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Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA

Alison H. Magill[a,*], John D. Aber[a], William S. Currie[b], Knute J. Nadelhoffer[c], Mary E. Martin[a], William H. McDowell[d], Jerry M. Melillo[e], Paul Steudler[e]

[a]*Complex Systems Research Center, University of New Hampshire, Durham, NH 03824, USA*
[b]*School of Natural Resources and Environment, University of Michigan, Ann Arbor, MI 48109, USA*
[c]*Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA*
[d]*Department of Natural Resources, University of New Hampshire, Durham, NH 03824, USA*
[e]*The Ecosystem Center, Marine Biological Laboratory, Woods Hole, MA 02354, USA*

Abstract

Humans have altered the global and regional cycles of nitrogen more than any other element. Alteration of N cycling patterns and processes in forests is one potentially negative outcome of accelerated N deposition worldwide. To assess potential impacts of N deposition on temperate forests, a series of chronic nitrogen additions in two contrasting forest types (red pine plantation and mixed hardwood stand) were designed as a core experiment of the Harvard Forest (HF) Long-term Ecological Research (LTER) program. This paper describes the chronic N experimental study site in detail and presents the long-term baseline measurements established at the beginning of treatments in 1988.

Results reported here continue or accelerate trends presented in previous papers. Losses of inorganic N remain high in the high N plots (higher in pines than hardwoods) and low N plots in the pine stand also have measurable DIN losses. Foliar and fine root N concentrations are elevated significantly. Mortality of red pine reached 56% by 2002 in the pine high N plot, and biomass accumulation has stopped altogether. The high N hardwood stand shows increased ANPP, but excess N availability and a severe drought in 1995 contributed to mortality of 72% of red maple trees by 2002. Species importance and litterfall patterns were altered in several plots after 1995. Roots, foliage and wood have diminished as net sinks for added N, re-emphasizing the role of soils in N retention. Two mechanisms for large net retention of added N were suggested in a review paper in 1998. Of these, abiotic immobilization is supported by a growing set of papers, while assimilation and re-exudation by mycorrhizae is suggested by increased DON concentrations.

Keywords: Ammonium nitrate; Biomass production; Foliar chemistry; Nitrogen deposition; Nitrogen saturation; Soil solution chemistry

1. Introduction

Our introductory paper for this special section (Aber and Magill, 2004) presents a brief review of the literature on global patterns of N deposition, and the long-term effects of this deposition on forests through nitrogen saturation. This paper describes the Chronic N Amendment Study at the Harvard Forest (HF) (Petersham, MA, USA) in detail and presents the long-term baseline measurements established at the beginning of treatments in 1988. Long-term measurements trace the retention and loss

* Corresponding author. Tel.: +1-603-862-4098; fax: +1-603-862-0188.
E-mail address: alison.magill@unh.edu (A.H. Magill).

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of added N, changes in pool size or concentration in different parts of the system, and the effects of N accumulation on plant and microbial processes, all in the context of the nitrogen saturation hypothesis (Aber et al., 1989, 1998). These basic measurements present the context for the detailed studies reported in other papers in the chronic N special section of this issue.

2. Methods

2.1. Study site and experimental design

The chronic nitrogen addition experiment is a core experiment of the Harvard Forest Long-term Ecological Research (LTER), and is located at the Harvard Forest in central Massachusetts (42°30′N, 72°10′W). Two contrasting forest stands were selected for this study, each with different land-use histories that have been well documented as part of the Harvard Forest silvicultural and land management records. The red pine (*Pinus resinosa* Ait.) plantation was established in 1926 and is located on the northwest side of Little Prospect Hill in a well-drained soil at 380 m. According to HF records, the site was previously pastured and planted in apple orchard until approximately 1900 when white pine, gray birch, pin cherry and red maple began seeding in. Undergrowth consisted of blueberry, raspberry and sweet fern. Site preparation for the red pine plantation took place in 1924–1925 when all trees were cut and the slash burned on site. Trees were planted in 1926 and successively weeded through 1938 to remove hardwood ingrowth. By 1944, trees had grown to a mean diameter breast height (DBH) of 10.9 cm and a mean height of 6.4 m. A fire in May 1957 burned close to the southwest corner of the stand and some trees were salvage logged in 1958.

The hardwood stand is located on the east side of Little Prospect Hill and dominated by black and red oak (*Quercus velutina* Lam.; *Q. rubra* L.) with varying amounts of black birch (*Betula lenta* L.), red maple (*Acer rubrum* L.), American beech (*Fagus grandifolia* Ehrh.) and black cherry (*Prunus serotina* Ehrh.). A large section of forest (approximately 10 ha) containing this stand was cleared or partially cut as part of a salvage to recover trees downed or badly damaged by the 1938 hurricane; 40–50% of the trees were estimated to be damaged. Post-salvage slash was piled but not burned and the stand allowed to regenerate without further site preparation. Additional trees from this stand were cut for firewood between 1942 and 1944. Harvard Forest timber cruise records from 1956 classified the area as a mix of red oak–black oak–American beech–red maple–white pine; average saw-log volume (oaks only) was estimated at 3005 boardfeet acre^{-1} or 17.52 m^3 ha^{-1}.

The dominant soil types are stony- to sandy-loams, classified as Typic Dystrochrepts according to soil pit descriptions completed in December 1995. The pine stand soil is a Montauk variant and the hardwood soil is a Canton variant. Historical records described the soils as a Brookfield fine sandy loam. Mean monthly temperatures range from 19 °C in July to −12 °C in January and average annual precipitation is 112 cm distributed relatively evenly throughout the year (climate data available at the Harvard Forest web site http://harvardforest.fas.harvard.edu/). Nitrogen deposition to the forest has been estimated at about 0.8 g m^{-2} per year (0.6 g m^{-2} per year wet and 0.2 g m^{-2} per year dry) by regional extrapolation from National Atmospheric Deposition Program sites (Ollinger et al., 1993), and at 0.66 g m^{-2} per year (including 0.22 g m^{-2} per year as dry deposition) by eddy covariance measurements at the tower site at the Harvard Forest (Munger et al., 1996 and http://www.as.harvard.edu/chemistry/hf/hfnitro.html).

Four 30 m × 30 m plots were established in each stand. Three were designated as control (no N added), low N (5 g N m^{-2} per year) and high N (15 g N m^{-2} per year). An additional plot in each stand was established as an N+S treatment (5 g N m^{-2} per year plus 7.4 g S m^{-2} per year). Amendments began in the spring of 1988. Because no significant differences were observed in baseline measurements between the low N and N+S plots over the first 11 years of the study, the sulfur additions were discontinued at the end of the1998 field season. The N+S plot is now treated as a replicate for the low N treatment. Fertilizer additions are divided into six equal doses and are applied at 4-week intervals from May to September as a concentrated solution of NH_4NO_3. Each plot is divided into 36-5 m × 5 m subplots and only the center 16 subplots are used for sample collection. In 1990

and 1991, isotopically enriched ^{15}N tracers were added to control and low-N plots of each forest stand. Isotopic methods and results to date are presented in Nadelhoffer et al. (2004).

2.2. Nitrate concentrations and fluxes

Samples of soil solution from mineral soil were collected approximately monthly, May through November, from four or five porous cup (tension) lysimeters installed in each plot at 60 cm depth. A tension of −5 KPa was applied to each lysimeter for approximately 24 h, samples were collected and a 20 ml subsample was frozen for subsequent analysis. Collections were attempted over a 7-month growing season, May through November. However, drought conditions precluded soil water collections in mid-summer for most years and reduced the total number of collections to three in 1995 and two in 2001. Samples of soil solution from the O horizon were collected with zero-tension lysimeters; results to date are presented in McDowell et al. (2004).

Lysimeter samples were filtered using a Whatman GF/F 0.7 μm filter and analyzed for NO_3-N and NH_4-N using a Bran & Luebbe (Technicon) TrAAcs 800 Autoanalyzer. Ammonium was analyzed using the Berthelot Reaction chemistry (Technicon Method 780-86T); nitrate was determined using hydrazine sulfate reduction (Technicon Method 782-86T). Detection limits for both nitrate-N and ammonium-N are 0.2 mg L^{-1} using these techniques. Dissolved inorganic nitrogen (DIN) was determined as the sum of nitrate and ammonium.

DIN flux below the rooting zone was calculated using monthly water drainage flux estimates from the PnET model (Aber et al., 1995a) parameterized with climatic data from the Harvard Forest. Flux estimates allow for calculation of total annual flux (sum of average monthly flux as g N m^{-2} per year) to complement mean annual concentration data (mean of average monthly concentration as mg N L^{-1}) for all years from 1989 through 2002. In addition, samples from 1999, 2000 and 2001 were bulked (due to low sample volume) by lysimeter within season and measured for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) using a Shimadzu TOC-5000 analyzer with a NDIR detector for DOC and an Antek chemiluminescent detector for TDN (Merriam et al., 1996). Dissolved organic nitrogen (DON) was calculated as follows: DON = TDN − DIN. Final numbers were averaged across all seasons and years to get a ''grand mean'' representing mean monthly concentration value. One-way ANOVA with Scheffe mean separation test was used to look at differences between treatments within the 1999–2001 sample period. Two-tailed *t*-test was used to compare grand means for the 1993–1996 data (Magill et al., 2000) and the 1999–2001 data.

2.3. Soil mass and carbon and nitrogen content

Measurements of soil carbon and nitrogen content were made in 1988, 1992, 1996, 1999 and 2002 on soils collected as part of either N mineralization studies, or root collections. Cores were collected to a depth of 10 cm (20 cm in 1999), split into organic and mineral horizons, sieved (3.5 mm) to remove roots and stones, air dried, and pulverized. Prior to CN analysis, samples were re-dried overnight at 70 °C then cooled in an evacuated desiccator before weighing. Sample weights were between 2–10 mg for organic soils and 10–30 mg for mineral soils. C and N concentrations were measured by dry combustion on five to nine samples per plot per collection.

