



Will more nitrogen enhance carbon storage in young forest stands in central Appalachia?



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ABSTRACT

Many temperate deciduous forests in the Eastern US are secondary, regrowing forests and have experienced decades of elevated inputs of acidic compounds and biologically available nitrogen (N) from the atmosphere. These young forests play an important role in the global carbon (C) cycle as C sinks, and it is possible that acidic deposition will influence the strength and longevity of this sink. We used the Fernow Experimental Forest Long Term Soil Productivity (LTSP) experiment near Parsons, WV to evaluate how 13 years of experimental N additions has affected ecosystem C storage and stand level dynamics in a young temperate deciduous forest. Specifically we examined whether N additions: (1) directly increased aboveground growth of regenerating trees but did so in a way that was independent of the indirect effects of soil acidification; (2) directly (independent of acidification effects) increased forest floor and soil C pools, and decreased the fine root C pool; and (3) lowered stand density and diversity. We also tested whether tree species were differentially affected by either N fertilization or soil acidification.

Thirteen years of ammonium sulfate additions to a regenerating deciduous forest stimulated C storage by 22% – even in a region with historically high levels of atmospheric N deposition. This response was driven primarily by a 27% increase in C storage in aboveground biomass and to a lesser extent by a 35% increase in C stored in the smaller forest floor pool. Despite the dominance of a single tree species (*Prunus pensylvanica*), the overall response may have been tempered by reductions in stand density and was only detectable when the changes in all species were included, rather than when only the changes in the dominant species were examined. Indirect acidification effects were found to increase C accumulation in the forest floor and decrease the number of different tree species.

In the short-term it is likely that N deposition will stimulate forest growth and C storage in young temperate deciduous forests. However, given the differential responses observed for longer-lived tree species versus the positive response for short-lived species, it appears that the long-term effects of N deposition on C storage in temperate deciduous forests may be different than the short-term effects, and may even be negative.

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1. Introduction

The residual terrestrial carbon (C) sink is the implied uptake by terrestrial ecosystems of the ~28% (~2.6 Pg C/yr) of anthropogenic carbon dioxide (CO₂) emissions that cannot be accounted for by atmospheric accumulation or oceanic uptake (Houghton et al., 1998; Le Quere et al., 2012; Pan et al., 2011). Currently, we think that a portion of this residual C uptake occurs on land in the northern temperate latitudes (Ciais et al., 1995). Furthermore, forests in these latitudes, such as those in the Appalachian Mountains

of Eastern US, may be responsible for a large portion of the sink (Pan et al., 2011; Schimel, 1995).

Several mechanisms have been proposed for the creation and maintenance of a temperate forest C sink. Increased forest growth following logging and abandoning of agricultural land, as well as the maintenance of secondary, regrowing forests by various silvicultural practices have been implicated as primary contributors to the enhanced C uptake (Goodale et al., 2002). Increased tree growth in response to increasing levels of atmospheric CO₂ may also be responsible for a portion of the C sink (Ainsworth and Long, 2005). Similarly, a fertilization effect from the biologically available nitrogen (N) in acidic deposition may have increased growth of N-limited forest trees (Aber and Driscoll, 1997; Thomas et al., 2010).

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Acidic deposition supplies ecosystems with biologically available forms of N at rates that are substantially greater than pre-industrial levels (Galloway et al., 1976, 1987). Despite recent reductions in acid deposition in the Eastern US due to the Clean Air Act, the levels of N in precipitation in this region are still above pre-industrial levels and likely will remain elevated for some time (Baumgardner et al., 2002; Dentener et al., 2006). Additionally, in other temperate forests, especially in Asia, levels of N deposition are likely to continue to rise in the future (Galloway et al., 2004). However, the effect of acidic deposition on the residual terrestrial C sink is more complex than the effects of forest regrowth or CO₂ fertilization because short-term benefits, such as the alleviation of N limitation, could be diminished, or reversed, by longer-term effects such as the depletion of base cations, lower soil pH, and higher levels of toxic aluminum (Al³⁺) (Aber et al., 1998).

In the last twenty years there has been a great deal of research on the effect of N deposition on forest C storage, and results vary. Modeling studies (Aber and Driscoll, 1997) and N addition experiments (Magill et al., 2004; Pregitzer et al., 2008), as well as more complex analyses combining models and field measurements (Magnani et al., 2007; Ollinger et al., 2008), have provided evidence that N deposition may enhance the terrestrial C sink – some of which occurs in forest soils. However, ¹⁵N tracer studies (Nadelhoffer et al., 1999; Templer et al., 2012) have questioned the likelihood of an increase in aboveground C storage due to elevated N inputs. Additionally, some field studies (Boggs et al., 2005; Magill et al., 1996) have shown a neutral or mixed response to N inputs, and some (Elias et al., 2009; May et al., 2005; McNulty et al., 2005) have even shown declines in forest growth due to N saturation with chronic N inputs. This range of results is likely due to the complex and non-linear responses that can develop during chronic acidification and their dependence on forest history and soil characteristics.

The timing of a shift from a positive to a negative response in aboveground tree C storage due to elevated N inputs may vary with forest type, land-use history, and soil properties. In general, it appears that coniferous forests are more sensitive to enhanced N inputs than deciduous forests (Aber et al., 1998; Magill et al., 2004) and older forests are less likely to utilize N inputs than young, fast-growing forests (Fenn et al., 1998; Vitousek and Reiners, 1975). A further complication is the likelihood that once N availability exceeds biological demand (N saturation), different soils will be more, or less, resilient to leaching of calcium (Ca²⁺) and magnesium (Mg²⁺), and to soil acidification. Specifically, trees growing in soils derived from predominantly sandstone parent materials will probably be more susceptible to negative effects than trees growing on soils derived from limestone containing parent material.

