

Temporal nitrogen dynamics in intensively managed loblolly pine early stand development

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ABSTRACT

Forest production is strongly dependent on nutrient uptake; however, sustainable management of intensively managed plantations requires an improved understanding of this relationship when fertilization occurs frequently across short rotations. Here, we studied temporal nitrogen (N) concentration ([N]) and content (Nc) dynamics under different silvicultural practices (herbicide, fertilization, and planting density) throughout early loblolly pine (*Pinus taeda*) stand development (5 years). We describe relationships of [N] and Nc of different stand components (foliage, branches, stem, roots, and competing vegetation) with carbon and biomass. Our results demonstrate that [N] of perennial loblolly tissues do not respond to silvicultural practices and progressively decrease through development. While foliar [N] was most responsive to resource availability, it was not consistent across time. Controlling competing vegetation was crucial to promote the use of site resources by the crop tree and increased loblolly Nc by >500%. However, increased N uptake and expedited growth is dependent upon fertilization early in stand development. At age 5, herbicide plus *reduced* and *full* fertilization rates exhibited similar aboveground Nc, which was 32% higher than with *herbicide only*. Increasing planting density resulted in increased above- and belowground loblolly Nc; however, increases in Nc were not proportional with increases in planting density. Net primary productivity and N uptake were linearly related, but age/development strongly controlled N use efficiency. Our study helps to understand complex relationships between N, biomass, and silvicultural practices during early stand development and demonstrates that temporal evaluation of nutrient dynamics is crucial to better understand loblolly pine growth, carbon sequestration potential, and to inform sustainable silvicultural practices across short rotations.

1. Introduction

Forest production is largely controlled by nutrient availability and uptake. The rising global demand for wood products implies a need for sustained productivity across forest rotations and continued silviculture refinement (Fox, 2000). The southeastern US is a prominent region for wood production, being responsible for about 17% of the global industrial wood supply (Prestemon et al., 2015). Recently, production forestry in southeastern US has partly transitioned from traditional (25–50+ years) to short rotations (~10 years), in which multiple amendments, such as herbicide and fertilization, are applied throughout the rotation to expedite stand development and biomass production (Coyle et al., 2016; Kline and Coleman, 2010; Munsell and Fox, 2010). Nitrogen (N) is considered the most limiting nutrient to forest production in temperate

latitudes (Fox et al., 2007; Reich and Oleksyn, 2004). Nitrogen uptake is highly dynamic and varies widely through a forest rotation, but especially during early stand development (Albaugh et al., 2008; Aubrey et al., 2012). Understanding the temporal relationship between nutrient demand and biomass production through stand development is critical to optimizing silviculture prescriptions to maximize nutrient uptake and productivity and minimize nutrient losses, thereby increasing economic and environmental sustainability of short-rotation production forestry.

Loblolly pine (*Pinus taeda*) is the primary candidate for wood production in the southeastern US because its overall productivity rivals all other tree species commercially adapted for the region while requiring lower economic investments (Coyle et al., 2016; Griffiths et al., 2019; Kline and Coleman, 2010). Loblolly pine has robust site requirements (Aubrey et al., 2012; Coyle et al., 2016), but nutrient amendments

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increase productivity in most cases (Coyle et al., 2016; Subedi and Fox, 2016). Reducing or eliminating competing vegetation through herbicide applications was assumed to suffice tree nutrient demand during early stand development in traditional forestry by favoring site resources to the crop tree (Fox et al., 2007). However, tree growth benefits from the combination of herbicide and fertilization during early stand development (Ferreira et al., 2020). While annual fertilization under intensive management can improve nutrient availability and expedite growth throughout the rotation (Subedi and Fox, 2016), fertilization in excess of uptake capacity reduces profits and may lead to offsite nutrient movement (Lee and Jose, 2005), which undermines the sustainability of forest production.

Foliar N concentration [N] and content (Nc) are common metrics to evaluate stand nutritional status and infer production efficiency (Albaugh et al., 2008; Barron-Gafford et al., 2003; Birk and Vitousek, 1986). However, foliar [N] concentrations are constrained within relatively narrow operating zones in perennial crops and may not change with additional nutrient availability (Aubrey et al., 2012), which limits its application for inferring N use efficiency (NUE) (Birk and Vitousek, 1986). Nitrogen uptake – measured by annual changes in Nc – on the other hand, is closely linked with biomass production and the relationship between N uptake and productivity is the preferred method to infer NUE (Albaugh et al., 2008; Birk and Vitousek, 1986; Chapin et al., 2011). Therefore, quantifying N balance through time has practical applications because it facilitates reasonable predictions of carbon sequestration and biomass productivity potential (Tang et al., 2018; Zhao et al., 2014). Evaluations of nutrient balances over early stand development are most critical because while trees positively respond to fertilization, fertilizer-use efficiency can be low (Albaugh et al., 2008; Aubrey et al., 2012). A thorough understanding of temporal nutrient dynamics and its relationship with biomass production under different nutrient availabilities in intensively managed forests remains lacking. Likewise, quantitative assessments of nutrient sink strength of competing vegetation are rare (Hough, 1982) and could help explain the dynamics of aggressive competition that causes substantial (>600%) reductions in loblolly pine early stand development when not controlled (Ferreira et al., 2020; Jokela et al., 2004; Neary et al., 1990). Moreover, observations of nutrient content and concentration dynamics in organs other than foliage in response to intensive management are relatively scarce, particularly for belowground.

Planting density influences intraspecific competition and alters biomass production and allocation and nutrient demand. Higher planting density may favor biomass allocation towards perennial (i.e., stem) in detriment of ephemeral (i.e., foliage) tissues (Subedi et al., 2012; Zhao et al., 2012). Due to differences in nutrient turnover rates between perennial and ephemeral tissues, particularly for mobile nutrients like N, such allocation changes may influence nutrient dynamics and demand (Griffin et al., 1995). Similar to accounting for ontogenetic effects when trying to understand biomass allocation responses to nutrient availability (Coyle and Coleman, 2005), nutrient allocation should also be evaluated with respect to stand age and development. The lack of side-by-side comparisons of temporal N dynamics in stands receiving different levels of resource amendments or exhibiting different levels of nutrient requirement due to increased planting density represents a major knowledge gap in intensively managed forests.

Here, we studied [N] and Nc in above and belowground loblolly pine tissues and aboveground competing vegetation throughout early stand development under different silvicultural practices (herbicide, fertilization, and planting density). Early results from this project indicated that (1) herbicide was critical to promote stand uniformity and increase productivity; (2) fertilization had an additional effect on growth, but annual application rates could be reduced; and (3) increased planting density along with repeated competition control and fertilization yield higher biomass with no effect on individual tree size (Ferreira et al., 2020). We assessed whether changes in [N] and Nc during early stand development followed changes in biomass and could help explain

differences in biomass production among treatments. Based on biomass responses, we hypothesized that (1) without herbicide, competing vegetation would acquire more N than loblolly trees; (2) herbicide alone would increase loblolly Nc relative to the non-fertilized non-herbicide control, but herbicide plus fertilization would result in higher Nc; and (3) similar Nc would be observed between reduced and full fertilization rates, reflecting their similarity in biomass. In addition to these hypotheses, we explored whether biomass increase in higher density stands is a result of a proportionally higher N uptake, changes in NUE, or both. And finally, we investigated the dynamics of [N] in different loblolly tissues to infer the influence of resource availability and stand development on N allocation and NUE.

