ABSTRACT

ZERPA, JOSE LUIS. Effects of Forest Floor Retention and Incorporation on Soil Nitrogen Availability in a Regenerating Pine Plantation. (Under the direction of H. Lee Allen and Jennifer Phelan).

In forest plantations, the period from harvest through replanting is when soils are most subject to changes in nutrient availability. Soil nitrogen (N) dynamics following harvest are commonly characterized by increased mineralization rates and extractable mineral N levels. These effects have been attributed to several factors including increased decomposition of forest floor and harvest residues from the previous rotation, increased soil temperature and moisture, and reduced N uptake caused by tree removal. More recently, it has been hypothesized that higher available N levels may result from reduced microbe immobilization due to lower levels of available carbon (C) from fresh litter inputs and root exudates following harvest. Thus, heterotrophic soil microbes, which are mainly responsible for N immobilization-mineralization, may be limited by energy sources and may not require as much N as before the harvest.

In loblolly pine plantations of the Southeast US, N and phosphorus (P) fertilizers are commonly used to increase wood production, which is realized in part by increasing the amount of foliage. Through litterfall, this foliage accumulates in the forest floor forming significant C and nutrient pools. The objectives of this project were to determine if post-harvest retention of the forest floor and its incorporation into the mineral soil could affect the magnitude and timing of N supply to the subsequent stand, and to examine if C limitations in the soil microbial population may be linked to these dynamics.

Forest floor decomposition and nutrient release, mineral soil C, N, and P pools, and foliar nutrition and tree growth of the regenerating stand were examined following harvest in a loblolly pine plantation on the Coastal Plain of North Carolina. Treatments included three forest floor retention levels (0, 15, and 30 Mg ha^{-1}) combined with two levels of incorporation (mixed, non-mixed) in a factorial design. After two years, the forest floor lost 84% and 78% of its mass, 80% and 69% of its N content, and 85% and 79% of its P content from the control and doubled treatments, respectively, using the 0 retention treatment as a reference. Total C and N pools in the mineral soil increased 20 and 21% respectively, and available C, N, and P pools increased 46, 47, and 49% respectively, by doubling the forest floor. A post-harvest flush of available soil N was observed throughout the first two growing seasons and doubling the forest floor caused a full year delay in peak N availability as compared to the removed treatment. The incorporation treatment had a transient effect, with available C, N, and P pools showing significantly higher levels only during the first month of sampling. Tree growth was not affected by forest floor retention treatments, but it was affected by the incorporation treatment showing 17 % more volume growth in the mixed treatment by year 3. In general, foliar nutrient concentrations increased at year 1 as compared to initial levels, but decreased to initial levels by year 3.

A laboratory experiment that measured the microbial respiration response to addition of C and water showed higher respiration responses to C additions from soils of the removed treatment, as compared to the control and the doubled treatments. Furthermore, additions of C decreased the extractable N, across field treatments and sampling dates, by 94% as compared to additions of water, confirming the strong control that C availability exerts on N release.

Effects of Forest Floor Retention and Incorporation on Soil Nitrogen Availability in a Regenerating Pine Plantation

by
Jose Luis Zerpa

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor in Philosophy

Forestry

Raleigh, North Carolina

2010

APPROVED BY:

H. Lee Allen Co-Chair of Advisory Committee	Jennifer Phelan Co-Chair of Advisory Committee
Shuijin Hu	Robert G. Campbell

BIOGRAPHY

Jose Luis Zerpa was born in 1971 in Mérida, Venezuela. He graduated from high school in 1987 and then went to Ohio, US to spend one year as a foreign exchange student. He entered the University of Los Andes in Mérida, Venezuela in 1989 and received a Bachelor of Science degree with a major in forestry in 1996. From 1996 to 1998 he worked for Smurfit Cartón de Venezuela managing eucalyptus, gmelina, and caribbean pine plantations on the west plains of Venezuela where intensive silviculture is practiced for the production of wood fiber for pulp and paper. In 1998 he moved to the United States and in 2003 he entered the graduate program in the Department of Forestry at North Carolina State University under the guidance of Dr. Lee Allen with an interest in the areas of silviculture and forest soils. In 2004 he was awarded the Hofmann Forest Graduate Fellowship, which allowed him to continue his research in these areas. In 2005 he completed his masters and continued with his doctorate program.

ACKNOWLEDGEMENTS

This research was funded by The Hofmann Forest Graduate Fellowship and The Forest Nutrition Cooperative through a special project supported by Campbell Group, Plum Creek Timber Company, Potlatch Forest Holdings, Rayonier, and Weyerhaeuser Company. Special thanks to Weyerhaeuser Co. for providing the site for the field study and field work support. I would like to thank all the people that help in this research project: my graduate committee particularly Lee Allen and Jennifer Phelan who provided invaluable guidance and support, and countless hours of stimulating discussion on this project. Also, Alicia Peduzzi, Wilson Edwards, Jessica Tisdale, Zakiya Leggett, and Eric Sucre for help with field and laboratory work.

TABLE OF CONTENTS

CHAPTER 3

CHAPTER 4

LIST OF TABLES

CHAPTER 1. Forest floor decomposition and nutrient release after establishing a second rotation loblolly pine plantation of the Southeast, US.

CHAPTER 2. Effects of post-harvest forest floor retention and incorporation on soil C, N, and P pools in a recently established loblolly pine plantation

CHAPTER 3. Effects of post-harvest forest floor retention and incorporation on tree growth and foliar nutrition in a recently established loblolly pine plantation

CHAPTER 4. Carbon limitation as a driver of post-harvest N availability in a loblolly pine plantation

LIST OF FIGURES

CHAPTER 1. Forest floor decomposition and nutrient release after establishing a second rotation loblolly pine plantation of the Southeast, US.

CHAPTER 2. Effects of post-harvest forest floor retention and incorporation on soil C, N, and P pools in a recently established loblolly pine plantation

CHAPTER 3. Effects of post-harvest forest floor retention and incorporation on tree growth and foliar nutrition in a recently established loblolly pine plantation

CHAPTER 4. Carbon limitation as a driver of post-harvest N availability in a loblolly pine plantation

CHAPTER 1

Forest floor decomposition and nutrient release after establishing a second rotation loblolly pine plantation of the Southeast US.

Abstract

Forest floor decomposition and nutrient release was determined during the first two years of a study with loblolly pine (*Pinus taeda* L.) in the coastal plain of North Carolina where two different levels of forest floor mass (15 and 30 Mg ha^{-1}) were retained from the previous rotation. Overall, decomposition and nutrient release from the forest floor was relatively fast, i.e. the forest floor lost 84% and 78% of its mass, 80% and 69% of its N content, and 85% and 79% of its P content from the control and doubled treatments, respectively. This suggests that forest floor decomposition and nutrient release is much faster in the early stages of development of second rotation loblolly pine stands, than at mid-rotation, or in older stands, which highlights the importance of this nutrient pool in early stand nutrition. In general, repeated measures analysis showed that the forest floor retention treatments had no significant effect on the proportions of forest floor mass lost and nutrients released throughout the sampling period indicating that forest floor decomposition is directly and linearly related to the level of forest floor retained between stand rotations.

Introduction

Litterfall inputs from forest stands accumulate over time to form a forest floor layer (Switzer and Nelson, 1972; Berg, 1986), which has been recognized as an important pool for supplying nutrients to the stands (Piatek and Allen, 2001; Berg and McClaugherty, 2003; Saint-Andre *et al.*, 2008). In unfertilized loblolly pine plantations of the Southeast US, nitrogen (N) and phosphorus (P) contained in the forest floor can be 1.7 to 3.2 and 1 to 1.7

times greater than the above-ground biomass N and P contents, respectively (Tew *et al.*, 1986; Markewitz *et al.*, 1998). The magnitude of these accumulations highlight the importance of the forest floor in the nutrition of the stand if these nutrients become available through decomposition and mineralization processes (Jorgensen *et al.*, 1980).

Past studies on loblolly pine plantations in the Southeast US have assessed forest floor mass loss and nutrient dynamics in older stands (Jorgensen *et al.*, 1980; Polyakova and Billor, 2007), and at mid-rotation (Lockaby *et al.*, 1995; Piatek and Allen, 2001; Gurlevik *et al.*, 2003) either by using the “sandwich” method, in which layers of nylon screen are placed every year over the forest floor, in the same location, and then collected at the end of the study, or the “litterbag” method, in which a known amount of litter, commonly fresh needles, is placed inside a mesh bag and let to decompose in the forest floor. These studies have reported forest floor mass losses ranging from approximately 60% of the original mass in 32 months to 50% in 12 months, which resulted in annual decay constants (k), from the exponential decay model by Olson (1963), ranging from 0.39 (Gurlevik *et al.*, 2003) to 0.78 (Polyakova and Billor, 2007) for 14 and 50-year-old stands, respectively.

Forest floor removal through shearing, piling, and burning was common with past site preparation practices. But currently, more widely used practices include strip shearing, bedding, and hardwood control with herbicides, all of which retain the forest floor on site. No decomposition and nutrient release information is available for the first years of growth on second rotation stands mainly because there have been very few studies where the forest floor is retained intact from one rotation to the next (Zerpa *et al.*, 2010).

Given that the productivity of loblolly pine plantations in the Southeast US is commonly limited by low soil nutrient availability (Fox *et al.*, 2007), fertilizer applications that increase leaf area and stemwood production have become common practice (Albaugh *et al.*, 2007). These fertilizations have resulted in greater levels of forest floor accumulation, because increases in litterfall are not matched by similar increases in forest floor decomposition and nutrient release (Gurlevik *et al.*, 2003). Increases of forest floor mass and N content due to fertilization in the range of 200% and 400%, respectively are not uncommon in very responsive sites of the Southeast US (Rojas, 2005). Current forest management practices aim at optimizing the utilization of site resources. Thus, it has become increasingly important to understand how this greater forest floor accumulation affects the nutrition of subsequent stands. Unfortunately, most residue management studies have compared complete forest floor removal treatments with forest floor accumulations levels typical of unfertilized stands, and the inclusion of tillage (bedding or disking) in several but not all treatment combinations confounds the interpretation of organic matter retention (Vitousek and Matson, 1985; Li *et al.*, 2003). Few reported studies (Smith et al. (2000), Mendham et al. (2003), Tutua et al. (2008), and Zerpa et al. (2010) for plantations of *Pinus radiata*, *Eucalyptus globulus*, *Pinus elliotti* x *Pinus caribaea* hybrid, and *Pinus taeda* respectively) have included treatments with organic matter additions above levels originally on the site, a condition which would more closely mimic forest floor accumulations obtained with current fertilization practices. But, while some information concerning the effects of increased forest floor retention on nutrient availability and productivity in mid-rotation loblolly pine plantations is available (Zerpa *et al.*, 2010), little is known about its effects on the nutrition of subsequent stands.

Our study presents a unique opportunity to determine the effects of different levels of forest floor retention on forest floor decomposition and nutrient release, at the early stages of growth of a second rotation stand in the Southeast US through annual measurements of remaining forest floor on site, thus capturing decomposition of the forest floor as a whole, including its litter, fermentation, and humus layers, and avoiding the artificial exclusion, created by the use of mesh in sandwich or litterbag methods, of larger detritivores such as springtails (collembolan) and earthworms (Berg and McClaugherty, 2003) which may affect the decomposition process.

Materials and methods

Site and Study Description

The study was established on Weyerhaeuser Company land in 2006 in the lower coastal plain of Pamlico County, North Carolina (35°6’2.00”N, 76°52’45.19”W) prior to harvesting a 33-yr old loblolly pine plantation. Ten-year (1998-2007) mean annual temperature is 17.5 °C with mean monthly temperatures ranging from 7.7 °C in January to 26.3 °C in July. Mean annual precipitation is 1,439 mm with a fairly uniform distribution throughout the year. January is the driest month with 77 mm, and August is the wettest month with 195 mm. The soil is a fine, mixed, subactive, thermic Aquic Hapludult of the Craven soil series with fair to good surface drainage. The A-horizon is a fine sandy loam with an average thickness of 10 cm, bulk density of 1.18 $g•cm^{-3}$, total C and N contents of 17,200 and 760 $kg•ha^{-1}$ respectively, and Mehlich III extractable P content of 7.8 $kg•ha^{-1}$. During the previous rotation, the harvested stand had received cumulative fertilizer additions of 670 kg N•ha^{-1} and 165 kg P•ha^{-1}, and had been commercial thinned at 15 and 25 years. The stand exhibited a site index of 24 m (25 years base age) and a density of 250-300 stems•ha^{-1}. The forest floor had accumulated to an ash-free mass of 15,600 kg•ha^{-1} and contained 8,000 kg C•ha^{-1}, 160 kg N•ha^{-1}, and 8.7 kg P•ha^{-1}. Logging was conducted with a boom-top excavator and trees were felled using the previous thinning roads to prevent disturbance of the forest floor and trafficking on the study plots.

Immediately following harvest, a complete randomized block study with 5 replications and 6- forest floor/mixing treatments was imposed on the site. The treatment design was a 3x2 factorial with 3 levels of forest floor retention (removed, control, doubled), and 2 levels of forest floor incorporation with the surface mineral soil (Mixed and Non-Mixed). Forest floor was raked from the removed plots and transported using tarps to the double plots where it was evenly distributed throughout the plots. Control plots were left with the original forest floor in place. To address the objectives of this study, only the control and the doubled forest floor retention treatments of the non-mixed set were considered. The plots size were 16.8m x 9.1m including buffer areas and the measurement plots were 12.2m x 4.9m. One month after the forest floor retention treatments were completed, 96-full sibs pine seedlings were planted per plot at 1.5m x 1.2m spacing for a total of 32 pines seedlings per measurement plot. The pine seedlings were treated with permethrin (Pounce) pesticide prior to planting to prevent damage by pales weevil, *Hylobius pales* (Herbst), and competing vegetation was controlled as needed with post-emergent herbicide. Treatment effects on these seedlings's growth are reported in chapter 3.

Forest Floor Sampling and Analysis

The forest floor was collected on March '06, immediately after treatment imposition, and for the next two consecutive years. At each sampling date, forest floor was collected at five randomly located points per plot, using a 30.5 cm diameter round sampler. The forest floor layer was cut until the mineral soil was reached. At the time of treatment imposition the forest floor was separated for each sampling location into three layers, litter (Oi), fermentation (Oe), and humus (Oa) according to the classification proposed by Guthrie and Witty (1982). Samples were composited by layer, providing three forest floor samples per plot. One year later, only two layers were still clearly differentiated. A combination of the layers Oi + Oe was collected separately from the Oa layer, providing two composite forest floor samples per plot. Two years after treatment imposition, no layer differentiation was possible, so one composite forest floor sample per plot was collected. Forest floor samples were oven dried at 70 °C to a constant mass, corrected to ash-free basis using the lost-on-ignition method described by Nelson and Sommers (1996) and scaled to a per hectare basis using the area of the forest floor sampler.

Oven dry forest floor samples were ground to pass through a 1mm mesh sieve and analyzed for total nitrogen (N) and carbon (C) concentration using a CHN elemental analyzer (CE Instruments-NC 2100, CE Elatech Inc., Lakewood, NJ). Total phosphorus (P), potassium

(K), calcium (Ca), magnesium (Mg), manganese (Mn), boron (B), copper (Cu), and zinc (Zn) concentrations were determined by dry ash digestion of 0.5 g of each sample with hydrochloric acid (Jones and Steyn, 1973) followed by analysis using an inductively coupled plasma atomic emission spectrometer (IPS-AES, Varian ICP, Liberty Series 2, Varian analytical instruments, Walnut Creek, CA).

The pine standard from the National Institute of Standards and Technology (standard reference material No. 1575) was used to ensure accuracy. All analyses were conducted with 10% sample duplication and a maximum coefficient of variation of 15% between duplicates was permitted for quality control. Although the final statistical analyses for forest floor mass, nutrient concentration and content were done using the sum of all forest floor layers, the laboratory analyses were done by layer to have a more accurate value of the nutrient concentrations and of the ash/mineral content correction. This was necessary because the Oi layer is usually collected free of mineral soil particles and has a very low ash content (<5%), as oppose to the Oa layer where ash content and contamination from mineral soil can be as high as 50%. Nutrient concentrations of the forest floor as a whole (using all layers of the forest floor) were calculated using a weighted average that accounted for the relative weight contributions of each layer to the forest floor.

Total nutrient content of the forest floor was calculated as concentration multiplied by forest floor mass. Forest floor mass, nutrient concentrations, and contents at each sampling time were divided by their initial amounts (at year 0) to express them as proportions remaining.

Data analysis

Repeated measures analysis was conducted using PROC MIXED (SAS, 2005) to determine the forest floor retention treatment, time, and interaction effects on forest floor nutrient concentrations, and the proportions remaining of forest floor mass and nutrient content. The null model likelihood ratio test was used to determine the need to specify a covariance structure to model the data. The Compound symmetry covariance structure was specified, when needed, based on the Akaike's (1987) information criterion (AIC), which assessed the goodness of fit of the predicted covariance matrix to the observed matrix. To determine treatment effects on forest floor nutrient concentrations on an annual basis, analyses of variance were performed on forest floor concentrations by year, and the Tukey's Studentized (HSD) test was used for treatment means comparison.

Results

Forest floor mass, nutrient concentrations and contents over the 2-year sampling period are summarized in table 1. Overall, decomposition and nutrient release from the forest floor was relatively fast i.e. the forest floor lost 84% and 78% of its mass (figure 1), 80% and 69% of its N content, and 85% and 79% of its P content from the control and doubled treatments, respectively during the sampling period. Carbon and all other macro and micronutrients concentrations were not affected by retention treatments (tables 1 and 2), with the exception of K and Cu, which throughout the sampling period maintained significantly higher concentrations in the control than in the doubled treatment. Based on these results, study averages of the proportion of carbon and macronutrients, and micronutrients concentrations are plotted in figures 2 and 3 respectively to show the effect of time on these variables. Through the assessment period, carbon concentrations remained constant, phosphorus and calcium concentrations had a slight increase at year 1, but by year 2 their concentrations were at the same level as at the beginning of the study. Nitrogen was the only nutrient that showed a significant and consistent increase in concentration as the forest floor decomposed, as opposed to potassium and magnesium, which showed a significant and consistent decrease (table 2, figure 2). The micronutrients boron and manganese also showed significant and consistent concentration decreases. Copper concentration had a slight increase only at year 1, and zinc had a slight increase only at year 2 (table 2, figure 3). Decomposition time had a different and significant effect on the forest floor retention treatment for C, Mg, and B concentrations. These interactions show the fact that concentrations for these 3 elements were higher in the control treatment at the beginning of the study, but ended being lower, or at the same level, as the doubled treatment towards the end of the sampling period. Proportion of nutrient contents in the forest floor were not significantly affected by retention treatments except for boron, which proportion remaining was higher in the doubled as compared to the control treatment. As expected, decomposition time had a significant effect on all forest floor nutrient pools (table 3), which showed significant nutrient release through the 2-year period. The study averages of carbon and macronutrients content remaining after 2 years ranged from 14% of the original pool size for K to 26% for N. Carbon and macronutrients release was in the following order: K = Mg > C = P = Ca > N (figure 4). The study averages of micronutrients content remaining after 2 years ranged from 11% of the original pool size for B to 22% for Zn, and were released in the following order: B > Mn > Cu = Zn (figure 5). Interactions between the proportions remaining of nutrient content and decomposition time were significant for N, P, and Zn following the same pattern as mass loss (figure 1) where the control treatment lost less mass in year 1, but more on year 2, as compared to the doubled treatment.

Discussion

The loss of 13 and 25 Mg ha^{-1} of forest floor mass, in a two-year-period, from the control and doubled forest floor retention treatments respectively, suggest faster decomposition rates than those previously reported for loblolly pine stands Unfortunately, annual samplings over a two-year period do not provide enough data points to adequately fit a decomposition model, such as Olson's exponential decay model (1963), in order to compare the slopes of decomposition curves with other published data. However, other decomposition studies in loblolly pine stands where measurements were carried out for approximately the same time (2 years) (Lockaby *et al.*, 1995; Piatek and Allen, 2001) have shown mass losses around 50%, much lower than the average 81% mass loss measured in our study. This faster decomposition may be attributed to environmental factors caused by the lack of a closed canopy in our study, such as higher direct solar radiation, which is know to aid the physical decomposition of litter tissue through photodegradation (Austin and Vivanco, 2006), and a higher water table as a result of lower tree demand for water given that this demand was removed with the harvest, and the current rotation is still too small to affect the level of the water table. A wet, but not water logged, forest floor would decompose faster by providing an ideal substrate to decomposers (Coleman *et al.*, 2004), as opposed to drier forest floors, which have been shown to decompose at slower rates (Cortina and Vallejo, 1994). The method of measuring decomposition, direct measurement of forest floor over time, in our case, versus litterbags in the other studies, may have also contributed to this difference in decomposition rates, since litter tissue inside nylon mesh is not exposed to larger decomposers such as springtails and earthworms which could accelerate the process.

Initial forest floor concentrations of C, K, and Cu were significantly higher in the control than in the doubled treatment (Table 1). This could be an unintended consequence of the treatment imposition. More twigs and small branches, with higher C concentrations, could have been left in the control plots as this forest floor was not as heavily manipulated as the one on the doubled treatment plots where forest floor removed from adjacent plots was brought in to double the amount retained. Potassium is a very mobile nutrient that can easily leach for intact plant tissue (Tew *et al.*, 1986). Thus, the lower K concentrations in the doubled treatment plots could also be explained by this manipulation given that the removed forest floor was raked, piled and transported in tarps from one location to another exposing this disturbed material to environmental conditions conducive to K loss. Concentrations of micronutrient such as Mn, Cu, and Zn tend to increase as the forest floor decomposes from litter to humus material (Gurlevik *et al.*, 2003). Cu concentrations, in particular, can be up to one order of magnitude higher in the humus layer as compared to the litter layer (Zerpa *et al.*, 2010), thus any small change in the amount of humus layer moved into the doubled treatment plots could have caused a difference in Cu concentration between the treatments.

Nutrients concentrations throughout the sampling period exhibited the expected patterns based on results from previous research, and on the mobility of nutrient in plant tissue (Berg and McClaugherty, 2003), with N showing a 30% increase as compared to its initial concentration, P, Ca, Cu, and Zn showing little change in concentration throughout the 2 years, and more mobile nutrients, such as K, Mg, B, and Mn showing 74, 74, 56, and 77% decreases respectively, as compared to their initial concentrations.

As expected, carbon release (figure 4) showed a very similar patter as forest floor mass loss (figure 1), as carbon oxidation is responsible for most of the forest floor weight loss at early stages of decomposition (Berg *et al.*, 1982; Polyakova and Billor, 2007). The release of 128 and 216 kg N ha^{-1} and of 7 and 14 kg P ha^{-1} from the control and doubled treatments respectively shows the contribution of the forest floor to the high levels of available nutrients commonly found in the soil in the early stages of stand development (Allen *et al.*, 1990), and the importance of increasing the retention of this pool for the nutrition of second rotation stands. In this study, decomposition and nutrient release were not significantly affected by the level of forest floor retained from one rotation to the next. It is important to note that direct comparisons of the doubled forest floor retention treatment with increased forest floor accumulations in fertilized stand might not be completely accurate because the layering of the forest floor is different in each case. Additionally, although this study is well replicated, it still lacks the site replication required to extrapolate results to a broader area or range of conditions. However, if these results hold true for fertilized stands with increased forest floor accumulation, then this pool could provide adequate nutrition to second rotation stand in early stages of development for a period of time that will be directly related to the amount of forest floor retained from the previous rotation. This, in turn could become a variable to consider for scheduling the first fertilizer application to the stand.

Conclusion

Results from this study suggest that forest floor decomposition and nutrient release is much faster in the early stages of development of second rotation loblolly pine stands than at mid-rotation or in older stands, which highlights the importance of this nutrient pool in early tree nutrition. This study also provides evidence that decomposition and nutrient release are not significantly affected by the level of forest floor retained from one rotation to the next. If this is the case, then the time during which early tree nutrition could rely on forest floor decomposition would be directly and linearly related to the level of forest floor retained.

References

Akaike, H., 1987. Factor Analysis and AIC. Psychometrika 52, 317-332.

Albaugh, T.J., Allen, H.L., Fox, T.R., 2007. Historical patterns of forest fertilization in the Southeastern United States from 1969 to 2004. South. J. Appl. For. 31, 129-137.

Allen, H.L., Dougherty, P.M., Campbell, R.G., 1990. Manipulation of Water and Nutrients - Practice and Opportunity in Southern U.S. Pine Forests. For. Ecol. Manage. 30, 437-453.

Austin, A.T., Vivanco, L., 2006. Plant litter decomposition in a semi-arid ecosystem controlled by photodegradation. Nature 442, 555-558.

Berg, B., 1986. Nutrient Release from Litter and Humus in Coniferous Forest Soils - a Mini Review. Scand. J. For. Res. 1, 359-369.

Berg, B., Hannus, K., Popoff, T., Theander, O., 1982. Changes in organic chemical components of needle litter during decomposition. Long-term decomposition in a Scots pine forest. Can. J. Bot. 60, 1310-1319.

Berg, B., McClaugherty, C., 2003. Plant Litter Decomposition, Humus Formation, Carbon Sequestration. Springer-Verlag Berlin Heidelberg, New York.

Coleman, D.C., Crossley, D.A.J., Hendrix, P.F., 2004. Fundamentals of Soil Ecology. Elsevier Academic Press, New York.

Cortina, J., Vallejo, V.R., 1994. Effects of clearfelling on forest floor accumulation and litter decomposition in a radiata pine plantation. For. Ecol. Manage. 70, 299-310.

Fox, T.R., Allen, H.L., Albaugh, T.J., Rubilar, R., Carlson, C.A., 2007. Tree nutrition and forest fertilization of pine plantations in the southern United States. South. J. Appl. For. 31, 5-11.

Gurlevik, N., Kelting, D.L., Allen, H.L., 2003. The effects of vegetation control and fertilization on net nutrient release from decomposing loblolly pine needles. Can. J. For. Res. 33, 2491-2502.

Guthrie, R.L., Witty, J.E., 1982. New designations for soil horizons and layers and the new Soil Survey Manual. Soil Sci. Soc. Am. J. 46, 443-444.

Jones, J.B., Jr., Steyn, W.J.A., 1973. Sampling, handling, and analyzing plant tissue samples. In: Walsh, L.M., Beaton, J.B. (Eds.), Soil Testing and Plant Analysis. Soil Sci. Soc. Amer., Madison, WI, pp. 249-270.

Jorgensen, J.R., Wells, C.G., Metz, L.J., 1980. Nutrient Changes in Decomposing Loblolly Pine Forest Floor. Soil. Sci. Soc. Am. J. 44, 1307-1314.

Li, Q.C., Allen, H.L., Wilson, C.A., 2003. Nitrogen mineralization dynamics following the establishment of a loblolly pine plantation. Can. J. For. Res. 33, 364-374.

Lockaby, B.G., Miller, J.H., Clawson, R.G., 1995. Influences of community composition on biogeochemistry of loblolly pine (Pinus taeda) systems. Am. Midl. Nat. 134, 176-184.

Markewitz, D., Richter, D.D., Allen, H.L., Urrego, J.B., 1998. Three Decades of Observed Soil Acidification in the Calhoun Experimental Forest: Has Acid Rain Made a Difference? Soil Sci. Soc. Am. J. 62, 1428-1439.

Mendham, D.S., O'Connell, A.M., Grove, T.S., Rance, S.J., 2003. Residue management effects on soil carbon and nutrient contents and growth of second rotation eucalyptus. For. Ecol. Manage. 181, 357-372.

Nelson, D.W., Sommers, L.E., 1996. Total Carbon, Organic Carbon, and Organic Matter. In: SSSA (Ed.), Methods of Soil Analysis Part 3 Chemical Methods. Soil Science Society of America, Inc., Madison, WI, pp. 1004-1005.

Olson, J.S., 1963. Energy Storage and the Balance of Producers and Decomposers in Ecological Systems. Ecology 44, 322-331.

Piatek, K.B., Allen, H.L., 2001. Are forest floors in mid-rotation stands of loblolly pine (Pinus taeda) a sink for nitrogen and phosphorus? Can. J. For. Res. 31, 1164-1174.

Polyakova, O., Billor, N., 2007. Impact of deciduous tree species on litterfall quality, decomposition rates and nutrient circulation in pine stands. For. Ecol. Manage. 253, 11-18.

Rojas, J.C., 2005. Factors influencing responses of loblolly pine stands to fertilization, Dissertation. Dep. Forestry. North Carolina State University, Raleigh, p. 147.

Saint-Andre, L., Laclau, J.P., Deleporte, P., Gava, J.L., Goncalves, J.L.M., Mendham, D.S., Nzila, J.D., Smith, C., du Toit, B., Xu, D.P., Sankaran, K.V., Marien, J.N., Nouvellon, Y., Bouillet, J.P., Ranger, J., 2008. Slash and Litter management Effects on *Eucalyptus* Productivity: a Synthesis Using a Growth and Yield Modelling Approach. In: Nambiar, E.K.S. (Ed.), Site Management and Productivity in Tropical Plantation Forests. Center for International Forestry Research (CIFOR), Proceedings of Workshops in Piracicaba (Brazil) 2004 and Bogor (Indonesia) 2006, pp. 173-189.

SAS, 2005. The SAS System for Windows Release 9.1.3. SAS Institute Inc., Cary, NC.

Smith, C.T., Lowe, A.T., Skinner, M.F., Beets, P.N., Schoenholtz, S.H., Fang, S.Z., 2000. Response of radiata pine forests to residue management and fertilisation across a fertility gradient in New Zealand. For. Ecol. Manage. 138, 203-223.

