

# CARBON BUDGET OF AN EASTERN NORTH CAROLINA POCOSIN FOLLOWING NUTRIENT GRADIENT MANIPULATION

By

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Wetlands store ~25-30% of the earth's soil pool of carbon. Seventeen percent of the US' wetlands are found in North Carolina (NC) where 70% of NC's freshwater wetlands are pocosin peatlands. Pocosins are known for thick peat layers that store substantial amounts of carbon and other nutrients, particularly nitrogen. Since the 1920s, 50% of NC's pocosins have been degraded for agricultural purposes and almost all of NC's pocosins have been altered at least once in some way. This research was carried out in a pocosin located on East Carolina University's West Research Campus (ECU-WRC) that drains into the Neuse and Tar Rivers, contributing to downstream river and estuary health by retaining large amounts of nutrients. The aim of this research was to determine how these freshwater ombrotrophic bogs budget and store carbon when the availability of phosphorus, the limiting nutrient, increases across a P-fertility gradient. CO<sub>2</sub> flux calculations were made using static greenhouse gas chambers and used as indicators of net ecosystem metabolism. Above- and belowground biomass were also collected to determine biomass C allocation. Soil samples were taken prior to the beginning of the experiment and then at 6 and 12 months afterwards to examine changes in soil nutrient concentrations. Changes in above- and belowground biomass allocation, C allocation in roots and shoots, CO<sub>2</sub> flux between treatments, and soil

nutrient stoichiometric ratios were compared to determine how increasing P availability in the soil affected the wetland's C budget and soil chemistry. Results show that increasing the limiting nutrient in pocosins initially decreases CO<sub>2</sub> flux, but only at low amounts of P applied. Additionally, results from biomass and soil analyses indicate that while P-fertility manipulations had local effects, there was a strong seasonal effect that the experiment did not cancel out. Demonstrating changes in carbon budgeting within a pocosin following nutrient application can provide insight into the fate of degraded peatlands and their potential influence on downstream freshwater resources, such as the ones that occur along the southeastern Coastal Plain of the U.S.



CARBON BUDGET OF AN EASTERN NORTH CAROLINA POCOSIN FOLLOWING  
NUTRIENT GRADIENT MANIPULATION

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the Faculty of the Department of Biology

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Master of Science in Biology

by

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## Chapter 1: Introduction

This study aimed to understand carbon movement and storage in a P-deficient eastern North Carolina (NC) pocosin under increased nutrient availability. Pocosins, Algonquin for “swamp-on-a-hill,” are freshwater ombrotrophic bogs characteristic to the southeastern United States (US), with the largest recorded expanse in NC (Richardson 2003). Several studies have been conducted using nitrogen fertility gradients in wetlands where N is limiting (e.g. Pastor et al. 1984, Pastore et al. 2015, Birk and Vitousek 1986), but similar studies assessing P-fertility in P-limited wetlands are rare (Walbridge 1991; Richardson 2012). Peatlands are good ecosystem models for examining nutrient controls over C cycling in wetlands because of their extreme soil environment with low pH, prolonged flooding or waterlogging, and low nutrient availability (Bridgham and Richardson 2003). Further, pocosins are important ecosystem models for nutrient controls and C cycling because of their large recalcitrant C pools and extent throughout NC (Richardson 1981).

Pocosins have been extensively developed since the 1700s, resulting in large losses of pocosin land to agriculture and other land uses (Lilly 1981). The loss and conversion of these wetlands could have significant impacts on C cycling and storage and it is important to understand the potential impacts. By measuring changes in gas flux, plant productivity, and soil nutrient stoichiometry in response to increasing P-fertility of pocosin soils, we were able to assess how changes in a soil nutrient availability gradient would affect nutrient cycling, C storage, and seasonal patterns of the system. With C cycling and storage moving to the forefront of wetland research in lieu of climate change, it is important to understand how nutrient controls and changes therein will

impact C pools, especially in the wetland class that constitutes a majority of NC's freshwater wetlands.

## **1.1 – Literature Review**

A literature search was performed to quantify and validate how understudied pocosins are compared to other peatlands, while also showing that P-fertility studies are also seemingly rare in these ecosystems. A bibliographic method using Google Scholar and Scopus, two of the most comprehensive reference databases was utilized to complement each database since neither are all-inclusive (Birch and Reyes 2018; Adriaanse and Rensleigh 2011). Google Scholar indexes references across multiple scholarly databases and is able to provide an accurate count of total articles and citations (Birch and Reyes 2018), while Scopus is a multidisciplinary database of Elsevier that indexes more than 20,000 peer-reviewed journals across the fields of science, technology, medicine, and social science (Elsevier B.V. 2018). Searches within these databases were conducted for articles with terms in the title, abstract, and keywords sections to compile reference citation counts (Table 1.1). With the earliest known documentation of pocosins from Tooker (1899), year restrictions were not used in the searches.

A list of the search terms used can be found in Table 1.1. We wanted to compare the reference citation counts for articles published regarding peatlands versus those regarding pocosins and then for nutrient studies within each wetland type, including those for nitrogen and then for phosphorus. The Scopus search produced 7 articles related to pocosins and nitrogen and 8 articles for pocosins and phosphorus. However,

it should be noted that from this search, 6 of the articles combined studies in N and P, with only 1 article related to nitrogen in pocosins and 2 exclusively related to phosphorus (Table 1.2). Overall, results show a severe lack of research within pocosins despite their importance as temperate peatlands in the southeastern United States. What's more, there are far fewer nutrient studies within pocosins as compared to comparable studies within peatlands in general.

## **1.2 - Pocosins in North Carolina**

Global wetlands only comprise about 6% of the earth's surface, with 14% of earth's wetlands found in the United States (DeLaune et al. 2013). It is estimated that wetlands store ~25-30% of the earth's soil pool of carbon (Mitsch et al. 2013). Peatlands, wetlands characterized by C-rich soils, total approximately 3% of the earth's surface yet constitute half of the world's wetlands and are distributed across millions of hectares in the US (Wang et al. 2015). Seventeen percent of the US's wetlands are found in North Carolina and most of NC's wetlands are exclusively located in its coastal plain (USGS 1997). Half of the freshwater wetlands found in NC are pocosins, also referred to as peat bogs, ombrotrophic wetlands known for their thick peat layers that store substantial amounts of carbon (Daniel 1981, Richardson 2003). Richardson (1981) estimated that the development of  $2.02 \times 10^5$  km<sup>2</sup> of pocosin land resulted in the release of  $7 \times 10^6$  t C yr<sup>-1</sup>.

The exact distribution of pocosins along the Coastal Plain of the southeastern US has not been mapped for most states (Richardson 2003), making it difficult to record their loss or development. However, for NC, studies show that 70% of the US' pocosins

are found in NC and span nearly 10,000 km<sup>2</sup> in the state (Richardson 2012). Wilson (1962) mapped the distribution and expanse of pocosins in the 41 NC counties that lie within 64 km of the coast and Richardson (1981) compiled the data to map the distribution of pocosins across the state (Figure 1.1). Researchers have since been able to use satellite imagery to expand the distribution estimate further inland (Richardson et al. 1981). In Pitt County, NC, where our research sites were located, pocosins were estimated to comprise 7.1% of the county's land area (Wilson 1962). At the time of this study however, it is unclear how much of the pocosin lands mapped in NC have been degraded since Wilson's survey was completed.

Pocosins are characterized by a highly variable vegetation and faunal communities as well as deep peat soils that serve as large carbon stores (Richardson 1983). These wetlands comprise the largest extent of true bogs in the southeastern United States and covered approximately 9,079 km<sup>2</sup> in NC in the 1970s (Richardson et al. 1981, Richardson 2003). To our knowledge, a more recent assessment of pocosin distribution in NC has not been conducted since Richardson's (1981) in 1979. Wells (1928) describes pocosins as occurring in broad, shallow basins along drainage basin heads and in flat uplands between rivers and sounds, above which they are usually elevated. These peatlands are often characterized by long hydroperiods with seasonal inundation during winter months, periodic burning, and sandy humus, muck, or peat soils (Richardson 2012). Cowardin et al. (1979) classified pocosins as palustrine wetlands, having no tidal influence. They can be classified as either short or tall pocosins based on the vegetation communities present, including trees (predominantly

pond pine, *Pinus serotina*), shrubs, mosses, and lichens that account for 30% or more of the ground cover (Cowardin et al. 1979).

The pocosin located on East Carolina University's West Research Campus (ECU-WRC) is predominantly a short pocosin with vegetation less than 6 m in height (Cowardin et al. 1979). Short pocosins account for the largest area of the NC wetland complex and resemble savanna-like grasslands with deep peat layers that contribute to their low nutrient availability and low soil pH (Richardson 2012). As noted by Daniel (1981), the sandy humus, mineral clay, and organic muck and peat soils found in these wetlands prevent runoff from draining quickly out of them. This allows soils to store excess nutrients (i.e. N and P) from input water sources and prevent downstream eutrophication (Richardson 1983). Additionally, during the summer, greater than 90% of water in pocosins is lost through evapotranspiration, often leaving soils exposed to air (Richardson 2012). During winter, output switches to runoff, leading to saturated or mildly flooded soils (Daniel 1981). Though pocosins do not usually have a direct upstream connection to surrounding bodies of water, they have been shown to have hydrologic connections to streams and estuaries through drainage systems (Richardson 2003). There is evidence following the development of pocosins in the Albemarle Sound region that these wetlands sequester nutrients and prevent excessive nutrient loading into rivers and estuaries, thus maintaining their health (Richardson 1981b; Richardson 1983; Gilliam and Skaggs 1981).

The global atmospheric concentration of CO<sub>2</sub> has increased annually (NOAA 2018) and is expected to lead to increased global temperatures. Wetlands are important to the global carbon cycle for their ability to sequester carbon through peat formation,

sediment deposition and plant biomass accumulation (Wilson et al. 2014). Globally, pocosins are estimated to store about 445 Pg C yr<sup>-1</sup> (Wang et al. 2015) and serve as important sources of CO<sub>2</sub> while simultaneously serving as sinks for CO<sub>2</sub> and methane (CH<sub>4</sub>; DeLaune et al. 2013). Despite being sinks for CH<sub>4</sub>, Bridgham and Richardson (1992) found that CH<sub>4</sub> emissions from pocosins are not a significant carbon flow pathway and are an insignificant contributor to global CH<sub>4</sub> emissions unlike other peatlands.

Peat serves as an important carbon sink and being such large sinks for C, pocosins could play a potentially significant role in carbon sequestration in response to regional climate change (Bridgham and Richardson 1992). Peat accretion occurs at a rate of 2.6 mm yr<sup>-1</sup> in some North Carolina pocosins, considerably higher than other wetland types (Richardson 2003), and sometimes 25 times higher than boreal pocosins (Wang et al. 2015). It has been estimated that 2529 million metric tons of peat containing 298 million tons of carbon to be stored in North Carolina's peatlands, with 82% of the total peat in NC found in pocosins (Otte 1981).

While the local effects of climate change are not completely understood, precipitation patterns are expected to change as a result, with the southeastern United States seeing heavier downpours, but longer drought periods (US EPA 2016). Wang et al. (2015) discuss how drought coupled with warming has been lowering water tables in peatlands for decades, degrading >11% of them through desiccation and how these changes in hydrology can further threaten carbon stores by turning peatlands from carbon sinks to carbon sources through increased microbial decomposition of peat. If the carbon sequestration potential of peatlands changes as a result of increased

temperatures and drought frequency, they could experience a net loss in carbon as CO<sub>2</sub> or CH<sub>4</sub>, further contributing to a positive feedback loop in the carbon cycle (Bridgham et al. 2008). Recent evidence from Wang et al. (2015) however, has shown that peatlands in Pocosin Lakes National Wildlife Refuge (PLNWR), located in eastern North Carolina, USA, experienced little impacts and even emitted lower CO<sub>2</sub> and stored carbon to a greater extent following a drought. The researchers then suggested that peatlands with different water table heights experience varying effects of drought (Wang et al. 2015). There is a lack of comprehensive studies in NC pocosins that aim to understand how regional climate change will specifically affect these wetlands.

### **1.3 - Phosphorus Cycling in Wetlands**

Though pocosins are deficient in nitrogen and phosphorus, concentrations of N far exceed those of P since pocosins are rain-fed and P occurs in a sedimentary nutrient cycle without a gaseous phase (Figure 1.2; Bridgham and Richardson 1992; Richardson 2003; Mitsch and Gosselink 2015). Phosphorus readily binds to calcium, iron and ferric compounds, aluminum, clay particles, and organic peat, rendering it relatively unavailable to plants (Mitsch and Gosselink 2015). P is typically removed from a wetland by one of three mechanisms: sorption onto already-existing substrates, storage in biomass, and the formation of new sediments and soils (Kadlec 1997). The first two mechanisms have a finite capacity and cannot contribute to sustained P removal, but accretion of new soils and sediments is a long-term solution for sustainable P retention (Richardson 1985). Interestingly, fires in pocosins also play a role in the ecology of the ecosystems, with concentrations of soil P increasing and remaining high

through the proceeding growing season following a burn (Bettis et al. 2009; Wilbur and Christensen 1983). Overall, many physicochemical characteristics of wetland soils and the soil/water interface play roles in the transformation of P between readily bioavailable inorganic orthophosphates and less-available organic P (Mitsch and Gosselink 2015).

Long-term fertilization studies within grasslands have shown that while N and P may both be limiting nutrients, uptake of one nutrient often relies on the soil concentration of the other (Pastor et al. 1984; Fornara et al. 2013). When concentrations of one nutrient increase past the point of limitation while other soil nutrient concentrations remain unchanged, nutrient toxicity becomes an issue. Nutrient toxicity, where root systems and the soil surrounding them become excessively saturated with a nutrient such as N or P, prevents the uptake of other nutrients by the plants and then decreases plant productivity (Pastore et al. 2015; Steffans et al. 2005). Over time, this can result in plant death and changes in system productivity that affect the overall function of the system. Long-term nutrient addition can also have adverse effects on soil microorganisms (van der Wal et al. 2009). This is a worthwhile consideration when undertaking nutrient addition studies such as this one.

Traditionally, after wetlands were developed for agriculture, fertilizers containing both N and P were applied to crops and grasslands based on nitrogen availability, not phosphorus availability, which lead to the excess application of P and an increase in soil P concentrations past that of what is required for plant growth (Whalen and Chang 2001; McDowell and Sharpley 2001). Phosphorus retention is one of the most important attributes of wetlands, because wetlands help buffer nutrient inputs, especially P inputs to downstream freshwater resources (Mitsch and Gosselink 2015; Reddy et al. 1999).

Research has shown that disruption and development along with sustained fertilization of peatlands leads to a reduced capacity for P retention and a dramatic increase in the amount of nutrients released from the wetland into tributaries (Goyette et al. 2018; Lane and Autrey 2016; Haque et al. 2018).

With the importance of pocosins for the health of downstream rivers and estuaries, it is important to understand the immobilization and mineralization dynamics of soil nutrients in response to changing environmental factors (Bridgham and Richardson 2003). This study focused on P-addition to a P-limited pocosin and the effects on C cycling within the system, one of the few studies to analyze this relationship. If plant productivity and C storage in plant biomass and soils could be increased through nutrient addition, this research could potentially provide insight into the effects of pocosin eutrophication on the C cycle within the system itself.

#### **1.4 - Greenhouse Gas Flux**

Methods for measuring greenhouse gas fluxes from soil vary widely from the popular and simple enclosure methods (i.e. static chambers) to micrometeorological methods (i.e. eddy covariance and accumulation, flux gradient) that in themselves vary in complexity (Cerri et al. 2013). Static greenhouse gas chambers are commonly used to sample greenhouse gas (CO<sub>2</sub>, N<sub>2</sub>O, and CH<sub>4</sub>) fluxes between soils and the atmosphere to better understand ecosystem emission of greenhouse gases due to their simple and cost-friendly designs (Collier et al. 2014, Cerri et al. 2013). Static gas chambers trap gases inside a headspace to allow for easy sampling to explore the differences between a set of treatments, or over seasons to over several years. They

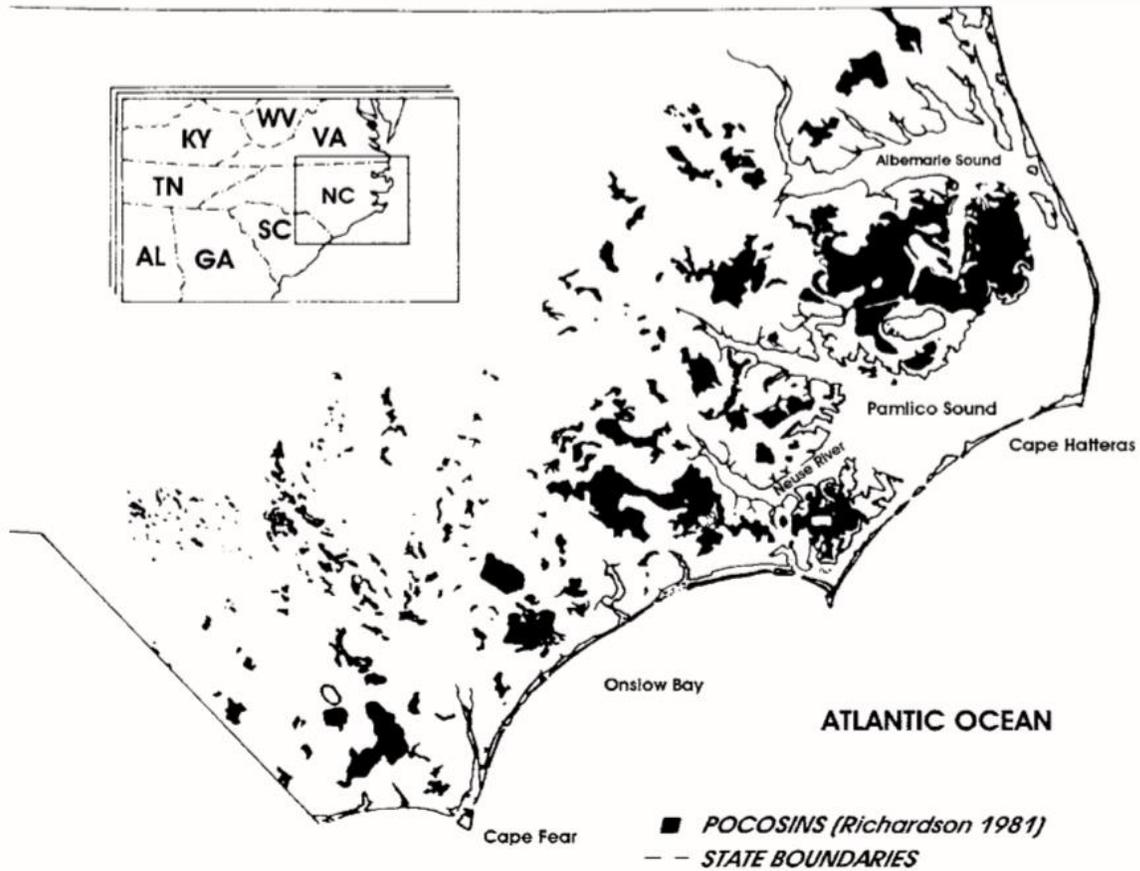
also offer opportunities for methodological variation to best suit the system being studied (Collier et al. 2014). Utilizing static gas chambers for CO<sub>2</sub> flux analysis in this experiment allowed us to study small plots with variable vegetation within the model system. Chambers must be made with nonreactive materials, such as PVC as suggested by Morse et al. (2012) and require anchors or soil collars placed into the soil to accommodate the soil flux (Parkin and Venterea 2010). Samples are commonly analyzed with a gas chromatograph (GC) or infrared (IR) laser (Collier et al. 2014) and the concentrations are used to determine the gas flux within the system. A similar approach was taken to that of Carter et al. (2011) with considerations taken from Collier et al. (2014) for this research.

One goal of this research was to calculate the annual carbon budget of a pocosin in NC in response to increased nutrient availability to better assess how C is allotted in the system. In contrast to saltmarshes where N is the limiting nutrient, P has been identified as the limiting nutrient in pocosins (White et al. 2012, Bettis, Sr. et al. 2009, Bridgham and Richardson 1993). Studies to quantify how elevated CO<sub>2</sub> and N inputs affect carbon sequestration and biomass allocation in saltmarshes showed a general trend towards increased biomass production with increased nutrient availability (Pastore et al. 2015, White et al. 2012). These studies suggest that if the limiting nutrient's availability increases in our study site, biomass production, and therefore carbon storage, will also increase.

