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Chronic nitrate additions dramatically increase the export of carbon and nitrogen from northern hardwood ecosystems

KURT S. PREGITZER[1,*], DONALD R. ZAK[2], ANDREW J. BURTON[1], JENNIFER A. ASHBY[1] and NEIL W. MACDONALD[3]
*[1]School of Forest Resources & Environmental Science, Michigan Technological University, Houghton, MI 49931; [2]School of Natural Resources & Environment, The University of Michigan, Ann Arbor, MI 48109; [3]Department of Biology, Grand Valley State University, Allendale, MI 49401; USA; *Author for correspondence (e-mail: kspregit@mtu.edu; phone: +1-906-487-2396; fax: +1-906-487-2915)*

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Abstract. A long-term field experiment was initiated to simulate chronic atmospheric N deposition, a widespread phenomenon in industrial regions of the world. Eight years of experimental nitrate (NO_3^-) additions (3 g NO_3^--N m^{-2} per year) to four different northern hardwood forests located along a 500 km geographic gradient dramatically increased leaching losses of NO_3^--N, dissolved organic carbon (DOC), and dissolved organic nitrogen (DON). During the last two water years, the average increase in solution NO_3^-- N and DON leaching from the NO_3^- -amended plots was 2.2 g N m^{-2}, equivalent to 72% of the annual experimental N addition. Results indicate that atmospheric N deposition may rapidly saturate some northern hardwood ecosystems across an entire biome in the upper Great Lakes Region of the USA. Changes in soil C and N cycling induced by chronic N deposition have the potential in this landscape to significantly alter the flux of DOC and DON from upland to aquatic ecosystems. Michigan Gradient study site characteristics are similar to those of European forests most susceptible to N saturation.

Introduction

Industrial and agricultural activity has doubled the amount of nitrogen (N) entering terrestrial and aquatic ecosystems in many portions of the Earth, a trend that is likely to continue into the next several decades (Vitousek 1994; Galloway 1995; Vitousek et al. 1997). This is particularly true in temperate forests of North America and Europe, whose productivity is sometimes limited by the availability of soil N (Vitousek & Howarth 1991). The relatively large amounts of N entering temperate forests from human sources suggest that these ecosystems may eventually reach N saturation, a point where forest productivity is no longer limited by soil N availability (Ågren & Bosatta 1988; Aber et al. 1989, 1998). Increases in N availability can directly affect the amount and chemical composition of plant litter, and this can modify the flow of C and N through the soil food web (Zak & Pregitzer 1998). Plant litter provides the energy (i.e., C) that structures soil food webs, and any changes in the production or chemistry of plant litter have the potential to modify the processing of organic compounds by soil microorganisms. Energy-rich organic compounds contained in plant litter fuel microbial growth, the byproducts of which are a set of complex organic compounds that form dissolved organic carbon (DOC) and dissolved organic nitrogen (DON).

DOC and DON produced in upland forest soils can leach below the rooting zone and move into aquatic ecosystems (McDowell & Likens 1988; Perakis & Hedin 2002), where this diverse set of organic compounds can influence ecosystem processes. Increased DOC and DON can decrease water transparency and alter aquatic biodiversity and ecosystem productivity (Kieber et al. 1989; Green & Blough 1994). Greater DOC and DON flux from terrestrial to aquatic ecosystems could also result in increased movement of heavy metals, chlorinated hydrocarbons, and nutrients into streams and lakes (Jardene et al. 1990; Qualls & Haines 1992; Guggenberger et al. 1994). Shifts in the ratios of organic/inorganic N exported from N-saturated terrestrial ecosystems might also significantly impact aquatic food webs, but this aspect of N saturation is only beginning to be studied (Currie et al. 1996).

We established a long-term field experiment to simulate chronic atmospheric N deposition in northern hardwood forests along a geographic gradient in Michigan to examine the effects of climate and atmospheric deposition on ecosystem processes (Burton et al. 1993). The majority of atmospheric N deposition (wet + dry) in this region is in the form of NO_3^--N (MacDonald et al. 1992; Liechty et al. 1993). Climate, atmospheric deposition, soil characteristics, forest growth and structure, and certain aspects of the N cycle on each of these four study sites have been reported elsewhere (MacDonald et al. 1991, 1992; Reed et al. 1994; Zogg et al. 1996, 2000; Brown et al. 2000). Here we report for the first time the long-term effects (0–8 years) of experimental additions of NO_3^--N on the concentrations and estimated fluxes of ammonium-N (NH_4^+-N), NO_3^--N, DOC and DON.

We initially hypothesized that experimental additions of NO_3^- would be immobilized by the microbial community in soil and would be subsequently mineralized as NH_4^+ through microbial turnover. We hypothesized that most of this NH_4^+ would then be taken up by the dominant vegetation and returned via litter to the soil. Through time, the added N would increase the availability of substrate for nitrification (Robertson 1982), and nitrification and subsequent NO_3^--N leaching would eventually result in N saturation. The alternative hypothesis in terms of NO_3^--N leaching was that the added NO_3^- would be immobilized by the microbial community, assimilated and cycled in the vegetation, or abiotically fixed in soil organic matter, resulting in little or no increase in NO_3^--N leaching over time.

We did not hypothesize a significant increase in the production and leaching of DOC and DON, predicting instead that the processes regulating organic C and N cycling and export would remain more or less unaltered. Our original DOC and DON hypothesis followed the theory that DOC concentrations and fluxes should be high under N-limited conditions and low under N-saturated conditions (Aber 1992). Alternative hypotheses were: (1) the ionic strength of the fertilizer would result in desorption of exchangeable DOC and DON leading to increased leaching of these organic compounds; or (2) increased NH_4^+ availability would inhibit lignolytic activity, resulting in the incomplete degradation of lignin and greater production of soluble polyphenols (DOC and DON; Fog 1988; Carreiro et al. 2000; Freeman et al. 2001; Solinger et al. 2001).

Methods

Study sites

The Michigan Gradient study was established in 1987 to examine the effects of climate and atmospheric deposition on ecosystem processes in the Great Lakes region. Four intensively monitored northern hardwood study sites are located along a 500 km climatic and N deposition gradient extending from northwestern Upper Michigan to southern Lower Michigan (Figure 1). The study was deliberately established to range from the northern edge of the northern hardwoods region in western Upper Michigan to the transition zone in the middle of the Lower Peninsula, encompassing most of the hemlock-white pine-northern hardwoods biome in the Great Lakes Region (Braun 1950). The dominant soil series across the gradient, Kalkaska sand (Typic Haplorthod), is Michigan's official State Soil and covers more than 300,000 ha in Michigan alone. These soils are typically dominated by second-growth northern hardwood forests, and this particular soil – forest type combination is very common in Michigan (Host and Pregitzer 1992). The four second-growth stands are approximately 90 years old and dominated by sugar maple (*Acer saccharum* Marsh.; Table 1). All four ecosystems are similar in terms of stand composition, structure, and soils, but differ in terms of temperature, N deposition and inherent levels of soil N availability (Table 1; Burton et al. 1991, 1996; MacDonald et al. 1992).

