Soil Carbon and Nitrogen Changes in Forests of Walker Branch Watershed, 1972 to 2004

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Changes in soil C and N concentrations and contents in four samplings during a 32-yr period on Walker Branch watershed in Tennessee were determined and compared with previously measured C and N fluxes and with changes in ecosystem C and N pools during this period. Soils showed significant increases in C and N concentrations in surface horizons from 1972 to 2004, and most of this increase occurred between 1972 and 1982. A previously observed decline in soil C and N contents between 1982 and 1993 was reversed in 2004 such that the latter increased to near 1982 values. The changes in soil C content could be approximately accounted for by previously measured litterfall and soil CO_2–C fluxes. Changes in soil N could not be accounted for by leaching, increments in vegetation, or by laboratory bias, changes during sample storage, or reasonable estimates of field sampling errors. We conclude that, although vegetation C and N pools increased steadily during the sampling period in most cases, changes in soil C and N pools on Walker Branch watershed are highly variable in both space and time, and there has been no unidirectional trend during the time period of this study.

On a global scale, soils contain two to three times as much C as either terrestrial vegetation or the atmosphere (Post et al., 1990; Schimel 1995; Schlesinger, 1997), and therefore even a slight change in soil C pools could have a major impact on global C budgets. Unfortunately, data on changes in soil C are very limited and not easily amenable to straightforward scaling up to a global level; we must rely on studies from selected sites with long-term records of soil change (e.g., Richter and Markewitz, 2001). Unfortunately, the limited number of such studies on soil C change have produced conflicting results. The decreases in soil C following the conversion of forests or grasslands to agriculture are well documented (Mann, 1986; Post and Kwon, 2000), but the potential for reaccumulation of C in these systems when they are allowed to revert back to forest or grassland is unclear. Some studies have shown large reaccumulations of C in soils following reversion of agricultural soils back to forest (e.g., Jenkinson, 1991), but in other cases little or no reaccumulation has been found (e.g., Compton et al., 1998; Compton and Boone, 2000; Richter et al., 1999). Schlesinger (1990) contended that the potential of soils to sequester C is too low to be an important factor mitigating atmospheric CO_2 increases. Post and Kwon (2000) calculated a rather low average value of soil C reaccumulation following agricultural abandonment (0.33–0.34 Mg C ha^{-1} yr^{-1}) that was similar to values previously calculated by Schlesinger (1990) with a much more limited database. Johnson and Curtis (2001) concluded, on the basis of a meta analysis of the literature, that forest harvesting followed by reforestation caused little or no change in soil C on average, regardless of the intensity of the harvest. Responses of individual sites showed considerable variation, however, and ranged from large net soil C losses (e.g., Turner and Lambert, 2000) to large net gains (Knoepp and Swank, 1997; Johnson and Todd, 1998).

Soil C changes must be accompanied by soil N changes, as these two elements are inextricably linked. The amounts of N that must be lost or gained to account for soil C changes are constrained by the range of observed C/N ratios in soils (~10–50) and the plants and microbial populations that they support. Field studies of soil N change have sometimes produced results that are consistent with the range of known ecosystem fluxes, such as uptake and leaching (e.g., Richter and Markewitz, 2001; Johnson et al., 2003), and in other cases, inexplicably large changes in soil N have been measured (Jenkinson, 1991; Knoepp and Swank, 1997; Johnson and Todd, 1998). Conflicting results for soil C and

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N change on sites within a few kilometers of each other have been observed on the Oak Ridge National Environmental Research Park in Tennessee. In one case, soils in a naturally regenerating deciduous forest showed inexplicably large increases in soil C and N during a 15-yr period (1980–1995) following harvesting (Johnson and Todd, 1998). In contrast, no significant changes in soil C or N were noted during 18 yr following the establishment of a loblolly pine (*Pinus taeda* L.) plantation on a nearby site, despite large increases in live biomass and forest floor components during this period (Johnson et al., 2003). Yet a different result was obtained in nearby Walker Branch watershed, where soil C and N concentrations increased between 1972 and 1982, then decreased between 1982 and 1993 (Johnson et al., 1988; Trettin et al., 1999). The declines in soil N content between 1982 and 1993 noted by Trettin et al. (1999) were inexplicably large, given known N leaching rates (Johnson and Todd, 1990). These results suggested a potential decline in soil fertility with attendant implications for long-term forest productivity on the watershed. Trettin et al. (1999) were cautious in extrapolating these changes across the longer term, however, and called for further sampling in the future to establish consistent trends, if indeed they exist.

This study represents the fourth decadal resampling of soils on Walker Branch watershed. Included in this study are analyses of 1972 and 2004 soils from most of the 24 original monitoring plots established on the watershed in 1972, expanding our database from the eight core plots sampled at intermediate times in the past (Johnson et al., 1988; Trettin et al., 1999). Our objectives were to: (i) assess, from the larger data set, how representative the results from the core plots were; (ii) determine if previously observed decreases in soil C and N between 1983 and 1993 in the core plots continued into the next decade; (iii) determine if continued soil C and N changes were associated with changes in vegetation growth; and (iv) determine if observed soil C and N changes could be accounted for by changes in vegetation and detritus pools and by previously measured C and N fluxes on the watershed.