2. Material and methods

This study is part of a watershed experiment that evaluated the effect of silvicultural treatments and best management practices on soil and water quality and biomass production of short-rotation loblolly pine stands (Ferreira et al., 2020; Griffiths et al., 2017). Site description, experimental design, and measurement protocols have been previously described in greater detail elsewhere (Ferreira et al., 2020; Griffiths et al., 2017). This study builds on previous biomass reports (Ferreira et al., 2020) and describes the relationship of carbon and biomass with [N] and Nc.

2.1. Site description

The experimental site is within the US Department of Energy (DOE) – Savannah River Site, a National Environmental Research Park managed by the USDA Forest Service under agreement with DOE, located on the Upper Coastal Plain physiographic region, near Aiken, SC (33°15' N; 81°36' W). The region has a humid subtropical climate, with annual precipitation averaging 1200 mm yr⁻¹ and a mean annual temperature of 18 °C. The experimental stands were in a gently sloped area, ranging from 90 to 130 m asl, with a fine-loamy, kaolinitic, thermic Plinthic Kandudult (Dothan series, Soil Survey Staff, 2014) dominant soil type. A second rotation loblolly pine stand planted in 1951 following the abandonment of row crop agriculture (Aubrey et al., 2019) was clearcut in 2012 to allow experiment establishment. Soil was mechanically prepared by subsoiling planting rows to 45 cm depth, and bare-root loblolly pine seedlings (ArborGen Mass Control Pollinated AGM 37) were hand-planted at the beginning of 2013.

2.2. Experimental design

Five treatments were established side-by-side following a complete randomized block design with eight replications. Treatments were a combination of different silviculture practices (competition control, fertilization, and planting density) as follows: Control – no herbicide and no fertilization (C); herbicide only (H); herbicide and half-reduced N and P fertilization rate (R); herbicide and full N and P fertilization (F); and increased planting density (60% higher) with herbicide and full N and P fertilization (D). The C, H, R, and F treatments were planted at ~3 × 2.5 m spacing (1346 plants ha⁻¹), while D was planted at ~3 × 1.5 m (2152 trees ha⁻¹). In each block, standard treatment plots (C, H, R, and F) contained 168 trees, and each plot had central measurement plots consisting of 48 trees and large end borders to accommodate destructive harvests. The D treatment plots consisted of 264 trees, with central measurement plots of 60 trees.

Competition in H, R, F, and D was controlled prior to planting with an application of imazapyr (Imazapyr 4SL) and glyphosate (Rodeo®). Immediately after planting, a second herbicide application with sulfometuron-methyl (Oust XP) and imazapyr (Arsenal®) was performed. Subsequently, Oust XP was applied annually until year 5 to minimize competition. Fertilization consisted of annual N and P inputs at varying rates; the first fertilizer application occurred immediately

after planting at 50 kg N ha⁻¹ and 56 kg P ha⁻¹; in year 2, ~100 kg N ha⁻¹ was applied; fertilization in year 3 consisted of ~100 kg N ha⁻¹ plus 26 kg P ha⁻¹; final fertilizer application occurred in year 4 at 196 kg N ha⁻¹. These fertilization rates were used in both *F* and *D* treatments and averaged ~110 kg N ha⁻¹ yr⁻¹ during fertilization period, equivalent to ~90 kg N ha⁻¹ yr⁻¹ across the 5-year observation span, which is an average rate frequently adopted in intensively managed stands (Borders and Bailey, 2001; Carlson et al., 2009; Coyle et al., 2016; Lee and Jose, 2005; Samuelson et al., 2004; Zhao et al., 2011). The *R* treatment received one-half the amount, while *C* and *H* treatments received no fertilizer. More detailed description of fertilization management (timing, rate, and source) can be found in Ferreira et al., 2020.

2.3. Growth and biomass measurements

Growth and biomass data and a thorough description of the sampling process can be found in Ferreira et al. (2020). A summary of biomass responses is presented in Supplemental Table 1. Briefly, diameter at ground level (dgl) and tree height (*H_t*) were measured annually for five years on all measurement plot trees. One representative tree was selected based on *H_t* for destructive harvesting from each treatment × block combination. Destructive harvests occurred every year, starting one year after planting for all treatments but *C*, which started one year later because of the very slow initial development. Felled trees (*n* = 192) were separated into needles, branches, stem, and roots. The biomass of each component was weighed in the field, and sub-samples were collected to determine the moisture content and convert to dry biomass.

Loblolly pine root biomass was quantified in all treatments except *C* because of the difficulty in distinguishing from competing vegetation roots. Root sampling protocol changed over time as roots developed and expanded in areal extent. In the first sampling, the whole seedling root system was excavated and quantified. For the second sampling, we placed a 0.75 × 0.75 m square frame over the tree stump and excavated the soil inside this area to a 90 cm depth. The third sampling followed this same protocol, but an extra 0.75 × 0.75 m square frame was placed diagonally between trees to account for peripheral roots, which were quantified following the previously described method. In the last two samplings, tree roots were excavated with a hydraulic spade (1 m diameter × 0.69 m deep), and the soil volume sampled was determined for each tree. Peripheral root determination followed the same method used in previous years with a 0.75 × 0.75 m square frame placed diagonally within the tree growing space. In all samplings, all the soil collected passed through a 12-mm sieve and retained coarse-roots were quantified; root subsamples were oven dried at 60 °C to obtain moisture content and convert to dry mass.

Competing vegetation was sampled annually by placing three 0.75 × 0.75 m square frames at random azimuths and distances from the center of each measurement plot. Competing vegetation rooted within the frame was harvested and oven dried at 60 °C until constant mass.

2.4. Nitrogen determination and calculations

Nitrogen concentration measurements started at year 2 and were carried out until age 5. A sub-sample of each tree component from destructive harvests and a composite sample of competing vegetation from each plot collected during each year were finely-ground (60-mesh) and used for carbon and N and ¹⁵N/¹⁴N ratio determination using an isotope ratio mass spectrometer elemental analyzer at UGA's Center for Applied Isotope Studies (in years 2, 3 and 4), while at the 5th year carbon and N concentrations were determined using a Flash EA 1112-NC elemental analyzer (CE Elantech Lakewood, NJ, USA). Concentrations were multiplied by dry biomass to obtain carbon and N content. The abundance of ¹⁵N (δ¹⁵N) in samples was used as a surrogate to infer the contribution of fertilizer to loblolly and competing vegetation N uptake, and was calculated as follows:

$$\delta^{15}\text{N}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 \quad (1)$$

where *R* = ¹⁵N/¹⁴N ratios in samples (*R_{sample}*) or atmospheric air N₂ certified standards (*R_{standard}*).

