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Characteristics of DOC Exported from Northern Hardwood Forests Receiving Chronic Experimental NO_3^- Deposition

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Abstract

Sugar maple (*Acer saccharum* Marsh.)-dominated northern hardwood forests of the Great Lakes Region commonly receive elevated levels of atmospheric nitrate (NO_3^-) deposition, which can alter belowground carbon (C) cycling. Past research has demonstrated that chronic experimental NO_3^- deposition (3 g N m^{-2} y^{-1} above ambient) elicits a threefold increase in the leaching loss of dissolved organic carbon (DOC). Here, we used DOC collected from tension-cup lysimeters to test whether increased DOC export under experimental NO_3^- deposition originated from forest floor or mineral soil organic matter (SOM). We used DOC radiocarbon dating to quantify C sources and colorimetric assays to measure DOC aromaticity and soluble polyphenolic content. Our results demonstrated that DOC exports are primarily derived from new C (<50-years-old) in the forest floor under both ambient and experimental NO_3^- deposition. Experimental NO_3^- deposition increased soluble polyphenolic content from 25.03 ± 4.26 to 49.19 ± 4.23 μg phenolic C mg DOC^{-1}, and increased total aromatic content as measured by specific UV absorbance. However, ncreased aromatic compounds represented a small fraction (<10%) of the total observed increased DOC leaching. In combination, these findings suggest that experimental NO_3^- deposition has altered the production or retention as well as phenolic content of DOC formed in forest floor, however exact mechanisms are uncertain. Further elucidation of the mechanism(s) controlling enhanced DOC leaching is important for understanding long-term responses of Great Lakes forests to anthropogenic N deposition and the consequences of those responses for aquatic ecosystems.

Key words: dissolved organic carbon; DOC; aromatics; radio-carbon; plant litter decomposition; soluble polyphenolics; nitrate (NO_3^-) deposition; UV absorbance

Introduction

Elevated atmospheric nitrogen (N) deposition is a common phenomenon in most terrestrial ecosystems (Galloway and Cowling 2002) and it can significantly alter the cycling of carbon (C) and

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nutrients (Berg and Matzner 1997). In the Great Lakes region of North America, where nitrate (NO_3^-) is the most important form of N deposition, experimental NO_3^- deposition has increased the export of dissolved organic carbon (DOC) from northern hardwood forests (Pregitzer and others 2004). DOC is of fundamental importance to soil organic matter (SOM) formation (McDowell and Likens 1988) and changes in the production or retention of DOC can influence long-term ecosystem C storage. Terrestrial DOC also can be exported to streams, rivers, and lakes where it can serve as an important source of energy and nutrients for aquatic heterotrophs (McDowell and Likens 1988; Perakis and Hedin 2002; Findlay 2005), facilitate the movement of heavy metals and other pollutants (Qualls and Haines 1992; Guggenberger and others 1994), and increase water color (Green and Blough 1994). Because DOC has a number of ecological consequences, it is critical to understand the source of increased DOC export in response to experimental NO_3^- deposition.

Atmospheric NO_3^- deposition could increase DOC export by altering the production or retention of DOC in either forest floor or SOM, but it is currently unclear which is the dominant source of DOC in forest ecosystems (Kalbitz and others 2000). Because the accumulation of SOM is slow (Schlesinger 1990; Zak and others 1990) and a significant portion of SOM is stabilized or recalcitrant (Paul and others 1997), DOC derived from stable SOM should be composed of relatively older C. In contrast, the turnover of forest floor is relatively rapid; suggesting that DOC produced from this pool would be younger in age and temporally dynamic due to predictable litter input events associated with autumn senescence in deciduous forests (Kalbitz and others 2000). As a consequence, DOC exports should quantitatively and qualitatively reflect the relative contribution of each C source, which could provide insight into the mechanisms controlling DOC export. For example, if chronic NO_3^- deposition stimulated microbial activity in stabilized SOM and increased DOC mobilization rates (Zech and others 1994), then the age of DOC would proportionally reflect that of stable SOM. Alternatively, if chronic NO_3^- deposition stimulated DOC export from fresh litter or forest floor, then the age of DOC produced should be much younger than that of stabilized SOM.

Some evidence suggests that high inorganic N availability can suppress the production of lignin-degrading extracellular enzymes (phenol oxidase and peroxidase) by white rot *Basidiomycota* and xylariaceous *Ascomycota*, which are primarily responsible for lignin degradation (Keyser and others 1978; Kirk and Farrell 1987). This suppression might result in competitive exclusion of the aforementioned fungal taxa and a shift towards incomplete lignin degradation by less efficient microbial physiologies (DeForest and others 2005). Such a mechanism could increase the leaching of soluble phenolic compounds associated with lignin degradation and account for the additional DOC exports reported by Pregitzer and others (2004). Reductions in ligninolytic enzyme activity in response to experimental NO_3^- deposition have been reported for northern hardwood forest soils (for example, DeForest and others 2004b; Gallo and others 2004; Waldrop and others 2004), yet those studies did not relate reductions in enzyme activity to expected changes in the qualitative chemistry of DOC exports.

