EFFECTS OF CHRONIC NITROGEN AMENDMENTS ON PRODUCTION OF DISSOLVED ORGANIC CARBON AND NITROGEN IN FOREST SOILS

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Abstract. Chronic N deposition has been hypothesized to affect DOC production in forest soils due to the carbon demand exerted by microbial immobilization of inorganic N. We tested this hypothesis in field experiments at the Harvard Forest, Petersham, Massachusetts, USA. During four years of sampling soil solution collected beneath the forest floor in zero-tension lysimeters, we observed little change in DOC concentrations (10-30 % increase, not statistically significant) associated with elevated N inputs, but did observe significant increases in DON concentrations. Both DOC and DON varied seasonally with highest concentrations in summer and autumn. Mean DON concentrations increased 200-300 % with the highest rate of inorganic N fertilization, and concentrations of DON were highest in samples with high inorganic N concentrations. We conclude that the organic chemistry of soil solution undergoes qualitative changes as a result of long-term N amendment at this site, with small changes in DOC, large increases in DON, and a decline in the C:N ratio of dissolved organic matter.

1. Introduction

Assessing the long-term impacts of anthropogenic N deposition is an important goal for forest ecosystem management. The impacts are potentially severe, and include decline in forest vigor as well as degradation of water quality (Aber *et al.*, 1989). The ability of a forest ecosystem to retain N and thus forestall the "N saturation" that can occur with continued high N inputs varies considerably from site to site or stand to stand (e.g., Kahl *et al.*, 1993; Magill *et al.*, 1997; Gundersen *et al.*, 1997). Understanding the mechanisms of N retention in forested ecosystems is essential to accurately predict the long-term consequences of N saturation, as well as the likelihood that a given site will undergo N saturation within a specific time frame.

Nearly complete retention of N has been observed in the Harvard Forest hardwood plots from 1988-1993 (Magill *et al.*, 1997), despite addition of 826 kg ha^{-1} N in the high-N treatments. This added N appears to have been retained in a non-exchangeable form in soil organic matter. The mechanism of N incorporation is unknown, but a plausible mechanism is immobilization by soil microbes (Aber *et al.*, 1993; Magill *et al.*, 1997). If microbial immobilization is responsible for this N retention, then decreases in the concentration and bioavailability of dissolved organic carbon (DOC) in soil solution were predicted to occur due to the increased energy demand associated with immobilization (Aber, 1992).

To test this hypothesis, we examined changes in soil solution chemistry associated with chronic N fertilization of hardwood and pine plots at the Harvard Forest. Concentration and flux of DOC and dissolved organic nitrogen (DON) were measured to assess the effects of increased N deposition on soil solution organic chemistry beneath the forest floor.

2. Methods and Materials

2.1. SITE DESCRIPTION

The study site is the Harvard Forest, Petersham, Massachusetts, USA (42° 30' N, 72° 10' W). Climate is mildly continental, with average temperatures of -7 °C in January and 19 °C in July. Precipitation averages 110 cm and is evenly distributed throughout the year (Currie et al., 1996). Snow typically covers the ground for several months during winter, but mid-winter thaws and rain storms are common. At the Harvard Forest, we studied soil solution in the chronic N plots (Aber et al., 1993, Magill et al., 1997). Plots (one per combination of vegetation and N deposition) are 30 x 30 m, with either hardwood (predominantly Quercus velutina and Q. alba) or pine (Pinus resinosa). The hardwoods are the result of regeneration at the site after clearcutting approximately 50 years ago; the pine was planted in 1926 after agricultural abandonment. Soils are derived from glacial till, and are rocky and well-drained Typic Dystrocrepts with C:N in the forest floor of 22-26 prior to manipulation. Soils of the pine plot were plowed prior to abandonment; those of the hardwood plots do not have an Ap horizon. The plots have received N additions since 1988. Current rates of addition are 150 kg N ha^{-1} yr^{-1} (high-N), 50 kg N ha^{-1} yr^{-1} (low-N), and control (no added N). Atmospheric deposition of N at the site is approximately 8 kg ha^{-1} yr^{-1} inorganic N in wet and dry deposition combined (Aber et al., 1993). Further descriptions of the study site, methods of N application, and results to date are given by Aber et al. (1993), Currie et al. (1996), and Magill et al. (1997).

2.2. SAMPLE COLLECTION AND ANALYSIS

Soil solution was collected from polyethylene zero tension lysimeters (5 in each of the 6 plots) installed beneath the forest floor in August 1992 (Currie et al., 1996). Lysimeters were sampled after major rain events; bottles were kept in place in the field at all times. Solution collected from the 5 lysimeters was combined by plot prior to analysis, yielding one sample per plot per event. Over the course of the study, approximately 400 samples were collected, corresponding to approximately 65 events in each of the 6 plots. 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 hr of collection. After filtration, samples were frozen prior to analysis.

