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Non-labile Soil ^{15}Nitrogen Retention beneath Three Tree Species in a Tropical Plantation

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ABSTRACT

Soil organic matter is the largest sink for N additions to forests. Species composition may affect soil N retention by altering the amount or proportion of added N stored in non-labile organic pools. We measured ^{15}N tracer retention in labile and non-labile pools of surface (0–20 cm) mineral soils, 7 yr after the tracer was applied to a 9 yr-old Puerto Rican tree plantation with replicated stands of three species (two N-fixers, one *Eucalyptus*, Euc). Laboratory incubations (13 mo) with repeated leaching separated total soil N into labile (inorganic N leached) and non-labile (total N minus leached N) pools, and a labile C treatment tested linkages between C availability and N retention. We hypothesized that species composition would alter the amount and proportion of recovered tracer N in non-labile organic matter. Surface soils contained 45% of the tracer, but the amount retained in labile and non-labile pools was similar among species. In contrast, the proportion of recovered tracer in non-labile pools was greater in soils beneath N-fixers (75%) than Euc (62%). Labile C additions increased the size of the non-labile tracer N pool. We conclude that tree species composition may affect long-term soil N retention by altering the proportion of N in slow-turnover, non-labile pools. Plants may also alter soil N retention by renewing labile C pools; a continuous supply of labile C increased the transfer of ^{15}N into non-labile organic matter.

Tree species composition affects forest biogeochemistry because species differ in their rates of nutrient and energy cycling (Zinke, 1962; Boettcher and Kalisz, 1990; Hobbie, 1992; Wardle et al., 1997; Binkley and Giardina, 1998). Species–ecosystem relationships link the fields of population and ecosystem ecology (Jones and Lawton, 1994) with major implications for soil fertility, C sequestration, and plantation productivity. Species characteristics may also affect retention of recent large increases in N fertilization (Matthews, 1994; Binkley et al., 1995) and atmospheric N deposition (Galloway et al., 1994).

In some recent case studies, forest N retention appears to depend in part on species composition. In New England, hardwood species had higher plant ^{15}N recovery (Nadelhoffer et al., 1995), total ecosystem N retetention (Magill et al., 2000), and net N mineralization rates (Finzi et al., 1998) than coniferous species. Many European beech (*fagus stuatica* L.)-dominated forests had lower N deposition and nitrate leaching than adjacent spruce (*Picea* A. Dietr.)-dominated forests (Rothe et al., 2001). In contrast, a synthetic analysis of over 300

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Abbreviations: AFE, atom fractional enrichment; ECEC, effective cation-exchange capacity; Cas, *Casuarina*; Euc, *Eucalyptus*; Leu, *Leucaena*; k_n, extraction efficiency; N_o, mass of labile N; N_a, the mass of tracer N in the labile pool; N_n, the mass of labile native soil N; $^{15}N_o$, AFE of the composite leachate sample; $^{15}N_a$, AFE of the tracer; $^{15}N_n$, AFE of the native soil; PNDFA, percent of tree N derived from the atmosphere; NPP, net primary productivity.

streams across the USA showed that conifer forests had lower stream-water nitrate concentrations than deciduous forests (Binkley, 2001). Among deciduous species, potential net nitrification was positively correlated with atmospheric N deposition in maple (*Acer* L.), but not beech stands (Lovett and Reuth, 1999) and lysimeter nitrate concentrations were greater in east-facing stands dominated by maple and cherry (*Prunus* L.) than in south-facing stands dominated by gum (*Nyssa sylvatica*) and beech (Peterjohn et al., 1999). In all of these studies, species effects were apparent, but were confounded by uncontrolled factors that covaried with species, such as land-use history, topography, prior soil conditions, or regional climate. Common garden experiments are needed to discern species effects on N loss and retention in plots with similar soils, topography, and climate (e.g., Johnson and Todd, 1988).

Tree species could affect N retention by several mechanisms, the most obvious being differential plant uptake of N. While this mechanism may be important, most ^{15}N-tracer experiments show that soils, rather than plants, are the largest sink for N added to forests (Mead and Pritchett, 1975; Heilman et al., 1982; Melin et al., 1983; Clinton and Mead, 1993; Preston and Mead, 1994a; Tietema et al., 1998; Nadelhoffer et al., 1999). Tree species could alter soil N retention if the quantity or quality of C inputs differs among species. Differences in C inputs could alter microbial immobilization of N (Jansson, 1958; Kelley and Stevenson, 1987; Hart et al., 1994), and differences in litter quality may affect the amount of N sequestered in non-labile humus (Melillo et al., 1989; Berg and Matzner, 1997). Large N inputs from N-fixing tree species may saturate soil N sinks and limit further N retention (Van Miegroet et al., 1990; Binkley et al., 1992), but N fixers may also promote non-labile organic matter formation (Kaye et al., 2000; Resh et al., 2001) and thus, long-term soil N sequestration.

