SOIL RETENTION AND SUBSEQUENT UPTAKE OF NITROGEN 2 YEARS FOLLOWING OPERATIONAL RATES OF FERTILIZATION IN LOBLOLLY PINE

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Abstract—In this experiment, ^{15}N-labelled fertilizers were used to trace nitrogen uptake, assimilation, and fate in four loblolly pine (*Pinus taeda*) stands fertilized with 224 kg ha^{-1} of elemental nitrogen (N) in the Southeastern United States. The fertilizer treatments included a non-fertilized control, urea, and three different enhanced efficiency N fertilizers. Two years after fertilization, soil from the upper 15 cm in each of five treatments from four different locations was collected. A greenhouse study was established to examine the availability of the residual fertilizer N in the soil. Two bare-root loblolly pine seedlings were planted in each of five replicate pots for a total of 200 seedlings in 100 pots. Total soil N, soil N availability, and seedling N uptake was measured periodically over 15 weeks, and the ^{15}N to ^{14}N ratio was analyzed on soil and seedlings prior to planting and at harvest. Significant (alpha = 0.05) increase in ^{15}N to ^{14}N ratio was found in seedlings 3 weeks after planting in the fertilized treatments. We determined that significant (alpha = 0.05) amounts of residual fertilizer N following operational fertilization was available to loblolly pine seedlings 2 years after application.

INTRODUCTION

Loblolly pine (*Pinus taeda*) is the most widely used plantation species in the Southeastern United States with over 16 million ha in plantations in 2010 (Wear and Greis 2012). Fertilization with nitrogen (N) and phosphorous (P) has been repeatedly shown to increase stem wood volume growth by increasing leaf area which captures more solar radiation and increases net photosynthetic production (Albaugh and others 1998, Carlson and others 2014, Vose and Allen 1988). Between 1998 and 2004, more than 400 000 ha of loblolly pine stands received mid-rotation fertilization with N and P annually (Albaugh and others 2007). With this level of investment in fertilization, it is important to understand how long applied N is available for plant uptake in these forested systems.

A research project was initiated in 2012 to investigate the fate of applied N in loblolly plantations across the Southeast. This study utilized urea enriched with the stable isotope 15N as a tracer (Raymond and others 2016). Urea and three different enhanced efficiency N fertilizers (EEFs) designed to reduce volatilization were tested and compared to an untreated control. After 1 year, Raymond and others (2016) found that 29–39 percent of the applied fertilizer N remained in the soil.

The objectives of this study were to: 1) determine if applied fertilizer N is plant-available in the soil 2 years after fertilization; and 2) determine if the application of enhanced efficiency fertilizers increases long term plant-available N in the soil more than urea.

METHODS

Field Study Experimental Design

Soils used in this study were collected from a subset of the locations established as part of a large regional fertilization study (Raymond and others 2016). The original study was established as a random complete-block design with five fertilizer treatments at 12 sites supporting mid-rotation loblolly pine plantations across the Southeastern United States. The five fertilizer treatments used in this study were: (1) urea; (2) urea impregnated with N-(n-Butyl) thiophosphoric triamide (NBPT); (3) urea impregnated with NBPT and coated with monoammonium phosphate (CUF); (4) polymer-coated urea (PCU); and (5) a control treatment with no fertilizer added. Urea (46-0-0) was used because it is the most common N fertilizer applied in the Southeastern United States. The enhanced efficiency fertilizers (EEFs) tested in this study were developed to reduce NH_3 volatilization and release fertilizer N slowly to the environment. The NBPT treatment (46-0-0) added NBPT at a rate of 26.7 percent by weight to urea granules to inhibit urease activity. The CUF treatment (39-9-0) also added NBPT to urea granules, which was then coated with an aqueous binder solution of boron and copper sulfate to slow N release. A final coating of CUF was

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added to provide phosphorus (P). The PCU (44-0-0) treatment encapsulated urea granules with a polymer coating containing pores designed to slowly release N (~80 percent) over 120 days. All N treatments were applied at an equivalent rate of 224 kg N ha^{-1}. Because the CUF treatment had P in a coating, P was applied in the other fertilizer treatments at the equivalent rate of 28 kg P ha^{-1} as triple superphosphate (TSP). The urea in all treatments was enriched with the stable isotope ^{15}N (0.5 atom percent). Each fertilizer treatment was broadcast applied by hand in individual 100-m^2 circular plots at each site on the same day between March 26 and April 8, 2012. Details of this study can be found in Raymond and others (2016).

