Influence of Forest Disturbance on Stable Nitrogen Isotope Ratios in Soil and Vegetation Profiles

Soil and plant stable N isotope ratios ($\delta^{15}$N) are influenced by atmospheric N$_2$ inputs and processes that regulate organic matter (OM) transformation and N cycling. The resulting $\delta^{15}$N patterns may be useful for discerning ecosystem differences in N cycling. We studied two ecosystems, longleaf pine (Pinus palustris Mill.)–wiregrass (Aristida stricta Michx.) (LLP) and Appalachian hardwood (AHW) forests in the US Southeast under different management regimes. In LLP, burning removes OM. In AHW, clearcutting creates large OM pulses of logging residue. Although burning removes OM and clearcutting creates a pulse addition of OM, both management regimes increase soil N availability and N$_2$–fixing plants. The LLP treatments included burning every 2 yr with N$_2$ fixers and reference fire exclusion sites without N$_2$ fixers. The AHW included 25-yr-old clearcut plots with and without N$_2$ fixers, and uncut reference without N$_2$ fixers. We hypothesized that: (i) compared with the reference, OM removal (LLP) would enrich $\delta^{15}$N values while OM addition (AHW) would deplete $\delta^{15}$N in soil and vegetation pools; and (ii) N$_2$ fixers would mitigate $\delta^{15}$N enrichment in LLP response and exacerbate $\delta^{15}$N depletion in AHW. We examined total $\delta^{15}$N in soil profiles, tree increment cores, and foliage. The LLP soil and vegetation $\delta^{15}$N values showed no treatment effect. In AHW, $\delta^{15}$N values in clearcut subsurface soils (20–60 cm) were lower than the reference, but N$_2$ fixers had no effect. Wood $\delta^{15}$N differed with treatment; N$_2$ fixers had no effect. Our data suggest that AHW soil profile $\delta^{15}$N patterns may indicate past disturbance; however, wood and foliar $\delta^{15}$N response is species specific. Additionally, N$_2$–fixing plants respond to ecosystem disturbance, but the data suggest that they play little role in soil or plant $\delta^{15}$N values.

Abbreviations: AHW, Southern Appalachian mixed hardwood forest; $\delta^{15}$N, stable nitrogen isotope ratio; LLP, Coastal Plain longleaf pine–wiregrass savanna; OM, organic matter; SOM, soil organic matter.

Soil N often limits primary productivity of temperate forest ecosystems. Plant N availability is regulated by inputs of atmospheric N and the decomposition and mineralization of soil organic matter (SOM) from leaf, wood, fine and coarse root inputs (Alban, 1982; Cole, 1995), and direct uptake by mycorrhizal associations (Hobbie et al., 1999, 2000). The stable N isotope ratios ($\delta^{15}$N) of plant tissue and SOM can provide insight into patterns of N cycling and availability, changes in SOM, inputs of fixed atmospheric N$_2$, and levels of C sequestration (Garten et al., 2007). Soil N isotope fractionation occurs during physical and biological processing of organic and inorganic N compounds, with thermodynamic and enzymatic processes generally discriminating against the heavier isotope (Nadelhoffer and Fry, 1988; Pardo et al., 2013). The result is the proportionally greater loss of N compounds with lower $\delta^{15}$N from the ecosystem through leaching, denitrification, or volatilization (Evans, 2007; Högberg, 1997) and the enrichment of the remaining organic material with $^{15}$N ($\delta^{15}$N values increase). The cumulative effect of these processes is a stable N isotope ratio that reflects not only atmospheric inputs (deposi-
tion and N₂ fixation) to the ecosystem but also the complex series of processes that transform organic and inorganic forms of N to plant-available compounds.

Studies measuring δ¹⁵N have shown that SOM becomes more enriched with δ¹⁵N with increasing depth through the soil profile (Evans, 2007; Högberg, 1997; Mariotti et al., 1980) due to increased residence time and vertical movement of SOM through the soil profile (Fredlund and Tieszen, 1997; Trumbore, 2000). Within an ecosystem, the resulting isotopic profiles represent site characteristics that may be related to forest species composition (Pardo et al., 2007a), site N status and net N transformations (Garten and van Miegroet, 1994; Pardo et al., 2002), soil temperature, water availability, and climate (Boeckx et al., 2005). Alteration of ecosystem δ¹⁵N can occur due to changes in OM inputs by disturbance, changes in atmospheric deposition or N₂ fixation (Högberg, 1997; Nadelhoffer and Fry, 1988), or shifts in vegetation cover (Fredlund and Tieszen, 1997; Mariotti et al., 1980; Tieszen, 1991). Vegetation δ¹⁵N values are affected by the source of available N (Högberg et al., 1999; Pardo et al., 2006) as well as by fractionation that occurs during transfer of N from mycorrhizal fungi to plants (Evans, 2001; Hobie et al., 2000) and during N incorporation into biomass (Pardo et al., 2013). In woody structures (e.g., tree rings), research findings conflict, with some studies concluding that δ¹⁵N values are reliable (Beghin et al., 2011; Bukata and Kyster, 2005) and others finding they are unreliable indicators of changes in N availability during tree growth (Hart and Classen, 2003; Sheppard et al., 2001; Sheppard and Thompson, 2000).

