

**Destination of Isotopic Nitrogen Fertilizer Under Varying  
Herbicide Regimes in a Mid-Rotation Loblolly Pine (*Pinus taeda* L.)  
Plantation in the Piedmont of Virginia, USA**

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ACADEMIC ABSTRACT

Mid-rotation fertilization and vegetation control are some of the most common silvicultural treatments in loblolly pine (*Pinus taeda* L.) plantations in the southeastern United States. Competing vegetation is commonly thought to sequester fertilizer nitrogen (N) and reduce the potential growth response to a mid-rotation fertilization treatment. This experiment aims to identify what proportion of applied N fertilizer is retained in the crop tree pine foliage, and the degree to which understory vegetation is competing for this resource. Our mid-rotation loblolly pine plantation received an application of <sup>15</sup>N fertilization (urea 365 kg/ha, at 46% N by weight, i.e. 168 kg/ha of N) and a portion of plots received an understory vegetation control (basal spray application of triclopyr; 13.6% active ingredient) treatment either before fertilization or not at all. One-year post-fertilization, <sup>15</sup>N contents within pine foliage, leaf fall/leaf litter, forest floor, and soil were measured, as was competing vegetation presence. There was significant variation in applied nitrogen acquisition among the different ecosystem components measured, with 0-15 cm soils retaining a majority at 32-37% added <sup>15</sup>N. Differences in fertilizer N acquisition in pine foliage between plots with and without understory vegetation control was marginally significant ( $p = 0.06$ ) with pine foliage in plots without understory vegetation capturing greater <sup>15</sup>N (4.3% greater). Red maple (*Acer rubrum*) and oak species (*Quercus* spp.) were the most common competitors but

neither had a uniquely pronounced effect on pine nitrogen sequestration. My data indicate that increasing competition reduces fertilizer N foliar concentrations in crop pine trees but at a modest rate and equally across species groups. An unrefined threshold determining when fertilizer N capture in crop pine trees was affected was found at 3.1 m<sup>2</sup>/ha of competing vegetation basal area. This site will continue to be monitored over time to assess fertilizer N retention in loblolly pine each year after fertilization and evaluate the fertilizer N capture within competing vegetation.

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GENERAL AUDIENCE ABSTRACT

Some of the most prevalent management practices for mid-rotation (age 15, i.e., roughly halfway through a crop cycle) loblolly pine (*Pinus taeda* L.) plantations in the southeastern United States are fertilization and vegetation control. Nitrogen (N) is consistently one of the most limiting factors to productivity. The addition of N via fertilization is therefore a common forestry practice. However, when a stand is fertilized, the added resource is partitioned and cycled throughout the ecosystem. It is presumed that the amount of fertilizer N obtained by crop trees in a plantation is dependent on the level of competing vegetation (i.e., weed-trees and shrubs) present on site. Controlling competing vegetation prior to fertilization may therefore be warranted under certain conditions. To date, the amount of competing vegetation where it begins to impact fertilizer uptake by the crop tree is unknown. This study aims to elucidate this competing vegetation threshold to better inform mid-rotation management of loblolly pine plantations. This study examined applied fertilizer N capture in ecosystem components with varying levels of understory vegetation, and found more fertilizer N in pine foliage when understory vegetation was completely removed prior to fertilization. No single understory hardwood weed species had a uniquely strong influence on crop tree productivity uptake. Plots that ranked in the upper third in competing vegetation presence did have significantly less foliar fertilizer N in the pine crop trees. Additional

replication of this study would be necessary to determine a universal threshold of competing vegetation which would trigger the removal of competing vegetation prior to fertilization.

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## 1. INTRODUCTION

Loblolly pine (*Pinus taeda* L.) is one of the primary timber species utilized in intensive silviculture in the southeastern US (Oswalt 2019), and nutrient limitations (most commonly nitrogen (N)) often limits productivity in these loblolly pine plantations (Vitousek 1986, 1991). Competing vegetation often plays a part in nutrient limitation of crop trees, and vegetation control is often employed as a tool to counteract this (Oppenheimer 1989). Despite the commonality of these treatments, the interaction of mid-rotation applications of fertilizer and vegetation control and its influence on crop tree pine fertilizer assimilation is still poorly understood.

Fertilizer regimes include considerations of the fertilizer source, rate, timing, and application method (Boswell et al. 1985). Urea, applied by the air or ground, is the most concentrated solid N fertilizer (Finch et al. 2014) and the primary source of N fertilizer in southern pine plantations due to its high N content (46% by weight), overall cheap cost per unit (Allen 1987, Fox et al. 2007), and role in recycling and translocating N in plants (Naz 2023).

Despite that fertilization has been shown to consistently increase productivity in pine plantations for decades (Fox et al. 2007, Raymond et al. 2016) there are many questions regarding crop tree assimilation of the fertilizer resource. The ultimate destination (i.e., the ecosystem component in which the fertilizer is found) of fertilizer N post-application in these plantation systems remains poorly documented. Furthermore, how fertilizer N destination is influenced by competitor abundance remains largely unknown (Raymond et al. 2016).

Fertilizer N is lost from plantation systems through such pathways as volatilization, denitrification (Shrestha *et al.* 2014), and leaching (Vitousek & Matson 1985a, Binkley *et al.* 1999, Meason *et al.* 2004, Aust & Blinn 2004). Subsequently, fertilizer N uptake efficiencies within loblolly pine plantations often vary and are substantially less than 100% of all fertilizer applied. For instance, a young pine plantation in Oklahoma, USA with a urea and diammonium phosphate fertilizer application was found to have recovered 6% to 25% of the applied N (Blaizer *et al.* 2006). An average 14.5% of fertilizer N was recovered in a 38 to 39-year old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) stand a decade post-application (Mead *et al.* 2008), while another Douglas-fir stand had recoveries in the region of 30% (Heilman *et al.* 1990). While assimilation rates of fertilizer N have been found to vary by system and by the age of the plantation, the influence of competing vegetation on this assimilation rate has not been studied extensively.

Stable isotopes, like  $^{15}\text{N}$ , are a tool for evaluating fertilizer capture and nutrient deficits (Hauck 1968).  $^{15}\text{N}$  enriched fertilizers allow us to follow the eventual fate of the applied nitrogen (Powlson & Barraclough 1993, Hauck 1968, Walker 1958). Stable isotopes also allow us to better understand the relationships of crop tree productivity and nutrient destination within the system (Sybert 2005, Birk & Vitousek 1985, Miller 1981). Utilizing  $^{15}\text{N}$ , we can identify fertilizer recovery similar to studies like Raymond *et al.* (2016) which used stable isotopes to assess fertilizer nitrogen use efficiency and the destination of N in the system.

Factors affecting nitrogen assimilation and best practices for nitrogen fertilizer application are important to study to improve fertilizer uptake efficiency and plantation

productivity. Nitrogen is a critical and often limiting nutrient for mid-rotation pine stands in the southeastern US, and its dynamics are influenced by a complex set of biotic and abiotic factors. By informing best management practices for fertilization regimes, we can ensure the long-term health and productivity of pine stands.

