Surficial gains and subsoil losses of soil carbon and nitrogen during secondary forest development

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Abstract

Reforestation of formerly cultivated land is widely understood to accumulate above- and belowground detrital organic matter pools, including soil organic matter. However, during 40 years of study of reforestation in the subtropical southeastern USA, repeated observations of above- and belowground carbon documented that significant gains in soil organic matter (SOM) in surface soils (0–7.5 cm) were offset by significant SOM losses in subsoils (35–60 cm). Here, we extended the observation period in this long-term experiment by an additional decade, and used soil fractionation and stable isotopes and radioisotopes to explore changes in soil organic carbon and soil nitrogen that accompanied nearly 50 years of loblolly pine secondary forest development. We observed that accumulations of mineral soil C and N from 0 to 7.5 cm were almost entirely due to accumulations of light-fraction SOM. Meanwhile, losses of soil C and N from mineral soils at 35 to 60 cm were from SOM associated with silt and clay-sized particles. Isotopic signatures showed relatively large accumulations of forest-derived carbon in surface soils, and little to no accumulation of forest-derived carbon in subsoils. We argue that the land use change from old field to secondary forest drove biogeochemical and hydrological changes throughout the soil profile that enhanced microbial activity and SOM decomposition in subsoils. However, when the pine stands aged and began to transition to mixed pines and hardwoods, demands on soil organic matter for nutrients to support aboveground growth eased due to pine mortality, and subsoil organic matter levels stabilized. This study emphasizes the importance of long-term experiments and deep measurements when characterizing soil C and N responses to land use change and the remarkable paucity of such long-term soil data deeper than 30 cm.

Keywords: land use change, loblolly pine, long-term experiment, reforestation, secondary forest development, soil fractionation, soil nitrogen, soil organic carbon

Introduction

Old-field secondary forests are thought to sequester carbon above and below ground, and thus mitigate anthropogenic carbon emissions. Odum's (1969) 'Strategy of Ecosystem Development' posited that succession is characterized not only by increasing biomass but also by accumulations of detrital organic matter (OM). Through leaching, fragmentation, and bioturbation, large OM pools on the soil surface (detrital leaf and woody organic matter) and in the soil profile (dead roots) might be expected to lead to an increase in surface soil C, but what of deeper soils? Globally, soils are estimated to store up to 2300 Pg C in the top 3 m (Jobbágy & Jackson, 2000), compared to only 600 Pg C

stored in vegetation biomass (Houghton, 2007). Given the enormous size of the soil C pool, understanding its sensitivities to management, disturbance, climate, and vegetation change is critical. Many studies have confirmed increases in surficial soil carbon following revegetation of old fields, with cropland to forest conversions resulting in an average of 34 g C m^{-2} yr^{-1} increase in soil carbon (Post & Kwon, 2000). Long-term and deep study of soil is essential to understanding and managing fertility and carbon sources and sinks following land use changes like deforestation, reforestation, and afforestation (Richter *et al.*, 2007).

Although the depth distribution of roots and soil carbon differs with plant cover type, more than one third of roots and more than one half of soil carbon are stored below 20 cm depth (Jobbágy & Jackson, 2000), suggesting that land use change may well affect C in subsoils. Yet despite this distribution of soil carbon and plant

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roots, most studies do not sample soils very deeply. Of approximately 360 studies included in two reviews on land use change effects on soil carbon, only 10% sampled more deeply than 30 cm (Post & Kwon, 2000; West & Post, 2002). Spatial variability and difficulty of sampling make soil a very challenging system to study, especially when attempting to account for relatively small temporal concentration changes in large soil volumes (Leuzinger & Hättenschwiler, 2013). However, studies that have examined soils below 30 cm have observed strong soil carbon responses to changes in temperature (Schwendenmann & Veldkamp, 2006), [CO_2] (Iversen et al., 2012), and land use change (Fontaine et al., 2004, 2007; Fimmen et al., 2008). Recognition of the importance of subsoil processes and responses appears to be growing (Schmidt et al., 2011).

One study uniquely equipped to quantify decadal change in the upper 60 cm of mineral soil in response to land use change and management disturbance is the Calhoun Long-Term Soil-Ecosystem Study (LTSE) in South Carolina, USA. Here, above- and belowground ecosystem components have been resampled and soil samples archived for over 50 years of forest growth and development on formerly cultivated fields. Based on observations during the first 40 years of forest development, previously published work from this experiment argued that forest development resulted in large and rapid accumulations of carbon in biomass and surficial detrital O-horizons, and transfers of nitrogen especially from subsoil (35 to 60 cm layer) to biomass and O-horizon (Richter et al., 1999, 2000; Billings & Richter, 2006). These studies observed relatively modest increases in OM in 0–7.5 cm soils and significant losses of carbon from subsoils at 35–60 cm (Richter et al., 1999) and attributed losses of subsoil OM to enhanced decomposition that exceeded rates of OM inputs (Richter et al., 1999).

Over a decade later, we now appreciate that these earlier studies captured the initial period of forest development from establishment through thinning phases of secondary pine forest development (sensu Peet & Christensen, 1987), and concluded during peak biomass and biomass-carbon storage (Mobley, 2011). In this study, we examine the dynamics of soil C and N during a later, transition, phase of forest development, during which overstory pine tree biomass decreased and, through natural successional processes, the forest community began to reorganize toward a mixed pine-hardwood forest.

The objectives of this study were to characterize changes in soil C and N throughout the development of this 50-year-old forest, including the transition phase of forest development from even-aged pine to mixed pine–hardwood, and to examine the soil fractions responsible for C and N gains or losses. We used density fractionation and stable isotopic and radio isotopic analysis of soil organic matter to investigate depth-dependent SOM gains and losses following land use change and through advanced stages of secondary forest development. We hypothesized that as aboveground forest growth slowed and tree mortality increased, the soil continued to gain C and soil N depletion slowed. As it is considered to be the most 'active' OM pool, we expected that light-fraction would be responsible for most mineral soil C and N changes during reforestation and that silt- and clay-associated OM would be relatively stable over time.

