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Changes in stable isotopic signatures of soil nitrogen and carbon during 40 years of forest development

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Abstract Understanding what governs patterns of soil $\delta^{15}N$ and $\delta^{13}C$ is limited by the absence of these data assembled throughout the development of individual ecosystems. These patterns are important because stable isotopes of soil organic N and C are integrative indicators of biogeochemical processing of soil organic matter. We examined $\delta^{15}N$ of soil organic matter ($\delta^{15}N_{SOM}$) and $\delta^{13}C_{SOM}$ of archived soil samples across four decades from four depths of an aggrading forest in southeastern USA. The site supports an old-field pine forest in which the N cycle is affected by former agricultural fertilization, massive accumulation of soil N by aggrading trees over four decades, and small to insignificant fluxes of N via NH_3 volatilization, nitrification, and denitrification. We examine isotopic data and the N and C dynamics of this ecosystem to evaluate mechanisms driving isotopic shifts over time. With forest development, $\delta^{13}C_{SOM}$ became depth-dependent. This trend resulted from a decline of ~2‰ in the surficial 15 cm of mineral soil to −26.0‰, due to organic matter inputs from forest vegetation. Deeper layers exhibited relatively little trend in $\delta^{13}C_{SOM}$ with time. In contrast, $\delta^{15}N_{SOM}$ was most dynamic in deeper layers. During the four decades of forest development, the deepest layer (35–60 cm) reached a maximum $\delta^{15}N$ value of 9.1‰, increasing by 7.6‰. The transfer of >800 kg ha^{-1} of soil organic N into aggrading vegetation and the forest floor and the apparent large proportion of ectomycorrhizal (ECM) fungi in these soils suggest that fractionation via microbial transformations must be the major process changing $\delta^{15}N$ in these soils. Accretion of isotopically enriched compounds derived from microbial cells (i.e., ECM fungi) likely promote isotopic enrichment of soils over time. The work indicates the rapid rate at which ecosystem development can impart $\delta^{15}N_{SOM}$ and $\delta^{13}C_{SOM}$ signatures associated with undisturbed soil profiles.

Keywords Stable isotopes · Reforestation · Soil profile

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Introduction

Stable isotopes are useful for investigating biogeochemical mechanisms that govern ecosystem C and N processes (Schlesinger 1997; Ehleringer et al. 2000; Robinson 2001; Pataki et al. 2003). Stable isotopes provide integrative measures of N and C cycling that help constrain budgets, identify sources and fates, and estimate the rates at which these elements are biogeochemically processed. As the relevance of soil organic matter (SOM) to global N and C cycles becomes more evident, stable isotopes of these elements are increasingly used to assess the details of soil and, by extension, ecosystem processes (Nadelhoffer and Fry 1988; Boutton 1996; Ehleringer et al. 2000; Billings et al. 2002; Loya et al. 2003).

Belowground mechanisms that control long-term N and C cycling are often difficult to discern, in part because soils support a complexity of interactions among a nearly unfathomable diversity of microorganisms, organic matter compounds and functional groups, mineral surfaces, and often heterogeneous physical, chemical, and biological conditions. Our abilities to elucidate some of these complexities notwithstanding (Schimel and Bennett 2004), the complex nature of biological, chemical, and physical interactions limits and challenges our understanding of how SOM is transformed over time.

The governing features of SOM cycling are also confounded by a notable absence of data describing how soil systems change during the development of individual ecosystems. For example, most experiments examining isotopic dynamics of SOM take place on time scales of hours, days, or months (Allen et al. 2000; Haile-Mariam et al. 2000; Finzi et al. 2002; Billings et al. 2004), or several years (Balesdent et al. 1998; Schlesinger and Lichter 2001). Other chronosequence studies have examined isotopic dynamics over very long periods of time (Amundson et al. 2003; Vitousek et al. 1989). Studies of SOM dynamics only rarely examine decades of ecosystem development (Richter et al. 1999; Jenkinson 1991; Richter and Markewitz 2001); those that do often employ the distinct C isotopic signatures of C_3 and C_4 vegetation to estimate SOM turnover (Balesdent and Mariotti 1996; Boutton 1996). Though such studies have helped us develop ideas about SOM dynamics, few studies have explored shifts of both $\delta^{15}N_{SOM}$ and $\delta^{13}C_{SOM}$ within the same soils throughout decades of an ecosystem's development. Ultimately, if we are to understand processes by which humans impact soil development, we need many more studies that span decades. Because stable isotopic signatures are integrative measures of ecosystem processes (Robinson 2001; Pataki et al. 2003), they can highlight the most influential processes that govern the biological and physical dynamics in a soil system over such periods (Nadelhoffer and Fry 1988; Ehleringer et al. 2000; Billings et al. 2002; Kramer et al. 2003; Krull and Skjemstad 2003).