Numerous temperate deciduous forests of the Eastern US are secondary, regrowing forests and may be vulnerable to the effects of elevated N inputs because they are not only underlain by base-poor, sandstone-derived soils but have also experienced a significant amount of timber harvest which can permanently remove large quantities of nutrient cations (Adams et al., 2000; Federer et al., 1989). Thus, although these young forests should initially be able to retain N due to a positive response in tree growth, once N saturation develops, the negative effects could come relatively quickly and be difficult to reverse.

In addition to the direct positive or negative changes on tree growth induced by chronically elevated N deposition, it is also possible that elevated N deposition could result in long-lived alterations in forest C sequestration by changing stand dynamics or species composition. Little research has focused on the effect of N inputs on stand-level changes in regenerating forests where differential species responses to nutrient level changes could alter competitive relationships and community structure in later succes-

sional stages (Adams, 2003). However, work in old fields (Stevens and Carson, 1999), and in grasslands and heathlands (Tilman, 1993; Vitousek et al., 1997) demonstrated that elevated N inputs can significantly change community level variables and lead to lower diversity. Research in temperate deciduous forests using a N deposition gradient (Boggs et al., 2005), forest inventory data (Thomas et al., 2010), and watershed fertilization (DeWalle et al., 2006; May et al., 2005) has shown that different tree species respond differently to elevated N. Also, more fertile sites have accelerated self-thinning resulting in fewer, larger trees than less fertile sites (Stevens and Carson, 1999). It therefore seems likely that, given enough time, acidic deposition will change stand dynamics and/or tree species composition in temperate deciduous forests in ways that could alter their capacity to sequester atmospheric C.

Although aboveground growth in temperate deciduous forests is often considered to be responsive to N additions, increasing N inputs can also alter belowground processes. For example, it has been observed that increased N availability in forests is associated with reduced fine root biomass and the amount of C stored in this pool (Jia et al., 2010; Nadelhoffer, 2000; Nadelhoffer et al., 1985). N inputs may also affect the composition of the soil microbial community and the expression of extracellular enzymes in ways that enhance C storage in the litter layer (DeForest et al., 2005; Fog, 1988; Knorr et al., 2005; Zak et al., 2008) and soil (Ramirez et al., 2012; Whittinghill et al., 2012) of forests. Thus, the response of the temperate deciduous forest C sink to N deposition will be the sum of both the above- and belowground pool responses.

The Fernow Experimental Forest Long Term Soil Productivity Experiment (LTSP) near Parsons, WV (Adams et al., 2004) provides a unique opportunity to evaluate how experimental N additions, and the associated soil acidification, have affected ecosystem C storage and stand level dynamics in a temperate deciduous forest during the first 13 years of forest regeneration. Using this experiment, we were able to test several hypotheses based on our current understanding of how N additions alter the ability of forests to sequester C. Specifically, we examined whether N additions: (1) directly increased aboveground growth of regenerating trees but did so in a way that was independent of the indirect effects of acidification (e.g., loss of nutrient cations, lower soil pH, and/or elevated Al³⁺); (2) directly (independent of acidification effects) enhanced forest floor and soil C pools, and lowered the fine root C pool; and (3) lowered stand density and diversity. We also examined whether tree species were differentially affected by either N fertilization or soil acidification.

2. Materials and methods

This study was done in the Fernow Experimental Forest LTSP plots near Parsons, WV (39°04'N, 79°41'W) (Adams et al., 2004). The location of the Fernow LTSP experiment has a SE aspect, and slopes between 15% and 31%. It is situated in the Allegheny Mountain subsection of the Appalachian Physiographic Province and has an elevation range of 798–847 m. Soils are classified as loamy-skeletal, mixed active, mesic typic Dystrudepts and the parent materials are sandstone colluvium, sandstone residuum, and weathered shale. Prior to the initiation of the experiment in 1996, the most recent logging activity occurred in ~1910, and most trees were ~85 years old. The forest community before the experiment started was classified as a central Appalachian mixed hardwood forest. *Acer saccharum*, *Quercus rubra*, *Acer pensylvanicum*, and *Prunus serotina* together accounted for 2/3 of the total importance value in this forest, and there were 584 stems/ha (0.06 stems/m²) and 312 metric ton/ha (31.2 kg/m²) of aboveground tree biomass. There was 1.5 kg/m² of forest floor, and the average C content of the forest floor was 45%. The top 15 cm of

mineral soil averaged a pH of 4.24 and 6.58% C content. Prior to initiation of the experiment, there was no “treatment” effect on soil C or N for any horizon. Likewise, there was not a pre-treatment “treatment” effect on aboveground biomass or foliar N concentration except for a trend in foliar N for *Acer rubrum* ($p=0.054$) (Adams et al., 2004).

The Fernow LTSP experiment is a randomized block design containing four blocks each with four plots that receive different treatments (Fig. 1). Each treatment plot is 0.4 ha in size (60.9 m on a side) and includes a 7.6-m wide buffer strip in which no samples were collected. The sampled area in each plot (referred to as a “growth plot”) is 0.2 ha in area. When the plots were established, a numbered grid of permanent stakes was added to facilitate plot location and consistent sampling (Adams et al., 2004).

