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Chronic experimental NO_3^- deposition reduces the retention of leaf litter DOC in a northern hardwood forest soil

Kurt A. Smemo [a,*], Donald R. Zak [a,b], Kurt S. Pregitzer [c]

[a] *School of Natural Resources and Environment, University of Michigan, 430 E. University Avenue, Ann Arbor, MI 48109-1115, USA*
[b] *Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109-1048, USA*
[c] *School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA*

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Abstract

In forests of the Great Lakes region, experimental NO_3^- deposition has suppressed soil respiration and enhanced DOC export. Reasons for these responses are unknown, but they could arise via two alternatives: (i) direct suppression of microbial activity by NO_3^- or (ii) indirect suppression of the microbial community via changes in litter biochemistry in response to greater N availability. To test the second alternative, we conducted a controlled laboratory experiment to examine how chronic experimental NO_3^- deposition affects the contributions of fresh leaf litter to microbial respiration and DOC export. The study reported here used manipulations of mineral soil and fresh leaf litter from a previously studied northern hardwood forest stand in northern Lower Michigan that has received 9 years of ambient and experimental (three times ambient) atmospheric NO_3^- deposition. We found that cumulative microbial respiration over the 6-week incubation was substantially greater in fresh litter plus mineral soil (20.2–13.4 mg C) versus mineral soil alone (4.4–4.1 mg C); however, experimental NO_3^- deposition had no effect on microbial respiration across the litter–mineral soil manipulations. DOC production ($\sim$75%) was primarily associated with leaching from fresh litter. In contrast, mineral soil was a significant sink for litter-derived DOC. Significantly, the mineral soil sink was less pronounced in soil receiving experimental NO_3^- deposition in which $\sim$30% more DOC was leached compared to the ambient NO_3^- deposition treatment. Furthermore, mineral soil was also both a source and sink for soluble phenolics; however, NO_3^- deposition suppressed a mineral–soil sink for phenolics derived from fresh leaf litter. These results suggest that increases in DOC export and declines in soil respiration in response to NO_3^- deposition in the field are not related to obvious changes in litter biochemistry or to the microbial metabolism of this material. Alternatively, these patterns may be linked to decreased abiotic sinks for litter-derived DOC in mineral soil, an unexpected ecosystem consequence of increased anthropogenic (NO_3^-) deposition.
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1. Introduction

Human activities, primarily the combustion of fossil fuels, have increased atmospheric inputs of nitrogen (N) to most of Earth's ecosystems and have subsequently increased the amount of biologically active N in the biosphere (Vitousek et al., 1997). Temperate forests, such as those in the northeastern United States and parts of Europe, commonly receive elevated rates of atmospheric nitrate (NO_3^-) deposition (Galloway, 1995), resulting in a variety of ecosystem consequences. In particular, chronic atmospheric NO_3^- deposition has the potential to directly affect microbial activity and initiate a series of physiological changes that alter cycling and storage of carbon (C) in soil (Berg, 1986; Berg and Matzner, 1997). Disruptions to the soil C cycle can, therefore, modify the source–sink relationship between the biosphere and atmosphere. Because ecosystem C export generally occurs as a gas (e.g. CO_2 from respiration) (Schlesinger and Andrews, 2000) or as dissolved organic matter (DOM) (McDowell and Likens, 1988), it is important to understand how chronic atmospheric NO_3^- deposition could affect microbial and physico-chemical processes that partly control these fluxes. In particular, a more robust and mechanistic understanding of the sources and sinks of DOM and the linkages between increased N availability and DOM dynamics is needed (Kalbitz et al., 2000; McDowell, 2003).

Despite the fact that DOM represents a small fraction of C outputs from terrestrial ecosystems (Neff and Asner, 2001), it constitutes a significant fraction of the C and N cycled within and among ecosystems (Qualls and Haines, 1991b; Perakis and

* Corresponding author. Tel.: +1 734 647 9808; fax: +1 734 936 2195.
E-mail address: smemo@umich.edu (K.A. Smemo).