Most analyses of species effects on N retention have focused on changes in actively cycling, labile N pools (inorganic or microbial biomass N). However, most soil N is not actively cycled by plants and microbes on annual time scales, and this large non-labile N pool may be an important sink for N additions. Nitrogen retained in a non-labile pool with a slow turnover time will not immediately affect water quality or plant production. On the other hand, N stored in labile pools may increase fluxes to plants and stream water. In agricultural systems, most fertilizer N is non labile after one growing season (Broadbent and Nakashima, 1967; Stanford et al., 1970; Smith et al., 1978; Smith and Power, 1985), but similar data are rare for forests (Preston and Mead, 1994b; Chang and Preston, 1998).

In this paper, we report the first direct test of tree species effects on ^{15}N-tracer retention using a replicated common garden experiment with three tree species (two N-fixers) and a 6 to 8 yr-old (hereafter 7 yr-old) ^{15}N addition. Rather than focusing on traditional plant-available N pools, we estimated species effects on non-labile pools of soil organic N. We hypothesized that tree species would affect the amount and proportion of added N that was retained in non-labile soil pools. To determine whether species effects resulted from differences in labile C inputs, we also conducted a labile C addition experiment.

There is no standard method to separate organic N into labile and non-labile pools. Previous studies have used physical (Strickland et al., 1992) and chemical (He et al., 1988) methods to fractionate soil C and N, assuming that aggregate size, organic matter density, or organic matter solubility was well correlated with C or N availability to microorganisms. Biological fractionations, such as the long-term incubations used in this study, allow the "in situ microbial and microarthropod community to define ecologically relevant SOM (soil organic matter) fractions" (Robertson and Paul, 1999).

MATERIALS AND METHODS

The study site is on the northern coast of Puerto Rico at the University of Puerto Rico's Toa Baja Agriculture Experiment Station (Parrotta et al., 1993, 1996; Parrotta, 1999). The plantation was organized as a completely randomized block (n = 3 blocks) experiment with six treatments per block. The treatments originally applied were monocultures and mixtures of *Eucalyptus robusta* J.E. Smith (Euc), N-fixing *Casuarina equisetifolia* J.R. & G. Forst. (Cas), and N-fixing *Leucaena leucocephala* (Lam.) de Wit (Leu). For research described here, we sampled only the monocultures at 9 yr of age. The stands were planted in 16 by 16 m^2 plots at a spacing of 1 by 1 m^2. Some general characteristics of the plantations are presented in Table 1.

Table 1. Some characteristics of biomass and soils in the plantations. From Parrotta (1999) and Parrotta et al. (1996).

		Tree species†		
	Plantation age	*Eucalyptus*	*Casuarina*	*Leucaena*
	yr			
Aboveground Biomass, g m^{-2}	4	6 250a	10 530b	7 180ab
Belowground Biomass, g m^{-2}	4	1 610a	2 360b	1 560a
Aboveground NPP‡, g m^{-2} yr^{-1}	1.5 to 3.5	2 240	3 850	3 090
Litterfall N, g m^{-2} yr^{-1}	1.5 to 3.5	4.2a	10.5b	19.3c
PNDFA§, %	1 to 3.5	0	40 to 60	100 to 40
Nitrogen fixation rate, g m^{-2} yr^{-1}	0 to 3.5	0	7.3	7.4
Soil pH¶	7.5	7.8	7.9	8.0
ECEC#, cmol$_c$ kg^{-1}	7.5	6.9	8.3	9.3

† Values from the same row with different lower case letters are statistically different ($P < 0.05$).
‡ Net primary productivity = annual dry wood mass increment plus dry litterfall mass.
§ Percent of tree nitrogen derived from the atmosphere.
¶ In deionized water at a 1:1 (soil mass/water volume) ratio.
# Effective cation exchange capacity = exchangeable Ca + Mg + K + Na + Al.

The soils are marine origin isohypothermic Typic Tropopsamments. Annual precipitation is 1600 mm and mean daily temperatures range from 23.8 °C in January to 29.4 °C in August. Inorganic N deposition at the nearest National Atmospheric Deposition Program (2001) site ranged from 0.1 to 0.3 g N m^{-2} over the past 15 yr (El Verde, 350 m higher and 60 km to the east). The plantations were established in September 1989 and from ages 6 to 32 mo, an aqueous solution of 10.0 atom% enriched $(^{15}NH_4)_2SO_4$ was added to subplots within each treatment at a rate of 1.0 (6, 12, and 18 mo) or 0.67 (24, 28, and 32 mo) g N m^{-2} for a total of 5 g N m^{-2}. This application regime was designed to measure N fixation rates by Cas and Leu (Parrotta et al., 1996). The ^{15}N subplots (3 by 3 m^2) included nine trees in the center and were trenched with plastic to 0.8 m. Soil within these plots had N isotope ratios of 150 to 200‰ at the time we sampled.

