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Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests

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Abstract. At the Harvard Forest, Massachusetts, a long-term effort is under way to study responses in ecosystem biogeochemistry to chronic inputs of N in atmospheric deposition in the region. Since 1988, experimental additions of NH_4NO_3 (0, 5 and 15 g N m^{-2} yr^{-1}) have been made in two forest stands: *Pinus resinosa* (red pine) and mixed hardwood. In the seventh year of the study, we measured solute concentrations and estimated solute fluxes in throughfall and at two soil depths, beneath the forest floors (Oa) and beneath the B horizons.

Beneath the Oa, concentrations and fluxes of dissolved organic C and N (DOC and DON) were higher in the coniferous stand than in the hardwood stand. The mineral soil exerted a strong homogenizing effect on concentrations beneath the B horizons. In reference plots (no N additions), DON composed 56% (pine) and 67% (hardwood) of the total dissolved nitrogen (TDN) transported downward from the forest floor to the mineral soil, and 98% of the TDN exported from the solums. Under N amendments, fluxes of DON from the forest floor correlated positively with rates of N addition, but fluxes of inorganic N from the Oa exceeded those of DON. Export of DON from the solums appeared unaffected by 7 years of N amendments, but as in the Oa, DON composed smaller fractions of TDN exports under N amendments. DOC fluxes were not strongly related to N amendment rates, but ratios of DOC:DON often decreased.

The hardwood forest floor exhibited a much stronger sink for inorganic N than did the pine forest floor, making the inputs of dissolved N to mineral soil much greater in the pine stand. Under the high-N treatment, exports of inorganic N from the solum of the pine stand were increased >500-fold over reference (5.2 vs. 0.01 g N m^{-2} yr^{-1}), consistent with other manifestations of nitrogen saturation. Exports of N from the solum in the pine forest decreased in the order NO_3-N > NH_4-N > DON, with exports of inorganic N 14-fold higher than exports of DON. In the hardwood forest, in contrast, increased sinks for inorganic N under N amendments resulted in exports of inorganic N that remained lower than DON exports in N-amended plots as well as the reference plot.

Introduction

Fluxes of organic solutes in the soils of humid forests play important roles in nutrient movement and pedogenesis (Dawson et al. 1978; Schoenau & Bettany 1987; Qualls et al. 1991). Year-round leaching fluxes distinguish the biogeochemistry of forests in humid climates from the biogeochemistry of other terrestrial ecosystems. In some other ecosystems the leaching of solutes from upper to lower soil horizons occurs during only part of the year, for example in Rocky Mountain forests (Arthur & Fahey 1993); in others illuviation is not typically significant, for example in semi-arid grasslands (Burke & Lauenroth 1996).

In forests of the northeastern U.S., the retention of anthropogenically-derived nitrogen from atmospheric deposition alters nutrient cycles in the forests themselves and affects surface-water quality by controlling nitrate levels and mitigating acidification (Eshleman & Hemond 1988; Johnson 1992). Quantification of the downward fluxes of inorganic and organic N compounds through soil horizons should show which horizons are responsible for the widely differing capacities of forest ecosystems to retain added inorganic N (compare Aber et al. 1993; Kahl et al. 1993). Determining the seasonality of N fluxes may further elucidate the processes responsible for N retention by each soil horizon.

Much of the N in forest soils is present in relatively stable humic substances formed during incomplete oxidation of forest litter (Stevenson 1982). Incomplete decomposition of foliar, root and woody litter leads to a buildup of organic residues on the soil surface (the forest floor), which typically amounts to 8000 g m^{-2} organic-matter mass in a mid-elevation Northern Hardwood forest 100 years after clear-cutting (Covington 1981). Some soluble organics are eluviated from the forest floor as solutes and sorbed in organo-mineral complexes in lower horizons. This transfer process could account for much of the plant nutrients and humic material in mineral soil (Fahey et al. 1985). Weathering products, heavy metals and organic pollutants can also be complexed and transported in soils by soluble organics (Schnitzer 1991).

In undisturbed forests, dissolved organic nitrogen (DON) typically composes the bulk (70 to 95%) of the total dissolved nitrogen (TDN) leached from the forest floor (Sollins & McCorison 1981; Fahey et al. 1985; Qualls et al. 1991). DON was also found to compose 95% of the N loss in streams from unpolluted, old-growth temperate forests in Chile (Hedin et al. 1995). In the few temperate forests studied in eastern North America, the fluxes of dissolved organic carbon (DOC) and DON transported from the forest floor to mineral soil have been estimated to represent from 5% to 24% of annual inputs of C in foliar litter and from 15% to 37% of annual inputs of N in foliar litter (Cole & Rapp 1981; McDowell & Likens 1988; Qualls et al.

1991; Vance & David 1991). The dominant fractions of the soluble organics are typically decay resistant organic acids. These acids result from condensation of partially decomposed plant and microbially-derived compounds into heteropolymers (Qualls & Haines 1992; Guggenberger & Zech 1994). Their sorption on mineral grains may differ among soils with different chemical and physical properties, for example texture (Jardine et al. 1989). Most studies have shown that the major fraction of DOC in leachate from forest floors in the eastern U.S. is retained in mineral soils (McDowell & Wood 1984; McDowell & Likens 1988; Qualls et al. 1991).

A manipulation experiment in progress at the Harvard Forest, Massachusetts provided the opportunity to measure fluxes of dissolved organics in two forest stands that have responded differently to long-term N amendments. Soils in a red pine stand and a mixed hardwood stand retained essentially all of the added nitrogen in the first three years of treatment, but after six years, N-amended plots in the pine stand were losing nitrate from the solum at high levels (Aber et al. 1993; Magill et al. 1996). Our objectives were as follows:

1. To determine the effects of long-term additions of inorganic N, mediated by forest vegetation type, on (a) concentrations of DOC, DON, and inorganic N in soil solution; (b) transport of these solute fractions from the forest floor to the mineral soil; and (c) loss of these solute fractions from the solums.
2. To calculate separate rates of annual retention of TDN (including biotic uptake) for the forest floor and mineral soil horizons of each forest stand.

We also sought to analyze soil-solution fluxes by season as a first step toward investigating the importance of seasonality in DOC:DON ratios and DOC and DON eluviation, illuviation and loss at the Harvard Forest. We expected that leaching from fresh litter would dominate Oa solution fluxes in the fall, resulting in high DOC:DON ratios, while in the summer the solubilization of decomposition products and secondary compounds would dominate, leading to lower DOC:DON ratios.

Materials and methods

Study site

Harvard Forest, in the central highlands of Massachusetts, is a site in the Long-Term Ecological Research (LTER) network sponsored by the U.S. National Science Foundation. Elevation ranges from 220 to 410 m; monthly mean temperatures are −7 °C in January and 19 °C in July. Average precipitation is approximately 110 cm yr^{-1}, distributed fairly evenly throughout the year (Van Cleve & Martin 1991). The snowpack is variable: it may begin to form

Table 1. Average litter chemistry for foliar litter at the Harvard Forest (Aber *unpublished data*). L, C and E refer to the proximate carbon fractions Lignin, Holocellulose, and Extractives (Ryan et al. 1990). Data were averaged over all N treatments within each stand, except for nitrogen, in which reference-plot averages are listed.

Stand	Species	Biomass (% of stand)[1]	L (%)	C (%)	E (%)	Nitrogen (%)
Red pine	Red pine	98	30	40	30	0.70
Mixed hardwood	Black oak/Red oak	78	31	39	30	0.86
Mixed hardwood	Red maple	5	27	38	35	0.47
Mixed hardwood	Black birch	8	35	45	20	1.65

Note: 1. Aber et al. 1993.

in the forest in December and last until early April, or it may melt completely and reform in midwinter.

This study took place on the Chronic N Plots at the Harvard Forest, in two forest stands occurring within 100 m of each other on very similar soils. The first is an even-aged red pine (*Pinus resinosa* Ait.) stand planted in 1926; the second is a mixed hardwood stand (*Quercus velutina* Lam., *Q rubra* L., *Betula lenta* L., *Acer rubrum* L.) approximately 50 years old, which naturally regenerated after clear-cutting. Lignin and nitrogen content of foliar litter from the dominant trees are quite similar in the two stands (Table 1). Soils are rocky and well-drained, formed from glacial till and contain well-defined O horizons (mor type). Soils in the pine stand are Montauk variants, described as coarse-loamy, mixed, frigid Typic Dystrochrepts. An Ap horizon is present in the pine stand. Soil Bw horizons transition to BC horizons at approximately 35 cm depth, and few roots extend below ca. 60 cm. Soils in the hardwood stand are Canton variants, coarse-loamy over sandy-skeletal, mixed, frigid Typic Dystrochrepts. Soil Bw horizons transition to BC horizons at approximately 50 cm depth, and few roots are present below ca. 70 cm (EcoTech Inc., Worcester, MA). Mineral horizons in the solums are extremely or very strongly acid, with pH in A horizons (measured in 1:2 solution of soil: 0.01 M $CaCl_2$) ranging from 3.7 to 4.0 and in B horizons from 4.2 to 4.7.

Nitrogen amendments have been made in these two stands since 1988. Nitrogen has been added in six equal applications per year, approximately once per month from early May through late September. In each stand there is one reference (no N addition), one low-N-addition (5 g N m^{-2} yr^{-1}) and one high-N-addition (15 g N m^{-2} yr^{-1}) plot (30 m × 30 m each). Fertilizer is sprayed onto the forest floor as 1.3 mol L^{-1} NH_4NO_3 in the low-N-addition and 4.0 mol L^{-1} NH_4NO_3 in the high-N-addition treatments. Though this design exhibits pseudoreplication within each stand, the design was chosen to maximize the size of the treatment plots so that ecosystem processes could be studied at a large scale and with minimal edge effects.

Experiment design and installation

Zero-tension lysimeters (ZTL's), five in each treatment per stand (30 total), were installed just beneath the forest floor (Oa horizon). Total thickness of the soil organic horizons, and thus ZTL depths beneath the litter surface, averaged 8.7 ± 2 cm (n = 60) in the pine stand and 8.5 ± 2 cm (n = 56) in the hardwood stand in August 1992. Porous cup tension lysimeters had been installed (30 total) in 1988, at 60 cm depth (measured from the top of the litter layer), which we refer to as the 'deep rooting zone'.