In this study, we investigated characteristics of the DOC exported from four northern hardwood forest stands in Michigan, USA that have received 10 years of experimental NO_3^- deposition (3 g NO_3^- N m^{-2} y^{-1}). Previous research demonstrated that chronic NO_3^- deposition increased DOC export more than threefold, relative to losses under ambient levels of N deposition (Pregitzer and others 2004). Our objectives were to determine whether chronic NO_3^- deposition altered (1) the contributions of forest floor or mineral soil organic matter to DOC export and (2) the chemical composition of DOC leaching from northern hardwood forests. To achieve these objectives, we used radiocarbon dating to ascertain potential sources of DOC (modern versus old C) and colorimetric assays to determine total phenolic and aromatic content of DOC exported from northern hardwood forests. We expected that the relatively young forest floor was the source of additional DOC exports reported by Pregitzer and others (2004) and that the concentration and chemistry of leached DOC would have a distinct seasonal signal associated with autumn senescence and spring flushing of DOC. We also hypothesized that if elevated DOC export was associated with incomplete lignin degradation and suppression of ligninolytic enzymes, then leached DOC under experimentally elevated N deposition would have a proportionally greater concentration of phenolic and aromatic compounds, when compared to DOC leached under ambient N deposition.

Methods

Site Description and Experimental Design

Dissolved organic carbon was collected from four northern hardwood forest stands in Michigan that

had received 10 years of experimental atmospheric NO_3^- deposition (Figure 1). These sugar maple (*Acer saccharum* Marsh.)-dominated stands span a 500–km climatic and N deposition gradient extending from the western Upper Peninsula of Michigan to the central Lower Peninsula (Figure 1). Soils are deep deposits of sandy glacial till (Typic Haplorthods) that are characterized by organic horizons (Oi and Oe) overlaying a thin organic-rich mineral A horizon and a spodic subsurface horizon. The forest floor is poorly developed (predominantly recognizable litter) due to rapid decomposition, and the active rooting zone is primarily above 75 cm. The forest overstory is dominated by *Acer saccharum* Marsh. and co-occurring northern hardwood species (for example, *Fagus grandifolia*, *Acer rubrum*, and *Quercus rubra*) (Burton and others 1991).

Each stand contains six 30–m × 30–m plots (with 10 m treatment buffers) in which three plots receive ambient N deposition (0.68–1.18 g N m^{-2} y^{-1}) and three receive ambient N deposition plus 3 g NO_3^- N m^{-2} y^{-1}. The additional NO_3^- was added in six equal aliquots (April-September) of pelletized $NaNO_3$ broadcast over the forest floor. Details of the experimental NO_3^- addition and further study site and edaphic information can be found in MacDonald and others (1993), Zogg and others (2000) and Pregitzer and others (2004).

DOC Sampling

Soil porewater was collected at 2–week intervals during the fall (late August to early December) of 2003 and 2004, and the spring (mid-April to early June) of 2004. Porous-cup ceramic tension lysimeters were located at a depth of 75 cm (below the primary rooting zone and into the C horizon) in each control and NO_3^- amended plot to collect soil water over the aforementioned time; lysimeters have been in place since 1987 in the control plots and since 1993 in the NO_3^- addition plots. Each plot contained four lysimeters, and the four samples were composited on a plot basis for each sampling date. A tension just over field capacity (up to 0.05 MPa) was applied to each lysimeter 2 weeks prior to each sampling. Soil water samples were removed from each lysimeter with a vacuum pump and all soil water was stored on ice for transport to the lab. Within 24–48 h of field collection, samples were passed through a 0.45 µ filter (Magna, nylon plain supported filters, Osmonics, Inc., Westborough, MA, USA), acidified with ultrapure HCl to pH less than 2, and stored in amber plastic bottles at 4°C prior to analysis.

Figure 1. Location of the four study sites in Michigan and the location of Michigan in the eastern United States.