In June 1998, we collected and composited three mineral soil samples ~0.2 m in diam. (inexact because a shovel was used) and exactly 0.2 m deep from each trenched ^{15}N plot. In addition, a core of known volume (0.0475 m in diam. and 0.2 m deep) was taken from each plot to estimate bulk density. The soils were double bagged and stored at 4 °C until the incubations began in July, 1998. Gravimetric water content was determined by drying at 105 °C for 48 h. Soil moisture at field capacity was determined by saturating a column of soil in a plastic tube with cheesecloth supporting the bottom of the column. Soil moisture content 48 h after the soil was saturated was considered field capacity. Total soil N and C were determined by dry combustion (LECO-1000, LECO Corporation, St. Joseph, MI). Inorganic C was determined by adding 6 *M* HCl and FeCl to a 0.5-g subsample in a serum bottle and measuring headspace pressure from CO_2 evolution (Wagner et al., 1998).

To separate the total soil N pool into a labile and a non-labile pool, a subsample (100-g mineral soil) of each field composite was incubated at 35 °C in plastic filters (Stanford and Smith, 1972; Nadelhoffer, 1990; Falcon Filter model 7111, Beckton Dickenson Labware, Lincoln Park, NJ) for 393 d. A glass fiber filter (Whatman GF/A, Whatman Ltd., Maidstone, UK) and an extra thick glass fiber filter (Gelman Sciences, Ann Arbor, MI) were placed beneath the soil and a third filter (Whatman GF/A) was placed above the soil to prevent dispersion (Motavalli et al., 1995). The filter units were sealed in airtight 2-L jars fitted with septa to allow sampling of headspace CO_2. Deionized water (20 mL) was placed in the bottom of each jar to maintain humidity and prevent soil drying. Every 2 wk the water was changed and the soil brought to field capacity with deionized water.

We leached the incubating soil at 0, 7, 17, 36, 79, 154, 217, 274, 330, and 393 d with a solution containing all essential nutrients except N (Nadelhoffer, 1990). For the samples receiving labile C additions, the solution also included 2 g L^{-1} sucrose. This C addition was comparable to twice annual belowground C inputs in a Hawaiian Euc plantation (Binkley and Ryan, 1998). At each leaching, N-free leaching solution (100 mL) was added to the top of the filter, allowed to equilibrate with the soil for 1 h, and drawn through the filter with a weak vacuum (−0.05 MPa) until leachate ceased to drip from the filter (<10 min). Leachates were frozen until analysis for NH_4^+ and $(NO_3^- + NO_2^-)$ by flow injection colorimetry. At the end of the incubation, a subsample (20 g) of the residual soil was extracted with 100 mL of 0.5 *M* K_2SO_4 to account for unleached inorganic N. We defined the labile N pool as the sum of all inorganic N $(NO_2^- + NO_3^- + NH_4^+)$ in leachates plus inorganic N extracted with K_2SO_4 immediately after the last leaching. Non-labile N was defined as total soil N minus labile N.

We determined the N isotope ratio of the leachate for each incubated soil by compositing 5 mL of leachate from each sampling date. The composite samples were diffused (Stark and Hart, 1996; Khan et al., 1998) for 7 d in 120-mL plastic containers. Devarda's alloy was added to convert NO_3^- in the samples to NH_4^+ and MgO was added to raise the pH and convert all NH_4^+ to NH_3. The NH_3 was collected on two acidified (10 ml of 2.5 *M* $KHSO_4$) filter paper disks (Whatman #1, Whatman Ltd., Maidstone, UK) sealed in Teflon tape. At the end of the diffusion, the acidified disks were dried over concentrated H_2SO_4 for 24 h and then stored in a desiccator until they were transferred to Sn capsules and analyzed on a VG isochrom-NA stable isotope ratio mass spectrometer (VG, Middlewich, UK). Samples with <85% N recovery were rediffused, but isotopic ratios of duplicate samples were always within 1% of each other. The $^{15}N/^{14}N$ ratio of the samples was corrected for N contamination in reagents by diffusing or applying directly to acidified disks, a standard of known enrichment and mass (175‰, 100 ug of N; Stark and Hart, 1996). The amount of tracer N residing in the labile fraction was determined using the following equations:

$$N_o = N_a + N_n \quad [1]$$

Rearranging $$N_n = N_o - N_a \quad [2]$$

$$N_o \times {}^{15}N_o = N_a \times {}^{15}N_a + N_n \times {}^{15}N_n \quad [3]$$