We parameterized treatment-specific allometric equations using dgl and Nc determinations for each tree component to estimate above- and belowground Nc across years 2 to 5 as follows:

$$Nc = aX^b \quad (2)$$

in which *Nc* is the N content of needles, branches, stem, and roots, respectively; *a* and *b* are the estimated model parameters (Supplemental Table 2), and *X* is the dgl. We used Eq. (2) to calculate individual tree Nc and scaled Nc to the plot-level by summing across all living individuals within each measurement plot. Total aboveground Nc in loblolly pine was obtained by summing needle, branch, and stem Nc.

By not accounting for N turnover or retranslocation, litterfall, or root-N (for *C*) or fine-root-N in all treatments, we were limited in our study to provide a comprehensive system-level NUE evaluation. Nonetheless, a proxy to infer NUE during early stand development was calculated as follows:

$$NUE = \Delta NPP / \Delta Nc \quad (3)$$

in which Δ*NPP* is the annual change in net primary productivity (*NPP*) – the annual net increase in needle, branch, and stem biomass – and Δ*Nc* is the annual change in Nc in these same tissues, which was used as a surrogate for annual N uptake.

2.5. Statistical analyses

We analyzed treatment effects through a robust repeated measures linear mixed model (SAS PROC MIXED v.9.1.3; SAS Inc., Cary, NC, USA) with Kenward-Roger degrees of freedom correction (Kenward and Roger, 1997) treating year as the fixed repeated factor, silvicultural treatment as a fixed factor, and the treatment-by-block combination (i.e., experimental unit) as the random subject factor. Each response variable ([N], δ¹⁵N, and Nc for each component, and NUE) was analyzed following the determination of the best fit for covariance structure (e.g., first-order autoregressive, Toeplitz, compound symmetry, and variance component) to model the correlation within experimental units over time. The best fit was determined using Akaike's information corrected criterion (AICc) (Burnham and Anderson, 1998). Simple main effects were assessed using the SLICE option in the LSMEANS statement in SAS (Littell et al., 2006; Schabenberger et al., 2000). Fisher's Least Significant Difference (LSD) contrasts with α = 0.05 were used in post hoc pairwise multiple comparisons. To evaluate treatments influence on NUE across all times, we compared the slopes of NPP × annual N uptake linear regressions, in which the slopes denote the NUE value, through a covariance analysis using SAS PROC MIXED. The influence of development on NUE was evaluated by both repeated measures linear mixed model ANOVA and NUE × aboveground biomass regression comparisons through covariance analysis.

3. Results

3.1. [N], δ¹⁵N, and Nc

There was annual variation in [N] of all loblolly tissues, while changes in [N] as influenced by treatments were only observed in the foliage (Table 1). Foliar [N] differed among treatments and changed through time, but these factors were not independent (i.e., year × treatment interaction; Table 1). The rank order of foliar [N] among treatments was not consistent across time (Fig. 1). Differences in foliar [N] among treatments occurred in years 2 and 4, in which *C* exhibited lower [N] than *H*, *R*, and *F*, and *C* and *H* exhibited lower [N] than *R*, *F*,

Table 1

Significance levels (p-value) of repeated-measures linear mixed model ANOVA for nitrogen concentration [N] and content (Nc) after five years of treatment application.

p-value	Foliage	Branches	Stem	Abvg.	Roots	Cpt. veg.
[N]						
Year (Y)	<0.0001	<0.0001	<0.0001	n/a	<0.0001	<0.0001
Treatment (T)	0.0196	0.2842	0.6512	n/a	0.9268	0.0013
Y × T	0.0153	0.7927	0.6203	n/a	0.9794	0.1316
δ¹⁵N						
Year (Y)	<0.0001	<0.0001	<0.0001	n/a	0.0008	<0.0001
Treatment (T)	<0.0001	<0.0001	<0.0001	n/a	0.0002	<0.0001
Y × T	0.0576	0.1211	0.0223	n/a	0.5199	0.0003
Nc						
Year (Y)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment (T)	<0.0001	<0.0001	<0.0001	<0.0001	0.0126	<0.0001
Y × T	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	0.7530

Abvg.: sum of needle, branch, and stem Nc; p < 0.05 values are highlighted in bold.

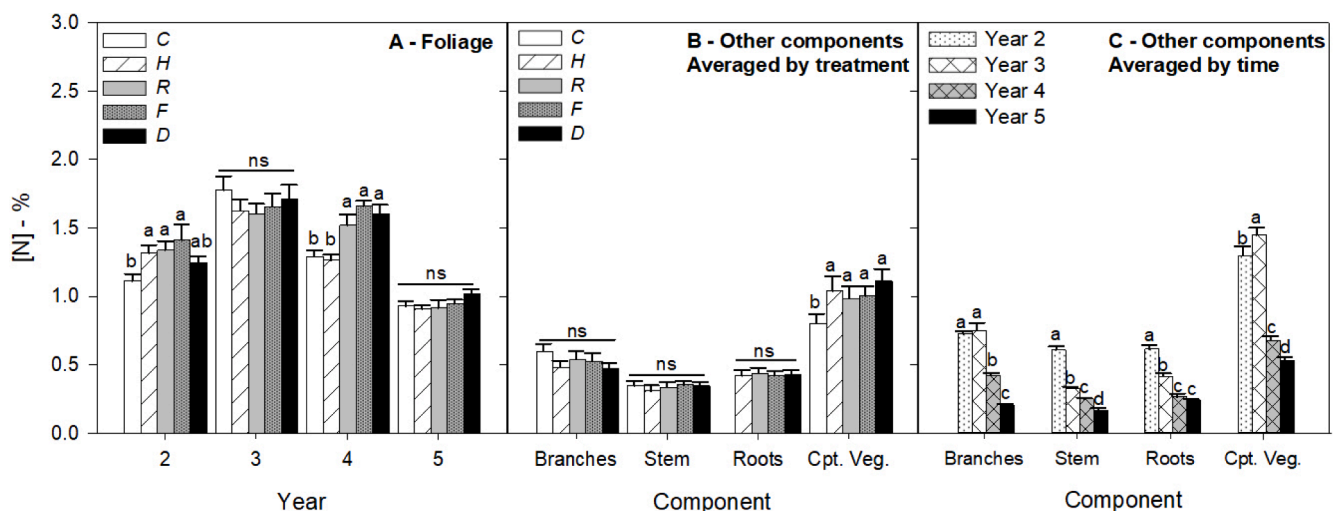


Fig. 1. Nitrogen concentration ([N], % – mean ± S.E., $n = 8$) of loblolly pine (A – Foliage; B and C – other components averaged by treatment and time, respectively) during four growing seasons influenced by the five treatments (C, H, R, F, and D) in South Carolina USA. Branches, stem, roots, and competing vegetation are averaged across treatments and year since the interaction year × treatment was not significant (Table 1). C: control; H: herbicide only; R: herbicide and half-reduced fertilization rate; F: herbicide and full fertilization; and D: increased stand density with herbicide and full fertilization. Cpt. Veg.: competing vegetation. Within each year or component, treatments or year with ns (not significant) or with the same letter are not significantly different (Fisher's LSD, $\alpha = 0.05$).

and D, respectively (Fig. 1A). For perennial loblolly components, there were no differences among treatments, but [N] decreased over time, in which branches, stem, and coarse roots exhibited 263, 262, and 157% lower [N] at age 5 compared with age 2, when averaged across treatments (Table 1; Fig. 1B; Fig. 1C).