^{14}C Dating of DOC

Dating of DOC was performed on filtered soil water collected on 6 dates during the fall of 2003. Samples collected during this period were composited across time in each plot ($n = 6$), producing 24 samples for ^{14}C dating (that is, 4 stands containing 6 plots). To concentrate DOC for ^{14}C dating, composite samples were then circulated through a spiral-wound, regenerated-cellulose, tangential-flow filtration (TFF) cartridge with a 1 kDa nominal molecular weight limit (Millipore, Inc., Billerica, MA, USA). Between samples, the TFF unit was flushed with 3 l of 0.5 M NaOH and 5 l of DI water. Two replicates of dilute sucrose solution were also concentrated using the same system to quantify ^{14}C contamination by the filtration system. ^{14}C content of the concentrated sucrose solution ($\Delta^{14}C$ = 125.6 ± 2.6‰) was not significantly different from the original material ($\Delta^{14}C$ = 132.6 ± 3.6‰). To address the potential for cross sample contamination among samples, we used the flushing protocol described above after concentrating the sucrose solution and then used the same process on DI water. DOC concentrations in both the water that passed thorough the membrane and the retained water were at or below the organic C detection limit, suggesting little to no cross sample contamination. Concentrated DOC samples for each plot, along with the sucrose samples, were lyophilized in preparation for ^{14}C analysis.

Dried DOC and SOM samples were analyzed for ^{14}C and ^{13}C content on an accelerator mass spectrometer at the Keck Carbon Cycle AMS facility (University of California at Irvine). Potential contributions of ^{14}C during processing and analysis were corrected by measurement and subtraction of ^{14}C-free coal. Isotope data are presented as $\delta^{13}C$, $\Delta^{14}C$, and ^{14}C age (YBP) following the conventions of

Stuiver and Polach (1977). Delta ^{14}C values represent the fractionation corrected ($\delta^{13}C$) per mil deviation of the sample $^{14}C/^{12}C$ ratio from the $^{14}C/^{12}C$ ratio of oxalic acid standard in 1950. Positive $\Delta^{14}C$ values are associated with modern (post 1950) C.

DOC Chemistry

DOC concentration in soil solution was determined by high-temperature catalytic oxidation using a Shimadzu TOC-5000A (Shimadzu Scientific Instruments, Columbia, MD, USA). Samples were acidified with HCl, 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 C by injection onto a platinum catalyst at 680°C. The DOC detection limit was 0.6 mg C l^{-1}.

Soil solution samples were then analyzed for total soluble polyphenolic content (as a fraction of total DOC) following the Folin-Ciocalteu method (Ohno and First 1998). We used standards ranging from 3 to 250 μmol l^{-1}, which were composed of equal amounts of ferulic, *p*-coumaric, *p*-hydroxybenzoic, vanillic, and syringic acids (Sposito 1989; DeForest and others 2005). Phenolic concentrations were determined colorimetrically (750 nm absorbance) in methacrylate cuvettes (1 cm pathlength) on a Spectronic GENESYS 20 Spectrophotometer (Thermo Electron Corp., Austin, TX, USA). Data were normalized for DOC content and expressed as micrograms of phenolic C per milligram of DOC (μg C mg DOC^{-1}).

To gain additional insight into the presence of aromatic hydrocarbons in DOC, filtered sub-samples were analyzed to determine the specific UV absorbance of DOC at a wavelength of 254 nm ($SUVA_{254}$) as an estimate of DOC aromatic content (Weishaar and others 2003). Soil solution UV absorbance at 254 nm was measured on a Spectronic UV spectrophotometer (Thermo Electron Corp., Austin, TX, USA). Absorbance was measured at room temperature with DI water as the blank. Duplicates of each sample were run and the DI blank was measured every ten samples. Absorbance at 254 nm was then normalized for DOC content to yield $SUVA_{254}$. $SUVA_{254}$ is obtained by dividing the absorbance at 254 nm (1 cm pathlength) by the [DOC] and is expressed as liters per milligram C per meter (l mg DOC^{-1} m^{-1}) following Weishaar and others (2003).

Statistical Analyses

Influence of study site and NO_3^- deposition on DOC age was investigated using a two-way ANOVA, in which site and NO_3^- deposition were crossed. A protected Fisher's LSD test was used to compare main effect and interaction means. To determine the influence of study site, NO_3^- deposition, and season on soil solution DOC concentration and phenolic/aromatic content, we used a split-plot Mixed-model procedure in SAS (proc Mixed). Forest site (n = 4), NO_3^- deposition (n = 2), and season (n = 2) were main effects, and [DOC], DOC phenolic content, and $SUVA_{254}$-DOC were considered repeated measures (sampling date) within each season. Plots (n = 6) were nested within each site to control for spatial similarity, and we used a Satterthwaite approximation procedure to reduce our degrees of freedom and correct for the unbalanced seasonal data (Neter and others 1996). Main effects and interaction means were compared using a Fisher's LSD test. Data used in these analyses were plot means calculated from the four lysimeters in each plot. We also used linear regression analyses to explore the relationship between soil solution DOC concentration, UV absorbance, phenolic content and $SUVA_{254}$. We compared slopes of regression functions for ambient and elevated NO_3^- deposition treatments with a residual sum of squares goodness of fit test (Neter and others 1996).