Due to the increased age of organic matter in mineral soil as a function of depth (Trumbore 2000), it is frequently assumed that the trend of increasing $\delta^{15}N_{SOM}$ and $\delta^{13}C_{SOM}$ with soil depth (Nadelhoffer and Fry 1988; Ehleringer et al. 2000; Amundson et al. 2003) is related to processes occurring during organic matter formation. Organic matter fractions containing generally older SOM are associated with relatively enriched isotopic signatures (Tiessen et al. 1984; Compton and Boone 2000; Billings et al. 2006). Several studies suggest that SOM subjected to increased degrees of microbial processing will exhibit ^{15}N and ^{13}C enrichment (Nadelhoffer and Fry 1988; Billings et al. 2002). Increasing $\delta^{15}N$ values with soil depths can also be associated with significant N uptake by mycorrhizal fungi and subsequent preferential transfer of ^{15}N-depleted compounds to vegetation (Högberg et al. 1996; Taylor et al. 1997; Kohzu et al. 1999; Hobbie et al. 1999, 2000), and could result in part from fungal transfers of N within a soil profile (Frey et al. 2000; Frey et al. 2003). Examining SOM patterns of $\delta^{13}C$ and $\delta^{15}N$ over decades of an ecosystem's development would help us understand the extent to which different mechanisms govern observed changes in C and N stable isotopes with soil depth.

In this study, we examine $\delta^{15}N_{SOM}$ and $\delta^{13}C_{SOM}$ within the soil of an old-field pine forest during four decades of forest ecosystem development. To our knowledge, this is the first study to examine the time trends of $\delta^{15}N_{SOM}$ and $\delta^{13}C_{SOM}$ at one site from a forest's establishment through canopy closure. We present data from soils archived on seven occasions, starting in 1962, five years after pine seedlings were planted. We also examine these parameters in O horizons, litterfall, foliage, and fine and coarse root biomass during the 1990s. These data are examined in the context of a series of studies exploring the effects of forest development on soil C and N following more than a century of agricultural use (Markewitz et al. 1998; Richter and Markewitz 1995; Richter et al. 1999, 1994, 2000).

The overwhelming feature of organic matter dynamics in these soils over four decades of forest development is the massive transfer of N from mineral soils to forest biomass and O horizons and the relatively minor changes in total organic C content in the mineral soil (Richter et al. 2000). From 1962 to 1997, the surface 60 cm of mineral soil experienced depletions of approximately 820 kg N ha^{-1} (~23.4 kg ha^{-1} $year^{-1}$); during this same time period, approximately 1,100 kg N ha^{-1} accumulated in the vegetation and forest floor due to plant uptake and litterfall (~31.4 kg ha^{-1} $year^{-1}$; Richter and Markewitz 2001). Concurrently, mineral-soil C experienced modest accumulations of approximately 1.5 Mg ha^{-1} (~42.9 kg ha^{-1} $year^{-1}$), whereas the forest's O horizon accumulated approximately 37.8 Mg ha^{-1} (~1,080 kg ha^{-1} $year^{-1}$). We use this information about C and N changes in Calhoun soils over four decades (Richter et al. 1999, 2000; Richter and Markewitz 2001) to help isolate processes that likely govern the observed depth-dependent patterns in soil $\delta^{15}N$ and $\delta^{13}C$ at Calhoun, and refer the reader to these previously published works for more detailed information on C and N accumulations throughout the forest's development.

Materials and methods

The Calhoun forest is in northwestern South Carolina (34.5°N, 82°W; Richter et al. 1994), at an elevation of about 200 m. The forest experiences mild winters and hot summers, with mean annual precipitation of about 1,250 mm (Richter et al. 2000). Prior to European settlement, the site supported a forest dominated by oak–hickory (*Quercus–Carya*). In the early decades of the 1800s, land in upstate South Carolina was cleared extensively for cotton. This site supported mainly crops of cotton until cultivation ceased after the growing season of 1954 (Richter and Markewitz 2001). Soils are derived from metamorphosed granitic gneiss and are classified as Ultisols, mainly of the Appling series (clayey, kaolinitic, thermic Typic Kanhapludults). Although long-term cultivation diminished O and A horizons, today there is a significant O horizon of forest detritus and an incipient and slowly redeveloping A horizon (Richter et al. 1999). Forest development is also affecting prominent depth-dependence in total N (Richter et al. 2000), pH and acidity (Markewitz et al.

1998), exchangeable cations (Richter et al. 1994; Markewitz and Richter 2000), organic and inorganic fractions of P (Richter et al. 2006b), and activity of Fe oxides (Richter et al. 2006a).

After 2 fallow years (1955–1956), 16 permanent plots at the site were planted with loblolly pine (*Pinus taeda*) seedlings in the winter of 1956–1957. To obtain the data presented here, we employed archived soils from eight of the long-term plots. Mineral soils were sampled in 1962, 1968, 1972, 1978, 1982, 1990, and 1997. Forest litterfall was sampled in 1991, O horizons and foliage were sampled in 1992, and fine and coarse roots were sampled in 1993. Archived samples are stored at Duke University.

Mineral soils were sampled at four depths (0–7.5, 7.5–15, 15–35, and 35–60 cm), except in 1968, when only the two most surficial depths were sampled. Punch tubes (2 cm diameter) were used to take at least 20 cores in each of the eight plots. The punch-tube samples were composited by depth within each plot. For this study, these samples were composited again across the eight plots, keeping years and depths separate, to generate 30 mineral soil samples for isotopic analyses.

