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DISSERTATION

RECALCITRANT NITROGEN POOL DYNAMICS IN FOREST AND GRASSLAND SOILS

Submitted by

Jason Philip Kaye

Graduate Degree Program in Ecology

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2000

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPEVISION BY JASON PHILIP KAYE ENTITLED RECALCITRANT NITROGEN POOL DYNAMICS IN FOREST AND GRASSLAND SOILS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Advisor

Department Head

ABSTRACT OF DISSERTATION

RECALCITRANT NITROGEN POOL DYNAMICS IN FOREST AND GRASSLAND SOILS

Most nitrogen (N) cycling research has focused on the relatively small pool of labile N that is cycled annually by plants and soil microorganisms. However, most ecosystem N is in soil organic pools that are not actively cycled by plants and microbes. The purpose of this dissertation was to measure the pool size, accumulation rate, and N sink potential of soil organic N pools that were not readily available to microorganisms (called non-labile or recalcitrant). I used soils from: 1) a subtropical plantation with three tree species and a 6 to 9 year-old ^{15}N addition, 2) a Taiga floodplain successional sequence with 1 to 500 + year-old terraces and an ^{15}N label added in the laboratory, and 3) a Great Plains soil C and texture gradient with a 2 year-old ^{15}N addition. I separated total soil N and ^{15}N into labile and non-labile pools using long-term incubations with repeated leaching.

In the tropical plantation, tree species did not affect non-labile N pools and 77 and 65 % of total soil N and ^{15}N were not labile, respectively. During Taiga floodplain succession, non-labile N pools increased by 2 g $N/m^2/yr$ for the first 50 years and then by 0.6 g $N/m^2/yr$ for the next 200 years. Thirty percent of the ^{15}N

added 3 weeks prior to the incubation was non-labile. In grassland soils, 80 and 50 % of total soil N and ^{15}N were not labile, respecitively. Soil C (r^2 = 0.72) correlated better with N pool sizes than soil texture (r^2 = 0.27). Across all three sites, non-labile N correlated with non-labile C (r^2 = 0.68) and labile N correlated with labile C (r^2 = 0.74).

Non-labile N pools increase in size rapidly and release N slowly. They will be an immediate, large, and long-term sink for N added to soils. Rapid sequestration of N into a slow-release pool may explain the asynchrony between N inputs and outputs in terrestrial ecosystems. Incorporation of N into non-labile pools may or may not require microbial immobilization and abiotic mechanisms of N retention warrant further study.

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I would like to thank my committee, Indy Burke, Gene Kelly, and Mike Ryan for steering my research and greatly improving this final product. Dan Reuss provided know-how, time, and lab space and Tony LeManager did most of the leaching and respiration measurements; this project would have taken twice the time without them. Jeb Barrett, Indy Burke, Chuck Rhoades, Xiaoming Zou, and John Parrotta, will be coauthors on some of these papers and they have contributed ideas, field time, and editing. Sally Dunphy guided me through the bureaucracy and was extremely helpful in general.

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CHAPTER 1

INTRODUCTION

For several decades, nitrogen (N) cycling research has focused on the relatively small pool of labile N that is made available to plants. This focus was clearly warranted because N limits plant production in most temperate ecosystems (Vitousek and Howarth 1991). Research on plant available N has been quite successful. We now understand that most plants use N that is converted from organic to inorganic forms by soil microorganisms and that net N mineralization depends on the amont of N immobilized by microbes (Jannson 1958, Paul and Juma 1981, Stark and Hart 1997). In addition, the availability of N to both plants and microbes depends on the quality (lignin: N ratio) of the organic material being decomposed (Melillo *et al.* 1982, Berg and McClaugherty 1989, Scott and Binkley 1997).

While our understanding of factors controlling plant available N is becoming complete, we still know very little about the N that is not available to plants. Most soil N (organic N; > 1000 mgN/kg soil) is not in plant-available pools (inorganic N; < 10 mgN/kg soil). Furthermore, the soil organic pool releases N slowly; only 1 to 3 % of total soil N is mineralized annually. Because organic N pools are large and release N slowly (i.e. relatively non-labile), their size may determine how much N can be stored in soil. Soil N storage relates directly to a number of important ecological issues, including:

1) Atmospheric N deposition

About 20 Tg/y of N is emitted to the atmosphere from fossil fuel combustion (Vitousek *et al.* 1997). Some of this atmospheric N is then deposited on terrestrial ecosystems at rates commonly ranging from 1 to 25 kg N/ha/yr. Initially, some ecologists were concerned that high rates of N deposition would saturate plant and soil N sinks, causing stream water nitrate concentrations to reach pollutant levels (Aber et al. 1989). However, field studies have shown that most of the deposited N is retained in the organic or mineral horizons of soils (Magill *et al.* 1997, Tietema *et al.* 1998, Aber *et al.* 1998, Nadelhoffer et al. 1999). Increasing N deposition tends to increase the amount of N retained in soils, not the amount of N leached (but see Tietema *et al.* 1998). Thus, the capacity of a system to incorporate and retain N in non-labile organic pools may determine when or whether that system will reach N saturation (Aber 1992, Fenn *et al.* 1998).

2) Fertilizer Efficiency

As with atmospheric deposition, fertilizer N additions do not necessarily increase the percentage of the fertilizer N reaching trees (Binkley *et al.* 1995, Magill *et al.* 1997). Many experiments have shown that only a fraction of fertilizer added to forests is incorporated into tree biomass (~10-40 %; Binkley *et al.* 1995) or microbial biomass (~15 %). A greater percentage (~40 to 90 %) is incorporated into the soil organic

matter where it is not immediately available to plants (Vitousek and Matson 1985). Some of the fertilizer N incorporated into soil organic matter may eventually be mineralized and taken up by trees. However, the fraction of this organic N that is eventually mineralized is not known. Understanding controls on non-labile N pool dynamics could improve fertilizer management.

3) Long-term nutrient availability

Inputs and outputs of N are not necessarily evenly distributed in time. Inputs vary greatly depending on fertilization, atmospheric N deposition, and biological N fixation. These pulse inputs may be incorporated into non-labile soil pools that then slowly release the N over long time scales. This temporal redistribution of N may impart long-term N availability to ecosystems with infrequent pulse inputs of N (Johnson 1992). Alternatively, the N retained in non-labile pools could be lost episodically following disturbance.

Why does organic N accumulate in soils? Why are microbes unable to mineralize all soil N? Two potential mechanisms are prevalent in the literature. The first mechanism is that soil organic N compounds can not be decomposed by microbes because of energetic limitations (Berg 1986, Berg and Matzner 1997). The N bound to non-labile organic matter (humus) is part of large, complex molecules that resemble lignin (Clinton *et al.* 1995, Hatcher *et al.* 1981). In order to decompose humus, heterotrophs must secrete exoenzymes that reduce the size of the molecules so they

can be incorporated into microbial cells (Meyer 1994). Exoenzymes are N-rich, energetically costly compounds so mineralization of any nitrogen bound to humus is thermodynamically limited. Furthermore, Berg and Matzner (1997) have suggested that adding N to soils promotes formation of non-labile N and C because N is a substrate for the condensation reactions that form humus. If this theory were correct, it would help explain why increased N amendments to ecosystems result in increased soil organic N pool sizes.

A second mechanism that may increase recalcitrance of soil organic N is physical protection through associations with clay and silt particles (Tisdall and Oades 1982, Hassink 1997). Soil colloids form aggregates around organic matter, which may limit microbial access to OM. Some work has shown that physical protection of organic matter limits C loss from agroecosystems and grasslands (Hassink 1997, Strickland *et al.* 1992).

Both physical protection and microbial thermodynamics likely contribute to N retention in most ecosystems. Consequently, differences in non-labile N pools among ecosystems probably result from differences in either organic C chemistry or soil texture. My dissertation focused on how soil C, soil texture, N inputs, and time control the size and turnover rate of non-labile soil N pools using three experiments. The first experiment (Chapter 2) assessed species effects on non-labile N accumulation by measuring the fate of ^{15}N applied to a tropical forest plantation. The second experiment (Chapter 3) explored the capacity of soils to retain N in non-labile soil pools over long time scales using a primary forest successional sequence in Alaska.

The third experiment (Chapter 4) measured effects of native soil C content and texture on the size of non-labile N pools and the retention of ^{15}N in grassland soils.

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CHAPTER 2

RETENTION OF ^{15}NITROGEN IN LABILE AND NON-LABILE SOIL POOLS BENEATH A SUBTROPICAL TREE PLANTATION

ABSTRACT

Recent ecosystem budget and ^{15}nitrogen tracer experiments have shown that soil organic matter is the largest sink for nitrogen (N) from both fertilizer and atmospheric deposition. The mechanisms by which inorganic N is incorporated into, and retained in soil organic N pools are poorly understood. We assessed the effects of three tree species (two N-fixers and one Eucalyptus species) on N retention in a 9-year-old tree plantation in Puerto Rico. Two N pools were observed: the bulk soil N, and ^{15}N labeled fertilizer added 6-9 years prior to our sampling. We used long-term laboratory incubations to determine how much soil N and ^{15}N were labile or non-labile. We hypothesized that soil ^{15}N retention would be greater beneath Eucalyptus and that most of the soil ^{15}N would be in non-labile pools. In addition, we treated soil with labile C to assess linkages between C availability, microbial activity, and N retention.

Tree species did not affect non-labile N pools; under all tree species, 75 % of total soil N and 65 % of ^{15}N were non-labile. Labile C additions consistently increased the size of the non-labile N pool and decreased the labile pool. While most soil N and ^{15}N were stored in non-labile pools, 100 g N/m^2 were labile and may be

susceptible to future mineralization, plant uptake, or leaching. Microbial immobilization and rapid turnover of microbial biomass are the most likely mechanisms by which the large labile pool is retained in soil.

INTRODUCTION

For several decades, nitrogen (N) cycling research has focused on the relatively small pool of labile N that is used by plants. This focus was clearly warranted because available inorganic N limits primary production in most temperate ecosystems (Vitousek and Howarth 1991). Yet, the focus on plant-available N has failed to explain several key aspects of the N cycle. One significant shortcoming is the failure to predict the fate of inorganic N added to the soil surface.

Several authors (Vitousek and Reiners 1975, Aber et al. 1989) suggested that plant and microbial uptake should be main mechanisms of N retention in young, N limited ecosystems. Trenching and harvesting experiments supported this theory because nitrate leaching often increased when plant uptake was eliminated (Likens et al. 1969, Vitousek et al. 1982). While N retention may be related to the presence of plants, ^{15}N tracer experiments do not support the hypothesis that N is retained in plants; most added ^{15}N is retained in soil organic matter (Mead and Pritchett 1975, Heilman et al. 1982, Melin et al. 1983, Clinton and Mead 1993, Preston and Mead 1994, Tietema et al. 1998, Nadelhoffer et al. 1999). These tracer studies also suggest that incorporation of added N into the organic N pool is quite rapid, occurring within 2 to 3 years of application.

Though soil is clearly the most important sink for fertilizer and atmospheric N deposition in forests, mechanisms of soil N retention are poorly understood. One fundamental question is whether the retained organic N remains sequestered or recycles quickly into inorganic pools. If the added N is sequestered in a non-labile pool with a slow turnover time, then N additions to terrestrial ecosystems will have little effect on biogeochemistry. On the other hand, N stored in labile soil pools may result in increased N flux to plants and streamwater. Most experiments to date have simply tracked the short-term fate of fertilizer N into plants and soil, without determining whether soil organic N is labile or non-labile.

In temperate ecosystems, soil retention of fertilizer N (Magill et al. 1997) and atmospheric N deposition (Peterjohn et al. 1999, Rothe et al. 2000) vary among tree species. Tree species may affect N retention by altering C inputs, N inputs, plant N uptake, or litter quality (Rothe et al. 2000). Interactions between species composition and soil N retention have not been measured in the tropics, despite projections that fertilizer applications will increase rapidly in tropical countries (Matthews 1994).

In this paper, we assess the effect of three tree species (two of which are N-fixers) on soil N retention a subtropical tree plantation. Two pools of N were observed: 1) the bulk soil organic N pool, and 2) a pool of ^{15}N labeled fertilizer added 6 to 9 years prior to our sampling. Our objectives were to determine: 1) how much of the N retained in soil was in non-labile pools, and 2) whether soil N retention was affected by tree species. Microbial N transformations depend in part on C availability

(Jansson and Persson 1982, Hart et al. 1994), so we also measured soil C pool dynamics and conducted a labile C addition experiment.

Study Site and Methods

The study site is located 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 (tree species) per block. The treatments originally applied were monocultures and mixtures of *Eucalyptus robusta* J.E. Smith, N-fixing *Casuarina equisetifolia* J.R. & G. Forst., and N-fixing *Leucaena leucocephala* (Lam.) de Wit. For research described here, we only sampled the monocultures at 9 years of age. The stands were planted in 16 m x 16 m plots at a spacing of 1 m x 1 m.

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. Upon plantation establishment (September 1989), an aqueous solution of 10.0 % enriched $(^{15}NH_4)_2SO_4$ was added to subplots within each of these treatments at a rate of 1.0 gN/m^2 every 6 months for 3 years for a total of 6 gN/m^2. The ^{15}N was originally added to measure N fixation rates by *Casuarina* and *Leucaena*. The ^{15}N subplots (3 m x 3 m) included 9 trees in the center of each plot and were trenched with plastic to 0.8 m. Soil within these plots had N isotope ratios of 150 to 200 per mil at the time we sampled.

In June 1998, we sampled the top 20 cm of mineral soil within the trenched ^{15}N plots of the monocultures. From each plot, three samples approximately 0.2 m in diameter and exactly 0.2 m deep were composited before analysis and incubation. In addition, a core of known volume (0.0475 m in diameter 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 hours. 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 hours 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, Michigan, USA). Because these soils contain significant C as carbonates, we determined the percentage of inorganic C by adding 6 N HCl and FeCl to a 0.5 g subsample in a serum bottle and measuring headspace pressure produced from CO_2 evolution (Wagner et al. 1998).

We separated labile and non-labile soil N and C pools with a long-term incubation experiment. We defined the labile N and C pools as that which was potentially mineralizable (Stanford and Smith 1972) by microorganisms during 1 year of incubation (Paustian and Bonde 1987) at 35°C (Campbell et al. 1993, Drinkwater et al. 1996) and field capacity soil moisture (Stanford and Epstein 1974). Two soil subsamples (100 g oven dry weight equivalent) from each field composite were incubated in plastic filters (Falcon Filter model 7111, Beckton Dickenson Labware, Lincoln Park, NJ). One of these subsamples was the control and the second received a

labile C addition (see below). Each filter unit included a glass fiber filter (Whatman GF/A) and an "extra thick" glass fiber pre-filter (Gelman Sciences) beneath the soil. A third glass fiber filter (Whatman GF/A) was placed above the soil to prevent dispersion (Motevalli et al. 1995a). The filter units were incubated in air-tight 2 L jars fitted with valves to allow sampling of headspace CO_2. Approximately 20 mL of deionized water were placed in the bottom of each jar to prevent soil drying. Every two weeks this water was changed and the soil brought to field capacity with deionized water.

To determine potentially mineralizable N (Stanford and Smith 1972), we leached the soil at 0, 7, 17, 36, 79, 154, 217, 274, 330, and 393 days 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 sucrose. This C addition was comparable to twice annual belowground C inputs in a Hawaiian *Eucalyptus* plantation (Binkley and Ryan 1998). At each leaching, 100 mL of the non-N leaching solution was added to the top of the filter, allowed to equilibrate with the soil for 1 hour, and then drawn through the filter with a weak vacuum (-0.05 MPa). The vacuum was applied until leachate ceased to drip from the filter (< 10 min). The leachate was frozen until analysis for NH_4^+ and NO_3^- by flow injection colorimetry. Inorganic N in the leachate was converted to mg N per kg oven dry soil and curves of the cumulative N leached versus incubation time were constructed. Curve fitting software (SigmaPlot version 4.00, SPSS Incorporated, Chicago) was used to fit an exponential curve of the form $N_c = N_0 (1\text{-}e^{-kt})$ where N_c is the cumulative N leached, k

is the reaction rate constant, N_o is the potentially mineralizable N (henceforth "labile" N), and t is the incubation time.

Non-labile N was determined by subtracting labile N (N_o) from total soil N. We determined the N isotope ratio of the leachate for each incubated soil by compositing 5mL of leachate from each sampling date. The composite samples were diffused (Stark and Hart 1996, Khan et al. 1998) for 7 days in 120 ml plastic containers. Dvarda'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 µl of 2.5 M $KHSO_4$) filter paper disks (Whatman #1) sealed in Teflon tape. At the end of the diffusion, the acidified disks were dried over concentrated H_2SO_4 for 24 hours and then stored in a desiccator until they were transferred to Sn capsules and analyzed for ^{15}N on a VG isochrom-NA stable isotope ratio mass spectrometer (VG, Middlewich, UK). Samples with less than 85 % N recovery were re-diffused, 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 using ^{15}N standards diffused from solution or applied directly to acidified disks (Stark and Hart 1996). The amount of added N (^{15}N labeled) residing in the labile fraction was determined using the following equations:

$$N_o = N_a + N_n \quad (1)$$

Rearranging

$$N_n = N_o - N_a \quad (2)$$

$$N_o{}^*{}^{15}N_o = Na^*{}^{15}Na + N_n{}^*{}^{15}N_n \quad (3)$$

Substituting from (2)

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

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

Where: N_o is mass of labile N, N_a is the mass of the added 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 added 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 added ^{15}N was in the pre-incubation soil. The amount of ^{15}N in the non-labile pool was determined by subtracting N_a from the amount of added N in the soil at the beginning of the incubation.

To determine the efficiency of our method for removing labile N, we measured microbial biomass N, extractable organic N, and extractable inorganic N in the soil at the beginning and end of 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 hour and then filtered (Whatman #1) and analyzed for NH_4^+ and NO_3^- by flow injection colorimetry. Microbial biomass N was determined by the chloroform fumigation-extraction technique (Brookes et al. 1985). In this method, extractable (100 mL of 0.5 M K_2SO_4) organic and inorganic N are determined before and after the soil has been subjected to a chloroform atmosphere (-15 mm Hg) for 5 days. 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 g oven dry soil fumigated) following fumigation minus total extractable N preceding the incubation.

