Ecosystems (2018) 21: 1623–1638
https://doi.org/10.1007/s10021-018-0244-2
ECOSYSTEMS

Total C and N Pools and Fluxes Vary with Time, Soil Temperature, and Moisture Along an Elevation, Precipitation, and Vegetation Gradient in Southern Appalachian Forests

Jennifer D. Knoepp,[1]* Craig R. See,[2,3] James M. Vose,[4] Chelcy F. Miniat,[1] and James S. Clark[5]

[1]*USDA Forest Service, Southern Research Station, Center for Forest Watershed Science, Coweeta Hydrologic Laboratory, 3160 Coweeta Lab Rd, Otto, North Carolina 28763, USA;* [2]*Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia 30602, USA;* [3]*Present address: Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, Minnesota 55108, USA;* [4]*USDA Forest Service, Southern Research Station, Center for Integrated Forest Science, North Carolina State University, Campus Box 8008, Raleigh, North Carolina 28695, USA;* [5]*Department of Statistical Science, Nicholas School of the Environment, Duke University, Durham, North Carolina 27706, USA*

ABSTRACT

The interactions of terrestrial C pools and fluxes with spatial and temporal variation in climate are not well understood. We conducted this study in the southern Appalachian Mountains where complex topography provides variability in temperature, precipitation, and forest communities. In 1990, we established five large plots across an elevation gradient allowing us to study the regulation of C and N pools and cycling by temperature and water, in reference watersheds in Coweeta Hydrologic Laboratory, a USDA Forest Service Experimental Forest, in western NC, USA. Communities included mixed-oak pine, mixed-oak, cove hardwood, and northern hardwood. We examined 20-year changes in overstory productivity and biomass, leaf litterfall C and N fluxes, and total C and N pools in organic and surface mineral soil horizons, and coarse wood, and relationships with growing season soil temperature and precipitation. Productivity increased over time and with precipitation. Litterfall C and N flux increased over time and with increasing temperature and precipitation, respectively. Organic horizon C and N did not change over time and were not correlated to litterfall inputs. Mineral soil C and N did not change over time, and the negative effect of temperature on soil pools was evident across the gradient. Our data show that increasing temperature and variability in precipitation will result in altered aboveground productivity. Variation in surface soil C and N is related to topographic variation in temperature which is con-

---

Received 20 December 2017; accepted 14 March 2018;

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s10021-018-0244-2) contains supplementary material, which is available to authorized users.

Author Contributions JK and JC helped design the experiment; JK, JC, and JV collected data; JK, CS, and CM analyzed data; JK wrote the first draft of the manuscript; CS, CM, JV, JC wrote, reviewed, and edited the manuscript.

All data collected in association with this manuscript will be posted at: http://coweeta.uga.edu/dbpublic/data_catalog.asp.

Corresponding author; e-mail: jknoepp@fs.fed.us

founded with vegetation community. Data suggest that climatic changes will result in altered aboveground and soil C and N sequestration and fluxes.

Key words: aboveground; belowground; C sequestration; fluxes; long-term data; N sequestration; pools.

INTRODUCTION

Forestry as a land use and the management of forests are increasingly becoming part of public policy decisions aimed at increasing C sequestration and mitigate greenhouse gas emissions (Lal and others 2003). Altering land use and land management can increase or decrease soil and biotic carbon pools (Davidson and Ackerman 1993; Guo and Gifford 2002), which make up an estimated 35% of the global terrestrial C pool—approximately 3060 Pg out of a total 8060 Pg—with the balance comprised of oil, coal, gas, and other fossil fuel deposits (Lal 2004). Soil C pool estimates range from 96 to 344 Mg C ha^{-1} in temperate to boreal forests, 110–236 Mg ha^{-1} in tropical to temperate grassland systems, and 643 Mg ha^{-1} in wetlands (Lal 2004). Forests are unique among terrestrial ecosystems, in addition to accumulating large soil C pools, they also accumulate large aboveground C pools with slow turnover rates. In northern hardwood and old-growth forests of the Pacific Northwest, USA, aboveground biomass pools comprise 40.5 and 64% of the total ecosystem C pool, respectively (Harmon and others 2004; Fahey and others 2005). Because both above- and belowground C pools turn over slowly, due to the long-lived nature of forest woody biomass and the relatively low frequency and intensity of soil disturbances (Luyssaert and others 2008), understanding forest C sequestration capacity requires both a long-term and an ecosystem perspective (Wardle and others 2004).

Much of our long-term view of how forested C pools change over time has been shaped by studies employing a space-for-time substitution, or chronosequence approach. Collectively, these studies show that for up to 100 years following disturbance, such as land use conversion or forest harvesting and regrowth, aboveground C pools rapidly recover and increase over time (Hooker and Compton 2003). Following disturbance, soil O-horizons can decrease due to increased decomposition rates from increased energy input and moisture availability followed by the accumulation of C before reaching an equilibrium (Federer 1984). In general, mineral soils accumulate C much more slowly than the O-horizon (Markewitz and others 2002). Replicating these results has been challenging, however. For example, Yanai and others (2000) resampled 23 northern hardwood forest sites included in a previous chronosequence study (Federer 1984) and found that this approach had limited success for understanding differences in forest floor mass over time due to high spatial variation. Shorter-term studies that sample the same forest plots over time (collections occurring over 15–30 years) show considerable variability with type of disturbance, forest species composition, climate variability, and interactions between litterfall, O-horizon, and mineral soil making general conclusions about patterns of long-term changes in soil C difficult (Knoepp and Swank 1994, 1997; Richter and others 1999; Johnson and others 2002; Knoepp and others 2005). More recently, research has shown that focusing on total C may obscure important changes to soil C distribution and stabilization over time (Hedde and others 2008; Crow and others 2009; Miltner and others 2012) due to changes in microbial communities and transformations (Carney and Matson 2005; Allison and others 2010; Oliverio and others 2017).

Ecosystem C sequestration capacity is regulated by the feedback mechanisms between above- and belowground C and N inputs and temperature and moisture conditions that regulate rates of organic matter decomposition and N mineralization (Knoepp and Swank 2002; Wardle and others 2004; Prescott 2005; Keiser and others 2013). Recent studies have addressed the complexity of these interactions (Bardgett and others 2005) exploring the role of plant species (Hobbie 2015), root morphology, physiology and mycorrhizal association (Bardgett and others 2014), and interaction of plant and microbial species and nutrient availability in organic matter decomposition and nutrient release (Gessner and others 2010; Hobbie 2015; Tipping and others 2016; Maaroufi and others 2017; Bani and others 2018). Aboveground C sequestration, that is, forest productivity (Cole 1995), is inextricably linked with N availability (Knicker 2011; Fernández-Martínez and others 2014), and increased productivity has been observed with the addition of N fertilizers (Elser and others 2007; Vadeboncoeur 2010) and along N deposition gradients (White and others 2004; Frangi and others 2005). Adding N increases aboveground vegetation C pools and may also increase total soil C pools due to reduced decomposition (Aber and others 1998; Eisenlord and others 2013; Frey and others 2014; Maaroufi and others 2017), particularly if the N addition is inorganic (Boot and others 2016). These studies demonstrate the importance of including N to fully understand patterns of ecosystem C sequestration.

Regional climate and microclimate within a landscape driven by macro- and micro-topography also affect soil C and N pool sizes and cycling rates and ultimately determine the maximum potential ecosystem C pool size (Stewart and others 2008). Globally, temperature controls belowground C allocation (Reich and others 2014). Locally, aboveground forest growth is regulated by available energy and water (Elliott and others 2015). Water availability has a direct effect on aboveground C sequestration with notable declines in vegetation growth and belowground C contributions and turnover under low soil moisture or drought conditions (Leppälammi-Kujansuu and others 2014; Ryan and others 2014; Luce and others 2016; Liese and others 2018). Soil moisture is also essential for soil nutrient cycling processes and plant uptake (Schlesinger and others 2015), and regulating N availability when temperature is not limiting (Knoepp and Swank 2002; Knoepp and Vose 2007). Predicted warming under climate change may alter the long-term topographic patterns of temperature and soil moisture within a given landscape, but the resulting impacts on soil C and N sequestration are not yet clear.

Across the USA, air temperatures and precipitation variability are increasing (Portmann and others 2009; Laseter and others 2012; Clark and others 2016). Long-term temperature and precipitation changes may interact with site topography and elevation to impact C and N pools. In this study we examined long-term temporal variation (1992–2013) in the C and N pools of an aggrading forest and their relationships to abiotic conditions along an elevation gradient at Coweeta Hydrologic Laboratory, Otto, NC, USA. Plots along the gradient differ in temperature and rainfall, and are representative of the major forest types of the southern Appalachian Mountain region. Within this long-term measurement period we hypothesized that: (H1) aboveground C and N accumulation, net primary productivity and litterfall flux increase with time and temperature and vary with precipitation; (H2) organic soil horizon (O-horizon) C and N pools are regulated by aboveground C and N inputs and can change over short time scales, but (H3) surface mineral soil C and N do not change over short time scales (25 years or less), instead differing among sites (vegetation) with abiotic (precipitation and temperature) variables.

MATERIALS AND METHODS

Plot Description

This study was conducted at the USDA Forest Service, Coweeta Hydrologic Laboratory, an experimental forest established in 1934 in the southern Appalachian Mountains of western North Carolina, USA, and a National Science Foundation Long-term Ecological Research site since 1980 (Figure 1). Annual precipitation is approximately 1900 mm and is above 100 mm in most months. The growing season extends from early May to early October. Mean monthly temperatures are highest in June through August (~ 20°C) and lowest in December through January (~ 5°C) (Laseter and others 2012).