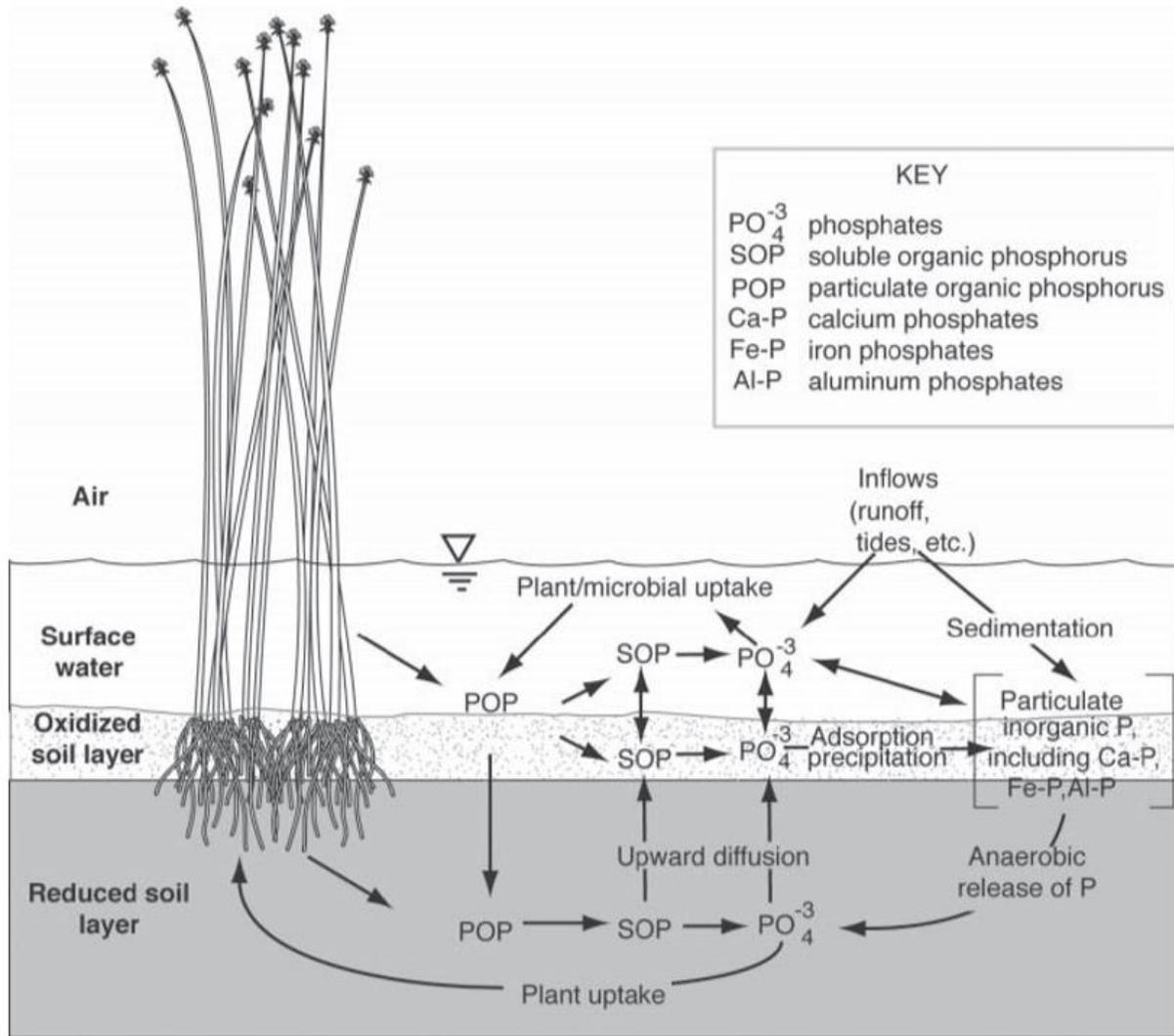
## 1.5 - Summary

Regardless of the possible ambiguity of results from nutrient fertility studies due to the inability to separate factors such as species differences, multiple limiting factors (as is the case for both N and P in pocosins), and species- vs. community-level effects (Walbridge 1991), this type of study can still provide useful information on system-wide trends on changes in soil nutrient stoichiometry, plant community function, and soil microbial function with changes in nutrient availability. The focus of this research was to understand nutrient enrichment effects on C storage and movement in a pocosin located in eastern NC. With P addition in this P-limited wetland, as with N addition in N-limited saltwater marshes, it was expected that CO<sub>2</sub> fixation by plants would increase, leading to increased C allocated and stored in plant biomass (Figure 1.3). Respiration rates were also expected to increase with increasing P availability due to microbial activity (not directly addressed in this study), but the increase in respiration was not predicted to surpass the rate of photosynthesis and lead to increased gas flux out of the system. Soil N:P ratios were expected to decrease with fertilization treatments through the course of the study with an increase in bioavailable orthophosphate. Soil organic carbon (SOC) was also predicted to increase with increased root biomass and C allocated to roots.

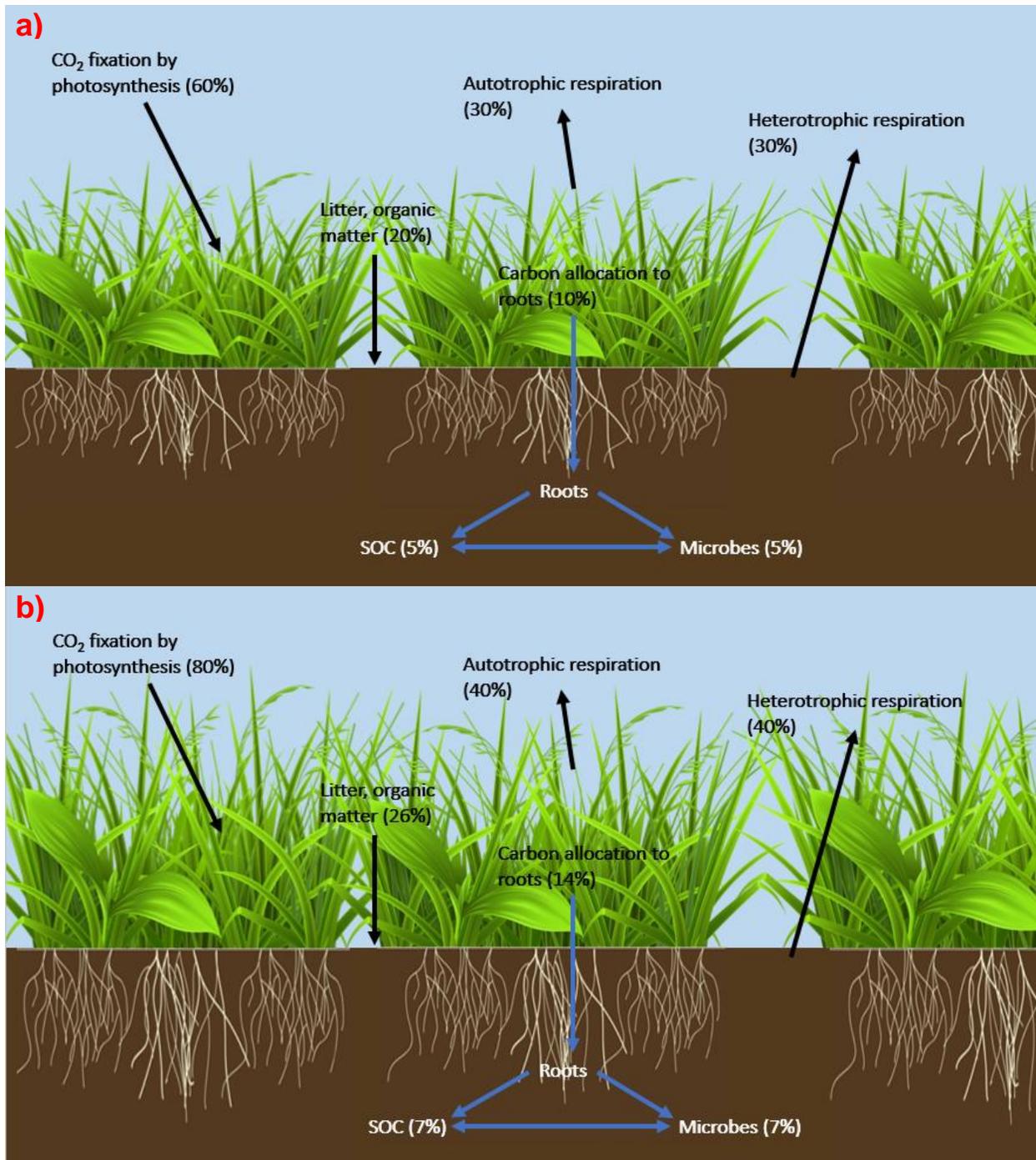
## 1.6 – Tables and Figures



**Figure 1.1** Distribution of pocosin bogs and Carolina bays in North Carolina. Pocosin distribution was estimated to be 908,000 ha. Map was compiled by Richardson (1981) from data collected in the 1950s by Wilson (1962).



**Figure 1.2** Illustration of the wetland phosphorus cycle. Adapted from Mitsch (2015).



**Figure 1.3** Schematic for carbon movement through the wetland system a) before P addition and b) after P addition. Percentages are only theoretical and aim to represent the portion of C from the whole moving through each compartment.

**Table 1.1** Literature search results comparing the number of references for peatlands and pocosins and nutrient studies within each.

| <b>Database</b> | <b>Search Terms</b>         | <b>No. Citations</b> |
|-----------------|-----------------------------|----------------------|
| Google Scholar  | peatland(s)                 | 81800                |
|                 | peatland(s) AND nutrient(s) | 33700                |
|                 | peatland(s) AND nitrogen    | 31900                |
|                 | peatland(s) AND phosphorus  | 17700                |
|                 | pocosin(s)                  | 13400                |
|                 | pocosin(s) AND nutrient(s)  | 1820                 |
|                 | pocosin(s) AND nitrogen     | 1230                 |
|                 | pocosin(s) AND phosphorus   | 790                  |
| Scopus          | peatland(s)                 | 7635                 |
|                 | peatland(s) AND nutrient(s) | 1046                 |
|                 | peatland(s) AND nitrogen    | 827                  |
|                 | peatland(s) AND phosphorus  | 423                  |
|                 | pocosin(s)                  | 85                   |
|                 | pocosin(s) AND nutrient(s)  | 12                   |
|                 | pocosin(s) AND nitrogen     | 7                    |
|                 | pocosin(s) AND phosphorus   | 8                    |

**Table 1.2** Literature search results for N and P in pocosins.

| Nitrogen | Phosphorus | Paper   |
|----------|------------|---|
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## **Chapter 2: CO<sub>2</sub> flux from a pocosin following addition of the limiting nutrient**

### **2.1 - Introduction**

This study examined how phosphorus availability in the soil affects carbon budgeting in an Eastern North Carolina pocosin, freshwater peatlands characterized by extreme soil conditions (Bridgham and Richardson 2003) by increasing the availability of the limiting nutrient. Similar studies have demonstrated how increasing nitrogen availability in saltwater marshes can affect carbon storage and nutrient dynamics in saltwater marshes (Pastore et al. 2015) while other studies have manipulated the soil environments of peatlands to assess changes in the immobilization-mineralization dynamics of C, N, and P (Bridgham and Richardson 2003 and 1992). For this research, the amount of phosphorus present in treatment plots located within a freshwater pocosin on the coastal plain of NC. The objective of this study was to measure carbon flux rates (as CO<sub>2</sub>) from the native grass community found in the study plots on East Carolina University's West Research Campus (ECU-WRC) in response to monthly P-fertilizer treatments. We hypothesized that under augmented nutrient conditions, flux rates in the ecosystem would decrease over an annual growing season as was seen in previous studies where N fertilizer was added to N-limited saltwater marshes (e.g. White et al 2012, Pastore et al 2015) and where P fertilizer was added to P-limited freshwater marshes (e.g. Richardson and Marshall 1986). Measurements were taken using clear static gas chambers, which allowed us to capture net ecosystem production (NEP) of CO<sub>2</sub> rather than respiration rates alone.

Different concentrations of P were used for different levels of treatment to measure the system's response to phosphorus enrichment on carbon immobilization in the native grass communities found in the WRC's pocosin (i.e. will increasing soil N:P ratios so that they more closely resemble the Redfield Ratio (Redfield et al. 1963) have positive or negative effects on plant communities?). While the amount of phosphorus in pocosin soils has been shown to be spatially variable (Wilbur and Christensen 2016), the overall concentration of phosphorus in pocosin soils is low with high N:P ratios (Bridgham and Richardson 1993). The outcome of P enrichment in this study aimed to understand how freshwater wetlands could play a role in increased carbon storage as atmospheric CO<sub>2</sub> levels and human activities that result in increased nutrient inputs (e.g., farming and fertilizing) increase.

The Redfield Ratio has been used as a model to determine which nutrient is limiting in an ecosystem based on the ratios of C:N:P, established to be 106:16:1. Even though the ratio of N:P should be 16:1 according to the Redfield Ratio (Redfield et al. 1963), Wang and Moore (2014) suggest it can vary according to plant functional types and in recent years the ratio has been suggested be closer to 117:14:1 (Benitez-Nelson 2000). Thus, phosphorus treatments were determined using this rationale and was applied in three different ratios, including the traditional phytoplanktonic Redfield Ratio of 16:1 (Redfield et al. 1963).

Historically, many pocosins in NC were disturbed through ditching and draining to access the fertile soil for crops and thus converted to large expanses of farmland across Eastern NC (Richardson 1981). Evidence shows the wetland located on the WRC was no exception to this disturbance after being extensively logged, mowed, and managed

under the Voice of America's (VOA) occupation of the property (Chester 2004). Following ECU's purchase of the property, a wetland delineation was performed by Kimley-Horn and Associates, Inc. and the WRC became a managed natural area for ecological restoration projects (Chester 2004). With the critical importance of wetlands and their protection and restoration, it is important to understand how restoring wetlands will affect their function. Pocosins are unique wetlands that are under-studied. Assessing how adding a limiting nutrient to the soil in these ecosystems affects CO<sub>2</sub> flux in or out of the system could provide assimilation rates and substantive information about the effects that ecosystem disturbance has had on these wetlands across Eastern NC. This study could also provide information on the fate of CO<sub>2</sub> flux in NC pocosins formally converted to farmland but are now under restoration. Both outcomes can help enhance present management of pocosin restoration at the ECU-WRC, as it's important to understand how the CO<sub>2</sub> fluxes in these systems might have changed following disturbance from farming and then restoration.

## **2.2 - Methods**

### **2.2.1 - Site history**

Situated on the former 2.60 km<sup>2</sup> Edward R. Murrow Transmitting Station Voice of America (VoA) site C is the East Carolina University West Research Campus (WRC) located approximately 5 miles west of Greenville, NC (Figure A-1). The site, originally 644 acres in size began operation in 1963 as a receiving-only site and was closed in March of 1995 (USDOS 2005). The property was leased to ECU in 2001 and the current land management plan for the property was brought about by a wetland delineation

performed in 2003 (Kimley-Horn and Associates, Inc.) that determined a large portion of the property is a wetland (Figure A-3; Hill 2007; Jagenow 2009).

In 1971, an agribusiness firm was allowed to bury toxic waste from a fire cleanup at a chemical storage warehouse in Farmville, NC in storage cells (USDOS 2005, Chester 2004). In 1994, the General Services Administration (GSA) sold 594 acres of VoA Site C to the state of North Carolina and to local governments, retaining the 2 km<sup>2</sup> parcel in the southern-central section of the site (Area F, Figure A-2) where the waste was buried (USDOS 2005, Chester 2004).

### **2.2.2 - Site description**

The West Research Campus, a 2.35 km<sup>2</sup> mineral flat with poorly drained soils located in North Carolina's central coastal plain (Figure A-1). The WRC sits at one of the highest elevations in Pitt County at 22-25 m above sea level (Figure A-4) and spans across, as well as drains into both the Neuse and Tar River basins (Chester 2004). A soil survey conducted by the United States Department of Agriculture (USDA) identified six soil types within the WRC: Coxville, Lynchburg, Goldsboro, Rains, Exum, and Bibb soils (USDA NRCS 2017, Figure A-5). According to the USDA NRCS (National Resources Conservation Service) report, Lynchburg and Rains soil types are dominant within the study site and are poorly drained soils with seasonal flooding and a water table at or just below the soil surface (USDA NRCS 2017). These two soil types are associated with elevated flatlands and Carolina Bay wetlands (USDA NCRS 2017). Pocosins are a type of Carolina Bay, but it should be noted that not all Carolina Bays can support pocosin ecosystems (Otte 1981). With the development of the VoA site in 1960, ditched access roads were installed for logging, with drainage ditches still present

around the study site today. Figure 2.1 shows the VoA site outside of Falkland, NC with its network of roads and tower locations in 1961. In the 1990s, the NC Forest Service was contracted to burn the site periodically to maintain a savanna-like grassland that supports many native carnivorous and fire-reliant plant species (Chester 2004). Chester (2004) identified a total of 242 plant species with 80% being herbaceous, 110 being facultative or obligate wetland species, 30 being fire-reliant, and 7 species being carnivorous.

### **2.2.3 - Experimental design**

A random block design was used to develop the experiment. Along the southwestern portion of the WRC in Area A (Figure A-2), four sites (experimental blocks) were chosen at random (Figure A-6), spanning between two pond-like areas that are seasonally inundated with the water level rising at or above the soil surface. Four, 4 m<sup>2</sup> plots were placed in each site and a smaller 1 m<sup>2</sup> quadrat was placed inside each plot to minimize edge effects and mark where sampling would actually occur (Figure A-7). Each site contained four treatment plots, each either receiving no P fertilizer (control), low, intermediate, or high amounts of P fertilizer. Levels of P fertilizer applied were determined using the Redfield Ratio (C:N:P is 106:16:1 or N:P is 16:1; Redfield 1963). Calculations used to determine the amount of P needed to bring the soil N:P ratios in each treatment plot up to these target ratios were based on concentrations of N and P in pocosin soils previously reported (Bridgham and Richardson 1993).

From March 2017 through March 2018, P fertilizer application was alternated every two weeks with gas and biomass sampling. Table 2.1 outlines response variables that were measured and analyzed as well as when sampling was scheduled versus

when it actually occurred to obtain data for these variables. Other related data, such as soil temperature, ambient air temperature, amount of light, plant and/or water volume within chambers, and weather conditions were recorded on sampling days. Weather for average daily air temperature, pressure at sea level, humidity, precipitation, and wind speed were retrieved from Weather Underground ([www.weatherunderground.com](http://www.weatherunderground.com)) since it utilizes citizen-led stations. It should be noted that early in September 2017 because of Hurricane Harvey, sampling days were delayed a week. Gas sampling scheduled for December 29, 2017 was canceled due to inclement weather (i.e. below freezing temperatures, high wind speeds) and only biomass samples were collected at this time.

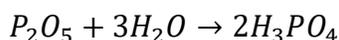
Gas samples were taken in duplicate in the field for each timepoint and designated as either A or B. A-samples were primarily used for analysis unless the sample's integrity was compromised (e.g., vial leakage), in which case the B-sample was used. Gas flux was calculated using the slope of the equation of the line for PPM CO<sub>2</sub> over time for each plot on each sampling day.

#### **2.2.4 - Phosphorous manipulation**

Each of the four plots located within a site were designated as either control (no fertilizer), low (18.33g fertilizer m<sup>-2</sup>), intermediate (36.67g fertilizer m<sup>-2</sup>), or high (55.0g fertilizer m<sup>-2</sup>) treatment plots. The amount of P applied was significantly greater than the amount of P applied as fertilizer to agricultural fields between the 1960s and 2014 (28-40 kg km<sup>-2</sup> yr<sup>-1</sup>; US EPA 2018) and found in wastewater (Richardson and Marshall 1986). P fertilizer was applied in amounts that would bring the N:P ratio of soils to 32:1 (low), 16:1 (intermediate), and 8:1 (high; Wang and Moore 2014). The amount of

fertilizer to apply to each plot was determined based on weight of  $P_2O_5$ . N:P ratios of pocosin soils tested in Bridgham and Richardson (1993) were used to calculate how much fertilizer would need to be applied to increase P concentrations in the soil to overcome limiting soil nutrient stoichiometric ratios reported by Wang and Moore (2014). The Bridgham and Richardson (1993) ratios were used because preliminary soil analyses were inconclusive due to testing limitations within the lab at the time.

Fertilizer was applied to each of the experimental plots 15 days prior to sampling. Bonide's® Garden Rich Triple Super Phosphate 0-45-0 fertilizer (MSDS No. 969) was mixed and dissolved with 1 L of water. The chemical equation for the reaction between the fertilizer and water is



Two mixes for one treatment were added to 7.6 L Flo-Master® multipurpose fertilizer sprayers and brought to a final volume of 7.6 liters. Half of this volume was placed in each sprayer was applied to two of the appropriate plots with frequent shaking of the sprayer to ensure homogenous application. Fertilizer was applied with regard to weather conditions to prevent storm events from immediately washing the phosphorus and fertilizer away.

After several fertilizer applications, it became apparent the sprayers were clogging due to fertilizer particulate still present in the intermediate and high mixes since the fertilizer did not completely dissolve, so watering cans were used to apply intermediate and high fertilizer treatments from September 2017 until the end of the study. The same principle of applying 3.8 L of the mix to each plot still applied, with

each watering can holding only 3.8 L of mix compared to the Flo-Master's® 7.6 L capacity.

## **2.2.5 - CO<sub>2</sub> flux analysis**

### ***2.2.5a - Plant productivity and gas sample collection***

Plant productivity was measured using CO<sub>2</sub> concentrations and flux rates in the study site once every four weeks immediately before taking above- and belowground biomass samples. Gas samples were collected following the protocol found in Collier et al. (2014), Parkin and Venterea (2010), and Carter et al. (2011). Acrylic static gas chambers fitted with two sampling ports and rubber septa (Figure 2.2) were used as the static chambers. Gas chambers were modified to be tall enough to accommodate vegetation present inside the collar. A sampling section was randomly chosen within the 1 m<sup>2</sup> quadrat in each plot and fitted with a soil collar immediately following fertilization to minimize soil disturbance before gas sampling.

Weather variables were accounted for as they could affect gas flux measurements and we wanted to be sure that any observed trends in CO<sub>2</sub> flux were not the product of changes in weather (i.e. Cerri et al. 2013; Carter et al. 2011; Harazono et al. 2015; Conen and Smith 1998). It was for this reason that gas sampling did not take place on days with storm events, excessive wind (> 10 mph) at the time of sampling, or extremely low temperatures.

On sampling days, initial and final soil temperatures were taken in each plot before and after sampling. The channels in each soil collar were filled with water and fitted with the chamber tops after measuring initial soil temperature.

One ambient air sample was taken at each of the 4 sites and 4 samples (taken in duplicate) were taken from each plot at 30-minute intervals using a 10 mL syringe fitted with a 3-way stopcock and 26-gauge hypodermic needle. Gas samples were injected and stored in 3.7 mL evacuated glass exetainers. Before taking each sample, the needle and syringe were used to mix the air inside the chamber with 5 draws and pushes of the plunger. Gas samples were analyzed in the lab using a LI-COR 7000 to determine the changes in the concentration of CO<sub>2</sub> (PPM) over time, which was used to determine the flux rate in g CO<sub>2</sub> m<sup>-2</sup> hr<sup>-1</sup>.

Gas samples were taken from two plots first following the scheme in Table 2.2 and the methods mentioned above before moving on to sample the next two plots following the same sampling method. The two plots measured first were alternated every sampling trip. Light measurements (lux and fc) were taken during gas sampling to calculate the photosynthetically active radiation (PAR), or amount of light available to plants for photosynthesis in case PAR was a confounding variable for gas flux due to seasonal changes in PAR.

