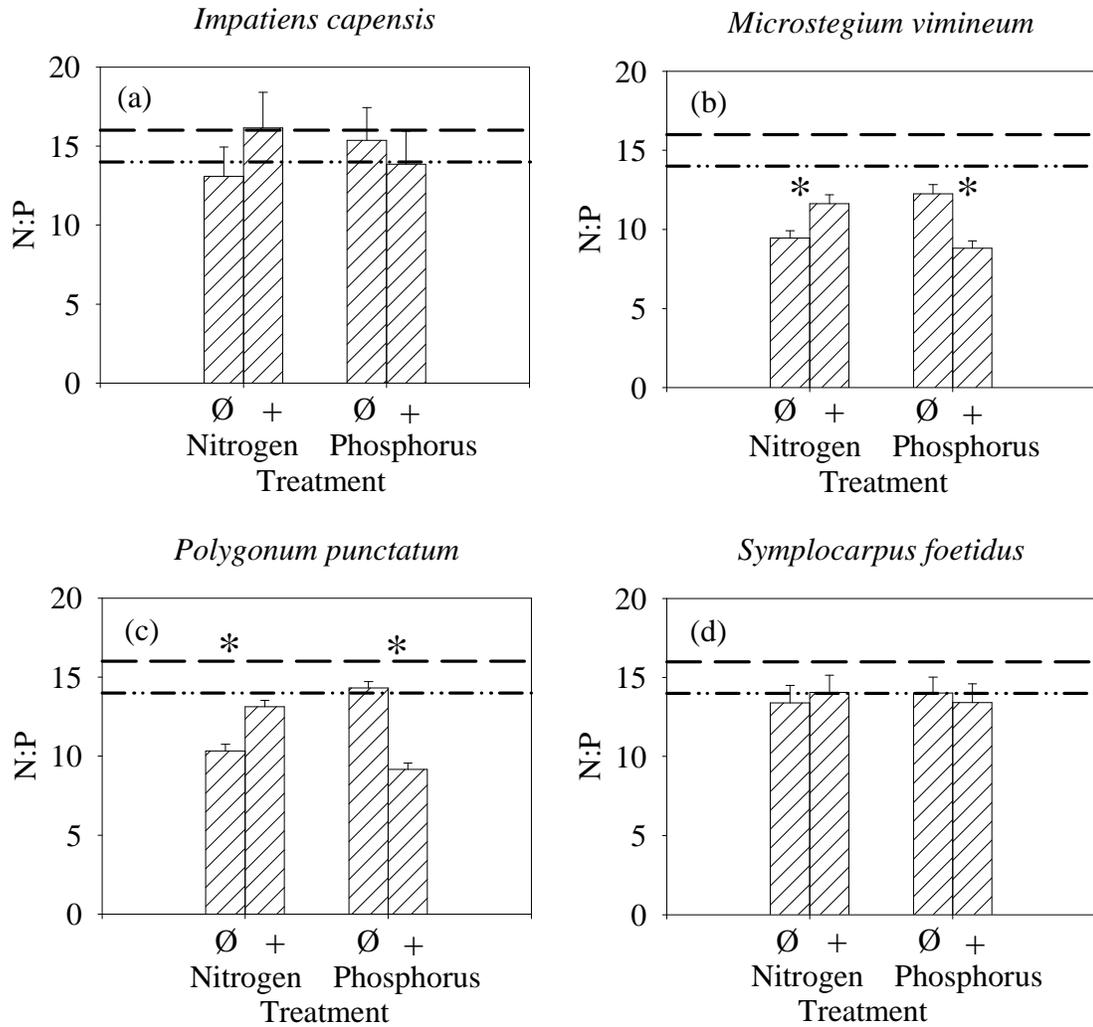
Figure 4-11. Nitrogen and phosphorus treatment effects on the N:P ratios in (a) Impatiens capensis, (b) Microstegium vimineum, (c) Polygonum punctatum, and (d) Symplocarpus foetidus. An asterisks (*) indicates a significant treatment effect for that nutrient at the 0.05 level. The horizontal lines in the N:P graph represent the cutoff values for nutrient limitation suggested by Koerselman and Mueleman (1996). Values above 16 represent phosphorus limitation while those below 14 represent nitrogen limitation. Values between 14 and 16 represent co-limitation by both nitrogen and phosphorus. Significant treatment effects due to nitrogen and phosphorus were found for Microstegium vimineum and Polygonum punctatum. Ø = no nutrient added; + = nutrient added.