We posed the following working hypotheses as a template for this study:

Hypothesis 1: Soil C and N data from the spatially expanded data set will show increased C and N concentrations between 1972 and 2004, as was the case for the previously measured eight core plots (Johnson et al., 1988; Trettin et al., 1999).

Hypothesis 2: Analysis of 2004 soils in the eight core plots will show declines in soil C and N concentration from 1993 to 2004, as was the case for the 1982 to 1993 period (Trettin et al., 1999).

Hypothesis 3: Declines in soil C and N will be associated with declines in forest growth.

Hypothesis 4: Changes in soil C and N can be explained by changes in vegetation and detrital C and N contents and known ecosystem C and N fluxes.

MATERIALS AND METHODS

Site

Walker Branch watershed is located on Chestnut Ridge within the Department of Energy Oak Ridge Reservation near Oak Ridge, TN. Before World War II, land use on the 97.5-ha watershed consisted of a mix of forest, sustenance agriculture, and open woodland grazing. After the federal government acquired it in 1942, the watershed was allowed to return to its natural state and has not been disturbed except naturally by fire and the invasion of insects such as the southern pine beetle (*Dendroctonus frontalis* Zimmermann). The watershed is underlain by Knox group dolomite, with several different formations occurring within the study area. Soils are mostly Ultisols (primarily Typic Paleudults) with occasional Inceptisols. The upland residual soils are developed from deep (up to 30 m) saprolite and saprolitic material, although areas of ancient and modern alluvium and colluvium of different ages also occur within the watershed boundary (Lietzke, 1994).

Major forest types were originally identified by Grigal and Goldstein (1971) and characterized predominately as upland hardwoods (*Quercus* spp., *Acer rubra, Carya* spp.) with stands dominated by chestnut oak (*Q. prinus* L.), with some intermixing of pine (*Pinus echineta* Mill. and *P. virginiana* Mill.) on ridges and old fields. Mesic coves and riparian zones are mainly yellow poplar (*Liriodendron tulipifera* L.) and American beech (*Fagus grandifolia* Ehrh.). Major changes in vegetation have taken place in many of these forest types, and some (in particular the pine type) no longer retain their original characteristics. The watershed is at approximately 300 to 350 m elevation, has 14.5°C mean annual temperature, and approximately 135 cm average annual precipitation, of which 43 to 48% is estimated to be lost as evapotranspiration (Henderson et al., 1978; Johnson et al., 1988).

In 1967, 298 vegetation inventory plots were established on the Walker Branch watershed following the protocol of Harris et al. (1973). Twenty-four of those plots were selected to represent four major forest types (pine, yellow poplar, oak–hickory, and chestnut oak) for an intensive study of nutrient cycling in 1972. As part of that study, standing biomass, forest floor, and soils were sampled from four to six subplots in each plot (Henderson and Harris, 1975; Henderson et al., 1978). A subset of eight of those plots was identified for intensive, temporal sampling and has served as the basis for assessing long-term changes in soil chemical properties and nutrient cycling (Johnson et al., 1988; Trettin et al., 1999). The eight intensively sampled core plots were originally selected on the basis of cover type, soil, and geomorphic settings that are characteristic of the watershed locally. Table 1 gives basic soil and vegetation characteristics of the eight core plots. With time, the vegetation composition of these plots has changed substantially in many cases, and Table 1 gives the vegetation composition of the major tree species (constituting >66% of the total biomass) as of 2004.

Field and Laboratory

In 2004 (January–March), the 24 plots previously sampled in 1972 were located and the soil resampled. The sampling procedure in 2004 included random selection of six 1-m^2 plots within a 12- by 12-m plot at each site location. At each sample location, all woody detritus >2.5-cm diameter was collected within 1 m^2. A 0.25-m ring was used to define the area in which wood <2.5 cm and Oi and Oa horizons were collected. The Oi litter was defined as recognizable by species while Oa was more highly decomposed forest floor (Oe material was included in the Oa sample). Soil cores were collected by bucket auger in 15-cm increments to a depth of 60 cm. Twigs, bark, and leaves were separated from Oi and Oa samples before drying. Wood, woody litter, and O horizon samples were oven dried to constant weight at 100°C, while soil samples were dried at 60°C. Litter samples were processed by grinding in a Tecator Cyclotec sample mill (Foss North America, Eden Prairie, MN). Samples were then stored in sealed glass jars until extraction and analysis.

All soils, including archived samples from 1972, 1982, and 1993, were included in the 2004 and 2005 chemical analyses. Soils were analyzed for total C and N using a dry combustion C and N analyzer (LECO Corp., St. Joseph, MI) at the Oklahoma State University Soil, Water, and Forage Analytical Laboratory (Stillwater, OK). All analyses included

Table 1. Basic soil and vegetation characteristics of the eight intensively sampled study plots. Current and former (1967 inventory) dominant vegetation is given. Species listed account for >70% of the biomass in order of importance.

