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SEASONAL CHANGES IN BIOMASS OF TWO COMMON FRESHWATER WETLAND PLANTS: STORAGE OF NUTRIENTS

A Thesis presented in partial fulfillment of requirements for the degree of Master of Science in the Department of Biology

University of Mississippi

Submitted by: Emily Kathryn McCann

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ABSTRACT

In this study, I quantified differences in above- and belowground biomass and storage of nutrients. I hypothesized that storage of phosphorous would be greater in belowground biomass in the non-growing season compared to the growing. I also hypothesized that the storage of phosphorous would be greater in aboveground biomass in the growing season compared to the non-growing season. Furthermore, I hypothesized that the total amount of nutrients in plant tissue would be greater in the growing season, than the non-growing season. *Typha latifolia* and *Carex lurida* were placed in mesocosms at the University of Mississippi Field Station (UMFS) and dosed with phosphorous. Each replicate experiment lasted one month and began with new plants and soil. At the end of the experiment, all plants were harvested and samples were collected for analyses of total inorganic phosphorous, and other macronutrients.

The results show that plants store more nutrients than are necessary for growth. Increasing the amount of phosphorous available to plants also increases the storage of other nutrients, such as magnesium and potassium. The addition of phosphorous also had an effect on the location of nutrients in *Typha latifolia*. Specimens of *T. latifolia*. that were dosed with phosphorous contained larger amounts in roots than shoots, and the opposite was observed in the control specimens. Also, dosed specimens of *T. latifolia* contained higher amounts of potassium in roots compared to shoots/leaves, whereas the control specimens contained higher amounts of potassium in shoots/leaves than roots. *Carex lurida* results show an increase in the amounts of

plant tissue nutrients in the non-growing season compared to the growing season. Conversely, *T. latifolia* contained more plant tissue nutrients in the growing season than the non-growing. Both species contained higher amounts of calcium, magnesium, and sulfur in shoots/leaves than roots.

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CHAPTER 1

INTRODUCTION

Chemical fertilizers are used by farmers to maximize plant growth and crop yield. In 2001, two million tons of phosphorous fertilizers were used in North American agriculture (Cooper and Moore, 2003). Farmers often apply more fertilizer than is necessary for crop production, resulting in the excess running off from fields into nearby water systems following rain. Unlike nitrogen, phosphorous cannot be released into the atmosphere, thus it remains in drainage waters (Kroger, 2007). Many of these contaminated drainage waters lead to large water bodies that can suffer from eutrophication due to the increase of these nutrients.

Eutrophication can occur due to the buildup of nutrient rich sediments, which increase algal growth and can therefore lead to hypoxic conditions. Hypoxia is oxygen deficiency, and can occur in both freshwater and saltwater environments (Rabotyagov et al. 2014). Large amounts of excess nutrients are from agricultural and urban runoff, which is known as nonpoint source pollution, NPS, and it can also affect drinking water supplies and recreational water (Thornton et al. 1999). Eutrophication is the most widespread water quality problem in the US. It accounts for almost half of the impaired lake areas and 60% of impaired river reaches in the US. It is also the most common pollution problem in US estuaries (Smith et al. 1999). Most of the hypoxic zones in the world are seasonal, occurring in spring and summer, and many are reoccurring. One of the largest reoccurring hypoxic zones is in the Gulf of Mexico, which spreads over 20,000 km², and has continued to grow since being first discovered in the 1970s

(Rabotyagov et al. 2014). The Gulf of Mexico "dead zone" is an example of eutrophication that has occurred due to concentrations of nutrients such as nitrogen and phosphorous (Pringle, 2003; Rabalais et al. 2002). Since preindustrial times, there is three times as much reactive phosphorous and nitrogen in the world's oceans. Most of these nutrients are from runoff from agricultural fields draining into lakes and streams which eventually drain into the ocean (Rabotyagov et al. 2014).

Wetlands are most commonly found at the interface of terrestrial ecosystems and open water. Characteristics of wetlands include the presence of water either at the surface or within the root zone, hydric soils, and vegetation adapted to wet conditions. Although natural wetlands recycle and process nutrients and energy, up until the last three decades, their importance was not recognized, and many were drained and destroyed. Globally over 50% of wetlands have been destroyed (Mitsch and Gosslink, 2007). Wetland loss is due to drainage for agriculture, forestry, housing development, mosquito control, residential and commercial use, waste disposal, and peat mining. Natural wetlands are important for nutrient cycling and mitigating pollution.

Constructed wetlands are being developed to provide filtration and processing of nutrients previously provided by natural wetlands. Constructed wetlands have been suggested as best management practices (BMP), or positive ways, to decrease the negative effects of potential agricultural pollutants to downstream receiving systems (Cooper and Moore 2003). The majority of nonpoint source pollution (NPS) contaminants originate in agricultural and urban areas. Nutrients, especially nitrogen and phosphorous, stimulate the growth of phytoplankton which may cover the surface of water bodies, reducing light penetration and therefore interfering with water uses by causing eutrophication. These nutrients are derived from fertilizers, and livestock operations (Cooper et al. 2003).

Drainage ditches surround many agricultural fields and are used to promote water removal following rainfall and controlled-release events. If drainage ditches are viewed as buffers, or as BMPs, between farmland and downstream receiving systems, then the water quality of agricultural runoff can be improved after storm events (Cooper et al. 2003). Since drainage ditches are already in place, utilizing them can be both beneficial and cost effective to farmers by increasing their nutrient sequestration potential through simple landscape manipulation (Kroger et al. 2012). It is most beneficial to use drainage ditches as constructed wetlands to remove excess nutrients associated with agricultural runoff. Ditches can have many of the same characteristics as wetlands, including many of the same aquatic plants (Cooper and Moore, 2003). Some of these excess nutrients are held in the wetland ecosystem and are recycled through plant growth and storage. When water leaves a wetland system, it is first filtered through soil, peats, and other substrates, removing nutrients before reaching connecting waters (Hamner 1997).

To effectively manage these systems it is important to increase the hydraulic retention time (HRT) to allow for increased contact time and nutrient retention. Plants are often used to increase the HRT, and reduce nutrient concentrations before reaching downstream systems (Kroger et al. 2012). Dense stands of wetland plants can decrease the water velocity time, causing solids to settle. Wetland plants also store more nutrients than are necessary for growth, further sequestering nutrients (Cronk and Fennessy, 2001).

CHAPTER 2

LITERATURE REVIEW

2.1 General life cycle of perennial wetland plants

Most wetland plants are perennial and live for more than one growth cycle. Typically, perennial wetland plants bloom over the spring and summer and enter a stage of dormancy over winter. At the beginning of the growing season, their shoots rapidly grow using stored energy from rhizomes and roots (Cronk and Fennessy, 2001). At the end of the growing season, plants lose their aboveground parts due to the breakdown of cell components leading to cell death, which is known as senescence (Bidlack and Jansky, 2014).

Plant non-growing season refers to late fall and winter when plants enter a stage of dormancy. Dormancy allows the plant to survive winter by limiting their growth, or stopping growth completely, until the spring. Leading to the dormant season, plants undergo several physiological and physical changes to prepare for winter. This process of preparation is known as acclimation, which occurs due to factors such as decreasing daylight, and lower temperatures (Raven et al. 2005).

2.2 Nutrient removal by wetland plants

Hunter et al. (2001) showed that vegetated areas remove more nitrogen and phosphorous than unvegetated areas. This study demonstrated that nitrate removal was 67% in vegetated

compared to non-vegetated mesocosms (29%). Mesocosms are outdoor experimental systems (tubs, small pools, horse troughs) used to incorporate natural temperature and moisture

fluctuations while having controlled elements, such as addition of nutrients, similar to a laboratory experiment. The amount of phosphorous removed was also higher in vegetated mesocosms (42%) than mesocosms without vegetation (20%).

Nutrient concentrations in aboveground vegetation tend to be highest in the early growing season (Johnston 1991). Vymazal et al. (1999) showed that *Phragmites australis* accumulated more nutrients in above ground biomass in nutrient enriched conditions than in poor nutrient systems. Cronk and Fennessy (2001) report that nutrient storage in live plant tissues is temporary: some of the nutrients are released through tissue sloughing, plant senescence, and/or decomposition at the end of the growing season. Studies have shown that the contribution plants make in removing nutrients in the spring and summer, is only temporary due to the loss of nutrients at senescence in fall and winter (Kao et al., 2003; Kroger et al., 2007; Menon and Holland, 2014). These studies indicate that plant senescence should be taken into account when calculating plant nutrient sequestration. Depending on the rate of decomposition of senesced plant parts, litter can retain large amounts of nutrients and release them over different periods (Kao et al. 2003). Kroger et al. (2007) demonstrated that a wetland plant, specifically *Leersia* oryzoides, takes up phosphorous during the growing season, but release it back into the water during the dormant season. This release in the dormant season may add to eutrophication in receiving waters.