Materials and methods

Site description

The Calhoun LTSE lies within the Sumter National Forest in the Piedmont of South Carolina, USA. The experimental site is characterized by gentle slopes (<3%) composed of Cataula series soils (fine, kaolinitic, thermic Oxyaquic Kanhapludults). These granite–gneiss-derived soils (including saprolite) are approximately 30 m deep over unweathered bedrock (Richter & Markewitz, 1995; Bacon et al., 2012). Sandy loam or loamy sand A-horizons (soil solids >75% sand, <10% clay by mass) range to 30 cm depth atop kaolinite- and sesquioxide-rich B horizons (47% sand, 38% clay) (Richter & Markewitz, 2001). The climate is subtropical (Mcknight & Hess, 2008): mean annual precipitation is 1185 mm; mean annual temperature is 15.2 °C (Union 8S Station, 1977–2006; National Climatic Data Center, 2008). The field experiment is organized in a randomized complete block design, with four blocks each containing four 0.1 ha plots of different loblolly pine (Pinus taeda L.) tree spacings (1.8, 2.4, 3.0, 3.7 m). For over 50 years, researchers have monitored the biogeochemical cycles of the developing loblolly pine forest – including C, N, P, cations, anions, and trace elements – through repeated sampling and archiving of soil and O-horizons and repeated tree biomass inventories (Richter & Markewitz, 2001).

The Calhoun LTSE is located on land that typifies the agricultural land use history of the southeastern USA Piedmont: clearing of mixed-hardwood stands by European colonists, >100 years of cultivation accompanied by soil erosion and substantial above- and belowground carbon loss, and field abandonment and replacement with even-aged loblolly pine by the mid-20th century (Metz, 1958; Richter & Markewitz, 2001). The experimental forest was planted in 1957 in two old cotton fields, previously limed and fertilized, as part of a US Forest Service pine productivity and spacing study (Metz, 1958; Balmer et al., 1975; Harms & Lloyd, 1981). Aboveground ecosystem development at the Calhoun LTSE proceeded typically for a southeastern USA old-field forest (Billings, 1938; Coile, 1940; Oosting, 1942). Live pine tree biomass accumulated rapidly during the establishment phase of forest growth (Peet & Christensen, 1987). Self-thinning of the pines began at approximately age 20, and woody debris began to accumulate

on the soil surface (Mobley, 2011; Mobley *et al.*, 2013). Starting at forest age 40 (circa 1997), live tree biomass began to decline as the forest entered the transition phase of development, in which growth of the remaining pines and the young hardwood understory was insufficient to compensate for the continued mortality of overstory pine trees (Mobley, 2011). By 2005, the loss of aboveground biomass had slowed (Mobley, 2011).

Soil collection

O-horizon (litter layer only, not including coarse woody detritus) was sampled in the 16 plots in 1992, 1997, and 2005 using 707 cm^2 circular samplers. Five samples were composited per plot. From the 1992 sampling (Urrego, 1993), only site-level mean organic matter and N masses of O-horizon are available, not the individual plot estimates, so uncertainties were estimated based on the among-plot CVs of 1997 and 2005 samples.

Mineral soils were collected at 4- to 8-year intervals in 1962, 1968, 1972, 1977, 1982, 1987, 1990, 1997, and 2005 by collecting 18 to 20 soil cores of 2 cm diameter from within each of 16 plots. Cores were separated into four depth increments (0–7.5 cm, surface; 7.5–15 cm and 15–35 cm, intermediate; and 35–60 cm, subsoil) and composited into one sample per depth increment per plot. In 1968, soils were only collected to 15 cm depth, and in 1990, soil was sampled in 8 of 16 plots.

The Calhoun LTSE does not benefit from a legacy of bulk density (D_b) data; therefore, we report soil C and N contents using a constant D_b over the course of forest development in the permanent plots. In 2011, extensive D_b sampling ($n = 128$) across all permanent plots in the Calhoun LTSE indicated that D_b at 0–7.5, 7.5–15, 15–35, and 35–55 cm was 1.22 ± 0.06, 1.43 ± 0.10, 1.46 ± 0.03, and 1.41 ± 0.03 g cm^{-3} (mean $\pm$ one standard deviation), respectively (Bacon *et al.* in preparation). These estimates are in close agreement with previous estimates for the permanent plots ($n = 72$ from four soil pits in the early 1990s; Richter *et al.*, 1994) and an extensive analysis of D_b to a depth of 35 cm under old-field pine ecosystems across Union County, South Carolina (Dunscomb, 1992). Simulations of potential D_b changes in the permanent plots from 1955 to 2011, and their impact on soil content calculations, indicate that estimates of C, N, and exchangeable cation contents differ by <10% when calculated with an equivalent soil mass model (which accounts for changing D_b; Ellert & Bettany, 1995; Wendt & Hauser, 2013) and when calculated assuming constant D_b (Bacon *et al.* in prep). We corrected the 1962–1997 soil C and N changes from Richter *et al.* (1999, 2000) (presented in above Site Description) using the updated D_b values from Bacon *et al.* (in prep).

For bulk soil C and N analyses, air-dried, pulverized soil of every depth from every plot was analyzed separately. The number of plots analyzed for bulk soil C and N varied by year: 13 in 1962; 8 each in 1977, 1982, and 1990; 16 in 1997 and 2005. To minimize use of irreplaceable archived soil samples, as well as to control analysis costs, isotopic analyses and fractionations were conducted on composite samples, wherein soils from 12 plots were combined into one sample per depth

for each sample year. Composited soils from 1962, 1982, and 2005 were used for soil density fractionation. Four plots were excluded from all composites due to insufficient mass of remaining sample.

Particle density and size fractionation

Our soil density fractionation protocol was modified from Sollins *et al.* (2009). A single light-fraction (LF) was defined at 1.85 g cm^{-3}, using a ratio of 20 g soil to 30 ml 1.9 g cm^{-3} clean (low C and N) sodium polytungstate [SPT; $Na_6(H_2W_{12}O_{40})$; Tungsten Compounds, Grub am Forst, Germany]. The remaining mineral soil was shaken with 30 ml of sodium hexametaphosphate (HMP; 5 g l^{-1}) at 100 rpm for 2 h, and then rinsed through a 53 μm sieve with 200–350 ml deionized water, yielding a > 53 μm fraction (sand) and a < 53 μm fraction (clay + silt; referred to as fine mineral, or FM, fraction). The rinse solution was captured with the FM fraction. The three fractions were dried at 60 °C, weighed, transferred into an agate mortar, and hand-pulverized with a pestle. The mass of HMP was subtracted from the mass of the FM fraction.

