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Stable Nitrogen and Carbon Pools in Grassland Soils of Variable Texture and Carbon Content

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Abstract

Nitrogen (N) inputs to many terrestrial ecosystems are increasing, and most of these inputs are sequestered in soil organic matter within 1–3 years. Rapid (minutes to days) immobilization focused previous N retention research on actively cycling plant, microbial, and inorganic N pools. However, most ecosystem N resides in soil organic matter that is not rapidly cycled. This large, stable soil N pool may be an important sink for elevated N inputs. In this study, we measured the capacity of grassland soils to retain ^{15}N in a pool that was not mineralized by microorganisms during 1-year laboratory incubations (called "the stable pool"). We added two levels (2.5 and 50 g N m^{-2}) of $^{15}NH_4^+$ tracer to 60 field plots on coarse- and fine-textured soils along a soil carbon (C) gradient from Texas to Montana, USA. We hypothesized that stable tracer ^{15}N retention and stable bulk soil (native + tracer) N pools would be positively correlated with soil clay and C content and stable soil C pools (C not respired during the incubation). Two growing seasons after the ^{15}N addition, soils (0- to 20-cm depth) contained 71% and 26% of the tracer added to low- and high-N treatments, respectively. In both N treatments, 50% of the tracer retained in soil was stable. Total soil C ($r^2 = 0.72$), stable soil C ($r^2 = 0.68$), and soil clay content ($r^2 = 0.27$) were correlated with stable bulk soil N pools, but not with stable ^{15}N retention. We conclude that on annual time scales, substantial quantities of N are incorporated into stable organic pools that are not readily susceptible to microbial remineralization or subsequent plant uptake, leaching losses, or gaseous losses. Stable N formation may be an important pathway by which rapid soil N immobilization translates into long-term N retention.

Key Words: Great Plains; laboratory incubation; ^{15}nitrogen; nitrogen retention; soil organic matter; tracer experiment.

Introduction

In 1977, Francis Clark reported a now classic field ^{15}nitrogen (N) tracer experiment in a shortgrass steppe ecosystem. The 5-year study led Clark (1977) to two important conclusions: (a) Plants were the dominant sink for the ^{15}N, retaining 60 % of the added nitrate; and (b) ^{15}N incorporation into stable humus was slow because the plant–microbe N cycle was tight. Subsequent experiments in the shortgrass steppe (Schimel and others 1986; Delgado and others 1996; Barrett and Burke 2002) and in forests (Tietema and others 1998; Nadelhoffer and others 1999b) 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 stable pools as Clark's (1977) data suggested?

Today, this question has increased relevance because human-derived N inputs have risen by 20% since Clark's paper was published (Galloway and others 1995). If new N inputs are slowly (less than

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5 years) incorporated into stable organic matter, then retained N will be susceptible to repeated microbial mineralization and associated leaching and gaseous N losses. In contrast, if some N inputs are quickly transferred into stable organic matter that is not readily susceptible to microbial mineralization, soils could sequester N for years to decades without significant loss from the ecosystem; there are no mechanisms for large annual losses of N in stable organic matter (cultivation and erosion may be exceptions).

The rate and magnitude of stable N retention may also have implications for stable carbon (C) sequestration in soils. Most mechanisms that promote stable N formation also stabilize soil C. For example, organic N, NH_4^+, and NO_2^- can react directly with soil organic C to form complex molecules (humus) that are thermodynamically costly for heterotrophs to decompose (Burge and Broadbent 1961; Nommik and Vahtras 1982; Meyer 1994). Microbial N turnover may catalyze humus-forming reactions or directly assimilate N and C into stable tissues (He and others 1988; Kaye and others 2002). Similarly, fine-textured soils may promote stable N and C formation by increasing aggregation (Tisdall and Oades 1982; Strickland and others 1992; Hassink and others 1993), NH_4^+ fixation in clay lattices (C is not affected in this case) (Nommik and Vahtras 1982), or chemical interactions between organic N, cations, and clay surfaces (Oades 1988; Sollins and others 1996).

Recent ^{15}N experiments have attempted to isolate one or more of these N immobilization processes on time scales ranging from minutes to years. Rapid (minutes to hours), presumably abiotic immobilization can account for up to 50% of the inorganic N added to live and sterilized soils (Berntson and Aber 2000; Johnson and others 2000; Dial and others 2001; Perakis and Hedin 2001). A similar quantity of N is immobilized by microbes in short-term (hours to days) ^{15}N experiments (Davidson and others 1990; Stark and Hart 1997); however, microbial biomass tends to be a transient N sink, declining significantly within days or weeks (Emmett and Quarmby 1991; Seely and Lathja 1997; Zogg and others 2000; Perakis and Hedin 2001). Long-term (months to years) field ^{15}N tracer studies show that soil organic matter persists as the largest ecosystem sink for ^{15}N (Preston and Mead 1994ba; Buchmann and others 1996; Tietema and others 1998; Nadelhoffer and others 1999a, b). Although these studies highlight soil organic matter as a major sink for N, they do not differentiate between labile and stable organic matter. To distinguish between recycling and stable N formation as mechanisms of soil N retention, soil ^{15}N must be partitioned into labile and stable pools (Delgado and others 1996; Chang and Preston 1998).