Mustafa and Scholz (2011) indicate that *Typha latifolia* is effective in removal of phosphorus and nitrogen from wastewater. The study also determined that more nutrients were stored in above ground biomass in the summer than winter. Conversely, more nutrients were

stored in belowground biomass, compared to aboveground biomass, in the winter. In studies of two coastal wetland plants, Chen (2011) also found more nutrients stored in belowground biomass in winter than summer.

2.3 Nutrient Resorption

Many wetland species have the ability to conserve nutrients through nutrient translocation. Species of Poaceae and Cyperaceae translocate carbohydrates and nutrients from leaves to roots, allowing them to overwinter and provide energy for growth in the next season. Translocation of nutrients can account for nutrient retention in plants. Temperate trees retain nutrients in root cortex cells over winter and translocate nutrients from roots to foliar tissue in the spring. Foliar phosphorous concentration in woody species reduces by over half during the course of the growing season. In the spring, foliar phosphorous is high (Cronk and Fennessy, 2001; Raven et al. 2005).

Killingbeck (1996) determined that woody perennial species have the ability to re-absorb nutrients before they senesce their leaves. Killingbeck defines nutrient resorption as "the process by which nutrients are mobilized from senescing leaves and transported to other plant tissues." This study discovered that resorption is highly proficient in plants that have reduced levels of nitrogen and phosphorus. Concentrations of 0.3% nitrogen and 0.01% phosphorus were found in senesced leaves (Killingbeck, 1996). It has also been determined that on average perennial species resorption efficiency is 52% for phosphorus and 50% for nitrogen. It has been further determined that resorption rates are not related to the amount of nutrients available for plants to use (Aerts, 1996).

A similar study was conducted in 2008 on four macrophytes: *Glycera maxima*, *Phragmites australis*, *Carex acutiformis* and *Typha angustifolia*. The study collected leaves from the species in July and senesced leaves were collected in September to determine nutrient resorption efficiency. The results showed a decrease in nutrient concentration of nitrogen, phosphorus and potassium in all species except for *C. acutiformis*, thus indicating a translocation of nutrients to below-ground biomass (Lawniczak, 2011). This study focuses on five macronutrients: phosphorous, calcium, magnesium, sulfur, and potassium.

2.4 Phosphorous and Plants

Phosphorous is one of the five nutrients analyzed for this study. Phosphorous (P) is required by almost all plant processes (Figure 1). It is vital in reactions including regulation of metabolic processes, activation of proteins, and energy transfer (Mikkelson, 2013). Furthermore, P is involved in photosynthesis, transformation of sugars and starches, nutrient movement in plants, and is also vital in transfer of genetic material since P is a main component in chromosomes and building blocks of genes. A large amount of P is required to develop new cells and to transfer the genetic code (International Plant Nutrition Institue,1999; Raven et al, 2005). Plants cannot grow without this nutrient, and it makes up about 0.2% of a plant's dry weight. Second to Nitrogen, Phosphorus is a frequent limiter of plant growth (Schachtman et al. 1998). Although many soils contain high amounts of P, it may be present in forms that are unusable to plants (figure 1). Plants can only utilize inorganic phosphorus (Pi), and 20-80% of P in soils is organic in form and unusable for plants. Therefore, farmers apply large amounts of P to crops due to the fact that 80% of applied P becomes unavailable to plants due to runoff, adsorption, or conversion to the organic form (Holford, 1997). The amount of P concentration in agricultural

plants ranges from 0.1 to 0.5 percent; once inside the plant it is stored in roots or in stems and leaves (International Plant Nutrition Institute, 1999).

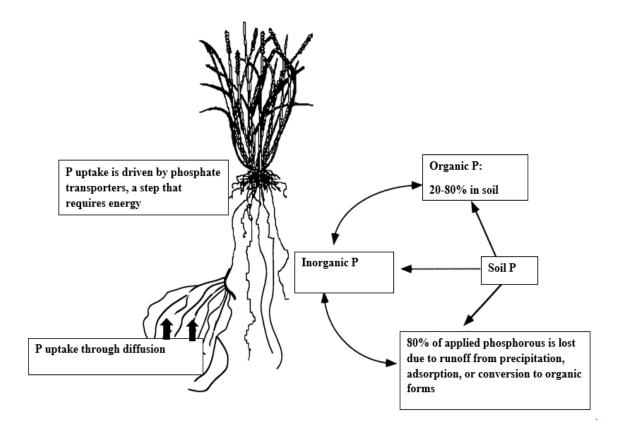


Figure 1. Phosphorus in soils and absorption by plants.

Phosphorus (P) uptake occurs mostly at young root tips and root hairs, typically through diffusion. P must first move through the apoplasm and into the "Casparian strip" until it reaches the stele of the root. The apoplasm is made up of the root walls, the cortical cells, and the open spaces between the two tissues. The movement of P from the apoplast to the stele is vital in transporting nutrients throughout the plant. This step requires energy-driven transport through phosphate transporters. Phosphate transporters are nutrient transport proteins, and research is

currently being done to determine ways to increase P uptake by these proteins and limit P application on crops (Mikkelson, 2013).

When the supply of inorganic Phosphorus (Pi) is high, plants store the excess amounts in older leaf tissues and in vacuoles. When Pi is low, plants grow more roots to increase their uptake from soil while translocating Pi from older leaves and removing storage of Pi from cortex, pith, and vacuoles. Studies have determined that plants with sufficient amounts of P absorbed Pi through roots, and transported it to younger leaves through the xylem. Significant translocation of Pi in phloem from older plant leaves to growing shoots has been observed in times when the amount of Pi in soils is inadequate (Schachtman et al. 1998).

2.5 Calcium and Plants

Calcium (Ca) in an essential element in plants. It is accessible to plants in the form of a cation Ca²⁺. Deficiency of calcium leads to shoots and tips of plants dying (Raven et al. 2005). Ca is especially essential for stability of cells by maintaining membrane structure. The strength of cell walls are increased by calcium addition. Ca forms cross-links within the cell matrix increasing the structural rigidity of the cell wall (Eastwood, 2002). When the concentration of Ca is low the cell walls become more pliable, and are easily ruptured (Hepler, 2005). It is involved in the movement of substances through cell membranes by activating Ca²⁺ permeable channels. Ca ions bind to acidic groups of membrane lipids where they can act as a second messenger and initiate plant response to environmental stimuli (Taiz et al. 2015). When Ca is low there can be a leakage of ions and metabolites. Calcium also acts as an enzyme cofactor and regulates stimulus responses (Nabors, 2004). Studies show that Ca also slows the loss of chlorophyll and tissue

senescence by enhancing cytokinin. Ca also is found in the mitochondria of plant cells. It regulates NADH dehydrogenase, and therefore regulates mitochondrial function (Hepler, 2005). NADH is reduced nicotinamide adenine dinucleotide and acts as an electron carrier in respiration (Bidlack and Jansky, 2014).

2.6 Potassium and Plants

Potassium (K) in plants typically is concentrated in the meristems and is responsible for enzyme activation (Bidlack and Jansky, 2014). It is a cofactor in osmosis and ionic balance, protein synthesis, and action of the stomata (Nabors, 2004). When potassium is deficient in plants necrotic spots occur, and the plant will also have narrow weak stems (Raven et al, 2005). K activates over 60 different enzymes that are required for plant growth. It changes that shape of the enzyme molecule and exposes the active site required for the reaction to take place. K also reduces organic anions and keeps the plant's pH neutral. Plants depend upon K for proper stomata function. Stomata opening and closing is essential for gas exchange, photosynthesis, and nutrient transport. K moves into guard cells that surround the stomata causing water to enter the cells. When water enters the guard cells the pores open and gas exchange occurs. When K levels are low the pores tightly close and prevent the loss of water through stomata. K is also responsible for osmosis in roots. Deficient K in roots cause plants to be less able to absorb water. K ions also maintain the production of ATP. When levels of K are low, photosynthesis is slowed down as well as the production of ATP. Furthermore, plants that do not have sufficient levels of K have lower translocation rates of nitrates, calcium, phosphates, magnesium and amino acids (IPNI, 1998).

Potassium (K) levels are typically high in soils, but largely in a form that is unavailable to plants. On average, 90-98% of K found in soils is in an insoluble form and unusable by plants. For plants to use K, it must be in a water soluble form. K uptake in plants is increased by soil moisture, oxygen presence and temperature. Higher soil moisture increases the soluble form of K and is more readily useable by plants. Air is necessary for root respiration and K uptake. The optimum soil temperature of K uptake is around 70 degrees Fahrenheit. Since levels of useable K in soils is low, farmers apply fertilizers such as potassium chloride or manure to reach optimum levels for crop growth (Rehn and Schmitt, 2002).