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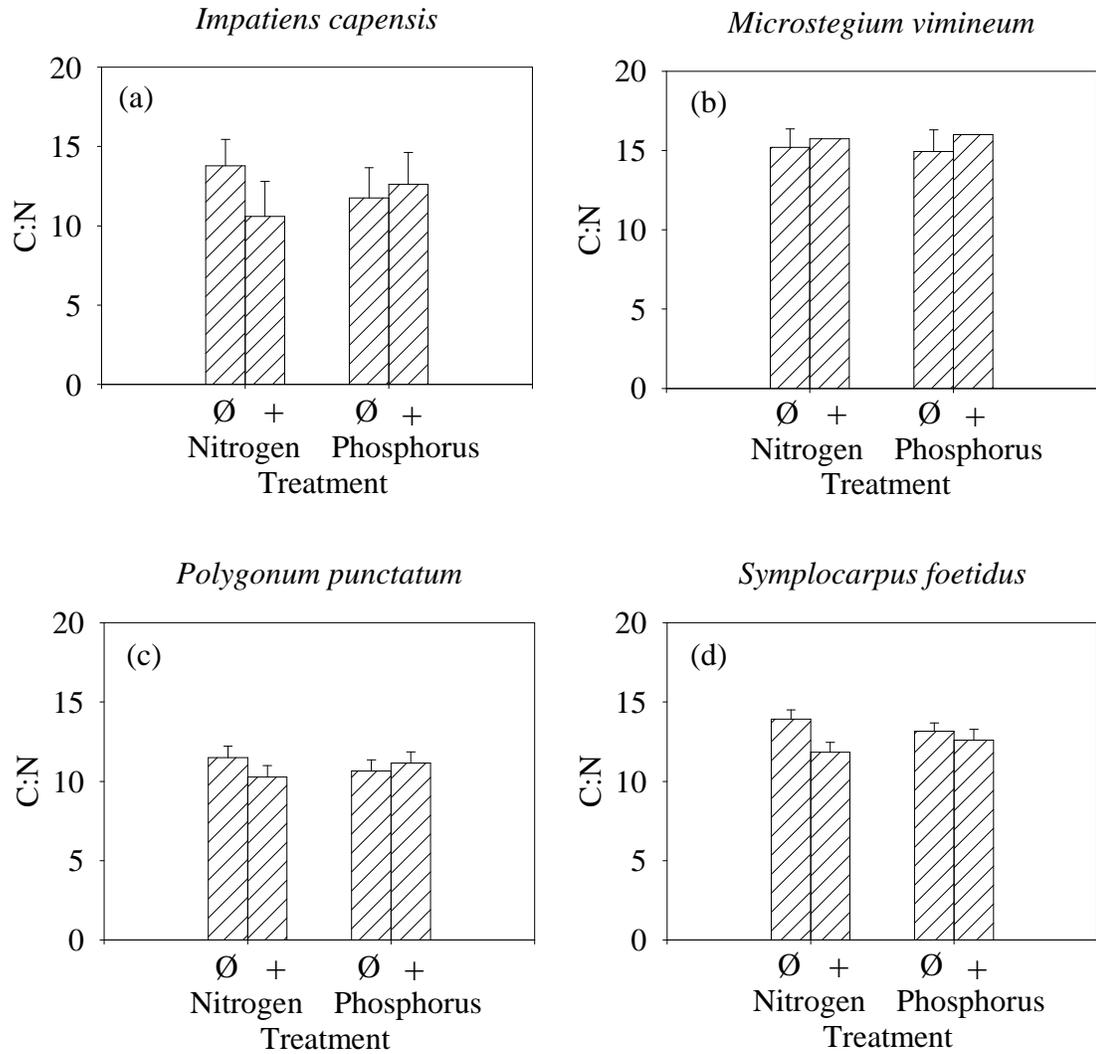
Figure 4-12. Nitrogen and phosphorus treatment effects on the C:N ratios in (a) Impatiens capensis, (b) Microstegium vimineum, (c) Polygonum punctatum, and (d) Symplocarpus foetidus. There were no significant treatment effects at the 0.05 level. Ø = no nutrient added; + = nutrient added.
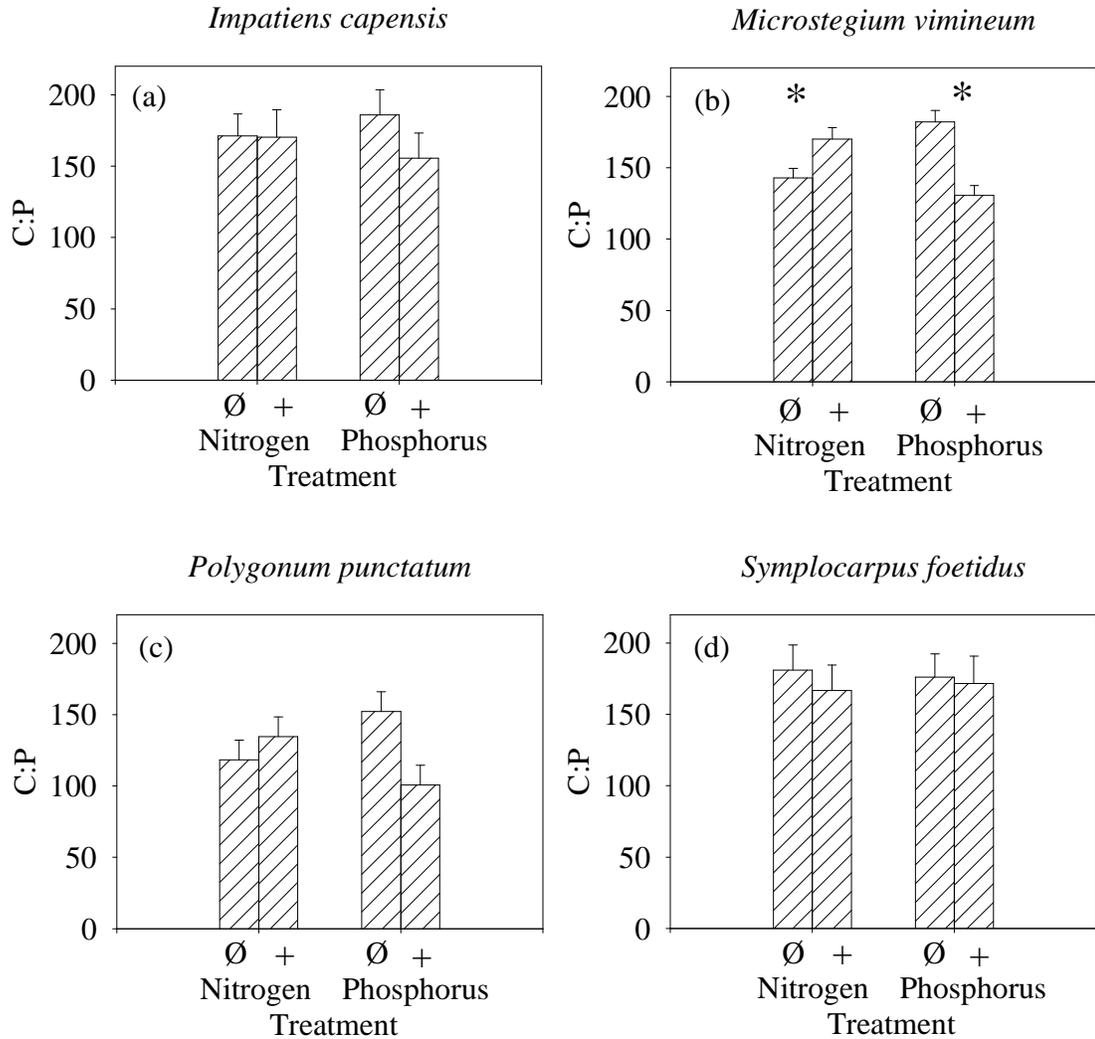
Figure 4-13. Nitrogen and phosphorus treatment effects on the C:P ratios in (a) Impatiens capensis, (b) Microstegium vimineum, (c) Polygonum punctatum, and (d) Symplocarpus foetidus. An asterisks (*) indicates a significant treatment effect for that nutrient at the 0.05 level. Signficant treatment effects at the 0.05 level were found for Microstegium vimineum. Ø = no nutrient added; + = nutrient added.
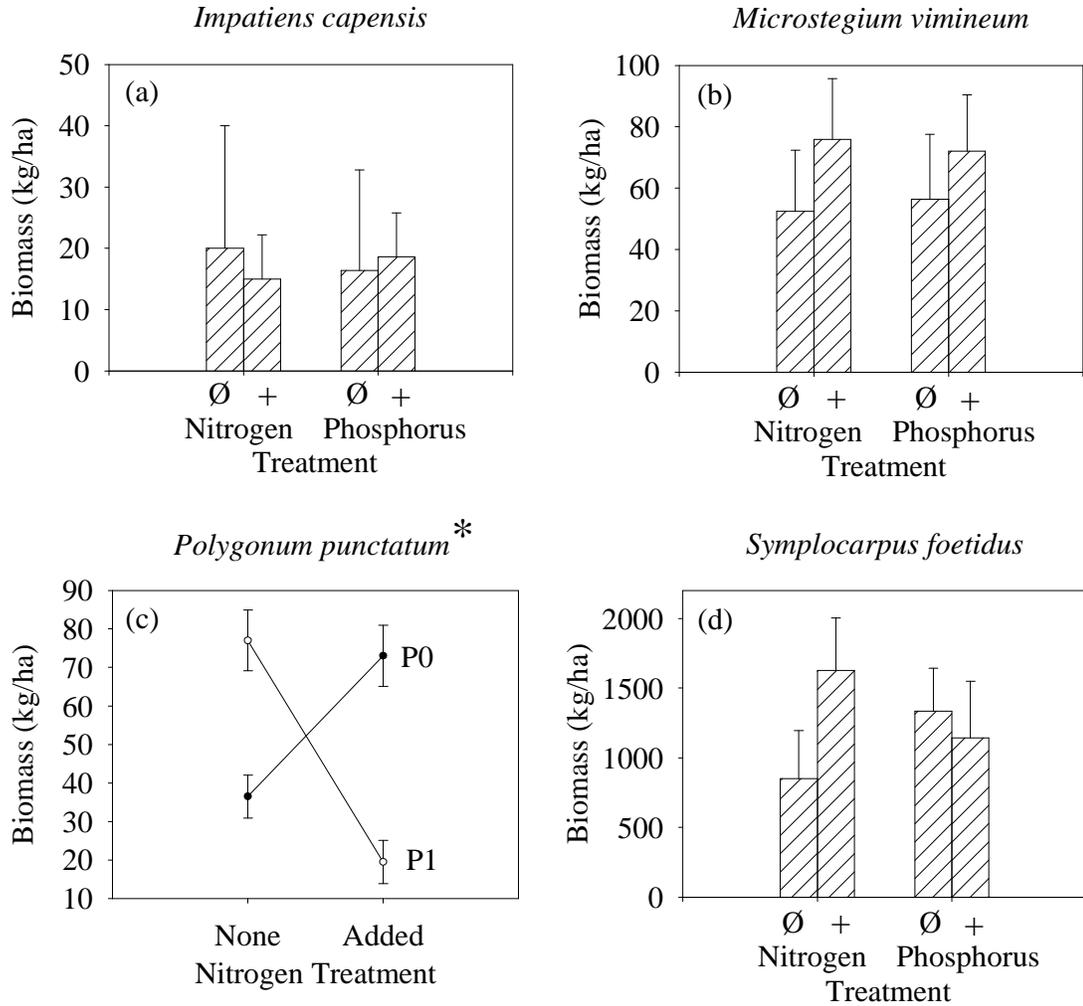
Figure 4-14. Nitrogen and phosphorus treatment effects on the biomass of (a) Impatiens capensis, (b) Microstegium vimineum, (c) Polygonum punctatum, and (d) Symplocarpus foetidus. Values with asterisks(*) above them indicate a significant treatment effect for that nutrient at the 0.05 level. Main effects are shown for Impatiens capensis, Microstegium vimineum, and Symplocarpus foetidus since the nitrogen*phosphorus interactions were insignificant. The nitrogen*phosphorus interaction is shown for Polygonum punctatum since it was found to be signficant. Ø = no nutrient added; + = nutrient added.

actually intersect, no broad statements about main effects can be made.

DISCUSSION

Differences in the N:P ratio and phosphorus NUE when considering all species combined may be attributed to significantly elevated concentrations of phosphorus in the biomass. The plants may have been experiencing luxury uptake of this nutrient. Since no change in biomass accompanied the increase in phosphorus uptake (indeed phosphorus application slightly decreased biomass, phosphorus is probably not the limiting nutrient limiting growth in this ecosystem. Nitrogen additions, however, slightly increased biomass suggesting that the plants may be limited by nitrogen.

Phosphorus concentrations were significantly increased by phosphorus fertilization, but neither the addition of nitrogen nor that of phosphorus created a significant change in nitrogen or carbon concentrations. However, the nitrogen was slightly higher when nitrogen was added. I had anticipated the concentration of nitrogen to increase with the addition of nitrogen and the concentration of phosphorus to increase with the addition of phosphorus. An increase in plant uptake relative to the supply of nutrients has been documented in other experiments as well (Hubbard *et al.* 1999).

I did not find significant changes in biomass due to the addition of nitrogen or phosphorus. Most fertilization experiments show that biomass is enhanced by the addition of the limiting nutrient (Schippers and Olff 2000; Güsewell and Verhoeven 2003). However, only 45 out of 121 plants studied by Güsewell, Koerselman, and Verhoeven (2003) increased in biomass when nitrogen or phosphorus was added. However, I only measured the aboveground biomass. Perhaps the belowground biomass rather than the aboveground was increased so that the plant could better attain the

nutrients. Change in biomass partitioning due to fertilization have been documented in previous studies (Claridge and Franklin 2002; Schippers and Olff 2000). Furthermore, Aerts (1999) found that plants often either allocate more biomass to their roots or increase their root length per unit root mass to overcome nutrient stresses.

There were no differences in percent cover or frequency due to nitrogen or phosphorus additions. Perhaps if I had fertilized the plants several times a difference in these two parameters would have been found. Also, I may have seen some differences if I had measured cover and frequency prior to fertilization and again after.

Nutrient loads revealed no significant changes due to nitrogen or phosphorus enrichment. This result makes sense when one considers the fact that no differences in biomass were detected.

There was no significant difference in nitrogen NUE when nitrogen or phosphorus was added. The phosphorus NUE was also unaffected by the addition of nitrogen. Yet it was significantly decreased when phosphorus was added. The phosphorus concentrations increased but did not produce a corresponding increase in biomass.

Moreover, neither the C:N ratio nor the C:P ratio was significantly affected when nitrogen or phosphorus was applied. In addition, the N:P ratio was not affected by the addition of nitrogen but it was significantly decreased when phosphorus was added since the phosphorus concentrations were elevated due to phosphorus fertilization. Once again this seems to suggest that overall the community is influenced more by phosphorus than by nitrogen.

Examining the results by species revealed some significant differences due to fertilization. Phosphorus did cause the phosphorus concentrations to significantly increase in *Microstegium vimineum* and *Polygonum punctatum*. Some suggest that if phosphorus is limited, then the phosphorus concentrations will increase in response to the added phosphorus (Tessier and Raynal 2003). Based on this assumption, I would conclude that phosphorus may actually be the limiting nutrient for these two species. However, the very definition of a limiting nutrient is that the addition of a nutrient or combination of nutrients limits plant growth. Thus, to simply say that phosphorus is limiting because it increased the uptake of the nutrient is not necessarily true especially since luxury uptake could be occurring.