The four treatments in the Fernow LTSP study are: (1) reference plots (Uncut) that were last harvested around 1910, and that received no experimental manipulation as part of the Fernow LTSP study; (2) cut plots (Ambient) that were subjected to a whole-tree harvest in 1996 in which all aboveground biomass was removed; (3) cut and fertilized plots (+N) that were whole-tree harvested in 1996 and since that time have received annual applications of 35 kg N/ha as ammonium sulfate to enhance N inputs and accelerate soil acidification; and (4) cut, fertilized, and limed plots (+N+Ca) that were treated the same as the +N plots and that have also received dolomitic lime every other year at an annual rate of 22.5 kg Ca/ha and 11.6 kg Mg/ha since the initiation of the study. Ambient inputs of NH_4^+ , NO_3^- , and SO_4^{2-} for 1996 at a nearby location were 2.83 kg N/ha, 5.26 kg N/ha, and 10.7 kg S/ha, respectively (NADP, 2000). By 2008, Ambient inputs of NH_4^+ , NO_3^- , and SO_4^{2-} at

the same location had fallen to 2.27 kg N/ha, 2.56 kg N/ha, and 6.16 kg S/ha, respectively (NADP, 2009).

In this study, we only considered harvested plots (Ambient, +N, +N+Ca) because we were concerned with the effects of acidic deposition on an aggrading forest as well as separating the N response from the acidity response. All of these treatment plots underwent a whole tree harvest in 1996 (complete removal of all aboveground biomass, which was the second major logging event for the forest) and were allowed to regenerate naturally. These plots are also located on soils derived from base-poor parent materials and in a region with a history of acidic deposition and logging (Adams et al., 2004). We included plots receiving lime additions because liming should mitigate the negative effects of acidification and allow us a unique opportunity to distinguish the direct effects of N inputs from the indirect effects of soil acidification caused by fertilizer additions.

To estimate and compare the ecosystem C storage in these regrowing forests, we measured the C pools in aboveground tree biomass, litterfall, the forest floor, mineral soil, and fine roots after 13 years of experimental treatments. All measurements were taken in summer of 2008 or 2009, except for litterfall, which was measured in autumn of 2009.

To determine the effect of the treatments on C storage in aboveground tree biomass, we measured diameter at breast height (DBH) of 3278 live trees in mid-summer 2009, noted the species, and used species-specific allometric equations to estimate tree biomass. To sample trees in the various treatments, we divided each plot into twelve squares of known area, randomly selected six of those squares, and recorded the DBH and species of all trees within the area of the square (Fig. 1). Converting DBH to biomass was done following Johnson et al. (2010). For each tree, we used Fernow-specific parameters, or parameters based on Brenneman et al. (1978), in the following allometric equation: $\text{biomass} = a(\text{DBH})^b$. Biomass estimates for each tree were then used to calculate aboveground C pools by following the general assumption that biomass had a 50% C content (Lamloom and Savidge, 2003; Thomas and Martin, 2012). We used a 50% conversion factor for all species to convert biomass to C. This assumption, while generally held, may result in C stock estimates being in error by ~5% (Thomas and Martin, 2012) and could be improved if interspecific differences, volatile C, and treatment effects are accounted for (Lamloom and Savidge, 2003; Thomas and Martin, 2012). However, individual estimates for C content of most species fall near one another and near 50%.

To detect any response of the forest floor C content to the treatments, we divided each plot into 192 squares and sampled the forest floor from the center of 12 randomly chosen squares per plot in mid-summer 2008 (Fig. 1). For each sample the entire forest floor (O_i , O_e , and O_d) was collected from a 10×10 cm area. Samples were dried (65°C for >48 h), weighed, ground, and analyzed for C concentration by Dumas combustion (Nelson and Sommers, 1982) using a Carlo Erba NA 1500 N, C, S elemental analyzer. We then calculated the average forest floor C content per unit area for each plot by using the C content and mass of the forest floor samples.

Litterfall was also measured to assess if any treatment differences in the forest floor mass might be attributable to treatment effects on the input of litter. We placed 5 plastic “laundry” baskets measuring 35.5×52 cm as litter collectors per plot. We anchored the litter collectors securely to the permanent growth plot stakes and randomly chose 5 of the 20 possible stakes for use (Fig. 1). The collectors were placed in the field before leaves began to fall in autumn 2009, and a single collection of leaves was made when all leaves had fallen. All baskets had small drainage holes drilled in the bottom; and leaves from each collector were dried (65°C for >48 h), weighed, and the average mass of litterfall per unit area was calculated for each plot.