2.7 Magnesium and Plants

Magnesium (Mg) is a main component in chlorophyll and an activator of enzymes (Raven et al, 2005). When deficient, dead spots occur on plant leaves (Bidlack and Jansky, 2014). Mg is responsible for the green color of leaves. Without Mg, chlorophyll cannot capture energy from the sun (Patterson, 2016). Mg is also the carrier of phosphorous in plants and enhances the uptake of phosphorous when applied as a fertilizer. The available form of Mg is the ionic form Mg⁺⁺. There are several factors that affect the availability of Mg in soils. Low levels of pH decrease the availability of Mg for plants, and soils that contain high levels of potassium or calcium provide less Mg to the plant (Spectrum Analytic Inc., 2016).

2.8 Sulfur and Plants

Sulfur (S) is an essential element in proteins, amino acids, and coenzymes of plants (Nabors, 2004). Plants require as much sulfur as they do phosphorous. S is found in cystine and

cysteine that make up cell proteins (Baird, 1991). It is also involved in chlorophyll formation and the conversion of nitrate to amino acids (Steward, 2010). The available form of sulfur for plants is SO₄. Transformation of S is similar to that of nitrogen. Sulfur that is available to plants can be transformed by bacteria to unusable forms. Harvesting and leaching also reduces the available S (Schulte and Kelling, 1992).

2.9 Typha latifolia

Known as the common cattail, it is found throughout most of the United States from Florida to Alaska and even into Mexico (Godfrey, 1979). It belongs to the Typhaceae family which consists of herbaceous perennial plants that live in fresh to slightly brackish wetlands. The cat-tail family is rhizomatous and often emergent in up to 1.5 meters of water. The flowers of Typhaceae are unisexual with both pistillate and staminate on the same plant. The pistillate spikes often persist into winter and foliage leaves are persistent. Each spike can produce thousands of seeds. The seeds are wind-dispersed and germinate under shallow water or on bare wet soils. Seedlings can clone rapidly by means of rhizomes in their first season and flower the second season. They often form large stands producing large amounts of biomass in thick persistent stands. Typha species are used in numerous ways throughout the world. The "fluff" from fruiting spikes is used for insulation, leaves are used for dwellings and furnishings. Typha is important as habitat and food for wildlife. It is also useful in removal of pollutants and is sold commercially in the US for habitat and wetland restoration (Smith, 2000). Typha latifolia (Figure 2) is commonly found in wetlands of the southeastern United States. It has also been proven to take up large amounts of nutrients (Mustafa and Scholz, 2011).

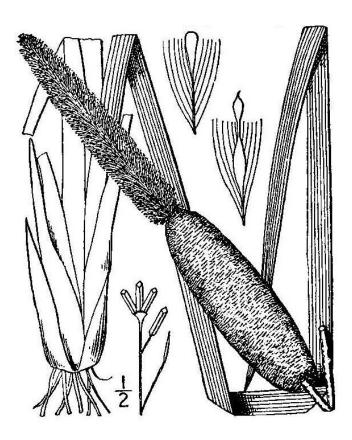


Figure 2. Typha latifolia line drawing (Britton and Brown (b), 1913).

2.10 Carex lurida

Belonging to the Cyperaceae family, the *Carex* genus consists of over 2000 species worldwide. *Carex* species are herbaceous, perennial, rhizomatous plants. Its culms are trigonous, with basal and cauline leaves. The inflorescences are terminal and flowers are unisexual. *Carex* is one of the largest genera of vascular plants in the world. Its distribution is almost worldwide, found most places except for the tropics and Southeast Asia. It is most commonly found in wet habitats, with water no more than 50cm deep in the growing season. Vegetative shoots have basal leaves and the stem-like aboveground portion is composed of overlapping sheaths. *Carex* species are important members of many peat deposits and are often used in moist habitats as

forage for wildlife and livestock. Grasslands also have a large amount of biomass in species of *Carex* (Ball et al, 2000). *Carex lurida* (Figure 3) is native to the southeastern United States. It has also been shown to uptake and retain nutrients, such as phosphorous (Menon and Holland, 2013).

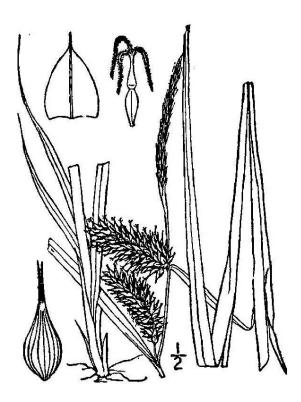


Figure 3. Carex lurida line drawing (Britton and Brown (a), 1913).

CHAPTER 3

OBJECTIVES AND HYPOTHESES

3.1 Objectives

Some scientific literature suggests that sequestration of nutrients by wetland plants is only temporary due to plant senescence (Kao et al. 2003; Kroger et al. 2007; Menon and Holland, 2014). These studies quantify release from plant senescence by collecting leaf litter during the growing season and measuring decomposition over time. However, other studies showed nutrient storage belowground in winter (Cronk and Fennessy, 2001; Raven et al. 2005). Previous work did not answer the question about how two wetland plants store nutrients throughout the seasons. This study examines the effectiveness of two common wetland plants in retaining five elements (including phosphorous) associated with agricultural runoff. I studied the nutrient storage of these plants following a simulated rainfall runoff event during both growing and non-growing crop seasons to assess their storage of phosphorous in above and belowground biomass. My objective was to determine the storage of plant nutrients in both growing and non-growing seasons.

3.2 Hypotheses

Hypothesis 1: Storage of phosphorous will be greater in belowground biomass in the non-growing season compared to the growing season.

Hypothesis 2. Storage of phosphorous will be greater in aboveground biomass in the growing season compared to the non-growing season

Hypothesis 3. Storage of other nutrients will be greater in the growing season in above ground biomass than during the non-growing season.

CHAPTER 4

METHODS

4.1 Research site and Experimental Set up

The research was conducted at the University of Mississippi Field Station (UMFS) which is located on a 800-acre site, 11 miles northeast of the University of Mississippi's Oxford campus, on County Road 202 (Figure 4; 34.432328°N, -89.38966°W). The Field Station is located within the Eoecene Hills of the interior coastal plains in the Southeastern United States. It contains both natural and constructed wetlands totaling over 200 experimental ponds (UM Field Station, 2016). The research was conducted in plastic drums that served as mesocosms. Mesocosms are outdoor experimental systems used to incorporate natural temperature and moisture fluctuations while having controlled elements, such as addition of nutrients, similar to a laboratory experiment. The drums were located outdoors, next to the UMFS greenhouse (Figure 5).

The species that were used in the experiment were collected from the UMFS pond 71 (Figure 2), which is located in the southeastern side of the field station. Pond 71 is spring fed and is dominated by species *Typha latifolia* and *Carex lurida* (Figures 6 and 7).

The soil was collected from UMFS, 200 yards west of the experiment site in an upland area free from any experimental runoff or contamination. The soil was sandy-loam, which is similar to the soil found in pond 71. The soil that was used was collected from the same location for each experiment.

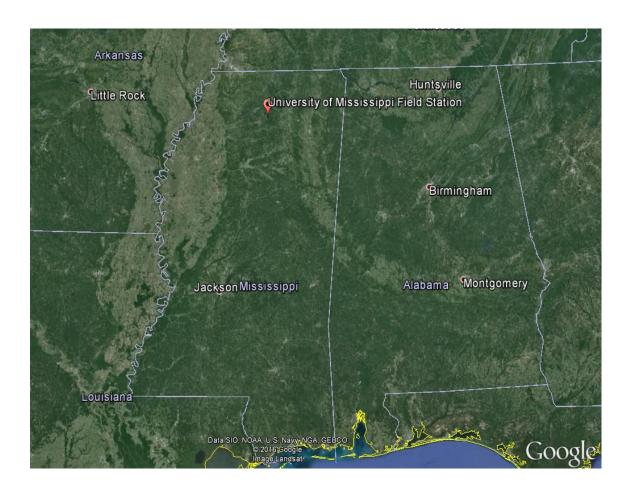


FIGURE 4: University of Mississippi Field Station, located in Abbeville, Mississippi (Google, January 2016).



FIGURE 5. University of Mississippi Field Station, Pond 71. Located in Abbeville, Mississippi (Google, January 2016).



Figure 6. Typha latifolia photo. (Russell, 2016).



Figure 7. Carex lurida photo. (Staunton, 2016).

The initial experiment was set up in June 2015, replicated in July 2015, reproduced in late September 2015, and replicated in late October 2015 to simulate growing and non-growing seasons, respectively. The species *Carex lurida* (shallow sedge), and *Typha latifolia* (cattail) were used in this experiment.