At each site, three control plots were established in 1987; three N-amended plots were subsequently established 1993. All plots were 30 m × 30 m in size, with a treated 10 m buffer on all sides. In each plot, four porous ceramic cup tension lysimeters were installed at a 75 cm depth (Model 1900 Soil Water Samplers, Soilmoisture Equipment Corp., Goleta, CA). This depth is in the lower portion of the Bs soil horizon. Previous studies at these sites have shown that most of the absorbing roots of sugar maple occur above this depth (Hendrick & Pregitzer 1996). Lysimeters were allowed to recover from installation disturbance for 1 year before soil solution sampling was initiated. Solid fertilizer was applied monthly during the growing season in six increments (0.5 g N m^{-2} as $NaNO_3$), for a total 3 g NO_3^--N m^{-2} per year. Nitrate was applied approximately every 4 weeks, beginning in late April/early May and ending each year in mid–late September. Nitrate applications began in the

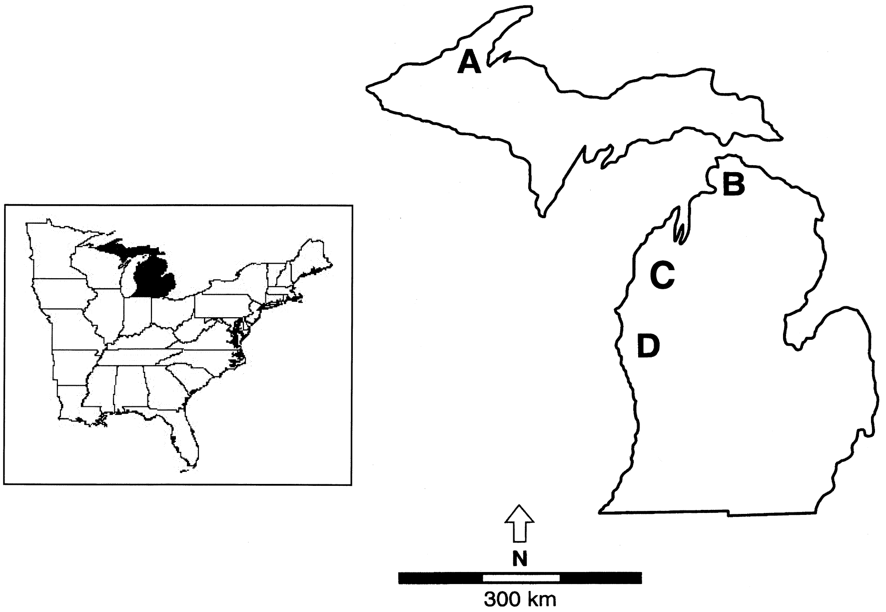

Figure 1. Location of the four study sites in Michigan. Inset shows the location of Michigan in the eastern USA.

spring of 1994 and continued for the duration of the study (8 years). The rate of simulated N deposition induced by our experimental treatments is comparable to current rates of atmospheric N deposition in portions of the northeastern US and Europe (Bredemeier et al. 1998; Fenn et al. 1998; MacDonald et al. 2002).

Soil solution sampling

Soil solutions were collected from ceramic cup tension lysimeters every 2 weeks during the spring (mid-April to early June) and fall (late August to early November) from 1994 to 2001 in both the control and NO_3^--amended plots. A tension just over field capacity (up to 0.05 MPa) was applied 2 weeks prior to each collection. Vacuum was not applied over winter or over summer. No water moves below the rooting zone of these soils during the summer. The first spring collection occurred before any fertilizer was added for that year, and the late fall lysimeter collections occurred 4 or more weeks after the last N additions for that year. Lysimeter solutions within a plot were composited on the date of collection. Samples to be analyzed for DOC, total N, NH_4^+-N, and NO_3^--N were filtered within 24–48 h of collection through a 0.45 micron filter (Magna, nylon plain supported filters, Osmonics, Inc., Westborough, MA), acidified with ultrapure HCl to pH < 2, and stored in plastic bottles at 4 °C

Table 1. Study site characteristics.

Characteristic	Site A	Site B	Site C	Site D
Latitude	46°52′N	45°33′N	44°23′N	43°40′N
Longitude	88°53′W	84°51′W	85°50′W	86°09′W
Net N mineralization (µg N per g soil)[a]	0.29b	0.46a	0.48a	0.32b
pH of A + E soil horizons[b]	4.6–5.1	4.6–5.3	4.4–4.5	4.3–5.2
pH of upper B soil horizons[b]	4.7–5.7	4.8–6.3	5.2–6.9	5.0–5.7
Forest floor (Oe + Oa) C:N ratio[c]	16.7	18.4	20.6	17.9
Mean annual precipitation, 1994–2001 (mm)[d]	821	828	856	793
Mean annual temperature, 1994–2001 (°C)[e]	4.8	6.1	6.9	7.6
Wet plus dry NO_3^--N (g m^{-2} per year)[f]	0.38	0.58	0.78	0.76
Wet plus dry total N (g m^{-2} per year)[f]	0.68	0.91	1.17	1.18
Total basal area, 2001 (m^2 ha^{-1})	35	33	33	36
Sugar maple basal area, 2001 (% of total)	91	86	79	71
Overstory age, 2001	94	88	89	93

[a] N mineralization data are from Zogg et al. (1996) for the top 10 cm of soil and organic matter occurring beneath the surface litter (Oi) layer. Net N mineralization was determined using the buried bag technique, with bags replaced at 5-week intervals from May through November 1994. Site means for N mineralization followed by a different letter are significantly different at the 0.05 level of probability.

[b] Soil pH values from MacDonald (unpublished data) from samples collected as described in MacDonald et al. (1991) for the control plots.

[c] Forest floor C:N data are for control plots and are calculated from MacDonald et al. (1991).

[d] Precipitation amounts were recorded using weighing rain gages (Model 5-780, Belfort Instrument Co., Baltimore, MD) located in open areas within 5 km of each site.

[e] Air temperature was measured at 2 m within all plots at all sites, using thermistors that were read every 30 min throughout the year, with averages recorded every 3 h using data loggers (EasyLogger Models 824 and 925, Data Loggers, Inc., Logan, UT).