    Dissolved organic C was analyzed as total C minus inorganic C using a Shimadzu TOC 5000 Total Organic Carbon analyzer with autosampler (Pt-catalyzed combustion at 680 °C). 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 (Merriam et al., 1996) from October 1994 to November 1996. Samples collected in 1994 were used to compare the two TDN methods; similar results were obtained with each (Merriam et al., 1996). Nitrate and NH$_4^+$ were measured with automated colorimetry (Technicon methods 782-86T and 780-86T, respectively); NO$_2^-$ was negligible (Currie et al., 1996). 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).

To assess the effects of vegetation type and rate of N application on DOC and DON concentrations, the results were analyzed with ANOVA as a 2x3 factorial design without replication (factors = vegetation and N application, with 2 levels for vegetation and 3 levels for N treatment). To assess differences in solution chemistry associated with season, the results were analyzed with ANOVA as a 3x3 factorial design without replication (factors = N application and season, with 3 levels each). Data were grouped into the following seasons: spring (March-May), summer (June-August), and autumn (September-November). Where significant differences were found, Tukey's test was used to make comparisons among means. Significance was established at $p < 0.05$; all results are reported on that basis. Fluxes were calculated using the method described in Currie *et al.*, (1996) on a calendar year basis; the method involves estimating monthly water losses from the forest floor using PnET II (Aber and Federer, 1992) and multiplying seasonal water flux 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.

3. Results

Concentrations of DOC in forest floor leachate varied significantly by season, with lowest concentrations (as low as 10 mg L^{-1}) in spring and highest concentrations in summer and autumn (up to 300 mg L^{-1}; Figure 1). Mean concentrations in summer and autumn were significantly higher than those for spring. Concentrations of DOC were elevated by approximately 8 mg L^{-1} (15-25 %) in high-N plots relative to the controls (Table I), but this difference was not statistically significant. The highest DOC concentrations were observed in the N-amended pine plots in the first year after lysimeter installation, when concentrations consistently exceeded 100 mg L^{-1} (Figure 1).

Concentrations of DON in forest floor leachate showed significant seasonal variation, following patterns similar to those of DOC (higher in summer and autumn than spring; Table I). Concentrations varied significantly with N treatment; high-N plots were significantly higher in DON concentration than controls, with a doubling or tripling of average concentrations (Table I). Concentrations of DON reached levels as high as 30 mg L^{-1}, but were typically no higher than 5-10 mg L^{-1} (Figure 1). Overall, there was a strong positive relationship between concentrations of DON and DIN (DON=0.14DIN - 0.04, r^2= 0.57, *p* < 0.0001).

Changes in the relative proportions of DOC and DON in solution resulted in a significant decrease in the C:N ratio (mass:mass) of dissolved organic matter with N treatment (Table I). Although the C:N ratio declined with N fertilization, it was not significantly affected by vegetation type. The C:N ratio showed significant seasonal variation, with summer and autumn different from spring.