### ***2.2.5b - Calculating flux***

For each experimental plot's time series, PPM CO<sub>2</sub> over time was plotted and evaluated for linearity in MS Excel Because NEP was being measured as opposed to respiration alone, data points from the time series were not excluded if they appeared to be outliers. Trace gas fluxes needed to be converted from volumetric to mass using the Ideal Gas Law ( $PV = nRT$ ) and molar mass of CO<sub>2</sub> (44.01 g mol<sup>-1</sup>). Average gas fluxes by treatment were calculated and graphed for each month and then plotted on a graph to observe any seasonal trends.

### 2.2.6 - Statistical analysis

SAS 9.4 software was used to perform a repeated measures ANOVA using the PROC MIXED procedure to determine the overall effect P-addition had on CO<sub>2</sub> flux from the system with sampling date being listed as the repeated variable. This design was chosen because samples were taken from the same 16 plots each month rather than from new plots within each site. Sites were listed as random block effects, since they were randomly chosen within the wetland situated on the WRC and marked the location of the 4 treatment plots held within each. Treatment and sampling dates were listed as separate fixed effects in the model and additionally crossed to determine if the way the response variable (CO<sub>2</sub> flux) changes through time depends on the treatment or the point in time during the sampling season.

Normality plots using studentized residuals did reveal that the data was normal, so data transformation was not needed. The default model-based degrees of freedom method, the containment method, was used since there were no partially-observed variables in the model. To determine the best variance-covariance structure for the model, the GLMM for treatment was run using different covariance structure methods for the repeated variable and the AIC scores were compared. Of the methods compared (autoregressive(1), heterogenous autoregressive(1), compound symmetry, heterogenous compound symmetry, and the default variance components), the compound symmetry (CS) covariance-structure provided the best model fit.

Though additional weather data was taken (i.e. average daily wind speed, average daily photosynthetically active radiation (PAR), air temperature), these factors were not considered in the model. Weather data is an intrinsic property of and therefore

confounded with the sampling day because the weather terms apply to all 4 treatments and not each individual one. However, repeated measures ANOVAs were run replacing treatment as a fixed effect with individual weather variables; sample date was not included in these models. The estimates of the weather factors' means from each model were compared to the estimates for the means of each sample date to see if there was any correlation between occurrences of more extreme estimates in the weather data and occurrences of more extreme mean estimates for sample dates.

### **2.3 - Results and Discussion**

The repeated measures ANOVA for treatment and sample date suggested an overall difference in CO<sub>2</sub> flux from the system as a function of P application ( $F_{3, 153} = 3.70$ ,  $p = 0.0132$ ) and sample date ( $F_{12, 153} = 5.65$ ,  $p < 0.0001$ ), but not for treatment dependent on sample date ( $F_{36, 153} = 0.83$ ,  $p = 0.7431$ ). Differences in the LSMs for the treatments showed the most difference occurring between the control and high treatments ( $t_{153} = -2.27$ ,  $p = 0.0245$ ) and the low and high treatments ( $t_{153} = 3.23$ ,  $p = 0.0015$ ). LSMs also showed that May ( $t_{153} = -2.34$ ,  $p = 0.0208$ ), July ( $t_{153} = 5.29$ ,  $p < 0.0001$ ), August ( $t_{153} = 5.65$ ,  $p < 0.0001$ ), and October ( $t_{153} = 2.77$ ,  $p = 0.0062$ ) were the most different from zero and thus saw significant changes in CO<sub>2</sub> fluxes.

These differences can be seen when graphing average flux per treatment for each month (Figure 2.3). It is necessary to pair these statistics with a graphical representation, because while the original hypothesis that P-addition to a P-limited pocosin would change the CO<sub>2</sub> flux from the system was supported, the treatments that we originally thought were going to decrease CO<sub>2</sub> flux from the system increased CO<sub>2</sub> flux. This could be due to nutrient toxicity where too much phosphorus in the form of

orthophosphoric acid ( $H_3PO_4$ ) was applied. This could have either raised the pH of the soil past the tolerable limit for the plants, increased the amount of available P in the soil past that of what the plants could tolerate, or decreased the N:P ratio so much that plants were starved for nitrogen.

Differences between the high treatment and control and low treatments were expected. As seen in Figure 2.3, throughout the study, low treatment plots appeared to have  $CO_2$  fluxes similar to or lower than those from the control plots. Both the control and low treatment plots had fluxes lower than those in the high plots throughout most of the study. This was especially evident in the warmer months of the growing season. While  $CO_2$  fluxes for each treatment across all 13 sampling months followed an expected seasonal trend where fluxes were higher during summer months and lower during the colder winter months,  $CO_2$  flux from the high treatment plots was considerably higher than that from the other 3 treatments during the warmer months. This was true for the months of May, July, August, and October, 4 months that had the highest average daily air temperatures from all sampling months.

While additional weather data was taken, those factors were considered intrinsic properties of and therefore confounding variables with the sampling date. Despite this, cross-referencing more extreme mean estimates for each weather variable to the sample dates during which those values occurred provided insight into which weather factors likely had the most effect on flux measurements. Most weather variables had little to no effect on  $CO_2$  flux measurements, lacking a clear pattern in the significance of the different values which were randomly interspersed along a scale. This was also true for light availability and photosynthetically active radiation (PAR), which had no effect on

flux despite the implications increased PAR has for increased plant productivity and rates of photosynthesis. However, wind speed and air temperature (Figure 2.4) during sampling appeared to have the most effect on gas flux measurements during this study. Recorded temperatures  $>22^{\circ}\text{C}$  and wind speeds  $>5$  kph had significant LSMs that corresponded to the four significant sampling months, May, July, August, and October.

## **2.4 - Conclusion**

While it does appear that fertilizing P-limited soils in a pocosin in Eastern NC affected the  $\text{CO}_2$  flux from the system, further studies should be done adjusting the amount of P applied to prevent acidification and nutrient toxification of the soil, leading to increased plant death, litterfall, and  $\text{CO}_2$  fluxes out of the system. It appears that the low treatment, while not statistically different from the control group, led to either a decrease in  $\text{CO}_2$  flux from the system during some months or at least a flux no different from the control group in others. This could indicate that if applied in the right concentrations, adding P to P-limited soils in freshwater wetlands could decrease  $\text{CO}_2$  flux and ultimately increase the carbon stored in the system as seen in studies where N was added to N-limited saltwater marshes (Pastore et al. 2015; White et al. 2012).

There are also implications about the effects that ditching, draining, and fertilizing pocosin wetlands in Eastern NC have had on  $\text{CO}_2$  flux changes in these systems and how the flux changes when these areas are restored. A drop in the water table following pocosin draining would increase litterfall as wetland plant species died. Combined with nutrient addition, this would present conditions similar to those in the intermediate and high treatment plots where plants started dying off before the fertilizer regime was completed. Control plots on the other hand, serve as examples of the system under

restoration since the area of the WRC where the study plots were located had been previously developed for use by the VoA.

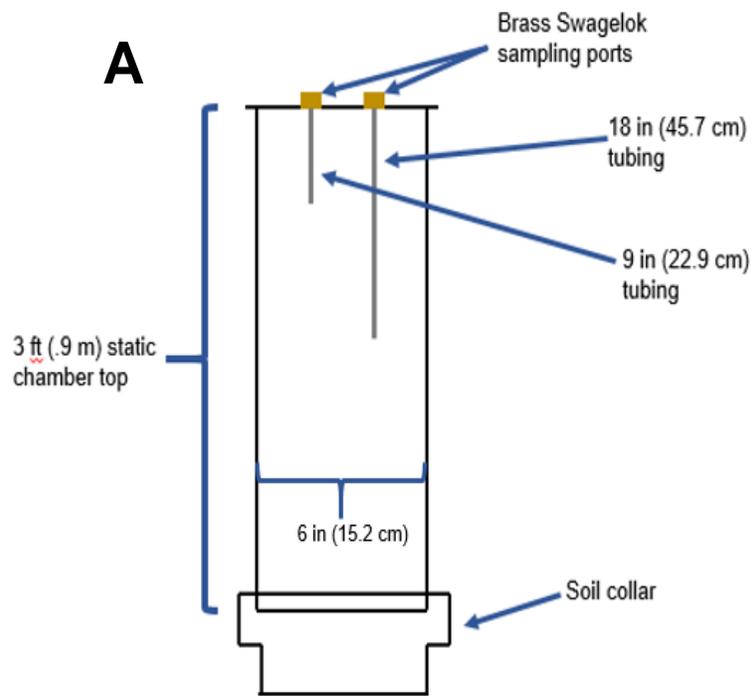
A longer study that spans over several growing seasons could also provide a clearer idea to what happens to CO<sub>2</sub> flux in the system if the limiting nutrient is applied long-term. This could rule out the effects of storm events or the severity of seasonal weather that might have affected gas fluxes within a month in this study. Additionally, shortening the time between fertilizing and sampling would be beneficial to observing whether P addition caused a priming effect with the soil microbes that was missed due to the set field schedule.

Overall, this study indicates that fertilizing a P-limited freshwater wetland with P fertilizer can decrease CO<sub>2</sub> flux from the wetland, leading to increased stored C in the soil that can potentially become sequestered C. Further research should be done to ascertain if there is a change in CO<sub>2</sub> flux seasonality and if P-addition leads to a priming effect in soil microbes.

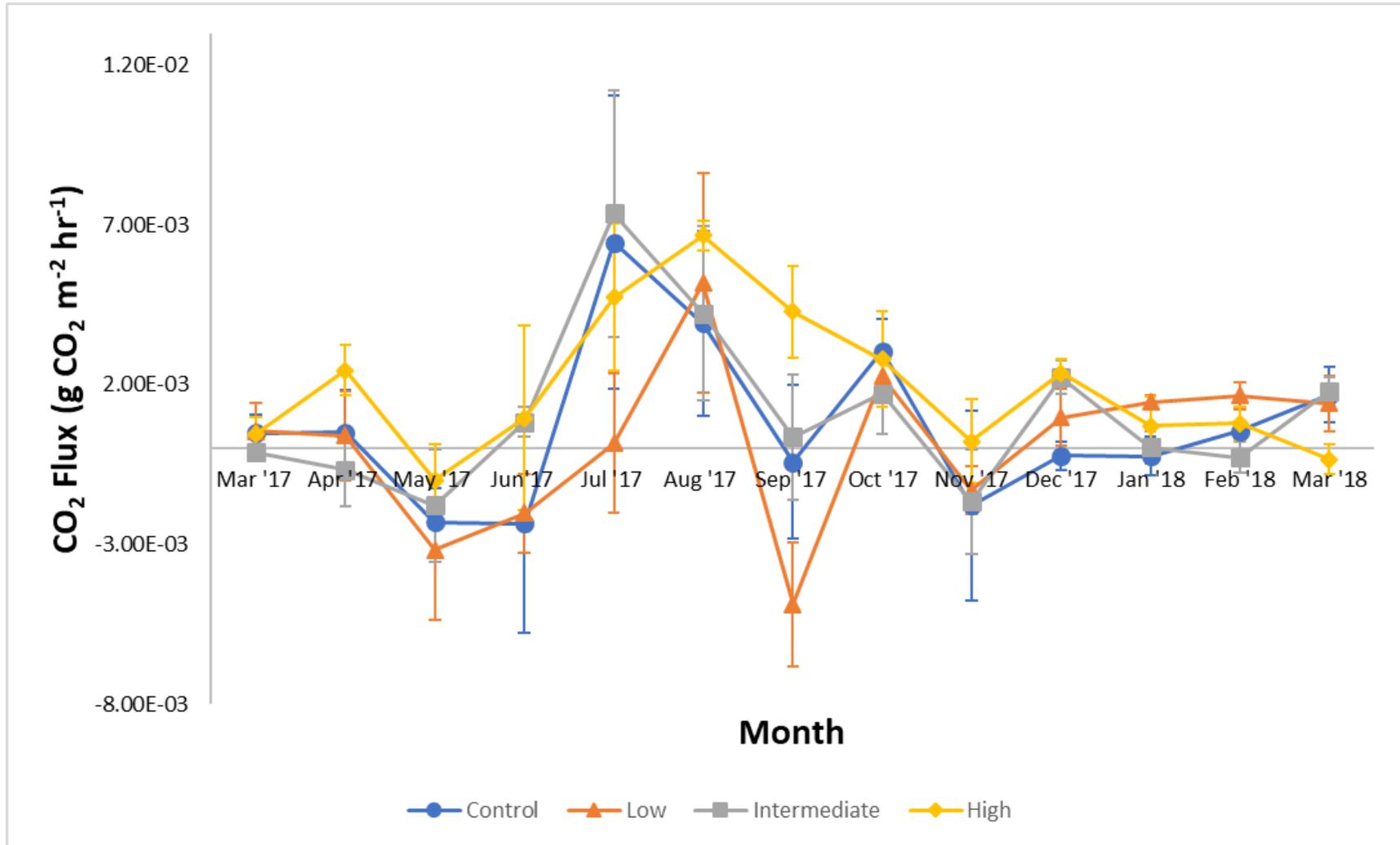
## 2.5 - Tables and Figures



**Figure 2.1** Aerial photo of VoA Site C after preparation of the area for construction and antennae installation (Daily Reflector May 3, 1961 Greenville, NC).



**Figure 2.2** Static gas chamber design. A) Chambers are 3 feet tall and 6 inches wide, fitted with two brass Swagelok sampling ports connected to tubing, and fit inside a soil collar with a channel. B) image of an actual chamber.



**Figure 2.3** CO<sub>2</sub> flux by treatment over the course of one sampling year. Error bars represent standard errors of the mean, n=4.

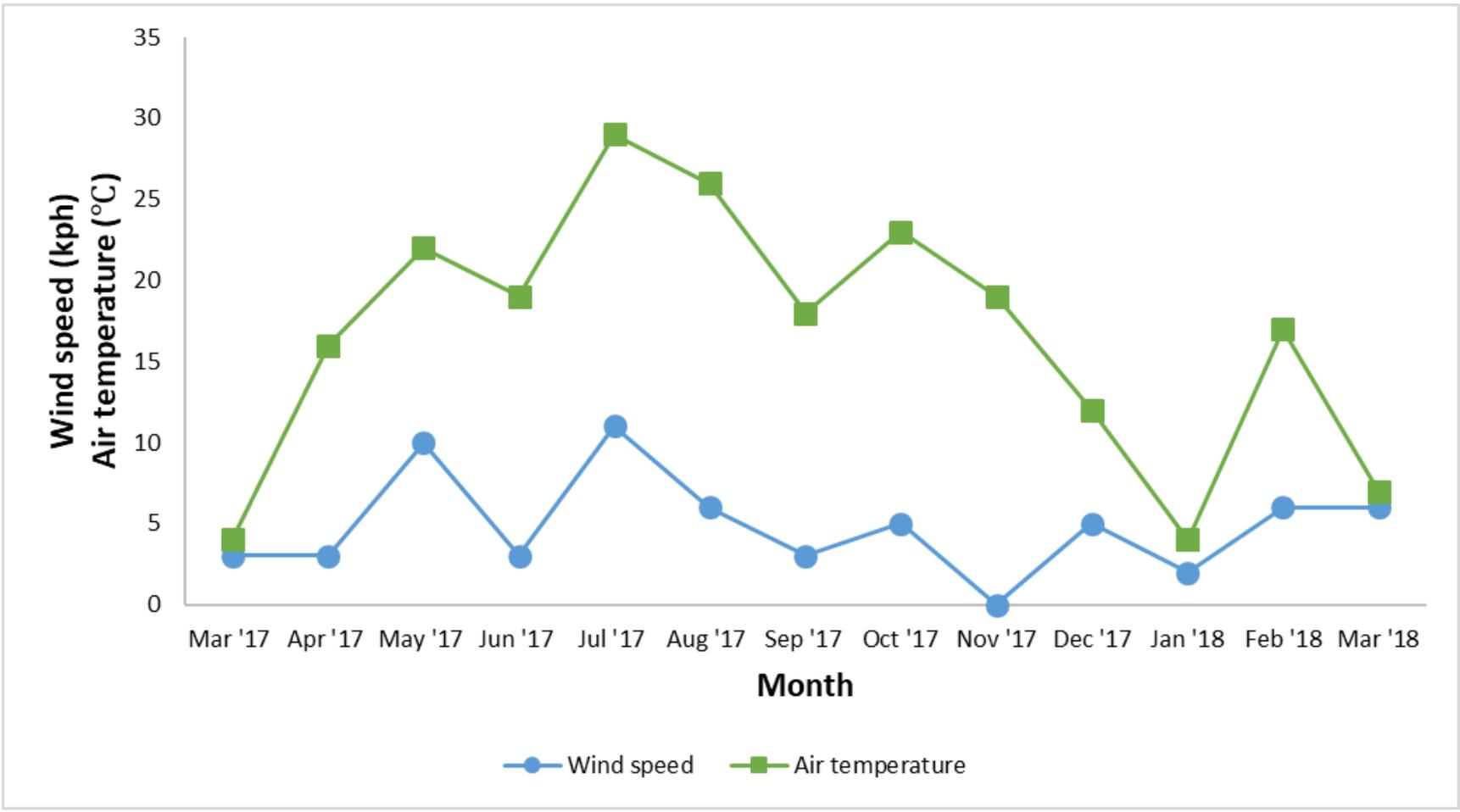


Figure 2.4 Average daily wind speeds and air temperatures for sample days throughout the sampling year (Weather Underground).

**Table 2.1** Field schedule outlining originally planned field dates and actual field dates listing the samples collected on each trip.

\* Field dates that were not originally planned but added in after the start of the field season.

† Field date that had to be pushed back due to Hurricane Harvey.

‡ Field date where CO<sub>2</sub> flux was not taken due to weather conditions.

| Field Dates         |                     | Treatment   | Response Variable   |                     |                      |              |
|---------------------|---------------------|-------------|---------------------|---------------------|----------------------|--------------|
|                     |                     | Fertilizing | Aboveground biomass | Belowground biomass | CO <sub>2</sub> flux | Soil N and P |
| Initial             |                     |             |                     |                     |                      | <b>X</b>     |
| <i>Planned</i>      | <i>Actual</i>       |             |                     |                     |                      |              |
| 3-3-17              | 3-3-17              | X           |                     |                     |                      |              |
| 3-17-17             | 3-17-17             |             | X                   | X                   | X                    |              |
| 3-31-17             | 4-1-17              | X           |                     |                     |                      |              |
| 4-14-17             | 4-14-17             |             | X                   | X                   | X                    |              |
| 4-28-17             | 5-1-17              | X           |                     |                     |                      |              |
| 5-12-17             | 5-15-17             |             | X                   | X                   | X                    |              |
| 5-26-17             | 5-26-17             | X           |                     |                     |                      |              |
| 6-9-17              | 6-9-17              |             | X                   | X                   | X                    |              |
| 6-23-17             | 6-26-17             | X           |                     |                     |                      |              |
| 7-7-17              | 7-7-17              |             | X                   | X                   | X                    |              |
| 7-21-17             | 7-21-17             | X           |                     |                     |                      |              |
| 8-4-17              | 8-3-17              |             | X                   | X                   | X                    | X            |
| 8-18-17             | 8-18-17             | X           |                     |                     |                      |              |
| 9-1-17 <sup>†</sup> | 9-8-17 <sup>†</sup> |             | X                   | X                   | X                    |              |
| 9-15-17             | 9-22-17             | X           |                     |                     |                      |              |
| 9-29-17             | 10-6-17             |             | X                   | X                   | X                    |              |
| 10-13-17            | 10-20-17            | X           |                     |                     |                      |              |
| 10-27-17            | 11-3-17             |             | X                   | X                   | X                    |              |
| 11-10-17            | 11-17-17            | X           |                     |                     |                      |              |
| 11-24-17            | 12-1-17             |             | X                   | X                   | X                    |              |
| 12-8-17             | 12-15-17            | X           |                     |                     |                      |              |
| 12-22-17            | 12-29-17            |             | X                   | X                   | ‡                    |              |
| 1-5-18              | 1-12-18             | X           |                     |                     |                      |              |
| 1-19-18             | 1-26-18             |             | X                   | X                   | X                    |              |
| 2-2-18              | 2-9-18              | X           |                     |                     |                      |              |
| 2-16-18             | 2-23-18             |             | X                   | X                   | X                    | X            |
|                     | 3-16-18*            | X           |                     |                     |                      |              |
|                     | 3-23-18*            |             | X                   | X                   | X                    |              |

**Table 2.2** Sample timing scheme for the collection of gas samples from multiple chambers at one time.

|                  | <b>T<sub>0</sub></b> | <b>T<sub>1</sub></b> | <b>T<sub>2</sub></b> | <b>T<sub>3</sub></b> |
|------------------|----------------------|----------------------|----------------------|----------------------|
| <b>CHAMBER 1</b> | 0                    | 30                   | 60                   | 90                   |
| <b>CHAMBER 2</b> | 5                    | 35                   | 65                   | 95                   |
| <b>CHAMBER 3</b> | 10                   | 40                   | 70                   | 100                  |
| <b>CHAMBER 4</b> | 15                   | 45                   | 75                   | 105                  |

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# **Chapter 3: Changes in biomass allocation and C storage after nutrient addition**

## **3.1 - Introduction**

Above- and belowground production in coastal wetlands are important for carbon accumulation and long-term carbon storage. Changes in biomass allocation in native wetland plant communities are possible indicators of ecosystem health, with belowground production being more sensitive to adverse conditions than aboveground production (Stagg et al. 2016). As in saltwater wetlands where increased nitrogen inputs increased plant productivity and carbon storage (Pastore et al. 2015), increased phosphorus inputs were expected to increase the primary production and carbon storage potential of this pocosin through increased carbon allocation and gross primary production (GPP) of above- and belowground biomass. The purpose of this study was to measure changes in carbon storage in above- and belowground biomass of grass communities in a pocosin located in Eastern NC following P-fertilizer treatments. This was accomplished by measuring changes in C and biomass allocation throughout the course of a sampling year. We also wanted to observe how changing nutrient inputs in the soil would affect the annual growing cycle and intrinsic seasonality of these plants.