Substituting from (2)

$$N_o \times {}^{15}N_o = N_a \times {}^{15}N_a + (N_o - N_a) \times {}^{15}N_n \quad [4]$$

Rearranging $$N_a = (N_o \times {}^{15}N_o - N_o \times {}^{15}N_n)/ ({}^{15}N_a - {}^{15}N_n) \quad [5]$$

where N_o is mass of labile N, N_a is the mass of the tracer N still in the labile pool, N_n is the mass of labile native soil N, $^{15}N_o$ is the concentration (atom fractional enrichment; AFE) of ^{15}N in the composite leachate sample, $^{15}N_a$ is the concentration of ^{15}N in the tracer N (AFE = 0.10) and $^{15}N_n$ is the concentration of ^{15}N in the native soil (AFE = 0.003663). Similar equations were used to determine how much of the tracer ^{15}N was in the preincubation soil. The amount of tracer N in the non-labile pool was determined by subtracting N_a from the amount of tracer N in the soil at the beginning of the incubation.

Microbial biomass N, extractable-organic N, and extractable-inorganic N were measured in the soil before and after the incubation. Inorganic N was determined by extracting a 20-g (oven-dry weight equivalent) subsample with 100 mL of 0.5 *M* K_2SO_4. The extract was shaken mechanically for 1 h and then filtered (Whatman #1) and analyzed for NH_4^+ and $(NO_3^- + NO_2^-)$ by flow injection colorimetry. Microbial biomass N was determined by the chloroform fumigation-extraction technique (Brooks et al., 1985). Extractable (100 mL of 0.5 *M* K_2SO_4) organic and inorganic N were determined before and after the soil was subjected to a chloroform atmosphere (−0.05 MPa) for 5 d. Total N in the extracts was determined by persulfate digestion (Cabrera and Beare, 1993) and analysis of NO_3^- by flow injection colorimetry. Chloroform-labile N was calculated as total extractable N (per gram of oven-dry soil fumigated) following fumigation minus total extractable N preceding the fumigation. Microbial biomass N was then calculated as chloroform labile N divided by 0.69 (an extraction efficiency that accounts for the fact that not all of the microbial biomass is released upon fumigation; Brooks et al., 1985).

Labile C was determined by capturing all soil respiration in the headspace of the incubation jars. The opened jars were

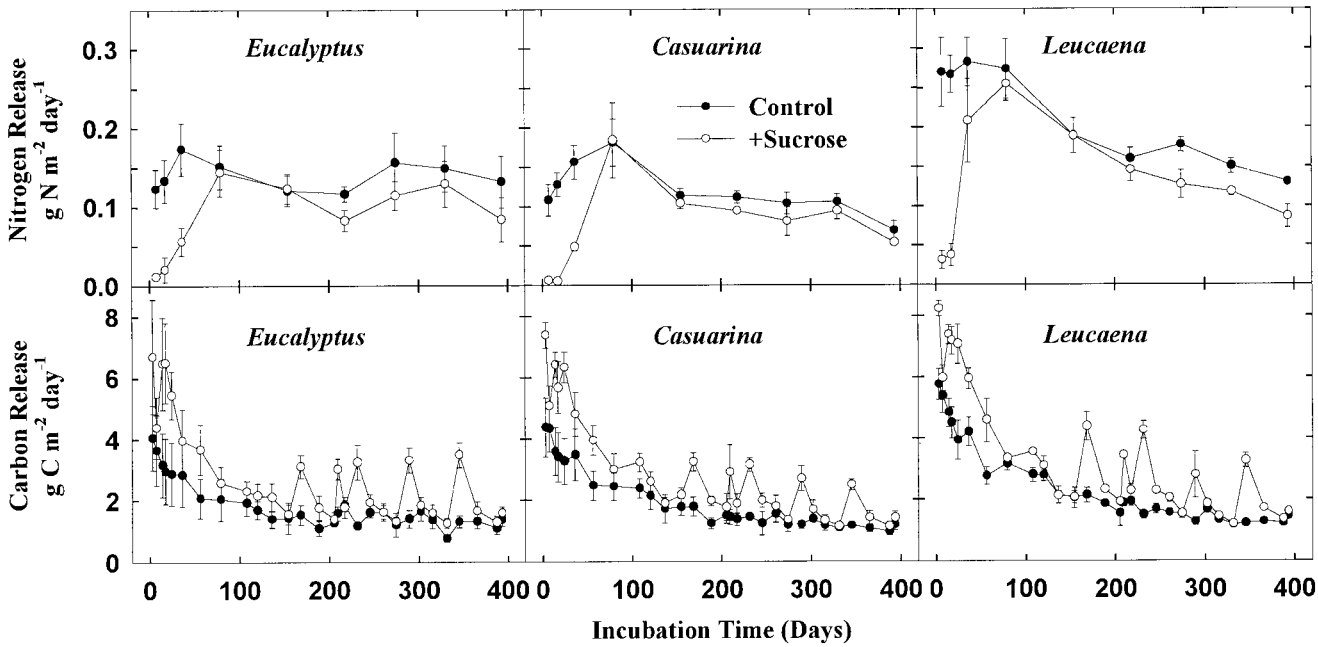

Fig. 1. Rates of N and C release throughout the incubation. Points are means (n = 3) ± one standard error.