The [N] of competing vegetation varied over time and was influenced by treatments (Table 1). Differences in competing vegetation [N] among treatments were most pronounced in year 2, but there was no year × treatment interaction ($p = 0.1316$). Averaged across treatments, competing vegetation [N] exhibited a decreasing trend after the second growing season (Fig. 1C). The [N] of competing vegetation was 1.3-fold lower in C compared to other treatments when averaged across time (Fig. 1B).

The $\delta^{15}\text{N}$ of all loblolly tissues and competing vegetation varied over time and between treatments (Table 1). Loblolly foliage and competing vegetation became progressively ^{15}N depleted (more negative) over time for all treatments (Fig. 2A and 2B, respectively). All other loblolly tissues exhibited similar $\delta^{15}\text{N}$ dynamics as of foliage (Supplemental Table 3). There was a clear distinction in $\delta^{15}\text{N}$ within loblolly and competing vegetation between non-fertilized and fertilized treatments through time (Fig. 2A and 2B, respectively). Overall, fertilized treatments were ^{15}N enriched compared with non-fertilized ones, and

differences were more pronounced (up to 6‰) in years 2 and 3 than in year 4. Within fertilized treatments, differences in $\delta^{15}\text{N}$ were of smaller magnitude and there was no difference in loblolly tissues among treatments, regardless of fertilizer rate or planting density (i.e., among R, F, and D) in year 4 (Fig. 2A; Supplemental Table 3).

The Nc of all tissues differed among treatments and increased through time, but time and treatment were not independent (i.e., year × treatment interaction; Table 1). Overall, C exhibited substantially lower Nc than all other treatments for all loblolly components throughout the experiment and D had the greatest Nc (Fig. 3), but statistical differences among treatments occurred only after year 3 (Supplemental Table 4). The lack of significance at year 2 may be a statistical artifact of the repeated measures two-way ANOVA, since the substantial changes in Nc through time results in a large least square difference threshold, which hampers statistical significance detection when numbers are proportionally lower. Interestingly, the proportional differences in foliar and branch Nc between C and other treatments decreased over time. For instance, H had 13- and 7-times higher foliar Nc than C at years 2 and 5, respectively (Supplemental Table 4; Fig. 3). The same decreasing trend in the proportional differences in foliar Nc was observed when comparing D with R or F – which did not differ – but at smaller magnitude (foliar Nc in D was ~1.7- and 1.3-times higher than in R or F

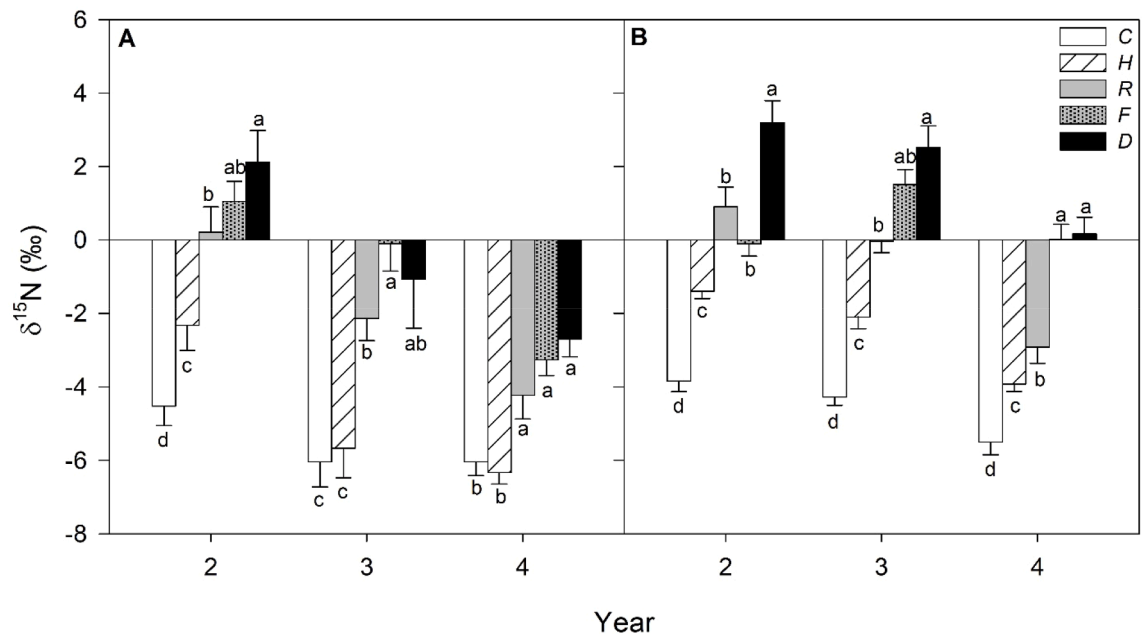


Fig. 2. ^{15}N abundance ($\delta^{15}\text{N}$) of loblolly pine (foliage; A) and competing vegetation (B) through ages 2 to 4 as influenced by the five treatments (C, H, R, F, and D) in South Carolina USA. C: control; H: herbicide only; R: herbicide and half-reduced fertilization rate; F: herbicide and full fertilization; and D: increased stand density with herbicide and full fertilization. Within each year, treatment means with the same letter are not significantly different (Fisher LSD test, $\alpha = 0.05$); the $\delta^{15}\text{N}$ of all loblolly pine components and respective statistical comparisons are presented in [Supplemental Table 3](#).

