

NITROGEN DISTRIBUTION WITHIN THE SOIL-PLANT-MICROBIAL SYSTEM IN RESPONSE TO PRE-THINNING FERTILIZATION TREATMENTS IN LOUISIANA

Michael A. Blazier and D. Andrew Scott¹

Abstract—Improvements in nitrogen (N) uptake efficiency and plantation growth require refined silvicultural systems that consider soil type, stand development, ecology, and their interactions. On four unthinned, mid-rotation loblolly pine plantations in Louisiana located on a gradient of soil drainage classes, soil, plant, and microbial N dynamics were measured in response to fertilization and understory vegetation control. Treatments consisted of an untreated control, N and phosphorus (P) fertilization, and N + P fertilization with herbicide understory suppression. Results indicated understory suppression was necessary to effectively promote increases in pine foliage N concentrations when stands had no prior history of herbicide application. Understory control was most effective in enhancing pine response to fertilization on a well-drained site. Soil N returned to background levels within 6 months of application at all sites, and microbial biomass N was relatively unaffected by fertilization and brush control at all sites.

INTRODUCTION

Loblolly pine plantations are commonly fertilized near mid-rotation, typically after thinning (Dickens and others 2003), to synchronize nutrient supply with plant demand, increase leaf area, and improve productivity. Chemical release treatments are also sometimes applied at this time to ensure that site resources are available for planted crop trees. However, further gains in production efficiency will require more than simply increasing resource inputs; they will require more comprehensive understanding and management of the interactions among crop trees, non-crop vegetation, soil properties, and applied nutrients (Jokela and others 2004). One method of affecting resource use efficiency is to alter the timing of various treatments, as is commonly practiced in row-crop agriculture.

Fertilization is conventionally conducted after thinning to ensure that only the remaining crop trees are exposed to the applied nutrients. However, the low-nutrient concentration, soluble carbon (C)-rich slash remaining on-site after thinning may facilitate increased microbial immobilization of nutrients, particularly of N. Additionally, basal areas of many of these stands are well below 35 m² ha⁻¹, which is the level identified by Jokela and others (2004) at which fertilization effectiveness declines for loblolly pine plantations in the Southeastern United States.

Alternatively, fertilizing mid-rotation stands prior to thinning may have several benefits. If N fertilizer is applied within 1 year prior to thinning, crop trees have sufficient time to exploit elevated soil N, which tends to persist for approximately 1 year (Blazier 2003). Competition for applied N from understory vegetation is relatively low due to light limitations caused by canopy closure (Dickens and others 2003). Higher pine biomass at the time of fertilization would promote high stand-level pine fertilizer uptake. This high pine fertilizer uptake could in turn reduce movement of applied nutrients below pine rooting zones via leaching on well-drained sites and denitrification on poorly drained sites. In addition, elevated nutrient levels within the fertilized trees may promote faster early growth following thinning.

This study was established to explore loblolly pine, understory vegetation, and microbial N uptake in response to fertilizer timing relative to mid-rotation thinning and understory vegetation suppression across a gradient of soil types in Louisiana. The objectives of this paper were to determine how pre-thinning fertilization and understory vegetation control affected extractable soil N, microbial biomass N (N_{mic}), understory vegetation N content, and crop tree foliar N concentrations across a gradient in site types.

METHODS

Study Sites

In September and October, 2003, four study sites were established in north, central, and southwest Louisiana. The sites were similar in age and management history, but they differed in soil drainage classes (table 1). The Lucky site was established in Bienville Parish, LA (32.3029° N, 92.9240° W), on a Betis loamy fine sand (thermic Lamellic Paleudult). The Dodson site was established in Jackson Parish, LA (32.0727° N, 92.6697° W) on a Bowie very fine sandy loam (thermic Plinthic Paleudult). The DeRidder site was established in Vernon Parish, LA (30.7742° N, 93.2493° W) on a Beauregard silt loam (thermic Plinthaquic Paleudult). The Oakdale site was established in Allen Parish, LA (30.8164° N, 92.6914° W) on a Caddo silt loam (thermic Typic Glossaqualf). All sites had originally been treated with chop-and-burn site preparation after harvest and planted with 1-0 seedlings.