fanned with ambient air for 1 h and sealed for periods from 2 d (beginning of the incubation) to 2 wk (end of the incubation) after which, the jar headspace was mixed and subsampled (2 mL) to determine CO_2 concentration by infrared gas analysis (LICOR-6200). Three sealed jars without soil were used as blanks to correct for ambient CO_2. Atmospheric pressure, air temperature, jar volume, subsample gas volume, and the oven-dry mass of the soil were used to convert headspace concentration to milligram C per kilogram of oven-dry soil. Labile C was defined as the sum of all CO_2-C respired during the incubation. Non-labile C was defined as total organic C minus labile C. Non-labile C pools were not estimated for the C addition experiment because the C addition would greatly confound results.

Species and labile C addition effects on N-pool sizes were analyzed using a split-plot analysis of variance with block, species, block × species (error term for species), labile C, and species × labile C, and residual error (error term for labile C). All hypotheses were tested at $\alpha = 0.05$. Data were log transformed when residual plots revealed unequal variance.

RESULTS

Nitrogen release rates from control (i.e., not sucrose amended) Euc and Cas soils varied little during the incubation while N release from control Leu soils was greatest during the first 100 d and declined thereafter (Fig. 1). In contrast, CO_2-C released from all control soils declined consistently. Sucrose amended samples had faster C mineralization rates and slower N leaching rates than control samples throughout the incubation. Despite great differences in N fixation (Table 1), neither total soil N pools nor non-labile soil N pools differed among tree species (Fig. 2). Labile soil N was greater in the Leu (80.4 ± 4.1 g N m^{-2}) treatment than Euc (59.1 ± 4.8 g N m^{-2}; $P = 0.03$) or Cas (50.4 ± 3.5 g N m^{-2}; $P = 0.01$) treatments (Fig. 2a). The sucrose addition increased non-labile N ($P = 0.01$) and decreased labile N ($P < 0.01$) by about 12 g N m^{-2} (a 4% increase

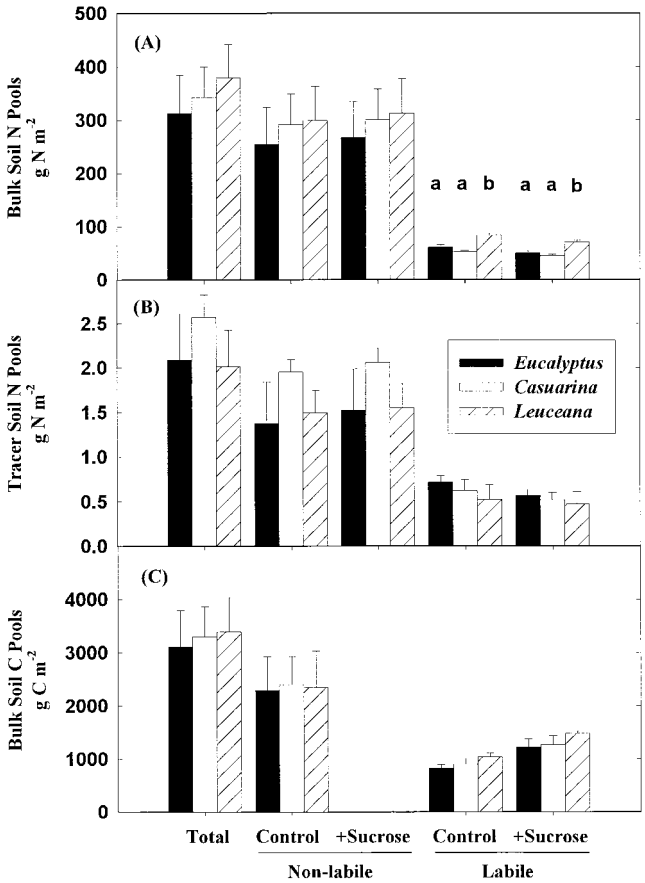

Fig. 2. Total, non-labile and labile (A) N, and (C) C in bulk soil and (B) tracer N pools. Bars (species) with different lowercase letters were statistically different ($P < 0.05$). Sucrose amended soils (+Sucrose) had more non-labile and less labile N than controls. Bars are means (n = 3) plus one standard error.

