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Effects of chronic nitrogen amendment on dissolved organic matter and inorganic nitrogen in soil solution

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Abstract

Increased atmospheric deposition of N to forests is an issue of global concern, with largely undocumented long-term effects on soil solution chemistry. In contrast to bulk soil properties, which are typically slow to respond to a chronic stress, soil solution chemistry may provide an early indication of the long-term changes in soils associated with a chronic stress. At the Harvard Forest, soil solution was collected beneath the forest floor in zero tension lysimeters for 10 years (1993–2002) as part of an N saturation experiment. The experiment was begun in 1988 with 5 or 15 g N m^{-2} per year added to hardwood and pine forest plots, and our samples thus characterize the long-term response to N fertilization. Samples were routinely analyzed for inorganic nitrogen, dissolved organic nitrogen (DON), and dissolved organic carbon (DOC); selected samples were also analyzed to determine qualitative changes in the composition of dissolved organic matter. Fluxes of DOC, DON, and inorganic N were calculated based on modeled water loss from the forest floor and observed concentrations in lysimeter samples. The concentration and flux of inorganic N lost from the forest floor in percolating soil solution are strongly affected by N fertilization and have not shown any consistent trends over time. On average, inorganic N fluxes have reached or exceeded the level of fertilizer application in most plots. Concentrations of DOC were unchanged by N fertilization in both the hardwood and pine stands, with long-term seasonal averages ranging from 31–57 mg l^{-1} (hardwood) and 36–93 mg l^{-1} (pine). Annual fluxes of DOC ranged from 30–50 g m^{-2} per year. DON concentrations more than doubled, resulting in a shift toward N-rich organic matter in soil solution percolating from the plots, and DON fluxes of 1–3 g m^{-2} per year. The DOC:DON ratio of soil solution under high N application (10–20) was about half that of controls. The organic chemistry of soil solution undergoes large qualitative changes in response to N addition. With N saturation, there is proportionally more hydrophilic material in the total DON pool, and a lower C:N ratio in the hydrophobic fraction of the total DOM pool. Overall, our data show that fundamental changes in the chemistry of forest floor solution have occurred in response to N fertilization prior to initiation of our sampling. During the decade of this study (years 5–14 of N application) both inorganic N and dissolved organic matter concentrations have changed little despite the significant biotic changes that have accompanied N saturation.

Keywords: N saturation; Forest floor; Soil solution; Nitrate; Ammonium; Dissolved organic nitrogen; Dissolved organic carbon

1. Introduction

Soil solution chemistry is often a sensitive indicator of biogeochemical processes in forests, responding

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quickly to various disturbances or stresses. Early experiments by Vitousek et al. (1979), for example, showed that forest cutting results in a rapid change in soil solution chemistry, particularly inorganic nitrogen. Much of the literature on the effects of disturbance on forest soil solution chemistry and fluxes over the past two decades has focused on inorganic nutrients (e.g. Edwards and Ross-Todd, 1979; Blood et al., 1991; Knight et al., 1991; Prescott, 1997). Recent work shows that dissolved organic matter (DOM) plays a significant role in many aspects of forest biogeochemistry such as weathering (Adamo and Violante, 2000), soil genesis (Lundstrom et al., 2000), and plant nutrition (Lipson and Nasholm, 2001). With this recognition of the importance of DOM in forest biogeochemistry, interest in understanding the dynamics of dissolved organic matter in the face of chronic or episodic disturbances has also increased, as has interest in understanding the linkages between dissolved organic matter and inorganic nutrient dynamics, particularly for nitrogen (Vitousek et al., 1998). Studies of the effects of increased nitrogen deposition on forest biogeochemistry provide excellent opportunities to examine these linkages. In many soils, the dominant form of N in transport is organic N prior to experimental manipulation of N inputs, but shifts to inorganic forms with increased anthropogenic N inputs, providing an opportunity to examine the role of N availability in DOM dynamics (Aber et al., 1998).

Dissolved organic matter in soil solution is generated by a variety of pathways, and understanding the primary drivers of DOM production is a major challenge for forest ecologists and biogeochemists (Kalbitz et al., 2000; McDowell, 2003). Both biotic and abiotic processes are important in regulating dissolved organic carbon (DOC) and nitrogen (DON) flux (Currie and Aber, 1997; Aitkenhead-Peterson et al., 2003). Detailed analysis of the components of DOM (amino acids, carbohydrates, amino sugars) indicates that most is of microbial origin, derived from the oxidative degradation of plant-derived organic matter and by production of microbial metabolites (Guggenberger et al., 1994; Schimel and Weintraub, 2003). Work by Møller et al. (1999) suggests that fungal community composition is an important controller of DOC production, and large increases in DON have been attributed to cell death and lysis after soil is treated with biocide (Yavitt and Fahey, 1984).