When competing vegetation control is conducted in combination with fertilization it tends to result in a greater pine crop tree growth response than when fertilizer is applied alone (Albaugh et al. 2012a). Albaugh et al. (2012a) saw a 31% increase in foliar nitrogen concentrations in stands with combined fertilizer and vegetation control applications compared to 24% and 1% increases, respectively, when the treatments were applied separately. Competing vegetation (hardwood basal area and shrub cover) decreased 81% in the combined treatment compared to an increase of 4% with fertilization alone (Albaugh et al. 2012a). The small competing vegetation growth increase in response to fertilization alone may suggest resources other than N, are limiting. Gurlevik et.al. (2004) found that field net N mineralization increased as a result of both vegetation control and fertilization, and there was a considerable positive interaction between the treatments. What remains unknown is to what extent varying quantities of competing vegetation limit the assimilation of fertilizer N into pine crop trees.

## LITERATURE REVIEW

Intensively managed tree plantations with high growth rates provide significant amounts of fiber and help to fulfill the rising demand for raw materials (Sedjo 2001). Intensively managed loblolly pine (*Pinus taeda* L.) plantations are a cornerstone of the

timber industry in the southeastern U.S. (Oswalt et al. 2019, Shepard et al. 2022). In the United States, plantation forests are largely concentrated in the South (Oswalt et al. 2014) occupying 12.8 million ha of mostly loblolly pine (Albaugh et al. 2007). The productive capacity of these forests can rise from less than  $2 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$  to more than  $10 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$  with intensive silvicultural management (Fox et al. 2007). Further, theoretical models and empirical field testing suggest that these southern pine plantations are capable of producing  $20 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$  over 15-year rotations (Allen et al. 2005, Fox et al. 2007). Future productivity gains must incorporate site-specific silvicultural treatments to address growth-limitations sustainably.

Nitrogen plays a vital role in the health of forests by promoting growth and enabling trees to convert sunlight into chemical energy to fuel their growth and make nutrients for leaves and other tree parts. Leaves with a limiting nitrogen supply are less productive mainly as a result of an effect on meristem size and cell count (Lambers et al. 2008). In the absence of nitrogen, trees stop growing, leafy branches die back, and entire trees die. N availability has a significant impact on photosynthesis since the majority of the N in a leaf is used by the photosynthetic machinery, and much of the remaining N is indirectly related to that function (Lambers et al. 2008).

Due to its frequently low abundance or inaccessible form, nitrogen is recognized as a common limiting factor to growth in mid-rotation loblolly pine stands and most restricting to plant development and productivity (Albaugh 2012b, Morgan & Connolly 2013). Forest soils are typically low in available nitrogen, and the quantity of trees and shrubs play an important role in reducing the supply of nitrogen in the soil (Lambers et al. 2008).

At mid-rotation, fertilization and the timing of understory vegetation control are some of the most important management decisions to be made by practitioners. In management practices, urea is the most common nitrogen fertilizer used in southern pine plantations due to its high N content (46%) and general minimal expense per unit (Allen 1987, Fox et al. 2007). Competing vegetation in the understory of pine plantations can sequester resources, namely nitrogen, limiting growth of crop pine species (Colbert et al. 1990, Amishev & Fox 2006). In commercial pine plantations, the main purpose of adding inorganic N fertilizer is to increase tree growth and ensure that the plantation remains profitable by promoting the health of existing trees, increasing the general health of our target species, altering diameter distributions, and to prevent competing vegetation from detrimentally impacting the crop trees in the stand (Boswell et al. 1985). In the southeast US, mid-rotation loblolly plantations frequently respond to fertilizer applications of nitrogen and phosphorus: over an eight-year period, 224 kg N ha<sup>-1</sup> and 56 kg P ha<sup>-1</sup> has been shown to increase wood output by an average of 3.50 m<sup>3</sup> ha<sup>-1</sup> year<sup>-1</sup> (Carlson et.al. 2014).

When understory vegetation is present, loblolly pine plantations experience reduced tree growth and increased mortality (Allen & Albaugh 2000). The heightened mortality is likely due to increased competition for resources, such as light, water, and nutrients. Reducing resources to a low level and tolerating limited amounts of resources are two competitive strategies species deploy when competition causes a decrease of a shared, limiting resource (Lambers et al. 2008). Abundant competition can slow growth and reduce the amount of nitrogen that is used by trees (Robinson 2001). In the mid-rotation stage of stand development, competing vegetation can also

grow into the canopy, reducing crop tree light capture, in addition to belowground resource acquisition (Nilsson et al. 1996).

Regardless of critical gains in forest productivity as a result of fertilization, the fate of fertilizer nitrogen in these plantation systems is largely unknown (Fox et al. 2007). Varying amounts of total competing vegetation as well as different competing species may uniquely modulate fertilizer nitrogen availability in pine plantations. Testing metrics of competition which consistently predict pine growth responses to mid rotation vegetation control has been challenging (Albaugh et al. 2012). In the total absence of competing vegetation, however, nitrogen fertilizer is most effective (Hanna 2000, Albaugh et al. 2012). Tracking nitrogen movement within a system is important for understanding where there are abundances and disparities of the nutrient and, further, how physiological differences among coexisting plants influence the acquisition of fertilizer N by crop tree pine. More specifically, previous studies (Albaugh et al. 2015b, Robinson et al. 2001, and Martin et al. 1999, Nilsson et al. 1996) are not representative of southeastern (piedmont) loblolly pine mid-rotation fertilization conditions.

Other ecosystem elements, which include litter, forest floor (organic horizon), and mineral soil, also sequester fertilizer N that is not lost from the ecosystem or assimilated by the crop trees (Birk and Vitousek 1986; Albaugh et al. 2004). Reduced fertilizer N uptake by desired crop trees leads to lower fertilizer nitrogen use efficiency, which is the ratio between the quantity of fertilizer N removed with the crop and the dose of fertilizer N applied (Havlin et al. 2014). Inability to accurately measure the cycling of fertilizer N and its final fate in forest ecosystems hinders our ability to estimate the proportion of fertilizer N that is assimilated by crop trees and lost from the system.

Current management practices often involve competition control at mid-rotation immediately followed by fertilization. The goal is to have herbicide reduce competing vegetation presence prior to fertilization thus allowing more of the fertilizer nitrogen to be acquired by the crop pine species. It is important to note that there is variability among the deployment and timing of these treatments in industry. Sometimes solely vegetation control is done, disregarding fertilization. This is mostly determined by the cost of the herbicide as, typically, herbicide is less expensive than fertilizer. Additionally, this suggested order of treatments may not always be followed depending on the availability of contractors and weather conditions.

Only two of the six nitrogen isotopes (mass numbers 14 & 15) occur naturally and are stable. Due to their essentially constant ratio in nature, the stable N isotopes ( $^{14}\text{N}$  and  $^{15}\text{N}$ ) have been utilized nearly exclusively as tracers for biological and related kinds of research (Boswell et.al. 1985). This practice of isotope tracing is allowing us to expand our knowledge and understanding of the fate of nitrogen in ecosystems, especially in crop pine production. This is a continued trend in research—stable isotopes are frequently used in conjunction with other analytical techniques to enhance comprehension of nutrient content and fertilizer reactivity (Miller 1981, Birk & Vitousek 1985, Sybert 2005, Raymond et al. 2016).

In mid-rotation loblolly pine plantations, nutrient availability and understory competing vegetation are two of the most important treatments to be considered. The efficacies of fertilizer and herbicide treatments in loblolly pine plantations tend to vary, and the causality is unknown. Independently, these mid-rotation treatments typically result in a positive growth response of the crop species, and combined the response is



expected to be additive (Albaugh et al. 2012). Competition in the understory of pine plantations depletes essential resources in the forest ecosystem that would otherwise be utilized by the crop tree species. With increased competition for resources on a nutrient or water limited site, there is reduced overall productivity, resulting in reduced growth and yield. Therefore, informing herbicide application and fertilization based on the quantity of understory competition is vital. Knowing this will (1) increase our comprehension of the ecosystem processes of intensively managed loblolly pine systems and (2) allow land managers to adapt management practices to optimize results.