Switzer, G., Nelson, L., 1972. Nutrient accumulation and cycling in loblolly pine (Pinus Taeda L.) plantation ecosystems: The first twenty years. Soil Sci. Soc. Am. Pro. 36, 143-147.
Tew, D.T., Morris, L.A., Allen, H.L., Wells, C.G., 1986. Estimates of Nutrient Removal, Displacement and Loss Resulting from Harvest and Site Preparation of a Pinus-Taeda Plantation in the Piedmont of North-Carolina. For. Ecol. Manage. 15, 257-267.

Tutua, S.S., Xu, Z.H., Blumfield, T.J., Bubb, K.A., 2008. Long-term impacts of harvest residue management on nutrition, growth and productivity of an exotic pine plantation of sub-tropical Australia. For. Ecol. Manage. 256, 741-748.

Vitousek, P.M., Matson, P.A., 1985. Disturbance, Nitrogen Availability, and Nitrogen Losses in an Intensively Managed Loblolly-Pine Plantation. Ecology 66, 1360-1376.

Zerpa, J.L., Allen, H.L., Campbell, R.G., Phelan, J., Duzan, H., 2010. Influence of variable organic matter retention on nutrient availability in a 10-year-old loblolly pine plantation. For. Ecol. Manage. 259, 1480-1489.

Table 1. Forest floor mass, nutrient concentrations and content in a recently established second rotation loblolly pine plantation in the Southeast, US under different forest floor retention treatments.

	Year 0		**Year 1**		**Year 2**	
	Control	**Doubled**	**Control**	**Doubled**	**Control**	**Doubled**
Mass						
(Mg ha^{-1})	15.63 (0.59)	31.70 (1.96)	11.34 (0.60)	19.80 (0.68)	2.57 (0.26)	7.07 (0.77)
Concentrations						
C (%)	51.1 (0.4) a	47.5 (0.9) b	49.0 (0.4) a	49.9 (1.0) a	48.5 (1.2) a	50.1 (0.3) a
N (%)	1.03 (0.04) a	0.98 (0.01) a	1.18 (0.05) a	1.18 (0.06) a	1.31 (0.06) a	1.34 (0.06) a
P (g kg^{-1})	0.56 (0.01) a	0.56 (0.02) a	0.62 (0.02) a	0.60 (0.02) a	0.52 (0.03) a	0.54 (0.01) a
K (g kg^{-1})	0.52 (0.01) a	0.48 (0.01) b	0.42 (0.01) a	0.41 (0.01) a	0.38 (0.02) a	0.36 (0.01) a
Ca (g kg^{-1})	7.0 (0.5) a	5.5 (0.2) a	7.1 (0.2) a	6.7 (0.2) a	6.1 (0.5) a	6.2 (0.2) a
Mg (g kg^{-1})	1.07 (0.05) a	0.89 (0.03) a	0.81 (0.02) a	0.70 (0.03) a	0.67 (0.04) a	0.74 (0.05) a
Mn (mg kg^{-1})	992 (56) a	818 (37) a	774 (61) a	684 (33) a	651 (110) a	707 (39) a
B (mg kg^{-1})	10.0 (1.1) a	7.3 (0.2) a	5.4 (0.1) a	6.4 (0.7) a	4.8 (0.1) a	4.6 (0.1) a
Cu (mg kg^{-1})	2.9 (0.1) a	2.3 (0.2) b	3.2 (0.2) a	2.9 (0.1) a	2.7 (0.4) a	2.3 (0.2) a
Zn (mg kg^{-1})	19.1 (0.6) a	18.2 (0.6) a	19.5 (0.9) a	18.1 (0.4) a	20.8 (1.4) a	22.2 (1.2) a
CN ratio	50.0 (1.9) a	48.3 (0.7) a	42.0 (2.1) a	42.4 (1.3) a	37.3 (1.6) a	37.8 (1.7) a
Content						
C (kg ha^{-1})	7974 (243)	15121 (1159)	5556 (287)	9896 (497)	1244 (122)	3550 (394)
N (kg ha^{-1})	161 (8)	312 (20)	133 (6)	235 (18)	33 (3)	96 (13)
P (kg ha^{-1})	8.7 (0.3)	18.0 (1.5)	7.0 (0.3)	11.9 (0.7)	1.3 (0.1)	3.8 (0.5)
K (kg ha^{-1})	8.1 (0.4)	15.2 (1.0)	4.7 (0.2)	8.2 (0.2)	1.0 (0.1)	2.5 (0.3)
Ca (kg ha^{-1})	110 (9)	175 (12)	80 (3)	132 (2)	15 (1)	43 (4)
Mg (kg ha^{-1})	16.7 (1.1)	28.5 (2.4)	9.1 (0.3)	13.8 (0.5)	1.7 (0.1)	5.2 (0.5)
Mn (kg ha^{-1})	15.6 (1.3)	25.7 (1.1)	8.8 (0.8)	13.5 (0.4)	1.6 (0.2)	4.9 (0.4)
B (kg ha^{-1})	0.158 (0.024)	0.233 (0.017)	0.061 (0.002)	0.127 (0.018)	0.012 (0.001)	0.033 (0.004)
Cu (kg ha^{-1})	0.045 (0.003)	0.073 (0.008)	0.036 (0.002)	0.058 (0.004)	0.007 (0.001)	0.016 (0.002)
Zn (kg ha^{-1})	0.298 (0.013)	0.579 (0.048)	0.220 (0.011)	0.358 (0.014)	0.052 (0.004)	0.154 (0.012)

Standard errors shown in parenthesis (n = 5); concentration means followed by the same letter within the same year are not significantly different by Tukey's Studentized range (HSD) test (P = 0.05)

Table 2. Summary of statistical significance (Pr > F) of forest floor retention treatments (FF) and sampling dates (Time) effects on forest floor carbon, macro and micro-nutrients concentrations during a two-year decomposition period in a second rotation loblolly pine plantation in the Southeast, US.

	Pr > F									
Source	C	N	P	K	Ca	Mg	Mn	B	Cu	Zn
FF	0.5955	0.9628	0.8236	**0.0187**	0.0504	0.0522	0.2092	0.2096	**0.0464**	0.7201
Time	0.9754	**<0.0001**	**0.0048**	**<0.0001**	0.0785	**<0.0001**	**0.0056**	**<0.0001**	0.0507	**0.0103**
FF x Time	**0.0086**	0.4688	0.7032	0.2691	0.0846	**0.0182**	0.2042	**0.0167**	0.8233	0.2979

Table 3. Summary of statistical significance (Pr > F) of forest floor retention treatments (FF) and sampling dates (Time) effects on forest floor mass remaining (FF mass), carbon, macro and micro-nutrients proportions remaining during a two-year decomposition period in a second rotation loblolly pine plantation in the Southeast, US.

	Pr > F										
Source	FF mass	C	N	P	K	Ca	Mg	Mn	B	Cu	Zn
FF	0.4926	0.4712	0.7650	0.4755	0.9747	0.3384	0.6872	0.7021	**0.0287**	0.5761	0.8731
Time	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
FF x Time	**0.0115**	0.1140	**0.0317**	**0.0398**	0.2081	0.4704	0.0695	0.1596	0.1045	0.8133	**0.0229**

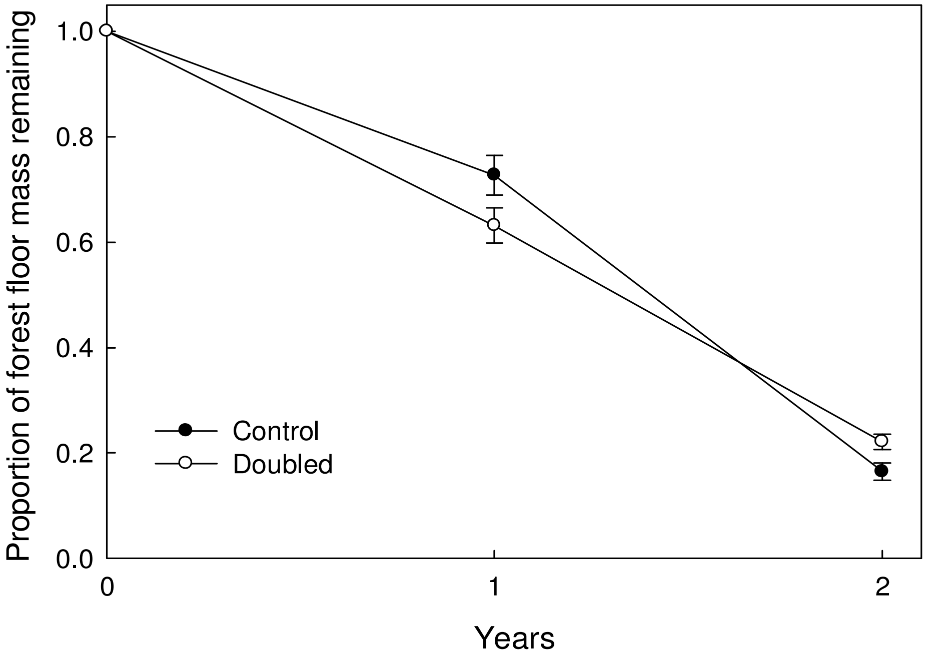

Figure 1. Proportion of forest floor mass remaining, in a recently established second rotation loblolly pine plantation in the Southeast, US. Bars are standard errors.

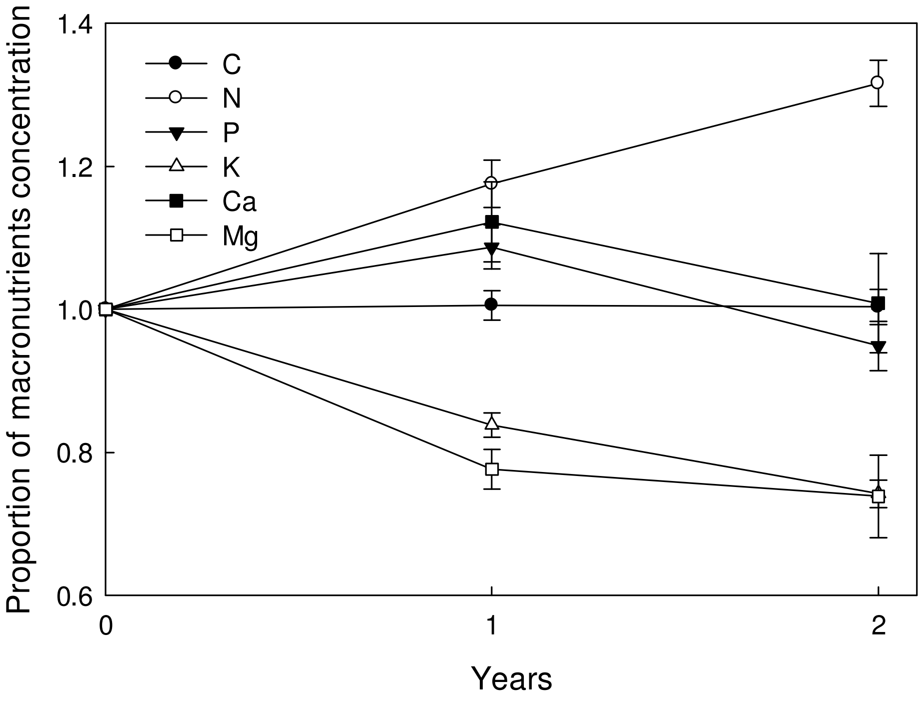

Figure 2. Proportion of initial forest floor carbon and macronutrients concentration, in a recently established second rotation loblolly pine plantation in the Southeast, US. Bars are standard errors.

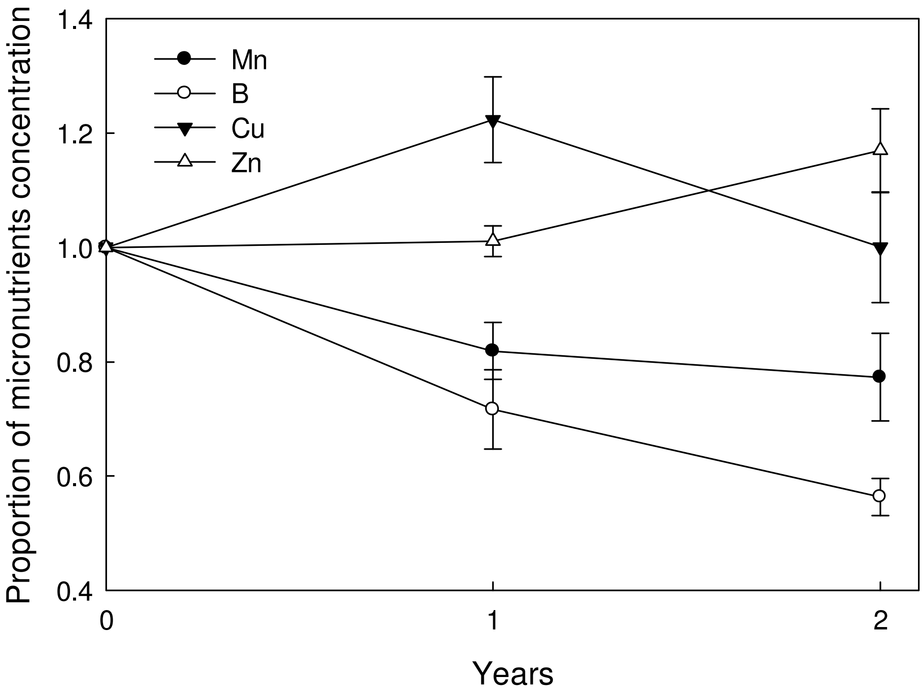

Figure 3. Proportion of initial forest floor micronutrients concentration, in a recently established second rotation loblolly pine plantation in the Southeast, US. Bars are standard errors.

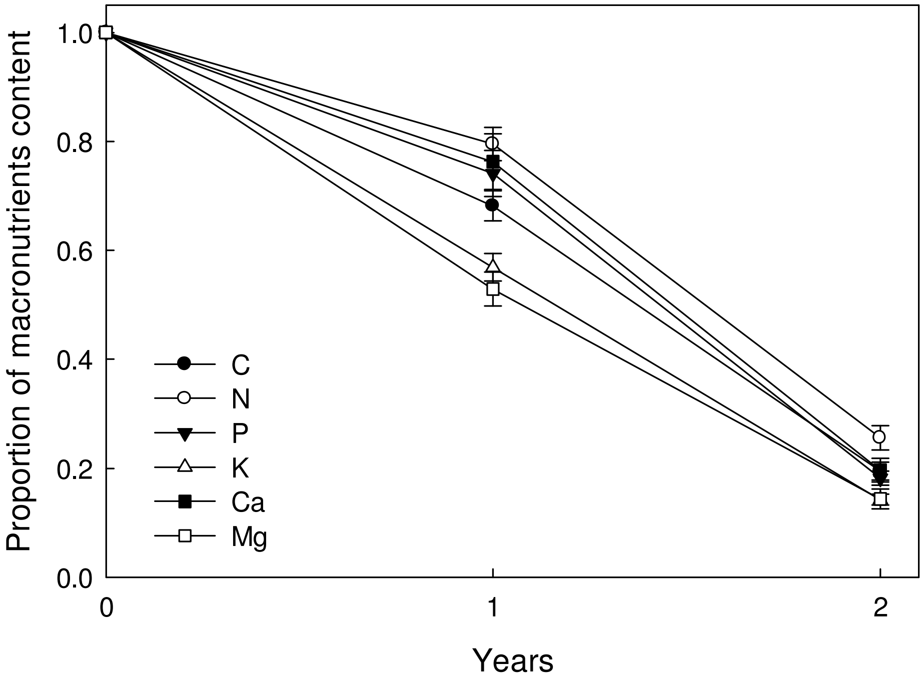

Figure 4. Proportion remaining of forest floor carbon and macronutrients content, in a recently established second rotation loblolly pine plantation in the Southeast, US. Bars are standard errors.

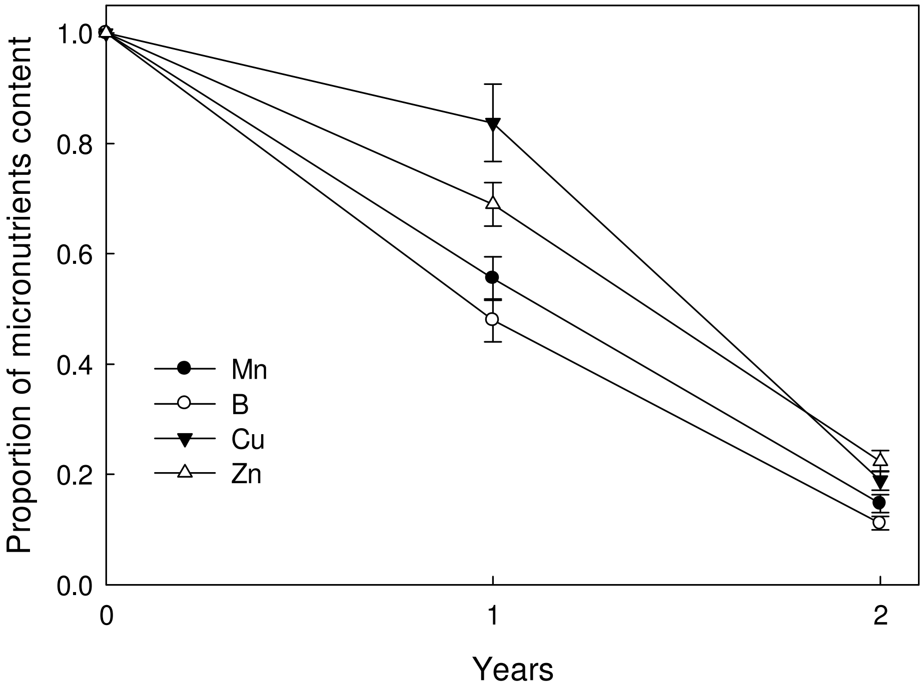

Figure 5. Proportion remaining of forest floor micronutrients content, in a recently established second rotation loblolly pine plantation in the Southeast, US. Bars are standard errors.

CHAPTER 2

Effects of post-harvest forest floor retention and incorporation on soil C, N, and P pools in a recently established loblolly pine plantation

Abstract

Mineral soil carbon, nitrogen, and phosphorus pools were determined in a loblolly pine plantation on the Coastal Plain of North Carolina, prior to and during two years following harvest. Treatments included three levels of forest floor mass retention (0, 15, and 30 Mg ha^{-1}) combined with two levels of incorporation (mixed, non-mixed) in a factorial design. The objective was to determine the effects of forest floor retention and the incorporation of forest floor into the mineral soil, on the size and changes of mineral soil C, N, and P pools. After two years, and using the 0 retention treatment as a reference, total C and N pools in the mineral soil increased 20 and 21% respectively, and available C, N, and P pools increased 46, 47, and 49% respectively, by doubling the forest floor. A post-harvest flush of soil available N (Assart effect) was observed throughout the two growing seasons and doubling the forest floor caused a full year delay in the times of maximum N availability as compared to the removed treatment. Incorporation had a transient effect, with available C, N, and P pools showing significantly higher levels only during the first month of sampling. Incorporation also increased total C and N pools in the mineral soil in the first month, but this effect was reversed after two years. Results from this study show that increasing forest floor retention has a direct positive effect on mineral soil C, N, and P pools sizes, and helps to better synchronize the site's N supply with stand demand by delaying the peak of maximum N soil availability.

Introduction

The importance of organic matter management in sustaining forest productivity has been highlighted by many studies and field installations across the globe, among them, the North American Long Term Site Productivity studies (LTSP) (Powers *et al.*, 2005), the network of studies coordinated by the Center for International Forestry Research (CIFOR) (Nambiar and Kallio, 2008), and several others (Binkley, 1984; Smith *et al.*, 2000; Zerpa *et al.*, 2010). In general, the influence of organic matter removals or additions on sustaining site productivity largely depends on the intensity and frequency of these manipulations and the initial size of the nutrient pools. In loblolly pine (*Pinus taeda* L.) plantations of the Southeast US, nitrogen (N) and phosphorus (P) fertilizer are used to increase wood production (Albaugh *et al.*, 2007), which is realized in part by increasing the amount of foliage. Through litterfall, this foliage accumulates in the forest floor, forming a significant nutrient pool (Tew *et al.*, 1986; Markewitz *et al.*, 1998). Increases in forest floor mass and N content of over 100% are possible following fertilization in highly responsive pine stands in the Southeast US. (Rojas, 2005) These accumulations highlight the importance of the forest floor in the nutrition of current and subsequent stands as nutrients become available through decomposition and mineralization processes (Jorgensen *et al.*, 1980).

Many studies have manipulated organic matter through harvesting and/or site preparation treatments in an effort to impose different levels of removal. These studies have provided important information, but are not particularly relevant to current pine plantation management where practices such as strip shearing, bedding, and vegetation control with herbicides retain rather than remove organic matter. Interestingly, studies where organic matter has been retained or added have shown either small positive effects (Mendham *et al.*, 2003; du Toit *et al.*, 2008; Smith *et al.*, 2008) or no effects (Hardiyanto and Wicaksono, 2008; Siregar *et al.*, 2008; Zerpa *et al.*, 2010) on mineral soil C and N pools. This lack of treatment effect could be the result of measuring too broad pools, such as total soil C and N, instead of more available pools that could be more immediately affected by treatment, and be more relevant to tree growth, or of measuring too late after treatment imposition, thereby missing the treatment effect at the early stages of stand development.

The post-harvest organic matter retention effects on soil P availability have not been as well documented as for N (du Toit *et al.*, 2008), but it is well known that P commonly limits tree growth on many sites (Fox *et al.*, 2007). Although P availability in soil solution is highly dependent on pH, and the mobility of its most common forms ($H_2PO_4^-$ and HPO_4^{2-}) is very different from nitrogen (NH_4^+ and NO_3^-), from a biological stand point, it is possible that forest floor retention treatments could affect N and P pools in similar ways and that factors controlling N availability at early stages of stand development may also control P availability. The C:N:P ratio for coniferous forest floors is approximately 720:12:1 while soil microbes, responsible for decomposing organic matter and mineralizing N and P, have an approximate ratio of 10:2:1 (Anderson and Domsch, 1980) suggesting a high demand for both nutrients as the carbon from the forest floor is oxidized through decomposition.

Mixing treatments that incorporate forest floor and slash with mineral soil through disking or bedding have resulted in stand productivity increases by improving physical properties and nutrient availability (Sanchez and Eaton, 2001). Evidence of accelerated organic matter decomposition with mixing treatments has been found by Sanchez, *et al.* (2003), and soil C and N concentrations have shown consistent increases in the first years after mixing forest floor to mineral soils (Sanchez *et al.*, 2000). Unfortunately, it has been difficult to isolate the effects of organic matter retention and mixing in previous studies, because the removal or retention treatments have been mechanized, thus including some level of soil disturbance (Li *et al.*, 2003), or the lack of a full factorial combination of treatments. It is expected that incorporation of the forest floor into the mineral soil should provide a better contact with the active microbial populations increasing initial nutrient immobilization.

Soil nitrogen dynamics following the harvest are commonly characterized by increased mineralization rates and extractable mineral N (Kimmins, 1997). These effects have been attributed to several factors including increased decomposition of forest floor and harvest residues from the previous rotation (Berg *et al.*, 1993; De Santo *et al.*, 1993; Sariyildiz and Anderson, 2003), increased temperatures (Kim *et al.*, 1995), higher soil moisture due to lower evapotranspiration rates (Barg and Edmonds, 1999), the post-harvest mixing of forest floor and slash material with the surface soil (Tamm, 1964; Kimmins, 1997), and reduced N uptake caused by tree removal (Burger and Pritchett, 1984; Vitousek and Andariese, 1986; Smethurst and Nambiar, 1989; Vitousek *et al.*, 1992). More recently, it has been hypothesized of that higher N levels may result from reduced microbe immobilization due to lower levels of available C from fresh litter inputs, root exudates, and throughfall following harvest (Hart *et al.*, 1994a; Bradley *et al.*, 2000; Li *et al.*, 2003). Thus, heterotrophic soil microbes, which are mainly responsible for N immobilization-mineralization, may be limited by energy sources and may not require as much nitrogen as before the harvest.

This post-harvest flush of available N, the "Assart" effect (Tamm, 1964; Kimmins, 1997) typically lasts between 1-5 years. During these first few years, the root systems of young plantations are not well developed and have not effectively occupied the available soil volume. Thus, the increased N availability, poorly timed with low plant uptake, can result in the conversion of available N into unavailable forms through complexation with metals, clays, organic matter and other ions, physical occlusion, or possibly leaching losses in sandy soils with low capacity to retain these ions (Likens *et al.*, 1970; Titus *et al.*, 1997) making the N unavailable to the trees.

Given that most soil organisms are heterotrophic (Hart *et al.*, 1994a; Bradley, 2001) and commonly limited by carbon (Alden *et al.*, 2001; Ekblad and Nordgren, 2002) it is hypothesized that by increasing the carbon pool through post-harvest forest floor retention, the heterotrophic microbial population will immobilize part of the available N pool, resulting in a delay in the peak of N availability in the soil. Therefore, there might be greater synchrony between nutrient supply from the soil and nutrient demand from the plantation; the nutrients would be released when the uptake capacity of the plantation is greater.

The objectives of our study are to determine the effects of different levels of forest floor retention, and its incorporation into mineral soil, on soil total C and N pools, and available C, N and P pools. The design and sampling scheme used allow the determination of main treatment effects and their interactions through time, thus providing insight on the effects of harvest and treatments on nutrient availability at the early stages of growth of a loblolly pine stand in the Southeast US.

Materials and Methods

Site and Study Description

The study was established on Weyerhaeuser Company land in 2006 in the Lower Coastal Plain of Pamlico County, North Carolina (35°6'2.00"N, 76°52'45.19"W) prior to harvesting a 33-yr old loblolly pine plantation. Ten-year (1998-2007) mean annual temperature is 17.5 °C with mean monthly temperatures ranging from 7.7 °C in January to 26.3 °C in July. Mean annual precipitation is 1,439 mm with a fairly uniform distribution throughout the year. January is the driest month with 77 mm, and August is the wettest month with 195 mm. The soil on this site is a fine, mixed, subactive, thermic Aquic Hapludult of the Craven soil series with fair to good surface drainage. The A-horizon is a fine sandy loam with an average thickness of 10 cm, bulk density of 1.18 $g•cm^{-3}$, total C and N contents of 17.2 and 0.76 $Mg•ha^{-1}$ respectively, and Mehlich III extractable P content of 7.8 $kg•ha^{-1}$. The harvested stand had received cumulative fertilizer additions of 670 kg $N•ha^{-1}$ and 165 kg $P•ha^{-1}$, and had been commercial thinned at 15 and 25 years. The stand exhibited a site index of 24 m

(25 years base age) and a density of 250-300 stems•ha^{-1}. The forest floor had accumulated to an ash-free mass of 15.6 Mg•ha^{-1} and contained 8 Mg C•ha^{-1}, 160 kg N•ha^{-1}, and 8.7 kg P•ha^{-1}. Logging was conducted with a boom-top excavator and trees were felled using the previous thinning roads to prevent disturbance of the forest floor and trafficking on the study plots.

Immediately following harvest, a complete randomized block study with 5 replications and 6- forest floor/mixing treatments was imposed on the site. The treatment design was a 3x2 factorial with 3 levels of forest floor retention (removed, control, doubled), and 2 levels of forest floor incorporation with the surface mineral soil (mixed and non-mixed). The forest floor treatments were imposed in mid March 2006. Forest floor was raked from the removed plots and transported using tarps to the double plots where it was evenly distributed throughout the plots. Control plots were left with the original forest floor in place. The mixing treatments were imposed in early April 2006 using a small tractor pulling a three-disk tiller on the first pass and a one-row disk tiller on the second and third pass to mix the forest floor with the mineral soil A-horizon.

The plots size were 16.8m x 9.1m including buffer areas and the measurement plots were 12.2m x 4.9m. Two weeks after the incorporation treatments were completed, 96-full sibs pine seedlings were planted per plot at 1.5m x 1.2m spacing for a total of 32 pines seedlings per measurement plot, with the objective of using them as a bioassay. This will be addressed in chapter 3.

Mineral soil sampling and analysis

Mineral soil samples from the A-horizon were collected at 5 randomly located points per plot following the schedule in table 1. Mineral soil collections were always made to the top of the B-horizon and the depth to the A-horizon was determined prior to treatment on all plots. This depth was very consistent with an abrupt boundary between the A- and the B-horizon. Three bulk density samples were also collected from the A-horizon in each plot using the core method (Grossman and Reinsch, 2002). The samples were composited by plot in the field, put in plastic bags and transported in refrigerated containers to the lab where they were sieved through 2 mm mesh size to remove roots and other large organic residues. The soil did not have a coarse fraction greater than the mesh size used.

The sieved samples were then stored at 4 °C until further analysis. Based on the schedule for analyses (Table 1), a sub-sample of mineral soil was left to air dry for pH, total C, and N analyses and Mehlich III extractions.

Determination of soil pH was done with a glass electrode (Mettler DL 12 Tritator, Mettler-Toledo, Inc., Hightstown, NJ) which measured the H^+ activity of slurry composed of 10 g of soil sample and 10 ml of deionized water (Thomas, 1996).

Total soil C and N concentrations were determined by dry combustion in a CHN elemental analyzer (CE Instruments-NC 2100, CE Elatech Inc., Lakewood, NJ).