Forest management practices, such as prescribed burning or forest cutting, alter aboveground (leaf litter and wood) and belowground (fine roots) inputs of OM to soils (Binkley et al., 1992; DeBano, 1990; Swank and Webster, 2014). The alteration of SOM quantity and/or quality, along with increased soil temperature and soil water availability, often leads to increased soil NH₄ and NO₃ availability due to increased soil microbial N transformations such as N mineralization and nitrification. As a result, these management practices may affect soil δ¹⁵N values, with initial changes most likely occurring in surface soils in response to the sudden changes in litter input. Additionally, following disturbance, many ecosystems have an increased abundance of early successional plant species associated with symbiotic N₂-fixing bacteria (Boring and Swank, 1984a), facilitating ecosystem recovery by introducing N into the system (Vitousek et al., 2002). Examples of this phenomenon include longleaf pine–wiregrass savannas, with diverse legumes in the herbaceous layer, and southern Appalachian hardwood forests with black locust (Robinia pseudoacacia L.), an N₂-fixing tree species (Boring and Swank, 1984b; Boring et al., 1988; Hendricks and Boring, 1999). Estimates of total N input due to N₂ fixation after disturbance in the longleaf pine forest is up to 16 kg N ha⁻¹ yr⁻¹ in the first year following burning (Cathey et al., 2010; Hainsds et al., 1999) due to the proliferation of up to 43 legume species representing nearly 120,000 stems per hectare. In the Appalachian hardwood forest, black locust became the dominant tree species within 2 yr following harvest, representing the largest pool of aboveground N and fixing an estimated 30 kg ha⁻¹ yr⁻¹ of atmospheric N₂ in the fourth year after harvest (Boring and Swank, 1984a, 1984b; Elliott et al., 2002). These N additions could potentially shift soil δ¹⁵N values because organisms that are actively fixing atmospheric N₂ have a distinctive signature, ranging from −3 to 0‰ (Evans, 2007), compared with a range of −5.9 to 2.7‰ in non-N₂–fixing species (Garten, 1993).

We examined ecosystem δ¹⁵N profiles in the soils and vegetation of a Coastal Plain longleaf pine–wiregrass savanna and a southern Appalachian mixed hardwood forest for responses to forest management and the potential of using this method to identify past forest disturbance. This information may be useful in prescribing forest management or restoration prescriptions. The commonly used management practices in these forests alter OM inputs and result in a significant increase in the abundance of N₂–fixing plant species (Boring and Swank, 1984a; Hainsds et al., 1999). We hypothesized that decreased (repeated prescribed burning) or large pulse (clearcutting) OM inputs to surface soils as well as the proliferation of N₂–fixing plants would result in altered δ¹⁵N values within the soil profile as well as in tree rings laid down during the period following disturbance characterized by increased N availability, N mineralization, and nitrification rates (Knoepp et al., 2014; Waide et al., 1988). Specifically, we expected that soil and wood δ¹⁵N would be more enriched in δ¹⁵N following OM removal by repeated burning compared with unburned reference forests due to increased transformation of the existing SOM. On the other hand, large pulse additions of OM as logging residue during clearcutting were expected to result in depleted soil δ¹⁵N values compared with reference sites due to the addition of OM with lower δ¹⁵N values compared with soils. Initially after disturbance, these changes should be most evident in surface soils and recently produced wood. However, with time, evidence of SOM δ¹⁵N enrichment or depletion would be evident deeper in the soil profile, resulting from decomposition, mineralization, denitrification, and other fractionation processes. We expected the period of disturbance to remain identifiable in the tree rings formed during periods of altered N availability and uptake. Further, we expected that the proliferation of N₂–fixing plants on both sites in response to disturbance and the input of OM with δ¹⁵N of ~0‰ would mitigate any response to OM losses through burning at LLP and exacerbate soil δ¹⁵N responses due to OM inputs from clearcutting at AHW.

MATERIALS AND METHODS

Site Description

Coastal Plain Longleaf Pine–Wiregrass Savanna

The Coastal Plain site was located in southwestern Georgia at the Joseph W. Jones Ecological Research Center at Ichauway (31.22° N, 84.48° W). The climate is characterized by a mean annual air temperature of 19°C and precipitation of 1356 mm (Goebel et al., 2001). The center contains ~6000 ha of longleaf pine–wiregrass savanna that has been managed with prescribed fire every 1 to 3 yr during the dormant season for the past 80 yr.
The landscape is a mosaic of burned and adjacent unburned areas that are a mixture of longleaf pine and *Quercus* spp. Soils are in the Ultisol and Entisol soil orders. Typical vegetation responses to burning include elimination of *Quercus* spp. and a proliferation of N₂-fixing legumes in the herbaceous layer (Hendricks and Boring, 1999); these legumes are unable to persist, due to competition, if a frequent fire regime is not maintained and are largely absent from areas unburned for 5 to 10 yr (Carroll et al., 1994; Hains et al., 1999).

Four sites were chosen at the Jones Center, all on sandy ridges with complex undulating slopes (2.0 ± 0.3%) ranging in elevation from 20 to 200 m. The plots were located on the Wagram (a loamy, kaolinitic, thermic Arenic Kandiudult) or Bigbee (a thermic, coated Typic Quartzipsamment) series (Goebel et al., 1998). Wagram soils are characterized by a solum thickness of 152 to 203 cm, depth to bedrock of >203 cm, and depth to seasonal high water table of >153 cm; while Bigbee soils are characterized by a solum thickness of 201 to 244 cm or more and a depth to bedrock of >244 cm (Soil Survey Staff, 2014). On each site, we selected paired plots: one in a prescribed burned area (burned every 2 yr) and one in a reference area and density) of black locust (*Robinia pseudoacacia* L.), a N₂-fixing tree (Elliott et al., 2002). This species does not persist during ecosystem development and is largely absent from mature forests (Elliott et al., 1997).

**Southern Appalachian Mixed Hardwood Forest**

The southern Appalachian hardwood site was located in the US Forest Service’s Coweeta Hydrologic Laboratory (35.3° N, 83.27° W), a 2250-ha experimental forest in western North Carolina. The climate is characterized by a mean annual air temperature of 13°C and precipitation of 1800 mm (Swift et al., 1988). The vegetation is characterized as mixed hardwood, and soils are in the Inceptisol or Ultisol soil orders. Typical vegetation response to timber removal in the southern Appalachians includes an increase in the importance (a combination of basal area and density) of black locust (*Robinia pseudoacacia* L.), a N₂-fixing tree (Elliott et al., 2002). This species does not persist during ecosystem development and is largely absent from mature forests (Elliott et al., 1997).