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. Before the jars were sealed, they were fanned with ambient air for 1 hour to provide a uniform background CO_2 concentration. Then the jars were sealed for periods from 2 days (beginning of the incubation) to 2 weeks (end of the incubation) after which the concentration of CO_2 in the headspace was determined using an infrared gas analyzer (LICOR-6200). The headspace was sampled by first mixing with a 50 mL syringe and then sampling 2 mL with a 10 mL syringe. Three sealed jars without soil were used as blanks to correct for ambient CO_2. Atmospheric pressure, air temperature, the volume of the jars, the volume of gas sampled, and the oven dry mass of the soil were used to convert headspace concentration to mg C per kg oven dry soil. Curves of cumulative C mineralized versus incubation time were constructed and analyzed as described for N. Non-labile C pools were not estimated for the C addition experiment because the C addition would greatly confound results.

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

RESULTS

Neither total soil N pools nor non-labile soil N pools differed among tree species (Fig. 2.1a). Labile soil N was greater in the *Leucaena* (371 mg/kg) treatment than *Eucalyptus* (271 mg/kg; $p = 0.03$) or *Casuarina* (224 mg/kg; $p = 0.01$) treatments. The sucrose addition increased the size of the non-labile N pool ($p = 0.01$) and decreased labile N ($p < 0.01$). The percentage of soil N that was not labile ranged from 73 to 80 % of total soil N but did not differ among species (Fig. 2.2a). Throughout the experiment and for all species and C treatments, more than 95% of the inorganic N leached was NO_3-N (data not shown). Tree species did not significantly affect any of the soil C pools (Fig. 2.1c). Labile C pool sizes ranged from 2974 to 3899 mg/kg soil and were about 30 % of total soil C (Fig 2c). The sucrose addition increased ($p < 0.01$) the labile C pool size by 30%.

About 35% of the ^{15}N labeled fertilizer remained in the mineral soil at the time we sampled (Fig. 2.3). Nitrogen in the leachate was always more enriched in ^{15}N than total soil N, and pre-incubation soil was always more enriched than post-incubation soil (data not shown). Leachate from *Leucaena* plots tended to be less enriched in ^{15}N than leachate from *Eucalyptus* plots (ANOVA $p = 0.09$; Fisher's LSD posthoc comparison $p = 0.06$). The size of the ^{15}N labeled pools did not differ among species (Fig. 2.1b), however, the proportion of ^{15}N that was non-labile was lower in *Eucalyptus* plots than in the *Casuarina* ($p = 0.03$) or *Leucaena* ($p = 0.03$) plots (Fig. 2.2b). 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 ($p < 0.01$).

Pre-incubation microbial biomass did not differ ($p = 0.12$) among species (Table 1). Microbial biomass following 393 days of incubation was similar to the pre-incubation 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 post-incubation soils, however, the sucrose amendment did decrease extractable inorganic N ($p = 0.04$).

Discussion

How much soil N is non-labile?

One of the most consistent results of recent "N saturation" research is that soil organic matter is the dominant sink for applied N. More than 2/3 of added N is typically retained in soil (Aber et al. 1998, Nadelhoffer et al. 1999), preventing leaching losses, cation losses, and soil acidification. The degree to which soil is a long-term sink may depend on whether the applied N resides in a non-labile or labile soil N pool; N stored in non-labile pool will not be as susceptible to re-mineralization and subsequent uptake or leaching. Our results suggest that most soil N and added ^{15}N are in non-labile pools and that soils will be an important long-term sink for anthropogenic N.

While non-labile N pools were clearly the dominant sink for N, we also isolated a large pool of labile N. Labile N pool sizes (100 g N/m^2) were much higher than typical field measurements of inorganic N pool sizes, net N mineralization, or microbial biomass N. Each of these pools accounts for less than 5% of total soil N; even their sum would be less than half of the pool that was labile to microbes during

our incubations. The fertilizer ^{15}N addition was even more labile than the bulk soil N. Previous incubation experiments have found similarly high percentages of labile N in forest soils. Fyles and McGill (1987) found that 12 to 43% of jack pine and 11 to 18 % of white spruce surface soils were potentially mineralizable based on 37-week incubations. Motavalli et al. (1995a, b) conducted 1-year incubations of 13 tropical forest soils classified into 7 different soil orders. The proportion of A horizon N that was potentially mineralizable ranged from 12.5% in an Oxisol to 30.3% in a Mollisol. Stanford and Smith (1972) and Campbell et al. (1981) found similarly high values for more than 40 agricultural soils in the U.S. and Australia. In contrast, Scott (1998) found potentially mineralizable (387 day incubations) values ranging from 1.3 to 2.4 % of total soil N in a temperate forest plantation. These values are lower than annual in situ net N mineralization rates for these soils and an order of magnitude lower than daily N mineralized in 30-day lab incubations (Scott 1998). Long-term ^{15}N experiments show results similar to long-term incubations. Preston and Mead (1994) measured the distribution of ^{15}N one and eight years after a fertilizer application to a lodgepole pine plantation. Soil organic N pools lost about 7 % of the fertilizer N per year between the year 1 and year 8 sampling, suggesting that much of the N originally incorporated into the organic pool was later mineralized.

If up to 40% of bulk soil N and fertilizer N is labile, how is N, both native and added, consistently retained in soil with minimal leaching and plant uptake? One possibility is that the large labile pool that we isolated is simply unused by microbes in the field (perhaps because of microclimatic limitations). This explanation seems

unrealistic in light of recent estimates of high gross N mineralization rates (10 or more times greater than net rates; Stark and Hart 1997). A second possibility is that high microbial demand for N limits net N mineralization and the availability of inorganic N. High gross N immobilization rates in undisturbed forests support this hypothesis (Stark and Hart 1997), and our data suggest that a continually renewed C supply promotes N retention via immobilization (Fig. 2.1). Aber et al. (1998) ruled out N immobilization by free-living microorganisms as a mechanism for N retention because the high C costs and high soil respiration rates required to sustain microbial biomass could not be reconciled with field data from a chronically fertilized forest. They suggested the yet untested hypothesis that soil N retention depended on N flux through mycorrhiza.

Though the specific mechanism can not be determined from existing data, our results clearly show that biological processes are responsible for up to 40% of the N retention in soils. While abiotic processes may contribute to N retention in 65 to 75 % of soil N (our non-labile pool) and they may be responsible for rapid incorporation of inorganic N into organic matter (Strickland et al. 1992), they can not explain the retention of a large labile pool of organic N in soil. Future retention of this large labile N pool will depend solely on whether net microbial mineralization makes the N available for leaching. While plants may "mine" (Johnson 1992a) this future N supply and reduce N leaching, long-term (> 5 years) ^{15}N and ecosystem budget experiments do not show a large flux of fertilizer N into trees (Preston and Mead 1994, Magill et al. 1997), further suggesting that microbes control long-term N retention.

Our results also have important implications for controls on plant-available N. More than 90 % of ecosystem N in forests resides in soils (Cole and Rapp 1981), yet most forests are N limited (Vitousek and Howarth 1991). The low availability of soil N to plants could result from low gross microbial mineralization due to poor substrate quality or microclimate, or strong competition between microbes and plants for soil N (Jansson and Persson 1982, Kaye and Hart 1997). Our results appear to rule out substrate quality as a cause for low N availability in forests; microbes have access to 10 times more N than they mineralize (net N mineralization) each year. Apparently, plant-microbe competition is the main mechanism controlling N availability to plants.

Do tree species affect N retention?

We found only minimal effects of tree species on soil N retention. The N-fixing tree *Leucaena* increased the size of the labile N pool, but non-labile pools of both native and added N did not differ between tree species (Figs. 2.1 and 2.2). The only significant effect of tree species on N was that a greater proportion of fertilizer ^{15}N was labile in *Eucalyptus* plots relative to the N-fixing species (Fig. 2.2). In a similar experiment in temperate forest plantations, Scott (1998) found no differences in potentially mineralizable N among tree species.

Tree species could influence soil N retention in several ways. Nitrogen fixing trees increase soil N inputs but use less soil N than non-N-fixing plants (Parrotta et al. 1993). This combination can promote net nitrification and N leaching (Binkley et al. 1982, Van Moegroet et al. 1990). Despite elevated leaching rates, soil N storage under N fixing trees is typically higher than under non-N-fixers (Fig. 2.1, Johnson 1992b).

Tree species vary widely in C inputs, which could affect N retention by promoting microbial immobilization (see next section). Tree species could alter N retention by changing the fraction of N that is labile or non-labile (i.e. humus quality) without altering the quantity of C or N inputs. Differences in humus quality could arise directly from species differences in litter quality (Berg and McClaugherty 1987, Melillo et al. 1989), or indirectly through litter quality effects on soil fauna (Zou 1993, Garcia-Montiel and Binkley 1998). Finally, species could alter N retention by changing soil structure and the physical protection of organic N in soil aggregates (Scott 1998, Hassink et al. 1993).

It is unclear why fertilizer N was more labile beneath *Eucalyptus* plots than beneath N-fixing trees. Large N inputs under the N-fixing trees (Parrotta et al. 1994) may have promoted N leaching rather than plant or microbial N uptake, decreasing the mean residence time of N in the labile pool. Similarly, increased worm densities under the N-fixer plots (X. Zou, unpublished data) could have increased organic matter turnover. However, both of these process would result in less fertilizer N retention, or at least lower labile fertilizer N pool sizes under N-fixers. We saw no effect of species on ^{15}N retention and only a non-significant trend in labile fertilizer N pool sizes.

Does labile C affect N retention?

Numerous case studies have shown that inorganic N pool sizes and microbial transformations of organic N depend strongly on C availability to microorganisms (Jansson 1958, Paul and Juma 1981, 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. Thus, to the extent that N losses are restricted to inorganic N, increasing labile C should increase N retention. Our results support this hypothesis. On every leaching date, and for all species, N leached from the labile C treatment was lower than that from controls (data not shown).

Higher N retention in the C-amended incubations could simply result from greater microbial biomass N. While microbial biomass tended to be greater in C amended soils, the relationship was not significant ($p = 0.14$). Even if this non-significant trend were real, the small increases in microbial biomass (carbon amended minus control = 3 to 5 mgN/kg soil) can not explain the large increase in soil N retention (20 to 70 mgN/kg soil). Even if we use the lowest k_n reported in the literature (0.18; Voroney et al. 1993), making our microbial biomass pool greater than 10 % of total soil N at the end of the incubation, microbial biomass can account for only 10 to 20 mgN/kg of the increase in N retention with labile C addition. 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 (organic N and NH_4^+).

Conclusions

Ecosystem budget and ^{15}N tracer experiments clearly show that soil is an important short-term sink for fertilizer N in forests. Magill et al. (1997), finding only a small amount of added N in extractable soil N pools, suggested that N was retained in a non-labile soil pool. To some extent our data support this theory; most bulk soil

N and ^{15}N labeled fertilizer were stored in a non-labile pools, suggesting that soil organic matter will be a long-term sink for most N added to forests. However, our data also showed that a substantial fraction (35 %) of added ^{15}N labeled fertilizer was labile 6 to 9 years after the fertilizer was added. This large labile pool of ^{15}N may be remineralized, taken up by plants, or leached in the future. High rates of microbial N immobilization and biomass turnover, rather than retention in biomass, are the most likely mechanisms by which large pools of labile N are retained in soils. Future research should assess relationships between microbial turnover and labile C availability in field, rather than laboratory settings.

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Table 1.1. Microbial biomass N and K_2SO_4 extractable organic and inorganic N from before and after a 393 day laboratory incubations with and without sucrose amendments. Soils were sampled beneath replicated plantations of *Eucalyptus* (E) and two N-fixing tree species, *Casuarina* (C) and *Leuceana* (L). Values are means (n = 3) and one standard error in parentheses. The only significant differences among treatments and species was an increase in post-incubation extractable inorganic N in C-amended soils (p =0.037).

Treatment	Species	Microbial Biomass N		Organic N		Inorganic N	
		Pre-incubation	Post-incubation	Pre-incubation	Post-incubation	Pre-incubation	Post-incubation
		mg N/kg soil					
Control	E	30.4 (5.2)	26.0 (7.1)	7.0 (1.9)	6.6 (1.7)	17.3 (5.1)	6.6 (2.6)
C-Amended	E		29.3 (7.3)		8.9 (1.0)		11.8 (4.0)
Control	C	28.6 (4.1)	24.1 (6.2)	6.7 (2.8)	5.7 (2.1)	17.8 (1.0)	6.9 (2.0)
C-Amended	C		29.1 (7.3)		6.2 (1.4)		12.0 (5.0)
Control	L	40.9 (0.6)	34.6 (3.5)	9.3 (2.1)	5.6 (2.1)	26.6 (1.0)	11.2 (4.1)
C-Amended	L		38.5 (2.7)		7.2 (0.9)		16.7 (8.8)

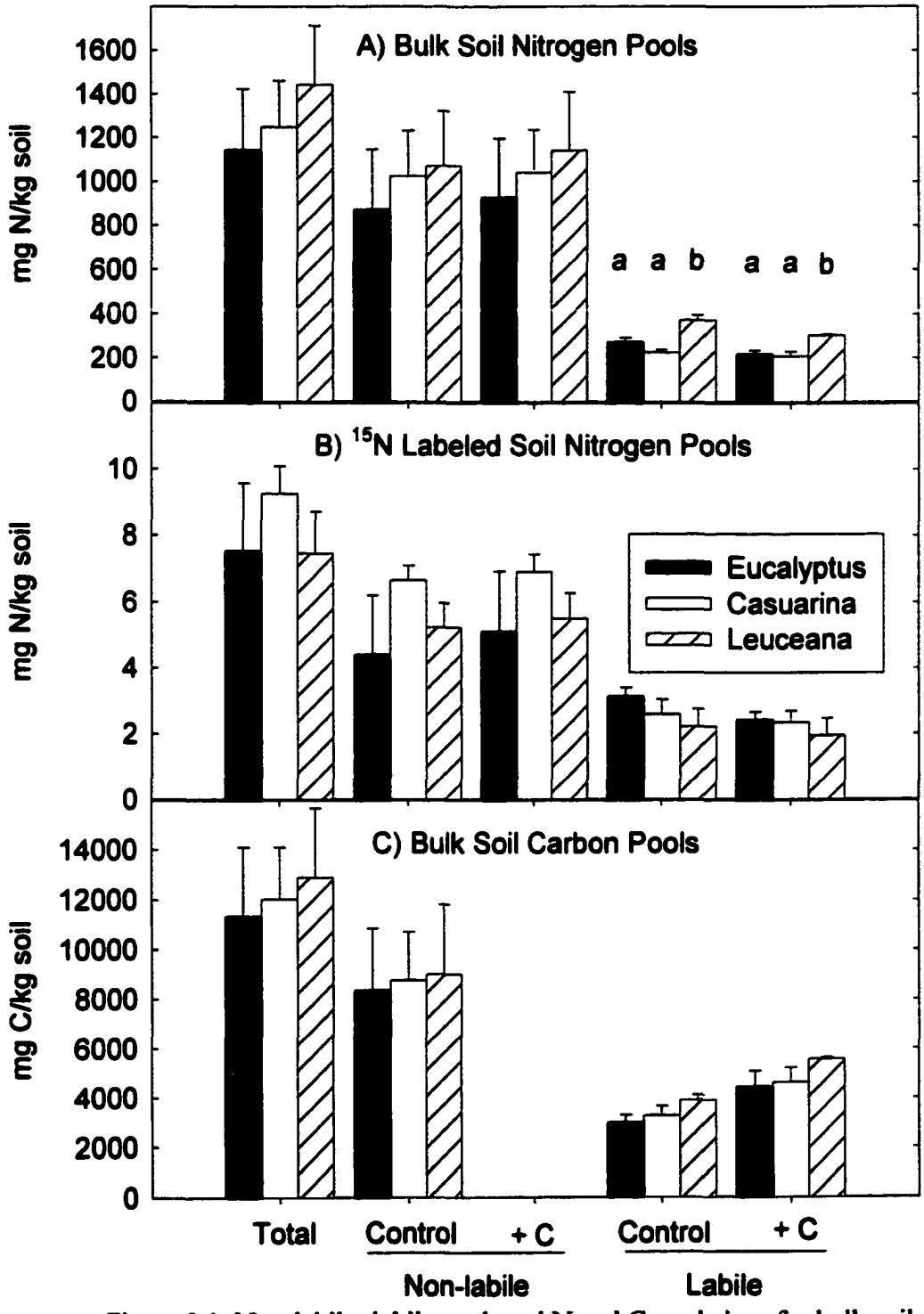

Figure 2.1. Non-labile, labile, and total N and C pool sizes for bulk soil (panels A and C) and ^{15}N labeled fertilizer (panel B). Bars (species) with different lowercase letters were statistically different ($p < 0.05$). Carbon amended soils (+ C) had more non-labile and less labile N than controls. Bars are mean plus one standard error.

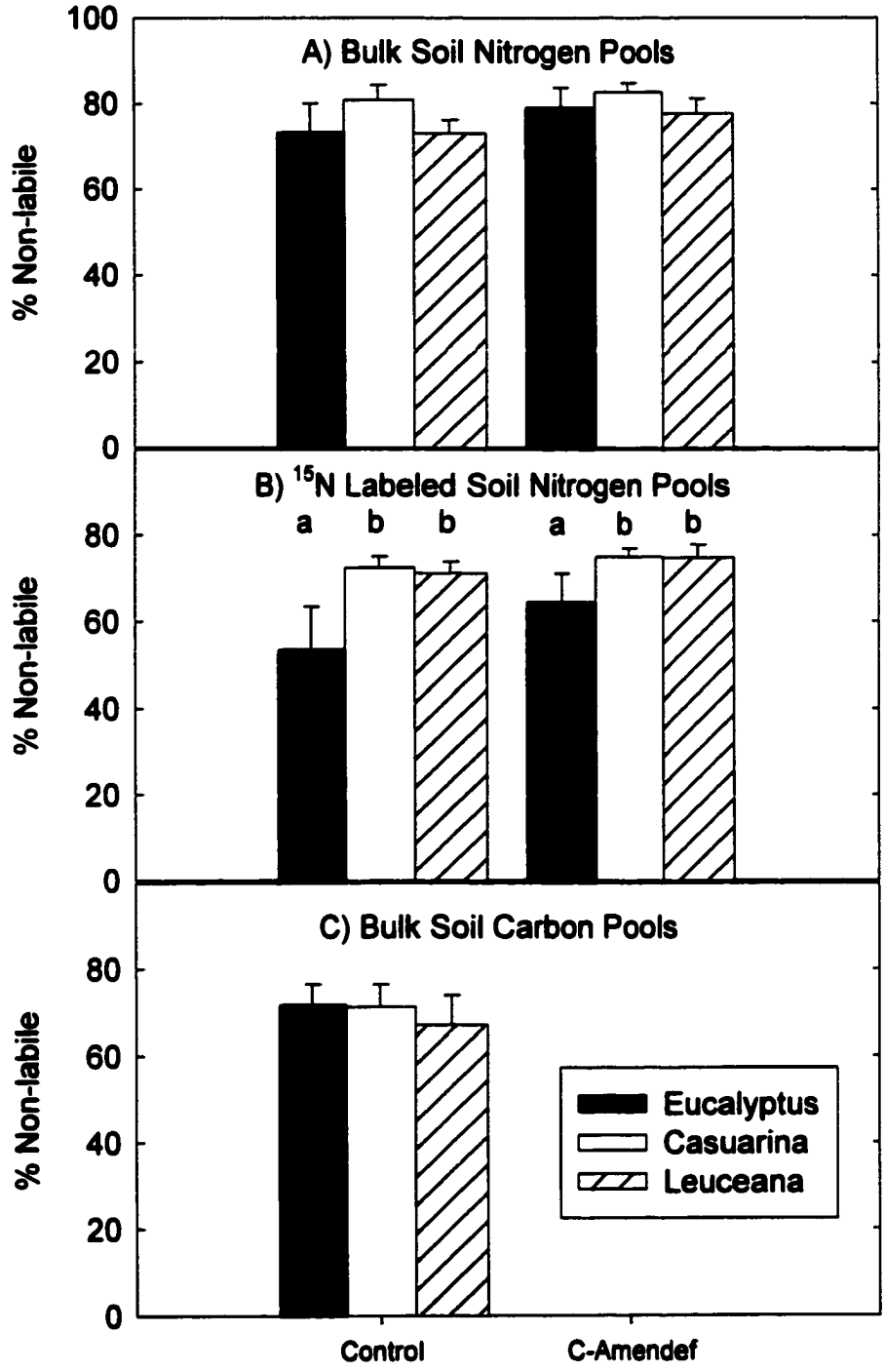

Figure 2.2. The proportion of bulk soil N (panel A), ^{15}N labeled fertilizer (panel B), and bulk soil C (panel C) that was non-labile. Bars (species) with different lowercase letters were statistically different ($p < 0.05$). The percentage of the total pool that was recalcitrant was larger in all carbon amended (C-amended) treatments than controls. Bars are mean plus one standard error.