Microbial biomass C and dissolved organic C were determined to assess for labile soil C pools. Microbial biomass C was determined on 2 M KCl extracts using the chloroform fumigation-extraction method described by Brookes et al.(1985). Dissolved organic C was determined from the non-fumigated extract. All the extracts were analyzed on a Schimadzu TOC analyzer.

Two approaches were used to assess for available N in the mineral soil; a snapshot approach in which the soil solution extractable N pool was determined at different time intervals, and an aerobic incubation of intact soil cores used as an index of potential net N mineralization (Hart *et al.*, 1994b). To determine the soil solution extractable N pool, fresh soil samples were extracted in 35 ml of 2M KCl by shaking at high speed for one hour and centrifuging for 15 minutes at 4,000 rpm. The centrifuged solutions were filtered using Whatman 42 ashless filters and analyzed for inorganic N with a Lachat Autoanalyzer (Quick-Chem 8000, Zellweger Analytics, Inc. Milwaukee, WI). These analyses were used as the initial values for the aerobic incubation of intact soil cores. This incubation was done for 28 day at 25 °C. Changes in the moisture content of the incubated soil cores were monitored every other day and deionized water was added with a hand sprayer when the moisture contents in the samples dropped by more than 5% below their initial levels. These incubated samples were extracted and analyzed using the same procedure described for the soil solution extractable N pool assessment. Potential N net mineralization was calculated by subtracting the initial extractable values of NO_3^--N and NH_4^+-N from the amounts extracted after incubation.

Extractable P from the mineral soil is highly dependent on the type and strength of the extracting solution used (Hedley *et al.*, 1982; Cross and Schlesinger, 1995), which is directly related to the pool's bioavailability. Three different methods, covering a range of availability, were used: an extraction with oxalic acid, a weak organic acid commonly found as root exudates (Ford *et al.*, 1985), an anionic exchange membranes P extraction, and a Mehlich III

P extraction. Oxalic acid-extractable P was determined using 10 g of fresh soil extracted with 100 ml of 3mM Oxalic acid. These extracts were analyzed for inorganic P using a Lachat Autoanalyzer. Anionic exchange membranes were used to extract available P in soil solution following a modified version of the method described by Myers et al (1999; 2005). A two-gram sample of fresh soil was shaken for 24 hours in a 125 ml wide-mouthed plastic bottle containing 100 ml of deionized water and a 2.5 x 6.25 cm anionic exchange membrane. The membrane was then removed from the bottle, washed under a stream of deionized water to remove any soil residue and shaken for 90 minutes in 50 ml of 0.5M HCl. This solution was then analyzed for inorganic P in a Lachat Autoanalyzer. Mehlich III extractable P was determined by extracting a 3.13 g air-dried soil sample in 25 ml of Mehlich III extracting solution. The solution was shaken for 5 minutes, filtered using Whatman 42 ashless filters and analyzed in an inductively coupled plasma atomic emission spectrometer (IPS-AES, Varian ICP, Liberty Series 2, Varian analytical instruments, Walnut Creek, CA).

Gravimetric soil moisture contents were determined for fresh and air-dried samples at every collection in order to correct for moisture content and express the soil pools on dry weight basis. All nutrient pools from the mineral soil were scaled to a per hectare basis using the depth of the A-horizon and the bulk density of the soil.

All analyses were conducted with 10% sample duplication and a maximum coefficient of variation of 15% between duplicates was permitted.

Data analysis

Repeated measures analysis of variance were performed to test for treatment, time, and their interaction effects on mineral soil pH, total C and N, and available C, N ,and P pools using PROC MIXED (SAS, 2005).The Spatial Power, Gaussian, and Spherical covariance structures were tested to model the observed data and to account for the unequal spacing of the sampling dates (Littell *et al.*, 2006). The Akaike's (1987) information criterion (AIC) was used for assessing the goodness of fit of the predicted covariance matrix to the observed matrix. For each variable, the covariance structure with the lowest AIC was selected to model the data.

The mixed model used was:

$$Y_{ijkm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + A_m + \varepsilon_{ijkm}$$

Where Y_{ijkm} = the response to treatment (i) and (j) in block (m) at time (k), μ = the overall mean, α_i = the fixed forest floor retention treatment effect (i=1,2,3), β_j = the fixed incorporation treatment effect (j=1,2), $(\alpha\beta)_{ij}$ = the fixed interaction effect of the forest floor retention treatment (i) with the incorporation treatment (j), γ_k = the fixed effect of time (k) which followed the schedule on table 1, $(\alpha\gamma)_{ik}$ = the fixed interaction effect of forest floor retention treatment (i) with time(k), $(\beta\gamma)_{jk}$ = the fixed interaction effect of incorporation treatment (j) with time (k), $(\alpha\beta\gamma)_{ijk}$ = the three-way fixed interaction effect of forest floor retention treatment (i) with incorporation treatment (j) with time (k), A_m = the random block effect (m=1,2,3,4,5), and ε_{ijkm} = the random residual error.

Significance was accepted at $p \leq 0.05$ for all analysis. The relationships between available C and N pools and between the methods to obtain extractable P from the mineral soil were examined with Pearson correlation coefficients using PROC CORR (SAS, 2005), and the general linear model (SAS, 2005) was used to described the levels of extractable N and Mehlich III P in the soil solution throughout the sampling period as a function of initial forest floor mass retained.

Results

Mineral soil (A-horizon) total carbon and total nitrogen increased, from pre-harvest to one month after treatment, an average of 8.3 Mg ha^{-1} and 426 kg ha^{-1} respectively for all treatments. During the post-treatment sampling period, the average total carbon was 22.8; 24.8; and 27.4 Mg ha^{-1} and the average total nitrogen was 1.12; 1.21; and 1.35 Mg ha^{-1} for the remove, control and doubled treatments, respectively (table 2, figures 1a and 4a) indicating a significant forest floor retention treatment effect on these variables (table 3). Not surprisingly, incorporation increased the total carbon and nitrogen content in the mineral soil from pre-harvest to one month after treatment by 24%, from 22.7 to 28.2 Mg ha^{-1}, and by 23% from 1.06 to 1.31 Mg ha^{-1}, respectively, but this trend was reversed by month 29 when total carbon and nitrogen in the mixed treatment were 22.5 Mg ha^{-1} and 1.11 Mg ha^{-1}, 10% and 13% lower than the non-mixed treatment, respectively. This dynamic resulted in statistically significant incorporation x time interactions for both variables, (table 3, figure 1b and 4b). Although at different levels, the patterns of total carbon and nitrogen changed similarly through time, thus the C:N ratio was not affected by forest floor treatment. However, the C:N ratio showed a significant time effect, dropping from 22.6 at pre-harvest to

20.0 at month 29, and a significant incorporation x time effect (table 3), which resulted in a separation of C:N ratios for the two incorporation treatments from the 15th month of sampling, when both treatments C:N ratio was around 19.8, to the 29th month, when the mixed treatment C:N ratio was 20.4 vs. 19.7 of the non-mixed treatment.

The dissolved organic carbon pool in the mineral soil decreased from pre-harvest sampling to one month after treatment by 60% from 142.3 to 57.5 kg ha^{-1}, by 53% from 143.9 to 67.4 kg ha^{-1}, and by 34% from 146.6 to 96.1 kg ha^{-1}, for the removed, control and doubled treatments respectively. The dissolved organic carbon during the sampling period showed consistently higher pool levels in the doubled treatment (85 kg ha^{-1}) as compared to the control (69 kg ha^{-1}) and the removed (58 kg ha^{-1}) treatments (tables 2 and 3, figures 2a). The higher levels of dissolved organic C shown in the mixed treatment (table 2, figure 2b) were principally due to higher levels on the doubled treatment, resulting in a significant forest floor x incorporation interaction (table 3).

The microbial biomass carbon through the sampling period showed consistently higher levels in the doubled treatment (451 kg ha^{-1}), as compared to the control (388 kg ha^{-1}), and the removed (336 kg ha^{-1}) treatments (table 2, figure 3a). The largest forest floor retention treatment differences were observed during the first four months of sampling, when the control and doubled showed an average of 25% and 48% more microbial biomass C than the 382 kg ha^{-1} measured in the removed treatment during this period. During the first month of sampling, the incorporation treatment significantly increased the microbial biomass carbon (figure 3b) in the control by 57% from 427 to 670 kg ha^{-1} and in the doubled by 71% from

436 to 744 kg ha^{-1} with no noticeable change for the removed treatment. This resulted in significant forest floor x incorporation and incorporation x time interactions (table 3). Mineral soil extractable N through the sampling period showed higher levels in the doubled treatment (14 kg ha^{-1}) as compared to the control (11 kg ha^{-1}) and the removed (10 kg ha^{-1}) treatments (table 2, figures 5a). The highest levels of extractable N occurred in the removed treatment (19 $kgha^{-1}$) during the first year and in the doubled treatment (28 kg ha^{-1}) during the second year, showing that increased forest floor retention caused a significant increase and a delay on the post-harvest flush of soil available N known as the "Assart effect". A linear model expressing the average extractable N measured during 29 months, as a function of initial forest floor mass retained showed an increase in extractable N of 0.14 kg ha^{-1} for every ton of forest floor retained after harvest, $R^2 = 0.81$ (figure 6). The main effect of incorporation on extractable N was not significant throughout the sampling period (table 3, figure 5b) although higher levels were observed for the mixed treatment during the first month of sampling, and for the non-mixed at month 4, resulting in a significant forest floor x incorporation x time interaction (table 3). For the mixed treatment, there was a negative correlation between the 3-month-average soil labile carbon, measured as dissolved organic C, and the 3-month-average soil extractable N (Pearson correlation coefficient $|r|=0.61$; $p = 0.02$; $n = 15$) (Figure 7). Potential mineralizable nitrogen showed a period of mineralization during the first eight months of sampling (March through November '06), then immobilization 15 months after treatment (June '07) and mineralization again 19 months after treatment (October '07). Through the entire sampling period, the removed treatment mineralized 3.62 kg N ha^{-1}, 65% and 180% more N than the control and doubled treatments,

respectively (tables 2 and 3, figure 8a). No significant effect was observed for the incorporation treatment (figure 8b), but the non-mixed treatment showed higher mineralization in months 2 and 4 resulting in a significant incorporation x time interaction (table 3).

Mehlich III extractable phosphorus through the entire sampling period (29 months) also showed consistently higher levels in the doubled (16.1 kg ha^{-1}) as compared to the control (11.7 kg ha^{-1}) and the removed (9.5 kg ha^{-1}) treatment (tables 2 and 3, figures 9a). A linear model expressing the average Mehlich III extractable P measured during 29 months, as a function of initial forest floor mass retained showed an increase in extractable P of 0.21 kg ha^{-1} for every ton of forest floor retained after harvest, $R^2 = 0.46$ (figure 10). Incorporation increased Mehlich III extractable P levels for all forest floor retention treatments only on the first month after treatment imposition (figure 9b), thereafter, control and removed treatments had no significant incorporation effect, and doubled showed lower Mehlich III extractable P on the mixed treatment. This dynamic resulted in a significant forest floor x incorporation x time interaction (table 3). Extractable phosphorus from the soil solution obtained with anion exchange membranes showed consistently higher levels in the doubled treatment (7.2 kg ha^{-1}) as compared to the control (5.2 kg ha^{-1}) and the removed (4.8 kg ha^{-1}) treatments through the sampling period (tables 2 and 3, figures 11a), in agreement with Mehlich III extraction values (figure12). Incorporation increased the anion exchange membrane extractable phosphorus by 29% from 5 to 6.47 kg ha^{-1} throughout most of the sampling period (table 3, figure 11b). Oxalic acid extractable P (figures 13a and 13b) showed similar results to those obtained with the anion exchange membranes, as confirmed by the high and significant correlation shown in figure 14. Soil pH was not affected by treatment and showed a small, but significant decrease from 5.5 one month after treatment to 5.2 fifteen months after treatment imposition (table 3).

Discussion

Total C and N pools (A-horizon) increased significantly from the pre-harvest (February ’06) to the first month after treatment assessment (April ’06) (figures 1 and 4). Up to 12.2 Mg ha^{-1} of fine root biomass have been reported by Adams et al. (1989), in a similar plantation of the Lower Coastal Plain, for the same sampling depth. Thus, fine root biomass decomposition after 2 months could partially explain this increase. Not surprisingly, the incorporation treatment had a significant interaction with time on total C and N pool in the mineral soil (table 3, figures 1b and 4b). Similar dynamics have been previously reported for loblolly pine (Sanchez *et al.*, 2003; Sanchez *et al.*, 2009) where the effects of incorporation on increasing soil carbon and nitrogen stocks were only noticeable in the short term. The forest floor treatments had a significant and sustained effect on total C and N pool in the mineral soil (A-horizon) during the sampling period (table 2, figures 1a and 4a) Sanchez *et al.* (2003) reported a similar effect during a similar time frame.

After 29 months, the forest floor on the doubled treatment had released 216 Kg ha^{-1} of nitrogen, and coincidentally, the difference in total soil N (A-horizon) between the removed and the double treatment in the non-mixed plots was 211 kg ha^{-1} indicating that after two years almost all (98%) of the N released from the forest floor was still in the mineral soil A-horizon.

The significant decrease in dissolved organic C in the mineral soil (A-horizon) from the pre-harvest assessment to the assessment done one month after treatment imposition (figure 2) may be explained by the drastic reduction in inputs of soil available C in the form of fresh litter inputs and root exudates from the previous pine stand which were removed by the harvest (Hart *et al.*, 1994a; Bradley *et al.*, 2000; Li *et al.*, 2003). The forest floor retention treatment had a significant and consistent effect on dissolved organic carbon from the mineral soil during the entire sampling period after harvest, showing an18% and 46% increase for the control (69 kg ha^{-1}) and double (85 kg ha^{-1}) treatments over the removed treatment (58 kg ha^{-1}) (figure 2). Not surprisingly, similar results were found of microbial biomass carbon (figure 3) given the increased C availability that resulted from increased forest floor retention.

The extractable N measured in the mineral soil (A-horizon) 29 months after harvest was 9.7, 11.2, and 14.3 kg ha^{-1} for the removed, control, and double treatments respectively. These values represent 0.87, 0.93, and 1.06% respectively of the total soil N and are 5.7, 6.7, and 8.2 times greater than the extractable N measured before harvesting the pine stand. This increase in post-harvest levels of available N in the mineral soil (assart flush) has been previously reported for loblolly pine (Vitousek and Matson, 1985; Li *et al.*, 2003). The summed area under the curve for extractable N during the first 8 months of sampling (first growing season) showed levels of available N of 74, 82, and 112 kg ha^{-1} for the removed, control, and doubled treatments respectively. The most similar treatments in Vitousek and Matson's (1985) study produced approximately 80 kg ha^{-1} of available N during the first growing season after harvest. Considering the entire sampling period, there was a significant positive and consistent forest floor effect on soil available N with the doubled and control treatments producing 51 and 11 % more available N than the removed treatment, respectively.

It is important to highlight the dynamics observed during the sampling period. If treatment comparisons are done for an individual sampling date, the removed treatment showed significantly higher levels of available soil N that the other two treatments, in the second month after treatment (figure 5a). This treatment ranking was reversed on subsequent dates (forest floor x time interaction effect, p-value < 0.0001) (table 3).

The fact that the removed treatment showed higher levels of soil available N two months after treatment may be linked to the low levels of soil labile carbon, measured as dissolved organic carbon, at the same time. This may be an indication that soil microbes were limited by labile carbon caused by the harvest (general increase of soil available N in all treatments) and the effects that forest floor retention has on maintaining higher levels of soil labile carbon (The treatments with less or no forest floor retention had lower levels of soil labile carbon and higher levels of soil available nitrogen, figure 7). It is worth noting that this correlation was significant only when the plots that had the forest floor mixed with the mineral soil were considered, as this incorporation may increase the effect that forest floor retention has on increasing soil labile carbon pools.

Mineralizable N levels during the sampling period can also be related with the levels of labile carbon maintained by the different forest floor retention treatments. The removed treatment mineralized more N than the other two treatments during the sampling period (table 2, figure 8a) showing that the higher levels of labile carbon obtained with increased forest floor retention were more conducive to N immobilization by the soil microbes. Additionally, no significant effect of the incorporation treatment was observed on potential net mineralizable N (table 3), but when analyzed by sampling date, the non-mixed treatment showed higher mineralization in months 2 and 4 (figure 8b) resulting in a significant incorporation treatment x time interaction (table 3), and indicating possible carbon limitations by the soil microbes on these treatment, and increased immobilization in the mixed treatments, where the carbon sources from forest floor where in close contact with soil microbes.

All measures of available P were affected in a similar manner by the forest floor retention treatments (table 2, figures 9 through 14) suggesting a common extraction pool for all three methods. The amounts of P extracted by these methods were in the order: Oxalic acid extractable ≈ AEM extractable < Mehlich III extractable, in agreement with the assumption that a weak organic acid would extract available P within the range of that extracted by an active P sink such as an exchange membrane, and the latter subsequently would extract less available P than a stronger acid with chelating agents such as Mehlich III (Mallarino and Atia, 2005).

Conclusions

Increasing forest floor retention had a stronger impact than the incorporation treatment, which had a very transient effect lasting less than four months. Increasing forest floor retention resulted in higher levels of available C, N, and P (figures 2, 6, and 10) over the sampling period (29 months), and increased the magnitude and delayed the peak of the Assart flush. Removing the forest floor seemed to have exacerbated the labile C limitation of the soil microbes which resulted in an early but short-term spike in available N two months after treatment imposition. The negative correlation between labile C and N availability found at this sampling date seem to support this hypothesis.

References

Adams, M., Pennell, K., Campbell, R., 1989. Fine root distribution in a young loblolly pine (Pinus taeda L.) stand: Effects of preplant phosphorus fertilization. Plant Soil 113, 275-278.

Akaike, H., 1987. Factor analysis and AIC. Psychometrika 52, 317-332.

Albaugh, T.J., Allen, H.L., Fox, T.R., 2007. Historical patterns of forest fertilization in the Southeastern United States from 1969 to 2004. South. J. Appl. For. 31, 129-137.

Alden, L., Demoling, F., Baath, E., 2001. Rapid method for determining factors limiting bacterial growth in soil. Appl. Environ. Microb. 67, 1830-1838.

Anderson, J.P.E., Domsch, K.H., 1980. Quantities of plant nutrients in the microbial biomass of selected soils. Soil Sci. 130, 211-216.

Barg, A.K., Edmonds, R.L., 1999. Influence of partial cutting on site microclimate, soil nitrogen dynamics, and microbial biomass in Douglas-fir stands in western Washington. Can. J. For. Res. 29, 705-713.

Berg, B., Berg, M.P., Bottner, P., Box, E., Breymeyer, A., Deanta, R.C., Couteaux, M., Escudero, A., Gallardo, A., Kratz, W., Madeira, M., Malkonen, E., Mcclaugherty, C., Meentemeyer, V., Munoz, F., Piussi, P., Remacle, J., Desanto, A.V., 1993. Litter mass loss rates in pine forests of Europe and Eastern United-States - some relationships with climate and litter quality. Biogeochemistry 20, 127-159.

Binkley, D., 1984. Does forest removal increase rates of decomposition and nitrogen release? For. Ecol. Manage. 8, 229-233.

Bradley, R.L., 2001. An alternative explanation for the post-disturbance NO_3^- flush in some forest ecosystems. Ecol. Lett. 4, 412-416.
Bradley, R.L., Titus, B.D., Hogg, K., Preston, C., Prescott, C.E., Kimmins, J.P., 2000. Assessing the controls on soil mineral-N cycling rates in managed coastal western hemlock ecosystems of British Columbia. J. Sustain. For. 10, 213-219.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837-842.

Burger, J.A., Pritchett, W.L., 1984. Effects of clearfelling and site preparation on nitrogen mineralization in a southern pine stand. Soil Sci. Soc. Am. J. 48, 1432-1437.

Cross, A.F., Schlesinger, W.H., 1995. A literature review and evaluation of the. Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. Geoderma 64, 197-214.

De Santo, A.V., Berg, B., Rutigliano, F.A., Aleani, A., Fioretto, A., 1993. Factors regulating early stage decomposition of needle litters in five different coniferous forests. Soil Biol. Biochem. 25, 1423-1433.

du Toit, B., Dovey, S.B., Smith, C.W., 2008. Effects of Slash and Site Management Treatments on Soil Properties, Nutrition and Growth of a Eucalyptus grandis Plantation in South Africa. In: Nambiar, E.K.S. (Ed.), Site Management and Productivity in Tropical Plantation Forests. Center for International Forestry Research (CIFOR), Proceedings of Workshops in Piracicaba (Brazil) 2004 and Bogor (Indonesia) 2006, pp. 63-77.

Ekblad, A., Nordgren, A., 2002. Is growth of soil microorganisms in boreal forests limited by carbon or nitrogen availability? Plant Soil 242, 115-122.

Ford, V., Torbert, J., Burger, J., Miller, O., 1985. Comparative effects of four mycorrhizal fungi on loblolly pine seedlings growing in a greenhouse in a Piedmont soil. Plant Soil 83, 215-221.

Fox, T.R., Allen, H.L., Albaugh, T.J., Rubilar, R., Carlson, C.A., 2007. Tree nutrition and forest fertilization of pine plantations in the southern United States. South. J. Appl. For. 31, 5-11.

Grossman, R.B., Reinsch, T.G., 2002. The Solid Phase, Bulk Density and Linear Extensibility. In: Dane, J.H., Topp, G.C. (Eds.), Methods of Soil Analysis Part 4 - Physical Methods. Soil Science Society of America, Madison, WI, pp. 201-228.

Hart, S.C., Nason, G.E., Myrold, D.D., Perry, D.A., 1994a. Dynamics of gross nitrogen transformations in an old growth forest: the carbon connection. Ecology 75, 880-891.

Hart, S.C., Stark, J., M., Davidson, E.A., Firestone, M.K., 1994b. Nitrogen Mineralization, Immobilization, and Nitrification. In: Weaver, R.W., Angle, J.S., Bottomley, P.S. (Eds.), Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 985-1018.

Hedley, M.J., Stewart, J.W.B., Chauhan, B.S., 1982. Changes in inorganic and organic soil-phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46, 970-976.

Jorgensen, J.R., Wells, C.G., Metz, L.J., 1980. Nutrient changes in decomposing loblolly pine forest floor. Soil. Sci. Soc. Am. J. 44, 1307-1314.

Kim, C., Sharik, T.L., Jurgensen, M.F., 1995. Canopy cover effects on soil nitrogen mineralization in northern red oak *(Quercus rubra)* stands in northern lower Michigan. For. Ecol. Manage. 76, 21-28.

Kimmins, J.P., 1997. Biogeochemistry, Cycling of Nutrients in Ecosystems. In: kimmins, J.P. (Ed.), Forest Ecology, a Foundation for Sustainable Management. Prentice Hall, Saddle River, N.J., pp. 71-129.

Li, Q.C., Allen, H.L., Wilson, C.A., 2003. Nitrogen mineralization dynamics following the establishment of a loblolly pine plantation. Can. J. For. Res. 33, 364-374.

Likens, G.E., Bormann, F.H., Johnson, N.M., Fisher, D.W., Pierce, R.S., 1970. Effect of forest cutting and herbicides treatement on nutrient budgets in the Hubbard Brook watershed-ecosystem. Ecol. Monogr. 40, 23-47.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberber, O., 2006. SAS for Mixed Models. SAS Publishing.

Mallarino, A.P., Atia, A.M., 2005. Correlation of a resin membrane soil phosphorus test with corn yield and routine soil tests. Soil Sci. Soc. Am. J. 69, 266-272.

Markewitz, D., Richter, D.D., Allen, H.L., Urrego, J.B., 1998. Three decades of observed soil acidification in the Calhoun Experimental Forest: has acid rain made a difference? Soil Sci. Soc. Am. J. 62, 1428-1439.

Mendham, D.S., O'Connell, A.M., Grove, T.S., Rance, S.J., 2003. Residue management effects on soil carbon and nutrient contents and growth of second rotation eucalyptus. For. Ecol. Manage. 181, 357-372.

Myers, R.G., Sharpley, A.N., Thien, S.J., Pierzynski, G.M., 2005. Ion-sink phosphorus extraction methods applied on 24 soils from the continental USA. Soil Sci. Soc. Am. J. 69, 511-521.

Myers, R.G., Thien, S.J., Pierzynski, G.M., 1999. Using an ion sink to extract microbial phosphorus from soil. Soil Sci. Soc. Am. J. 63, 1229-1237.

Nambiar, E.K.S., Kallio, M.H., 2008. Increasing and Sustaining Productivity in Tropical Forest Plantations: Making a Difference through Cooperative Research and Partnerships. In: Nambiar, E.K.S. (Ed.), Site Management and Productivity in Tropical Plantation Forests: Workshop Proceedings, 22-26 November 2004 Piracicaba, Brazil, and 6-9 November, Bogor, Indonesia Center for International Forestry Research (CIFOR), Bogor, Indonesia., pp. 205-228.

Powers, R.F., Scott, D.A., Sanchez, F.G., Voldseth, R.A., Page-Dumroese, D., Elioff, J.D., Stone, D.M., 2005. The North American long-term soil productivity experiment: Findings from the first decade of research. For. Ecol. Manage. 220, 31-50.

Rojas, J.C., 2005. Factors Influencing Responses of Loblolly Pine Stands to Fertilization, Dissertation. Dep. Forestry. North Carolina State University, Raleigh, p. 147.

Sanchez, F.G., Carter, E.A., Klepac, J.F., 2000. Soil carbon and soil physical properties response to incorporating mulched forest slash. New Zeal. J. For. Sci. 30, 150-168.

Sanchez, F.G., Carter, E.A., Klepac, J.F., 2003. Enhancing the soil organic matter pool through biomass incorporation. Biomass Bioenerg. 24, 337-349.

Sanchez, F.G., Carter, E.A., Leggett, Z.H., 2009. Loblolly pine growth and soil nutrient stocks eight years after forest slash incorporation. For. Ecol. Manage. 257, 1413-1419.

Sanchez, F.G., Eaton, R.J., 2001. Sequestering carbon & improving soils - Benefits of mulching and incorporating forest slash. J. Forest. 99, 32-36.

Sariyildiz, T., Anderson, J.M., 2003. Interactions between litter quality, decomposition and soil fertility: a laboratory study. Soil Biol. Biochem. 35, 391-399.

SAS, 2005. The SAS System for Windows Release 9.1.3. SAS Institute Inc., Cary, NC.

Smethurst, P.J., Nambiar, E.K.S., 1989. Role of weeds in the management of nitrogen in a young *Pinus radiata* plantation. New Forest. 3, 203-224.

Smith, C.T., Lowe, A.T., Skinner, M.F., Beets, P.N., Schoenholtz, S.H., Fang, S.Z., 2000. Response of radiata pine forests to residue management and fertilisation across a fertility gradient in New Zealand. For. Ecol. Manage. 138, 203-223.

Smith, T.E., Osborne, D.O., Simpson, J.A., 2008. Inter-rotation Management Impacts on Growth and Soil Properties in Hybrid Pine Plantations on Sandy Soils in Subtropical Australia. In: Nambiar, E.K.S. (Ed.), Site Management and Productivity in Tropical Plantation Forests. Center for International Forestry Research (CIFOR), Proceedings of Workshops in Piracicaba (Brazil) 2004 and Bogor (Indonesia) 2006, pp. 93-106.

Tamm, C.O., 1964. Determination of nutrient requirements of forest stands. Int. Rev. For. Res. 1, 115-170.
Tew, D.T., Morris, L.A., Allen, H.L., Wells, C.G., 1986. Estimates of nutrient removal, displacement and loss resulting from harvest and site preparation of a Pinus taeda plantation in the Piedmont of North Carolina. For. Ecol. Manage. 15, 257-267.

Thomas, G.W., 1996. Soil pH and Soil Acidity. In: Sparks, D.L. (Ed.), Methods of Soil Analysis, Part 3, Chemical Methods. Soil Science Society of America, Madison, WI, pp. 475-490.

Titus, B.D., Roberts, B.A., Deering, K.W., 1997. Soil solution concentrations on three white birch sites in central Newfoundland following different harvesting intensities. Biomass Bioenerg. 13, 313-330.

Vitousek, P.M., Andariese, S.W., 1986. Microbial transformations of labelled nitrogen in a clearcut pine plantation. Oecologia 68, 601-605.

Vitousek, P.M., Andariese, S.W., Matson, P.A., Morris, L., Sanford, R.L., 1992. Effects of harvest intensity, site preparation, and herbicide use on soil-nitrogen transformations in a young loblolly-pine plantation. For. Ecol. Manage. 49, 277-292.

Vitousek, P.M., Matson, P.A., 1985. Disturbance, nitrogen availability, and nitrogen losses in an Intensively managed loblolly-pine plantation. Ecology 66, 1360-1376.

Zerpa, J.L., Allen, H.L., Campbell, R.G., Phelan, J., Duzan, H., 2010. Influence of variable organic matter retention on nutrient availability in a 10-year-old loblolly pine plantation. For. Ecol. Manage. 259, 1480-1489.

Table 1. Sampling and analyses schedule performed on the mineral soil during the first two years of a forest floor retention and incorporation study in the Coastal Plain of the Southeast, US.