Sites sampled for this experiment were in a recovering clearcut watershed and an adjacent reference watershed. The former was clearcut, bole removal only, with all stems >5 cm cut during site preparation, in 1976 to 1977 and was 25 yr old (post-harvest) at the time of sampling; the latter was unmanaged since partial cutting occurred in 1927, leaving approximately 75 yr since disturbance at the time of sampling (Swank et al., 1988). Both watersheds have slopes of 23 to 81%, ranging in elevation from 720 to 1065 m. All sample plots were located in the Chandler soil series (a coarse-loamy, micaceous, mesic Typic Dystrudept) (Soil Survey Staff, 2014) characterized by the presence of a Bw horizon, with total depth of the solum ranging from 50 to 100 cm and depth to bedrock of >180 cm. We selected six plots with a 10-m radius, which included six overstory trees, in the recovering clearcut watershed with similar slope position and aspect.

Vegetation composition included tulip poplar (*Liriodendron tulipifera* L.) and chestnut oak (*Quercus prinus* L.) and/or white oak (*Q. alba* L.); oak samples were combined for all statistical analyses and are referred to as *Quercus* spp. Three plots had black locust present within the plot, and three plots had no visible presence of black locust trees within the plot or within 5 m of the plot edge (n = 6). In the reference watershed, we established three plots 20 m in radius, which included six overstory trees, with species composition similar to the clearcut plots but excluding black locust (n = 3; total n = 9); the larger plot size was required to allow inclusion of a similar number of individual trees.

**Longleaf Pine Savanna Sample Collection**

We sampled O horizon and mineral soils, tree-ring increment cores, foliage, and roots from the replicate sites representing burned and unburned management treatments for total N δ¹⁵N analysis. The O horizon and mineral soil samples were collected at three to six random locations within each plot. All samples were composited by plot for analysis. The O horizon samples were collected using 10-cm polyvinyl chloride (PVC) rings and divided into Oi, Oe, and Oa layers, except in the LLP burned site where no Oa layer was present (n = 24). Soils were collected in incremental depths similar to site soil profile descriptions, using a 2.5-cm soil probe, at 0- to 2.5-, 2.5- to 10-, 10- to 20-, and 20- to 30-cm depths below the mineral soil surface (n = 32). Soils from each plot were composited by plot before analysis.

We collected one tree-ring increment core from each of the six dominant trees, using a 5-mm-diameter stainless steel increment borer. Core length generally comprised the entire radius of the tree. The cores were allowed to air dry for 48 h, then sealed in separate bags and refrigerated until dissection. Increment cores were visually dated and segmented based on disturbance period, representing years before (16–23) and since (0–15) the last fire on the unburned reference area.

Upper canopy leaves were collected with pole pruners and hand shears from the six dominant trees in each site and plot during June and July of 2002 (n = 48). The species sample varied by treatment; loblolly pine was sampled on both burned and unburned reference plots, while *Quercus* spp. were present on unburned plots only. All canopy foliar samples were composited by species by plot. Foliar samples from the four to six dominant N₂-fixing legume species were also collected from the burned LLP site within a 2-m radius of the plot center point; legumes were absent in the unburned reference plots. Fine-root samples (<2-mm diameter) were collected from four random locations within each plot using a 6-cm auger to depths of 0 to 10, 10 to 20, and 20 to 30 cm. Soil plus roots were refrigerated until the roots were manually separated from the soil, washed, and oven dried at 50°C. Bulk root samples from each plot were composited by depth (n = 24).

**Hardwood Forest Sample Collection**

We sampled O horizon and mineral soils, increment cores, foliage, and roots from the replicate sites representing uncut ref-
ere, clearcut with black locust, and clearcut without black locust AHW for $\delta^{15}N$ analysis of the total N. The O horizon and mineral soil samples were collected at three to six random locations within each plot. All samples were composited by plot for analysis. The O horizon samples were collected using 10-cm PVC rings and divided into Oi, Oe, and Oa layers ($n = 27$). Soils were collected in incremental depths similar to site soil profile descriptions, using a 2.5-cm soil probe, at 0- to 10-, 10- to 20-, 20- to 40-, and 40- to 60-cm depths. Soils from each plot were composited by depth ($n = 36$) and sieved to <2 mm.

We collected two tree-ring increment cores from each of the six dominant trees on each plot, using a 5-mm-diameter stainless steel increment borer. Increment cores from clearcut sites (with six dominant trees on each plot, using a 5-mm-diameter stainless steel increment borer) were dissected into three segments—0- to 10-, 10- to 20-, and 20- to 30-yr segments.

Upper canopy leaves were collected from the six dominant trees in each site and plot with extendable pole pruners and/or by shooting branches out of the canopy with a 20-gauge shotgun during June and July of 2002 ($n = 54$). Quercus spp. and tulip poplar were present on all plots; black locust was present on only the clearcut plots with N$_2$ fixers. All canopy foliar samples were composited by species by plot. Fine-root samples (<2-mm diameter) were collected from four random locations within each plot using a 6-cm auger to depths of 0 to 10, 10 to 20, 20 to 40, and 40 to 60 cm. Soil plus roots were refrigerated until the roots were manually separated from the soil, washed, and oven dried at 50°C. Bulk root samples from each plot were composited by depth ($n = 27$).

Sample Preparation and Analysis

All soil and plant tissue samples were dried at 50°C to a constant mass. Soil, wood segments, and plant samples were pulverized to a fine powder with a ball mill (SPEX 8000-D Mixer/ Mill, SPEX SamplePrep). Plant tissue (excluding wood) was homogenized and ground to <1 mm using a Wiley mill (Model 4, Thomas Scientific). Wood samples were pretreated with commercial 3% H$_2$O$_2$, adjusted to pH 12 with NaOH, vacuum filtered, and rinsed three times with deionized H$_2$O (Sheppard, and without N$_2$ fixers) were dissected into three segments—0- to 10-, 10- to 20-, and >20-yr segments—and cores from references sites were dissected into 0- to 10-, 10- to 20-, and 20- to 30-yr segments.