Results

DOC age

Radiocarbon analysis demonstrated that modern C (<50-years-old) was the primary source of DOC during the fall in both treatments (Table 1). This suggests that the forest floor was the primary source of DOC in this northern hardwood forest. Moreover, experimental NO_3^- deposition did not significantly affect DOC age (F = 2.16, P = 0.162). Mean $\Delta^{14}C$ values of DOC averaged across sites were 20.46 ± 19.85‰ in the ambient N treatment and 51.70 ± 11.89‰ in the elevated N treatment, clearly indicating modern C sources in both treatments. Study site was a significant factor (F = 3.45, P = 0.044) controlling DOC age, where mean DOC ages were oldest at the northernmost site (A; $\Delta^{14}C$ = 5.15 ± 5.5‰) and southernmost site (D; $\Delta^{14}C$ = 1.54 ± 23.49‰) and youngest in the middle sites (site B $\Delta^{14}C$ = 56.47 ± 9.23‰; site C $\Delta^{14}C$ = 81.17 ± 31.51‰). This pattern was driven by two ambient N treatment (control) plots at sites A and D that each contained a significant amount of pre-1950 C (Table 1). In general, DOC age in the ambient N treatment was more variable than the elevated N treatment (Figure 2). In the chronic NO_3^- treatment, no sample had a pre-modern C signature, whereas pre-modern values occurred in the ambient treatment at sites D and C.

Table 1. Accelerator Mass Spectrometry Analysis Results from Autumn 2003 DOC Samples

NO^{3-} deposition treatment	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)[1]	^{14}C age (BP)[2]
Site A			
Ambient N	−24.35 (0.41)	2.92 (6.71)	> modern (0)
Elevated N	−27.76 (0.31)	7.37 (9.54)	> modern (0)
Site B			
Ambient N	−28.77 (1.69)	40.89 (10.24)	> modern (0)
Elevated N	−30.11 (0.27)	72.05 (8.89)	> modern (0)
Site C			
Ambient N	−29.15 (3.949)	81.22 (64.18)	50.00 (50)
Elevated N	−31.93 (1.22)	93.15 (33.30)	> modern (0)
Site D			
Ambient N	−27.32 (2.981)	−31.95 (32.26)	323.33 (226.59)
Elevated N	−26.32 (0.90)	45.94 (14.21)	> modern (0)

Values are from four northern hardwood forest sites receiving both ambient (Ambient N) and experimental NO_3^- deposition (Elevated N) and represent mean values (±SE in parentheses; n = 4) for each site (n = 4) by NO_3^- deposition treatment (n = 2). NO_3^- deposition treatment effect was highly insignificant while site had an overall significant effect ($P < 0.05$) on DOC age.

[1] $\Delta^{14}C$ is the $\delta^{13}C$ fractionation corrected relative difference between the sample and the reference material activity and corrected for decay of the standard between 1950 and present.

[2] ^{14}C age denotes years before 1950. C assimilated after 1950 is >modern.

DOC Concentration and Chemistry

Chronic experimental NO_3^- deposition increased the export of DOC from these northern hardwood forest sites (Table 2 and Figure 3A) across sites and seasons, closely resembling the pattern reported by Pregitzer and others (2004). The soil solution DOC concentration in response to the chronic N deposition was 2.9 times (18.47 mg C l^{-1}) that of the ambient treatment (6.40 mg C l^{-1}) when averaged across all sites. Study site was also a significant main effect (Table 2), mainly due to soil solution DOC concentrations in site B that were 2.5 times those of the other sites. Season (fall versus spring) had a significant effect on DOC concentrations in soil solution, as did seasonal interactions with study site and NO_3^- deposition treatment (Table 2). DOC concentrations were generally highest in the spring (13.65 mg C l^{-1}) compared to fall (11.22 mg C l^{-1}), but the seasonal effect was mostly influenced by the elevated N treatment in site B, which increased the overall DOC concentration disparity between NO_3^- deposition treatments (Figure 3A).

Across all sites and seasons, experimental NO_3^- deposition effectively doubled the total soluble phenolic content of DOC from 25.03 ± 4.26 to 49.19 ± 4.23 µg phenolic C mg DOC^{-1}; chronic NO_3^- deposition also increased the $SUVA_{254}$ from 2.8107 ± 0.3157 l mg DOC^{-1} m^{-1} to 4.10 ± 0.3092 l mg DOC^{-1} m^{-1} (Table 2 and Figures 3B and 3C). We observed no significant interaction between study site and NO_3^- deposition treatment on DOC phenolic content and $SUVA_{254}$ (Table 2).