The pocosin found on ECU's WRC is predominantly a short pocosin with vegetation less than 6 m in height (Cowardin et al. 1979). Short pocosins account for the largest area of the NC wetland complex and resemble savanna-like grasslands with deep peat layers that contribute to their low nutrient availability and low soil pH (Richardson 2012). Peat accretion occurs at a rate of 2.6 mm per year in some North Carolina pocosins (Richardson 2003), considerably higher than other wetland types and

sometimes 25 times higher than boreal pocosins (Wang et al. 2015). Peat soils have a mineral sublayer that consists of heavy clays to sandy humus derived from marine sediments and surface layers of organic mucks and peats (Barnes 1981; Richardson 2012).

The accretion of peat is dependent on plant biomass that dies and becomes part of the surface leaf litter, or in the case of roots, soil organic matter (SOM). Fertilization has been shown to increase the amount of belowground biomass in a wetland system (Pastore et al. 2015), which can potentially aid wetlands' ability to accrete peat. This is important, because peat is a large store of recalcitrant carbon. Five hundred and twenty-nine million metric tons of peat containing 298 million tons of carbon have been estimated to be stored in North Carolina's peatlands, with 82% of the total peat in NC found in pocosins (Otte 1981). This study could indicate how increased nutrient inputs can alter peat accretion rates through litter deposition in these wetlands by using changes in C allocation within biomass as indicators of increased C input to soil through root and shoot die-off.

Because of their extreme soil environments that can have negative feedbacks on plant production, peatlands are great model systems for examining nutrient controls over carbon cycling (Bridgham and Richardson 2003). Assessing how the availability of P in pocosin soils affects the C allocation in plant biomass and primary productivity of pocosin plant communities can provide insight into exogenous nutrient availability drives the immobilization-mineralization dynamics of C in these wetlands.

### **3.1.1 - Plant community**

From April 2002 to June 2004, Chester (2004) compiled a species list of plant species found and identified at ECU's WRC. The final list consisted of 242 species representing 63 families, but due to unidentifiable species the total number could include up to 260 plant species (Chester 2004). Of the species identified, 110 were facultative or obligate wetland species. Seven of the species identified represent 3 genera (*Drosera*, *Sarracenia*, and *Utricularia*) of carnivorous/insectivorous plants with unique adaptations that allow them to survive in nutrient-poor soils (Chester 2004). About 80% of the species found in the WRC are herbaceous (Chester 2004).

Utilizing the floristic assessment from Chester (2004), plant communities specifically within our 16 plots were identified to the genus level as to adapt sampling protocols to their physiological characteristics (i.e. growing patterns). Of the plants identified in our plots, most were rhizomatous sedge species. *Arundinaria gigantea* (Walter) Muhl., or Giant Cane, was also common in all of the sites and with the progression of the growing season and fertilization, spread more prolifically through the plots.

### **3.1.2 - Rhizome propagation**

The sampling protocol for belowground biomass (see below) established required monthly destructive samples to be taken from within each plot. This caused some concern about the effects on the plant community left within the plot and made it necessary to identify the plant species that made up the plant communities in each plot in order to determine how the plants' roots grow.

The most common species found in the plots belong to the Cyperaceae (sedge) family and more specifically, the genus *Carex*; these species are known for being rhizomatous. Another dominant plant species in and around the plots was the giant cane (*Arundinaria gigantea*), which also uses thick rhizomes to grow. Giant cane is native to the southeastern United States and is a reedy, rhizomatous member of the *Poaceae* family with thick and tough rhizomes that it uses to propagate (Magee 2002). It typically grows between 1 and 6 m tall, but is stunted in the study site, reaching only around 0.6 to 0.9 m in most areas of the WRC. If not properly managed, giant cane can become weedy or invasive, displacing desirable or other native vegetation (Anderson & Oakes 2011). Rhizomes of the plants in the plots made it difficult to install the soil collars (Chapter 2), often preventing them from fitting into the ground without assistance.

Rhizomes are modified stems that are important for asexual reproduction and plant propagation in clonal plant species and are often used by plants to regenerate in the new growing season (Sakamaki and Ino 2006). While Bai et al. (2009) showed that severing rhizomes can cause rhizome biomass to decrease, this effect was not observed until the second growing season and Sakamaki and Ino (2006) showed that rhizome fragments can give rise to new plant populations that grow remarkably well. For this reason, destructive sampling is not expected to affect above- or belowground biomass of adjacent plants in the plots. There was even evidence that cutting the rhizomes in our plots, as Sakamaki and Ino (2006) found, gave rise to new plants (Figure 3.1).

## **3.2 - Methods**

### **3.2.1 – Site Description**

East Carolina University's West Research Campus (ECU-WRC) is a 2.35 km<sup>2</sup> mineral flat with poorly drained soils located in North Carolina's central coastal plain (Figure A-1). The WRC is among one of the highest elevated locations in Pitt County at 22-25 m above sea level (Figure A-4) and spans across, as well as drains into both the Neuse and Tar River basins (Chester 2004). The last soil survey conducted in Pitt County by the USDA shows the presence of 2 soil types within the chosen experimental sites, including Lynchburg and Rains soils (USDA NRCS 2017, Figure A-5). These two soil types are associated with elevated flatlands and Carolina Bay wetlands. They are poorly drained soils that experience seasonal flooding with a water table at or just below the soil surface (USDA NCRS 2017). With the development of the VoA site in 1960, ditched access roads were installed to allow logging, with the ditches still present around the study site today.

### **3.2.2 – Experimental design**

A random block design was established along the southwestern portion of the WRC in Area A (Figures A-2). Four sites were chosen at random (Figure A-6) spanning between two pond-like areas that are seasonally inundated with the water level rising at or above the soil surface. Four, 4 m<sup>2</sup> plots were placed in each site and a smaller 1 m<sup>2</sup> quadrat was placed inside each plot to minimize edge effects and mark where sampling would actually occur (Figure A-7). Each site contained four treatment plots, each either receiving no P fertilizer (control), low, intermediate, or high amounts of P fertilizer. Levels of P fertilizer applied were determined using the Redfield Ratio (C:N:P is

106:16:1 or N:P is 16:1; Redfield 1963). Calculations used to determine the amount of phosphorus needed to bring the soil N:P ratios in each treatment plot up to these target ratios were based on concentrations of N and P in pocosin soils previously reported (Bridgham and Richardson 1993).

From March 2017 through March 2018, phosphorus fertilizer application was alternated every two weeks with gas and biomass sampling. Biomass samples were taken from subplots marked within each 1 m<sup>2</sup> sampling quadrat to ensure that the same area was not sampled twice within the same sampling year.

### **3.2.3 - Biomass sampling**

Above- and belowground biomass samples were used to determine how pocosin plants were storing carbon in response to changes in nutrient availability and how biomass allocation between aboveground biomass (AGB) and belowground biomass (BGB) and their respective alive/dead components changed. Immediately following gas sampling (Chapter 2), AGB samples from inside each soil collar were collected and separated into living and dead biomass within a week of sample collection. Thirty cm deep soil core samplers (7.2 cm diameter) were then used to take belowground biomass cores from each collar to a depth of 25 cm, except for the days when 6- and 12-month soil samples were taken. When taking soil samples in addition to biomass samples, the core was used to take biomass samples from depths of 0-5 cm, 5-15 cm, and 15-25 cm. Soil was later sieved from the roots using a 1 mm sieve. Roots from the 3 depths in one plot were homogenized as we disregarded any potential differences..

Belowground core samples were rinsed using tap water and a 3 mm sieve to remove bulk and rhizosphere soil within a week of collection. The remaining root mass

was then separated into living and dead biomass according to buoyancy (Stagg et al. 2016). All AGB and BGB samples were dried in an oven at 60°C for 3 days until reaching a stable dry weight. Dried biomass samples were then ashed at 550°C in a Muffle furnace for 3 hours (Hoogsteen et al. 2015) to determine C lost on ignition (LOI) and ash weights were taken upon cooling. Total C in biomass was determined by subtracting sample ash weight from sample dry weight.

### **3.2.4 - Statistical analysis**

SAS 9.4 software was used to perform a repeated measures ANOVA using the PROC MIXED procedure to determine the overall effect P-addition had on carbon storage and biomass allocation in above- and belowground plant biomass as well as the ratio of alive : dead biomass in roots and shoots and the ratio of total aboveground to total belowground biomass. This design was chosen because destructive samples were taken from within the same 16 treatment plots each month rather than from new plots within the site and sites were random blocks in the experimental design. In the mixed models run, sampling date was listed as the repeated variable and sites were listed as random block effects. Treatment and sampling dates were listed as separate fixed effects in the model and were additionally crossed to determine if how the dependent variables changed through time was dependent on the treatment. Output from the analyses of above- and belowground biomass data were grouped by “alive” or “dead” when applicable to analyze the treatment’s effect on carbon content of these biomass portions.

Above- and belowground C allocation data were natural-log transformed to normalize the distribution of the residuals and better satisfy the assumption of

homogeneity of variances while accounting for non-linearity in the response variable first observed in the model; transformations of the other variables tested were not needed. The Kenward Roger degrees of freedom method was used for mixed models testing the effect of treatment on C allocation, because it accounts for small sample bias and is best suited for mixed models with partially observed data, both of which were present in the biomass data from lack of sample due to season (i.e. less AGB during the winter) or loss of sample. The default containment degrees of freedom method was used for the remaining models since missing data was not prominent enough to be detrimental to the analyses. Some data was lost either to human error during processing or a lack of biomass present in the sampling area (occurred with aboveground biomass); samples with negative carbon contents were deleted, since this indicated an error in sample handling. To prevent erroneous results and bias, sampling months with >50% missing data for any of the dependent variables tested were excluded from the analysis.

To determine the variance-covariance structure best-suited for the repeated variable in the model, multiple models were run with different covariance structures and their AIC scores were then compared. Because the autoregressive (AR) model represents random processes and are used to describe processes that vary in time, the AR(1) covariance structure or its heterogenous counterpart, ARH(1) provided the best fits for each model. The covariance structure that produced the model with the lowest AIC score and met the convergence criteria was used, which led to both AR(1) or ARH(1) being used in the mixed models.

### 3.3 - Results and Discussion

Repeated measures ANOVAs run to test the effect of fertilizing and sample date on the amount of dead and alive roots and shoots ( $\text{g m}^{-2}$ ) within the plots indicated that treatment and sample date had effects on dead aboveground and dead belowground biomass while sample date only had an effect on alive belowground biomass (Table 3.1). For both alive and dead belowground biomass ( $\text{g m}^{-2}$ ), differences between least squared means (LSMs) showed a significant difference between the high treatment and the low treatments (Table 3.2). May was also significantly different than most other months for belowground biomass, which corresponds to a slight peak in dead and alive BGB (Figure 3.2).

Analyses of the effect of treatment and sample date on the ratio of alive : dead above- and belowground biomass indicated a significant sample date effect for both alive : dead AGB and alive : dead BGB (Table 3.1). Alive : dead AGB also changed through time as a result of treatment (Table 3.1). The ratio of alive : dead AGB remained variable among treatments over time, but generally declined throughout the sampling year (Figure 3.3). This supports the death of aboveground biomass seen as fertilization treatments persisted in the study (Figure 3.4) that is indicative of the fertilizer's negative effects on the plant community, which was contrary to the hypothesis that fertilization would enhance plant productivity and biomass production.

Mixed models testing the effects of treatment, sample date, and treatment\*sample date on total AGB ( $\text{g m}^{-2}$ ) suggested that only sample date had a significant effect, while treatment and sample date had significant effects on total BGB ( $\text{g m}^{-2}$ ; Table 3.1). This reflects seasonal patterns in AGB growth and death that were

not altered by treatment and BGB growth and death that followed seasonal patterns while also experiencing significant effects as a result of fertilization. Pairwise differences for total BGB show that the high treatment plots were most different from the low and intermediate plots (Table 3.2).

Interestingly, total BGB experienced a slight decline in the months towards the end of the growing season (Sep. 2017 and Oct. 2017) before seeing an increase from Nov. 2017 through the end of Dec. 2017 (Figure 3.10b). However, total AGB only experienced a slight peak in May 2017, before leveling back off for the rest of the sampling year (Figure 3.5a). The peak in total AGB in May 2017 corresponds to two similar peaks for alive and dead AGB during the same month while the increase in total BGB in the early winter months (Nov. 2017 through Dec. 2017) correspond to simultaneous increases in alive and dead BGB (Figures 3.5c and 3.5d). While total AGB and BGB appear to be increasing during their respective time periods, the peaks do not appear to correspond in increases in solely alive or dead biomass. Despite significant changes in AGB and BGB throughout the sampling year as a function of either sampling date or treatment, the fixed effects had no significant effect on total AGB : total BGB.

The repeated measures ANOVAs did not suggest an overall difference in C storage in plant tissues as a function of treatment or treatment dependent on sample date for either above- or belowground biomass and their alive/dead portions. The models showed however, that C storage in the plants' tissues was dependent on sample date (Table 3.3). This data does not support the original hypothesis that C allocation to either above- or belowground plant biomass would change with increasing

P availability. It does however, support that allocation would follow a seasonal trend regardless of treatment level.

These differences can be seen when graphing average C content for both above- and belowground biomass during each sampling month (Figure 3.6). Carbon content for both alive and dead aboveground biomass followed a similar trend across all treatments where a consistent decrease in carbon allocation can be seen between March 2017 and June 2017. Carbon allocation increases to amounts higher or similar to those during March 2017 in July 2017. While C allocation increases across treatments for alive and dead aboveground biomass, the carbon content in the dead biomass remains lower than that of the alive biomass until January 2018.

Carbon content for dead and alive belowground biomass also followed a similar trend across all treatments, but one that was markedly different from that seen in the aboveground biomass. Carbon allocation between alive and dead biomass was relatively similar through most of the sampling season, though dead belowground biomass had slightly higher C content. During October 2017, belowground biomass experienced a drastic increase in C content, which quickly returned to levels seen in previous months by November 2017 and a slight dip in C allocation from the first sampling date in December 2017 until January 2018. From January 2018 until the last sampling day in March 2018, C content converged among treatments and between alive and dead biomass.

### 3.4 - Conclusion

Fertilization treatments had the greatest effect on belowground biomass compared to the aboveground biomass. Aboveground biomass was more influenced by sample date, indicating a strong relationship between AGB ( $\text{g m}^{-2}$ ) and season that is not easily changed with nutrient addition. The ratio of alive : dead AGB did change through time dependent on treatment, but was also affected by sample date, which further contributes to there being a strong seasonal trend in AGB production and death. The change in alive : dead AGB through time with treatment is likely due to nutrient toxicity. Plants within intermediate and high treatment plots showed signs of nutrient stress with yellowing or browning leaves as fertilizing progressed.

While belowground biomass ( $\text{g m}^{-2}$ ) was affected by nutrient addition and sample date compared AGB, the seasonal influence on total BGB was still present as treatment over time did not affect the response variable. Alive : dead BGB was only affected by sample date, further pointing to a strong seasonal trend in BGB production and death that was not affected by treatment over this short sampling period. When considering the effect of treatment on alive : dead AGB that might point to nutrient toxicity in the plants as fertilization progresses, the lack of treatment effect on alive : dead BGB indicates that aboveground tissues were likely burned from the fertilizer application. Regardless, the fertilizer's effect on AGB tissues did not appear to hinder plant production since total AGB : total BGB were not affected by treatment or sample date and alive AGB was only affected by sample date, not treatment.

Although C allocation in biomass appeared to remain uninfluenced by P fertilization in P-limited soils, further studies should be conducted for longer periods of

time and over several growing seasons to see if C allocation changes over time after more than one growing season. While the data shows slight trends in C allocation through a growing season, it is evident that there is a strong seasonal effect on biomass C content and this effect should be explored further.

Another factor attributed to the slight decline in C allocation throughout the growing season is nutrient toxicity. Plants within intermediate and high treatment plots showed signs of nutrient stress with yellowing leaves and an increase in dead biomass as fertilizing went on. Despite this, there is no evidence that plant die-off from fertilizing affected the C content of above- or belowground biomass, both dead and alive (Figure 3.6). Additional studies with lower amounts of P applied across all treatment plots could provide insight into whether this apparent lack of trend can be contributed to nutrient toxicity or a seasonal pattern that plants are able to maintain, even after nutrient addition.

This study indicates that C allocation in plants characteristic of pocosins has a strong seasonal pattern that is not easily affected by nutrient addition. While adding P to a P-limited wetland in the right amounts may decrease CO<sub>2</sub> flux in the short-term (Chapter 2), it does not increase above- or belowground biomass in the same time frame. It is likely that nutrient addition would need to occur over several growing seasons before more C is allocated to plant tissues. Lower CO<sub>2</sub> fluxes paired with no change in C allocation can possibly be attributed to soil microbes utilizing applied P faster than plants.

Overall, this study indicated that fertilizing a P-limited freshwater wetland with P fertilizer has no significant effect on C allocation in plant biomass, which could have led

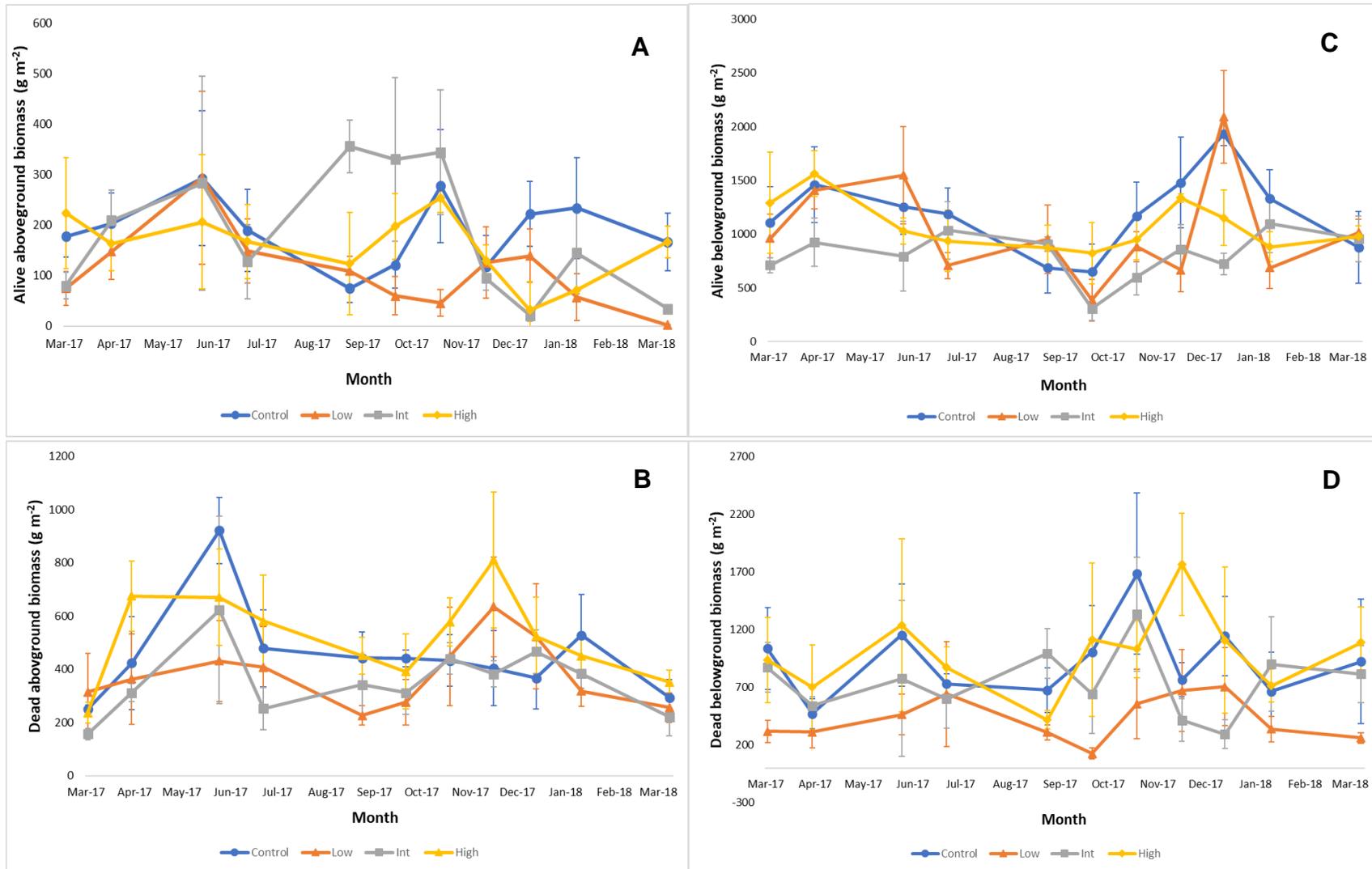
to increased stored C in plants and ultimately the soil and increased C sequestration over time. Further studies should be done to assess whether the seasonal trend in C allocation in plant tissues observed can be changed to allow for increased C storage over time and if there is a soil microbe priming effect.