In 1991, we established 20 m × 40 m plots (original plots) at five locations representing a gradient in vegetation, elevation, climate, and N deposition within the Coweeta Basin. All plots were located within reference watersheds (WS) 18 and 27 (with no cutting since 1923) and represent the four major vegetation community types within the Coweeta basin (Elliott and Vose 2011) and an elevation range from 788 to 1389 m (Table 1). Plots on WS 18 included dry mixed-oak pine (OP), cove hardwoods (*Liriodendron tulipifera, Quercus prinus, Carya spp.*; CH), and low elevation mesic mixed-oak (LO) plots. WS 27 plots were high elevation mesic mixed-oak (HO), and northern hardwoods (*Betula alleghaniensis, Quercus rubra, Betula lenta, Tilia*

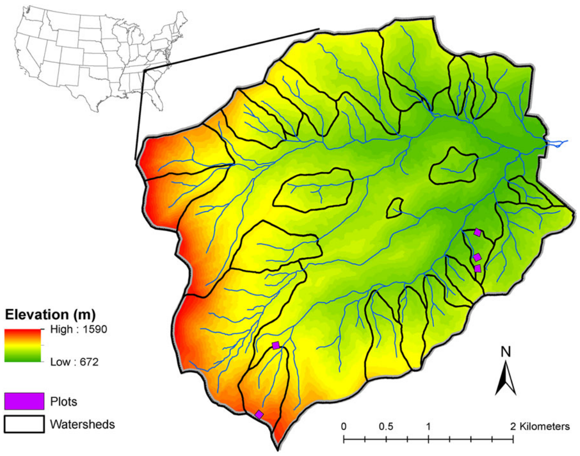

Figure 1. Location of Coweeta Hydrologic Laboratory within the contiguous USA and plot locations within Coweeta; elevation gradient is color coded as designated in legend.

Table 1. Plot Descriptions

Site	Oak pine (OP)	Cove Hdwd (CH)	Low oak (LO)	High oak (HO)	No. Hdwd (NH)
Elevation (meters)	788	801	860	1094	1389
Precipitation (cm)	187	189	191	238	238
Temperature (°C)	12.9	12.3	12.7	11.2	9.6
Vegetation type	Mixed-oak pine	Cove hardwoods	Mixed-oak	Mixed-oak	No. hardwoods
Dominant species	*Pinus rigida* *Quercus coccinea* *Quercus prinus* *Carya spp.* *Kalmia latifolia*	*Liriodendron tulipifera* *Quercus prinus* *Carya spp.*	*Quercus prinus* *Carya spp.* *Quercus rubra* *Rhododendron maximum*	*Quercus prinus* *Quercus rubra* *Carya spp.* *Rhododendron maximum*	*Betula alleghaniensis* *Quercus rubra* *Betula lenta* *Tilia heterophylla*

Mean annual air temperature and precipitation inputs.

heterophylla; NH) vegetation community types. Soils are predominately in the Ultisols and Inceptisols orders with profiles characterized by a well-developed mor-type O-horizon (Oa-horizon 2–4 cm), A horizon (10–22 cm), transitional horizon (AB or BA; 10–30 cm), and total soil pedon depth ranging from 60 to 100 cm. Measurements on each plot included overstory species composition and productivity, litterfall mass and chemistry, forest floor mass and chemistry, coarse wood mass and chemistry, and surface soil chemistry; initiation of measurement varied for each parameter. In 1998, plot size was expanded to 80 × 80 m (expanded plots) to allow a greater number of measurements and to capture spatial variability; the direction of plot expansion was determined by topography and overstory vegetation composition. Table 1 provides information about dominant vegetation, elevation, aspect, slope, and soil series on each plot; see the Coweeta LTER web site (http://coweeta.ecology.uga.edu/webdocs/1/index.htm) for additional information.

All plots were instrumented with air temperature (1.3 m above the ground), soil temperature (5, 20, 50 cm), and soil moisture (0–30, 30–60 cm) sensors. All data were collected on a Campbell Scientific, Inc. automated data logger (Model CR10x (1991–2013) Campbell Scientific, Logan, Utah) or CR1000 (2013 to present). Air and soil temperature sensors were located at the center bottom of the original plot; mean hourly temperatures were recorded on a data logger and downloaded monthly. Air (Campbell thermistor model 207) and soil [5 and 20 cm; Campbell thermistor model 107 (1992–2001), copper–constantan thermocouple (2001–present)] temperature measurements began in 1992; 50 cm soil temperature measurements began in 2000. Precipitation inputs were measured using standard rain gauges located within the Coweeta Hydrologic Laboratory basin. Precipitation input for each site was corrected using established relationships between specific locations within the basin and individual or multiple rain gauges (Swift and others 1988). We used growing season mean minimum soil (5 cm) temperature (GS T_{5cm}) and growing season precipitation inputs (PPT_{GS}) to explore climatic regulation of C and N pools and fluxes.

Measurement of C and N Pools and Inputs

Aboveground Biomass

We measured the accumulation of woody overstory aboveground C and N in two ways, overstory net primary productivity (ONPP, increase in woody mass per year, excludes mortality) and aboveground biomass (measured biennially, wood plus litterfall). Beginning in 1992 all trees in each original plot greater than 2 m in height were identified to species, tagged, and measured at breast height (DBH; 1.3 m above ground level). These measurements were continued every 2 years during the dormant season until 2014. In 1998, when the plot size was increased, tree measurements included all individuals in the expanded 80 m × 80 m plot. We estimated total aboveground biomass (Mg ha^{-1}) as well as total C and total N in the woody tissue of the overstory tree species using site- and region-specific allometric equations for each species (Martin and others 1998; Elliott and Swank 2008). To estimate overstory total aboveground biomass pools we added measured annual litterfall pools, as described below. Shrub layer (including overstory species < 2 m in height and evergreen shrub species, *Rhododendron maximum* and *Kalmia latifolia*) and herbaceous layer biomass were not measured. Estimates of evergreen shrub biomass in WS 18 and WS 27 as determined in permanent vegetation plots are 4 and 8% of the total aboveground biomass, respectively (Elliott, personal communication). We estimated annual woody aboveground overstory net primary productivity (ONPP) increment, the annual amount of new C in the overstory wood, as the sum of the increase in woody mass of each living tree plus ingrowth of new trees greater than 1 cm DBH, in two adjacent measurement periods divided by the number of years between measurements.

Litterfall Input

Litterfall collectors (ten, 0.84 m^2 litter traps per plot) were installed in spring 1994 within the original plot and sampled quarterly from 1994 to 2014. During the third quarter collection (October–December), when deciduous trees lose their leaves, traps were emptied monthly then composited by trap for the quarter. Samples from each trap were oven-dried at 60°C to a constant weight, sorted by litter component, and weighed. Litter components included deciduous leaves, rhododendron leaves, coniferous leaves, fine wood, and other material which included seeds and bark, and so on; all components were not present on all plots.

Litter component samples from each plot were composited quarterly across traps for chemical analysis. Composited samples were ground to less than 1 mm and analyzed for total C and N using a Perkin Elmer 2400 CHN Analyzer (1994–2005) or Flash EA 1112 (2005–2014). We calculated the quarterly C and N fluxes of each litter component by multiplying the average plot dry mass (average of 10 litter traps) by the composited C and N concentrations. C and N fluxes of each component were summed to calculate total litterfall flux for the quarter; quarterly fluxes were summed for annual flux. We used deciduous leaf litterfall (which represented between 65 and 73% of the total annual litterfall C and 64–71% of total litterfall N) to examine changes over time, differences among plots, and relationships between litterfall, environmental factors, and other C and N pools.

Coarse Wood

Coarse wood (CW), defined as wood on the ground greater than 10 cm in diameter and 1 m in length, was inventoried in 1998, 2003, 2008, and 2013, on each expanded plot. Inventory began in the dormant season and required 6–9 months for completion. In the 1998 CW inventory we used the *x*, *y* coordinates of both ends of each piece of wood within the 10 m grid system of the expanded plot to calculate the length. The diameter of both ends of all pieces was measured using calipers, along with a center diameter on all pieces greater than 3 m in length. In 2003, we measured the length of each piece to the nearest 5 cm, and using calipers, the diameter at each end and in the center if total length was above 3 m; a metal tag was placed in approximately the center of each piece of CW for future inventories. In 2008 and 2013, tagged CW was relocated, re-measured, and checked for change in decay class (described below); new CW was tagged and measured as described above.

During measurement each piece of CW was assigned a decay class, 1–5, using a classification system modified from Harmon and others (1986) for Douglas-fir. Class 1 represents newly fallen trees with small (< 2.5 cm) branches present and bark intact. At the other extreme, Class 5 is elliptical in shape, often has sunk into the O-horizon, lacks intact outer wood, and is soft in texture. Total wood volume was calculated as a truncated cone for decay classes 1–4. Class 5 wood required diameter measurement in two dimensions to calculate the volume of a truncated elliptical cone.

During the summer of 2004 we selected 30 pieces of CW in each plot to sample for wood density, and total C and N concentrations. Individual pieces were selected using a weighted randomized sampling scheme, selecting proportionally more pieces from dominant decay classes. Cross sections were collected from each log using a chainsaw. Each cross section was oven-dried and weighed. The area of each cross section, in decay classes 1–4, was determined by making multiple measurements of the diameter and thickness of each piece to calculate volume. Class 5 cross sections did not remain intact after cutting; we determined the cross-sectional area by making multiple measurements of both cut surfaces on the sample log from which the cross section was removed. After weighing, each cross section was ground, mixed well, and analyzed as previously described for total C and N. Total plot coarse wood volume represents the sum of all wood pieces measured on each plot. We used the mean wood density for each decay class across all five plots to calculate total coarse wood mass; site specific, decay class total C and N concentrations were used to calculate total C and total N in kg ha^{-1}. Some plots did not have CW in all decay classes, that is, there were no class 1 logs in either OP or LO, in those cases we used the mean bulk density and C and N concentrations for that decay class from all plots.