In this study, we used long-term laboratory incubations to separate a 2-year-old ^{15}N addition into labile and stable pools. Our main goal was to estimate the magnitude of ^{15}N retention in stable soil organic matter. The field ^{15}N addition included two levels of tracer N added to paired coarse- and fine-textured soils along a latitudinal gradient in the Great Plains (Barrett and Burke 2002). We exploited regional-scale gradients in texture and soil C to address two secondary research questions: (a) Does stable N retention increase in soils with higher C content and finer texture? and conversely, (b) do stable soil C pool sizes (C not respired during the incubation) increase with increasing stable N retention or finer soil texture?

Materials and Methods

Study Area

In the spring of 1996, five research sites were established along a latitudinal gradient in the semiarid region of the US Great Plains (Table 1). At each site, distinct coarse- and fine-textured soil types were identified. Mean annual air temperature decreased and mean annual precipitation generally increased (National Climate Data Center 1996) moving from north (Montana) to south (Texas) along the gradient (Table 1). Soil C and N content varied greatly among the sites depending on latitude and soil texture. The three southern sites are shortgrass steppe, and the two northern sites are northern mixed prairie (Dodd 1979; Epstein and others 1996). Aboveground net primary production (ANPP) based on simulation modeling and remote sensing was 150–200 g C m^{-2} at all sites, although variability arises from temporal fluctuations in precipitation (Parton and others 1989; Sala and others 1988; Burke and others 1997; Paruelo and others 1997). The land-use history of all sites included domestic grazing, but none of them were currently or previously cultivated. Ambient wet N deposition at all sites was approximately 2 g N m^{-2} (National Atmospheric Deposition Program 2000).

^{15}N Additions

Two levels of ^{15}N-labeled ammonium sulfate were applied to three replicate 1.0-m^2 plots located on coarse- and fine-textured soils at each of the five sites (total number of plots = 5 sites × 2 texture classes × 2 N levels × 3 replicates = 60) (Table 1). The date of ^{15}N application at each site (Table 1)

Table 1. Study Site Characteristics and Soil (0 to 20 cm) Properties

Site	State	USDA Soil Classification	MAP (cm)	MAT (°C)	Sand (%)	Silt (%)	Clay (%)	pH	Organic C (g C m^{-2})	Total N (g N m^{-2})	Date ^{15}N Applied
Muleshoe National Wildlife Refuge	TX	Thermic calciustoll	45	14.3	13	34	52	6.2	3180	320	3/18/96
		Aridic paleustalf	45	14.3	48	26	24	7.8	2290	255	
Comanche National Grasslands	CO	Ustollic haplargid	41	11.9	19	42	38	8.1	2750	282	5/14/96
		Ustipsamment	41	11.9	53	27	20	7.5	2130	226	
Pawnee National Grasslands	CO	Aridic argiustoll	37	9.1	26	48	26	7.7	3030	337	5/11/96
		Torripsamment	39	9.0	60	25	15	6.7	2440	266	
Thunder Basin National Grassland	WY	Ustollic paleargid	31	7.9	33	33	34	7.5	4260	324	5/09/96
		Ustic torriorthent	33	7.9	42	26	22	6.7	2110	194	
Fort Keogh Livestock and Range Research Laboratory	MT	Ustollic haplargid	35	7.1	12	47	41	8.0	5530	353	4/30/96
		Ustollic haplargid	35	7.1	64	21	15	7.9	4190	289	

MAP, 30-y mean annual precipitation; MAT, 30-y mean annual temperature
Soil characteristics at each site include two rows of data for paired fine-textured (upper row) and coarse-textured (lower row) soils.

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 (Barrett and Burke 2002). The two N addition levels were 2.5 g N m^{-2} (11.78 g of 11.1 atom% ^{15}N – $(NH_4)_2SO_4$) and 50.0g 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 and others 1987; Burke and others 1997). The high-N treatment was intended to overwhelm N sinks to determine the N retention capacity of the soil. Nitrogen was added in solution, simulating 0.3 cm of precipitation. Each plot was trenched to 0.3 m and lined with aluminum skirting to minimize lateral N transport. Wire cages (approximately 0.05-m mesh) excluded large herbivores.

Soil Sampling and Laboratory Analyses

After two growing seasons (late August 1997), we collected two cores (0.05 m in diameter and 0.2 m deep) at random points within each plot. The cores were composited, air-dried, weighed, and sieved (2 mm); a subsample was then 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; LECO, St. Joseph, MI, USA), and atom% ^{15}N was measured on an ANCA 2020 mass spectrometer (Europa Scientific, Cincinnati, OH, USA).