We used an endmember mixing calculation to estimate the contribution of N$_2$–fixing plant foliage to the $\delta^{15}N$ value of the forest floor at LLP and AHW (Christophersen et al., 1990; Deutsch et al., 2012; Haag et al., 2000). We presumed that the reference non-N$_2$–fixing plant foliage and N$_2$–fixing plant foliage were the only contributors of $^{15}N$ to the forest floor; all calculations were made using plot means. At LLP, the reference plant material was loblolly pine foliage and the N$_2$–fixing plant $\delta^{15}N$ value was the mean of all N$_2$–fixing legumes collected. Because of the forest floor structure in which longleaf pine needles remain elevated on the wiregrass while other leaf material falls through, we used the Oe horizon $\delta^{15}N$ value as the final N pool (Hendricks et al., 2002). At AHW, we used the mean $\delta^{15}N$ values for Quercus spp. and tulip poplar foliage as the reference plant material and black locust foliage as the N$_2$–fixing contributor to estimate the $\delta^{15}N$ of the Oi horizon. We estimated the proportion of total N contributed by N$_2$–fixing plant foliage using published values of forest floor mass (Knoepp et al., 2000; Markewitz et al., 2002). Estimation of N$_2$–fixing plant material in the mineral soil was not possible. The Oa horizon and mineral soil $\delta^{15}N$ values show evidence of enrichment, violating the mixing model assumption that both endmembers are nonreactive and conservative (Christophersen et al., 1990).

Estimates of atmospheric inorganic N deposition were obtained from National Atmospheric Deposition Program (NADP) sites located near or at the study sites (http://nadp.sws.uiuc.edu/). We used the mean NH$_4^+$ + NO$_3^-$ deposition (kg ha$^{-1}$) from 1998 to 2006 to include years before and after sample collection.

Statistical Analysis

We examined the effect of treatment on soils by site as a two-factor, split-plot design on soil profile total soil C, total soil N, $\delta^{15}N$ values, and total soil $^{15}N$ content using PROC Mixed in SAS (SAS Institute, 2013). Treatment was a fixed effect with two levels at LLP (burn with N$_2$–fixation and unburned reference) and three levels at AHW (clearcut without N$_2$–fixation, clearcut with N$_2$–fixation, and uncut reference); we included plot within site as a random effect. Both sites had seven soil horizons; the LLP Oa horizon was absent at the burn with N$_2$–fixation treatment. We tested the effect of treatment and wood core segments at LLP as a two-factor split-plot design; treatment and incre-
ment core segment were fixed effects. There were two treatments and two wood core segments (0–15 and 16–23 yr before collection). Treatment, core segment, and species effects were tested at AHW as a three-factor split-plot design with three treatments, three core segments (0–10, 10–20, and 20–30 yr before collection), and two tree species (Quercus spp. and tulip poplar).

We tested the effect of treatment only on longleaf pine foliage $^{15}$N at LLP as a split-plot design and the effect of treatment and tree species (Quercus spp. and tulip poplar) as a two-factor split-plot design at AHW. Treatment and soil depth effects on bulk fine-root tissue $^{15}$N were analyzed as a two-factor, split-plot design at both LLP and AHW (SAS Institute, 2013).

The plot mean for each sample type served as the experimental unit. All data were normally distributed. We used a post-hoc means separation test (Tukey–Kramer adjusted means) to determine significant differences among mean $^{15}$N values within significant main effects and interactions.

**RESULTS**

**Ecosystem Nitrogen Inputs**

The estimated contribution to the LLP forest floor Oe horizon of foliar N by N$_2$–fixing legumes was 24% (standard error = 9.8), representing approximately 1.1 kg N ha$^{-1}$ yr$^{-1}$ or about 0.05% of the total ecosystem soil N. The estimated input of inorganic N as wet atmospheric deposition to LLP (1998–2006) is 4.9 kg ha$^{-1}$ yr$^{-1}$ (http://nadp.sws.uiuc.edu/). In the AHW ecosystem, the N$_2$–fixing tree, black locust, contributed an estimated 21% (standard error = 4.3) of the total N in the Oi horizon of the forest floor, representing an estimated 3.9 kg N ha$^{-1}$ yr$^{-1}$ or about 0.12% of the total soil N. The estimated input of inorganic N as wet atmospheric deposition to AHW (1998–2006) is 6.6 kg ha$^{-1}$ yr$^{-1}$ (http://nadp.sws.uiuc.edu/).

**Soil Profile Total Stable Nitrogen Isotope Ratio and Total Carbon and Nitrogen Concentrations**

Within the LLP ecosystem, burning without the proliferation of N$_2$–fixing plants was not observed; therefore, we tested only the effect of burning plus N$_2$ fixation on $^{15}$N patterns through the soil profile. We found no significant difference in $^{15}$N values between the reference and prescribed burn with N$_2$ fixation (Table 1; Fig. 1a). The $^{15}$N value differed significantly among soil depths; the O horizons were more depleted in $^{15}$N than the mineral horizons (Fig. 1a), and soils became more enriched in $^{15}$N with depth. Both reference and burning treatments had the same pattern of change in $^{15}$N within the soil profile, with no treatment $\times$ depth interaction (Table 1; Fig. 1a).

At LLP, total C and N concentrations (g kg$^{-1}$) differed significantly among soil layers, but the treatment effect was significant only on total N (Table 1). The greatest C concentrations were in the Oi and Oe horizons in the reference plots and in the Oi horizon in the prescribed burn treatment (Table 2). The total N concentration was greatest in the Oe horizon in both treatments (Table 2), with no significant difference among mineral soil layers. The treatment effect on total N was significant (Table 1).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Soil $^{15}$N</th>
<th>Soil C conc.</th>
<th>Soil N conc.</th>
<th>Wood $^{15}$N</th>
<th>Foliage $^{15}$N</th>
<th>Root $^{15}$N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (T)</td>
<td>1</td>
<td>0.03</td>
<td>0.00</td>
<td>0.20</td>
<td>0.00</td>
<td>0.86</td>
</tr>
<tr>
<td>Depth (D)</td>
<td>6</td>
<td>482.23</td>
<td>&lt;0.001</td>
<td>360.89</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Core segment (C)</td>
<td>1</td>
<td>0.03</td>
<td>0.00</td>
<td>0.20</td>
<td>0.00</td>
<td>0.86</td>
</tr>
<tr>
<td>T $\times$ D</td>
<td>5</td>
<td>2.10</td>
<td>0.07</td>
<td>5</td>
<td>0.15</td>
<td>0.98</td>
</tr>
<tr>
<td>T $\times$ C</td>
<td>5</td>
<td>2.10</td>
<td>0.07</td>
<td>5</td>
<td>0.15</td>
<td>0.98</td>
</tr>
</tbody>
</table>

**Table 1. Mixed model analysis of $^{15}$N responses in longleaf pine–wiregrass forest ecosystems examining the effects of treatment (burned vs. unburned) and soil depth (Oi through 20–30-cm depth) on soil $^{15}$N concentration (g kg$^{-1}$), the effects of treatment and increment core segment (0–15 and 16–23 yr) on wood $^{15}$N, the effects of treatment on longleaf pine foliage $^{15}$N (single factor, split-plot design), and the effects of treatment and soil depth on root $^{15}$N. Treatment was a fixed effect; plot within treatment was a random effect.**
1); however, there were no significant differences between treatments within the O horizon or mineral soil layers (Table 2). The treatment × soil layer interaction was not significant for either C or N concentration.