The ZTL and sample-bottle assembly were designed to allowed access to the collection bottle for removal to the laboratory and replacement for each sample collection. Each ZTL body, which drained a surface area of 154 cm^2, was cut from a rectangular polyethylene container and packed with glass beads and acid-washed silica gravel. ZTLs were placed at least 1 m from tree stems, in locations representative of the forest-floor heterogeneity, which included the presence or absence of understory canopy, herbaceous vegetation and woody litter. The forest floor was not cut or otherwise disturbed; ZTL's were installed through horizontal tunnels excavated in the mineral soil from downslope positions. Each ZTL drained into a 1 L polyethylene bottle housed in a wooden box that preserved surrounding soil structure while providing easy access.

A throughfall (TF) collector was placed about 1 m from each ZTL (30 total). We used polyethylene funnels (14 cm. diam.) fitted with 2 mm nylon mesh to exclude debris. Funnels drained into liners which were held in opaque, dark plastic bottles staked firmly on the litter surface. Funnels were set in the field in April and returned to the laboratory for cleaning at approximately 6 week intervals. Precipitation was collected in a polyethylene bucket in a clearing at the Harvard Forest, approximately 1 km from the site of the experiment. No vegetation or buildings were present within a cone 20° from horizontal surrounding the precipitation collector (Galloway & Likens 1978).

Sample collection and preservation

Precipitation was collected in periods of 24 hours or less. We sampled TF and forest-floor (Oa horizon) leachate on an event basis in many cases by installing clean TF liners and clean ZTL collection bottles for forecasted rain events. However, using two sets of collection bottles we sampled Oa leachate continuously, leaving the second set in place between event collections. Buckets and ZTL bottles were acid-washed and rinsed with deionized water (DIW) for each collection. For each TF collection we placed a new, DIW-rinsed and air-dried polyethylene liner in each bottle. All TF samples were collected within 8 days (d) of liner installation. The median length of

time that ZTL samples remained in the field before collection was 5 d, with a maximum of 24 d. Tension lysimeters were drawn down to 0.34 MPa vacuum and sampled after 24 hr once per month.

Disturbance effects are sometimes seen following installation of zero-tension lysimeters, although the duration and importance of such effects are not fully understood (Shepard et al. 1990; Mitchell et al. 1994; Johnson et al. 1995; Currie 1995). Here, to minimize disturbance effects, we consider only samples collected more than one year after ZTL installation (Litaor 1988). ZTL installation was completed in mid-August 1992. Lysimeter data reported here cover one litterfall year (Qualls et al. 1991), from October 1993 through September 1994. This period covered the seventh year of N amendments in these plots (May to September 1994). No samples were collected from December to mid-April because of snow cover. We collected Oa leachate during spring snowmelt by installing ZTL collection bottles in late March 1994 (40 cm snowpack depth) and retrieving the samples 21 d later after the snowpack had melted. For consistency, the TF and deep-rooting-zone data we report here cover the same time period, October 1993 to September 1994. The precipitation data we report here, however, was collected in the prior year, October 1992 to September 1993.

Samples were transported on ice to the University of New Hampshire, where they were filtered immediately or refrigerated at 2 °C overnight. Samples were vacuum-filtered through ashed (1 hour at 425 °C) Whatman GF/F glass-fiber filters (nominal pore size 0.7 um) within 36 hr of collection. Filtered samples were frozen in high-density polyethylene storage bottles, where they remained for 1 to 6 months prior to analysis. Flocculation of organics during freezing of post-filtered samples was not visible and was not considered significant; R. G. Qualls had quantified flocculation as $\leq 2\%$ of DOC in similar samples (*personal communication*).

In most TF and ZTL collections, samples were bulked by stand and treatment prior to analysis. Samples were bulked in order to maximize the spatial sampling within plots and the temporal coverage within each season while keeping analytical costs manageable. Bulking was also necessary in many cases because of the low quantities of ZTL solution recovered. In two collections of TF and ZTL solutions, samples from each of the 5 replicate collectors per stand and treatment plot remained unbulked for analysis. Samples retrieved from tension lysimeters remained unbulked in all cases because collections were made only once per month.

Laboratory analyses

The following analyses were performed on all sample types on frozen subsamples after thawing. We measured DOC as total organic carbon in filtered

samples, through catalytic oxidation at 680 °C with a Shimadzu TOC 5000 unit (TC-IC method). Nitrate-N was measured by the automated hydrazine sulfate reduction method (Technicon Method 782-86T); nitrite-N was negligible, as revealed by high pressure liquid chromatography, consistent with the findings of Qualls et al. (1991). We measured ammonium-N by the automated Berthelot reaction method (Technicon Method 780-86T). Zinc sulfate was added to the working reductor in the hydrazine reduction method (10 mL of 3% $ZnSO_4$ in 500 mL working reductor) to mitigate humic-acid interference.

Total dissolved nitrogen (TDN) was measured by alkaline persulfate digestion converting all nitrogen to nitrate (Solorzano & Sharp 1980), with measurement of nitrate-N as above. This method for TDN measurement is described in detail by Merriam et al. (1996). Dissolved organic N (DON) was then calculated as shown in equation (1).

$$DON = TDN - NO_3\text{-}N - NH_4\text{-}N \quad (1)$$

We determined the lower limit of quantitation (LOQ) for our analytical methods (Greenberg et al. 1992) to be 0.15 mg N L^{-1} for NH_4-N, 0.13 mg N L^{-1} for NO_3-N and DON, and 1.0 mg C L^{-1} for DOC.

Statistical analyses and flux calculations

Statistical analyses were performed separately on data from bulked and unbulked collections of lysimeter solution from the Oa horizon. First, data were tested for normality using the Shapiro-Wilk test (Stata Corporation 1993). When necessary, appropriate logarithmic transformations were made. In the two unbulked sample sets, one-way analyses of variance (ANOVA) were performed on solute concentration data. ANOVA was performed within each collection date and forest stand, with level of N treatment being the tested effect. On bulked sample sets, two-way analyses of variance were performed on solute concentration data. ANOVA was performed within each stand, with season and level of N treatment as the two main effects. Where significant effects existed, pairwise comparisons for post-hoc determination of significant differences between means among levels of N treatment were made, using Tukey's Honestly Significant Difference (HSD) test (Systat Inc. 1992). In the bulked sample sets these tests required a time-for-space substitution, with multiple samples within a season considered independent (David & Gertner 1987).

Where we report concentration data, volume-weighting was not used. For flux calculations, it was necessary to use volume-weighted mean chemistry because significant relationships were present between sample volumes collected and some aspects of solution chemistry for TF, ZTL, and tension lysimeter samples.

Numerous assumptions are made in the calculation of solute fluxes from solution chemistry data. Chief among them in our calculation of deep-rooting-zone fluxes is the assumption that the solution obtained in 60-cm deep, porous-cup lysimeters held at a tension of 0.34 MPa for 24 hr accurately reflects the tension and residence time of the water percolating downward through the soil at that depth (Litaor 1988). Solute fluxes in TF and soil solution were calculated as follows. First, samples were grouped by season based on collection date (April, May and June = spring; July, August and September = summer; October and November = fall). For solution collected in the Oa horizon, volume-weighted mean solute concentrations were then calculated within each season from data representing samples previously bulked within each N treatment plot. For solution from the deep rooting zone, since samples had remained unbulked for analysis, volume-weighting was performed on data from each lysimeter separately within each season. Within each season, results were then averaged across lysimeters in each treatment plot.

Volume-weighted mean concentrations of solutes in each season were multiplied by monthly hydrologic fluxes estimated for TF and soil solution. Hydrologic fluxes in TF were estimated from precipitation measurements (Harvard Forest weather station data). We derived regressions between precipitation and throughfall quantities at the Harvard Forest for the pine and hardwood stands separately, based on 14 event collections in which data from all 15 throughfall collectors were combined per stand. Hydrologic fluxes in soil solution were estimated with the PnET-II ecosystem and hydrologic model (Aber & Federer 1992; Aber et al. 1996). We ran the model on a monthly timestep using aggregated daily precipitation and daily maximum and minimum temperatures at Harvard Forest for 1 October 1993 to 30 September 1994 (Harvard Forest weather station data). Hydrologic fluxes in December were included in the estimations of solute fluxes for the fall. Hydrologic fluxes in the deep rooting zone from January through April were multiplied by solute concentrations measured the first week of May (the earliest sample of the year). Hydrologic fluxes in Oa leachate from January through April were multiplied by solute concentrations measured in Oa leachate during spring snowmelt. These estimation methods may provide low bounds on solute fluxes during winter, because solute concentrations during spring snowmelt were the lowest of the year. If solute concentrations during winter were higher, particularly in years when the forest floor did not freeze, then solute fluxes during winter could be proportionally higher than our estimates.

Results

Chemistry of precipitation and throughfall

In the 10 wet deposition events we collected, rainfall amount varied from 0.2 to 3 cm and pH ranged from 3.4 to 4.6. Mean concentration and standard error for NO_3-N was 0.53 ± 0.1 mg N L^{-1}, for NH_4-N was 0.31 ± 0.07 mg N L^{-1}, and for DOC was 1.8 ± 0.7 mg C L^{-1}. Mean concentration of DON was below our limit of quantitation.

In throughfall (TF), seasonal mean concentrations of DOC ranged from 11 to 60 mg C L^{-1}, while those for DON ranged from 0.24 to 1.1 mg N L^{-1} (Table 2). Peak concentrations of DOC and DON occurred in June (data not shown). In the prior year, peaks of DOC were observed in June and July, while peaks of DON were observed in June (data not shown). Pollen, present in TF samples from both stands in June, was removed during filtering. No effects related to N amendment were noted in summary statistics of solute concentrations or in the ratio of DOC:DON in throughfall. Results reported here are therefore averaged among plots within each forest stand.