[f] Atmospheric deposition data from MacDonald et al. (1992).

until analysis. Concentrations of NH_4^+-N, NO_3^--N, DOC and DON were determined for each sample date.

Laboratory analyses

Nitrate-N, NH_4^+-N and persulfate-N were determined via colorimetric analysis on an O.I. Analytical Flow Solution 3000 analyzer (O.I. Analytical, College Station, TX). DON concentrations were determined as the difference between persulfate-N and NO_3^--N plus NH_4^+-N. DOC was analyzed by high-temperature catalytic oxidation using a Shimadzu TOC-5000A (Shimadzu Scientific Instruments, Columbia, MD) by injecting 100 µl of sample onto a Pt-on-alumina catalyst at 680 °C. Samples were acidified with HCl to pH 2, sparged with carrier gas (ultra-pure grade air, 99.9959% oxygen) for 4 min to remove carbonate or bicarbonate, and then analyzed for non-purgeable organic carbon. Detection limits for NH_4^+-N, NO_3^--N, and DON were 0.01, 0.05, and 0.05 mg N l^{-1}; the detection limit for DOC was 0.60 mg C l^{-1}.

Flux estimates and statistical analyses

Soil and air temperature and precipitation are continuously measured at each of the study sites (Brown et al. 2000; Burton et al. 2000). Estimates of water flux for each sampling period were calculated from air temperature and precipitation inputs using the water balance method (Thornthwaite & Mather 1957). These were multiplied by soil solution NH_4^+-N, NO_3^--N, DOC, and DON concentrations for the same period and summed to determine soil solution fluxes in g m^{-2} for each water year. Water years consisted of samples collected during the fall of 1 year and the subsequent spring. They represent water collected over a continuous groundwater recharge period during which little additional NO_3^--N is added to the plots. Water flux for the overwinter period was assigned the concentration of the first collection in the spring. It was not uncommon for the last spring and first few fall collections to have a calculated water flux of zero.

Plot flux estimates and average concentrations for each water year were statistically analyzed using univariate repeated measures analysis of variance (ANOVA), with study site and NO_3^--N addition as main effects and concentrations and fluxes measured during the seven water years considered as repeated measurements. The data were treated as a temporal series and intervals were equal (data were by water year). Probability levels change only slightly when multivariate repeated measures are used or when the Greenhouse-Geiser and Huynh-Feldt adjusted P-values are used instead of the unadjusted univariate P-values. Significant effects for NO_3^-, DOC and DON were the same for both univariate and multivariate repeated measures ANOVA.

Column study

A laboratory soil column study was conducted to determine whether DOC concentrations observed in NO_3^--amended plots could result from ionic exchange (i.e., a ‘salt effect’). In December 1997, three replicate sets of four intact soil cores (12 cores total) were removed from soils in the plots at Site A using lengths of PVC pipe (30.5 cm × 5.1 cm inner diameter). Cores were wrapped in plastic and stored at 4 °C overnight. The following day, the top and bottom ends of the core were lined with 1 cm quartz wool (cleaned by ignition at 450 °C) and sealed to a plastic Buchner funnel with silicone glue. On the third day, cores were mounted vertically to collect leachates. Cool room temperatures were maintained throughout the experiment (11–13 °C) to avoid DOC production resulting from enhanced microbial activity and to closely approximate field conditions. Each core within a set of the four was randomly assigned one of the four $NaNO_3$ solutions (0.4, 25.0, 50.0, and 100.0 mg l^{-1} $NaNO_3$-N; representing conductivities of 6.2, 192, 372, and 750 µS cm^{-1}, respectively). The 0.4 mg N l^{-1} solution represented typical NO_3^--N concentrations in precipitation at all sites (Liechty et al. 1993). Solutions were gently poured onto the columns by hand in 50 ml aliquots over a 12 h period, beginning within 46 h from the time the cores were extracted in the field and ending with a fourth application (200 ml total) at 58 h after collection. Four sets of leachate solutions (~50 ml each) were collected from the cores and filtered through a 0.22 μm nylon filter, acidified to $\text{pH} < 3$, and stored in plastic bottles at 4 °C until analysis for DOC. Leachate collections were completed within 69 h after core removal in the field. Total DOC mass (mg DOC-C) leached from each replicate soil column was calculated by multiplying DOC concentration by the volume of leachate collected from the column. Treatment means were compared using a one-way ANOVA.

Results

Soil solution DOC, NO_3^--N, NH_4^+-N and DON concentrations

Chronic NO_3^- additions caused highly significant increases in the soil solution concentrations of DOC, NO_3^--N and DON (Table 2 and Figure 2). Concentrations of all three constituents increased over time in the NO_3^--amended plots, but varied only slightly from year-to-year in the control plots (Figure 2), resulting in significant within subject effects for water year and the water year × NO_3^- addition interaction (Table 2). Over the 8-year study, soil solution concentrations in NO_3^--amended plots were 2.3 times those in control plots for DOC, 3.2 times control values for DON, and more than 20 times control levels for NO_3^--N. Soil solution concentrations of NH_4^+-N were not affected by the chronic NO_3^- additions (Table 2 and Figure 2).

The response of DOC, NO_3^--N and DON concentrations to NO_3^- additions was consistent at all of the sites; thus, there were no site × NO_3^- addition interactions (Table 2). Figure 3 demonstrates this result for the last two water years. All four sites responded to chronic NO_3^- additions the same way, with NO_3^--amended plots always exhibiting much greater concentrations of DOC, NO_3^--N and DON than control plots. This was true over the entire duration of the study. Nitrate-N and DON concentrations on the NO_3^--amended plots tended to be greater at sites B and C than at sites A and D (significant study site effect in Table 2; results for last two water years depicted in Figure 3). Study sites B and C have higher inherent N availability (Table 1; Zogg et al. 1996). Therefore, greater inherent soil N availability consistently resulted in the highest rates of NO_3^--N and DON production.

DOC:DON ratios decreased over time on the NO_3^--amended plots, but the data were noisy. Over the 7 years, DOC:DON ratios on the NO_3^--amended plots were 27% lower than those on the control plots (20.7 vs. 28.4); differences among mean ratios were not statistically significant, $P = 0.47$.

Table 2. Repeated measures ANOVA table for soil solution concentrations of DOC, NO_3^--N, NH_4^+-N and DON collected from control and NO_3^--amended plots at four northern hardwood study sites.