Within the AHW site, treatment, clearcutting alone, and clearcutting with N2–fixing plants and soil depth had significant effects on soil δ15N values. The treatment × depth interaction was also significant (Table 3). The pattern of increasing soil δ15N with depth was evident in all treatments; however, the degree of enrichment with depth varied by treatment (Table 3; Fig. 1b), with a significant difference in δ15N occurring in the subsoil. Reference site subsols at both the 20- to 40- and 40- to 60-cm layers had δ15N values greater than either clearcut site, with or without N2–fixing plants; clearcut sites did not differ. Surface mineral soil (0–10 and 10–20 cm) and O horizon δ15N values did not differ among treatments.

At AHW, treatment, soil layer, and treatment × soil layer interactions significantly affected total C concentrations (Table 3). Soil layer had a significant effect on soil N concentrations; treatment effect was not significant (Table 3). In clearcut treatments, Oe and Oa horizon C concentrations were greater than those of the uncut reference; however, in the 10- to 20-cm soil layer, reference plot C concentrations were greater than those in the clearcut with N2–fixation treatment (Table 4). In reference sites, the Oi horizon had the greatest total C concentration, which declined until the mineral soil layers. In clearcut treatments, however, the Oi and Oe horizons had the greatest C concentrations compared with the Oa horizon and mineral soil layers (Table 4). Nitrogen concentrations were greatest in the Oe horizon in the reference plots and in both the Oe and Oa horizons in the treated sites. There were no significant treatment effects on N concentrations in organic or mineral soil layers.

**Wood Stable Nitrogen Isotope Ratio**

At LLP, wood δ15N was not affected by the burning treatment (Table 1; Fig. 2a). Increment core segments differed in δ15N values; wood laid down 0 to 15 yr before collection was significantly more depleted in 15N than wood from 16 to 25 yr before collection.

At AHW, there was both a significant treatment and tree species effect on wood δ15N (Table 3; Fig. 2b). Wood collected from clearcut without N2–fixation sites was more depleted in 15N than either clearcut with N2–fixation or reference sites. Tulip poplar was significantly more depleted than *Quercus* spp. The treatment × tree species interaction was not significant. Increment core segments did not differ significantly and there was no treatment × core segment interaction (Table 3).

**Foliage and Root Stable Nitrogen Isotope Ratios**

Longleaf pine foliage δ15N in LLP did not respond to treatment; foliage collected on reference plots had a value of −3.1‰ δ15N (Table 1; Fig. 2a). The LLP fine-root δ15N values did not
respond to effects of treatment or soil depth (Table 1; Fig. 2a). On AHW, both *Quercus* spp. and tulip poplar foliage did not respond to treatment. However, there was a significant tree species effect on δ¹⁵N values (Table 3; Fig. 2b); *Quercus* spp. foliage from the uncut reference site had an average δ¹⁵N value of −2.0‰, while reference plot tulip poplar foliage averaged −3.7‰. In AHW, both treatment and soil depth had significant effects on fine-root δ¹⁵N values (Table 4; Fig. 2b), with greater enrichment (greater δ¹⁵N values) in reference plot root samples; roots growing in the Oa horizon had lower δ¹⁵N values than roots in mineral soil layers. The treatment × soil depth interaction was not significant.

### DISCUSSION

**Effects of Forest Disturbance on Soil Profile Stable Nitrogen Isotope Ratio**

Soil profiles in a relatively undisturbed forest typically have a pattern of δ¹⁵N enrichment with depth in the soil profile. This pattern was evident at both LLP and AHW reference sites and is consistent with research conducted in many parts of the world, from tropical rainforests in Borneo (Kitayama and Iwamoto, 2001) and old growth forests in Chile (Boeckx et al., 2005) to findings in the southeastern United States (Pardo et al., 2002) and northeastern United States (Billings and Richter, 2006) and the numerous studies reviewed by Tiunov (2007). Research studies have attributed these patterns to ecosystem N transformation rates, N losses, and mineral N sorption. Processes that produce plant-available N, such as N mineralization and nitrification, discriminate against the heavier ¹⁵N; the result is plant tissue, including wood and foliage, that is depleted in ¹⁵N (lower δ¹⁵N) com-

### Table 2. Treatment means of total soil C and total soil N in longleaf pine–wiregrass ecosystems. Treatments included prescribed burning with N₂-fixing species and reference; soil layers included the O horizons and the mineral soil collected by depth.