Overall, soil recovery in the three fractions was excellent, within 1% of initial bulk soil mass. Masses of soil particle size components varied among depths according to the mean soil textures of the site (Table 1), with the fine mineral size fraction increasing from 15.7% of soil mass at 0–7.5 cm to 54.1% of soil mass at 35–60 cm, and sand conversely decreasing from 83.2% of soil mass at 0–7.5 cm to 44.9% of soil mass at 35–60 cm.

Bulk soil samples from individual plots from all sample years were analyzed for %C and %N on a CE Elantech Flash EA 1112 Elemental Analyzer (CE Elantech, Inc., Lakewood,

Table 1 Mean masses of three mineral soil components – light-fraction organic matter (LF, floated at density of 1.85 g cm^{-3}), fine mineral (FM, clay and silt particle size fraction, passed 53 μm sieve), and sand particle size fraction (retained on 53 μm sieve) – at four soil depths of the Calhoun LTSE, SC, USA. Coefficients of variation represent variation among 3 sample years (1962, 1982, and 2005)

Soil Component	Depth (cm)	Percent of initial bulk soil mass	Coefficient of Variation (%)
LF, <1.85 g cm^{-3}	0–7.5	1.07	51
	7.5–15	0.52	9.3
	15–35	0.23	7.7
	35–60	0.088	27
FM, <53 μm	0–7.5	15.7	3.6
	7.5–15	17.6	2.8
	15–35	28.8	5.2
	35–60	54.1	1.5
Sand, >53 μm	0–7.5	83.2	0.20
	7.5–15	82.0	0.34
	15–35	70.9	1.9
	35–60	44.9	3.8

NJ, USA) at Duke University. Composited bulk soils from 1962 to 1997 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 in 2005 (Billings & Richter, 2006). LF and FM soil fractions were analyzed for %C and %N, and LF and FM fractions plus the 2005 bulk soils were analyzed for $\delta^{13}C$ and $\delta^{15}N$, with a coupled continuous-flow elemental analyzer isotope ratio mass spectrometer (EA-IRMS) system coupled to a Carlo Erba model 1108 EA, interfaced to a Thermo-Finnigan Delta Plus XP IRMS, at the USDA ARS Regional Facility in Albany, CA. Radiocarbon analysis on all samples was conducted at the Keck AMS Facility at University of California-Irvine, with the 1962–1990 samples analyzed in 1998 (Richter et al., 1999), and 1997–2005 samples analyzed in 2011. Based on preliminary analysis, sand size soil fractions were deemed too low in %N to achieve reliable combustion and element detection, and so were not further analyzed. To test for effects of analysis date or laboratory on the data, four bulk soil composite samples from the 2005 stable isotope analysis and two samples from the 1998 radiocarbon analysis were reanalyzed in 2011. Stable isotope remeasurements were within 1‰, and radiocarbon remeasurements were within 40‰.

Statistical analyses

For bulk soil changes at each soil depth over time, we used two-tailed, paired (by plot) two-sample t-tests comparing 1962 soil C and N values with values from 1997 and 2005 at the four soil depths and for the 0–60 cm profile (for 1997 and 2005 comparisons: $n = 16$ for 0–7.5 cm; $n = 15$ for 7.5–15 cm, 15–35 cm, and 35–60 cm; $n = 13$ for 0–60 cm profile).

Results

O-horizon

In the forest's first 40 years, between 1957 and 1997, it accrued approximately 13.0 kg C m^{-2} ± 0.44 SE in living tree biomass (Richter et al., 1999; Richter & Markewitz, 2001). At the same time, O-horizon stocks accumulated to 3.8 kg C m^{-2} (± 0.18 SE) by 1997 and then decreased to 2.7 kg C m^{-2} (± 0.12) by 2005. O-horizon N reached 0.073 kg N m^{-2} (± 0.003) in 1997 (Richter et al., 1999), then decreased to 0.064 kg N m^{-2} (± 0.003) in 2005. The O-horizon C : N ratio decreased from the 1997 value of 52.3 (± 1.86; Richter et al., 2000) to 42.5 (± 1.11) in 2005.

Bulk mineral soil change

The aboveground and O-horizon changes corresponded to depth-dependent changes in mineral soil. At approximately forest age 40, 32 g DOC m^{-2} yr^{-1}

± 8.9 washed in to the mineral soil from the O-horizon, and less than 1.0 g DOC m^{-2} yr^{-1} ± 0.07 leached out below 60 cm soil depth (Markewitz et al., 1998). Surface mineral soil (0–7.5 cm) carbon increased by 103 g C m^{-2} ± 22.8 over 40 years, or ~4 g m^{-2} yr^{-1} (Richter et al., 1999). Yet over the same time period, sufficient C was lost from the 35–60 cm depth (442 g C m^{-2} ± 77.3) to result in a significant net loss of 0–60 cm soil C of 271 g C m^{-2} ± 97.3 ($P = 0.0322$ in two-tailed, paired t-test of 1962 vs. 1997 soil C). Over the same 40 years, the 0–60 cm soil nitrogen pool was significantly depleted by 83.9 g N m^{-2} ± 7.8 ($P < 0.0001$ in two-tailed, paired t-test of 1962 vs. 1997 soil N) as 109 g N m^{-2} ± 3.7 was translocated to tree biomass and O-horizons (Richter et al., 2000; Richter & Markewitz, 2001). Billings & Richter (2006) documented associated and large shifts in $\delta^{15}N$ in soil organic nitrogen over this period.

From 1997 to 2005, patterns of soil C and N change continued to vary by soil depth. The increasing trajectory of surface soil C and N continued such that by 2005, surface soil carbon had significantly increased to
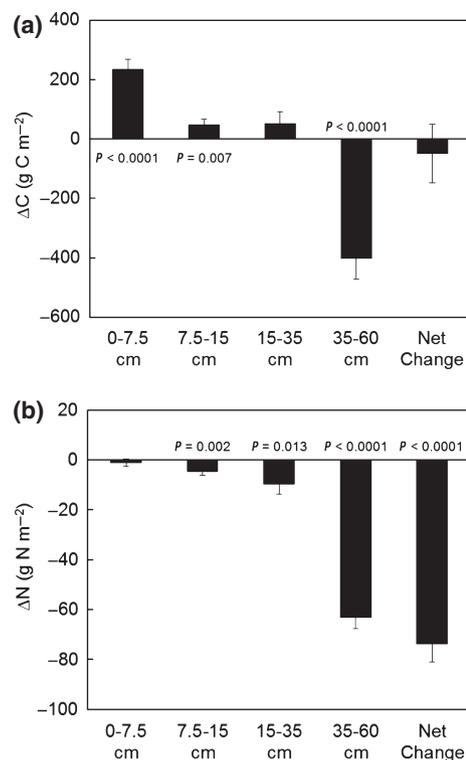

Fig. 1 Change in (a) mineral soil carbon storage and (b) mineral soil nitrogen storage, from 1962 to 2005 at four depth increments and in the full 0–60 cm profile. Standard error bars represent among-plot variation. P-values indicate significant differences in a paired t-test comparing 2005 values with 1962 values.