Microstegium vimineum did slightly increase in biomass due to both nitrogen and phosphorus additions but since neither change is significant, it is difficult to determine which nutrient is limiting growth. Claridge and Franklin (2002) fertilized this same plant with all purpose (N-P-K 20-20-20) fertilizer and found that the biomass increased significantly with the addition of the fertilizer. The biomass of *Microstegium vimineum* increased slightly when I added nitrogen and phosphorus suggesting that this species may be limited by both nutrients. However, the N:P ratios were below 14, the cutoff value representing nitrogen limitation suggested by Koerselman and Meuleman (1996). Thus, I believe that this species is limited by nitrogen. This is especially important for *Microstegium vimineum* since this plant is an introduced species. The interaction of nitrogen and phosphorus was found to cause a significant difference in the biomass of *Polygonum punctatum*. However, since this interaction is rank-order the relationship of nitrogen and phosphorus to the biomass change cannot be distinguished. An increase in

plant biomass is often observed when the limiting nutrient is added. However, Güsewell, Koerselman, and Verhoeven (2003) found results that out of 121 plants studied, only 45 had an increase in biomass due to the addition of nitrogen or phosphorus. There were no differences in nitrogen or carbon concentrations in any of the species due to the addition of nitrogen or phosphorus.

Moreover element ratios were inspected so that species responses due to nitrogen or phosphorus could be further extrapolated. The N:P ratios of *Microstegium vimineum* and *Polygonum punctatum* were both significantly increased by the application of nitrogen. The N:P ratios of both these species were also decreased significantly when phosphorus was applied. The N:P ratios of the other species were not significantly influenced by nitrogen or phosphorus. C:N ratios are often used to determine if nitrogen is limited in quantity or readily available in ecosystems. It was anticipated that the C:N ratios of all species would decrease in the plots receiving nitrogen if this was the limiting nutrient. All of the species except *Microstegium vimineum* decreased somewhat when nitrogen was added. Thus, these results suggest that *Impatiens capensis, Polygonum punctatum,* and *Symplocarpus foetidus* were taking up nitrogen thereby decreasing the C:N ratio. Investigation of C:P ratios revealed that the addition of nitrogen was not expected to produce a change in the C:P ratios. However, the C:P ratio in *Microstegium vimineum* biomass was significantly increased when nitrogen was added. This species is extremely plastic (i.e. can adapt easily to changes in environmental conditions) (Claridge and Franklin 2002). Perhaps it produced more carbon when nitrogen was added due to an increase in photosynthesis causing an increase in the C:P ratio. Indeed, the carbon concentration in this species was slightly increased by the addition of nitrogen and this is

probably causing the C:P ratio to change. The C:P ratio in *Microstegium vimineum* was also significantly decreased by the addition of phosphorus since the phosphorus concentration increased significantly.

No differences were observed in percent cover or frequency by species when nitrogen or phosphorus was applied. Joyce (2001) studied the effects of nitrogen fertilization on the vegetation in a flood-meadow. He found a wide variety of results. Fertilization caused some grass species to increase in cover and some to be unaffected at all. However, the majority of species actually decreased in cover. Perhaps the plants respond slowly to added nutrients. I may have been able to detect significant differences if I continued to fertilize the plants for several growing seasons.

CONCLUSIONS

When looking at the effect of fertilization on the entire plant community, only phosphorus caused significant changes. The N:P ratio and phosphorus NUE of the entire plant community were significantly decreased when phosphorus was applied due to the significantly higher concentrations of phosphorus taken up by plant. *Microstegium vimineum* and *Polygonum punctatum* were the only species that experienced significant changes due to fertilization. Moreover, these shifts involved nitrogen and phosphorus only (i.e. P concentrations and N:P and C:P ratios). Thus, the addition of nutrients may affect the normal nutrient cycling with respect to these two species in particular.

More long-term fertilization experiments must be conducted to determine the whether forested riparian wetlands are limited by nitrogen, phosphorus, or a combination of the two nutrients. The effects of chronic nutrient additions on plant communities should be further investigated so that better management decisions can be made. By

studying these systems more thoroughly, others may be able to better predict the

consequences of agricultural development using computer modeling techniques.

Chapter 5: The Effects of Nutrient Enrichment on *Symplocarpus foetidus*

INTRODUCTION

Nonpoint source pollution caused by excess nutrients is a continuing problem in the United States (Carpenter et al. 1998). The level of nutrients entering waterways is continuing to increase as agricultural and suburban areas encroach upon natural ecosystems. Controlling nitrogen and phosphorus loading is particularly important since these two nutrients often lead to eutrophication.

The vegetation in these areas is especially important since plants actively take up nutrients. Silvan *et al.* (2004) found that the vegetation in a constructed wetland buffer was the primary factor mitigating nitrogen and, to a lesser degree, phosphorus entering from nearby forestry production.   Studying the direct effects of nitrogen and phosphorus on wetland species found in natural riparian wetland buffers is necessary so that the role of vegetation in nutrient retention can be determined and may ultimately lead to better management practices.

Symplocarpus foetidus (L.) Salisb. ex Nutt (skunk cabbage) is an herbaceous species that has unique characteristics. Its respiration produces heat up to a temperature of 72°F in the winter when air temperatures are low and is one of the first plants to emerge in the spring (Eastman 1995). Its large leaves make it easily identifiable taxonomically and the high surface area of the leaves can potentially allow the plant to harvest a large amount of sunlight for photosynthesis. Moreover, it has thick roots that extend deep into the soil profile (approximately equal to the height of the plant- usually about 0.5 m) making them difficult to remove from the soil (personal observation).

Indeed, the roots contract slightly acting to pull the plant deeper in the soil by several millimeters every year (Eastman 1995). These roots are even thought to persist for centuries (Eastman 1995). Because of their characteristic roots and large leaves, I thought that this plant had a high potential for attenuating large amounts of nutrients. Despite these unique traits, there is little, if any, literature investigating the nutrient limitation and nutrient uptake of this species. I conducted an experiment to examine the role of *Symplocarpus foetidus* in nutrient uptake by fertilizing these plants *in situ* in a forested riparian wetland not receiving direct additions of nutrients.

I anticipated that the addition of nitrogen and phosphorus would increase the nutrient concentrations in the leaves since more nitrogen would be available for uptake. I also thought biomass would increase when the limited nutrient(s) was added since this is a typical response shown by fertilized plants. It was also thought that the leaf area, plant width, and plant height would increase since an increase in biomass would probably mean an increase in the size of the leaf and plant in general. Total nitrogen and carbon and inorganic nitrogen forms (ammonium and nitrate) were also measured in the soil. Concentrations o all forms of nitrogen were anticipated to increase due to the application of nitrogen.

METHODS

In June of 2003, forty *Symplocarpus foetidus* plants were selected to be fertilized in a forested riparian wetland that was not exposed to high nutrient concentrations (reference site). Selection of the plants was based on similarities in general size, the number of leaves, and height. Ten plants were randomly assigned to each of the four

treatments, which included (1) the addition of nitrogen, (2) the addition of phosphorus, (3) addition of both nitrogen and phosphorus, and (4) no additions.

Nitrogen was added in the form of urea fertilizer (N-P-K (46-0-0 ratio))and phosphorus was added in the form of triple superphosphate (N-P-K (0-46-0)). One hundred and fifty grams of the assigned treatment was applied twice to account for any possible delays in the plants response to the additional nutrients. The first application occurred on June 19, 2003 (the middle to end of the 2003 leaf growth season for *Symplocarpus foetidus*) and the second was on April 6, 2004 (the beginning of the 2004 leaf growth season for *Symplocarpus foetidus*). The area chosen for this experiment was frequently flooded. The fertilizer was placed below the soil surface to a depth of 20 cm using a soil corer to prevent the loss of fertilizer during these flooding events. Holes were also dug under plants not receiving fertilizer to account for any possible influences. The holes were then sealed with soil.

I examined several parameters to determine if fertilization affected the general growth of the plants. The maximum plant height and width and the length of the youngest leaf, which was tagged on the first measurement date, were measured. Measurements of tagged leaves were taken on May 12, 2004 and again after two weeks. Leaf area indices (LAI) were also measured 20 cm from the base of each plant and 5 cm above the ground using a LI-COR 1 m quantum sensor with an LI-250 light meter (LI-COR, Lincoln, Nebraska). LAI is a dimensionless number representing the area of foliage per unit area of ground surface and is therefore related to plant biomass.

The plant leaves were harvested at the end of the 2004 growing season prior to senescence and placed in a 60°C oven until constant weight was achieved. The samples

were then weighed and homogenized using a Wiley Mill. Total phosphorus was

extracted using a modified Kjeldahl digestion method (5 mL of sulfuric acid was added to

each sample instead of the 3.5 mL called for in the Kjeldahl digestion protocol) and

analyzed on a Flow Injection Analyzer (Lachat QuikChem 8000, Milwaukee,

Wisconsin). Total nitrogen and carbon were also determined via dry combustion using a

LECO Tru-Spec CN Analyzer (St. Louis, Missouri).

Soil samples were also collected to ensure that the fertilizer had remained at the

site of application and available for plant use. Two samples were obtained underneath

each plant to a depth of 20 cm using a handheld soil extractor. The two samples were

then combined to make one sample per plant, air dried, and homogenized using a mortar

and pestle. A LECO Tru-Spec CN Analyzer (St. Louis, Missouri) was used to ascertain

the total nitrogen and carbon in the samples. Inorganic nitrogen in the form of

ammonium and nitrate was determined by first extracting the nitrogen using a 2 M KCl

solution (Mulvaney 1996) and then analyzing it on a Flow Injection Analyzer (Lachat

QuikChem 8000, Milwalkee, Wisconsin). C:N ratios were computed using the total

nitrogen and carbon data on a percentage basis. Soil phosphorus concentrations were not

determined mainly because I did not have the available time or the necessary resources to

complete the analyses. Phosphate determinations via the dilute acid extraction procedure

(also known as North Carolina or Mehlich-1 P test) require digesting the samples with a

strong acid in a specialized hood (Kuo 1996). The specialized hood required to safely

complete the analyses was unavailable for use until a later time because it was being used

by other researchers. Phosphorus is transported mainly through surface processes by

adhering to soil particles and traveling in surface runoff. Since I placed the fertilizer

below the soil surface, I felt that the loss due to possible flooding would be minimal.