Fluxes of DOC and DON in soil solution followed the patterns seen in concentrations, with the highest fluxes typically found in high-N plots. Fluxes of DOC ranged from 27 to 75 g m^{-2} yr^{-1}, with highest values for high-N pine plots (Table II). Fluxes of DON ranged from 0.7 to 3.6 g m^{-2} yr^{-1}, and values were highest in the high-N treatments from both stands.
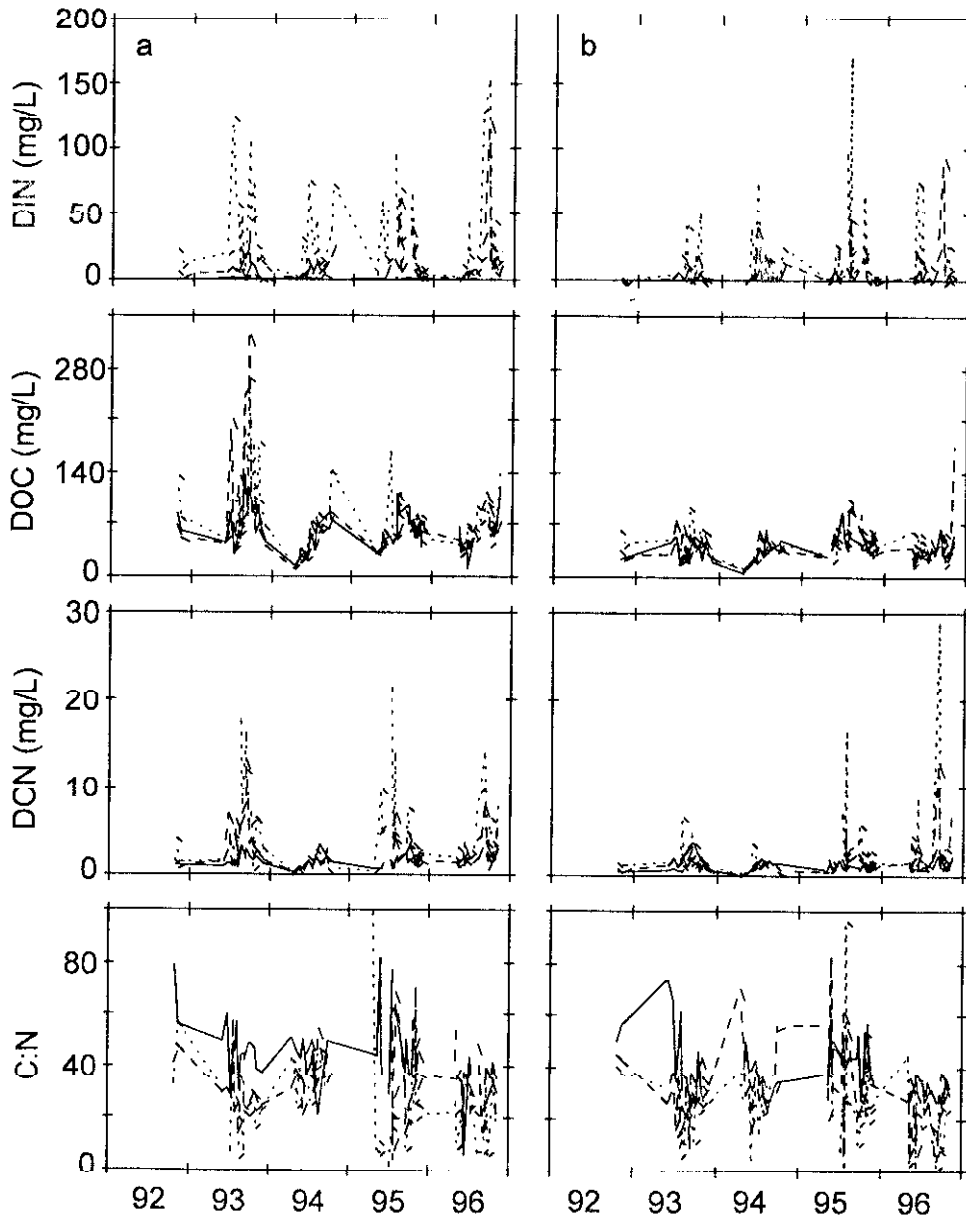
Fig. 1. Concentrations of inorganic N (DIN), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and the ratio DOC:DON (C:N; mass:mass) in soil solution collected from zero-tension lysimeters (forest floor leachate) in the pine (a) and hardwood (b) stand from 1992-1996. Solid line indicates control, dashed line the low-N treatment (50 kg ha^{-1} yr^{-1}), and dotted line the high-N treatment (150 kg ha^{-1} yr^{-1}).

TABLE I

Mean concentration of DOC and DON (mg L^{-1}) and mean DOC:DON (mass:mass) in forest floor solution collected from pine (P) and hardwood (H) plots at the Harvard Forest with zero tension lysimeters from 1992-1996. Since 1988, N has been applied to the plots at rates of 0 (control), 50 (Low-N), and 150 (High-N) kg ha^{-1} yr^{-1} (Magill et al., 1997).

		Season			N-treatment		
	Stand	Spring	Summer	Autumn	Control	Low-N	High-N
DOC	Pine	36.5	69.3	86.3	60.8	75.5	76.6
	Hardwood	31.4	43.5	43.9	35.7	42.7	46.1
DON	Pine	1.50	3.19	3.21	1.46	2.68	458
	Hardwood	1.05	2.02	2.07	0.98	1.67	3.02
DOC:DON	Pine	39.6	32.9	37.6	43.6	36.4	28.7
	Hardwood	42.4	30.6	31.4	40.1	32.8	25.6

TABLE II

Flux of DOC and DON (g m^{-2} yr^{-1}) by N treatment and stand type from 1993-1996. Fluxes are calculated as the seasonal average concentration×seasonal water flow estimated by PnET II (Aber and Federer, 1992) for each year.