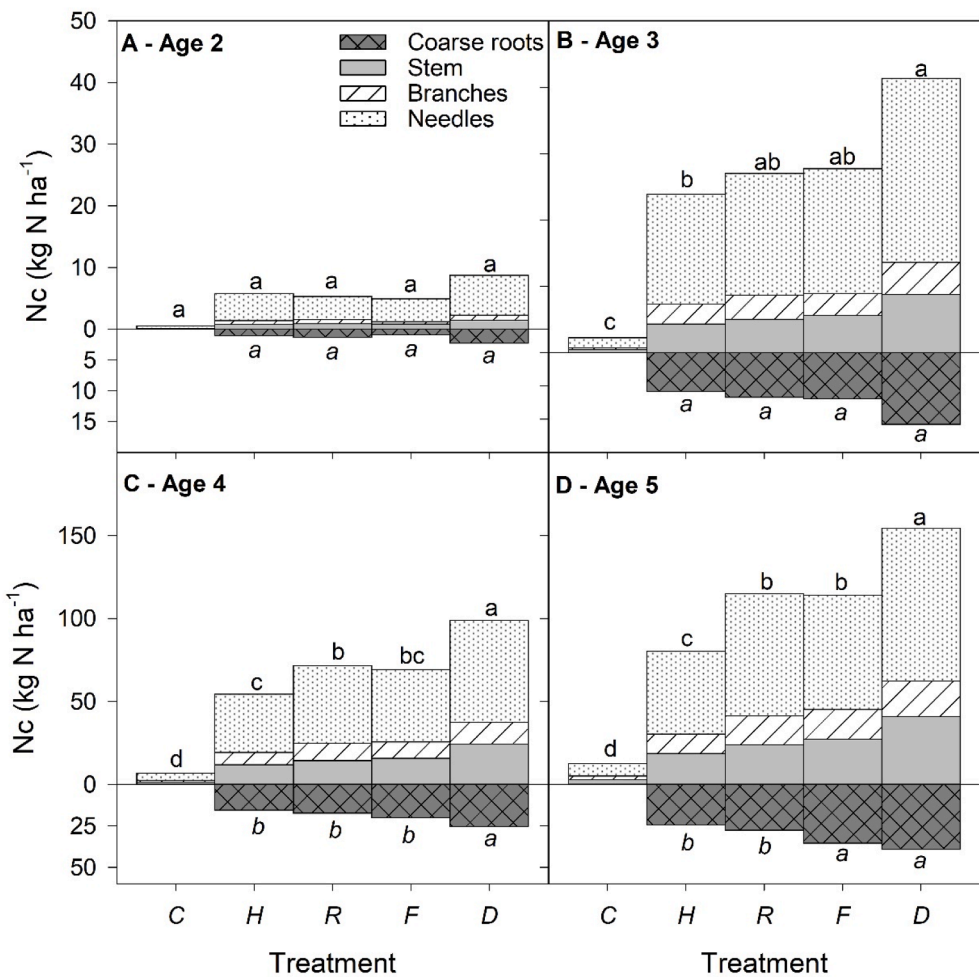


Fig. 3. Nitrogen content (Nc - kg N ha⁻¹) of above and belowground loblolly pine components through ages 2 to 5 as influenced by the five treatments (C, H, R, F, and D) in South Carolina USA. C: control; H: herbicide only; R: herbicide and half-reduced fertilization rate; F: herbicide and full fertilization; and D: increased stand density with herbicide and full fertilization. Zero on the y axis represents groundline. Within each year, treatment means with the same letter (lowercase letters refer to Nc in aboveground components combined; italic lowercase letters refer to belowground Nc) are not significantly different (Fisher LSD test, $\alpha = 0.05$); a thorough component breakdown statistical comparison is presented in [Supplemental Table 4](#).

at year 2 and 5, respectively). For stem Nc, *H* vs. *C* and *D* vs. *R* or *F* proportional differences were of similar magnitude, but temporal variations in the proportional differences among treatments were less expressive with no clear upward or downward trends observed. In contrast, an opposite upward trend with Nc proportional differences becoming more pronounced with time was observed for all loblolly components when comparing *R* or *F* with *H*.

Foliage accounted for most aboveground N (>60%) throughout the first five growing seasons, regardless of treatment. There were little changes in N partitioning among aboveground components, in which slight increases in branch- and stem Nc (3 – 9% across treatments for either component) occurred over time at the expense of needle Nc (Supplemental Table 4; Supplemental Fig. S2). Coarse roots had higher Nc than stem for all treatments throughout the first five growing seasons, except *D* at age 5. The only difference between *R* and *F* was for root Nc, in which *F* exhibited ~1.3-times higher root Nc compared with *R* (Fig. 3). All treatments had > 550% aboveground Nc than *C*. At age 5, *D* exhibited the highest aboveground Nc (154 kg N ha⁻¹), which was 1.35-, 1.93-, and 12.23-times higher than *R* or *F*, *H*, and *C*, respectively. Fertilized treatments (*R* and *F*) were on average ~1.4-fold greater than *H* for all aboveground tissues combined (Fig. 3; Fig. 4).

Competing vegetation Nc was higher than loblolly aboveground Nc in year 2 in all treatments (Fig. 4A). By year 3, there was a similar Nc in competing vegetation and loblolly trees in all treatments but *C*. Differences among treatments in competing vegetation Nc were observed only at year 3, when competing vegetation Nc in *C* was ~2.2-times higher than in the other treatments (Fig. 4B). Competing vegetation Nc decreased in years 4 and 5 compared with initial years in all treatments.

Loblolly pine in amended treatments (*H*, *R*, *F*, and *D*) acquired more N than competing vegetation in years 4 and 5 (Fig. 4C; D). Competing vegetation contained more N than loblolly trees in *C* until age 5, when they did not differ. After five growing seasons, loblolly pine in *H*, *R* or *F*, and *D* had 11-, 12-, and 16-times more Nc than competing vegetation (Fig. 4D).

3.2. NUE

There was a strong linear relationship between N uptake and NPP (i.e., slope = NUE) for all treatments (Fig. 5A). The relationship between N uptake and NPP in *C* was distinguished from others because of the very slow initial growth, but differences in slopes (i.e., NUE) were only observed when *H* was contrasted with *R*, *F* or *D*, in which *H* had higher NUE than fertilized treatments (*R*, *F* or *D*; Supplemental Table 5). On an annual basis, *H* exhibited the highest relative NUE increase, exhibiting the highest NUE at years 4 and 5 (Fig. 5B). There were strong ($R^2 > 0.97$) power-law relationships between development (i.e., aboveground biomass) and NUE, with slope < 1 for all treatments (Fig. 5C – linearized; Supplemental Table 4). Differences in regression slopes were observed for all treatment pairwise contrasts, except for *F* vs. *D*. Among treatments, *H* and *C* exhibited the highest (0.33) and lowest (0.17) slopes, respectively (Supplemental Table 5).