Source	df	SS	MS	F	$P>F$
DOC concentration					
Between subjects					
Study site	3	1429	476	6.70	0.004
NO_3^- addition	1	3028	3030	42.59	<0.001
Study site × NO_3^- addition	3	49	16	0.23	0.876
Error	15	1067	71		
Within subjects					
Water year	6	636	106	12.71	<0.001
Water year × study site	18	366	20	2.44	0.003
Water year × NO_3^- addition	6	835	139	16.68	<0.001
Water year × study site × NO_3^- addition	18	272	15	1.81	0.036
Error	90	751	8		
NO_3^--N concentration					
Between subjects					
Study site	3	194	65	1.61	0.230
NO_3^- addition	1	622	622	15.49	0.001
Study site × NO_3^- addition	3	173	58	1.44	0.272
Error	15	602	40		
Within subjects					
Water year	6	424	71	9.84	<0.001
Water year × study site	18	331	18	2.56	0.002
Water year × NO_3^- addition	6	392	65	9.08	<0.001
Water year × study site × NO_3^- addition	18	313	17	2.42	0.003
Error	90	647	7		
NH_4^+-N concentration					
Between subjects					
Study site	3	0.004	0.001	1.154	0.360
NO_3^- addition	1	0.002	0.002	1.403	0.255
Study site × NO_3^- addition	3	0.006	0.002	1.496	0.256
Error	15	0.019	0.001		
Within subjects					
Water year	6	0.012	0.002	1.626	0.149
Water year × study site	18	0.026	0.001	1.209	0.271
Water year × NO_3^- addition	6	0.007	0.001	0.933	0.475
Water year × study site × NO_3^- addition	18	0.025	0.001	1.165	0.307
Error	90	0.109	0.001		
DON concentration					
Between subjects					
Study site	3	6.15	2.05	7.34	0.003
NO_3^- addition	1	22.15	22.15	79.22	<0.001
Study site × NO_3^- addition	3	2.74	0.91	3.26	0.051
Error	15	4.19	0.28		

Table 2. (*continued*)

Source	df	SS	MS	F	$P>F$
Within subjects					
Water year	6	29.77	4.96	12.80	< 0.001
Water year × study site	18	25.03	1.39	3.59	< 0.001
Water year × NO_3^- addition	6	21.58	3.60	9.28	< 0.001
Water year × study site × NO_3^- addition	18	16.16	0.90	2.32	0.005
Error	90	34.88	0.39		

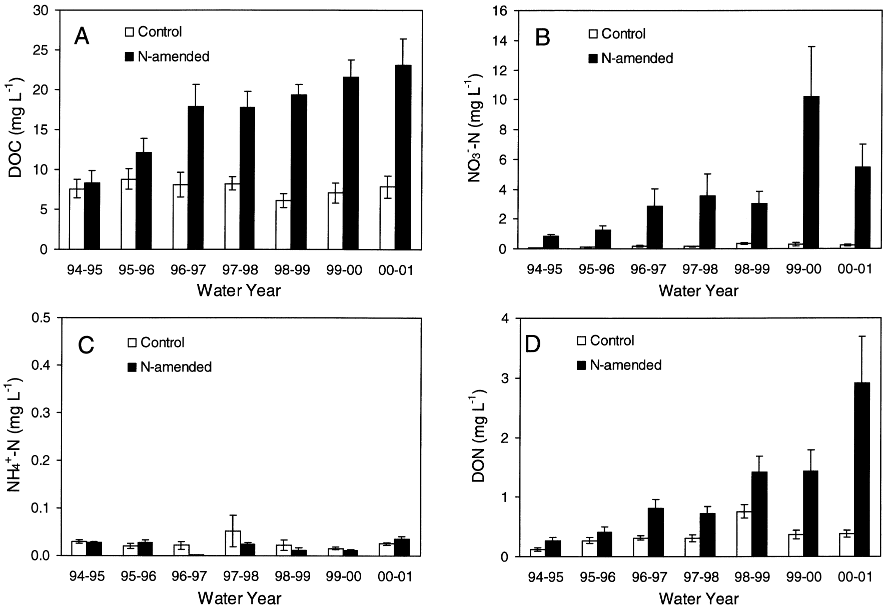

Figure 2. Time trends in DOC (A), NO_3^--N (B), NH_4^+-N (C) and DON (D) concentrations for control and NO_3^--amended plots. Error bars indicate 1 standard error for all plots receiving a treatment across all four sites (n = 12).

Flux estimates

Chronic NO_3^- additions also caused highly significant increases in the soil solution fluxes of DOC, NO_3^--N and DON (Table 3 and Figure 4). Similar to concentrations, these fluxes had a strong increasing trend on the NO_3^--amended plots but varied only slightly from year-to-year on the control plots, resulting in significant within subject effects for water year and the water year × NO_3^- addition interaction (Table 3). During the last two water years, the average increase in combined solution NO_3^--N and DON losses from the N-amended plots across all the study sites was 2.2 g N m^{-2}, equivalent to 72% of our annual N additions.

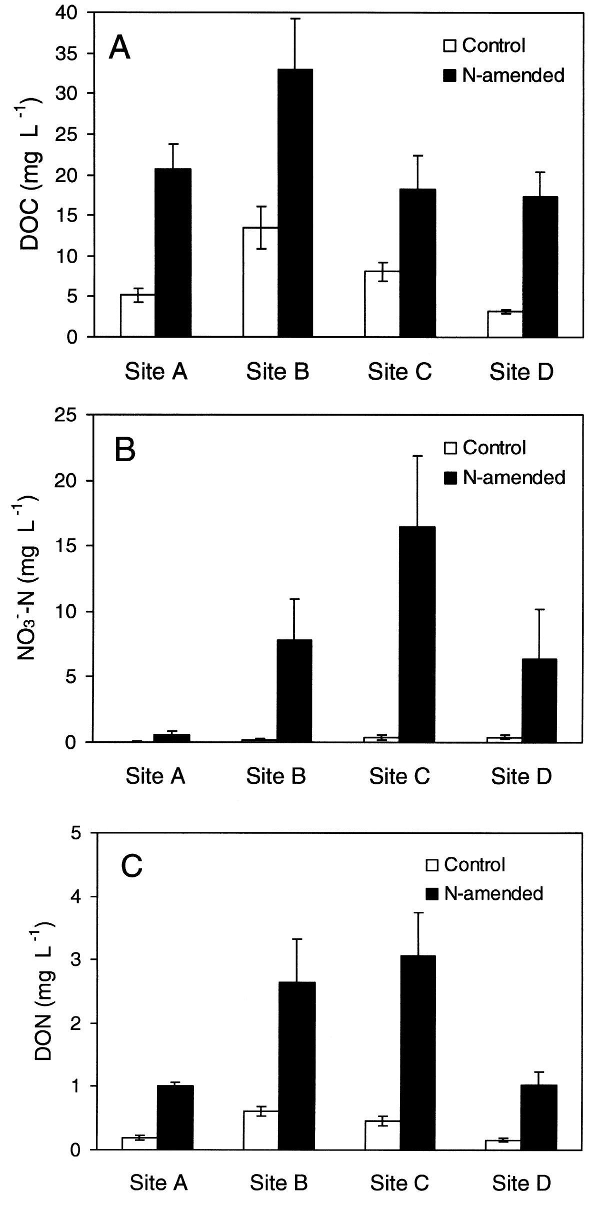

Figure 3. Patterns among sites in DOC (A), NO_3^--N (B) and DON (C) concentrations for the 1999–2000 and 2000–2001 water years. Error bars indicate the standard error for three plots per treatment at each site.