Chemistry of soil solution in reference plots

In reference plots, solute concentrations in Oa leachate showed broadly similar temporal patterns in the two forest types, though DOC and DON averaged greater in the pine stand than in the hardwood stand in all seasons (Table 3). DOC concentration peaked in October in the red pine forest and in July in the mixed hardwood forest (Figure 1). DON concentration peaked in August in the pine forest and July in the hardwood forest (Figure 2). The lowest concentrations of DOC and DON occurred during spring snowmelt in both stands. Ratios of DOC:DON showed no apparent difference between forest stands in the spring, but appeared higher in the pine stand in summer and fall. Concentrations of NO_3^- in Oa leachate peaked in the spring in both forest types, while concentrations of NH_4^+ were highest in summer (Table 3). In both spring and summer, concentrations of NO_3^- in Oa leachate appeared higher in the pine than in the hardwood stand.

Coefficients of variation for DOC and DON, in the two sets of unbulked samples of Oa leachate, ranged from 19% to 60% of the means in the reference plots (Table 4). DOC concentrations in Oa leachate were negatively correlated with water fluxes across sample collection dates ($p < 0.05$); however the variances in DOC concentration explained by this effect were very low (r^2 = 0.04 in each stand). For DON the correlations were not statistically significant.

Comparisons between the pine and hardwood reference plots in data from the unbulked sample sets of Oa leachate reinforced some but not all of the

Table 2. Solute concentrations in throughfall samples from October 1993 through September 1994. Means and standard errors are shown among collection dates within each season. Sample numbers appear in parentheses, in which each sample represents five combined, replicate field samples. (A). Red pine stand. (B). Hardwood stand.

Season	NH_4-N [mg N L^{-1}]	NO_3-N [mg N L^{-1}]	DOC [mg C L^{-1}]	DON [mg N L^{-1}]	DOC:DON mass ratio
A. Throughfall: Red pine stand					
Fall:	0.17 ± 0.060(9)	0.94 ± 0.17(9)	13 ± 1.2(9)	0.35 ± 0.065(9)	41 ± 5.1(8)
Spring:	0.60 ± 0.14(15)	1.6 ± 0.25(15)	37 ± 5.8(15)	1.1 ± 0.24(15)	33 ± 2.2(13)
Summer:	0.61 ± 0.13(18)	1.1 ± 0.21(18)	24 ± 2.6(18)	0.61 ± 0.096(18)	44 ± 4.3(16)
B. Throughfall: Hardwood stand					
Fall:	<0.15 ± 0.022(9)	0.69 ± 0.12(9)	11 ± 1.6(9)	0.24 ± 0.048(9)	45 ± 3.7(6)
Spring:	0.31 ± 0.056(15)	0.93 ± 0.14(14)	60 ± 17(15)	1.1 ± 0.26(14)	40 ± 5.4(11)
Summer:	0.53 ± 0.093(18)	0.78 ± 0.14(18)	16 ± 2.2(18)	0.59 ± 0.11(18)	33 ± 4.2(16)

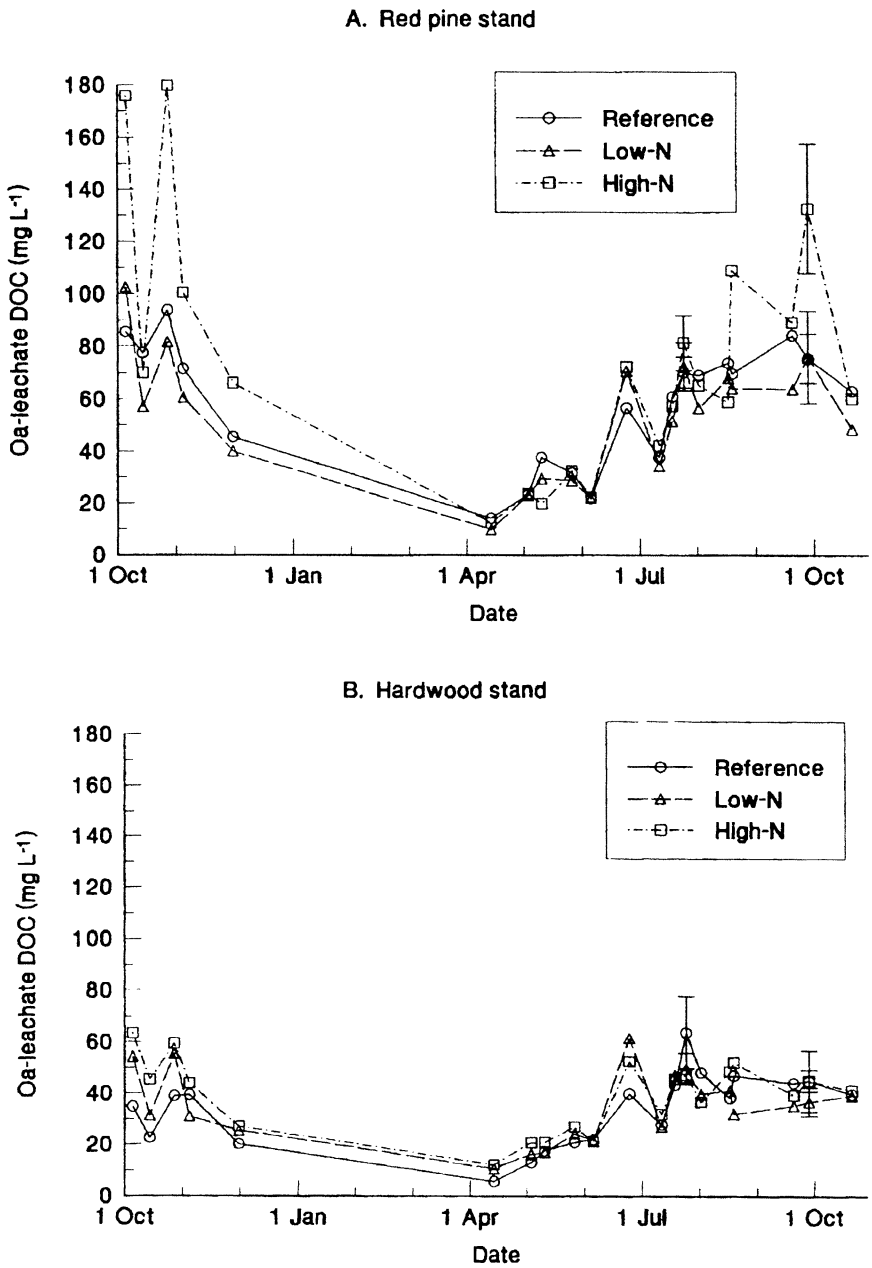

Figure 1. Concentrations of dissolved organic carbon (DOC) in lysimeter samples from the Oa horizon collected from 5 October 1993 to 21 October 1994. Results from reference, low-N addition and high-N addition plots are shown. Values indicated represent chemistry of bulked samples from 5 replicate collections within each treatment. Error bars represent standard errors from the two dates on which samples remained unbulked for analysis. A. Red pine stand. B. Hardwood stand.

Table 3. Solute concentrations in lysimeter samples in the Oa horizon from October 1993 through September 1994. 'Ref.' indicates reference plots, while 'low-N' and 'high-N' indicate plots in which 5 g N m^{-2} yr^{-1} and 15 g N m^{-2} yr^{-1} were applied to the forest floor. Means and standard errors are shown among collection dates within each season. Numbers of sample collections appear in parentheses, in which each sample represents five bulked, replicate field samples. Different letters beside the results for DOC:DON ratios denote significant differences ($p < 0.05$) among N treatments within each forest type and season. (A). Red pine stand. (B). Hardwood stand.

Season	Plot	NO_3-N [mg N L^{-1}]	NH_4-N [mg N L^{-1}]	DON [mg N L^{-1}]	DOC [mg C L^{-1}]	DOC:DON mass ratio
A. Oa leachate: Red pine stand						
Fall:	Ref.	0.13 ± 0.028(5)	0.24 ± 0.075(5)	1.7 ± 0.13(5)	75 ± 8.2(5)	44 ± 2.4(5)a
	Low-N	2.0 ± 0.28(5)	0.66 ± 0.18(5)	2.6 ± 0.42(5)	68 ± 11(5)	27 ± 1.2(5)b
	High-N	8.1 ± 1.1(5)	8.3 ± 1.8(5)	4.6 ± 1.2(5)	118 ± 25(5)	23 ± 2.5(4)b
Snowmelt:	Ref.	0.88	<0.15	0.28	14	51
	Low-N	1.3	0.23	0.31	9.7	31
	High-N	1.5	0.99	0.28	12	43
Spring:	Ref.	0.73 ± 0.27(5)	<0.15 ± 0.057(5)	0.80 ± 0.20(5)	34 ± 6.3(5)	45 ± 2.2(5)a
	Low-N	4.0 ± 1.6(5)	1.7 ± 0.85(5)	0.96 ± 0.17(5)	35 ± 9.1(5)	35 ± 4.0(5)b
	High-N	19 ± 7.4(5)	11 ± 5.3(5)	0.99 ± 0.25(5)	34 ± 9.8(5)	39 ± 3.0(3)ab
Summer:	Ref.	0.66 ± 0.20(8)	0.46 ± 0.25(8)	1.8 ± 0.27(8)	68 ± 4.9(8)	40 ± 3.3(8)a
	Low-N	7.0 ± 1.6(8)	3.6 ± 0.91(8)	1.6 ± 0.15(8)	61 ± 4.7(8)	40 ± 3.0(8)a
	High-N	15 ± 4.4(8)	11 ± 3.3(8)	1.9 ± 0.25(8)	79 ± 11(8)	40 ± 3.0(5)a

Table 3. Continued.