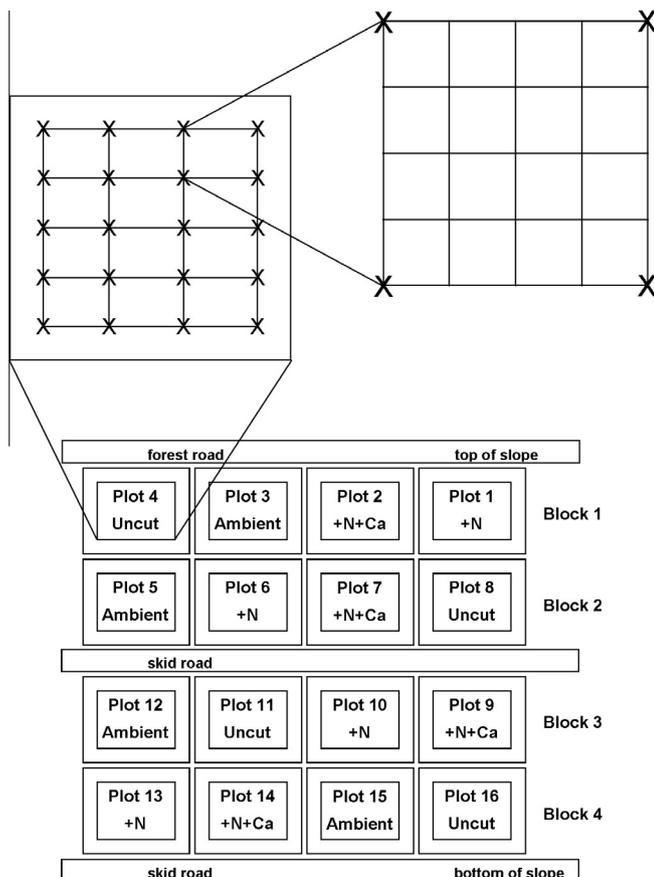


Fig. 1. The LTSP plots at Fernow Experimental Forest. The x's in the expanded views represent the permanent stakes, and the expanded views illustrate how plots were divided into 12 or 192 squares for sampling purposes (Adams et al., 2004).

We collected and analyzed soil samples to determine the effect of the treatments on the soil C pool in the top 5 cm of soil. Soil cores (2.3 cm in diameter and 5 cm long) were collected in one sampling effort over the course of one week in mid-summer 2008 at 16 locations per plot, selected by dividing the plot into 192 squares and randomly choosing 16 (Fig. 1). At each location, the top 5 cm of the mineral soil was sampled due to very rocky soils. The soil cores were sieved to remove all roots and rocks, dried (65 °C for >48 h), ground, and analyzed for C concentration using a Carlo Erba NA 1500 N, C, S elemental analyzer. The average soil C mass per unit area in the top 5 cm of soil was then calculated using the C content values and reported soil bulk density values from Adams et al. (2004).

We also measured the effect of treatments on the fine root (<2 mm diameter) C pool in the top 10 cm of soil by collecting soil cores from each plot in the Fernow LTSP and sorting out and analyzing the roots. We only went down to 10 cm because of very rocky soil. We collected 15 soil cores per plot, each 4.5 cm in diameter and 10 cm long, by dividing the plots into 192 squares and randomly choosing 5 locations where we collected 3 cores (Fig. 1). All cores were collected in one sampling effort over the course of one week in mid-summer 2009, and, for consistency, all fine roots from each soil core were removed from the soil cores by the same individual. The roots were then dried (65 °C for >48 h), weighed, ground, and analyzed for C concentration following the same procedure used for forest floor and mineral soil samples. Total fine root C in the top 10 cm of soil per unit area was then calculated for each sample and averaged per plot.

The species census data collected during the aboveground tree biomass estimation was also used to compare community diversity and composition, and to look for species-specific effects of the treatments. Species richness and the Shannon's Index (SI) of diversity were calculated for each plot. The relative importance value (RIV), an average of the relative basal area and relative abundance for each species, was calculated across all plots and treatments and for each plot separately. Finally, the average size of individual trees and the number of trees per unit area were calculated for all species and for each species separately.

We used analysis of variance (ANOVA) to determine if increasing N inputs altered the amount of C stored in various pools of an aggrading temperate deciduous forest. For each variable measured, we set up a two-way ANOVA with the main factors being treatment and block (no within block replication prevents the inclusion of a treatment * block interaction). Each plot per treatment was a single replicate ($n = 4$) and the average value for each variable was used for each plot. If significant effects of either factor (treatment or block) were found, then *post hoc* Tukey–Kramer tests were run. In order to test whether the effect of treatment depended on species, we set up an additional ANOVA for the dependent variables biomass per tree, tree density, and RIV including species as a factor with the four most common species being considered. In this ANOVA, we included treatment, species, block, and treatment * species as effects. An alpha level of 0.05 was used throughout to assess statistical significance and p values <0.1 were considered trends. Assumptions of ANOVA, including normality of residuals and homogeneity of variances were tested, and any necessary transformations were performed. All statistical tests were performed using SAS JMP version 10.

3. Results

After 13 years of continuous treatment there are detectable differences in soil acidity and N availability. Specifically, the soil pH was significantly lower in the +N treatments (3.76) than in the Ambient (4.12) or +N+Ca (4.01) treatments ($p = 0.01$). Additionally,

foliar N for *Prunus pensylvanica* (the dominant tree species) was higher in both plots receiving additional N than in the Ambient plots ($p < 0.01$). Specifically, *P. pensylvanica* foliage in the Ambient plots averaged 2.5% N, while in the +N and +N+Ca plots the averages were 3.13% and 3.02% N, respectively (Fowler, 2014).

In response to these treatments we found that total measured C storage (aboveground + forest floor + fine roots top 10 cm + mineral soil top 5 cm) in a regrowing temperate deciduous forest was greater in plots receiving additions of ammonium sulfate (Fig. 2). Total measured C storage was 22% higher in +N plots and 18% higher in +N+Ca plots ($p = 0.02$). These differences correspond to an additional 1140 g C/m² in the +N treatment and 950 g C/m² in the +N+Ca treatment relative to the Ambient treatment. If we consider the total amount of N added across the duration of the treatments, then the +N treatment resulted in an additional C sequestration of 25 g C/g N added and the +N+Ca treatment resulted in an additional C sequestration of 21 g C/g N added.

The greater C storage in plots receiving ammonium sulfate was driven by higher aboveground tree biomass C ($p = 0.045$) and a higher forest floor C content ($p < 0.01$). There were no significant changes in fine root C pools ($p = 0.38$) or soil C pools ($p = 0.28$) (Fig. 3). The C in aboveground tree biomass was 27% greater in the +N and 25% greater in the +N+Ca plots when compared to Ambient plots (Fig. 3). The C pool in the forest floor was 35% greater in the +N plots (Fig. 3). The top 5 cm of mineral soil averaged 5.1% C and the forest floor averaged 35.6% C.