RESULTS

The application of nitrogen produced a significant increase in both the leaf area index (Figure 5-1; Table 5-1) and concentrations of nitrogen in the leaves (Figure 5-2a; Table 5-1). The phosphorus treatment had a significantly positive effect on the phosphorus concentrations in the leaves (Figure 5-2b; Table 5-1). Nevertheless, leaf carbon concentrations were unaffected by either nitrogen or phosphorus (Figure 5-2c).

In addition, there were some differences found for element ratios when treatments were applied. The N:P ratios were significantly increased when nitrogen was added (Figure 5-3a; Table 5-1). However, both the N:P (Figure 5-3a; Table 5-1) and C:P ratios (Figure 5-3b; Table 5-1) were significantly decreased when phosphorus was added. The C:N ratios (Figure 5-3c; Table 5-1), on the other hand, were not altered by the nutrient treatments.

The interaction of nitrogen and phosphorus produced a significant effect on leaf width (Figure 5-4; Table 5-2). The application of nitrogen and phosphorus together caused the plant width to be significantly greater than when nitrogen alone was applied. Surprisingly, fertilization did not affect plant height (Figure 5-5a; Table 5-2), the length of the youngest leaf (Figure 5-5b; Table 5-2), or biomass (Figure 5-6; Table 5-2).

The total nitrogen in the soil was significantly increased when nitrogen was applied (Figure 5-7a; Table 5-3). The application of nitrogen also significantly increased both plant ammonium (Figure 5-7b; Table 5-3) and nitrate (Figure 5-7c; Table 5-3) in the
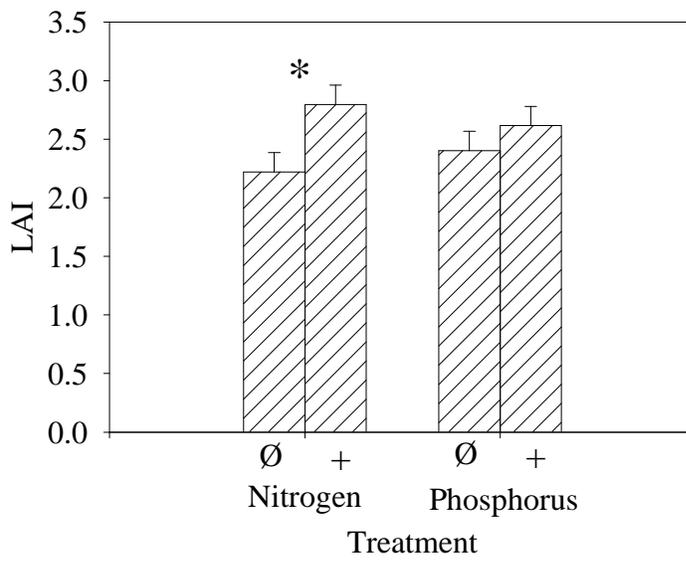
Figure 5-1. Nitrogen and phosphorus treatment effects on the leaf area indices (LAI) of
Symplocarpus foetidus. **Values with asterisks (*) above them indicate a significant treatment effect**
for that nutrient at the 0.05 level. The LAI increased significantly when nitrogen was applied, but
was unaffected by the application of phosphorus. Ø = no nutrient added; + = nutrient added.

Parameter	N	P	N*P
LAI	$6.10_{(1,36)}$*	$0.84_{(1,36)}$	$0.14_{(1,36)}$
Biomass	$0.01_{(1,36)}$	$3.25_{(1,36)}$	$0.00_{(1,36)}$
Nitrogen	$4.60_{(1,36)}$*	$0.56_{(1,36)}$	$0.08_{(1,36)}$
Phosphorus	$1.74_{(1,36)}$	$8.36_{(1,36)}$**	$0.55_{(1,36)}$
Carbon	$1.14_{(1,35)}$	$0.02_{(1,35)}$	$1.34_{(1,35)}$
N:P	$9.17_{(1,36)}$**	$21.49_{(1,36)}$***	$0.02_{(1,36)}$
C:P	$2.17_{(1,36)}$	$10.26_{(1,36)}$**	$0.06_{(1,36)}$
C:N	$3.36_{(1,36)}$	$0.95_{(1,36)}$	$0.02_{(1,36)}$

Table 5-1. Tests of fixed effects for LAI, biomass, nitrogen, phosphorus, and carbon concentrations, and N:P, C:P, and C:N ratios in *Symplocarpus foetidus*. Values are the F values and the numbers in parentheses are the numerator and denominator degrees of freedom, respectively. N is the nitrogen treatment effect; P is the phosphorus treatment effect; N*P is the nitrogen*phosphorus interaction effect. An asterisks (*) indicates significance at the following levels: * = <0.05; ** = < 0.01; * = < 0.0001.**
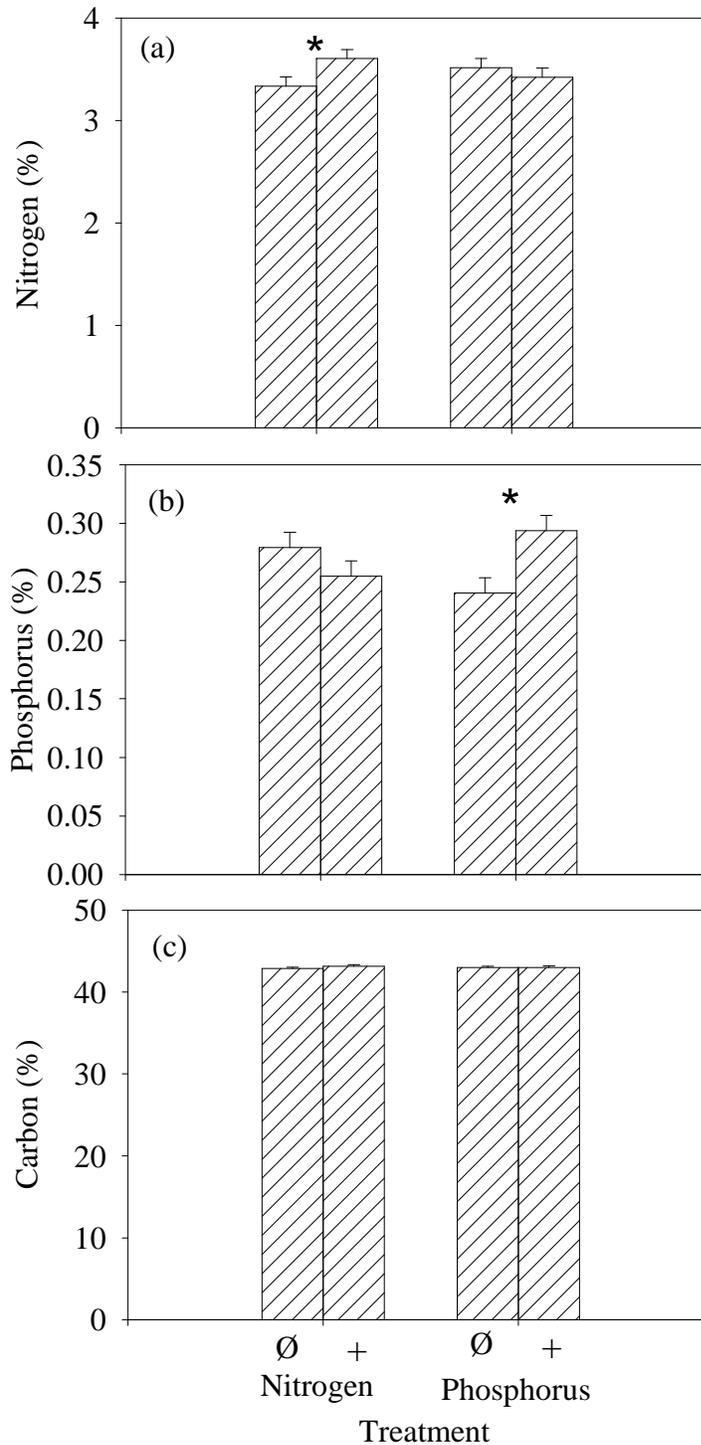
Figure 5-2. Nitrogen and phosphorus treatment effects on the concentrations of (a) nitrogen, (b) phosphorus, and (c) carbon in the leaves of *Symplocarpus foetidus*. Values with asterisks (*) between them indicate a significant treatment effect for that nutrient at the 0.05 level. The concentration of nitrogen increased significantly when nitrogen was applied and the concentration of phosphorus increased significantly when phosphorus was added. Carbon concentrations were unaffected by fertilization. Ø = no nutrient added; + = nutrient added.
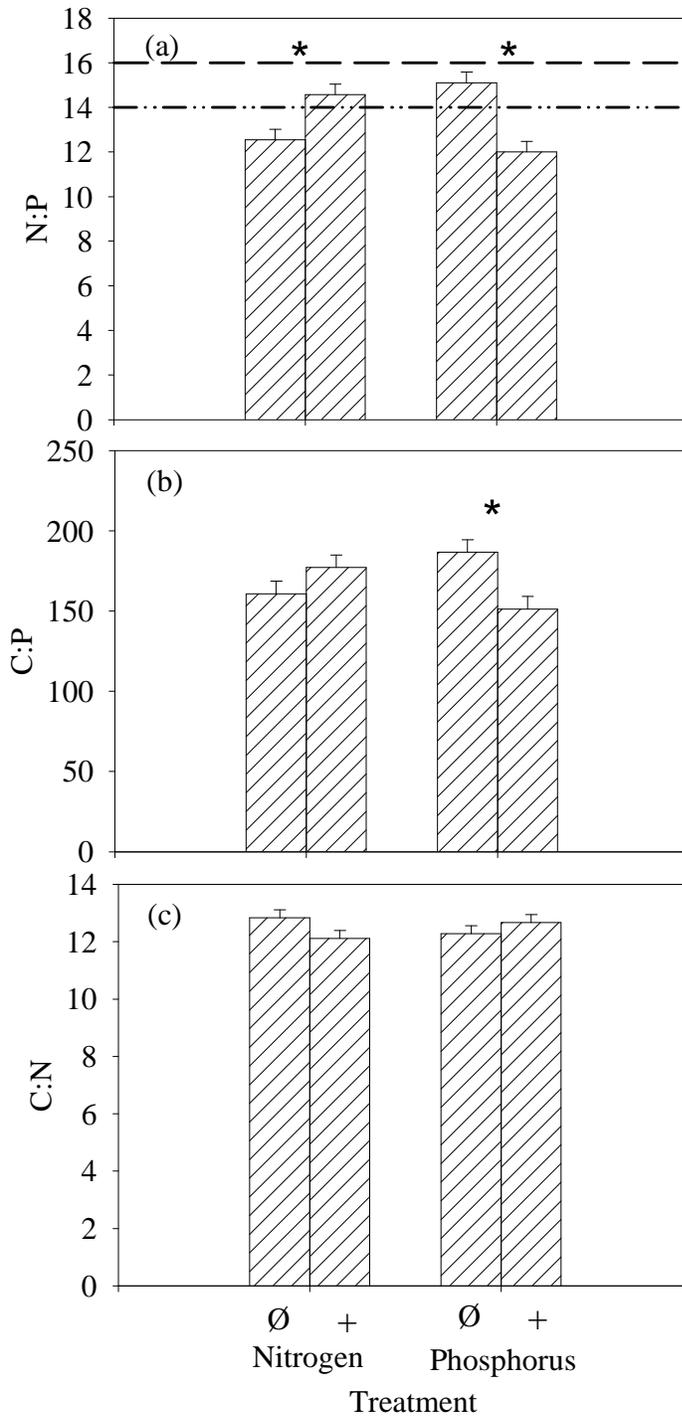
Figure 5-3. Nitrogen and phosphorus treatment effects on the (a) N:P, (b) C:P, and (c) C:N element ratios. Values with asterisks (*) above them indicate a significant treatment effect for that nutrient at the 0.05 level. The horizontal lines in the N:P graph represent the cutoff values for nutrient limitation suggested by Koerselman and Mueleman (1996). Values above 16 represent phosphorus limitation while those below 14 represent nitrogen limitation. Values between 14 and 16 represent co-limitation by both nitrogen and phosphorus. The application of nitrogen had no effect on the C:N or C:P ratios, but significantly increased the N:P ratio. The application of phosphorus had not effect on the C:N ratio, but significantly decreased the C:P and N:P ratios. Ø = no nutrient added; + = nutrient added.
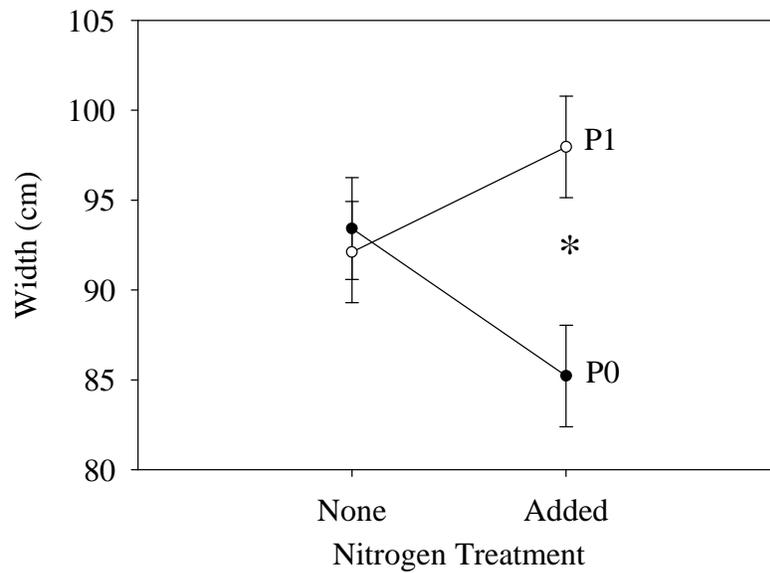
Figure 5-4. Nitrogen and phosphorus treatment effects on plant width. The interaction of nitrogen*phosphorus was found to be significant using the tests of fixed effects and so is presented here. Values with asterisks (*) above them indicate a significant treatment effect for that nutrient at the 0.05 level. The addition of both nitrogen and phosphorus caused the plant width to be significantly higher than when just nitrogen was added. Ø = no nutrient added; + = nutrient added.