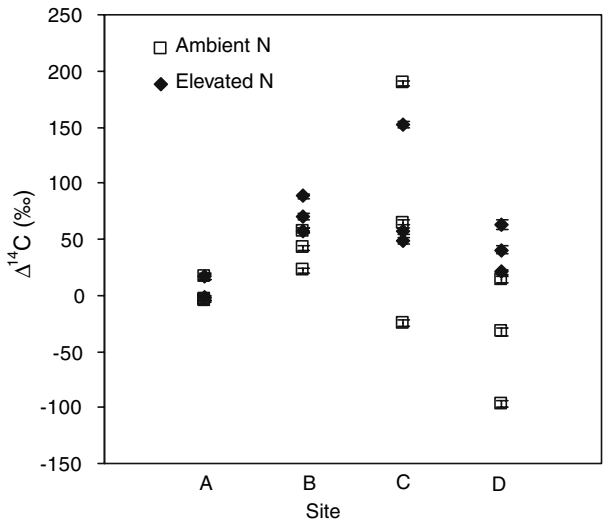

Figure 2. Fall 2003 $\Delta^{14}C$ (‰) values for individual plots ($n = 3$) within each northern hardwood forest study site and NO_3^- deposition treatment ($n = 2$). Error bars represent ± instrument certainty for each measurement.

Regression analysis demonstrated that DOC concentration was correlated with UV absorbance. However, the slope of the regression was significantly ($F = 8.99$, $P = 0.0002$) altered by chronic experimental NO_3^- deposition (Figure 4), suggesting that DOC was chemically different under chronic NO_3^- deposition. Season had a significant effect on the soil solution concentration of total soluble phenolics, but not on the $SUVA_{254}$ of DOC (Table 2 and Figure 3B, C). Phenolic concentrations were highest in the spring (43.5 ± 3.81 µg

Table 2. Results from a Repeated Measures Mixed-Model for Soil Solution DOC Concentration, Total Soluble Phenolic Content of DOC, and SUVA 254 of DOC Collected from Four Northern Hardwood Forest Study Sites

	DOC concentration				DOC phenolic content				$SUVA_{254}$ of DOC			
	ndf	ddf	*F*	*P* > F	ndf	ddf	*F*	*P* > *F*	ndf	ddf	*F*	*P* > *F*
Between subjects												
Study site	3	16.1	3.14	0.0544	3	17.9	0.48	0.679	3	20.4	0.71	0.5546
NO_3^- addition	1	16.1	10.25	0.0055	1	18.3	16.2	0.0008	1	22.3	8.55	0.0078
Study site × NO_3^- addition	3	16.1	0.91	0.4562	3	17.9	0.62	0.614	3	20.4	0.57	0.6424
Within subjects												
Season	1	220	12.08	0.0006	1	217	11.83	0.0007	1	221	0.68	0.4101
Season × study site	3	220	8.15	<0.0001	3	217	2.26	0.0823	3	221	0.92	0.4339
Season × NO_3^- addition	1	220	22.73	<0.0001	1	217	1.73	0.1894	1	221	0.01	0.9361
Season × study site × NO_3^- addition	3	220	10.97	<0.0001	3	217	1.48	0.22	3	221	0.38	0.7686

phenolic C mg DOC^{-1}) compared to fall (30.7 ± 3.23 μg phenolic C mg DOC^{-1}); however, this pattern was not consistent across sites and was influenced by the high spring-time phenolic content of DOC exported from the northern sites and low fall phenolic content in southern sites (Figure 3B).

Discussion

Despite continued measurement of elevated DOC export in these northern hardwood forest stands, experimental NO_3^- deposition has little apparent effect on the source of soil solution DOC. We base this conclusion on the results presented in Table 1, which illustrate that leached DOC in the autumn was primarily of modern origin, regardless of NO_3^- deposition treatment. We expected ^{14}C age to correspond with DOC source because annual litter inputs to these northern hardwood forest soils are approximately 400 g m^{-2} and forest floor mass is approximately 4000 g m^{-2} (MacDonald and others 1993), suggesting a mean residence time of approximately 10 years. Thus, all C in the forest floor has accumulated since 1950. Incorporation of forest floor C into stable SOM in temperate forest soils can be rapid in aggrading forests that were formerly fields and pastures (Gaudinski and others 2000). However, our northern hardwood forest sites were not plowed or grazed, and stable SOM therefore has accumulated slowly over approximately 11,000 years of soil development. SOM accumulation rates also are known to decrease with time after forest establishment, further decreasing the rate of modern litter contributions to stable SOM (Richter and others 1999). We assumed, then, that a significant fraction of the SOM pool is stable or recalcitrant and has therefore accumulated prior to 1950 (Paul and others 1997). DOC derived from the stabilized portion of SOM would therefore be highly depleted in ^{14}C, and that signal should be apparent in the radiocarbon data (Karltun and others 2005). Depleted ^{14}C measurements in the ambient N treatment of sites C and D likely reflect DOC leaching from soil C pools with a range of turnover times (Tipping and others 2005). Because our ^{14}C measurements were made in the autumn, it is unclear if the same pattern would be observed in other seasons when the relative contribution of DOC sources might vary.