Season	Plot	NO_3-N [mg N L^{-1}]	NH_4-N [mg N L^{-1}]	DON [mg N L^{-1}]	DOC [mg C L^{-1}]	DOC:DON mass ratio
B. Oa leachate: Hardwood stand						
Fall:	Ref.	0.14 ± 0.064(5)	<0.15 ± 0.018(5)	1.0 ± 0.19(5)	31 ± 4.1(5)	32 ± 3.6(5)ab
	Low-N	0.24 ± 0.12(5)	<0.15 ± 0.006(5)	1.1 ± 0.16(5)	40 ± 6.4(5)	37 ± 2.0(5)a
	High-N	0.90 ± 0.36(5)	0.78 ± 0.13(5)	1.8 ± 0.23(5)	48 ± 6.4(5)	27 ± 1.6(5)b
Snowmelt:	Ref.	<0.13	<0.15	<0.13	5.7	>44
	Low-N	<0.13	<0.15	0.15	11	70
	High-N	<0.13	0.28	0.32	12	38
Spring:	Ref.	0.43 ± 0.12(5)	<0.15 ± 0.037(5)	0.54 ± 0.11(5)	23 ± 4.6(5)	42 ± 2.1(5)a
	Low-N	6.2 ± 4.4(5)	3.4 ± 3.3(5)	0.91 ± 0.23(5)	28 ± 8.5(5)	37 ± 3.7(4)a
	High-N	16 ± 8.9(5)	8.5 ± 5.4(5)	1.4 ± 0.62(5)	28 ± 6.2(5)	28 ± 1.7(3)a
Summer:	Ref.	0.38 ± 0.10(8)	0.20 ± 0.046(8)	1.4 ± 0.11(8)	45 ± 3.6(8)	32 ± 1.4(8)a
	Low-N	3.4 ± 1.5(8)	1.9 ± 0.87(8)	1.1 ± 0.12(7)	39 ± 2.7(8)	34 ± 1.9(7)a
	High-N	5.8 ± 2.3(8)	3.6 ± 1.2(8)	1.5 ± 0.21(8)	43 ± 2.4(8)	27 ± 2.8(7)a

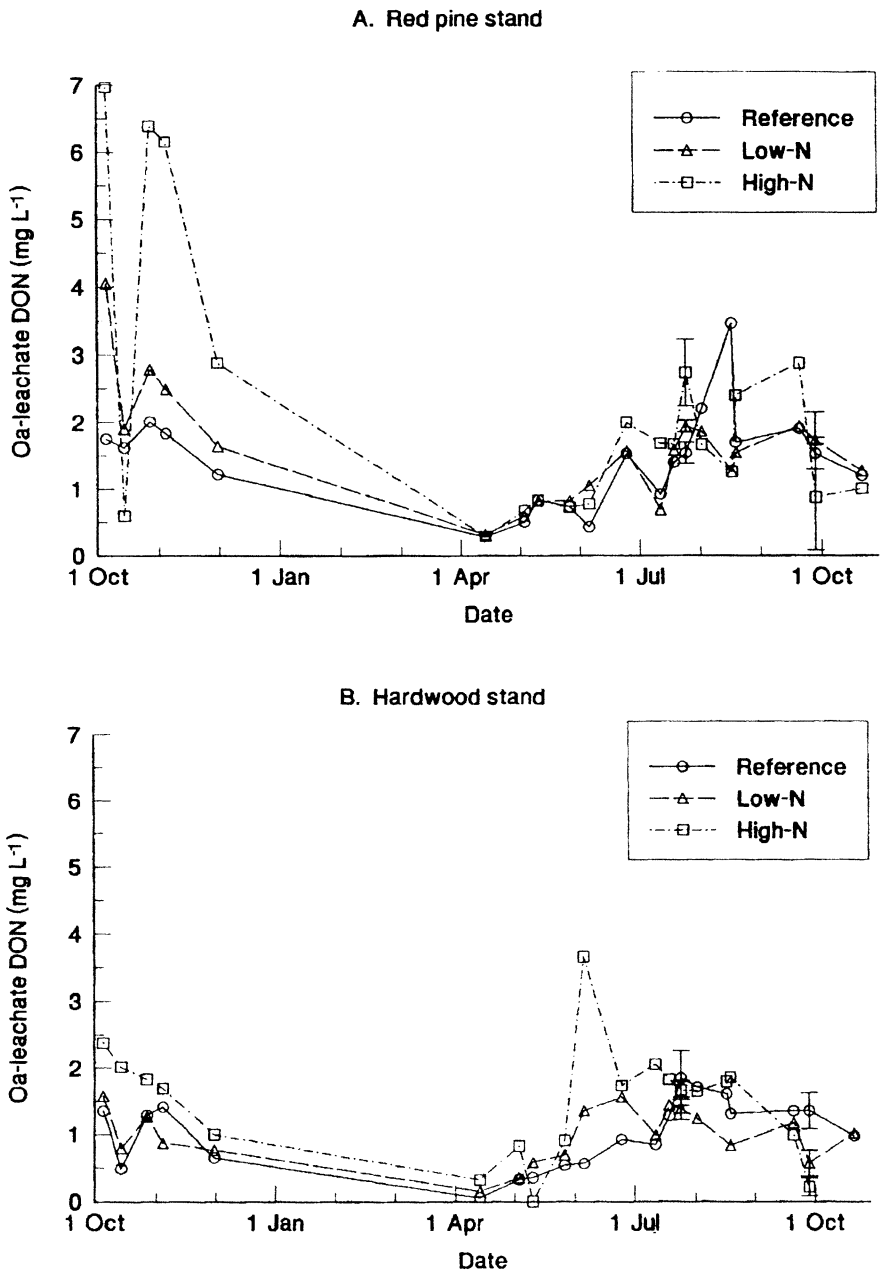

Figure 2. Concentrations of dissolved organic nitrogen (DON) in lysimeter samples from the Oa horizon collected from 5 October 1993 to 21 October 1994. Treatment plots, replication and error bars are as in Figure 1. A. Red pine stand. B. Hardwood stand.

Table 4. Solute concentrations in lysimeter samples in the Oa horizon from two collections in which replicate samples remained unbulked for analysis. Means and standard errors are shown (number of samples in parentheses). Different letters denote significant differences among N treatments ($p < 0.05$) within each stand and collection date. A. Red pine stand. B. Hardwood stand.

Date and plot	NO_3-N [mg N L^{-1}]	NH_4-H [mg N L^{-1}]	DOC [mg C L^{-1}]	DON [mg N L^{-1}]	DOC:DON [mass ratio]	DON/TDN percent
A. Red pine stand						
25 July 1994						
Ref.	0.37 ± 0.05(5)	0.15 ± 0.02(5)	70 ± 5.8(5)a	1.5 ± 0.16(5)a	46 ± 1.2(5)a	75 ± 1.7(5)a
Low N	3.4 ± 0.62(5)	1.5 ± 0.39(5)	72 ± 9.1(5)a	1.9 ± 0.086(5)ab	37 ± 3.1(5)ab	30 ± 5.4(5)b
High N	7.9 ± 1.8(5)	4.3 ± 1.0(5)	81 ± 10(5)a	2.7 ± 0.50(5)b	31 ± 2.9(5)b	19 ± 2.7(5)c
28 Sept. 1994						
Ref.	0.045 ± 0.003(5)	0.11 ± 0.04(5)	75 ± 9.4(5)a	1.5 ± 0.24(5)a	50 ± 1.6(5)	90 ± 2.0(5)a
Low N	16 ± 3.9(4)	6.1 ± 2.6(2)	76 ± 18(5)a	1.7 ± 0.43(5)a	*	7.6 ± 2.1(5)b
High N	41 ± 6.2(5)	29 ± 6.5(5)	133 ± 25(5)a	*	*	0.82 ± 0.70(5)c
B. Hardwood stand						
25 July 1994						
Ref.	0.22 ± 0.04(5)	0.12 ± 0.02(5)	64 ± 14(5)a	1.8 ± 0.41(5)a	35 ± 1.1(5)a	81 ± 5.1(5)a
Low N	0.36 ± 0.03(5)	0.097 ± 0.02(5)	50 ± 6.2(5)a	1.4 ± 0.17(5)a	36 ± 1.6(5)a	75 ± 2.3(5)a
High N	0.64 ± 0.06(4)	0.59 ± 0.37(4)	47 ± 2.8(4)a	1.7 ± 0.14(4)a	29 ± 1.6(4)b	60 ± 5.3(4)b
28 Sept. 1994						
Ref.	0.070 ± 0.01(5)	0.10 ± 0.008(5)	45 ± 12(5)a	1.4 ± 0.27(5)a	32 ± 2.9(5)a	87 ± 2.7(5)a
Low N	6.5 ± 2.7(4)	3.6 ± 1.9(2)	37 ± 5.5(4)a	0.57 ± 0.19(4)ab	42 ± 4.6(3)a	18 ± 12(4)b
High N	17 ± 4.4(4)	9.8 ± 3.3(4)	45 ± 4.2(4)a	*	*	1.0 ± 0.67(4)c

Note:
* Because we calculate DON by difference from TDN, at very low ratios of DON/TDN the precision in DON is unreliable.

Table 5. Solute concentrations in lysimeter samples in the deep rooting zone from October 1993 through September 1994. Means and standard errors are shown (number of lysimeters in parentheses).

Plot	DOC [mg C L^{-1}]	DON [mg N L^{-1}]	TDN [mg N L^{-1}]	DOC:DON mass ratio
Red pine stand				
Reference	26 ± 5.2(5)	0.78 ± 0.28(5)	0.81 ± 0.32(5)	41 ± 7.9(5)
Low-N addition	24 ± 3.4(5)	0.89 ± 0.46(5)	1.9 ± 1.1(5)	41 ± 9.8(5)
High-N addition	22 ± 3.3(5)	0.96 ± 0.50(5)	8.8 ± 4.2(5)	36 ± 14(5)
Hardwood stand				
Reference	21 ± 3.9(5)	0.63 ± 0.23(5)	0.67 ± 0.26(5)	46 ± 12(5)
Low-N addition	24 ± 3.4(5)	0.67 ± 0.20(5)	0.73 ± 0.22(5)	48 ± 12(5)
High-N addition	21 ± 3.0(5)	0.62 ± 0.16(5)	0.92 ± 0.40(5)	44 ± 9.1(5)

patterns apparent in the data covering the full year. The higher concentrations of DOC in the pine forest, which showed clearly in the time series and seasonal means, were clearly reflected in the unbulked set collected in September but not in the set collected in July (Table 4). Apparent differences in DON concentrations were not reflected in data from the two unbulked collections, but the ratios of DOC:DON appeared higher in the pine plot than in the hardwood plot in both unbulked sets.

In the deep rooting zone, differences between the stands were much less pronounced than the corresponding differences in Oa leachate. Concentrations of DOC and DON in reference plots averaged only slightly higher in the pine versus the hardwood stand (Table 5).