We separated the total soil N pool into labile and stable pools using long-term laboratory incubations with repeated leaching (Stanford and Smith 1972). Physical (Hassink and others 1993) and chemical (He and others 1988) techniques can be used for such fractionations, but we were specifically interested in determining whether added N was isolated from the plant–microbe internal N cycle. Laboratory incubation is the only fractionation technique in which N availability to microbes is directly estimated (Robertson and Paul 1999).

A subsample (50 g air-dried) from each field composite was incubated at optimal temperature (35°C) (Campbell and others 1993; Drinkwater and others 1996) in plastic filters (Nadelhoffer 1990) (Falcon Filter model 7111; Becton Dickinson Labware, Lincoln Park, NJ, USA). A glass fiber filter (Whatman GF/A, Whatman Inc., Ann Arbor, MI, USA), an "extra thick" glass fiber prefilter (Gelman Sciences, Ann Arbor, MI, USA), and a layer of glass wool were placed beneath the soil. A third glass fiber filter (Whatman GF/A, Whatman, Inc., Ann Arbor, MI, USA) was placed above the soil to prevent dispersion (Motavalli and others 1995). The filter units were sealed in airtight 2-L jars fitted with septa. Approximately

20 ml of deionized water was placed in the bottom of each jar to prevent soil drying. Every 2 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 (Stanford and Smith 1972; Nadelhoffer 1990). At each leaching, 100 ml of the N-free leaching solution was added to the top of the filter, allowed to equilibrate with the soil for 1 h, 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 (less than 10 min). Leachates were frozen until analysis for NH_4^+ and (NO_2^- + NO_3^-) by flow injection colorimetry and converted to g N m^{-2} using leachate (plus soil water) volume, initial dry mass of the incubated soil, and mean bulk density of the two field cores. At the end of the incubation, a subsample (20 g) of the residual soil was extracted with 100 ml of 0.5 M K_2SO_4 to account for unleached inorganic N. We defined the labile N pool as the sum of all inorganic N (NO_2^- + NO_3^- + NH_4^+) in leachates plus inorganic N extracted with K_2SO_4 immediately after the last leaching. Stable N was defined as total N minus labile N.

We determined the atom% ^{15}N of the leachate (NH_4^+ and NO_3^- combined) for each incubated soil by compositing 5 ml of leachate from each sampling date. The composite samples were diffused (Stark and Hart 1996; Khan and others 1998) for 6 days in 120-ml plastic containers. Dvarda's alloy converted NO_3^- in the samples to NH_4^+ and MgO raised the pH, converting 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 no. #41) sealed in Teflon tape. At the end of the diffusion, the acidified disks were dried over concentrated H_2SO_4 (24 h), stored in a desiccator, transferred to tin capsules, and analyzed for ^{15}N on the same instrument used for soil ^{15}N analyses. The atom% ^{15}N of samples was compared to ^{15}N standards and corrected for N in diffusion reagents using the pool dilution method of Stark and Hart (1996). The mass of tracer N residing in the labile pool was calculated using the following equations:

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

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

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

(4) Substituting from Eq. (2)

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

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 the 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, $^{15}N_n$ is the atom % ^{15}N enrichment of the added N, and $^{15}N_a$ is the atom% ^{15}N enrichment of the native soil (0.368% based on two samples per site). Similar equations were used to calculate the mass of tracer N in the soil prior to incubation. The amount of tracer N in the stable pool was determined by subtracting N_a from the amount of tracer N in the soil prior to incubation.

The labile C pool size was estimated by capturing all carbon dioxide (CO_2) in the headspace of the incubation jars. Before the jars were sealed, they were fanned with ambient air for 1 h 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; LICOR, Lincoln, NE, USA). 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, jar volume, sampled gas volume, dry soil mass, and bulk density were used to convert headspace concentration to g C m^{-2}. Labile C was defined as the sum of all CO_2-C respired during the incubation. Stable C was defined as total C minus labile C.

A three-way factorial analysis of variance (ANOVA) was used to analyze effects of N level (high and low), texture (coarse or fine), and site (five sites in Table 1) on stable and labile soil N and C pools. Simple and multiple linear regression identified correlations between soil N and C pools and clay content. All hypotheses were tested at α = 0.05.