0.81% C ± 0.045 SE ($P < 0.0001$ in two-tailed, paired *t*-test of 1962 vs. 2005 soil C) from the 1962 value of 0.55% C ± 0.042 (Fig. 2). Soil C concentrations at intermediate soil depths changed little from 1997 to 2005 (Fig. 2). On a mass basis, the surface soil gain (+234 g C m^{-2} ± 33.4) and subsoil carbon loss (−403 g C m^{-2} ± 69.2) canceled each other (Fig. 1a). Total 0–60 cm SOC storage summed to 3100 g C m^{-2} ± 106 in 2005, compared to 3220 g C m^{-2} ± 184 in 1962.

By 2005, surface soil %N had recovered to 1962 levels, and intermediate-depth soils (7.5–35 cm) nearly so. Subsoil N concentrations stopped decreasing after 30 years (Richter *et al.*, 2000) and remained steady through 2005 (Fig. 3). The C : N ratio of the 0–60 cm bulk soil declined from its 1990 peak of 21.1 ± 1.73 SE to 16.4 ± 0.34 by 2005. On a mass basis, there was virtually no net change in surface 0–7.5 cm soil N (−1.1 g N m^{-2} ± 1.44), but the intermediate depths of 7.5–15 cm and 15–35 cm lost 4.7 ± 1.40 and 9.8 ± 3.94 g N m^{-2}, respectively, and the deepest soil sampled lost 63.1 g N m^{-2} ± 4.54 from (Fig. 1b). Overall, the 0–60 cm soils lost 29% of soil N over nearly 50 years, decreasing from 266 g N m^{-2} ± 11.7 in 1962 to 190 g N m^{-2} ± 6.1 in 2005.

As the forest grew, pronounced depth-dependent patterns developed in mineral soil δ^{13}C, Δ^{14}C, and δ^{15}N. Values of δ^{13}C were progressively depleted in surface and intermediate soil layers, approaching values of pine leaf and root litter (−26.5 to −28.5‰; Billings & Richter, 2006), but were unchanged in subsoils

Fig. 3 Change in bulk mineral soil nitrogen concentrations at four depth increments during old-field forest development at the Calhoun LTSE, SC, USA, updated from [Richter *et al.* (2000); previously published data encompassed in gray shading]. Standard errors represent among-plot variation. Asterisks indicate significant differences between 1962 and 2005 in paired *t*-tests.

(Fig. 4; Billings & Richter, 2006). Surface soil Δ^{14}C values increased with atmospheric $\Delta^{14}CO_2$ between 1962 and 1968, and did not begin to decrease until after 1990 (Fig. 5; Richter *et al.*, 1999). Surface and intermediate-depth soils remained greatly enriched above 1962 Δ^{14}C levels through 1997. Subsoil Δ^{14}C increased to 50‰ in
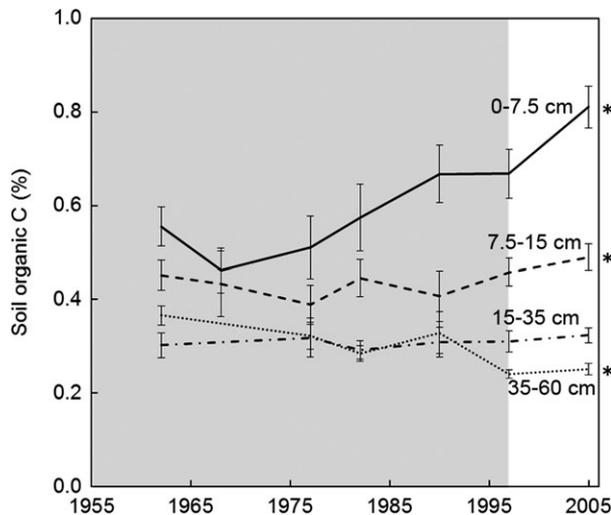
Fig. 2 Change in bulk mean mineral soil carbon concentrations at four depth increments during old-field forest development at the Calhoun LTSE, SC, USA, updated from Richter *et al.* (1999; previously published data encompassed in gray shading). Standard error bars indicate among-plot variation. Asterisks indicate significant differences between 1962 and 2005 in paired *t*-tests.
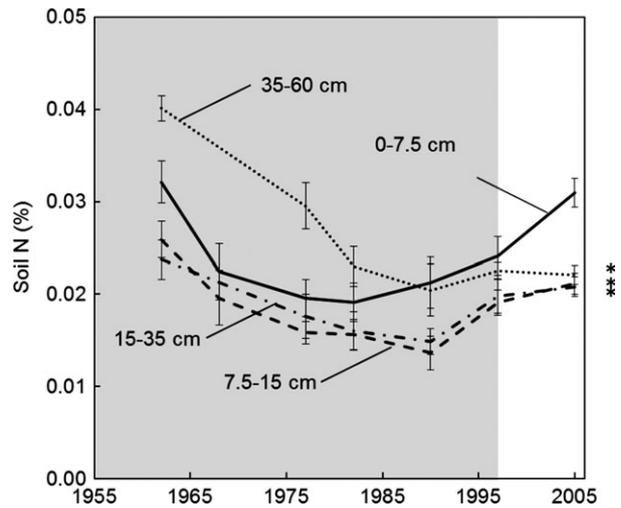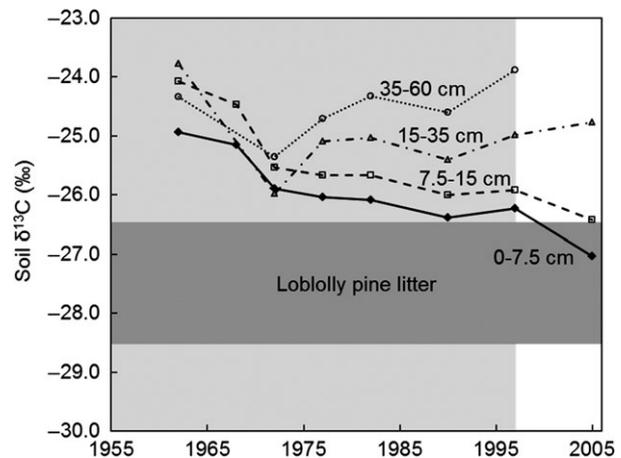
Fig. 4 Change in bulk mineral soil organic matter δ^{13}C signatures of four depth increments during old-field forest development at the Calhoun LTSE, SC, USA, updated from [Billings & Richter (2006); previously published data encompassed in light gray shading]. Value for 2005 35–60 cm was excluded during quality control.
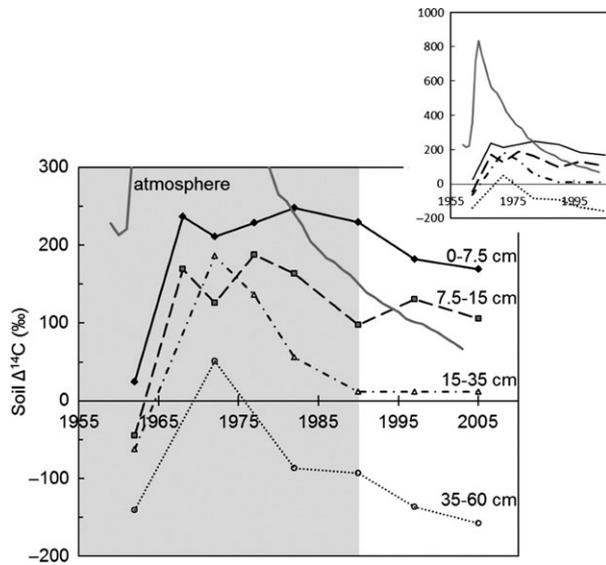
Fig. 5 Trend of bomb-^{14}C incorporation into four mineral soil depth increments at the Calhoun LTSE, SC, USA, updated from [Richter *et al.* (1999); previously published data encompassed in gray shading]. The dark gray line indicates atmospheric ^{14}C signature (Levin & Kromer, 2004). Inset shows full extent of atmospheric ^{14}C bomb spike.
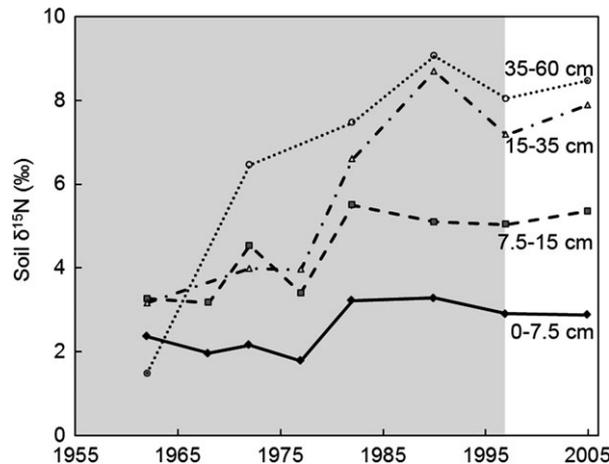