Although our data demonstrate that autumn DOC exports from this northern hardwood forest originate from the same C pool irrespective of N deposition treatment, analysis of DOC aromatic and polyphenolic content suggests that DOC originating from the ambient N treatment was chemically distinct from that of the elevated N treatment (Table 2; Figures 3B, C, 4). In the elevated N treatment, DOC had a relatively higher concentration of soluble phenolics and aromatic constituents than in the ambient treatment. Phenolic compounds, as a subset of total aromatics, are abundant in soils and can be important regulators of soil processes (Hattenschwiler and Vitousek 2000). Phenols are a basic structural unit of lignin and can be degraded by soil microbial communities. For example, white rot *Basidiomycota* aid in the degradation of phenolic compounds via the release of ligninolytic extracellular enzymes (Fog 1988; Carreiro and others 2000). Sugar maple litter, although low in lignin, is high in soluble phenolics (Lovett and others 2004), which can be physically leached or released as a byproduct of microbial decomposition. Turnover and decomposition of

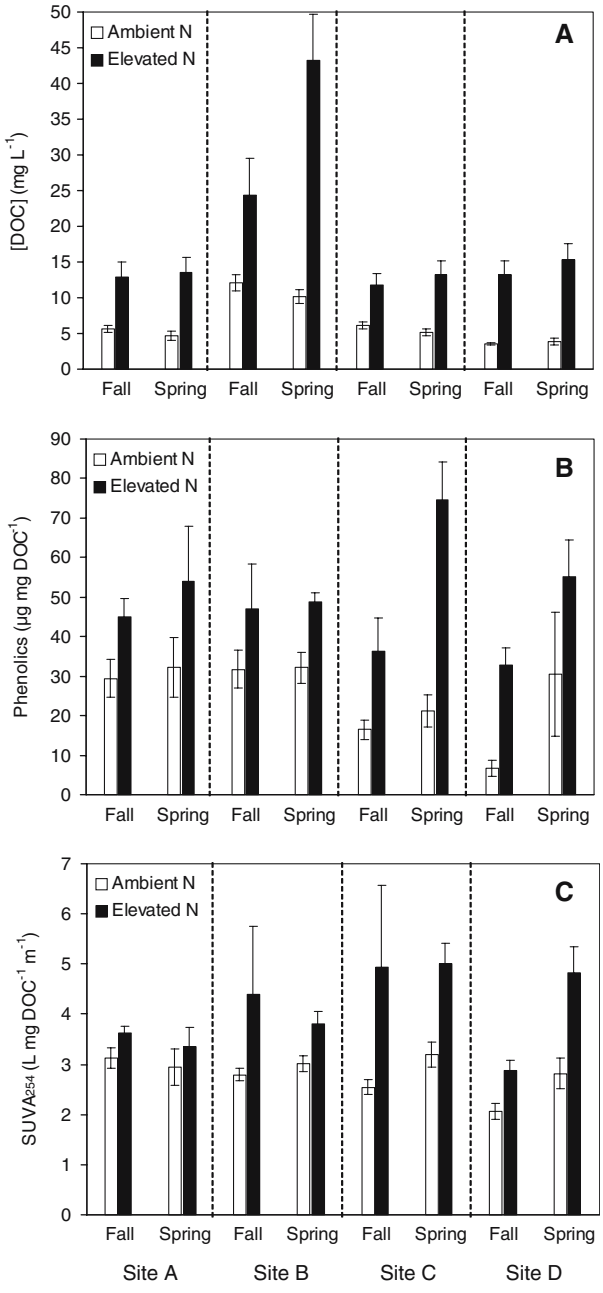

Figure 3. Fall (2003 and 2004) and Spring (2004) **A** DOC concentrations in soil solution, **B** soluble phenolics in DOC, and **C** $SUVA_{254}$ of DOC from four northern hardwood forest sites receiving ambient and experimental NO_3^- deposition. Values are LS means (±SE) for treatment plots within sites (n = 3).

fine roots (<1 mm), which also are high in lignin (~37%) and add about 290 g m^{-2} y^{-1} to SOM, might be another important source of aromatic compounds (Burton and others 2004; Eikenberry 2004). Thus, the increases in DOC aromatic and polyphenolic content we measured might be caused by changes in either sources or sinks for