Treatments

The effects of fertilization on the soil-plant-microbial system of unthinned loblolly pine plantations were tested by comparing a N + P fertilization treatment to an untreated control. The fertilizer treatment consisted of 135 kg N ha⁻¹ and 13 kg P ha⁻¹ applied as a urea and DAP mixture. Fertilizer was applied with shoulder-mounted spreaders in April, 2004. At each site, fertilization was done within 1 week prior to a precipitation event to promote uniformity of fertilizer dissolution. The effects of understory vegetation suppression were tested by comparing a basal bark spray of a 5 percent solution of triclopyr to

¹ Assistant Professor, Hill Farm Research Station, Louisiana State University AgCenter, Homer, LA 71040; and Research Soil Scientist, USDA Forest Service, Southern Research Station, Pineville, LA 71360, respectively.

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Table 1—Location, stand age in 2003, soil characteristics, and management history of four study sites established in loblolly pine plantations in north, central, and southwestern Louisiana

Location	Age	Soil series	Soil drainage	Previous release ^a	Previous fertilization ^b
Lucky	12	Betis	Well-drained	None	None
DeRidder	15	Beauregard	Moderately well	1997 ^c	170 Kg Dap Ha ⁻¹
Dodson	13	Bowie	Moderately well	None	None
Oakdale	15	Caddo	Poorly drained	None	280 Kg Dap Ha ⁻¹

^a All sites had originally been treated with a chop and burn site preparation.

^b Both fertilizer applications were in 1997.

^c The site was treated with 415 g imazapyr and 70 g sulfometuron ha⁻¹ in 1997.

an untreated control. Triclopyr was applied with backpack sprayers in March, 2004. The fertilizer × herbicide treatments were applied as a split-plot treatment structure, with fertilizer as the whole-plot treatment and the herbicide treatment as the subplot treatment. However, a no-fertilizer × herbicide treatment combination was not conducted in this phase of the study. Plots were 0.20 ha in size, and subplots were 0.10 ha. Each plot is separated by at least 8 m, and each subplot is separated by 5 m to ensure independence of treatments. The experimental design was an incomplete block design, with soil texture differences at each site used as a blocking factor. Treatments were replicated 3 times at each site.

Measurements

In February and September, 2004, foliage was sampled for nutrient analysis. In February, five trees were sampled per plot, and three trees per subplot were sampled in September. A 12-gauge shotgun was used to extract branches from the upper, middle, and lower portions of the crowns. Ten fascicles per flush were collected from each sampled branch. Fascicles from each flush were combined to create composite samples for each plot (in February) or subplot (in September). Samples were oven-dried at 70 °C to constant weight, and foliage N was determined with a Leco-2000 C/N/S analyzer (Leco Instruments, St. Joseph, MI). Foliage N values per plot or subplot were then determined by calculating the weighted average of foliage N from each crown position and flush, with the ratio of foliage to branch mass in each crown third used as the weighting factor. The ratio of foliage to branch mass was determined from data collected on destructively harvested trees, described below.

In September 2004, a destructive harvest of 10 trees per site was conducted to develop site-specific models that predict dry weight of each aboveground biomass component (table 2). Trees that represented the range of diameters (as based on d.b.h. measurements taken on all study trees in August 2004) and fertilization treatments at each site were selected for harvest. Destructively harvested trees were selected from within subplots. Prior to felling, total height, height to live crown, and d.b.h. were measured. Trees were then felled, all branches were cut from the stem and separated by crown position (upper, middle, lower), and the stem was cut into 1-m bolts. Fresh weights of crown biomass (branch + foliage) from each crown position and stem bolts were weighed in the field. Three random branches were subsampled per crown position. Branch and foliage mass of these subsamples were separated, and fresh branch and foliage weights were measured.