	Dates								
Analyses and Measurement	Feb 06	Apr 06	May 06	Jun 06	Jul 06	Nov 06	Jun 07	Oct 07	Aug 08
	Collection time (months after treatment)								
	-1	1	2	3	4	8	15	19	29
pH	●	●					●		
Total C	●	●					●		●
Microbial biomass C	●	●		●	●	●	●	●	
Dissolved organic C	●	●		●	●	●	●	●	
Total N	●	●					●		●
2 M KCl extractable N	●	●	●	●	●	●	●	●	●
Potential net mineralizable N	●	●	●	●	●	●	●	●	
Mehlich III extractable P	●	●			●		●		●
AEM* extractable P		●	●	●	●	●	●		
3mM Oxalic acid extractable P	●	●	●	●	●				

* AEM = Anionic exchange membranes

Table 2. Treatment means for mineral soil variables measured during the first two years of a forest floor retention and incorporation study in the Coastal Plain of the Southeast, US. Orthogonal contrasts comparing the retention and incorporation treatments against control and non-mixed respectively, were derived from the repeated measures analysis.

Treatment factors >	Forest floor Retention					Forest floor incorporation with mineral soil		
Treatment levels >	Removed		Control	Doubled		Non-Mixed	Mixed	
Variable	Mean	P-value contrast vs. Control treatment	Mean	Mean	P-value contrast vs. Control treatment	Mean	Mean	P-value contrast vs. Non-Mixed treatment
Total C (Mg ha^{-1})	22.8	0.12	24.8	27.4	**<0.05**	24.4	25.6	0.26
MBC (kg ha^{-1})	336	**<0.05**	388	451	**<0.05**	382	401	0.32
DOC (kg ha^{-1})	58.4	**<0.05**	69.0	85.1	**<0.01**	67.3	74.3	0.06
Total N (Mg ha^{-1})	1.12	0.13	1.21	1.35	**<0.05**	1.21	1.25	0.39
Ext. N (kg ha^{-1})	9.71	0.08	11.2	14.3	**<0.01**	11.6	11.8	0.81
Pot.Min. N (kg ha^{-1})	3.62	0.12	2.20	1.28	0.31	2.95	1.79	0.12
C:N	20.5	0.82	20.6	20.3	0.64	20.4	20.6	0.61
Mehlich P (kg ha^{-1})	9.46	0.30	11.7	16.1	0.05	11.4	13.4	0.26
AEM P (kg ha^{-1})	4.82	0.64	5.22	7.17	**<0.05**	5.00	6.47	**<0.05**
Oxalic P (kg ha^{-1})	1.89	0.47	2.31	2.80	0.39	1.96	2.71	0.12

MBC = Microbial biomass carbon; DOC = Dissolved organic carbon; Ext. N = 2M KCl extractable N; Pot.Min. N = Potential net mineralizable N; Mehlich P = Mehlich III extractable P; AEM P = Anionic exchange membranes extractable P; Oxalic P = 3mM Oxalic acid extractable P.

Table 3. P-values from repeated measures analyses on the mineral soil variables measured during the first two years of a forest floor retention (FF) and incorporation (Incorp.) study in the Coastal Plain of the Southeast, US.

Dependent Variable	Effect						
	FF	Incorp.	Time	FF*Incorp.	FF*Time	Incorp.*Time	FF*Incorp.*Time
pH	0.87	0.79	**<0.0001**	0.30	0.99	0.64	0.08
Total C content (kg ha^{-1})	**<0.01**	0.26	**<0.01**	0.37	0.33	**<0.0001**	0.17
Dissolved organic C (kg ha^{-1})	**<0.0001**	0.06	**<0.0001**	**<0.05**	**<0.05**	0.12	0.25
Microbial biomass C (kg ha^{-1})	**<0.001**	0.32	**<0.0001**	**<0.05**	0.30	**<0.01**	0.53
Total N content (kg ha^{-1})	**<0.01**	0.39	**<0.001**	0.32	0.17	**<0.0001**	0.28
2M KCl extractable N (kg ha^{-1})	**<0.0001**	0.81	**<0.0001**	0.22	**<0.0001**	**<0.0001**	**<0.05**
Potential net mineralizable N (kg ha^{-1})	**<0.05**	0.12	**<0.0001**	0.90	0.25	**<0.01**	0.92
C:N	0.90	0.61	**<0.0001**	0.76	0.38	**<0.01**	0.88
Mehlich III extractable P (kg ha^{-1})	**<0.05**	0.26	**<0.0001**	0.85	0.29	**<0.001**	**<0.05**
Anionic exchange membrane extractable P (kg ha^{-1})	**<0.05**	**<0.05**	**<0.0001**	0.14	0.21	**<0.05**	0.67
3mM Oxalic acid extractable P (kg ha^{-1})	0.29	0.12	**<0.0001**	0.98	0.15	**<0.01**	0.87

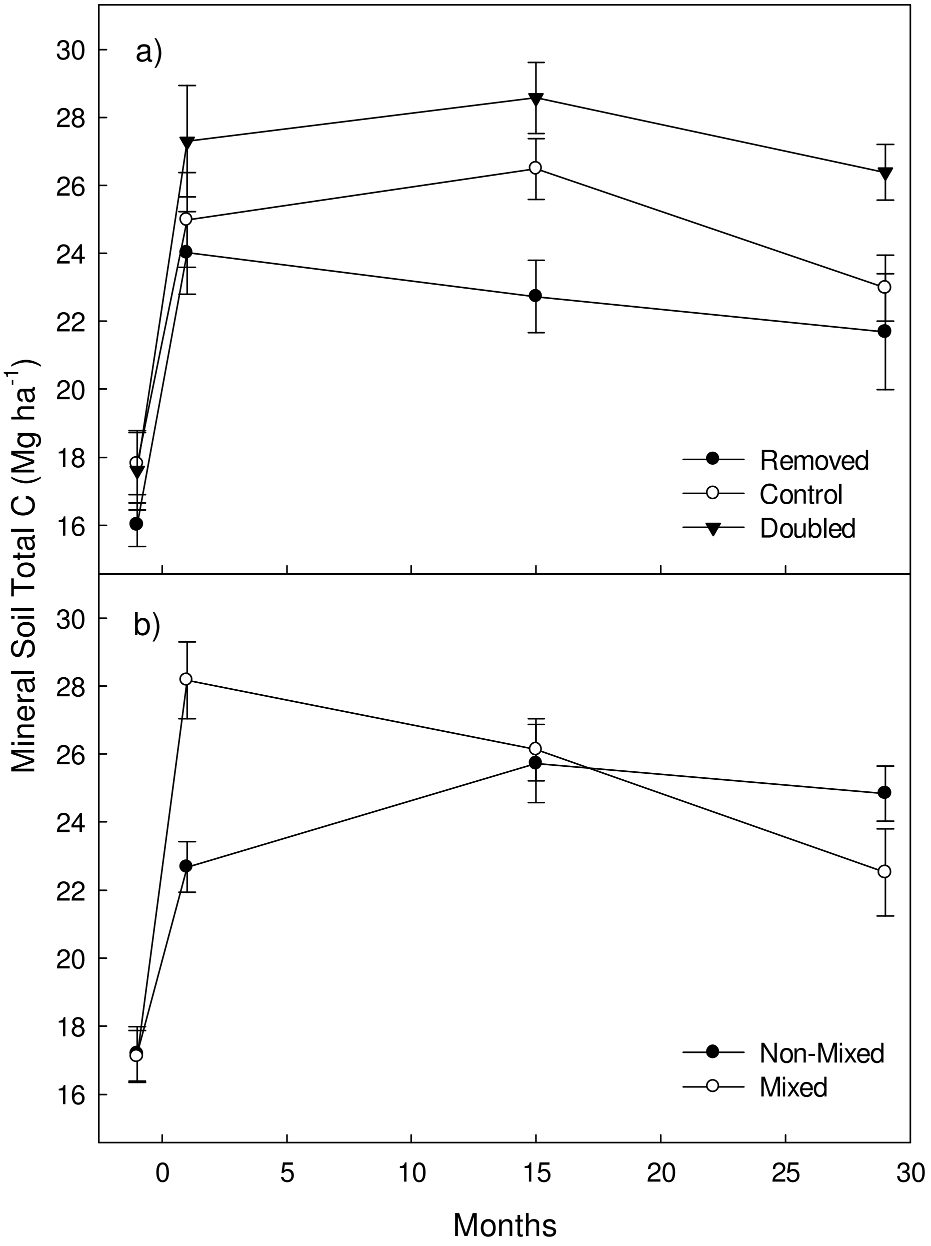

Figure 1. Mineral soil (A-horizon) total carbon content 29 months after treatment. Month -1 indicates the pre-harvest assessment. The forest floor retention treatments a) Removed = 0 kg C ha^{-1}, Control = 7,974 kg C ha^{-1}, and Doubled = 15,121 kg C ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

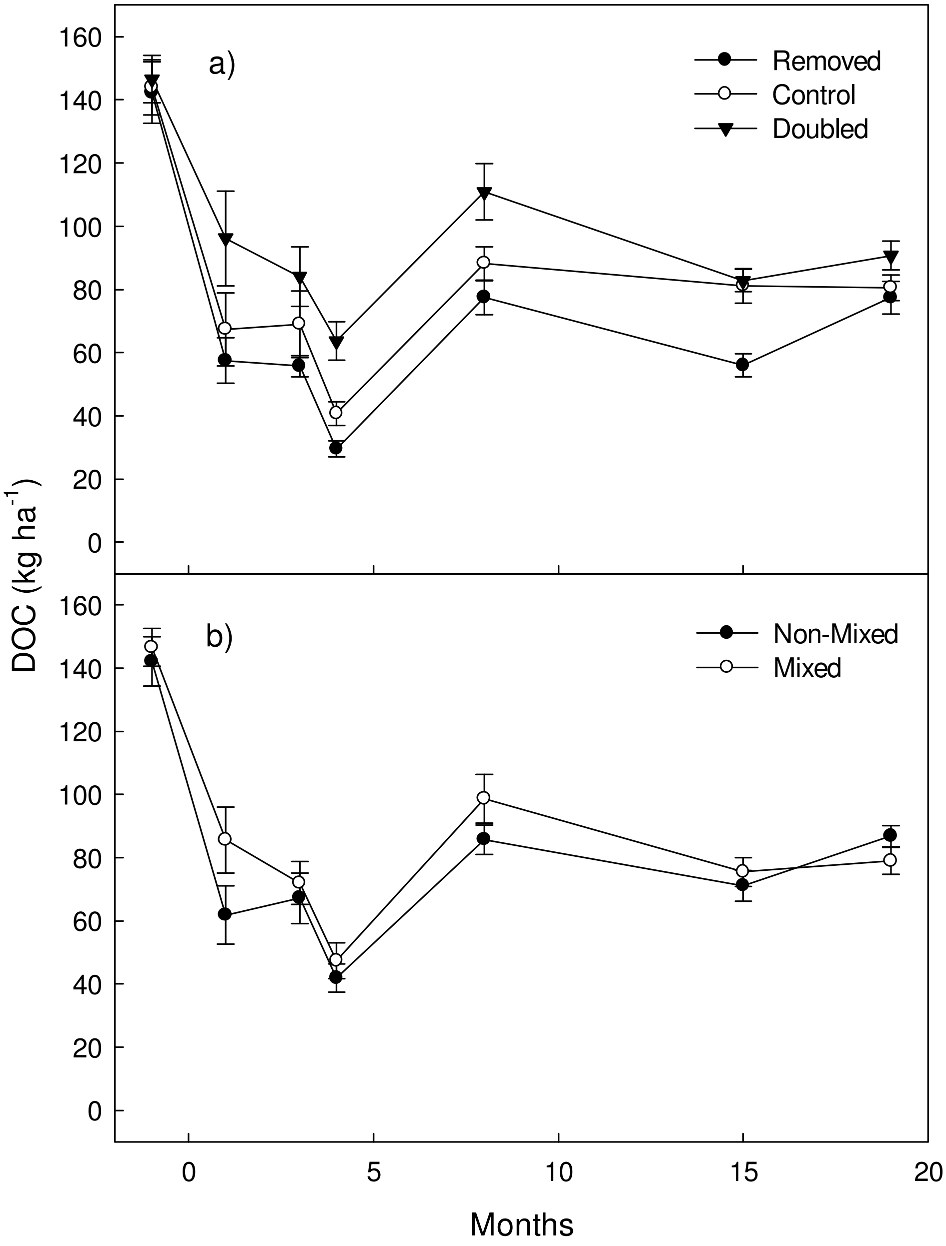

Figure 2. Dissolved organic carbon from the mineral soil (A-horizon) 19 months after treatment. Month -1 indicates the pre-harvest assessment. The forest floor retention treatments a) Doubled = 31,700 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Removed = 0 kg ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

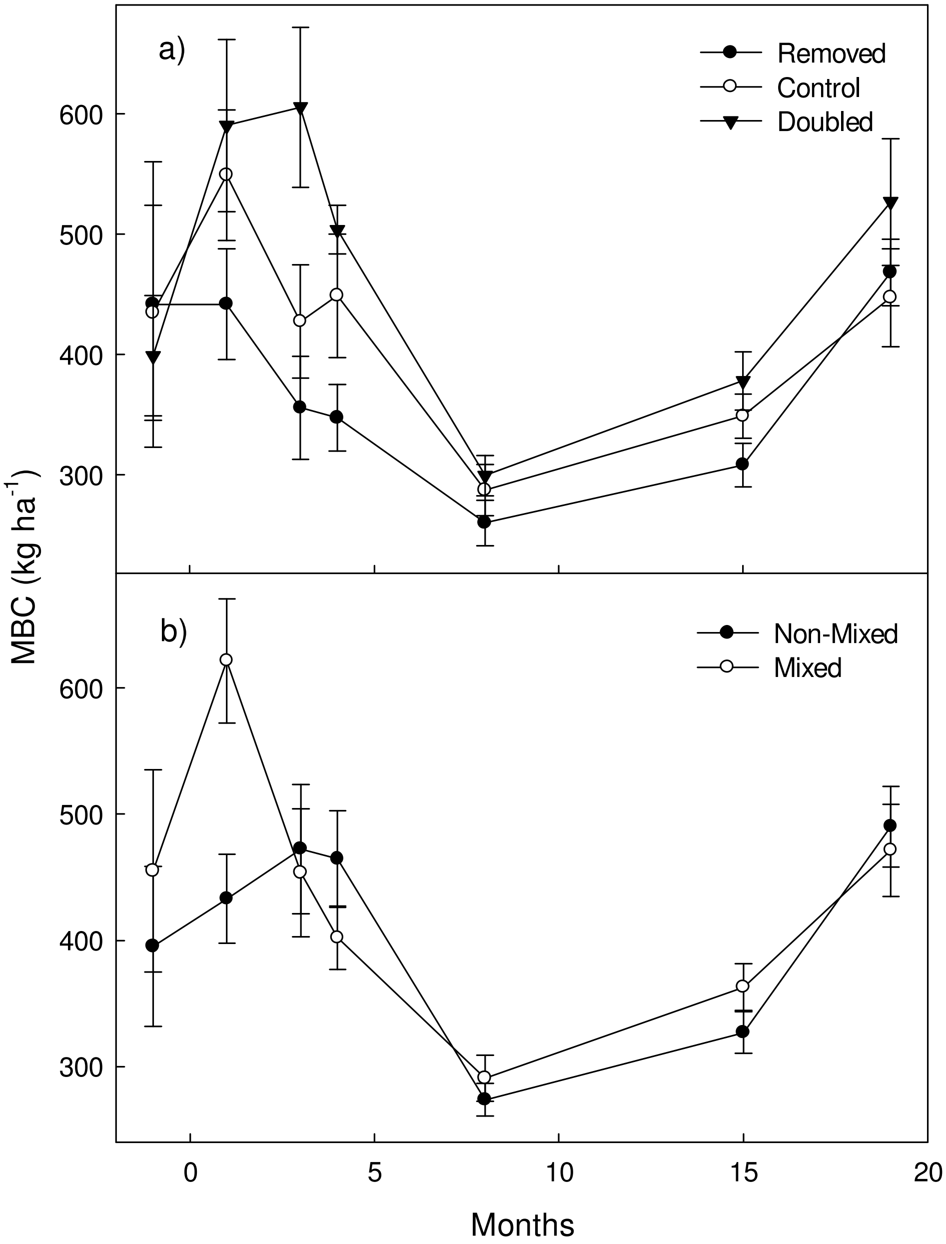

Figure 3. Microbial biomass carbon from the mineral soil 19 months after treatment. Month -1 indicates the pre-harvest assessment. The forest floor retention treatments a) Doubled = 31,700 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Removed = 0 kg ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

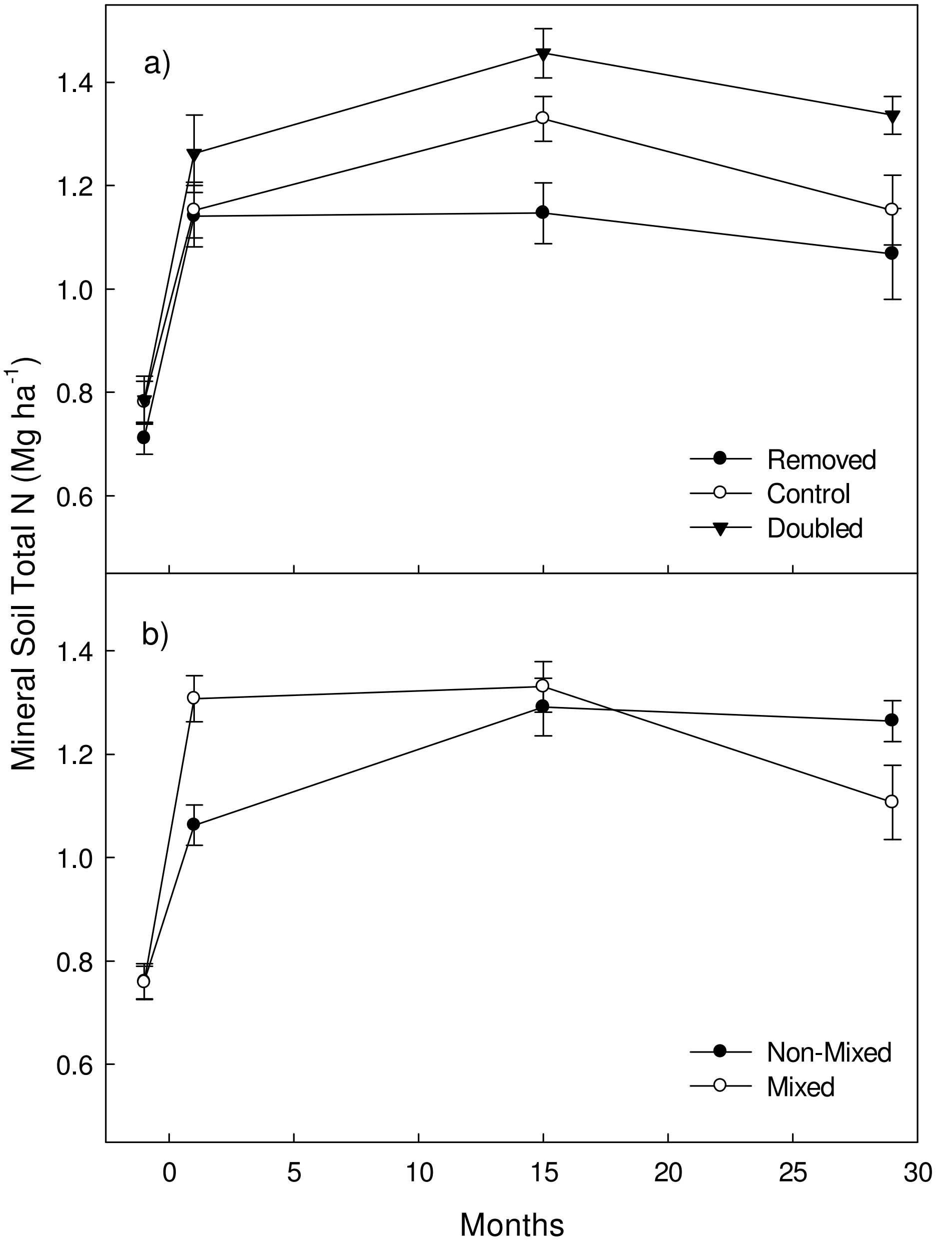

Figure 4. Mineral soil (A-horizon) total nitrogen content 29 months after treatment. Month -1 indicates the pre-harvest assessment. The forest floor retention treatments a) Removed = 0 kg N ha^{-1}, Control = 160 kg N ha^{-1}, and Doubled = 312 kg N ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

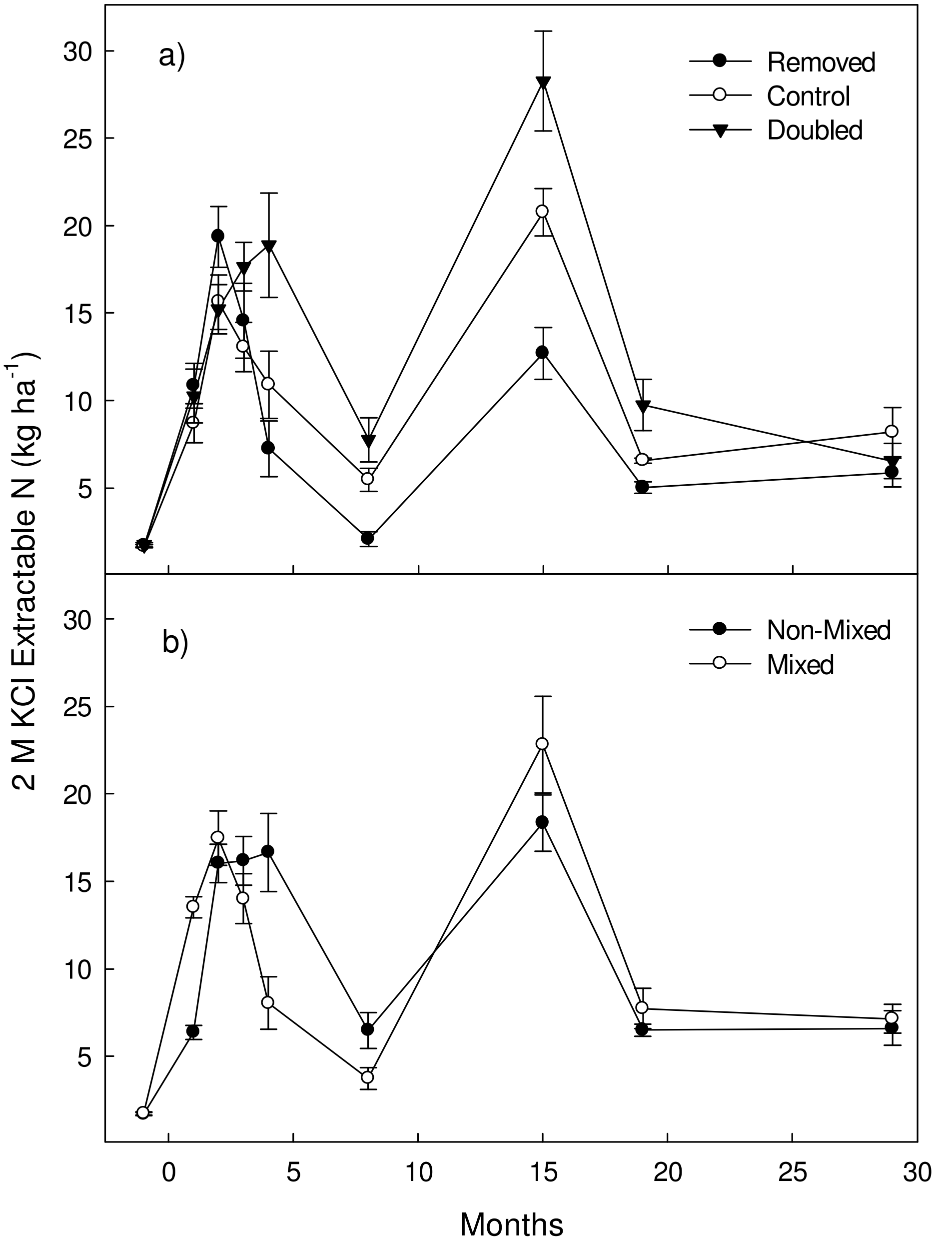

Figure 5. Mineral soil (A-horizon) extractable nitrogen pool 29 months after treatment. Month -1 indicates the pre-harvest assessment. The forest floor retention treatments a) Doubled = 31,700 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Removed = 0 kg ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

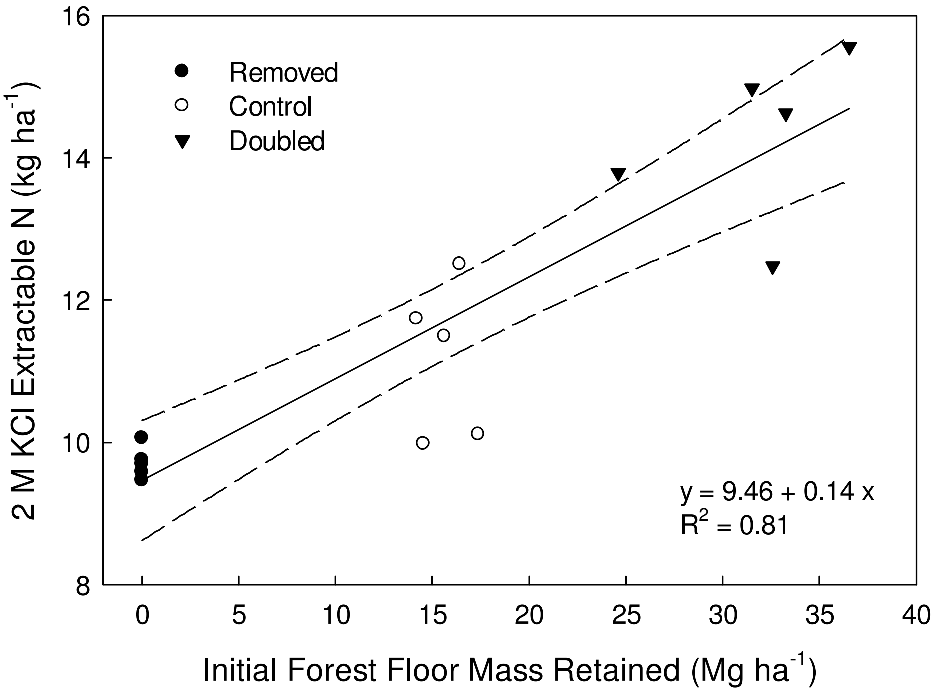

Figure 6. Average extractable N measured in the mineral soil (A-horizon) during 29 months, as a function of initial forest floor mass retained. The forest floor retention treatments: Doubled = 31,700 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Removed = 0 kg ha^{-1} were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US.

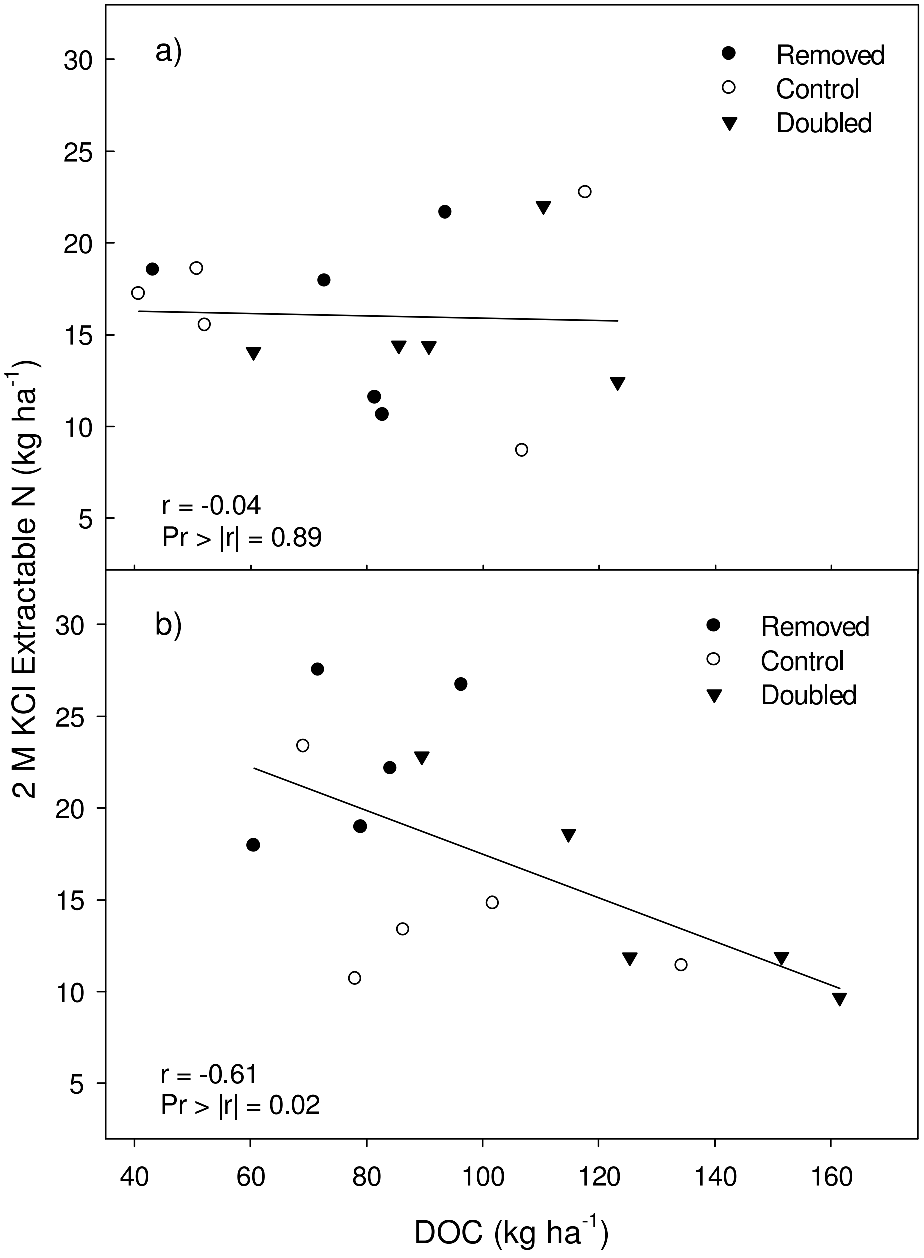

Figure 7. Relationship between extractable N and dissolved organic C from the mineral soil (A-horizon) in a) Non-Mixed plots, and b) Mixed plots, two months after treatment. The forest floor retention treatments: Doubled = 31,700 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Removed = 0 kg ha^{-1} and the incorporation treatments: Mixed, and Non-Mixed were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US.