Plot Treatment	Year							
	1993		1994		1995		1996	
	DOC	DON	DOC	DON	DOC	DON	DOC	DON
Pine								
Reference	54	1.2	46	1.2	45	1.0	45	1.4
Low N	72	2.6	44	1.2	50	1.6	52	1.7
High N	75	3.6	56	1.3	54	3.2	45	2.9
Hardwood								
Reference	33	0.7	28	0.8	33	0.8	27	0.8
Low N	31	1.1	28	0.8	37	1.1	35	1.8
High N	42	1.8	31	1.0	40	1.9	36	2.9

4. Discussion

We originally had hypothesized that N saturation would be accompanied by a decrease in DOC concentration and flux due to the immobilization of added N by free-living soil microbes and the subsequent depletion of C available to fuel the immobilization process (Aber, 1992). Our results directly contradict that hypothesis; we observed a significant

effect of N treatment on DOC concentrations in the hardwood plot, but the response was a moderate (and statistically insignificant) increase in DOC concentrations with high N application rather than the predicted decrease (Table I). Based on one year (1993-1994) of the four years of data presented here, Currie *et al.*, (1996) did not observe a statistically significant change in DOC or DON concentrations, although they did observe a significant change in DOC:DON. Our longer record shows there also has been a long-term change in DON concentrations associated with N treatment, but no change in DOC concentrations. In a comparison of 5 sites (NITREX project), Gundersen *et al.*, (1997) also concluded that DOC flux was not clearly related to N status at their control sites, but found that N addition did not consistently affect DON production. Rustad *et al.*, (1996) found no statistically significant changes in DOC concentrations with 4 yr of high N treatment of a spruce stand in Maine, USA. Guggenberger (1994) found that DOC fluxes in soil solution are highest in a site receiving the highest inputs of N in acid deposition, and suggested that this high DOC flux was the result of higher microbial activity due to increased N availability. In summary, neither our data nor data from other field experiments support the initial hypothesis of Aber (1992) that DOC concentrations will decline under conditions of N saturation.

Laboratory experiments, however, do show that DOC production by forest soils can be sensitive to N status. Gödde *et al.* (1996) found that in a comparison of six spruce stands, DOC produced during laboratory incubations of both cores and sieved soils was significantly lower in stands with high total N and low C:N in soil organic matter. At Harvard Forest, similar results have been obtained. DOC production by N-saturated soils (pine plot, high-N treatment collected in 1991) was less than half that of control and low-N samples when sieved soils were incubated for 35 days in laboratory microlysimeters (Benjamin, 1996). This discrepancy between field and laboratory results may be most easily explained by the lack of vegetation in the microlysimeters. In the lab, in the absence of vegetation or fully functioning tree roots, sources of DOC in high-N soils were depleted rapidly (Gödde *et al.*, 1996). We infer that in the field, this decline in DOC production by forest soil might be masked by a corresponding increase in DOC production by plant processes such as decomposition of recently fallen plant litter, root exudation, sloughing and decomposition of fine roots, or decomposition of mycorrhizal fungi.

The relative importance of various sources of DOC in soil solution is largely unknown. Guggenberger *et al.*, (1994) found that both oxidative degradation of plant-derived organic matter and production of microbial metabolites contribute to DOC in soil solution. Isotopic analysis (^{14}C) of DOC suggests that much of it is of recent origin, the result of photosynthetic fixation during the past 40 years (Schiff *et al.*, 1990). The striking disagreement between field and laboratory data for Harvard Forest points out the potentially large significance of recent plant photosynthate in providing DOC to soil solution, and warrants further investigation.

Our results do not provide conclusive evidence regarding the mechanism of N retention in forest soils experiencing heavy N loading. The original hypothesis that DOC should decline under conditions of N saturation was based on the premise that soil solution reflects the status of soil organic matter dynamics, and that soil organic matter would be depleted of labile materials with the increased C demand posed by N immobilization by soil microbes. One or both parts of our initial premise might be wrong. Rather than reflecting soil organic matter dynamics, DOC in soil solution might be more responsive to C balance in vegetation than previously thought. Alternately, N immobilization might be an abiotic

process or due to immobilization of added N by mycorhizzal fungi rather than by free-living soil microorganisms.

The marked change in DOC:DON ratio (from above 40 to 26-29) which we observed under heavy N loading (Table I) shows that large qualitative shifts in the organic chemistry of soil solution have occurred. Other studies have shown that this ratio typically ranges from 40 to 12 (Qualls and Haines, 1991; Sollins and McCorison, 1981; Gundersen et al., 1997), and thus the mean values that we report are within the range of those previously published. Fertilization has resulted in a 25-67 % increase in foliar N content on the high-N treatments (Magill et al., 1997). This change in litter chemistry might have contributed to the change in soil solution chemistry by altering the soluble products of leaf decomposition, but such linkages are largely speculative.