This study aims to identify fertilizer  $^{15}\text{N}$  acquisition disparities at mid-rotation under varying competing vegetation control regimes and presence values. Our objectives are as follows: (1) estimate the relative presence of  $^{15}\text{N}$  fertilizer sequestered by crop loblolly pine foliage at mid-rotation; (2) measure variability in crop tree  $^{15}\text{N}$  sequestration between plots with and without competing vegetation and among varying vegetation densities; (3) measure the effect of competing vegetation aboveground biomass presence on crop tree  $^{15}\text{N}$  capture; and (4) estimate the relative “competitiveness” of competing vegetation by species. We expected there to be (1) greater kg/ha of fertilizer nitrogen captured by crop loblolly pine trees compared to other ecosystem parts, (2) greater proportion of fertilizer nitrogen sequestered by the pine trees when competing vegetation was removed, and (3) that species identity would not be an influential predictor of the acquisition of fertilizer N by crop tree foliage.

## 2. METHODS

### 2.1 Site Description

This study was conducted at the Appomattox-Buckingham State Forest (37°26'06.4"N 78°39'10.0"W) in Virginia, USA. Average annual precipitation is 118.3 centimeters, average growing season temperatures range from 11 to 24°C, and winter temperatures range from -2 to 10°C (US Climate Data 2023).

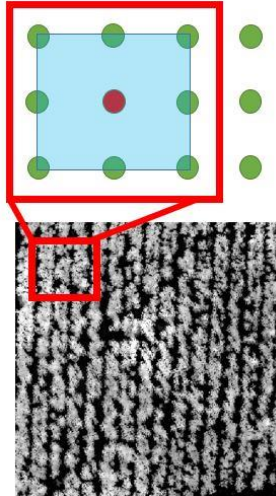
The site is comprised of Spears Mountain silt loam (Fine, mixed, semiactive, mesic Typic Hapludults) and Littlejoe silt loam (Fine, mixed, subactive, mesic Typic Hapludults)—both of which are considered well-drained with an index surface runoff of low to high. The profile is most commonly silt loam in the first horizon for roughly 15cm followed by clay for the next 75cm (USDA-NRCS (2020) Web Soil Survey). The study was established in a mid-rotation loblolly pine plantation (ages 14-16 at time of sampling), planted at an approximate 3m by 3m spacing. The competing vegetation found in the midstory and understory include red maple (*Acer rubrum* L.), chestnut oak (*Quercus montana* W.), black oak (*Q. nigra* L.), Virginia pine (*Pinus virginiana* Mill.), loblolly pine, yellow-poplar (*Liriodendron tulipifera* L.), blackgum (*Nyssa sylvatica* L.), hickory (*Carya* spp Nutt.), black cherry (*Prunus serotina* Ehrh.), maple-leaf-viburnum (*Viburnum acerifolium* L.), eastern red cedar (*Juniperus virginiana* L.), tree-of-heaven (*Ailanthus altissima* Mill.), sassafras (*Sassafras albidum* Nutt.), winged sumac (*Rhus copallinum* L.), serviceberry (*Amelanchier arborea* F.Michx.), sourwood (*Oxydenrum arboretum* L.), flowering dogwood (*Cornus florida* L.), American beech (*Fagus grandifolia* Ehrh.), American chestnut (*Castanea dentata* Michx.), and *Vaccinium* spp L.

The current experiment is overlaid atop a former chemical site preparation study installed in 2005 that resulted in experimentally varied levels of understory competition presence today. The former study investigated chemical (imazapyr and sulfometuron methyl) site preparation regimes on loblolly pine growth into mid-rotation (Byers 2021). The gradient of understory competing vegetation basal area which resulted from the chemical site preparation study facilitated an investigation of the competitiveness of competing vegetation for fertilizer in a mid-rotation loblolly pine stand.

## *2.2 Experimental Design*

Data were collected from 48 pre-existing measurement plots (approximately 34 m<sup>2</sup>). Measurement plots were nested within treatment plots (approximately 79 m<sup>2</sup>). Treatment plots extended about 1.5 m outside of the measurement plot—half the distance to the next tree (Figure 1). Having measurement plots nested within treatment plots was intended to reduce asymmetrical root ingrowth of measurement-plot boundary trees so that we could better simulate stand-wide fertilization and herbicide treatments within the measurement plot.

Fertilization was applied at a rate of 365 kg/ha of liquified urea with a N content of 46% i.e., 168 kg/ha of N. The fertilizer solution was created by dissolving roughly a 3.78-liter bag of urea granules in 3.78 liters of warm water to create about a 7.5-liter solution to be applied on a single plot. Chemical control was applied using Pathfinder II ®, 13.6% triclopyr, applied as a basal spray application with 30 cm to 45 cm vertical bands of chemical to the lower stem of all competitors. Both fertilization and chemical control treatments were applied via basal spray by hand-crews with backpack sprayers.



**Figure 1.** Aerial view of measurement plot (~34 m<sup>2</sup>) and treatment area (~79 m<sup>2</sup>) diagram in a mid-rotation *Pinus taeda* L. plantation in Buckingham County, Virginia. Circles (green/red) represent planted trees. The red circle represents the focal tree from which data (foliage) were collected. The light blue box represents the measurement area where data collection took place, and the red square represents the treatment area where the chemical control applications and <sup>15</sup>N fertilizer were applied.

The treatments for the current study were as follows: (1) fertilized January 2021, chemically controlled January and March 2021 (i.e., pre-fertilization); (2) fertilized January 2021, chemically controlled February 2022 (i.e., post-fertilization); (3) fertilized January 2021, no chemical control applied (i.e., no vegetation control). Treatments were assigned using random stratification to assure each new treatment reflected the gradient of existing understory competition. A comprehensive timeline can be found in Table 1.

**Table 1.** Project timeline of applications and processes for the current study concerning applied fertilizer <sup>15</sup>N, chemical control regimes, and varying levels of understory competing vegetation in a mid-rotation *Pinus taeda* L. plantation in Buckingham County, Virginia.

| Date                  | Occurrence  |
|-----------------------|---|
| January/March 2021    | Herbicide (triclopyr; 13.6% active ingredient) applied as basal spray to 1/3 of the stand         |
| January 2021          | Fertilizer (urea) applied to entire stand   |
| September 2021        | Leaf litter traps constructed and installed into a subset of plots within the stand               |
| February 2022         | Herbicide (triclopyr; 13.6% active ingredient) applied as basal spray to another 1/3 of the stand |
| March 2022            | Pine foliage sampled from focal trees stand-wide  |
| March 2022            | Litter traps retrieved from subset of the stand   |
| March 2022            | Forest floor material sampled stand-wide  |
| April/May/June 2022   | Soil sampled stand-wide   |
| June 2022             | Bulk density value retrieved from subset of the stand   |
| July 2022             | Competing vegetation biomass sampled from the 1/3 of the stand without herbicide treatment        |
| June/July/August 2022 | Sampled material dried, ground, ball-milled, and processed through an isotopic mass spectrometer  |

### 2.3 Data Collection

Since this experiment is part of a long-term study, trees could not be harvested. Pre-treatment competing vegetation basal area was collected to determine a base-state for understory growth. The basal area for the competing vegetation was separated equally based on current understory vegetation levels across plots. The thresholds included “none” at 0 m<sup>2</sup>/ha, “low” at 0-0.8 m<sup>2</sup>/ha, “medium” at 0.8-3.1 m<sup>2</sup>/ha, and “high” at 3.1-18.3 m<sup>2</sup>/ha.