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Hedin, 2002; Cleveland et al., 2004) and drives heterotrophic metabolism in aquatic ecosystems (McDowell et al., 1998). In northern hardwood forests, DOM in soil solution can have a variety of plant and microbe-mediated sources. Plant root exudates, throughfall, and leaching from litter and soil humus comprise plant-derived sources (Cleveland et al., 2004), whereas microbial biomass and the decomposition of organic matter in the forest floor provide additional sources (Kalbitz et al., 2000; Cory et al., 2004). DOM is also consumed within the mineral soil and forest floor through microbial metabolism and sorption to mineral and organic surfaces (Dalva and Moore, 1991; Qualls and Haines, 1991b, 1992; McBride, 1994; Kalbitz et al., 2005). These source–sink relationships create a complex cycle that can result in an ecosystem functioning as a net sink or a net source of DOM. Anthropogenic NO_3^- deposition can further complicate this dynamic by altering the function of plants and soil microorganisms. For example, studies in temperate forest ecosystems have shown increases (Moldan et al., 1995), decreases (Vestgarden, 2001), or no change (Sjöberg et al., 2003) in DOM export in response to experimental N deposition, suggesting that ecosystem responses are somewhat idiosyncratic (Gundersen et al., 1998).

A recent study demonstrated that chronic experimental NO_3^- deposition increases the ecosystem export of dissolved organic carbon (DOC) and nitrogen (DON) in sugar maple (*Acer saccharum* Marsh.)-dominated northern hardwood forest stands (Pregitzer et al., 2004), thus contradicting hypotheses predicting decreased DOC export with increased N availability (Aber et al., 1998; Gundersen et al., 1998; McDowell et al., 1998). Moreover, Burton et al. (2004), studying these same forest stands, found that experimental NO_3^- deposition suppressed soil respiration (net CO_2 flux), a finding that has been reported elsewhere (Haynes and Gower, 1995; Bowden et al., 2004). It is unclear what caused these responses and whether they are associated with functional changes in mineral soil, forest floor, or changes in the biochemistry and quantity of plant litter.

Although past results demonstrate that experimental NO_3^- deposition influences hydrologic and gaseous losses of soil C from northern hardwood forests (Burton et al., 2004; Pregitzer et al., 2004), subsequent studies have found that experimental NO_3^- deposition did not alter mineral soil microbial respiration or biomass (Zak et al., in press), nor did it influence fine root longevity, respiration, or biomass (Burton et al., 2004). Therefore, it appears that the observed suppression of soil respiration and the increase in DOC export following experimental NO_3^- deposition cannot be attributed to physiological changes in roots or microbial communities inhabiting mineral soil, but instead may be attributed to a fundamental change in the way DOC is produced or consumed in the forest floor (Zak et al., in press).

Here, we present results from a controlled laboratory experiment that examined how chronic experimental NO_3^- deposition in a northern hardwood forest ecosystem can affect the separate and combined contributions of fresh leaf litter and soil organic matter to microbial respiration and DOC production. Our experimental design allowed us to distinguish the influence of fresh leaf litterfall from organic matter in mineral soil. It is important to make this distinction, because fresh litterfall is the biggest contribution to annual DOC export (Pregitzer et al., 2004) and the fact that organic matter in mineral soil can act as a sink for litter-derived DOC. We hypothesized that inputs of fresh leaf litter during autumn were the primary source of the additional DOC produced under elevated NO_3^- deposition. We further hypothesized that previously documented declines in lignolytic activity under elevated NO_3^- deposition (DeForest et al., 2004a,b) should cause a decline in microbial respiration and greater net production of DOC. Alternatively, increased DOC export could simply be a consequence of greater leaf litter input to soils as a result of higher plant productivity, or N-induced production of DOC in soil and leaf litter.

2. Materials and methods

2.1. Study site and experimental design

Soil and freshly senesced leaf litter were collected from a northern hardwood forest stand in the northern Lower Peninsula of Michigan, USA (44°23′N, 85°50′W) that has received 9 years of experimental atmospheric NO_3^- deposition. The site consists of six 30 m × 30 m sugar maple-dominated plots in which three plots received ambient N deposition (1.17 g N m^{-2} yr^{-1}; hereafter referred to as control treatment) and three received ambient N deposition plus 3 g NO_3^-–N m^{-2} yr^{-1} (hereafter referred to as NO_3^- amended). The experimental NO_3^- was added over the growing season in six aliquots of pelletized $NaNO_3$ (0.5 g N m^{-2} $month^{-1}$). Surface soil in this stand consists of a thin (1–2 cm) Oi horizon over mineral A and E horizons; the forest floor is not well developed. This simplified the separation of mineral horizons from organic horizon. Additional information about the study site and the details of the experimental N addition can be found in MacDonald et al. (1993), Zogg et al. (2000) and Pregitzer et al. (2004).