Chemistry of soil solution in N-amended plots

Concentrations of DOC and DON in Oa-leachate in the N-amended plots showed similar time-series patterns to those in the reference plots in both forests (Figures 1 and 2). In data covering the full year of Oa-leachate chemistry, neither DOC nor DON showed significant responses to N treatment, but DOC:DON ratios did show significant responses (Table 6). Seasonal effects were observed in both forests in concentrations of DOC, DON, and the ratio of DOC:DON (Table 6).

When broken down by season, in the pine forest the mean ratios of DOC:DON were significantly lower under N amendment in spring (low N treatment only) and in fall (Table 3). In the hardwood forest there was a significant effect only in fall. In fall, the higher concentrations of DOC and

Table 6. P-values resulting from two-way analyses of variance performed on solute concentrations ($n = 57$ red pine stand, $n = 57$ hardwood stand). Samples were collected in lysimeters in the Oa horizon from October 1993 through September 1994.

Forest stand	Effect	DOC	DON	DOC:DON mass ratio
Red pine	N treatment	0.21	0.17	<0.0005
	Season	<0.0005	<0.0005	0.002
Hardwood	N treatment	0.20	0.22	<0.0005
	Season	<0.0005	0.001	0.006

DON in the pine forest versus the hardwood appeared accentuated under N amendment (Table 3). No such accentuation of differences was observed in spring or summer.

The unbulked sample sets showed effects of N amendments on concentrations of DON but not DOC (Table 4). The two forest types showed different responses in concentrations of DON on the two different dates. In July, DON concentration increased significantly with N amendment in the pine stand but remained unchanged in the hardwood stand. In September, DON concentration was not related to N amendment in the pine stand but was significantly lower with N amendment in the hardwood stand. In both forests, higher fluxes of inorganic N under N amendment caused lower ratios of DON:TDN in Oa leachate (Table 4).

In nearly every combination of stand, season, and level of N amendment, concentrations of NH_4^+ were lower than concentrations of NO_3^- in Oa leachate, indicating a preferential sink for NH_4^+ in the forest floor of both stands. Seasonal patterns in the effects of N amendments on NH_4^+ concentrations in Oa-leachate, however, were very similar to those on NO_3^- concentrations.

N amendments accentuated some patterns in the leaching of inorganic N from the forest floor that were present in reference plots. In the spring, when NO_3^- concentrations in Oa leachate were highest in reference plots, N-amended plots showed high concentrations of both NO_3^- and NH_4^+ (Table 3). This apparent enhancement of inorganic N leaching persisted into summer and fall in both stands, though it was more evident in the pine stand. Concentrations of NO_3^- in N-amended plots in the pine stand were higher than those in the hardwood by a factor of 2 or greater in summer and 8 or greater in fall.

In the deep rooting zone, concentrations of DOC showed no differences between stands in the N-amended plots (Table 5). DON also showed no strong effects of N amendments in either stand. TDN, in contrast, showed a markedly different response between the two stands. Under N amendments, TDN was greatly elevated in the pine stand but little affected in the hardwood.

Solute fluxes in throughfall

Regressions of throughfall (TF) quantity versus precipitation (PPT) quantity are given in equation (2) for the red pine stand and equation (3) for the hardwood stand. TF and PPT quantity are expressed in equivalent units such as mm.

$$TF = (0.80PPT) - 0.12 \qquad r^2 = 0.91,\ n = 14 \tag{2}$$

$$TF = (0.76PPT) - 0.059 \qquad r^2 = 0.93,\ n = 14 \tag{3}$$

On a flux basis, NO_3-N was the dominant form of N in TF, accounting for just over half of the TDN flux in each forest type (Table 7). DON accounted for 27% of the TDN flux in the pine stand and 29% of the TDN flux in the hardwood stand.

Solute fluxes in soil in reference plots

In reference plots, fluxes of DOC, DON, and inorganic N leached from the forest floor to the mineral soil were greater in the pine than in the hardwood forest both annually and within each season (Table 7; Figures 3 and 4). DON composed the bulk of the TDN flux in Oa leachate in summer and fall while inorganic N dominated in winter and spring. Although differences could not be tested statistically, in both stands DON fluxes appeared to increase and inorganic N fluxes to decrease as spring progressed to summer (Figure 4). On an annual basis, DON composed 56% of the TDN flux in the pine stand and 67% of the TDN flux in the hardwood stand.

Annual fluxes of DOC and DON in the deep rooting zone, like those in Oa leachate, were higher in the pine relative to the hardwood stand. Annual fluxes of DON from the deep rooting zone composed 98% of the TDN fluxes in both the pine and the hardwood reference plots.

Solute fluxes in soil in N-amended plots

In the responses of solute fluxes to N amendments, differences between forest types varied through the year. For DON fluxes in Oa leachate the clearest differences occurred in the fall, when there was already a large difference between the two reference plots, and when DON fluxes were by far the largest components of the TDN fluxes. DON fluxes increased with increasing level of N amendment in each stand, and differences between the pine and hardwood forests in fall also increased (Figure 4). Patterns in fall fluxes of DOC were less clear, though the difference between forest types appeared to be maintained under the N amendments (Figure 3).

Table 7. Annual solute fluxes covering the litterfall year October 1993 through September 1994. Reference, low-N and high-N addition plots are as in Table 3.

Flux or flux difference	Plot	NO_3-N [g m^{-2} yr^{-1}]	NH_4-N [g m^{-2} yr^{-1}]	DON [g m^{-2} yr^{-1}]	TDN [g m^{-2} yr^{-1}]	DOC [g m^{-2} yr^{-1}]
Wet deposition[1]	All	0.513	0.346	0.064	0.923	1.38
Red pine stand						
Throughfall[1]	All	0.696	0.223	0.348	1.27	13.9
From Oa	Reference	0.604	0.138	0.953	1.70	39.8
From Oa	Low N addition	3.25	1.30	1.14	5.68	36.8
From Oa	High N addition	9.40	6.80	1.56	17.8	50.3
From deep rooting zone	Reference	<0.001	0.010	0.536	0.549	16.7
From deep rooting zone	Low N addition	0.506	0.033	0.437	0.968	15.1
From deep rooting zone	High N addition	5.16	0.029	0.362	5.56	13.7
Illuviated & retained[2]	Reference	0.604	0.128	0.417	1.15	23.1
Illuviated & retained	Low N addition	2.74	1.27	0.703	4.71	21.7
Illuviated & retained	High N addition	4.24	6.77	1.20	12.2	36.6

Table 7. Continued.

Flux or flux difference	Plot	NO_3-N [g m^{-2} yr^{-1}]	NH_4-N [g m^{-2} yr^{-1}]	DON [g m^{-2} yr^{-1}]	TDN [g m^{-2} yr^{-1}]	DOC [g m^{-2} yr^{-1}]
Hardwood stand						
Throughfall[1]	All	0.488	0.181	0.268	0.938	11.7
From Oa	Reference	0.199	0.104	0.611	0.915	22.5
From Oa	Low N addition	2.08	1.23	0.697	4.03	26.6
From Oa	High N addition	3.65	2.20	1.08	6.91	28.7
From deep rooting zone	Reference	0.002	0.003	0.319	0.324	12.3
From deep rooting zone	Low N addition	0.002	0.016	0.457	0.475	17.0
From deep rooting zone	High N addition	0.082	0.015	0.354	0.452	14.5
Illuviated & retained[2]	Reference	0.197	0.101	0.292	0.591	10.2
Illuviated & retained	Low N addition	2.06	1.21	0.240	3.56	9.60
Illuviated & retained	High N addition	3.57	2.18	0.726	6.46	14.2

Notes:
1. Throughfall fluxes exclude winter (January through March), and do not include stemflow.
2. 'Illuviated & retained' refers to material illuviated into mineral soil and retained there, calculated as the flux from the Oa horizon minus the flux from the deep rooting zone.

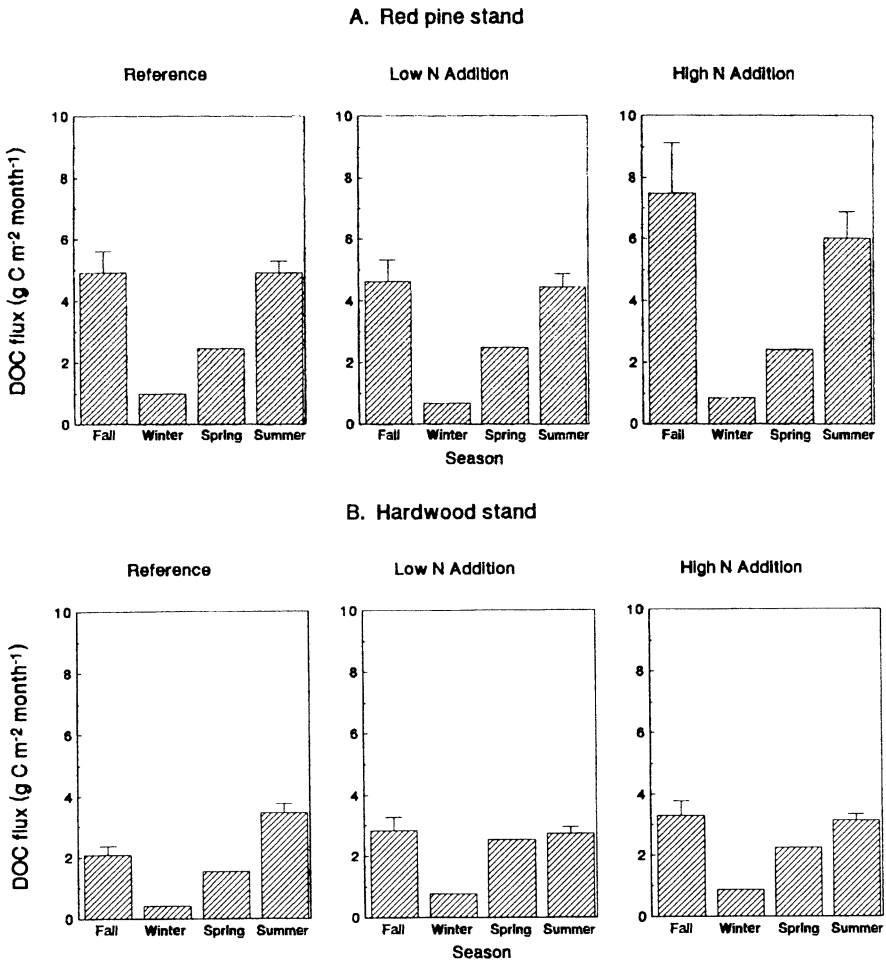

Figure 3. Seasonal fluxes of DOC (dissolved organic carbon) eluviated from the Oa horizon. Bars represent seasonal volume-weighted average concentrations multiplied by seasonal hydrologic fluxes through the bottom of the Oa horizon. Error bars represent fluxes arising from standard errors of mean concentrations. Error bars are absent from winter and spring fluxes because the single bulked snowmelt collection did not allow estimate of variances in snowmelt concentrations. A. Red pine stand. B. Hardwood stand.