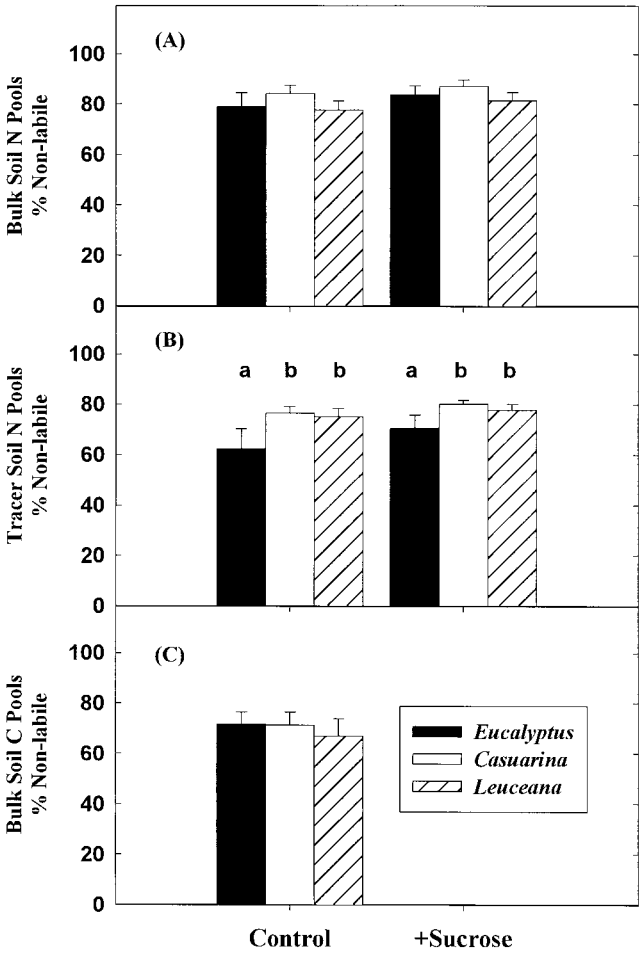

Fig. 3. The proportion of (A) bulk soil N, (B) retained tracer N, and (C) bulk soil C that was non-labile. Bars (species) with different lowercase letters were statistically different ($P < 0.05$). The percentage of the total pool that was non-labile was larger in all sucrose-amended (+Sucrose) treatments than controls. Bars are means ($n = 3$) plus one standard error.

and 16% decrease, respectively; Fig. 2a). Non-labile N ranged from 78 to 84% of total soil N but did not differ among species (Fig. 3a). Throughout the incubation, for all species and both sucrose treatments, >95% of the inorganic N leached was NO_3^--N. We did not detect tree species effects on any of the soil C pools (Fig. 2c). Labile C pool sizes (820–1047 g C m^{-2}) were ~30% of total soil organic C (Fig. 2c). Sucrose amendments increased ($P < 0.01$) labile-C pool sizes by 30%.

For all tree species, 2 to 2.5 g N m^{-2} of the added ^{15}N labeled fertilizer (~45% of the original 5 g N m^{-2} added) remained in the surface mineral soil after 7 yr (Fig. 2b). Nitrogen in the leachate was always more enriched in ^{15}N than total soil N, and preincubation soil was always more enriched than postincubation soil (data not shown). Despite large differences in labile and non-labile ^{15}N pool sizes (means differed by 15–40%), we did not detect statistical differences among species ($P > 0.45$ for both labile and non-labile pools; Fig. 2b). The percentage of ^{15}N that was non-labile was lower in Euc plots (62%) than in the Cas (76%; $P = 0.03$) or Leu (75%; $P = 0.03$) plots (Fig. 3b). The sucrose addition increased the size of the non-labile ^{15}N pool ($P = 0.03$) and decreased the size of the labile ^{15}N pool ($P < 0.01$).

Preincubation microbial biomass did not differ ($P = 0.12$) among species (Table 2). Microbial biomass following 393 d of incubation was similar to the preincubation value and was not affected by either tree species ($P = 0.30$) or sucrose amendments ($P = 0.14$). Extractable organic and inorganic N did not differ among tree species in pre- or postincubation soils, however, the sucrose amendment increased extractable inorganic N ($P = 0.04$) by 1.4 g N m^{-2}.

DISCUSSION

One of the most consistent results of recent N-tracer experiments is that soil organic matter is the dominant long-term (1–3 yr) sink for applied N. More than two-thirds of added N is typically retained in soil (Aber et al., 1998; Tietema et al., 1998; Nadelhoffer et al., 1999), reducing N leaching, cation losses, and soil acidification. Because plant N uptake is smaller than soil retention, species effects on N retention will likely derive from species effects on soils. This study yielded three insights into the role of species composition in soil and forest N retention:

1. Most of the N retained in soil was non-labile: 7 yr after ^{15}N was applied to our site, 62 to 75% of the tracer still in the surface mineral soil was non-labile.
2. Tree species can alter the proportion of N retained in non-labile pools: Euc plots had a smaller proportion of non-labile tracer N than N-fixer plots.
3. Labile-C additions affect N transfer into non-labile pools: Sucrose additions increased the size of the non-labile tracer-N pool for all species.