In order to have a representative sample of ecosystem components, data were collected from the stand as follows: focal tree loblolly pine foliage (red circle in Figure 1) from three heights within the canopy (lower, middle, and upper) as well as its diameter at breast height (DBH; 1.37m), foliage from 0.093m<sup>2</sup> leaf litter traps, forest floor material (including leaves, duff, and organic material) down to a mineral layer, soil to 15cm and 30cm depths, and bulk density. A subset of 25 leaf litter traps were randomized, setting single traps in different plots representing each treatment, and then were set in randomized directions and distances from the focal tree. Four soil increment and forest floor locations were devised in randomized directions and distances from the focal tree, one in each quadrant within the plot. These quadrant samples were then combined to make one plot sample. DBH for trees at least 1.37m in height, basal diameters for trees less than 1.37m in height, origin type (seed-in vs stump-sprout), and species were recorded for all unplanted stems within the measurement plot. DBH estimates were converted to aboveground biomass estimations using species-specific equations found in Jenkins et al. (2003) for trees over 2.5cm at DBH. For the planted pine, biomass values for the focal tree were multiplied by the number of trees within the treatment plot, and the values were then scaled to a per hectare basis based on the treatment plot approximations. For small stems and seedlings, biomass equations from Williams and McClenahan (1984) were utilized. At the time competing species data were collected, two-thirds of the plots no longer had understory vegetation, the entire experiment area had been fertilized with <sup>15</sup>N a year prior.

Collected samples were processed in a lab to estimate the biomass and <sup>15</sup>N concentrations. The samples were oven dried at 65°C and ground into a powder. The

samples were further refined in a ball-mill machine (Retsch® MM 200) at an oscillation-rate of 25/second for 1 minute 45 seconds per sample. Then representative sub-samples of 5 µg were used for total N and <sup>15</sup>N abundance evaluations by mass spectrometry. To calculate how much <sup>15</sup>N was in each sample, the samples were placed into an automated continuous flow Mass Spectrometer, Model ANCA-SL (Europa Scientific) following the methods of Mulvaney (1993), Barrie and Prosser (1996) and Fenilli et al. (2007). Each sample was separately run through the isotopic sampler and the resulting values of these processed samples were utilized in scaling the data up to a per-hectare basis using the following equation:

$$[1] \% \text{ Fertilizer in Component} = ( \delta^{15}\text{N}_f - \delta^{15}\text{N}_i ) / ( \delta^{15}\text{N}_a - \delta^{15}\text{N}_i )$$

<sup>15</sup>N presence sample estimation formula where  $\delta^{15}\text{N}_f$  is the final detected <sup>15</sup>N ratio in the sample,  $\delta^{15}\text{N}_i$  is initial <sup>15</sup>N presence before fertilizer application, and  $\delta^{15}\text{N}_a$  is the <sup>15</sup>N acquired in the sample once fertilized.

Total foliar N content (applied and already available; kg/ha) was then calculated by dividing the ‘%N’ output from the mass spectrometer by 100 and multiplying the result by the foliage biomass (kg/ha). Fertilizer N in foliage (kg/ha) was calculated by multiplying the total foliar N by the result of equation [1].

#### 2.4 Data Analysis

Statistical analyses were performed using JMP® (Version Pro16, SAS Institute Inc., Cary, NC, 1989–2021). Mixed effects analysis of variance and covariance with ‘plot’ as a random effect were applied to test response variables of <sup>15</sup>N concentrations among system components (i.e. focal tree foliage, soil 0-15cm, soil 15-30cm, leaf fall, and forest floor) with the alpha value set at 0.05. An arcsine transformation was

performed on variables in percentage units. Importance values (sum of relative frequency, and relative dominance (biomass), and relative density) were calculated to evaluate the effect of understory competing vegetation presence on focal tree capture of  $^{15}\text{N}$ . The importance values were included in mixed effects models, crossed with competing vegetation biomass and basal area values to view the response of pine foliar N capture.

### 3. RESULTS

#### 3.1 *Stand-wide Nitrogen Presence*

$^{15}\text{N}$  was found in all tested forest material components one-year post-fertilization. Of the sampled ecosystem components, total sampled system-wide fertilizer N retention was not significantly different between plots that retained competing vegetation and those that did not ( $p = 0.95$ , Table 2). Among components, N acquisition varied significantly among material types ( $p < 0.0001$ , Table 2). Averaged across treatments, soil (0-15 cm) had significantly greater  $^{15}\text{N}$  acquisition than soil (15-30 cm), pine foliage, forest floor, and leaf fall material types (Table 3). Soil (15-30 cm), pine foliage, and forest floor material types had similar  $^{15}\text{N}$  acquisition values and were not significantly different from each other. The lowest proportion of obtained  $^{15}\text{N}$  was in the leaf fall (Table 3).



**Table 2.** Analysis of variance summary table of the interaction of material type (focal tree foliage, soil 0-15 cm, soil 15-30 cm, leaf fall, forest floor) and treatment (with and without competing vegetation present) on stand-wide total sampled (2022) system <sup>15</sup>N acquisition in a mid-rotation *Pinus taeda* L. plantation in Buckingham County, Virginia (n = 277).

| Source                  | F Ratio | Std Error | Prob > F |
|-------------------------|---------|-----------|----------|
| Material Type           | 54.19   | 1.45      | <.0001   |
| Treatment               | 0.003   | 1.01      | 0.95     |
| Material Type*Treatment | 1.16    | 1.48      | 0.33     |

**Table 3.** Pairwise comparisons of the variable “Material Type”. The response was arcsine transformed and then back-transformed to reflect actual percent proportions of total sampled system <sup>15</sup>N acquisition in a mid-rotation *Pinus taeda* L. plantation in Buckingham County, Virginia. Levels not connected by the same letter represent significant differences of  $p \leq 0.05$  (n = 277).

| Material Type |   |   |   | Percent (%) of total fertilizer applied in Material Type |
|---------------|---|---|---|--|
| Soil 0-15 cm  | A |   |   | 34.3   |
| Soil 15-30 cm |   | B |   | 20.9   |
| Pine Foliage  |   | B |   | 18.2   |
| Forest Floor  |   | B |   | 17.6   |
| Leaf Fall     |   |   | C | 1.4  |