Organic Horizon

The organic soil horizon (O-horizon) was collected for mass and nutrient determinations in 1994/1995, 2003/2004, 2008/2009, and 2013. Years 1994 and 1995, 2003 and 2004, 2008 and 2009 were paired, with one collection taking place in winter and one in summer; we used the mean of these collections to represent 1 year, 1994, 2003, and 2008. In 1994, samples were collected at six randomly selected locations (4 in winter 1995 and 2 in summer 1995); along four 10 m transects extending from each corner and parallel to the 40 m axis outside the original 20 m × 40 m plot ($n = 5$ plots × 4 transects × 6 locations = 120 sample locations). During subsequent collections (2003, 2008, and 2013) we randomly selected eight x, y coordinates within the expanded plot and collected two samples per location (2 m apart) using a 0.09 m^2 quadrant (2003 and 2008, $n = 5$ plots × 2 collections × 8 coordinates × 2 samples = 160 sample locations; 2013, $n = 5$ plots × 8 coordinates × 2 samples = 90 sample locations). The quadrant was placed on the forest floor and the material was cut using a knife, to mineral soil. O-horizon layers, Oi, Oe, Oa, and wood (1–10 cm diameter), were collected separately (four layers per sample location, all layers were not present at all locations); wood (any woody material $<$ 10 cm diameter at any stage of decomposition) from all horizons was composited into one sample. In 1994/1995, wood was collected in the horizon it was found. Wood was not collected in 2003, we corrected O-horizon sample weights using wood present in the 2004 collection. Each sample collected was placed in a paper bag, oven-dried and weighed as described above. In 1994, samples were weighed and ground for chemical analysis separately ($n = 480$); in 2003, 2008, and 2013 samples were weighed separately but composited by x, y coordinates prior to grinding for chemical analysis (2003 and 2008, $n = 320$; 2013, $n = 180$). O-horizon total C and total N content were calculated using the mean mass for each location multiplied by the C and N concentrations. Total O-horizon mass, and C and N contents represent the sum of all three horizons for each sample location; wood was excluded due to high variability. The variation of C and N pool sizes among coordinates was used to calculate variability for each collection year.

Mineral Soil C and N Pools

We collected surface mineral soil (0–10 cm) periodically between 1992 and 2000 in the original plot (Knoepp and Swank 1998; Knoepp and others 2008) as part of ongoing N mineralization measurements. Soils were collected using a 10-cm-long 4.3-cm-diameter PVC core, from sample locations randomly located along 4, 20-m transects in the original plots; only soils collected during the dormant season were analyzed (1992–2000, $n = 4$ per year × 6 years). Soils were air-dried, sieved to less than 2 mm, and ground to a powder; total N and total C were determined as previously described. In 2008 and 2013, we sampled the expanded plot to continue with the long-term total soil C and N data and altered sampling protocols to estimate C and N pools for the entire soil profile. From eight locations randomly selected for O-horizon sample collection as described above, we sampled soils from 0 to 10 cm (comparable to the soils collected on the original plot), 10 to 30 cm, and 30+ cm, down to the saprolite layer or 95 cm, whichever came first. We collected composite soil samples from the two O-horizon sample quadrats, one on either side of the randomly selected x, y coordinate (2 samples × 8 coordinates = 16 sample locations), using a 2.5 cm soil probe (each sample included 5–8 individual samples); maximum depth sampled was recorded for each sample location. Soils were air-dried, sieved, and analyzed for total C and N as described above. In 2010, we determined bulk density for the soil profile within the expanded plot at eight randomly selected locations. We used a 10-cm-long 4.3-cm-diameter PVC core for surface soils (0–10 cm) and a 5-cm-long by 5-cm-diameter core attached to a slide hammer and collected cores from the center of the 10–30 cm and 30+ cm depth. Soils were dried, weighed, sieved, and weighed again to measure bulk density of the less than 2 mm fraction. We used bulk density values to calculate the total soil C and N pools.

Statistical Analysis

Plots ($n = 5$) were selected along a gradient in temperature and precipitation and represent the major forest communities present within the Coweeta Basin and across the southern Appalachians. To test our first hypothesis, we used an analysis of covariance [PROC Mixed, SAS (2013)] to determine if response variables varied by year after accounting for GS $T_{5\mathrm{cm}}$ and $\mathrm{PPT}_{\mathrm{GS}}$. We used a 1-tailed test for year and temperature because we expected a priori that response variables would increase over time and with temperature; and a 2-tailed test for precipitation. To test our second hypothesis, we used an analysis of covariance [PROC Mixed SAS (2013)] to determine if soil organic horizon C and N pools varied by year after accounting for GS $T_{5\mathrm{cm}}$, $\mathrm{PPT}_{\mathrm{GS}}$, and aboveground C and N inputs. We used the mean GS $T_{5\mathrm{cm}}$, $\mathrm{PPT}_{\mathrm{GS}}$, and litterfall inputs from 3 years prior to O-horizon collection. We used a 1-tailed test for year and aboveground C and N inputs because we expected a priori that organic soil horizon C and N pools would increase over time, and with increasing temperature and inputs. We used a 2-tailed test for precipitation.

To test our third hypothesis, we used an analysis of covariance [(PROC Mixed, SAS (2013)] to determine if mineral soil C and N pools varied by year, accounting for GS $T_{5\mathrm{cm}}$ and $\mathrm{PPT}_{\mathrm{GS}}$. We used a 2-tailed test for year, and a 1-tailed test for temperature and precipitation because we expected a priori that mineral soil C and N pools would not change over time, but would decline with increasing temperature, and increase with increasing precipitation.

Our primary goal to examine temporal patterns of C and N pools and fluxes while accounting for the effect of GS $T_{5\mathrm{cm}}$ and $\mathrm{PPT}_{\mathrm{GS}}$ is described above. Our secondary goal was to identify relationships between C and N pools and fluxes and climatic variables, GS $T_{5\mathrm{cm}}$ and $\mathrm{PPT}_{\mathrm{GS}}$, factors confounded with plot due to their locations along an elevation gradient. We conducted an additional analysis to detrend the temporal response of C and N pools and fluxes from climatic variability using residuals (predicted–actual) values from a mixed analysis (SAS 2013) with year as a fixed and plot as a random variable. After identifying significant relationships using correlation procedure (Proc Corr, SAS 2013), residuals were plotted against climate to determine if interannual variability was attributed to climatic conditions. We used a similar approach to examine relationships between O-horizon C and N pools, climatic data, and litterfall C and N inputs. Significant correlations between residuals and climate or other variables are reported with $p < 0.1$. Results of this statistical approach are presented in the Supplemental materials.

RESULTS

Climate Along the Gradient

Mean growing season minimum air temperature (GS T_{air}) varied across the gradient with the low elevation oak pine community being the warmest at 18.7°C to the high elevation northern hardwood community being the coolest at 12.5°C. Growing season minimum surface soil temperature (GS $T_{5\mathrm{cm}}$) followed the same pattern and ranged from 16.8°C in OP to 13.8°C in NH (Figure 2A). Total growing season precipitation ($\mathrm{PPT}_{\mathrm{GS}}$, cm) was also lowest in the low elevation oak pine community at 112 cm and highest in the northern hardwood community at 134 cm (Figure 2B). We used both GS $T_{5\mathrm{cm}}$ and $\mathrm{PPT}_{\mathrm{GS}}$ to examine mechanisms for changes in C and N pools and fluxes over time. As reported previously, GS T_{air} has been increasing over time (Oishi and others unpublished; Caldwell and others 2016; Elliott and others 2015; Laseter and others 2012).

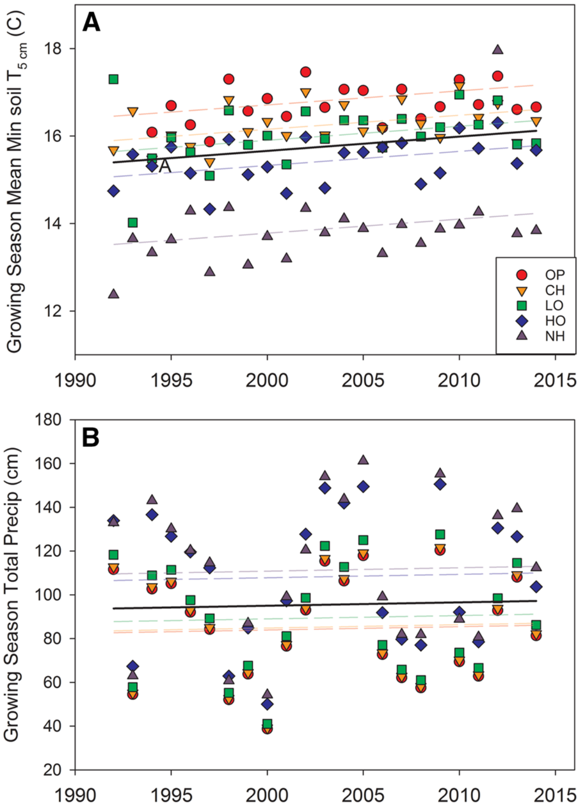

Figure 2. Growing season mean minimum soil temperature (*T*; °C) measured at 5 cm soil depth (**A**) and growing season total precipitation input (cm) (**B**) of study plots, mixed-oak pine (*filled circle*), cove hardwood (*filled inverted triangle*), low elevation mixed-oak (*filled square*), high elevation mixed-oak (*filled diamond*), and northern hardwood (*filled triangle*). *Solid line* shows pattern of change over time overall plots ($n = 5$) and faded *dashed line* for individual plots.