Results

Bulk Soil N, and C Pools

Rates of N (leached) and C (respired) released from the bulk soil (native + tracer) pool declined rapidly during the first 100 days of the incubation and then

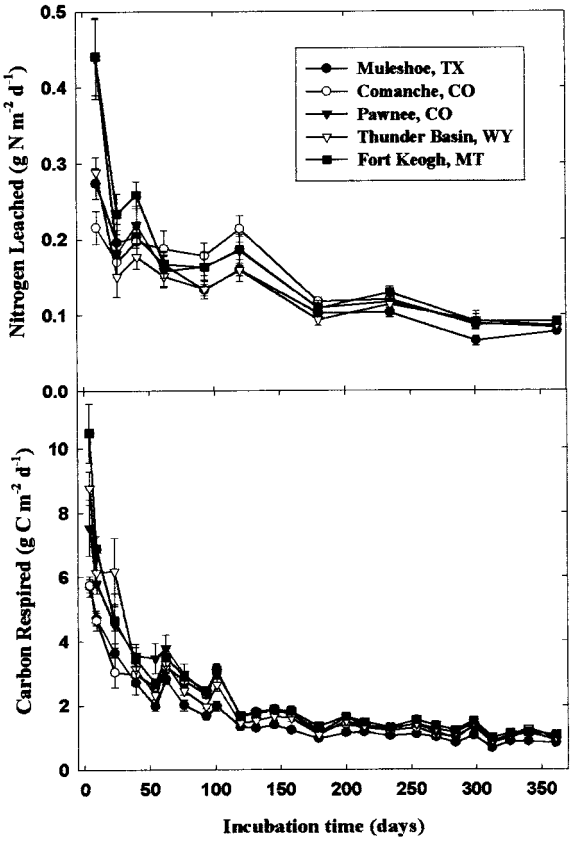

Figure 1. Inorganic nitrogen (N) leached and carbon (C) respired during long-term laboratory incubations of soils from five Great Plains grasslands. Data are means and standard errors across all soil texture and ^{15}N addition levels.

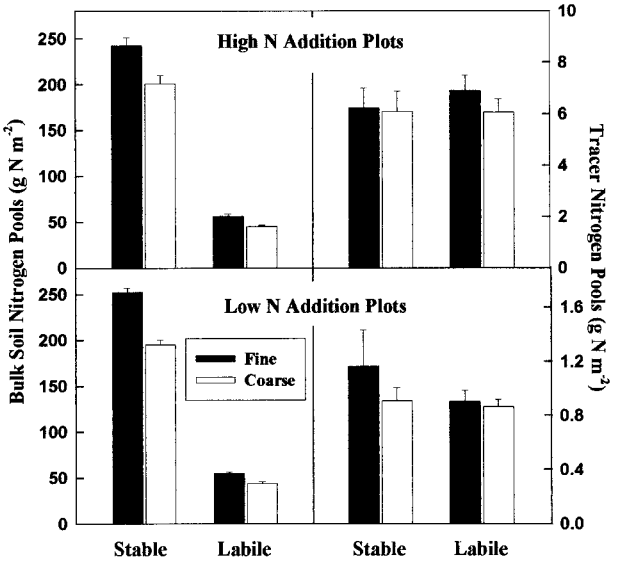

Figure 2. Pool sizes of labile and stable soil nitrogen (N) in grassland soils. Left-hand graphs show bulk soil N (native + tracer); right-hand graphs show ^{15}N-labeled tracer pools. Soils were collected 2 years after the tracer addition. For tracer N pools, there were no significant treatment effects. For the bulk soil, fine-textured soils had more stable N ($P < 0.001$) for both high-N and low-N additions. There was a significant site × texture interaction for labile N pools; all sites had more ($P < 0.001$) labile N in fine-textured soils, except Texas ($P = 0.25$). Bars are means plus one standard error.

remained constant for the last 200 days (Figure 1). Nitrate accounted for more than 95% of the N leached during the incubation (data not shown). Fine-textured soils contained 20% more stable N ($P < 0.001$) (Figure 2) and 30% more stable C ($P < 0.001$); (Figure 3) than coarse-textured soils. For labile N and C pools, there was a significant site × 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 ($P = 0.25$), but coarse-textured soils had more labile C than fine-textured soils ($P = 0.03$). In both texture classes and at all sites, labile and stable N and C did not differ between high (50 g N m^{-2}) and low (2.5 g N m^{-2}) addition treatments ($P > 0.46$). About 80% of bulk soil N was stable (Figure 4), and this percentage was not affected by texture ($P = 0.64$) or N addition level ($P = 0.17$). A smaller percentage of soil C was stable in coarse-textured soils than in fine-textured soils ($P < 0.001$) (Figure 4).

Although soil texture had a statistically significant effect on bulk soil N and C pools when analyzed as a discrete variable, regional-scale correlations between soil clay content and labile ($r^2 = 0.23$) and stable ($r^2 = 0.27$) N pool sizes were not strong (Figure 5). Soil C content explained 49% of the variability in labile N and 72% of the variability in stable N (Figure 6). When both soil C and clay were included in multiple regression models, clay was not a significant ($P > 0.79$) predictor of N pool sizes (data not shown). The ratio stable N:total N did not correlate with clay ($P = 0.79$) or C ($P = 0.72$) content (data not shown).