<table>
<thead>
<tr>
<th>Soil layer</th>
<th>Reference</th>
<th>Prescribed burn with N₂ fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total C, g kg⁻¹</td>
<td></td>
</tr>
<tr>
<td>Oi horizon</td>
<td>484.7 (2.3) A†</td>
<td>485.5 (1.8) A</td>
</tr>
<tr>
<td>Oe horizon</td>
<td>415.0 (17.0) A</td>
<td>394.4 (46.4) B</td>
</tr>
<tr>
<td>Oa horizon</td>
<td>260.1 (22.3) B</td>
<td></td>
</tr>
<tr>
<td>0–2.5 cm</td>
<td>35.8 (2.0) C</td>
<td>30.4 (8.1) C</td>
</tr>
<tr>
<td>2.5–10 cm</td>
<td>13.9 (2.4) C</td>
<td>14.3 (3.3) C</td>
</tr>
<tr>
<td>10–20 cm</td>
<td>8.6 (1.9) C</td>
<td>8.8 (1.9) C</td>
</tr>
<tr>
<td>20–30 cm</td>
<td>5.6 (1.4) C</td>
<td>6.6 (1.9) C</td>
</tr>
<tr>
<td>Total N, g kg⁻¹</td>
<td>5.3 (0.3) B</td>
<td>3.8 (0.4) AB</td>
</tr>
<tr>
<td>Oe horizon</td>
<td>9.8 (1.2) A</td>
<td>6.7 (1.6) A</td>
</tr>
<tr>
<td>Oa horizon</td>
<td>7.2 (0.5) AB</td>
<td></td>
</tr>
<tr>
<td>0–2.5 cm</td>
<td>1.3 (0.0) C</td>
<td>1.0 (0.2) BC</td>
</tr>
<tr>
<td>2.5–10 cm</td>
<td>0.5 (0.1) C</td>
<td>0.7 (0.1) C</td>
</tr>
<tr>
<td>10–20 cm</td>
<td>0.7 (0.2) C</td>
<td>0.7 (0.1) C</td>
</tr>
<tr>
<td>20–30 cm</td>
<td>0.2 (0.0) C</td>
<td>0.3 (0.0) C</td>
</tr>
</tbody>
</table>

† Mean with standard error in parentheses. Means within a profile (column) followed by the same uppercase letter are not significantly different.
Table 4. Treatment means of total soil C and total soil N in Appalachian hardwood forest ecosystems. Treatments included clear-cut, clear-cut with N₂-fixing species, and reference; soil layers included O horizons and mineral soil collected by depth.

<table>
<thead>
<tr>
<th>Soil layer</th>
<th>Reference</th>
<th>Clear-cut without N₂ fixation</th>
<th>Clear-cut with N₂ fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td>O horizon</td>
<td>444.0 (3.02) At</td>
<td>458.1 (1.12) A</td>
<td>457.9 (1.57) A</td>
</tr>
<tr>
<td>O horizon</td>
<td>384.2 (7.97) Bb</td>
<td>434.91 (3.37) Aa</td>
<td>428.1 (3.80) Aa</td>
</tr>
<tr>
<td>O horizon</td>
<td>238.6 (20.67) Cb</td>
<td>393.9 (8.54) Ba</td>
<td>317.8 (16.98) Ba</td>
</tr>
<tr>
<td>0–10 cm</td>
<td>37.0 (6.76) D</td>
<td>35.1 (2.37) C</td>
<td>26.1 (5.85) C</td>
</tr>
<tr>
<td>10–20 cm</td>
<td>23.4 (3.20) Da</td>
<td>15.7 (2.72) Cab</td>
<td>12.5 (1.54) Cb</td>
</tr>
<tr>
<td>20–40 cm</td>
<td>18.0 (4.14) D</td>
<td>12.2 (0.71) C</td>
<td>12.3 (3.08) C</td>
</tr>
<tr>
<td>40–60 cm</td>
<td>13.8 (4.85) D</td>
<td>6.4 (0.61) C</td>
<td>6.4 (1.00) C</td>
</tr>
<tr>
<td>O horizon</td>
<td>9.91 (0.43) B</td>
<td>9.20 (0.45) B</td>
<td>9.71 (0.34) B</td>
</tr>
<tr>
<td>O horizon</td>
<td>13.0 (0.89) A</td>
<td>16.4 (0.49) A</td>
<td>16.8 (0.38) A</td>
</tr>
<tr>
<td>O horizon</td>
<td>10.7 (1.13) AB</td>
<td>16.2 (0.62) A</td>
<td>15.1 (0.86) A</td>
</tr>
<tr>
<td>0–10 cm</td>
<td>2.2 (0.60) C</td>
<td>1.4 (0.13) C</td>
<td>1.2 (0.27) C</td>
</tr>
<tr>
<td>10–20 cm</td>
<td>1.3 (0.28) C</td>
<td>0.7 (0.13) C</td>
<td>0.6 (0.09) C</td>
</tr>
<tr>
<td>20–40 cm</td>
<td>0.9 (0.28) C</td>
<td>0.5 (0.05) C</td>
<td>0.5 (0.13) C</td>
</tr>
<tr>
<td>40–60 cm</td>
<td>0.7 (0.30) C</td>
<td>0.2 (0.10) C</td>
<td>0.2 (0.11) C</td>
</tr>
</tbody>
</table>

† Mean with standard error in parentheses. Means within a profile (column) followed by the same uppercase letter are not significantly different. Means within a soil layer (row) followed by the same lowercase letter are not significantly different.

pared with the soil organic matter pool (Boeckx et al., 2005; Garten and van Miegroet, 1994; Kahmen et al., 2008; Pardo et al., 2007b; Templer et al., 2007).

We hypothesized that site disturbance through the implementation of forest management practices would result in enriched or depleted δ¹⁵N values due to the removal or pulse addition of OM, changes in rates of N transformations, and N loss and may leave evidence in the δ¹⁵N values of the soil profile. Nadelhoffer and Fry (1988) found that, after 28 yr of litterfall additions or exclusions, the additions resulted in depleted soil organic matter δ¹⁵N values while the exclusion resulted in enriched soil ¹⁵N. They attributed this response to the addition of leaf litter with a lower isotopic ratio than the bulk SOM, as well as the isotopic enrichment of existing SOM that occurs during decomposition. The greatest responses occurred in the surface 0 to 10 cm. To the contrary, we found no effect of either OM removal or OM additions on surface soil δ¹⁵N values compared with reference sites. However, the OM pulse of logging residue, 25 yr before sample collection, resulted in depleted δ¹⁵N values only in subsurface soils from 20 to 60 cm.