In 1999, a separate set of samples was collected specifically for determining soil bulk density. A total of 49 samples were collected from each plot using a 2.5 cm diameter soil corer. Samples were collected volumetrically, and an estimate of horizon depth was obtained. Each sample was separated into O, A and B horizons, dried and weighed to obtain a bulk density measurement. Samples were not sieved prior to weighing and therefore include roots. Final values were reported as g m^{-2}.

An estimate of soil mass for the organic horizon and top 10 cm of the mineral soil was needed to convert C and N concentration to total pools. In previous papers, a mean dry soil core weight value (CoreDryWt) was calculated for 270–288 cores per horizon from each plot, collected between 1988 and 1991 as follows: [total sieved core weight (g)] × [wet weight to dry weight conversion]/[area of soil core collector (m^2)]. The 1988–1993 data were compared with soil mass estimates calculated on 1999–2002 soil cores and with the bulk density numbers collected in 1999. Least

significant difference (LSD) was used to compare mass values across time given as:

$$s\frac{\sqrt{2}}{n}t_{h(n-1)}$$

where s is the within-sample estimate of σ_0 and $h(n-1)$ is the number of degrees of freedom of this estimate (Snedecor and Cochran, 1978; Miller and Miller, 1988).

2.4. Foliar chemistry

Fresh foliage was collected during the last week of July or first week of August each year from 1989 through 1999. Samples from 12 or 20 trees were pooled into four or five samples in the hardwood and pine stand, respectively, for all samples collected through 1998. In 1999, leaves or needles were not pooled. Red pine was the only species collected in the pine stand. In the hardwood stand, black/red oak (hereafter referred to collectively as black oak) and red maple were collected all years; black birch was not collected after 1998. Only black oak foliar chemistry is reported here as it is the dominant species, comprising 60–80% of annual litterfall across all treatments. Previous data were reported in Magill et al., 2000.

Fresh foliage samples were air-dried, then ground using a Wiley mill with a 1 mm mesh screen; fresh weights were not recorded. Ground samples were dried overnight at 70 °C and analyzed for nitrogen, lignin, and cellulose content using near-infrared (NIR) spectroscopy (McLellan et al., 1991; Bolster et al., 1996).

2.5. Litterfall

Foliar litterfall was collected three times per year, on or near 1 June, 1 September and 15 November, from each of nine plastic baskets (0.2345 m^2 in size) per plot. Litter samples were sorted by species, dried for 48 h at 70 °C, weighed and ground using a Wiley mill with a 1 mm mesh screen. Ground samples were dried overnight at 70 °C and analyzed for nitrogen, lignin, and cellulose content using near-infrared (NIR) spectroscopy (McLellan et al., 1991; Bolster et al., 1996). Only a partial collection was made in 1988 (November) and is therefore not reported in this dataset. Litterfall biomass reported in figures includes leaves only; litter biomass used in NPP calculations includes all aboveground material except bark. One-way ANOVA with Scheffe mean separation test was used to determine changes in litterfall mass over time and between treatments.

2.6. Woody biomass production and mortality

All trees greater than 5 cm in diameter were tagged at 1.5 m above ground level in 1988 with numbered aluminum tags and DBH was measured 2.5 cm above the tag in October 1988. Tree diameters were re-measured in November 1990, November 1993, January 1997, March 2000 and March 2003. As measurements represent year-end standing biomass, spring measurements refer to biomass at the end of the previous year, i.e. January 1997 = 1996; March 2000 = 1999; March 2003 = 2002. Aboveground woody biomass was calculated from tree diameter measurements using allometric equations (Pastor et al., 1984; Nadelhoffer et al., 1985). In 1996, 1999 and 2002, ingrowth trees were tagged and tallied.

Visual observations of tree death made it apparent that there was a need for a mortality analysis. For each measurement year, we determined whether each tree was alive or dead from field observations or by comparing change in DBH between years. If DBH decreased or stayed the same for two consecutive measurement periods, that tree was considered dead in the first year that DBH did not increase. If DBH did not increase for one measurement period but increased the following measurement period, the tree was considered alive (Table 1). Once year of death had been determined, and ingrowth trees tallied, three combinations of aboveground woody biomass were compared:

$$\text{live} + \text{dead} + \text{ingrowth} = \text{total cumulative wood biomass production}$$

$$\text{live} + \text{dead} - \text{ingrowth} = \text{total cumulative production of trees present in 1988}$$

$$\text{live} - \text{dead} + \text{ingrowth} = \text{total live woody biomass}$$

The first two measurements include all dead trees in the calculation with the DBH at year of death carried through to 2002. Ingrowth includes trees that were less than 5 cm DBH in 1988 but had grown to greater than

Table 1
Sample tree measurements to show the method used to determine year of tree death

Year	Tree 1 DBH	Tree 1 status	Tree 2 DBH	Tree 2 status	Tree 3 DBH	Tree 3 status
1988	10.0	Alive	10.0	Alive	10.0	Alive
1990	10.3	Alive	10.3	Alive	10.3	Alive
1993	10.6	Alive	10.6	Alive	10.3	Dead
1996	10.8	Alive	10.4	Alive	10.3	Dead
1999	10.8	Dead	10.7	Alive	10.3	Dead
2002	10.7	Dead	10.9	Alive	10.3	Dead

5 cm by 1996, 1999 or 2002. Ingrowth was not tallied prior to 1996.

Net primary production of woody biomass is the change in total cumulative woody biomass between time periods. NPP was calculated as total woody biomass (Eq. (1), above) for each measurement year minus initial total woody biomass from 1988 and the percent increase for the 14-year period determined. Mean annual wood production was determined by dividing each 2- or 3-year productivity value by the number of years to get a 1-year estimate. Wood values were added to measured mean annual litterfall resulting in a value for total annual biomass accumulation.

2.7. Fine roots

Fine root biomass and nitrogen content were measured in 1988 and 1999/2001. Methods used in 1988 are presented in Magill et al. (1997); 1999 and 2001 samples were collected as follows: Pine control, low N+S and high N, and hardwood control, low N and high N, were collected in 1999. A sampling error made it necessary to resample the pine low N in 2001. Ten soil cores from each plot were taken to a depth of 20 cm into the mineral soil, then split into six horizons in the field: Oi, Oe, Oa, M0–5, M5–10, M10–20. Split samples were placed into plastic bags, transported to the laboratory in coolers and refrigerated prior to processing.

Root samples were sorted within 14 days after collection. A single sample was removed from refrigeration, sieved through a 2 mm sieve and all roots that remained in the top of the sieve were separated. Once sieved, the sample was hand picked of all additional distinguishable roots longer than 0.5 cm. The roots were washed in deionized water and placed in an aluminum weigh boat. Root samples were dried for 48 h at 70 °C. After drying, roots were cooled in a desiccator and weighed. Roots greater than 2 mm were recorded as ''coarse'' roots and 2 mm or less as ''fine'' roots. All species were included and live and dead roots were not distinguished.

To prevent the need for time-consuming intensive sorting of the entire sieved portion of the soil core, a subsample weighing approximately 1/4 of the sieved soil weight was intensively sorted, the root biomass scaled to the whole core and added to the total root mass. Subsamples were frozen for a maximum of 6 months prior to sorting, then removed one at a time and allowed to thaw at room temperature for 30–60 min. Samples were placed on a piece of white paper and all visible roots removed with tweezers. Roots were then washed, dried and weighed in the same manner as the primary samples but not retained for chemical analysis after weighing.

Root samples were pulverized, dried at 70 °C then analyzed for carbon and nitrogen content on a Carlo Erba or Perkin-Elmer CHN analyzer. Horizons were analyzed separately, then averaged into organic (Oa + Oe), upper mineral (M0–5 + M5–10) and lower mineral (M10–20) for final data calculations.

3. Results and discussion

3.1. Nitrogen inputs and losses

Background nitrogen deposition at the Harvard Forest (0.66–0.8 g N m^{-2} per year) is moderate for the northeastern United States (Munger et al., 1996; Ollinger et al., 1993; Lovett and Lindberg, 1993) and substantially lower than many experimental sites in Europe (Dise and Wright, 1995; Dise et al., 1998; Gundersen et al., 1998). We assume that deposition has

remained relatively constant over the course of the experiment, as total US emissions of NO_x have not changed during the experimental period (http://www.epa.gov/oar/aqtrnd00/nitrodox.html). Ammonium nitrate additions to the chronic N plots have increased total N inputs by approximately 6- and 18-fold.

Previous papers reported nitrate leaching below the rooting zone as one of the first indicators of N saturation; this paper reports total DIN (nitrate + ammonium). The pine and hardwood stands have shown very different patterns of response to N additions over the 15 years of N additions, and the degree of interannual variability differs if soil solution DIN measurements are expressed as mean annual concentrations (mg N L^{-1}), or as total annual flux (g N m^{-2} per year).

DIN concentrations increased first in the pine stand high N plot (Fig. 1a). Measurable DIN concentrations occurred in the pine low N plots in 1993 and have been detected in most years since. DIN was not detected in the hardwood high N plot until 1995, and remains below detection limit in the hardwood low N and low N+S plots (Fig. 1b).