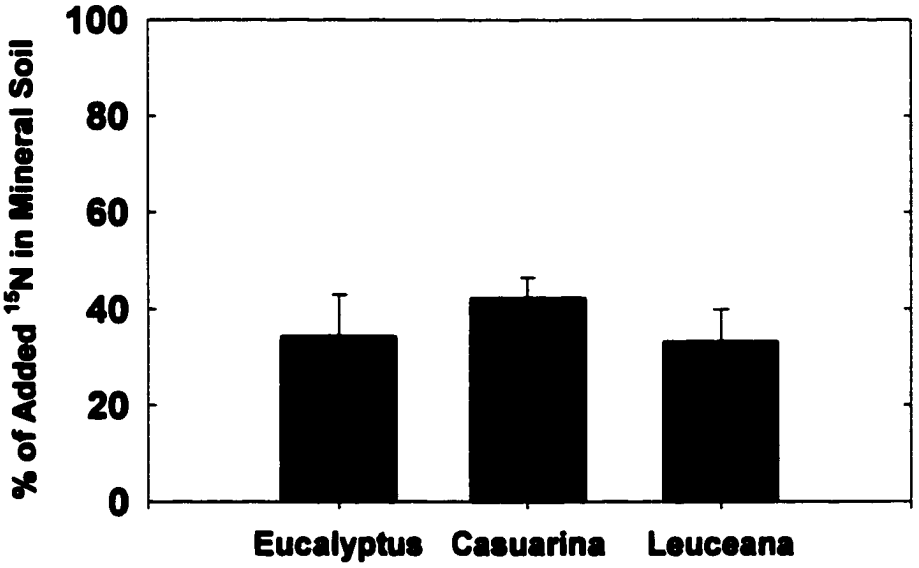

Figure 2.3. The percentage of added ^{15}N labeled fertilizer retained in the top 20 cm of mineral soil 6 to 9 years after the fertilizer was applied. Bars are means plus one standard error and there were no differences among species.

CHAPTER 3

CONTROLS ON LONG-TERM SOIL NITROGEN RETENTION: THEORY AND DATA FROM PRIMARY FLOODPLAIN SUCCESSION

ABSTRACT

Fertilization and atmospheric deposition have increased N inputs to many terrestrial ecosystems. Soil organic matter is the largest long-term sink for this N, but mechanisms of soil N retention are poorly understood. We used a successional sequence on floodplains of the Tanana River near Fairbanks, Alaska to assess rates and mechanisms of N retention in soils ranging from 1 to 500+ years old. We used 1-year laboratory incubations with repeated leaching to separate total soil N into labile and non-labile pools. Both N pools increased rapidly during the first 50 years of primary succession, but then labile N reached a maximum while non-labile and total N continued to increase. Soil C pools showed similar trends, and non-labile N was correlated strongly with non-labile C ($r^2 = 0.95$). From 84 to 95 % of soil N was non-labile during our incubations. Although, the labile N pool declined as a proportion of total N, this was still large on an areal basis (up to 38 g N/m^2). The mass of labile N per gram soil C decreased over time in both the forest floor ($r^2 = 0.85$) and mineral soil ($r^2 = 0.71$). An ^{15}N addition experiment showed that soils had the capacity to retain much more N than accumulated naturally during succession. The ^{15}N was incorporated rapidly into a non-labile N pool by an abiotic substrate (N) limited

reaction. Our results suggest that most soil N is retained in a non-labile organic pool that can accumulate rapidly but is not readily accessible to microbial mineralization. Theoretically, long-term soil N retention should be controlled by net ecosystem production, changes in the soil organic matter pool size, and the soil C:N ratio, but not by changes in plant or microbial uptake.

INTRODUCTION

The accumulation of nitrogen (N) during primary succession has become paradigmatic in forest ecosystem ecology (Crocker and Major 1955, Syers et al. 1970, Sollins et al. 1983, Walker 1989). While the rates and timing of N accumulation vary greatly, all chronosequence studies to date have shown greater N in intermediate or late successional ecosystems than in the youngest ecosystems. Implications of this pattern for long-term nutrient availability, species composition, and primary productivity have been studied extensively (Van Cleve et al. 1993, Vitousek et al. 1989, Vitousek and Farrington 1997, Walker et al. 1986). The pattern may also have implications for current "N saturation" research (Aber et al. 1998), but connections between chronosequence studies and potential ecosystem N retention have not been thoroughly explored.

Ecosystems store N in organic pools that increase over successional time, but usually not infinitely (Vitousek and Howarth 1991). Eventually N inputs balance N outputs on average (Vitousek and Reiners 1975), even though inputs and outputs may not be synchronized. If N inputs and outputs are asynchronous, what determines the rate of change in ecosystem N storage? One possibility is that N inputs accumulate in

a non-labile pool and the rate of release from this pool controls losses. Soil organic matter contains more than 90 % of ecosystem N in many forests. This pool is a large sink for N fertilizer (Nadelhoffer et al. 1999) and it may store N in non-labile pools that slowly release N long after episodic N inputs occur.

To tested the hypothesis that non-labile soil pools accumulate N rapidly, we measured non-labile N accumulation in a primary successional sequence on floodplains of the Tanana River in Alaska (Van Cleve et al. 1971, Walker 1989, Viereck et al. 1993a). Primary succession begins on silt and sand bars deposited by recent floods and proceeds through shrub, deciduous forest, and coniferous forest ecosystems across 500 years. In addition, we conducted a laboratory ^{15}N addition experiment to test whether the capacity of soils to retain N was different from actual, observed rates of N accumulation during succession.

Previous research at this site suggested that forest floor net N mineralization was lower in late successional stages than in mid-successional stages (Van Cleve et al. 1993a,b). The decline in N mineralization was thought to relate to both microclimate and the quality of white spruce litter relative to litter of willow, alder, and poplar trees that precede spruce. To determine whether a single species could alter the size of labile N pools, we compared soil collected from the base of spruce trees with samples from surrounding mixed-species stands.

STUDY SITE AND METHODS

The Floodplain Ecosystems

The soils were collected from the Bonanza Creek Long-Term Ecological Research site near Fairbanks, Alaska. The soils, vegetation, and climate of the site have been described in detail elsewhere (Adams 1999, Viereck et al. 1993a, www.lter.alaska.edu). Briefly, the Tanana is a glacial-fed river that floods annually in spring and summer following snowmelt. When floods deposit sediment greater than 0.5 m above the typical water level, willow (*Salix* spp.) and horsetail (*Equisetum* spp.) proliferate and reduce erosion such that subsequent floods increase the height of the terrace. Young terrace soils are low in organic C and N and high in $CaCO_3$ and $CaSO_4$ (Marion et al. 1993). Within 5 years of vegetation establishment balsam poplar (*Populus balsamifera* L.) and thinleaf alder (*Alnus tenuifolia* Nutt.) cover increase and after 5 to 10 years, a shrub thicket of alder and willows increases the C and N content of the soil and produces the first contiguous organic soil horizon. Balsam poplar lives longer and grows taller than alder and willow and increases in height of poplar cause a transition from shrub thicket to open shrub-poplar forest to closed poplar forest (Walker et al. 1986). Eighty to 100 years following terrace establishment, deciduous forests are overgrown by white spruce (*Picea glauca* (Moench) Voss) forests with thick (10 - 20 cm) organic horizons and a continuous moss understory.

Patterns of succession beyond the first cohort of white spruce (~200 years) are unclear. Early descriptions of succession (Drury 1956, Viereck 1970) suggested that decreased soil temperatures, due increased organic horizon thickness, lead to the development of permafrost, which in turn caused declines in white spruce and the

emergence of black spruce (*Picea mariana* (Mill.) B.S.P.). However, recent work suggests that the transition to black spruce may result from altered hydrologic and fire regimes (Mann et al. 1995), rather than changes in organic horizon thickness alone. Well drained stands of white spruce may or may not convert to black spruce, while poorly drained, fire prone "back swamp" stands convert to black spruce following secondary succession. Black spruce stands contain sparse tree stems and an understory dominated by *Vaccinnium* spp., *Alnus crispa*, and *Salix* spp. Mean annual temperature on the floodplains is -3.3°C but can range from –50 to 35°C. Mean annual precipitation is 269 mm (Viereck et al. 1993b).

Soil Sampling and Laboratory Incubations

In August, 1998, we sampled soils adjacent to permanent plots established by the Bonanza Creek LTER project, and on bare silt and sand bars greater than 1 m above the river (Table 1). The bare sand and silt bars were completely devoid of vegetation and most likely established during the previous flood year, though their actual age could range from < 1 year to 2 years. The exact ages of the LTER black spruce sites is unknown and could range from 500 years to several thousand years (Mann et al. 1995). We use 500 years as a conservative estimate. Details regarding the location, soils, and vegetation of these sites are available at www.lter.alaska.edu.

At each site, we collected three mineral soil cores (4 cm in diameter x 20 cm deep) from a 30 m transect that originated 10 meters from a corner post of the permanent LTER plot. On terraces that had a continuous forest floor layer (30 years and older), we also collected three 10 cm x 10 cm sections of the entire organic

horizon. On the open willow terraces, we doubled the sampling intensity because of potential heterogeneity caused by shrub islands. On sites that contained white spruce (FP2, 3, and 4), we collected an additional set of soils directly beneath the three largest white spruce near the control cores. Spruce trees ranged from 3.5 cm (diameter at breast height) at FP2 sites to 42 cm on FP4 sites.

The soils were double bagged and stored at 4°C until the incubations were initiated one month later. The composite samples were weighed and sieved (4 mm mesh) and a subsample was dried at 105°C (mineral soil) or 70°C (forest floor) for 48 hours. Bulk density of the mineral soil was determined using the mass of the composite and the volume of three soil cores. To convert forest floor N concentrations to pool sizes per unit area, we used density (mass/area) data collected on the LTER control plots in 1989 (www.lter.alaska.edu data file BNZD00076) or from Van Cleve et al. (1993) for the black spruce sites. The 1989 forest floor sampling was extensive (10 samples per terrace) and showed no changes in mass with terrace age. 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 hours 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, Michigan, USA) of oven dry, ground subsamples. Inorganic soil C was determined by adding 6 N HCl with $FeCl_2$ to a dry, ground subsample, and measuring CO_2 produced using a pressure transducer (Wagner et al. 1998).

To separated the total soil N pool into a labile pool and a non-labile pool we used a long-term laboratory incubation approach. A subsample (50 g mineral soil; 15 g forest floor) from each field composite was incubated at 30°C in plastic filters (Nadelhoffer 1990; Falcon Filter model 7111, Beckton Dickenson Labware, Lincoln Park, NJ). This temperature produced the largest net N mineralization rates in short-term laboratory incubations of these soils (Klingensmith and Van Cleve 1993). A glass fiber filter (Whatman GF/A) and an "extra thick" glass fiber pre-filter (Gelman Sciences) were placed beneath the soil and a third glass fiber 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 gasses. Approximately 20 mL of deionized water were placed in the bottom of each jar to prevent soil drying. Every two weeks this water was changed and the soil brought to field capacity with deionized water.

To determine the pool size of labile N, we leached the soil at 1, 14, 29, 43, 84, 111, 179, 238, 295, and 356 days with a solution containing all essential nutrients except N (Nadelhoffer 1990). At each leaching, 100 mL of the non-N leaching solution was added to the top of the filter, allowed to equilibrate with the soil for 1 hour, and then drawn through the filter with a weak vacuum (-0.05 MPa). The vacuum was applied until leachate ceased to drip from the filter (< 10 min). The leachate was frozen until analysis for NH_4^+ and NO_3^- 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. Inorganic N in the

leachate was converted to mg N per kg oven dry soil, summed across all leaching dates, and added to K_2SO_4 extractable N to estimate the total labile N pool size. Non-labile N was determined by subtracting labile N from total soil N.

Microbial respiration was determined by capturing CO_2 in the headspace of the incubation jars. Before the jars were sealed, they were fanned with ambient air for 1 hour to provide a uniform background CO_2 concentration. Then the jars were sealed for periods from 2 days (beginning of the incubation) to 3 weeks (end of the incubation) after which the concentration of CO_2 in the headspace was determined using an infrared gas analyzer (LICOR-6200). The headspace was sampled by first mixing with a 35 mL syringe and then sampling 2 mL with a 10 mL syringe. Ten sealed jars without soil were used as blanks to correct for ambient CO_2. Atmospheric pressure, air temperature, the volume of the jars, the volume of gas sampled, and the oven dry mass of the soil were used to convert headspace concentration to mg C per kg oven dry soil.

An ^{15}N addition experiment was conducted on soils from young poplar forests (FP2) and mature white spruce forests (FP4) sites. We added 18, 25, 50, and 150 mg N to ~50 g soil as 99, 79,79, and 25 % $^{15}NH_4Cl$. The lowest addition approximately doubled the labile organic N pools from these soils and the larger additions were intended to identify thresholds for the capacity of the soil to retain N. After the ^{15}N addition, the soils were incubated for 3 weeks, and then leached on the same schedule described above. At the end of the incubation, a 5 g subsample was extracted three times each with 25 mL of 0.5 M K_2SO_4 and 25 mL of water to remove residual

inorganic ^{15}N. We shook the sample with extractant for 0.5 hours, then added several drops of 0.25 M $CaCl_2$ (to flocculate clay), centrifuged at 10000 rpm for 10 minutes, and then discarded the supernatant. The soil and $^{15}NH_4$ Cl solutions were analyzed for total N and ^{15}N at the Stable Isotope Laboratory, Utah State University, U.S.A. The mass of ^{15}N retained in the soil was calculated from the mass of N in the sample at the end of the experiment (post washing), the ^{15}N enrichment of the sample at the end of the experiment, the ^{15}N enrichment of the added N (measured, not calculated), and the ^{15}N enrichment of soil not receiving N additions (assumed 0.003663 atom fractional enrichment).

We analyzed both the quality of N in organic matter (grams of labile or non-labile N per gram soil organic C, where organic C is a proxy for organic matter content) and the quantity of N in soil (g N/m^2). We used linear and non-linear regression with terrace age as the independent variable to determine the effect of ecosystem age on N and C response variables. The regression model that produced the lowest sum of squares was used. We did not include black spruce sites in regression analyses because the age of black spruce forests was unknown and because the transition to black spruce may relate more to stochastic disturbance events than predictable vegetation changes. We compared black spruce sites to other stages using one-way ANOVA's with dominant vegetation as the main effect. We compared samples collected under white spruce trees to controls using a nested ANOVA with successional stage, replicate within stage (error term for stage), spruce presence, spruce x stage interaction, and stand x spruce within stage (error term for spruce and

spruce x stage) as model effects. A similar nested ANOVA was used to assess the effect of vegetation type and N addition level on ^{15}N retention. ANOVA analyses were done on log-transformed data when residual plots revealed unequal variance.

RESULTS

Both the quality and quantity of soil N changed with terrace age (Figs. 3.1 and 3.2). The amount of labile N per kg soil C (an index of organic matter quality) declined ($r^2 = 0.85$) with terrace age in the forest floor (Fig. 3.1). In the mineral soil, labile N per kg C increased from unvegetated silt and sand bars to willow-alder vegetation but then declined ($r^2 = 0.71$). From 85 to 94 % of the total soil N was non-labile during the incubations.

Labile pools of N ranged from 13 to 38 g N/m^2 on forested terraces (Fig. 3.2; forest floor plus top 20 cm of mineral soil). Both labile ($r^2 = 0.77$) and non-labile ($r^2 = 0.76$) N pools were best described by an exponential curve with a non-zero y-intercept. Labile (~0.4 g N/m^2/yr) and non-labile (~2 gN/m^2/yr) pools increased until year 50, then labile pools leveled off while non-labile N continued to increase with time (~0.6 gN/m^2/yr). Soil C followed similar trends (Fig. 3.2). The amount of C and N in labile ($r^2 = 0.91$) and non-labile ($r^2 = 0.95$) pools were strongly correlated (Fig. 3.3).

Black spruce soils (Fig. 3.2) had less labile N than all other vegetated ecosystems ($p < 0.03$ for all systems except willow-alder: $p = 0.052$). Conversely, non-labile N was greater in black spruce mineral soils than in any other successional stage ($p < 0.01$). Only about 1 % of the N in the mineral soil and 3 % of the N in the forest floor were labile from the black spruce soils during the incubation. We did not

detect differences in labile N (per gram C) between controls and soil sampled directly beneath spruce trees (Fig. 3.4).