Greenhouse Experimental Design

The four study locations included in this greenhouse experiment were co-located with the PINEMAP Throughfall Exclusion by Fertilization Experiment (Pinemap.org). The stands were located in the Georgia Piedmont (33°37′35″ N, 82°47′54″ W), Florida Coastal Plain (30°12′22″ N, 83°52′12″ W), Oklahoma Upper Coastal Plain (34°01′47″ N, 94°49′23″ W), and Virginia Piedmont (37°27′37″ N 78°39′50″ W) (fig. 1). The stands ranged in age from 4 to 9 years, and stand density ranged from 789 to 1610 trees ha^{-1}. Will and others (2015) provides detailed site, stand, and climatic descriptions.

In April of 2014, 2 years after initial N fertilization applications, soil from the upper 15 cm of the mineral soil (excluding the forest floor) was collected from each of the five fertilizer treatments. This soil was sieved using a 12-mm screen to remove rocks and roots and to homogenize the sample. Five replicate 4-L plastic pots were filled with soil from each site by treatment combination for a total of 100 pots. Two 1-0 bare-root loblolly pine seedlings were planted in each pot on May 24, 2014. One pot from each site by treatment combination was randomly assigned to each of five benches (blocks) in the greenhouse. Pots were watered every 2 days until drainage was visible from the bottom of the pots. After 21 days, the smaller of the two seedlings in each pot was cut at the ground line. The new growth on the terminal was separated for ^{15}N analysis. The remaining seedlings were grown in the greenhouse for an additional 12 weeks.

Measurements

Seedling height and ground-line diameter were taken at 0, 21, 69, and 112 days. Soil N concentration and ^{15}N were assessed at 0 and 112 days. Tissue N concentration and ^{15}N assessments were also measured at 0, 21, and 112 days.

Tissue samples from the seedlings were dried in a forced air oven at 60 °C. After drying, all samples were coarse

Figure 1—PINEMAP Throughfall Exclusion by Fertilization Experiment study locations where soil was collected. The outlined area on the map represents the natural range for loblolly pine (Pinemap.org).

ground in a Wiley Mill to pass a 2-mm sieve. The organic samples were then homogenized to a fine powder with a ball mill (Retsch® Mixer Mill MM 200, Haan, Germany) for 1 minute at 25 revolutions per second (rps). Mineral soil samples were ball milled for 2 minutes at 25 rps. After ball milling, individual homogenized samples were put in separate tin capsules and weighed on a Mettler-Toledo© MX5 microbalance (Mettler-Toledo, Inc., Columbus, OH, USA). These individually weighed samples were analyzed to determine the $^{15}N/^{14}N$ isotope ratio and total N on a coupled elemental analysis-isotope ratio mass spectrometer (IsoPrime 100 EA-IRMS, Isoprime© Ltd., Manchester, UK) All grinding, ball milling, and weighing equipment was cleaned after each sample with ethanol to reduce contamination.

An N availability index using cation and anion exchange membranes was estimated over the period from day 21 to day 112. Cation exchange membranes and anion exchange membranes (GE Power and Water, Trevose, PA, USA) were used to measure ammonium and nitrate concentrations, respectively, and to provide an index of cumulative N availability in the soil solution over the course of their deployment (Cheesman and others 2010). One anion and one cation exchange membrane (10 cm * 5cm) was inserted vertically in each pot after one seedling had been harvested. Exchange membranes were left for 6 weeks and then removed, extracted, and returned to the pots for the final 6-week period. Laboratory procedure followed those described by Cooperband and Logan (1994). Extracts were analyzed for ammonium and nitrate N concentrations using a TRAACS 2000 analytical console (Bran & Luebbe, Norderstedt, Germany).

RESULTS AND DISCUSSION

Initial total soil N was highly variable and ranged from 0.036 percent in the CUF treatment in Georgia to 0.143 percent in the control in Oklahoma (fig. 2). At all sites except Florida, the control treatment had higher total soil N than the fertilized treatments (fig. 2). If we assume that a 15-cm furrow slice weighs 2000 Mg ha^{-1}, then 720 to 2860 kg ha^{-1} of N is present in these soils. As stated earlier from Raymond and others (2016), 30 percent of the applied fertilizer was present in the soil after 1 year or approximately 60 kg ha^{-1} of fertilizer N. Therefore similar to the findings of Miller (1981), natural sources of total soil N exceed fertilizer N by a factor of 12 in the lowest case and 47 in the highest case.