DIN fluxes vary more than concentrations, as concentrations tend to be higher in years when soil moisture is more plentiful. Soils were too dry for successful collection of lysimeter samples for much of the summer in 1995, 1997, 1998, 1999 and 2001; nitrate concentrations were generally low in these years, with the exception of 1999. As a result, estimated flux values (Fig. 1c) vary more than five-fold in

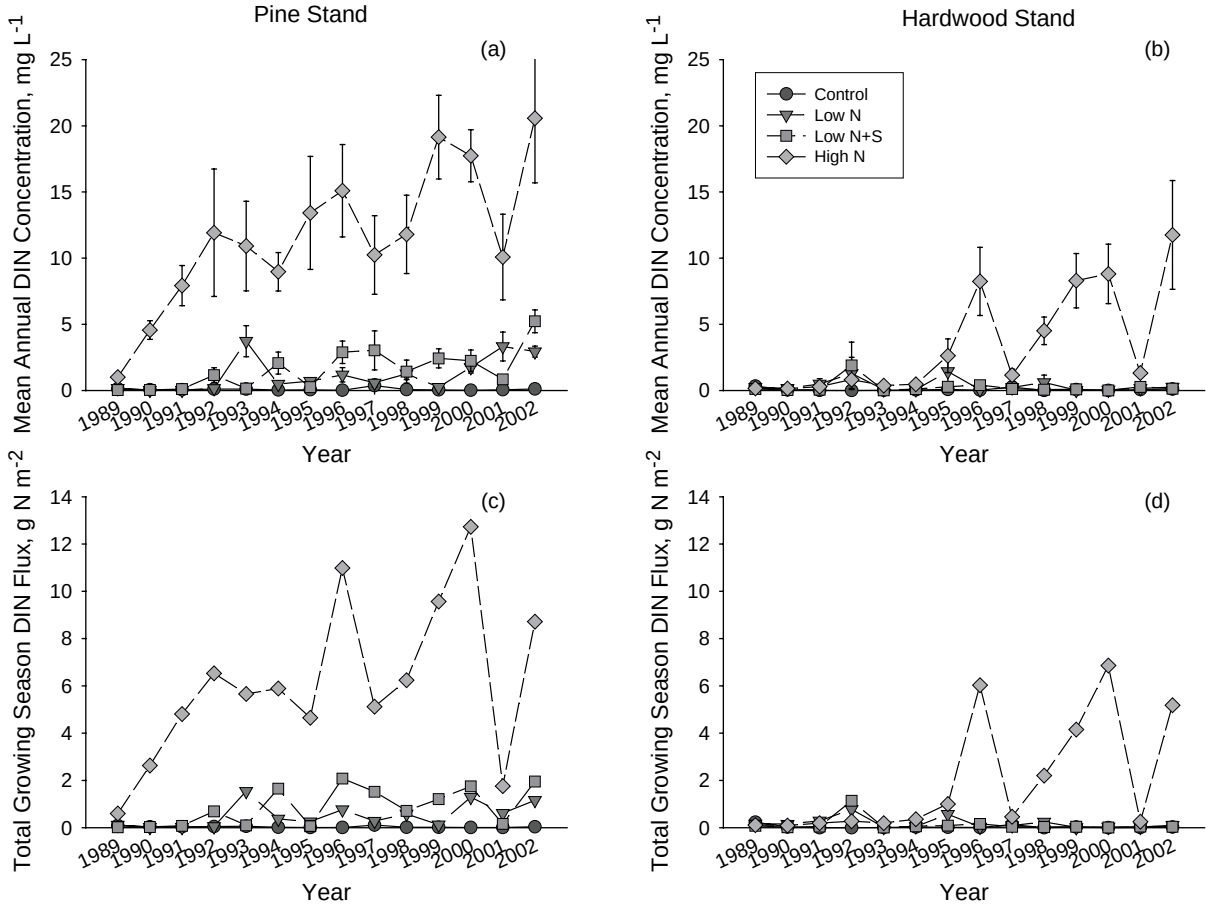

Fig. 1. Mean growing season DIN concentration and flux in tension lysimeters below the rooting zone (60 cm). Top panels are DIN concentration and bottom panels are DIN flux for the pine stand (a and c) and hardwood stand (b and d). DIN flux was calculated as concentration times drainage as estimated by the PnET Model (Aber et al., 1995b).

the high N plot in the pine stand, where concentrations vary less than three-fold.

Although fertilizer additions are as NH_4NO_3, leaching of N is assumed to be primarily as nitrate and therefore previous papers reported nitrate values only. However, the relative importance of nitrate and ammonium varies widely with treatment. In control plots, DIN is greater than 80% ammonium whereas in treated plots, the high leaching rates of nitrate reduce ammonium importance to 6% and 18% for the pine high N and hardwood high N plots, respectively. This change in distribution is likely due to a combination of factors including plant uptake and retention of NH_4 on exchange sites, while NO_3 is leached from fertilized plots where availability exceeds demand.

Increased mobility of nitrate would be expected to increase losses of base cations as well, eventually affecting soil and foliar chemistry (Reuss and Johnson, 1986; Driscoll et al., 1987; Van Breeman et al., 1984). Previous papers have reported increased movement of calcium, magnesium and potassium between the organic horizon and mineral soil in all treated plots (Currie et al., 1999), and significant decreases in both the Mg:N and Ca:Al ratios in foliage (Magill et al., 1997; Aber et al., 1995a). The longer-term effects of chronic N additions on nutrient cation fluxes, foliar element ratios and biochemical stress indicators have been presented by Minocha et al. (2000).

Measurements of dissolved organic forms of nitrogen (DON) and carbon (DOC) were reported in Magill et al. (2000). Fig. 2 shows DOC, DON and DOC:DON ratios of 1999–2001 samples as one mean value per plot (grand mean) for the collection period, representing monthly concentrations. High DIN concentrations made it difficult to determine DON in treated plots; DON is a small number determined from the difference between two large values, resulting in the need to calculate one grand mean from a scattered data set. Future analyses will require development of a method for reduction of DIN prior to DON analysis, such as membrane filtration or ion exchange columns.

DOC was hypothesized to decrease with increased N availability, but there has been no significant change in DOC concentrations after 15 years of N additions. No change in DOC was also found in soil solution collected from zero-tension lysimeters beneath the organic horizon in the hardwood high N plot, and

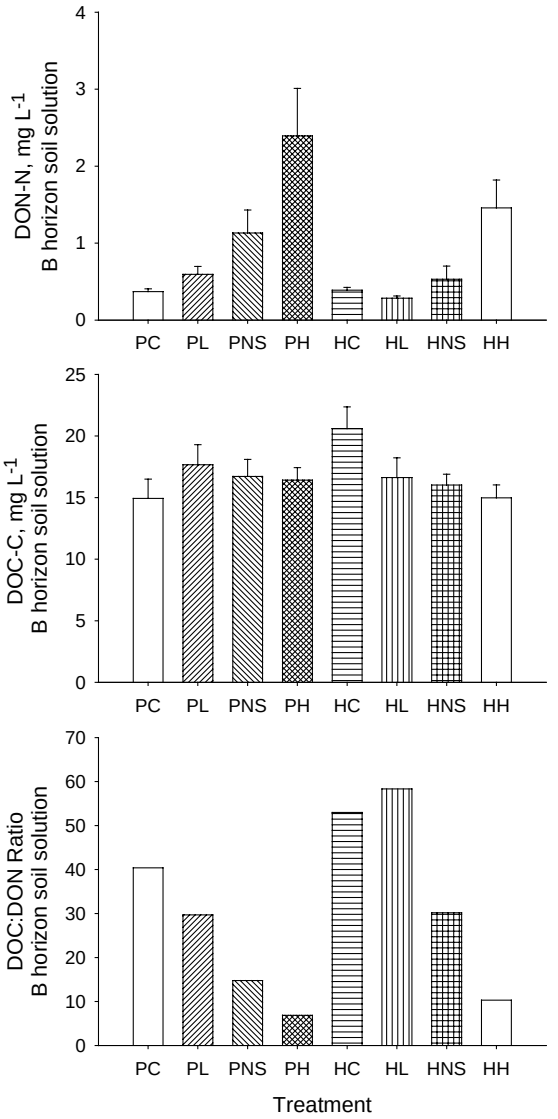

Fig. 2. DON (top panel), DOC (middle panel) and DOC:DON ratios (bottom panel) in tension lysimeters below the rooting zone (60 cm). Values are means of cumulated samples collected from spring 1999 through spring 2001.

modest increases (35%) rather than the predicted decline were found in the pine high N plot (McDowell et al., 2004). DON increased in the high N plots of both stands by 1996 (Magill et al., 2000). The 1999–2001 mean DON concentration in the pine high N is significantly higher than controls, with no significant differences in the hardwood stand. The *t*-test compar-

ison of grand means from 1993 to 1996 and 1999 to 2001 showed no significant change in DON concentration over time within treatment.

Conversion of DIN to DON is a possible mechanism of N retention in these plots. A study by Dail et al. (2001) looked at biotic and abiotic $^{15}NO_3$ retention in soils collected from the hardwood stand but outside the treated plots. They recovered 28% (live) to 50% (sterile) of the added $^{15}NO_3$ in K_2SO_4 extractable DON, demonstrating the importance of DON formation as a mechanism of N assimilation. Increased NO_3 concentrations in soil solution mean that substrate for DIN assimilation is plentiful. Qualls et al. (2002) measured greater than 95% retention of DOC and DON inputs to mineral soils in a forested watershed at the Coweeta Hydrologic Laboratory. Additional measurements of mineral soil adsorption capacity, and polyphenol or tannin complexation (e.g. Yu et al., 2002; Azhar et al., 1986) are necessary to determine the mechanisms of DON retention on the chronic N plots; direct measurements of retention (as the difference in DON concentrations between O horizon and mineral horizon soil solution) are restricted by the low sample size and intermittent water availability.