Soils beneath white spruce and poplar-alder stands retained up to 30 % of the ^{15}N added prior to the yearlong incubations and spruce soil retained less N per unit C than poplar-alder soil ($p = 0.01$). Increasing the mass of ^{15}N added to the soil increased the amount of ^{15}N retained ($r^2 = 0.67$; Fig. 3.5) mass, but decreased the percentage of ^{15}N retained. Soil respiration did not correlate with the amount of ^{15}N added (Fig. 3.5) or retained ($p > 0.16$, data not shown).

Discussion

Non-labile Nitrogen Pools

Over long time scales, the capacity of an ecosystem to retain N inputs will depend on the relative importance of losses via leaching and denitrification, and retention in biomass and soil organic matter (Vitousek and Reiners 1975, Fenn et al. 1998). Denitrification, while important for greenhouse gas production, does not tend to be large enough to affect ecosystem N retention (Magill et al. 1997). Similarly plant and microbial biomass N are usually a small fraction of total ecosystem N (Cole and Rapp 1981, Smith and Paul 1990). On the Tanana floodplain for example, white spruce and poplar forests have 300-600 g N/m^2 in soil (to 0.8 m) and about 27 g N/m^2 in aboveground biomass (Van Cleve et al. 1983). Thus, over decades or centuries, the vast majority of N inputs, whether they originate from alder fixation on the Tanana floodplains, or N deposition in New England or Europe, will either be leached or retained in soil.

Our data demonstrated that the majority of soil N that accumulated in soil (85 to 95 %) was not accessible to microbes during one-year incubations with optimal microclimate. This non-labile pool accumulated N rapidly through ecosystem development, corroborating Walker's (1989) estimate that surface soils on Tanana River floodplains accumulate 2 to 5 g $N/m^2/yr$ of N during early succession. Soil N accumulated more slowly later in succession, but high N retention in black spruce soils demonstrated that altered hydrology can affect soil N retention.

If the soil N that accumulated rapidly during primary succession is not susceptible to microbial mineralization, then its retention is largely an abiotic phenomenon resulting from physical protection in aggregates (Strickland et al. 1992, Borchers and Perry 1991) or chemical fixation to clay or organic matter (Nommik and Vahtras 1982). The floodplain soils contain minimal clay (mean = 3.7 % clay), and we found a strong relationship between non-labile C and non-labile N (Fig. 3.3), suggesting that reactions with organic matter are the most likely mechanism promoting N retention.

Our ^{15}N experiment supported the theory that N was rapidly incorporated into non-labile organic pools; up to 35 % of the ^{15}N we added was non-labile during the incubation. The amount of ^{15}N retained in the soil was 10 to 20 times the measured annual rate of non-labile N accumulation during succession (Fig. 3.2). This N could have been incorporated into microbial biomass and then into non-labile organic matter following microbial death, but our respiration measurements do not support this hypothesis (Fig. 3.5); N additions did not increase C respired (Fig. 3.5). Even if N

additions increased microbial growth efficiency [(C in biomass/(C in biomass + C respired)] to 0.6 (Hart et al. 1994), assimilation of 1 g of N would have respired more than 4 g of C (assuming microbial C:N of 5) which we would have easily detected. Thus, fixation by clay, physical protection by aggregates, and microbial transformations are all unlikely mechanisms for retention of the ^{15}N.

We suggest that the large ^{15}N retention we observed resulted from chemical reactions between the added NH_4^+ and organic matter (Burge and Broadbent 1961, Mortland and Wolcott 1965, Johnson 1992). While the exact reactions have not been identified, NH_4^+ likely reacts with quinones and phenols in reactions that consume oxygen, increase with pH, and increase with soil C content (Burge and Broadbent 1961, Nommik and Vahtras 1982, Axelsson and Berg 1988). Our soils have relatively high pH (~7) that may have facilitated reactions between $^{15}NH_4^+$ and organic matter. Increased N retention with increasing N inputs (Nommik 1970, Axelsson and Berg 1988) suggests that the reactions that promote N retention are, to some extent, substrate (N) limited. Our data suggest that abiotic incorporation of N into organic matter follows zero order kinetics (Fig. 3.5).

Whether or not atmospheric N deposition reacts with organic matter in the same manner as our large laboratory N additions is unclear. Johnson (1992) suggested that trees are an important sink for small and constant N additions (atmospheric deposition) and soil organic matter becomes an important sink for larger, infrequent N additions (fertilizer and lab additions). NMR spectra may support the hypothsis that N dose affects retention mechanisms because low and high N additions result in different

spectra (Preston et al. 1982, Thorn and Mikita 1992, Clinton et al. 1995). The low pH of many forest soils may also inhibit NH_4^+-organic matter reactions, however, several studies have observed fixation at low pH (Axelsson and Berg 1988, Schimel and Firestone 1989, Johnson et al. 2000). Clearly, we are far from understanding the importance of organic matter-N reactions under field conditions. We suggest that future research should focus on low-dose field studies across a range of sites (cf. Johnson et al. 2000) to assess the real-world importance of abiotic N retention.

Labile Nitrogen Pools

We have emphasized the non-labile N pool throughout this discussion because it will be the most important global sink for N in forest ecosystems. However, we also isolated a large pool of N (up to 38 g/m^2) that was susceptible to microbial mineralization. This labile N pool size is much larger than most ecosystem N fluxes, for example, white spruce trees use 2.5 g $N/m^2/yr$ (Van Cleve et al. 1983) and leaching from intact forest ecosystems is typically less than 1 $g/m^2/yr$ (Vitousek and Melillo 1979). We expect that this labile N pool may respond rapidly to disturbance and changing climate, and thus may be an important short-term source or sink for N (Agren and Bosatta 1988, Fenn et al. 1998). The size of labile N pools should be related to the size of the labile C pool because microbial residues with constrained C:N ratios are a large component of labile organic matter (Fig. 3.3, Smith and Paul 1990). Tree species may affect labile C availability and N turnover on the Tanana floodplains (Flannagan and Van Cleve 1983, Van Cleve et al. 1993, Schimel et al. 1998). Declines in organic matter quality with time support this hypothesis (Fig. 3.1),

however, at the ecosystem level (Fig. 3.2) we did not detect a decline in the labile N pool size with time. In both of these analyses (Figs. 3.1 and 3.2), species changes are confounded with changes in time. When we controlled for time by sampling directly beneath and outside of spruce canopies on the same terrace, we found no evidence for reductions in labile N.

Implications for N retention in Forest Ecosystems

Our data suggest that non-labile soil organic matter pools accumulate native N and added ^{15}N rapidly, but release this N slowly. Similar results have been obtained on a wide range of soils using various fractionation techniques (Table 2). In addition, many studies have shown or simulated rapid incorporation of ^{15}N into soil without determining the lability of the ^{15}N (Shimel and Firestone 1989, Tietema et al. 1998, Currie and Nadelhoffer 1999, Nadelhoffer et al. 1999, Johnson et al. 2000). If soil organic matter in a wide range of ecosystems accumulates N rapidly but releases it slowly, then steady state models of N retention may be misleading.

Vitousek and Reiners (1975) suggested that nitrogen retention should be related to organic matter increment (net ecosystem production, NEP) because N not stored in organic matter would be leached. This theoretical model was based on the assumption that late in ecosystem development NEP approaches zero, at which point N inputs must balance N outputs and ecosystem N storage must approach a steady state. Another important assumption of this model is that the stoichiometry of organic matter is constant.

Theoretically, the steady state assumption of ecosystem N storage must be true on average. However, if the non-labile soil N pool accumulates N rapidly and releases it slowly, then in practice, steady state N retention may never actually be observed in ecological experiments. Carefully constructed input-output budgets support this asssertion; N inputs always exceed N ouputs except in rare cases with N fixing trees or very high N deposition (Johnson et al. 1993, Dise and Wright 1995, Bredemeier et al. 1998). We suggest that the non-labile N pool sequesters pulse N additions and releases them either slowly over long time scales, or episodically after a disturbance that may occur long after the pulse N input. This asynchrony between N inputs and outputs occurs because soil organic matter has a flexible stoichiometry, that is, the C:N ratio changes. This can be seen across the successional sequence, as Figure 3.3 has a significant y-intercept, and in the ^{15}N addition experiment where 30 to 80 g/m^2 were retained with no new C inputs (Fig. 3.5).

Because the non-labile N pool can rapidly sequester N by changing stoichiometry, we suggest results from N addition studies within a given ecosystem will have dramatically different results from comparative analyses across ecosystems. For example Vitousek (1977) measured N concentrations in streams draining ecosystems of various ages in New England and found a positive correlation between ecosystem age and N concentration. We hypothesize that ^{15}N additions to each of these ecosystems, even old ecosystems with zero NEP, would could have been retained in non-labile soil pools.

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Table 3.1. Dominant vegetation and ages of the terraces used in this study. From Adams (1999).

Ecosystem type	LTER Name	Age in 1998	Age Source	Symbol
Bare sand and silt deposit	Not LTER	1,1,1	Assumed	s
Willow with alder shrubs	FP1A,B,C	18,13,18	Aerial photo	wa
Young poplar/alder understory	FP2A,B,C	53,43,68	Aerial photo	pa
Mature poplar/young white spruce	FP3A,B,C	123,128,98	Oldest tree	ps
Mature white spruce/relict poplar	FP4C	153	Oldest tree	sp
Mature-uneven aged white spruce	FP4A,B	271,216	Oldest tree	w
Mature black spruce	FP5A,C,D	500 +	Assumed	b

Table 3.2. Selected studies separating recent ^{15}N additions into labile and non-labile pools.

Reference	% of ^{15}N non-labile	Method	Age of ^{15}N	Soil Type
Stanford et al. 1970	45	554 hr autoclave	14 mo	Hapludult
Chichester et al. 1975	54 to 65 47 to 54	26 wk incubation	1 wk	Hapludult Paleudalf
Smith et al. 1978	43 to 70 73 to 79	24 wk incubation	3 to 6 mo 3 yr	Vertisol Alfisol
Paul and Juma 1981	80	15 wk incubation	1 growing season	Chernozem
He et al. 1988	46	Humin fraction	1 wk	Argiudoll
Strickland et al. 1992	> 50	Density fractionation and slurry incubation	2 mo	Haplaquod Dystropept Argiustol Dystrandept Haplumbrept

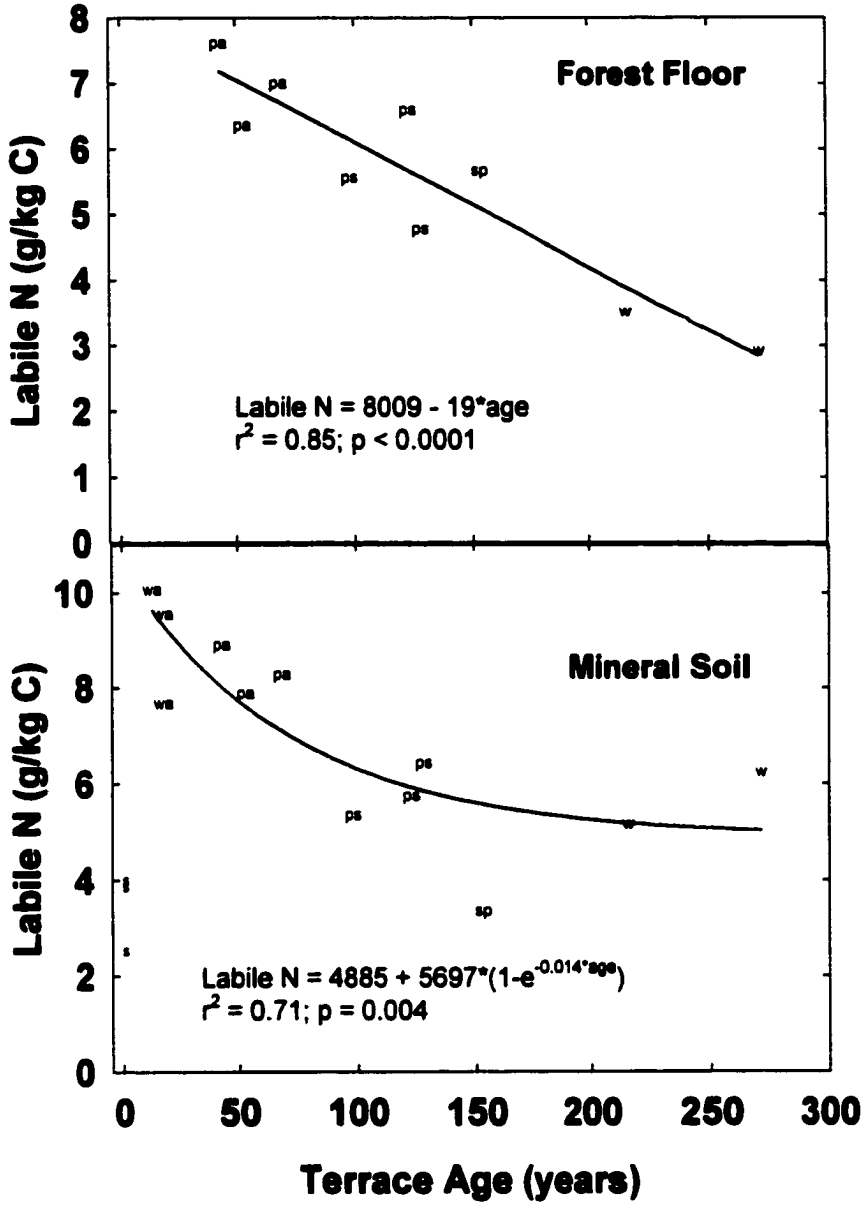

Figure 3.1. Organic matter quality (labile nitrogen per kilogram soil C) in soils of different ages. Symbols denote dominant vegetation: s = silt and sand bar, wa = willow-alder, pa = poplar-alder, ps = poplar-spruce, sp = spruce-poplar, and w = white spruce. Silt and sand bars were not included in the mineral soil regression.

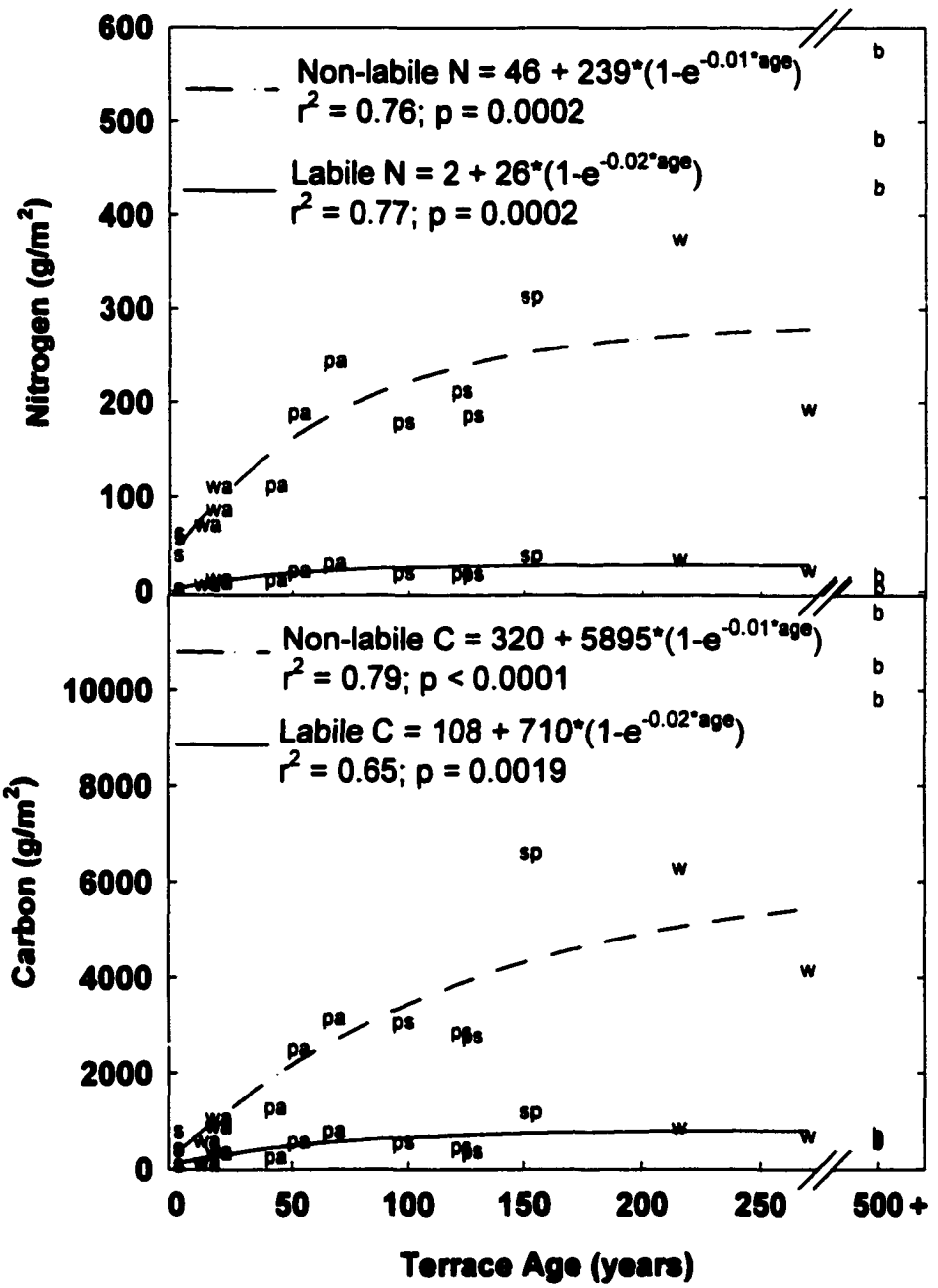

Figure 3.2. Nitrogen (N) and Carbon (C) accumulation in labile and non-labile soil pools. Symbols denote dominant vegetation: s = silt and sand bars, wa = willow-alder, pa = poplar-alder, ps = poplar-spruce, sp = spruce-alder, w = white spruce, and b = black spruce. Black spruce sites were not included in regression analyses.