Most disturbances or manipulations tend to increase the concentration of DOC and DON in forest soil solution (Kalbitz et al., 2000), with effects that are typically mediated by microbial processes and last a year or 2. Forest cutting, organic matter additions, liming, acidification, and hurricanes have each been shown to increase DOM concentration and flux in forest floor solution (Sollins and McCorison, 1981; Guggenberger, 1994; Johnson et al., 1995; McDowell et al., 1996; Qualls et al., 2000; Kalbitz et al., in press). In contrast to the consistent increases in DOM seen with other disturbances, studies of the effects of N fertilization on DOM dynamics have yielded inconsistent results. In Europe, the NITREX sites showed little change in DOC and DON production and export in soil solution with addition of N for 4–6 years (Emmett et al., 1998; Gundersen et al., 1998; Stuanes and Kjønass, 1998; Raastad and Mulder, 1999). Similarly, laboratory incubations of soils collected from sites with 8–29 years of N fertilization showed no effects of N fertilization on DOC or DON production (Sjöberg et al., 2003). In contrast, a chronic N fertilization experiment in southern Norway showed that after 9 years of NH_4NO_3 additions (at 3 and 9 g m^{-2} per year) there was a significant difference between control and treatment plots in both DOC and DON concentrations (Vestgarden et al., 2001). Forest floor DOC concentrations declined from 80.3 mg l^{-1} in control plots to 24.9 and 38.5 mg l^{-1} in the low and high N plots, respectively; similar results were obtained for DON. In Massachusetts, after 7 years of chronic nitrogen addition, McDowell et al. (1998) and Yano et al. (2000) reported no significant change in DOC concentrations, or the fraction of DOC that was biodegradable. They did, however, observe large increases in DON concentrations (double or triple the control values). The reasons for these widely divergent results (no change in DOC or DON at one set of European sites; declines in both DOC and DON at another; and no change in DOC with large increases in DON at a North American site) are unknown, suggesting that there is much yet to be learned about the processes driving production and uptake of DOC and DON in forest soils.

Most of the literature on the response of DOM to forest disturbance is based on sampling soil solution for a few years. The extent to which disturbance responses are maintained over intervals longer than a few years is largely unknown. Here we present data from ten years of sampling soil solution at the Harvard Forest chronic N experiment. We hypothesized that DOC and DON concentrations and flux would show progressive changes in response to the declines in ecosystem health predicted to occur with chronic N fertilization.

2. Materials and methods

2.1. Study site

The Harvard Forest, Petersham, Massachusetts, USA (42°30′N, 72°10′W) is the site of the chronic N experiment. The region has undergone extensive changes in vegetation and land use over the last two centuries, with a cycle of forest clearing, agricultural or woodlot use, abandonment, and forest regrowth (Foster, 1992). Average monthly temperatures range from −7 °C in January and 19 °C in July; precipitation is evenly distributed throughout the year and averages 1100 mm (Currie et al., 1996). Although snow often covers the ground for several months during winter, mid-winter thaws, soil freezing, and rain storms also occur. The chronic N study plots are 30 m × 30 m, with either hardwood (predominantly *Quercus velutina* and *Q. rubra*) or pine (*Pinus resinosa*). The hardwood site regenerated after clearcutting in the 1940s; the pine was planted in 1926 after agricultural abandonment. The pine plots were plowed prior to establishment of the pine plantation, but the hardwood plots were not. Soils are rocky and well-drained Typic Dystrochrepts derived from glacial till. Nitrogen fertilization started in 1988, and has continued in every subsequent growing season. Addition rates are 15 g N m^{-2} per year (high N), 5 g N m^{-2} per year (low N), and control (no added N); N is added as NH_4NO_3 applied with a backpack sprayer (Magill et al., 1997). Atmospheric deposition of inorganic N is approximately 0.8 g m^{-2} per year in wet and dry deposition combined (Aber et al., 1998). Plots are designated as pine high N, low N, and control and hardwood high N, low N, and control. Additional information on the study site, methods of N application, and soil solution chemistry can be found in Aber et al. (1993, 1998), Currie et al. (1996), Magill et al. (1997, 2000), McDowell et al. (1998), Minocha et al. (2000) and Yano et al. (2000).

2.2. Lysimeter sampling

Soil solution was collected from polyethylene zero tension lysimeters installed beneath the forest floor in August 1992, 4 years after the initiation of N fertilization (Currie et al., 1996). Each plot contains five lysimeters that were sampled after major rain events; bottles were kept in place in the field at all times. From 1993 to 1998, soil solution collected from the five lysimeters was combined by plot prior to chemical analysis, yielding one sample per plot per event. From 1999 to 2002, samples were collected and analyzed from individual lysimeters to allow a statistical analysis of variability among plots. Volume-weighted means were also calculated for these years to provide comparability with bulked samples collected from 1993 to 1998. During the 10-year study period, over 155 events were sampled, resulting in a total of over 1500 individual samples analyzed. Samples were transported on ice to the University of New Hampshire, where they were filtered through pre-combusted Whatman GF/F glass fiber filters within 36 h of collection. After filtration, samples were frozen prior to analysis.