Overstory Net Primary Productivity (ONPP) and Aboveground Biomass

ONPP (measurement of wood growth only) ranged from 3.9 Mg ha^{-1} y^{-1} on CH to 4.7 Mg ha^{-1} y^{-1} on LO and is increasing by approximately 0.1 Mg C ha^{-1} y^{-1} ($F_{1,29} = 17.81$; $p < 0.001$; Figure 3A) supporting our first hypothesis. $\mathrm{PPT}_{\mathrm{GS}}$ was a positive covariate with ONPP ($p = 0.02$), but GS $T_{5\mathrm{cm}}$ had no effect ($p = 0.43$).

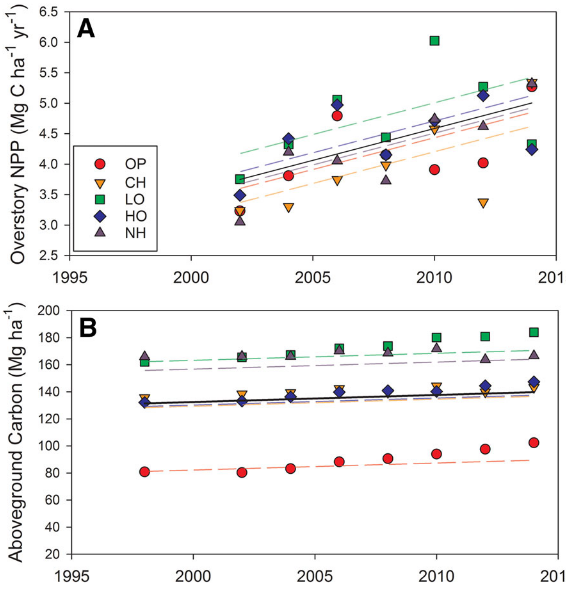

Figure 3. Overstory net primary productivity (ONPP) (**A**) and overstory total carbon pool (**B**) in study plots, mixed-oak pine (*filled circle*), cove hardwood (*filled inverted triangle*), low elevation mixed-oak (*filled square*), high elevation mixed-oak (*filled diamond*), and northern hardwood (*filled triangle*). *Solid line* shows pattern of change over time overall plots and faded *dashed line* for individual plots.

Aboveground biomass C (wood plus litterfall) did not increase with time ($F_{1,34} = 0.31$; $p = 0.58$) (Figure 3B). Total biomass ranged from an average of 86 Mg ha^{-1} in OP to 167 Mg ha^{-1} in LO. As with ONPP, GS $T_{5\text{cm}}$ did not explain a significant amount of variation among plots ($p = 0.35$), whereas PPT$_{\text{GS}}$ had a positive effect ($p = 0.01$).

Aboveground biomass N did not change with time ($F_{1,34} = 0.48$; $p = 0.49$) and was greatest at LO (mean = 406 kg ha^{-1}) and least at NH (mean = 254 kg ha^{-1}). GS $T_{5\text{cm}}$ was not a significant covariate for biomass N (p =0.66); PPT$_{\text{GS}}$ was a significant positive covariate (p =0.04).

Litterfall

Litterfall total C and total N fluxes increased over time and were affected by GS $T_{5\text{cm}}$ and PPT$_{\text{GS}}$, supporting our first hypothesis. Annual litterfall increased with time at a rate of 24 kg C ha^{-1} y^{-1} ($F_{1,94}$= 9.14; $p < 0.001$) (Figure 4A). Litterfall C flux ranged from 1951 kg C ha^{-1} in OP to 1475 kg C ha^{-1} in NH. GS $T_{5\text{cm}}$ was a significant positive covariate for C flux ($p < 0.001$); the warmer the soil temperature the greater the litterfall C flux. PPT$_{\text{GS}}$ did not explain variation in litterfall C flux among years ($p = 0.21$).

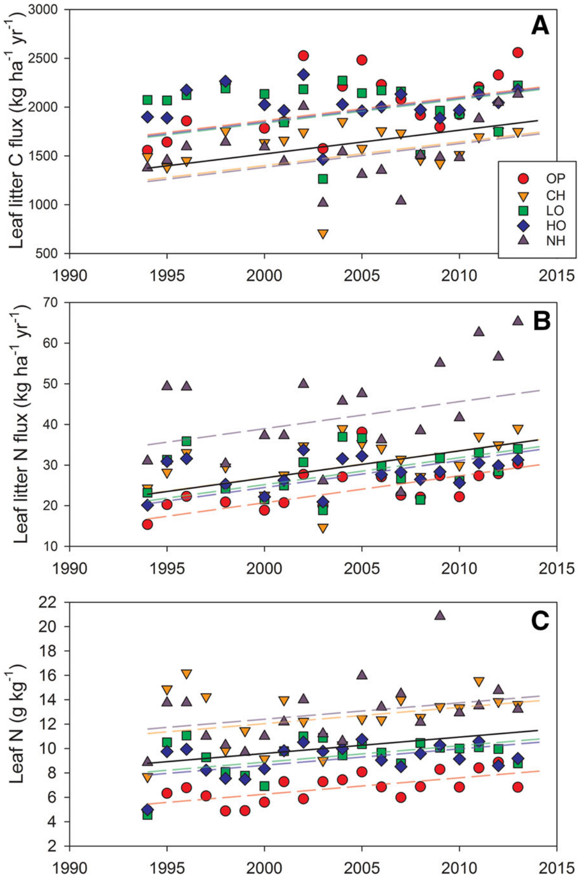

Figure 4. Deciduous leaf litterfall C (kg ha^{-1} y^{-1}) (**A**), deciduous leaf litterfall N (kg ha^{-1} y^{-1}) (**B**), leaf litter N concentration (g kg^{-1}) (**C**) of study plots, mixed-oak pine (*filled circle*), cove hardwood (*filled inverted triangle*), low elevation mixed-oak (*filled square*), high elevation mixed-oak (*filled diamond*), and northern hardwood (*filled triangle*). *Solid line* shows pattern of change over time overall plots and faded *dashed line* for individual plots.

Litterfall total N flux increased over time ($F_{1,94} = 8.92$; $p = 0.004$) (Figure 4B), by an average of 0.70 kg N ha^{-1} y^{-1}. Litterfall N flux was least in OP (23 kg N ha^{-1} y^{-1}), and greatest in NH (41 kg N ha^{-1} y^{-1}). GS $T_{5\text{cm}}$ had a negative effect on N flux ($p = 0.02$), and PPT$_{\text{GS}}$ was a positive covariate ($p = 0.01$); the cooler and wetter the site, the greater litterfall N flux was.

Leaf litterfall N concentrations increased over time ($F_{1,94} = 3.53$, $p = 0.03$) (Figure 4C). Concentrations ranged from 6.71 g kg^{-1} in OP to 1.29 g kg^{-1} in NH. GS $T_{5\text{cm}}$ did not contribute to variability in N concentrations ($p = 0.42$), whereas PPT$_{\text{GS}}$ was a positive covariate ($p = 0.001$).

O-horizon and Coarse Wood Pools

Total O-horizon C and N pools showed no pattern of change over time (C, $p = 0.88$; N, $p = 0.38$), and variation in O-horizon total C and N was not attributed to litterfall C or N flux, leading us to reject our second hypothesis. O-horizon C pools averaged 11.4 Mg ha^{-1}, ranging from 9.3 Mg ha^{-1} in LO to 14.0 Mg ha^{-1} in OP. Variation in O-horizon C was not well explained by $\mathrm{PPT}_{\mathrm{GS}}$ ($p = 0.10$) or GS $T_{5\mathrm{cm}}$ ($p = 0.28$). O-horizon N pools averaged 0.3 Mg N ha^{-1}, ranging from 0.20 Mg ha^{-1} in CH to 0.41 Mg ha^{-1} in NH. In contrast to C pools in the O-horizon, both GS $T_{5\mathrm{cm}}$ ($p = 0.03$) and $\mathrm{PPT}_{\mathrm{GS}}$ ($p = 0.06$) had a negative effect on total N pools.

Total CW volume and mass did not vary over time, (volume; year, $F_{1,14} = 4.04$, $p = 0.06$, mass; year, $F_{1,14} = 3.49$, $p = 0.08$). Total CW mass estimates ranged from 17 Mg ha^{-1} in OP to 45 Mg ha^{-1} in NH. Both GS $T_{5\mathrm{cm}}$ and $\mathrm{PPT}_{\mathrm{GS}}$ had a negative effect on both CW volume (GS $T_{5\mathrm{cm}}$, $p < 0.001$; $\mathrm{PPT}_{\mathrm{GS}}$, $p < 0.001$) and mass (GS $T_{5\mathrm{cm}}$, $p = 0.001$; $\mathrm{PPT}_{\mathrm{GS}}$, $p = 0.002$).

Mineral Soil Total C and N

Total surface mineral soil C and N pools showed no pattern of change over time (Figure 5A; C, $p = 0.57$; N, $p = 0.59$) leading us to accept our third hypothesis. Total C content ranged from 24 Mg ha^{-1} in OP to 58 Mg ha^{-1} in NH, and total N ranged from 0.8 Mg ha^{-1} in OP to 3.7 Mg ha^{-1} in NH. C/N ratio also showed no pattern of change over time ($p = 0.64$) and ranged from 31 in OP to 16 in NH. The cooler the soil temperature, the greater the mineral soil C ($p < 0.001$), N ($p = 0.01$) and C/N ratio ($p = 0.04$) were. $\mathrm{PPT}_{\mathrm{GS}}$ did not explain variation in soil C or N pools.