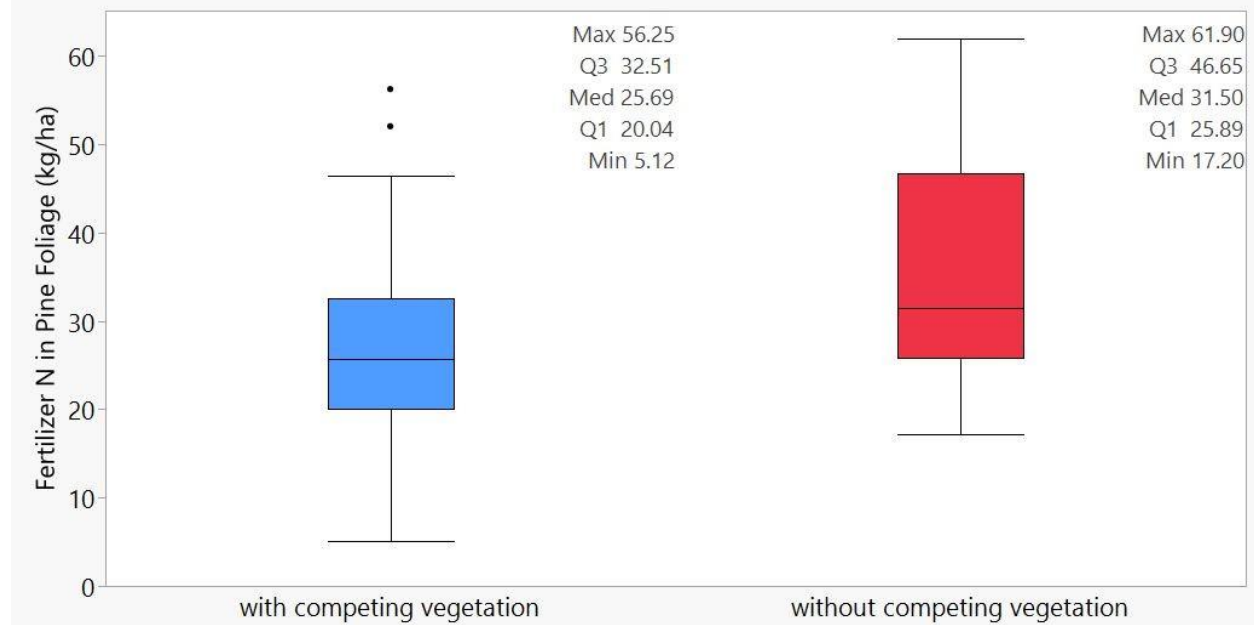
Testing each material type separately to consider how much fertilizer N was retained in the individual material type in plots with or without the presence of competing vegetation, proved predominantly insignificant. Soil sequestered applied N at both 0-15 cm and 15-30cm increment depths did not vary with the presence of competing vegetation ( $p = 0.43$  &  $0.59$  respectively). The presence of competing vegetation on this scale also proved insignificant on forest floor fertilizer nitrogen acquisition ( $p = 0.33$ ).

Competing vegetation incidence did not necessarily have an effect in creating a weighty response in leaf fall fertilizer nitrogen acquisition ( $p = 0.16$ ).

### 3.2 Competing Vegetation Influence

Loblolly pine tree foliar  $^{15}\text{N}$  acquisition was marginally greater when competing vegetation was absent ( $p = 0.06$ ) (Figure 2). Competing vegetation basal areas (0, low, medium, high) throughout the stand showed a significant effect on the response variable of applied nitrogen sequestered in pine foliage ( $p = 0.01$ ) (Figure 3).

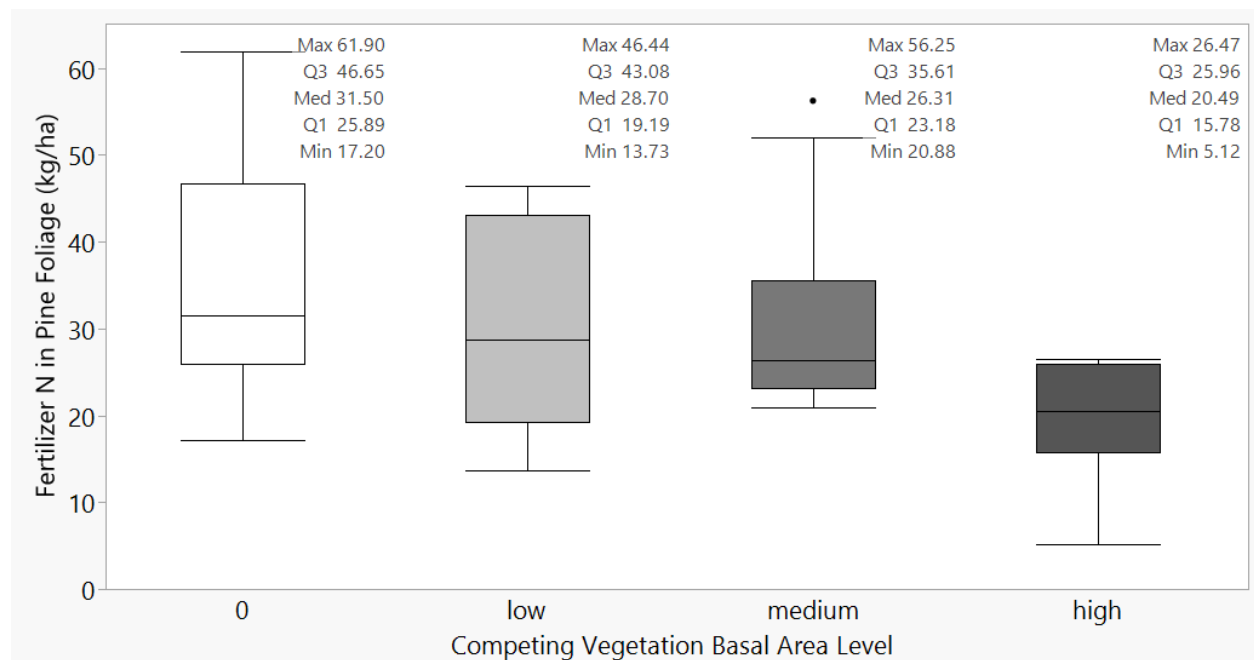
Specifically, levels '0' and 'high' were significantly different (Table 5).



**Figure 2.** The average applied fertilizer  $^{15}\text{N}$  ratio acquired by crop pine tree foliage between plots that retained competing vegetation and those that did not at time of sampling (2022), in a mid-rotation *Pinus taeda* L. plantation in Buckingham County, Virginia ( $n = 48$ ).

**Table 4.** Analysis of variance summary table of the association of crop tree *Pinus taeda* L. foliage (sampled 2022) fertilizer N acquisition and different levels (0 m<sup>2</sup>/ha, “low” 0-0.8 m<sup>2</sup>/ha, “medium” 0.8-3.1 m<sup>2</sup>/ha, “high” 3.1-18.3 m<sup>2</sup>/ha) of understory competing vegetation basal areas (sampled 2020). Samples were collected in a mid-rotation *Pinus taeda* L. plantation in Buckingham County, Virginia. “BA Levels” were determined by evenly dividing present competing vegetation basal areas into quantifiable categories (n = 48).

| Source   | DF | Sum of Squares | Mean Square | F Ratio | Prob > F |
|----------|----|----------------|-------------|---------|----------|
| BA Level | 3  | 1479.52        | 493.17      | 4.13    | 0.0115   |
| Error    | 44 | 5252.76        | 119.38      |         |          |
| C. Total | 47 | 6732.28        |             |         |          |