In 1992, forest floor samples were collected via four or five randomly placed, 0.13-m^2 sampling locations in eight of the permanent plots. These samples were composited within each of the eight plots and divided into three layers, representing the O_i, O_e, and O_a horizons. In 1991 litterfall was collected with five, 0.7-m^2 traps in each of the eight plots. Foliage was collected in 1992 as a composite sample of ten harvested trees, each of which was sampled in upper, middle, and lower crown positions. In 1993, fine and coarse roots were sampled from the eight plots with a 6-cm-diameter core taken to 15-cm depth. As with the mineral soils, O horizon and biomass samples were composited across plots to generate composite samples with $n = 8$ ($n = 10$ for tree foliage).

All soil and biomass samples were analyzed for $\delta^{15}N$ and $\delta^{13}C$ on a Finnigan Delta$^+$ mass spectrometer (Finnigan MAT, Germany) coupled to a Carlo Erba elemental analyzer (NA1500 CHN combustion analyzer; Carlo Erba Strumentazione, Milan) via a Finnigan Conflo II Interface at the University of Arkansas Stable Isotope Laboratory. To assess analytical repeatability, we analyzed duplicates of approximately 25% of all samples. All mineral soil duplicates exhibited maximum differences in $\delta^{15}N$ and $\delta^{13}C$ values of 0.3‰. Standards (acetanilide and spinach) were analyzed after every ten samples; analytical precision of the instrument was ± 0.13 for $\delta^{13}C$ and ± 0.21 for $\delta^{15}N$ (SDs). Results are reported as values of either individual samples or, where appropriate, the mean of duplicate values. We do not present the C and N content data obtained while analyzing these soils for $\delta^{15}N$ and $\delta^{13}C$ because these composited samples have been analyzed previously for [C] and [N] on an elemental analyzer specifically dedicated to such tasks (Markewitz et al. 1998; Richter and Markewitz 1995; Richter et al. 1999, 1994, 2000). Instead, we discuss isotopic data in the context of the N and C data that have been presented and discussed in these earlier works. Soil $\delta^{15}N$ and $\delta^{13}C$ data were regressed against date of sampling to test for slopes significantly different from zero. For all analyses, statistical significance was determined at $P < 0.05$.

Results and discussion

Land use history and soil $\delta^{13}C$ and $\delta^{15}N$

In 1962, mineral soil $\delta^{13}C$ and $\delta^{15}N$ values at all measured depths were relatively similar, ranging from −23.8 to −24.9‰ and from 1.5 to 3.3‰, respectively (Figs. 1, 2). The similar soil $\delta^{13}C$ and $\delta^{15}N$ values at all depths early in this forest's development reflect the historical agricultural use of this land. Soil supporting cotton frequently exhibits $\delta^{13}C$ values between −23.9 and −21‰ (Conteh et al. 1998; Leavitt et al. 1996). When planted with cotton, these soils were regularly cultivated and well mixed throughout the plow layer (presumed to be up to 20-cm depth), with crop residues incorporated into the surface horizons; data from deeper horizons

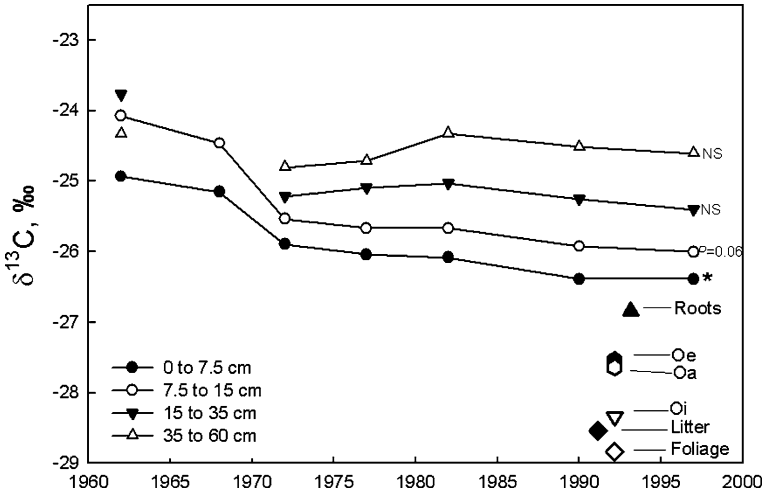

Fig. 1 Soil $\delta^{13}C$ values (‰) at four depths in the soil profile (0–7.5, 7.5–15, 15–35, and 35–60 cm) during four decades of forest development at the Calhoun Experimental Forest, South Carolina. Each point represents either the mean of two samples, or an individual measured value, of archived soil samples composited by depth from at least 20 punch tubes at each of eight permanent plots (means of approximately 160 punch tube samples). *Asterisk* indicates $P < 0.05$

reflect decomposing root biomass and illuviated material. Such soil management practiced for up to 150 years resulted in a profile that reflects the $\delta^{13}C$ of cotton stubble and roots throughout the upper 60 cm.