Table 3. Repeated measures ANOVA table for soil solution fluxes of DOC, NO_3^--N, NH_4^+-N and DON collected from control and NO_3^--amended plots at four northern hardwood study sites.

Source	df	SS	MS	*F*	*P* > *F*
DOC flux					
Between subjects					
Study site	3	161	53.7	5.34	0.011
NO_3^- addition	1	336	336.1	33.41	<0.001
Study site × NO_3^- addition	3	21	7.0	0.69	0.571
Error	15	151	10.1		
Within subjects					
Water year	6	95	15.8	9.41	<0.001
Water year × study site	18	161	8.9	5.34	<0.001
Water year × NO_3^- addition	6	64	10.7	6.37	<0.001
Water year × study site × NO_3^- addition	18	75	4.2	2.49	0.002
Error	90	151	1.7		
NO_3^--N flux					
Between subjects					
Study site	3	9.8	3.28	2.17	0.134
NO_3^- addition	1	31.4	31.41	20.77	<0.001
Study site × NO_3^- addition	3	8.9	2.97	1.96	0.163
Error	15	22.7	1.51		
Within subjects					
Water year	6	17.5	2.91	8.03	<0.001
Water year × study site	18	16.6	0.92	2.54	0.002
Water year × NO_3^- addition	6	16.8	2.80	7.70	<0.001
Water year × study site × NO_3^- addition	18	15.5	0.86	2.37	0.004
Error	90	32.7	0.36		
NH_4^+-N flux					
Between subjects					
Study site	3	0.00021	0.00007	1.22	0.336
NO_3^- addition	1	0.00001	0.00001	0.23	0.638
Study site × NO_3^- addition	3	0.00032	0.00011	1.84	0.183
Error	15	0.00088	0.00006		
Within subjects					
Water year	6	0.00188	0.00031	5.61	<0.001
Water year × study site	18	0.00276	0.00015	2.74	<0.001
Water year × NO_3^- addition	6	0.00094	0.00016	2.79	0.015
Water year × study site × NO_3^- addition	18	0.00202	0.00011	2.01	0.017
Error	90	0.00503	0.00006		
DON flux					
Between subjects					
Study site	3	0.258	0.086	3.41	0.045
NO_3^- addition	1	1.698	1.698	67.26	<0.001
Study site × NO_3^- addition	3	0.054	0.018	0.71	0.559
Error	15	0.379	0.025		

Table 3. (*continued*)

Source	df	SS	MS	*F*	$P > F$
Within subjects					
Water year	6	0.537	0.090	9.33	< 0.001
Water year × study site	18	1.427	0.079	8.26	< 0.001
Water year × NO_3^- addition	6	0.477	0.080	8.28	< 0.001
Water year × study site × NO_3^- addition	18	0.856	0.048	4.96	< 0.001
Error	90	0.864	0.010		

The response of DOC, NO_3^--N and DON fluxes to NO_3^- additions also was consistent among all of the sites, thus there were no study site × NO_3^- addition interactions (Table 3). Soil solution fluxes of NH_4^+-N were not affected by NO_3^- addition (Table 3 and Figure 4(C)). The relatively high DOC fluxes in the 1996–1997 water year resulted from large amounts of water moving below the rooting zone during this relatively wet year; fluxes are also relatively high in the control plots for that water year.

With the exception of NH_4^+-N, there was a clear trend for an increase in the difference between control and NO_3^--amended plots in NO_3^--N, DOC and DON fluxes over time (Figure 4), suggesting that chronic NO_3^- additions influence the belowground C cycle and that the study sites rapidly approached N saturation. Averaged across sites, the amount of NO_3^--N lost in soil solution increased from 7% of the amount added in 1994–1995 to 57% of the amount added during the 1999–2000 and 2000–2001 water years. Both DOC and DON losses in NO_3^--amended plots also increased over time (Figure 4), with DOC levels in the NO_3^--amended plots more than 3 times those in the control plots by the 2000–2001 water year ($P < 0.001$). Average DON losses in the NO_3^--amended plots were more than 6 times those in the control plots by the 2000–2001 water year ($P < 0.001$). Differences in DOC and DON concentrations between the control and NO_3^--amended plots were so great as to be visible to the naked eye when collecting samples in the field by the 1996–1997 water year (i.e., darker soil solutions in the NO_3^--amended plots).

As mentioned above, sites B and C have higher inherent N availability (Table 1) and it is interesting to note that NO_3^--N and DON leaching losses in plots receiving NO_3^- additions occurred sooner and were greater at these sites compared to sites A and D, which have lower inherent N availability (data not shown, but flux trends mirror concentration trends, which are depicted for the last two water years in Figure 3).

Column study

Results from the soil column experiment designed to test the alternative hypothesis that fertilizer ionic strength would influence the mass of DOC in soil

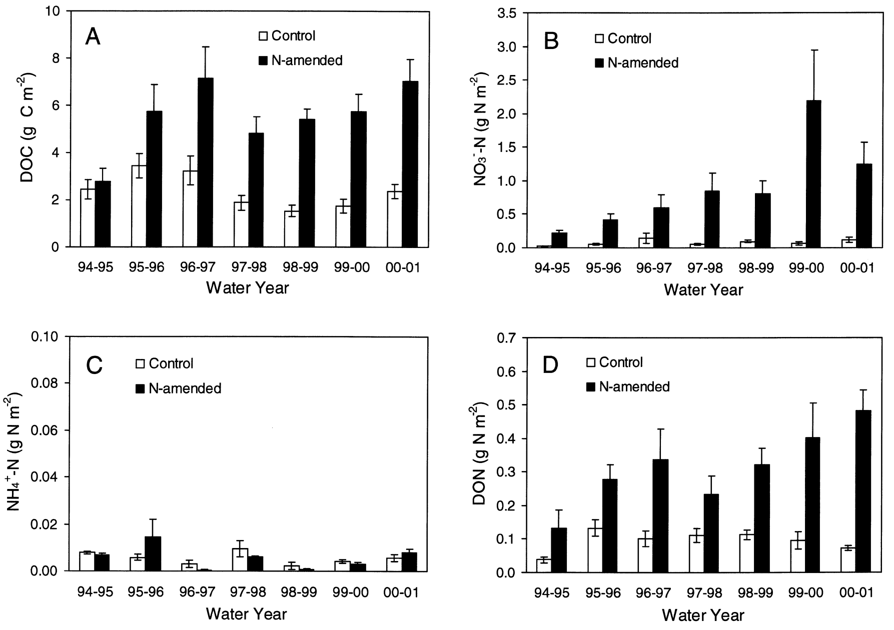

Figure 4. Time trends in DOC (A), NO_3^--N (B), NH_4^+-N (C) and DON (D) fluxes for control and NO_3^--amended plots. Error bars indicate 1 standard error for all plots receiving a treatment across all four sites ($n = 12$).