3.2. Direct measurement of soil C and N pools

Estimating net N retention in soils by direct measurement is generally confounded by extreme spatial variability in soil organic C and N pools. We have previously estimated soil N storage by difference calculations and recovery of added ^{15}N (Magill et al., 2000; Nadelhoffer et al., 1999, 2004; Aber et al., 2003). However, given the very high estimated values for N retention in soils in the high N plots (totaling over 150 g N m^{-2} in the high N hardwood stand by 2002) we could expect differences to appear in estimates of soil pools derived from bulk samples.

In the pine stand, O horizon C:N ratio is significantly higher in treated plots (Fig. 3a), suggesting reduced, rather than increased, N storage. Measured C:N ratios in the organic horizon did not vary consistently across treatments over time in the hardwood plots (Fig. 3b). Apparent differences between control and treated plots in 1988 and 1996 are contradicted by data from 1992 and 1999 suggesting that there is more noise than signal and that no change in O horizon C:N has occurred in the hardwood stand. A comparison of percent N and percent C show no consistent trends or significant differences between or within treatments over the 15-year measurement period (data not shown).

Calculations of total C and N pools are dependent on estimates of total soil horizon mass. The apparent lack of change in soil C and N concentration could be offset by changes in total horizon mass, due to either changes in litter inputs to the organic horizon (see below), or reductions in the rate of litter decay with N additions (Magill and Aber, 1998); increased O horizon mass would increase calculated soil N retention.

Initial O horizon mass varied by less than 10% across plots in the 1988–1991 samples (Aber et al., 1993). Estimates of mean organic horizon mass (Fig. 4a) show year-to-year differences that cannot be attributed to altered rates of litter input (See Figs. 7a and 8a) or litter decay. Interannual variation in mean values, excluding the 1999 bulk density sampling (1999bd), is consistent across treatments and stands, suggesting that systematic differences in differentiation of horizons occurred in the different sample years, as samples were collected for different purposes each year. While such variations are often encountered and discussed at meetings, they are less often reported in the literature. Trends in the ratio of treated to control plot O horizon mass across years (Fig. 4b) show no significant changes, again with the exception of the 1999bd samples which were significant at the 0.05 level using the least significant difference test (Snedecor and Cochran, 1978). The 1999–2002 samples in the pine low N, low N+S and high N, averaging about 20% higher than controls, become significant at the 0.10 level.

We suggest that the differences in methods used in the 1999bd sampling make comparisons with other years unsupportable. Data collected in all other years indicates that there has been no detectable net increase in N storage in the organic horizon in the hardwood stand over the treatment period. Assuming the increase in organic horizon mass in the pine stand is consistent with decreased litter decay rates (Magill and Aber, 1998), a 20% increase ($\sim$1000 g m^{-2}) in organic horizon mass times N concentrations of $\sim$2% gives a potential storage of 20 g N m^{-2} over the first 15 years of treatment. This averages to 1.3 g N m^{-2} per year which is slightly less than 10% of total additions in the high N plot. This minimal and barely

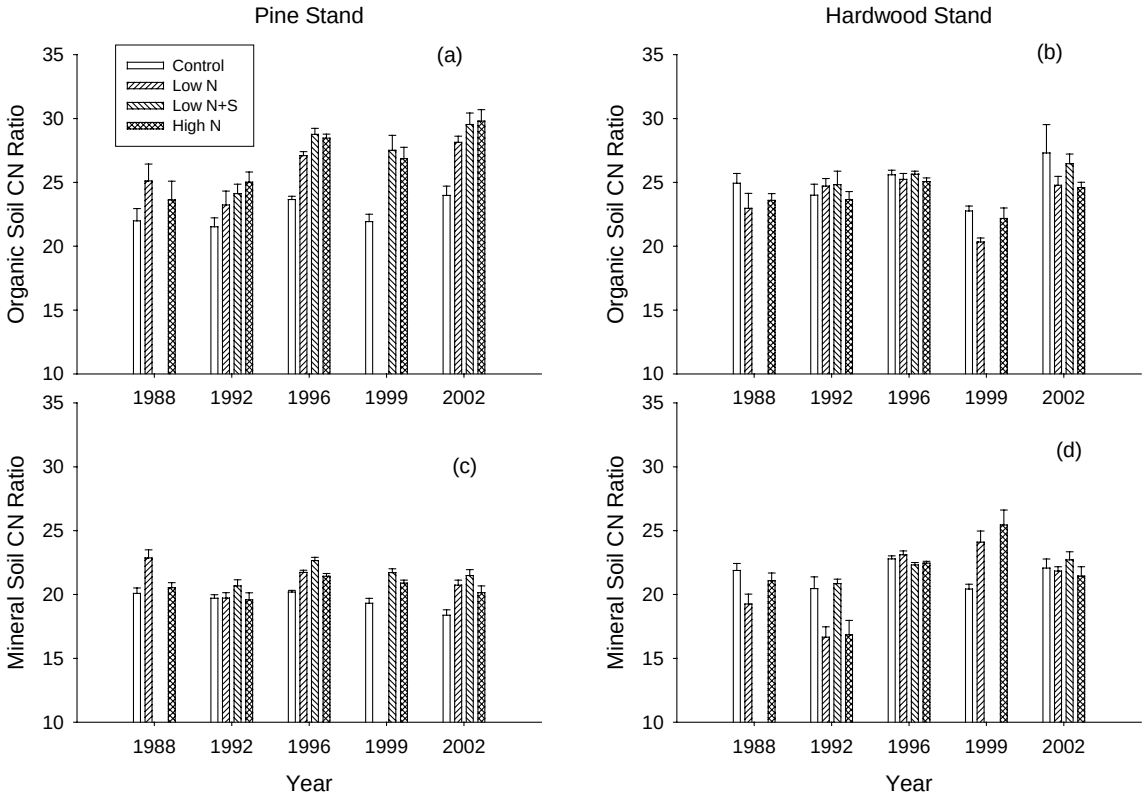

Fig. 3. Soil carbon to nitrogen ratios from five sample collections over 15 years of N additions. Top panels are organic horizon and bottom panels are mineral horizon for the pine stand (a and c) and hardwood stand (b and d).

significant value is in line with organic horizon lysimeter data, which also suggest no net retention in the organic horizon (McDowell et al., 2004). Estimates based on difference calculations (budgets) and on ^{15}N distributions suggest approximately 70% of added N is retained in soils; most or all of this must occur in the mineral soil.

Accurate estimates of mineral soil organic matter content are even more difficult to measure than the organic horizon due to spatial variability and incomplete sampling by depth. Our measurements of C:N ratios in mineral soils show no clear change or significant differences over time (Fig. 3c and 3d). However, both DOM sorption (Jardine et al., 1989; Kaiser and Zech, 2000; Qualls and Haines, 1991) and abiotic transformations of DIN (e.g. Johnson et al., 2000; Dail et al., 2001; Berntson and Aber, 2000) may be important for the entire depth of the rooting zone. Distributing the sink for N retention over such a large soil mass makes detection of changes in pool size by bulk sampling nearly impossible.

3.3. Biomass production and N concentration

The nitrogen saturation hypothesis (Aber et al., 1989, 1998) predicts that the final stages of N saturation lead to tree decline and even death. In Europe, forest decline has been seen as a result of long-term additions of pollutants including nitrogen, yet there are few areas in North America where the long-term cumulative effects of ambient N deposition have adversely impacted forest health. As with other ecosystem compartments on the chronic N plots, response of trees has been different for the two stands, due primarily to stand age and pre-treatment site characteristics.

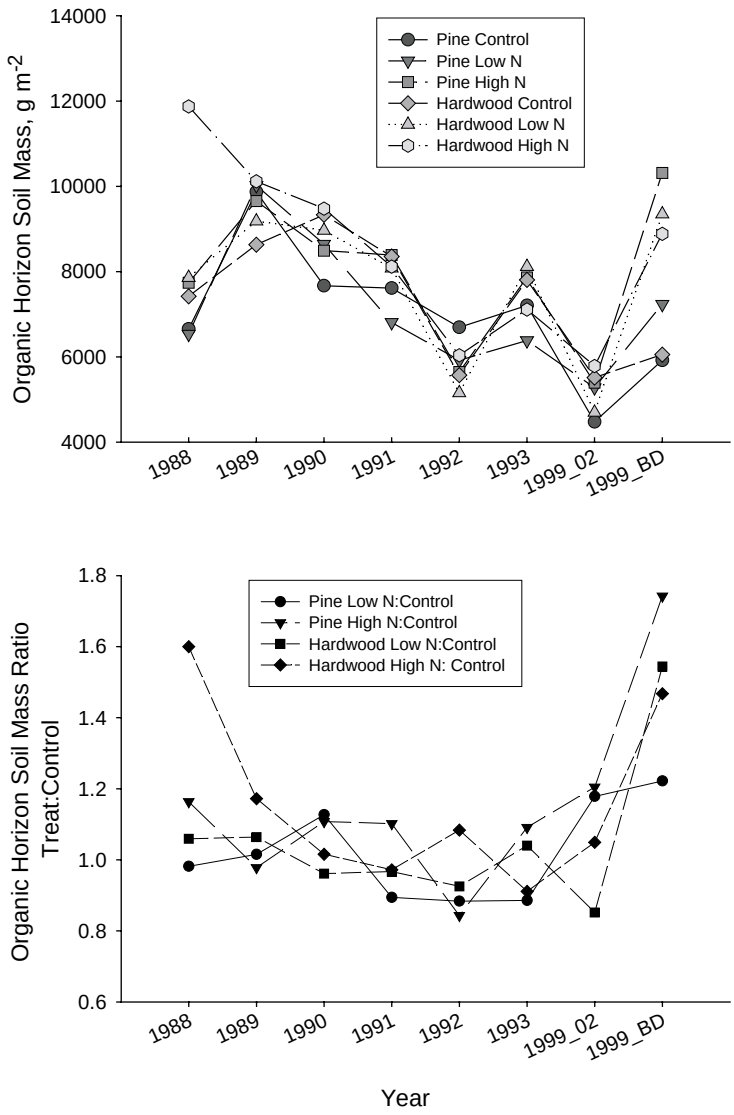

Fig. 4. Organic horizon mass by treatment (top panel) and ratio of treated:control mass values for eight collections over the 15-year treatment period. Values are means of all samples collected within each year. 1999_02 values are a mean of 1999, 2001 and 2002 soil collections; 1999BD are the 1999 bulk density collections.