Values of $\delta^{15}N_{SOM}$ in 1962 also reflect the legacy of cotton farming. Cultivated soil often does not exhibit soil ^{15}N enrichment with depth (Karamanos and Rennie 1980; Nadelhoffer and Fry 1988; Högberg 1997), in contrast with undisturbed soils. Because plant biomass tends to possess $\delta^{15}N$ signatures depleted relative to SOM (Nadelhoffer and Fry 1988, 1994; Högberg 1997; Robinson 2001; Amundson et al. 2003), mixing crop residues into the soil maintains relatively depleted $\delta^{15}N_{SOM}$ in these surface layers. The deepest layer (35–60 cm) likely possesses relatively ^{15}N-depleted signatures due to residues from the annual turnover of ^{15}N-depleted crop roots. Additionally, soils at depth may exhibit relatively depleted $\delta^{15}N$ signatures of illuviated material. Such illuviation effects have been observed in other agricultural systems (Delwiche and Steyn 1970; Nadelhoffer and Fry 1994). Illuviation could be promoted in agricultural soils with enhanced aggregate fragmentation and downward movement of cotton residue from above. The application and mixing of fertilizers into the soil profile and uptake of this fertilizer by vegetation also contributed to relatively ^{15}N-depleted signatures in 1962. Anthropogenic fertilizers generally have $\delta^{15}N$ signatures close to 0‰ (Hübner 1986).

Forest development and soil $\delta^{13}C$ and $\delta^{15}N$

By 1977, mineral soil $\delta^{13}C$ and $\delta^{15}N$ were depth-dependent, a trend that intensified throughout the rest of the forest's development. Surficial layers were the most depleted in both ^{13}C and ^{15}N (Figs. 1, 2). $\delta^{13}C_{SOM}$ in this layer declined significantly, from −24.9 to −26.2‰ ($P = 0.01$), while $\delta^{15}N_{SOM}$ in the surface layer remained constant throughout the forest's growth. In the three deeper layers, $\delta^{15}N_{SOM}$ increased significantly ($P = 0.04$, 0.02, and 0.05 for 7.5–15, 15–35, and 35–60 cm, respectively), by a maximum of 7.5‰ in the deepest layer. $\delta^{15}N_{SOM}$ values in the two deepest layers were significantly more enriched than the more surficial soils, and were not significantly different from each other. In contrast, deeper horizons experienced no significant change in $\delta^{13}C_{SOM}$ values with forest development. Depth-dependency exhibited by $\delta^{15}N_{SOM}$ and $\delta^{13}C_{SOM}$ from 1977 up to and including 1997 thus results from contrasting trends, with $\delta^{15}N_{SOM}$ increasing deeper in the profile and remaining stable at the surface, and $\delta^{13}C_{SOM}$ declining at the surface and remaining relatively constant deeper in the profile.

The decline of $\delta^{13}C_{SOM}$ in the 0- to 7.5-cm layer is attributed to the incorporation of forest biomass into this layer during forest development. Litterfall and roots from pines possessed $\delta^{13}C$ signatures of −28.5 and −26.8‰ (Fig. 1), respectively. These values are consistent with other published forest values (Buchmann et al. 1997) and are slightly more negative than most reported cotton values (−22 to −26‰; Wullschleger et al. 1991). These differences appear to be reflected in soils, with those supporting pine forests generally possessing more negative $\delta^{13}C_{SOM}$ values than those supporting cotton (Leavitt et al. 1996; Conteh et al. 1998; Quideau et al. 2003). Though the differences between these crops' $\delta^{13}C$ values are slight relative to the $\delta^{13}C$ shifts experienced with C_3/C_4 conversions, the shift is evident in these data as SOM derived from pine biomass develops in the surface layer. In contrast, relatively constant $\delta^{13}C_{SOM}$ deeper in the profile (e.g., 15–60 cm) suggests minimal incorporation of recently produced pine residues in these layers. These data support Richter et al. (1999), who estimated net C accumulation in the surficial 15 cm of mineral soil to be approximately 145 g m^{-2} at this site from 1962 to 1997 and to be insignificant deeper in the profile, congruent with estimated rates of C input deeper in the soil profile vs. those in the top 7.5 cm (Richter and Markewitz 2001).

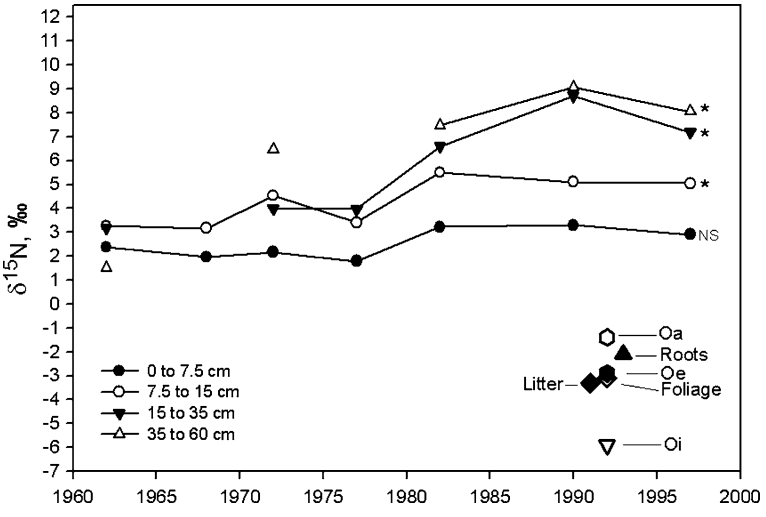

Fig. 2 Soil $\delta^{15}N$ values (‰) at four depths in the soil profile (0–7.5, 7.5–15, 15–35, and 35–60 cm) during four decades of forest development at the Calhoun Experimental Forest, South Carolina. Each point represents either the mean of two samples, or an individual measured value, of archived soil samples composited by depth from at least 20 punch tubes at each of eight permanent plots (means of approximately 160 punch tube samples). *Asterisks* indicate $P < 0.05$