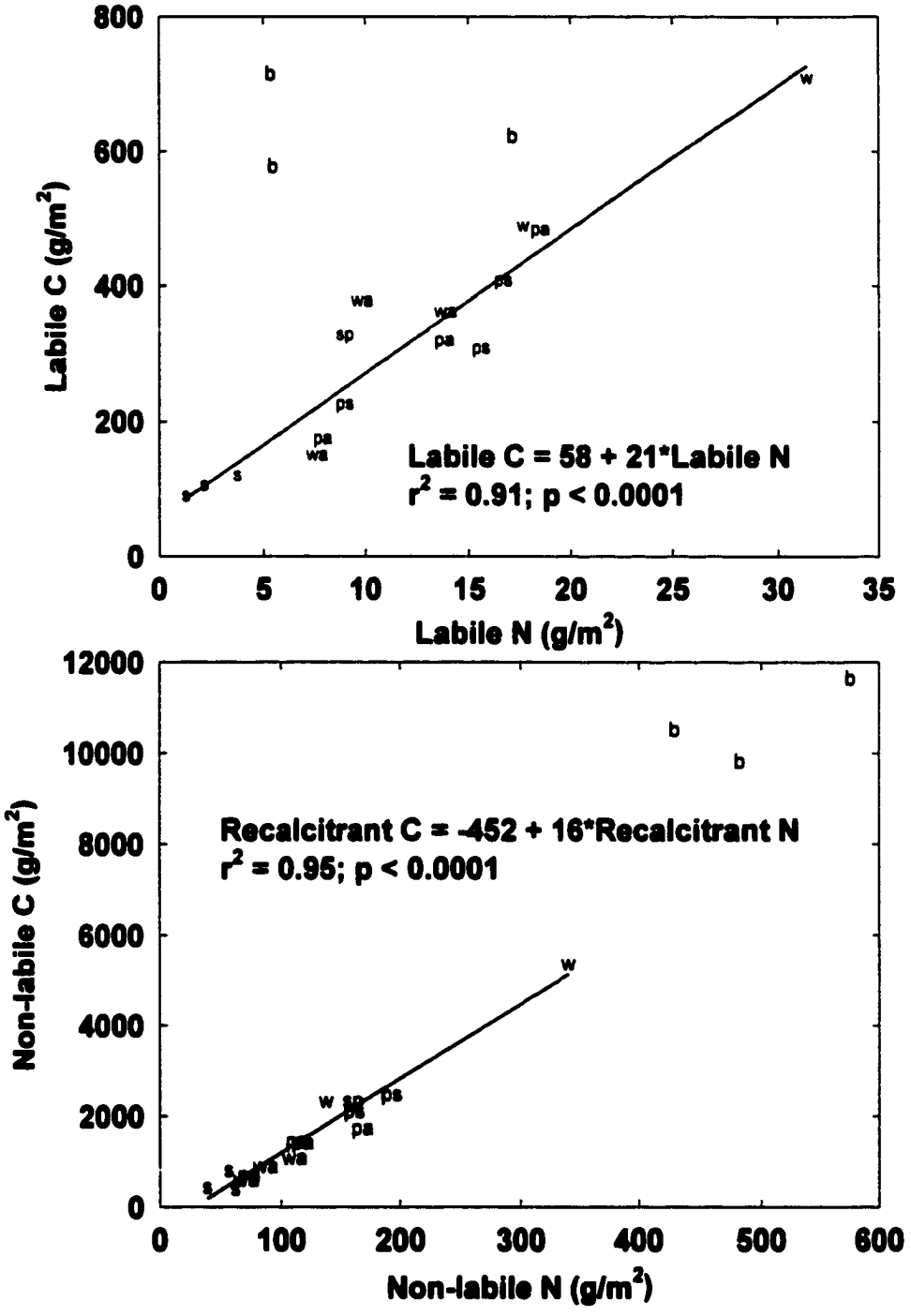

Figure 3.3. The relationship between mineral soil labile (top) or non-labile (bottom) nitrogen and carbon. Symbols denote dominant vegetation: s = silt and sand bars, wa = willow-alder, pa = poplar-alder, ps = poplar-spruce, sp = spruce-poplar, w = white spruce, and b = black spruce. Black spruce data were not included in regression analyses

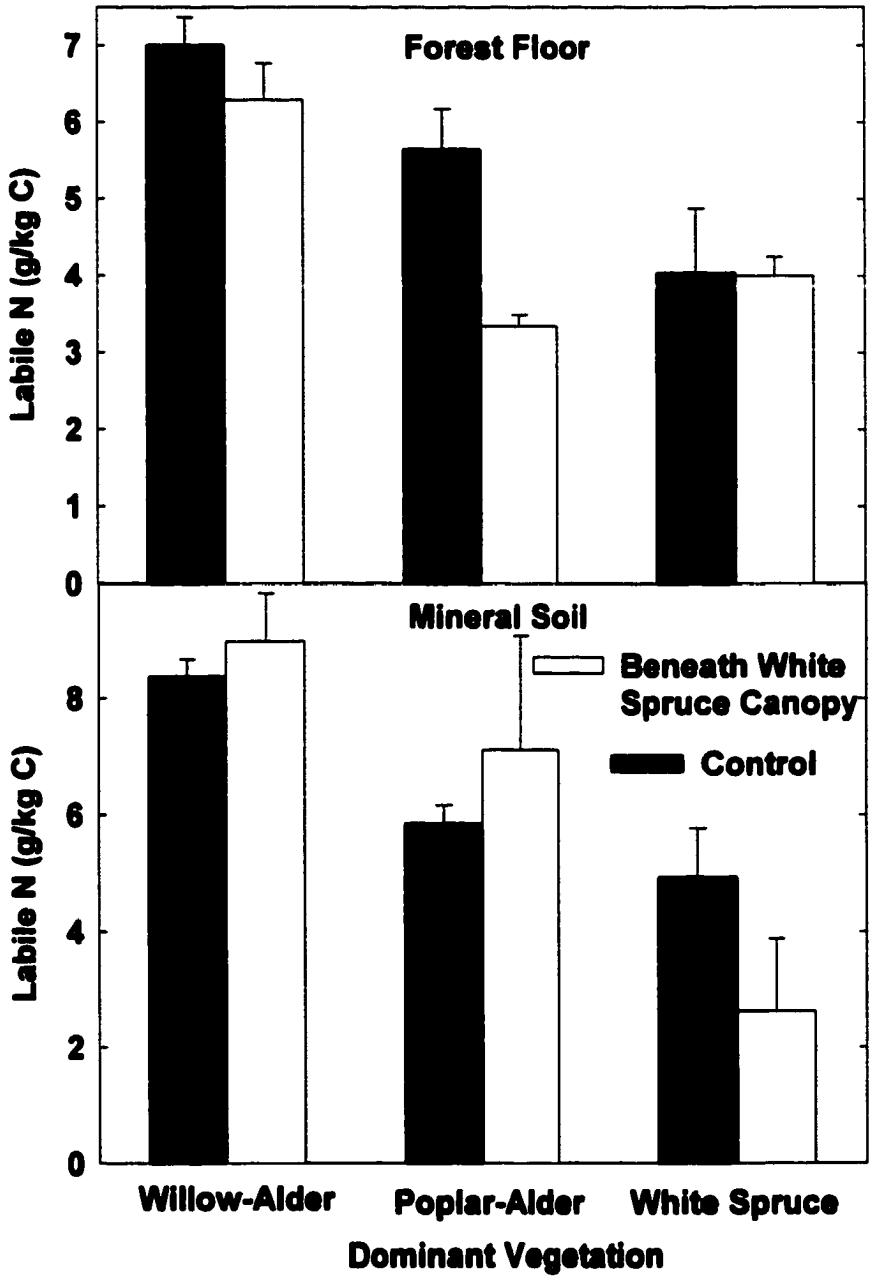

Figure 3.4. Labile nitrogen (per gram initial soil C) from samples collected at the base of white spruce trees and control samples that were collected without respect to canopy type. We did not detect differences between control and spruce-biased samples ($p > 0.17$). Bars are means plus one standard error.

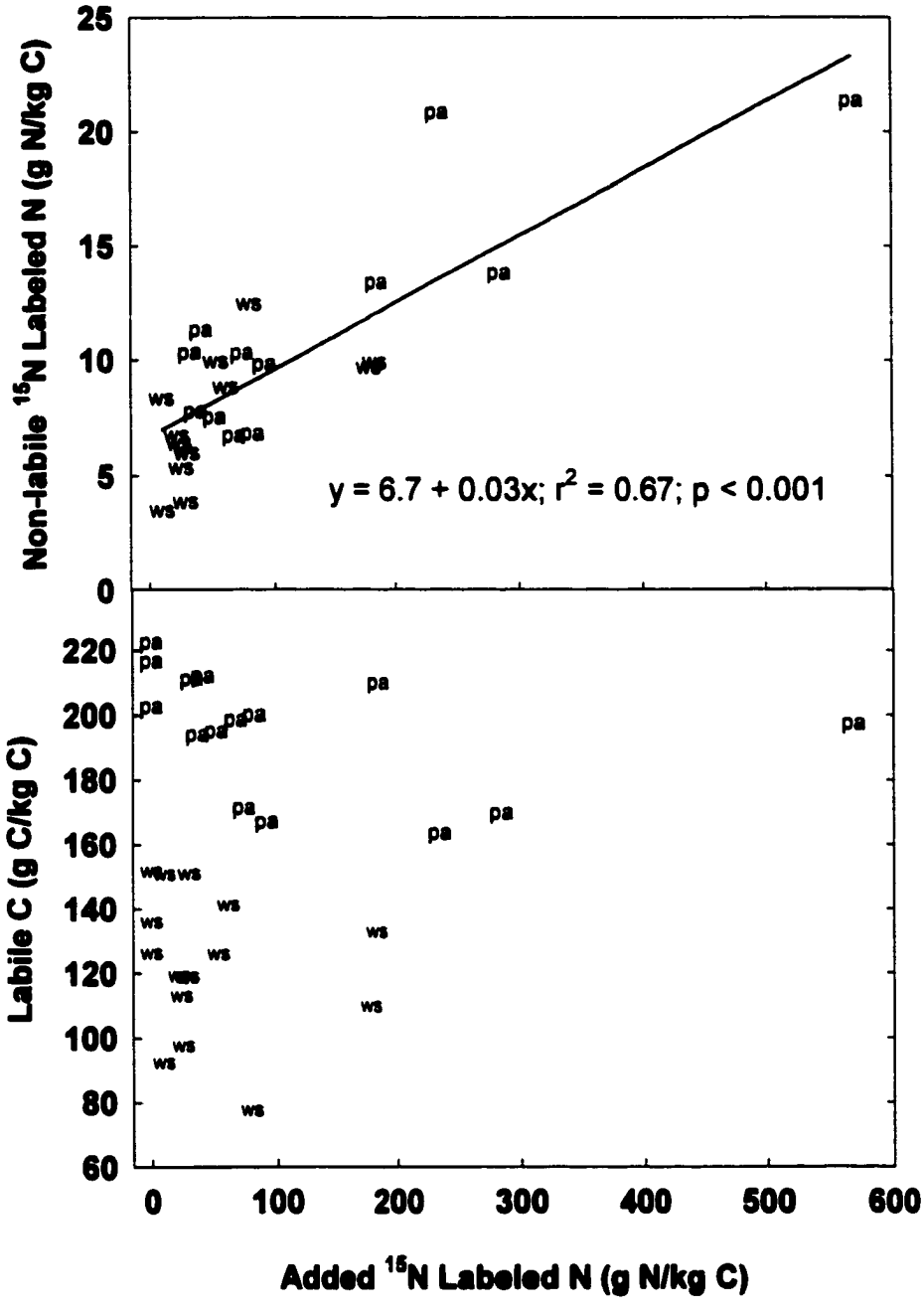

Figure 3.5. The relationship between the amount of ^{15}N labeled N added to soil and the amount of ^{15}N labeled N that was non-labile (top panel) or the amount of soil C that was labile (bottom panel). All data are expressed per unit total soil C before the incubation. Correlations between added ^{15}N and labile C were not significant. Symbols denote dominant vegetation: pa = poplar-alder ws = white spruce.

CHAPTER 4

RAPID INCORPORATION OF ^{15}NITROGEN INTO NON-LABILE SOIL NITROGEN POOLS IN GREAT PLAINS GRASSLANDS

ABSTRACT

Nitrogen (N) inputs to terrestrial ecosystems are increasing and most of these inputs are sequestered in a soil organic pools that are poorly understood. While N retention research has focused on actively cycling pools (plants, microbes, and inorganic N), most ecosystem N resides in soil organic matter that is not cycled by microbes and plants on annual time scales. This large soil N pool may be a significant sink for fertilizer and atmospheric N deposition. In this paper, we determined the N retention capacity of a soil N pool that was not mineralized by microorganisms during one-year laboratory incubations (called the non-labile pool). We added two levels of ^{15}N to coarse and fine textured soils along a gradient of organic matter content in the Great Plains, USA. We hypothesized that soils with high soil C content and fine texture would have the most N and ^{15}N in the non-labile pool. Two years after the ^{15}N was added, 50% of the soil ^{15}N resided in the non-labile pool. The large N addition (50 g/m^2) had no effect ($p > 0.46$) on the labile pool of native soil N. Soil texture and carbon content did not correlated with sequestration of non-labile ^{15}N. Soil C (r^2 = 0.72) explained more variability in non-labile bulk soil N than did the percent of soil

in silt and clay sized particles ($r^2 = 0.27$). These results suggest that N is rapidly incorporated in to a pool that releases N slowly and that soils may be immediate and long-term sinks for N fertilizer and atmospheric deposition.

INTRODUCTION

In 1977, Francis Clark reported on a now classic field ^{15}N tracer experiment in a shortgrass steppe ecosystem. The 5-year study lead Clark (1977) to two important conclusions: plants were the dominant sink for the ^{15}N labeled nitrate (retaining 60 % of the added N), and ^{15}N would only slowly be incorporated into humus that was non-labile to microbes because the plant-microbe N cycle was a tight. Subsequent experiments in the shortgrass steppe (Schimel et al. 1985, Barrett and Burke 2000) and in forests (Tietema et al. 1998, Nadelhoffer et al. 1999) have not corroborated Clark's first finding; plants have not been the dominant sink for ^{15}N in recent tracer studies. Clark's second conclusion has received much less attention. Are N additions slowly incorporated into non-labile pools as Clark (1977) concluded?

There are several mechanisms by which N additions could be rapidly converted to non-labile organic forms (Johnson 1992, Sollins et al. 1996). Soil organic C can react with organic N (microbial and plant necromass) to form complex molecules (i.e. humus) that are thermodynamically costly for heterotrophs to decompose (Meyer 1994). If humus-forming condensation reactions are substrate limited, N retention in non-labile organic matter may depend on soil C content. Similarly, inorganic N (ammonium or nitrite) can react directly with organic matter to form humic substances that are thought to be non-labile (Nommik and Vahtras 1982, Johnson 1992). These

reactions increase with increasing organic C concentration in soil (Burge and Broadbent 1961).

Fine textured soil particles may promote the formation of non-labile soil N pools in at least two ways. Silt and clay particles can physically protect N from mineralization by forming aggregates around the organic matter (Tisdall and Oades 1982, Strickland et al. 1992, Hassink 1997). Aggregation limits microbial access to encapsulated organic matter. In addition, organic matter can be "complexed" with clay when positively charged organic matter becomes associated with negatively charged clay surfaces (Sollins et al. 1996) or when negatively charged organic matter becomes associated with cations attached to clay (Oades 1988). Nitrogen associated with the organo-mineral complex will be stabilized in the process.

In this paper, we examined the fate of a two-year-old ^{15}N addition to several grassland ecosystems to determine whether the added N was labile or non-labile to microorganisms during one year laboratory incubations. We used an experiment established by Barrett and Burke (2000) in which two levels of ^{15}N labeled fertilizer were added to paired coarse and fine textures soils along an organic C gradient in the Great Plains. Two years after the N was applied we determined: 1) the size of labile and non-labile bulk soil N pools, 2) the size of labile and non-labile pools of ^{15}N labeled fertilizer, and 3) whether soil carbon and texture affect the pools in 1) and 2). We hypothesized that the amount of bulk soil N and ^{15}N in non-labile pools would be higher in soils with higher C content and finer texture.

MATERIALS AND METHODS

Study Area

In the spring of 1996, 5 research sites were established along a latitudinal gradient in the semiarid region of the U.S. Great Plains. Soil C content ranged from 2300 g C/m^2 in coarse textured soils at the southernmost site (Panhandle of Texas) to 5500 g C/m^2 in fine textured soils at the northernmost site (southeastern Montana), but soil N content varies little across the gradient (Table 1). The three southern sites are located in shortgrass steppe and the two northern sites are in the northern mixed prairie (Dodd 1979, Epstein et al. 1996). Thirty-year mean annual temperature decreased from 14.3 °C in the south to 7.1 °C in the north and mean annual precipitation ranged from 45 cm in the south to 35 cm in the north (NCDC 1998). Aboveground net primary production based on simulation modeling and remote sensing ranges from 150 to 200 g C m^{-2} at all sites, though variability arises from temporal fluctuations in precipitation (Parton et al. 1989, Sala et al. 1988, Paruelo et al. 1997, Burke et al. 1997).

^{15}N Additions

In the spring of 1996, two levels of ^{15}N labeled ammonium sulfate were applied to three replicated 1.0 m^2 plots located on coarse and fine textured soils at each of the five sites (total number of plots = 5 sites x 2 texture classes x 2 N levels x 3 replicates = 60; Table 1). The date of ^{15}N application at each site (Table 1) coincided with the start of the growing season (Paruelo and Lauenroth 1995), so plant N demand was high and differences in plant phenology did not confound initial ^{15}N recovery. Nitrogen additions consisted of 2.5 g N m^{-2} [11.78 g of 11.1 atom % ^{15}N - $(NH_4)_2SO_4$]

and 50.0 g N m^{-2} [235.71 g of 1.8 atom % ^{15}N - $(NH_4)_2SO_4$]. The low-N treatment represents typical growing season plant N uptake for these sites (Parton et al. 1987, Burke et al. 1997). The high-N treatment was intended to overwhelm the N sinks to determine the N retention capacity of the soil. Nitrogen treatments were added in solution, simulating a 0.3 cm precipitation event. Each plot was trenched to 0.3 m and lined with aluminum skirting to minimize lateral transport of N. Wire cages (ca. 0.05 m mesh) excluded large herbivores.

Soil sampling and laboratory analyses

After two growing seasons (late August, 1997), we collected two 0.05 m diameter cores at random points within each plot. The cores were composited, air dried, weighed, sieved through a 2 mm screen, and ground on a ball mill. Bulk density was estimated from the soil weight and core volume. Total soil C and N were determined by dry combustion (LECO CHN-1000 analyzer, St. Joseph, MI), and the atom % ^{15}N was determined on an ANCA 2020 mass spectrometer (Europa Scientific, Cincinnati, OH).

We separated the total soil N pool into an labile pool and an non-labile pool using long-term laboratory incubations. A subsample (50 g air dry) from each field composite was incubated at optimal temperature (35°C; Campbell et al. 1993, Drinkwater et al. 1996) in plastic filters (Nadelhoffer 1990; Falcon Filter model 7111, Beckton Dickenson Labware, Lincoln Park, NJ). A glass fiber filter (Whatman GF/A), an "extra thick" glass fiber pre-filter (Gelman Sciences), and a layer of glass wool were placed beneath the soil and a third glass fiber filter (Whatman GF/A) was placed

above the soil to prevent dispersion (Motevalli et al. 1995). The filter units were sealed in airtight 2 L jars fitted with septa. Approximately 20 mL of deionized water were placed in the bottom of each jar to prevent soil drying. Every two weeks this water was changed and the soil brought to field capacity with deionized water.