Table 2—Regression coefficients for equations used in prediction of foliage, branch, and stem biomass of mid-rotation loblolly pine at four study sites (identified by nearest town) in north, central, and southwestern Louisiana

Dependent Variable ^a	Parameter estimates			Statistics ^b	
	b ₀	b ₁	b ₂	FI	SE
Lucky					
FOLWT ^c	0.018	2.155	—	0.94	0.60
BRWT ^d	0.104	4.414	-3.213	0.99	0.33
STEMWT ^c	0.266	1.926	—	0.99	1.65
Dodson					
FOLWT ^d	23.535	2.144	-2.733	0.99	0.50
UBRWT ^c	0.232	2.223	—	0.99	0.05
FBRWT ^c	0.057	1.826	—	0.96	1.37
USTEMWT ^c	1.023	1.555	—	0.99	1.85
FSTEMWT ^c	6.695	0.808	—	0.99	2.95
DeRidder					
UFOLWT ^c	3.4×10 ⁻⁶	5.277	—	0.99	0.37
FFOLWT ^c	0.005	2.769	—	0.98	0.63
BRWT ^c	6.4×10 ⁻⁴	3.534	—	0.97	0.64
STEMWT ^d	0.189	1.787	0.324	0.99	1.21
Oakdale					
FOLWT ^c	0.021	2.070	—	0.98	0.45
BRWT ^c	0.017	2.428	—	0.94	2.21
STEMWT ^d	3.1×10 ⁻⁴	1.175	3.403	0.97	5.37

^a FOLWT = foliage dry weight for fertilized and unfertilized trees (kg), BRWT = branch dry weight for fertilized and unfertilized trees (kg), STEMWT = stem dry weight for fertilized and unfertilized trees (kg), UBRWT = branch dry weight for unfertilized trees (kg), FBRWT = branch dry weight for fertilized trees (kg), USTEMWT = stem dry weight for unfertilized trees (kg), FSTEMWT = stem dry weight for fertilized trees (kg), UFOLWT = foliage dry weight for unfertilized trees (kg), FFOLWT = foliage dry weight for fertilized trees (kg).

^b FI = fit index, SE = standard error of the estimate.

^c Model form: $Y = b_0 \times dbh^{b_1}$.

^d Model form: where Y = foliage, branch, or stem dry weight (kg), dbh = diameter at breast height (cm), and ht = total tree height (m).

Disks approximately 2.54-cm thick were cut from the top and bottom of each stem bolt, and fresh weight of disks was measured (Blazier and others 2002). Branch, foliage, and stem subsamples were oven-dried to constant weight at 70 °C and weighed. The dry to fresh weight ratio of all samples was determined and averaged for each crown third of each tree. The ratio of foliage to branch mass was also determined for all crown subsamples. Total dry foliage and branch weights were estimated for each destructively harvested tree by multiplying the fresh weight of each crown position by its average dry to fresh weight and foliage to branch weight ratios and summing the dry weight estimates of each crown position. Model fitting procedures will be discussed below. N contents of branch, foliage, and stem subsamples were determined using a Leco-2000 C/N/S analyzer. Tree dimensions used as model inputs were measured in September or October at each site. Unfortunately, some of the plots at the Lucky site were mistakenly thinned before tree dimensions could be measured.

In September, 2004, understory vegetation was sampled in three random 0.04-ha subsample plots per subplot. Total height of hardwood trees, total number of stems, and species composition was recorded in each subsample plot. Herbaceous vegetation was clipped within a 1-m² quadrat randomly placed in each subsample plot. These variables were then used as inputs in models developed by Stagg and Scott (2006) to predict understory vegetation biomass and N content.

Soil NH₄⁺ and NO₃⁻ were sampled using a punch auger to collect soil in March, May, August, and September, 2004. In each subplot, 3 soil samples from the top 20 cm of soil were collected from 2 randomly placed subsample areas. All soil samples were composited for each subplot. Once samples were air-dried to constant weight, soil NH₄⁺ and NO₃⁻ were quantified by the diffusion-conductivity method (Carlson 1978, Carlson and others 1990) using a TL-550A Ammonia/Nitrate Analyzer (Timberline Instruments, Inc., Boulder, CO) following extraction with a 2 M solution of KCl (Mulvaney 1996). Total extractable N was determined by summing NH₄⁺ and NO₃⁻ values for each observation.