Stable soil C pools increased with stable N ($r^2 = 0.67$) and soil clay content ($r^2 = 0.48$) (Figure 7). Labile C pool sizes were not correlated with clay content ($r^2 = 0.02$; $P = 0.32$), and the ratio stable C:total C increased with increasing clay content (stable C:total C = 0.66 + 0.4×(soil clay content); $r^2 = 0.38$; $P < 0.001$).

Tracer N Pools

Two growing seasons after the ^{15}N addition, surface soil (0–20 cm) tracer retention in the low N plots

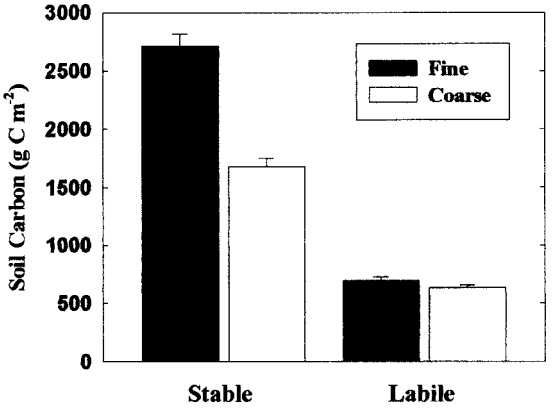

Figure 3. Pool sizes of labile and stable soil carbon (C) in grassland soils. Fine-textured soils had more stable C than coarse-textured soils ($P < 0.001$). There was a significant site × texture interaction for the labile pool; all sites had more labile C in fine-textured soils except Texas, which had more labile C in coarse-textured soils ($P = 0.03$). Bars are means and one standard error.

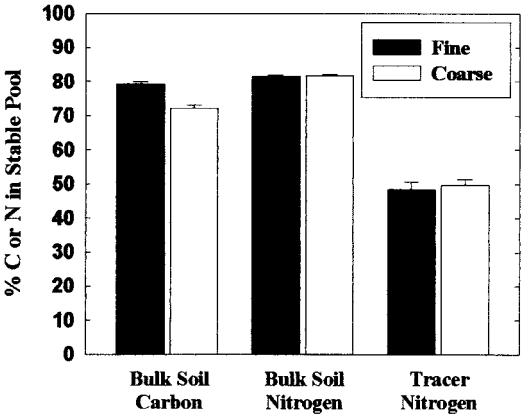

Figure 4. The percentage of total soil carbon (C) (*left*), total soil nitrogen (N) (*center*), and retained ^{15}N-labeled tracer N (*right*) that was stable. Soils were collected 2 years after the tracer addition. Bulk soil pools had a greater percentage of stable N than tracer pools ($P < 0.001$), and fine-textured soil had a greater percentage stable C than coarse-textured soils ($P < 0.001$). Bars are means and one standard error.

was 1.8 ± 0.6 g N m^{-2} (mean ± standard deviation [SD]), or approximately 71% of the 2.5 g N m^{-2} originally added. High-N plots contained 12.8 ± 5.0 g N m^{-2} of tracer N, or 26% of the 50 g N m^{-2} originally added. For both N treatments, 50% of the tracer retained in soil was stable, and soil texture had no effect on either the amount (labile $P = 0.14$, stable $P = 0.68$) (Figure 2) or proportion ($P = 0.40$) (Figure 4) of tracer N that was labile or stable. Neither soil C nor clay content correlated significantly with labile or stable tracer N pools or the fraction of retained tracer N that was stable (data not shown). The best predictor of the amount of tracer N in the labile pool was the total amount of tracer N in the soil after two growing seasons and immediately prior to incubation (Figure 8).

Discussion

The Magnitude of Stable ^{15}N Retention

The labile and stable N pools that we isolated are potentially retained by distinctly different pathways. Labile soil organic N is susceptible to microbial mineralization and the potential leaching and gaseous losses that accompany N mineralization. Retention of this labile N depends on repeated, efficient recycling of the mineralized N via immobilization into plant, microbial, or abiotic sinks. The stable pool, by definition, is less susceptible to microbial mineralization and subsequent leaching or gaseous losses. On annual time scales, there are no major loss vectors for stable organic matter in un-