DISCUSSION

Forest ecosystems contain large pools of total C and play an important role in global C and N cycles and C sequestration (Dixon and others 1994; Lal 2005). We examined up to 20 years of variation in total C and N pools and fluxes across the four major vegetation communities along an elevation gradient in the southern Appalachian Mountains. We examined three hypotheses: (H1) aboveground C and N accumulation, net primary productivity and litterfall flux increase over time and with temperature/precipitation; (H2) organic soil horizon (O-horizon) C and N pools change over these short time scales and are regulated by aboveground C and N inputs; and (H3) surface mineral soil C and N do not change over short time scales but differ among sites with abiotic (precipitation and temperature) variables. Our data showed that ONPP and litterfall fluxes increased over time; O-horizon and mineral surface layer C and N pools, and coarse wood volume and mass did not change over time. Growing season precipitation was positively correlated with variability in ONPP and aboveground C and N pools (biomass). Total litterfall C flux was positively correlated with variability in soil temperature; N flux was negatively correlated with temperature and positively correlated with precipitation. O-horizon and mineral soil C and N pools showed no change over time, and litterfall C or N inputs had no effect on O-horizon C and N. Mineral soil C and N were negatively correlated with temperature across the elevation gradient, and overall, cooler sites had greater soil C and N pools.

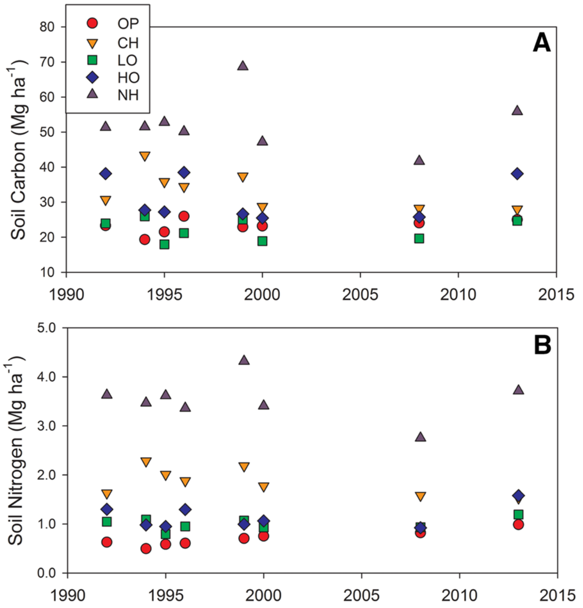

Figure 5. Mineral surface soil (0–10 cm) total C (Mg ha^{-1}) (**A**) and total N (Mg ha^{-1}) (**B**) over time in study plots, mixed-oak pine (*filled circle*), cove hardwood (*filled inverted triangle*), low elevation mixed-oak (*filled square*), high elevation mixed-oak (*filled diamond*), and northern hardwood (*filled triangle*).

Overstory Net Primary Productivity and Litterfall Flux Increased with Time and Temperature and Varied with Precipitation

In our study, ONPP, (Figure 3A) and total C and N litterfall fluxes (Figure 4A) increased over time suggesting these are aggrading forest ecosystems. In the alternative analysis (see Supplemental Material) aboveground biomass C and N also increase with time. We attribute this difference to the relatively short period of measurements (16-years) in terms of forest growth, and a plot size of 0.64 ha in which the mortality of individual trees can affect the outcome. ONPP, overstory biomass C and N pools, and litterfall C and N fluxes increased with growing season precipitation increased. Litterfall N fluxes decreased with soil temperature. The interaction of temperature and precipitation in regulating above- and belowground productivity has been shown in many forested ecosystems (Friedrichs and others 2009; Leppälammi-Kujansuu and others 2014; Elliott and others 2015; Martin-Benito and Pederson 2015). Others have also described the positive relationships between both NPP and litterfall with temperature (Kang and others 2006; Raich and others 2006). We found no relationship between ONPP and temperature, and our analysis of productivity residuals showed interannual variability was not correlated with soil temperature (Figures S1, S2). The lack of detectable response is perhaps due to our bi-annual measurements, relatively small plot size, or differences in the vegetation response. The positive correlation between litterfall N concentrations and $\mathrm{PPT}_{\mathrm{GS}}$ is similar to foliar N responses measured by Crowley and others (2012) along a western NY/PA to Maine gradient that included 718 sites and 12 tree species. Foliar N concentrations also increased with precipitation; N deposition also increases along the elevation gradient at our site and across the eastern US mountains (Swift and others 1988; Swank and Vose 1997; Knoepp and others 2008; Fox and others 2011), the factors are confounded and cannot be separated; however, they suggest that eastern temperate forests are N limited and continue to show a positive growth response to N deposition.

Organic Soil Horizon C and N Pools Do Not Change Over Time and are Not Regulated by the Total Quantity of Aboveground C and N Inputs

We measured temporal increases in litterfall C and N and although sample collection years varied significantly, we found no change in O-horizon C or N pools over time, likely due to high spatial variability and infrequent collections. Unlike studies showing a negative relationship between temperature and O-horizon C due to increased decomposition rates (Raich and others 2006), we found no effect of either GS $T_{5\mathrm{cm}}$ or $\mathrm{PPT}_{\mathrm{GS}}$ on O-horizon C content. GS $T_{5\mathrm{cm}}$ was negatively correlated with O-horizon N, but lack of sample year variability or correlations between residuals and temperature suggests this relationship was due to differences among plots. Earlier work at Coweeta has shown that O-horizon mass increased 28% over 20 years due to declining nutrient concentrations and decomposition rates (Knoepp and Swank 1994; Knoepp and others 2005). During the 20 years of measurement in the present study we found no directional changes in O-horizon mass over time even though litterfall mass and N concentrations were increasing. Our findings contradict that of Garten (2009), who found increased O-horizon mass following experimental increases in litterfall inputs but are consistent with the findings of the DIRT (detritus input and removal treatment) experiment which found no change in soil organic C content after doubling litter input (Crow and others 2009). Federer (1984) examined a northern hardwood forest chronosequence in which stands ranged from 1 to 100 years since disturbance. He estimated that the O-horizon reached a maximum mass after 60–65 years although resampling efforts by Yanai and others (2000) suggest that results from chronosequence studies may lead to erroneous conclusions. It is possible that O-horizon mass and decomposition rates, measured in the early 1970s (Cromack and Monk 1975; Yount 1975), had not reached equilibrium following the mid-1920s forest harvest (Douglass and Hoover 1988) resulting in the O-horizon increase previously found by Knoepp and others (2005) and Knoepp and Swank (1994). This suggests that although interannual variation in temperature and moisture impacts litterfall C and N, neither had significant effect on total O-horizon C and N contents and findings do not support our hypothesis.

Surface Mineral Soil C and N Do Not Change Over Short Time Scales But Differ Among Plots (Vegetation) with Abiotic (Precipitation and Temperature) Variables

Surface mineral soil total C and N concentrations or contents did not change over the 20-year sample period. We found no significant effect of GS $T_{5\mathrm{cm}}$ or $\mathrm{PPT}_{\mathrm{GS}}$ on soil C or N; however, patterns across all plots (Figure 6) suggest that total C and N decreased with increasing soil temperature. This pattern coupled with the differences among plots suggests that total C and N pools are regulated by the long-term interactions of vegetation and climate. As such, plots and the soils in them differ in C content and cycling processes and reach an equilibrium based on soil forming factors (Jenny 1941), referred to by Stewart and others (2008) as the C saturation point. Franzluebbers and others (2001) examined soil organic matter processes across a range of temperature and moisture regimes. They

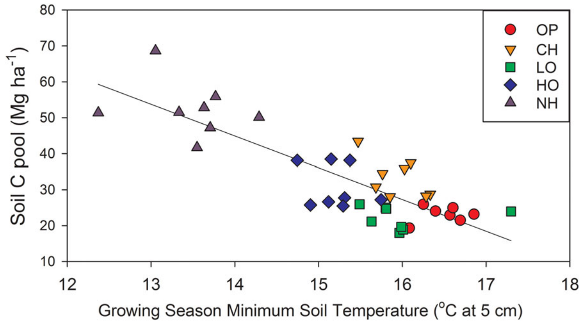

Figure 6. Relationship between mineral surface soil (0–10 cm) C pool (Mg ha^{-1}) and growing season mean minimum soil temperature for all study plots, mixed-oak pine (*filled circle*), cove hardwood (*filled inverted triangle*), low elevation mixed-oak (*filled square*), high elevation mixed-oak (*filled diamond*), and northern hardwood (*filled triangle*). Regression line shows the relationship for all data.

found that soils in thermic regions had lower total C content compared to frigid regions; however, the C present was more biologically active. This supports our finding that both vegetation and climate play a crucial role in soil C, not only in terms of quantity but also quality and cycling.

Soil C represents a major portion of total ecosystem C. Soil C comprised between 25 (mixed-oak pine) and 43% (northern hardwoods) of the total ecosystem C in our study plots (Figure 7). This is similar to the 36% reported in Pacific Northwest old-growth Douglas-fir stands (Harmon and others 2004) and 43% in a northeastern forest (Fahey and others 2005). These C estimates include root biomass calculated by allometric equations (present study) or directly sampled using quantitative pit (Fahey and others 2005), or soil coring (Harmon and others 2004) methods. Belowground C pools respond in complex ways (Davidson and Ackerman 1993; Guo and Gifford 2002) to changing vegetation, site productivity, climate, and microbial communities (Carney and Matson 2005; Allison and others 2010; Keiser and others 2013; Oliverio and others 2017) including altered distribution and stabilization of soil C that may be masked in measurements of total C (Hedde and others 2008; Crow and others 2009; Miltner and others 2012). As a result, examination of forest soil C changes over time to determine the C sequestration potential under a changing climate has yielded variable results. Ten-year measurements in high elevation southern Appalachian spruce-fir forests found sites were at steady state, with 46% of the total C in the mineral and organic soils (50 cm depth) (Van Miegroet and others 2007). Other repeated sampling studies in the region have shown that while deep soil layers may contain more stable or recalcitrant C, the upper mineral soils may be accumulating (Kelly and Mays 2005) or losing C over time (Knoepp and others 2014).