We estimate that aboveground tree biomass stored a total of 3.04 kg C/m² (234 g C/m²/yr) in the Ambient treatment plots, 3.87 kg C/m² (298 g C/m²/yr) in the +N plots, and 3.82 kg C/m² (294 g C/m²/yr) in the +N+Ca plots (Fig. 3). When compared to the Ambient treatment, the change in C storage in aboveground tree biomass due to the +N treatment was 0.83 kg C/m² or 18 g C/g N added, and that for the +N+Ca treatment was 0.78 g C/m² or 17 g C/g N. Using a 50% conversion factor, values for biomass would be twice those for C.

The greater response of C storage in aboveground tree biomass to additions of ammonium sulfate was the net effect of a greater average size of individual trees ($p = 0.01$) that may have been partially offset by an insignificant reduction in tree density ($p = 0.14$) (Fig. 4). The average tree size was 62% greater in the +N and 73% greater in the +N+Ca plots when compared to Ambient plots. Estimates of tree densities were 0.67, 0.53, and 0.50 stems/m² for the Ambient, +N, and +N+Ca plots, respectively. There was no change due to treatment in the mass of autumn litterfall, which ranged from 0.22 to 0.24 kg/m²/yr ($p = 0.74$).

With respect to tree species composition, there were 21 different species encountered, 4 of which accounted for more than 5% each of the total RIV (Table 1). These species were *P. pensylvanica* (PRPE), *Liriodendron tulipifera* (LITU), *Betula lenta* (BELE), and

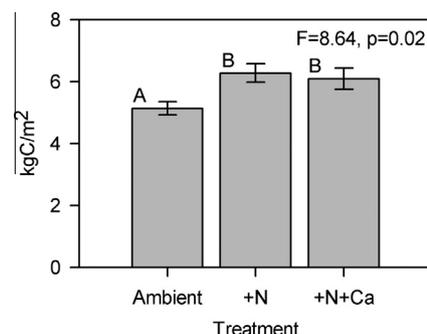


Fig. 2. Average total measured C pool after 13 years of treatment. Total measured C pool includes aboveground tree biomass, the forest floor, fine roots in the top 10 cm, and the top 5 cm soil. Error bars are one standard error, and bars with different letters had significantly different means at $p < 0.05$ in *post hoc* analysis.

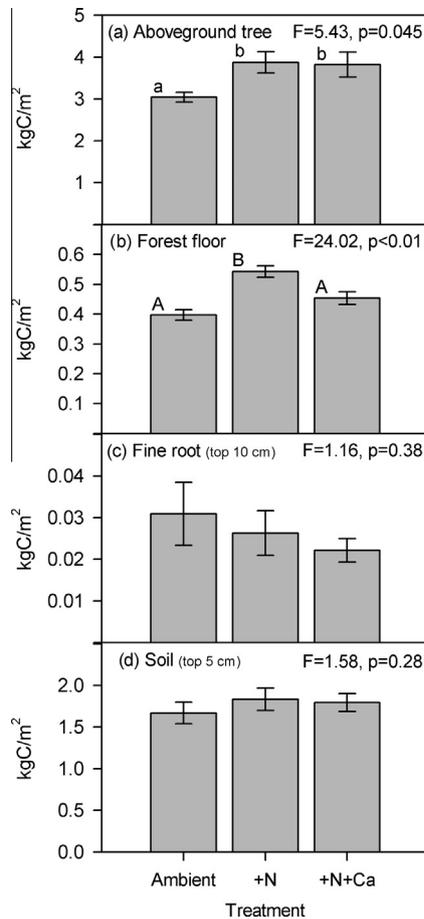


Fig. 3. Average C pools after 13 years of treatment. Error bars are one standard error, values with different capital letters are significantly different ($p < 0.05$), and those with different lowercase letters show a trend towards differences ($p < 0.1$) in *post hoc* analyses.

P. serotina (PRSE). *P. pensylvanica* was the most dominant tree in the LTSP plots, with 51.9% of the total RIV. At the scale of the total area sampled in each plot (6 “squares” per plot \times 81 m² per square = 0.486 ha per plot), the species richness of live trees was significantly lower in the +N treatment which had ~2 fewer species than the Ambient and +N+Ca plots ($p = 0.02$) (Fig. 5). Two species that were absent only in the +N plots were *A. saccharum* (ACSA) and *Quercus prinus* (QUPR). However, there was no difference in tree diversity as measured by Shannon’s Index due to the treatments ($p = 0.89$). Shannon’s Index values ranged from 1.57 to 1.68.

The effect of the treatments on biomass per tree depended on species when the four most important (each >5% of total RIV)

Table 1

A list of all species found, the abbreviation used to represent each of them, and the total relative importance value (RIV) for each. The relative importance value for each species was calculated regardless of treatment.