Microbial biomass nitrogen (N_{mic}) was sampled using a punch auger to collect soil in September, 2004. In each subplot, 3 soil samples from the top 10 cm of soil were collected from 2 randomly placed subsample areas. All soil samples were composited for each subplot. Soil samples were kept at 5 °C during transport and storage. Samples remained in storage a maximum of 1 week prior to analysis. N_{mic} was assessed by the chloroform fumigation-incubation method (Horwath and Paul 1994). Procedures included a 10-day pre-incubation of soil samples at 25 °C followed by fumigation with alcohol-free CHCl₃ vapor for 24 hours. Extraction of NH₄ was conducted with 2 M KCl, and NH₄ in extracts was analyzed by the diffusion-conductivity method (Carlson 1978, Carlson and others 1990) using a TL-550A Ammonia/Nitrate Analyzer. Samples were incubated at 25 °C for 10 days. A proportionality constant of 0.68 was used to convert NH₄-N to N_{mic} (Shen and others 1984).

Data Analysis

During biomass model development, the influence of fertilization on the relationship between each biomass component and tree dimensions (height, diameter, crown width) was investigated using procedures described by Blazier and others (2002).

Categorical (dummy) variables were incorporated into the models to account for fertilizer and brush control influences. When the dummy variables were found to be significant, separate models for prediction of a biomass component were estimated for fertilized and non-fertilized study trees. When the dummy variables were not significant, data were pooled and a single model for prediction of a biomass component was estimated for fertilized and non-fertilized study trees.

After the need for separate or single models was assessed with analyses of dummy variables, a stepwise procedure was performed on each model using a significance level of P = 0.15 to ensure that only variables that significantly affected foliage and stem weight were included in the regression equations. Residual analyses were then performed on each model to investigate any significant departures from linearity. Cook's distance and DFFITS tests were conducted to search for any outliers that substantially influenced each model (Neter and others 1996). After stepwise procedures, residual analyses, and outlier tests were completed, the NLIN procedure of the SAS System (SAS Institute Inc., Cary, NC) was used to estimate regression coefficients for each nonlinear biomass model.

Analyses of all response variables except soil N were conducted by analysis of variance using the MIXED procedure of the SAS System with site, block, treatment, and the interaction between site and treatment as fixed effects. The treatment effect was comprised of three levels: control (CONT), fertilizer-only (NP), and fertilizer + herbicide (NPBC). This definition of the treatment effect was necessary due to the missing no-fertilizer × herbicide treatment combination in this phase of the study. A significance level of P = 0.10 was used to increase the power of the test given the inherent relatively high variability of soil and plant N quantification. Soil N was analyzed with a repeated measures model with an autoregressive correlation structure with: (1) site, (2) block, (3) treatment, (4) month, and (5) the interactions between treatment, site, and month as fixed effects. When an ANOVA indicated significant treatment effects, treatment means were calculated and separated by the DIFF and SLICE options of the LSMEANS procedure. The DIFF option provided multiple comparisons of treatment means by invoking t-tests to determine significant differences between all possible treatment combinations. The SLICE option provided t-tests of treatment means in which the effect of one treatment is evaluated at each level of another treatment. The SLICE option was used to investigate treatment main effects when significant 2-way interactions were found. N_{mic} and soil N values were log-transformed for analysis after the null model likelihood ratio test revealed significant heterogeneity in variances within the dataset.

RESULTS AND DISCUSSION

No significant pre-treatment differences among treatments in foliage N concentrations were revealed by the February, 2004 foliage sampling. However, foliage N concentrations of the trees on the poorly drained Oakdale site were significantly greater than those of the trees on the other sites (table 3). Average foliage N concentrations were greater than the critical level of 1.2 percent for loblolly pine foliage N (Dickens and others 2003) at all sites, while the average N concentration of the Oakdale site trees exceeded the critical value by about 0.2 percent.