3.4. Woody biomass

Previous papers (Magill et al., 1997, 2000) reported only total accumulated woody biomass production as an indication of effects of treatments on tree growth. Those values did not include ingrowth of trees that had reached >5.0 cm DBH since 1988, or report changes in live biomass by accounting for mortality. Figs. 5 and 6 compare each plot within the pine and hardwood stands, using three different calculations for aboveground standing biomass. The reported number for "live + dead − ingrowth" is equivalent to previously reported values (Magill et al., 2000) and although this method is sufficient for determining cumulative productivity, it is inadequate for assessing changes in live biomass, especially in stands where mortality is high.

The percent mortality was calculated by stem as the fraction of total stems no longer living (Tables 2 and 3)

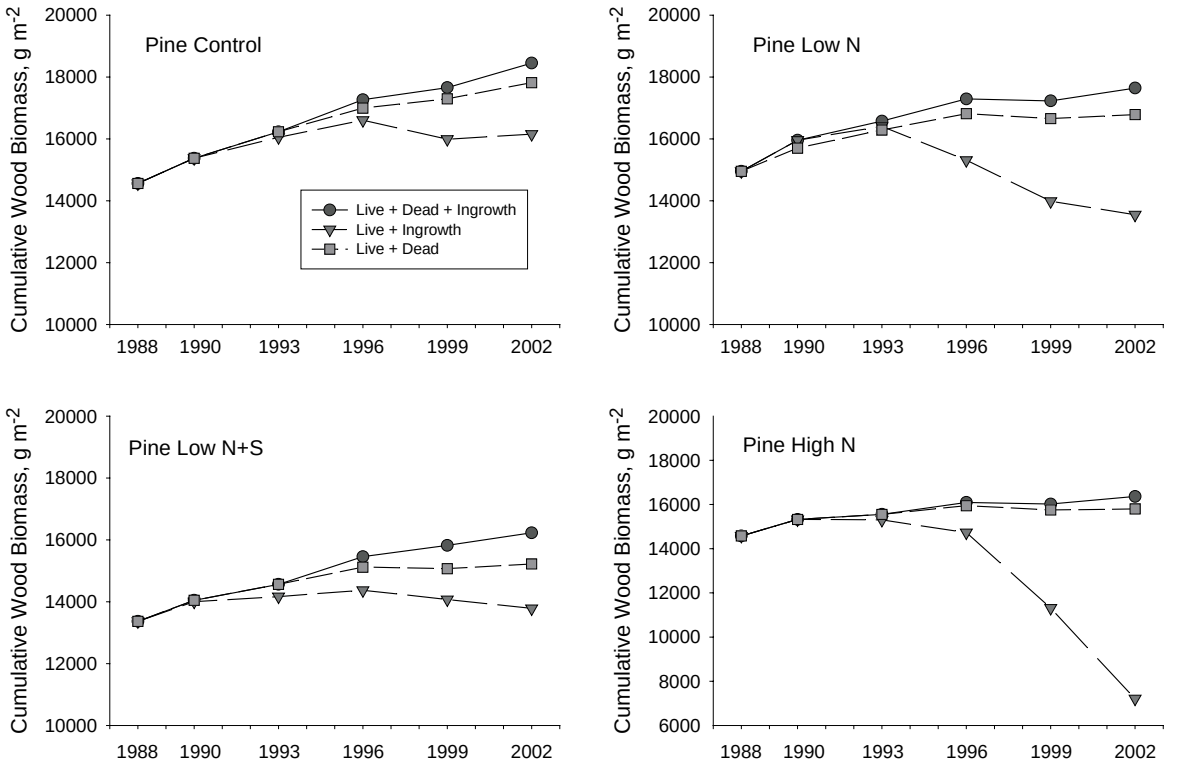

Fig. 5. Comparison of different calculation methods for determining wood growth in the pine stand. See text for description of different values.

and by mass as the fraction of biomass in dead trees, (Figs. 5 and 6) represented as the difference between the circles (L + D + I) and triangles (L − D + I). In the pine stand, percent mortality by mass was highest in the high N plot, reaching 56% by 2002. The pine low N was 23%, the pine low N+S, 15% and the pine control, 12%. A comparison of mortality by stem shows that 56% of the stems that died were equal to 56% of the mass, indicative of the even-aged plantation (Table 2). Conversely, in the hardwood high N, although 49% of the trees had died (Table 3), these comprised only 17% of tree biomass (Fig. 6). This relationship was true for the other plots within the hardwood stand as well. The hardwood control had a higher percent mortality for both mass and stem than either of the low N plots, suggesting that the low N addition to this stand has had a beneficial effect on tree health and that initial site quality was limiting to growth.

Declines in productivity or increases in mortality have been reported for other coniferous evergreen forests in the US (Aber et al., 1995a; McNulty et al., 1996), and increases in growth in response to removal of N and S from throughfall have been seen in Europe (e.g. Beier et al., 1998; Boxman et al., 1998). At the Solling site in Germany, earlier studies linked forest decline to reduced Mg:N ratios in foliage (Schulze, 1989), a response which was seen in the chronic N plots through 1997 (Magill et al., 1997; Minocha et al., 2000). Additional indicators of tree mortality were seen in measurements of physiological characteristics and included decreased foliar retention time, a reduction in rates of net photosynthesis despite a near doubling of foliar N concentration, and the accumulation of biochemical indicators of physiological stress (Minocha et al., 2000; Bauer et al., 2004). Together, these results suggest that the cumulative effects of N deposition at moderate-to-high levels may have negative impacts on biomass production and overall tree health in temperate forest systems.

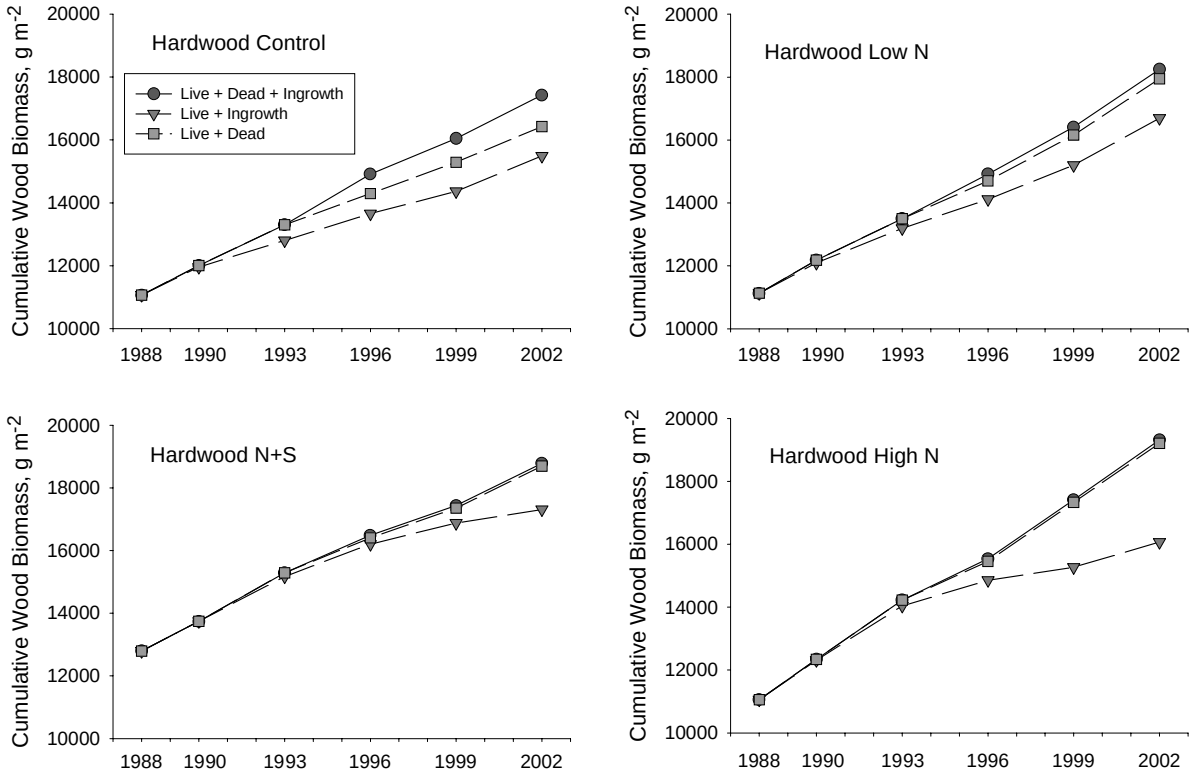

Fig. 6. Comparison of different calculation methods for determining wood growth in the hardwood stand. See text for description of different values.