We collected soil and fresh leaf litter following leaf senescence in October 2003. In each plot, we collected and composited 10 random fresh litter samples (0.25 m × 0.25 m) in order to ensure plot coverage and representation of all overstory tree species. Leaf litter was air-dried for 1 week and sorted by species. We then reduced leaf size using a one-hole paper punch to extract circular, evenly sized sub-samples from each leaf. In each plot, we also randomly collected four cores

Table 1
Leaf litter and edaphic characteristics for ambient N and experimental NO_3^- deposition (elevated N) plots

NO_3^- deposition treatment	Annual dry litterfall ($g\ m^{-2}\ yr^{-1}$)[a]	Pre-incubation soil ($mg\ C/N\ g^{-1}$ soil)		Pre-incubation leaf litter ($mg\ C/N\ g^{-1}$ leaf tissue)		Pre-incubation sand ($mg\ C/N\ g^{-1}$ soil)	
		C*	N*	C	N	C	N
Ambient N	488	26.6 (1.8)	1.8 (0.0)	476 (3.7)	9.0 (0.8)	0.1 (0.01)	0.0 (0.0)
Elevated N	534	31.3 (1.7)	2.1 (0.0)	469 (6.3)	9.1 (0.4)		

Values are treatment means ($n=3$) with standard errors in parentheses for litterfall mass, and carbon (C) and nitrogen (N) content of soil and litter. Significant effect of experimental NO_3^- deposition ($P \leq 0.05$) is denoted by *.
[a] Data from Pregitzer et al. (unpublished data).

constructed microcosms containing combinations of litter (no litter versus litter present) and soil organic matter (sterilized acid-washed sand versus native soil). Microcosms were constructed using leaf litter and mineral soil collected in plots receiving ambient ($n=3$) and experimental ($n=3$) NO_3^- deposition. They were fabricated using 60 ml syringes fitted with 2.7 μm nylon filters and ~1 cm of quartz wool (Fig. 1). Soil and/or leaf litter was placed in each syringe and an additional 1 cm of quartz wool was placed over the litter or soil surface to inhibit dispersion of material while DOC was leached from the microcosms. We added soil and/or leaf litter to each microcosm according to the following combinations: soil without litter, leaf litter without soil, and soil plus leaf litter (Fig. 1). Microcosms contained 40 g of soil (31.07 ± 0.53 g OD soil; $n=36$) or sand (38.25 ± 0.17 g OD soil; $n=21$) at field capacity. Leaf litter was added in proportion to the average leaf litter species composition of the study plots (85% sugar maple, 8% American beech (*Fagus grandifolia*), and 7% other species). Leaf litter was also added according to the measured average annual litterfall mass values (Table 1) for control and N amended treatments over the past 5 years (Pregitzer et al., unpublished data) and scaled to the area of the microcosm. Each microcosm was incubated in a 1 l glass Mason jar sealed with an air-tight lid containing a rubber septum for sampling headspace gases. To compare C fluxes from soil and litter sources, results for microbial respiration and DOC leaching are reported as total cumulative C (mg) respired or leached over the 6-week incubation.

Our overall experimental design consisted of two NO_3^- deposition treatments (ambient versus experimental NO_3^- deposition), and the three litter–soil organic matter manipulations (soil, litter, soil + litter; Fig. 1) imposed on the aforementioned NO_3^- deposition treatments. Nitrate deposition treatments are replicated three times in the field, and we constructed three laboratory replicates of the litter–soil organic matter manipulations for each plot receiving ambient and experimental NO_3^- deposition; thus, the combination of field and laboratory treatments produced a total of 54 microcosoms. We also incubated three microcosms containing only sterilized acid-washed sand to control for microbial activity and/or leaching of DOC from the sand added to the leaf litter without soil manipulation. Microcosms were incubated for 6 weeks in the dark at 13 °C, which was the average field temperature at the time of our sampling.

2.2. Microbial respiration, leaching, and analytical techniques

Microbial respiration was measured weekly (i.e. six measurements) by removing samples of headspace gas from each incubation jar. The CO_2 concentration of the gas sample was then determined using a Trace 2000 Series gas chromatograph (Thermo Electron Corp., Austin, TX) equipped with a Porapak Q column (50/80 mesh; Waters Chromotography, Millipore Corp., Milford, MA) and a thermal conductivity detector. We used the accumulation of CO_2–C over the incubation period and non-linear least squares regression to derive the first-order rate constant k (day^{-1}) for microbial respiration and to estimate the cumulative amount of respired C (sensu Zak et al., 1999).