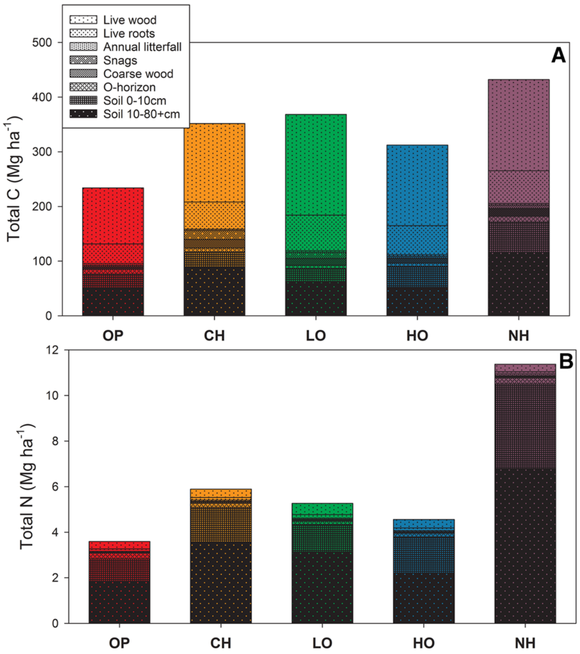

Figure 7. Total C (**A**) and total N (**B**) mass (Mg ha^{-1}) present in all pools measured and estimated during 2013/2014 for all study plots, mixed-oak pine (OP), cove hardwood (CH), low elevation mixed-oak (LO), high elevation mixed-oak (HO) and northern hardwood (NH).

Soil N plays a vital role in ecosystem productivity (Cole 1995; Knicker 2011) and soil C processing and turnover rates (Aber and others 1998; Du and others 2014; Boot and others 2016). Although there are few reports of the soil profile total N, our estimates (ranging from 3.2 Mg ha^{-1} in mixed-oak pine to 10.9 Mg ha^{-1} in northern hardwood, Figure 7) are similar to soil profiles in northern hardwood sites in New Hampshire (6.2–9.4 Mg ha^{-1}) (Vadeboncoeur and others 2012). Soil represented the largest total N pool with 86% of the N in the low elevation mixed-oak and 96% in the northern hardwood plot (Figure 7). This is a greater proportion of total ecosystem N than was found in northern hardwood forests of northeastern USA, with estimates of approximately 70% (Hooker and Compton 2003; Yanai and others 2013). Aboveground site productivity is often restricted by and correlated with soil N availability (Fernandez and others 2000; White and others 2004). Our data do not support this, study plots with the lowest N availability (mixed-oak pine and low elevation mixed-oak), as indicated by litterfall N concentration, had the lowest and highest aboveground biomass pool, respectively (Knoepp and Swank 1998; Knoepp and others 2000).

Ecosystem Regulation of C Sequestration

Forest ecosystems accumulate C and N in aboveground long-lived woody material and in belowground coarse roots, O-horizon, and mineral soils, the result of complex interactions between aboveground and belowground diversity, inputs, microbial community composition, and decomposition processes (Wardle and others 2004; Crow and others 2009; Qiao and others 2014; Bani and others 2018). Overstory woody biomass C ranged from 97 Mg ha^{-1} in mixed-oak pine to 175 Mg ha^{-1} in the low elevation mixed-oak (Figure 7); soil profile C pools (O-horizon plus mineral soil to 90+ cm) were similar, ranging from 84 Mg ha^{-1} in the mixed-oak pine to 182 Mg ha^{-1} in the northern hardwood plot. These pool sizes are similar to estimates of other complete ecosystem studies as are the distributions of total C to above and belowground; soil pools make up between 40 and 43% of the total ecosystem C (Dixon and others 1994; Hooker and Compton 2003; Harmon and others 2004; Fahey and others 2005; Van Miegroet and others 2007). Conversely, in comparison with the few research studies reporting total ecosystem N pools (Hooker and Compton 2003; Yanai and others 2013), our study plots sequester a greater proportion of ecosystem N belowground. Patterns of above- and belowground N accumulation may have resulted from greater N deposition in the northeast (NADP 2017) stimulating vegetation growth (Fernandez and others 2000) and increasing soil N leaching losses (Brookshire and others 2007) or due to differences in forest plant and microbial species composition (Cross and Perakis 2011; Bani and others 2018), and resultant patterns of organic matter decomposition (Zak and others 2008; Jacobs and others 2018).

Variation in C storage among ecosystems has been attributed to differences in overstory canopy structure, tree form, and mycorrhizal association (Frangi and others 2005; Jacobs and others 2018), C quality (Hooper and others 2000; Midgley and others 2015), C:element ratio stoichiometry (that is, N concentrations and availability) effects on decomposition and food webs (Hessen and others 2004; Tipping and others 2016), environmental regulation of plant productivity (Raich and others 2006), and microbial community and processes regulating organic matter decomposition (Akselsson and others 2005; Keiser and others 2013; Maaroufi and others 2017). Forest ecosystem N represents inputs of atmospheric N as deposition or N_2-fixation, the release of N from soil organic matter derived from leaf, wood, fine and coarse root inputs during decomposition and mineralization (Alban 1982; Cole 1995), and direct uptake of organic N by mycorrhizal associations (Hobbie and others 1999, 2000). In general, temperate forest ecosystems have low N background conditions (Aber and others 1998) and research has shown that N additions may increase overstory and belowground C sequestration by altering microbial C processing rates (Du and others 2014). Lovett and Goodale (2011) describe two prerequisites for ecosystem N uptake, the capability of vegetation and soils to process N inputs (kinetic) and the presence of N sinks in soils and vegetation (capacity). A positive growth response to N additions indicates the presence of N capacity, whereas a stream N response indicates the exceedance of kinetic processing (Lovett and Goodale 2011). All plots in our study had increasing ONPP, increasing overstory N pools, and increasing litterfall C and N fluxes over time suggesting the continued presence of N capacity. However, the presence of nitrate N in soil solution below the rooting zone ($>$ 60 cm) suggests plot differences in N kinetic processing (Knoepp and others 2000, 2008). An N sequestration model developed by Berg and Dise (2004) supported the concept of N capacity, sites with N-rich litter sequestered more N in response to N availability. This is supported by our results in which deciduous leaf litter N concentrations (Figure 4) are greatest in plots with greater total soil N (Figure 5), and total ecosystem N (Figure 7). We also found that litterfall N concentrations increased over time, suggesting soil N availability is increasing and N kinetic conditions are changing. Previous research at Coweeta provides additional evidence that N kinetic conditions are changing including, long-term increases in stream inorganic N concentrations and export from reference watersheds (Swank and Vose 1997; Argerich and others 2013), decreased retention of atmospheric N deposition (Adams and others 2014), and shifting seasonal patterns of stream N exports (Webster and others 2016). Cumulatively, these results suggest that some forest communities within Coweeta may be in initial stages of ecosystem N saturation.

This study showed that overstory productivity increased over time. Patterns of litterfall C and N suggest that productivity is correlated with both temperature and precipitation; however, we did not detect an effect of temperature in net primary productivity based on biennial growth measurements collected over a 16-year period. We found no relationship between O-horizon total C or N pools and litterfall inputs, soil temperature, or precipitation. Surface soil C and N pools did not change over time, however, soil total C decreased with increasing temperature over the 600 m gradient in a pattern confounded with changing vegetation community. Our long-term measurements of C and N pools and fluxes lead us to conclude that plant productivity is sensitive to short-term changes in climate, while belowground processes do not respond on short time scales. Finding similar patterns across a gradient in vegetation community, temperature, and precipitation suggests that changes in climate would eventually alter forest ecosystem C and N pools and cycling.

ACKNOWLEDGEMENTS

We thank the investigators of Coweeta LTER 3 (1990–1996) for contributions to the design of the original gradient study, the technicians and graduate students who have made measurements on these plots periodically throughout the last 20+ years, and Coweeta Analytical Lab managers, Dr. Barbara (Kitti) Reynolds, James Deal and Cindi Brown who have insured high quality control of all analyses. We also thank Drs. Rich Bowden and Luke Nave for helpful comments on the manuscript. This work was funded by NSF Grants DEB0218001 and DEB0823293 to the Coweeta LTER program at the University of Georgia and by USDA Forest Service, Southern Research Station, Coweeta Hydrologic Laboratory project funds. Any opinions, findings, conclusions, or recommendations expressed in the material are those of the authors and do not necessarily reflect the views of the USDA Forest Service.

Compliance with Ethical Standards

Conflict of interest The authors declare that experiments complied with the current laws of the USA and there is no conflict of interest.

REFERENCES

Aber J, McDowell WH, Nadelhoffer K, Magill A, Berntson GM, Kamakea M, McNulty S, Currie WS, Rustad L, Fernandez I. 1998. Nitrogen saturation in temperate forest ecosystems. BioScience 48:921–34.

Adams MB, Knoepp JD, Webster JR. 2014. Inorganic nitrogen retention by watersheds at fernow experimental forest and coweeta hydrologic laboratory. Soil Sci Soc Am J 78:S84–94.

Akselsson C, Berg B, Meentemeyer V, Westling O. 2005. Carbon sequestration rates in organic layers of boreal and temperate forest soils—Sweden as a case study. Glob Ecol Biogeogr 14:77–84.

Alban DH. 1982. Effects of nutrient accumulation by aspen, spruce, and pine on soil properties. Soil Sci Soc Am J 46:853–61.

Allison SD, Wallenstein MD, Bradford MA. 2010. Soil-carbon response to warming dependent on microbial physiology. Nat Geosci 3:336–40.

Argerich A, Johnson S, Sebestyen S, Rhoades C, Greathouse E, Knoepp J, Adams M, Likens G, Campbell J, McDowell W. 2013. Trends in stream nitrogen concentrations for forested reference catchments across the USA. Environ Res Lett 8:014039.

Bani A, Pioli S, Ventura M, Panzacchi P, Borruso L, Tognetti R, Tonon G, Brusetti L. 2018. The role of microbial community in the decomposition of leaf litter and deadwood. Appl Soil Ecol. 126:75–84.