Plot	Soil series	Classification	Dominant species	Former dominant species
26	Fullerton, Tarklin	Typic Paleudult, Typic Fragiudult	yellow poplar (60%), shortleaf pine (14%)	shortleaf pine (56%), yellow poplar (43%)
42	Fullerton silt loam	Typic Paleudult	chestnut oak (34%), red oak (23%), dogwood (14%)	chestnut oak (36%), red oak (31%), black oak (8%)
91	Bodine cherty silt loam	Typic Paleudult	chestnut oak (37%), hickory (20%), white oak (14%)	hickory (42%), chestnut oak (34%), white oak (6%)
98	Claiborn silt loam	Humic Paledult	yellow poplar (41%), white oak (35%)	yellow poplar (46%), white oak (27%)
107	Fullerton cherty silt loam	Typic Paleudult	black oak (49%), hickory (21%)	hickory (48%), black oak (35%)
179	Fullerton silt loam	Typic Paleudult	chestnut oak (79%)	chestnut oak (59%), white oak (11%)
237	Fullerton silt loam	Typic Paleudult	chestnut oak (39%), white oak (35%)	white oak (41%), chestnut oak (22%), red oak (11%)
281	Claiborn silt loam	Humic Paleudult	yellow poplar (64%), beech (32%)	yellow poplar (63%), beech (19%)

blanks, standards, and 10% duplication. Quality assurance checks comparing previous analyses for C and N (PerkinElmer CHN analyzer; Trettin et al., 1999) revealed a 5% bias (lower in 2004 analyses), and for that reason only the 2004 analyses were used in this study.

The change in biomass was calculated from measurements of diameter at breast height on 0.0404-ha nested plots (Edwards et al., 1989), using allometric equations established by Harris et al. (1973). Vegetation nutrient contents were calculated from these mass data and nutrient concentrations obtained by destructive sampling on Walker Branch watershed (Henderson et al., 1978) and the Chestnut Ridge whole tree harvest site (Johnson et al., 1982). More detailed descriptions of these procedures are found in the above publications.

Statistical Analyses

Changes in C and N concentrations and C/N ratios in the individual 24 plots between 1972 and 2004 were analyzed using unpaired Student's *t*-tests. Changes in the average of all plots were analyzed using paired Student's *t*-tests on the average values for each plot. The effects of plot, year, and depth on soil concentrations in the eight intensively sampled core plots were analyzed using SAS PROC GLM software (SAS Institute, Cary, NC). Pairwise comparisons of average C concentrations, N concentrations, and C/N ratios in each horizon of the eight core plots were conducted using least significant differences at $P \leq 0.05$ (Carmer and Swanson, 1973). The analyses of ecosystem C and N contents were conducted using plot as a replicate because only one estimate of tree C and N content was available per plot. The effects of plot and year on ecosystem contents were also analyzed by SAS PROC GLM software, and pairwise comparisons of C and N contents of various ecosystem components were conducted using least significant differences (Carmer and Swanson, 1973). Statistical significance was assigned to probability levels of $P \leq 0.05$.

RESULTS

Changes in Soil Carbon and Nitrogen Concentrations

The changes in soil C, N, and soil C/N ratios in the 0- to 15- and 45- to 60-cm depths for the original plots between 1972 and 2004 are shown in Fig. 1. For the 0- to 15-cm depth, samples from the 1972 collection were available for reanalysis for 23 of the original 24 core plots, and for the 45- to 60-cm depth, 19 samples from 1972 were available for reanalysis; samples from the remaining plots had been depleted by other analyses. Recall that only 2004 analyses were used in these comparisons.

There was a general increase in C, N, and the C/N ratio during the 1972 to 2004 sampling period. In the 0- to 15-cm depth, C concentrations increased in 21 of 23 plots (two being unchanged), all statistically significant changes within individual plots (10 of 23) showed increases, and the average of all plot averages also showed a significant increase. Similarly, N concentrations in the 0- to 15-cm depth increased in 20 of 23 plots, all statistically significant changes (9 of 23) showed increases, and the average of all plot averages also showed a significant increase. For C/N ratio in the 0- to 15-cm depth, 18 of 23 plots showed increases, but only four of these were statistically significant, and the average of all plot averages showed a slight but significant increase. In the 45- to 60-cm depth, C concentrations showed increases in 14 of 19 plots, six of which were statistically significant in the positive direction; however, plot averages did not show a significant change. Nitrogen concentrations in the 45- to 60-cm depth increased in only five of 19 plots, two of which were statistically significant, and there was no significant change in plot averages. Carbon/nitrogen ratios in the 45- to 60-cm depth increased in 17 of 19 plots, seven of which were statistically significant. Carbon/nitrogen ratios in the 45- to 60-cm depth significantly decreased in one plot, but the average of all plot averages showed a significant increase.

Changes in C, N, and C/N ratios in the eight intensively sampled core plots are shown in Fig. 2 to 4 and statistical analyses are shown in Table 2. As noted above, the intermediate depths (15–30 and 30–45 cm) were not sampled in 1972 and therefore the full profiles for these plots can only be analyzed from 1982 on. Thus, the statistical results are broken into two categories: the 1972 to 2004 data, including four samplings but only the 0- to 15- and 45- to 60-cm depths, and the 1982 to 2004 data, including three samplings and all depths.