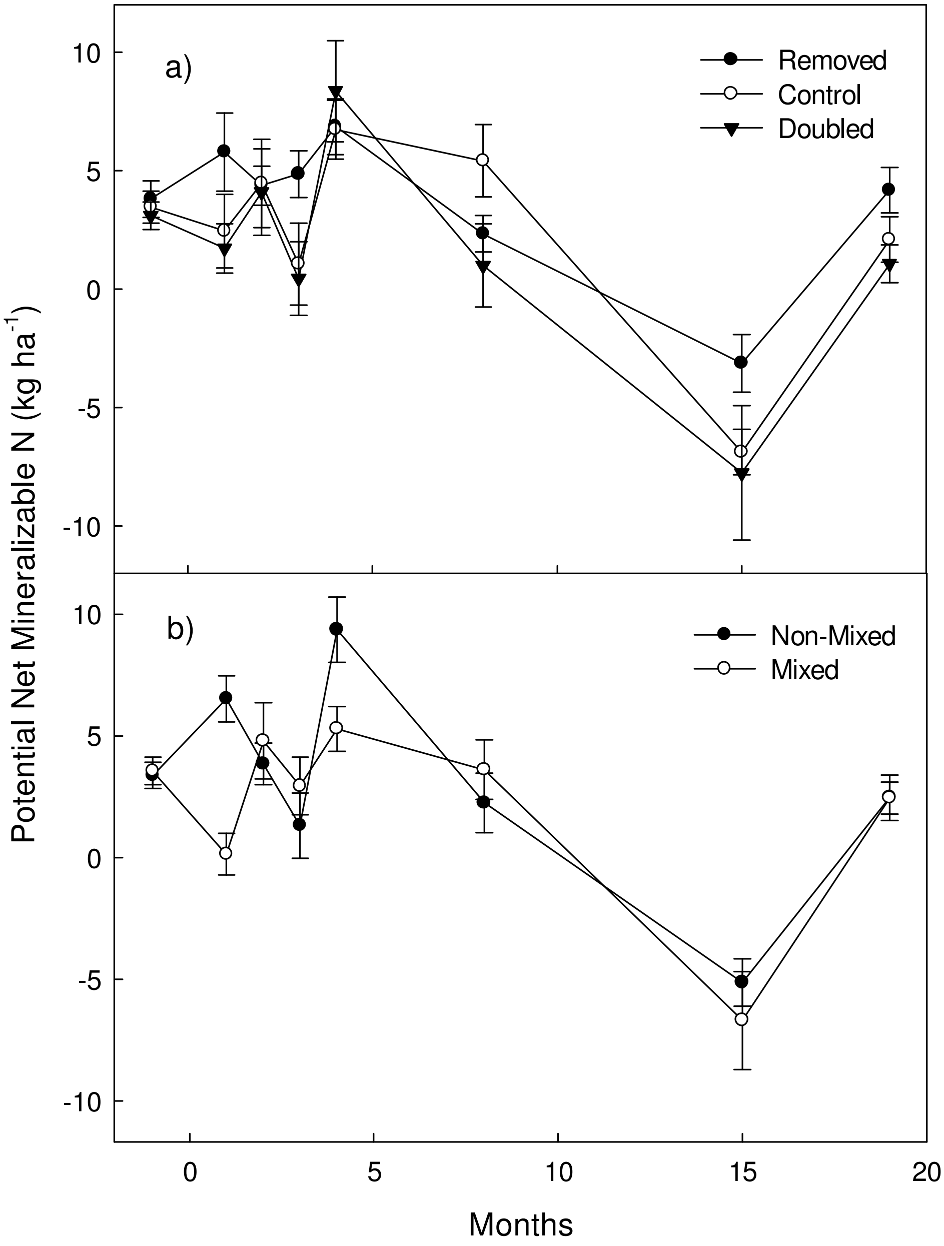

Figure 8. Mineral soil (A-horizon) potential net mineralizable nitrogen 19 months after treatment. Month -1 indicates the pre-harvest assessment. The forest floor retention treatments a) Doubled = 31,700 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Removed = 0 kg ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

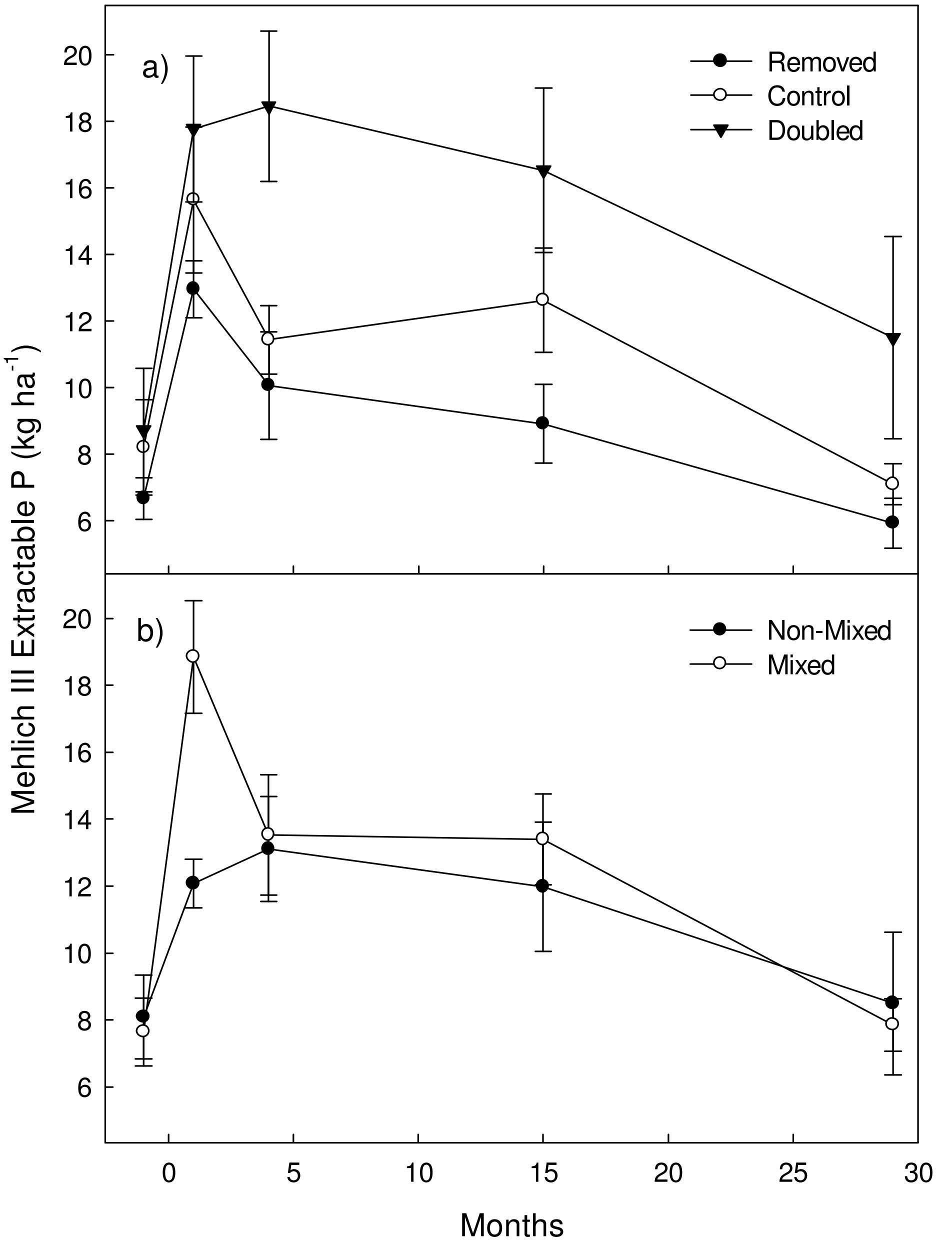

Figure 9. Mineral soil (A-horizon) Mehlich III extractable phosphorus 29 months after treatment. Month -1 indicates the pre-harvest assessment. The forest floor retention treatments a) Doubled = 31,700 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Removed = 0 kg ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

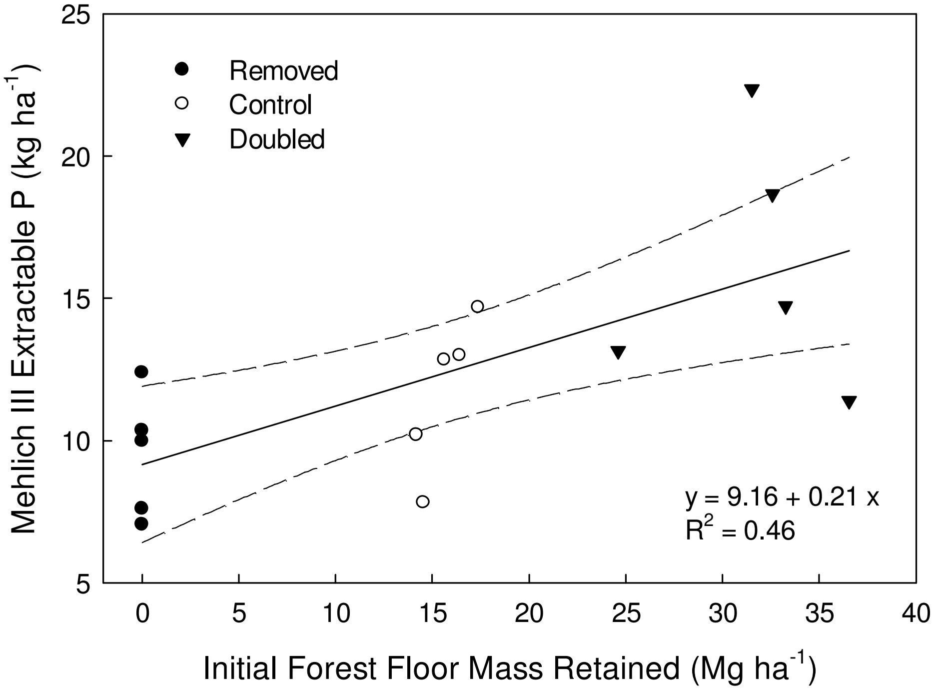

Figure 10. Average Mehlich III extractable P measured in the mineral soil (A-horizon) during 29 months, as a function of initial forest floor mass retained. The forest floor retention treatments: Doubled = 31,700 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Removed = 0 kg ha^{-1} were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US.

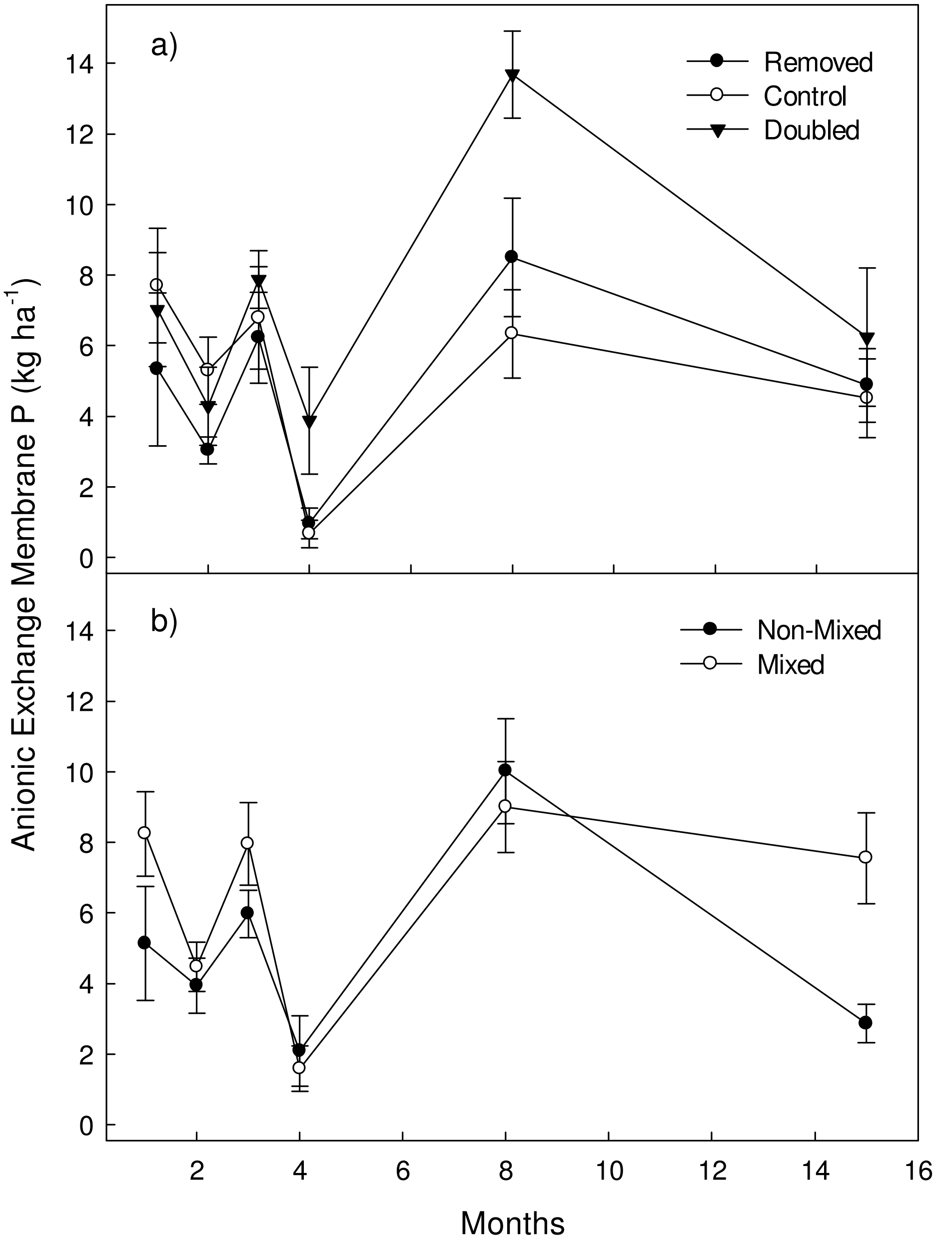

Figure 11. Exchange membrane extractable phosphorus from the mineral soil (A-horizon) 15 months after treatment. The forest floor retention treatments a) Doubled = 31,700 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Removed = 0 kg ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

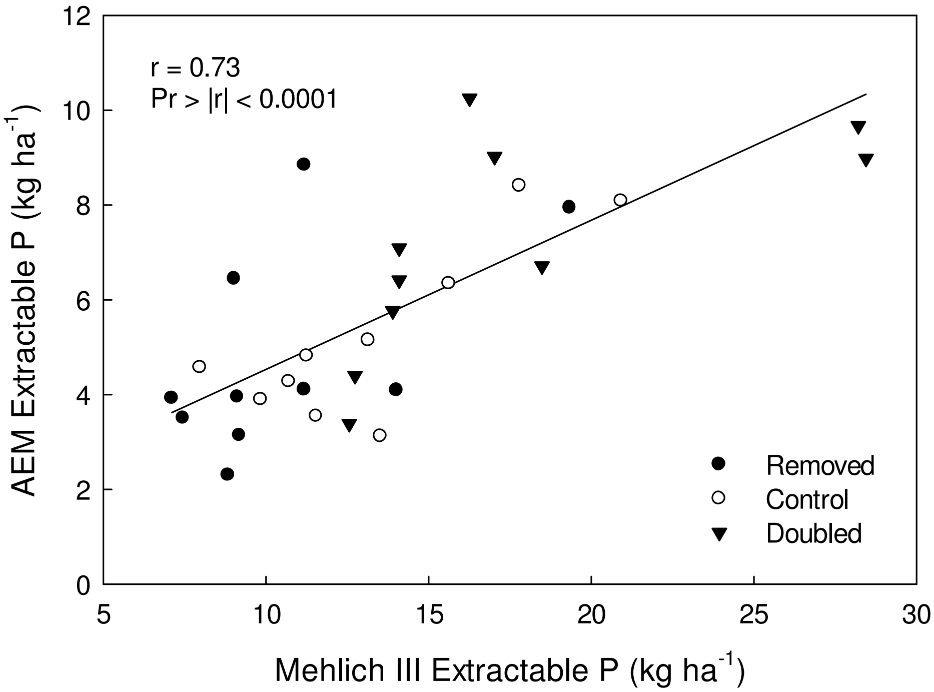

Figure 12. Relationship between two methods to obtain extractable phosphorous from the mineral soil: Anionic exchange membrane (AEM) extractable P vs. Mehlich III extractable P. Plot means measured during the first fifteen months of a forest floor retention and incorporation study in a loblolly pine stand in the Southeast, US.

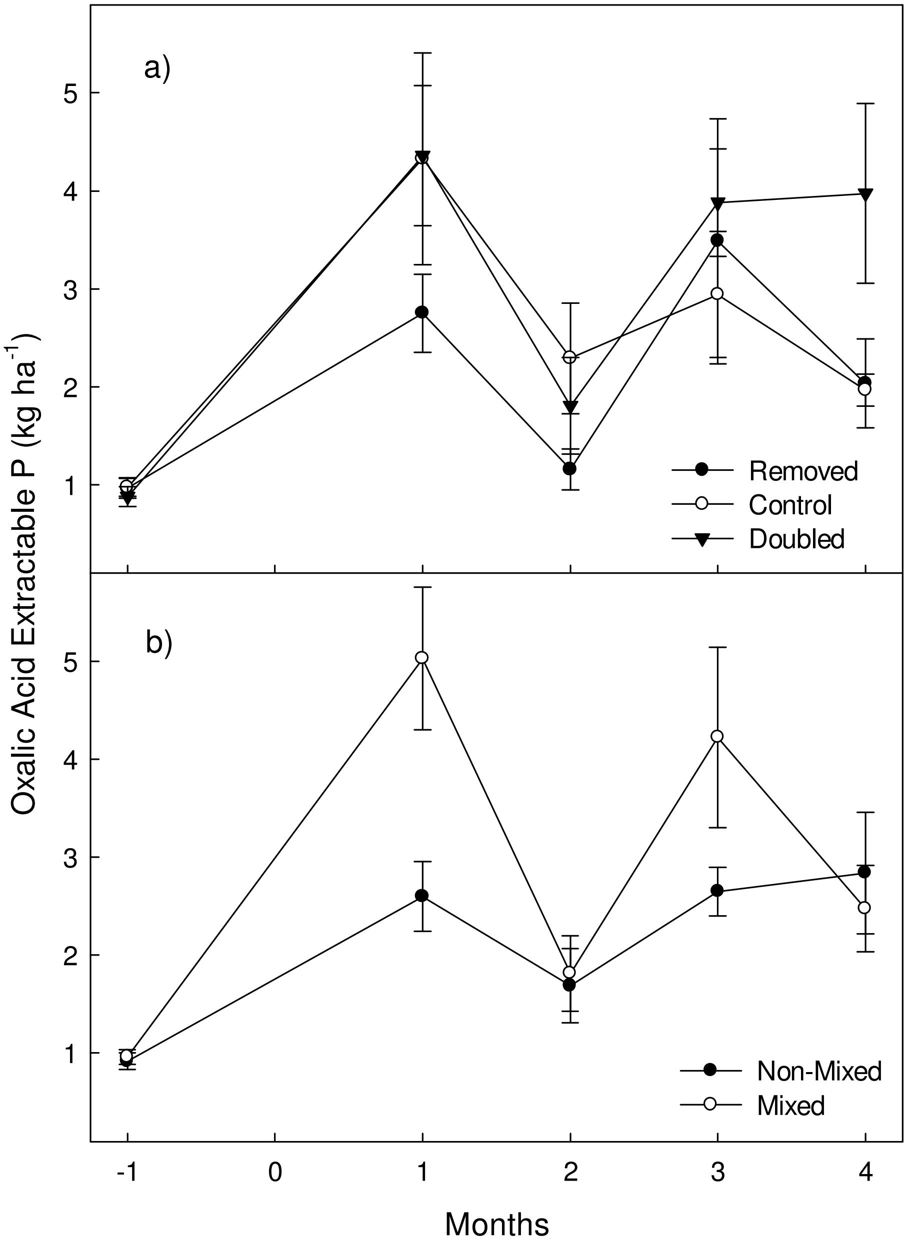

Figure 13. Oxalic acid extractable phosphorus from the mineral soil (A-horizon) 4 months after treatment. Month -1 indicates the pre-harvest assessment. The forest floor retention treatments a) Doubled = 31,700 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Removed = 0 kg ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed after harvesting the previous stand in a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

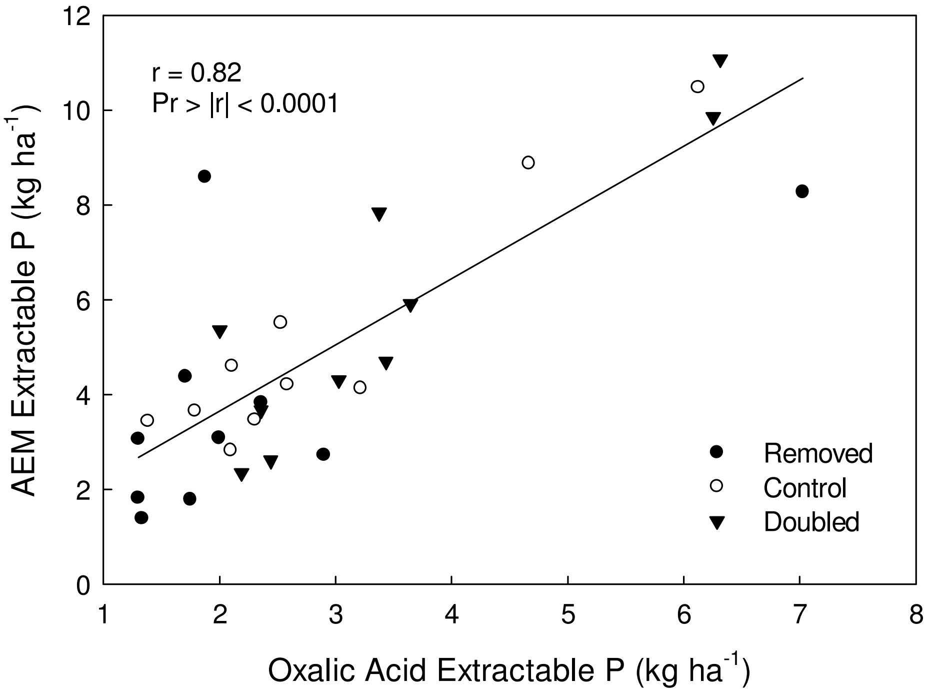

Figure 14. Relationship between two methods to obtain extractable phosphorous from the mineral soil: Anionic exchange membrane (AEM) extractable P vs. 3mM Oxalic acid extractable P. Plot means measured during the first four months of a forest floor retention and incorporation study in a loblolly pine stand in the Southeast, US.

CHAPTER 3

Effects of post-harvest forest floor retention and incorporation on tree growth and foliar nutrition in a recently established loblolly pine plantation

Abstract

Tree growth and foliar nutrition were examined in a loblolly pine stand on the Coastal Plain of North Carolina following imposition of several forest floor treatments, including three retention levels (0, 15, and 30 Mg ha^{-1}) combined with two levels of incorporation (mixed, non-mixed). Our objective was to use tree growth and foliar nutrition as indicators of changes caused by the imposed treatments. Tree growth was not affected by forest floor retention treatments, but it was affected by the incorporation treatment, showing 17 % more volume growth in the mixed treatment by year 3. In general, foliar N, P, Ca, Mg, Mn, Cu, and Zn concentrations increased by year 1 as compared to initial levels, but decreased to initial levels by year 3. Foliar K showed a consistent increase, and foliar B a consistent decrease through the sampling period. Foliar N, P, Cu, and Zn concentrations were significantly lower in the removed treatment as compared to the control and doubled treatments at year 1. No significant differences in foliar nutrition were observed between the control and the doubled treatments during the sampling period. At this early stage of stand development, the positive effects on nutrient availability caused by increasing forest floor retention were only observed, transiently, in foliar nutrition. The positive effect of the incorporation treatment on tree growth was likely due to improved physical conditions in the rooting environment.

Introduction

The importance of organic matter management in sustaining forest productivity has been highlighted in many studies including the North American Long Term Site Productivity studies (LTSP) (Powers *et al.*, 2005), the network of studies coordinated by the Center for International Forestry Research (CIFOR) (Nambiar and Kallio, 2008), and several others (Binkley, 1984; Smith *et al.*, 2000; Zerpa *et al.*, 2010). In general, the influence of organic matter removals or additions on stand productivity largely depends on the intensity and frequency of these manipulations and the initial size of the nutrient pools. In loblolly pine (*Pinus taeda* L.) plantations of the Southeast US, nitrogen (N) and phosphorus (P) fertilizer are used to increase wood production (Albaugh *et al.*, 2007), which is realized, in part, by increased foliage production. Through litterfall, this foliage accumulates in the forest floor forming a significant nutrient pool (Tew *et al.*, 1986; Markewitz *et al.*, 1998). Increases in forest floor mass and N content of over 100% are possible following N + P fertilization in highly responsive pine stands in the Southeast US. (Rojas, 2005) These accumulations highlight the importance of the forest floor in the nutrition of current and subsequent stands as nutrients become available through decomposition and mineralization processes (Jorgensen *et al.*, 1980).

In many studies, organic matter has been manipulated through harvesting and/or site preparation treatments in an effort to impose different levels of removal. These studies have provided important information, but are not particularly relevant to current pine plantation management where practices such as strip shearing, bedding, and vegetation control with herbicides retain and/or incorporate rather than remove organic matter. Incorporation of forest floor and slash with mineral soil through disking or bedding can increase productivity by improving physical properties and nutrient availability (Sanchez and Eaton, 2001). Evidence of accelerated organic matter decomposition and increased soil C and N concentrations with mixing has been found by Sanchez et al., (2000). Unfortunately, it has been difficult to isolate the effects of organic matter retention and mixing in previous studies because they have lacked the full factorial combination of treatments (Sanchez *et al.*, 2003) or the removal have been mechanized and therefore have included soil disturbance (Li *et al.*, 2003).

The effects of these manipulations on tree growth and foliar nutrition have been variable. Several studies have shown small positive effects of increased organic matter retention on tree growth (Smith *et al.*, 2000; Mendham *et al.*, 2003) particularly on low fertility sites. Several studies have shown only foliar nutrition effects (du Toit *et al.*, 2008; Hardiyanto and Wicaksono, 2008), and these effects seem to depend on the nutrient and the time of the assessment, and others have shown a significant effect on growth, but no effect on foliar nutrition (Zerpa *et al.*, 2010).

Our objectives were to determine the effects of different levels of forest floor retention, and its incorporation into mineral soil, on tree growth and foliar nutrient concentrations during the first three years of a second rotation loblolly pine stand in the Southeast US. We were interested in relating changes in growth and foliar nutrition with the observed changes in soil nutrient pools reported in chapter 2.

Materials and Methods

Site and Study Description

The study was established on Weyerhaeuser Company land in 2006 on the lower coastal plain in Pamlico County, North Carolina (35°6’2.00”N, 76°52’45.19”W) prior to harvesting a 33-yr old loblolly pine plantation. Ten-year (1998-2007) mean annual temperature is 17.5 °C with mean monthly temperatures ranging from 7.7 °C in January to 26.3 °C in July. Mean annual precipitation is 1,439 mm with a fairly uniform distribution throughout the year. January is the driest month with 77 mm, and August is the wettest month with 195 mm. The soil on this site is a fine, mixed, subactive, thermic Aquic Hapludult of the Craven soil series with fair to good surface drainage. The A-horizon is a fine sandy loam with an average thickness of 10 cm, bulk density of 1.18 g•cm^{-3}, total C and N contents of 17.2 and 0.76 Mg•ha^{-1} respectively, and MehlichIII extractable P content of 7.8 kg•ha^{-1}. The harvested stand had received cumulative fertilizer additions of 670 kg N•ha^{-1} and 165 kg P•ha^{-1}, and had been commercial thinned at 15 and 25 years. The stand exhibited a site index of 24 m (25 years base age) and a density of 250-300 stems•ha^{-1}. The forest floor had accumulated an ash-free mass of 15.6 Mg•ha^{-1} and contained 8 Mg C•ha^{-1}, 160 kg N•ha^{-1}, and 8.7 kg P•ha^{-1}. Logging was conducted with a boom-top excavator and trees were felled using the previous thinning roads to prevent disturbance of the forest floor and trafficking on the study plots. Immediately following harvest, a complete randomized block study with 5 replications and 6- forest floor/mixing treatments was imposed on the site. The treatment design was a 3x2 factorial with 3 levels of forest floor retention (removed, control, doubled), and 2 levels of forest floor incorporation with the surface mineral soil (mixed and non-mixed). The forest floor treatments were imposed in mid March 2006. Forest floor was raked from the removed plots and transported using tarps to the double plots where it was evenly distributed throughout the plots. Control plots were left with the original forest floor in place. The mixing treatments were imposed in early April 2006 using a small tractor pulling a three disk tiller on the first pass and a one-row disk tiller on the second and third pass to mix the forest floor with the mineral soil down to 10 cm.