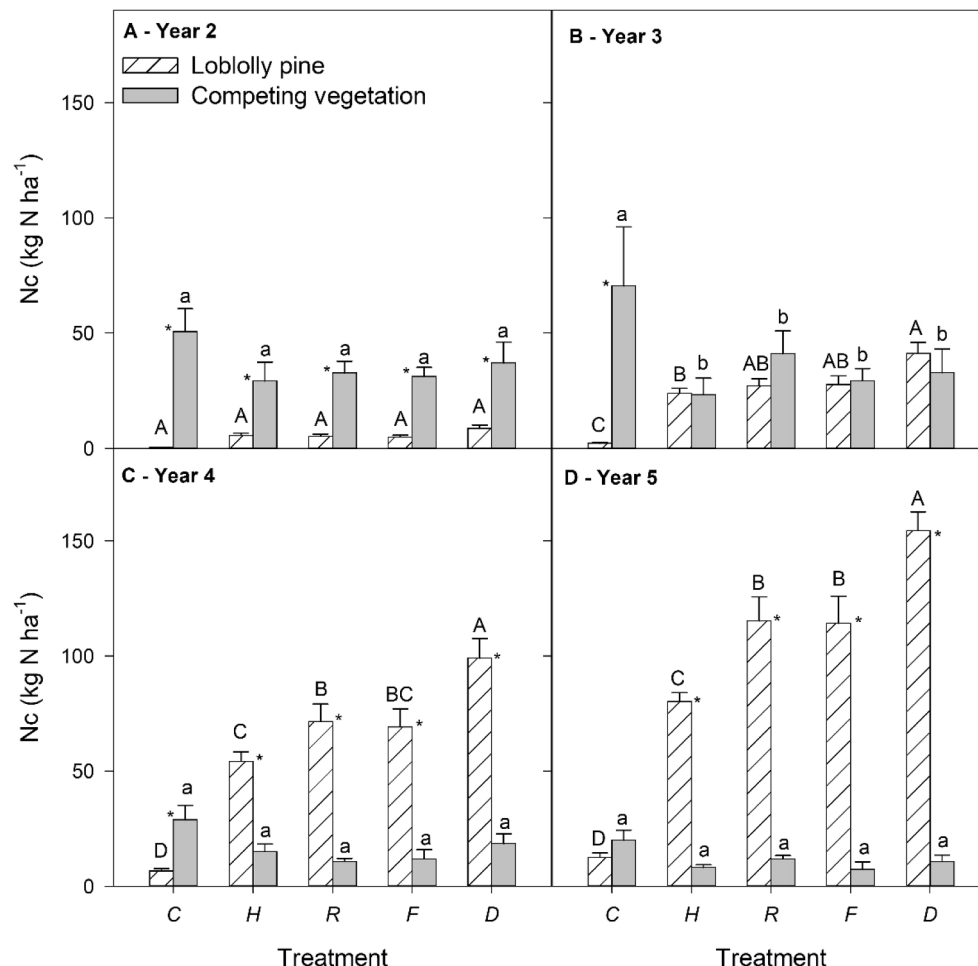


Fig. 4. Nitrogen content (Nc, kg N ha⁻¹) in aboveground vegetation (loblolly pine and competing vegetation) throughout experimental years 2 to 5 in South Carolina USA. *C*: control; *H*: herbicide only; *R*: herbicide and half-reduced fertilization rate; *F*: herbicide and full fertilization; and *D*: increased stand density with herbicide and full fertilization. Within each time, treatments with the same letter (upper and lower case for loblolly pine and competing vegetation, respectively) are not significantly different, while * indicates Nc differences between competing vegetation and loblolly pine (Fisher's LSD, $\alpha = 0.05$).

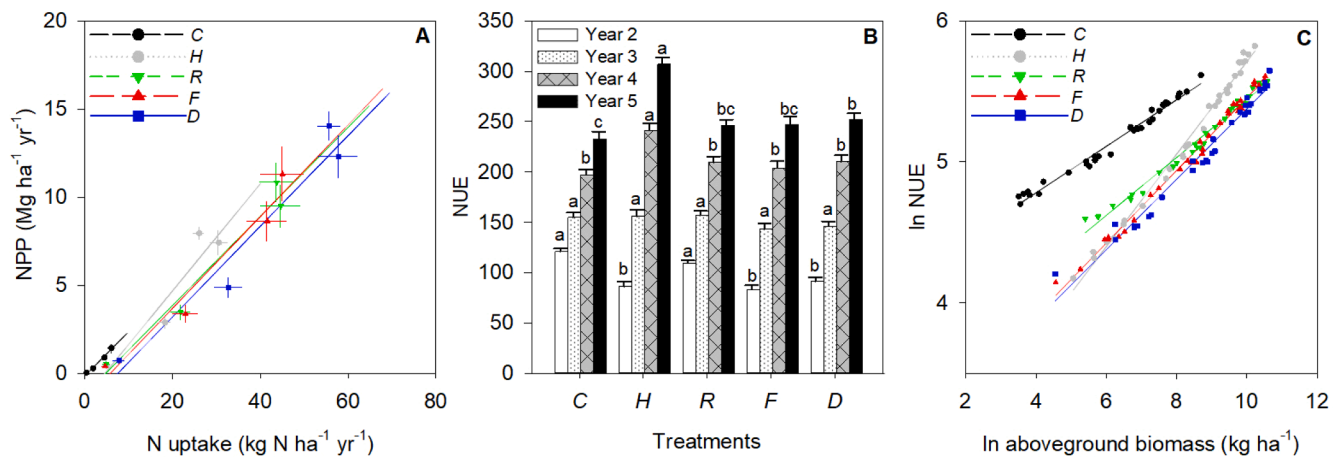


Fig. 5. Nitrogen use efficiency (NUE) calculated as changes in aboveground net primary productivity (NPP) as function of nitrogen (N) uptake – annual change in aboveground Nc (A), the annual ratio of NPP and N uptake (Eq. (3); B), and relationship between development (i.e., aboveground biomass) and NUE (C) during four years of treatment (C, H, R, F, and D) application in South Carolina USA. C: control; H: herbicide only; R: herbicide and half-reduced fertilization rate; F: herbicide and full fertilization; and D: increased stand density with herbicide and full fertilization. Error bars represent standard error ($n = 8$). Treatments with the same letter at the same year are not significantly different (Fisher's LSD, $\alpha = 0.05$). Regression parameter estimates and goodness-of-fit, and slope comparisons for A and B panels are presented in Supplemental Table 5.

4. Discussion

4.1. [N] is a poor indicator of treatment-induced impacts on nutrient status and NUE

Our results demonstrate that [N] of perennial loblolly tissues did not respond to silvicultural practices (Table 1; Fig. 1B). While foliar [N] was most responsive to resource availability, it was not consistent across time (Fig. 1A). For all treatments and throughout the observation period, loblolly foliar [N] was within the range of species previously reported values (Albaugh et al., 2010). Loblolly foliar [N] has been reported to vary less under different resource levels compared with hardwood species (Cobb et al., 2008). Increasing N availability and uptake did not induce major changes in [N] during early stand development. Rather, it increased overall biomass production (including needles), and changes in Nc mostly reflect the biomass response to silviculture treatments (Supplemental Table 1; Ferreira et al., 2020). There was a significant age-related influence on [N] for perennial tissues (Fig. 1C), while foliar [N] also varied over time, but with no clear directional temporal trend (Fig. 1A). The lack of a temporal consistency of foliar [N] to resource availability, observed here and elsewhere (Aubrey et al., 2012; Barron-Gafford et al., 2003; Cobb et al., 2008; Maggard et al., 2016), suggests that single year foliar [N] snapshots alone might not yield a robust assessment of overall loblolly nutrient status. Metrics to allow effective comparison of NUE must be of broad applicability, being consistent through developmental stages and across different levels of resource availability. The small differences in [N] with different resource levels, plus the age-related influence, corroborate that foliar [N] has little power to infer NUE in production forestry (Birk and Vitousek, 1986).