In summer, when differences in fluxes of DON in Oa leachate between the two reference plots were minimal, differences between the stands remained smaller and DON fluxes showed no clear pattern with N amendments. Due to large increases in the fluxes of inorganic N under N amendments in summer versus spring, DON fluxes accounted for markedly diminishing fractions of TDN fluxes. Fluxes of DOC, as in the fall, remained higher in the pine stand but showed no clear responses to N amendments.

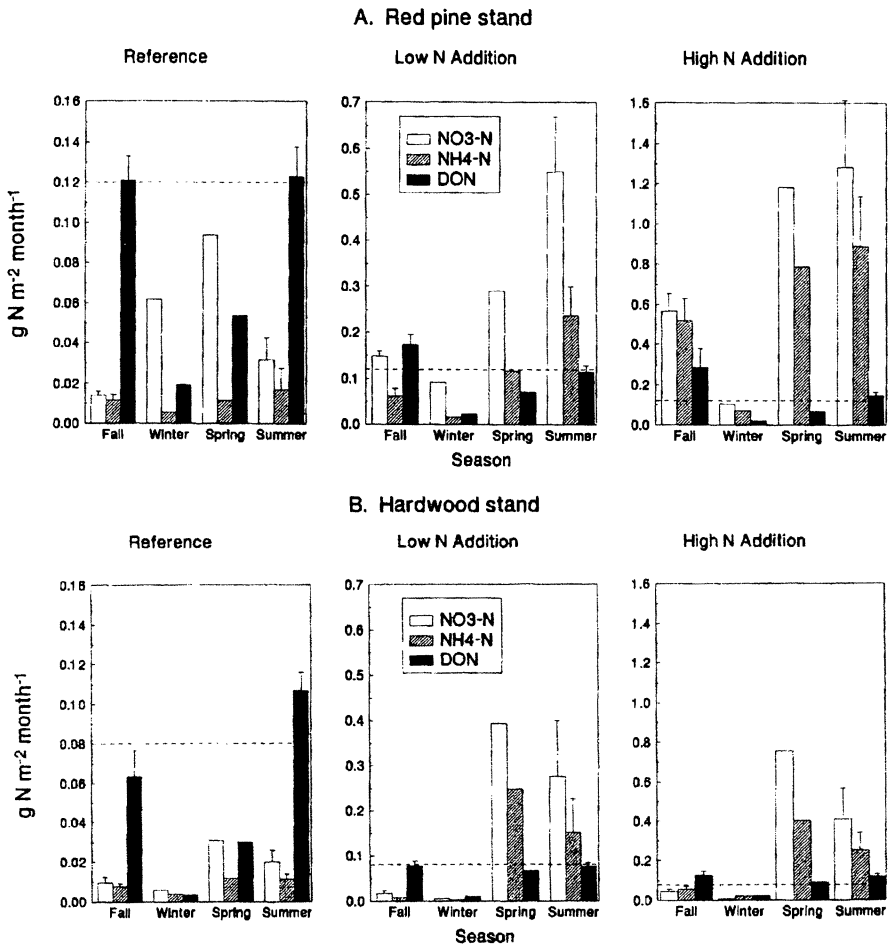

Figure 4. Seasonal fluxes of N eluviated from the Oa horizon. Error bars represent fluxes arising from standard errors of mean concentrations (absent from winter and spring fluxes because snowmelt was sampled only on one date). Note the extreme differences in scale among plots within each stand: reference lines are provided to facilitate direct comparison among reference and N addition plots. A. Red pine stand. B. Hardwood stand.

For annual fluxes of DON in Oa leachate, a regression against the level of N amendments (Figure 5) was significant ($p = 0.018$) in the pine stand although not in the hardwood stand ($p = 0.10$). For DOC, fluxes were elevated in three of the four N-treated plots relative to references, but regressions against the level of N amendments showed no statistical significance.

The two forest types showed important differences in fluxes of inorganic N in Oa leachate. On an annual basis, fluxes of nitrate were much higher in the pine stand relative to the hardwood, as were fluxes of ammonium in the

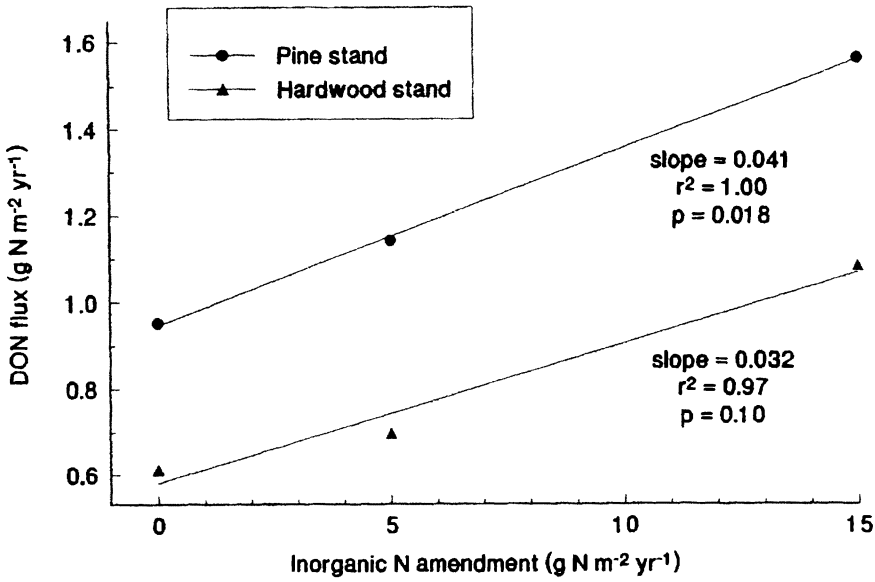

Figure 5. Annual fluxes of DON in lysimeter samples from the Oa horizon versus fluxes of inorganic N amendments.

high-N plots. Also, fluxes or inorganic N appeared to behave differently in the two forest types from spring to summer. In the hardwood stand, fluxes of NO_3^- and NH_4^+ appeared to decrease from spring to summer in N-amended plots, repeating the temporal trend present in the reference plots of both stands. In the pine stand, in contrast, the fluxes of inorganic N appeared to increase from spring to summer in the N-amended plots. The breakdown of leaching fluxes by season thus seems to show that sinks in the forest floor for amendments of inorganic N grew stronger at a much greater rate in the hardwood stand than in the pine stand as spring progressed to summer.

In the deep rooting zone, the slight differences between stands in fluxes of DOC and DON in reference plots were not duplicated under N amendments. In the reference and treated plots taken together, there did not appear to be any differences related to vegetation in DOC or DON fluxes. Nor did there appear to be any patterns related to N amendment.

Nitrogen retention

The fraction of TDN input that was retained by the forest floor in N-amended plots was much greater in the hardwood stand than in the pine stand (Figure 6). Approximately half of the annual TDN input to the forest floor of the hardwood stand in each treated plot was retained there (including plant uptake).

Of the TDN that leached from the hardwood forest floor in treated plots, approximately 90% was retained in the mineral soil (including plant uptake). In the pine stand, in contrast, TDN output from the forest floor was approximately equal to input (within 10%) in each treated plot. Of the TDN that leached from the forest floor in the pine stand, only 70 to 80% was retained in the mineral soil.

For each form of N and in both forest types, the annual quantity of N retained in mineral soil after transport from above was greater in high-N addition plots than in reference plots (Table 7). Relative retention of DON in mineral soil was similar in the two stands. Absolute retention of DON in mineral soil was greater in the pine than in the hardwood stand because fluxes of DON transported from the forest floor were greater (Table 7).

Discussion

Fluxes of dissolved organics increased as throughfall solution entered and passed through the forest floor as Oa leachate. The highest concentrations and greatest downward fluxes of dissolved organics typically occur in the forest floor or the upper mineral horizon (McDowell et al. 1988; Qualls et al. 1991). In lower soil horizons, fluxes of organics are reduced because of retention mechanisms such as sorption in mineral soil (McDowell & Wood 1984; Jardine et al. 1989). This general pattern was evident in our concentrations and fluxes and provides a framework for interpreting the effects of N amendments and differences in forest vegetation on the transport of dissolved organics.

We noted important differences in the transport of DOC in two forest stands where the dominant vegetation differed. This result corroborated and extended previous findings in eastern North America. Higher DOC concentrations in coniferous forests were found when coniferous and hardwood forests were studied simultaneously (David & Driscoll 1984; Cronan & Aiken 1985). This same pattern also appeared when concentrations of DOC were compared across studies. Mean DOC in Oa leachate from coniferous forests, each covering varying periods of 3 months or longer and excluding winter and snowmelt collections, ranged from 40 to 50 mg C L^{-1} (Cronan & Aiken 1985; Koprivnjak & Moore 1992; David & Driscoll 1984). In hardwood forests, the analogous mean concentrations ranged from about 20 to 40 mg C L^{-1} (Cronan & Aiken 1985; McDowell & Likens 1988). Our results provided further evidence to establish the importance of dominant vegetation in controlling DOC concentrations, while extending the differences to fluxes of DOC over an annual period. Our results also provide an initial indication that the difference may extend to concentrations and fluxes of DON.