The relatively stable $\delta^{15}N_{SOM}$ values in the surface layer also reflect the incorporation of forest litterfall and rhizodeposition into this layer. Most biomass and organic horizons possessed $\delta^{15}N$ values depleted by several parts per thousand relative to mineral soil (Fig. 2), consistent with numerous other studies (Nadelhoffer and Fry 1988, 1994; Amundson et al. 2003). The incorporation of this ^{15}N-depleted material in the surface layer likely limited the development of ^{15}N enrichment with time, as was observed deeper in the profile where organic inputs were minimal (Richter et al. 1999, 2000).

Mechanisms of depth-dependence for soil $\delta^{13}C$ and $\delta^{15}N$

Increases in $\delta^{13}C_{SOM}$ observed with depth will likely persist well into the future, for several reasons. First, the combustion of ^{13}C-depleted fossil fuels has diluted the $\delta^{13}C$ of atmospheric CO_2 by about 1.3‰ (until 1993; Friedli et al. 1987; Trolier et al. 1996; Ehleringer et al. 2000; Tu and Dawson 2005). This ^{13}C-depleted C is incorporated into vegetation and subsequently into SOM, generating a depleted $\delta^{13}C$ signature in surface soils relative to the older, more ^{13}C-enriched material deeper in the profile. Though the impact of fossil fuel combustion on the $\delta^{13}C$ values of atmospheric CO_2 cannot explain the extent of the depth-dependence of $\delta^{13}C_{SOM}$ observed in these soils, it likely has contributed to the pattern. $\delta^{13}C_{SOM}$ values also may become enriched through time due to increased contributions of organic compounds derived from microbial cells, which tend to be more ^{13}C-enriched relative to microbial substrate (Ehleringer et al. 2000; Tu and Dawson 2005). Decomposition processes themselves also likely result in some C isotopic fractionation. Though direct evidence of microbial discrimination against ^{13}C during substrate breakdown is not abundant (but see Silfer et al. 1992; Hobbie et al. 2004), linear relationships between $\delta^{13}C_{SOM}$ and $\ln[C_{SOM}]$ observed in other studies (Nadelhoffer and Fry 1988) suggest that such fractionation may occur, congruent with the non-significant trends generated by Calhoun soils (Fig. 3). Even slight fractionations could account for observable shifts in $\delta^{13}C_{SOM}$ when applied over decades (Högberg et al. 2005).

The trend of increasing $\delta^{15}N_{SOM}$ values deeper in the soil profile during forest development contrasts with the relatively modest changes in $\delta^{13}C_{SOM}$ for these layers. We suggest two key features of these soils that likely explain their $\delta^{15}N_{SOM}$ values: (1) preferential excretion of ^{15}N-depleted compounds by heterotrophic organisms, particularly release of N by ectomycorrhizal (ECM) fungi to vegetation; and (2) to a lesser extent, the fractionation associated with mineralization of organic N in these soils. N isotope fractionation occurs during microbial growth and dissimilation of nitrogenous compounds (Macko and Estep 1984; Checkley and Miller 1989; Hoch et al. 1996). With a degree of ^{15}N discrimination greater for dissimilatory compared to assimilatory processes for heterotrophic organisms, microbial biomass would become progressively ^{15}N-enriched over time (Delwiche and Steyn 1970; Minagawa and Wada 1984; Nadelhoffer and Fry 1994). Isotopic enrichment of microbial biomass relative to other organic matter in ecosystems has been widely observed; these trends are particularly strong for ECM fungi (Handley et al. 1996; Högberg et al. 1996; Hoch et al. 1996; Hobbie et al. 1999, 2000, 2005; Henn and Chapela 2001). Kohzu et al. (1999) report an isotopic enrichment factor (ε) for ^{15}N of ECM fungi relative to wood of 6.1 ± 0.4‰ (SD), and Hobbie et al. (2005) suggest values of mycorrhizal fractionation against ^{15}N during N transfer to the host plant of approximately 8–10‰. ECM fungi represent a significant proportion of microbial diversity in these soils (R. Vilgalys and J. Jackson, personal communication), and likely are responsible for a significant fraction of pine N acquisition. With significant ECM symbiosis, plant tissues and subsequent plant litterfall become correspondingly ^{15}N-depleted relative to ECM fungal tissues (Högberg