Each replicate experiment lasted one month and began with new plants and soil. Twenty plastic drums (55 gallon), cut in half, were used as planters (Figure 8). At the beginning of each experiment the planters were cleaned and filled with new soil. Then the plant species were collected from pond 71, cleaned of all residual sediments, weighed and then planted in barrels. Once planted the specimens were allowed to acclimate for three weeks before dosing occurred (Menon and Holland 2013). C. lurida was planted in ten drums and T. latifolia in ten. Five drums of each species served as controls. The controls received unchlorinated ground-water. The other drums were dosed with phosphorous dissolved in unchlorinated ground-water (Figure 9). Dosing occurred using a 19 liter aquarium doser. Each dose consisted of 18 liters of 2.5 mg/l of phosphorus to simulate a rainfall event. The phosphorus concentration was selected based on the concentration of phosphorus (0.01 to 3.0 mg P L -1) commonly found in agricultural runoff (Frossard et al. 2000). Throughout the experiment the planters were watered weekly with unchlorinated groundwater to ensure soil saturation. At the end of the experiment all plants were harvested, cleaned of sediments, and weighed. Specimens were brought back to the laboratory for analyses. For the month of September, Typha latifolia specimens were not successful following transplantation. For this reason, the month of September is not included in the analyses of Typha latifolia.



Figure 8. Empty mesocosms, UMFS

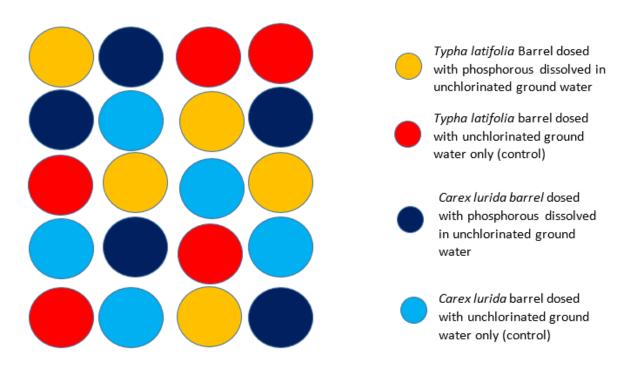


Figure 9. Experimental set-up.

4.2 Laboratory Analyses

Above- and belowground biomass were measured in the laboratory in the UM Biology Department in Oxford, MS. Clean specimens were sorted by species. Above-and belowground structures were separated, weighed and dried in a drying oven at 85 degrees Celsius for 48 hours to determine above and below ground biomass.

Samples of root and stem tissue were collected, prior to drying, from each specimen and sent to the University of Georgia Soil, Plant, and Water laboratory, in Athens, GA, to be analyzed for total inorganic phosphorous (TIP) and four other nutrients.

4.3 Statistical Analyses

A multi-factor mixed effect ANOVA was used to compare means of plant nutrients among months, between location on plant (root or leaves), between phosphorus treatment, control versus dosed, and with interactions among those three factors. Specimen was treated as a random factor in the ANOVA, resulting in a split-plot analysis with location on plant as the within-plot factor, and month and phosphorous treatment as between-plot factors. Separate analyses were used for each plant species, for each of the five response variables: percent phosphorus, percent magnesium, percent calcium, percent sulfur, and biomass. Significance was accessed using α =0.05. Adjusted (least-squares) means and standard errors were calculated for the significant effects. For significant interactions or significant main effects of month, means were compared using a priori contrast. All analyses were performed using the lmer() function in the lmerTest package of R version 3.1.

CHAPTER 5

RESULTS

5.1 Typha latifolia

5.1.1 Phosphorous

The results of the ANOVA showed the phosphorus-treated specimens contained more phosphorous than the control specimens ($F_{1.24}$ = 4.57, p=0.04282, Figure 10). There was a trend toward a P treatment by plant location interaction, whereby the treated specimens contained more P in roots than aboveground parts, whereas the control specimens had similar P concentration in roots and aboveground parts ($F_{1.24}$ = 4.0862, p=0.05452, figure 10).

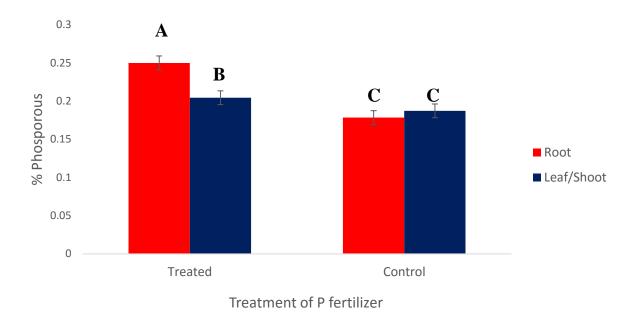


Figure 10. Mean above-belowground percent P compared for treated and control specimens of *Typha latifolia* (±SE, n=60). Means that share letters were not significantly different.

Month and location had no effect on P concentration (p=0.20146).

5.1.2 Calcium

There was a significant three-way interaction of the treatment of phosphorous on the storage location of calcium between months ($F_{2.24}$ =7.984, p=.0021974, Figure 11). The leaves/shoots had higher means than the roots. Higher amounts of Ca were found specifically in the growing season (June and July), than the non-growing month (October). The specimens that served as control had higher amounts of Ca.

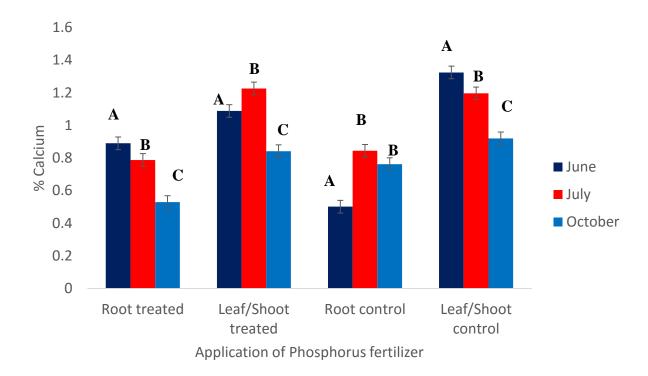


Figure 11. *Typha latifolia* above- and belowground percent Ca of treated and control specimens compared over months (\pm SE, n=60). Means that share letters were not significantly different across the post-hoc significant effect contrasts within each location x treatment combination.

5.1.3 Potassium

There was a significant three-way interaction of the treatment of phosphorous on the storage location of potassium between months (F_{2.24}=3.9483, p=0.03293, Figure 12). There was more potassium (K) in June and July than October. Specimens that were treated with phosphorous contained more K than the control specimens. The control specimens contained more K in roots in the non-growing than the growing, and conversely contained more K in the

shoots in the growing than the non-growing season. The treated specimens had higher concentrations of K in the growing season than the non-growing season.

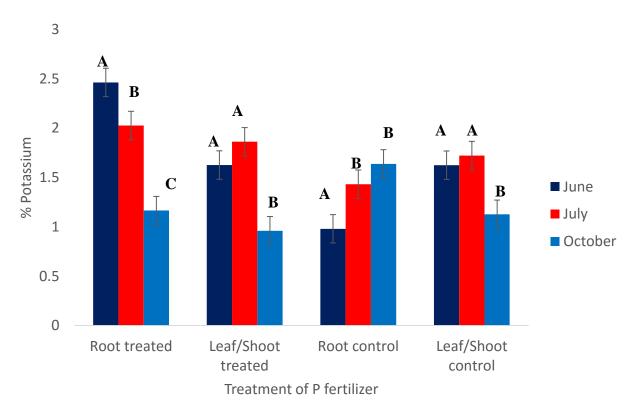


Figure 12. *Typha latifolia* above- and belowground percent K of treated and control specimens compared over months (\pm SE, n=60). Means that share letters were not significantly different across the post-hoc significant effect contrasts within each location x treatment combination.

5.1.4 Magnesium

Results show a significant effect of treatment of phosphorous and storage of magnesium (Mg) over months ($F_{2.48}$ =5.0747, p=.01002, Figure 13). Specimens contained more Mg in the growing season (June and July) than the non-growing (October). The treated specimens

contained more Mg in June than the control. Conversely the control specimens contained more Mg in October than the treated specimens.

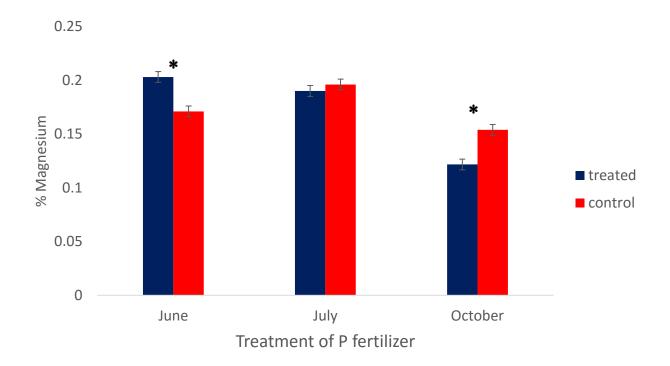


Figure 13. Percent Mg of treated and control specimens, of *Typha latifolia*, compared over months (\pm SE, n=60).