Prior to each 1-week incubation period, microcosms were leached with 40 ml of 0.05 M $CaCl_2$ and the soil solution volume subsequently replaced with 10 ml of a N-free nutrient solution (Stanford and Smith, 1972) to prevent nutrient limitation of microbial activity. Vacuum was applied to bring soil to approximate field capacity. The leachate was collected in an amber polyethylene bottle, passed through a 0.45 μm nylon filter, and then analyzed for DOC on a Shimadzu TOC-Vcp series total organic C analyzer equipped with an ASI-V series auto-sampler (Shimadzu Corp., Kyoto, Japan). Subsamples of the leachate were analyzed for total soluble polyphenolics following the Folin–Ciocalteu method (Ohno and First, 1998), using standard concentrations ranging from 3 to 250 $\mu mol\ l^{-1}$ (Sposito, 1989) of equal amounts of ferulic,

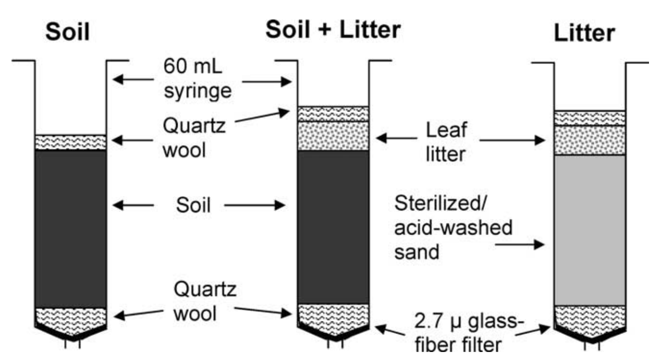

Fig. 1. Illustration of the experimental microcosm design and the three litter–soil organic matter manipulations. Litter–soil manipulations ($n=3$) were imposed on plots receiving ambient ($n=3$) and experimental ($n=3$) NO_3^- deposition in a well-characterized northern hardwood stand in northern Lower Michigan. Each litter–soil manipulation was replicated three times in each experimental plot, producing 54 microcosms in this study.

p-coumaric, *p*-hydroxybenzoic, vanillic, and syringic acid. Phenolic concentrations were determined colorimetrically (750 nm absorbance) on a Spectronic GENESYS 20 Spectrophotometer (Thermo Electron Corp., Austin, TX). All leachate data were compiled to determine cumulative mass of DOC and phenolic C leached from each microcosm over the 6-week incubation period. The same data were used to create accumulation curves and calculate first-order rate constants for the net production of DOC and soluble phenolics in leaf litter and soil.

2.3. Statistical analysis

Data were analyzed using an analysis of covariance (ANCOVA) within the general linear model (GLM) procedure of SAS (v8.5, SAS Institute, Inc., Cary, NC) to determine if experimental NO_3^- deposition had an effect on microbial respiration, DOC export and net production of soluble phenolics in soil and fresh litter. In our model, litter–soil organic matter manipulations ($n=3$; soil, litter, soil + litter) were nested within NO_3^- deposition treatments ($n=2$; ambient, experimental NO_3^- deposition). Data used in the model were plot means ($n=3$) for each litter–soil organic matter treatment. Leaf litter production is slightly greater under experimental NO_3^- deposition, and we adjusted the amount of litter in microcosms to parallel this effect. Therefore, we used initial litter mass as a covariate in our analysis to determine whether greater DOC leaching resulted from greater litter production or via a change in the manner in which fresh leaf litter is metabolized by the microbial community. We compared main effect and interaction means using a Fisher's protected LSD test. Significance for all tests was accepted at $\alpha=0.05$.

3. Results

3.1. Microbial respiration

Nitrogen deposition as a main effect had no significant impact on microbial respiration. Cumulative respired C over 6 weeks was 12.72 ($\pm$1.27) mg across ambient NO_3^- deposition treatment and 13.15 ($\pm$1.37) mg across the experimental NO_3^- deposition treatment. Microbial respiration differed significantly ($P \leq 0.001$) across the three litter–soil organic matter manipulations and was highest in the soil + litter manipulation (19.77 $\pm$ 0.73 mg C) compared to litter (14.29 $\pm$ 0.37 mg C) and soil (4.29 $\pm$ 0.20 mg C). We found no significant interaction between N deposition treatments and litter–soil manipulations (Fig. 2). In the ambient NO_3^- deposition treatment, cumulative respired C was greater in the litter (13.4 $\pm$ 0.42 mg C) and soil + litter manipulations (19.3 $\pm$ 1.05 mg C), compared to the soil manipulation (4.4 $\pm$ 0.27 mg C). The same response also occurred in the experimental NO_3^- deposition treatment with values in the litter (15.1 $\pm$ 0.48 mg C) and soil + litter (20.2 $\pm$ 1.07 mg C) exceeding those in the soil manipulation (4.1 $\pm$ 0.31 mg C). Cumulative respiration was additive across the litter–soil manipulations; the sum of soil respiration and litter respiration