The effects of plot, depth, and year were significant for C and N concentrations, whether analyzed for the 1972 to 2004 data set (0–15- and 45–60-cm depths only) or the 1982 to 2004 data set (all depths; Table 2). The interaction term plot × year was not significant for C or N concentration in the 1972 to 2004 data set, indicating that there were no significant differences in changes with time among plots. The plot × year term was significant for N but not for C in the 1982 to 2004 data set. The interaction term depth × year was significant for both C and N in the 1972 to 2004 data set, indicating that responses differed by depth, but

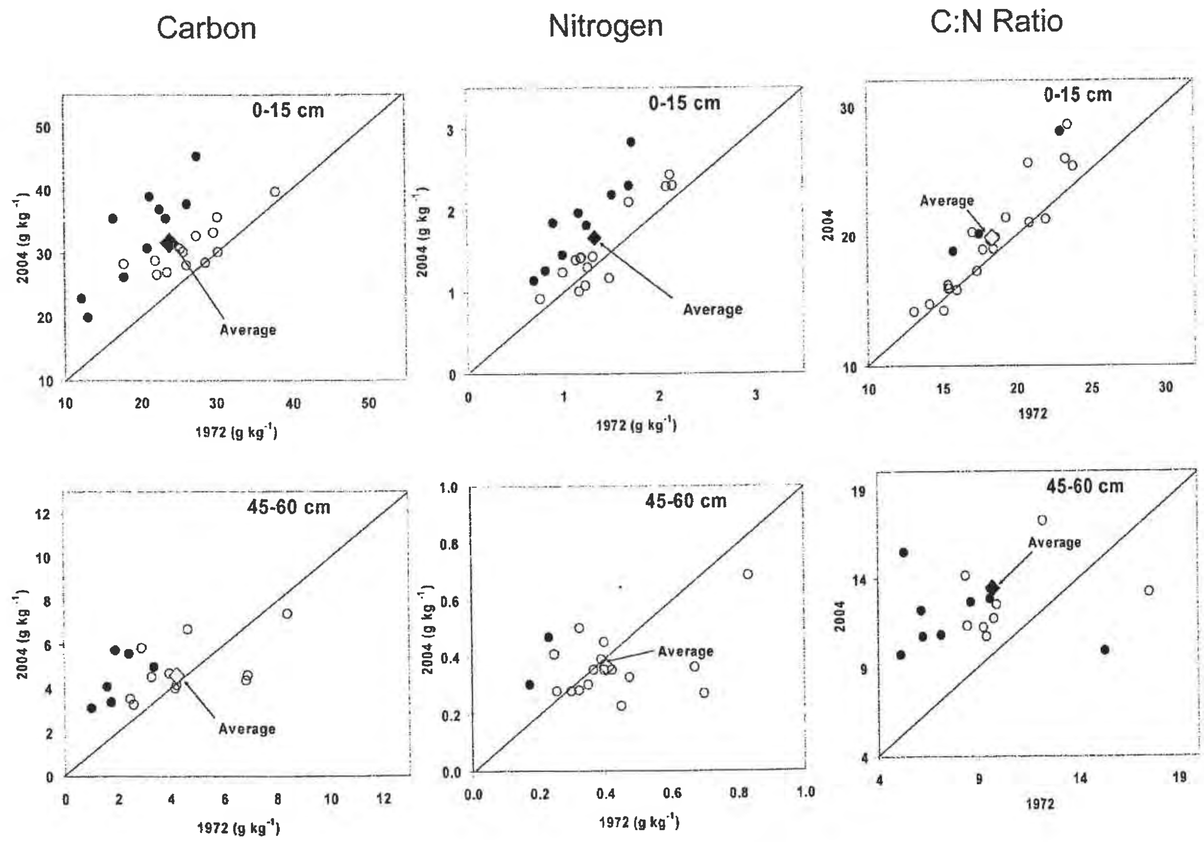

Fig. 1. Soil C and N concentrations and C/N ratios in the A (0–15 cm) and Bt (45–60 cm) horizons in the 24 core plots measured in 1972 and 2004 on Walker Branch watershed, Tennessee. Darkened circles indicate statistically significant differences for unpaired Student's *t*-tests within an individual plot and open circles show nonsignificant changes. Diamonds show changes in average plot values, with darkened diamonds indicating statistically significant differences for paired Student's *t*-tests.

the depth × year term was not significant for either C or N in the 1982 to 2004 data set. Plot × depth × year was significant for N in both data sets and for C in the 1982 to 2004 data set. Plot, depth, and year were significant for C/N ratio in both data sets, as were all measured interaction terms, indicating significant variations in responses among plots and depths with time.

The patterns of C and N change at individual depths reflected the increases between 1972 and 2004 observed for the larger data set (Fig. 1–3) and the pattern of decline from 1983 to 1993 previously noted by Trettin et al. (1999) (Fig. 2 and 3). Some individual plots varied from this general pattern, but the average values for all plots clearly reflected these general changes: in the 0- to 15-cm depth, C and N concentrations increased significantly between 1972 and 2004 and decreased significantly between 1982 and 1993 (Fig. 2 and 3). Similarly, in the 15- to 30- and 30- to 35-cm depths, C and N concentrations decreased significantly between 1982 and 2004. In the 45- to 60-cm depth, C concentration decreased significantly between 1982 and 1993, but N concentrations did not change significantly. Between 1993 and 2004, the pattern of declines reversed in most cases: soil C concentrations increased significantly relative to 1993 values at all depths and N concentrations increased significantly in the 0- to 15- and 15- to 30-cm depths (Fig. 2 and 3). Nitrogen concentrations in the 30- to 45- and 45- to 60-cm depths did not change significantly between 1993 and 2004.