Table 2
Species distribution and total mortality by stem count for the red pine stand at the end of the 2002 growing season

	Total	Black birch	Black cherry	Red pine	Striped maple	Other species
Control						
Dead trees	25	2	0	23	0	0
Total trees	121	17	4	87	9	4
Percent mortality	20.7	11.8	0.0	26.4	0.0	0.0
Low N						
Dead trees	48	0	0	48	0	0
Total trees	137	6	4	117	7	3
Percent mortality	35.0	0.0	0.0	41.0	0.0	0.0
Low N+S						
Dead trees	26	2	0	23	1	0
Total trees	109	5	6	78	13	7
Percent mortality	23.9	40.0	0.0	29.5	7.7	0.0
High N						
Dead trees	65	0	0	64	0	1
Total trees	116	6	2	94	6	8
Percent mortality	56.0	0.0	0.0	68.1	0.0	12.5

The other species category includes species that are low in abundance and not found in every plot: American beech, black oak, black gum, Eastern hemlock and red maple.

Table 3
Species distribution and total mortality by stem count for the hardwood stand at the end of the 2002 growing season

	Total	American beech	Black birch	Black oak	Red maple	White birch	White pine	Other species
Control								
Dead trees	46	0	4	26	7	3	2	4
Total trees	173	5	21	97	27	5	8	10
Percent mortality	26.6	0.0	19.0	26.8	25.9	60.0	25.0	40.0
Low N								
Dead trees	39	3	4	16	9	5	0	2
Total trees	206	46	22	72	47	8	2	3
Percent mortality	18.9	6.5	18.2	22.2	19.1	62.5	0.0	66.7
Low N+S								
Dead trees	29	0	11	7	3	2	2	2
Total trees	156	1	41	45	45	4	15	9
Percent mortality	18.6	0.0	26.8	15.6	6.7	50.0	13.3	22.2
High N								
Dead trees	92	0	7	39	40	3	1	4
Total trees	188	5	12	106	55	3	4	5
Percent mortality	48.9	0.0	58.3	36.8	72.7	100.0	25.0	80.0

The other species category includes species that are low in abundance and not found in every plot: black cherry, Eastern hemlock, striped maple and white oak.

3.5. Litterfall

Long-term changes in total litterfall are reported for the pine stand (Fig. 7a) and hardwood stand (Fig. 8a). Although there are no long-term significant differences in total litter mass, it is apparent that after the 1995 drought trees in both the pine and hardwood high N plots were affected. Total litter mass was highest in the high N plots through 1995, but decreased and remained lower than the other treatments from 1996 through 2002. In order to examine the potential for change in species composition with mortality, we looked at the percent of total litterfall contributed by each species.

In the pine stand, red pine litter consistently comprises 80–85% of total litterfall annually but there is a marked increase in litterfall of lower canopy species after 1997 (Fig. 7, panels b–d). Striped maple, black birch and black cherry all increase three- to four-fold over pre-1997 litterfall mass measurements in the pine high N. As red pine death occurs, openings in the canopy allow for more light to reach the lower canopy creating favorable conditions for increased growth of understory species. This trend is not significant and is masked by the high number of hardwood species in the pine control plot where understory tree litterfall also increases. Again, this may indicate that due to stand age (>75 years), the red pines are transitioning from an aggrading to degrading forest.

In the hardwood stand, the affect of the 1995 drought is most apparent in the change in red maple litterfall in the high N plot (Fig. 8, panel b). Red maple was consistently around 24% of total litterfall through 1995 but by 1997 comprised only 6% of litterfall. These data compare with the tree mortality by stem data (Table 3) where by 2002 nearly 75% of the red maple trees had died. Importance of black oak increases with red maple death but other species such as black birch are not affected (Fig. 8, panels c and d). (See Magill et al. (2000) for a discussion of the potential for drought to alter both the shedding of foliage and rates of nitrate leaching as seen in Fig. 1).

3.6. Aboveground production

Table 4 summarizes NPP between 1988 and 2002 as net production in 2- or 3-year intervals and as 14-year means. In the pine stand, mean annual net wood production was highest in the control plot. Wood production decreased 31%, 27% and 54% relative

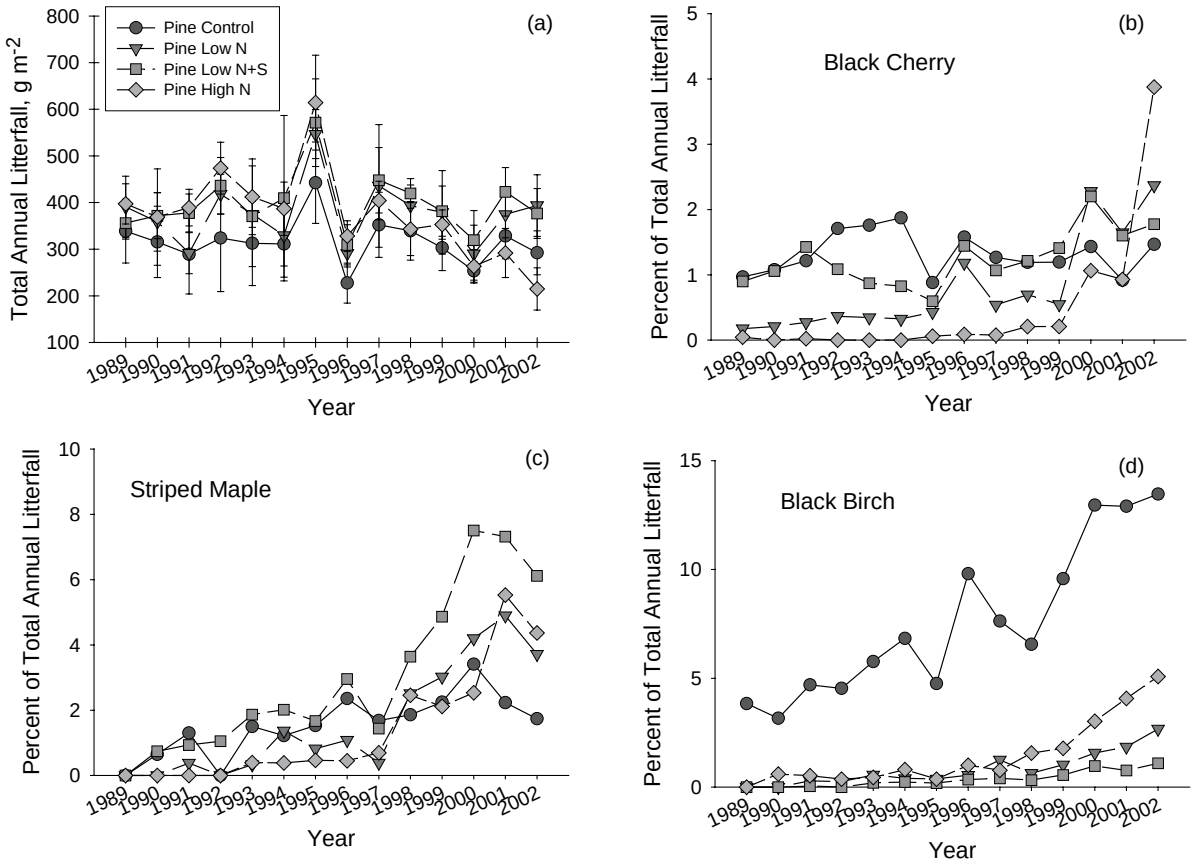

Fig. 7. Total annual litterfall in the pine stand (panel a) and changes in percent contribution of individual species to total litterfall over time: black cherry (panel b), striped maple (panel c), and black birch (panel d). Values are for leaves/needles only; other materials not included.

to control for the low N, low N+S and high N plots, respectively. Relative partitioning of total NPP between wood and litterfall varied greatly between treatments. Total annual production was split evenly between litter and wood in the control plot (47% from wood) whereas only 25% of total annual NPP was wood in the high N plot. This trend was seen by 1996 (Magill et al., 2000) and has continued through 2002. Since 1996, foliar production has decreased, as measured by decreases in red pine litterfall. Less production combined with decreased foliar lifespan (Bauer et al., 2004) has resulted in significantly reduced leaf area (Fig. 9). Combined with reduced photosynthetic potential in foliage and the accumulation of forms of N not related to the photosynthetic process (Bauer et al., 2004) severe carbon stress appears to reduce both foliar and wood production in the pine stand.

The hardwood stand has responded differently over the treatment period. Measurements through 1996 showed a near 50% greater ANPP in the high N plot relative to control (Magill et al., 2000) and the 14-year mean was 30% greater. The low N and low N+S treatments were 11% greater and 6% lower than control, respectively. For all treatments, wood contributed more to total annual NPP than litterfall and was consistently close to 60% of the total.