The carbon concentration [C] is relatively constant within plant tissues (Ma et al., 2018; Martin et al., 2015); thus, having [N] that is bound within a species-specific operating range is assumed to be a strategy to support the internal carbon:N (C:N) stoichiometry to satisfy physiological requirements regardless of nutrient availability (Aubrey et al., 2012; Ingestad and Agren, 1991; McGroddy et al., 2004; Sardans et al., 2012). This process has been documented across forests worldwide (McGroddy et al., 2004), including loblolly and multiple hardwood plantation forests (Aubrey et al., 2012). Nonetheless, our data demonstrate that [N] in woody tissues progressively decreased (Fig. 1C), while [C] remained static (data not shown), which collectively results in a dynamic internal C:N equilibrium (Supplemental Fig. 3) that varied

~250% within specific tissues in short periods of time through early forest development (Fig. 1C). Such dynamics is similar to what has been described in plants under elevated levels of CO₂, in which wider C:N ratio is observed as a result of an adaptive higher NUE (Stitt and Krapp, 1999; Tissue et al., 1997) and [N] varies with developmental stage (Coleman et al., 1993), but it is poorly documented in production forestry. Yet, our data suggest the process is both age and development related (Fig. 5B; 5C), since there is a clear difference in development between treatments, especially between C and other treatments (Ferreira et al., 2020), while little difference is observed with respect to [N] (Fig. 1) or C:N stoichiometry (Supplemental Fig. 3). These results have critical implications for comparing NUE and carbon sequestration potential dynamics of uneven-aged stands or at different developmental stages in multiyear forest rotations.

4.2. Herbicide, fertilization, and planting density effects on N balance and production efficiency

Overall, the influence of silvicultural practices on Nc parallel the observed biomass responses (Supplemental Table 1; Ferreira et al., 2020). In traditional southeastern US loblolly pine forestry (25–50+ years rotations), there was an assumption that organic mineralizable N could supply N for trees during early stand development because N demand is relatively low (Fox et al., 2007). Thus, simply controlling competing vegetation would provide adequate N to achieve expedited tree growth, and fertilization would be most effective at mid-rotation (Albaugh et al., 2007; Fox et al., 2007). Here, we showed that herbicide is critical and seems to be the factor alone responsible for the greatest Nc gain. In addition, while not directly testing it (e.g., through a full-factorial herbicide × fertilization), our data demonstrate that competing vegetation is an efficient competitor for fertilizer-derived N (Fig. 2B), implying that competition control is a silviculture practice of utmost importance in either fertilized or unfertilized forest plantations (Rubilar et al., 2018). With no silvicultural treatment (C), competing vegetation accounted for more or similar Nc in aboveground vegetation than loblolly throughout the first five years (Fig. 4). We do not have N estimations for the belowground components of competing vegetation, but these results indicate that competing vegetation acquired substantial amounts (if not most) of available site N, which limited the amount of N available for loblolly uptake and thus loblolly growth (Ferreira et al., 2020). In treatments receiving herbicide (H, R, F, and D), loblolly outperformed competing vegetation at year 3 (D) or 4 (H, R, and F). At age

5, *H*, *R*, *F*, and *D* had from 6- to 12-times more aboveground Nc than *C*, paralleling differences in biomass (Supplemental Table 1; Ferreira et al., 2020).

While herbicide substantially increased N uptake by loblolly trees, the use of fertilization early in the rotation in intensively managed stands had an additional effect on plant Nc (Supplemental Table 4; Fig. 3) and further expedited growth (Ferreira et al., 2020). While we cannot attest the specific N source (e.g., fertilizer or inherent soil N) in fertilized loblolly plants since we did not measure fertilizer- $\delta^{15}\text{N}$ nor available soil $\delta^{15}\text{N}$ and due to multiple technique limitations, isotope kinetic fractionations, and complexity of the N cycle in perennial plants (Chalk, 2018; Craine et al., 2015; Höglberg, 1997; Mead and Preston, 1994), the clear distinction in $\delta^{15}\text{N}$ between non-fertilized and fertilized treatments and the similar pattern and magnitude of $\delta^{15}\text{N}$ in the different loblolly tissues (Fig. 2A; Supplemental Table 3) suggest an active influence of fertilization on N uptake and tissue construction since early ages. The progressive increase in proportional differences of Nc between *R* or *F* vs. *H* over time for all loblolly components suggests an intensifying nutrient limitation through time in *H* that began early in the rotation at this site. At age 5, *R* and *F* exhibited similar aboveground Nc, which was 32% higher than in *H*. The only difference between *R* and *F* was for root Nc; it was ~1.3-times higher in *F* (Fig. 3). We are limited in our study to infer whether this difference in N allocation belowground will have any implication to biomass production throughout the rotation, but the small Nc differences between *R* and *F* suggest that annual fertilization rates could be reduced during early stand development to increase sustainability while attaining similar productivity (Ferreira et al., 2020). Interestingly, because Nc is autocorrelated with biomass, these results challenge the assumption of lower belowground allocation under higher nutrient availability (Haynes and Gower, 1995), which also have been questioned in other resource manipulative production forestry studies (Coleman et al., 2004; Coyle et al., 2008; King et al., 1999).

The influence of fertilization on loblolly pine early stand development is somewhat consistent across the Southeast US (Ferreira et al., 2020). The change in the response pattern from traditional forestry to current intensively managed stands may result from improved genotypes, deployment of silvicultural practices, or both. Yet, here we demonstrate that increases in Nc are less than the amount of N applied, either for *R* or *F*. For instance, *R* and *F* exhibited 38 and 45 kg N ha⁻¹ more than *H*, respectively, while ~225 and 450 kg N ha⁻¹ were applied throughout the first five years. These proportions are presumably underestimated and the gap is undoubtedly lower since our data did not include litterfall or fine root or fine root turnover, which together, but particularly fine root Nc, can account for a considerable proportion (up to ~50%) of the N balance during early stand development (Adegbidi et al., 2005; Aubrey et al., 2012). Nonetheless, the difference in the total N applied and loblolly Nc reinforces that annual rates frequently used (Borders and Bailey, 2001; Carlson et al., 2009; Coyle et al., 2016; Lee and Jose, 2005; Samuelson et al., 2004; Zhao et al., 2011) during early stand development could be reduced.