To determine the labile N pool size, we leached the soil at 1, 10, 26, 41, 62, 93, 120, 180, 235, 301, and 363 days with a solution containing all essential nutrients except N (Nadelhoffer 1990). At each leaching, 100 mL of the non-N leaching solution was added to the top of the filter, allowed to equilibrate with the soil for 1 hour, and then drawn through the filter with a weak vacuum (-0.05 MPa). The vacuum was applied until leachate ceased to drip from the filter (< 10 min). The leachate was frozen until analysis for NH_4^+ and NO_3^- 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. Inorganic N in the leachate was converted to mg N per kg oven dry soil, summed across all leaching dates, and added to K_2SO_4 extractable N to estimate the total labile N pool size. Non-labile N was defined as total N minus labile N.

We determined the atom % ^{15}N of the leachate for each incubated soil by compositing 5mL of leachate from each sampling date. The composite samples were diffused (Stark and Hart 1996, Khan et al. 1998) for 6 days in 120 ml plastic containers. Dvarda'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 (5 µl of 2.5 M $KHSO_4$ per disk) filter paper disks (Whatman #41)

sealed in Teflon tape. At the end of the diffusion, the acidified disks were dried over concentrated H_2SO_4 for 24 hours and then stored in a desiccator until they were transferred to Sn capsules and analyzed for ^{15}N on the same instrument used for soil ^{15}N analyses. The atom % ^{15}N of the samples was corrected for N in diffusion reagents using the pool dilution method of Stark and Hart (1996). The mass of added N residing in the labile pool was calculated using the following equations:

$$N_o = N_a + N_n \quad (1)$$

Rearranging $$N_n = N_o - N_a \quad (2)$$

$$N_o{}^*{}^{15}N_o = N_a{}^*{}^{15}N_a + N_n{}^*{}^{15}N_n \quad (3)$$

Substituting from (2) $$N_o{}^*{}^{15}N_o = N_a{}^*{}^{15}N_a + (N_o - N_a){}^*{}^{15}N_n \quad (4)$$

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

Where: N_o is mass of labile N, N_a is the mass of the added N still in the labile pool, N_n is the mass of labile native soil N, $^{15}N_o$ is the atom % ^{15}N enrichment in the composite leachate sample, $^{15}N_a$ is the atom % ^{15}N enrichment of the added N, and $^{15}N_n$ is the atom % ^{15}N enrichment of the native soil (0.368 % based on two samples per site). Similar equations were used to determine how much of the added ^{15}N was in the unleached soil. The amount of ^{15}N in the non-labile pool was determined by subtracting N_a from the amount of fertilizer N in the soil at the beginning of the incubation.

Microbial respiration was determined by capturing all CO_2 in the headspace of the incubation jars. Before the jars were sealed, they were fanned with ambient air for 1 hour to provide a uniform background CO_2 concentration. The jars were sealed for

periods from 2 days (beginning of the incubation) to 3 weeks (end of the incubation) after which the concentration of CO_2 in the headspace was determined using an infrared gas analyzer (LICOR-6200, City, State). The headspace was sampled by first mixing with a 35 mL syringe and then sampling 2 mL with a 10 mL syringe. Ten sealed jars without soil were used as blanks to correct for ambient CO_2. Atmospheric pressure, air temperature, the volume of the jars, the volume of gas sampled, the oven dry mass of the soil, and bulk density were used to convert headspace concentration to g C per m^2.

We used a three way factorial ANOVA to analyze effects of N level (high and low), texture (coarse or fine), and site (5 sites in Table 1) on non-labile and labile soil N and C pools. We used simple and multiple linear regression to identify correlations between soil C and soil texture (as represented by the % Silt + Clay) and soil N and C pools. All hypotheses were tested at $\alpha = 0.05$.

RESULTS

Bulk Soil Nitrogen and Carbon Pools

Fine textured soils contained 20 % more non-labile N ($p < 0.001$; Fig. 4.1) and 30 % more non-labile C ($p < 0.001$; Fig. 4.2) than coarse textured soils. For labile N and C pools, there was a significant site x texture interaction; all sites except Texas had more labile N ($p < 0.001$) and C ($p < 0.001$) in fine textured soils. In Texas soils, texture did not affect labile N pool sizes ($p = 0.25$) and coarse textured soils had more labile C ($p = 0.03$) than fine textured soils. In both texture classes and at all sites, labile and non-labile N and C did not differ between high (50g N/m^2) and low (2.5 g

N/m^2) addition treatments ($p > 0.46$). About 20 % of bulk soil N was labile (Fig. 4.3) and this percentage was not affected by texture ($p = 0.64$) or N addition level ($p = 0.17$). A greater percentage of soil C was labile in coarse textured soils than in fine textured soils ($p < 0.001$; Fig. 4.3).

While soil texture had a statistically significant effect on N and C pools when analyzed as a discrete variable (preceding paragraph), soil particle size (% silt + clay) explained only 18 and 27 % of the variability in labile and non-labile N pool sizes, respectively (Fig. 4.4). Soil C content was a much better correlate, explaining 49 % of the variability in labile N and 72 % of the variability in non-labile N (Fig. 4.5). When both soil C and texture were included in multiple regression models, the texture term was not a significant ($p > 0.79$) predictor of N pool sizes (data not shown). In addition, labile N was correlated with labile C ($r^2 = 0.46$) and non-labile N was correlated with non-labile C ($r^2 = 0.67$; Fig. 4.6).

Isotopically Labeled Fertilizer N Pools

Fifty percent the ^{15}N labeled fertilizer N residing in the soil was non-labile, and soil texture had no effect on either the amount (labile $p = 0.14$, non-labile $p = 0.68$, Fig. 4.1) or proportion ($p = 0.40$, Fig. 4.3) of added N that was labile or non-labile. Neither soil C nor soil texture correlated with labile or non-labile fertilizer N pool sizes (data not shown). The best predictor of the amount of ^{15}N in the labile pool was the amount of ^{15}N initially in the soil (Fig. 4.7), but for the high N addition plots, labile ^{15}N did correlated ($r^2 = 0.31$) with the size of the labile C pool (Fig. 4.8).

Discussion

Was ^{15}N slowly incorporated into non-labile soil pools as Clark's (1977) data suggested, or were non-labile pools important short-term sinks for added N? Just 2.5 years after N fertilizer was added, 50% of the ^{15}N retained in soil was non-labile (Fig. 4.2). Furthermore, we could not detect differences in labile N pool sizes between soils receiving 2.5 g N/m^2 and those receiving 50 g N/m^2. While microbial turnover may have converted the inorganic fertilizer into organic N forms, microbial immobilization in the absence of other retention mechanisms would have simply lead to the accumulation of labile organic N (microbial necromass, Brooks et al. 1985, Bonde and Roswell 1987). We conclude that microbial turnover was coupled with N retention mechanisms that rapidly (within two years) converted labile N or inorganic N into non-labile forms.

We hypothesized that pool sizes of non-labile bulk soil N and added ^{15}N should be greatest in soils with fine texture and high C content. These hypotheses were corroborated by the bulk soil data; non-labile N pools were positively correlated with the percentage of fine particles in soil and the soil C pools (Figs. 4.4 and 4.5). However, the hypothesis was not supported by the ^{15}N data where the only significant correlations we observed were between the labile ^{15}N pool and the labile C pool (Fig. 4.8). The best predictor of ^{15}N pool sizes was the amount of ^{15}N in the soil, suggesting that spatial variability in the added ^{15}N signature is still the dominant factor determining variability in ^{15}N pool sizes. In the low N plots 25 to 100 % of the added N was retained in soil and in the high N plots 5 to 50 % of the added N was in soil

(Fig. 4.7, x-axis). It is unclear why mechanisms that promoted this wide range of soil N retention would not affect the proportion of N that is in non-labile pools.

At least part of the discrepancy between Clark's (1977) results and our results may be due to the type of N added. We added $^{15}NH_4^+$, which may be more prone to react directly with organic matter (Nommik and Vahtras 1982) than NO_3^-. In addition, plants may compete more effectively with microbes for NO_3^- than NH_4^+ (Kaye and Hart 1997), explaining the low plant recovery on our plots (Barrett and Burke 2000) relative to Clark's (1977) plots.

Schimel et al. (1986) added ^{15}N labeled urea to shortgrass steppe on two topographic positions. The backslope position had less clay and soil C and a footslope position and the later retained more ^{15}N, even 10 years after the application (Delgado et al. 1996). At the 10 year sampling, 42 % and 58 % of the N retained in the footslope and backslope soils, respectively, were in a "passive" pool thought to turnover every 200 to 1500 years. Barrett and Burke (2000), using the same plots that we sampled, found that soil ^{15}N retention correlated positively clay content and soil C on several (but not all) collection dates within one year of the ^{15}N application. One year after the application 45 and 32 % of the N retained in low and high N plots, respectively was in particulate organic matter. In grasslands, the particulate organic matter pool is though to have an "intermediate" turnover time (decades), though the pool is variously referred to as slow, decomposable, or stabilized with respect to decomposition (Cambardella and Elliot 1992).

Thus, in case studies (this study, Schimel et al. 1986, Delgado et al. 1996, Barrett and Burke 2000) when N was added as NH_4^+ or urea to the shortgrass steppe, most N is retained in soil, greater soil C and finer texture tend to increase N retention, and a large fraction of soil ^{15}N is in pools non-labile to microbes on annual time scales. Our results also agree with agricultural studies showing that 40 to 80 % of ^{15}N labeled fertilizer is not readily labile to future crop rotations (Chichester et al. 1975, Smith et al. 1978, Duxbury et al. 1991). Correlations between texture, soil C, and N retention have also been observed in forests (Seely and Lajtha 1997).

In our study, soil texture only appeared to be important to N retention in-so-much-as it is weakly correlated with soil C ($r^2 = 0.30$, data not shown). Correlations between non-labile N pools and soil texture were weak (Fig. 4.4) relative to correlations with C (Fig. 4.5), and texture was not correlated with N pools in multiple regression models that included soil C. At least one reason for the poor texture-N correlations was the low bulk density of very fine textured soils. For example, the Texas site had the greatest percentage of clay in the soil (~50 % clay) and high concentrations of C and N. However, because bulk density was lowest at this site, total C and N content were as low as in many coarse textured soils. Because of these inconsistencies, we suggest that soil C, rather than proximate determinants of soil C such as soil texture, will be the best predictor of soil N retention. Non-labile soil C should be consistently correlated with non-labile soil N (Fig. 4.6) because of the low and relatively constant C:N ratio of humus (Kaye et al. 2000).

Conclusions

Over relatively small time scales (2 years) substantial quantities of N fertilizer were removed from pools that were readily available to microbes and shunted into non-labile pools. These non-labile pools comprised 80 % of the total soil N and 50 % of the fertilizer N. While microbial immobilization may provide the important first step of converting inorganic N into organic forms, microbial turnover cannot explain large pools of native and recently added N in pools that are non-labile. Humus formation, aggregation, and complexation with clays are the most likely mechanisms by which labile N becomes non-labile. In our analysis, none of these factors were correlated with non-labile ^{15}N pools, but soil C content seemed to be the most important determinant of how much native soil N was non-labile. Factors that alter soil C content including, land-use history, soil texture, soil temperature, primary productivity, and decomposition may affect both short- and long-term N retention in non-labile soil pools.

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We thank John Stark and Myq Larson for ^{15}N analyses and Dan Reuss and Anthony LeManager for laboratory assistance. This research was supported by McIntire Stennis appropriations to Colorado State University.

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Table 4.1. Chacteristics of study sites. Sand, silt, clay, pH, soil C, and soil N are for fine and coarse textured soils (0-20 cm).

Site	State	lat.[1]	long.[1]	MAP[2] cm	MAT[2] °C	% sand	% silt	% clay	pH	Organic C g C m^{-2}	Total N g N m^{-2}	date N applied
Muleshoe National Wildlife Refuge	TX	33°57'	102°46'	45	14.3	13	34	52	6.2	3180	320	3/18/96
						48	26	24	7.8	2290	255	
Comanche National Grasslands	CO	37°22'	102°46'	41	11.9	19	42	38	8.1	2750	282	5/14/96
						53	27	20	7.5	2130	226	
Pawnee National Grasslands	CO	40°37'	103°41'	37	9.1	26	48	26	7.7	3030	337	5/11/96
						60	25	15	6.7	2440	266	
Thunder Basin National Grasslands	WY	43°28'	105°03'	32	7.9	33	33	34	7.5	4260	324	5/09/96
						42	26	22	6.7	2110	194	
Fort Keogh Livestock and Range Research Laboratory	MT	46°30'	105°88'	35	7.1	12	47	41	8.0	5530	353	4/30/96
						64	21	15	7.9	4190	289	

[1]lat. = latitude and long. = longitude

[2]MAP = mean annual precipitation and MAT = mean annual temperature

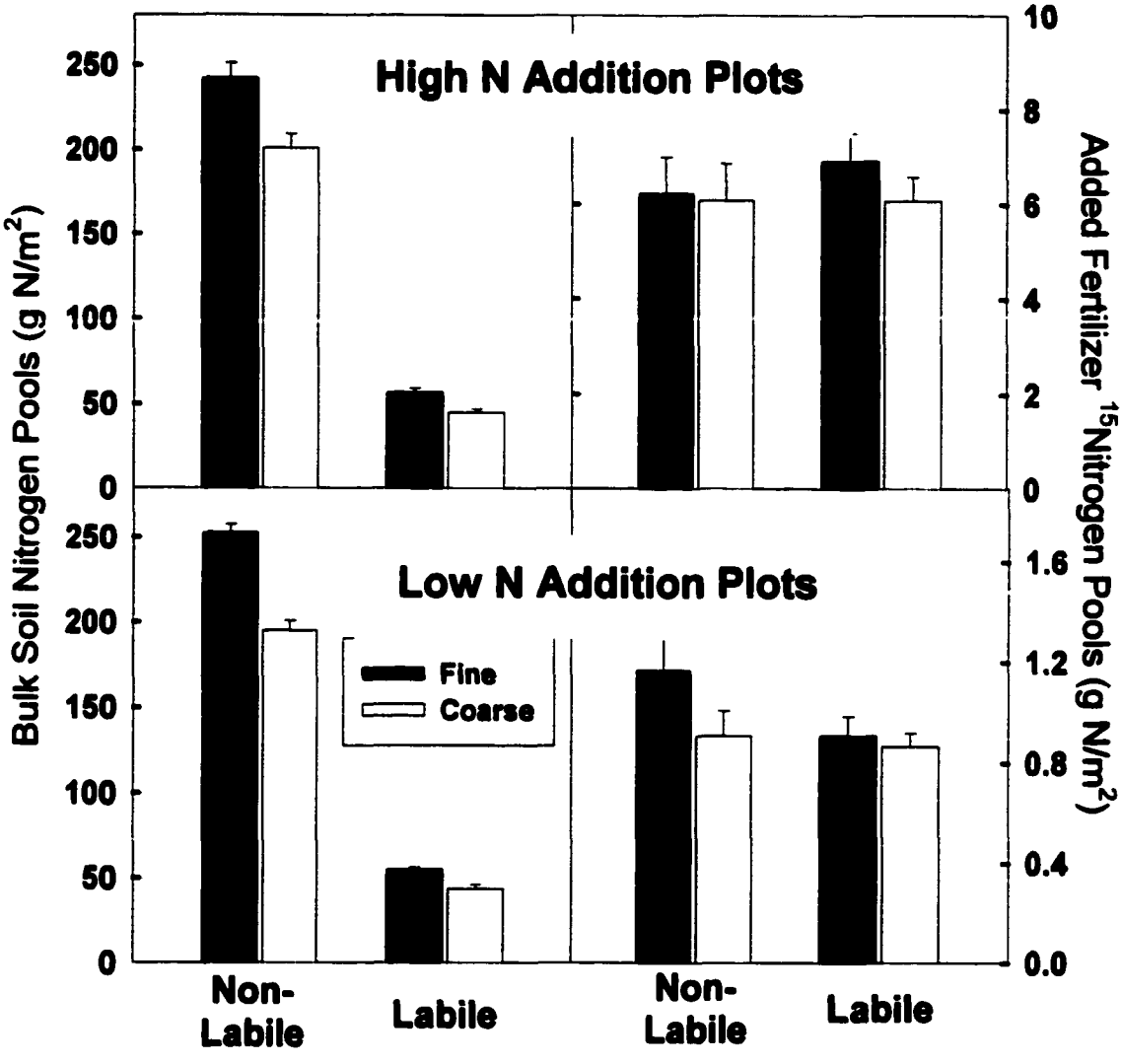

Figure 4.1. Pool sizes of labile and non-labile soil nitrogen (N) in grassland soils. Left-hand graphs show bulk soil N (native + fertilizer) and right-hand graphs show an ^{15}N labeled fertilizer pool. High N addition plots received 2.5 gN/m^2 (top graphs) and high N plots received 50 gN/m^2 (bottom graphs). For the ^{15}N fertilizer pool there were no significant treatment effects. For the bulk soil N, fine textured soils had more unavailable N ($p < 0.001$) for both high and low N additions. There was a significant interaction between site and texture for available N pools; all sites had more ($p < 0.001$) available N in fine textured soils except Texas, for which there was no texture effect ($p = 0.25$). Bars are mean plus one standard error.