Parameter	N	P	T	N*P	N*T	P*T	N*P*T
Length	$0.49_{(1,63)}$	$0.64_{(1,63)}$	$10.05_{(1,63)}$**	$0.58_{(1,63)}$	$0.10_{(1,63)}$	$0.01_{(1,63)}$	$0.179_{(1,63)}$
Width	$0.19_{(1,63)}$	$4.53_{(1,63)}$*	$4.28_{(1,63)}$*	$6.86_{(1,63)}$*	$0.03_{(1,63)}$	$0.00_{(1,63)}$	$0.26_{(1,63)}$
Height	$0.54_{(1,63)}$	$0.06_{(1,63)}$	$1.04_{(1,63)}$	$0.01_{(1,63)}$	$0.00_{(1,63)}$	$0.04_{(1,63)}$	$0.00_{(1,63)}$

Table 5-2. Tests of fixed effects results for length, width, and height of *Symplocarpus foetids*. Values are F values and the numbers in parentheses are the numerator and denominator degrees of freedom, respectively. N is the nitrogen treatment effect; P is the phosphorus treatment effect; T is the time effect; N*P is the nitrogen*phosphorus interaction effect; N*T is the nitrogen*time interaction; P*T is the phosphorus*time interaction; N*P*T is the nitrogen*phosphorus*time effect. An asterisks (*) indicates significance at the following levels: * = <0.05.
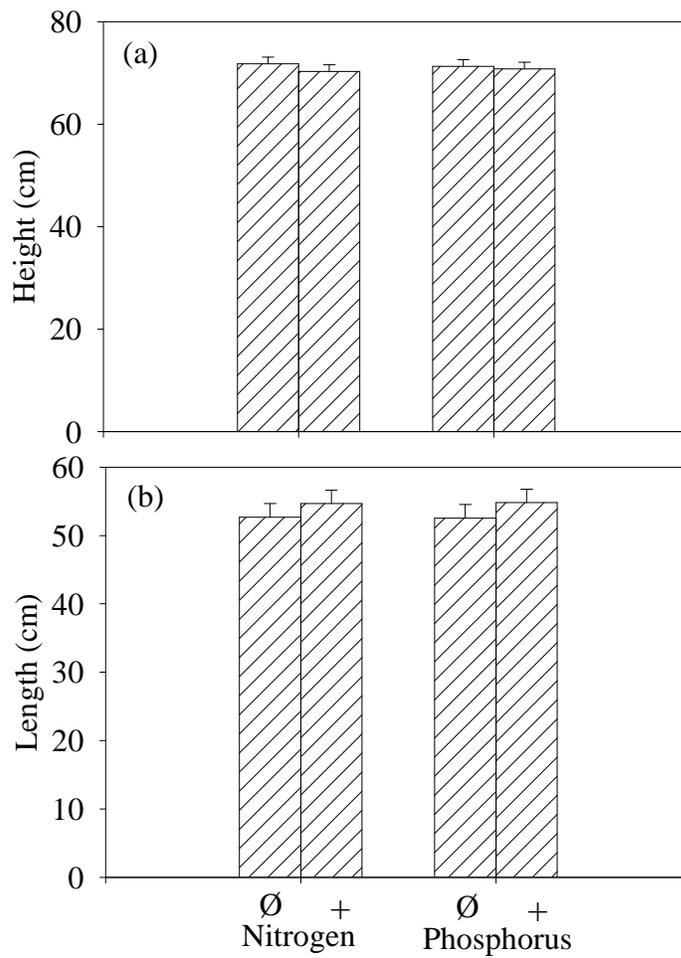
Figure 5-5. Nitrogen and phosphorus effects on (a) plant height and (b) the length of the youngest leaf. There were no significant treatment effects at the 0.05 level. Ø = no nutrient added; + = nutrient added.
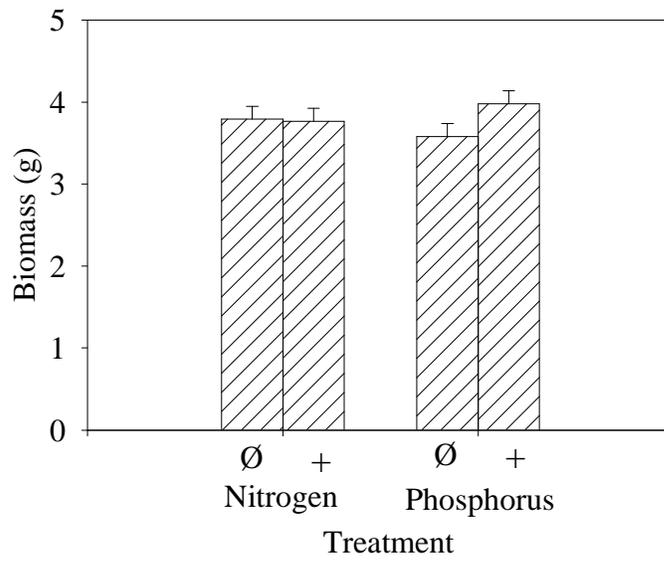
Figure 5-6. Nitrogen and phosphorus effects on plant biomass. There were no significant treatment effects at the 0.05 level. Ø = no nutrient added; + = nutrient added.
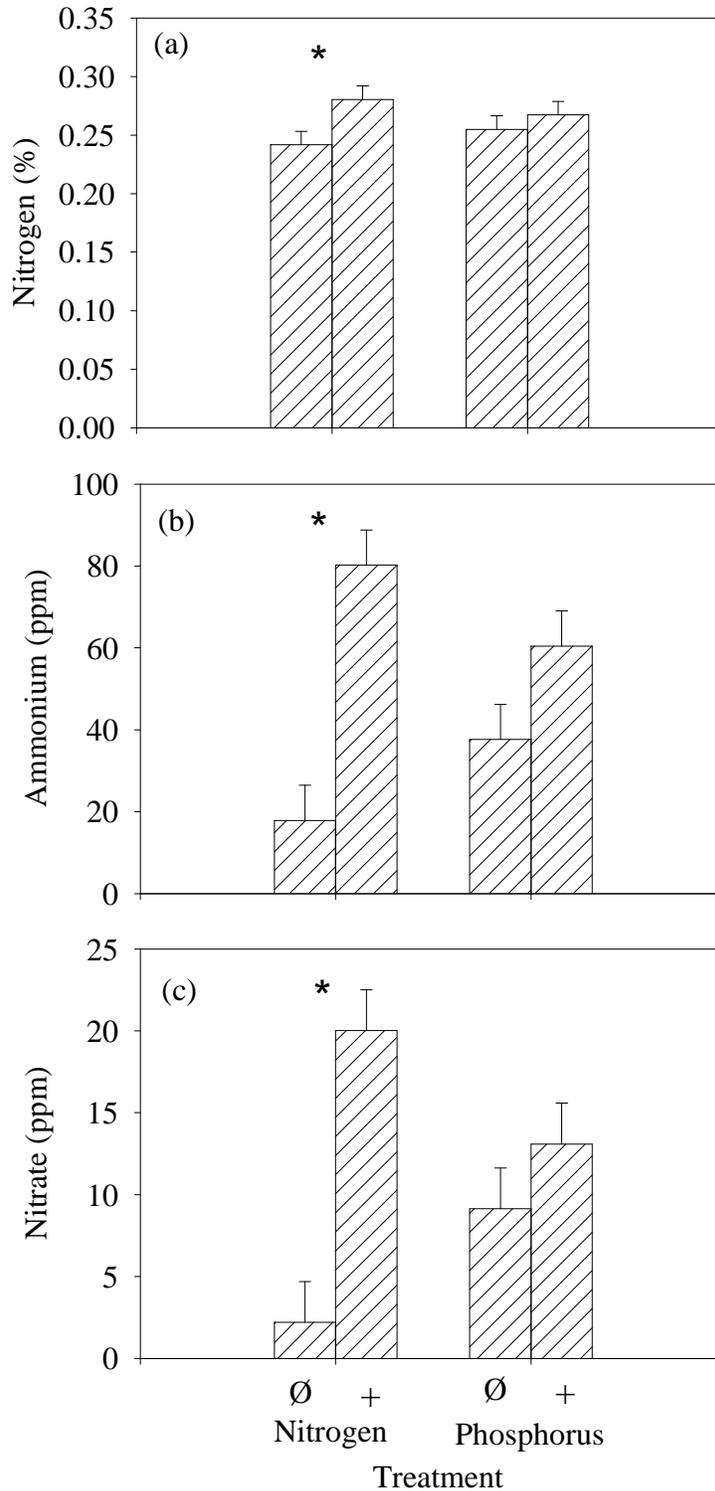
Figure 5-7. **Nitrogen and phosphorus treatment effects on (a) total nitrogen, (b) ammonium, and (c) nitrate concentrations in soil. Values with asterisks (*) between them indicate a significant treatment effect for that nutrient at the 0.05 level. The addition of nitrogen significantly increased the concentrations of all the nitrogen species. Ø = no nutrient added; + = nutrient added.**