The treatment plots were 16.8 x 9.1 m including treated buffers. Measurement plots were 12.2 x 4.9 m. Two weeks after the incorporation treatments were completed, 96-full sibs pine seedlings were planted per plot at 1.5m x 1.2m spacing for a total of 32 pines seedlings per measurement plot. The pine seedlings were treated with permethrin (Pounce™) pesticide prior to planting to prevent damage by pales weevil, *Hylobius pales* (Herbst), and non pine vegetation was controlled annually with glyphosate.

Tree measurements and foliar sampling and analysis

Groundline diameter and total height were measured on May ’06 (one month after planting), and then on February ’07 and December ’08. Diameter^2 * height was used as a volumetric index to compare treatments.

Foliar samples were collected in April ’06 and then again in February ‘07 and December ‘08. The first collection, done at time of planting, was a composited foliage sample from 30 randomly chosen seedlings. For subsequent collections the foliage was obtained from the first flush produced during the current growing season from the upper third of the live crown of 5 dominant or co-dominant trees in each plot. Twenty fascicles from each selected tree were collected for a total of 100 fascicles per plot. The samples were dried at 70°C, ground to pass through a 1mm mesh sieve, and analyzed for C and N concentration using a CHN elemental analyzer (CE Instruments-NC 2100, CE Elatech Inc., Lakewood, NJ). Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), boron (B), copper (Cu), and zinc (Zn) concentrations were determined through dry ash digestion of 0.5 g of ground, oven-dry foliage with hydrochloric acid (Jones and Steyn, 1973) followed by analysis using an inductively coupled plasma atomic emission spectrometer (IPS-AES, Varian ICP, Liberty Series 2, Varian analytical instruments, Walnut Creek, CA).

Mineral soil total N content, 2M KCl extractable N content, and extractable Mehlich-III P, previously reported on chapter 2, were used in correlation analysis with the growth and foliar nutrients, and as possible explanatory variables for changes in foliar N and P concentrations measured 1 year after treatment imposition.

Data analysis

Analyses of variance were performed on foliar nutrient concentrations and tree measurement by sampling date, and orthogonal contrasts were used for treatment means comparison. Repeated measures analyses were conducted using PROC MIXED (SAS, 2005) to determine the forest floor retention and incorporation treatments, time, and interaction effects on tree measurements and foliar nutrient concentrations. The null model likelihood ratio test was used to determine the need to specify a covariance structure to model the data. An unstructured covariance was specified, when needed, based on the Akaike's (1987) information criterion (AIC), which assessed the goodness of fit of the predicted covariance matrix to the observed matrix.

The mixed model used was:

$$Y_{ijkm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + A_m + \varepsilon_{ijkm}$$

Where Y_{ijkm} = the response to treatment (i) and (j) in block (m) at time (k), μ = the overall mean, α_i = the fixed forest floor retention treatment effect (i=1,2,3), β_j = the fixed incorporation treatment effect (j=1,2), $(\alpha\beta)_{ij}$ = the fixed interaction effect of the forest floor retention treatment (i) with the incorporation treatment (j), γ_k = the fixed effect of time (k), $(\alpha\gamma)_{ik}$ = the fixed interaction effect of forest floor retention treatment (i) with time(k), $(\beta\gamma)_{jk}$ = the fixed interaction effect of incorporation treatment (j) with time (k), $(\alpha\beta\gamma)_{ijk}$ = the three-way fixed interaction effect of forest floor retention treatment (i) with incorporation treatment (j) with time (k), A_m = the random block effect (m=1,2,3,4,5), and ε_{ijkm} = the random residual error.

The general linear model (SAS, 2005) was used to model foliar N and P concentrations as a function of mineral soil total N content, 2M KCl extractable N content, and extractable Mehlich-III P measured 1 year after treatment imposition. Relationships among these variables were examined, within incorporation treatment and also for all plots combined, using Pearson correlation coefficients through PROC CORR (SAS, 2005). Significance was accepted at $p \leq 0.05$ for all analysis.

Results

Tree height, diameter, and volume growth were not affected by forest floor retention treatments (tables 1 and 2), however, diameter and volume growth increased, 8% ($p<0.05$) and 17% ($p=0.06$), respectively, in the mixed treatment at year 3 (table 1). Tree growth also showed increased divergence between the two incorporation treatments with time, p-value of incorporation x time interaction = 0.0528 (table 2).

Foliar concentrations at time of planting were as follows: C = 53.6 %; N = 1.05 %; P = 1.34 g kg^{-1}; K = 5.31 g kg^{-1}; Ca = 2.47 g kg^{-1}; Mg = 0.89 g kg^{-1}; Mn = 173 mg kg^{-1}; B = 53.5 mg kg^{-1}; Cu = 2.33 mg kg^{-1}; and Zn = 33 mg kg^{-1}. Significant forest floor retention, incorporation, time, and/or time x treatment interaction effects were observed for several nutrients, particularly N, P, and Cu (tables 2 and 3). From the time of planting to one year later, the average increase in foliar concentrations for these nutrients in the control and the doubled treatment, was 68%, 21%, and 48% respectively, while the increase for the removed treatment was significantly less or non-existent. Foliar concentrations of all nutrients decreased to initial levels by year 3 with the exceptions of K, which, regardless of treatment, showed a consistent average increase from 5.31 to 6.42 g kg^{-1}, and B, which had a consistent average decrease from 53.5 to 10.9 mg kg^{-1} through the sampling period (table 3). The forest floor retention treatment ranking for foliar Mg changed from year 1 to year 3 resulting in significant interactions with time (table 2). Foliar Mg in the removed treatment, at year 3, was 0.83 g kg^{-1}, 11% less than the control treatment. No significant differences in foliar nutrition were observed between the control and the doubled treatments during the sampling period (table 3).

Correlation were examined within incorporation treatment, and also for all plots combined, given the significant forest floor retention x incorporation treatment interaction found in some of the variables (table 2). With the exception of Cu in the mixed treatment, no significant correlations were observed among tree growth and foliar nutrient concentrations (table 4). Foliar N and P were positively and significantly correlated with each other and with foliar Ca, Cu, and Zn, and negatively correlated with foliar Mg. Foliar N was also correlated with foliar K. There were positive significant correlations between both foliar N and foliar P and mineral soil total and available N pools. Foliar P was positively and significantly correlated with Mehlich III extractable P. In general these correlations between foliar concentrations and mineral soil nutrients were stronger, or occurred exclusively, in the non-mixed treatment (table 4).

The linear response in foliar N across the forest floor retention treatments, at year 1, was modeled as a function of total soil N content (figure 1). For the non-mixed plots (figure 1a) this model indicates a 0.09% increase in foliar N per 100 kg ha^{-1} increase in total N content, as a result of increased forest floor retention ($R^2 = 0.41$). A similar model for the linear response in foliar N as a function of 2M KCl extractable N content, for the non-mixed plots (figure 2a), indicates a 0.03% increase in foliar N per kg ha^{-1} increase in 2M KCl extractable N pool in the mineral soil ($R^2 = 0.44$). Lastly, a linear model describing foliar P concentrations from the non-mixed plots, across the forest floor retention treatments, as a function of Mehlich III extractable P from the mineral soil (figure 3a) indicates a 55 mg kg^{-1} increase in foliar P per kg ha^{-1} increase in Mehlich III extractable P pool in the mineral soil ($R^2 = 0.53$). The proportion of the variation in foliar N and P explained by mineral soil variables was lower for the mixed plots (figures 1b, 2b, and 3b)

Discussion

Tree growth was not affected, after three years, by the significant changes in nutrient availability caused by the forest floor retention treatments reported in chapter 2. It appears that even the lowest nutrient levels observed in the removed treatment were sufficient for current stand demand, and that all trees were still benefiting from the “assart flush” (Kimmins, 1997). Fertilization studies in young pine plantations do not always show a response to increased nutrition (NCSFNC, 1995), therefore it is not surprising that, at this early stage of stand development, our trees have not responded to increased retention of an organic nutrient pool, that must decompose and mineralize to become available for plant uptake.

In contrast to the lack of forest floor retention treatment effect, the incorporation treatment had a significant positive effect on tree growth (table 2). This is likely a result of improved physical condition i.e. aeration, caused by the mixing treatment, because incorporation did not significantly affect soil nutrient availability (see chapter 2).

Similar tree growth responses were found by Kelting et al. (2000) in a loblolly pine bioassay study in the Lower Coastal Plain of South Carolina, with site preparation treatments that included organic matter removal. Their multilinear regression analysis concluded that oxidation depth was more important than N mineralization in explaining the variation in tree growth at an early age.

The incorporation treatment x time interaction observed on tree volume (p=0.0528) (table 2) suggests that improved rooting environment conditions in the mixed treatment may have lasting and increasing benefits for tree growth.

Forest floor retention and incorporation effects on foliar concentrations were, in general, transient, as most effects were only significant at year 1 and foliar concentrations returned to the levels found at planting by year 3. Exceptions were foliar K which consistently increased and B which consistently decreased through time. This transient effect on foliar concentrations could be the result of increased leaf area and growth, which created a dilution effect as the stand developed (Aronsson and Elowson, 1980; Adams and Allen, 1985). Foliar N, P, Cu, and Zn were significantly higher at year 1 in the control and doubled treatments that retained some level of forest floor, (Table 3). Coincidentally, at year 1, these same nutrients showed higher concentrations in the non-mixed treatment than in the mixed treatment, where tree growth was greater. This may provide further support to the dilution effect caused by tree growth.

Changes in foliar N, and P, at year 1, could be partially explained, in the non-mixed treatment, by mineral soil N and P pools, as indicated by the positive and significant slopes on figures 1a, 2a, and 3a. The relative location of the forest floor retention treatments along these relationships between mineral soil variables and foliar nutrition, confirm the positive and significant effect that increased forest floor retention has had on improving soil nutrient availability and foliar nutrition. No discernable relationships between foliar nutrient concentrations and mineral soil nutrient pools were observed on the mixed treatment (figures 1b, 2b, and 3b) given the confounding effect that mixing may have had on nutrient availability and the dilution of foliar nutrients caused by the increased tree growth observed in this treatment.

Evaluating the effects of forest floor retention and incorporation treatments on soil nutrient availability through tree growth and foliar nutrition, at this early stage of stand development is difficult, as these variables represent the final expression of processes such as forest floor decomposition, microbial mineralization-immobilization of nutrients, root development, and are influenced by changes in soil temperature, water availability, and carbon inputs caused by the harvest. All these processes and their complex interactions might mask the effect of treatments at this early age. The forest floor and mineral soil variables measured in this study, and reported in chapters 1 and 2, offer a detailed view of treatment effects on site nutrient supply. These treatment effects may have been missed if only tree measurements and foliar analysis had been used as dependent variables, as occurs in most silvicultural trial.

References

Adams, M., Allen, H., 1985. Nutrient proportions in foliage of semi-mature loblolly pine. Plant Soil 86, 27-34.

Akaike, H., 1987. Factor analysis and AIC. Psychometrika 52, 317-332.

Albaugh, T.J., Allen, H.L., Fox, T.R., 2007. Historical patterns of forest fertilization in the Southeastern United States from 1969 to 2004. South. J. Appl. For. 31, 129-137.

Aronsson, A., Elowson, S., 1980. Effects of irrigation and fertilization on mineral nutrients in scots pine needles. Ecol. Bull., 219-228.

Binkley, D., 1984. Does forest removal increase rates of decomposition and nitrogen release? For. Ecol. Manage. 8, 229-233.

du Toit, B., Dovey, S.B., Smith, C.W., 2008. Effects of Slash and Site Management Treatments on Soil Properties, Nutrition and Growth of a Eucalyptus grandis Plantation in South Africa. In: Nambiar, E.K.S. (Ed.), Site Management and Productivity in Tropical Plantation Forests. Center for International Forestry Research (CIFOR), Proceedings of Workshops in Piracicaba (Brazil) 2004 and Bogor (Indonesia) 2006, pp. 63-77.

Hardiyanto, E.B., Wicaksono, A., 2008. Inter-rotation Site Management, Stand Growth and Soil Properties in Acacia mangium Plantations in South Sumatra, Indonesia. In: Nambiar, E.K.S. (Ed.), Site Management and Productivity in Tropical Plantation Forests. Center for International Forestry Research (CIFOR), Proceedings of Workshops in Piracicaba (Brazil) 2004 and Bogor (Indonesia) 2006, pp. 107-122.

Jones, J.B., Jr., Steyn, W.J.A., 1973. Sampling, Handling, and Analyzing Plant Tissue Samples. In: Walsh, L.M., Beaton, J.B. (Eds.), Soil Testing and Plant Analysis. Soil Sci. Soc. Amer., Madison, WI, pp. 249-270.

Jorgensen, J.R., Wells, C.G., Metz, L.J., 1980. Nutrient changes in decomposing loblolly pine forest floor. Soil. Sci. Soc. Am. J. 44, 1307-1314.

Kelting, D., Burger, J., Patterson, S.C., 2000. Early loblolly pine growth response to changes in the soil environment. New Zeal. J. For. Sci. 30, 206-224.

Kimmins, J.P., 1997. Biogeochemistry, Cycling of Nutrients in Ecosystems. In: kimmins, J.P. (Ed.), Forest Ecology, a Foundation for Sustainable Management. Prentice Hall, Saddle River, N.J., pp. 71-129.

Li, Q.C., Allen, H.L., Wilson, C.A., 2003. Nitrogen mineralization dynamics following the establishment of a loblolly pine plantation. Can. J. For. Res. 33, 364-374.

Markewitz, D., Richter, D.D., Allen, H.L., Urrego, J.B., 1998. Three decades of observed soil acidification in the Calhoun Experimental Forest: has acid rain made a difference? Soil Sci. Soc. Am. J. 62, 1428-1439.

Mendham, D.S., O'Connell, A.M., Grove, T.S., Rance, S.J., 2003. Residue management effects on soil carbon and nutrient contents and growth of second rotation eucalyptus. For. Ecol. Manage. 181, 357-372.

Nambiar, E.K.S., Kallio, M.H., 2008. Increasing and Sustaining Productivity in Tropical Forest Plantations: Making a Difference through Cooperative Research and Partnerships. In: Nambiar, E.K.S. (Ed.), Site Management and Productivity in Tropical Plantation Forests: Workshop Proceedings, 22-26 November 2004 Piracicaba, Brazil, and 6-9 November, Bogor, Indonesia Center for International Forestry Research (CIFOR), Bogor, Indonesia., pp. 205-228.

NCSFNC, 1995. Response of Young Loblolly Pine to Nitrogen, Phosphorus, and Potassium Fertilization. Department of Forestry. College of Forest Resources. North Carolina State University, Raleigh, NC. Report No. 34, p. 23.

Powers, R.F., Scott, D.A., Sanchez, F.G., Voldseth, R.A., Page-Dumroese, D., Elioff, J.D., Stone, D.M., 2005. The North American long-term soil productivity experiment: Findings from the first decade of research. For. Ecol. Manage. 220, 31-50.

Rojas, J.C., 2005. Factors Influencing Responses of Loblolly Pine Stands to Fertilization, Dissertation. Dep. Forestry. North Carolina State University, Raleigh, p. 147.

Sanchez, F.G., Carter, E.A., Klepac, J.F., 2000. Soil carbon and soil physical properties response to incorporating mulched forest slash. New Zeal. J. For. Sci. 30, 150-168.

Sanchez, F.G., Carter, E.A., Klepac, J.F., 2003. Enhancing the soil organic matter pool through biomass incorporation. Biomass Bioenerg. 24, 337-349.

Sanchez, F.G., Eaton, R.J., 2001. Sequestering carbon & improving soils - Benefits of mulching and incorporating forest slash. J. Forest. 99, 32-36.

SAS, 2005. The SAS System for Windows Release 9.1.3. SAS Institute Inc., Cary, NC.
Smith, C.T., Lowe, A.T., Skinner, M.F., Beets, P.N., Schoenholtz, S.H., Fang, S.Z., 2000. Response of radiata pine forests to residue management and fertilisation across a fertility gradient in New Zealand. For. Ecol. Manage. 138, 203-223.

Tew, D.T., Morris, L.A., Allen, H.L., Wells, C.G., 1986. Estimates of nutrient removal, displacement and loss resulting from harvest and site preparation of a Pinus taeda plantation in the Piedmont of North Carolina. For. Ecol. Manage. 15, 257-267.

Zerpa, J.L., Allen, H.L., Campbell, R.G., Phelan, J., Duzan, H., 2010. Influence of variable organic matter retention on nutrient availability in a 10-year-old loblolly pine plantation. For. Ecol. Manage. 259, 1480-1489.

Table 1. Growth in young loblolly pine trees measured in a forest floor retention and incorporation study in the Coastal Plain of the Southeast, US. Orthogonal contrasts compare the retention and incorporation treatments against control and non-mixed respectively. Standard errors shown in parenthesis (n = 5). GD = groundline diameter, Ht = height; Vol = Volume.

Treatment factors >		Forest floor Retention					Forest floor incorporation with mineral soil		
Treatment levels >		Removed		Control	Doubled		Non-Mixed	Mixed	
Variable	Year	Mean	Contrast vs. Control	Mean	Mean	Contrast vs. Control	Mean	Mean	Contrast vs. Non-Mixed
GD (cm)	1	0.84 (0.02)	0.27	0.78 (0.02)	0.89 (0.02)	0.06	0.79 (0.01)	0.88 (0.01)	0.06
	3	5.94 (0.11)	0.63	5.86 (0.13)	6.20 (0.12)	0.17	5.76 (0.11)	6.21 (0.09)	**<0.05**
Ht (cm)	1	45 (1)	0.21	41 (1)	46 (1)	0.08	42 (1)	46 (1)	**<0.05**
	3	277 (5)	0.37	268 (5)	286 (5)	0.17	266 (5)	287 (4)	0.06
Vol (GD^2 x Ht) (cm^3)	1	42 (3)	0.30	34 (2)	48 (3)	0.11	35 (2)	47 (2)	0.10
	3	12487 (512)	0.57	11962 (539)	13988 (584)	0.12	11724 (455)	13756 (433)	0.06

Table 2. P-values from repeated measures analyses on tree growth and foliar nutrient concentrations measured during the first three years of a forest floor retention and incorporation study in the Coastal Plain of the Southeast, US. GD = groundline diameter, Ht = height; Vol = Volume.

Dependent Variable	Effect						
	FF	Incorporation	Time	FF*Incorporation	FF*Time	Incorporation* Time	FF*Incorporation* Time
GD (cm)	0.26	**<0.05**	**<0.0001**	0.92	0.47	0.05	0.95
Ht (cm)	0.31	**<0.05**	**<0.0001**	0.98	0.51	0.10	0.99
Vol (GD^2 x Ht) (cm^3)	0.24	0.05	**<0.0001**	0.99	0.25	0.05	0.99
C %	0.49	0.93	**<0.05**	0.83	0.52	0.25	0.40
N %	**<0.0001**	**<0.0001**	**<0.0001**	**<0.01**	**<0.0001**	**<0.0001**	**<0.01**
P (g kg^{-1})	**<0.01**	**<0.01**	**<0.0001**	0.23	**<0.0001**	**<0.0001**	**<0.01**
K (g kg^{-1})	0.81	0.82	**<0.001**	0.32	0.95	0.82	0.38
Ca (g kg^{-1})	0.52	0.44	**<0.0001**	**<0.01**	0.74	0.26	0.65
Mg (g kg^{-1})	0.43	**<0.05**	**<0.0001**	0.19	**<0.05**	0.70	0.70
Mn (mg kg^{-1})	0.88	0.46	**<0.0001**	0.60	0.82	**<0.001**	0.87
B (mg kg^{-1})	0.18	0.81	**<0.0001**	0.94	0.52	0.18	0.43
Cu (mg kg^{-1})	**<0.05**	**<0.01**	**<0.0001**	0.32	0.06	**<0.05**	0.63
Zn (mg kg^{-1})	**<0.01**	0.46	**<0.0001**	0.08	0.15	0.36	0.13

Table 3. Foliar nutrient concentrations in young loblolly pine trees measured in a forest floor retention and incorporation study in the Coastal Plain of the Southeast, US. Orthogonal contrasts compare the retention and incorporation treatments against control and non-mixed respectively. Standard errors shown in parenthesis (n = 5)

Treatment factors >		Forest floor Retention					Forest floor incorporation with mineral soil		
Treatment levels >		Removed		Control	Doubled		Non-Mixed	Mixed	
Variable	Year	Mean	Contrast vs. Control	Mean	Mean	Contrast vs. Control	Mean	Mean	Contrast vs. Non-Mixed
C %	1	53.1 (0.11)	0.78	53.2 (0.17)	53.2 (0.13)	0.93	53.1 (0.12)	53.2 (0.09)	0.28
	3	52.6 (0.19)	0.14	53.0 (0.23)	52.9 (0.18)	0.68	52.9 (0.17)	52.8 (0.17)	0.55
N %	1	1.37 (0.04)	**<0.0001**	1.76 (0.10)	1.76 (0.08)	0.93	1.81 (0.08)	1.44 (0.04)	**<0.0001**
	3	1.40 (0.03)	0.84	1.40 (0.02)	1.38 (0.02)	0.36	1.38 (0.02)	1.41 (0.02)	0.28
P (g kg^{-1})	1	1.37 (0.04)	**<0.001**	1.59 (0.07)	1.65 (0.06)	0.29	1.66 (0.05)	1.41 (0.03)	**<0.0001**
	3	1.39 (0.02)	0.44	1.36 (0.02)	1.39 (0.03)	0.33	1.37 (0.02)	1.39 (0.02)	0.51
K (g kg^{-1})	1	5.96 (0.13)	0.97	5.95 (0.09)	5.85 (0.21)	0.62	5.92 (0.10)	5.92 (0.14)	0.99
	3	6.49 (0.13)	0.68	6.39 (0.14)	6.37 (0.24)	0.96	6.45 (0.18)	6.38 (0.09)	0.74
Ca (g kg^{-1})	1	3.31 (0.08)	0.52	3.39 (0.09)	3.46 (0.10)	0.55	3.40 (0.08)	3.37 (0.07)	0.81
	3	2.17 (0.07)	0.90	2.15 (0.07)	2.20 (0.10)	0.68	2.11 (0.06)	2.24 (0.07)	0.18
Mg (g kg^{-1})	1	1.15 (0.02)	0.09	1.09 (0.02)	1.07 (0.03)	0.50	1.07 (0.03)	1.13 (0.01)	**<0.05**
	3	0.83 (0.03)	**<0.05**	0.93 (0.03)	0.88 (0.03)	0.25	0.86 (0.03)	0.90 (0.02)	0.25
Mn (mg kg^{-1})	1	1067 (40)	0.57	1093 (39)	1087 (29)	0.90	1122 (26)	1043 (29)	**<0.05**
	3	407 (21)	0.82	414 (25)	423 (24)	0.78	400 (22)	429 (14)	0.28
B (mg kg^{-1})	1	15.2 (0.65)	0.76	15.6 (0.84)	17.5 (1.34)	0.15	16.4 (0.87)	15.8 (0.79)	0.52
	3	10.9 (0.63)	0.41	10.4 (0.35)	11.4 (0.39)	0.10	10.4 (0.33)	11.4 (0.41)	**<0.05**
Cu (mg kg^{-1})	1	2.50 (0.13)	**<0.0001**	3.36 (0.27)	3.56 (0.21)	0.28	3.64 (0.19)	2.64 (0.12)	**<0.0001**
	3	1.45 (0.09)	0.98	1.44 (0.20)	1.55 (0.39)	0.75	1.58 (0.26)	1.38 (0.14)	0.46
Zn (mg kg^{-1})	1	45 (3)	**<0.05**	52 (2)	56 (2)	0.13	52 (2)	50. (2)	0.30
	3	30 (1)	**<0.05**	34 (1)	34 (2)	0.96	33 (1)	33 (1)	0.99

Table 4. Pearson correlation coefficients from a year-old loblolly pine plantation in the Lower Coastal Plain of the Southeast, US. regenerated under different forest floor retention treatments

Variables		Mixed Trt. Plots	Foliar Concentrations										Mineral Soil Pools (A-horizon)		
			C	N	P	K	Ca	Mg	Mn	B	Cu	Zn	Tot. N	Ext. N	Ext. P
Tree Growth	Vol	All	0.19	-0.11	-0.11	0.29	0.03	-0.06	-0.20	-0.13	-0.01	0.02	0.09	0.11	0.17
		Mixed	0.40	0.41	0.24	0.52	0.16	-0.43	-0.22	-0.34	**0.58**	0.16	-0.01	0.01	-0.05
		Non-Mixed	-0.27	-0.20	-0.08	-0.37	-0.18	-0.18	0.18	0.39	-0.15	-0.23	0.20	0.12	0.54
Foliar Conc.	C	All		-0.13	-0.13	-0.18	0.01	0.11	-0.31	-0.11	-0.06	-0.07	0.12	-0.01	0.04
		Mixed		-0.27	-0.13	-0.15	-0.23	-0.28	-0.41	-0.48	-0.11	-0.23	0.01	-0.49	-0.30
		Non-Mixed		0.08	0.03	-0.23	0.20	0.12	-0.15	0.16	0.19	0.07	0.17	0.49	0.18
	N	All			**0.91**	0.13	0.19	**-0.56**	0.23	0.10	**0.97**	0.53	**0.40**	0.25	0.11
		Mixed			**0.67**	**0.77**	**0.73**	-0.30	0.07	0.17	**0.92**	**0.67**	**0.53**	**0.63**	0.10
		Non-Mixed			**0.92**	-0.23	-0.03	-0.50	-0.02	-0.02	**0.96**	**0.55**	**0.64**	**0.67**	0.28
	P	All				-0.03	0.30	**-0.39**	0.17	0.17	**0.88**	**0.61**	0.42	0.18	0.20
		Mixed				0.34	**0.85**	-0.01	-0.08	0.30	**0.58**	0.74	**0.58**	0.35	0.51
		Non-Mixed				-0.35	0.05	-0.31	-0.04	0.05	**0.90**	**0.62**	**0.63**	**0.61**	**0.73**
	K	All					**0.38**	0.04	0.05	-0.18	0.19	0.30	0.12	0.19	**-0.48**
		Mixed					0.46	-0.30	-0.06	-0.22	**0.75**	0.39	0.47	0.49	-0.17
		Non-Mixed					0.30	0.21	0.23	-0.14	-0.18	0.21	-0.27	-0.45	**-0.84**
	Ca	All						0.34	-0.16	0.14	0.25	**0.71**	0.30	0.28	-0.02
		Mixed						-0.08	0.11	0.42	**0.69**	**0.84**	**0.64**	**0.60**	0.19
		Non-Mixed						**0.58**	-0.51	-0.12	0.05	**0.61**	0.01	-0.16	-0.16
	Mg	All							-0.27	-0.19	**-0.52**	0.06	-0.16	-0.17	-0.08
		Mixed							-0.19	-0.03	-0.45	-0.11	-0.29	-0.28	-0.07
		Non-Mixed							-0.18	-0.22	-0.42	0.20	-0.21	-0.43	-0.15
	Mn	All								**0.42**	0.25	0.01	0.02	0.30	-0.01
		Mixed								0.25	0.08	-0.05	-0.01	**0.57**	0.26
		Non-Mixed								0.57	-0.01	-0.03	0.14	0.19	-0.12
	B	All									0.12	0.23	0.13	-0.18	**-0.42**
		Mixed									0.11	0.42	0.15	0.49	-0.17
		Non-Mixed									0.05	0.06	0.13	0.28	0.13
	Cu	All										**0.57**	**0.38**	0.27	0.06
		Mixed										**0.68**	0.45	**0.63**	0.05
		Non-Mixed										**0.60**	**0.66**	**0.68**	0.23
	Zn	All											0.52	**0.44**	0.07
		Mixed											**0.67**	**0.69**	0.26
		Non-Mixed											0.44	0.30	-0.01
Min. Soil Pools	Tot. N	All												**0.56**	**0.40**
		Mixed												**0.59**	0.25
		Non-Mixed												**0.58**	0.49
	Ext. N	All													0.34
		Mixed													0.24
		Non-Mixed													0.49

Significance for all plots (n = 30) r = 0.36 for $p < 0.05$ and r = 0.46 for $p < 0.01$; for Mixed and Non-Mixed plots (n = 15) r =0.51 for $p < 0.05$ and r = 0.64 for $p < 0.01$; Vol = tree volume, Tot. N = Total N, Ext. N = 2M KCl extractable N, Ext. P = Mehlich III extractable P.