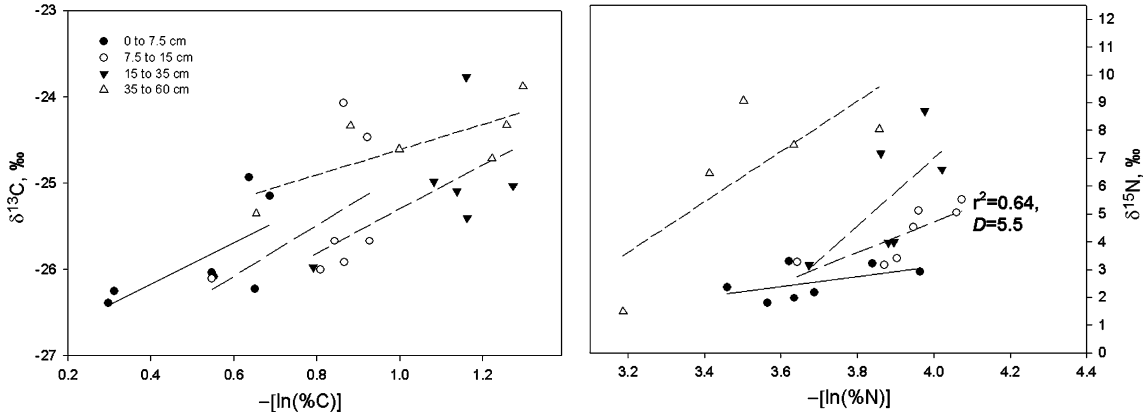

Fig. 3 Soil $\delta^{13}C$ (‰) versus the negative natural logarithm of percent C (*left-hand panel*) and $\delta^{15}N$ (‰) versus the negative natural logarithm of percent N (*right-hand panel*) for four depths in the soil profile (0–7.5, 7.5–15, 15–35, and 35–60 cm) during four decades of forest development at the Calhoun Experimental Forest, South Carolina. Discrimination factor (*D*) is calculated for that soil layer where the relationship is statistically significant. See text for details

et al. 1996; Henn and Chapela 2001). In the three deeper layers of the soil profile, incorporation of plant litterfall is relatively slight, resulting in SOM composition in deeper layers becoming increasingly influenced by the ^{15}N enrichment of microbial biomass, likely dominated by ECM. Though microbial biomass may comprise a relatively small fraction of total SOM, the significant ^{15}N enrichment of microbial tissue, particularly that of ECM fungi, can be a large influence on $\delta^{15}N_{SOM}$ (Högberg et al. 1996). The increasing $\delta^{15}N_{SOM}$ in deeper layers can be explained by the accretion over time of recalcitrant materials derived from microbial (especially fungal) cells (Paul and Clark 1996). This effect is mitigated in surface layers, where plant inputs are more significant.

We also suggest that preferential mineralization of ^{15}N-depleted organic compounds (Nadelhoffer and Fry 1988, 1994) has contributed to the rapid establishment of depth-dependent $\delta^{15}N_{SOM}$ patterns in these soils, even though the isotopic fractionation associated with SOM mineralization may be slight relative to other N cycling processes [N isotope fractionation (ϵ) ranging up to 5‰; Robinson 2001]. The enzymes responsible for transforming organic substrates such as nucleotides and nucleic acids, proteins, and free amino acids into NH_4^+ (Killham 1994; Schimel and Bennett 2004) likely discriminate against ^{15}N-containing molecules to a small degree (Nadelhoffer and Fry 1994; Högberg 1997; Robinson 2001), but even a slight fractionation factor occurring during decades of massive transfers of N from mineral soil into forest biomass and O horizons (Richter and Markewitz 2001) could be sufficient to increase $\delta^{15}N_{SOM}$ by 6–8‰ in deeper soil layers (e.g., in 15- to 35-cm and 35- to 60-cm layers).

The observed pattern of soil $\delta^{15}N$ cannot be explained by N inputs to the forest from external sources. Fractionation can occur during N_2 fixation, but only to a relatively small extent (ϵ of 0–6‰; Shearer and Kohl 1989; Högberg 1997; Robinson 2001), and N_2 fixation in these soils is presumed to be minimal (Richter et al. 2000; Richter and Markewitz 2001). Though atmospheric deposition may have totaled >200 kg ha^{-1} over four decades (Richter et al. 2000), any changes in $\delta^{15}N$ values of these inputs cannot explain the observed increase in soil $\delta^{15}N$ with depth. Given the high biotic demand for N at the site (Richter et al. 2000), a change in $\delta^{15}N$ of atmospheric deposition would likely result in a shift in vegetation $\delta^{15}N$ and, subsequently, in surficial soil layers. In contrast, the large shifts in $\delta^{15}N$ occurred deeper in the soil profile.