2.3. Analytical techniques

Dissolved organic carbon was analyzed using a Shimadzu Total Organic Carbon analyzer with autosampler (Pt-catalyzed combustion at 680 °C; Shimadzu 5000 or TOC-V; detection limit 0.1 mg l^{-1}). Total dissolved N (TDN) was measured by alkaline persulfate digestion (Solorzano and Sharp, 1980) from October 1992 to September 1994, and high-temperature Pt-catalyzed combustion from October 1994 to November 2002 (Merriam et al., 1996). The two methods provide similar results (Merriam et al., 1996), although detection limits (0.03 mg l^{-1}) are lower for high-temperature combustion than persulfate (0.1 mg l^{-1}). Nitrate and NH_4^+ were measured with automated colorimetry (Technicon methods 782-86T and 780-86T, respectively; detection limit is 0.2 mg l^{-1} for each method). Dissolved organic N was estimated as the difference between concentrations of TDN and dissolved inorganic N (DIN; the sum of NH_4^+-N and NO_3^--N). Very high concentrations of DIN relative to DON made detection of DON difficult in many of the samples. Approximately 20% of samples had undetectable levels of DON.

The dissolved organic matter in soil solution was characterized using the fractionation scheme described by Kaushal and Lewis (2003). Soil solution was bulked by treatment during June and August 2000 and 2001, and a total of 24 samples were fractionated. The bulked water was filtered with a glass fiber filter (Whatman GF/F), acidified to pH 2 with sulfuric acid, and passed through columns containing XAD-8 resin, which retains hydrophobic acids, neutrals, and bases (Leenheer and Huffman, 1976). The hydrophobic fraction (which can be considered a measure of the humic fraction of dissolved organic matter) was then eluted with 0.1 N NaOH and analyzed for DOC and DON content using thermal combustion and persulfate oxidation, respectively. The concentration of DOC and DON in the hydrophilic (non-humic) fraction was estimated as the differences between total DOC and DON, and that found in the hydrophobic fraction.

2.4. Data analysis

Fluxes were calculated using the method described in Currie et al. (1996) and McDowell et al. (1998) on a calendar year basis. Percolation of water through the forest floor was modeled using PnET II (Aber and Federer, 1992); flux of nitrogen or carbon was calculated by multiplying seasonal water percolation by seasonal mean concentrations in soil solution. For winter, solution concentrations were estimated from the average of the last autumn sample and the first spring sample for the appropriate year (McDowell et al., 1998). Data were grouped into winter (December–February), spring (March–May), summer (June–August) and autumn (September–November).

Treatment effects were assessed statistically for 4 years (1999–2002) during which up to five individual lysimeter samples were collected from sub-plots within each of the treatment plots. Two-way ANOVA (treatment × season) was used to assess the effects of N fertilization and season on the concentrations of DIN, DON, and DOC in soil solution of the two stands. The data were transformed ($\log(n+1)$) prior to analysis to reduce skewness and kurtosis. The Kolmogorov–Smirnov test was also used to obtain a non-parametric analysis of the effects of N treatment on solution concentrations. One-way ANOVA was used to assess the effect of treatment on the percentage of total DOC and DON found in the hydrophobic fraction, and the C:N ratio of both the hydrophobic and hydrophilic fractions of dissolved organic matter. This analysis was conducted separately for each stand. Tukey's test was used for post-hoc comparisons of means for cases in which ANOVA revealed significant treatment effects. To estimate trends in concentrations and fluxes over time, linear regression analysis was applied to annual averages in the 10-year data sets. For all statistical analyses a significance level of $P < 0.05$ was used.

3. Results

3.1. Dissolved inorganic nitrogen

Concentrations of NH_4^+ and NO_3^- did not show any consistent trends over the 10-year study period in either the hardwood or pine plots (Fig. 1). Ammonium and nitrate both increased substantially in the hardwood high N plot from 1993 to 1996, for example, but dropped almost as much from 1996 to 2001. Soil solution from the low N treatments was consistently around 4 mg l^{-1} NH_4-N, and 6 mg l^{-1} NO_3-N, and also showed no strong trends over time in either the hardwood or pine plots. Flux from the forest floor showed no consistent interannual patterns across treatments, suggesting relatively little influence of year-to-year variation in climate on fluxes. 1996, for example, was the highest DIN flux for the hardwood high N plot, but one of the lowest flux years for the pine high N plot. High N and low N plots showed similar variations in annual flux over the 10-year sampling period, with the standard deviation equal to about 20% of the mean in the pine plots, and 40% of the mean in the hardwood plots (Table 1). Average annual flux of NO_3-N was consistently higher than NH_4-N in all the experimental treatments, and approached or exceeded the amount of inorganic N added to the plots (Table 1).

There was a large effect of N treatment on DIN concentrations in soil solution, as well as strong seasonal variation in concentrations (Table 2). Patterns were consistent across season, with high N