leachate were not significant. Mean (±standard deviation) DOC-C collected from the cores treated with four different $NaNO_3$ solutions (0.4, 25.0, 50.0, and 100.0 mg l^{-1} $NaNO_3$-N) were 27.7 (±15.5), 32.9 (±8.9), 33.6 (±12.3) and 26.9 (±5.9) mg C l^{-1}, respectively ($P = 0.83$).

Discussion

Rapid N saturation

The four study sites rapidly approached N saturation (Figures 2 and 4) and responded to the chronic NO_3^- additions the same way, suggesting that the mechanisms controlling C and N cycling are the same in all four forests. Furthermore, the two sites with higher inherent N availability at the beginning of the experiment (sites B and C, Table 1) approached N saturation more rapidly, results which support the long-standing hypothesis that N saturation develops more rapidly in ecosystems with greater initial soil N availability (Aber et al. 1989).

Perturbation of normal C and N cycling caused by N additions to our study sites is of particular concern because the response was induced over a short time period by N additions (3 g N m^{-2} per year) that are not excessive relative to rates of atmospheric deposition experienced in the northeastern USA (Fenn et al. 1998). Our N additions are only 2.5 times ambient deposition (1.2 g N m^{-2} per year) for our southernmost sites, and these N additions quickly caused advanced stages of N saturation to occur in a very common Lakes States ecosystem, perhaps presaging a widespread pattern in this region given current levels of anthropogenic atmospheric NO_3^- deposition. The fact that these four ecosystems lost an average of 72% of the added N in the last two water years is remarkable; we accept our original hypothesis that experimental additions of NO_3^- would increase NO_3^--N leaching and cause N saturation.

Gundersen et al. (1998) demonstrated that forests with the highest risk of NO_3^--N leaching had forest floor C/N ratios less than 25. A more recent summary of NO_3^--N leaching from 181 forests distributed across Europe demonstrated that forests with organic horizon (forest floor) C/N ratios < 25, soil pH < 4.6 (B horizon) and atmospheric N inputs > 3 g N m^{-2} per year exhibited the highest rates of NO_3^- leaching and were most susceptible to N saturation (MacDonald et al. 2002). The mean C/N ratios of the Oe–Oa (forest floor) soil horizons on each of our four study sites were: 16.7, 18.4, 20.6 and 17.9 for sites A, B, C and D, respectively (Table 1). A/E soil horizon pH's ranged from 4.3 to 5.2 and B soil horizon pH's ranged from 4.7 to 6.9 across the four study sites (Table 1). Thus, with experimental N additions of 3 g N m^{-2} per year, our study sites have similar characteristics to the forests most susceptible to rapid N saturation in Europe. Apparently, acid, N-rich soils receiving high atmospheric N deposition are very susceptible to N saturation and large losses of NO_3^- via leaching.

Our results contrast sharply with those at Harvard Forest, where most of the experimentally added N has been retained within the ecosystem (Aber et al. 1998). Perhaps these divergent results are due to differences in land use history (Aber et al. 1998), soil texture (Torn et al. 1997), soil acidity (MacDonald et al. 2002), the dominant type of vegetation and subsequent litter quality (litter C/N ratio), the form of N added (NH_4NO_3 vs. $NaNO_3$), or inherent differences in initial N availability (Aber et al. 1998). Differences in response among our four study sites (sites B and C vs. sites A and D; Figure 3) clearly suggest that progression toward N saturation is directly related to inherent N availability as Aber et al. (1989) originally hypothesized.

DOC and DON production and leaching

There has been increasing interest in understanding how atmospheric N deposition effects the production and leaching of DOC and DON from terrestrial ecosystems, because these fluxes have sometimes been ignored, and because both DOC and DON play key roles in aquatic ecosystems (McDowell et al. 1998; Campbell et al. 2000a; Perakis & Hedin 2002). The experimental NO_3^-

additions we made dramatically (> 6 times) elevated the export of DON from all four forests. DON production and export are directly tied to perturbations of the N cycle in these sandy Michigan soils and DON must be quantified to understand the effects of N deposition on N cycling in this landscape. DOC exports were also greatly elevated (> 3 times) by the chronic NO_3^- additions. Both DOC and DON leaching losses in NO_3^--amended plots increased over time (Figure 4), suggesting that the NO_3^- additions have altered availability of the substrates that lead to formation of DOC and DON, or that NO_3^- additions directly altered the processing of organic matter by soil microbial communities, or both. We reject our original hypothesis that chronic NO_3^- additions would have little effect on the production and export of DOC and DON.

DOC and DON trends in the NITREX (Moldan et al. 1995) and Harvard Forest (Currie et al. 1996; Currie & Aber 1997) experiments are not as clear and pronounced as our results. However, in both instances, DOC and DON export following N addition was quantitatively greater, although the difference was not always statistically significant. In NITREX, we calculate (from Moldan et al. 1995) a 14% average increase in DOC export across all 3 years following the addition of 3.5 g m^{-2} NH_4-NO_3-N per year. At Harvard Forest, Currie et al. (1996) report that fluxes of DOC were elevated in three of the four N-treated plots relative to controls and ratios of DOC:DON and DON:inorganic N were significantly affected by N additions. Rustad et al. (1993), Zech et al. (1994), McDowell et al. (1998) and Magill et al. (2000) provide additional evidence that increased N availability results in greater DOC export from forest ecosystems. However, Vestgarden et al. (2001) reported that N additions of 3 and 9 g N m^{-2} per year reduced both DOC and DON concentrations in B horizon soil solutions in a Scots Pine Forest in Norway after 9 years of a single dose of fertilizer each year. In most observational and experimental studies conducted in forests, increased N availability results in more DOC and DON production and leaching, but clearly exceptions occur.