Table 3—Foliage nitrogen concentrations (%) of mid-rotation loblolly pine at four study sites (identified by nearest town) in north, central, and southwestern Louisiana in response to untreated control (CONT), fertilization with nitrogen and phosphorus (NP), and fertilization with nitrogen and phosphorus with triclopyr used for understory brush control (NPBC)^a

Site	February 2004			September 2004		
	CONT	NP	NPBC	CONT	NP	NPBC
Lucky	1.23a	1.22a	1.22a	0.95c	1.18b	1.32a
Dodson	1.24a	1.17a	1.17a	1.06b	1.24ab	1.21a
DeRidder	1.16a	1.24a	1.24a	0.92b	1.25a	1.06a
Oakdale	1.40a	1.32a	1.32a	1.13b	1.14b	1.26a

^a For each month, means within a row followed by a different letter differ significantly at $P < 0.10$.

The site \times treatment effect was significant in September, 2004 (table 3). At the Oakdale and Dodson sites, foliage N concentrations were significantly increased only by the combined fertilization and brush control treatment. At the moderately well-drained DeRidder site, foliage N concentrations were comparably increased by the NP and NPBC treatments. At the well-drained, sandy Lucky site, fertilization increased foliage N concentrations by a significantly greater magnitude with understory suppression. Albaugh and others (2003) similarly found variable responses to mid-rotation vegetation suppression and fertilization treatments across well- and poorly drained sites.

Understory vegetation trends may partially explain differences in foliage N concentrations at the four sites (fig. 1). The Oakdale and Dodson sites were characterized by relatively high understory biomass, so understory biomass may have severely reduced pine uptake of applied nutrients. Understory biomass may have been more problematic in this study than with operational fertilization, because our fertilization

rates were 25 percent lower than commonly used N and P rates (NCSFNC 2002). Although understory biomass at the Lucky and DeRidder sites was comparable, pine foliage N concentrations were significantly enhanced by the NPBC treatment only at the Lucky site. Because tree nutrient uptake and growth potential are lower on well-drained soils due to poorer moisture availability, understory suppression may have been more important in promoting pine N uptake at the sandy Lucky site (Powers and Reynolds 1999).

The lack of significantly lower understory biomass between the NP and NPBC treatments at the Lucky site (fig. 1) is possibly due to the presence of similarly high numbers of southern red oak (*Quercus falcate* Michx.) stems in both treatments. Southern red oak was relatively resistant to the maximum labeled rate of triclopyr used in this study. Similarly, the seemingly anomalous understory biomass trends observed at the Oakdale site were partially influenced by Chinese tallow (*Sapium sebiferum* L.) and sweetgum (*Liquidambar styraciflua* L.) trees > 8 cm in d.b.h. that were resistant to basal spray of triclopyr as well as southern red oak. Understory biomass estimates may be biased at the Oakdale site due to the presence of hardwood trees > 6 m in height, because the model used was not developed for trees > 4 m tall. In future years of this study, destructive harvests of hardwood trees in excess of 4 m in height will be conducted to make the models more accurate for these sites.

The site \times treatment and month \times treatment interactions were significant in the analyses of soil NO_3^- , NH_4^+ , and total extractable N (table 4). Total extractable N of the Oakdale site was significantly greater than that of all other sites, and the Lucky site had significantly lower extractable N than the DeRidder site. All soil N variables significantly differed only in May, and significant differences among treatments were found at only the Lucky and DeRidder sites. At Lucky, total extractable N and NH_4^+ were significantly greater in May in response to the fertilization treatments, but no differences were found between the NP and NPBC treatments. Interestingly, at the DeRidder site total N, NH_4^+ , and NO_3^- were significantly increased by only the NP treatment. The lack of soil N response at the Oakdale and Dodson sites may be indicative of a large native pool of N, a relatively low N fertilization rate for this study, and high variability of soil N after fertilization. The return of extractable soil N to native levels within 6 months of fertilization at