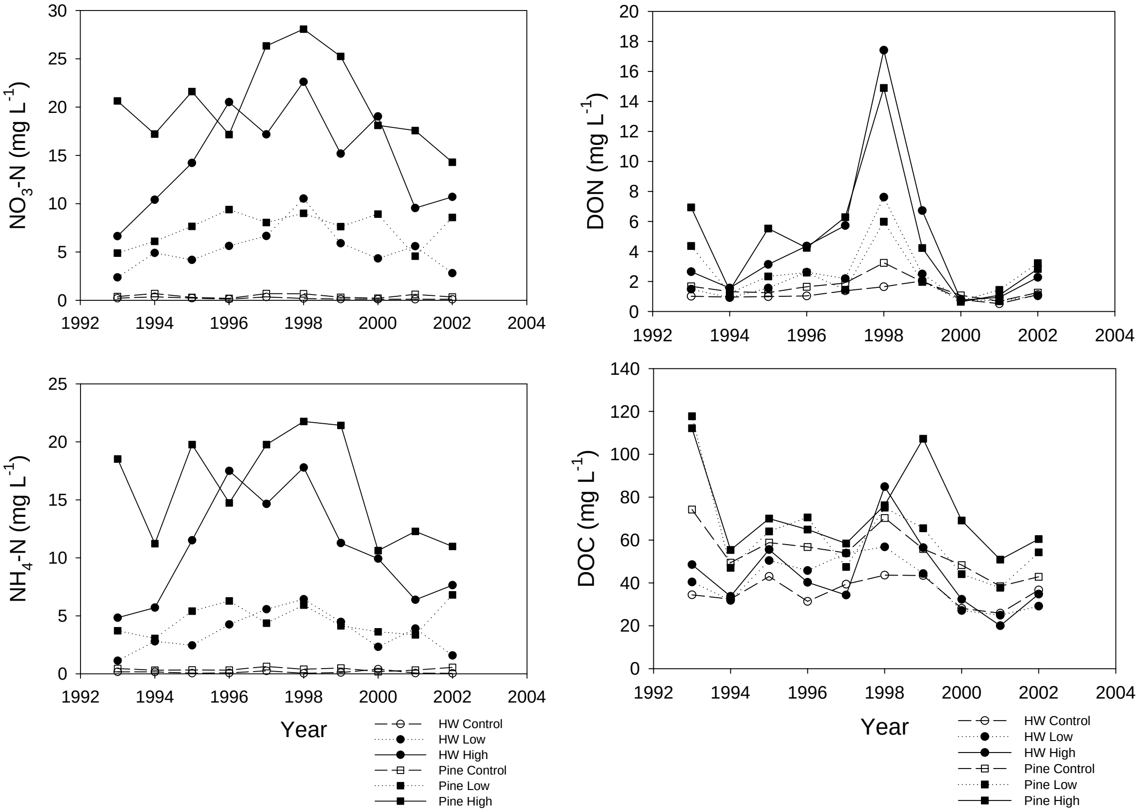

Fig. 1. Concentrations of NO_3, NH_4, DOC, and DON in forest floor solution collected from zero tension lysimeters at Harvard Forest 1993–2002.

treatments > low N > controls in every season in both hardwood and pine plots. Concentrations of DIN were highest in summer, with an average of 32 mg l^{-1} DIN in the hardwood high N plot and 48 mg l^{-1} in the pine high N plot; lower values were obtained in spring and autumn (Table 2). Nitrogen fertilization had a statistically significant effect on N concentrations in both the hardwood and pine stands (Table 3). Season had a statistically significant effect on DIN concentrations in both stands, and interaction between treatment and season was also significant (Table 3).

Table 1
Flux of ammonium (NH_4^+), nitrate (NO_3^-), dissolved organic nitrogen (DON), and dissolved organic carbon (DOC) in soil solution collected beneath the organic horizon in zero tension lysimeters from 1993 to 2002[a]

Stand	Treatment	NH_4-N	NO_3-N	DON	DOC
Hardwood	Control	0.10 (0.07)	0.15 (0.07)	0.80 (0.20)	28.8 (3.7)
	Low N	1.60 (0.70)	2.51 (0.93)	1.21 (0.74)	27.6 (7.4)
	High N	5.91 (2.37)	8.61 (3.15)	2.37 (1.10)	30.2 (9.5)
Pine	Control	0.24 (0.06)	0.33 (0.15)	1.09 (0.29)	40.2 (8.5)
	Low N	2.60 (0.53)	4.72 (0.89)	1.59 (0.75)	43.7 (14.1)
	High N	9.57 (2.08)	12.44 (2.74)	2.71 (1.86)	53.5 (13.4)

[a] All values are means (standard deviation) in g m^{-2} per year. Low N treatment is 5 g N m^{-2} per year, and high N treatment is 15 g m^{-2} per year.

Table 2
Mean concentration (mg l^{-1}) of inorganic N and dissolved organic matter by stand and season for 1993–2002[a]

Stand	Treatment	DIN			DON			DOC			DOC:DON		
		SP	SU	AU	SP	SU	AU	SP	SU	AU	SP	SU	AU
Hardwood	Control	0.36	0.42	0.19	0.99	1.21	1.12	33.4	38.7	35.6	34	32	32
	Low	6.90	11.6	7.34	1.29	2.83	1.92	31.7	47.5	41.8	25	17	22
	High	19.9	31.8	24.1	2.25	4.20	3.09	32.0	56.7	42.8	14	14	14
Pine	Control	0.64	1.39	0.48	0.93	1.79	1.79	36.1	58.4	66.0	39	33	37
	Low	5.98	13.2	15.1	1.38	2.35	3.26	42.1	63.1	76.6	31	27	23
	High	7.34	47.6	35.0	1.92	6.70	4.65	41.8	67.9	92.5	22	10	20

[a] Seasons are spring (SP), summer (SU), and autumn (AU); treatments are control, low (5 g N m^{-2} per year) and high (15 g N m^{-2} per year). Sample size = 30–50 sample dates for each cell. DIN: dissolved inorganic N ($NH_4 + NO_3$); DON: dissolved organic nitrogen; DOC: dissolved organic carbon. DOC:DON is mass to mass and is unitless.