Species	Abbreviation	Overall RIV
<i>Prunus pensylvanica</i>	PRPE	0.519
<i>Liriodendron tulipifera</i>	LITU	0.128
<i>Betula lenta</i>	BELE	0.124
<i>Prunus serotina</i>	PRSE	0.075
<i>Acer rubrum</i>	ACRU	0.042
<i>Acer pensylvanicum</i>	ACPE	0.023
<i>Quercus rubra</i>	QURU	0.016
<i>Aralia spinosa</i>	ARSP	0.016
<i>Robinia pseudoacacia</i>	ROPS	0.014
<i>Magnolia acuminata</i>	MAAC	0.010
<i>Magnolia fraseri</i>	MAFR	0.009
<i>Acer saccharum</i>	ACSA	0.005
<i>Carpinus caroliniana</i>	CACA	0.004
<i>Ostrya virginiana</i>	OSVI	0.004
<i>Tilia americana</i>	TIAM	0.003
<i>Sassafras albidum</i>	SAAL	0.002
<i>Fraxinus americana</i>	FRAM	0.002
<i>Quercus prinus</i>	QUPR	0.001
<i>Betula alleghaniensis</i>	BEAL	0.001
<i>Oxydendrum arboreum</i>	OXAR	0.001
<i>Ilex montana</i>	ILMO	<0.001

species (*P. pensylvanica*, *L. tulipifera*, *B. lenta*, and *P. serotina*) were considered ($p = 0.04$) (Fig. 6). Furthermore, while the slope of the *P. pensylvanica* and *P. serotina* biomass responses to N additions were positive, those of *L. tulipifera* and *B. lenta* were negative or neutral (Fig. 6). When only these four species were considered, the treatment effect on biomass per tree was no longer detectable ($p = 0.18$). There was also an effect of species on the biomass of individual trees ($p < 0.01$), with *P. pensylvanica* having about three times greater biomass per tree than the other species which were not different from one another. Excluding *P. pensylvanica* from the analysis yielded similar results with the effect of treatment depending on species for the remaining three species ($p = 0.047$), yet no treatment effect ($p = 0.29$) or species effect ($p = 0.16$).

For both tree density and RIV, the effect of treatment did not depend on species ($p = 0.83$, $p = 0.39$, respectively). There was no detectable treatment effect on RIV ($p = 0.19$), but there was a trend toward a lower tree density of the four most dominant species due to ammonium sulfate addition ($p = 0.06$). Both tree density and RIV differed by species ($p < 0.01$, both), however, with *P. pensylvanica* having larger values than the other species, which did not differ from one another. There were over twice as many *P. pensylvanica* trees per area as the other three species, and *P. pensylvanica* accounted for about five times as much of the RIV as the other species.

The only statistical model for which there were significant deviations from a normal distribution for residuals was the one

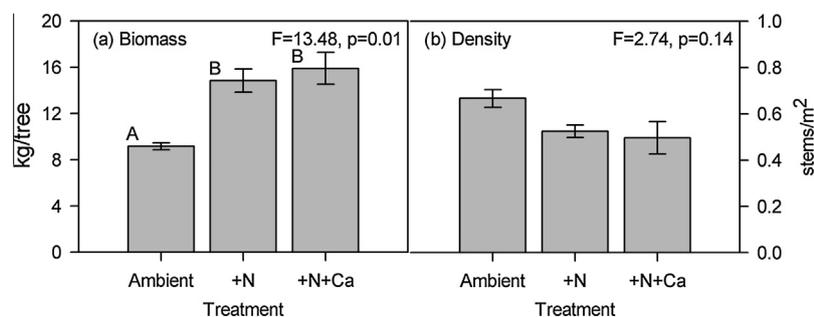


Fig. 4. Average biomass per tree and density of trees per area after 13 years of treatment, regardless of species. Error bars are one standard error, and bars with different letters were found to have different means in *post hoc* analysis.

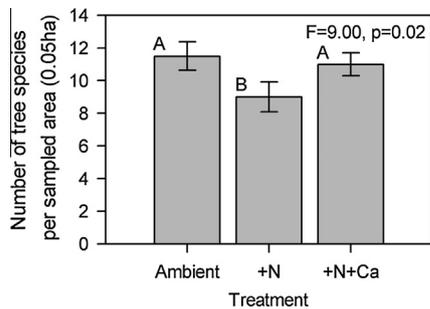


Fig. 5. Average species richness of trees in total area sampled per plot after 13 years of treatment. Error bars are one standard error, and bars with different letters had significantly different means in *post hoc* analysis.

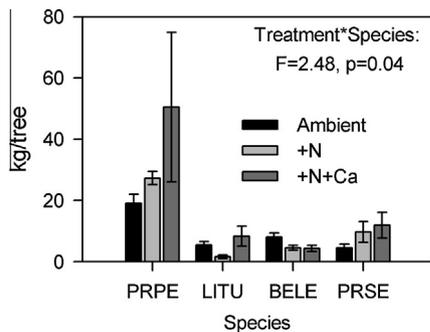


Fig. 6. Average biomass per tree after 13 years of treatment by species. Each of the species which individually make up more than 5% of the total RIV are considered. Error bars are one standard error. Data graphed are untransformed data, but statistics were done on log transformed data. (PRPE = *Prunus pensylvanica*, LITU = *Liriodendron tulipifera*, BELE = *Betula lenta*, PRSE = *Prunus serotina*).

including species as a factor and having individual tree biomass as a dependent variable. In this case, a log transformation resulted in a distribution of residuals which was not significantly different from normal, and statistics were done on the log transformed data. We found block effects to be significant only for soil C pools ($p = 0.02$). Block 1 had significantly higher C content than Block 4, neither of which differed from Blocks 2 or 3.