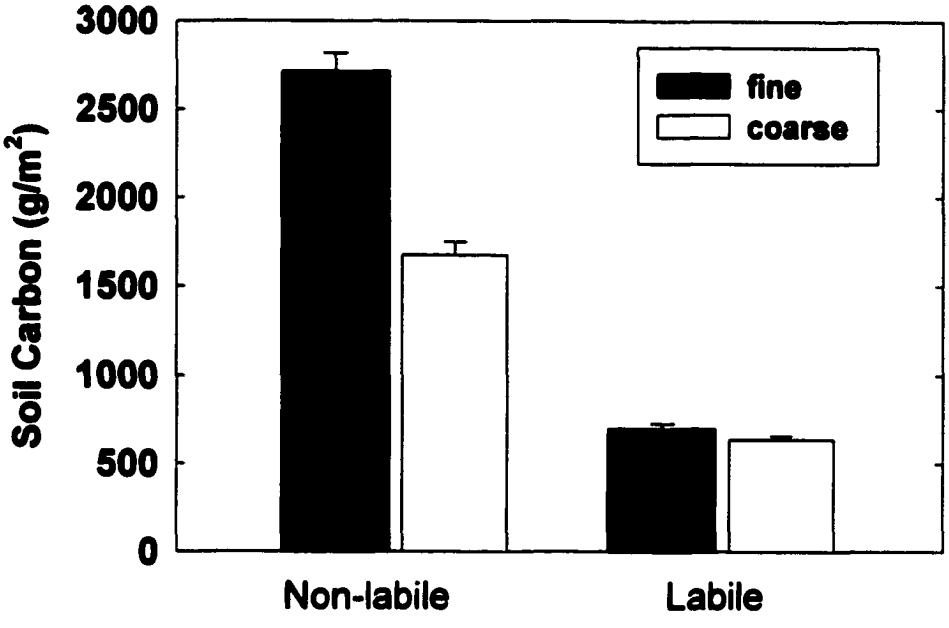

Figure 4.2. Pool sizes of soil carbon (C) that were unavailable or available to microbes during one year laboratory incubations. Fine textured soils had more unavailable C than coarse textured soils ($p < 0.001$). There was a significant interaction between site and texture for the available pool; all sites had more available C in fine textured soils except Texas, which had more availalble C in coarse textured soils ($p = 0.03$). Bars are means and one standard error.

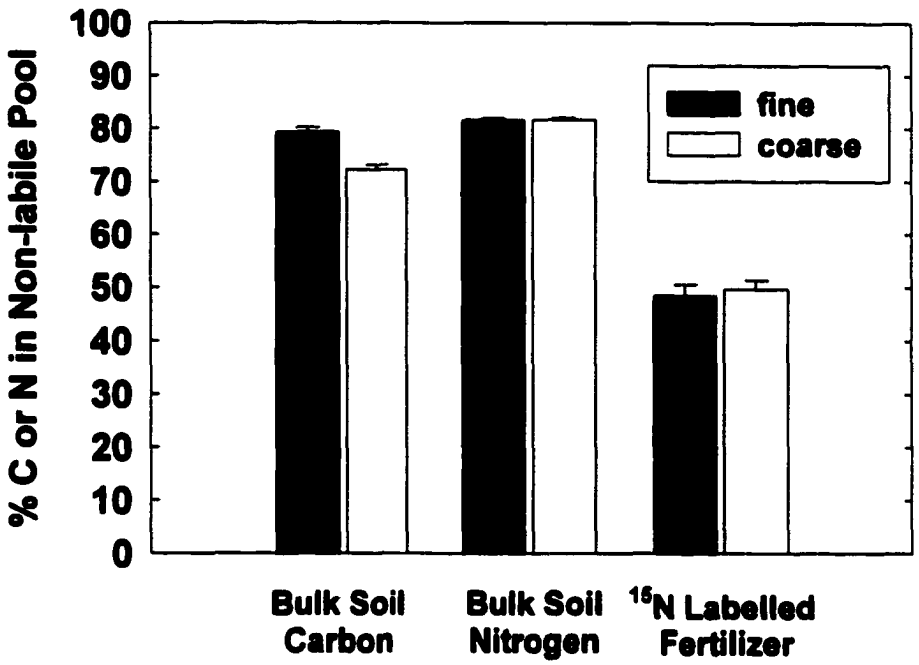

Figure 4.3. The percentage of total soil C (left), total soil N (middle), and ^{15}N labelled fertilizer (right) that was non-labile. The percentage of total N that was non-labile N was greater for the bulk soil than for the ^{15}N labeled fertilizer ($p < 0.001$), and fine textured soil had a greater fraction of non-labile C than coarase textured soils ($p < 0.001$). Bars are means and one standard error.

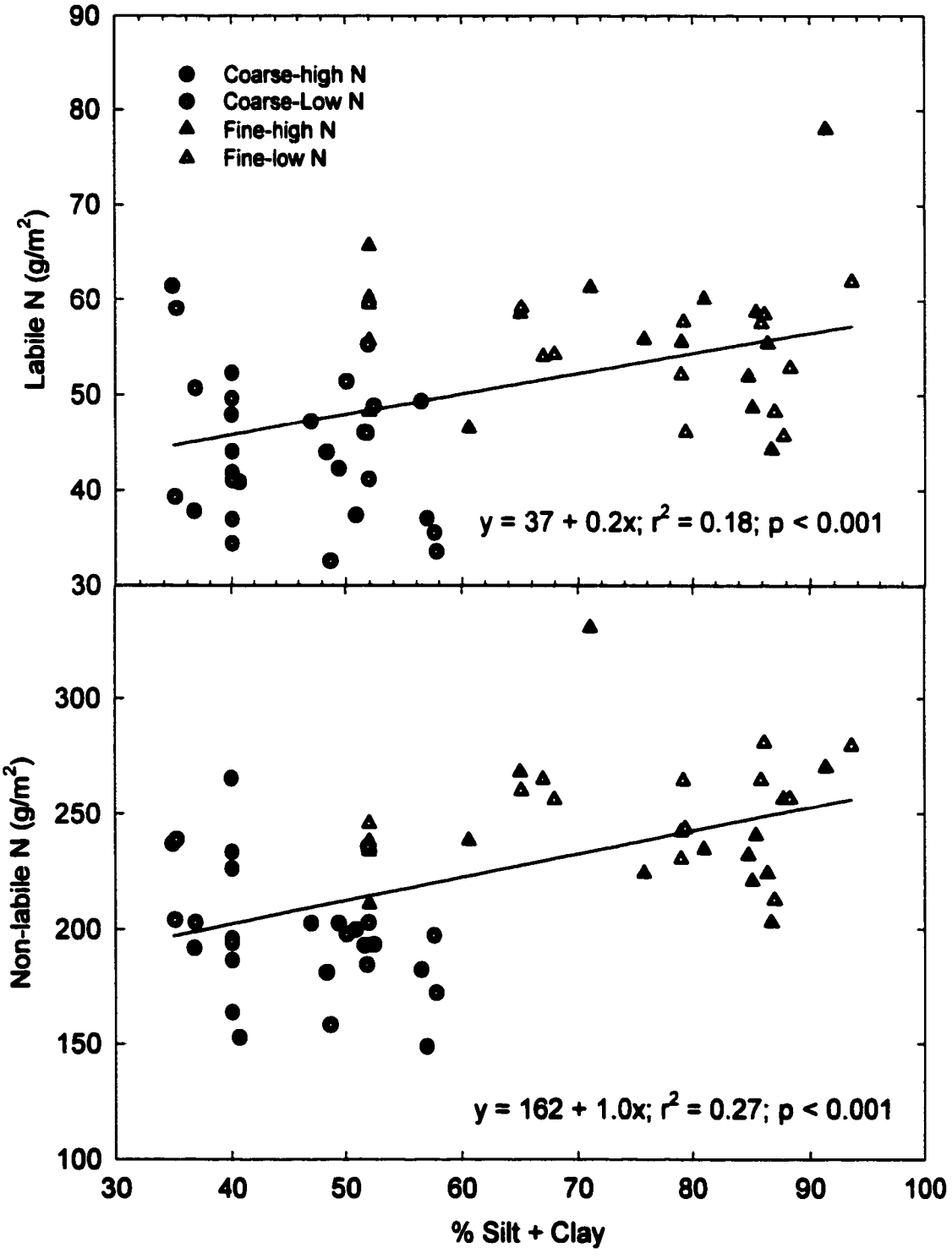

Figure 4.4. The relationship between the amount of fine soil particles (% Silt + Clay) in soil and the amount of soil N that was labile or non-labile. Symbols denote whether data comes from high or low N addition plots and whether the plot was considered coarse or fine textured in ANOVA analyses (see Fig. 1).

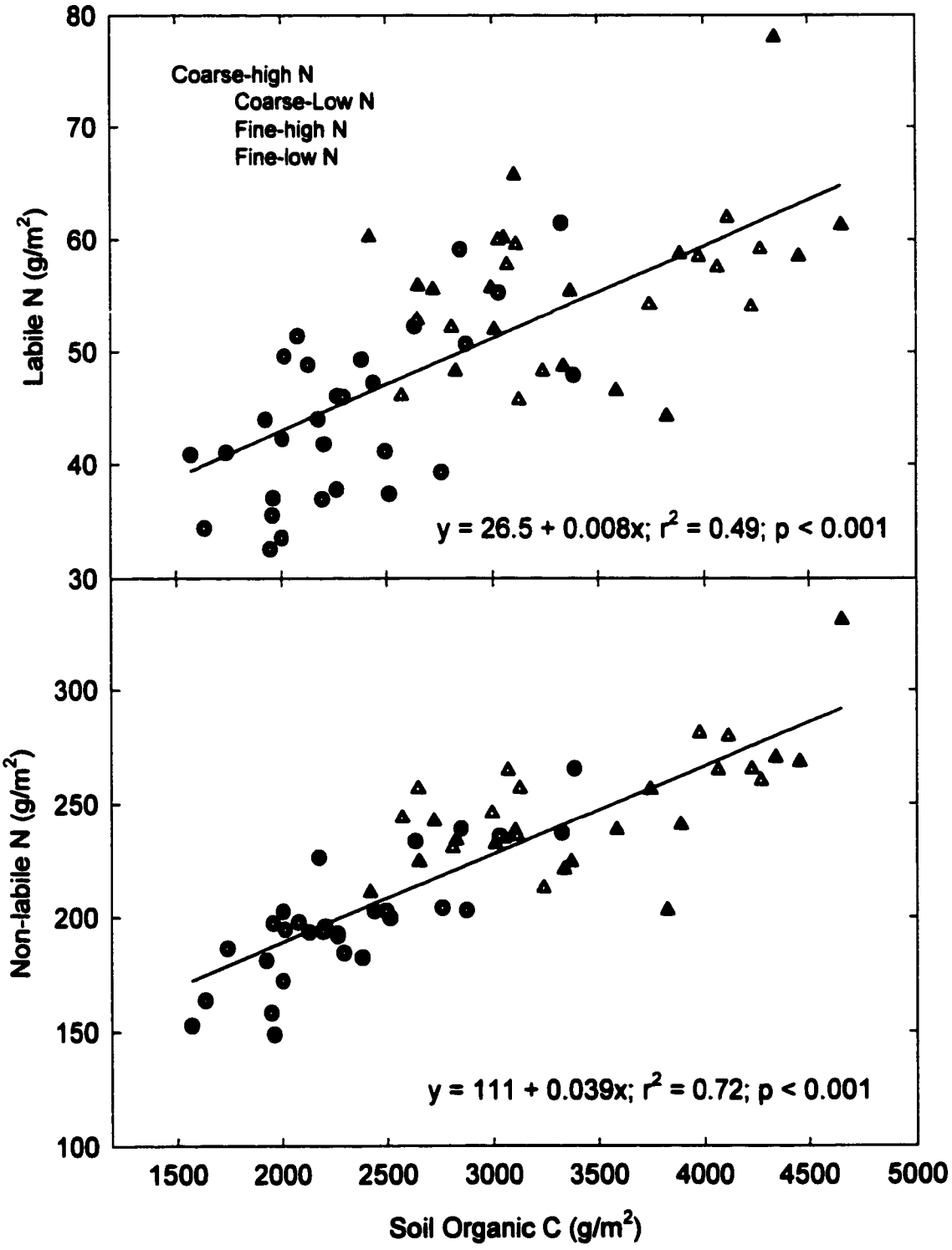

Figure 4.5. The relationship between soil carbon (C) and the amount of soil N that was labile or non-labile. Symbols denote whether data comes from high or low N addition plots and whether the plot was considered coarse or fine textured in ANOVA analyses (see Fig. 1).

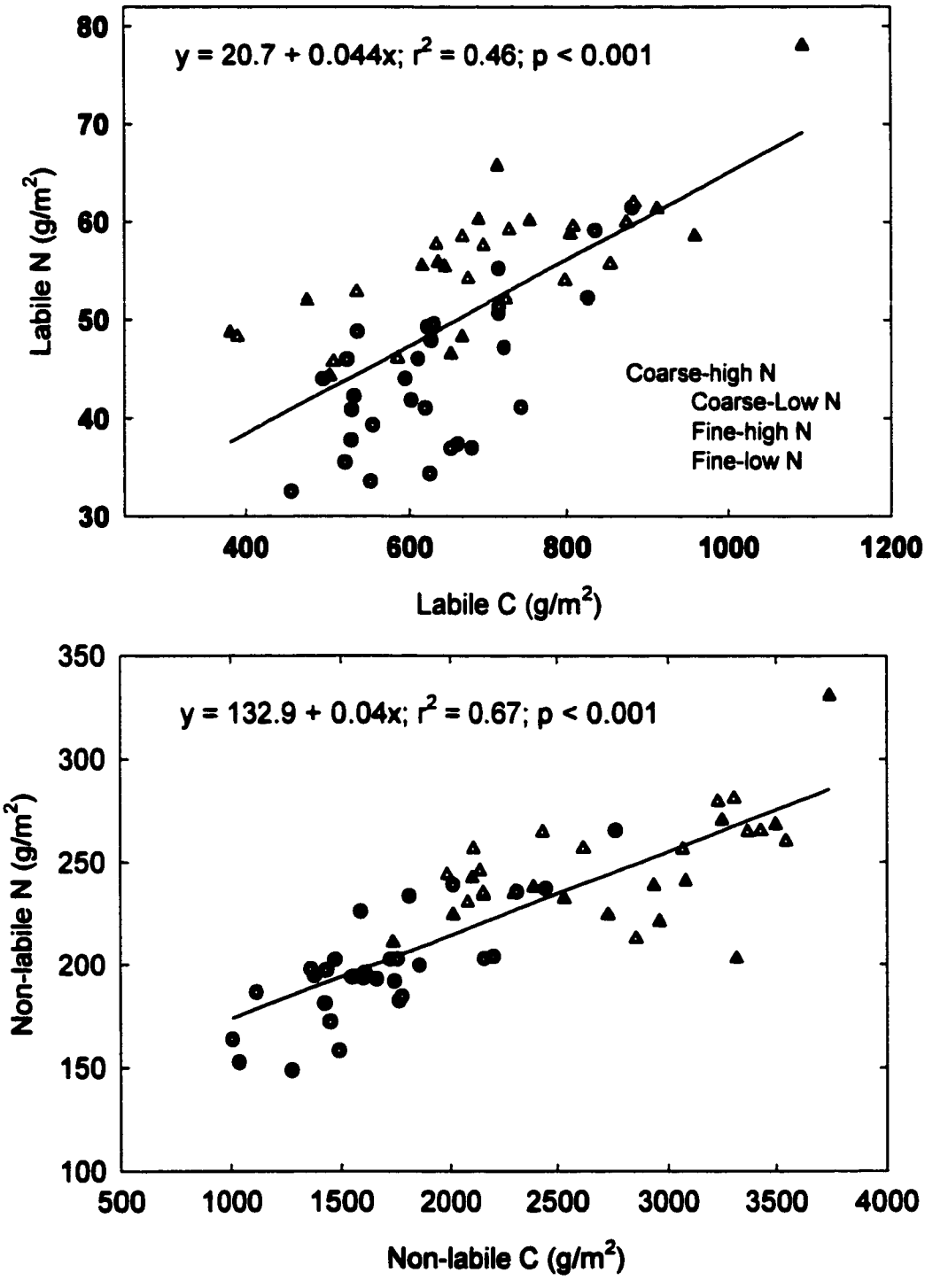

Figure 4.6. The relationship between soil carbon (C) and nitrogen (N) pools that were labile or non-labile. Symbols denote whether data comes from high or low N addition plots and whether the plot was considered coarse or fine textured in ANOVA analyses (see Fig. 1).

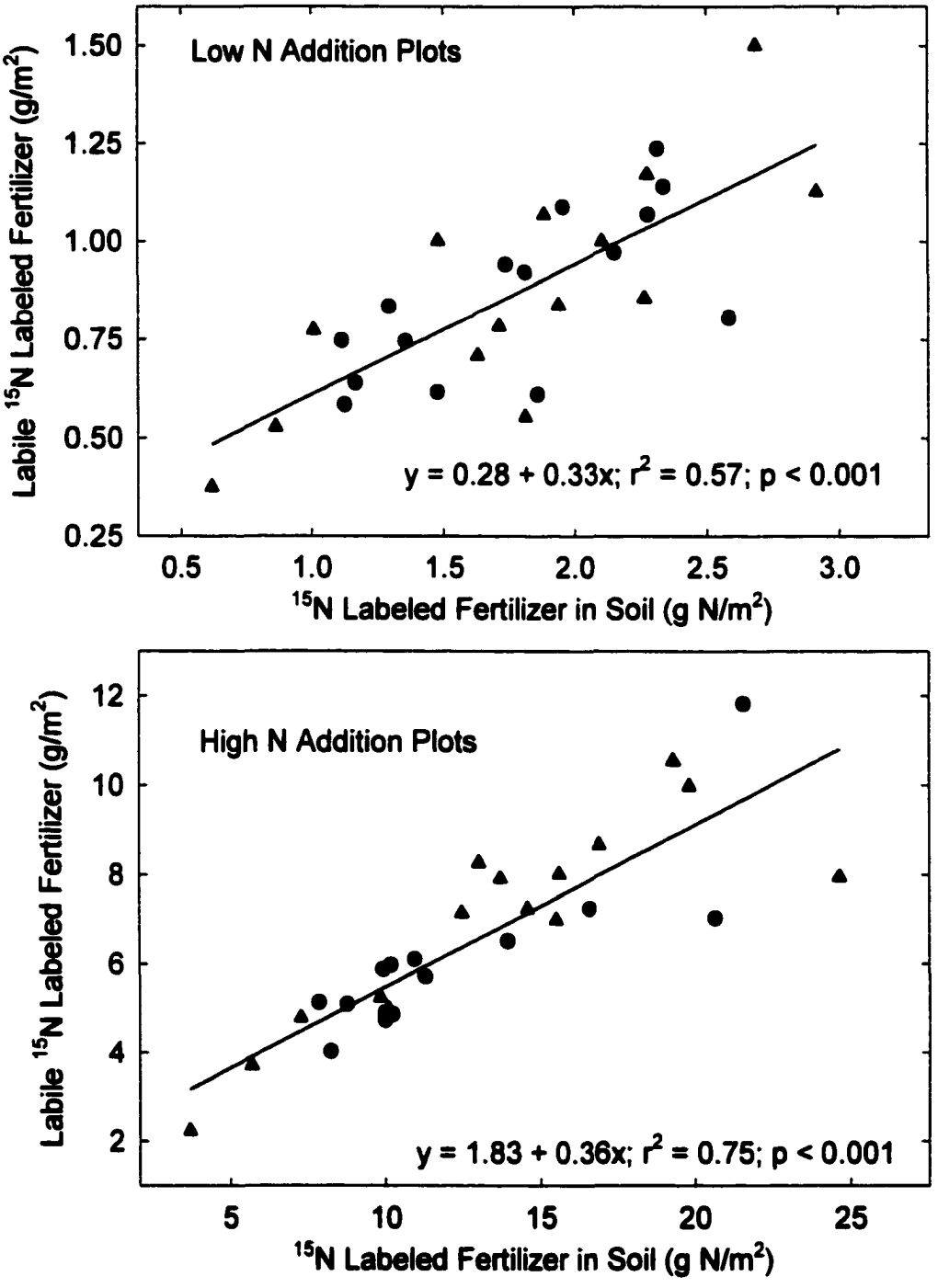

Figure 4.7. The relationship between the total amount of ^{15}N labeled fertilizer in the soil and the amount of ^{15}N labeled fertilizer that labile. Low and high N additions were 2.5 and 50 g N/m^2, respectively. Symbols denote whether the plot was considered coarse (triangles) or fine (circles) textured in ANOVA analyses (see Fig. 1).