Fig. 6 Change in bulk mineral soil organic matter δ^{15}N signatures of four depth increments during old-field forest development at the Calhoun LTSE, SC, USA, updated from [Billings & Richter (2006); previously published data encompassed in gray shading].

1972 and then decreased to its initial 1962 value of 150‰ by 1997. Values of δ^{15}N were unchanged in surface soils, with progressively larger enrichments from surface to subsoils (Fig. 6; Billings & Richter, 2006). After 1997, there was little change in soil δ^{13}C, Δ^{14}C, or δ^{15}N (Figs 4, 5 and 6).

Soil fraction change: C and N

Light-fraction organic matter (LFOM) quantity and change were largest in surface soils. Across the three sample years subject to fractionation (1962, 1982, and 2005), LFOM averaged 1.3% of soil mass in surface soil, decreasing with depth to <0.1% of soil mass at 35–60 cm (Table 1). From 1962 to 2005, LFOM in surface soils increased from 0.8 to 1.7% of soil mass (data not shown). As a result, light-fraction carbon (LFC) doubled in surface soils from 1962 to 2005, from 2.4 mg C g^{-1} soil to 4.8 mg g^{-1} (Fig. 7). Light-fraction carbon accumulations at other depths were of smaller magnitudes. Fine mineral-associated carbon (FMC) varied little among depths, ranging from 1 to 2 mg C g^{-1}. FMC did fluctuate over time: from 1962 to 1982, FMC decreased by 0.2–0.4 mg C g^{-1} soil in each of the soil depths. From 1982 to 2005, 0–35 cm soils recovered to near-1962 levels, while 35–60 cm FMC mass remained relatively unchanged. Thus, over the full measurement period of 1962–2005, 35–60 cm subsoils lost 0.3 mg FMC g^{-1} soil, or 15% of 1962 subsoil FMC.

Nitrogen in soil fractions varied with depth and over time. Like total LFOM, LFN decreased with soil depth. Although light-fraction N (LFN) did not change at any depth from 1962 to 1982, a surface LFN increase from
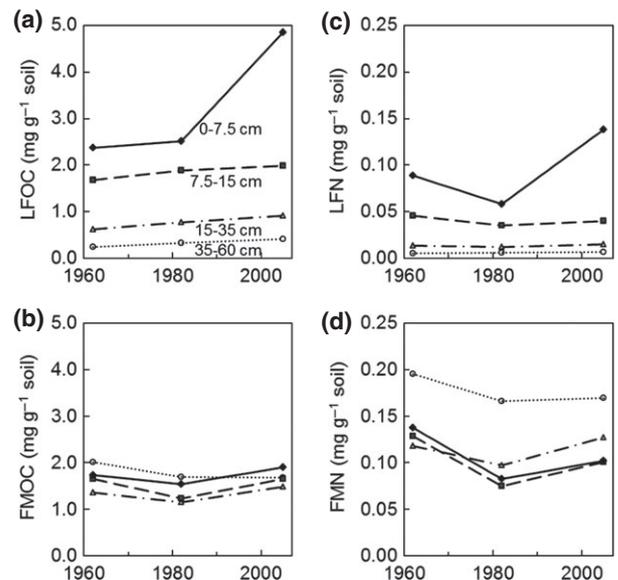

Fig. 7 Trends in masses of C and N of two soil fractions and four soil depths over 43 years of resampling at the Calhoun LTSE, SC, USA. Left panels show trends in C content and right panels show trends in N content. The solid black line represents 0–7.5 cm soils, and the lines become steadily more broken with deeper soil depth. Top panels (a) and (b) show trends in light-fraction C and N (LFC, LFN) over time; bottom panels (c) and (d) show trends in fine mineral-associated C and N (FMC, FMN).