Carbon/nitrogen ratios increased slightly with time in the 0- to 15-cm depth and were significantly greater in 2004 than in 1972 (Fig. 4). In the 15- to 30-, 30- to 45-, and 45- to 60-cm depths, the C/N ratio decreased between 1983 and 1993 and increased again between 1993 and 2004 in a manner similar to C and N concentrations. The changes in the C/N ratio in the lower depths reflected the fact that C concentrations changed more than N concentrations with time. Between 1982 and 1993, average C concentrations decreased by 39, 43, and 41% in the 15- to 30-, 30- to 45-, and 45- to 60-cm depths, respectively, whereas average N concentrations decreased by only 29, 22, and 1% at these depths. Similarly, between 1993 and 2004, average C concentrations increased by 60, 45, and 65% in the 15- to 30-, 30- to 45-, and 45- to 60-cm depths, respectively, whereas average N concentration increased by only 23% in the 15- to 30-cm depth and did not increase at all in the 30- to 45- and 45- to 60-cm depths (−4 and 0% changes, respectively).

Ecosystem Carbon and Nitrogen Contents

Average ecosystem C and N contents for the eight intensively sampled plots are shown in Fig. 5 and statistical analyses for ecosystem C and N changes are presented in Table 3. Table 4 gives ecosystem C and N increments for 1983 to 1993, 1993 to 2004, and 1983 to 2004.

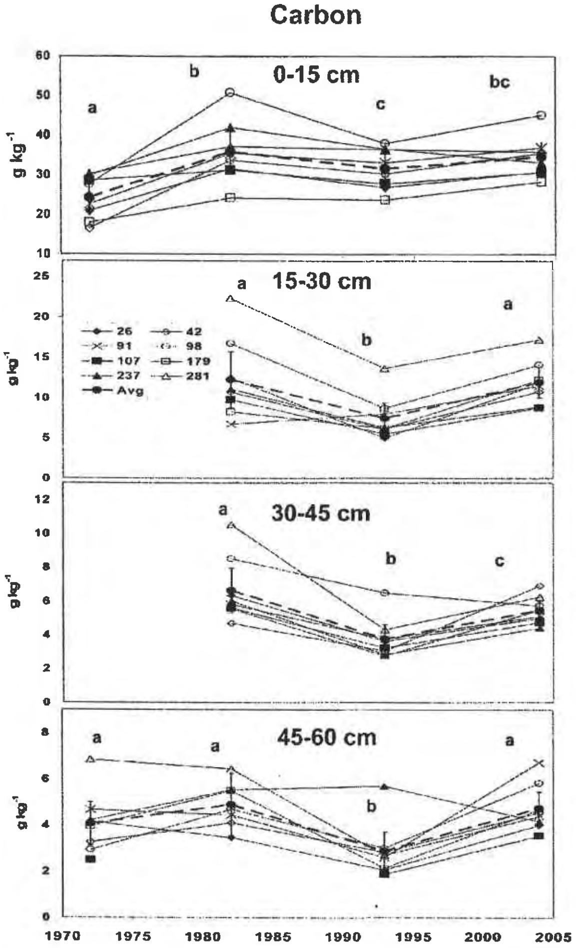

Fig. 2. Soil C concentrations in the eight core plots of Walker Branch watershed, Tennessee, between 1972 and 2004 (for the 0–15- and 45–60-cm depths) and between 1982 and 2004 for the intermediate depths. Average values not sharing the same letters are significantly different from one another (LSD, $P \leq 0.05$).

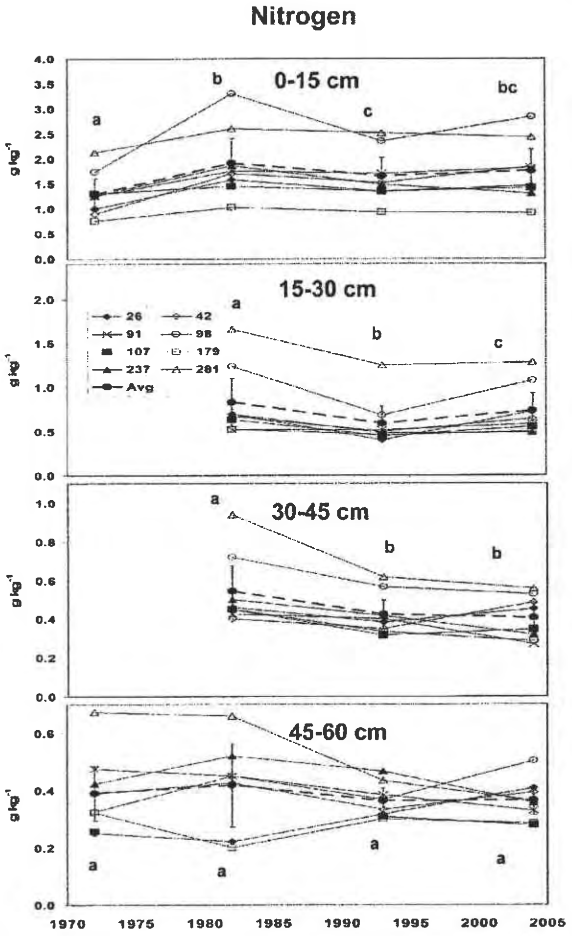

Fig. 3. Soil N concentrations in the eight core plots of Walker Branch watershed, Tennessee, between 1972 and 2004 (for the 0–15- and 45–60-cm depths) and between 1982 and 2004 for the intermediate depths. Average values not sharing the same letters are significantly different from one another (LSD, $P \leq 0.05$).