There was a significant difference between the storage of Mg in roots and leafs/shoots $(F_{1.48}=24.5742, p=9.324e-06, Figure 14)$. The leaves/shoots contained more Mg than the roots.

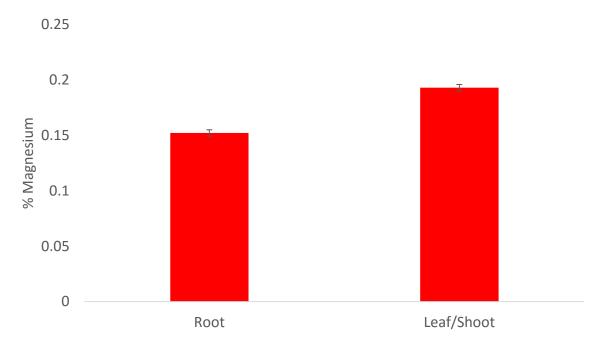


Figure 14. *Typha latifolia* above- and belowground percent Mg (±SE, n=60).

5.1.5 Sulfur

The treatment of P had no significant effect (p= 0.982). The results show a significant difference between the amount of Sulfur (S) between the different months (F_{2.24}=9.6595, p=0.0008363, Figure 15). The growing months contained more S than the non-growing month. The month of July had the highest amount of S.

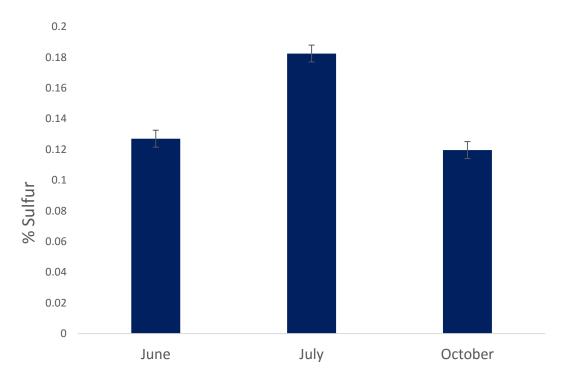


Figure 15. Percent S, in *Typha latifolia*, compared over months (±SE, n=60).

There is a significant difference between location of S in the plants ($F_{1.24}$ =19.9409, p=0.0001617, Figure 16). The leaf/shoots contained more S than the roots.

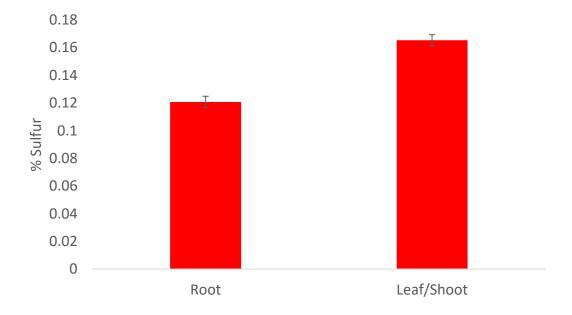


Figure 16. *Typha latifolia* above- and belowground percent S (±SE, n=60).

5.1.6 Mass

The treatment of P had no significant effect on growth of roots and shoots/leaves (p= 0.8489). The results show a significant difference in mass locations from root to shoot/leaf. (F_{1.24}=16.8278, p=0.0004069, Figure 17), where the leaf/shoots had a higher mass than the roots.

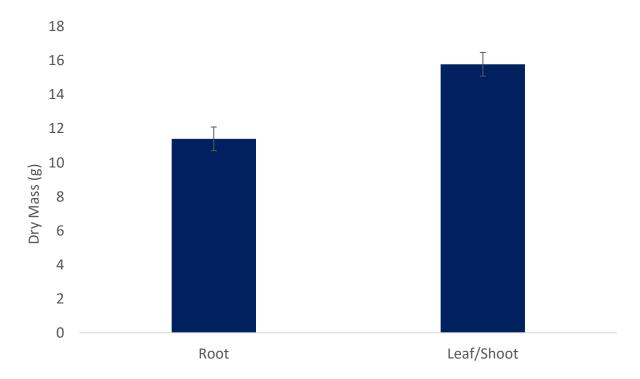


Figure 17. *Typha latifolia* above- and belowground mass (±SE, n=60).

There was no significant difference in the mass between dosed and control specimens (p=0.4589). There was also no significant difference in above- and below ground biomass between months (p=0.9415).

5.2 Carex lurida

5.2.1 Phosphorous

The treatment of P had no significant effect (p=0.738624). Results show a significant difference in the storage of phosphorous (P) between months ($F_{3.32}$ =13.2863, p= 8.417e-06, Figure 18). The non-growing months had higher concentrations of phosphorous than the growing months.

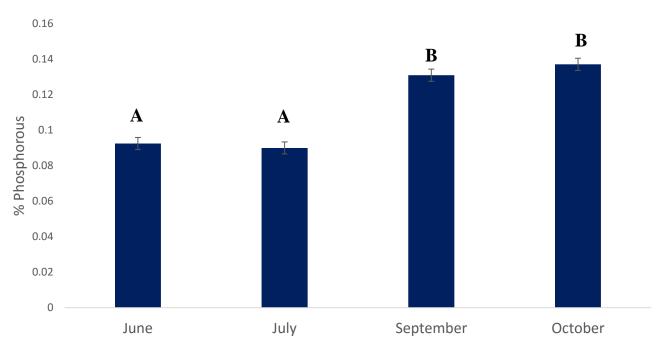


Figure 18. Percent P compared over months, of *Carex lurida* (±SE, n=60). Means that share letters were not significantly different.

There is a significant difference in the location of phosphorous in the plant ($F_{1.32}$ =11.6093 p=.001788, Figure 19). The leaves/shoots contained a higher concentration of P than the roots.

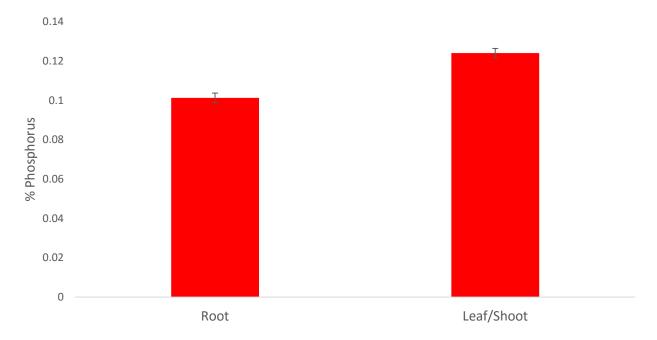


Figure 19. *Carex lurida* above- and belowground percent P (±SE, n=60).

There is no significant difference in the location of P between months (p=0.35).

5.2.2 Calcium

The treatment of P had no significant effect (p= 0.87331). The results show a difference in the location of calcium between months ($F_{3.32}$ =5.014, p=.005816, Figure 20). There were higher amounts of Ca in the leaves/shoots than the roots for the month of July, conversely there was a higher concentration of Ca in the roots than the leaves/shoots in September.

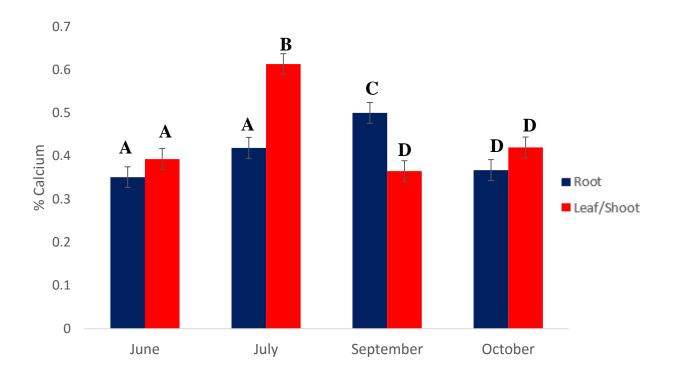


Figure 20. Carex lurida above- and belowground percent Ca compared over months (\pm SE, n=60). Means that share letters were not significantly different.

5.2.3 Potassium

The treatment of P had no significant effect (p=0.5325). There is a significant difference between months and the storage of potassium ($F_{3.32}$ =12.394, p= 1.524e-05, Figure 21). There are higher amounts of K in the non-growing months than the growing.