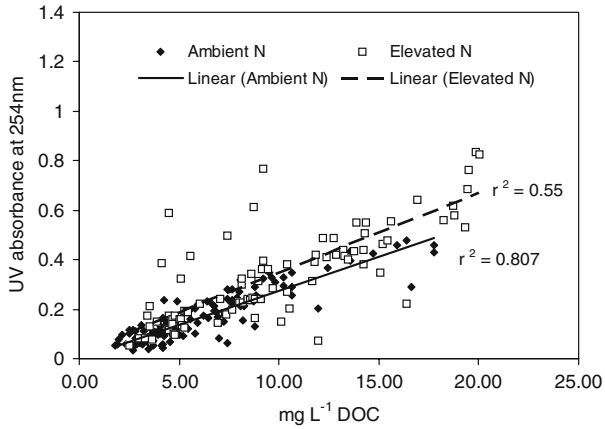

Figure 4. Regression analysis of soil solution DOC concentration at 75 cm depth versus UV absorbance at 254 nm. Individual points are across-season plot values for samples within each treatment having a DOC concentration in the range of 0–20 mg l^{-1}.

these compounds, thereby providing clues to the mechanism behind elevated DOC export. Nonetheless, our estimates of polyphenolic and aromatic content may have error associated with them. The Folin Ciocalteau reagent is a general reagent and also is used for protein analysis, presenting the possibility that our assay over or underestimated the actual phenolic content of DOC. Because phenolics are a subset of the total aromatic content, we would expect a correlation between $SUVA_{254}$ and soluble phenolics. This relationship was strong for the elevated N treatment (r^2 = 0.613). However, we observed a weak relationship between soluble phenolics concentration and $SUVA_{254}$ in the ambient N treatment (r^2 = 0.099), which resulted from a high degree of variability in soluble phenolic concentrations. Given the constraints of our assay, DOC in the ambient treatment may contain a wider array of organic compounds.

We hypothesized that leaching and decomposition of freshly fallen labile litter would be the single largest source of DOC in this ecosystem. The predominantly sugar maple litter in our sites is low in lignin (Melillo and others 1982) and is rapidly decomposed (Waldrop and others 2004), and direct production of DOC from fresh litter is known to be important (Tipping and others 2005). We recently demonstrated that fresh leaf litter was a greater source of DOC and aromatic compounds than SOM (Smemo and others 2006). If freshly fallen litter is the largest source of DOC, we expect that DOC export and the amount of aromatic compounds in DOC would be highest in the spring, corresponding to late fall senescence and soil flushing after snow melt (Qualls and Haines 1991; Kaiser and others

2001). Our results support this idea (Table 2 and Figure 3A–C) and suggest that seasonal fresh litter inputs might dominate annual DOC budgets, although patterns were strongly driven by site B. In a laboratory study, we found no effect of experimental NO_3^- deposition on the production of DOC in fresh litter (Smemo and others 2006), casting doubt on the role of DOC production as a primary driver of our observed DOC export patterns. The current study did find a significant effect of experimental NO_3^- deposition on seasonal DOC and aromatic compound concentrations (highest in spring), but the significant site interactions suggest that the pattern was not robust. Thus, we cannot conclude that experimental NO_3^- deposition has altered the processes controlling DOC production in fresh litter and therefore the observed patterns of elevated DOC export. Rather, chronic NO_3^- deposition appears to increase DOC export by altering processes in the forest floor or mineral soil.

Experimental NO_3^- deposition could alter DOC aromatic content by increasing total litter production (that is, plant productivity) and therefore available substrates for decomposers and physical leaching. Alternatively, elevated N could create differences in litter biochemistry that modify rates and pathways of microbial decomposition. However, on-going work in these northern hardwood forest sites has demonstrated that leaf litter production did not differ between treatments (Burton and others unpublished data) and litter biochemistry has not fundamentally changed in response to chronic NO_3^- additions (Eikenberry 2004). Nitrate additions could stimulate the production of sugar maple fine roots, which are high in lignin, but previous work has shown that this is not the case (Burton and others 2004). We therefore conclude that increased DOC export is not associated with the quantity of DOC produced in the forest floor. Instead, less efficient lignin degradation might cause aromatic by-products of lignin depolymerization to accumulate, thereby changing the quality of DOC leached from the forest floor.

A plausible explanation for the increased DOC export and DOC aromatic content is a reduction in one or more sinks for forest floor-derived DOC that has high concentrations of aromatic compounds. For example, analyses of soil and fresh litter from the same northern hardwood forests showed that chronic experimental NO_3^- deposition significantly suppressed the ability of SOM to act as a sink for DOC and soluble phenolic compounds (Smemo and others 2006). This SOM sink for litter-derived DOC has been demonstrated in other studies (for example, McDowell and Likens 1988; Dalva and Moore 1991; Cory and others 2004), although it is not certain whether the response is associated with microbial metabolism or physico-chemical processes. Although DOC adsorption to mineral surfaces is an important process in many ecosystems, it is unclear how experimental NO_3^- deposition would alter this retention mechanism; thus, a microbial response is a more plausible explanation. Experimental NO_3^- deposition might increase DOC export by disrupting the microbial processes in SOM that degrade forest floor-derived DOC. This mechanism has been suggested before (for example, DeForest 2005), and strong evidence suggests that high N availability suppresses the production of ligninolytic enzymes involved in lignin degradation by some soil fungi (Carreiro and others 2000; Saiya-Cork and others 2002; Sinsabaugh and others 2002; DeForest and others 2004a, b; Gallo and others 2004). Taken together, the aforementioned evidence suggests that this response results from a decline in the microbial utilization of leached phenolic compounds that are produced via incomplete lignin degradation in the forest floor. This would explain why suppression of ligninolytic enzyme activity has been measured in mineral soil despite no significant increase in the production of soluble polyphenolics in soil (DeForest and others 2004a, 2005). Furthermore, this would explain how microbial processes in SOM could be altered without increasing the contribution of old C to DOC exports.