As is often the case (e.g., Cole and Rapp, 1981), vegetation dominated the ecosystem C pools in these plots and strongly affected the changes in net ecosystem C contents with time. The decline in soil C content between 1982 and 1993 (−16.9 Mg ha^{-1}), however, combined with the decline in detritus (dead vegetation plus forest floor) C contents (−11.2 Mg ha^{-1}) caused a significant decrease in ecosystem C content (−24.9 Mg ha^{-1}) during this period despite a small increase (3.2 Mg ha^{-1}) in live vegetation C content. Although most soil C was contained in the upper 15 cm, total losses of soil C from 1982 to 1993 were dominated by C losses in the 15- to 60-cm depths (Fig. 5). Between 1993 and 2004, total ecosystem C content increased (40.7 Mg ha^{-1}), largely because of large increases in live vegetation C content (20.4 Mg ha^{-1}) combined with increases in both soil (13.3 Mg ha^{-1}) and detritus (7.5 Mg ha^{-1}) C contents. During the period 1982 to 2004, ecosystem C content increased by 15.8 Mg ha^{-1} because of increases in live vegetation C content (23.2 Mg ha^{-1}), which offset net decreases in detritus (−3.8 Mg ha^{-1}) and soil (−3.7 Mg ha^{-1}) C pools during this period.

Mineral soil content dominated the ecosystem N pools in these plots, as is also often the case in forest ecosystems (Cole and Rapp, 1981), and changes in ecosystem N contents with time were strongly affected by changes in soil N. Between 1982 and 1993, net declines in mineral soil N content (−774 kg ha^{-1}), most of which occurred in the 15- to 60-cm depths, were the major cause of the declines in ecosystem N content (−806 kg ha^{-1}), more than offsetting the small net increases in live vegetation N content (14 kg ha^{-1}). Declines in detritus N content between 1982 and 1993 (−46 kg ha^{-1}) were small in comparison to declines in soil N content. Between 1993 and 2004, soil N increased by 258 kg ha^{-1}, but this was not statistically significant. Increases in vegetation (89 kg ha^{-1}) and detritus (124 kg ha^{-1}) N contents were significant. During the 1982 to 2004 period, there were no significant changes in the N content of any ecosystem component except live vegetation and litter.

DISCUSSION

Hypothesis 1 (soil C and N data from the spatially expanded data set will show increased C and N concentrations between 1972 and 2004) was supported by the results of this study. The general increases in C and N concentrations in the 0- to 15-cm

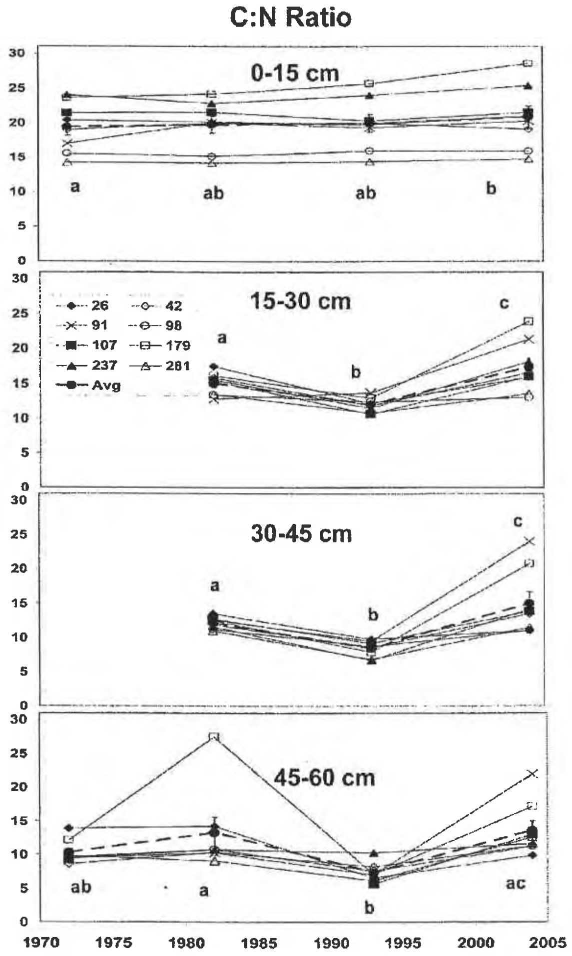

Fig. 4. Soil C/N ratios in the eight core plots of Walker Branch watershed, Tennessee, between 1972 and 2004 (for the 0–15- and 45–60-cm depths) and between 1982 and 2004 for the intermediate depths. Average values not sharing the same letters are significantly different from one another (LSD, $P \leq 0.05$)

depth of the eight core plots was reflected in the larger data set. In the 45- to 60-cm depth, data from the eight core plots showed a less clear trend, with both increases and decreases between 1972 and 2004, and this was also reflected in the larger data set. Thus, the data from the eight core plots was representative of the larger sample taken across the landscape.

Neither Hypothesis 2 (analysis of 2004 soils will show continued declines in soil C and N) nor Hypothesis 3 (declines in soil C and N will be associated with declines in forest growth) was supported by the results of the 2004 sampling. The downward trends in soil C and N between 1982 and 1993 were either reversed (in the case of C) or halted (in the case of N) between 1993 and 2004, and vegetation biomass increased substantially and significantly during the same period.