1982 to 2005 resulted in 50% gain of 0.05 mg N g^{-1} in surface soil over 43 years (Fig. 7). LF C : N ratios increased from 1962 to 1982 at all depths, and were little changed from 1982 to 2005 (Table S1). From 1962 to 1982, 0.02–0.05 mg FMN per g soil was lost from each depth. From 1982 to 2005, 0.08 mg FMN g^{-1} soil was replaced in the surface and intermediate soil layers, approximately half of the initial loss. Fine mineral-associated N (FMN) depletion in subsoil amounted to 0.03 mg N g^{-1} soil, or 15% of 1962 levels. FMN losses from the 0–60 cm soil profile amounted to 12% of 1962 FMN. FM C : N ratios at 0–15 cm increased from 1962 to 1982, and then were unchanged from 1982 to 2005 (Table S1). FM C : N at 15–60 cm was unchanged over the measurement period.

Across all depths and dates, the sum of values for FM and LF fractions accounted for 77.5% of bulk soil carbon and 76.5% of bulk soil nitrogen. The unaccounted soil C and N may be attributed to OM remaining in the unmeasured sand fraction (Tables 1, 2), dilution of C and N signals due to residual SPT in analyzed samples, or OM mobilized into the acidic SPT solution and discarded (Crow *et al.*, 2007; Throop *et al.*, 2013). Based on our extensive rinsing of SPT with DI water and 99–101% soil mass recovery, we consider loss of soil during fractionation and dilution of signal by residual SPT to be relatively minor. Previous studies have shown that up to 25% (Swanston *et al.*, 2005; Crow *et al.*, 2007; Marin-Spiotta *et al.*, 2009; Schulze *et al.*, 2009; Throop *et al.*, 2013) of soil organic matter can be lost during the density fractionation process, depending upon the method used to rinse the samples and recover the soil fractions (Kramer *et al.*, 2009; Sollins *et al.*, 2009). Our subsequent analysis accounting for losses of C and N to SPT waste, sorbed to sand grains, or trapped on filters during rinsing found 3–9% of bulk soil C and 9.5–13.5% of bulk soil N to have mobilized

into the SPT solution (Table 2). OM trapped on glass fiber filters accounted for less than 2%, and the sand size fraction accounted for less than 3%, of bulk soil C or N. Accounting for these C and N losses increased overall recoveries to 85.9% of bulk soil C and 88.5% of bulk soil N (Table 2).

Soil fraction change: isotopic values

The LFOM δ^{13}C signatures were relatively uniform among depths, but the depth-weighted mean values decreased from −25.8‰ in 1962 to −27.0‰ in 1982 to −27.6‰ in 2005 (Table S1, Fig. 8). In contrast, the δ^{13}C signatures of fine mineral-associated organic matter (FMOM) behaved similarly to bulk soil, starting out at a relatively uniform −23.5‰ at all depths in 1962, and then developing depth dependence as depletion of δ^{13}C decreased with increasing soil depth such that subsoil FMOM δ^{13}C remained unchanged while surficial FMOM δ^{13}C was depleted from −23.5‰ in 1962 to −25.3‰ in 1982 to −26.5‰ in 2005 (Fig. 8).

Neither LFOM nor FMOM δ^{15}N signatures changed over time. LFOM δ^{15}N values were relatively uniform across depths as well, with a depth-weighted mean of +3.5‰ across the three sample dates. FMOM δ^{15}N varied among depths, with the surface soil most depleted (mean +4.5‰), and the subsoil most enriched (+7.6‰; Table S1).

At all depths, LFOM showed strong enrichment in Δ^{14}C from 1962 to 1982, followed by depletions from 1982 to 2005. The Δ^{14}C signatures of LFOM were ~0‰ at all but 15–35 cm depth in 1962 (Fig. 8). LFOM signatures spiked as high as +232.4‰ in 1982 surface soil. The deepest soil depth maintained the highest LFOM Δ^{14}C in 2005, at +152.3‰. FMOM showed a contrasting pattern in which Δ^{14}C enrichment from 1962 to 1982 decreased with increasing soil depth, with subsoil Δ^{14}C

Table 2 Recovery of C and N in three soil fractions (LF, FM, and sand) and two loss pathways – LF remaining on glass fiber filters after rinsing (filter) and C and N mobilized into SPT solution (SPT) – during a methodological test of OM recovery and loss carried out after the main experiment was concluded. Units are mg C or N per g bulk soil, except for Total columns that are in percent of initial bulk soil C or N. Standard Deviation represents variation in total recovery among *n* = 3 sample years (1962, 1982, 2005)

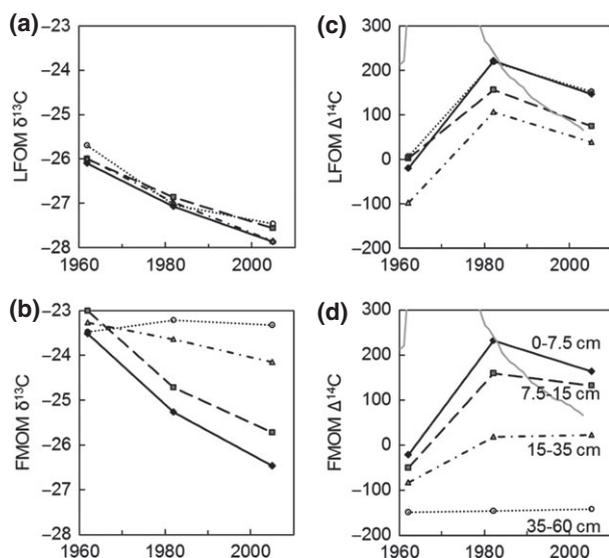|	Depth (cm)	Bulk Soil	Fractions			Losses		Total Recovery	
			LF	FM	Sand	Filter	SPT	Percent	SD
C	0–7.5	6.7	3.2	1.7	0.2	0.1	0.6	87.3	3.5
	7.5–15	4.9	1.9	1.5	0.1	0.1	0.4	82.0	2.8
	15–35	3.0	0.9	1.5	0.1	–	0.1	84.0	3.2
	35–60	3.1	0.4	2.3	0.0	–	0.1	90.1	3.8
N	0–7.5	0.30	0.08	0.12	0.004	0.003	0.04	85.1	4.3
	7.5–15	0.23	0.03	0.12	0.000	0.001	0.03	82.3	1.6
	15–35	0.21	0.01	0.15	0.000	–	0.02	87.9	3.2
	35–60	0.29	0.01	0.25	0.000	–	0.03	98.7	1.7