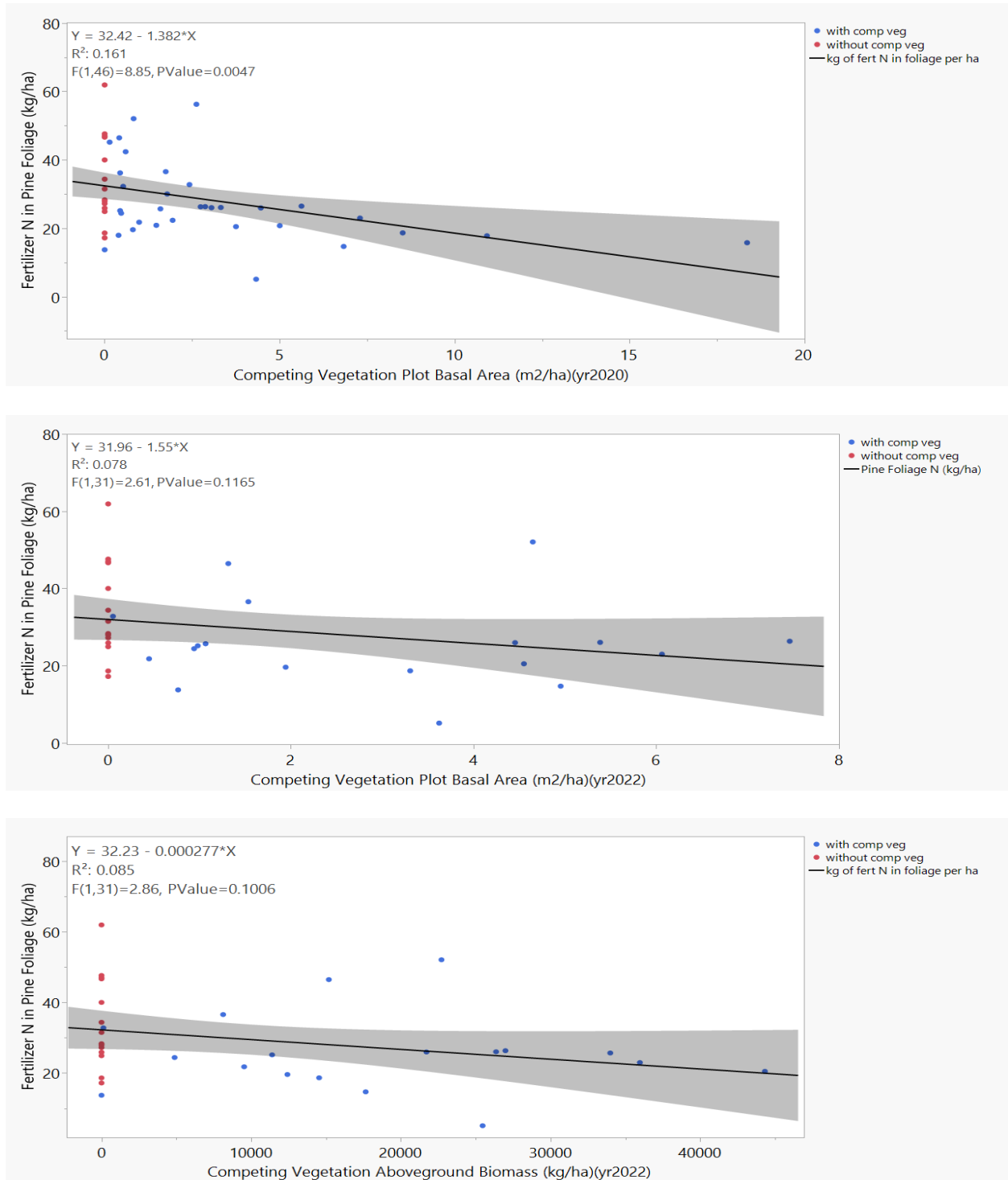


**Figure 3.** Relationship among levels of present competing vegetation basal area (sampled 2020) categories (0 m<sup>2</sup>/ha, “low” 0-0.8 m<sup>2</sup>/ha, “medium” 0.8-3.1 m<sup>2</sup>/ha, “high” 3.1-18.3 m<sup>2</sup>/ha) and fertilizer <sup>15</sup>N acquisition by the crop tree loblolly pine foliage (sampled 2022) in a mid-rotation *Pinus taeda* L. plantation in Buckingham County, Virginia (n = 48).

**Table 5.** Post-hoc test analyzing the effect of understory competing vegetation basal area levels (0 m<sup>2</sup>/ha, “low” 0-0.8 m<sup>2</sup>/ha, “medium” 0.8-3.1 m<sup>2</sup>/ha, “high” 3.1-18.3 m<sup>2</sup>/ha) on crop tree *Pinus taeda* L. foliage <sup>15</sup>N sequestration (sampled 2022) in a mid-rotation plantation in Buckingham County, Virginia. “BA Levels” were determined by evenly dividing present competing vegetation basal areas into quantifiable categories (sampled 2020). Levels not connected by the same letter represent significant differences of  $p \leq 0.05$  (n = 48).

| BA Level |   |   | Least Squares Mean (kg/ha) |
|----------|---|---|----------------------------|
| 0        | A |   | 34.23                      |
| medium   | A | B | 31.42                      |
| low      | A | B | 30.33                      |
| high     |   | B | 19.53                      |

As competing vegetation (2020) basal area increased, pine foliage acquisition of nitrogen decreased (Figure 4. top). For 2022 competing vegetation samples, basal area had an insignificant effect on pine foliage <sup>15</sup>N capture (Figure 4. middle). The relationship between pine foliar <sup>15</sup>N and current competing vegetation biomass was not significant, however (Figure 4. bottom).