Table 2. Microbial biomass N and K_2SO_4 extractable organic and inorganic N before and after 393-d laboratory incubations with (+Sucrose) and without (Control) sucrose additions. Soils were from replicated plantations of *Eucalyptus* (Euc) and two N-fixing trees, *Casuarina* (Cas) and *Leuceana* (Leu). Values are means ($n = 3$) and one standard error in parentheses. The only significant difference among treatments was an increase in post incubation inorganic N in sucrose-amended soils ($P = 0.04$).

		Microbial biomass N		Organic N		Inorganic N	
Treatment	Species	Preincubation	Postincubation	Preincubation	Postincubation	Preincubation	Postincubation
		g N m^{-2}					
Control	Euc	8.4 (1.3)	7.1 (1.8)	1.9 (0.5)	1.8 (0.5)	4.7 (1.3)	1.8 (0.7)
+Sucrose	Euc		8.0 (1.8)		2.4 (0.3)		3.2 (1.0)
Control	Cas	7.9 (1.1)	6.6 (1.8)	1.8 (0.7)	1.6 (0.6)	4.9 (0.3)	1.9 (0.5)
+Sucrose	Cas		8.0 (2.0)		1.7 (0.4)		3.3 (1.4)
Control	Leu	10.9 (0.2)	9.1 (0.7)	2.5 (0.6)	1.5 (0.6)	7.1 (0.5)	2.9 (1.0)
+Sucrose	Leu		10.3 (1.0)		1.9 (0.2)		4.3 (2.2)

Most Soil Nitrogen Was Non-Labile

Previous incubation-based estimates of non-labile N pool sizes in forest (Fyles and McGill, 1987; Motavalli et al., 1995; Scott, 1998) and agricultural soils (Stanford and Smith, 1972; Campbell et al., 1981) were similar to ours. In addition, several laboratory experiments with recent (<6 mo) ^{15}N additions recovered more than 45% of added ^{15}N in chemical or physical fractions that are considered non-labile (Stanford et al., 1970; He et al., 1988; Strickland et al., 1992; Chang and Preston, 1998). Bioassay experiments produced similar results; plants used only a small fraction of fertilizer retained in soil more than 6 mo (Power and Legg, 1984; Webster and Dowdell, 1985; Preston and Mead, 1994a, 1994b; Chang et al., 1999).

Older ^{15}N additions, such as ours, are rare. Preston and Mead (1994a) measured the distribution of ^{15}N 1 and 8 yr after a fertilizer application to a lodgepole pine (*Pinus contorta* Dougl. ex Loud.) plantation. Between Years 1 and 8, soil N pools lost ~50% of the fertilizer N they initially retained. In perennial grasslands, total soil ^{15}N recovery after 5 yr decreased (Webster and Dowdell, 1985), increased (Clark, 1977) or stayed constant (Smith and Power, 1985). More (and longer) case studies are required before we can generalize about field retention of ^{15}N in soils. In addition, studies that document non-labile ^{15}N recovery throughout the soil profile would augment the data reported here for surface soils. We recovered 45% of the tracer N in the top 20 cm of mineral soil and 62 to 76% of this recovered N was non-labile, thus, one-third of the total tracer application was stored in non-labile surface soils. The total non-labile soil tracer recovery is probably larger than this because deeper soils and forest floor material likely contain significant pools of non-labile tracer N.

While non-labile N pools were clearly the dominant sink for N in our experiment, we also isolated a pool of labile N (up to 90 g N m^{-2}) that was larger than typical field measurements of inorganic N pool sizes, annual net N mineralization, or microbial biomass N. Each of these pools accounts for <5% of total soil N; even their sum would be less than half of our measured labile pool. The retention of such a large pool of labile soil N implies that either: (i) microbes don't mineralize all of the labile N because of energetic (labile C) or microclimatic constraints on growth, or (ii) high microbial demand for N limits net N mineralization and subsequent plant uptake or leaching. Recent estimates of high gross N mineralization (10 or more times greater than net rates; Stark and Hart, 1997) and immobilization rates support the latter hypothesis.