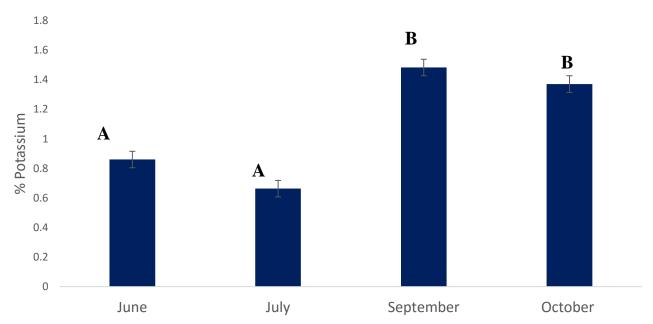


Figure 21. Percent K, in *Carex lurida*, compared over months (±SE, n=60). Means that share letters were not significantly different.

There is a significant difference between the location of K on the plant ($F_{1.32}$ =38.621, p=5.84e-07, Figure 22), with a higher amount of K in the leaf/shoot than the root.

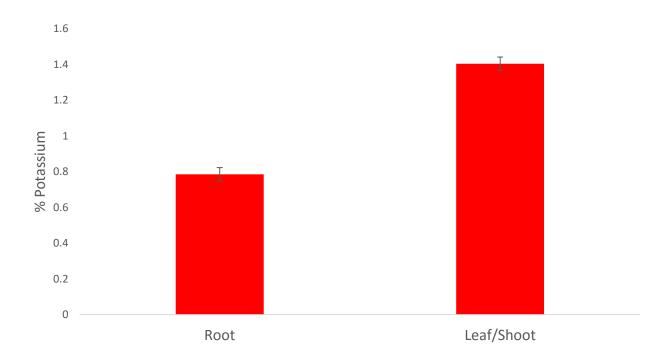


Figure 22. *Carex lurida* above- and belowground percent K (±SE, n=60).

5.2.4 Magnesium

The treatment of P had no significant effect (p= 0.991913). Results show a significant difference between location of Magnesium (Mg) and month (F_{3.32}=4.0594, p=0.014934, Figure 23). There was no significant difference in the concentration of Mg over the seasons, but there was an increase in storage of Mg in roots from growing season to non-growing. In the growing season higher concentrations of Mg were in the leaves/shoots. Similar concentrations of Mg were found in leaves/shoots and roots in the non-growing season.

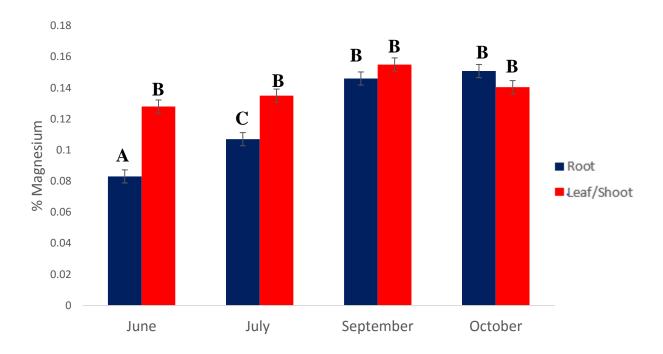


Figure 23. *Carex lurida* above- and belowground percent magnesium compared over months (±SE, n=60). Means that share letters were not significantly different.

5.2.5 Sulfur

The treatment of P had no significant effect (p= 0.3319411). There is a significant difference in the amount of Sulfur (S) between months (F_{3.32}=9.4080, p=.0001318, Figure 24). There are higher amounts of S in the non-growing months than the growing. There is also a significant increase in S from June to July.

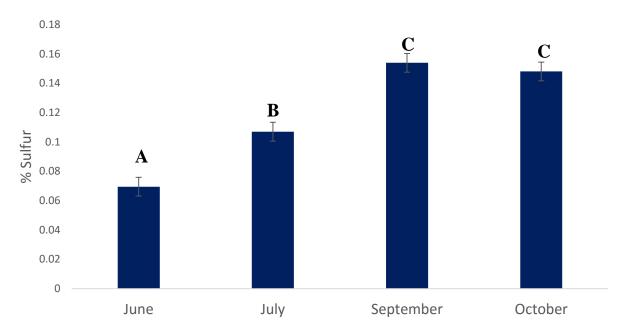


Figure 24. Percent S, in *Carex lurida*, compared over months (±SE, n=60). Means that share letters were not significantly different.

The results show a significant difference between the location of S ($F_{1.32}$ =4.9284, p= 0.0336263, Figure 25). There is more S in the leaves/shoots than the roots.

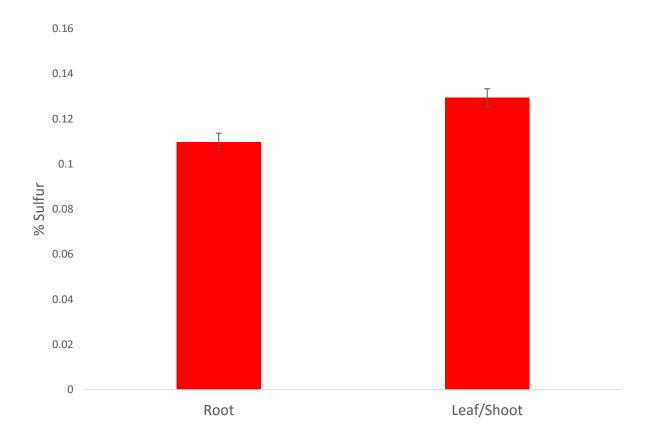


Figure 25. *Carex lurida* above- and belowground percent S (±SE, n=60).

5.2.6 Mass

There is a significant interaction of mass between months and location on the plant $(F_{3.32}=8.3952, p=0.0002, Figure 26)$. There was an increase in root mass from June to July, but no significant change in mass of leaves/shoots. September and October showed no significant difference in storage location, but did show a significant decrease in overall mass from September to October.

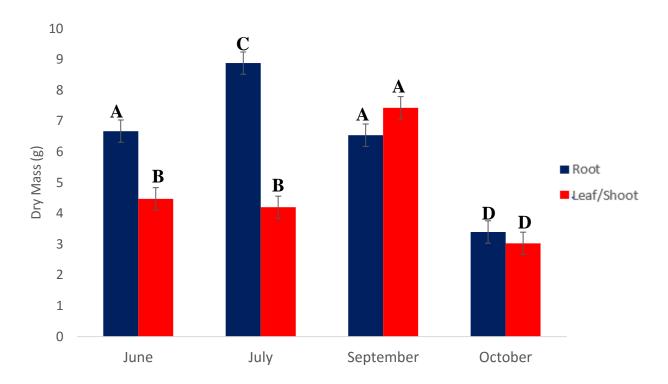


Figure 26. *Carex lurida* above- and belowground mass compared over months (±SE, n=60). Means that share letters were not significantly different.

5.3 Weather Results 2015

The average temperature for the 2015 growing and the non-growing season showed an increase from normal and 2014's temperature (Table 1). The months of October and November were especially high with averages of 1.3 and 4.7 degrees Fahrenheit from normal temperatures. The average daily precipitation showed a slight increase from normal (Table 2). Both June and September had decreases in amount of precipitation.

Monthly Average Temperature Report (F)								
Month (2015)	Observed	Normal	Depart from normal	Last year's (2014)				
May	71.5	70.7	0.8	71.3				
June	79.2	78.1	1.1	78.8				
July	83	81.4	1.6	76.5				
August	78.8	80.8	-2.00	80.5				
September	74.7	74.1	0.6	75.5				
October	64.3	63	1.3	65				
November	57.6	52.9	4.7	46.8				

Table 1. Average Temperature per month (NOAA, 2016).

Monthly Average Precipitation Report (inch/day)								
Month (2015)	Observed Rain (inch)	Normal	Depart from normal	Last year's (2014)				
May	0.29	0.18	0.11	0.14				
June	0.11	0.15	-0.04	0.28				
July	0.33	0.13	0.2	0.2				
August	0.21	0.11	0.1	0.04				
September	0.01	0.11	-0.1	0.11				
October	0.13	0.13	0	0.27				
November	0.21	0.16	0.05	0.15				

Table 2. Average precipitation per day (NOAA, 2016).

CHAPTER 6

DISCUSSION

6.1 Application of Phosphorous

The application of phosphorous had no significant effect on *Carex lurida*, but had a significant effect in *Typha latifolia*. *Typha latifolia* that was dosed with phosphorous had larger amounts of P in both above and belowground biomass than the control (Figure 10), showing plants store more nutrients than are necessary for growth (Cronk and Fennessy, 2001). Dosed specimens of *Typha latifolia* also had larger amounts of potassium (Figure 13), and magnesium (Figure 14) than control specimens. Magnesium is a carrier of phosphorous in plants (Spectrum Analytic Inc., 2016), and potassium is required to translocate phosphorous (IPNI, 1998). My results corroborate the fact that plants with higher amounts of phosphorous contain larger amounts of magnesium and potassium to carry and translocate the phosphorus throughout the plant.