Bardgett RD, Bowman WD, Kaufmann R, Schmidt SK. 2005. A temporal approach to linking aboveground and belowground ecology. Trends Ecol Evol 20:634–41.

Bardgett RD, Mommer L, De Vries FT. 2014. Going underground: root traits as drivers of ecosystem processes. Trends Ecol Evol 29:692–9.

Berg B, Dise N. 2004. Validating a new model for N sequestration in forest soil organic matter. Water Air Soil Pollut Focus 4:343–58.

Boot CM, Hall EK, Denef K, Baron JS. 2016. Long-term reactive nitrogen loading alters soil carbon and microbial community properties in a subalpine forest ecosystem. Soil Biol Biochem 92:211–20.

Brookshire ENJ, Valett HM, Thomas SA, Webster JR. 2007. Atmospheric N deposition increases organic N loss from temperate forests. Ecosystems 10:252–62.

Caldwell PV, Miniat CF, Elliott K, Swank WT, Brantley ST, Laseter SH. 2016. Declining water yield from forested mountain watersheds in response to climate change and forest mesophication. Glob Change Biol 22:2997–3012.

Carney KM, Matson PA. 2005. Plant communities, soil microorganisms, and soil carbon cycling: does altering the world belowground matter to ecosystem functioning? Ecosystems 8:928–40.

Clark JS, Vose JM, Luce CH. 2016. Forest drought as an emerging research priority. Glob Change Biol 22:2317.

Cole DW. 1995. Soil nutrient supply in natural and managed forests. Plant Soil 168–169:43–53.

Cromack J, K., Monk CD. 1975. Litter production, decomposition, and nutrient cycling in a mixed hardwood watershed and a white pine watershed. In: Howell FG, Gentry JB, Smith MH, Eds. Mineral cycling in southeastern ecosystems proceedings. Augusta (GA): US Energy Research and Development Administration, pp 609–24.

Cross A, Perakis SS. 2011. complementary models of tree species-soil relationships in old-growth temperate forests. Ecosystems 14:248–60.

Crow SE, Lajtha K, Filley TR, Swanston CW, Bowden RD, Caldwell BA. 2009. Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. Glob Change Biol 15:2003–19.

Crowley KF, McNeil BE, Lovett GM, Canham CD, Driscoll CT, Rustad LE, Denny E, Hallett RA, Arthur MA, Boggs JL, Goodale CL, Kahl JS, McNulty SG, Ollinger SV, Pardo LH, Schaberg PG, Stoddard JL, Weand MP, Weathers KC. 2012. Do nutrient limitation patterns shift from nitrogen toward phosphorus with increasing nitrogen deposition across the northeastern United States? Ecosystems 15:940–57.

Davidson EA, Ackerman IL. 1993. Changes in soil carbon inventories following cultivation of previously untilled soils. Biogeochemistry 20:161–93.

Dixon RK, Brown S, Houghton RA, Solomon A, Trexler M, Wisniewski J. 1994. Carbon pools and flux of global forest ecosystems. Science (Washington) 263:185–9.

Douglass JE, Hoover MD. 1988. History of Coweeta. In: Swank WT, Crossley DA Jr, Eds. Forest hydrology and ecology at Coweeta. New York: Springer. p 469.

Du Y, Guo P, Liu J, Wang C, Yang N, Jiao Z. 2014. Different types of nitrogen deposition show variable effects on the soil carbon cycle process of temperate forests. Glob Change Biol 20:3222–8.

Eisenlord SD, Freedman Z, Zak DR, Xue K, He Z, Zhou J. 2013. Microbial mechanisms mediating increased soil C storage under elevated atmospheric N deposition. Appl Environ Microbiol 79:1191–9.

Elliott KJ, Miniat CF, Pederson N, Laseter SH. 2015. Forest tree growth response to hydroclimate variability in the southern Appalachians. Glob Change Biol 21:4627–41.

Elliott KJ, Swank WT. 2008. Long-term changes in forest composition and diversity following early logging (1919–1923) and the decline of American chestnut (*Castanea dentata*). Plant Ecol 197:155–72.

Elliott KJ, Vose JM. 2011. The contribution of the Coweeta Hydrologic Laboratory to developing an understanding of long-term (1934–2008) changes in managed and unmanaged forests. For Ecol Manag 261:900–10.

Elser JJ, Bracken ME, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–42.

Fahey T, Siccama T, Driscoll C, Likens G, Campbell J, Johnson C, Battles J, Aber J, Cole J, Fisk M. 2005. The biogeochemistry of carbon at Hubbard Brook. Biogeochemistry 75:109–76.

Federer CA. 1984. Organic matter and nitrogen content of the forest floor in even-aged northern hardwoods. Can J For Res 14:763–7.

Fernández-Martínez M, Vicca S, Janssens IA, Sardans J, Luyssaert S, Campioli M, Chapin FSIII, Ciais P, Malhi Y, Obersteiner M. 2014. Nutrient availability as the key regulator of global forest carbon balance. Nat Clim Change 4:471–6.

Fernandez IJ, Simmons JA, Briggs RD. 2000. Indices of forest floor nitrogen status along a climate gradient in Maine, USA. For Ecol Manag 134:177–87.

Fox S, Jackson B, Jackson S, Kauffmann G, Koester MC, Mera R, Seyden T, Van Sickle C, Chipley S, Fox J, Hicks J, Hutchins M, Lichtenstein K, Nolan K, Pierce T, Porter B. 2011. Western North Carolina report card on forest sustainability. Asheville, NC: USDA Forest Service, Southern Research Station. pp 1–198.

Frangi JL, Barrera MD, Richter LL, Lugo AE. 2005. Nutrient cycling in Nothofagus pumilio forests along an altitudinal gradient in Tierra del Fuego, Argentina. For Ecol Manag 217:80–94.

Franzluebbers AJ, Haney RL, Honeycutt CW, Arshad M, Schomberg HH, Hons FM. 2001. Climatic influences on active fractions of soil organic matter. Soil Biol Biochem 33:1103–11.

Frey S, Ollinger S, Nadelhoffer K, Bowden R, Brzostek E, Burton A, Caldwell B, Crow S, Goodale C, Grandy A. 2014. Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. Biogeochemistry 121:305–16.

Friedrichs DA, Trouet V, Büntgen U, Frank DC, Esper J, Neuwirth B, Löffler J. 2009. Species-specific climate sensitivity of tree growth in Central-West Germany. Trees 23:729.

Garten CT. 2009. A disconnect between O horizon and mineral soil carbon–implications for soil C sequestration. Acta Oecol 35:218–26.

Gessner MO, Swan CM, Dang CK, McKie BG, Bardgett RD, Wall DH, Hättenschwiler S. 2010. Diversity meets decomposition. Trends Ecol Evol 25:372–80.

Guo LB, Gifford R. 2002. Soil carbon stocks and land use change: a meta analysis. Glob Change Biol 8:345–60.

Harmon ME, Bible K, Ryan MG, Shaw DC, Chen H, Klopatek J, Li X. 2004. Production, respiration, and overall carbon balance in an old-growth Pseudotsuga-Tsuga forest ecosystem. Ecosystems 7:498–512.

Harmon ME, Franklin JF, Swanson FJ, Sollins P, Gregory SV, Lattin JD, Anderson NH, Cline SP, Aumen NG, Sedell JR, Lienkaemper GW, Cromack JK, Cummins KW. 1986. Ecology of coarse woody debris in temperate ecosystems. In: MacFadyen A, Ford ED, Eds. Advances in ecological research. Cambridge: Academic Press. p 133–302.

Hedde M, Aubert M, Decaëns T, Bureau F. 2008. Dynamics of soil carbon in a beechwood chronosequence forest. For Ecol Manag 255:193–202.

Hessen DO, Ågren GI, Anderson TR, Elser JJ, de Ruiter PC. 2004. Carbon sequestration in ecosystems: the role of stoichiometry. Ecology 85:1179–92.

Hobbie EA, Macko SA, Shugart HH. 1999. Interpretation of nitrogen isotope signatures using the NIFTE model. Oecologia 120:405–15.

Hobbie EA, Macko SA, Williams M. 2000. Correlations between foliar δ15N and nitrogen concentrations may indicate plant-mycorrhizal interactions. Oecologia 122:273–83.

Hobbie SE. 2015. Plant species effects on nutrient cycling: revisiting litter feedbacks. Trends Ecol Evol 30:357–63.

Hooker TD, Compton JE. 2003. Forest ecosystem carbon and nitrogen accumulation during the first century after agricultural abandonment. Ecol Appl 13:299–313.

Hooper DU, Bignell DE, Brown VK, Brussard L, Mark Dangerfield J, Wall DH, Wardle DA, Coleman DC, Giller KE, Lavelle P. 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks: we assess the evidence for correlation between aboveground and belowground diversity and conclude that a variety of mechanisms could lead to positive, negative, or no relationship-depending on the strength and type of interactions among species. BioScience 50:1049–61.

Jacobs LM, Sulman BN, Brzostek ER, Feighery JJ, Phillips RP. 2018. Interactions among decaying leaf litter, root litter and soil organic matter vary with mycorrhizal type. J Ecol 106:502–13.

Jenny H. 1941. Factors of soil formation: a system of quantitative pedology. New York, NY: McGraw-Hill Book Company Inc.

Johnson DW, Knoepp JD, Swank WT, Shan J, Morris LA, Van Lear DH, Kapeluck PR. 2002. Effects of forest management on soil carbon: results of some long-term resampling studies. Environ Pollut 116:S201–8.

Kang S, Lee D, Lee J, Running SW. 2006. Topographic and climatic controls on soil environments and net primary production in a rugged temperate hardwood forest in Korea. Ecol Res 21:64–74.