3.2. Dissolved organic nitrogen and dissolved organic carbon

Similar to the results for inorganic nitrogen, the concentration and flux of DON showed no consistent trends over the 10-year study period (Figs. 1 and 2). Concentrations and fluxes of DON in the high N plots were particularly high in 1998, a year in which DIN fluxes were not markedly higher than average for most plots. Using regression analysis, we found that average annual DON concentrations were correlated with DIN concentrations in hardwood high N and low N plots, as well as the pine high N plot. In those plots DIN concentrations explained 45–60% of the variation in DON concentrations over the 10-year sampling period. For the remaining plots (hardwood control, pine control, and pine low N), relationships between DON and DIN concentrations were not statistically significant.

Average DON fluxes in the high N plots were triple those of the controls, with values as high as 2.7 g m^{-2} per year (Table 1). N treatment had a statistically significant effect on DON concentration in both hardwood and pine stands (Table 3). In the control plots of both stands, DON represented almost 75% of total dissolved nitrogen flux; the proportion declined to about 12% in the high N plots (Table 1). For most plots, DON concentrations were typically lowest in the spring, and highest in the summer. Season had a statistically significant effect on DON concentrations in both the hardwood and pine stands (Table 3).

Dissolved organic carbon did not follow the same patterns as dissolved organic nitrogen. Overall, there was little change in DOC concentration and flux in response to N fertilization, despite the large changes in DON (Figs. 1 and 2). Concentrations of DOC increased by at most 50% from control to high N, but usually no more than 20% (Table 2). These

Table 3
Results of ANOVA examining the effects of N treatment and season on the concentrations of DIN, DON, and DOC in soil solution at the Harvard Forest chronic N plots[a]

Stand	Factor	DIN		DON		DOC	
		F-value	*P*	*F*-value	*P*	*F*-value	*P*
Hardwood	Treatment	169.1	0.000	53.98	0.000	0.502	0.629
	Season	19.00	0.000	4.580	0.011	2.306	0.120
	Treatment × season	3.200	0.013	28.65	0.000	2.005	0.118
Pine	Treatment	293.4	0.000	72.53	0.000	2.000	0.136
	Season	14.05	0.000	6.869	0.001	13.147	0.000
	Treatment × season	7.881	0.000	1.697	0.150	2.241	0.063

[a] Individual samples from five sub-plots per treatment plot collected from 1999 to 2002 were used in the analysis. All data were $\log(n+1)$ transformed prior to analysis to reduce skewness and kurtosis.

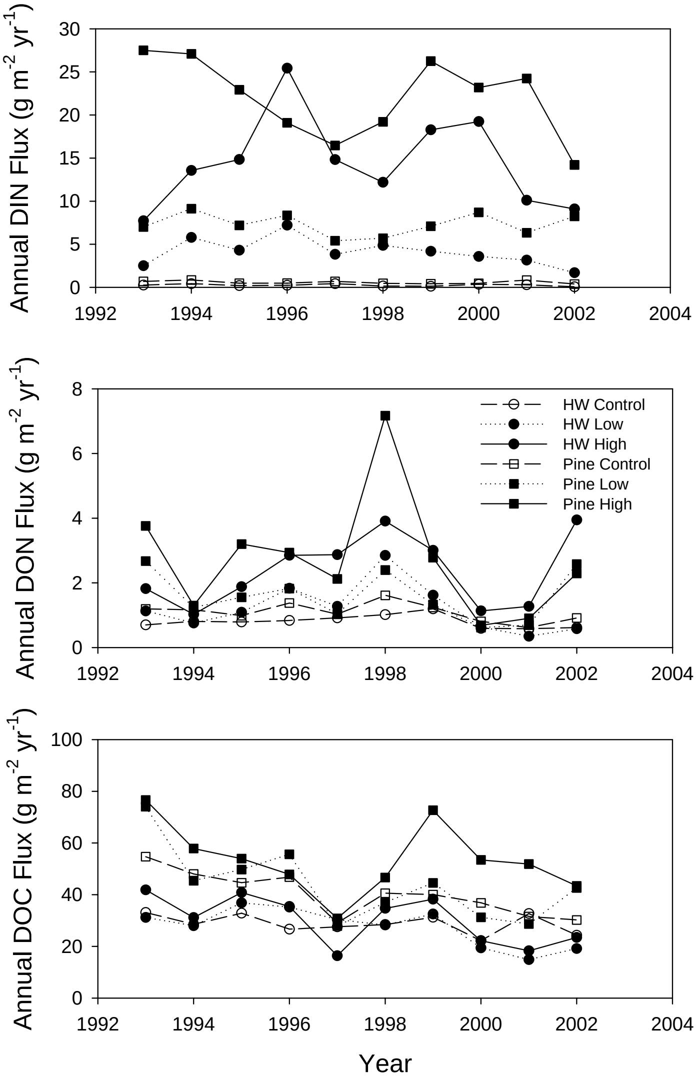

Fig. 2. Flux of dissolved inorganic nitrogen, dissolved organic carbon, and dissolved organic nitrogen in forest floor solution collected from zero tension lysimeters at Harvard Forest from 1993 to 2002.

differences were not statistically significant (Table 3). Similar results were obtained when using the non-parametric Kolmogorov–Smirnov test to determine the effects of N treatment on DOC. Season had a statistically significant effect on DOC concentrations in the pine stand, but not the hardwood; highest concentrations were found in summer and autumn (Table 3). Flux of dissolved organic carbon showed statistically significant declines over time in half of the plots (hardwood low N, and pine control and low N). In these plots, time explained 45–69% of the variance in annual DOC flux. In the other plots, DOC flux also declined over our 10-year sampling period (negative slope in the regression analysis), but the trends were not statistically significant.