The loblolly Nc observed in our study are similar to other intensively managed young plantations in the Southeast US for both standard (Adegbidi et al., 2005; Aubrey et al., 2012) and increased planting densities (Barron-Gafford et al., 2003). The increase in planting density yielded higher Nc for all loblolly components compared with standard spacing treatments. Increases in Nc with higher planting density parallel biomass gains (Fig. 3), which were ~30% and 10% for aboveground and belowground Nc components, respectively (e.g., *D* vs. *F*). Since biomass and Nc gains were of similar magnitude but not proportional with planting density increases (~60%), the results suggest an early sign of intraspecific competition hampering both N uptake and biomass production, even though no indication of growth dominance was observed (Ferreira et al., 2020).

More than 60% of aboveground Nc was contained in the foliage across all treatments during the four growing seasons (Supplemental

Fig. S2), exhibiting a different allocation pattern compared with biomass, which changed dominance from foliage biomass in years 1 and 2 to woody tissues afterwards (Ferreira et al., 2020). In fact, Nc allocation also shifts from foliar toward woody tissues yet at a much smaller rate than biomass, with small variations through time and among treatments (Supplemental Fig. S2). Paralleling our observations but in an older plantation, Zhao et al. (2014) reported that foliage and branches accounted for up to 65% of aboveground N, while being responsible for <25% of total biomass in 12-year-old loblolly pine stands. Root Nc was higher than stem Nc throughout early stand development for all treatments but *D*, in which stem Nc was higher than root Nc at year 5 (Supplemental Table 4; Fig. 3). These results suggest the need to account for belowground pools when evaluating vegetation nutrient balance or a whole-plant NUE assessment (Aubrey et al., 2012), which is particularly important because belowground components are typically overlooked in forestry studies, and fewer studies have addressed nutrient allocation belowground. Changes in N allocation from foliage to woody components have implications for N cycling during and across rotations, since the N present in woody tissues suggest longer-term N sequestered, less N turnover, and potential export at harvesting (Griffin et al., 1995). The contrasting biomass and N allocation might be explained by foliage replacement state with previous cohort senescence while stem biomass continued to accumulate. However, the foliage replacement does not seem to be the only or primary explanation for the contrasting biomass and N allocation, particularly because litterfall was minimal until year 4 (data not shown). The distinct [N] dynamics in needles and woody tissues (Fig. 1A; 1B) may also influence the contrasting biomass and Nc allocation through early stand development, while the decrease in [N] in woody tissues concurrently with woody biomass gain (Supplemental Table 1; Ferreira et al., 2020) minimize shifts in Nc proportional allocation.

We observed a strong age influence on NUE, regardless of treatments (Fig. 5B), while treatment differences were smaller. Among treatments, *H* had the highest NUE over time (higher slope; Fig. 5A), *C* had overall the lowest relative NUE increase (i.e., the difference from year 2 to 5), and very little differences were observed between *R*, *F*, and *D*. Therefore, no clear relationship between competition and resource availability levels and NUE could be inferred, and NUE differences were determined by relative annual NUE shifts within treatments, which was most expressive for *H* (Fig. 5B). The distinct allocation of biomass and Nc over time plus the progressive decrease of [N] in woody tissues can partially explain NUE increase through time. Higher NUE through loblolly pine development has also been observed in other studies (Albaugh et al., 2008; Aubrey et al., 2012), but it is expected to reach a plateau or decrease after a certain age (Albaugh et al., 2008). The power-law relationships between development (i.e., aboveground biomass) and NUE with slopes lower than unity (Fig. 5C; Supplemental Table 5) suggests that not only age but development also influences NUE (Coleman et al., 1993), and corroborates that NUE should increase more slowly as trees develop. Unfortunately, to our knowledge, there are few studies demonstrating temporal changes in NUE to warrant rotation-length comprehensive comparisons in intensively managed stands, which is a current knowledge gap in these forests that develop most rapidly. As previously discussed, loblolly pine was considered to be most responsive to fertilization at mid-rotation in traditional loblolly pine silviculture (Albaugh et al., 2007; Fox et al., 2007). The progressive increase in NUE combined with higher N demand as trees grow bigger support the assumption of greater responsiveness to fertilization at mid-rotation. However, expedited growth during early stand development is still dependent upon fertilization, and the increasing differences in Nc between *R* or *F* vs. *H* over time (Fig. 3; Supplemental Table 4) suggest nutrient limitation earlier than mid-rotation (Ferreira et al., 2020). Maximum growth documented rates for the southeastern US (Borders and Bailey, 2001; Bryars et al., 2013; Zhao et al., 2016) are still far from region potential (Farnum et al., 1983). Our results indicate that early stand development is a critical period to improving NUE and that

expediting early growth increases site uptake capacity and results in a higher NUE more rapidly, which should be targeted to reduce the gap between potential and achieved growth rates.

5. Conclusions

Our results demonstrate that temporal evaluation of nutrient dynamics is crucial to better understand loblolly pine growth, carbon sequestration potential, and guide silvicultural practices. Changes in Nc during early stand development in response to silvicultural treatments that influence resource access (i.e., herbicide), resource availability (i.e., fertilization), and resource demand (i.e., density) parallel changes in biomass. Despite treatment-induced increases in Nc, [N] was tightly constrained in perennial (i.e. woody) tissues and decreased through development regardless of silvicultural practice. Silvicultural treatments did influence foliar [N], but these responses were inconsistent through time, which suggest limitations on the use of single year [N] snapshots alone to assess nutrient status of loblolly pine stands. Without herbicide, competing vegetation acquired most available N. While herbicide was crucial to promote the uptake of site resources by the crop tree and increase Nc of loblolly pine, increased N uptake and expedited growth (Ferreira et al., 2020) depended upon fertilization early in the rotation. However, a substantial portion of N applied may not have been acquired, and the small differences between *R* and *F* suggest that fertilization could be reduced during early stand development. Increasing planting density resulted in increased loblolly Nc and higher biomass. However, increases in Nc and biomass were not proportional to planting density. Finally, our data demonstrate distinct allocation of biomass and Nc patterns as well as progressive decrease of [N] in woody tissues during early stand development, which partially explain NUE temporal dynamics and have critical implications for comparing NUE of stands at different developmental stages. Our study underscores important dynamics that occur during early stand development and can be applied to improve the sustainable management of intensively managed, short-rotation forests. Studies across multiple full rotations, different loblolly pine genotypes, and different geographic locations with temporal evaluations are yet needed to better understand NUE dynamics and develop more sustainable silviculture.

CRedit authorship contribution statement

Gabriel W.D. Ferreira: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Benjamin M. Rau:** Conceptualization, Methodology, Data curation, Formal analysis, Resources, Writing - review & editing. **Doug P. Aubrey:** Conceptualization, Formal analysis, Supervision, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Research data

Plot level data associated with this article can be assessed from the Dryad Digital Repository (Ferreira et al., 2021).

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2020.118890>.

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