It can be difficult to compare ANPP and mortality estimates across studies as different methods may be used and data sets are often incomplete (Clark et al., 2001). Most studies measure woody biomass and some measure litter, but often different litterfall fractions are included. Few studies address mortality separately, including instead estimates of total woody biomass accumulation as we have done in Eq. (1). At

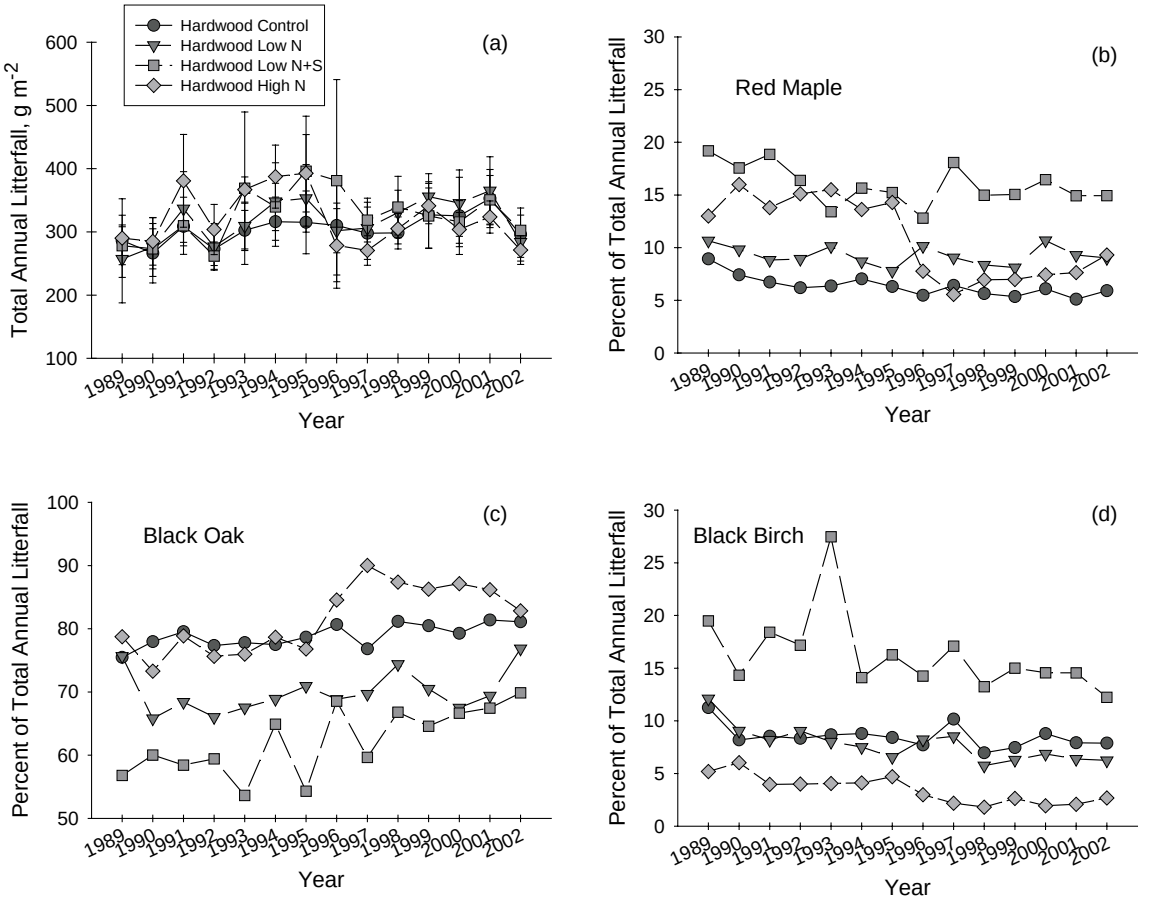

Fig. 8. Total annual litterfall in the hardwood stand (panel a) and changes in percent contribution of individual species to total litterfall over time: red maple (panel b), black oak (panel c), and black birch (panel d). Values are for leaves/needles only; other materials not included.

the Bear Brooks Watershed in Maine, ANPP and mortality were measured in a beech–maple–yellow birch–red spruce stand receiving nitric and sulfuric acid additions for three growing seasons: 1989, 1990 and 1991(Magill et al., 1996). Mortality was 10%, 23% and 31% of cumulative production for the control, low N and high N plots, respectively, indicating a trend toward higher mortality with N additions.

3.7. Foliar chemistry

Foliar N concentration is reported for the two dominant species: red pine in the pine plots (Fig. 10a) and black oak in the hardwood plots (Fig. 10b). Both species showed the same overall pattern of leaf N concentrations: high N > low N = N+S > control ($P < 0.05$). Red pine foliage showed the most dramatic change from controls, increasing by nearly 100 and 50% in the high N and low N plots, respectively, by 1999. Hardwood foliar N levels increased as well, but differences were lower than red pine when compared with control plot foliage (approximately 33% and 15% increases in high and low N plots for black oak).

Both species showed significant interannual variability in lignin concentration in both green and senescent foliage (Fig. 10c–d) with little influence of N additions in either case. Foliar cation concentrations decreased through 1996 (Minocha et al., 2000). Preliminary results from foliage collected in 1999 and 2002 show that calcium concentrations in treated plots are significantly lower than controls for both stands,

Table 4
Aboveground net primary productivity from 1988 through 2002 as g m^{-2}. Trees were measured post-growing season for the year indicated

	Pine stand				Hardwood stand			
	Control	Low N	Low N+S	High N	Control	Low N	Low N+S	High N
Cumulative Wood NPP (g m^{-2})								
1990	814	1003	680	745	938	1051	951	1281
1993	1666	1623	1197	978	2233	2365	2495	3165
1996	2705	2339	2091	1515	3846	3784	3696	4480
1999	3098	2276	2454	1446	4974	5278	4646	6356
2002	3890	2690	2863	1785	6360	7116	5995	8259
Percent increase since 1988	27	18	21	12	57	64	47	75
14-year means								
Mean annual wood production	278	192	204	127	454	508	428	590
Mean annual litter production	310	373	392	379	291	317	312	331
Total annual biomass accumulation	588	565	596	507	746	826	740	921

Wood NPP values are the measurement year biomass minus 1988 (initial) woody biomass. Litterfall was measured annually and includes all materials except bark: leaves or needles, twigs, fruits and flowers. See Section 2 for detailed description of calculations.

indicating that depletion of important nutrients is continuing (data not shown). Changes in cation content of foliage, roots and soils are currently under investigation.

Table 5
Fine root mass and nitrogen content for samples collected in 1999; the low N plot was sampled in 2001

	Pine stand				Hardwood stand		
	Control	Low N	Low N+S	High N	Control	Low N	High N
	1999	2001	1999	1999	1999	1999	1999
Fine root percent N							
Organic	1.6	2.2	1.7	2.0	1.2	1.4	1.6
S.E.	*0.05*	*0.06*	*0.08*	*0.06*	*0.08*	*0.10*	*0.03*
Mineral 0–10	1.1	1.8	1.6	1.6	0.8	0.9	1.2
S.E.	*0.05*	*0.09*	*0.11*	*0.07*	*0.03*	*0.04*	*0.07*
Mineral 10–20	0.8	1.6	1.3	1.4	0.6	0.6	0.9
S.E.	*0.08*	*0.10*	*0.08*	*0.08*	*0.04*	*0.03*	*0.05*
Fine root biomass (g m^{-2})							
Organic	152.3	248.0	219.9	118.7	359.5	269.1	260.3
S.E.	*18.2*	*28.8*	*29.4*	*11.9*	*49.5*	*46.6*	*26.6*
Mineral 0–10	263.4	278.6	292.1	266.9	292.9	282.0	241.8
S.E.	*29.4*	*37.3*	*17.6*	*22.7*	*27.3*	*49.3*	*23.5*
Mineral 10–20	198.4	177.2	178.2	207.3	142.7	150.0	150.6
S.E.	*30.4*	*29.3*	*19.7*	*21.1*	*10.6*	*19.7*	*18.3*
Total N content (g N m^{-2})							
Organic	2.4	5.4	3.7	2.4	3.8	4.1	4.3
Mineral 0–10	2.8	4.9	4.7	4.2	2.4	2.6	2.9
Mineral 10–20	1.6	2.9	2.3	3.0	0.9	1.0	1.4
Total to 10 cm	5.2	10.3	8.4	6.6	6.2	6.8	7.1
Total to 20 cm	6.8	13.2	10.6	9.6	7.1	7.8	8.5

Values are means of 10 cores per plot, with standard error in italics. Total N content calculated as percent N × biomass.

Fig. 9. View of the control (top panel), low N (middle panel) and high N (bottom panel) canopy in the pine stand at the Harvard Forest (images courtesy of Christian Arabia).

3.8. Fine roots

Measurements of fine roots (<2.0 mm) collected in 1991 (Magill et al., 1997) showed no change in biomass but an increase in percent N with fertilizer additions, resulting in an overall increase in total fine root N content in the treated plots. The same pattern of response was observed in 1999/2001 for all three horizons measured (Fig. 11, Table 5). In the pine stand, C:N ratio of low N and high N plot fine roots were significantly lower than the control for both mineral horizons. In the hardwood stand, the high N fine root C:N ratio was significantly lower than control roots for all horizons. Increased N concentration was the driver behind changes in C:N ratio as carbon content was not found to change significantly. Roots from cores collected outside the experimental plots but within the same forest stands (reference cores) were not significantly different from controls in either stand. This comparison was made partly due to concerns that foot-traffic on the plots could impact soil and root properties.

N concentration in fine roots typically decreases with depth in the soil profile (e.g. Burke and Raynal, 1994). This pattern is seen in all hardwood plots and the pine control but has been eliminated in the pine low N and high N plots where C:N ratio averaged around 25 for all three horizons (Fig. 11), again due to higher N concentrations.