It is equally unlikely that losses of N via NH_3 volatilization, NO_3^- leaching, or denitrification drive the observed patterns of progressively enriched $\delta^{15}N$ signatures in these soils, although N isotope fractionation can be appreciable with these processes (ϵ up to 60‰ for volatilization; Wellman et al. 1968; Delwiche and Steyn 1970; Mariotti et al. 1981; Hübner 1986; Yoshida et al. 1989; Nadelhoffer and Fry 1994; Handley et al. 1996; Robinson 2001). In highly acidic soils such as these (Markewitz et al. 1998), NH_3 volatilization is not likely to play a role in gaseous N loss. The low rates of nitrification and low concentrations of NO_3^- in soil waters of the Calhoun pine forest indicate that NO_3^- losses are also not an important feature of the N cycle (Markewitz et al. 1998; Richter et al. 2000; Richter and Markewitz 2001). Gaseous loss of N via denitrification is also taken to be small given very well-aerated conditions in surficial soil layers; moreover, denitrification is likely very small relative to nitrification rates in most forests (Bowden et al. 1990), so we expect that denitrification is not an important factor affecting N isotopic signatures in these soils.

Other features that could potentially induce N isotope fractionation include discrimination against ^{15}N during plant uptake of inorganic N (Kohl and Shearer 1980; Högberg 1997) and microbial uptake of NH_4^+ and NO_3^- (Delwiche and Steyn 1970; Wada and Hattori 1978). None of these processes is likely to explain $\delta^{15}N_{SOM}$ patterns observed with depth and time. Discrimination against ^{15}N during plant N uptake when ample N supplies exist can sometimes result in relative depletion in ^{15}N of plant biomass (Kohl and Shearer 1980; Evans and Ehleringer 1993; Högberg 1997), and such a process would result in ^{15}N-depleted plant material, as observed at this site and many others (Amundson et al. 2003). However, in natural systems where N is often limiting, discrimination against inorganic ^{15}N during plant uptake is rare (Robinson 2001; Evans 2001). Given the pronounced N deficiency considered to affect pine trees at Calhoun, the vegetative demand for N is high relative to N supply (Richter et al. 2000), so we expect that discrimination against ^{15}N during plant uptake to be insignificant. Another process that can result in N isotope fractionation within a system is microbial uptake of NH_4^+ and NO_3^- (ϵ ranging from 13 to 20‰; Delwiche and Steyn 1970; Wada and Hattori 1978; Robinson 2001). Such a process, however, would likely result in enrichment of inorganic N supplies relative to microbial biomass; several studies have observed the opposite trend (Hoch et al. 1996; Högberg 1997).

Understanding how $\delta^{15}N_{SOM}$ shifts with declines in mineral soil organic N during the forest's development can help us understand the extent to which microbial processing such as transfers of N via ECM fungi and mineralization have affected these data. Between 1962 and 1997, mineral soil organic N in these and nearby plots declined by approximately 63, 35, 35, and 125 μg g^{-1} in the 0- to 7.5-cm, 7.5- to 15-cm, 15- to 35-cm, and 35- to 60-cm layers, respectively (calculated from Richter et al. 2000; Richter and Markewitz 2001). The slope coefficients of regression equations of $-$[ln([N])] versus $\delta^{15}N_{SOM}$ represent the isotopic discrimination factor [D (‰)] (Nadelhoffer and Fry 1988). The D was 5.5‰ for the 7.5- to 15-cm layer (Fig. 3). In deeper layers, where $\delta^{15}N_{SOM}$ also increased significantly during the study period, regressions of $-$[ln(%N)] versus $\delta^{15}N_{SOM}$ exhibit trends that suggest similar or higher values of D,

though these trends are non-significant at $\alpha = 0.05$. In closed systems, D indicates the extent to which organic N transformations result in ^{15}N-enrichment of SOM (Nadelhoffer and Fry 1988); though the Calhoun forest has received biologically significant N inputs from atmospheric deposition (Richter and Markewitz 2001), incorporation of atmospheric inputs into lower mineral soils is likely negligible, permitting us to assess D for these layers. The relatively high values of D in this study are consistent with the amount of mineralization experienced by these soils and the ECM dominance of microbial diversity (Richter and Markewitz 2001; R. Vilgalys and J. Jackson, personal communication).

We can test the isotopic fractionation needed to generate the N isotopic shift observed in these soils over this forest's development (6.5‰ in the deepest layer from 1962 to 1997) by employing the Raleigh principle [Mariotti et al. (1981), as described in Högberg et al. (2005)]:

$$\delta_s = \delta_{s0} + \varepsilon \cdot \ln(f)$$

where δ_s represents the δ^{15}N of the substrate (here, soil organic N) in 1997, the last date of sampling, δ_{s0} represents the δ^{15}N of the substrate in 1962, ε represents the isotopic fractionation effect, and f represents the fraction of original substrate remaining in 1997. Substituting 8.0 and 1.5‰ for δ_s and δ_{s0}, respectively, and inserting 0.51 for f, we obtain a value of −9.7‰ for ε. Such a fractionation effect is not unreasonable given the massive transfer of N from the soil profile into vegetation mediated by ^{15}N-discriminating mycorrhizal fungi and the large extent of net N mineralization experienced by these profiles' SOM.

Conclusion

Depth-dependent patterns of $\delta^{15}N_{SOM}$ and $\delta^{13}C_{SOM}$ that are similar to those commonly observed in undisturbed profiles developed during four decades under an aggrading, old-field pine forest. In a steady state, soil δ^{15}N ultimately reflects the isotopic signature of N inputs to and losses from the system. In an aggrading ecosystem such as the Calhoun forest, examining the impact of N cycling processes within a system on soil δ^{15}N, as well as the δ^{15}N of inputs and losses, is also critical for understanding $\delta^{15}N_{SOM}$ patterns.