The fact that soil C and N contents did not differ between 1982 and 2004 may suggest that the significant declines in 1993 were simply due to sampling or analytical error. Analytical error is an extremely unlikely factor because all old samples were reanalyzed. Changes in C and N concentration during sample storage are also an unlikely explanation for the 1993 decreases because such effects should be more pronounced in the 1982 than in the 1993 samples, yet the 1982 samples had higher C and N concentrations after more than a decade longer in storage. Field sampling bias is a possible source of error, as always. For example, if the 1993 soils were sampled more deeply than the 1982 or 2004 samples, they would be diluted with lower C and N concentrations from below and have lower values. Soils on Walker Branch do not have smectitic clays, and thus natural shrink–swell potential is low. We feel that the maximum error in our sampling depth could be 2 cm, and used this to calculate the potential for error if samples taken in 1993 were taken 2 cm too deep. We assumed the following:

$$(C_i + 2) = [15(C_i) + 2(C_j)]/17 \qquad [1]$$

where C_i = the concentration at depth i, C_j = concentration at depth j, which is the horizon below i, and $(C_i + 2)$ = the calculated concentration if the sample was taken 2 cm too deep. In essence, this calculation assumes that the concentration measured by sampling 2 cm too deep equals 88% (15 of 17 cm) of the concentration of the true 0- to 15-cm depth plus 12% (2 of 17 cm) of the concentration of the horizon below. The results of this error analysis fell far short of explaining the observed differences in soil C and N concentrations with time: this calculation could account for a change of only 3 to 7% of measured values for C and 3 to 8% of measured values for N. Even had sampling depth been a factor, it does not seem likely that *all* samples were taken more deeply in 1993, causing the consistent pattern of lower C and N in that year. Similarly, it seems very unlikely that spatial variability can account for the 1993 decline, given the consistent nature of the decline among most plots and depths. Thus, we judge that field sampling error is an unlikely cause of the observed declines in soil C and N in 1993; we must entertain the possibility that the observed changes in soil C and N are real and attempt to reconcile these changes with estimated changes in vegetation and detritus pools as well as previously measured C and N fluxes on the watershed.

Table 2. Analysis of variance results for changes in soil C and N in the eight intensively sampled core plots using all replicate samples within each plot.

Plot and depth	df	C		N		C/N ratio	
		F ratio	*P*	*F* ratio	*P*	*F* ratio	*P*
1972, 1982, 1993, and 2004 samplings, 0–15- and 45–60-cm depths only							
Plot (P)	7	6.09	<0.001	34.89	<0.001	28.85	<0.001
Depth (D)	1	1107.95	<0.001	1108.48	<0.001	713.10	<0.001
Year (Y)	3	13.02	<0.001	13.13	<0.001	20.59	<0.001
P × Y	21	0.78	0.740	1.54	0.063	2.20	0.002
D × Y	3	9.14	<0.001	14.18	<0.001	13.84	<0.001
1982, 1993, and 2004 samplings, all depths							
P	7	9.14	<0.001	54.58	<0.001	25.41	<0.001
D	3	721.07	<0.001	642.74	<0.001	205.05	<0.001
Y	2	16.80	<0.001	14.23	<0.001	96.43	<0.001
P × Y	14	1.00	0.448	2.22	0.007	6.57	<0.001
D × Y	6	1.09	0.365	2.09	0.053	8.38	<0.001

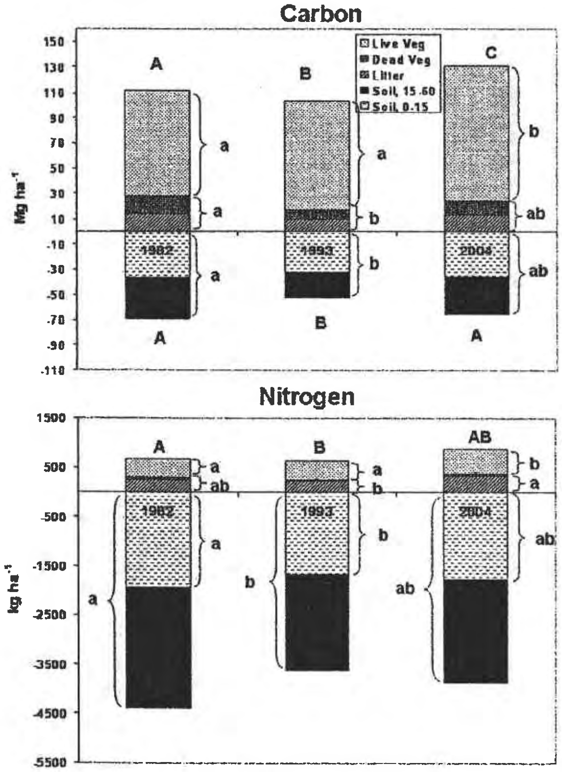

Fig. 5. Average ecosystem C and N contents in the eight intensively sampled plots of Walker Branch watershed, Tennessee. Bars not sharing the same uppercase letters on top represent total ecosystem contents that are significantly different from one another. Bars not sharing the same lowercase letters for a segment represent differences in live vegetation, detritus (litter + dead vegetation), or soil contents in 0 to 15 or 0 to 60 cm that are significantly different from one another.

Table 3. Analysis of variance values for average changes in ecosystem C and N contents between 1982 and 2004.