The average concentrations of DON that we have measured in the control plots (1.0-1.5 mg L^{-1}) at Harvard Forest are similar to those observed at other sites. The average concentration of DON collected beneath the forest floor over multiple years and sites (hardwood and conifer) by the Integrated Forest Study in the United States was 0.4 to 2.1 mg L^{-1} (Johnson and Lindberg 1992); an identical range in concentration was obtained for coniferous forests in Sweden by Stuanes et al., (1995). Flux values that we report in the control plots also are comparable to those reported by the Integrated Forest Study (Johnson and Lindberg, 1992) and NITREX (Gundersen et al., 1997).

Neither the chemical structure nor the density of identifiable functional groups is known for the DON produced in response to inorganic N fertilization at Harvard Forest. Without such information, it is difficult to assess the ecological significance of this additional DON. If the added DON is comprised of compounds such as amines, then it is potentially available to plant roots or microbes growing in the mineral soil (Kaye and Hart, 1997). If it contains more refractory compounds, then long-term accumulation of this DON in the soil profile could occur. No net increase in DON concentration or flux from the mineral soil occurs under conditions of N saturation (Currie et al., 1996), suggesting that either biotic uptake or abiotic adsorption (cf. McDowell and Wood, 1984) maintains a constant DON flux from the solum despite the increased flux from the forest floor.

Acknowledgments

References

Aber, J.D.: 1992, TREE 7, 220.
Aber, J.D. and Federer, C. A.:1992, Oecol. 92, 463.
Aber. J.D., Magill, A., Boone, R., Melillo, J.M., Steudler, P. and Bowden, R.: 1993, Ecol. Applic. 3, 156.

Aber, J.D., Nadelhoffer, K.J., Steudler, P. and Melillo, J.M.: 1989, *BioScience* **39**, 378.

Benjamin, S.S.: 1996, *Effects of Nitrogen Status on Dissolved Organic Carbon Dynamics in Forest Soils*, Thesis, University of New Hampshire, Durham.

Currie, W.S., Aber, J.D., McDowell, W.H., Boone, R.D. and Magill, A.H.: 1996, *Biogeochemistry* **35**, 471.

Gödde, M., David, M.B., Christ, M.J., Kaupenjohann, M. and Vance, G.F.: 1996, *Soil Biol. Biochem.* **28**, 1181.

Guggenberger, G.: 1994, *Environ. Internat.* **20**, 31.

Guggenberger, G., Zech, W. and Schulten, H.: 1994, *Org. Geochem.* **21**, 51.

Gundersen, P., Emmett, B.A., Kjønaas, O.J., Koopmans, C.J. and Tietema, A.: 1997, *Forest Ecol. Manag.*, in press.

Johnson, D.W. and Lindberg, S.E.: 1992, *Atmospheric Deposition and Forest Nutrient Cycling*, Springer Verlag, New York.

Kahl, J.S., Norton, S.A., Fernandez, I.J., Nadelhoffer, K.J., Driscoll, C.T. and Aber, J.D.: 1993, *Environ. Sci. Techol.* **27**, 565.

Kaye, J.P. and Hart, S.C.: 1997, *TREE* **12**, 139.

Magill, A.H., Aber, J.D., Hendricks, J.J., Bowden, R.D., Melillo, J.M. and Steudler, P.A.: 1997, *Ecol. Applic.* **7**, 402.

McDowell, W.H. and Wood, T.: 1984, *Soil Sci.* **137**, 23.

Merriam, J., McDowell, W.H. and Currie, W.S.: 1996, *Soil Sci. Soc. Am. J.* **60**, 1050.

Qualls, R G. and Haines, B.L.: 1991, *Soil Sci. Soc. Am. J.* **55**, 1112.

Rustad, L.E., David, M.B., Mitchell, M.J., Nadelhoffer, K.J. and Fuller, R.B.: 1996, *Soil Sci. Soc. Am. J.* **60**, 1933.

Schiff, S.L., Aravena, R., Trumbore, S.E. and Dillon, P.J.: 1990, *Water Resourc. Res.* **26**, 2949.

Sollins, P. and McCorison, F.M.: 1981, *Water Resourc. Res.* **17**, 1409.

Solorzano, L. and Sharp, J.H.: 1980, *Limnol. Oceanogr.* **25**, 751.

Stuanes, A.O., Kjonaas, O.J. and Van Miegroet, H.: 1995, *For. Ecol. Manage.* **71**, 99.