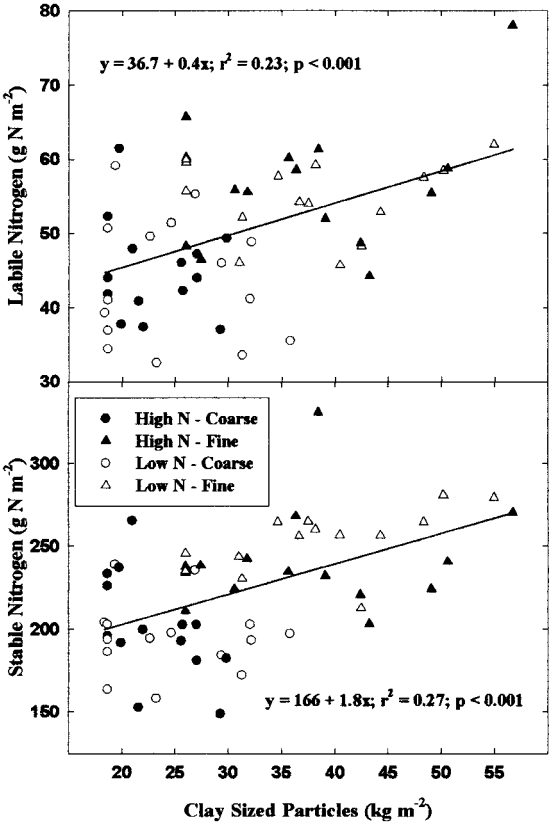

Figure 5. The relationship between the mass of clay-sized soil particles and pool sizes of labile or stable bulk soil (native + tracer) nitrogen (N). Symbols denote whether data come from high or low tracer N addition plots and whether the plot was considered coarse- or fine-textured in ANOVA analyses (Figure 2).

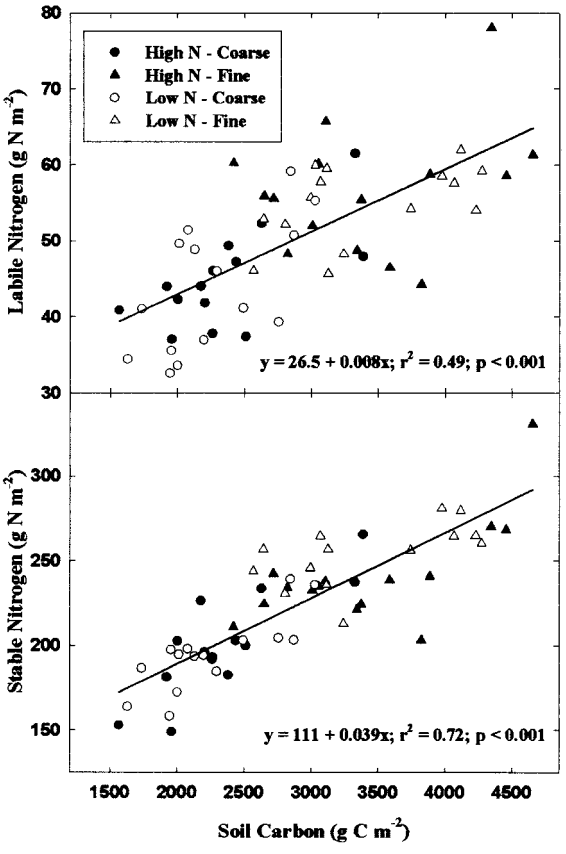

Figure 6. The relationship between soil carbon (C) content and pool sizes of labile or stable bulk soil (native + tracer) nitrogen (N). Symbols denote whether data come from high or low tracer N addition plots and whether the plot was considered coarse- or fine-textured in ANOVA analyses (Figure 2).

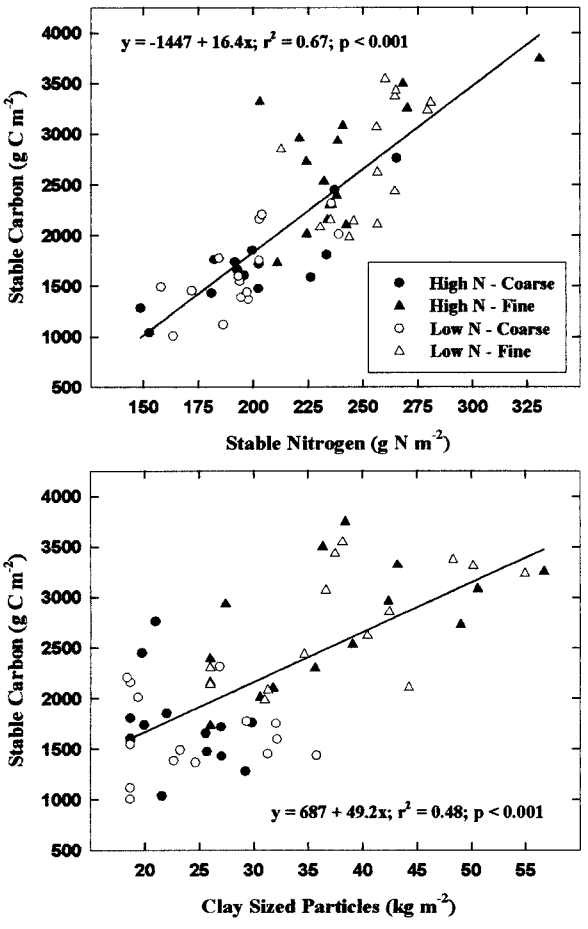

Figure 7. The relationship between the stable carbon (C) pool size and the bulk soil (native + tracer) stable nitrogen (N) pool size (top) or the mass of clay sized soil particles (bottom). Symbols denote whether data come from high or low tracer N addition plots and whether the plot was considered coarse- or fine-textured in ANOVA analyses (Figure 2).

plowed soils, so this pool should represent a relatively long-term sink for added N. Our incubations show that stable ^{15}N retention was substantial in grassland soils; 2 years after the tracer was applied, half of the retained N resided in the stable pool.