Fig. 8 Carbon stable isotopic and radio isotopic signatures of two soil fractions and four soil depths during forest development at the Calhoun LTSE, SC, USA. Left panels (a) and (c) show δ^{13}C signatures; (b) and (d) show Δ^{14}C signatures on the right. Top panels (a) and (b) show trends in light-fraction OM (LFOM). Bottom panels (c) and (d) show trends in fine mineral-associated OM (FMOM). The solid black line represents the surface 0–7.5 cm soils, and the lines become steadily more broken with deeper soil depth. The gray line in (b) and (d) shows atmospheric Δ^{14}C signature (Levin & Kromer, 2004).

remaining relatively unchanged at $< -142\%$ (Fig. 8). FMOM Δ^{14}C was relatively unchanged at all depths from 1982 to 2005.

Discussion

Depth dependent forest development effects on soil organic matter

Aboveground changes in forest biomass strongly impacted belowground C and N cycling. Over the first 40 years, the forest progressed through the establishment and thinning development phases, rapidly accumulating tree biomass (Peet & Christensen, 1987). This rapid tree growth accumulated substantial biomass C and N, and was accompanied by dramatic changes in soil C and N pool size and depth distributions. After an initial lag period of approximately 20 years, surface mineral soils gained SOC steadily, while the subsoil lost C (Richter et al., 1999). Also in the first 40 years, the forest translocated 31.5% of mineral soil N to aboveground pools, with most of that N coming from 35–60 cm subsoil layer (Richter et al., 2000).When forest biomass peaked in the mid-1990s, the forest entered the transition phase of development, characterized by large

accumulations of coarse woody detritus as overstory trees died and live biomass accumulation slowed (Mobley, 2011; Mobley et al., 2013). This work shows that as biomass demand for mineral soil N decreased, subsoil N stabilized and even began to increase; subsoil C concentrations also stabilized during this time (Figs 2, 3). After 48 years of secondary forest development through the establishment, thinning, and transition phases, we estimate the net change in 0–60 cm mineral SOC to be zero.

Fractionation of archived soils allowed us to examine these patterns of C and N gain and loss in more detail. Light-fraction C and N were quite dynamic in surface soils, losing C and N initially, and then gaining large quantities of C and N later in forest development (Fig. 7). Almost the entire gain in bulk surface soil C was due to accumulation of light-fraction OM (Table S1). We observe that subsoil N losses during early forest development were largely from subsoil fine mineral OM. Although we could not assign all of the subsoil C loss to a measured soil fraction, at least some of the loss was from the fine mineral fraction, and there was no evidence of loss from the subsoil light-fraction.

In the decade following peak biomass, surface soils have continued to gain considerable C and N while subsoil C and N pools remained largely unchanged. Values of δ^{13}C for the surface bulk soils in direct contact with the O-horizon continue to decline toward values of fresh pine litter (-29%), while subsoil δ^{13}C values have remained little altered over time, averaging -24.6% (Fig. 4; Billings & Richter, 2006). The δ^{15}N value of subsoil OM has also stabilized, after increasing from $<2\%$ to $> 8\%$ over the first 35 years due to fractionating uptake by mycorrhizal pine root systems (Billings & Richter, 2006). Immediately after the Δ^{14}C of atmospheric CO_2 peaked in 1964, surface soil Δ^{14}C values leapt to 240‰; steady soil Δ^{14}C values through 1990 indicated that this ^{14}C-labeled SOM was retained over that time. On the other hand, the spike in Δ^{14}C value of SOM between 7.5 and 60 cm followed within a decade of the atmospheric peak, but this elevated Δ^{14}C was transient at depth, demonstrating relatively rapid decomposition. These patterns probably reflect the large depth-dependent differences in fine root turnover (Gaudinski et al., 2001) and in mass of ^{14}C incorporated.

Isotopic signatures of LFOM showed incorporation of pine-derived C into LF at all depths (Fig. 8). Depth-dependent patterns of FMOM δ^{13}C and Δ^{14}C indicated that pine-derived C was incorporated into mineral associations in surface soils where C inputs are highest, despite low concentrations of reactive minerals, but not in deeper soils where reactive minerals are more abun-

dant (Richter *et al.*, 2006; Li *et al.*, 2008; Li, 2009; Mobley, 2011).

Mechanisms of depth dependent soil C gains and losses

Inputs and decomposition have shifted nonlinearly over time since reforestation. An initial loss of surface 0–7.5 cm SOC implies that decomposition outpaced inputs in the early years of reforestation. After approximately 20 years, inputs to surface soils finally exceeded decomposition and surface mineral SOC began to accumulate. The lag time was even longer at depth: 35–60 cm subsoil C did not stabilize until 40 years after reforestation. Surface 0–7.5 cm mineral soils continue on a trajectory of steady increase at 10.4 g C m^{-2} yr^{-1}, and show no sign of slowing. The subsoil C pool is currently stable, and its future trajectory an open question.