The above mechanism implies that chronic NO_3^- addition has decreased the flow of C among soil pools and could help explain, in part, other observed treatment responses in these forests such as suppressed soil respiration (Burton and others 2004) and reduced soil microbial biomass (DeForest and others 2004b). However, we are unable to conclude that this is the primary mechanism controlling observed patterns of DOC export. Increases in the mass of leached phenolics in response to chronic experimental NO_3^- deposition are not sufficient to account for the measured mass increase in DOC exports. Total soluble phenolics, in general, contributed approximately 1 mg C l^{-1} in the elevated N treatment (<10%). This is small considering that DOC concentrations are on average 6.60 mg C l^{-1} in the ambient treatment and 18.47 mg Cl^{-1} in the elevated N treatment.

A final explanation for the observed increase in DOC export is the influence of pH on the solubility and mobility of DOC. It is well established that the solubility of DOC is positively correlated with pH (Kalbitz and others 2000, and references therein) and DOC adsorption to mineral surfaces has been

shown to decrease as soil pH values exceed 6.0 (Kaiser and others 1996). Indeed, many studies in Europe and North America have proposed recovery from acid deposition as a mechanism for catchment-scale increases in surface water DOC (Driscoll and others 2003; Evans and others 2005), whereas others have discussed and rejected acid deposition recovery as the primary mechanism (Worrall and others 2004; Findlay 2005). In our experiment, soil porewater pH for the 2004 water year was significantly greater ($P = 0.019$) in the experimental NO_3^- deposition treatment (6.07 ± 0.03; $n = 90$) compared to the ambient N deposition treatment (5.93 ± 0.05; $n = 85$). However, the range of pH values within the ambient N deposition treatment was greater than the range of values across both treatments. Moreover, N deposition produced the greatest DOC export form Site B, but the ambient N deposition treatment in that site had a significantly greater pH ($P = 0.002$; 6.30 ± 0.14) than the NO_3^- deposition treatment (5.77 ± 0.08). A pH response to $NaNO_3$ additions, therefore, cannot explain our observed DOC export responses or why those responses are much stronger than those seen in the NITREX (Moldan and others 1995) and Harvard Forest (Currie and others 1996) studies, which used NH_4NO_3.

Although long-term trends are still uncertain, our data suggest that the observed increase in DOC leaching rate in this ecosystem has slowed and is now at a new equilibrium. Pregitzer and others (2004) reported that DOC exports began to increase in the second year of treatment and that the relative increase rose steadily over the next six treatment years. For the last two water years, they found that soil solution DOC concentrations under experimental NO_3^- deposition were slightly more than 3 times that of ambient treatment. Calculations for the three seasons (1.5 water years) investigated in this study demonstrate that soil solution DOC concentration in the chronic NO_3^- deposition treatment were 2.9 times greater than ambient. It is possible that the influence of elevated NO_3^- deposition on the processes controlling DOC export has reached its maximum as these forests have become more N saturated (Pregitzer and others 2004).

Conclusions and Implications

Results from this study suggest that experimental NO_3^- deposition has not altered sources of DOC in northern hardwood forest soils, despite increased DOC export. DOC is primarily derived from new C in the forest floor, although that DOC has proportionally more phenolic and aromatic compounds. This implies a fundamental alteration in the processes that degrade litter in the forest floor or the processes controlling the fate of DOC produced in the forest floor. These results are significant because they point to N-induced changes in the soil food web that may have implications for long-term C storage; however, it is clear that this is not the primary mechanism responsible for observed patterns of DOC export in response to experimental NO_3^- deposition. Further investigation into the effects of NO_3^- deposition on DOC export needs to address the source/sink relationship between the forest floor and SOM, as well as the influence of pH on DOC sorption and mobility. The effects of anthropogenic N deposition on the transport of DOC to aquatic ecosystems may already be apparent as has been reported in the lower Hudson River watershed (Findlay 2005), and the results presented here underscore our need to understand how terrestrial processes influence both the quantity and quality of C inputs to aquatic ecosystems.

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