Parameter	N	P	N*P
Total Nitrogen	$5.73_{(1,36)}$*	$0.59_{(1,36)}$	$0.27_{(1,36)}$
Nitrate	$25.57_{(1,36)}$**	$1.26_{(1,36)}$	$1.02_{(1,36)}$
Ammonium	$26.53_{(1,36)}$**	$3.54_{(1,36)}$	$2.73_{(1,36)}$
Total Carbon	$6.24_{(1,36)}$*	$0.02_{(1,36)}$	$0.02_{(1,36)}$
C:N	$41.40_{(1,36)}$**	$0.73_{(1,36)}$	$0.04_{(1,36)}$

Table 5-3. Tests of fixed effects results for soil. Values are F values and the numbers in parentheses are the numerator and denominator degrees of freedom, respectively. N is the nitrogen treatment effect; P is the phosphorus treatment effect; T is the time effect; N*P is the nitrogen*phosphorus interaction effect. An asterisks (*) indicates significance at the following levels: * = <0.05; ** = < 0.0001.

soil. However, carbon concentrations in the soil actually significantly decreased with the

addition of nitrogen (Figure 5-8; Table 5-3).
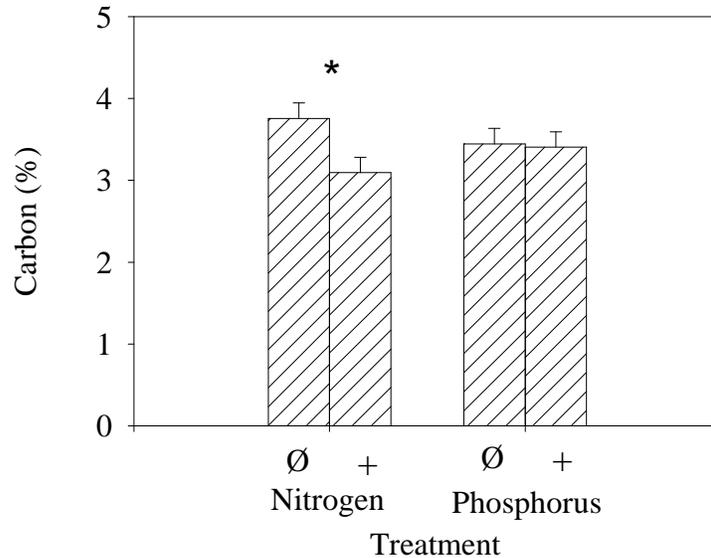
Figure 5-8. Nitrogen and phosphorus treatment effects on the carbon concentrations in soil. Values with asterisks (*) between them indicate a significant treatment effect for that nutrient at the 0.05 level. The addition of nitrogen significantly decreased carbon concentrations. Ø = no nutrient added; + = nutrient added.

Because of these changes in nutrient concentrations associated with added nitrogen, the

C:N ratio of the soil also significantly decreased due to the nitrogen treatment (Figure 5-

9; Table 5-3).

DISCUSSION

The leaf area of the plants increased due to the addition of nitrogen. This result

was of great interest since it is related to plant biomass. I also thought that plant height

and width along with the length of the youngest leaf would increase in response to the

added nutrients. Hubbard *et al.* (1999) studied the influence of swine lagoon effluent on

six wetland plant species. They found that some of the species, especially broad-leaved

plants, that were exposed to the swine effluent increased in leaf area, plant height, and
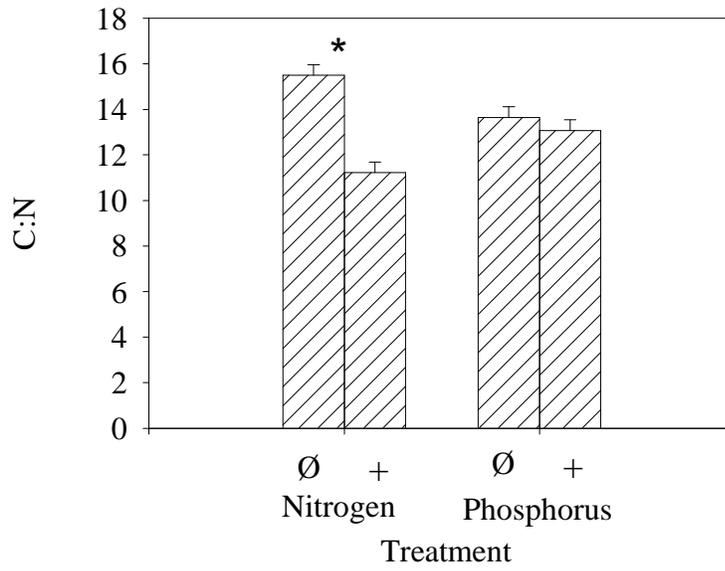
**Figure 5-9. Nitrogen and phosphorus treatment effects on the C:N ratios in soil.   Values with asterisks (*) above them indicate a significant treatment effect for that nutrient at the 0.05 level.   The addition of nitrogen significantly decreased C:N ratios.   Ø = no nutrient added; + = nutrient added.**

growth index, which is a calculation involving plant height and width (Hubbard et al. 1999). However, I may not have found a change in plant height and width or the length of the youngest leaf because *Symplocarpus* foetidus may respond slowly to the additions of nutrients. In addition, its large roots and corm may store large stocks of nutrients. This increase in leaf area has important implications since the plants are effectively increasing the area exposed to sunlight and able to photosynthesize.

In addition, the nitrogen and phosphorus in the leaves significantly increased in response to added nitrogen and phosphorus, respectively. This increase in plant uptake relative to the supply of nutrients has been documented in other experiments as well (Hubbard *et al.* 1999). I expected to find a difference in carbon concentrations due to nitrogen and/or phosphorus additions. I thought that whatever nutrient(s) was limiting plant growth would cause a change in plant biomass, which would result in an increase in carbon production and hence carbon concentrations. However, maybe the absolute carbon amounts increased instead of the concentrations.

Both the N:P and C:P ratios were anticipated to decrease when phosphorus was added. It was believed that higher phosphorus availability would cause uptake to increase and ultimately lead to higher concentrations in the leaves. These higher phosphorus concentrations then caused both the N:P and C:P ratios to decrease. N:P ratios were also influenced by nitrogen. Once nitrogen was added, the concentrations of nitrogen in the leaves increased, yielding larger N:P ratios. Koerselman and Meuleman (1996) proposed that N:P ratios greater than 16 indicate phosphorus limitation, those below 14 represent nitrogen limitation, and those values in between reflect co-limitation by nitrogen and phosphorus. The N:P ratio increased from 12.5 to 14.6 when nitrogen

was added. This result suggests that nitrogen addition resulted in a shift from nitrogen limitation to co-limitation by nitrogen and phosphorus. Similarly, when phosphorus was added, the plants shifted from 15.1 to 12.0 reflecting since they had higher concentrations of phosphorus in their leaves. No differences in C:N ratios were found but the addition of nitrogen did decrease it slightly.

Although I did not find any differences in biomass due to the treatments, other studies have concluded the same results. Only 45 out of 121 plants studied by Güsewell, Koerselman, and Verhoeven (2003) increased in biomass when nitrogen or phosphorus was added. Since *Symplocarpus foetidus* have high belowground biomass, perhaps this fraction of the biomass rather than the aboveground was increased so that the plant could better attain the nutrients. Changes in biomass partitioning due to fertilization have been documented in previous studies (Schippers and Olff 2000; Claridge and Franklin 2002).