N availability index based on the ion exchange membranes was also highly variable (fig. 3), and there were no significant differences (alpha = 0.05) among treatments or sites. The overall site means follow the same trend as total initial total soil N with Florida having the lowest means and Oklahoma the highest. These results support conclusions by many prior researchers (Brady and Weil 2013, Johnson and Todd 1988, Miller and Gardiner 1997) that conventional total soil N and N availability indexes are not very useful for testing or predicting long-term fertilizer response.

The elevated ^{15}N to ^{14}N ratios (fig. 4) of the fertilized soil compared to the controls for each site at day 0 indicate that fertilizer N was still present in the soil 2 years after application. Analysis of ^{15}N to ^{14}N ratios in the new growth on the terminal of seedlings after 21 days indicated significantly elevated ^{15}N above control levels in all of the fertilized treatments (fig. 5). The Georgia and Oklahoma sites had higher ^{15}N in the ESN and NBPT treatments over the urea and CUF. The proportion of N from fertilizer was calculated using the method of Nadelhoffer and Fry (1994). The residual fertilizer N taken up by the seedlings in the 21-day new growth was small compared to the overall N uptake. The percent of the total N taken up by the seedlings derived from the residual ^{15}N-labeled fertilizer averaged 1.1 percent and ranged from 0.25 percent for the urea treatment from Florida to 3.2 percent for the PCU and NBPT treatments from Oklahoma. These results demonstrate that some of the ^{15}N fertilizer applied 2 years prior to the study is still present in a form that is available for loblolly pine uptake. However, when the initial proportion of fertilizer N in the soil is compared to the mean proportion of fertilizer N in the new growth for each site (fig. 6), it is evident that proportionally less fertilizer N is being take up than is present in the soil. This suggests that the fertilizer N is immobilized in some fashion either by heterotrophs, in other organic compounds, or bound as NH_4^+ to clay or humus (Brady and Weil 2013). Further investigation is needed to determine the means of fertilizer N immobilization and whether more of the fertilizer N can be made plant-available over time.

CONCLUSIONS

Conventional measures of total and “available” soil N are not very useful for evaluating long-term fertilizer response. Fertilizer N is still present in the soil 2 years after fertilization. Some of the fertilizer N present in the soil is plant-available.

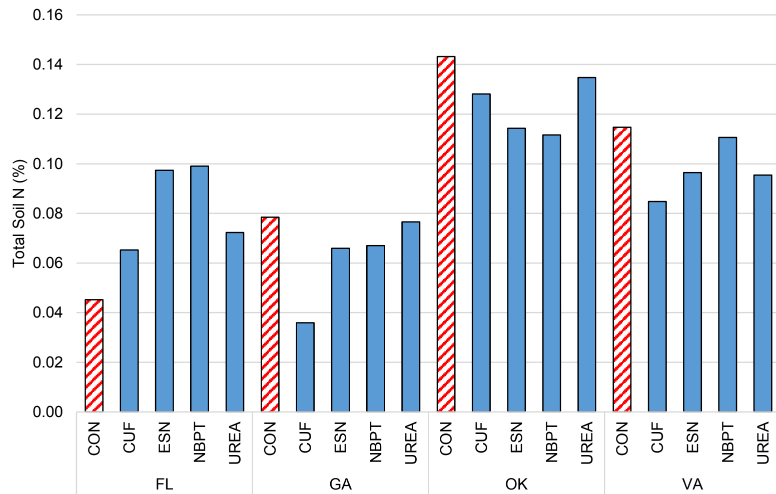

Figure 2—Total soil nitrogen percent at day 0 for each site by fertilizer treatment combination 2 years after fertilizer application to the soil. The hatched bars highlight the control treatment at each site.

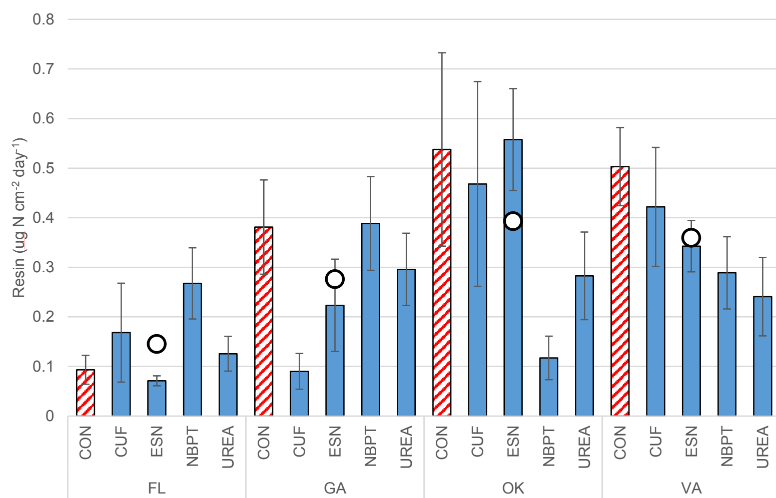

Figure 3—Mean soil nitrogen availability index determined with ion exchange membranes from day 21 to day 112 in ug N cm^{-2} day^{-1}. The hatched bars highlight the control treatment at each site. Error bars indicate the standard error (n = 5), and the circles indicate the site mean.