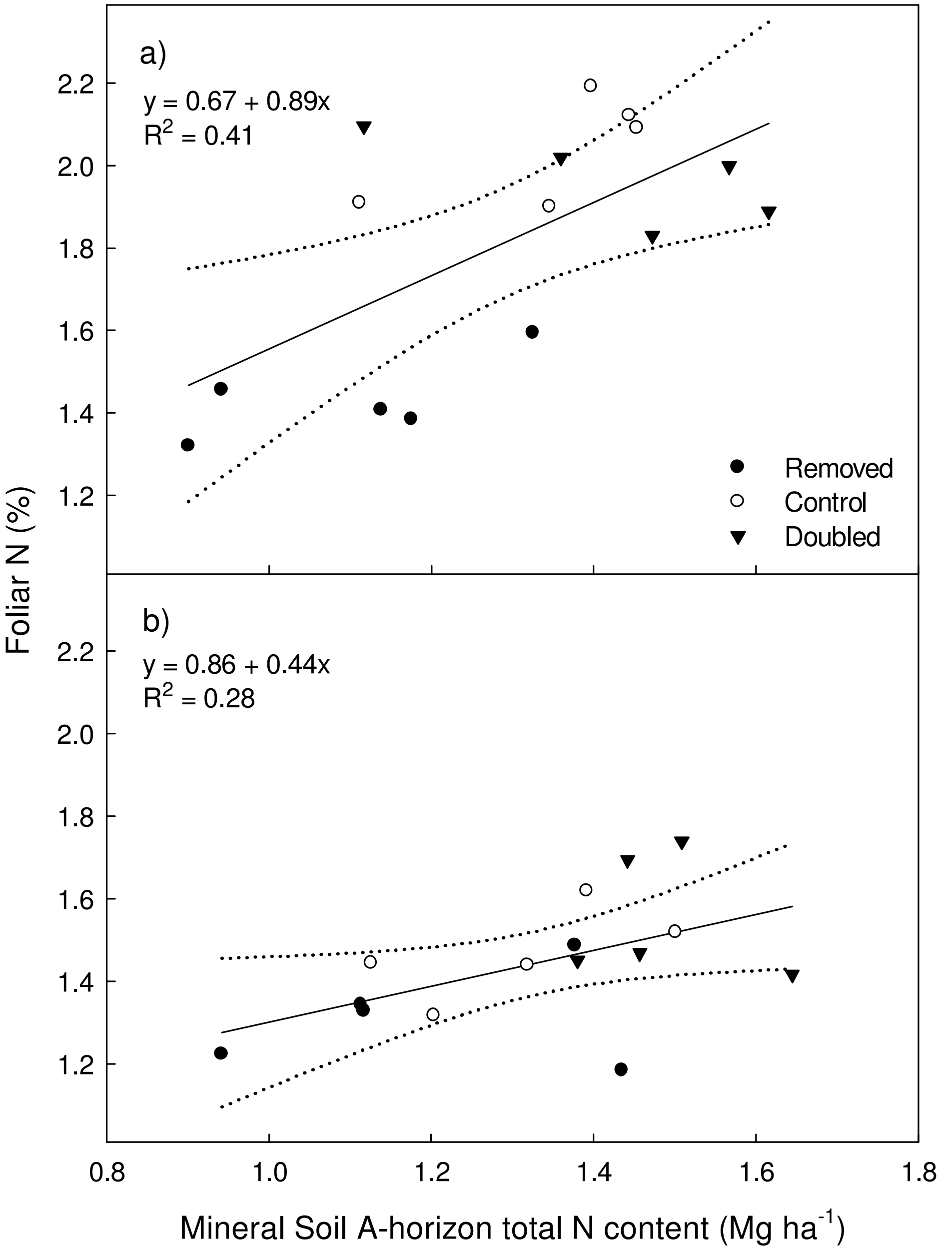

Figure 1. Foliar N concentration from a 1-year-old second rotation loblolly pine stand in the Lower Coastal Plain of the Southeast, US, as a function of mineral soil (A-horizon) total N content. Forest floor retention treatments: Removed = 0 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Doubled = 31,700 kg ha^{-1} were imposed prior to planting. a) Non-Mixed plots and b) Mixed plots.

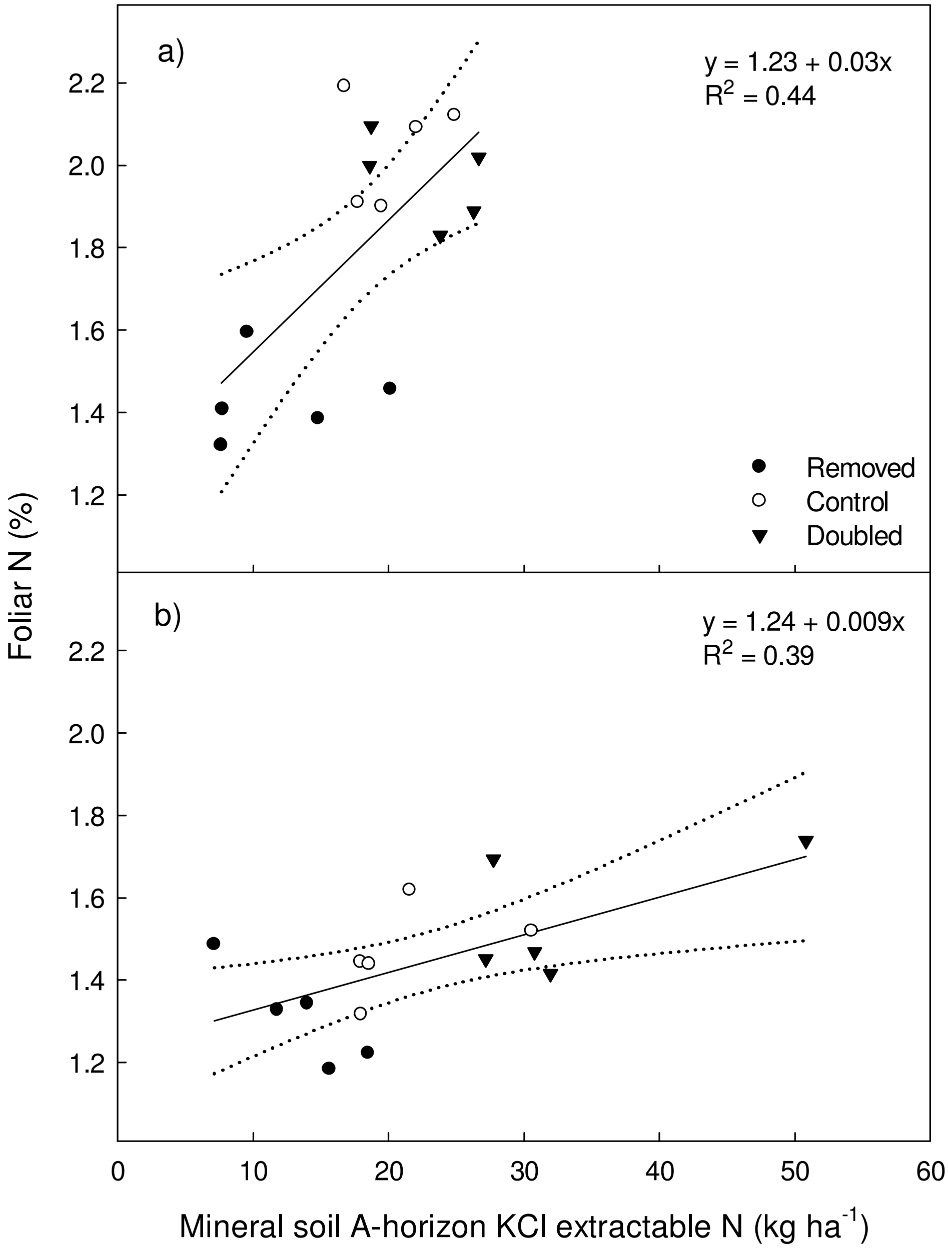

Figure 2. Foliar N concentration from a 1-year-old second rotation loblolly pine stand in the Lower Coastal Plain of the Southeast, US, as a function of mineral soil (A-horizon) KCl extractable N content. Forest floor retention treatments: Removed = 0 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Doubled = 31,700 kg ha^{-1} were imposed prior to planting. a) Non-Mixed plots and b) Mixed plots.

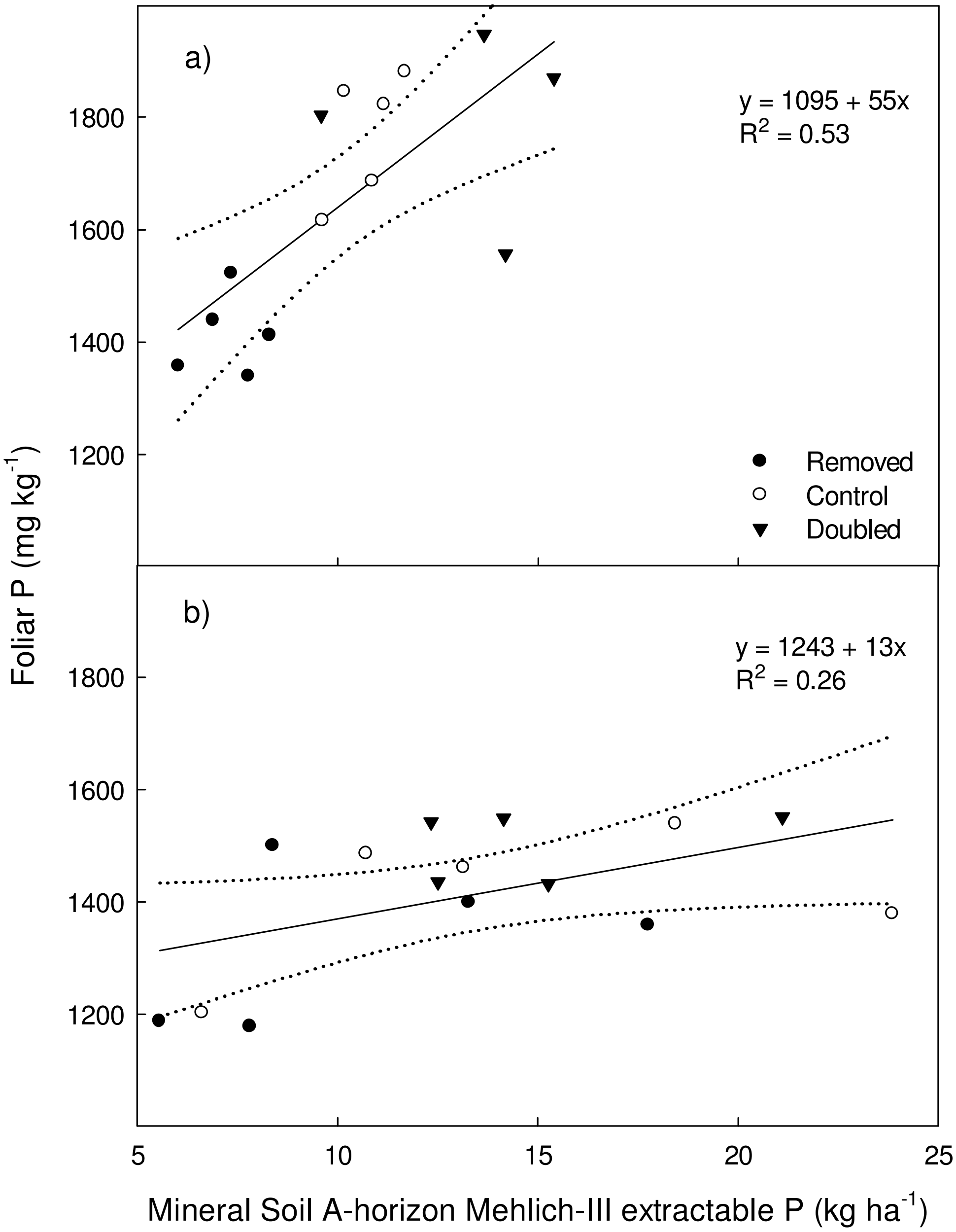

Figure 3. Foliar P concentration from a 1-year-old second rotation loblolly pine stand in the Lower Coastal Plain of the Southeast, US, as a function of mineral soil (A-horizon) Mehlich-III extractable P content. Forest floor retention treatments: Removed = 0 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Doubled = 31,700 kg ha^{-1} were imposed prior to planting. a) Non-Mixed plots and b) Mixed plots. An outlier data point from the Doubled treatment with Mehlich-III extractable P = 37 kg ha^{-1} and foliar P = 1813 mg kg^{-1} was removed from figure a).

CHAPTER 4

Carbon limitation as a driver of post-harvest N availability in a loblolly pine plantation

Abstract

A carbon limitation assay was conducted in the laboratory using soil samples from a field study established after harvesting a loblolly pine stand in the Coastal Plain of North Carolina, with three forest floor retention treatments (removed = 0 $Mg*ha^{-1}$, control = 15 $Mg*ha^{-1}$, and doubled = 30 $Mg*ha^{-1}$) combined with two levels of incorporation (mixed, non-mixed). The retention treatments resulted in significantly different levels of soil available carbon and nitrogen, as well as N release pattern in the first two years of sampling i.e. the point of maximum N availability occurred in the first year after harvest for the treatment with no forest floor retention, and in the second year for the treatments where forest floor was retained. Thus, this assay examined carbon deficiencies that could influence N immobilization by the soil microbes, as a way to explain the increase in post-harvest N availability, as well as the timing of N release observed in the field. The assay measured soil respiration response to additions of labile carbon, and de-ionized water as a control. Respiration response was used as an indicator of carbon limitation by the soil microbes. After carbon additions the respiration response was 37% higher in the removed treatment as compared to the control and doubled, in the period that coincided with the peak of maximum N availability shown by the removed treatment in the field, and extractable N from the carbon amended soils showed a 94% decreased as compared to the water only treatment suggesting that in these pine stands the post-harvest increase in N availability (Assart effect) is in part controlled by soil carbon availability.

Introduction

In forest plantations, the period from harvest through replanting is when soils are most subject to changes in organic matter and nutrient availability (Nambiar and Kallio, 2008). Soil nitrogen (N) dynamics following harvest are commonly characterized by increased mineralization rates and extractable mineral N (Kimmins, 1997). These effects have been attributed to several factors including increased decomposition of forest floor and harvest residues from the previous rotation (Berg *et al.*, 1993; De Santo *et al.*, 1993; Sariyildiz and Anderson, 2003), increased temperatures (Kim *et al.*, 1995), higher soil moisture due to lower evapotranspiration rates (Barg and Edmonds, 1999), the post-harvest mixing of forest floor and slash material with the surface soil (Tamm, 1964; Kimmins, 1997), and reduced N uptake caused by tree removal (Burger and Pritchett, 1984; Vitousek and Andariese, 1986; Smethurst and Nambiar, 1989; Vitousek *et al.*, 1992). More recently, it has been hypothesized that higher N levels may result from reduced microbial immobilization of N due to lower levels of available C from fresh litter inputs, root exudates, and throughfall following harvest (Hart *et al.*, 1994; Bradley *et al.*, 2000; Li *et al.*, 2003). This hypothesis is based on the energy requirements for N immobilization. Most of the soil N is found in organic forms, not immediately available for microbial or plant uptake. Nitrogen immobilization usually begins outside the microbial cells by the action of enzymes such as proteinases and peptidases, which facilitate the hydrolysis of simple organic compounds into peptides and amino acids. The latter can be easily assimilated by microbes for their own protein synthesis and there is evidence that plants can also use these simple organic compounds as an N source (Turnbull *et al.*, 1996; Schmidt and Stewart, 1999). This first step in microbial N uptake involves the production of extra cellular enzymes and two membrane transports, all of which have energy requirements. Once inside the cell, the major pathway for microbial N assimilation is the glutamate dehydrogenase pathway. The glutamate dehydrogenase enzyme uses different coenzymes (NADH or NADPH) to allow organisms to control the release or uptake of NH_4^+ (Paul and Clark, 1996). Through this metabolic pathway the tricarboxylic acid cycle plays an important role producing the coenzymes (NADH) and providing the source of energy required for these reactions to occur., At the same time, the inputs into the TCA cycle must come from a labile source of carbon from outside the microorganisms (Ahmad and Helleburt, 1991) (figure 1).

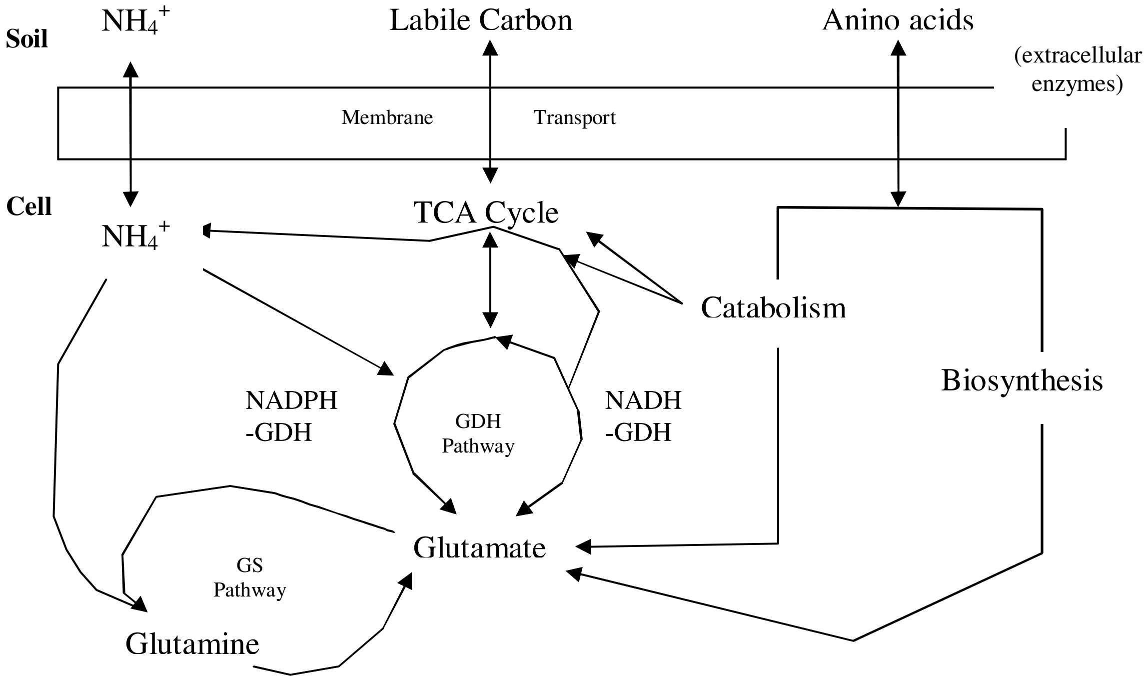

Figure 1. Major Pathways of ammonium assimilation and mineralization. NADPH-GDH, NADH-GDH glutamate Dehydrogenase with respective coenzymes; GS glutamine synthetase-glutamate synthase (Adapted from Ahmad and Helleburt, 1991.)

Thus, if harvest causes a decrease in labile carbon, then heterotrophic soil microbes, which are mainly responsible for N immobilization-mineralization, may be limited by energy sources and consequently will not require as much nitrogen as before the harvest.

The post-harvest flush of available N, the "Assart" effect (Tamm, 1964; Kimmins, 1997) typically lasts between one to five years. During these first few years, the root systems of young plantations are not well developed and have not effectively occupied the available soil volume. Thus, the increased N availability, poorly timed with low plant uptake, can result in the conversion of available N into unavailable forms through complexation with metals, clays, organic matter and other ions, physical occlusion, or possibly leaching losses in sandy soils with low capacity to retain these ions (Likens *et al.*, 1970; Titus *et al.*, 1997).

With these observations as background, a field study was established in the Lower Coastal Plain of North Carolina to determine if post-harvest forest floor retention and incorporation could influence the size and the dynamics of mineral soil carbon and nitrogen pools. Results from this field study showed a linear increase in soil available N with forest floor retention, and a full year delay in the point of maximum N availability between the treatment with no and 2x retention (see chapter 2).

Based on these field results, we designed a laboratory assay with objectives to determine if soil microbes were limited by carbon, and if so, to determine how this limitation was affected by the field treatments, to examine if the timing of this limitation was related to the available N dynamics observed in the field, and if N immobilization could be influenced by ameliorating this carbon limitation.

Methods

Site and Field Study Description

The field study, from where soil samples were collected for the carbon limitation assay, was established in the Lower Coastal Plain of Pamlico County, North Carolina. Immediately following harvest of a 33-yr old loblolly pine plantation, a complete randomized block study with 5 replications and 6- forest floor/mixing treatments was imposed on the site. The treatment design was a 3x2 factorial with 3 levels of forest floor retention (removed, control, doubled) imposed in mid March 2006, and 2 levels of forest floor incorporation with the surface mineral soil (mixed and non-mixed) imposed in early April 2006. More detailed descriptions of the site, the study design, and the treatments imposition are presented in chapters 2 and 3.

Mineral soil sampling

Mineral soil samples from the A-horizon were collected at 5 randomly located points per plot at 1, 4, 8, 15, and 19 months after the mixing treatment imposition. The samples were composited by plot in the field, put in plastic bags and transported in refrigerated containers to the laboratory where they were sieved through 2 mm mesh size to remove roots and other large organic residues. The soil did not have a coarse fraction greater than the mesh size used. Mineral soil collections were always made to the top of the B-horizon and the depth to the A-horizon was determined prior to treatment on all plots. This depth was very consistent with an abrupt boundary between the A- and the B-horizon. Three bulk density samples were also collected from the A-horizon in each plot using the core method (Grossman and Reinsch, 2002).

Carbon Limitation Assay

Additions of a labile source of carbon to soils commonly result in an exponential increase in the soil respiration rate. This increase continues until carbon or some mineral nutrient becomes limiting to further microbial growth (Nordgren, 1992). This laboratory assay quantified carbon limitations on soil microbes by measuring microbial respiration in response to labile carbon addition. Additionally it isolated the respiration response due to carbon limitations by controlling environmental variables such as soil moisture and temperature that could influence the respiration rate in the field. The underlying assumption for this assay is that the maximum respiration response to carbon addition is proportional to the limitation of the microbial population to carbon. Thus, field treatments where labile carbon is more limiting should show a stronger respiration response to carbon additions than those where labile carbon is less or non-limiting.

In order to compare the maximum respiration responses among treatments, the labile carbon addition must be large enough to insure that microbial growth will reach the maximum possible growth under the given soil conditions. A preliminary experiment adding glucose-C in the range of 0.7, to 7.7 μg C g soil^{-1} to soils from this site showed that an addition of between 3.2 and 7.7 μg C g soil^{-1} was high enough to ensure a maximum microbial respiration response.

The carbon limitation assay consisted of measuring the respiration response of two treatments: pure de-ionized water, used as a control (figure 2), and carbon at a dose of 7.7 mg glucose-C solution g^{-1} soil (figure 3). Both treatments were delivered in 10 ml to 40 g of soil sample. This amounted to a total of 60 samples per collection date: (3 forest floor retention field treatments x 2 incorporation field treatments x 5 field replicates x 2 carbon limitation assay treatments). Soils samples were weighed inside a 500 ml mason jar and, after the additions, were loosely covered with plastic wrap, and let to incubate in the dark at 22° C. The jars were capped with an air-tight lid fitted with a septum approximately two hours prior to each respiration measurement. A 1 ml gas sample from each jar's headspace was drawn using a syringe and analyzed for CO_2 concentration using a Li-Cor 6262 infrared gas analyzer (Li-Cor 6262, Lincoln, NE). Respiration rate was calculated by multiplying the sample's CO_2 concentration by the headspace volume of the mason jar and dividing by the moisture corrected soil sample weight and the time during which CO_2 accumulated in the head space of the closed jar.

Changes in the moisture content of the soil samples were monitored after each respiration measurement and de-ionized water was added with a hand sprayer when the moisture content dropped by more than 5%.

Respiration was measured at 48 hour intervals until a drop in rate was observed in all treatments, with the exception of the 4 month sampling when it was measured until rates returned to initial levels (figure 3).

Soil Extractable N

Extractable N was determined at the end (6 weeks after C and water additions were initiated) of the month 1 and month 15 carbon limitation assays to determine the effect of carbon additions on N availability. In addition, extractable N from the water only assay also served as a long term aerobic N mineralization test for field treatment effects. Fresh soil samples (10 g) were extracted in 35 ml of 2M KCl by shaking at high speed for one hour and centrifuging for 15 minutes at 4,000 rpm. The centrifuged solutions were filtered using Whatman 42 ashless filters and analyzed for inorganic N with a Lachat Autoanalyzer (Quick-Chem 8000, Zellweger Analytics, Inc. Milwaukee, WI). Results of soil extractable N pools in the field were presented in chapter 2 and were used here to relate temporal changes in N availability with soil respiration responses to carbon additions obtained from the laboratory assay. These extractions followed the same method described here.

Microbial Biomass C

Microbial biomass C from fresh soil samples was used to standardize the respiration responses in the carbon limitation assay based on the significant positive effect that the forest floor retention treatments had on microbial C (see chapter 2) and on the assumption that the respiration rate after glucose amendments is proportional to the microbial biomass (Anderson and Domsch, 1978; Nordgren, 1992). Microbial biomass C was determined on 2 M KCl extracts using the chloroform fumigation-extraction method described by Brookes et al.(1985). Organic C from these extracts was analyzed on a Schimadzu TOC analyzer.

Gravimetric soil moisture contents were determined for each field samples to express the extractable N and microbial biomass C pools on dry weight basis. These values were then scaled to a per hectare basis using the depth of the A-horizon and soil bulk density (see chapter 2).

Data analysis

Repeated measures analyses were conducted using PROC MIXED (SAS, 2005) to examine the effects of forest floor retention and incorporation treatments, time, and interaction effects on maximum soil respiration responses and extractable N after water and carbon additions. An unstructured covariance was specified based on the Akaike's (1987) information criterion (AIC), which assessed the goodness of fit of the predicted covariance matrix to the observed matrix.

The mixed model was:

$Y_{ijkm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + A_m + \varepsilon_{ijkm}$

where Y_{ijkm} = the response to treatment (i) and (j) in block (m) at time (k), μ = the overall mean, α_i = the fixed forest floor retention treatment effect (i=1,2,3), β_j = the fixed incorporation treatment effect (j=1,2), $(\alpha\beta)_{ij}$ = the fixed interaction effect of the forest floor retention treatment (i) with the incorporation treatment (j), γ_k = the fixed effect of time (k), $(\alpha\gamma)_{ik}$ = the fixed interaction effect of forest floor retention treatment (i) with time(k), $(\beta\gamma)_{jk}$ = the fixed interaction effect of incorporation treatment (j) with time (k), $(\alpha\beta\gamma)_{ijk}$ = the three-way fixed interaction effect of forest floor retention treatment (i) with incorporation treatment (j) with time (k), A_m = the random block effect (m=1,2,3,4,5), and ε_{ijkm} = the random residual error.

Additionally, analyses of variance were performed on maximum soil respiration responses and extractable N after water and carbon additions by sampling date, and orthogonal contrasts were used for treatment means comparison. Significance was accepted at $p \leq 0.05$ for all analyses.

Results

Representative examples of the maximum respiration response to water and carbon addition are presented in figures 2 and 3, respectively. The maximum soil respiration response to carbon additions averaged 31, 22, and 21 times greater than the response to water additions for the removed, control, and doubled treatments respectively, and 24 times greater for both mixed and non-mixed treatments (table 1). Almost 100% of applied C had been respired after 480 hours of incubation with no significant field treatment differences in the amount respired.

Significant forest floor retention, incorporation, and time effects were observed for maximum respiration response after water and carbon addition throughout the sampling period (Table 2). The higher respiration responses to water addition observed in the mixed treatment (table 1, figure 4b) were principally due to higher levels on the doubled treatment, resulting in a significant forest floor x incorporation interaction (table 2).

The maximum respiration responses to water additions were significantly higher in the doubled treatment than the other two retention treatments at months 8 and 15 (table 1, figure 4a). Mixing resulted in significantly higher respiration responses only at month 15 (table 1, figure 4b). In contrast, the maximum respiration responses to carbon additions were significantly higher in the removed treatment than the other two retention treatments at 4 month (table 1, figure 5a). Mixing also resulted in significantly higher respiration responses to carbon additions at 4 months (table 1, figure 5b).

Extractable N results, post-carbon limitation assay, are presented in table 3. In general extractable N, after water addition, was higher at month 1 than at month 15 resulting in a significant main time effect (table 2). Also, after water addition, significantly higher levels of extractable N were found in the removed treatment followed by the control and doubled treatments 1 month after the field treatments were imposed. This treatment ranking was reversed at month 15 resulting in a significant forest floor x time interaction effect (table 2). A similar dynamic occurred between the incorporation treatments. Extractable N was higher in the non-mixed treatment at month 1, but higher in the mixed treatment at month 15 after field treatments resulting in a significant incorporation x time interaction effect (table 2).

Extractable N levels after carbon additions were one tenth of the levels obtained after water addition (table 3). Only a significant time effect was observed for this variable (table 2), which describes a drop in extractable N levels from 2.6 kg N ha^{-1} at month 1, to 0.8 kg N ha^{-1} at month 15 after treatment.

Discussion

In general, the respiration responses to water additions were higher in the doubled treatment (figure 4a) indicating more labile C was available for respiration in this treatment. This is supported by the field results of mineral soil total C, dissolved organic C, and microbial biomass C, all of which were positively and significantly affected by doubling the forest floor retention (Chapter 2). In contrast, the respiration responses to C additions were, in general, higher in the removed treatment suggesting that C addition ameliorated a greater labile C limitation in this treatment than in the treatments where the forest floor was retained (table1).

The respiration responses to C additions, across field treatments and sampling dates, were between 11 and 35 times greater than the baseline respiration with water additions (Table 1). Similar respiration responses to C addition were reported by Allen and Schlesinger (2004) for a Piedmont soil under a mid-rotation loblolly pine stand suggesting that microbial growth, and possibly N immobilization, may be limited by C availability, at different ages in this type of forest.

Additions of C from this laboratory assay amounted to approximately 9.0 Mg C ha^{-1} applied all in one labile dose. This was, on average, 36% of the total C pool, 23 times the average microbial biomass C, and 132 times the average dissolved organic C pools found in the mineral soil. In contrast, the forest floor retention treatments amounted to 0, 1x and 2x a pool of 7.7 Mg C ha^{-1} of organic material of which only a small fraction is immediately available to soil microbes and the remaining must decompose before becoming available. This assay proved useful in detecting field treatment differences in microbial respiration responses despite the large difference in the proportional contributions of C between the field and the assay treatments.