### 3.5 - Tables and Figures

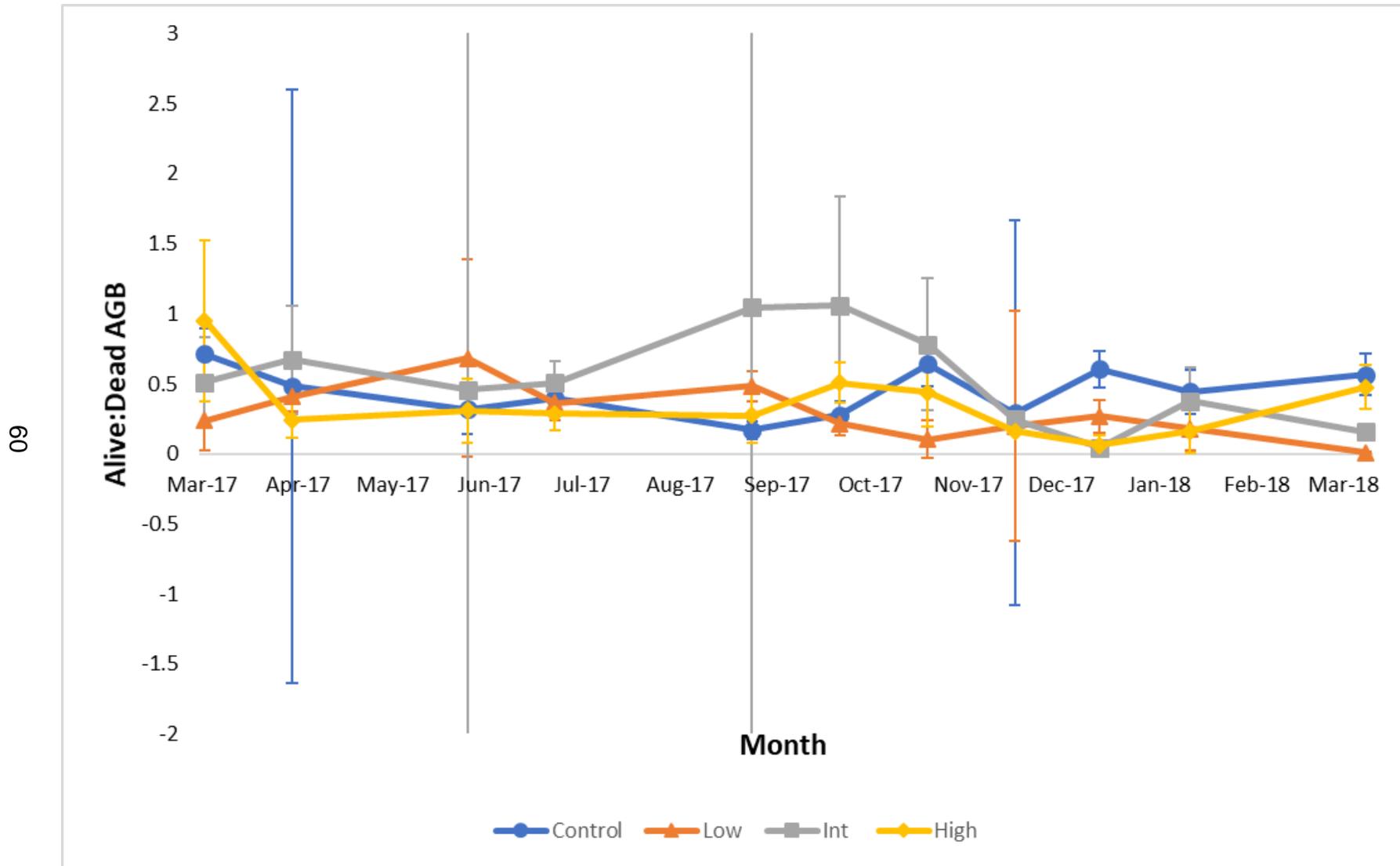


**Figure 3.1** New rhizome growth into a soil core hole from previous sampling (date: 23Feb18).

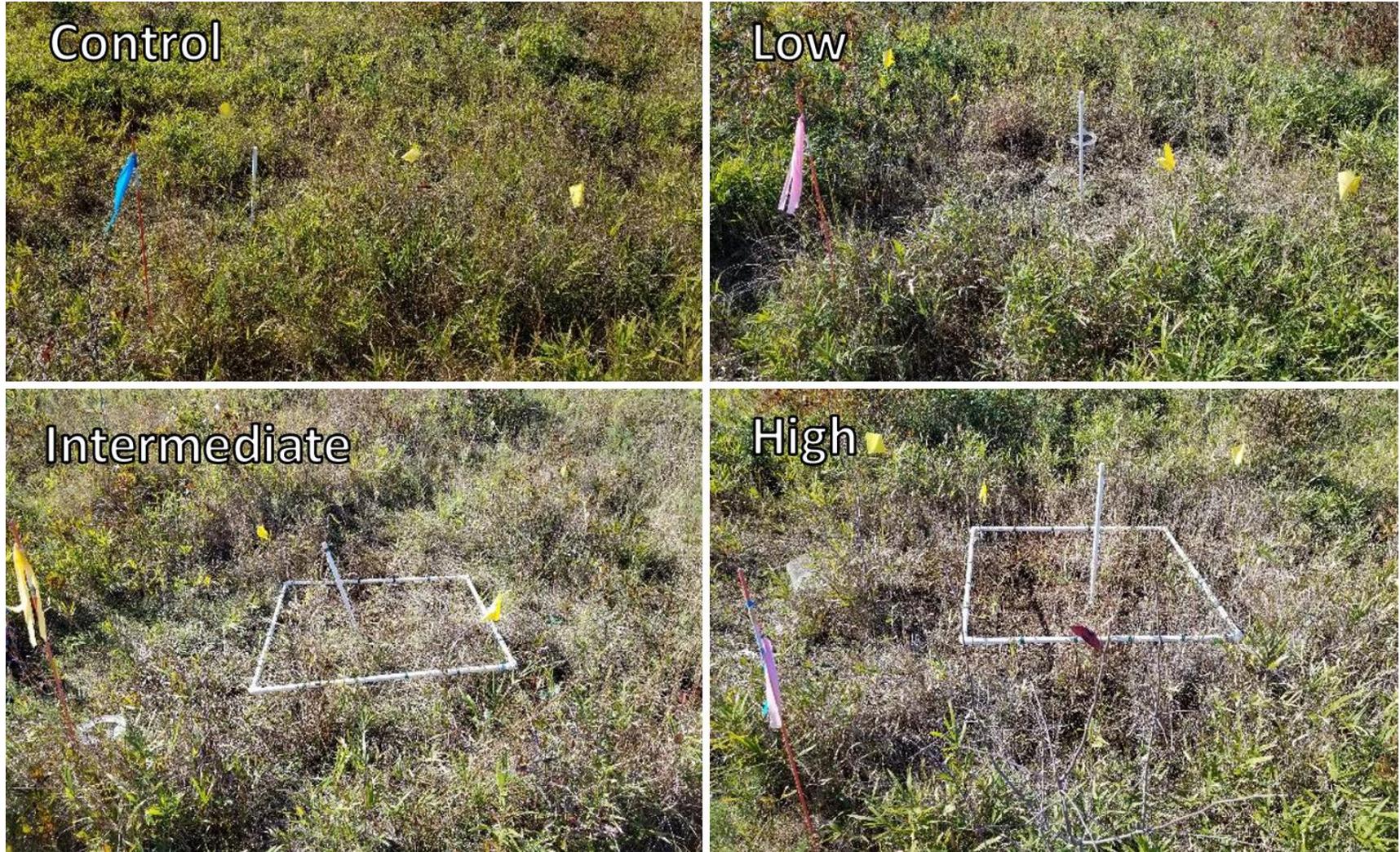




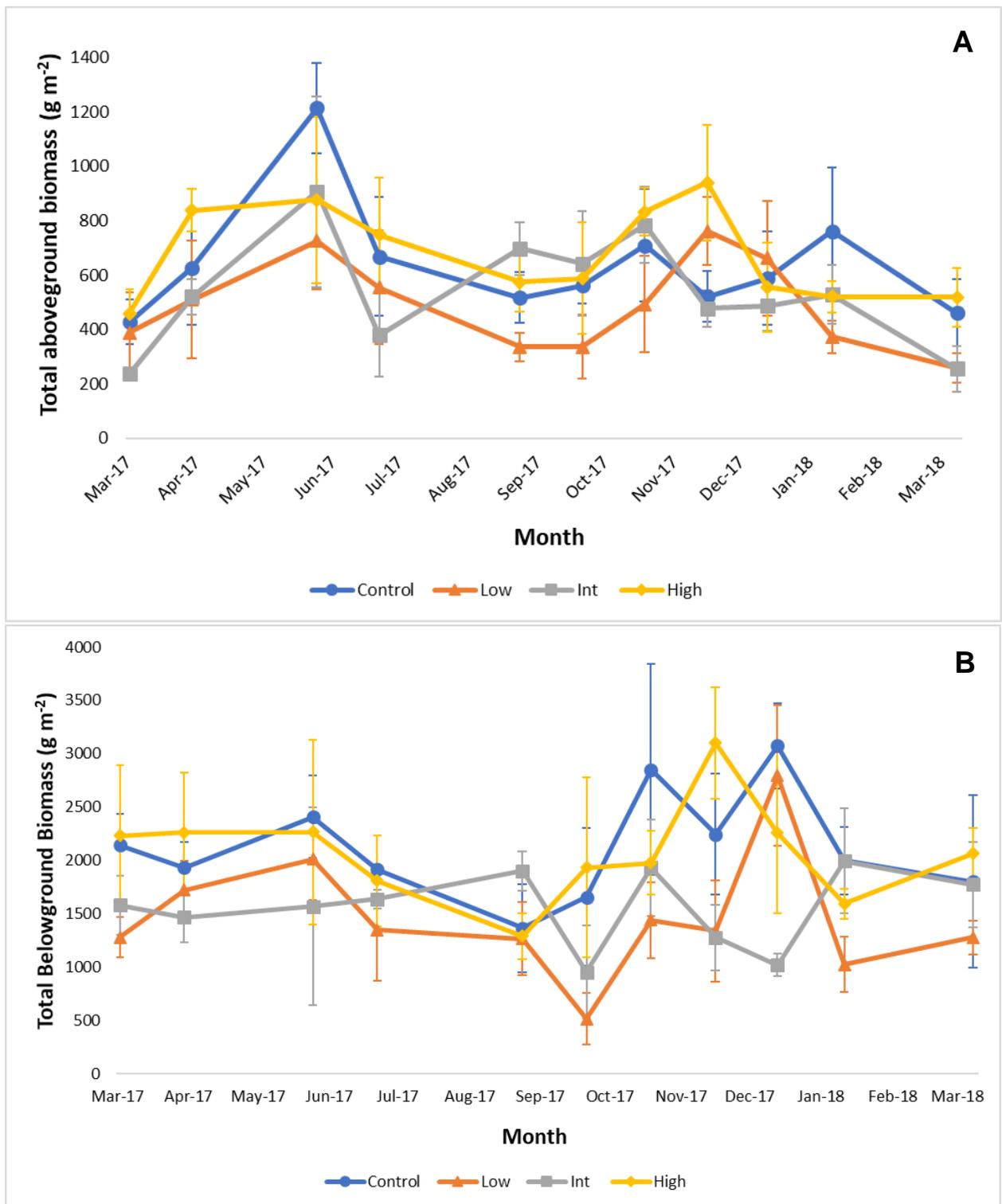
**Figure 3.2** Changes in a) alive AGB, b) dead AGB, c) alive BGB, and d) dead BGB through the sampling season. Error bars represent standard error of the mean,  $n=4$ . There are peaks in alive and dead BGB in Nov. 2017-Dec. 2017 and a slight peak in May 2017. Both alive and dead AGB experienced peaks in May 2017 and the early winter months of 2017., *Int* represents the intermediate treatment.



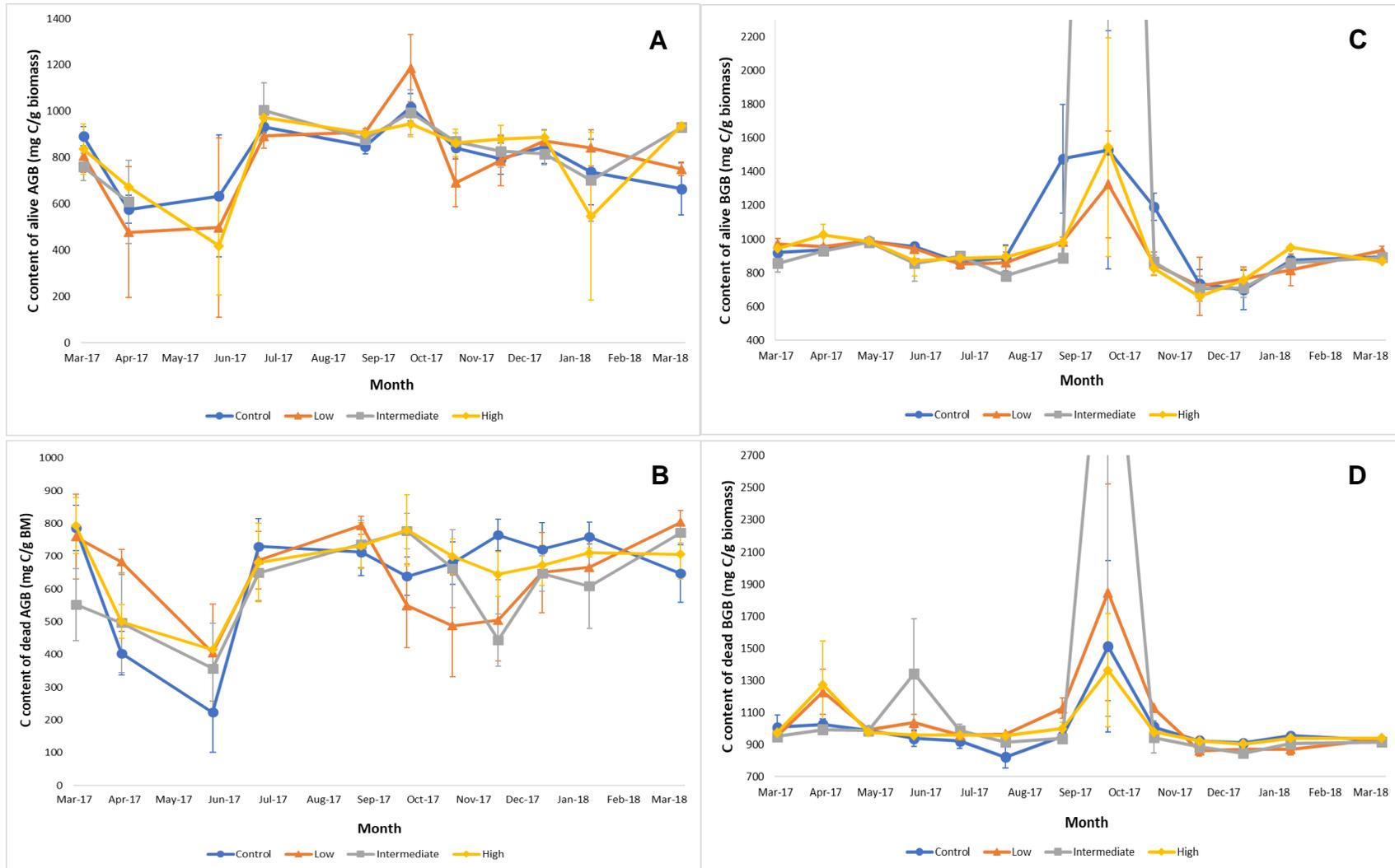
**Figure 3.3** Trend in Alive : Dead AGB through the sampling season with variation within treatments, but a general decline in the ratio overall. Error bars represent standard error of the mean, n=4. Standard errors for the intermediate treatment were off the graph for some months and values are as follows: Jun-17 SE±5.06 and Sep-17 SE±3.27.



**Figure 3.4** Differences in aboveground biomass within treatment plots after 8 months of fertilizer treatment. “Control,” “Low”, “Intermediate,” and “High,” refer to treatment levels of the plots pictured.



**Figure 3.5** Changes in a) total AGB and b) total BGB through the sampling season showing peaks in AGB in May 2017 and peaks for BGB in Nov. 2017 through December 2017. *Int* represents the intermediate treatment. Error bars represent standard error of the mean,  $n=4$ .



**Figure 3.6** Changes in C content in a) alive AGB, b) dead AGB, c) alive BGB, and d) dead BGB in mg C per g biomass through the sampling year. Error bars represent standard error of the mean, n=4. In c) intermediate for Oct. 2017 was  $9079.47 \pm 8183.18$  mg C/g biomass and in d) intermediate for Oct. 2017 was  $4058.03 \pm 2981.69$  mg C/g biomass.

**Table 3.1** Summary statistics for repeated measures ANOVAs results for statistically significant effects of treatment, sample date, and treatment\*sample date on above- and belowground biomass weights and C allocation. Results with  $p < 0.05$  are shaded.

| Variable              | Treatment |        |         |         | Sample date |        |         |          | Treatment*Sample date |        |         |         |
|-----------------------|-----------|--------|---------|---------|-------------|--------|---------|----------|-----------------------|--------|---------|---------|
|                       | Num DF    | Den DF | F Value | Pr >  F | Num DF      | Den DF | F Value | Pr >  F  | Num DF                | Den DF | F Value | Pr >  F |
| <i>Dead AGB</i>       | 3         | 129    | 3.31    | 0.0222  | 11          | 129    | 4.49    | < 0.0001 | 33                    | 129    | 0.73    | 0.8551  |
| <i>Dead BGB</i>       | 3         | 163    | 3.09    | 0.0287  | 13          | 163    | 7.15    | < 0.0001 | 39                    | 163    | 0.82    | 0.7702  |
| <i>Alive BGB</i>      | 3         | 164    | 2.22    | 0.0873  | 13          | 164    | 24.94   | < 0.0001 | 39                    | 164    | 1.10    | 0.3379  |
| <i>Alive:Dead AGB</i> | 3         | 110    | 1.18    | 0.3210  | 11          | 110    | 3.72    | 0.0002   | 33                    | 110    | 2.52    | 0.0002  |
| <i>Alive:Dead BGB</i> | 3         | 162    | 0.61    | 0.6063  | 13          | 162    | 3.27    | 0.0002   | 39                    | 162    | 0.89    | 0.6623  |
| <i>Total AGB</i>      | 3         | 132    | 1.92    | 0.1295  | 11          | 132    | 4.63    | < 0.0001 | 33                    | 132    | 0.89    | 0.6404  |
| <i>Total BGB</i>      | 3         | 165    | 3.57    | 0.0154  | 13          | 165    | 29.15   | < 0.0001 | 39                    | 165    | 1.36    | 0.0948  |

**Table 3.2** Pairwise comparisons from PROC MIXED repeated measures ANOVA showing significant differences between treatments on alive and dead above- and belowground biomass quantities.

| <b>Variable</b>  | <b>Comparison</b> | <b>DF</b> | <b>t Value</b> | <b>Pr &gt;  t </b> |
|------------------|-------------------|-----------|----------------|--------------------|
| <b>Alive BGB</b> | low/high          | 164       | 2.08           | 0.0387             |
|                  | intermediate/high | 164       | 2.17           | 0.0312             |
| <b>Dead BGB</b>  | low/high          | 163       | 3.03           | 0.0028             |
| <b>Dead AGB</b>  | low/high          | 129       | 2.50           | 0.0135             |
|                  | intermediate/high | 129       | 2.86           | 0.005              |
| <b>Total BGB</b> | low/high          | 165       | 3.10           | 0.0022             |
|                  | intermediate/high | 165       | 2.21           | 0.0287             |

**Table 3.3** Statistics from the PROC MIXED repeated measures ANOVA showing significant effects of sample date on C storage in alive and dead AGB and BGB biomass portions. Treatment and treatment\*sample date did not have significant effects on C allocation and are not shown. Data was natural log transformed to normalize the distribution of the residuals.

|                  | <b>Num DF</b> | <b>Den DF</b> | <b>F Value</b> | <b>Pr &gt;  F </b> |
|------------------|---------------|---------------|----------------|--------------------|
| <b>Alive AGB</b> | 10            | 17.3          | 2.63           | 0.0376             |
| <b>Dead AGB</b>  | 10            | 30.1          | 2.59           | 0.0214             |
| <b>Alive BGB</b> | 12            | 28.4          | 11.46          | <0.0001            |
| <b>Dead BGB</b>  | 12            | 31            | 13.65          | <0.0001            |

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# **Chapter 4: Nutrient addition effects on soil nutrient stoichiometry in an NC pocosin**

## **4.1 - Introduction**

### **4.1.1 - Soil survey**

The most current soil survey data provided by the US Department of Agriculture on their interactive web soil survey indicates that the different soil types identified at the WRC are various sandy loams associated with coastal areas. Two soils, Lynchburg and Rains, are present where the study sites for this research as indicated by the soil survey (USDA NRCS 2017).

Lynchburg soils are reported to be found at elevations of 0-31 m above sea level in Atlantic Coast flatwoods (marine terraces) with a slope of 0-2%. They are classified as poorly drained soils with a water table at or close to the soil's surface, approximately 15-46 cm below, and low availability to store water in the soil profile. Goldsboro soil is a minor component of Lynchburg soil, comprising about 8% and is also associated with marine terraces and broad interstream divides on these terraces (USDA NRCS 2017).

Rains soils can be found at elevations between 20-160 ft (6-49 m) above sea level in Carolina bays on marine terraces, flat areas on marine terraces, and in interstream divides between these landforms. They are defined as poorly drained soils with a water table at or just below the soil surface, approximately 0-12 in (0-33 cm) and a high ability to store water in the soil profile. Lynchburg soils make up 8% of Rains soils, while Pantego, a ponded soil type associated with a concave across-slope shape, comprises 6% of Rains soils (USDA NRCS 2017).

#### **4.1.2 - Site soil and geological history**

The geological development and growth of Coastal Plain of Eastern NC pocosin peatlands show that over the last million years, sea level has varied from 200 ft (61 m) above to as much as 400 ft (122 m) below its current level due to the expansion and contraction of the polar ice caps (Daniel 1981). The presence of scarps and ridges along the coastal plain of NC indicates that the rise in sea level was not continuous, but experienced pauses long enough to cause these formations. When the last great expansion began 75,000 years ago, sea level was higher than it currently is, and the shoreline was situated along what is called the Suffolk Scarp (Figure 4.1). Following the Wisconsin Ice Age 15,000 years ago, sea level fell well past its current height, exposing large expanses of the continental shelf and allowing streams to form and carve complex drainage patterns (Daniel 1981).

For the past several thousand years, sea level has been slowly rising, drowning the rivers and streams that had formed along the continental shelf. Stream velocities started to decline, allowing for the buildup of sediment and thus vegetation and water tables rose, creating damper soils. As a result, about 8,900 year ago, peatlands began forming on interstream divides and in low drainage areas as the conditions created from rising sea levels allowed for peat accumulation (Daniel 1981). Today, peatlands envelope interstream uplands and are found on flat, clay soils in shallow basins (Richardson 2012).