6.2 Change over seasons

Plants typically contain fewer nutrients in the fall and winter as they enter a dormant stage, and higher concentration of nutrients in early growing season (Johnston, 1991). Dormancy occurs due to decreasing daylight and lower temperatures (Raven et al. 2005). My expectations were that storage of phosphorous would change locations during the season. However, my

results show the storage of phosphorous between above-and belowground biomass for *Typha latifolia* over the seasons was not significant. In *Typha latifolia* the storage of calcium, potassium, magnesium, and sulfur were greater in the growing months of June and July than the non-growing month of October (Figures 12, 13, 14, 16)

Carex lurida contained larger amounts of nutrients in the non-growing season than the growing. In the months of September and October Carex lurida contained higher amounts of phosphorous, calcium, potassium, and sulfur compared to June and July (Figures 21, 22, 24, 25, 28), specifically with larger amounts of nutrients in the specimens harvested in September than in October. For the non-growing seasons the specimens were collected in the beginning of October and November. Both months had temperatures that were higher than normal (Table 1). The month of October had an increase of 1.3 degrees Fahrenheit from normal, with a maximum temperature of 76 degree Fahrenheit. November had an increase of 4.7 degrees Fahrenheit from normal, with a maximum temperature of 68 degrees Fahrenheit. With higher temperatures than normal the plants may not have responded to environmental cues to begin translocating nutrients to belowground biomass from aboveground biomass (Cronk and Fennessy, 2001), and were still actively using nutrients for photosynthesis and growth.

6.3 Storage of nutrients

Carex lurida contained more phosphorous in leaves/shoots than roots (Figure 22). Phosphorous (P) is required for photosynthesis, and typically found in large amounts in leaves (Raven et al. 2005). When the supply of P in soils is high, plants store the excess in older leaf tissue and vacuoles (Schachtman et al, 1998). The control specimens of *Typha latifolia* contained

more P in leaves/shoots than roots, while the dosed specimens, although they contained more P than the control, contained more P in roots than leaves/shoots. This could be due to insufficient sampling size as a result of the death of specimens from the month of September, or the specimens could have been storing more P in roots to prepare for vegetative growth. Large amounts of P are needed to transfer the genetic code (IPNI, 1999), and none of the specimens were developing sexually.

Typha latifolia contained more calcium, Ca, in leaves/shoots than in roots. This was similarly observed in Carex lurida with the exception of the month of September (Figure 12 and 24). Ca is important in mitochondrial function. Leaves of plants that are undergoing photosynthesis would need larger amounts of Ca (Hepler, 2005).

Potassium, K, ions are important in the production of ATP, and adequate supplies of K are needed for photosynthesis to proceed, and for the opening and closing of stomata (IPNI, 1998). *Carex lurida* results show more amounts of Potassium, K, in leaves than in roots (Figure 26), indicating high levels of photosynthesis occurring. There was a significant difference between dosed and control specimens of *Typha latifolia*, and location of K (Figure 13). Specimens treated with P contained more overall storage of K in roots compared to shoots/leaves, this suggests the specimens were storing the excess K in roots. Control specimens of *T. latifolia* contained more K in roots in October, while June and July contained more K in shoots/leaves. Without extra supplies of K the specimens were using what was available in above ground tissue to undergo photosynthesis, until October when greater amounts of nutrients were being stored in roots in preparation for winter.

Results from *Typha latifolia* specimens show there were greater amounts of Magnesium, Mg, in shoots/leaves than in roots (Figure 15), as does *Carex lurida* (Figure 27), with the

exception of the month of October. *Typha latifolia* contains larger amounts of Mg in the growing season than the non-growing season (Figure 14). Mg is a main component in chlorophyll, and is therefore a requirement for photosynthesis (Patterson, 2016). High levels of Mg in leaves rather than roots indicate use of Mg in photosynthesis, rather than storage such as what is seen in *Carex lurida* in the month of October when greater amounts of Mg are found in roots than leaves/shoots (Figure 27). Both *Carex lurida* and *Typha latifolia* contain more sulfur, S, in aboveground biomass than belowground biomass (Figure 17, and 29), which is to be expected, since sulfur is an important element in the formation of chlorophyll (Steward, 2010).

CHAPTER 7

SUMMARY AND CONCLUSION

The original intent of this research was to investigate how two wetland plant species store phosphorous between seasons. After the experiment was concluded results for the amounts of other nutrients were also determined. The first hypothesis was that the storage of phosphorous would be greater in belowground biomass in the non-growing season compared to the growing season. The results were not significant for either species in reference to phosphorous. The second hypothesis was that the storage of phosphorous would be greater in aboveground biomass in the growing season compared to the non-growing season. The results were not significant for either species in reference to phosphorous. So neither hypothesis can be proven true. Although there was no significance for phosphorous storage, the results did yield significance for other nutrients.

Both *Carex lurida*, and *Typha latifolia* showed significant differences in the storage of calcium, with both storing more calcium in above ground biomass than belowground biomass in the growing season, and more calcium in belowground biomass than the aboveground biomass in the non-growing season. This is also seen in the results from *Carex lurida* and the storage of magnesium, where there was more storage in the roots in the non-growing season than the growing.

This study also showed that plants store more nutrients in the non-growing season than the growing. Although they may be taking in larger amounts of nutrients from the water system

in the early growing season they are readily using them and not storing them as they would be in the fall and winter. This study also shows that the dosing of phosphorous not only affects the storage of other nutrients, but also affects the location of the storage of those nutrients.

7.1 Recommendations for Future Research

In order to learn more about the storage of phosphorous in above- and belowground biomass between seasons, it is recommended to repeat the experiment increasing the number of specimens. A similar experiment should be conducted, but for a longer period that extends into winter, such as December or January, to see the full extent of the non-growing season. To further understand the effect of phosphorous on the storage and location of other nutrients within plants it is recommend a similar experiment be conducted using more replicates and varying amounts of phosphorous.

7.2 Significance of the Study

The important findings of this study were that there was a significant difference in the storage of nutrients between above- and belowground biomass between months. The findings also show that plants that have excess amounts of phosphorous store them differently than under normal conditions. The study shows that specimens with increased amounts of phosphorous in the water take up more phosphorous and store more in aboveground biomass, even in the non-growing season. Although these specimens continue to take up nutrients in the non-growing season, it is in lower amounts than the growing. Therefore the aboveground biomass in the non-growing season would still contain large amounts of phosphorous, and cutting the above ground

vegetation, by farmers and landowners, of both *Carex lurida* and *Typha latifolia* in the non-growing season, could release larger amounts of nutrients, specifically phosphorous, into the water system than previously estimated. This increase in nutrients could lead to higher levels of eutrophication of downstream receiving systems.

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APPENDIX

```
Analysis of Variance Table of type III with Satterthwaite
approximation for degrees of freedom
                                    Mean Sq NumDF DenDF F.value Pr(>F)
                           Sum Sq
Treatment
                        0.0121999 0.0121999
                                              1
                                                    24 4.5750 0.04282 *
month
                        0.0025368 0.0012684
                                              2
                                                    24 0.4757 0.62721
Location
                        0.0049575 0.0049575
                                              1
                                                    24 1.8591 0.18538
                        0.0043509 0.0021755 2
0.0108964 0.0108964 1
Treatment:month
                                                    24 0.8158 0.45418
                                                    24 4.0862 0.05452 .
Treatment:Location
                        0.0091414 0.0045707
                                               2
month:Location
                                                     24 1.7140 0.20146
Treatment:month:Location 0.0041323 0.0020661
                                               2
                                                   24 0.7748 0.47198
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table 3. ANOVA results for interactions of *Typha latifolia* with Phosphorous as response variable.