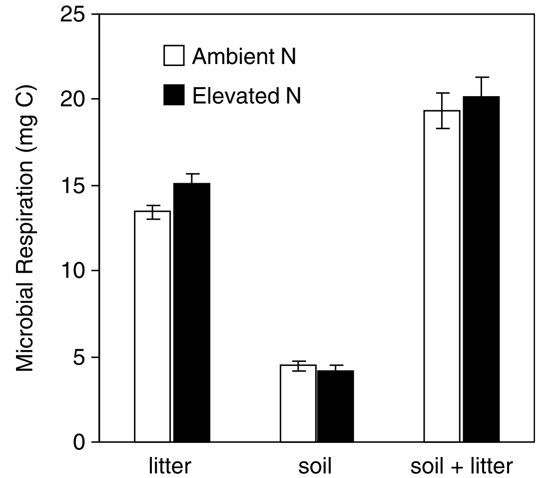

Fig. 2. Effect of experimental NO_3^- deposition treatment and litter–soil manipulation on cumulative microbial respiration in forest soil and leaf litter. Values are least square means ($n=3$) $\pm$ SE of the N deposition-litter manipulation treatment combinations. Elevated N was had no significant effect in any of the leaf litter–soil organic matter treatments.

is not significantly different from the soil + litter respiration ($P \leq 0.05$). Microbial respiration was highest ($\sim$75% of total respiration) in the fresh litter, yet short-term interactions between soil and leaf litter appear to be unimportant as a control on microbial respiration rates. Moreover, we found no significant effect of NO_3^- addition or litter–soil manipulation on the first-order rate constants for microbial respiration (Table 2).

3.2. Leachate chemistry

Experimental NO_3^- deposition did not have an overall significant effect on net DOC production in the microcosms. Mean net DOC production was 7.41 ($\pm$0.72) mg C in the ambient NO_3^- deposition treatment and 8.20 ($\pm$0.67) mg C in the experimental NO_3^- deposition treatment. Fresh litter was the largest net source of leached DOC and accounted for 11.17 ($\pm$0.46) mg C over the 6-week incubation. Soil was a much smaller net source of leached DOC and accounted for 3.70 ($\pm$.24) mg C. In contrast to the cumulative respiration

Table 2
Effect of experimental NO_3 deposition on the first order rate constant (k) for microbial respiration, net DOC production, and net soluble phenolic production

Treatments	k_{resp} (day^{-1})	k_{DOC} (day^{-1})	k_{phenol} (day^{-1})
Soil			
Ambient N	0.016 (0.004)	0.078 (0.005)	0.202 (0.053)
Elevated N	0.028 (0.013)	0.052 (0.004)	0.102 (0.022)
Sand + litter			
Ambient N	0.039 (0.0030)	0.129 (0.012)	0.135 (0.016)
Elevated N	0.036 (0.004)	0.106 (0.006)	0.147 (0.0156)
Soil + litter			
Ambient N	0.036 (0.004)	0.092 (0.003)	0.094 (0.017)
Elevated N	0.045 (0.006)	0.077 (0.011)	0.101 (0.018)

Values are LS means for treatment interactions with standard errors in parentheses. No significant differences were found for main or interaction treatment effects.

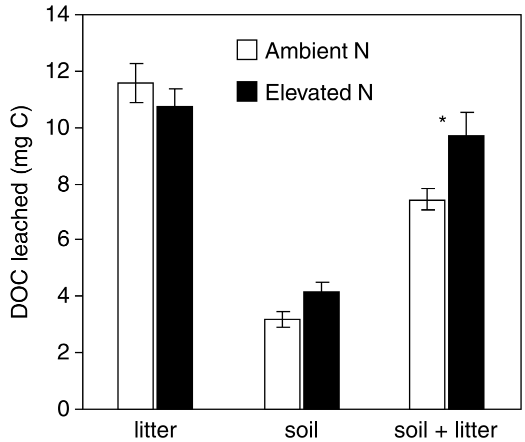

Fig. 3. Effect of experimental NO_3^- deposition treatment and litter–soil manipulation on cumulative DOC leaching in forest soil and leaf litter. Values are least square means ($n=3$)$\pm$SE of the N deposition–litter manipulation treatment combinations. Significant interaction effect ($P \leq 0.01$) is denoted by *.