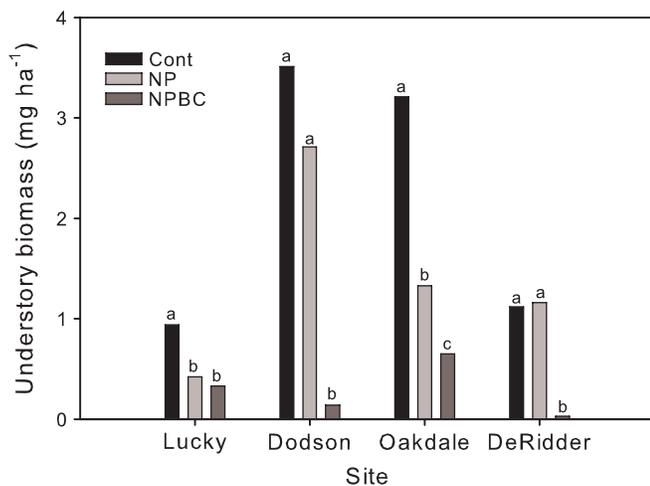


Figure 1—Understory vegetation biomass in mid-rotation loblolly pine plantations at four study sites (identified by nearest town) in north, central, and southwestern Louisiana in response to untreated control (Cont), fertilization with nitrogen and phosphorus (NP), and fertilization with nitrogen and phosphorus with triclopyr used for understory brush control (NPBC).

Table 4—Soil nitrogen concentrations (mg kg⁻¹) in mid-rotation loblolly pine plantations at four study sites (identified by nearest town) in north, central, and southwestern Louisiana in response to untreated control (CONT), fertilization with nitrogen and phosphorus (NP), and fertilization with nitrogen and phosphorus with triclopyr used for understory brush control (NPBC)^a

Treatment	NO ₃ ⁻			NH ₄ ⁺			Total extractable N		
	CONT	NP	NPBC	CONT	NP	NPBC	CONT	NP	NPBC
Lucky									
March	1.58a	1.79a	1.25a	3.85a	4.07a	3.56a	5.43a	5.87a	4.81a
May	2.79a	6.84a	5.82a	7.07b	15.19a	19.57ab	9.86b	22.02a	25.39a
August	1.65a	1.98a	2.30a	2.76a	4.04a	3.62a	4.41a	6.02a	5.92a
Dodson									
March	3.71a	3.31a	3.09a	3.02a	4.01a	4.02a	6.74a	7.32a	7.11a
May	3.74a	4.82a	7.88a	9.34a	18.05a	15.28a	13.09a	22.87a	23.16a
August	4.99a	2.40a	3.40a	3.25a	4.45a	4.03a	8.24a	6.86a	7.43a
DeRidder									
March	1.88a	2.10a	1.65a	5.26a	4.70a	4.49a	7.13a	6.80a	6.14a
May	4.87b	40.69a	7.67b	5.29b	35.78a	7.06b	10.16b	76.47a	14.72b
August	1.52a	0.82a	1.30a	5.47a	3.72a	5.58a	7.00a	4.54a	6.89a
Oakdale									
March	4.14a	4.21a	3.71a	5.89a	5.82a	5.19a	10.02a	10.02a	8.90a
May	2.82a	5.86a	11.27a	12.45a	39.63a	34.86a	15.28a	45.49a	46.13a
August	3.13a	8.33a	4.33a	9.59a	15.99a	10.98a	12.73a	24.32a	15.31a
September	6.60a	7.17a	8.46a	11.84a	11.89a	11.32a	18.44a	19.06a	19.78a

^a For each form of nitrogen, means within a row followed by a different letter differ significantly at P < 0.10.

all sites highlights the rapid movement of applied N into soil, plant, or microbial pools in these unthinned mid-rotation stands.

The fate of the applied N was unclear. By the end of the growing season, none of the measured N pools were significantly greater on the fertilized plots than on the unfertilized plots (fig. 2). The N fertilization rate was relatively low within the context of the total N budget for these sites. Furthermore, pine root biomass N was not quantified, and the methods for understory biomass N were not site-specific. Refinement of measurement procedures may detect more N in subsequent years of this study. The lack of differences in total pine biomass N was partially attributable to the variation in tree density inherent to unthinned mid-rotation stands. Interestingly, although the understory vegetation biomass contained relatively little N, the presence of understory vegetation significantly impeded increases in foliage N concentrations at the Oakdale and Dodson sites. N_{mic} was unaffected by fertilization and understory control through the end of the growing season, which may indicate that the treatments did not substantially affect soil C and N dynamics. More N was present in the N_{mic} pool than in the extractable N pool at the Oakdale, Dodson, and DeRidder sites, which may indicate that NO₃⁻ and NH₄⁺ availability in mid-rotation stands could be increased by practices that promote release of N from this pool.