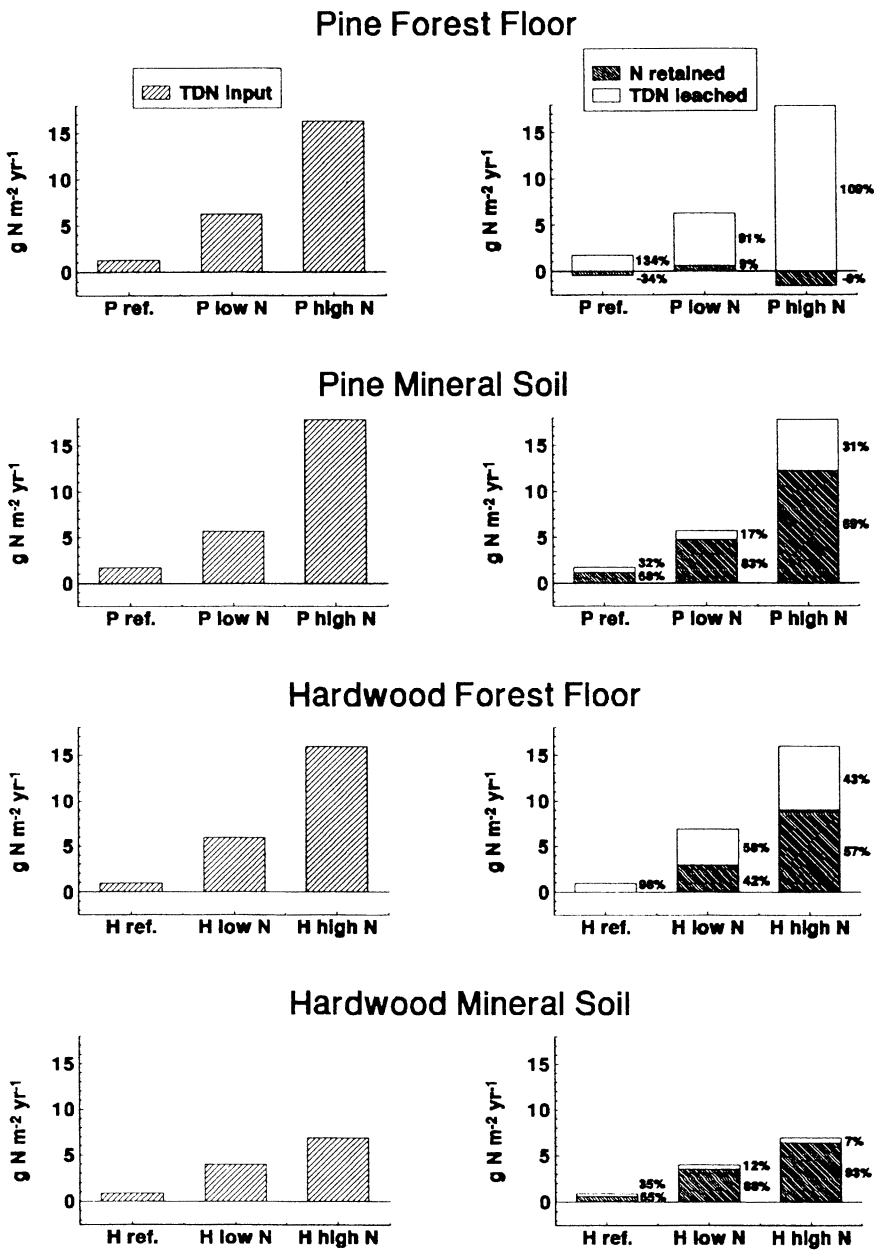

Figure 6. Absolute amounts of total dissolved nitrogen (TDN) that were input to, retained by, and leached from organic and mineral soil horizons in the seventh year of NH_4NO_3 additions. 'TDN input' represents throughfall (TF) inputs to the forest floor and, on treated plots, fertilizer additions. 'TDN input' to the mineral soil represents fluxes of TDN in Oa-leachate. 'TDN leached' refers to estimated annual fluxes of TDN transported downward at two depths. 'N retained' was calculated by difference and refers to N retention by the ecosystem, including plant uptake, accounted for in each soil component. Percentage numbers show the portion of TDN input to each soil component that was leached or retained.

Our estimates of annual fluxes of DOC in Oa leachate in mixed hardwood forest appeared to be representative of those in the region. At the Harvard Forest we calculated a flux of 22 g C m^{-2} yr^{-1} in the reference plot, compared with 26 g C m^{-2} yr^{-1} in a predominantly hardwood forest at Hubbard Brook, NH (McDowell & Likens 1988). Seasonal patterns in DOC concentration in forest-floor leachate at Hubbard Brook were also similar to those at Harvard Forest (McDowell & Likens 1988). A flux of 40 g C m^{-2} yr^{-1} was measured in the Oa leachate of a mixed deciduous forest at Coweeta, NC, where foliar litterfall mass is ca. 30% higher, mean temperature in July averages 2 °C higher, and annual precipitation averages ca. 60% higher than at Harvard Forest (Qualls et al. 1991; Van Cleve & Martin 1991; Magill et al. 1996). We know of no other calculations of annual fluxes in a coniferous forest in eastern North America with which to compare our estimate of 40 g C m^{-2} yr^{-1}.

Concentrations of DOC and DON in the deep rooting zone appeared to be controlled by characteristics of the mineral soil. Field-measured concentrations of DOC in mineral soil vary widely, and the equilibrium points of sorption isotherms also vary widely among mineral soils (Moore et al. 1992). Concentrations similar to those we observed at Harvard Forest (14 to 17 mg C L^{-1}) have been observed elsewhere in coniferous and hardwood forests (Koprivnjak & Moore 1992; Dalva & Moore 1991). DON concentrations at the Harvard Forest were higher than those reported in the rooting-zone solutions of three lodgepole pine ecosystems in Wyoming (0.17 to 0.51 mg N L^{-1}; Fahey & Yavitt 1988), and much higher than those reported in the B horizon of a hardwood forest at Coweeta, NC (0.03 to 0.07 mg N L^{-1}; Qualls & Haines 1991).

Production of dissolved organics in the forest floor

Differential eluviation of DOC and DON from the forest floor of the pine versus the hardwood forest remained after 7 years of high, artificial inputs of inorganic N. Increases in the fluxes of DON in Oa leachate which we observed in N-amended plots must have been caused by higher production of DON in the forest floor, because DON fluxes in throughfall did not vary with N amendments.

Some of the dissolved organics at either soil depth may have been root exudates. However, calculations of the annual fluxes of organic C and N in root exudates have produced estimates much lower than those we measured for DOC and DON in soil solution. For the Northern Hardwood forest at Hubbard Brook, root-exudate C flux was calculated to be 0.4 g C m^{-2} yr^{-1} (Smith 1976), while root-exudate (organic) N flux was calculated to be 0.01

g N m^{-2} yr^{-1} (Bormann et al. 1977). The fluxes we measured in Oa leachate were ca. 80-fold higher than those estimated for root exudation.

Leaching of organic N from fresh, foliar litter probably did not account directly for the higher DON fluxes. Qualls et al. (1991) showed that the leaching of soluble compounds from fresh, foliar litter could not account for either the chemical characteristics or the annual fluxes of DON in Oa leachate. Soluble humic or pre-humic substances typically compose major fractions of dissolved organics in Oa leachate (Qualls et al. 1991; Guggenberger & Zech 1994). It appears unlikely, however, that the organics in Oa leachate could be accounted for wholly by solubilization from the bulk humus in the Oa horizon. The ratios of DOC:DON in reference plots ranged from 32:1 to 51:1, whereas the C:N ratios of forest floor material in these plots ranged from 22:1 to 26:1. In a Norway spruce forest in Germany, Guggenberger & Zech (1994) found the lignin component of Oa leachate to be more highly oxidized than lignin in the Oi, Oe, or Oa horizons, and the carbohydrate component of Oa leachate to be more easily attributed to plant origins (versus microbial) than was the solid-phase material in the forest floor.

Soluble humic substances can be produced by reactions among products of incomplete decomposition, for example, condensation reactions occurring after partial oxidation of polyphenols by phenoloxidases (Stevenson 1982; McClaugherty 1983; Liu et al. 1985). Such a mechanism of DON production would be limited by the quantity of either organic N substrates or polyphenols (Northup et al. 1995), or by rates of oxidation. Increased organic N substrates could derive from foliar or fine root litter, both of which showed increased litter N fluxes in the N-amended plots at Harvard Forest (Magill et al. 1996).

Controls on the seasonality of DOC and DON concentrations in Oa leachate appear to be related to the timing of both the decomposition of detritus extant in the forest floor, and inputs of new litter. Decomposition products should be produced at a greater rate when temperatures are higher in late summer. High concentration of dissolved organics in summer could be directly related to such an effect (concentration-dilution effects of varying water fluxes was not highly explanative in our data). High concentrations in fall could be due partly to hydrologic flushing of accumulated decomposition products. If lower ratios of DOC:DON indicate the presence of dissolved organics that are more highly decomposed (Melillo et al. 1982), we would expect the ratios of DOC:DON to be the lowest of the year in summer. In mean concentrations by season, this was the case in reference plots in both stands and in treated plots in the hardwood stand. Timing of the peak concentrations of organics may also have been partly controlled by new inputs of decomposition substrates in fresh litter. Differences between the pine and hardwood forests in annual DOC and DON fluxes can be attributed largely to

differences in fall fluxes. Concentrations under the different vegetation types are similar in spring, begin to diverge in late June, and diverge much further September through November (Figures 1 and 2).

The apparent increases in DON production under N amendments could also be attributed to processes occurring either in summer, in fall, or both. The differences in annual DON fluxes among treated plots within each vegetation type can best be accounted for by differences in DON fluxes in the fall (Figure 4). However, lower ratios of DOC:DON were significantly related to N amendments in the unbulked set of samples collected in July. We can not currently distinguish between the importance of the decomposition of extant detritus versus new litter inputs in controlling DON fluxes from the forest floor under chronic N amendments. In order to do so, more detailed studies would need to be undertaken concerning the nature of the DON in Oa leachate, the mechanisms of its production, the residence time of the N, or the source pool of the DON.