The addition of nitrogen significantly increased all of the nitrogen components in the soil (total nitrogen, ammonium, and nitrate). This expected result indicates that the fertilizer was not washed out of the system. It has been shown that wetland soils often provide long-term storage of nutrients (Bischoff et al. 2001). Moreover, both nitrification and denitrification are important mechanisms of nutrient retention and removal in the soil (Mitsch and Gosselink 1993). Unexpectedly, the addition of nitrogen also impacted the concentrations of carbon – negatively. This result could be due to several things. Perhaps the added nitrogen actually caused some of the carbon in the form of organic matter to breakdown and thus was removed from the system. Also, it is known that *Symplocarpus foetidus* plants are thermoregulators (Seymour and Baylock 1999; Ito *et al.* 2003). For a period of roughly two weeks in the winter months these plants produce heat.

Maybe the addition of nitrogen acted to promote the stimulation of microbes involved in decomposition or somehow increased the length of time that the plant released heat. Since increased temperatures can promote the breakdown of organic matter through decomposition, perhaps it led to the removal of more organic matter. However, it could just be due to random error. The C:N ratios were also significantly decreased by the application of nitrogen. This change has important implications for soil microbes.

CONCLUSIONS

Increased nitrogen and phosphorus caused distinct changes in *Symplocarpus foetidus* to occur. The plants responded to these excess nutrients by taking up more nitrogen and phosphorus. By doing so their N:P and C:P ratios were also altered. The plant shifted more towards co-limitation by nitrogen and phosphorus when nitrogen was added but towards nitrogen limitation when phosphorus was added. This has important implications for the cycling of nutrients in the ecosystem as a whole since this ratio can affect soil processes and microbial communities. Determining the long-term impact of excess nutrients on wetland ecosystems will lead to better management practices and a greater understanding of the role of vegetation in nutrient retention within agricultural watersheds.

Chapter 6: Synthesis and Conclusions

The ability of forested riparian wetlands to mitigate nutrients and chemicals has been well documented. The role of vegetation in this buffering capacity, however, has been long overlooked as a major influencing factor. Indeed, the vegetation has long been considered to be less important than other factors, mainly soils, since it was thought to provide only temporary retention of nutrients through uptake. However, the impact of plants in the processing of excess nutrients can no longer be neglected. Results from the combination of observational studies and fertilization experiments that I conducted suggest that excess nutrients impacted the plant nutrient concentrations and consequently the plant component of nutrient cycling. In addition, the plant community had lower species diversity, richness, and evenness in the agriculturally influenced wetland when compared to the non-enriched wetland.

The N:P ratios of herbaceous vegetation at both systems were investigated to determine which nutrient (or combination of nutrients) was limiting. Observational studies revealed that the overall N:P ratios of both sites were less than 14 suggesting that the wetlands were limited by nitrogen. Drought appears to influence the N:P ratios in herbaceous vegetation. The N:P ratio was lower in 2002 (drought year) than 2003 ("normal" year) at both sites possibly indicating that during periods of drought, nitrogen limited sites may actually become further restricted by nitrogen. Moreover, the N:P ratio was significantly higher at the reference site than at the agricultural site in 2002 but was only slightly higher in 2003. The agricultural site used in my research was found to be largely influenced by groundwater. Therefore, during a drought there would be a lower

supply of nitrogen entering the system and available for plant use. While counterintuitive, this result could mean that drought may cause enriched nitrogen limited sites to have lower N:P ratios.

The direct application of nitrogen and phosphorus to vegetation found at the reference site provided results that may be important in plant conservation and management. The fertilization of all plants in vegetative plots produced significant results due to phosphorus additions when all species were examined together by treatment. The concentrations of phosphorus in the aboveground biomass were significantly increased while both the phosphorus NUE and the N:P ratios were significantly decreased when phosphorus was applied. These results could be due to luxury uptake of phosphorus. If phosphorus was limiting this plant community, one would expect the overall plant biomass to increase when this nutrient was added. Although not significant, aboveground biomass decreased slightly when phosphorus was applied and increased slightly with the addition of nitrogen. Thus, this supports the conclusion that the reference is nitrogen limited.

Symplocarpus foetidus may be a good candidate for use in created riparian wetlands used specifically for nutrient mitigation since those found at the agricultural site had significantly higher concentrations of nitrogen and phosphorus in 2003 than those at the reference site. In the *Symplocarpus foetidus* fertilization experiment, the plant uptake of nitrogen and phosphorus were both increased in leaf tissue with the addition of nitrogen and phosphorus, respectively. The LAI was also significantly increased by the addition of nitrogen indicating a change in growth pattern. In addition, the percent cover was significantly higher when only nitrogen was applied compared to when both nitrogen

and phosphorus were applied. Although not significant, the frequency of occurrence was also slightly higher in plots receiving nitrogen fertilizer. The cover and frequency estimates that were determined in the observational study for *Symplocarpus foetidus* further support these findings since both parameters were significantly higher at the agricultural site than at the reference site.

While both percent cover and frequency may be influenced by light, nitrogen, and water, it appears that the growth of this species is particularly influenced by water supply and nitrogen availability. In 2003, the frequency of *Symplocarpus foetidus* was almost 100% in all of the plots at the agricultural site, except those in the plot furthermost upstream. In this plot the frequency was 62.5%. This is important since this upstream area is extremely narrow in width and consequently has a high amount of light penetrating the canopy. If this species were limited mainly by light in this environment, I would expect it to have the highest cover and frequency in this plot. Furthermore, the surface runoff from the agricultural fields does not drain into this upper region nor does it receive a lot of groundwater input when compared to some of the other plots. Since nitrogen is transported via these two mechanisms, the nitrogen supply may be more limited in this upper region. This suggests that nitrogen and water availability may be the main factors limiting the growth of this species in this environment. Thus, if natural areas containing *Symplocarpus foetidus* are considered for use in mitigating nitrogen, it can be anticipated that this species would respond by increasing in growth, cover, and possibly frequency as long as the source of water was not limited.

The influence of excess nutrients on *Microstegium vimineum*, a non-native species, was of particular interest. By fertilizing plant communities I was able to

determine which nutrient was limiting growth and colonization of individual species within the ecosystem. N:P ratios indicated that this species was limited by nitrogen. A biomass response was not found, but did increase slightly with the addition of nitrogen and phosphorus. The N:P ratios in this species' aboveground biomass that were determined in the observational study further supported that nitrogen was the limiting nutrient. Therefore, *Microstegium vimineum* would most likely spread in habitats that were impacted by excess nitrogen.

Additionally, overall comparisons between the study sites in 2003 revealed that the concentrations of nitrogen, phosphorus, and carbon were significantly higher in tree leaf litter, but not in aboveground herbaceous vegetation, at the agricultural site. It has been documented by other researchers that the groundwater nitrate concentrations at the agricultural site increase with depth. Since tree roots grow deeper than the herbaceous plant roots, the trees take up groundwater that is deeper in depth and therefore has more nutrients, particularly nitrogen. Also, the trees may be allocating these resources to their aboveground biomass more so than their herbaceous counterparts. The herbaceous plants in contrast may be allocating the acquired nutrients to belowground biomass in an effort to further extract nutrients from the soil. The impact of nutrients on the belowground biomass of plants must be studied to verify this possibility. Further studies of herbaceous and tree litter must also be performed to determine if elevated nutrients in plant tissues alter soil nutrient processes like decomposition and denitrification rates. For instance, the elevated concentrations in the plant litter may actually promote soil mineralization rates since more nitrogen is available to stimulate the microbes involved in this process.

Furthermore, the NUEs of nitrogen and phosphorus were both significantly higher in the agricultural tree litter than the reference litter. This suggests that trees in nutrient-rich environments may be less efficient at retaining nutrients from leaves than those in non-impacted areas. High NUE could therefore be an adaptation to low nutrient availability. Further research investigating the relationship of elevated nutrients and NUEs is needed.

This research also indicates that trees found in nutrient enriched sites resorb nutrients better during the latter period of senescence. The leaf litter at the agricultural site had higher concentrations at the beginning of the 2003 fall season and significantly decreased during the senescent period whereas those concentrations in the reference litter remained relatively low and stable. By the end of the season the agricultural trees had resorbed the excess nutrients in the leaves leaving their concentrations approximately equal to those at the reference site. The excess nutrients in the agricultural litter may therefore be altering the nutrient cycling of litter.

The ability of herbaceous aboveground biomass to take up nutrients was found to vary by species. This result may be due to several factors including, but not limited to, the following: (1) certain plant species may be more adept at harvesting and utilizing nutrients; (2) species may differ in their allocation of nutrients to different parts of the plant (belowground versus aboveground) due to annual variation (i.e. climactic changes) or differing life histories (i.e. species primarily focused on competition versus those focused on reproduction); and (3) plants may take up more nutrients than necessary for plant sustenance (i.e. "luxury uptake"). Yearly variation in nutrient concentrations was also found. This reflects the importance of studying patterns of nutrient cycling over

several years. I suspect that the data from 2003 is more indicative of normal nutrient cycles within the plant communities rather than the data from 2002, which was a drought year.

Furthermore, the influence of excess nutrients on the plant community structure within forested riparian wetlands must be determined so that these buffers may be better managed to ensure the continued mitigation of pollutants. The agricultural wetland in this study was typified by lower overall species diversity, richness, and evenness when compared to the reference site. This implies that some plants within these nutrient enriched wetlands may be replaced by species that are better able to cope with excess nutrients.

Forested riparian wetlands provide ample opportunities for nutrient retention to occur. By continuing to study the relationships of plants, water, and soils in these ecosystems we will be better able to predict their responses to increased fertilization.   It is clear that the plant community structure and the plant nutrient dynamics are affected by elevated nutrients. Finding ways to preserve and maintain the vegetation in these in these areas is of utmost importance to maintaining the ecosystem services they provide. In conclusion, the main findings of this research were:

- Agricultural sites may have lower species diversity, richness, and evenness due to elevated nutrients.