3.3. Composition of dissolved organic matter

The ratio of DOC:DON in forest floor solution did not change over the 10-year study period for any of the plots (no statistically significant relationship between DOC:DON and year). There were large

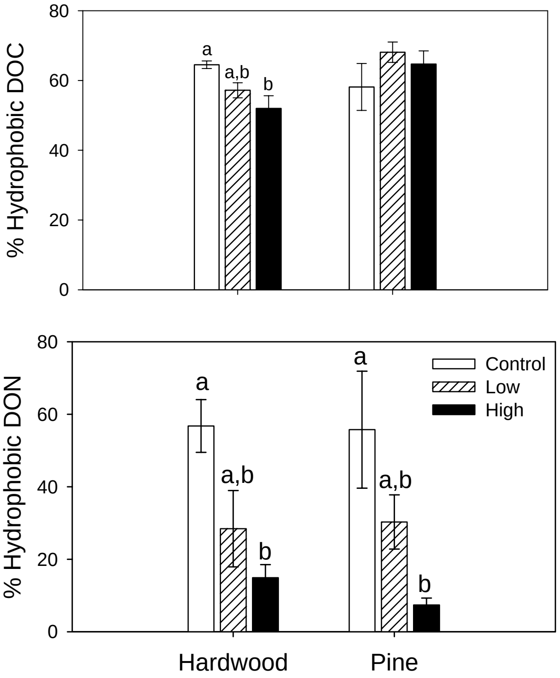

Fig. 3. Effects of N treatment on percentage of total DOC and DON found in the hydrophobic fraction of dissolved organic matter. Forest floor solutions were collected in June and August 2000 and 2001. Bars show averages, and error bars are standard error of the mean.

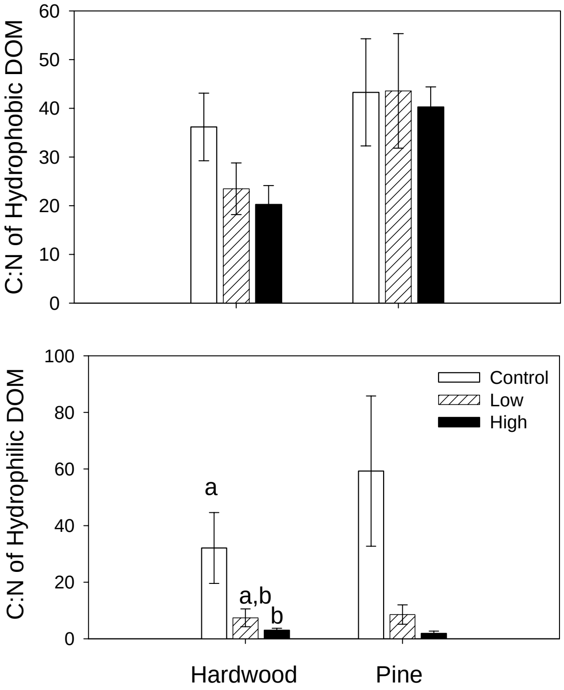

Fig. 4. Effects of N treatment on percentage of total DOC and DON found in the hydrophilic fraction of dissolved organic matter. Forest floor solutions were collected in June and August 2000 and 2001. Bars show averages, and error bars are standard error of the mean.

and consistent declines in DOC:DON as a function of N treatment, with values of the high N plots almost half those of controls (Table 2). There were consistent seasonal differences as well, with highest DOC:DON values in spring and lowest values in autumn for every plot.

The DON increase resulting from N fertilization was found primarily in the hydrophilic fraction of dissolved organic matter; the proportion of total DON found in the hydrophobic fraction declined from over 55% to less than 15% in both hardwood and pine high N (Fig. 3). Similarly, large changes were observed in the C:N ratio of the hydrophilic fraction of dissolved organic matter, which declined from 30 to 3 in the hardwood plot, and from 60 to 2 in the pine plot (Fig. 4). The percentage DOC found in the hydrophobic fraction was relatively unchanged with fertilization, although there was a small but statistically significant decline (from 65 to 52% of total DOC) in the hardwood plots (Fig. 3). Overall, our results show that there have been both quantitative and qualitative changes in DON resulting from N fertilization.