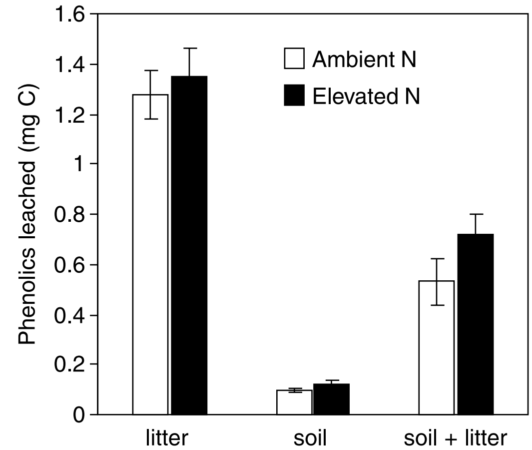

Fig. 4. Effect of experimental NO_3^- deposition treatment and litter–soil manipulation on cumulative soluble phenolic leaching in forest soil and leaf litter. Values are least square means ($n=3$)$\pm$SE of the N deposition–litter manipulation treatment combinations. Elevated N had no significant effect in any of the leaf litter–soil organic matter treatments.

data, DOC leaching was not additive across the individual litter–soil organic matter manipulations and the soil + litter manipulation produced only 8.56 ($\pm$0.54) mg C, demonstrating that soil was a significant sink for litter-derived DOC. The interaction between the N deposition treatment and litter–soil manipulation was significant ($P \leq 0.01$), but that significance was driven by the soil + litter treatment (Fig. 3). Although experimental NO_3^- deposition had no effect on the mass of DOC leached from soil or litter, it did significantly decrease ($P \leq 0.01$) the ability of the soil to act as a sink for litter-derived DOC (Fig. 3). In the experimental NO_3^- deposition treatment, the litter + soil manipulations lost 9.67 ($\pm$0.97) mg C as DOC, compared to 7.44 ($\pm$0.39) mg under ambient NO_3^- deposition. We also found that neither experimental NO_3^- addition treatment nor litter–soil organic matter manipulation had a significant effect on rate constants (k) for net DOC production (Table 2).

Nitrate deposition treatment (main effect) had no overall significant effect on the leaching of soluble phenolics; values were 0.63 ($\pm$0.10) mg phenolic C under ambient NO_3^- deposition and 0.73 ($\pm$0.11) mg C under experimental NO_3^- deposition. Similar to the patterns for DOC leaching, fresh litter was the largest net source of soluble phenolics (1.31 ($\pm$0.07) mg C) over the 6-week incubation followed by soil + litter (0.62 ($\pm$0.06) mg C) and soil (0.11 ($\pm$0.01) mg C). Soils appear to be both a source and sink of phenolic compounds, and although not statistically significant, NO_3^- deposition appeared to dampen the capacity of the soil to act as a sink for soluble phenolics (Fig. 4). Soluble phenolics, as a subset of total DOC, accounted for ~10% of the total mass of DOC leached from litter and ~3% of DOC leached from soil, whereas soluble phenolics were ~7% of the DOC produced in soil + litter. Nitrate deposition had no measurable effect on the cumulative production or loss of soluble phenolic compounds from fresh leaf litter, nor did it alter first-order rate constants (k) (Table 2).

4. Discussion

4.1. Controls on DOC production and export

Experimental NO_3^- deposition alters the processes that control DOC fluxes in northern hardwood forest ecosystems of the upper Great Lakes region by reducing DOC sinks, not by changing DOC sources. Although fresh leaf litter was a greater source of DOC than soil organic matter in our study, experimental NO_3^- deposition did not alter the amount of DOC leached from either leaf litter or soil (Fig. 3). It is therefore unlikely that NO_3^- additions altered the availability of substrates that are precursors to DOC formation (Pregitzer et al., 2004) resulting from microbial decomposition or a physico-chemical process. Instead, DOC export may be controlled by a soil–leaf litter interaction whereby experimental NO_3^- deposition decreases the capacity of the mineral soil to act as a sink for litter-derived DOC. This mineral soil or soil organic matter sink for litter and/or humus-derived DOC has been shown in a variety of studies (e.g. McDowell and Likens, 1988; Dalva and Moore, 1991; Cory et al., 2004; Kalbitz et al., 2005), yet it is not certain if this sink is associated with microbial immobilization or physico-chemical processes. Moreover, the manner in which NO_3^- deposition could mechanistically alter this sink is unclear even though similar responses to N addition have been demonstrated in other ecosystems (Moldan et al., 1995; Currie et al., 1996).