Fine root mass in the organic horizon (Oe + Oa) of the pine high N plot was almost 50% lower than the low N and low N+S treatments and 20% lower than the control whereas hardwood low N and high N fine root mass were both 25% lower than control. These differences were not significant, likely due to inherent heterogeneity of root distribution, and there were no differences in fine root biomass of either mineral horizon. Reported values for total (live + dead) fine root biomass range from 100 to 2000 g m^{-2} (Baron-Gafford et al., 2003; Joslin and Henderson, 1987; Ruess et al., 1996; Kelting et al., 1995), an indication of not only the variability between species and ecosystems but also the variation in collection techniques. Declining fine root biomass has been identified as a key response to N saturation and forest decline in European and North American studies (e.g. Persson et al., 1998). A study of fine root turnover, soil respiration and root biomass is currently underway

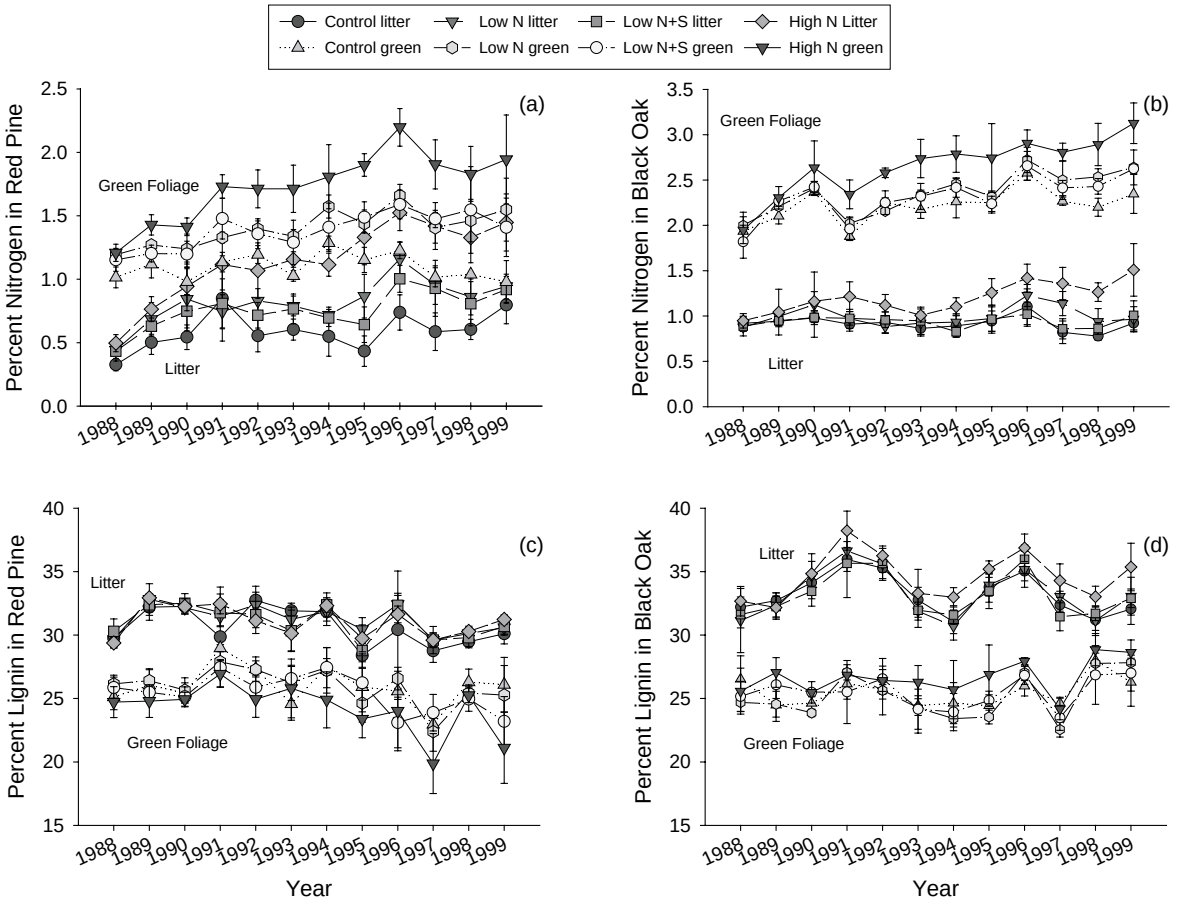

Fig. 10. Interannual variation in nitrogen and lignin concentrations in green and senesced foliage in the pine stand. Top panels are percent nitrogen and bottom panels are percent lignin for red pine foliage or litter (a and c) in the pine stand or black oak foliage or litter (b and d) in the hardwood stand.

at the chronic N plots where roots were separated into live and dead, <1 mm size class. Initial data show that live fine root biomass is declining with treatment in the pine stand but not in the hardwood stand, although hardwood high N live biomass is lowest of the four treatments (L. Rustad, personal communication).

Root nitrogen pool calculations are presented in Table 5. Previously reported data included roots only to 10 cm in the mineral horizon (Magill et al., 1997). With the addition of 10–20 cm depth root measurements, total N storage in roots increases 25% for pine control, low N and low N+S and 50% for pine high N. These data agree with an elimination of the gradient in decreasing N concentration with depth (see above). Increase in total root N pool size is only 15–20% in the hardwood stand as N concentrations are much lower in the mineral horizon roots. Root N pool sizes are similar to others reported in the literature. Ruess et al. (1996) measured root N content in several Alaskan upland forests and measured N pools of 7.9 and 2.6 g N m^{-2} for a birch-aspen and a white spruce stand, respectively.

4. Comparisons with previous work and other studies from the chronic N plots

Many of the findings reported in this paper continue or accelerate trends seen in earlier data summaries of the chronic N study. Losses of inorganic N remain

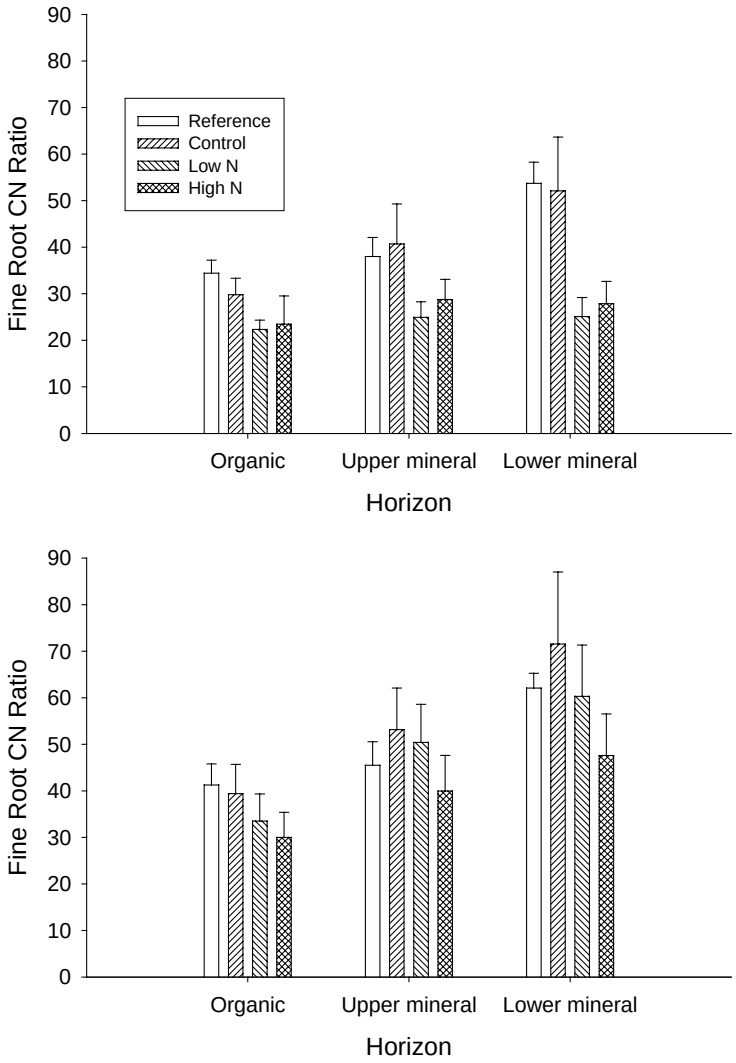

Fig. 11. Fine root C:N ratio by treatment and horizon for the pine stand (top panel) and hardwood stand (bottom panel). Reference values are cores collected from untreated areas outside the control plot boundaries. All samples collected in 1999 except pine low N which was sampled in 2001. Values are means with standard deviations as error bars.

high in the high N plots (higher in pines than hardwoods) and low N plots in the pine stand also have measurable DIN losses. Foliar and fine root N concentrations are elevated significantly. Trends in tree decline in the pine high N plot have accelerated and complete mortality in that stand is likely in the near future. Canopy condition has declined in the low N pine plots, and mortality is beginning to increase. The high N hardwood stand shows increased ANPP, but the co-occurrence of drought and significant mortality of red maple in the understory is indicative that the vegetation may have decreased resistance against environmental stresses. Stress impacts are apparent with the alteration of foliar production patterns in both stands.

The leveling off of increases in foliar N concentrations in roots and foliage, along with flat or declining wood production in all but the high N hardwood plot, suggests once again that soils must be the major sink for added N that is retained. As in an earlier paper

(Nadelhoffer et al., 1999), recent ^{15}N analyses support this general conclusion (Nadelhoffer et al., 2004).

In 1998, we revisited our initial hypotheses regarding the progress of N saturation in forests, suggesting two alternative explanations for high N retention in soils without measurable increases (and possibly significant decreases) in soil respiration rates. One of these, the chemical reaction between inorganic nitrogen and soil organic matter, has been demonstrated to occur at significant rates under ambient conditions (Dail et al., 2001; Johnson et al., 2000; Berntson and Aber, 2000). Davidson et al. (2003) have proposed a mechanism by which this could occur.

The second mechanism proposed was assimilation by mycorrhizae followed by re-exudation into the soil solution as N-rich organic compounds (perhaps extra-cellular enzymes). Results from the chronic N plots are varied. DON flux has increased, and the DOC:DON ratio has decreased in soil solutions below the rooting zone and beneath the organic soil; an increase in non-humic compounds are reported for fluxes below the organic horizon as well (McDowell et al., 2004). While these results indicate an increased cycling of organic N with mycorrhizal assimilation and turnover as a possible mechanism, Frey et al. (2004) found that both active fungal biomass and mycorrhizal diversity were lower in treated plots. Further examination of the specific mechanisms controlling organic nitrogen cycling is needed, as both the chemical immobilization of N into soil organic matter and the assimilation and exudation of N by mycorrhizae remain poorly known and quantified processes. A better understanding of the kinetics and saturation capacity of each is required if we are to develop a more quantitative and predictive understanding of N saturation in forests.

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