At the 4 month assay, nearly 100% of the added C had been respired by the soil microbes after 480 hours. The respiration rates were still higher than starting levels (figure 3) indicating a priming effect (Bingeman *et al.*, 1953; Fontaine *et al.*, 2003) caused by the addition of labile C.

Although at different magnitudes, respiration responses to both water and carbon additions were higher in soils sampled during the first field year as compared to the second field year (table 2, figures 4 and 5) indicating higher C limitations in the first year. The soil samples from the first year's collections had lower moisture contents than those collected in the second year, although these differences were not significant they could partially explain this time trend in respiration responses to water additions. Dissolved organic carbon, used as a measure of extractable labile carbon in soil solution (chapter 2) was lower in the first year, as compared to the second, particularly during the first 4 months after field treatments. These lower levels of labile carbon would explain the observed higher respiration responses to C additions during the first year after treatment.

Significantly higher levels of extractable N (laboratory water assay) were observed in the removed treatment, at month 1, as compared to the control and doubled treatments (table 3). Lower labile carbon pools, higher extractable N pools, and higher net N mineralization rates were also observed in field samples from this treatment taken at this time (chapter 2), as well as higher respiration responses to laboratory carbon additions (figure 5). This suggests that immediately after harvest available N levels were higher where there was less labile carbon for soil microbes to immobilize N in agreement with Vitousek and Matson (1984) who found that microbial immobilization was an important mechanism for retaining N in regenerating forests.

Interestingly, these dynamics were reversed at month 15 when, after water additions, the doubled treatment showed significantly higher extractable N compared to the control and removed treatments (significant forest floor x time interaction, table 2). By this time the decomposition of the forest floor was apparently contributing to maintaining higher extractable N levels as confirmed by the results from fresh soil samples (chapter 2). A similar explanation may apply to mixing in that it brings the labile C sources from the forest floor into closer proximity to the mineral soil microbial populations which could potentially use the C and increase N immobilization. The significant incorporation x time interaction with water addition (table 2) indicates higher extractable N at 1 month in the non-mixed treatment, which was where less labile carbon, measured as dissolved organic carbon (chapter 2) was found.

Additions of C decreased the extractable N, across field treatments and sampling dates, by 94% as compared to additions of water, confirming the strong control that C availability exerts on N release (Vitousek and Matson, 1984)

Conclusions

The laboratory assay conducted showed very strong respiration responses to carbon additions across all field treatments indicating a generalized carbon deficiency in this recently harvested loblolly site. Furthermore, it showed significantly stronger carbon limitations where no forest floor was retained, in the period that coincided with the point of maximum field N availability for this treatment, indicating a significant forest floor retention treatment effect on carbon availability and suggesting that the observed carbon limitation exerted a strong control on microbial N immobilization. This was confirmed by the dramatic reduction in extractable N levels obtained after carbon additions.

References

Ahmad, I., Helleburt, J.A., 1991. Enzymology of nitrogen assimilation in mycorrhiza. Method. Microbiol. 23, 182-202.

Akaike, H., 1987. Factor analysis and AIC. Psychometrika 52, 317-332.
Allen, A.S., Schlesinger, W.H., 2004. Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. Soil Biol. Biochem. 36, 581-589.

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. 10, 215-221.

Barg, A.K., Edmonds, R.L., 1999. Influence of partial cutting on site microclimate, soil nitrogen dynamics, and microbial biomass in Douglas-fir stands in western Washington. Can. J. For. Res. 29, 705-713.

Berg, B., Berg, M.P., Bottner, P., Box, E., Breymeyer, A., Deanta, R.C., Couteaux, M., Escudero, A., Gallardo, A., Kratz, W., Madeira, M., Malkonen, E., Mcclaugherty, C., Meentemeyer, V., Munoz, F., Piussi, P., Remacle, J., Desanto, A.V., 1993. Litter mass loss rates in pine forests of Europe and Eastern United-States - some relationships with climate and litter quality. Biogeochemistry 20, 127-159.

Bingeman, C.W., Varner, J.E., Martin, W.P., 1953. The effect of the addition of organic materials on the decomposition of an organic soil. Soil Sci. Soc. Am. Pro. 29, 692–696.

Bradley, R.L., Titus, B.D., Hogg, K., Preston, C., Prescott, C.E., Kimmins, J.P., 2000. Assessing the controls on soil mineral-N cycling rates in managed coastal western hemlock ecosystems of British Columbia. J. Sustain. For. 10, 213-219.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837-842.

Burger, J.A., Pritchett, W.L., 1984. Effects of clearfelling and site preparation on nitrogen mineralization in a southern pine stand. Soil Sci. Soc. Am. J. 48, 1432-1437.

De Santo, A.V., Berg, B., Rutigliano, F.A., Aleani, A., Fioretto, A., 1993. Factors regulating early stage decomposition of needle litters in five different coniferous forests. Soil Biol. Biochem. 25, 1423-1433.

Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biol. Biochem. 35, 837-843.

Grossman, R.B., Reinsch, T.G., 2002. The Solid Phase, Bulk Density and Linear Extensibility. In: Dane, J.H., Topp, G.C. (Eds.), Methods of Soil Analysis Part 4 - Physical Methods. Soil Science Society of America, Madison, WI, pp. 201-228.

Hart, S.C., Nason, G.E., Myrold, D.D., Perry, D.A., 1994. Dynamics of gross nitrogen transformations in an old growth forest: the carbon connection. Ecology 75, 880-891.

Kim, C., Sharik, T.L., Jurgensen, M.F., 1995. Canopy cover effects on soil nitrogen mineralization in northern red oak *(Quercus rubra)* stands in northern lower Michigan. For. Ecol. Manage. 76, 21-28.

Kimmins, J.P., 1997. Biogeochemistry, Cycling of Nutrients in Ecosystems. In: kimmins, J.P. (Ed.), Forest Ecology, a Foundation for Sustainable Management. Prentice Hall, Saddle River, N.J., pp. 71-129.

Li, Q.C., Allen, H.L., Wilson, C.A., 2003. Nitrogen mineralization dynamics following the establishment of a loblolly pine plantation. Can. J. For. Res. 33, 364-374.

Likens, G.E., Bormann, F.H., Johnson, N.M., Fisher, D.W., Pierce, R.S., 1970. Effect of forest cutting and herbicides treatement on nutrient budgets in the Hubbard Brook watershed-ecosystem. Ecol. Monogr. 40, 23-47.

Nambiar, E.K.S., Kallio, M.H., 2008. Increasing and Sustaining Productivity in Tropical Forest Plantations: Making a Difference through Cooperative Research and Partnerships. In: Nambiar, E.K.S. (Ed.), Site Management and Productivity in Tropical Plantation Forests: Workshop Proceedings, 22-26 November 2004 Piracicaba, Brazil, and 6-9 November, Bogor, Indonesia Center for International Forestry Research (CIFOR), Bogor, Indonesia., pp. 205-228.

Nordgren, A., 1992. A method for determining microbially available N and P in an organic soil. Biol Fert Soils 13, 195-199.

Paul, E.A., Clark, F.E., 1996. Soil Microbiology and Biochemistry. Academic Press, San Diego, CA.

Sariyildiz, T., Anderson, J.M., 2003. Interactions between litter quality, decomposition and soil fertility: a laboratory study. Soil Biol. Biochem. 35, 391-399.

SAS, 2005. The SAS System for Windows Release 9.1.3. SAS Institute Inc., Cary, NC.
Schmidt, S., Stewart, G.R., 1999. Glycine metabolism by plant roots and its occurrence in Australian plant communities. Aust. J. Plant Physiol. 26, 253-264.

Smethurst, P.J., Nambiar, E.K.S., 1989. Role of weeds in the management of nitrogen in a young *Pinus radiata* plantation. New Forest. 3, 203-224.

Tamm, C.O., 1964. Determination of nutrient requirements of forest stands. Int. Rev. For. Res. 1, 115-170.

Titus, B.D., Roberts, B.A., Deering, K.W., 1997. Soil solution concentrations on three white birch sites in central Newfoundland following different harvesting intensities. Biomass Bioenerg. 13, 313-330.

Turnbull, M.H., Schmidt, S., Erskine, P.D., Richards, S., Stewart, G.R., 1996. Root adaptation and nitrogen source acquisition in natural ecosystems. Tree Physiol. 16, 941-948.

Vitousek, P.M., Andariese, S.W., 1986. Microbial transformations of labelled nitrogen in a clearcut pine plantation. Oecologia 68, 601-605.

Vitousek, P.M., Andariese, S.W., Matson, P.A., Morris, L., Sanford, R.L., 1992. Effects of harvest intensity, site preparation, and herbicide use on soil-nitrogen transformations in a young loblolly-pine plantation. For. Ecol. Manage. 49, 277-292.

Vitousek, P.M., Matson, P.A., 1984. Mechanisms of nitrogen retention in forest ecosystems - a field experiment. Science 225, 51-52.

Table 1. Maximum soil respiration response (µg C mg MBC^{-1} hr^{-1}) after water and carbon additions, in a forest floor retention and incorporation study in the Coastal Plain of the Southeast, US established immediately after harvesting a loblolly pine plantation. Orthogonal contrasts compare the retention and incorporation treatments against control and non-mixed respectively. Standard errors shown in parenthesis (n = 5).

Treatment factors >		Forest floor Retention					Forest floor incorporation with mineral soil		
Field Treatment >		Removed		Control	Doubled		Non-Mixed	Mixed	
Lab treatment	Month	Mean	Contrast vs. Control	Mean	Mean	Contrast vs. Control	Mean	Mean	Contrast vs. Non-Mixed
Water	1	3.7 (0.7)	0.48	3.2 (0.3)	3.8 (0.3)	0.36	3.6 (0.5)	3.5 (0.3)	0.93
	4	4.1 (0.6)	0.51	3.7 (0.3)	3.9 (0.3)	0.65	3.7 (0.5)	4.1 (0.2)	0.40
	8	3.9 (0.6)	0.39	4.4 (0.3)	5.7 (0.5)	**<0.05**	4.4 (0.4)	5.0 (0.4)	0.20
	15	1.6 (0.1)	0.05	1.9 (0.1)	2.4 (0.3)	**<0.05**	1.7 (0.1)	2.2 (0.2)	**<0.01**
	19	1.1 (0.1)	0.11	1.5 (0.2)	1.5 (0.1)	0.99	1.3 (0.1)	1.4 (0.2)	0.42
C	1	61 (9)	0.38	51 (9)	42 (4)	0.42	56 (8)	46 (4)	0.29
	4	114 (9)	**<0.01**	82 (7)	85 (7)	0.71	81 (6)	107 (7)	**<0.01**
	8	130 (12)	0.31	113 (10)	126 (12)	0.45	115 (10)	131 (7)	0.23
	15	71 (10)	0.12	52 (9)	53 (8)	0.92	50 (6)	67 (7)	0.09
	19	38 (5)	0.20	26 (4)	42 (8)	0.09	35 (5)	35 (5)	0.99

Table 2. P-values from Repeated measures analyses on maximum soil respiration response (μg C mg MBC^{-1} hr^{-1}) and 2M KCl extractable N (kg ha^{-1}) after water and carbon additions, in a forest floor retention and incorporation study in the Coastal Plain of the Southeast, US established immediately after harvesting a loblolly pine plantation. FF = Forest floor retention field treatments; Incorp = Incorporation field treatment.

Dependent Variable	Effect						
	FF	Incorp.	Time	FF* Incorp.	FF*Time	Incorp. *Time	FF* Incorp.*Time
Max. microbial respiration after additions of WATER	**<0.01**	**<0.05**	**<0.0001**	**<0.01**	0.15	0.40	0.17
Max. microbial respiration after additions of C	**<0.01**	**<0.05**	**<0.0001**	0.41	0.57	0.07	0.72
2M KCl extractable N after addition of WATER	0.06	0.71	**<0.0001**	0.10	**<0.0001**	**<0.05**	**<0.01**
2M KCl extractable N after addition of C	0.08	0.63	**<0.0001**	0.81	0.07	0.17	0.64

Table 3. Treatment means of 2 M KCl extractable N (kg ha^{-1}) after water and carbon additions, in a forest floor retention and incorporation study in the Coastal Plain of the Southeast, US established immediately after harvesting a loblolly pine plantation. Orthogonal contrasts compare the retention and incorporation treatments against control and non-mixed respectively. Standard errors shown in parenthesis (n = 5).

Treatment factors >		Forest floor Retention					Forest floor incorporation with mineral soil		
Field Treatment >		Removed		Control	Doubled		Non-Mixed	Mixed	
Lab treatment	Month	Mean	Contrast vs. Control	Mean	Mean	Contrast vs. Control	Mean	Mean	Contrast vs. Non-Mixed
Water	1	50.7 (3.7)	**<0.001**	31.6 (3.5)	26.7 (3.9)	0.29	38.4 (2.8)	34.2 (4.9)	0.27
	15	14.7 (1.4)	**<0.05**	20.7 (1.8)	30.7 (3.3)	**<0.01**	19.1 (1.8)	25.0 (2.9)	**<0.05**
C	1	3.0 (0.7)	0.07	1.6 (0.3)	3.3 (0.5)	**<0.05**	3.0 (0.4)	2.3 (0.5)	0.32
	15	0.5 (0.1)	0.15	0.9 (0.2)	0.9 (0.2)	0.84	0.6 (0.1)	0.9 (0.2)	0.11

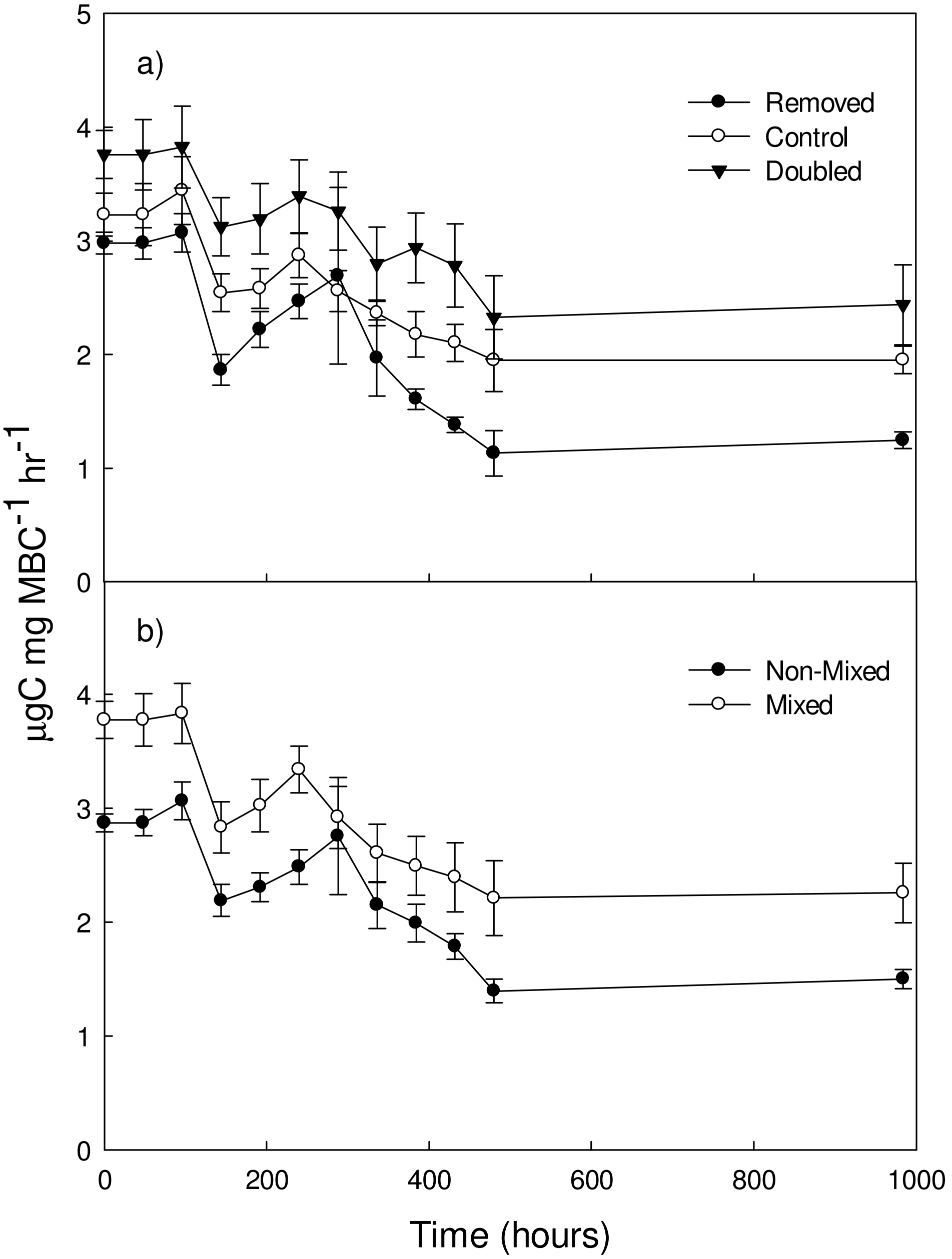

Figure 2. Mineral soil respiration rates measured in the laboratory after addition of 0.25 mL of de-ionized water g soil^{-1} four months after the imposition of field treatments. The forest floor retention treatments a) Removed = 0 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Doubled = 31,700 kg ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed immediately after harvesting a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

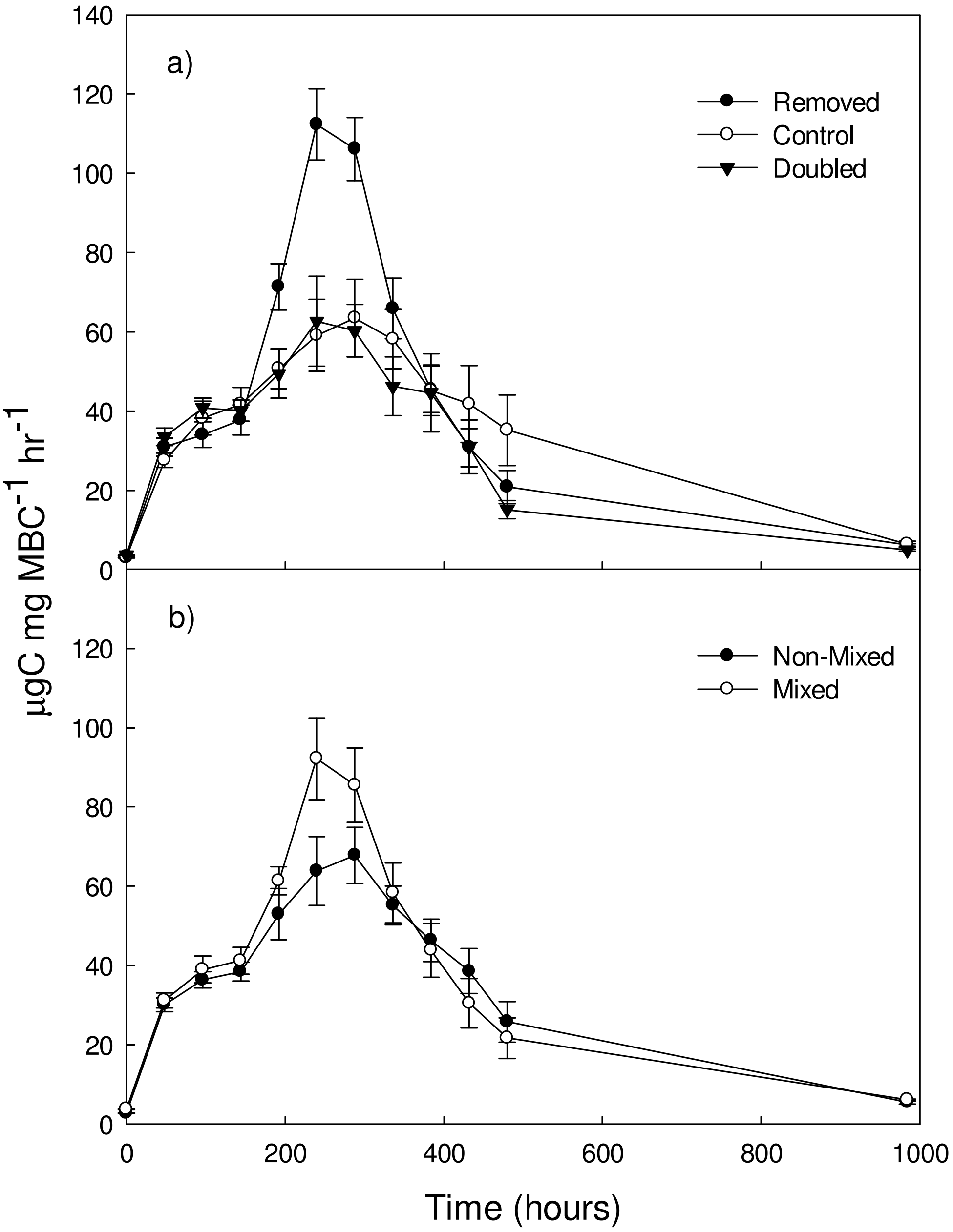

Figure 3. Mineral soil respiration rates measured in the laboratory after addition of 7.7 mg glucose C and 0.25 mL of de-ionized water g soil^{-1} four months after the imposition of field treatments. The forest floor retention treatments a) Removed = 0 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Doubled = 31,700 kg ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed immediately after harvesting a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

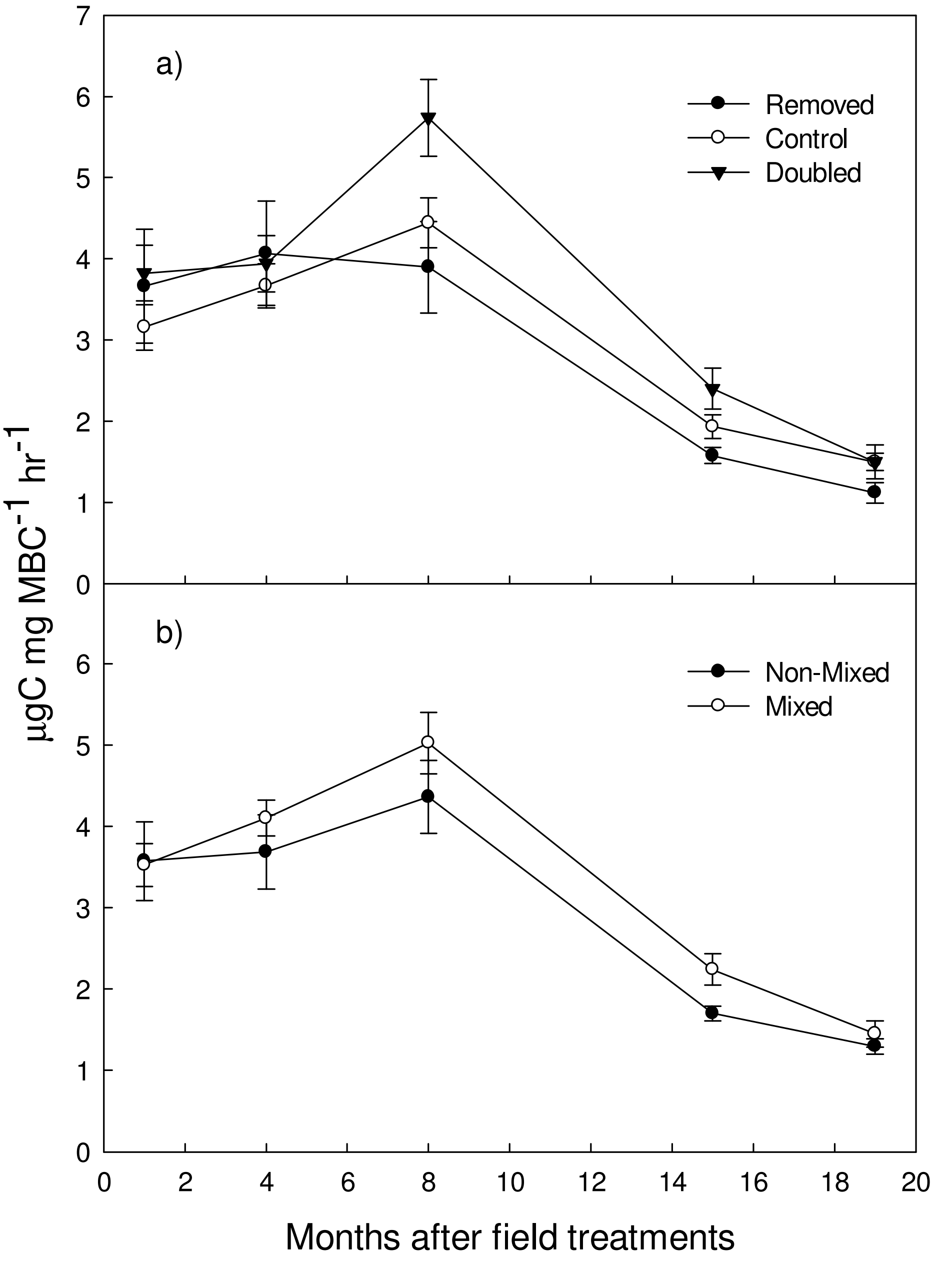

Figure 4. Mineral soil maximum respiration rates measured in the laboratory after addition of 0.25 mL of de-ionized water g soil^{-1}. The forest floor retention treatments a) Removed = 0 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Doubled = 31,700 kg ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed immediately after harvesting a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.

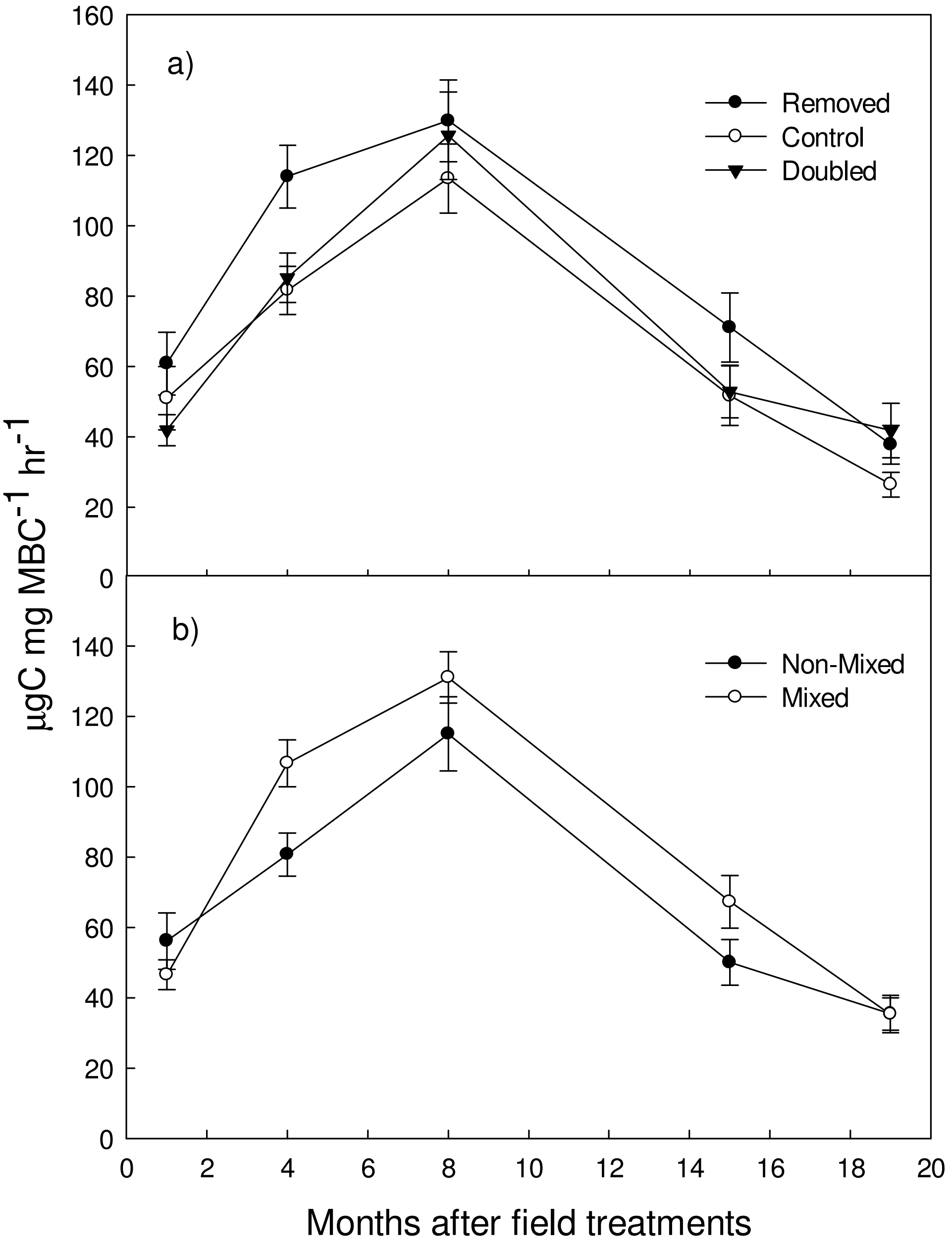

Figure 5. Mineral soil maximum respiration rates measured in the laboratory after addition of 7.7 mg glucose C and 0.25 mL of de-ionized water g soil^{-1}. The forest floor retention treatments a) Removed = 0 kg ha^{-1}, Control = 15,600 kg ha^{-1}, and Doubled = 31,700 kg ha^{-1} and the incorporation treatments b) Mixed, and Non-Mixed were imposed immediately after harvesting a loblolly pine plantation in the Southeast, US. Error bars = 1 S.E.