In addition to altering OM inputs, ecosystem disturbance often results in the proliferation of N₂-fixing plants, such as in our forests, and may increase N inputs and decrease soil δ¹⁵N (Bai et al., 2013; Johnson et al., 2005). Active N₂–fixing plants have δ¹⁵N values approaching 0‰, ranging from ~3 to 0‰ (Bai et al., 2013; Fry, 1991). We proposed that the increased abundance of active N₂–fixing plants would mitigate the soil ¹⁵N enrichment response resulting from OM losses due to prescribed burning and increase ¹⁵N depletion resulting from OM inputs after clearcutting due to the addition of ¹⁵N-depleted organic material. The N₂–fixing plants in our systems showed evidence of active fixation, with δ¹⁵N values of ~1.1‰ in legume foliage found in the longleaf pine forest and ~0.6‰ in black locust foliage in the Appalachian hardwood forest. Mixing models estimated that the contribution of N₂–fixing foliage to the forest floor represented 24% of N in the Oe horizon of the longleaf pine forest and 21% in the Oi horizon in the hardwood forest. This represents approximately 22% at LLP and 59% at AHW of the annual N (NO₃ + NH₄) input in wet atmospheric deposition.

Past research suggests that prescribed burning removes O horizon OM and may alter the fine-root distribution but typically has no effect on total mineral soil C and N concentrations (Addington et al., 2006; Knoepp et al., 2004; Ojima et al., 1994). Our study had similar results; the Oa horizon was absent due to repeated burning, with no effect on the mineral soil C concentration (Table 2). The lack of a soil response perhaps suggests altered patterns of OM inputs, such as the inputs of black carbon (Boring et al., 2004), a long-lived C source, or increased inputs from belowground sources (Ojima et al., 1994). In other research examining the effect of prescribed burning in the Coastal Plain longleaf pine ecosystem, Wilson et al. (1999) found that repeated burning decreased N inputs due to reduced inputs of higher quality Quercus spp. litter; however, this reduction was mitigated by the presence of N₂–fixing legumes (Hendricks and Boring, 1999). Thus, while repeated prescribed burning altered both the quantity and quality of OM inputs, we found no effect on total C or N concentrations in either the O horizon or mineral soil layers.

We hypothesized that prescribed burning would result in the enrichment of soil δ¹⁵N due to decreased OM inputs (Nadelhoffer and Fry, 1988), the volatilization of organic N (Evans, 2007; Saito et al., 2007), and increased organic N transformation rates (Högberg, 1997) and that this response would be mitigated by the presence of N₂–fixing plants. In studies of prescribed burn impacts on ¹⁵N distribution in bishop pine (Pinus muricata D. Don) stands in California, Grogan et al. (2000) found that in burned sites the δ¹⁵N values of non-N₂–fixing plant materials and surface soils were similar, while at unburned sites plants were depleted in ¹⁵N. We confirmed the decrease in surface OM inputs to the soil as a result of burning (Table 2); however, studies in Coastal Plain longleaf pine (Wilson et al., 1999, 2002) have suggested that, while soil N transformation rates increase after burning, repeated burning may decrease the overall site N despite N inputs by N₂ fixation. Our data support the proposed mitigating effect of the inputs from N₂–fixing legumes on N losses because we found no difference between reference and burned plot O horizon N concentrations (Table 2). Past estimates of N₂ fixation were 16 kg N ha⁻¹ yr⁻¹ (Cathey et al., 2010; Ha dams et al., 1999); our estimate of legume leaf litter N inputs was much lower, at 1.1 kg N ha⁻¹ yr⁻¹; however, this does not include root inputs or estimates of plant N₂ fixation. Mitigation of the response by N₂ fixation may also explain the consistent pattern of δ¹⁵N enrichment that we found throughout the soil profile, a pattern that does not reflect current or past prescribed burning (Fig. 1a). However, the
In contrast, forest clearcutting practices that cut all woody vegetation (>5 cm) but only remove tree boles leave a substantial amount of logging residue on-site that, along with high fine-root mortality, can result in short-lived increases in soil total C and N (Johnson et al., 2002; Knoepp and Swank, 1997; Mattson and Swank, 1989). The clearcut watershed had 121 Mg ha\(^{-1}\) (dry weight) of logging residue on the site following harvest, resulting in a significant increase in surface (0–10 cm) soil C compared with the reference watershed for 5 yr and in two of seven collections during the next 26 yr of periodic sampling (Knoepp et al., 2014). The total soil N concentration was greater in clearcut sites for 1 yr only. In the current study, 25 yr after treatment, we found that total C concentrations in Oe and Oa horizons were greater in both clearcut treatments compared with the reference sites; however, there was no effect on organic horizon C/N ratios (data not shown). Changes in total C along with documented changes in vegetation composition (Boring et al., 2014) suggest a long-term change in both litterfall chemistry and decomposition rates. Additionally, we found that total C concentrations in the 10-to 20-cm mineral soils was significantly greater in the reference plots (Table 4), suggesting a shift in SOM dynamics long after disturbance.

The proposed effects of clearcut harvest on soil \(^{15}\)N values depend on whether this treatment is viewed as the removal or addition of organic matter. Pardo et al. (2002) attributed increased O horizon and surface soil \(^{15}\)N for 3 yr following clearcut harvest to decreased litterfall inputs along with increased rates of SOM decomposition, nitrification, and NO\(_3\) leaching; \(^{15}\)N values returned to preharvest values within 15 yr of treatment. On the contrary, we predicted soil \(^{15}\)N depletion following harvest at AHW in response to the input of logging residue, high root mortality (Mattson and Swank, 1989), and the rapid recovery of leaf litter inputs (Boring et al., 2014; Elliott et al., 2002). Mattson and Swank (1989) measured significantly reduced live root mass for 5 to 8 yr after harvest compared with an uncut reference; leaf litter mass and leaf area index were equal to preharvest levels in a similar amount of time (7–8 yr) (Elliott et al., 2002). As predicted, we measured significantly depleted soil \(^{15}\)N values, but only in the subsoil layers of the soil profile (>40 cm), in both clearcut treatment sites 25 yr after treatment (Fig. 2). In treated plots, soil \(^{15}\)N values indicate no enrichment throughout the profile (Fig. 1b), suggesting the presence of OM that was less transformed by microbial processes. This could result from the movement of OM through the soil profile and/or the input of fine roots (Fig. 2b) and occurred despite increased rates of soil N mineralization and nitrification (Knoepp et al., 2014; Waide et al., 1988) and NO\(_3\) leaching (Swank et al., 2014) for >20 yr following harvest.