A decreasing mineral soil DOC sink could be attributed to lower microbial utilization of readily available substrates in fresh leaf litter. If this were the case in our experiment, we should have observed a decrease in microbial activity under high N availability, thus decreasing the sink strength of the mineral soil and resulting in greater DOC export; however, our results are not consistent with this expectation. High N availability has been shown to directly inhibit microbial degradation of organic matter by suppressing the production of lignolytic enzymes (e.g. phenol oxidase and peroxidase) by white-rot fungi (basidiomycota), which help degrade lignin compounds in soil (Fog, 1988; Carreiro et al., 2000; DeForest et al., 2004a,b; Pregitzer et al., 2004). Although, this makes sense mechanistically and supports our initial hypothesis, we would also expect to see an associated decrease in microbial respiration and an increase in the concentration of unmetabolized soluble phenolics in leachate from soil and litter receiving experimental NO_3^- deposition. However, this was not the case in our study; experimental NO_3^- deposition did not significantly alter microbial respiration in litter or mineral soil (Fig. 2), nor did it alter production and export of soluble phenolics (Fig. 4) regardless of the litter–soil organic matter manipulation. Hence, these results do not support the hypothesis that experimental NO_3^- deposition alters DOC production via changes in the function of the microbial community or that the observed patterns in DOC export are biological in origin. Our results alternatively suggest that experimental NO_3^- deposition alters the physical and chemical adsorption capacity of mineral soil and soil organic matter, thereby diminishing an important ecosystem sink for DOC and potentially reducing the formation of soil organic matter. It is also possible that NO_3^- deposition primarily exerts its influence on microbial activity in forest floor (Park et al., 2002), which was not a component of this study.

The conclusion that physical adsorption is controlling DOC export on our system is consistent with other studies that have suggested sorption of DOC to mineral surfaces as the primary sink for DOC in mineral soil (McDowell and Likens, 1988; Qualls and Haines, 1992; Currie et al., 1996; Cory et al., 2004), and might be particularly true for aromatic compounds derived from lignin depolymerization (Kalbitz et al., 2005). Moreover, the unsaturated condition in our microcosms suggests little mineral soil and litter interaction between leaching events. DOC is therefore removed from soil water rapidly during leaching, providing further evidence for an abiotic sink that is suppressed by chronic experimental NO_3^- deposition. Despite evidence for this reduced sink, it is unclear how increased N availability could alter this process. It is possible that the effect is not due to N availability, but to the increase in ionic strength (salt effect) as a result of the $NaNO_3$ used to simulate NO_3^- deposition. Anions such as NO_3^- could compete directly with DOC for adsorption sites, yet evidence for this process is lacking (Kalbitz et al., 2000; Pregitzer et al., 2004). Moreover, inherent in our study design, we added no additional NO_3^- to our incubations and freely available NO_3^- decreased (*unreported data*) as a result of continued leaching during the incubation period, thus minimizing the potential importance of this mechanism. Excess Na^+ would only reduce organic matter solubility (Kalbitz et al., 2000), thereby resulting in less DOC export in contrast to the increase reported here. Our results therefore support the conclusion of Pregitzer et al. (2004) and reject the notion that DOC export is enhanced by a fertilization-associated salt effect.

Adsorption and/or microbial utilization of DOC in mineral soil also might be suppressed because DOC derived from plant litter under experimental NO_3^- deposition is biochemically distinct from litter produced under ambient NO_3^- deposition. Greater N availability could lead to changes in nutrient uptake and photosynthetic efficiency by plants, ultimately controlling the quantity and biochemistry of litter inputs to the soil and altering substrates that can be leached or microbially decomposed. Increases in litter quality (low tissue C:N and lignin) under high N availability could then create a feedback on microbial community composition and function and increase the rate at which organic matter is decomposed (Fog, 1988; Carreiro et al., 2000), ultimately influencing the quantity and quality of DOC leached from fresh litter. Our results are not consistent with this mechanism (Table 2). Experimental NO_3^- deposition does not appear to alter the quantity or phenolic content of DOC entering mineral soil. Although experimental NO_3^- deposition has increased the quantity of litter entering the forest floor, N content of that litter is not significantly different between ambient and NO_3^- amended treatments (Table 1). A study of leaf litter biochemistry from the same northern hardwood forest hardwood stand also demonstrated that experimental NO_3^- deposition had no effect (Eikenberry et al,. unpublished data). Therefore, it is unlikely that the DOC export patterns we have documented are controlled by differences in leaf litter biochemistry.