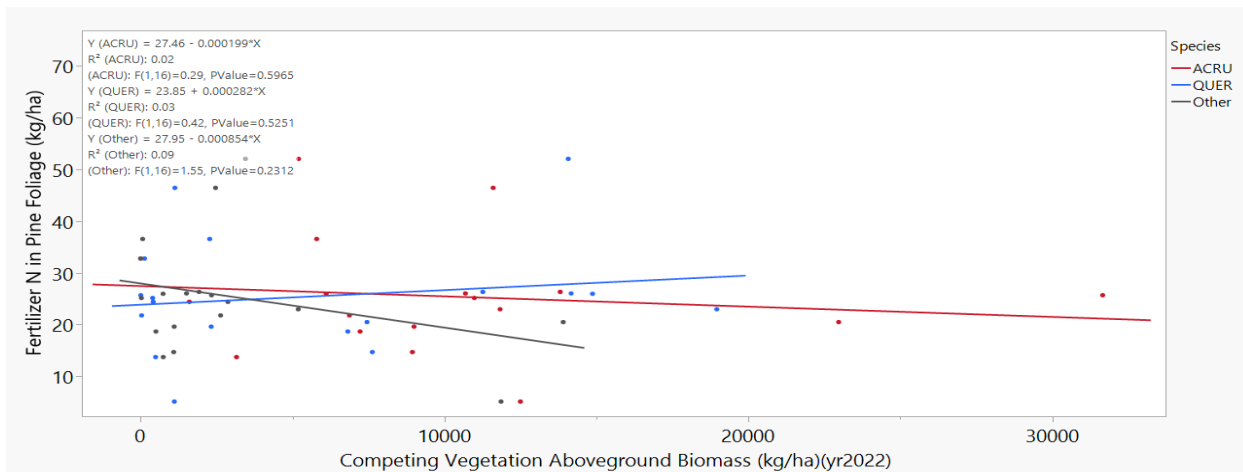
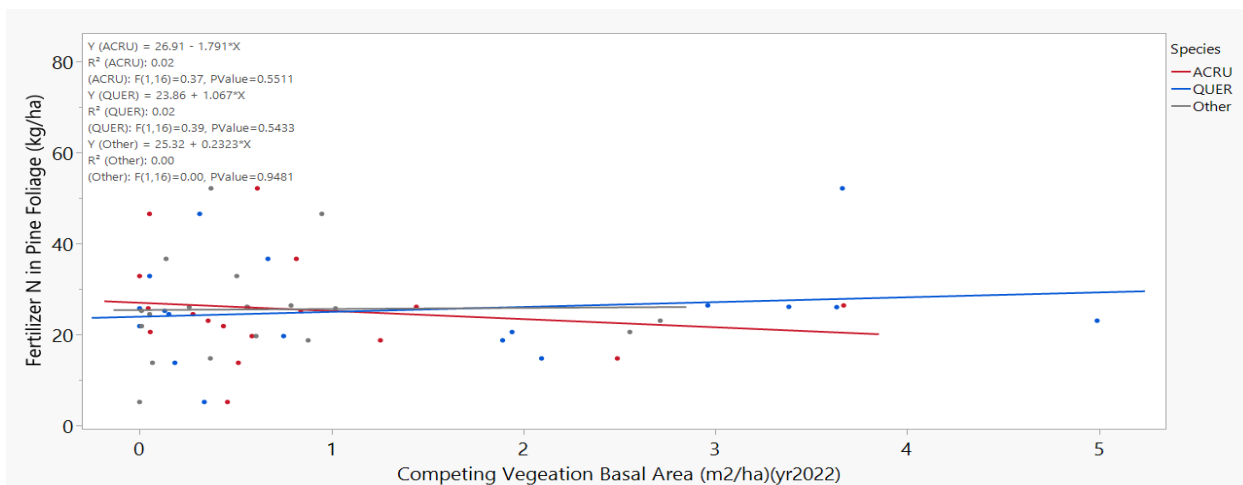
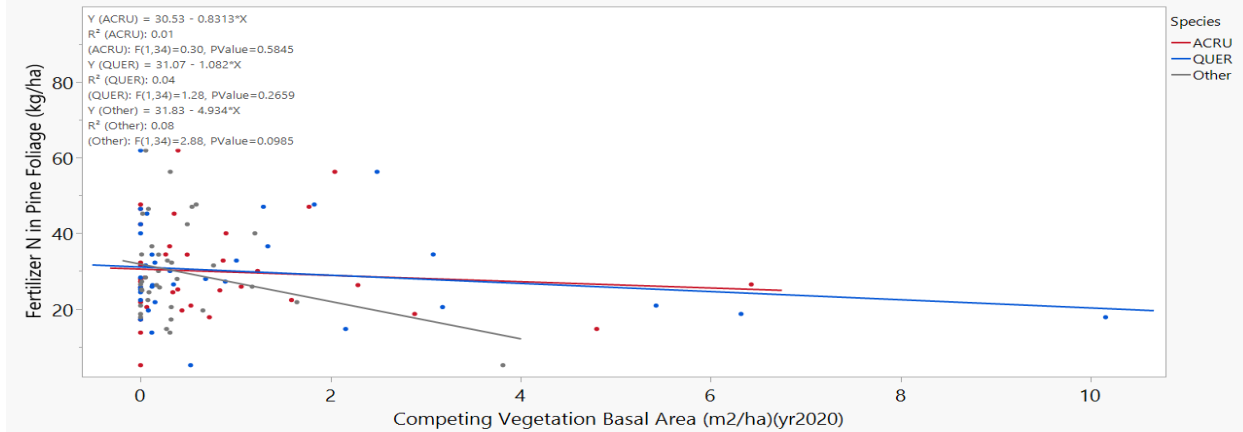


**Figure 4.** (Top & Middle) Relationship between competing vegetation basal area (sampled 2020 & 2022 respectively) and  $^{15}\text{N}$  fertilizer capture in crop tree *Pinus taeda* L. foliage (sampled 2022) in a mid-rotation pine plantation in Buckingham County Virginia (2020 n = 48; 2022 n = 33). (Bottom) Relationship between competing vegetation aboveground biomass (sampled 2022) and  $^{15}\text{N}$  fertilizer capture in crop tree *Pinus taeda* L. foliage in a mid-rotation pine plantation in Buckingham County, Virginia (n = 33).

*Acer rubrum* and *Quercus* spp. had greater importance values (IMPVs) compared to other species (Table 6); however, their aboveground biomass amounts were not significantly related to foliar nitrogen acquisition in loblolly pine ( $p = 0.58$  and  $0.50$  for *Acer rubrum* and *Quercus* spp. respectively) (Figure 5. bottom). Expanding the analysis to include all other species showed no effect on pine fertilizer N acquisition (species:  $p = 0.72$ , aboveground biomass (kg/ha):  $p = 0.40$ , and species x aboveground biomass (kg/ha):  $p = 0.37$ ). Competing vegetation basal area collected in 2022 shows similar insignificance (Figure 5. top).

**Table 6.** Importance value (IMPV) estimations of competing vegetation species throughout a mid-rotation *Pinus taeda* L. plantation in Buckingham County, Virginia. Importance values are a sum of relative frequency (%)—proportion of plots with species present across stand—relative dominance (%)—proportion of aboveground biomass—and relative density—proportion of total stem count by species ( $n = 799$ ).

| Species                        | Relative Frequency | Relative Dominance | Relative Density | Importance Value (IMPV) |
|--------------------------------|--------------------|--------------------|------------------|-------------------------|
| <i>Castanea dentata</i>        | 1.01               | 0.10               | 0.38             | 1.49                    |
| <i>Fagus grandifolia</i>       | 1.01               | 0.10               | 0.25             | 1.36                    |
| <i>Nyssa sylvatica</i>         | 10.10              | 2.47               | 7.88             | 20.46                   |
| <i>Juniperus virginiana</i>    | 3.03               | 0.03               | 0.75             | 3.81                    |
| <i>Prunus serotina</i>         | 4.04               | 1.12               | 0.88             | 6.03                    |
| <i>Cornus florida</i>          | 1.01               | 0.01               | 0.13             | 1.14                    |
| <i>Carya</i> spp.              | 10.10              | 1.36               | 5.13             | 16.59                   |
| <i>Acer rubrum</i>             | 17.17              | 52.89              | 44.68            | <b>114.74</b>           |
| <i>Prunus serotina</i>         | 4.04               | 0.02               | 1.38             | 5.44                    |
| <i>Quercus</i> spp.            | 18.18              | 31.41              | 23.15            | <b>72.75</b>            |
| <i>Pinus</i> spp.              | 15.15              | 1.87               | 9.26             | 26.28                   |
| <i>Sassafras albidum</i>       | 2.02               | 0.00               | 0.38             | 2.40                    |
| <i>Amelanchier arborea</i>     | 2.02               | 0.00               | 0.25             | 2.27                    |
| <i>Oxydenrum arboreum</i>      | 1.01               | 0.25               | 0.50             | 1.76                    |
| <i>Ailanthus altissima</i>     | 1.01               | 0.44               | 2.50             | 3.95                    |
| <i>Rhus copallinum</i>         | 2.02               | 0.03               | 0.25             | 2.30                    |
| <i>Liriodendron tulipifera</i> | 7.07               | 7.90               | 2.25             | 17.22                   |
| Total                          | 100.00             | 100.00             | 100.00           | 300.00                  |



**Figure 5.** (Top & Middle) Relationship between competing vegetation basal area (subsamped 2020 & 2022 respectively), species, and fertilizer  $^{15}\text{N}$  acquisition in the crop tree *Pinus taeda* L. foliage (2020 n = 110; 2022 n = 54). (Bottom) Relationship between competing vegetation aboveground biomass (subsamped in 2022), species, and fertilizer  $^{15}\text{N}$  acquisition in the crop tree loblolly pine foliage in a plantation at mid-rotation in Buckingham County Virginia (n = 54).