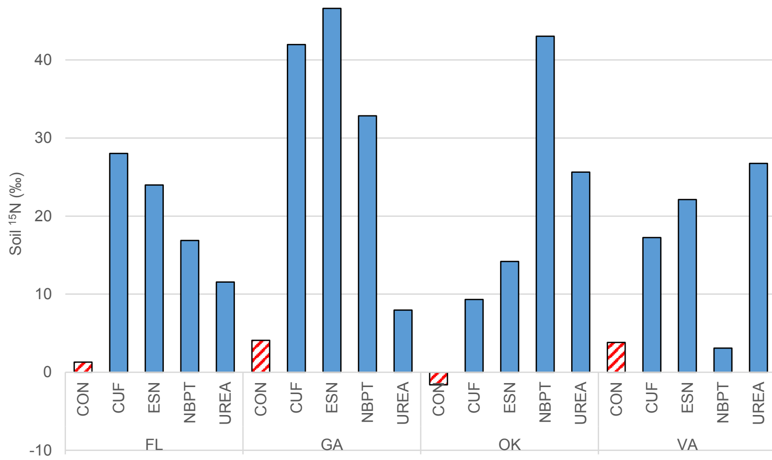

Figure 4—Soil ^{15}N to ^{14}N ratios at day 0 of the greenhouse experiment for each site by fertilizer treatment combination 2 years after fertilizer application to the soil. The hatched bars highlight the control treatment at each site.

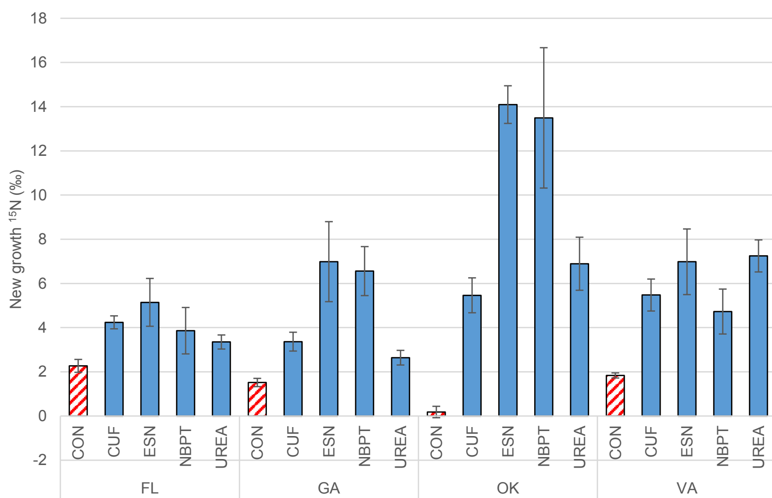

Figure 5—Mean ^{15}N to ^{14}N ratios for tissue from the growing terminal over the first 21 days. The hatched bars highlight the control treatment at each site. Error bars indicate the standard error (n = 5).

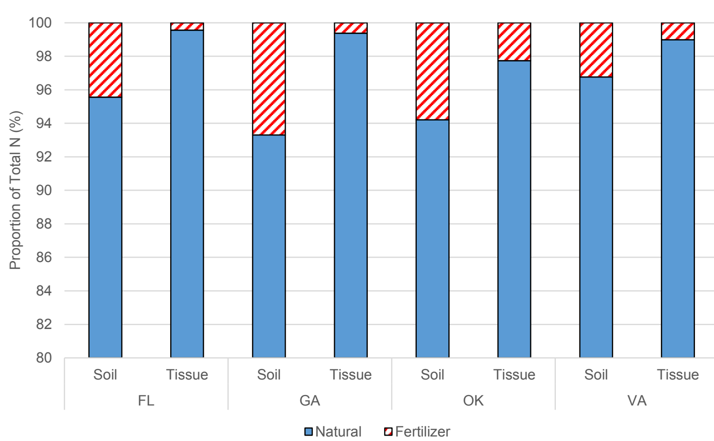

Figure 6—A side-by-side comparison of the proportion of fertilizer N to total N in the soil and new plant tissue for each site.

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