Tree Species Affect Nitrogen Retention

Stands with N-fixing trees contained a greater proportion of retained tracer N in non-labile soil pools than Euc stands. Several mechanisms could increase the proportion of non-labile tracer N under N-fixers relative to Euc. Large N inputs and small plant N uptake by the N-fixing trees (Table 1) may have promoted leaching losses of labile tracer N which would increase the proportion of retained tracer that was non-labile. Conversely, Euc soil may have retained a larger pool of labile tracer N through efficient recycling between labile plant, soil, and microbial pools (Clark, 1977). The trend toward smaller labile tracer N pools in N-fixer plots was not significant, suggesting that increases in non-labile tracer pools also contributed to the increased proportion of non-labile tracer retained under N-fixers.

Nitrogen-fixers could increase non-labile N pools by altering the quantity or quality of C or N inputs to soils. Differences in C quantity could alter humification rates (i.e., non-labile N formation) by increasing microbial activity (see below) and differences in C quality could alter the availability of humus precursors such as lignin (Melillo et al., 1989; Stevenson, 1994; Berg, 2000). Similarly, large N inputs may increase humus formation, either because N is a substrate for humus forming reactions or because N inhibits formation of enzymes that decompose lignin (Fog, 1988; Berg and Matzner, 1997; Berg, 2000; Carrierro et al., 2000). Two recent studies in tropical forest plantations (including ours) showed that N-fixing trees inhibit the decomposition of old (7–17 yr) soil C relative to Euc (Kaye et al., 2000; Resh et al., 2001). The large C and N inputs in our N-fixer plots (Table 1) could have promoted humus formation and increased the proportion of tracer N shunted into non-labile pools.

Whatever the mechanism, if tree species affect the proportion of N retained in non-labile pools then forest composition may affect long-term N retention in soils. Our non-labile pool will be less susceptible to remineralization, leaching, and plant uptake than the labile pool. Species that increase the proportion of N in this non-labile pool should increase the time N is stored in soil.

Labile Carbon Affects Non-Labile Nitrogen

Microbial transformations of organic and inorganic N depend strongly on C availability to microorganisms (Jansson, 1958; Kelley and Stevenson, 1987; Hart et al., 1994). In general, labile C additions promote microbial growth, which increases microbial demand for N and increases gross N immobilization of inorganic N. While these short-term effects of C on microbial immobilization are well documented, it is less clear whether microbial N uptake promotes long-term N retention. Our results showed that microbial N uptake and subsequent microbial death increase the transfer of inorganic and labile tracer N into non-labile pools. On every leaching date and for all species, N leached from the sucrose-amended samples was lower than controls (Fig. 1). By the end of the incubation, sucrose additions had increased non-labile N pools by 12 g N m^{-2}.

Higher N retention in the sucrose-amended incubations could simply result from greater microbial biomass N. There was a non-significant ($P = 0.14$) increase in biomass N in sucrose-amended plots, but even if this trend were real, the small increases in microbial biomass (sucrose-amended minus control <1.5 g N m^{-2}) cannot explain the large increase in soil N retention (~12 g N m^{-2}). Even if we use the lowest extraction efficiency,

k_n, reported in the literature (0.18; Voroney et al., 1993), making our post incubation microbial biomass N >10% of total soil N, microbial biomass can account for <4.5 g N m^{-2} of increased N retention with sucrose additions.

These results support the hypothesis (Stark and Hart, 1997) that microbial turnover, rather microbial storage in biomass, is a major mechanism of N retention in soil. By maintaining high gross immobilization rates, microbes convert mobile forms of N (especially NO_3^-) into less mobile forms (microbial biomass and necromass N). Our results further document that microbial turnover fosters the production of non-labile organic N. This could result from increased formation of non-labile microbial tissues (He et al., 1988), microbial production of humus precursors (Stevenson, 1994), or interactions between microbial byproducts and clay surfaces (Strickland et al., 1992). Microbial facilitation of non-labile N formation provides and important link between short-term (24 h) ^{15}N immobilization experiments (Stark and Hart, 1997) and long-term (months to years) ^{15}N tracer retention (Tietema et al., 1998; Nadelhoffer et al., 1999).

CONCLUSIONS

Previous studies suggested that tree species composition affected N retention (Magill et al., 2000; Peterjohn et al., 1999; Rothe et al., 2001). However, in these experiments the relationship between species composition and N retention was equivocal because the experimental design included confounding factors that covaried with species composition. In this study, species composition was experimentally manipulated and tree species clearly affected the proportion of retained tracer that was non-labile. Across all species, most bulk soil N and recovered tracer N were stored in a non-labile pools, suggesting that soil organic matter will be a long-term sink for N added to forests. Three mechanisms may be responsible for the increased proportion of non-labile tracer in N-fixer plots: (i) greater labile tracer N losses, (ii) increased non-labile organic matter formation resulting from increased N inputs, or (iii) increased microbial transfer of tracer N from labile to non-labile pools, as simulated by our sucrose addition experiment.

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