4.2. Sources and controls of soil respiration

Soil respiration is controlled by the physiological activity and interactions of plant roots and soil microorganisms, and many factors controlling these processes are directly affected by anthropogenic NO_3^- deposition. For example, increased N availability can alter fine root biomass (Haynes and Gower, 1995), specific root respiration (Burton et al., 1996; Ryan et al., 1996), and the rate of mycorrhizal infection (Wallenda and Kottke, 1998; Treseder and Allen, 2000; Lilleskov et al., 2002). After 8 years of experimental NO_3^- deposition, Burton et al. (2004) found that soil respiration (net CO_2 flux) was reduced by 15%. For this to occur, at least one source of soil respiration would have to be suppressed. Nevertheless, studies in the same forest stands found no significant effect of experimental NO_3^- deposition on fine root biomass or respiration (Burton et al., 2004), suggesting that fine roots in mineral soil did not contribute to a decline in soil respiration. Similarly, NO_3^- deposition also did not alter microbial respiration in mineral soil (Zak et al., in press) despite a significant decrease in soil microbial biomass (DeForest et al., 2004a,b). In the present study, NO_3^- deposition did not alter microbial respiration in mineral soil, nor did it alter the metabolism of fresh leaf litter. Therefore, we must reject the hypothesis that microbial respiration in fresh leaf litter is suppressed by experimental NO_3^- deposition and is consequently driving the reduction in soil respiration reported by Burton et al. (2004). The remaining alternative is that chronic NO_3^- deposition has altered microbial activity in forest floor (partially decomposed litter from previous years) (Park et al., 2002), which is responsible for a decline in soil respiration and an increase in DOC production.

Although we attempted to separate the individual contributions of soil organic matter and fresh leaf litter, our study design excluded partially decomposed litter from previous years (old forest floor) as a way of distinguishing the influence of fresh litter and mineral soil on DOC production and respiration. This differentiation was important for understanding C cycling in temperate sugar maple-dominated forests that receive annual pulses of readily degradable leaf litter and which are currently experiencing elevated NO_3^- deposition. For example, it has been suggested that fresh leaf litter is the most important source of DOC in deciduous forest soils (Qualls and Haines, 1991a; Park et al., 2002) and this study showed that leaf litter was the greatest source of microbial respiration. However, it is possible that microbial response to NO_3^- deposition in forest floor is responsible for reductions in soil respiration and increases in DOC. Because forest floor contains organic matter acted on by decomposing microorganisms for an entire year, the most labile substrates have already been leached or metabolized leaving behind more recalcitrant substrates, such as lignin, that is more resistant to microbial decay. It is in this pool where N availability may decrease the activity and ultimately the respiration of lignin-degrading organisms by suppressing the production of extracelluar enzymes (Fog, 1988; Frankland, 1998; DeForest et al., 2004a,b). Our study design necessitated separation of leaf litter from soil, homogenization of mineral soil and organic matter, and exclusion of plant roots. One obvious artifact of this approach is the lack of mycorrhizal fungi activity associated with fine roots; hence, we were unable to measure how these fungal communities may have responded to the experimental treatments. Considering the obvious contribution of fungal respiration to soil CO_2 flux and the apparent impact of N deposition on the mycorrhizae (Wallenda and Kottke, 1998; Treseder and Allen, 2000; Lilleskov et al., 2002), this aspect of ecosystem response to NO_3^- deposition deserves further attention. A decrease in the biomass of mycorrhizal fungi resulting from high N availability is a plausible explanation for the suppression of soil respiration in response to experimental NO_3^- deposition. Although we purposely excluded roots from our incubations to measure saprophytic activity in root-free soil, our ability to draw conclusions from our results assumes that there are no interactions between roots and soil microbial communities such as root exudates. Thus, unquantified root responses to chronic NO_3^- deposition could have lead to inherent differences between laboratory and field responses.

4.3. Conclusions

Overall, the results of this study imply that increased export of DOC and the suppression of soil CO_2 flux in response to experimental NO_3^- deposition are unrelated to obvious differences in litter biochemistry or alterations of the microbial controls on DOC fluxes from fresh leaf litter and mineral soil. Alternatively, our results suggest that DOC export may be controlled by abiotic sinks for litter-derived DOC in mineral soil, and that experimental NO_3^- deposition alters the strength of that sink and decreases sequestration of DOC into soil organic matter. Furthermore, decreases in soil respiration may have originated from microbial activity in portions of the forest floor or mineral soil not examined in this study. Although our results suggest that these patterns are not related to microbial activity in fresh leaf litter and surface mineral soil, future research needs to address long-term effects of NO_3^- deposition on the processes controlling incorporation of DOC into soil organic matter and how soil microbial communities mediate those processes.

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