Our data are consistent with shorter incubation experiments (Smith and others 1978; Preston 1982; Smith and Power 1985; Chang and others 1997) and with ^{15}N tracer experiments using chemical and physical organic matter fractionation techniques (Stanford and others 1970; Delgado and others 1996; Chang and Preston 1998; Barrett and Burke 2002). In all cases, stable N accounted for more than 40% of N retention on annual time scales. Shorter ^{15}N exposures (days to months) resulted in a similar amount of stable N retention (more than 40% of soil ^{15}N) (Broadbent and Nakashima 1967; Chichester and others 1975; He and others 1988; Strickland and others 1992; Chang and Preston 1998); stable organic matter may be an immediate and long-term sink for N.

Several less direct lines of evidence imply an important role for stable N retention. Bioassay (Jansson 1963; Preston and Mead 1994b; Chang and others 1999) and field ^{15}N tracer experiments (Preston and Mead 1994ab; Buchmann and others 1996; Nadelhoffer and others 1999a) show that plants use only a small fraction of fertilizer that has been in soil for more than 6 months. Similarly, microbial immobilization of inorganic ^{15}N may be important initially, but ^{15}N retained in microbial biomass typically declines within several weeks of tracer application (Emmett and Quarmby 1991; Seely and Lathja 1997; Zogg and others 2000; Perakis and Hedin 2001). Low ^{15}N recovery in plants, declining

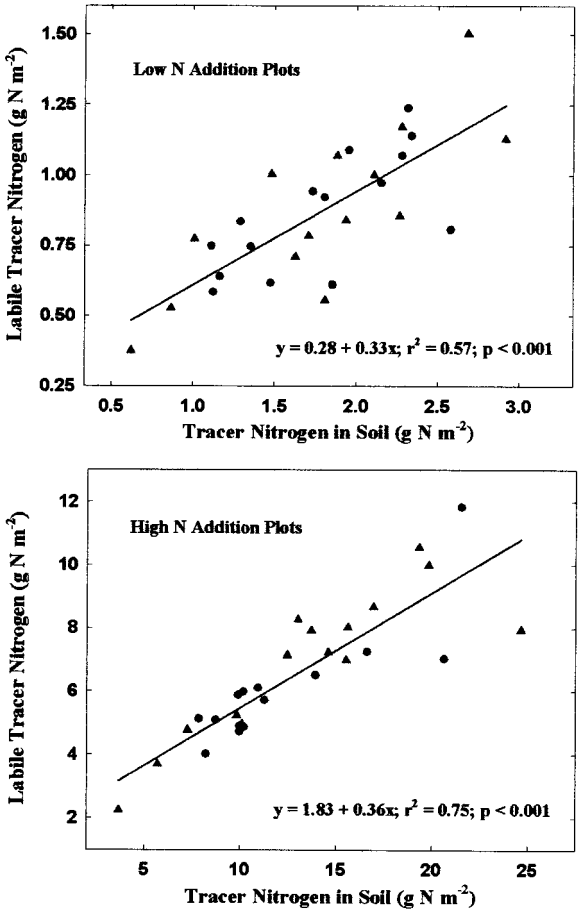

Figure 8. The relationship between the total amount of ^{15}N-labeled tracer nitrogen (N) in the soil and the amount of ^{15}N-labeled tracer that was labile. Soils were collected 2 years after plots received low (2.5 g N m^{-2}) and high (50 g N m^{-2}) tracer N additions. Symbols denote whether the plot was considered coarse-textured (▲) or fine-textured (●) in ANOVA analyses (see Figure 2).

recovery in microbes, and small N leaching losses (Gundersen and Rasmussen 1995; Wright and others 1995; Nadelhoffer and others 1999a, b; Aber and others 1998) in conjunction suggest low remineralization rates for N retained in organic matter (Perakis and Hedin 2001; but see Preston and Mead 1994 a, b).

Texture and C Controls on Stable N Pool Sizes

We hypothesized that stable bulk soil and tracer N pools would be largest in soils with fine texture and high C content. These hypotheses were corroborated by the bulk soil data; fine-textured soils contained more stable N than coarse-textured soils (Figure 2), and stable N pools were positively correlated with soil clay and C content (Figure 5 and 6). However, the hypothesis was not supported by the ^{15}N addition experiment; stable tracer N retention was not affected by soil texture or C content (Figure 2). The best predictor of labile and stable tracer N pool sizes was the total amount of tracer N in the soil prior to the incubation (that is, soil tracer N retention 2 years after the tracer was added) (Figure 8). It is unclear why mechanisms that promoted a wide range of soil N retention (Figure 8, x-axes) did not affect the proportion of retained tracer in stable pools.