Keiser A, Knoepp JD, Bradford M. 2013. Microbial communities may modify how litter quality affects potential decomposition rates as tree species migrate. Plant Soil 372:167–76.

Kelly J, Mays P. 2005. Soil carbon changes after 26 years in a Cumberland Plateau hardwood forest. Soil Sci Soc Am J 69:691–4.

Knicker H. 2011. Soil organic N-An under-rated player for C sequestration in soils? Soil Biol Biochem 43:1118–29.

Knoepp JD, Coleman DC, Crossley DA Jr, Clark JS. 2000. Biological indices of soil quality: an ecosystem case study of their use. For Ecol Manag 138:357–68.

Knoepp JD, Reynolds B, Crossley DA Jr, Swank WT. 2005. Long-term changes in forest floor processes in southern Appalachian forests. For Ecol Manag 220:300–12.

Knoepp JD, Swank WT. 1994. Long-term soil chemistry changes in aggrading forest ecosystems. Soil Sci Soc Am J 58:325–31.

Knoepp JD, Swank WT. 1997. Forest management effects on surface soil carbon and nitrogen. Soil Sci Soc Am J 61:928–35.

Knoepp JD, Swank WT. 1998. Rates of nitrogen mineralization across an elevation and vegetation gradient in the southern Appalachians. Plant Soil 204:235–41.

Knoepp JD, Swank WT. 2002. Using soil temperature and moisture to predict forest soil nitrogen mineralization. Biol Fertil Soils 36:177–82.

Knoepp JD, Swank WT, Haines BL. 2014. Long- and short-term changes in nutrient availability following commercial sawlog harvest via cable logging. In: Swank WT, Webster JR, Eds. Long-term response of a forest watershed ecosystem: clearcutting in the southern Appalachians. London: Oxford University Press. p 57–84.

Knoepp JD, Vose JM. 2007. Regulation of nitrogen mineralization and nitrification in southern Appalachian ecosystems: separating the relative importance of biotic vs. abiotic controls. Pedobiologia 51:89–97.

Knoepp JD, Vose JM, Swank WT. 2008. Nitrogen deposition and cycling across an elevation and vegetation gradient in southern Appalachian forests. Int J Environ Stud 65:389–408.

Lal R. 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123:1–22.

Lal R. 2005. Forest soils and carbon sequestration. For Ecol Manag 220:242–58.

Lal R, Follett RF, Kimble JM. 2003. Achieving soil carbon sequestration in the United States: a challenge to the policy makers. Soil Sci 168:827–45.

Laseter SH, Ford CR, Vose JM, Swift JLW. 2012. Long-term temperature and precipitation trends at the Coweeta Hydrologic Laboratory, Otto, North Carolina, USA. Hydrol Res 43:890–901.

Leppälammi-Kujansuu J, Salemaa M, Kleja DB, Linder S, Helmisaari H-S. 2014. Fine root turnover and litter production of Norway spruce in a long-term temperature and nutrient manipulation experiment. Plant Soil 374:73–88.

Liese R, Lübbe T, Albers NW, Meier IC. 2018. The mycorrhizal type governs root exudation and nitrogen uptake of temperate tree species. Tree Physiol 38:83–95.

Lovett GM, Goodale CL. 2011. A new conceptual model of nitrogen saturation based on experimental nitrogen addition to an oak forest. Ecosystems 14:615–31.

Luce CH, Vose JM, Pederson N, Campbell J, Millar C, Kormos P, Woods R. 2016. Contributing factors for drought in United States forest ecosystems under projected future climates and their uncertainty. For Ecol Manag 380:299–308.

Luyssaert S, Schulze E-D, Börner A, Knohl A, Hessenmöller D, Law BE, Ciais P, Grace J. 2008. Old-growth forests as global carbon sinks. Nature 455:213–15.

Maaroufi NI, Nordin A, Palmqvist K, Gundale MJ. 2017. Nitrogen enrichment impacts on boreal litter decomposition are driven by changes in soil microbiota rather than litter quality. Sci Rep 7:4083.

Markewitz D, Sartori F, Craft C. 2002. Soil change and carbon storage in longleaf pine stands planted on marginal agricultural lands. Ecol Appl 12:1276–85.

Martin-Benito D, Pederson N. 2015. Convergence in drought stress, but a divergence of climatic drivers across a latitudinal gradient in a temperate broadleaf forest. J Biogeogr 42:925–37.

Martin JG, Kloeppel BD, Schaefer TL, Kimbler DL, McNulty SG. 1998. Aboveground biomass and nitrogen allocation of ten deciduous southern Appalachian tree species. Can J For Res 28:1648–59.

Midgley MG, Brzostek E, Phillips RP. 2015. Decay rates of leaf litters from arbuscular mycorrhizal trees are more sensitive to soil effects than litters from ectomycorrhizal trees. J Ecol 103:1454–63.

Miltner A, Bombach P, Schmidt-Brücken B, Kästner M. 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111:41–55.

NADP. 2017. (NRSP-3). National Atmospheric Deposition Program Office, Illinois State Water Survey, 2204 Griffith Dr., Champaign, IL 61820.

Oliverio AM, Bradford MA, Fierer N. 2017. Identifying the microbial taxa that consistently respond to soil warming across time and space. Glob Change Biol 23:2117–29.

Portmann RW, Solomon S, Hegerl GC. 2009. Spatial and seasonal patterns in climate change, temperatures, and precipitation across the United States. Proc Natl Acad Sci 106:7324–9.

Prescott CE. 2005. Do rates of litter decomposition tell us anything we really need to know? For Ecol Manag 220:66–74.

Qiao Y, Miao S, Silva LCR, Horwath WR. 2014. Understory species regulate litter decomposition and accumulation of C and N in forest soils: a long-term dual-isotope experiment. For Ecol Manag 329:318–27.

Raich JW, Russell AE, Kitayama K, Parton WJ, Vitousek PM. 2006. Temperature influences carbon accumulation in moist tropical forests. Ecology 87:76–87.

Reich PB, Luo Y, Bradford JB, Poorter H, Perry CH, Oleksyn J. 2014. Temperature drives global patterns in forest biomass distribution in leaves, stems, and roots. Proc Natl Acad Sci 111:13721–6.

Richter DD, Markewitz D, Trumbore SE, Wells CG. 1999. Rapid accumulation and turnover of soil carbon in a re-establishing forest. Nature 400:56–8.

Ryan MG, Vose JM, Hanson PJ, Iverson LR, Miniat CF, Luce CH, Band LE, Klein SL, McKenzie D, Wear DN. 2014. Forest processes. Climate change and United States forests. Berlin: Springer. pp 25–54.

SAS. 2013. SAS/STAT 9.3 User's Guide. Cary, NC USA: SAS Institute Inc.

Schlesinger WH, Dietze MC, Jackson RB, Phillips RP, Rhoades CC, Rustad LE, Vose JM. 2015. Forest biogeochemistry in response to drought. Glob Change Biol 22:2318–28.

Stewart CE, Paustian K, Conant RT, Plante AF, Six J. 2008. Soil carbon saturation: evaluation and corroboration by long-term incubations. Soil Biol Biochem 40:1741–50.

Swank WT, Vose JM. 1997. Long-term nitrogen dynamics of Coweeta forested watersheds in the southeastern United States of America. Glob Biogeochem Cycles 11:657–71.

Swift LW Jr, Cunningham BB, Douglass JE. 1988. Climatology and hydrology. In: Swank WT, Crossley DA Jr, Eds. Forest hydrology and ecology at Coweeta. New York, NY: Springer. p 35–55.

Tipping E, Somerville CJ, Luster J. 2016. The C: N: P: S stoichiometry of soil organic matter. Biogeochemistry 130:117–31.

Vadeboncoeur MA. 2010. Meta-analysis of fertilization experiments indicates multiple limiting nutrients in northeastern deciduous forests. Can J For Res 40:1766–80.

Vadeboncoeur MA, Hamburg SP, Blum JD, Pennino MJ, Yanai RD, Johnson CE. 2012. The quantitative soil pit method for measuring belowground carbon and nitrogen stocks. Soil Sci Soc Am J 76:2241–55.

Van Miegroet H, Moore PT, Tewksbury CE, Nicholas NS. 2007. Carbon sources and sinks in high-elevation spruce-fir forests of the Southeastern US. For Ecol Manag 238:249–60.

Wardle DA, Bardgett RD, Klironomos JN, Setala H, van der Putten WH, Wall DH. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629–33.

Webster J, Knoepp J, Swank W, Miniat C. 2016. Evidence for a regime shift in nitrogen export from a forested watershed. Ecosystems 19:1–15.

White LL, Zak DR, Barnes BV. 2004. Biomass accumulation and soil nitrogen availability in an 87-year-old *Populus grandidentata* chronosequence. For Ecol Manag 191:121–7.

Yanai RD, Arthur MA, Siccama TG, Federer CA. 2000. Challenges of measuring forest floor organic matter dynamics: repeated measures from a chronosequence. For Ecol Manag 138:273–83.

Yanai RD, Vadeboncoeur MA, Hamburg SP, Arthur MA, Fuss CB, Groffman PM, Siccama TG, Driscoll CT. 2013. From missing source to missing sink: long-term changes in the nitrogen budget of a northern hardwood forest. Environ Sci Technol 47:11440–8.

Yount JD. 1975. Forest-floor nutrient dynamics in southern Appalachian hardwood and white pine plantations ecosystems. In: Howell FG, Gentry JB, Smith MH, Eds. Mineral cycling in southeastern ecosystems proceedings. Augusta, GA: U.S. Energy Research and Development Administration, p 898.

Zak DR, Holmes WE, Burton AJ, Pregitzer KS, Talhelm AF. 2008. Simulated atmospheric NO_3—deposition increases soil organic matter by slowing decomposition. Ecol Appl 18:2016–27.