#### 4. DISCUSSION

This study evaluated the variability in the retention of fertilizer N among ecosystem components and crop tree uptake of applied fertilizer N under variable understory densities and abundances in a mid-rotation pine plantation in the piedmont region of Virginia, US. We expected to find (1) greater kg/ha of fertilizer nitrogen captured by crop loblolly pine trees compared to other ecosystem parts; (2) a greater proportion of fertilizer nitrogen sequestered by the pine trees when competing vegetation was removed; and (3) that species identity would not be an influential predictor of the acquisition of fertilizer N by crop tree pine. Our findings suggest the first 0-15cm of soil retains the most fertilizer N, pine foliage sequestration of fertilizer N is moderately increased when understory competing vegetation is controlled, and that the species identity of the competing vegetation understory is insignificant in affecting pine foliage fertilizer N uptake.

The greatest accumulation of applied fertilizer nitrogen among the components measured was found in the 0-15 cm increment of soil (roughly 34%; followed by 15-30 cm increment soils at 21% and pine foliage at 18%) and this was not significantly influenced by the vegetation control treatments. <sup>15</sup>N sequestration being greatest in soils was expected considering the application happened one year prior to sampling and the soil is the access point from which vegetation will acquire the fertilizer (Mallory & Griffin 2007). Raymond et al. (2016) had similar findings with the greatest amount of recovered fertilizer N being in the soil (28.4% with urea; 30.6%-38.8% with urea as an enhanced efficiency fertilizer) and the pine trees (34.8%; 38.5%-49.9%, respectively). Our experiment did retain less fertilizer nitrogen in the loblolly pine trees comparatively



—where Raymond et al. (2016) recorded relatively similar amounts of fertilizer N in both the soil and pine trees. Our study found a larger difference in the pine foliage and soil with pine foliage obtaining significantly less than the soil at 0-15 cm increments (roughly 18% acquisition in pine foliage). However, this discrepancy may be that of our study sampled only the pine foliage, whereas Raymond et al. (2016) sampled additional aspects of the tree including root biomass.

The total of fertilizer N, i.e., the sum of measured N in sampled ecosystem components, was relatively equal with and without competing vegetation present. This result suggests that the commonly held assumption that vegetation killed post-fertilization will result in a pulse of released N is unlikely as the vegetation seemingly did not act as a large fertilizer sink in system-wide fertilizer N retention. However, this insignificance may be due to a small sample size, the limited temporal scope of the study, and a relatively modest gradient of competing vegetation presence. Other potential sources of error within this study include the calculation of foliar mass which is based on DBHs and allometric equations. Potential, unaccounted for sources of nitrogen losses and pools within the system include fine and coarse roots (10.8% & 1.7%, Raymond et al. 2016), bark and coarse branches (0.4% & 3.2%, Raymond et al. 2016) competing vegetation leaf area (20% in conifer seedlings; Hangs et al. 2003), leaching and volatilization (ammonia loss 14.4%-36%, Kissel et al. 2004). Our estimates of total N sequestered, however, indicate relatively little N lost from the system (~10%).

The pine foliage <sup>15</sup>N estimations were marginally greater when competing vegetation was controlled and no longer present. This trend suggests that eliminating

competing vegetation entirely increased resources available to be acquired by crop trees. Similar findings were reported in Albaugh et.al. (2004, 2012, & 2015) where elimination of understory vegetation increased the crop pine tree growth.

We had conflicting outcomes among similar tests comparing the abundance of competing vegetation (basal area  $\text{m}^2/\text{ha}$  (measured 2020) or biomass  $\text{kg}/\text{ha}$  (measured 2022)). We measured a significantly negative relationship between pine foliage  $^{15}\text{N}$  and pre-treatment (2020) basal area at constructed levels as well as a significantly negative linear relationship between pine foliage  $^{15}\text{N}$  and pre-treatment continuous basal area. We also, however, measured an insignificant relationship between pine foliage  $^{15}\text{N}$  and post-treatment (2022) basal area, as well as an insignificant relationship between pine foliage  $^{15}\text{N}$  and post-treatment aboveground biomass. This could mean the effect of the competing vegetation on pine foliage  $^{15}\text{N}$  acquisition may take longer to manifest or the effect is slight over the range of competing vegetation values present in our study. If the contrasting finding is related to the slight, albeit real, effect, the contradiction may be related to the smaller sample size used in the post-treatment (2022) biomass and basal area analysis. A smaller sample size had to be used as competition control treatment reduced the number of plots with living competing vegetation present.

The insignificance and large variance of the biomass relationship could also be attributed to the allometric biomass equations not including a measurement of sample height or weight. The insignificance of our post-treatment independent variables on pine  $^{15}\text{N}$  acquisition might also suggest that competition for N is not the most limiting site attribute, but rather something else such as water or light. Mead (2005) found competition control resulted in large benefits of >25% volume gains for short-rotation

crops (<12–15 years). It may be that although the presence of competing vegetation varied among treated plots, the presence may not have exceeded a critical threshold regarding competing vegetation biomass in this experiment. The Forest Productivity Cooperative has historically recommended controlling competing vegetation prior to fertilization once the competition exceeds  $4.6 \text{ m}^2 \text{ ha}^{-1}$ . Our experiment possessed relatively few plots (2020  $n = 11$ ; 2022  $n = 6$ ) with basal areas exceeding this threshold. Our conflicting evidence of competing vegetation's influence on pine foliage  $^{15}\text{N}$  may be a result of different measurement timings or differences in sample size between and/or within explanatory variables. In lieu of a strict biomass threshold for competing vegetation control, our study suggests any vegetation control will be beneficial due to the negative relationship between crop tree  $^{15}\text{N}$  assimilation and increasing competition (basal area). Our 2020 categorical basal area data for competing vegetation showed an approximate basal area threshold may be claimed at  $3.1 \text{ m}^2/\text{ha}$ .

Assessing the present competing vegetation by species resulted in no meaningful trend affecting nitrogen sequestration into the crop tree foliage. This suggests competitiveness for the fertilizer resource is approximately the same for all species. Thomas et.al. (1999) had a similar finding where traditional "functional types" had little usefulness in forecasting how different species would react to silvicultural practices, and in response to fertilization species richness lessened. Similarly, Morris et.al. (1993) suggested that pine growth amid competition was not a function of the competitors themselves but instead induced differences in soil resource and light conditions. We found similar evidence that competition effects are resource based

more so than species-based. Our data suggests management objectives for competition control should focus on overall abundance rather than composition.

## 5. CONCLUSIONS & MANAGEMENT IMPLICATIONS

The main findings of this study were: (1) there were minor positive correlations between crop pine species fertilizer N acquisition and vegetation control and decreasing understory vegetation densities; and (2) the effect of speciating the understory competing vegetation to determine fertilizer N acquisition efficacy in the crop pine trees is inconsequential. In this experiment, effects of understory vegetation basal area on pine  $^{15}\text{N}$  capture are mixed likely due to the small sample size of the 2022 data. This study's competing vegetation biomass evaluations were insignificantly related to crop tree pine's acquisition of fertilizer N. However, when able, management objectives should still include mid-rotation vegetation control as there is a positive response of  $^{15}\text{N}$  assimilation into crop tree foliage with less competing vegetation. Additional research for this project will include identifying the competing vegetation  $^{15}\text{N}$  foliar concentrations as well as examining pine foliage  $^{15}\text{N}$  responses one year after post-fertilization vegetation control. Future directions should be to expand the number of sites studied and the gradient of competing vegetation presence. In doing so, one may observe potential ideal thresholds for vegetation control when combined with N fertilization.

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