Interestingly, our estimates of stable soil tracer retention in the high N plots were correlated with estimates of rapid abiotic N immobilization in the same soils ($r^2 = 0.63$; $P < 0.01$) (J. E. Barrett, J. P. Kaye, D. W. Johnson, and I. C. Burke unpublished). Abiotic immobilization in these soils did not correlate with soil C or texture (Barrett and others 2002), and it is possible that the stable pools isolated in our incubations result directly from abiotic immobilization that occurred immediately after tracer addition. Repeated sampling of stable pool sizes over minutes to years would be necessary to test this hypothesis.

We know of no other regional-scale study of texture and C controls on stable tracer N retention. Regional and cross-site studies do reveal correlations between total (labile plus stable) soil N retention and soil C, soil C:N ratios, or soil texture (Nohrstedt and others 1996; Emmett and others 1998; Barrett 1999; Johnson and others 2000). Similar results have been observed within individual sites. In a coastal forest in Massachusetts, coarse sandy soils with low C content retained less ^{15}N than loamy sands with higher C content (Seely and Lathja 1997). The backslope position of a grassland catena had less clay and soil C than a footslope position, and the latter retained more ^{15}N (Schimel and others 1986) even 10 years after the application (Delgado and others 1996). At the 10-year sampling, 42% and 58% of the N retained in the footslope and backslope soils, respectively, were in a stable pool thought to turnover every 200 to 1500 years. In our study, the effects of clay and C content on stable N retention may have been confounded by differences in parent material, clay mineralogy, C quality (that is, differences in humic compounds), or field microbial activity among the sites (Barrett and others 2002).

Texture and N Controls on Stable C Pools

The notion that fine-textured soils stabilize soil organic C is paradigmatic in grassland and agricultural

ecology (Oades 1988). The idea is explicit in ecosystem models (Parton and others 1987) and has been observed at numerous individual sites (Delgado and others 1996; Collins and others 2000). In contrast, labile C pools that are not associated with soil clay particles correlate poorly with soil texture across multiple sites (Giardina and others 2001; Hassink and others 1993). Our results are consistent with both of these findings, since labile C pool sizes were not correlated with soil clay content across the regional gradient ($r^2 = 0.02$; $P = 0.32$); the Texas site had less labile C in fine-textured soils, whereas other sites showed the opposite trend (Figure 3) and stable C increased with increasing clay content (Figure 7). Regional gradients in soil texture may correlate with the largest pool of soil C (the stable pool); however, soil texture does not necessarily correlate with labile pools that respond most rapidly to land-use and climate change.

Several investigators have suggested that N additions alter stable C pools either by decreasing lignolitic enzyme production or by increasing humification rates (Berg and Matzner 1997; Carrierro and others 2000). We did not detect a significant N-level effect on stable C pools, but the flux (if it existed) was likely below the detection limits of our method. Using the measured stable C:N ratios (Figure 7), soil tracer retention (Figure 8), and the fraction of tracer that was stable (Figure 4), we estimate that our high-N treatment increased the stable C pool size by a maximum of 4%. In contrast to the experimental N addition, bulk soil stable N and C were strongly correlated across the regional gradient (Figure 7); thus, steady-state stable N and C pools appear to be associated with predictable, though not constant, stoichiometry. In our data, soils with low stable N content had narrower C:N ratios than soils with high stable N content (Figure 7; significant negative y-intercept; $P < 0.001$), but regional N deposition studies show the opposite trend for total soil (as opposed to stable) C:N ratios (Emmett and others 1998). Although regional N deposition and stable soil C sequestration may be linked through a predictable soil C:N ratio (Schimel 1998; Nadelhoffer and others 1999b), constant stoichiometry (soil C:N) is unlikely.

Conclusions

Previous N cycling and N retention research focused on the small pools of inorganic and labile organic N that are actively cycled by plants and microbes. Our results demonstrated that on annual time scales, active recycling of labile N accounted for only half of the ^{15}N retained in grassland soils. Stable N formation was an equally important pathway of N retention. These results are consistent with numerous field tracer experiments showing that plants and microbes have limited access to ^{15}N sequestered in soil organic matter.

Despite its importance as a terrestrial N sink, mechanisms and rates of stable N formation are still poorly understood. In this study, we exploited large gradients in soil C and texture in an attempt to isolate controls on stable ^{15}N retention. We did not detect correlations between these factors and stable ^{15}N pools; however, bulk soil stable N pools were correlated with stable soil C pools and clay content, as expected.

Significant N retention in stable pools provides and important mechanistic link between rapid microbial and abiotic N immobilization studies and long-term field tracer experiments showing persistent soil N retention. Further insight could be gained through experiments that measure short- and long-term stable N retention (minutes to days) of a single N tracer. Linking short- and long-term fates of N (Chang and Preston 1998) will isolate the time scales over which recycling and stable N formation dominate N retention processes.

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