4. Discussion

4.1. N saturation

Our results show that the organic soil horizons in both the pine and hardwood plots have been effectively "saturated" (Aber et al., 1989) with N for at least the past decade, when our sampling began. For the pine plots, average output of inorganic N in forest floor solution exceeded N inputs by fertilization for both application rates (Fig. 5). For the hardwood plots, outputs were similar to or exceeded inputs when the dissolved organic N resulting from fertilization was included in the N outputs (Fig. 5). This "saturation" of the forest floor must have occurred quickly, within no more than four years of initial application. We cannot

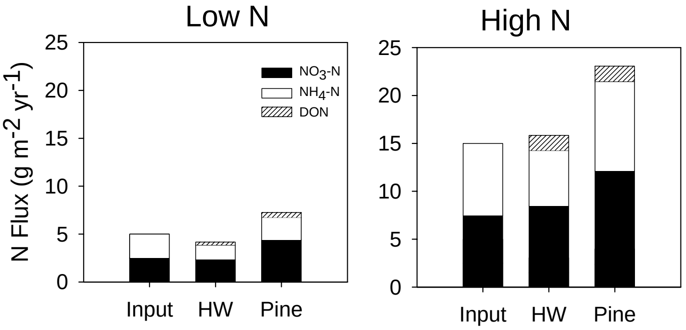

Fig. 5. Average flux of N from the forest floor relative to fertilizer inputs, 1993–2002. Fluxes in the N treatment plots of each stand are net fluxes due to treatment; values obtained in the control plots have been subtracted from the treatment values.

be sure of the exact timing, as our study commenced in the fifth year of N application. We emphasize that our results are for the forest floor alone, as soil solution from 60 cm depth in the mineral soil showed significant lags between N application and the appearance of N in soil solution (Magill et al., 2000, 2004).

We believe that there has been a cascading effect as fertilizer N has moved through the hardwood plots, based on results presented here for the forest floor and those described elsewhere for mineral soil solution collected at 60 cm depth (Magill et al., 2000, 2004). Outputs of DIN from the forest floor of the hardwood high N plot have been high and relatively constant from 1993 to 2002 at 10–20 mg/l (Fig. 1). These results for the forest floor are in sharp contrast to those for mineral soil solution in the hardwood high N plot, which showed little effect of N fertilization prior to 1996, and did not exceed 10 mg l^{-1} until 2002 (Magill et al., 2000, 2004). This suggests a cascading effect of added N, with additions overwhelming forest floor retention mechanisms at some point in the first 4 years, but not beginning to exceed N retention capacity in the mineral soil until after 8 years of fertilization at 15 g m^{-2} per year. The lag between saturation of the organic horizons and the underlying mineral soil thus appears to be at least 4 years, and is due to a significant N sink in the mineral soil. The magnitude of this mineral soil N sink in the hardwood stand appears to be considerable. In the hardwood low N plot, for example, inorganic N inputs averaged 4 g m^{-2} per year from the organic horizon during 1993–2002 (Table 1), but DIN flux in mineral soil averaged less than 0.5 g m^{-2} per year throughout the same period (Magill et al., 2004). This indicates an annual N sink of as much as 3.5 g m^{-2} per year, or a cumulative N sink of as much as 35 g m^{-2} in the mineral soil.

The large excess of N outputs over inputs in the forest floor of the pine high N plot suggests that ‘‘mining’’ of N from soil organic matter may be occurring to support the observed flux in forest floor solution. Nitrogen outputs in excess of added fertilizer could be due to increases in N mineralization, decreases in plant or microbial assimilation of N, or some combination of both. Reduced N uptake by trees in the latter part of the experiment seems likely; tree vigor has declined dramatically over time in the pine high N plot, with noticeable tree death by 2000. In 2002, a complete census of the plots showed that total biomass of red pine in the control plot had declined by 12% from 1988 to 2002 in the control, 23% in the low N, and 56% in the high N plots; similar trends were also apparent for stem density (Magill et al., 2004). Soil respiration in the field was significantly less in the pine high N plot than the control in 2001, and microbial respiration in laboratory-incubated soils was 60% lower in high N plots than controls (Bowden et al., 2004). These observations suggest that declines in N uptake by both trees and bacteria have played a significant role in the net depletion of N occurring in the fertilized pine plots. With reduced N uptake, N produced by mineralization and leaching of soil organic matter is more susceptible to loss from the organic horizon in soil solution.

The importance of obtaining a long-term data set is clearly shown in Figs. 1 and 2. Over the entire decade of study, no long-term trends were evident. Earlier work suggested that both DIN and DON concentrations were increasing over time at the site (McDowell et al., 1998), but this conclusion now appears to be incorrect when our early results are placed in the context of the long-term data set. We know of no other forest soil manipulation in which solution data have been recorded from the organic horizon continuously for a decade, but suspect that our results would be typical for other sites or other experimental manipulations. We believe that interannual variability should receive greater attention in future studies, as our results indicate that misleading conclusions can be drawn from a data set of only a few years in duration.

It should be noted that our estimates of annual N fluxes in soil solution include losses during winter. At the site, soils typically remain unfrozen beneath a snow cover, and water moves through the soil profile during winter. This water flux is reasonably well characterized by the hydrologic component of the PnET model. We have not directly sampled soil solution in the winter, however, and are thus relying on an estimated concentration of winter soil solution based on measured concentrations in fall and spring. This adds some uncertainty to our estimates of N flux, and probably biases our results toward high N flux if N loss from the forest floor is driven by temperature.