DOC and DON alternative hypotheses

It could be argued that greater export of DOC and DON partially resulted from desorption of DOC due to the addition of Na^+ and NO_3^- ions (i.e., a salt effect). However, several lines of evidence suggest that our observations of greater DOC and DON export from NO_3^--amended plots are not the result of this mechanism. First, we found no effect of ionic strength on the DOC concentration of leachate from the soil column leaching experiment designed to test the salt effect hypothesis, as was true in a similar study (Reemtsma et al. 1999). Secondly, losses of DOC from control plots did not correspond to soil solution salt strength (data not shown). Finally, the chronic NO_3^- fertilizer additions have depressed soil respiration and altered soil enzyme activity in ways that clearly suggest the belowground C cycle has been fundamentally altered (Burton, Zak and Pregitzer unpublished data). We believe increases in DOC/DON export result from changes in the production of organic substrates and their processing by the soil food web, not from the cation/anion exchange reactions fostered by fertilizer additions. We do not imply that abiotic N cycling and retention are not important, but rather that the increases in DOC/DON in this study appear to be biologically driven.

Our second alternative hypothesis was that increased soil NH_4^+ inhibits lignolytic activity, resulting in the incomplete degradation of lignin and greater production of DOC and DON. Zogg et al. (2000) found on these same study sites that most $^{15}NO_3^-$-N added to the soil was immediately immobilized by the microbial community. However, subsequent long-term, stand-level additions of $^{15}NO_3^-$-N to the soil show that most of the $^{15}NO_3^-$-N moves from the microbial community into the overstory canopy (Zak et al. unpublished data). It is possible our chronic NO_3^- additions cause increased N uptake, return of N-enriched litter to the forest floor, subsequent microbial release of elevated levels of NH_4^+ and then suppression of lignin degradation (Fog 1988; Carreiro et al. 2000; Freeman et al. 2001), resulting in increased levels of soil phenolics and greater production of DOC and DON. Canopy N content is significantly elevated in all of the NO_3^--amended plots across all four study sites (Pregitzer et al. unpublished data). The alternative hypothesis that increased soil NH_4^+ inhibits lignolytic activity appears viable at this time and deserves further investigation.

Implications

Chronic experimental additions of NO_3^--N greatly increased the production and leaching of NO_3^--N, DOC and DON over the course of the 8-year study. N saturation of all four northern hardwood ecosystems occurred quickly across the 500 km geographic study gradient, although the mechanisms controlling similar patterns of altered C and N cycling remain unknown. The fact that simulated N deposition dramatically increased DOC and DON production is particularly interesting because these complex organic compounds play key roles in regulating the food webs of streams, rivers and lakes, and because DOC and DON have often been ignored in studies of N deposition. Our results are clear in this regard and other recent studies also suggest N deposition increases DOC and DON production (McDowell et al. 1998; Yano et al. 2000; Campbell et al. 2000a, b). Detailed examinations of C and N processing by the soil food web are now necessary in order to elucidate the unknown mechanisms controlling N saturation in these ecosystems. It is clear that chronic experimental NO_3^--N additions quickly saturated and fundamentally altered the C and N cycles in these northern hardwood ecosystems – ecosystems which are very common in the Great Lakes region of the USA.

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References

Aber J.D. 1992. Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. Trends Ecol. Evol. 7: 220–224.

Aber J.D., Nadelhoffer K.J., Steudler P. and Melillo J.M. 1989. Nitrogen saturation in northern forest ecosystems. Bioscience 39: 378–386.

Aber J., McDowell W., Nadelhoffer K., Magill A., Berntson G., Kamakea M., McNulty S., Currie W., Rustad L. and Fernandez I. 1998. Nitrogen saturation in temperate forest ecosystems. BioScience 48: 921–934.

Ågren G.I. and Bosatta E. 1988. Nitrogen saturation of terrestrial ecosystems. Environ. Pollut. 54: 185–197.

Braun E.L. 1950. Deciduous Forests of Eastern North America. Macmillan, New York.

Bredemeier M., Blanck K., Xu Y.J., Tietema A., Boxman A.W., Emmett B., Moldan F., Gundersen P., Schleppi P. and Wright R.F. 1998. Input–output budgets at the NITREX sites. For. Ecol. Manage. 101: 57–64.

Brown S.E., Pregitzer K.S., Reed D.D. and Burton A.J. 2000. Predicting daily mean soil temperature from daily mean air temperature in four northern hardwood forest stands. For. Sci. 46: 297–301.

Burton A.J., Ramm C.W., Reed D.D. and Pregitzer K.S. 1991. Use of multivariate methods in forest research site selection. Can. J. For. Res. 21: 1573–1580.

Burton A.J., Pregitzer K.S. and MacDonald N.W. 1993. Foliar nutrients in sugar maple forests along a regional pollution–climate gradient. Soil Sci. Soc. Am. J. 57: 1619–1628.

Burton A.J., Pregitzer K.S., Zogg G.P. and Zak D.R. 1996. Latitudinal variation in sugar maple fine root respiration. Can. J. For. Res. 26: 1761–1768.

Burton A.J., Pregitzer K.S. and Hendrick R.L. 2000. Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. Oecologia 125: 389–399.

Campbell J.L., Hornbeck J.W., McDowell W.H., Buso D.C., Shanley J.B. and Likens G.E. 2000a. Dissolved organic nitrogen budgets for upland, forested ecosystems in New England. Biogeochemistry 49: 123–142.

Campbell J.L., Eagar C., McDowell W.H. and Hornbeck J.W. 2000b. Analysis of nitrogen dynamics in the Lye Brook Wilderness area, Vermont, USA. Water Air Soil Pollut. 122: 63–75.

Carreiro M.M., Sinsabaugh R.L., Repert D.A. and Parkhurst D.F. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81: 2359–2365.

Currie W.S. and Aber J.D. 1997. Modeling leaching as a decomposition process in humid montane forests. Ecology 78: 1844–1860.

Currie W.S., Aber J.D., McDowell W.H., Boone R.D. and Magill A.H. 1996. Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. Biogeochemistry 35: 471–505.

Fenn M.E., Poth M.A., Aber J.D., Baron J.S., Bormann B.T., Johnson D.W., Lemly A.D., McNulty S.G., Ryan D.F. and Stottlemyer R. 1998. Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. Ecol. Appl. 8: 706–733.

Fog K. 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. 63: 433–462.

Freeman C., Ostle N. and Kang H. 2001. An enzymatic 'latch' on a global carbon store. Nature 409: 149.

Galloway J.N. 1995. Acid deposition: perspectives in time and space. Water Air Soil Pollut. 85: 15–24.