Changes in soil \(^{15}\)N values within the soil profile were similar in AHW clearcut alone and clearcut with \(N_2\) fixation (Fig. 1b and 2b). The lack of an effect of \(N_2\) fixation suggests that inputs of up to 30 kg N ha\(^{-1}\) yr\(^{-1}\) from \(N_2\) fixation (Boring...
Subsoil (Bai et al., 2013). Additionally, studies have shown greater \( ^{15}N \) water content, soil temperature, and soil physical characteristics but rather by N fractionation processes such as rates of N transformations, the proportion of gaseous vs. leaching losses, soil water content, soil temperature, and soil physical characteristics (Bai et al., 2013). Additionally, studies have shown greater \( ^{15}N \) enrichment throughout the soil profile in the microaggregate soil fraction than in macroaggregates (Huygens et al., 2008). This was attributed to OM protection by clay minerals as well as differences in microbial processing, microbial turnover, and the accumulation of microbial compounds. Our analyses were conducted on bulk soil samples, possibly representing more recent OM inputs; separation of soils into micro- and macroaggregates may yield different results.

**Effects of Forest Disturbance on Wood Stable Nitrogen Isotope Ratio**

Dendrochemical analysis has been successful in linking changing patterns of wood chemistry with changes in soil nutrient availability (Sheppard et al., 2001) and soil pH with time (Guyette et al., 1992; Shortle et al., 1995). Research examining the use of tree-ring N and \( ^{15}N \) to identify changes in soil N availability with time have had mixed results. Bukata and Kyser (2005) found that tree-ring \( ^{15}N \) was a useful indicator of N sources following forest cutting. However, it has also been shown that N is mobile between tree rings (Hart and Classen, 2003) and that trees must be actively taking up N when changes in soil N availability occur (Beghin et al., 2011). We examined the potential of using wood \( ^{15}N \) as an indicator of changes in soil N availability following forest management and with time and obtained inconsistent results in LLP and AHW. In LLP, contrary to our hypothesis, we found no treatment effect on wood \( ^{15}N \) (Table 1). However, \( ^{15}N \) was more depleted in recent core increment segments (Table 1; Fig. 2a), suggesting decreasing available soil N with time in both reference and treated plots. This contradicts the findings of Wilson et al. (1999, 2002), whose results suggest that repeated burning would maintain greater N availability compared with the unburned sites. In AHW, treatment effects on wood \( ^{15}N \) were significant, suggesting a treatment effect on the sources of plant-available N. However, both tulip poplar and *Quercus* spp. were more depleted on clearcut plots without N\(_2\) fixers present, while clearcut with N\(_2\) fixation and uncut reference sites were similar, making the interpretation of treatment effects difficult. Core segments did not differ, leading us to conclude that N availability or perhaps N quality has remained relatively constant for reference and treated plots with or without N\(_2\) fixation (Table 3; Fig. 2b) since forest cutting. This is supported by measurement of long-term increases in N availability (Knoepp et al., 2014) and stream NO\(_3\)–N after harvest with no change in the reference (Swank et al., 2014).

**Nitrogen Availability Effects on Stable Nitrogen Isotope Ratios in Plant Components**

Research in the northeastern United States found that foliar \( ^{15}N \) responses to N availability varied with tree species (Pardo et al., 2007b; Templer et al., 2007). A review by Höghberg (1997) and more recently research by Pardo et al. (2013) and Kalcsits et al. (2014) discussed the importance of plant species composition and mycorrhizal associations on patterns of \( ^{15}N \) in the soil profile and different plant components. We found no foliar \( ^{15}N \) response to treatment at either LLP or AHW (Fig. 2a and 2b; Tables 1 and 3). Previous research showed that both prescribed burning and clearcut harvest resulted in elevated rates of N transformations (Knoepp et al., 2014; Wilson et al., 2002); however, the rates previously measured in our ecosystems were much lower than those measured by Pardo et al. (2007a) and Templer et al. (2007). Our current findings suggest that foliar \( ^{15}N \) values are regulated by tree species differences. Both prescribed burning and clearcut harvest resulted in an altered overstory composition. Prescribed burning at LLP eliminates *Quercus* spp., leaving longleaf pine as the only overstory tree species. Clearcutting at AHW shifted the overstory from a stand dominated by *Quercus* spp. to tulip poplar (Elliott et al., 2002). Species differences in \( ^{15}N \) values could result from mycorrhizal associations because *Quercus* spp. associate with an ectomycorrhiza and tulip poplar with an arbuscular mycorrhiza (Hobbie et al., 2000); in LLP, both longleaf pine and *Quercus* spp. are ectomycorrhizal.

We hypothesized that the impacts of forest management on the soil \( ^{15}N \) profile and foliar and wood \( ^{15}N \), through OM removal (\( ^{15}N \) enrichment) or addition (\( ^{15}N \) depletion), would be mitigated or exacerbated, respectively, by the presence of N\(_2\)–fixing vegetation following disturbance. We found no soil \( ^{15}N \) response to repeated burning in the longleaf pine–wiregrass ecosystem and no response in soil C or N concentrations. However, we could not fully test this hypothesis due to the lack of a prescribed burn treatment without N\(_2\)–fixing legumes. Wood core segment \( ^{15}N \) values did not respond to treatment. Appalachian hardwood soil \( ^{15}N \) as well as total C and N concentrations showed a significant response to forest cutting; treatment × soil profile depth interactions were also significant. However, the presence of the N\(_2\)–fixing tree, *R. pseudoacacia*, had no effect. Additionally, while wood \( ^{15}N \) showed a treatment response, the impact of N\(_2\) fixation was not discernible, and changes with time since harvest were not significant. Our data suggested that soil \( ^{15}N \) profiles may be useful in discerning previous disturbance, although responses vary with forest ecosystem and the utility of wood \( ^{15}N \) profiles varies among tree species. Finally, we found that while N\(_2\)–fixing species play an important role in ecosystem recovery and N inputs, their effect on ecosystem \( ^{15}N \) response is far from generalizable.
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