Flushing of solutes from the forest floor

Fluxes of water through the forest floor appeared to exert control on the eluviation fluxes of organic and inorganic solutes. The flux of TDN in Oa leachate in the high-N plot of the pine stand equaled 109% of the TDN flux input to the forest floor. We consider the excess 9% to be within the uncertainty of the annual flux calculations, but excess eluviation of TDN could have been caused by above-average precipitation. In the litterfall year October 1993 through September 1994, precipitation was 142 cm (Harvard Forest weather station data), compared with an annual average of 110 cm for the site (Van Cleve & Martin 1991). No significant correlations were found between DON, NO_3-N or NH_4-N and collected sample volumes in either stand. The lack of any such relationship suggests that TDN flux should be above average if hydrologic flux is above average, since there were no concentration-dilution effects.

Another example of the importance of water movement controlling solute eluviation appears in spring snowmelt. The low concentrations of dissolved organics during snowmelt at the Harvard Forest reflects an important difference in the seasonal pattern of precipitation between forests in the northeastern U. S. and *Pinus contorta* ecosystems in Wyoming, one of the few locations in which DOC and DON fluxes have also been measured (Fahey et al. 1985; Yavitt & Fahey 1986; Fahey & Yavitt 1988; Arthur & Fahey 1993). In the northern Rocky Mountains, rainfall is normally low from June through September (Van Cleve & Martin 1991). The forest floor often dries out in summer; spring snowmelt flushes solutes that have accumulated in the forest floor as a result of decomposition during the previous summer and fall (Arthur &

Fahey 1993). During snowmelt, concentration of DOC was 32 mg C L^{-1} and concentration of DON was 1.3 mg N L^{-1} (Yavitt & Fahey 1986; Fahey et al. 1985). In contrast, rainfall at the Harvard Forest is evenly distributed throughout the year, and the snowpack does not ordinarily form before December. When transpiration declines in the fall, hydrologic fluxes in the forest floor and mineral soil increase. This may result in the downward flushing of some decomposition products that accumulate in upper soil horizons in the summer and that continue to be produced through the fall. In the winter, the snowpack may melt and re-form in midseason, and rain often falls on the snowpack throughout the winter (Harvard Forest weather station data). The forest floor may be well flushed by late March and early April when spring snowmelt occurs, yielding low solute concentrations in Oa leachate. The importance of such seasonal flushing may vary annually or vary within the region; Cronan & Aiken (1985) measured concentrations of DOC in March of ca. 30 mg C L^{-1} in Adirondack Park, NY.

Even though N amendments were applied only from May through September, concentrations of inorganic N in Oa leachate at Harvard Forest were elevated (relative to reference) during spring snowmelt in the N-amended plots of the pine stand. This may indicate weak retention mechanisms for N in the forest floor of the pine stand in fall. Alternatively, if stronger N sinks were present in the hardwood stand in early spring, then inorganic N stored in the snowpack may have been retained by the forest floor of the hardwood stand during snowmelt but not by the forest floor in the pine stand.

Nitrogen saturation

The pine and hardwood forests showed markedly different responses in the retention of inorganic N in the forest floor. This is illustrated by the unbulked collection set from 25 July 1994 (Table 4). These samples were collected over a period of 6 days during which 6.8 cm of rain fell and the average maximum daily temperature was 30 °C. In the pine stand, total concentration of inorganic N (NO_3-N + NH_4-N) in Oa leachate ranged from 0.52 mg N L^{-1} in the reference plot to 12 mg N L^{-1} in the high-N plot. In the analogous plots of the hardwood stand the total inorganic N ranged from 0.34 to only 1.2 mg N L^{-1}. The forest floor of the hardwood stand exhibited a much stronger sink for inorganic N than did the forest floor of the pine stand. The elevated N sink in amended plots over reference plots can be attributed primarily to increased uptake by vegetation, which was much greater in the hardwood stand than in the pines (Magill et al. 1996). Differences in uptake demand by the vegetation could also explain why sinks for inorganic N in the forest floor grew stronger in the hardwood stand but not in the pine stand

as spring progressed to summer. Nitrification, which has increased under N amendments in the pine forest floor (Magill et al. 1996), contributed to N leaching by converting NH_4 to NO_3 which is a more mobile form of N. From July to September, under N amendments the rate of eluviation of inorganic N equaled or nearly equaled the N fertilization rate in the forest floor of the pine stand.

Leaching of inorganic N from the deep rooting zone was also much greater in the pine stand than in the hardwood, even though the mineral soil showed greater absolute retention of inorganic N in the pine stand. As mentioned above, plant uptake could be responsible for much of the inorganic N sink in mineral soil, but increases in plant uptake of N under N amendments were smaller in the pine forest than in the hardwood. Thus, increased rates of net microbial immobilization (Nadelhoffer et al. 1995) or some other means of inorganic N retention operated in the mineral soil. Substrates available for microbial processing in the mineral soil include fine root litter, woody root litter, or fractions of the DOM leached from the forest floor and retained in the mineral soil (Qualls & Haines 1992). The mean ratio of DOC fluxes to DON fluxes illuviated and retained in mineral soil was 39:1 in the pine stand 31:1 in the hardwood stand. The ratios of C:N in mineral soil organic matter were much lower, 23:1 and 20:1 respectively (Magill et al. 1996). Overall, the mineral soil in the pine stand was subject to a much greater flux of N input than was the mineral soil in the hardwood stand, uptake demand by vegetation was lower, and additional sinks for inorganic N were superseded. The solum of the pine stand under the high-N treatment leached inorganic N at a flux about 50-fold higher than the solum of the hardwood stand under equivalent treatment (Table 7).

The differences in N leaching in these two forests can be understood in terms of the theory of nitrogen saturation. Given a forest ecosystem that is cycling N tightly, when additional inputs of N surpass the ability of the system to store or cycle the excess, significant changes to the N cycle occur that may have deleterious consequences for the forest or for resource quality "downstream" (Agren & Bosatta 1988; Aber et al. 1989; Gundersen 1991). Export of dissolved inorganic N from the solum would be an effect of N saturation. Both stands at Harvard Forest exhibit tight cycling of inorganic N in the absence of amendments, evidenced by the virtual absence of inorganic N in solution in the deep rooting zone of the reference plots (Aber et al. 1993; this study, Table 7). Results of the present study suggest that net sinks of N in the forest floor play a key role in the saturation status of a forest ecosystem; the high rate of leaching of inorganic N from the forest floor in the pine stand is a symptom of N saturation.

Another key finding in the present work was that small, absolute differences in the input-output budgets of inorganic N for the forest floor in reference plots correlated with major differences in N leaching patterns under N amendment. Fluxes of inorganic N leached from the forest floor equaled 81% of the inputs of inorganic N in throughfall in the pine stand and 45% of that in the hardwood stand (Table 7). Outputs of inorganic N from the O horizon in the pine stand were thus much closer to inputs even in the absence of fertilization.

Summary

Was it vegetation type *per se* that caused the differences in leaching in the reference plots, and the differences in response to N amendments? In this case, vegetation type appears to have played a major if not dominant role. In reference plots, ratios of C:N in soil were nearly equivalent between the pine and hardwood stands, and net rates of N mineralization were similar. But the hardwood vegetation showed greater uptake of N than did the pines in the reference plots, and the hardwood vegetation showed greater increases in N uptake under N amendments (Aber et al. 1993; Magill et al. 1996).

Comparisons with other studies, however, make the answer less clear. Our results suggest that nitrate amendments to plots in a Northern Hardwood forest (aged about 40 yr) in eastern Maine should have been largely retained, but Nadelhoffer and others (1995) found significant leaching: greater than 50% of the added N was exported from below the solum. Likewise, preliminary results from the Adirondack Manipulation and Modeling Project (AMMP; $(NH_4)_2SO_4$ amendments) did not reinforce our own (Mitchell et al. 1994, Figure 4). In deep lysimeters (50 cm), nitrate concentrations increased over references in two of three forests dominated by deciduous hardwoods, but showed no increases in a *Pinus resinosa* forest. In an analysis of N cycling in European forests, Gundersen (1995) found a pattern that did reinforce our results with respect to vegetation types. With increasing fluxes of N in throughfall among 64 forests, N flux in litterfall showed a continued increase in deciduous forests but reached a maximum at much lower fluxes in coniferous forests. The deciduous forests were better able to respond to high N availability through higher rates of N cycling in litterfall. The manifestation of this pattern on a case by case basis is no doubt impacted by land use history, disturbance history, and interactions between vegetation and soil.

In undisturbed temperate forests in Chile, Hedin et al. (1995) found that DON was the major form of dissolved N exported from watersheds in streamwater and that ammonium dominated over nitrate in streams. The authors suggested that nitrate at concentrations higher than both ammonium and DON in watershed streams may be symptomatic (in the absence of dis-

turbance) of forests subject to anthropogenic N deposition. Our results at Harvard Forest support such a view when the fluxes in our reference and treated plots are compared, emphasizing not only N deposition but the role of N saturation. As Hedin et al. (1995) found in exports from watersheds in Chile, we found in exports from the reference-plot solums at Harvard Forest: N was transported primarily in dissolved organic form. By analyzing inputs and outputs from the forest floor and the mineral soil separately, we also found that in reference plots DON production and leaching was the dominant means of vertical transport of dissolved N from organic to mineral horizons (98% of TDN flux). Although a fraction of DON may be bioavailable in some terrestrial ecosystems (e.g. Northup et al. 1995), DON in solution in temperate forests is thought to be composed primarily of humic substances (Qualls & Haines 1991), unavailable for plant use.

Under long-term N amendments, transport fluxes of DON from the forest floor were exceeded by the leaching of bioavailable (inorganic) N. Leaching of nitrate exceeded leaching of ammonium, though equal amounts of each were present in the N amendments. In the N-saturated forest (pine) this occurred in solution in the deep rooting zone as well, making the pattern in solute exports from the solum as a whole decrease in the order NO_3-N > NH_4-N > DON. In the seventh year of N amendments, DON exports from the solum of the pine forest ranged from 98% (reference) to 45% (low-N) and 7% (high-N) of TDN. The hardwood forest, which responded with strong, increased N sinks including vegetative uptake, was still exporting N from the solum primarily as DON (96% and 78% of TDN) at both levels of N treatment.

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