4.2. *Implications for mechanisms of DOM production*

Dissolved organic carbon flux from the organic horizons of both the pine and hardwood stands is remarkably insensitive to the biological changes associated with N saturation. The concentration of DOC in soil solution reflects the balance between a wide variety of competing processes that produce and consume DOC. These include abiotic leaching of soil organic matter and recent litter, production of DOC during decomposition of litter and soil organic matter, microbial respiration of DOC, and rhizosphere processes such as root exudation and decomposition of dead roots (McDowell, 2003). A number of these processes have likely been changed by chronic N fertilization. Fungal community composition is known to have an effect on DOC production (Møller et al., 1999), and fungal importance in the microbial community as well as phenol oxidase production have decreased at Harvard Forest in response to N addition (Frey et al., 2004). This reduction in phenol oxidase production by white-rot fungi has probably decreased DOC production (Møller et al., 1999). Above-ground litter inputs have declined over time in all the pine plots, and litter has shifted toward a higher proportion of more readily decomposed hardwood leaves as the dying pines are replaced by early successional species (Magill et al., 2004). The microbial component of soil respiration has declined with N additions (Bowden et al., 2004), suggesting reduced uptake of the DOC needed to fuel microbial respiration and a net increase in DOC concentrations. In the face of these biotic changes, it appears that there must be counterbalancing changes in the rates of DOC production and consumption to maintain a relatively stable DOC concentration and flux. Alternately, abiotic leaching of soil organic matter may also be contributing to the relatively constant DOC fluxes. Leaching reflects overall soil organic matter content, which has not undergone detectable change in the 14 years of the chronic N study (Magill et al., 2004).

Large increases in DON concentration and flux without correspondingly large changes in DOC suggest that DOC and DON dynamics have been decoupled under conditions of N saturation (McDowell, 2003). Increased production of the N-rich fraction of DOM, or decreases in its consumption, must be occurring with N saturation to produce the changes we have observed. Under ambient N inputs, DOM lost from the forest floor in soil solution is carbon-rich, with a C:N ratio of 35–45 (mass:mass), depending on stand type and season (Table 2). This is on the high end of values found in other temperate forested ecosystems, which range from 20 to 40 (Michalzik et al., 2001). N fertilization lowers the C:N ratio of DOM at Harvard Forest to 13 in the hardwood high N plot and 20 in the pine high N plot, well below values reported for other temperate forests (Michalzik et al., 2001). Increases in the hydrophilic fraction of DON drive the overall shift toward N-rich DOM, suggesting that compounds such as amino acids and amino sugars have increased in concentration due to N saturation (Fig. 3). This conclusion is further supported by the steep decline in C:N ratio of the hydrophilic fraction, which under conditions of N saturation reaches levels indicative of a high amine content (C:N below 5; Fig. 4).

In an earlier paper we had hypothesized that mycorrhizal assimilation of DIN and exudation of amine-rich DON that is subsequently adsorbed in mineral soil might be an important pathway for N conservation within the soil profile under conditions of N saturation (Aber et al., 1998). Of the likely N retention mechanisms, it was the only one which fit the constraints imposed by field observations of CO_2 flux, DOC flux, and plant uptake. Our data are consistent with this hypothesis, as net DON flux has increased and its composition has shifted toward the low DOC:DON ratio typical of amine-rich organic matter. Although we suspect that increased DON production by mycorrhizae is driving the increased DON flux at Harvard Forest, N addition might also decrease DON mineralization (through reduced lignase production; Carreiro et al., 2000) or stimulate abiotic formation of DON from nitrate (Dail et al., 2001). Development and application of techniques for analysis of gross DON production, analogous to the ^{15}N measurements of gross mineralization and nitrification rates that are now widely employed in studies of N cycling in soils (Stark and Hart, 1997), will be needed to gain a mechanistic understanding of controls on DON flux in soils (McDowell, 2003).

4.3. Synthesis and conclusions

Our long-term data show quite clearly that the forest floor in both the hardwood and pine plots has been N-saturated for at least a decade, highlighting the importance of the mineral soil in retaining N and slowing the onset of ecosystem-level N saturation. N saturation appears to cascade through forest ecosystems, first overwhelming N retention mechanisms in the forest floor and subsequently overwhelming those in the mineral soil. Quantifying dissolved organic carbon dynamics in the face of N saturation has helped to constrain the pathways by which N is retained at this site. With N application, we had originally hypothesized that a decline in the concentration and flux of DOC in forest floor solution would occur due to the energetic costs of microbial uptake and assimilation of N (Aber, 1992). This hypothesis appeared to be incorrect after the first several years of data (McDowell et al., 1998), and now we have shown unequivocally in this paper that the hypothesized declines have not occurred. The failure of this hypothesis, when combined with other supporting data on biotic responses to N addition, suggested novel pathways that might be responsible for N retention (Aber et al., 1998).

Our long-term results of the Harvard Forest chronic N study provide useful insights into linkages between the nitrogen cycle and the production and consumption of dissolved organic matter in forest soils. Our data show that DOC flux from the forest floor is remarkably insensitive to N status in both hardwood and pine stands, yet DON flux responds strongly to increased N inputs. This suggests that there are fundamental differences in the pathways producing and/or consuming DOC and DON in forest soils, despite their obvious similarities. They also highlight our fundamental ignorance of the chemical nature, origin, and ecological significance of dissolved organic nitrogen in forest soils.

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