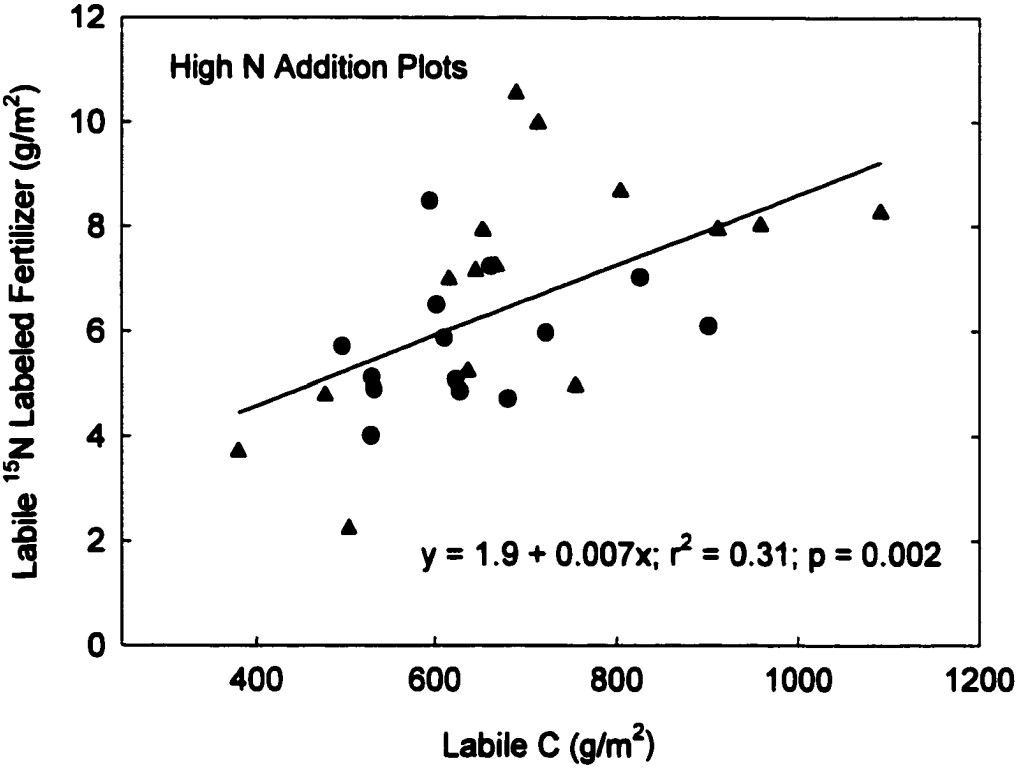

Figure 4.8. The relationship between labile carbon (C) and labile ^{15}N labeled fertilizer. The plots received 50 g N/m^2 of fertilizer. Symbols denote whether the plot was soncidered coarse (triangles) or fine (circles) textured in ANOVA analyses (see Fig. 1).

CHAPTER 5

SYNTHESIS

This dissertation addressed several simple, but fundamental, questions in ecosystem ecology: 1) How much soil N is non-labile?, and 2) What is the role of this non-labile pool in retention of native N, fertilizer N, and atmospheric N deposition? The answers to these questions have far-reaching implications for water quality and long-term ecosystem function in sites with elevated N inputs. They are also fundamental to our understanding of N cycling in undisturbed ecosystems. For this chapter, I pooled data from the preceding three chapters into a synthetic analysis. My objective was to identify general patterns in non-labile and labile N pool sizes that held true across a range of diverse sites.

Non-labile soil N pools

In Chapters 3 and 4, I showed that the non-labile N pool size were correlated with non-labile C pool sizes in the Alaska and Great Plains soils. I was interested in whether a universal relationship could be derived for all sites, and whether this relationship would show a flexible C:N ratio (i.e. significant y-intercept). There was a significant relationship between non-labile N and C ($r^2 = 0.68$) across the tropical plantation, grassland, and Taiga successional soils (Fig. 5.1) and the y-intercept was positive ($p < 0.001$). However, the slope of the relationship varied among sites;

grassland soils gained less non-labile N per unit C than either forested site. The proportion of N that was non-labile did not correlate with total or non-labile soil C.

To determine the role of non-labile soil N pools in retention of fertilizer and atmospheric N deposition, I used ^{15}N labels of various ages. In the Alaskan Taiga soils (Chapter 3), I added ^{15}N just 3 weeks prior to laboratory incubations. In the grassland (Chapter 4) soils ^{15}N was added to field plots 2 years prior to the incubations and in the tropical plantation soils (Chapter 2), the ^{15}N was added in the field 6 to 9 years prior to the incubations. Because different levels of ^{15}N were added I synthesized these data based on the fraction of ^{15}N or bulk soil N that was non-labile during the incubations. While the fraction of bulk soil N that was non-labile was relatively constant among sites (range = 77 to 90 %), the fraction of ^{15}N that was non-labile varied greatly, and increased with time since N application (Fig. 5.2). This pattern could either result from incremental losses of labile N or from incorporation of labile N into the non-labile pool over time. In the grassland soils, the former is true because total soil N retention declined over time (Barrett and Burke 2000), at least in the high N plots. At the other sites, we have only a point estimate of the amount of ^{15}N in soil.

Labile soil N pools

In Chapter 2, I suggested that much more N is available to microbes (labile) than we previously thought. I emphasized the role of microbial turnover in retaining labile N in soils and showed that adding labile C could promote N retention by increasing microbial turnover. In Chapters 3 and 4, I further suggested that labile N

pools could be correlated because microbial residues with constrained C:N ratios make up a large fraction of labile N. Thus, to the extent that microbes in all environments have similar C:N ratios, there should be a general relationship between labile N and labile C. Indeed, labile C explained 74 % of the variance in labile N across all sites (Fig. 5.3). Unexplained variance likely resulted from the variable C:N ratios of labile organic matter inputs by plants.

Conclusions

Most theoretical (Agren and Bosatta 1988, Aber et al. 1989) and empirical (Tietema 1998, Stark and Hart 1997) research on soil N retention has focused on microbial N transformations. High rates of nitrification are thought to promote N leaching (Vitousek et al. 1982, Aber et al 1998) because NO_3^- is more mobile in soils than NH_4^+. Conversely, high rates of microbial N immobilization are thought to inhibit N leaching by converting NO_3^- into organic forms (Stark and Hart 1997). These processes are certainly important to N retention. However, my results suggest that N retention is not simply a function of microbial turnover; N that is not readily available to microorganisms is also a large sink for N added to soils. Furthermore, as the primary successional sequence and ^{15}N addition experiments showed, incorporation of N into non-labile pools can be rapid. Rapid accumulation of an non-labile soil N pool is consistent with recent research showing that soil can accumulate several hundred kg/ha of fertilizer N without substantial leaching losses (Christ et al. 1995, Ring 1995, Magill et al. 1997).

Future research on N retention in soils will need to focus on traditional retention mechanisms related to microbial turnover and on less traditional mechanisms by which N is rapidly incorporated into pools that are no labile. My data (Figs. 5.1 and 5.3) suggest that the amount of C in soil is closely linked with both labile and non-labile N pools. Field experiments with chronic N and C additions are required to test whether the relationship between N and C pool sizes is mechanistic and not simply correlative.

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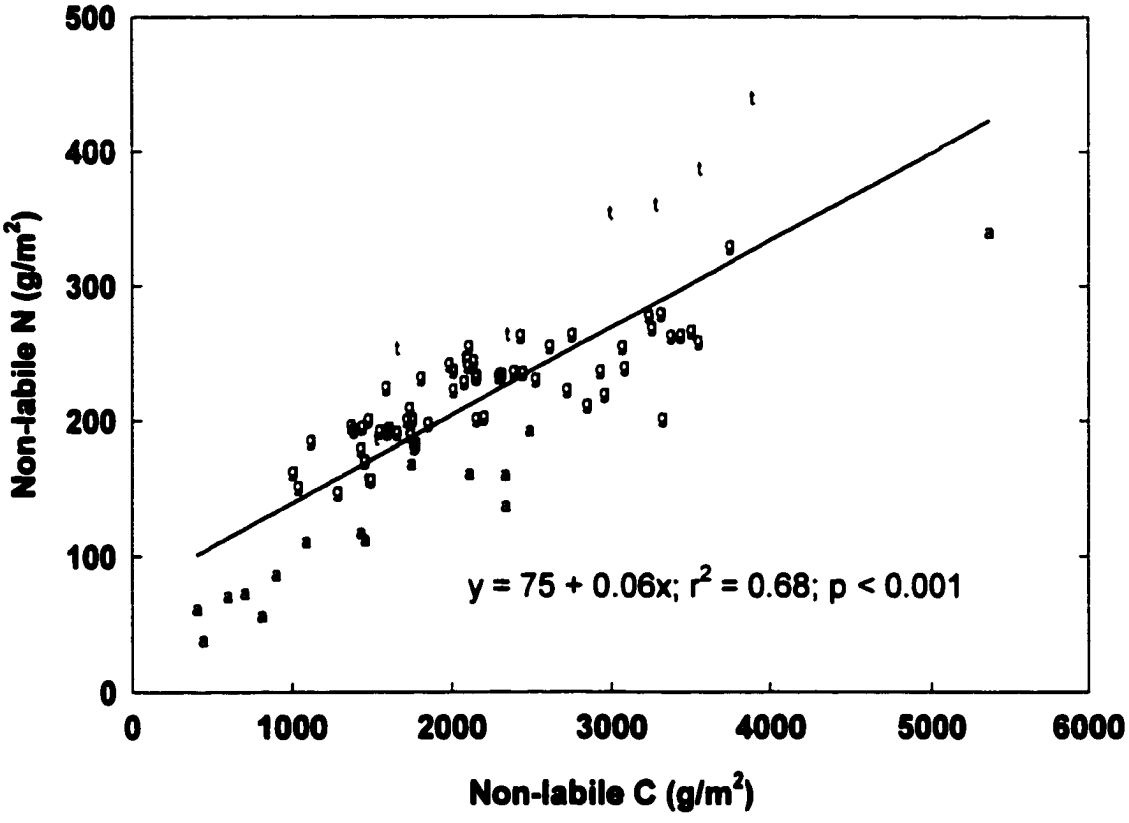

Figure 5.1. The relationship between non-labile nitrogen (N) and carbon (C) at three sites. Symbols denote Alaskan floodplains (a), Great Plains grasslands (g), and tropical plantations (t). The y-intercept was greater than zero ($p < 0.0001$)

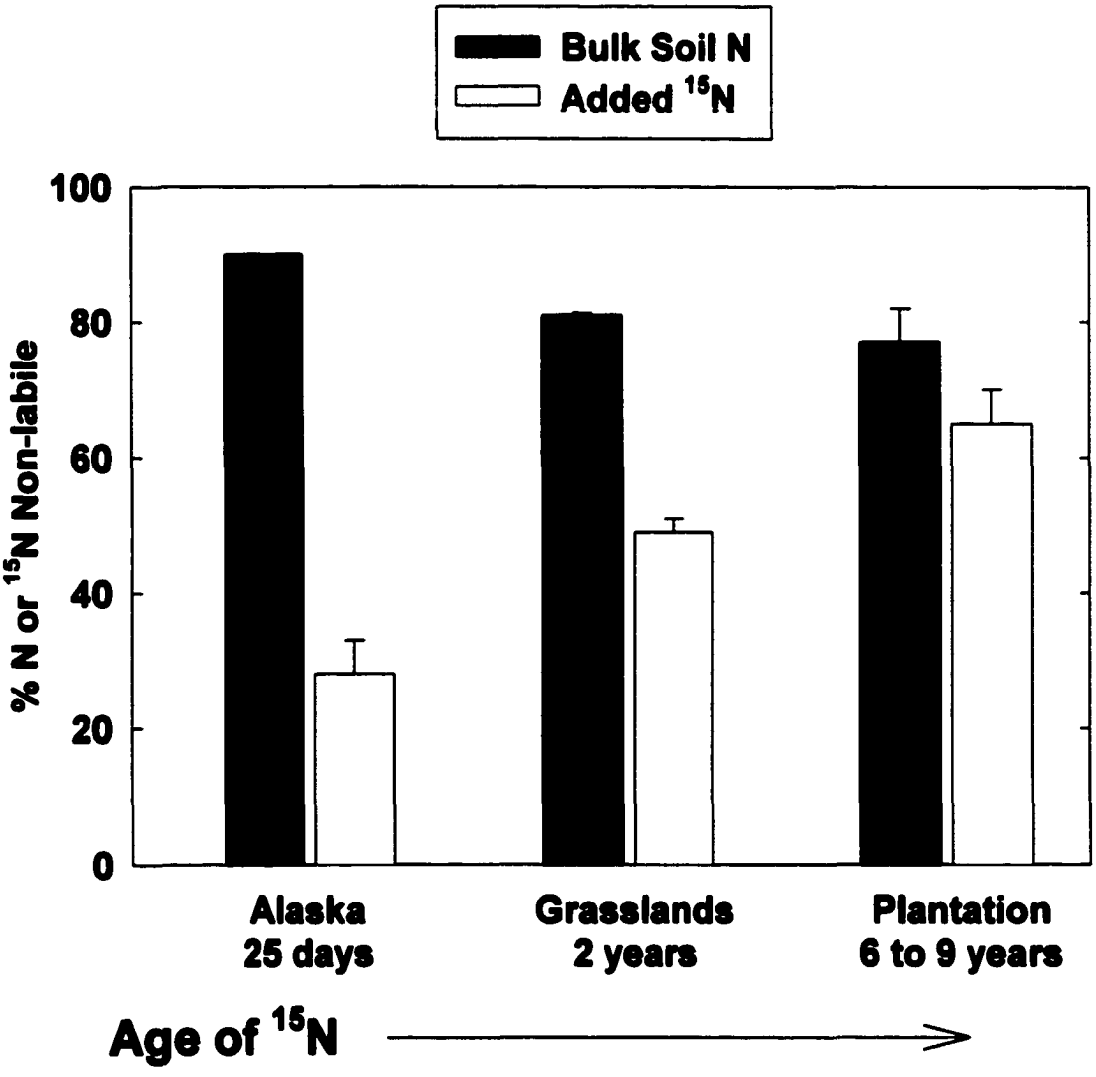

Figure 5.2. The percentage of total soil N or ^{15}N that was non-labile during 1-year laboratory incubations. The x-axis is the site and time between the ^{15}N addition and the beginning of the incubation

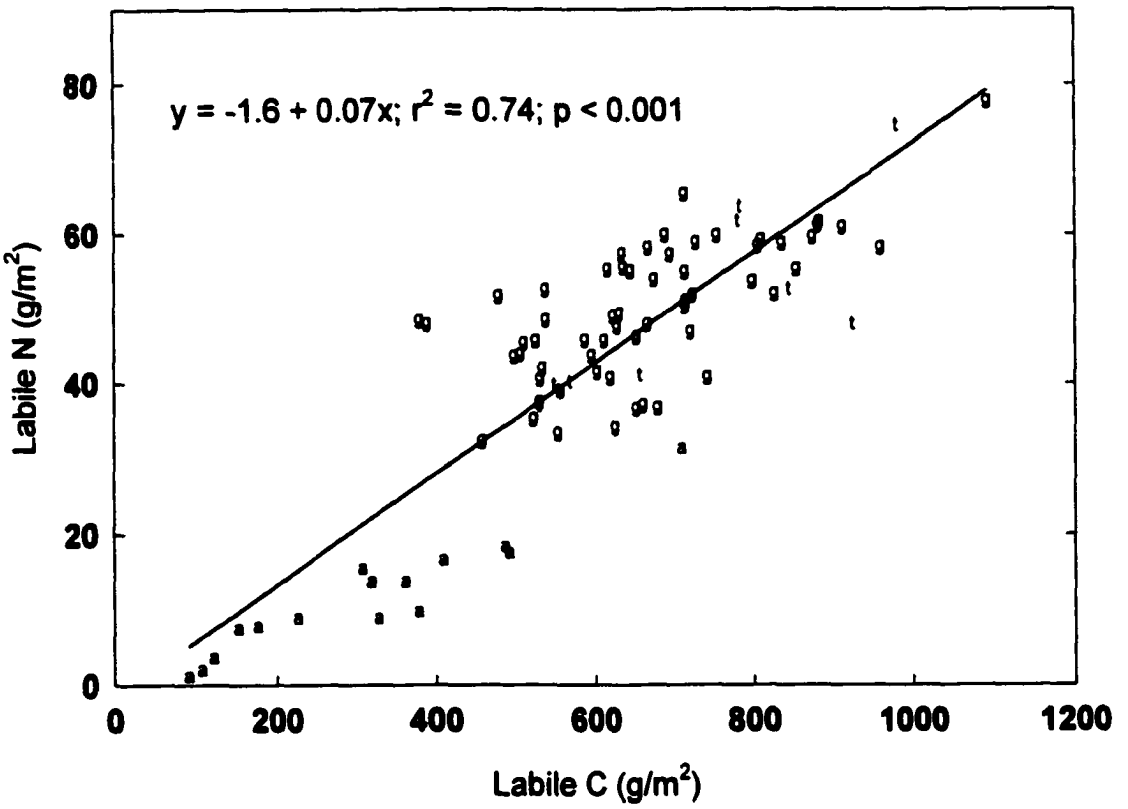

Figure 5.3. The relationship between labile nitrogen (N) and carbon (C) at three sites. Symbols denote Alaskan floodplains (a), Great Plains grasslands (g), and tropical plantations (t).