The research site, about 30-40 km west from the Suffolk Scarp (Figure 4.1), is situated in a large river valley and floodplain system in what used to be the lower coastal plain 75,000 years ago when the shoreline lay along the scarp (Ingram and Otte

1981). Soil survey data for the site is supported by its geological history. Today, the pocosin being studied lies upland of and drains into the Neuse and Tar Rivers (Chester 2004) with no visible stream input (Figure A-4) and rain as its only source of water.

#### **4.1.3 – P and pocosins**

Pocosins are freshwater ombrotrophic wetlands commonly referred to as evergreen shrub bogs that are found spanning across the Southeastern United States from southern Virginia to northern Florida on broad, flat river basins near estuaries (Richardson 1983; Brinson 1991). The soils in these wetlands are characterized by low nutrient availability and peat layers that can be anywhere from a few cm to several meters deep (Bettis, Sr. et al. 2009) with underlying mineral and sandy humus soils (Richardson 2012). It has been estimated that NC's peatlands, using geological and fire history of the area, contain 529 million metric tons of carbon-rich peat (Richardson 2012). Though pocosins have nutrient poor soils that are deficient in P and N, they are typically P-limited, with N:P ratios decreasing with pocosin succession from short to tall pocosin to gum swamp (Bridgham and Richardson 1993; Walbridge 1991).

Development of NC swamps dates back to the 1700s. Swamps were originally ditched and drained initially for timber production and later in the early to mid-1900s, agriculture (Lilly 1981). Lilly (1981) reports that almost all of the swamp land in NC has been logged and drained to some extent at least once. Despite being ecologically important for several rare and endangered plant and animal species and holding high floral and faunal diversity (Richardson 1983; Brinson 1991), only about 5% of pocosins are protected from federal and state use (Richardson et al. 1981). The ditching of pocosins changes their hydrology from a diffuse runoff pattern into nearby streams to

runoff that is more directly channeled to discrete discharge points with increases in peak and low flows (Daniel 1981). Pocosin land developed for agriculture also requires large amounts of lime to increase soil pH and fertilizer to increase soil nutrients for crop production (Richardson 1983). The channelization of pocosin outflow combined with increased nutrient inputs to the soil often means that downstream systems are receiving higher amounts of dissolved nutrients and suspended sediment, which has been well-studied in streams and rivers into which pocosins drain, but not on the subsequent estuaries (Brinson 1991). Barber et al. (1979) studied the influence of agricultural development on peat-covered soils on regional water quality. This study suggested that drastically altered pocosins cannot buffer their downstream estuaries against freshwater influx or saltwater intrusion. Skaggs et al. (1980) showed that conversion of pocosin to farmland increased TN and TP outputs to downstream systems threefold. Additional studies have shown that as fertilization of disturbed pocosins continues, the capacity of these peatlands to store P long-term became limited (Burke 1975; Duxbury and Peverly 1978).

East Carolina University's West Research Campus (ECU-WRC), where the study sites are located, is a 2.35 km<sup>2</sup> mineral flat in North Carolina's central coastal plain (Figure A-1). The land the research campus now sites on was extensively logged, mowed, and managed while the Voice of America (VoA) occupied the property (Chester 2004). Drainage ditches and access roads that were installed to drain the wetland and allow logging are still present around the WRC today. Following purchase of the West Research Campus by East Carolina University, a wetland delineation was performed and determined the site to be mostly pocosin (Chester 2004; Kimley-Horn and

Associates, Inc.). Since the delineation, the research campus has become the site of ecological restoration projects and has been managed for restoration through natural succession (Chester 2004).

Several studies have examined the relationships between nutrient availability and ecosystem function using fertility gradients in N-limited systems (Pastor et al. 1984; Birk and Vitousek 1986), but studies of P-limited ecosystems and fertility gradients therein have been rare in comparison (Walbridge 1991). Pocosins in themselves are understudied wetlands that are unique with their extreme soil environments (Bridgham and Richardson 2003). Assessing how increasing the limiting nutrient in pocosin soils affects soil nutrient stoichiometry can provide insight into potential changes in ecosystem services.

This study specifically focused on ecosystem changes in response to P-addition to a pocosin under restoration. By assessing changes in soil nutrient stoichiometry, we could identify seasonal changes in plant and soil microbe function as well as functional changes in response to treatment and treatment over time. We also wanted to know if P-addition would increase soil organic carbon (SOC) content through increase plant productivity and root biomass allocation.

## **4.2 - Methods**

### **4.2.1 – Experimental design**

Along the southwestern portion of the WRC in Area A (Figures A-2), four sites were chosen at random (Figure A-6), spanning between two pond-like areas that are seasonally inundated with the water level rising at or above the soil surface. Four, 4 m<sup>2</sup>

fertilization plots were placed in each site with smaller 1 m<sup>2</sup> sampling quadrats placed inside to minimize edge effects during sampling.

Each site contained four plots, each either receiving no P fertilizer (control), low, intermediate, or high amounts of P fertilizer. Levels of P fertilizer applied were determined using the Redfield Ratio (C:N:P is 106:16:1 or N:P is 16:1; Redfield 1963) where the intermediate treatment would bring the N:P ratio of the soil to 16:1, the low treatment to a ratio of 32:1, and the high treatment to a ratio of 8:1. Calculations used to determine the amount of phosphorus needed to bring the soil N:P ratios in each treatment plot up to these target ratios were based on amounts of N and P in pocosin soils reported by Bridgham and Richardson (1993). The amount of P applied was significantly greater than the amount of P applied as fertilizer to agricultural fields between the 1960s and 2014 (28-40 kg km<sup>-2</sup> yr<sup>-1</sup>; US EPA 2018) and found in wastewater (Richardson and Marshall 1986). Preliminary soil samples had been taken from the WRC to determine N:P ratios, but soil concentrations of P produced unreliable results.

#### **4.2.2 - Soil sample collection**

From March 2017 through March 2018, phosphorus fertilizer was applied every four weeks and sample were taken two weeks after fertilizing. Samples were collected using soil cores 7.2 cm in diameter and placed in gallon-sized Ziploc bags for transport back to the lab. Soil samples were transported in a cooler to keep temperatures stable. Two initial soil samples (0 months) were taken from within each of the four sites at depths of 0-5 cm (surface) and 5-15 cm (mid) and then homogenized. Additional soil samples were taken with biomass cores from the subplots marked inside of the 1 m<sup>2</sup>

sampling quadrat at 6 and 12 months from depths of 0-5 cm (surface), 5-15 cm (mid), and 15-25 cm (deep). Cores were taken back to lab and later processed to remove the soil from biomass samples.

#### **4.2.3 - Sample preparation**

The soil samples from each site taken at each depth were homogenized. Subsamples from each of the homogenized samples weighing 25-30 g were taken and dried at 60°C for 5 days or until reaching a stable weight. The rest of the samples not taken for analysis were stored at -20°C. Samples were ground using a mortar and pestle and stored in scintillation vials at -20°C until nutrient extraction.

For soil samples at 6 and 12 months, biomass cores were sieved with a 1-3 mm sieve (size dependent on sample moisture) to separate soil from root biomass. Soil was collected from the sieve onto foil, placed in Ziploc bags, and stored at 4°C before weighing it out. Within a week of sieving, at least 25 g of soil from each sample was weighing out into aluminum weigh boats to dry at 60°C for 5 days or until reaching a stable weight. If there was still sample left after weighing out 25 g it was stored at -20°C. Once reaching a stable dry weight, soil samples were ground with a mortar and pestle and stored in scintillation vials at -20°C until extraction.

#### **4.2.4 – Available N**

$\text{NH}_4^+$  and  $\text{NO}_3^-/\text{NO}_2^{2-}$  (referred to as nitrates or  $\text{NO}_x$ ) in the soil was determined by shaking 5 g of oven-dry soil with 50 mL of 2 M KCl in a polypropylene bottle for 1-hr on a reciprocating shaker (Mulvaney 1996). Considerations were taken from Jones and Willett (2006) on the best sample preparation and extraction method to use. After shaking, samples were allowed to settle for 30 minutes, or until the supernatant was

clear. Samples were then filtered using Whatman No. 42 filter paper and filtrates stored in polypropylene bottles at 4°C or -20°C if analysis was not to occur for more than a day. Sample filtrates were analyzed in the Environmental Research Lab (ERL) at East Carolina University using a SmartChem 170 discrete autoanalyzer (Unity Scientific, Milford, MA) for  $\text{NH}_4^+$  and then  $\text{NO}_x$ .

#### **4.2.5 - Phosphorus**

The ignition method outlined by Andersen (1975) was used to determine total phosphorus (TP) of soil samples. Samples were weighed (0.15-0.2 g) and ashed at 550°C for 1 hr to convert organic P to inorganic P. Ashed sample weights were recorded. TP was extracted from the sample by boiling the residue in 25 mL of 1N HCl for 15 minutes in 125 mL Erlenmeyer flasks. After boiling, samples were diluted to 100 mL with 1N HCl.

Inorganic P was determined using the HCl extraction method outlined above, but samples were not ashed. Both TP and IP extracts were allowed to settle for 30 minutes or until the supernatant was clear. Samples were then filtered using Whatman No. 42 filter paper and filtrates stored in polypropylene bottles at 4°C or -20°C if samples were not going to be analyzed for more than one day. Samples were analyzed at ECU's ERL using a SmartChem 170 (Unity Scientific, Milford, MA).

Organic P was calculated by subtracting inorganic P found in the HCl extraction from TP found in the ignition method in the unignited soil (Vasilas et al 2013).

Wuenscher et al. (2015) compare 14 soil phosphorus extraction methods and report HCl and ignition extraction methods to have the highest P recovery.

#### **4.2.6 – Total Carbon and Total Nitrogen**

Carbonate content in soils were tested using the aqueous HCl method described in Komada et al. (2008). Because it was suspected that carbonate content in our soils would be low or nonexistent, 30 samples were chosen at random from all sample dates, sites, and treatments and tested for the presence of carbonates. While there was some inorganic carbon present in the samples, it was less than 1% of the sample weight and soils from the sites are not characteristically calcareous (USDA NRCS 2017). It has also been noted that carbonate removal with HCl can overestimate IC content in soils (Wotherspoon et al. 2015). Taking these factors into consideration, it was determined that TC could be considered as representative of OC.

Samples for total C and total N analysis were weighed and sent in glass scintillation vials to the Environmental and Agriculture Testing Service at NC State University (Raleigh, NC) to be determined by combustion analysis.

#### **4.2.7 - Statistical analysis**

SAS software version 9.4 was used to run a repeated measures ANOVA using the PROC MIXED procedure to determine the overall effect that addition of P to the wetland under study had on soil nutrient concentrations and stoichiometry. This design was chosen because samples were taken from the same sites and plots at the designated sampling dates. Due to the experiment's random block design, sites were listed as random block effects and treatment, sample date, and treatment\*sample date were listed as fixed effects. Treatment and sampling date were crossed in the model to determine if how the dependent variables changed through time was dependent on the

treatment. Output was grouped by depth of the soil sample to analyze any effects of treatment or sample date throughout the sampled soil profile.

The variance-covariance structure best-suited for the repeated variable in each model was chosen by running multiple models with different covariance structures and then comparing the subsequent AIC scores. The covariance structure that produced the lowest AIC score and met the convergence criteria was used. For most of the dependent variables, the compound symmetry (CS) covariance structure, where all variances and covariances are equal was used. The rest of the dependent variables were best represented by the variance components (VC) structure, where each variance is different and covariance equal 0.

### **4.3 - Results and Discussion**

Summary statistics for statistically significant interactions can be found in Table 4.1. Repeated measures ANOVAs indicated strong seasonal effects for TP in the surface and mid layers and for  $\text{PO}_4^{3-}$  at all 3 depths. Treatment had the largest effect on TP and  $\text{PO}_4^{3-}$  in the surface layers, which was to be expected with the method of fertilizer application.  $\text{PO}_4^{3-}$  was found to be affected by treatment in the deep layer (15-25 cm), but this does not correspond to changes in TP or organic P in the deeper soils. Because the fertilizer was dissolved in water before applied, forming  $\text{H}_3\text{PO}_4$  that would be converted to  $\text{PO}_4^{3-}$  in the ecosystem, it is likely that the  $\text{PO}_4^{3-}$  leached through the soil to the deeper layers. This however, would affect the concentration of TP in the deep layer, which is not seen. The increase in  $\text{PO}_4^{3-}$  and the lack of change in TP in soil from 15-25 cm suggests a more complicated interaction either between root biomass (though sparse compared to the median soil layers), soil microbes, or both.

Pairwise comparisons for the 3 P portions show the most difference occurring between the control and intermediate, control and high, and low and high treatments in the surface layer for TP and  $\text{PO}_4^{3-}$  and the control and high treatment for organic P in the surface layer and  $\text{PO}_4^{3-}$  in the mid layer (Table 4.2). Additionally,  $\text{PO}_4^{3-}$  had statistical differences among treatments in the deep soil layer where OP and TP did not. This correlates to the statistically significant change in  $\text{PO}_4^{3-}$  with treatment and sample date in the deep layer that is not seen in the other 2 P portions. Total, inorganic, and organic portions of P were not affected by treatment through time and organic P was not statistically affected by treatment or sample date at any depth in the soil sampled. These results were as expected since fertilizer was applied as  $\text{PO}_4^{3-}$ , which would increase TP and not organic P concentrations. Organic P is biologically unavailable and must go through transformation before it is usable to plants and microbes in the ecosystem (Dunne and Reddy 2005), so it is not surprising that, when usable  $\text{PO}_4^{3-}$  was more readily available from fertilization treatments, organic P fractions were not affected.

Total nitrogen in the soil was only affected by treatment through time at the deep level (Table 4.1). Increases in TN concentrations can be seen in the surface and mid soil layers between the samplings at 0 and 6 months across all the treatments, with increases continuing from 6 to 12 months in the surface layers of the intermediate and high treatments (Table 4.3). Increases in TN content were also seen between 6- and 12-month samplings in the mid and deep layers of control plots and deep layers of the low treatment. Overall, it appears that TN content in the soil at all sampled levels, though not statistically significant is a result of sample date and changes in the

ecosystem possibly due to variable weather and environmental factors (i.e. precipitation, disturbance or lack of) and the subsequent changes in plant and microbial activity.

$\text{NH}_4^+$  increased from the 0- to 6-month samplings in the mid soil layers across all treatments and between the 6- and 12-month samplings across all depths and treatments (Table 4.3). The repeated measures ANOVAs showed that  $\text{NH}_4^+$  was affected by sample date at all 3 sampled depths but was not affected by treatment or treatment through time. The observed seasonal changes point to a strong seasonal pattern in nitrogen mineralization that fertilizing did not change over one growing season.

$\text{NO}_x$  on the other hand, was not affected by sample date, only by treatment in the surface layer. Pairwise comparisons show that  $\text{NO}_x$  was most different between the control treatment and the low, intermediate, and high treatments at the surface level and the control and low treatment between 15-25 cm (Table 4.2). Looking at changes in nitrate concentrations between sampling points (Table 4.3) reveals that  $\text{NO}_x$  concentrations decrease across all sites and treatments from 0 to 6 months except in the surface layer of the high treatment. Nitrate concentrations then increase from 6 to 12 months in the mid soil layer of the low, intermediate, and high plots. This indicates that treatment likely increases nitrification rates in the oxidized soil layer containing the greatest portion of root biomass in conjunction with sample date.

Soil N:P ratios decreased from the 0- to 6-month sampling dates in the surface and middle soil layers for all treatment plots except the control (Table 4.3). These ratios further decreased from 6 to 12 months across all treatments and depths (except the

deep layer in the intermediate plots). By the 12-month sampling, N:P ratios in most of the plots indicated that N became the limiting nutrient. Furthermore, PROC MIXED models showed that soil N:P ratios at all three depths were affected by treatment and by sample date in the mid layer (Table 4.1). The effect of sample date on N:P ratios in the mid layer is likely due to seasonal changes in plant and microbial activity in the soil as the soil depth from 5-15 cm is where most of the plant roots were located. Sample date was close to significantly significant in the deep layer, which might also be explained by plant and microbial activity because while some roots were present from 15-25 cm, there was far less root biomass present compared to the middle soil layer sampled (Figure 4.2). The deep layer was also most affected by seasonal changes in the water table, which would affect microbial activity and nutrient movement in these soils. Pairwise comparisons show (Table 4.2) the most difference between the control treatment and three treatment levels in the surface layer, the control treatment and three treatment levels as well as the low and high treatments in the mid layer, and the control treatment and intermediate and high treatments as well as the low and intermediate treatments in the deep layer.

Unexpectedly, there were no significant changes in soil total organic carbon as a function of treatment, sample date, or treatment through time and no significant differences in TOC among any treatments at any soil depth. While this shows that fertilizing did not affect C mineralization, it also means that C immobilization was not changed. This result, in combination with the changes in flux rate with treatment (Chapter 2) and lack of change in C allocation in root and shoot biomass (Chapter 3)

points to soil microbial communities as the main drivers for the changes in nutrient dynamics that are seen.

#### **4.4 - Conclusion**

While treatment did significantly affect  $\text{PO}_4^{3-}$  concentrations and N:P ratios in the wetland soil across most treatments and depths, it did not seem to have much of an effect on the other soil nutrient concentrations and had no effect on TOC. Sampling date was largely responsible for changes in TN,  $\text{NH}_4^+$ , and nitrates likely due to changes in plant and soil microbe activities and other environmental factors such as precipitation and disturbance. Lack of disturbance is a potential culprit because pocosins experience natural, low intensity burns frequently (every 3-5 years), which help maintain the shrub-scrub plant communities and health of the ecosystem by changing the soil nutrient stoichiometry and maintaining fire-reliant plant species that are found there (Wilbur and Christensen 1983; Christensen et al. 1981; Chester 2004). The pocosin under study, though contracted to be burned, has not been burned in several years evidenced by fire scares on resident pine trees (Figure 4.3).

Changes in the soil nutrient stoichiometry could also be due to in large part to soil microbes. Richardson and Marshall (1986) showed that while P fertilization over one year did not affect nutrient uptake to and ratios in plant tissues, it did increase P concentrations in soil microorganisms. Soil microbes have a greater influence on new additions of P into pocosins in the first year of fertilization (Richardson and Marshall 1986) than originally thought. While plant growth is limited to the nutrients available to plants, microorganism growth and activity is also limited to the available nutrients.

Microbes play a large role in nutrient mineralization and immobilization in soils, thus affecting what is available to plants.

The lack of change seen in the TOC content of the soil might be related to the cycling of organic matter in the soil. This study only accounted for the total C stock in the soil without considering different fractions of C that compose soil organic stocks such as lignin, cellulose, and easily-decomposed compounds like sugars, starches, and proteins (Sahu et al. 2017). Fertilization could have affected the SOM and SOC content by increasing the rate of turnover within the labile C pool through increased plant and microbe productivity, something that would not necessarily be reflected in soil TOC concentrations.

Interestingly, our results showed a treatment-related increase in nitrates in the mid soil layers of the treatment plots (Table 4.3) that despite not being statistically significant, is likely still ecologically significant. Root biomass was concentrated in the mid soil layers with only a small fraction of belowground biomass found in the 15-25 cm soil cores. In most of the sites, observed during monthly biomass sampling and coring (Chapter 3), the water table never dropped below the depth of the biomass cores (30 cm) and only came up to right below the soil surface during the coldest winter months (November 2017 – February 2018) when the evapotranspiration rate dropped. This means the mid soil layer was oxidized for most of the sampling year, allowing for nitrification rates to increase. This seasonal trend, when coupled with P addition, is likely the reason nitrification rates in the treatment plots exceeded those in the untreated control plots throughout the sampling year.

N:P ratios from this study were far from the Redfield Ratio of N:P = 16:1 (Table 4.3; Redfield 1963) or the N:P ratios that have since been suggested to be more characteristic of wetland soils (i.e. Wang and Moore 2014; Benitez-Nelson 2000). After 6 months of P addition, intermediate and high treatment plots had N:P ratios indicative of N-limited soils. This became even more true by month 12 and then applied to the low treatment plots as well. Plant communities found in pocosins are adapted to extreme soil conditions (Richardson 2012) and the compiled plant list from our study sites (Chester 2004) reports several plant species that are tolerant to low nutrient availability, especially low P. The dramatic increase in bioavailable P in the soils likely led to nutrient toxicity resulting in a decrease in plant growth and increase in plant death (Figure 4.4).

Overall, these results, while compelling in some respects, point to the need for a longer study that extends over several growing seasons and incorporates soil microbe assays. A factorial experimental design is suggested where N and P are added in varying concentrations together and separately. This will test how addition of both nutrients to different degrees affects soil stoichiometric ratios and is more likely to prevent fertilization from changing which nutrient is the limiting factor.





**Figure 4.2** Soil profile from a core approximately 22 cm deep. The higher concentration of roots in the first 15 cm of soil is evident by the darker soil color with few roots being present in the lighter sandy mineral soil further down.



**Figure 4.3** Fire scars on a pine tree located near the sites. The darker, scarred bark is indicative of a controlled burn performed by the US Forest Service. Heights of surrounding loblolly pine saplings (~1-1.5 m) suggest they are around 2 years old. Taking the presence of red maples in the wetland (not pictured), which were 1-3 m in height into consideration, the last burn in the sites occurred between 3-5 years prior to the start of this project.



**Figure 4.9** Differences in aboveground biomass within treatment plots after 8 months of fertilizer treatment showing the effects of P inputs lowering the N:P ratio so that N has become limiting.