CONCLUSIONS

Although insufficient time has elapsed since fertilization to assess pine growth responses to fertilization, this study's early results provide some information about the stand and soil conditions that influence the effectiveness of fertilizing unthinned mid-rotation loblolly pine plantations. When reducing N and P fertilization rates to a level 25 percent lower than conventional rates, understory biomass suppression was necessary to promote pine uptake of applied N at sites that had no history of herbicide treatment. The beneficial impact of understory suppression in conjunction with fertilization was most pronounced on the well-drained Lucky site. The significant gains in foliage N concentrations seen in this phase of the study may impart growth benefits to these stands after thinning, but the variable density of unthinned stands may cause high variability in stand growth responses to fertilization. This study will continue to monitor pine, understory, and microbial biomass and nutrient and growth dynamics to understand such trends should they occur.

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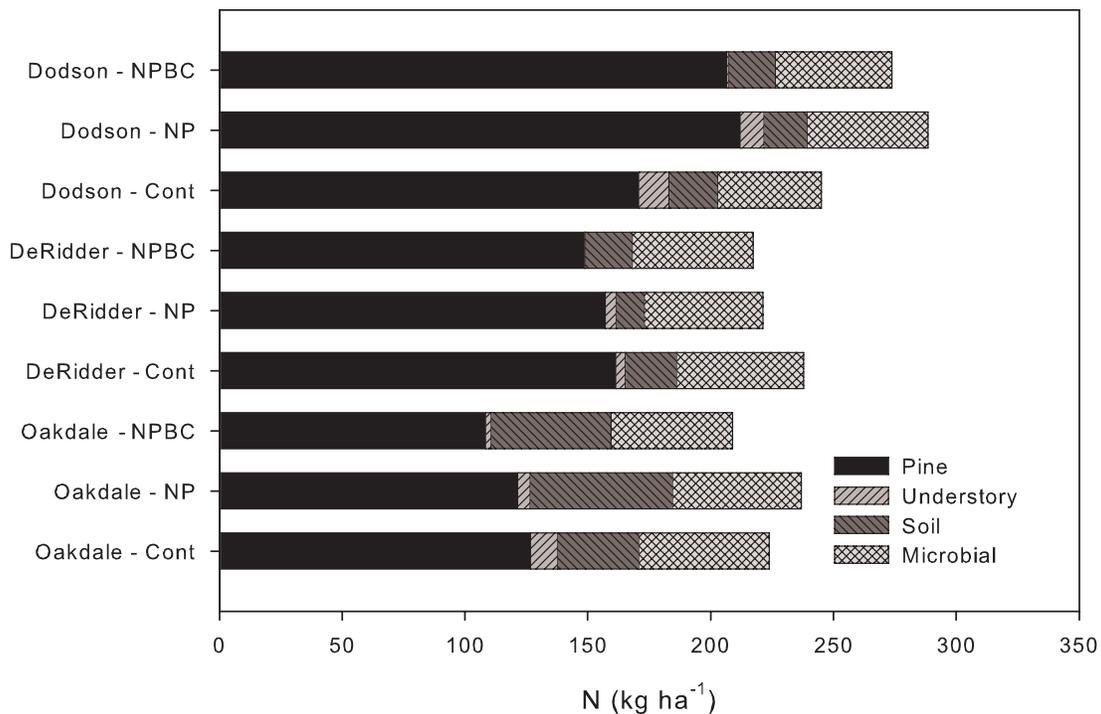


Figure 2—Total nitrogen contents of aboveground loblolly pine biomass, aboveground understory biomass, microbial biomass, and extractable nitrogen at four study sites (identified by nearest town) in north, central, and southwestern Louisiana in response to untreated control (Cont), fertilization with nitrogen and phosphorus (NP), and fertilization with nitrogen and phosphorus with triclopyr used for understory brush control (NPBC) in August-September, 2004.

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