ECM transfers of isotopically light N to plants, in conjunction with the (relatively slight) N isotope fractionation during organic N mineralization and the great magnitude of N transfers between ecosystem pools (Fig. 4), likely have resulted in isotopically light N moving to vegetation, the forest floor, and back into redeveloping A horizons, all accompanied by a corresponding enrichment in ^{15}N pools in the lower A, the E, and upper B horizons. This effect is particularly evident in deeper soil layers due to the minimal inputs and subsequent lack of recirculation of soil organic N there relative to surface layers (Fig. 5). Accretion of isotopically enriched, recalcitrant compounds derived from microbial cells, particularly ECM fungi, during this ecosystem's development, likely governed these patterns.

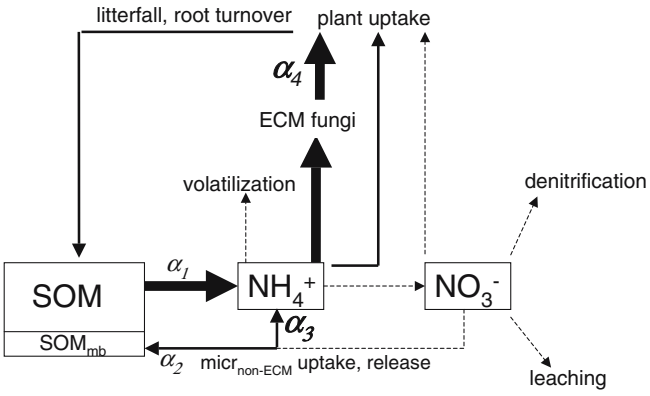

Fig. 4 Diagram of four-decade N cycle at the Calhoun Experimental Forest, South Carolina. *Width* of *solid arrows* suggests magnitude of flux. *Dashed arrows* indicate processes that are insignificant at the site. *Font size* differences of α reflect differences in isotopic fractionation associated with mineralization [slight fractionation (α_1)], microbial N assimilation [slight fractionation (α_2)], microbial N dissimilation [greater fractionation (α_3)], and ectomycorrhizal (*ECM*) transfers of N to vegetation [greater fractionation (α_4)]. α is not denoted for processes associated with dashed arrows, though N isotope fractionation during these processes can be appreciable. See text for discussion of atmospheric N deposition and Fig. 5 for details of plant returns to soil organic matter (*SOM*). SOM_{mb} Microbial biomass component of SOM, α N isotope fractionation factor, $micr_{non\text{-}ECM}$ microbial uptake and release other than ECM processes

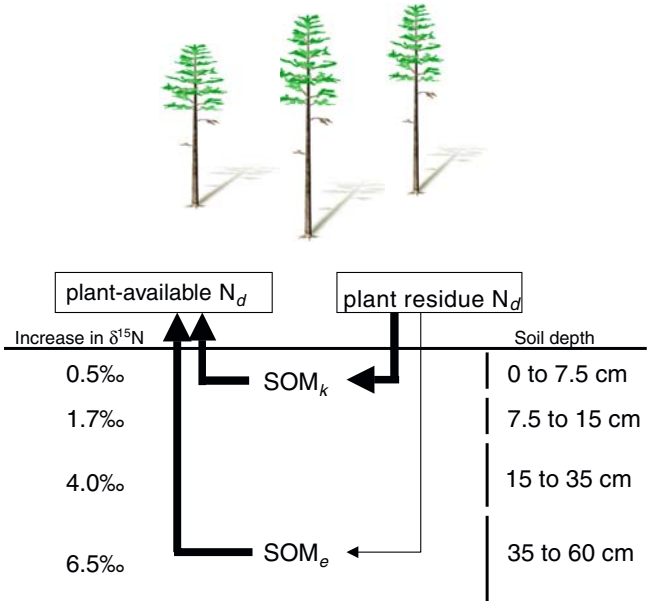

Fig. 5 Conceptual model of N circulation within the soil profile and vegetation during four decades at the Calhoun Experimental Forest, South Carolina. For clarity, only arrows representing 0- to 7.5-cm and 35- to 60-cm layers are indicated, representing the two most contrasting soil layers. *Subscript k* indicates relatively constant δ^{15}N values, *d* indicates ^{15}N-depleted material, and *e* indicates ^{15}N-enriched material. δ values (‰) represent the increase in $\delta^{15}N_{SOM}$ from 1962 to 1997 associated with each soil depth

Though the N isotopic fractionation associated with SOM mineralization may be slight, we also suggest that the massive net mineralization of SOM during 40 years of forest development resulted in significant ^{15}N enrichment deeper in the soil profile. The minimal amount of plant material being incorporated into deeper layers of the soil profile is consistent with the $\delta^{13}C_{SOM}$ values in these horizons. These isotopic signatures emphasize both the relatively rapid establishment of ^{15}N and ^{13}C enrichment with forest development, as well as the governing role of SOM transformations and microbial biomass residues in determining these patterns.

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