- The trees found in agricultural settings may be larger due to excess nutrients.

- Fragmentation of wetlands in agricultural settings may reduce the total litter input of these systems.

- Tree leaf litter at agricultural sites has elevated nutrient concentrations.

- Nutrient loading may not be higher at agricultural sites due to lower litter input caused by fragmentation.

- It appears that both communities are nitrogen limited.

- Elevated nutrient concentrations in deep groundwater at agricultural sites may result in higher concentrations in tree leaf litter but not in herbaceous vegetation.

- Trees in nutrient-rich environments may retain a smaller proportion of nutrients from leaves than those in non-impacted areas (or that there is an upper limit to resorption rate).

- *Symplocarpus foetidus* may be nitrogen-limited in forested riparian wetlands.

Appendix A

Table A-1. List of the scientific and common names, species codes, families, and wetland indicator status for herbaceous plants studied at both sites.

(Page 1 of 3)

Scientific Name	Common Name	Species Code	Family	Wetland Indicator Status
Apios americana	Groundnut	APAM	Fabaceae (Pea family)	FACW
Arisaema triphyllum (L.) Schott	Jack in the pulpit	ARTR	Araceae (Arum family)	FACW-
Boehmeria cylindrica(L.) Sw.	Smallspike false nettle	BOCY	Urticaceae (Nettle family)	FACW+
Carex intumescens Rudge	Greater bladder sedge	CAIN12	Cyperaceae (Sedge family)	FACW+
Carex lurida Wahlenb.	Shallow sedge	CALU5	Cyperaceae (Sedge family)	OBL
Cinna arundinacea L.	Sweet woodreed	CIAR2	Poaceae (Grass family)	FACW+
Claytonia virginica	Virginia spring beauty	CLVI	Portulacaceae (Purslane family)	FACU
Dioscorea villosa L.	Wild yam	DIVI	Dioscoreaceae (Yam family)	FAC+
Duchesnea indica (Andr.) Focke	Indian strawberry	DUIN	Rosaceae (Rose family)	FACU-
Fragaria virginiana Duchesne	Virginia strawberry	FRVI	Rosaceae (Rose family)	FACU

(Continued)

Scientific Name	Common Name	Species Code	Family	Wetland Indicator Status
Impatiens capensis Meerb.	Jewelweed	IMCA	Balsaminaceae (Touch-me-not family)	FACW
Juncus effusus L.	Common rush	JUEF	Juncaceae (Rush Family)	FACW+
Leersia oryzoides (L.) Sw.	Rice cutgrass	LEOR	Poaceae (Grass family)	OBL
Microstegium vimineum (Trin.) A. Capus	Nepalese browntop	MIVI	Poaceae (Grass family)	FAC
Onoclea sensibilis L.	Sensitive fern	ONSE	Dryopteridaceae (Wood fern family)	FACW
Osmunda cinnamomea L.	Cinnamon fern	OSCI	Osmundaceae (Royal fern family)	FACW
Panicum clandestinum (L.) Gould	Deer-tongue grass	PACL	Poaceae (Grass family)	FAC+
Panicum dichotomum L.	Fall panicgrass	PADI	Poaceae (Grass family)	FACW-
Parthenocissus quinquefolia (L.)	Virginia creeper	PAQU	Vitaceae (Grape family)	FACU
Panicum virgatum L,	Switchgrass	PAVI2	Poaceae (Grass family)	FAC
Peltandra virginica (L.) Schott	Green arrow arum	PEVI	Araceae (Arum family)	OBL
Pilea pumila (L.) Gray	Canadian clearweed	PIPU	Urticaceae (Nettle family)	FACW
Polygonum arifolium L.	Halberdleaf tearthumb	POAR	Polygonaceae (Buckwheat family)	OBL
Polygonum persicaria L.	Spotted ladysthumb	POPE	Polygonaceae (Buckwheat family)	FACW
Polygonum punctatum Ell.	Dotted smartweed	POPU	Polygonaceae (Buckwheat family)	OBL

(Continued)

Scientific Name	Common Name	Species Code	Family	Wetland Indicator Status
Polygonum sagittatum L.	Arrowleaf tearthumb	POSA	Polygonaceae (Buckwheat family)	OBL
Sagittaria latifolia Willd.	Broadleaf arrowhead	SALA2	Alismataceae (Water-plantain family)	OBL
Scutellaria lateriflora L.	Blue skullcap	SCLA	Lamiaceae (Mint family)	FACW+
Schoenoplectus tabernaemontani (K.C. Gmel.) Palla	Softstem bulrush	SCTA2	Cyperaceae (Sedge family)	OBL
Smilax rotundifolia L.	Roundleaf greenbrier	SMRO	Smilacaceae (Catbrier family)	FAC
Symplocarpus foetidus (L.) Salisb. ex Nutt.	Skunk cabbage	SYFO	Araceae (Arum family)	OBL

Table A-2. List of the scientific and common names, species codes, families, and wetland indicator status for woody plants studied at both sites.

Scientific Name	Common Name	Species Code	Family	Wetland Indicator Status
Acer rubrum L.	Red maple	ACRU	Aceraceae (Maple family)	FAC
Betula nigra L.	River birch	BENI	Betulaceae (Birch family)	FACW
Ilex opaca Ait.	American holly	ILOP	Aquifoliaceae (Holly family)	FACU+
Ilex verticillata (L.) Gray	Common winterberry	ILVE	Aquifoliaceae (Holly family)	FACW+
Liquidambar styraciflua L.	Sweetgum	LIST	Hamamelidaceae (Witch-hazel family)	FAC
Lyonia ligustrina (L.) DC.	Maleberry	LYLI	Ericaceae (Heath family)	FACW
Nyssa sylvatica Marsh	Blackgum	NYSY	Nyssaceae (Sour gum family)	FAC
Quercus alba L.	White oak	QUAL	Fagaceae (Beech family)	FACU-
Quercus bicolor Willd.	Swamp white oak	QUBI	Fagaceae (Beech family)	FACW+
Quercus rubra L.	Red oak	QURU	Fagaceae (Beech family)	FACU-

Appendix B

SAS Programs

Example of mean calculations and t-tests used throughout Chapters 2, 3, and 4

DM 'LOG;CLEAR;OUTPUT;CLEAR';

OPTIONS LS=80 PS=75 PAGENO=1;

data soil;

input Plot$ Site$ no2no3ppm   nh3ppm;

datalines;

<Insert data here>;

proc sort data=soil;

by param site;

quit;

proc ttest data=soil;

class site;

var mmean;

by param;

quit;

proc means data=soil n mean stderr;

by param site;

var mmean;

output out=soilmeans mean=soilmean stderr=soilse;

quit;

```
proc print data=soilmeans;

quit;
```

Example of Tukey-Kramer Procedure used in Chapter 3

DM 'LOG;CLEAR;OUTPUT;CLEAR';

OPTIONS LS=90 PS=100 PAGENO=1;

data litter;

input site$ plot$ date$ Species$ Litterg day$ Npct;

classday=day;

datalines;

<insert data here>;

TITLE1 'Npct';

title2 'Full model';

proc mixed data=litter;

class site species;

model Npct = site species site*species/ddfm=kr outp=resids;

lsmeans site species site*species/ adjust=tukey diff=all cl;

ods output lsmeans=lsmean1;

ods listing exclude diffs; ods output diffs=diff1;

ods output tests3=stat1;

quit;

proc sort data=litter;

by site;

quit;

title2 'by site';

proc mixed data=litter;

```
class site species;

model Npct = site species site*species/ddfm=kr outp=resids;

by site;

lsmeans species/pdiff adjust=tukey diff=all cl;

ods output lsmeans=lsmean1;

ods listing exclude diffs; ods output diffs=diff1;

ods listing exclude lsmeans;

ods output tests3=stat1;

%include 'a:pdmix800.sas';

%pdmix800(diff1,lsmean1,alpha=.05,sort=yes);

quit;
```

Example of repeated measures analysis used in Chapter 3 for determination of

concentration changes in tree leaf litter during the period of senescence

```
DM 'LOG;CLEAR;OUTPUT;CLEAR';

OPTIONS LS=80 PS=75 PAGENO=1;

data litter;

input site$    plot$   Date$  Nkgperha Litterg day;

classday=day;

datalines;

<insert data here>;

proc mixed data=litter;

class site day plot;

model Nkgperha = site day site*day /ddfm=satterth outp=resids;

random plot;

lsmeans site|day/adjust=tukey diff=all cl slice=day;

ods output lsmeans=lsmean1;

ods listing exclude diffs; ods output diffs=diff1;

ods output tests3=stat1;

quit;

proc plot data=resids vpercent=50;

plot resid*pred/vref=0;

quit;

data resids;

set resids;
```

```
aresid=ABS(resid);

run;

proc corr spearman data=resids;

var aresid pred;

quit;

proc univariate data=resids plot normal;

var resid;

quit;

proc print data=lsmean1;

quit;

proc print data=diff1;

quit;

proc print data=stat1;

quit;
```

Example of PROC MIXED procedure used to analyze the factorials in the

fertilization experiments in Chapters 4 and 5;

```
DM 'LOG; CLEAR; OUTPUT; CLEAR';

OPTIONS LS=90 PS=100 PAGENO=1;

data syfofert;

INPUT Ntrt$ Ptrt$ rep$ N C bio;

DATALINES;

<insert data here>;

proc mixed data=syfofert;

class Ntrt Ptrt rep;

model N = Ntrt Ptrt Ntrt*Ptrt/ddfm=satterth outp=resids;

lsmeans Ntrt Ptrt Ntrt*Ptrt/ adjust=tukey diff=all cl;

ods output lsmeans=lsmean1;

ods listing exclude diffs; ods output diffs=diff1;

ods output tests3=stat1;

quit;

proc plot data=resids vpercent=50;

plot resid*pred/vref=0;

quit;

data resids;

set resids;

aresid=ABS(resid);

run;
```

```
proc corr spearman data=resids;

var aresid pred;

quit;

proc univariate data=resids plot normal;

var resid;

quit;

proc print data=lsmean1;

quit;

proc print data=diff1;

quit;

proc print data=stat1;

quit;
```

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