4. Discussion

Fertilization with ammonium sulfate resulted in an increased input of both bioavailable N and S. In this study, we interpreted any changes due solely to ammonium sulfate additions to be only a consequence of enhanced N, rather than enhanced S availability. We feel this is reasonable and parsimonious because: (1) N is present at much greater levels than S in temperate deciduous forest trees (Vitousek et al., 1988); (2) the lack of available N often limits, or co-limits, growth in terrestrial forests (Elser et al., 2007; Lebauer and Treseder, 2008; Reich et al., 1997); (3) in regions with elevated S deposition (such as West Virginia), atmospheric S inputs exceed forest S requirements (Vitousek et al., 1988); and (4) a previous fertilization experiment at the Fernow Experimental Forest found that mature trees were N limited in 1970 (Auchmoody and Smith, 1977).

Overall, the results from this study support the hypothesis that additional inputs of N to a young forest can, at least initially, enhance C sequestration by stimulating C_g C storage in aboveground tree growth and in the forest floor – even for a forest located in a region with historically high levels of atmospheric N deposition. Treatment comparisons further suggested that enhanced storage in aboveground biomass was a direct result of N on tree growth, whereas enhanced C storage in the forest floor resulted from the indirect effects of N addition on soil or plant

factors that could be mitigated by lime additions. In contrast to our expectations, N additions (either with or without lime) did not significantly alter the amount of C in fine roots in the top 10 cm of soil in a year with precipitation equal to the 50-year average or the C content in the upper 5 cm of mineral soil.

Quantitatively, the average enhancement rate of C storage in aboveground tree biomass between 1996 and 2009 was ~60–64 g C/m²/yr (~17 g C/g N), and was similar to rates (~50 g C/m²/yr) reported for a hardwood forest in Michigan after 10 years of N fertilization with 30 kg N/ha/yr as NH₄NO₃ (Pregitzer et al., 2008). Furthermore, these results were qualitatively consistent with other studies of hardwood forests in Massachusetts (Magill et al., 2004), Wisconsin (Nadelhoffer et al., 1985), and throughout the Eastern US (Thomas et al., 2010) that all reported a stimulation of forest tree growth due to N addition or greater levels of soil N availability – although the magnitude of change reported in these studies varies widely from ~5 g C/g N in Massachusetts to ~61 g C/g N for forests throughout the Eastern US.

A greater C storage in the forest floor in response to additions of only ammonium sulfate, combined with the lack of any difference in litterfall mass, suggests a reduction in litter decomposition in the +N plots. This interpretation is also consistent with slower litter decay rates measured in a nearby watershed (Fernow WS 3) that has received ammonium sulfate additions since 1989 in the same annual amounts as the Fernow LTSP experiment (Adams and Angradi, 1996). However, a unique insight from this study was that the treatment-induced change in the amount of forest floor C was not detectable when lime was added along with ammonium sulfate (+N+Ca plots). This liming effect provides evidence that changes in litter decay can result from soil acidification rather than the direct effect of N additions alone. And since other studies reporting a reduction in forest floor decomposition with N addition did not have a liming treatment (Hobbie, 2008; Magill and Aber, 1998; Pregitzer et al., 2008), it is possible that at least some of their results were an indirect effect of N addition due to soil acidification.

In contrast with the results from several other studies, we did not detect lower amounts of fine root biomass in the top 10 cm of soil (Nadelhoffer, 2000; Nadelhoffer et al., 1985) or elevated levels of C in the upper 5 cm of mineral soil in N-fertilized plots (Edwards et al., 2011; Pregitzer et al., 2008). However, a study of chronic additions of ammonium sulfate to a more mature forest in a nearby watershed (Fernow WS 3 last cut in 1970) suggests that the expected differences in both fine roots and soil C may emerge in time. A recent comparison (Peterjohn, unpublished data) between Fernow WS 3 and an adjacent, unfertilized forest of similar age (Fernow WS 7) found that the fertilized forest had ~50% fewer fine roots to a depth of 15 cm (~150 vs. ~300 g/m²), and although the soil C pools in the upper 5 cm of mineral soil in Fernow WS 3 were not significantly greater ($p = 0.14$), the C concentrations were (~5.8 vs. ~5.3% C; $p = 0.047$). It is likely that our method of measuring fine roots in this study was too coarse spatially and temporally to see through natural variation in fine root dynamics and observe treatment effects. However, it does provide an estimate of the small size of this pool relative to the others measured. It is also possible that treatment-induced changes in root or soil C occurred deeper within the soil.

Our estimate of forest C storage (aboveground + roots + soils) per unit of N input (~21–25 g C/g N) fell below other estimates. For example, Erisman et al. (2011) reviewed studies of total C storage in forests and heathlands and found a range of 35–65 g C/g N. Earlier reviews (Liu and Greaver, 2009; Reay et al., 2008) estimated that C storage responses to N addition fall in the range of 40–200 g C/g N added. However, the 200 g C/g N added value (Magnani et al., 2007) has been questioned and the range is now considered to be 30–75 g C/g N added (de Vries et al., 2008; Sutton et al., 2008) – still well above our estimate. Low values of total C storage per unit of N

input for our experiment, and for any fertilization experiment using large N additions relative to ambient inputs, are understandable since the photosynthetic capacity and increment growth of forest trees are known to saturate above a certain level of N input (Fleischer et al., 2013; Mitchell and Chandler, 1939). Thus, especially in regions with high amounts of background N deposition, comparisons of unfertilized forests to those receiving high doses of added N should yield a conservative estimate of C storage per unit of N addition.