Green S.A. and Blough N.V. 1994. Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. Limnol. Oceanogr. 39: 1903–1916.

Guggenberger G., Glaser B. and Zech W. 1994. Heavy metal binding by hydrophobic and hydrophilic dissolved organic carbon fractions in a Spodosol A and B horizon. Water Air Soil Pollut. 72: 111–127.

Gundersen P., Callescen I., deVries W., 1998. Nitrate leaching in forest ecosystems is related to forest floor C/N ratios. Environ. Pollut. 102: 403–407.

Hendrick R.L. and Pregitzer K.S. 1996. Temporal and depth-related patterns of fine root dynamics in northern hardwood forests. J. Ecol. 84: 167–176.

Host G.E. and Pregitzer K.S. 1992. Geomorphic influences on ground-flora and overstory composition in upland forests of northwestern lower Michigan. Can. J. For. Res. 22: 1547–1555.

Jardene P.M., Wilson G.V., McCarthy J.F., Luxmoore R.J., Taylor D.L. and Zelazny L.W. 1990. Hydrogeochemical processes controlling the transport of dissolved organic carbon through a forested hillslope. J. Contam. Hydrol. 6: 3–19.

Kieber D.J., McDaniel J. and Mopper K. 1989. Photochemical source of biological substrates in sea water: Implications for carbon cycling. Nature 341: 637–639.

Liechty H.O., Mroz G.D. and Reed D.D. 1993. Cation and anion fluxes in northern hardwood throughfall along an acidic deposition gradient. Can. J. For. Res. 23: 457–467.

MacDonald N.W., Burton A.J., Jurgensen M.F., McLaughlin J.W. and Mroz G.D. 1991. Variation in forest soil properties along a Great Lakes air pollution gradient. Soil Sci. Soc. Am. J. 55: 1709–1715.

MacDonald N.W., Burton A.J., Liechty H.O., Witter J.A., Pregitzer K.S., Mroz G.D. and Richter D.D. 1992. Ion leaching in forest ecosystems along a Great Lakes air pollution gradient. J. Environ. Qual. 21: 614–623.

MacDonald N.W., Witter J.A., Burton A.J., Pregitzer K.S. and Richter D.D. 1993. Relationships among atmospheric deposition, throughfall, and soil properties in oak forest ecosystems. Can. J. For. Res. 23: 2348–2357.

MacDonald J.A., Dise N.B., Matzner E., Armbruster M., Gunderson P. and Forsius M. 2002. Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. Glob. Change Biol. 8: 1028–1033.

Magill A.H., Aber J.D., Bernston G.M., McDowell W.H., Nadelhoffer K.J., Melillo J.M. and Steudler P. 2000. Long-term nitrogen additions and nitrogen saturation in two temperate forests. Ecosystems 3: 238–253.

McDowell W.H. and Likens G.E. 1988. Origin, composition, and flux of dissolved organic carbon in the Hubbard Brook valley. Ecol. Monogr. 58: 177–195.

McDowell W.H., Currie W.S., Aber J.D. and Yano Y. 1998. Effects of chronic nitrogen amendments on production of dissolved organic carbon and nitrogen in forest soils. Water Air Soil Pollut. 105: 175–182.

Moldan F., Hultberg H., Nystrom U. and Wright R.F. 1995. Nitrogen saturation at Gardsjon, southwest Sweden, induced by experimental addition of ammonium nitrate. For. Ecol. Manage. 71: 89–97.

Perakis S.S. and Hedin L.O. 2002. Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. Nature 415: 416–419.

Qualls R.G. and Haines B.L. 1992. Biodegradability of dissolved organic matter in forest throughfall, soil solution and stream water. Soil Sci. Soc. Am. J. 56: 578–586.

Reed D.D., Pregitzer K.S., Liechty H.O., Burton A.J. and Mroz G.D. 1994. Productivity and growth efficiency in sugar maple forests. For. Ecol. Manage. 70: 319–327.

Reemtsma T., Bredow A. and Gehring M. 1999. The kinetics of organic matter release from soil by salt solutions. Eur. J. Soil Sci. 50: 53–64.

Robertson G.P. 1982. Factors regulating nitrification in primary and secondary succession. Ecology 65: 1561–1573.

Rustad L.E., Fernandez I.J., Fuller R.D., David M.B., Novdin S.C. and Halteman W.A. 1993. Soil solution response to acidic deposition in a northern hardwood forest. Agric. Ecosyst. Environ. 47: 117–134.

Solinger S., Kalbitz K., Matzner E. 2001. Controls on the dynamics of dissolved organic carbon and nitrogen in a Central European deciduous forest. Biogeochemistry 55: 327–349.

Thornthwaite C.W. and Mather J.R. 1957. Instructions and tables for computing potential evapotranspiration and the water balance. Publ. Climatol. 10: 185–311.

Torn M.S., Trumbore S.E., Chadwick O.A., Vitousek P.M. and Hendricks D.M. 1997. Mineral control of soil organic carbon storage and turnover. Nature 389: 170–173.

Vestgarden L.S., Abrahamsen G. and Stuannes A.O. 2001. Soil solution response to nitrogen and magnesium application in a Scots pine forest. Soil Sci. Soc. Am. J. 65: 1812–1823.

Vitousek P.M. 1994. Beyond global warming: ecology and global change. Ecology 75: 1861–1876.

Vitousek P.M. and Howarth R.W. 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13: 87–115.

Vitousek P.M., Aber J.D., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W., Schlesinger W.H. and Tilman D.G. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7: 737–750.

Yano Y., McDowell W.H. and Aber J.D. 2000. Biodegradable dissolved organic carbon in forest soil solution and effects of chronic nitrogen deposition. Soil Biol. Biochem. 32: 1743–1751.

Zak D.R. and Pregitzer K.S. 1998. Integration of ecophysiological and biogeochemical approaches to ecosystem dynamics. In: Groffman P.M. and Pace M.L. (eds), Proceedings of the Seventh Annual Cary Conference: Successes, Limitations, and Frontiers in Ecosystem Science. Springer, New York, pp. 372–403.

Zech W., Guggenberger G. and Schulten H.-R. 1994. Budgets and chemistry of dissolved organic carbon in forest soil: effects of anthropogenic soil acidification. Sci. Total Environ. 152: 49–62.

Zogg G.P., Zak D.R., Burton A.J. and Pregitzer K.S. 1996. Fine root respiration in northern hardwood forests in relation to temperature and nitrogen availability. Tree Physiol. 16: 719–725.

Zogg G.P., Zak D.R., Pregitzer K.S. and Burton A.J. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. Ecology 81: 1858–1866.