```
Analysis of Variance Table of type III with Satterthwaite
approximation for degrees of freedom
                         Sum Sq Mean Sq NumDF DenDF F. value
Treatment
                        0.01282 0.01282 1
                                               24
                                                     0.445 0.5111762
                                           2
                                                24 10.449 0.0005444 ***
month
                        0.60231 0.30115
                                          1
                        2.13477 2.13477
                                               24 74.066 8.459e-09 ***
Location
                        0.12155 0.06078
0.06055 0.06055
Treatment:month
                                          2
                                                24
                                                    2.109 0.1433360
Treatment:Location
                                          1
                                                24
                                                    2.101 0.1601791
month:Location
                        0.18708 0.09354
                                           2
                                                24
                                                     3.245 0.0565633 .
Treatment:month:Location 0.46025 0.23013
                                          2
                                                24
                                                     7.984 0.0021974 **
```

Table 4. ANOVA results for interactions of *Typha latifolia* with Calcium as response variable

Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom

	Sum Sq	Mean Sq	Numbe	DenDF	F. value	Pr(>F)	
Treatment	0.44275	0.44275	1	24	1.7379	0.19985	
month	1.44626	0.72313	2	24	2.8385	0.07825	
Location	0.23925	0.23925	1	24	0.9391	0.34217	
Treatment:month	1.23501	0.61751	2	24	2.4239	0.10995	
Treatment:Location	1.11477	1.11477	1	24	4.3758	0.04721	Ŕ
month:Location	0.44340	0.22170	2	24	0.8702	0.43166	
Treatment:month:Location	2.01172	1.00586	2	24	3.9483	0.03293	*

Table 5. ANOVA results for interactions of *Typha latifolia* with Potassium as response variable.

Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom

	Sum Sq	Mean Sq	NumDF	DenDF	F. value	Pr(>F)	
Treatment	0.000061	0.0000608	1	48.001	0.0605	0.80672	
month	0.035834	0.0179168	2	48.001	17.8246	1.624e-06	***
Location	0.024701	0.0247014	1	48.001	24.5742	9.324e-06	***
Treatment:month	0.010202	0.0051010	2	48.001	5.0747	0.01002	W
Treatment:Location	0.003170	0.0031704	1	48.001	3.1541	0.08208	
month:Location	0.002248	0.0011238	2	48.001	1.1180	0.33529	
Treatment:month:Location	0.003731	0.0018653	2	48.001	1.8557	0.16739	

Table 6. ANOVA results for interactions of *Typha latifolia* with Magnesium as response variable.

```
Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom
```

	Sum Sq	Mean Sq	Numbe	DenDF	F. value	Pr(>F)	
Treatment	0.0000007	0.0000007	1	24	0.0005	0.9828689	
month	0.0285252	0.0142626	2	24	9.6595	0.0008363	***
Location	0.0294433	0.0294433	1	24	19.9409	0.0001617	***
Treatment:month	0.0003213	0.0001607	2	24	0.1088	0.8973358	
Treatment:Location	0.0002707	0.0002707	1	24	0.1834	0.6723230	
month:Location	0.0027077	0.0013539	2	24	0.9169	0.4133005	
Treatment:month:Location	0.0055829	0.0027914	2	24	1.8905	0.1728011	

Table 7. ANOVA results for interactions of *Typha latifolia* with Sulfur as response variable.

Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom

	Sum Sq	Mean 5q	Numbe	DenDF	F. value	Pr(>F)	
Treatment	0.612	0.612	1	24	0.0370	0.8489855	
month	8.725	4.362	2	24	0.2642	0.7699882	
Location	277.801	277.801	1	24	16.8278	0.0004069	***
Treatment: month	27.209	13.604	2	24	0.8241	0.4506680	
Treatment:Location	9.352	9.352	1	24	0.5665	0.4589776	
month:Location	1.995	0.997	2	24	0.0604	0.9415110	
Treatment: month: Location	4.214	2.107	2	24	0.1276	0.8807601	

Table 8. ANOVA results for interactions of *Typha latifolia* with dry mass as response variable.

Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom

	Sum Sq	Mean Sq	NumDF	DenDF	F. value	Pr(>F)	
Treatment	0.000099	0.0000992	1	32	0.1133	0.738624	
month	0.034916	0.0116386	3	32	13.2863	8.417e-06	异异异
Location	0.010170	0.0101696	1	32	11.6093	0.001788	**
Treatment:month	0.001007	0.0003357	3	32	0.3832	0.765756	
Treatment:Location	0.000617	0.0006170	1	32	0.7044	0.407537	
month:Location	0.002917	0.0009724	3	32	1.1100	0.359453	
Treatment:month:Location	0.000056	0.0000187	3	32	0.0213	0.995685	

Table 9. ANOVA results for interactions of *Carex lurida* with Phosphorous as response variable.

Analysis of Variance Table of type III with Satterthwaite approximation for degrees of freedom

	Sum Sq	Mean Sq	Numbe	DenDF	F. value	Pr(>F)	
Treatment	0.000469	0.000469	1	32	0.0259	0.873143	
month	0.151923	0.050641	3	32	2.7963	0.055967	
Location	0.029149	0.029149	1	32	1.6095	0.213709	
Treatment:month	0.009143	0.003048	3	32	0.1683	0.916996	
Treatment:Location	0.015380	0.015380	1	32	0.8492	0.363662	
month:Location	0.272413	0.090804	3	32	5.0140	0.005816	**
Treatment:month:Location	0.030038	0.010013	3	32	0.5529	0.649926	

Table 10. ANOVA results for interactions of Carex lurida with Calcium as response variable.

```
Analysis of Variance Table of type III with Satterthwaite
approximation for degrees of freedom
                         Sum Sq Mean Sq NumDF DenDF F. value
                                                                Pr(>F)
                                0.0782
Treatment
                         0.0782
                                            1
                                                  32
                                                       0.398
                                                                0.5325
                         7.3015
                                 2.4338
                                            3
                                                  32 12.394 1.524e-05 ***
month
                                 7.5842
                                                     38.621 5.840e-07 ***
Location
                         7.5842
                                            1
                                                  32
Treatment:month
                         0.0440
                                 0.0147
                                            3
                                                  32
                                                       0.075
                                                                0.9732
Treatment:Location
                         0.0005
                                 0.0005
                                            1
                                                  32
                                                       0.003
                                                                0.9603
month:Location
                         0.0873
                                 0.0291
                                            3
                                                  32
                                                       0.148
                                                                0.9300
Treatment:month:Location 0.0702 0.0234
                                            3
                                                  32
                                                       0.119
                                                                0.9482
```

Table 11. ANOVA results for interactions of *Carex lurida* with Potassium as response variable.

```
Analysis of Variance Table of type III with Satterthwaite
approximation for degrees of freedom
                                    Mean Sq NumDF DenDF F. value
                           Sum Sq
Treatment
                        0.0000001 0.0000001
                                               1
                                                    32 0.0001 0.991913
                                                    32 12.0093 1.981e-05 ***
month
                        0.0249259 0.0083086
                                               3
Location
                        0.0063392 0.0063392
                                                        9.1627
                                               1
                                                    32
                        0.0009685 0.0003228
Treatment:month
                                               3
                                                    32
                                                        0.4666
Treatment:Location
                        0.0003971 0.0003971
                                               1
                                                    32
                                                        0.5740
                                                                0.454217
month:Location
                        0.0084254 0.0028085
                                               3
                                                    32 4.0594
                                                                0.014934 *
Treatment:month:Location 0.0006017 0.0002006
                                               3
                                                    32 0.2899 0.832334
```

Table 12. ANOVA results for interactions of *Carex lurida* with Magnesium as response variable.

```
Analysis of Variance Table of type III with Satterthwaite
approximation for degrees of freedom
                                    Mean Sq NumDF DenDF F. value
                           Sum Sq
                                                                   Pr(>F)
Treatment
                         0.001504 0.0015044
                                                        0.9705 0.3319411
                                                1
                                                     32
month
                         0.043751 0.0145837
                                                3
                                                     32
                                                         9.4080 0.0001318 ***
Location
                         0.007640 0.0076396
                                                1
                                                     32
                                                        4.9284 0.0336263 *
Treatment:month
                         0.001110 0.0003700
                                                3
                                                     32
                                                        0.2387 0.8687261
Treatment:Location
                         0.000499 0.0004990
                                                1
                                                     32 0.3219 0.5744306
month:Location
                         0.006025 0.0020085
                                                3
                                                     32 1.2957 0.2927903
Treatment:month:Location 0.001351 0.0004502
                                                3
                                                     32 0.2904 0.8319669
```

Table 13. ANOVA results for interactions of *Carex lurida* with Sulfur as response variable.

```
Analysis of Variance Table of type III with Satterthwaite
approximation for degrees of freedom
                         Sum Sq Mean Sq NumDF DenDF F.value
                                                               Pr(>F)
Treatment
                          1.468
                                 1.468
                                                 32
                                                    0.4289 0.5172050
                                           1
                                                 32 7.8134 0.0004735 ***
                                            3
month
                         80.254
                                26.751
Location
                        45.983 45.983
                                           1
                                                 32 13.4304 0.0008890 ***
                                  6.285
Treatment:month
                        18.855
                                            3
                                                 32 1.8356 0.1605456
                                                 32 0.5319 0.4710973
Treatment:Location
                         1.821
                                 1.821
                                           1
                                                 32 8.3952 0.0002935 ***
month:Location
                         86.230
                                28.743
                                            3
Treatment:month:Location 21.935
                                 7.312
                                            3
                                                 32 2.1355 0.1151438
```

Table 14. ANOVA results for interactions of *Carex lurida* with dry mass as response variable.

VITA

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