Interestingly, if values for C storage efficiency are assumed to be constant over the range of actual atmospheric N deposition as in Thomas et al. (2010), then recent declines in N emissions (Kim et al., 2006) and deposition (Burns et al., 2011) observed in the Eastern US due to the Clean Air Act should diminish this region's contribution to the terrestrial C sink. Likewise, any reductions of N deposition in Europe resulting from the Gothenburg protocol should also diminish the terrestrial C sink in forests of that region, and the majority of the N-stimulated C sink in temperate forests is likely to shift towards Asia where N deposition most likely will continue to increase for the foreseeable future (Dentener et al., 2006). More generally, if actions were taken to lower the impacts of acid deposition and associated N deposition in affected temperate forests, then one undesirable consequence might be a diminished terrestrial C sink, and a resultant increase in the atmospheric fraction of CO₂ emissions from human activities.

Among the more long-lasting impacts of enhanced N inputs to forest ecosystems can be changes in the structure, composition, and diversity of forest trees. Regarding changes in stand structure, it was interesting to note that the ~66% increase in the amount of C stored in the wood of individual trees due to N additions (Fig. 4) may have been partially offset by an insignificant difference in stand density which resulted in only a ~33% increase in the amount of C stored in the wood on a per unit area basis (Fig. 3 if wood is 50% C). This offset indicates that measurements on an individual tree basis could be misleading and may inflate estimates of C storage actually realized on a per area basis. The acceleration of self-thinning – transition to larger and fewer trees – in more fertile sites is well known (Harper, 1977; Stevens and Carson, 1999) and suggests that its potential to diminish the impact of N-stimulated growth on overall C storage in aboveground biomass may be widespread.

With respect to the composition of trees, of the 21 tree species found in the Fernow LTSP plots, pin cherry (*P. pensylvanica*) was clearly dominant. This species was the most abundant, had the largest individual trees, and accounted for at least 40% of the total relative importance value (RIV) on each treatment plot. Surprisingly, however, despite the predominance of *P. pensylvanica*, the overall treatment effects on biomass per tree were only detectable when the changes in all species were included (Fig. 3a), rather than when we examined only the changes in the dominant species (Fig. 6). This surprising result is likely due to the higher, but real, variation found in the biomass of *P. pensylvanica* growing in limed plots.

The response of tree species other than *P. pensylvanica* may be especially important because *P. pensylvanica* is a short lived (25–30 years), early successional species in temperate deciduous forests of the Eastern US (Marks and Bormann, 1972; Marks, 1974). Thus, the response of later successional species in these regenerating stands may significantly influence the long-term effects of N additions on overall C storage as these forests age. Indeed, no *P. pensylvanica* was found in the mature forest that was cut to initiate the LTSP experiment (Adams et al., 2004). The effect of N addition on the 3 most dominant, longer-lived species (*B. lenta*, *L. tulipifera*, and *P. serotina*) was significantly different than that for *P. pensylvanica*. *L. tulipifera* appears to show a negative response to soil acidification that may be reversible through the addition of lime. Although lime additions have not been made in the nearby watershed fertilization experiment (Fernow WS 3), it has been documented that individual

L. tulipifera trees grew more slowly than those in a reference watershed (Fernow WS 7) after 7 years of ammonium sulfate additions (DeWalle et al., 2006; May et al., 2005). In contrast, the biomass of *B. lenta* in the Fernow LTSP plots was negatively affected by N additions with, or without, lime additions suggesting that the direct effect of N was more important for this species than any indirect effect due to N-induced soil acidification.

With respect to species diversity at the scale of the total area sampled in each plot (0.486 ha), this is the only experimental study we are aware of that has documented a N-induced reduction in the richness of trees species (Fig. 5). While not dramatic (a reduction of ~2 species in the +N plots), this result supported other observations from the Fernow Experimental Forest that reveal: (1) temporal reductions in tree species diversity in areas with a high, but not with a low, site index (Schuler, 2004); and (2) an inverse relationship in two reference watersheds between tree species richness in small plots (10-m radius) and a measured index of soil N availability ($r = -0.63$; Peterjohn, unpublished). It is also interesting to note that no treatment effect was found for Shannon's Index of diversity, and plots receiving both ammonium sulfate and lime showed no detectable change in species richness. Importantly, the apparent effect of lime additions suggests that reductions in the number of tree species was an indirect effect of N additions on soil acidification or other factors that could be ameliorated through the addition of lime, such as the replacement of base cations and/or a reduction in the levels of soluble Al³⁺.

Comparisons to the pre-treatment values for biomass, species composition, and soil variables are of limited value since these values were measured in a forest made up of 85 year old trees and the current forest is made up of 13 year old trees. The number of stems per area in the current forest was much greater, and the total aboveground tree biomass much lower, for instance, as would be expected. The forest floor mass, soil pH, and soil C content were also different, but not different enough to raise suspicion. The species composition was also different due to different stand age, but all major species present in the current forest were found in the pre-treatment seedbank (Adams et al., 2004).

In summary, 13 years of ammonium sulfate additions to a regenerating deciduous forest stimulated its ability to store C, even in a region with historically high levels of atmospheric N deposition. This response was driven primarily by increased C storage in aboveground biomass and to a lesser extent by increased C stored in the forest floor. Despite the dominance of a single tree species, the overall response may have been tempered by reductions in stand density and was only detectable when the changes in all species were included, rather than when only the changes in the dominant species were examined. Unlike other studies, indirect effects of N additions that could be mitigated by lime additions were found to influence C accumulation on the forest floor as well as the number of tree species, suggesting that the interpretation of results from fertilization experiments need to consider the indirect effects of soil acidification that may co-occur with the direct effects of N additions. Finally, given the differential responses observed for longer-lived tree species versus the positive response for short-lived species, it appears that the long-term effects of N deposition on C storage in temperate deciduous forests may be different than the short-term effects and may even be negative.

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