**Table 4.1** Summary statistics for repeated measures ANOVAs results for statistically significant effects of treatment, sample date, and treatment\*sample date on nutrient concentrations and ratios at 3 depths. Results with  $p < 0.05$  are shaded.

| Fixed effect                      | Variable  | 0-5 cm |        |         |         | 5-15 cm |        |         |         | 15-25 cm |        |         |         |
|-----------------------------------|---|--------|--------|---------|---------|---------|--------|---------|---------|----------|--------|---------|---------|
|                                   |   | Num DF | Den DF | F Value | Pr >  F | Num DF  | Den DF | F Value | Pr >  F | Num DF   | Den DF | F Value | Pr >  F |
| <i>Treatment</i>                  | <i>TP</i>   | 3      | 24     | 4.79    | 0.0094  | 3       | 24     | 1.13    | 0.3564  | 3        | 21     | 1.20    | 0.3349  |
|                                   | <i>PO<sub>4</sub><sup>3-</sup></i>                            | 3      | 24     | 3.49    | 0.0312  | 3       | 24     | 1.98    | 0.1432  | 3        | 21     | 5.83    | 0.0046  |
|                                   | <i>NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>2-</sup></i> | 3      | 24     | 21.49   | <0.0001 | 3       | 24     | 0.75    | 0.5356  | 3        | 21     | 2.01    | 0.1428  |
|                                   | <i>N:P</i>  | 3      | 24     | 17.89   | <0.0001 | 3       | 24     | 14.86   | <0.0001 | 3        | 21     | 7.52    | 0.0013  |
| <i>Sample date</i>                | <i>TP</i>   | 1      | 24     | 9.66    | 0.0048  | 1       | 24     | 5.87    | 0.0233  | 1        | 21     | 4.01    | 0.0583  |
|                                   | <i>PO<sub>4</sub><sup>3-</sup></i>                            | 1      | 24     | 4.62    | 0.0419  | 1       | 24     | 4.8     | 0.0385  | 1        | 21     | 4.44    | 0.0473  |
|                                   | <i>NH<sub>4</sub><sup>+</sup></i>                             | 1      | 24     | 33.47   | <0.0001 | 1       | 24     | 14.79   | 0.0008  | 1        | 21     | 13.21   | 0.0016  |
|                                   | <i>N:P</i>  | 1      | 24     | 1.22    | 0.2801  | 1       | 24     | 12.56   | 0.0017  | 1        | 21     | 1.12    | 0.3625  |
| <i>Treatment*<br/>Sample date</i> | <i>TN</i>   | 3      | 24     | 1.36    | 0.2777  | 3       | 24     | 1.61    | 0.2140  | 3        | 21     | 3.67    | 0.0287  |

**Table 4.2** Pairwise comparisons between treatments from repeated measures ANOVAs testing the effects of treatment, sample date, and treatment\*sample date on different soil nutrient concentrations and ratios at three depths.

| <b>Variable</b>                                   | <b>Depth</b> | <b>Comparison</b>    | <b>DF</b> | <b>t Value</b> | <b>Pr &gt;  t </b> |
|---|--------------|----------------------|-----------|----------------|--------------------|
| <b>TP</b>   | 0-5 cm       | control/high         | 24        | -3.54          | 0.0016             |
|   |              | control/intermediate | 24        | -2.48          | 0.0204             |
|   |              | low/high             | 24        | 2.41           | 0.0241             |
| <b>PO<sub>4</sub><sup>3-</sup></b>                | 0-5 cm       | control/high         | 24        | -2.88          | 0.0082             |
|   |              | control/intermediate | 24        | -2.20          | 0.0376             |
|   |              | low/high             | 24        | 2.16           | 0.0412             |
|   | 5-15 cm      | control/high         | 24        | -2.14          | 0.0426             |
|   | 15-25 cm     | control/high         | 21        | -3.70          | 0.0013             |
|   |              | control/intermediate | 21        | -2.51          | 0.0205             |
|   |              | low/high             | 21        | 3.10           | 0.0054             |
| <b>organic P</b>                                  | 0-5 cm       | control/high         | 24        | -2.14          | 0.0425             |
| <b>NO<sub>3</sub>/NO<sub>2</sub><sup>2-</sup></b> | 0-5 cm       | control/high         | 24        | 7.08           | <0.0001            |
|   |              | control/intermediate | 24        | 6.76           | <0.0001            |
|   |              | control/low          | 24        | 5.38           | <0.0001            |
| <b>N:P</b>  | 0-5 cm       | control/high         | 24        | 6.24           | <0.0001            |
|   |              | control/intermediate | 24        | 6.06           | <0.0001            |
|   |              | control/low          | 24        | 5.61           | <0.0001            |
|   | 5-15 cm      | control/high         | 24        | 6.11           | <0.0001            |
|   |              | control/intermediate | 24        | 5.33           | <0.0001            |
|   |              | control/low          | 24        | 3.35           | 0.0027             |
|   |              | low/high             | 24        | -2.76          | 0.0106             |
|   | 15-25 cm     | control/high         | 21        | 3.81           | 0.0010             |
|   |              | control/intermediate | 21        | 4.19           | 0.0004             |
|   |              | low/intermediate     | 21        | -2.32          | 0.0308             |

**Table 4.3** Soil nutrient concentrations and ratios for all measured nutrients that were significantly influenced by treatment, sample date, or treatment\*sample date. TOC and organic P are not reported since they did not experience any significant changes during the experiment. 0 mos. refers to the initial soil sampling date held before fertilization began. “Surface” = 0-5 cm, “mid” = 5-15 cm, and “deep” = 15-25 cm. Mean±(SE), n=4.

| Treatment    | Depth   | TP (mg/g soil)              |                |                | PO <sub>4</sub> <sup>3-</sup> (mg/g soil) |                   |                    | TN (mg/g soil) |             |             |
|--------------|---------|-----------------------------|----------------|----------------|---|-------------------|--------------------|----------------|-------------|-------------|
|              |         | Initial                     | 6 mos.         | 12 mos.        | Initial                                   | 6 mos.            | 12 mos.            | Initial        | 6 mos.      | 12 mos.     |
| initial      | surface | 0.10 (0.02)                 |                |                | 0.05 (0.002)                              |                   |                    | 1.68 (0.64)    |             |             |
|              | mid     | 0.08 (0.003)                |                |                | 0.04 (0.004)                              |                   |                    | 1.10 (0.07)    |             |             |
| control      | surface |                             | 0.28 (0.15)    | 0.89 (0.78)    |   | 0.07 (0.01)       | 0.53 (0.47)        |                | 3.45 (0.96) | 3.05 (1.10) |
|              | mid     |                             | 0.18 (0.09)    | 0.85 (0.72)    |   | 0.06 (0.02)       | 0.55 (0.49)        |                | 2.28 (0.72) | 3.23 (1.55) |
|              | deep    |                             | 0.17 (0.10)    | 0.81 (0.71)    |   | 0.06 (0.02)       | 0.51 (0.47)        |                | 2.05 (1.20) | 2.58 (1.50) |
| low          | surface |                             | 0.97 (0.40)    | 1.62 (0.64)    |   | 0.51 (0.29)       | 0.96 (0.52)        |                | 3.43 (0.93) | 3.25 (0.56) |
|              | mid     |                             | 0.17 (0.04)    | 0.81 (0.52)    |   | 0.07 (0.01)       | 0.64 (0.46)        |                | 2.00 (0.47) | 1.90 (0.42) |
|              | deep    |                             | 0.13 (0.04)    | 0.70 (0.54)    |   | 0.04 (0.007)      | 0.57 (0.46)        |                | 1.15 (0.38) | 1.68 (0.52) |
| intermediate | surface |                             | 1.40 (0.57)    | 2.59 (1.14)    |   | 0.92 (0.45)       | 1.85 (1.06)        |                | 2.80 (0.67) | 2.95 (0.49) |
|              | mid     |                             | 0.62 (0.37)    | 1.11 (0.88)    |   | 0.19 (0.06)       | 0.93 (0.77)        |                | 2.35 (0.99) | 1.68 (0.50) |
|              | deep    |                             | 0.48 (0.27)    | 0.73 (0.60)    |   | 0.12 (0.04)       | 0.62 (0.52)        |                | 1.68 (0.88) | 1.40 (0.68) |
| high         | surface |                             | 1.73 (0.72)    | 3.38 (0.71)    |   | 1.25 (0.51)       | 2.12 (0.97)        |                | 2.68 (0.38) | 3.33 (0.37) |
|              | mid     |                             | 0.58 (0.27)    | 1.38 (0.54)    |   | 0.37 (0.20)       | 1.01 (0.56)        |                | 2.23 (0.50) | 1.98 (0.29) |
|              | deep    |                             | 0.31 (0.11)    | 0.82 (0.55)    |   | 0.17 (0.04)       | 0.66 (0.50)        |                | 1.78 (0.23) | 1.25 (0.37) |
| Treatment    | Depth   | NH <sub>4</sub> (mg/g soil) |                |                | NO <sub>x</sub> (mg/g soil)               |                   |                    | N:P            |             |             |
|              |         | Initial                     | 6 mos.         | 12 mos.        | Initial                                   | 6 mos.            | 12 mos.            | Initial        | 6 mos.      | 12 mos.     |
| initial      | surface | 0.0067 (0.003)              |                |                | 8.54E-4 (6.04E-5)                         |                   |                    | 15.2 (3.6)     |             |             |
|              | mid     | 0.0038 (0.001)              |                |                | 7.90E-4 (8.90E-5)                         |                   |                    | 14.2 (0.5)     |             |             |
| control      | surface |                             | 0.0096 (0.003) | 0.023 (0.009)  |   | 4.41E-4 (7.28E-5) | 3.68E-4 (5.62E-5)  |                | 16.4 (2.7)  | 14.8 (4.7)  |
|              | mid     |                             | 0.0072 (0.003) | 0.019 (0.009)  |   | 3.13E-4 (9.19E-5) | 2.63E-4 (8.74E-5)  |                | 14.4 (1.9)  | 11.1 (3.3)  |
|              | deep    |                             | 0.0073 (0.004) | 0.019 (0.010)  |   | 4.48E-4 (1.60E-5) | 2.07E-4 (5.06E-5)  |                | 11.6 (2.4)  | 8.8 (2.8)   |
| low          | surface |                             | 0.015 (0.005)  | 0.024 (0.004)  |   | 2.07E-4 (6.15E-5) | 1.20E-4 (5.10E-5)  |                | 4.1 (0.5)   | 2.6 (0.7)   |
|              | mid     |                             | 0.0082 (0.002) | 0.018 (0.004)  |   | 1.57E-4 (3.60E-5) | 2.37E-4 (4.25E-5)  |                | 10.8 (0.7)  | 4.9 (1.5)   |
|              | deep    |                             | 0.0043 (0.001) | 0.013 (0.004)  |   | 1.35E-4 (3.85E-5) | 1.44E-4 (8.18E-5)  |                | 8.5 (1.3)   | 6.7 (1.9)   |
| intermediate | surface |                             | 0.010 (0.003)  | 0.019 (0.005)  |   | 1.12E-4 (2.87E-5) | 9.53E-5 (1.87E-5)  |                | 3.1 (1.1)   | 1.6 (0.5)   |
|              | mid     |                             | 0.0067 (0.002) | 0.014 (0.005)  |   | 1.78E-4 (2.78E-5) | 1.84E-4 (2.09E-5)  |                | 5.8 (1.4)   | 4.2 (1.4)   |
|              | deep    |                             | 0.0056 (0.002) | 0.014 (0.005)  |   | 2.02E-4 (8.13E-5) | 1.90E-4 (6.16E-5)  |                | 4.1 (0.8)   | 4.8 (1.2)   |
| high         | surface |                             | 0.0077 (0.002) | 0.019 (0.002)  |   | 9.74E-5 (6.57E-5) | 8.15E-5 (1.08E-5)  |                | 2.9 (1.4)   | 1.1 (0.3)   |
|              | mid     |                             | 0.0069 (0.003) | 0.014 (0.005)  |   | 1.75E-4 (3.26E-5) | 2.66E-4 (1.54E-04) |                | 5.7 (1.7)   | 2.1 (0.6)   |
|              | deep    |                             | 0.0069 (0.003) | 0.0097 (0.004) |   | 2.54E-4 (5.79E-5) | 1.10E-4 (5.54E-5)  |                | 7.0 (1.2)   | 2.9 (0.9)   |

## 4.6 – References

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## Chapter 5: Conclusion

This research sought to contribute to the current understanding of C budgeting in southeastern US pocosins in response to changes in P-fertility. Investigating this feedback loop could prove important to understanding how an understudied wetland type that has been extensively developed will respond to climate change, increased land use, and more importantly, how this response might affect downstream river and estuary health over time.

Pocosins make up the largest portion of North Carolina's freshwater wetlands with most of the pocosins in the United States found in NC (Daniel 1981; Richardson 2012). With their extreme soil environments, pocosins are a good model for nutrient fertility studies and how increasing nutrient fertility gradients change in response to changing environmental conditions, especially considering that pocosins in NC are sinks and not sources for CH<sub>4</sub> (Bridgham and Richardson 2003; Bridgham et al. 2008; Walbridge 1991).

This research showed that increasing P-fertility of P-deficient pocosin soils does decrease CO<sub>2</sub> flux from the system, but only if P fertilizer is applied in low concentrations otherwise, CO<sub>2</sub> flux from the system was increased past that of the control plots (see Chapter 2). Phosphorus fertilizer application did not affect C allocation in plant tissues, though it had greater effects on overall root biomass content than on shoot biomass portions (see Chapter 3). Additionally, increasing P-fertility increased bioavailable inorganic P in the soil and nitrate concentrations, but had no significant effects on other soil nutrient concentrations (see Chapter 4). Overall, both biomass and soil nutrient stoichiometry appeared to be more affected by seasonal changes. N:P soil

ratios did increase significantly throughout the experiment as expected with the continuation of fertilizer treatments, but they increased to a point, even in low treatment plots by month 12, where N was limiting, likely leading to nutrient toxicity and plant death.

We concluded that the addition of P fertilizer can change system productivity and dynamics, but only if fertilizer is applied in low enough concentrations less frequently and for periods longer than one growing season due to plant-microbe competition as seen in previous research (e.g. Richardson and Marshall 1986; Bridgham and Richardson 2003). As seen in other studies (e.g. Pastore et al. 2015), it is unlikely that any changes in ecosystem's nutrient cycling will continue unless nutrient addition is maintained due to nutrient co-limitation and plant-microbe competition. Changes in CO<sub>2</sub> flux occur naturally over the course of a growing season due to changes in environmental conditions and soil microbe activity. Human activity can also greatly alter CO<sub>2</sub> fluxes in or out of a system. This allowed us to measure CO<sub>2</sub> flux changes in the research sites in response to seasonal variation in weather conditions and P-fertility over the course of only one year during which the experiment was conducted. However, changes within plant communities and the soil and their C storage potential in response to fertilization require longer than one growing season to occur (Chapters 3 and 4). This presents a challenge for studies that only occur over one year, because while increasing P-fertility may appear to increase CO<sub>2</sub> flux into a system and therefore C storage potential, biomass and soil responses do not indicate that more C is being stored and that the C storage potential is not changing. Therefore, short-term studies

such as this one does not provide enough evidence for changes in C storage potential in response to an increase in P-fertility.

As global climate change continues, wetlands will become more important to focus on due to their existing large recalcitrant C pools and potential ability to become either sources or sinks of greenhouse gases such as CO<sub>2</sub> and CH<sub>4</sub> with the resulting changes in environmental factors (Mitsch et al. 2012; Bridgham et al. 2008).

As East Carolina University continues to manage the pocosin found on the WRC for restoration (e.g., prescribed burns), it is important to understand how this dynamic system can potentially change under changing environmental conditions. Pocosins found in the southeastern United States are markedly different to their boreal and tropical counterparts in how they release or store greenhouse gases (Bridgham and Richardson 2003; Brinson 1991). The studied pocosin was once ditched, drained, and developed for government use and considering that all pocosins within NC have been developed at least once, with certain pocosin lands experiencing varying degrees of development or federal protection than others, and it is important to understand how NC pocosins will respond to changing climate and certain environmental conditions (Chester 2004; Lilly 1981; Richardson et al. 1981).

As noted by Bridgham and Richardson (2003), there have been few studies on carbon cycling in warm climate peatlands like the pocosin under study in this research, despite their potential importance in contributing to either a positive or negative feedback loop for climate change by serving as sources as sinks of C. Additionally, studies of P-fertility gradients in P-limited ecosystems are rare compared to studies that manipulate N-fertility gradients (Walbridge 1991). With the lack of studies on pocosins

and their biogeochemistry, more should be known about factors that affect pocosin health and function considering their significant in the C cycle and on the health of downstream tributaries and estuaries.

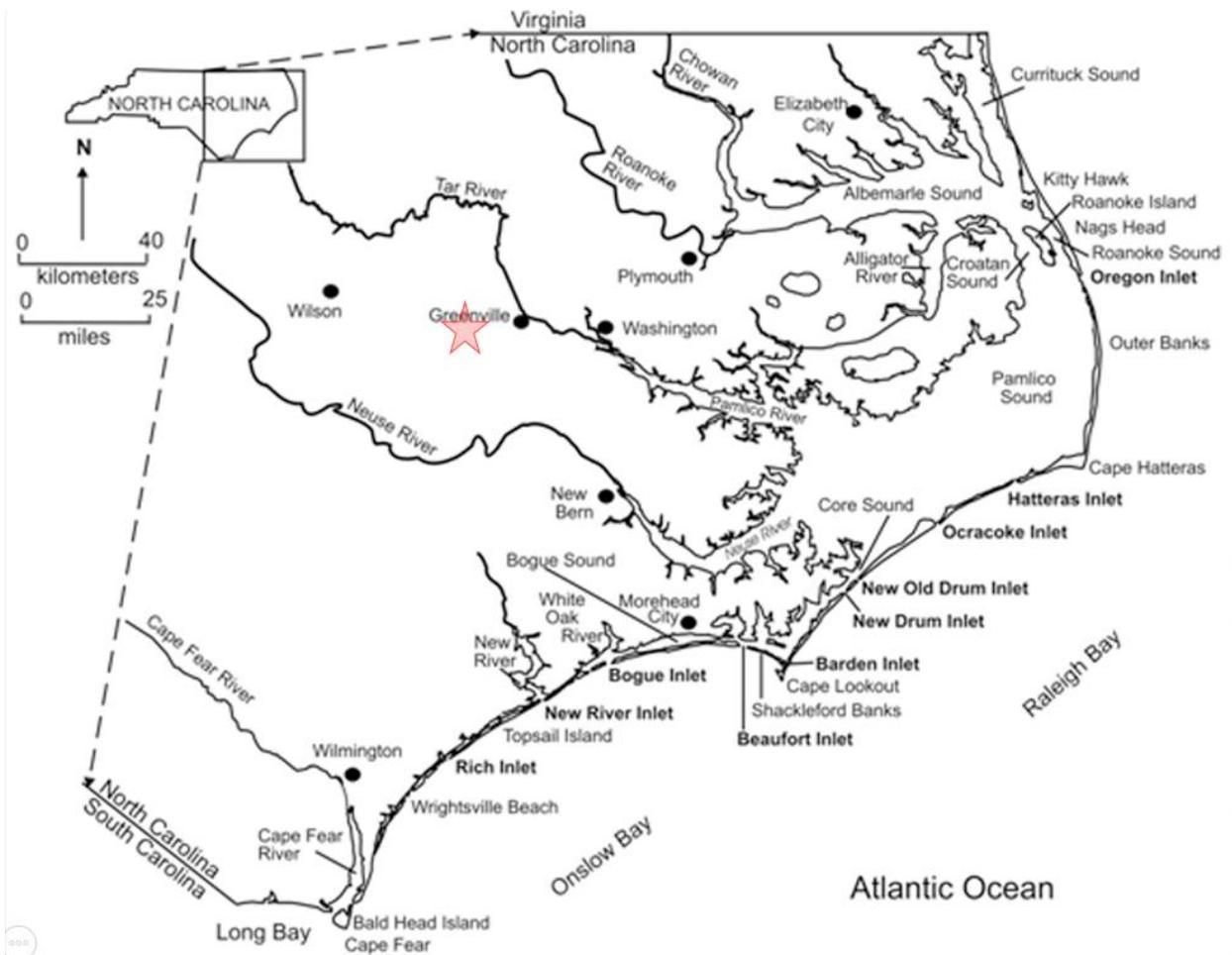
Future research in this topic should be directed at long-term P-gradient studies within pocosins, concentrating on C movement through the plant biomass and storage in the soil. Studies concerning microbial interactions within soil could also help to better understand how these communities affect nutrient dynamics and controls in the system when the limiting nutrient is added. Nitrogen and phosphorus concentrations in plant tissues should also be assessed to see how P-fertility changes nutrient concentrations and storage in their biomass to better understand how increasing the limiting nutrient affects nutrient cycling and sequestration within plant biomass.

Understanding nutrient cycling dynamics in pocosins is essential to understanding how best to manage them and protect them, even after they have been developed. Continuing to manage the WRC as a natural area could promote plant and animal communities unique to the area (Chester 2004), while also providing an important C sink as global and regional climates continue to change. Studies such as this could provide further insight into better management practices in order to preserve the wetland over time.

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**Appendix:  
Supplemental West Research Campus  
Maps and Figures**



**Figure A-1** Location of the WRC in North Carolina's Coastal Plain. The research campus is located about 5 miles west of Greenville, NC.