Component	df	C		N	
		F ratio	*P*	*F* ratio	*P*
Live vegetation					
Plot	7	3.80	0.018	4.94	0.007
Year	3	12.59	<0.001	12.33	0.001
Dead vegetation					
Plot	7	2.26	0.092	2.49	0.069
Year	2	1.00	0.393	1.06	0.372
Forest floor					
Plot	7	2.55	0.069	3.22	0.033
Year	2	2.60	0.112	4.08	0.042
Detritus (dead vegetation + forest floor)					
Plot	7	2.69	0.055	3.61	0.020
Year	2	3.18	0.073	4.94	0.024
Soil					
Plot	7	9.3	<0.001	16.23	<0.001
Year	2	12.01	0.001	4.76	0.028
Total ecosystem					
Plot	7	6.28	0.001	20.48	<0.001
Year	2	22.68	<0.001	6.19	0.012

Hypothesis 4 (changes in soil C and N can be explained by known ecosystem C and N fluxes) was supported in the case of C but not in the case of N. The magnitude of the average observed soil C loss between 1982 and 1993 (–1.5 Mg ha^{-1} yr^{-1}; Table 4) is approximately half as large as the estimated CO_2–C respiration from soil organic matter estimated in a chestnut oak stand on Walker Branch (–0.8 Mg ha^{-1} yr^{-1}; Edwards et al., 1989) and the error bounds for the two measurements probably overlap. The magnitude of the average observed soil C gain between 1993 and 2004 (1.2 Mg ha^{-1} yr^{-1}) equals about half of leaf litterfall C inputs (2.1–2.4 Mg ha^{-1} yr^{-1}; Edwards et al., 1989) and C from root turnover adds to that input. Thus, the changes in soil C during the sampling period are not inconsistent with known C fluxes on the watershed. On the other hand, the magnitude of the soil N decline between 1982 and 1993 (–70.4 kg ha^{-1} yr^{-1}) is large relative to other ecosystem N fluxes. For example, accumulation in vegetation during this period (1.3 kg ha^{-1} yr^{-1}) equaled only a fraction of this amount, and leaching during the same period should not exceed 10 kg ha^{-1} yr^{-1}, given previous soil solution measurements on the watershed (Johnson and Todd, 1990). One could postulate that litter blowing (Comiskey et al., 1977) could account for some of the changes—that is, if a fraction of litterfall blew offsite from the core plots. This could account for only a maximum of 30 kg ha^{-1} yr^{-1}, which is the maximum rate of litterfall N return observed among these plots (Johnson and Todd, 1990). Thus, we are unable to account for the apparent decreases in soil N between 1982 and 1993 with any known N fluxes.

Neither soil nor ecosystem N contents in 2004 is significantly different from those in either 1982 or 1993, allowing us to attribute the statistically significant declines in soil N in 1993 to Type I error. Binkley et al. (2000) reviewed many studies documenting apparent soil N changes and concluded that most could be attributed to sampling or analytical error (an exception being a harvesting study near Walker Branch; Johnson and Todd, 1998). On the other hand, we should not bury data suggesting large soil N changes simply because the magnitudes of these changes do not fit our preconceived notions of how the N cycle should operate. The latter is especially true in view of recent developments in N

Table 4. Ecosystem C and N increments in the eight core plots during the sampling period.

Component	Total increment			Annual increment		
	1982–1993	1993–2004	1982–2004	1982–1993	1993–2004	1982–2004
Carbon	Mg ha^{-1}			Mg ha^{-1} yr^{-1}		
Live vegetation	3.2	20.4*	23.2*	0.3	1.8*	1.1*
Dead vegetation	−7.1	3.5	−3.6	−0.6	0.3	−0.2
Litter	−4.1	4.0	−0.2	−0.4	0.4	0.0
Soil	−16.9*	13.3*	−3.7	−1.5*	1.2*	−0.2
Total	−24.9*	40.7*	15.8*	−2.3*	3.7*	0.7*
Nitrogen	kg ha^{-1}			kg ha^{-1} yr^{-1}		
Live vegetation	14	89*	103*	1.3	8.1*	4.7*
Dead vegetation	−22	13	−9	−2.0	0.2	−0.4
Litter	−23	111*	88*	−2.1	10.1*	4.0*
Soil	−774*	258	−517	−70.4*	23.4	−23.5
Total	−806*	471	−344	−73.2*	42.8	−15.2

* Increments are significantly different from zero (i.e., difference in ecosystem contents during the specified time period were significant) at $P \leq 0.05$.

cycling research that require substantial paradigm shifts as to how that cycle truly operates (e.g., Schimel and Bennett, 2004).

CONCLUSIONS

Soil C and N concentrations and contents fluctuated during the 32-yr sampling interval of this study, showing both increases and decreases across decadal intervals. The magnitude of fluctuations in soil C can be accounted for by previously measured fluxes of C via litterfall and soil CO_2–C efflux. The magnitude of fluctuations in soil N content, however, cannot be accounted for by changes in vegetation or detritus N storage, atmospheric deposition, or leaching, nor can they be reasonably attributed to laboratory or field sampling bias. We must conclude that trends in soil C and N pools on the Walker Branch watershed are not only highly variable across space (both among plots and between Walker Branch and other nearby studies; Johnson and Todd, 1998; Johnson et al., 2003), but also with time. There does not seem to be any unidirectional trend during the time period of this study, and once again, we conclude that further sampling in the future is called for.

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