In subsoils at 35–60-cm, the coincident patterns of SOC depletion, large removals of N, and the shifts in C and N isotopic signatures, all during the period of aboveground biomass accumulation and decline, raise several possibilities about mechanisms that may link these patterns. Considerable SOC losses despite root C inputs suggest root-induced mechanisms for SOM loss, wherein physical or chemical effects of tree roots resulted in loss of subsoil OM (Cheng & Kuzyakov, 2005). One potential mechanism is a drying effect (Cheng & Kuzyakov, 2005), by which the conversion of a relatively shallow-rooted agricultural ecosystem to a more deeply rooted forest with greatly elevated transpiration exposed subsoil OM and microbes to increased aeration, increased variability in soil moisture, and an overall reduction in soil moisture (Richter *et al.*, 1999; Cheng & Kuzyakov, 2005). This may have increased microbial turnover and decomposition rates (Cheng & Kuzyakov, 2005). Additional root effects that may have influenced subsoil C include the priming mechanisms of preferential substrate utilization or microbial activation (Cheng & Kuzyakov, 2005). Preferential substrate utilization results when soil microbes prefer nutrient-rich soil organic matter to nutrient-poor rhizosphere C when nutrients are limiting. In the case of microbial activation, labile root C inputs may have stimulated rhizosphere microbial communities directly or indirectly, increasing decomposition of SOM, all under more aerobic environments than under cultivation.

Ecosystem and laboratory studies have shown increased fluxes of belowground carbon to result in both soil carbon loss (Fontaine *et al.*, 2004, 2007; Fontaine & Barot, 2005; Carney *et al.*, 2007) and increased uptake of N by plants (Dijkstra *et al.*, 2009; Drake *et al.*, 2011). Priming of soil C decomposition due to enhanced N demand is a phenomenon that has come to be appreciated in recent elevated-CO_2 studies (Carney *et al.*, 2007; Drake *et al.*, 2011). A conceptually similar pattern was observed by Billings & Ziegler (2008), where incubations of Duke FACE soils showed increasing C losses to microbial respiration with increasing N limitation. In this study, we find evidence for substantial modern C inputs to depth, which are not being incorporated into stable fractions, and we have clear evidence of high demand for soil N. The spatial linkage between the soil C and soil N changes further supports drying and priming hypotheses for soil C loss.

Implications for reforestation and afforestation

A recent inventory of the world's long-term soil experiments observed that less than 10% examined effects of land use change on forests, and also that continents (e.g., South America and Africa) and soils (e.g., Oxisols, Ultisols) undergoing large-scale land use transitions had notably few long-term soil change studies (Richter & Yaalon, 2012). Our study of five decades of reforestation of a subtropical-climate forest after over a century of agricultural use provides important empirically based insight into soil changes that may accompany ongoing reforestation and afforestation activities in subtropical and tropical regions of the world. Laganiere *et al.* (2010) suggested that SOC response to re- and afforestation at subtropical sites (like the Calhoun LTSE) was more similar to that of tropical sites than to other temperate zone sites. As Shi *et al.* (2013) have shown soils in the tropics to be more prone to losing SOC with reforestation or afforestation, it may be that highly weathered, nutrient-poor soils are particularly prone to root-induced SOM losses during forest establishment.

Our study underlines the importance of the O-horizon to estimates of SOC change following field to forest land use change (Laganiere *et al.*, 2010; Poeplau *et al.*, 2011; Bárcena *et al.*, 2014). Whereas recent meta-analyses have suggested that the O-horizon accounts for 29–47% of total SOC increase on timescales from 22 to 100 years (Laganiere *et al.*, 2010; Poeplau *et al.*, 2011; Shi *et al.*, 2013), our long-term observations show O-horizon to be even more important, contributing 89% of SOC change in the top 30 cm, or 100% if the 35–60 cm depth and its SOC loss are included. The O-horizon also served as a reservoir for 82% of the N removed from the mineral soil by the forest between 1962 and 2005.

The O-horizon SOM pool is, of course, relatively susceptible to recirculation and loss due to disturbance, and thus its C and N storage is likely to be of shorter duration than that of mineral soil. It is important to note that changes to O-horizon inputs or pool size also cascade into mineral soils. Another long-term study found that removal of the O-horizon for 50 years

caused losses of mineral soil C in both light and mineral-associated fractions, whereas litter addition increased mineral soil C in light-fraction only (Lajtha *et al.*, 2014). Both of these long-term studies suggest that disturbances or land use changes that reduce organic matter inputs, even temporarily, result in rapid mineral SOC losses that are very slow to recover after inputs resume.

Our study clearly demonstrates that the subsoil is dynamic and ecologically relevant on decadal timescales. At the Calhoun LTSE, the result is that nearly 50 years of SOC gains in the surficial mineral soils are canceled by mineral subsoil C losses. Furthermore, our understanding of soil N dynamics also depends on depth of sampling. Looking only to 35 cm depth, we would have observed that organic and mineral soils had gained 49.2 g N m^{-2}, a 39% increase, by 2005, and might have speculated that N deposition or fixation was responsible. Looking to 60 cm, however, we observed a 5% net loss of 14.1 g N m^{-2} from 0 to 60 cm mineral soil, which when compared to our estimate of tree biomass N accumulation of 25.9 g N m^{-2}, or 10% of 1962 0–60 cm soil N, indicates a much tighter ecosystem N cycle. Shallow sampling may explain unaccounted soil N increases with reforestation in other studies as well (Li *et al.*, 2012).

Conclusions on SOM response to forest development

Long-term monitoring of soil organic matter down to 60 cm tells a fundamentally important story of soil C and N dynamics, and even still we find questions regarding the biogeochemistry of the soil and the ecosystem as a whole. We propose that land use change from field to forest drove a cascade of changes through the ecosystem and soil profile that enhanced microbial activity and SOC decomposition in subsoils. These changes include drying effects, wherein tree rooting and transpiration increased the oxygenation of subsoils and accelerated decomposition of pre-existing SOM. Preferential substrate utilization may also be at work as strong N demand by tree roots spurred degradation of relatively nutrient-rich SOM, perhaps with energy provided by root-derived C (Cheng & Kuzyakov, 2005).

The establishment and thinning phases of forest development were particularly taxing on soil C and N pools, especially in subsoils where removals exceeded new inputs. Yet when aboveground biomass accumulation slowed and the forest began to transition from even-aged pine to mixed pine–hardwood stands with lower biomass, subsoil C and N finally stabilized. It will be important to observe these soils' depth dependent trajectories over future decades of forest development. After five decades of observation, we find some

support for Odum's predictions of increasing OM with forest development in O-horizons, but only in the most surficial of mineral soils, and not yet for subsoils. It is very clear from these results that the full soil profile is a highly dynamic component of the ecosystem, with pronounced depth dependent processing of OM, such that accumulations and losses of carbon above ground are on different temporal trajectories than changes below ground.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Properties of bulk SOM and two SOM fractions in three sample years at four depths in the soil profile of the Calhoun LTSE, SC, USA. Microsoft Word document.