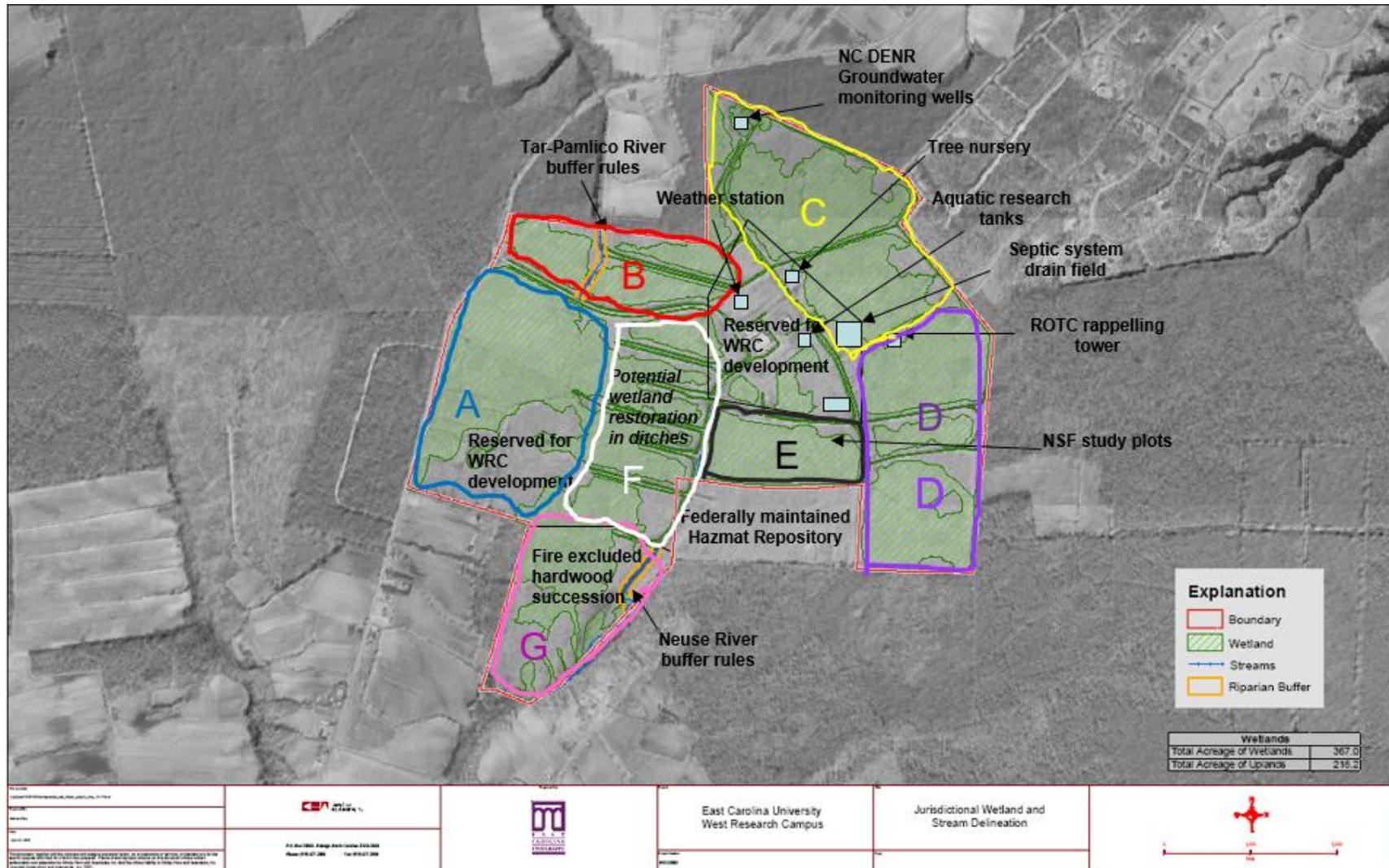
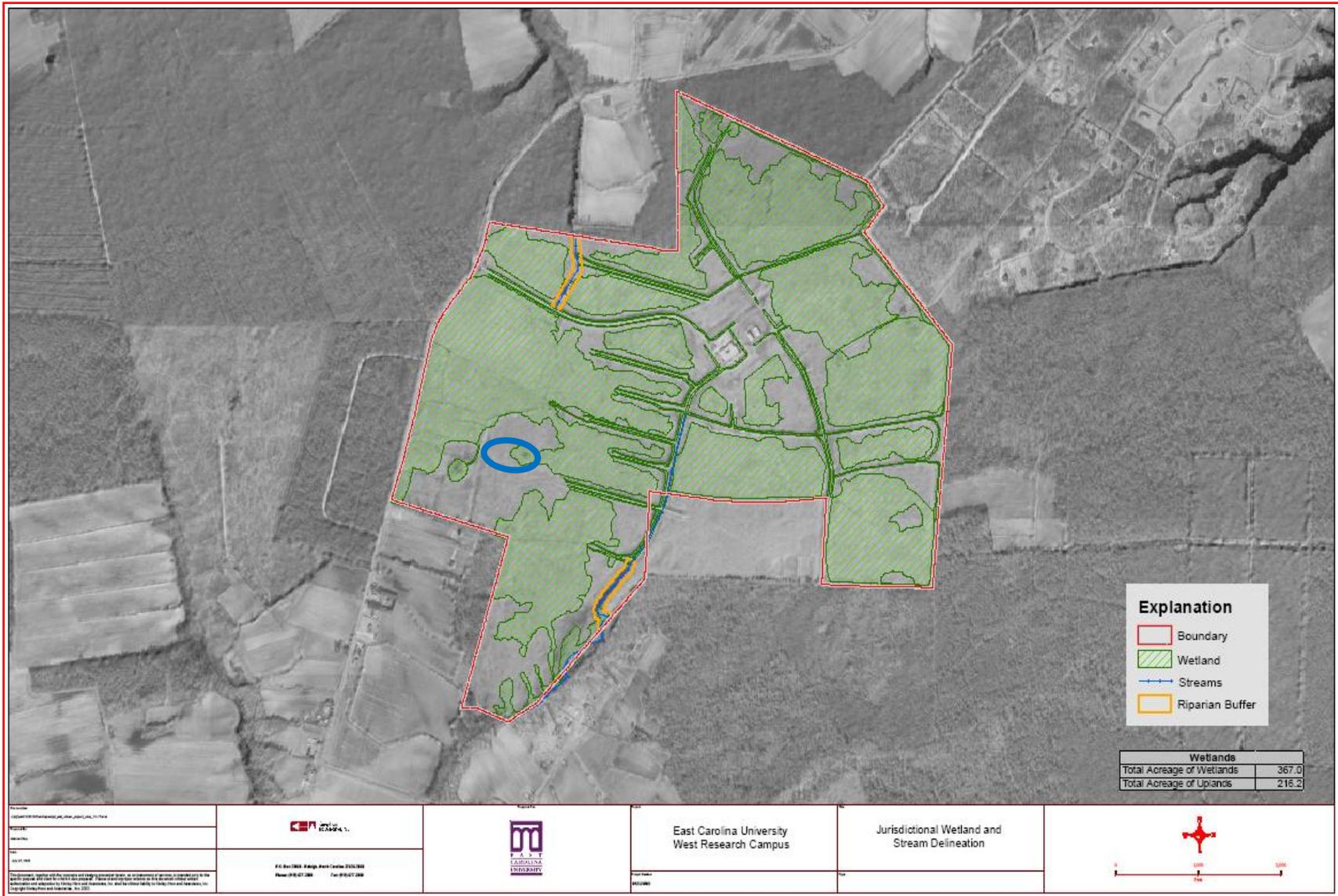
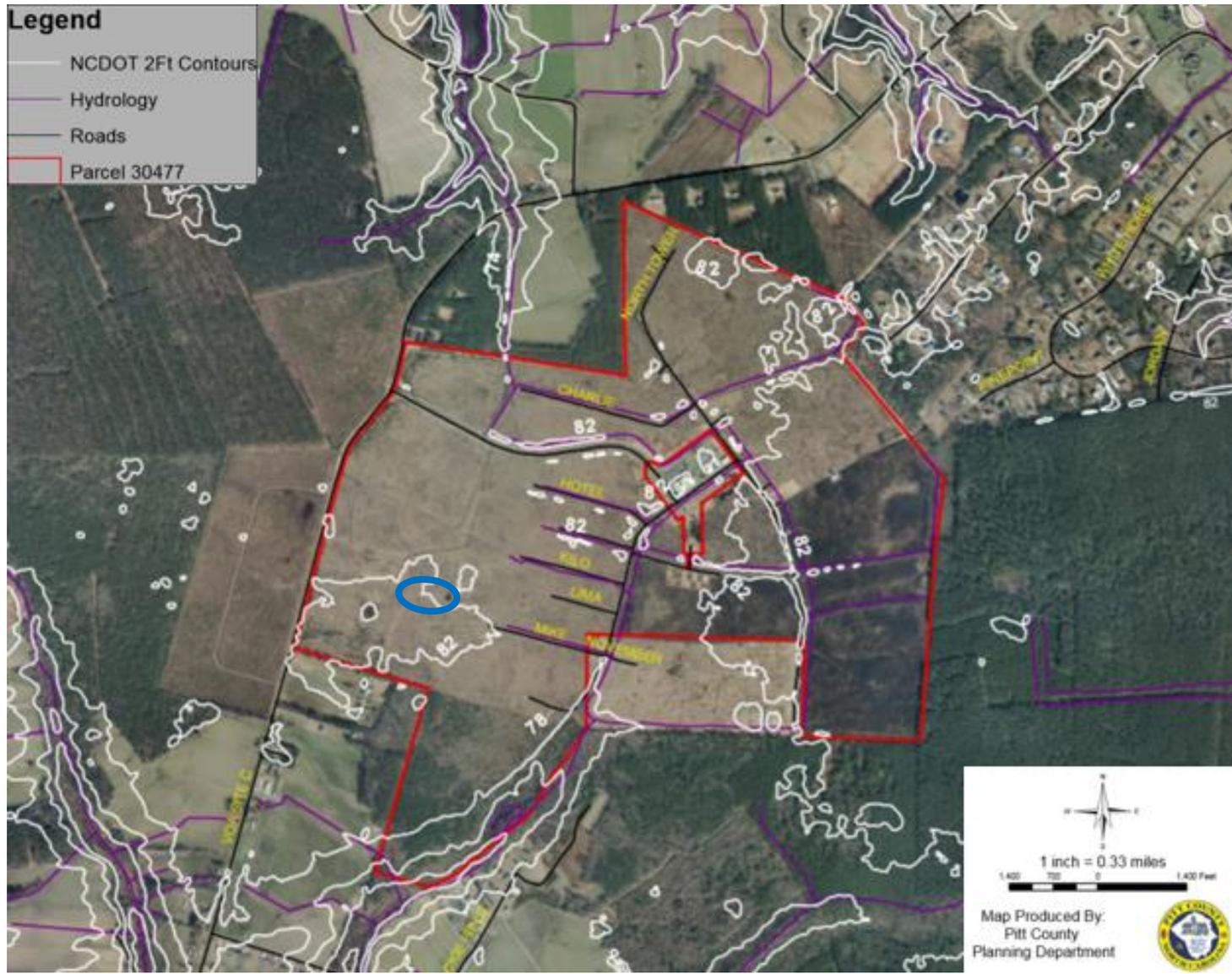


Figure A-2 Land use map of the ECU-WRC with sections outlined and their purpose listed. Study sites were located in Area A.



**Figure A-3** Wetland delineation map of the ECU-WRC. Study sites were located in Area A and are outlined in blue. Wetland area is shaded in green.



**Figure A-4** Hydrology and topography map of the WRC. The ECU-WRC sits at the highest elevation in Pitt County at 22-25 m above sea level. Site locations are outlined in blue and are at one of the highest points on the site at 78-82 ft (24-25 m) above sea level.

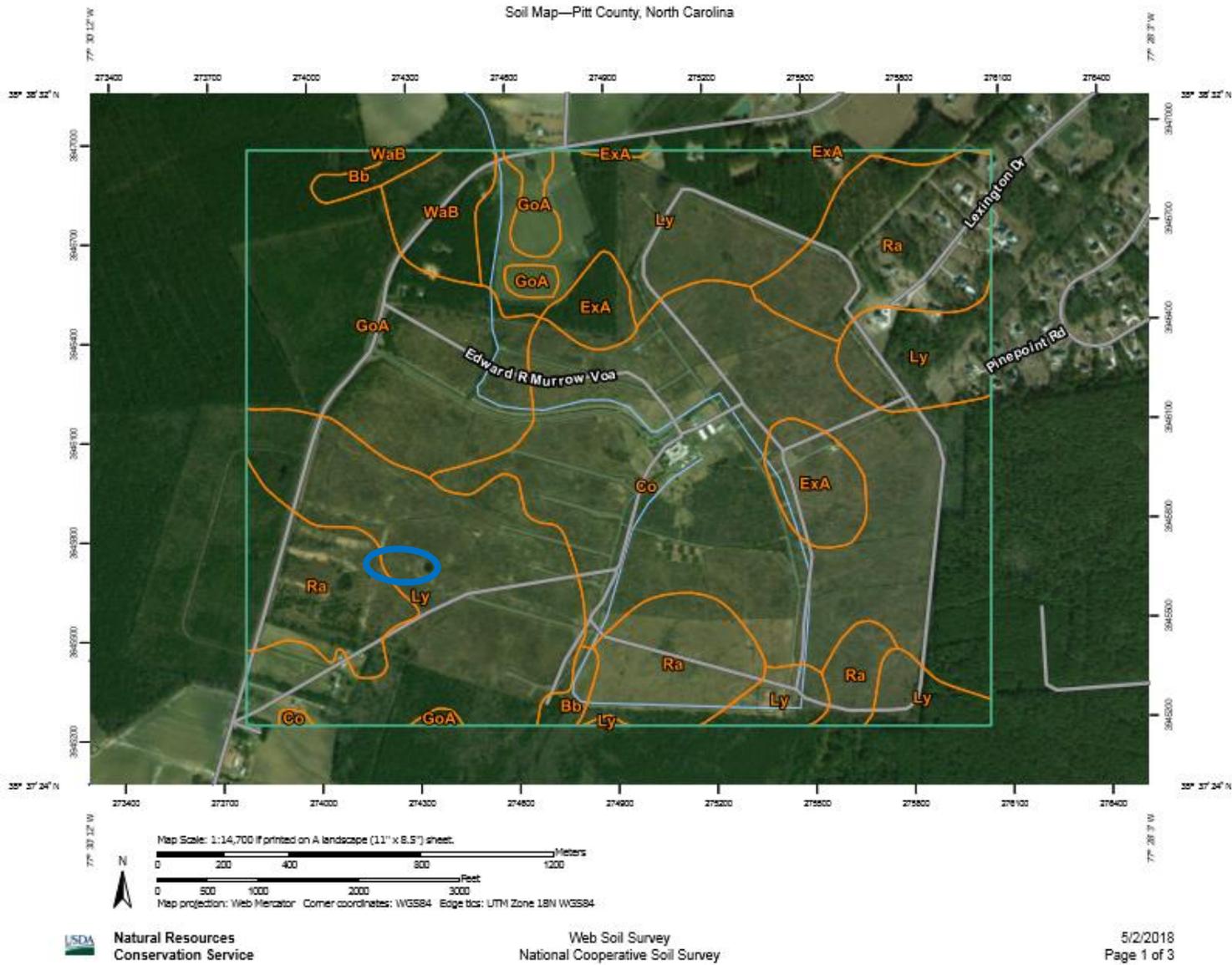
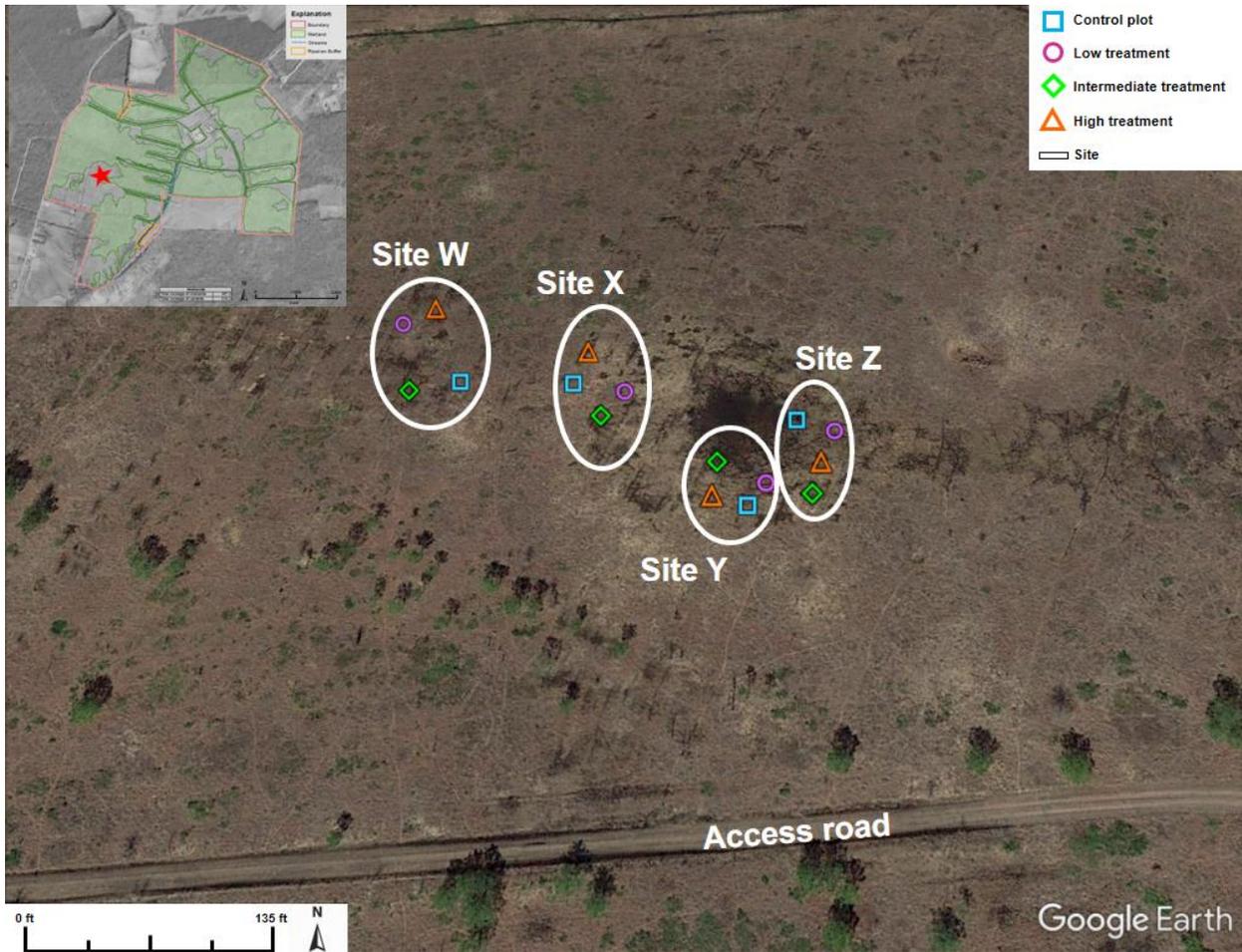
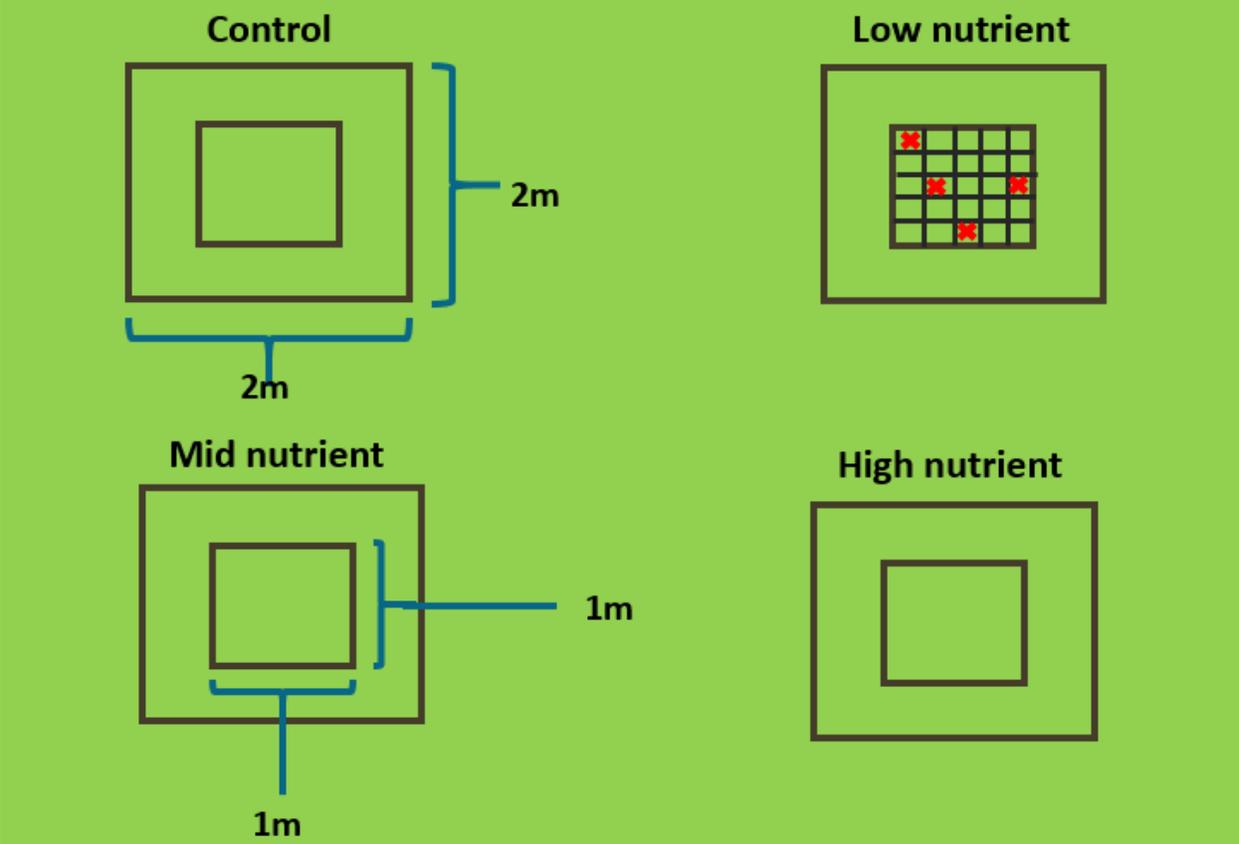


Figure A-5 Soil survey map from the USDA-NCRS (2017). Research sites are outlined in blue and consist of Rains and Lynchburg type soils.



**Figure A-6** Location of the 4 study sites and 4 treatment plots within each site in the WRC.



**Figure A-7** Representation of the experimental design of a single site (or block) and the four treatment plots located within that one site. Red X's represent the destructive sampling of subplots inside the 1m<sup>2</sup> quadrat, with one sample being taken each month; there are 25 total subplots in each experimental plot.

