A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis

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Abstract

Forest soils, rather than woody biomass, are the dominant long-term sink for N in forest fertilization studies and, by inference, for N from atmospheric deposition. Recent evidence of significant abiotic immobilization of inorganic-N in forest humus layers challenges a previously widely held view that microbial processes are the dominant pathways for N immobilization in soil. Understanding the plant, microbial, and abiotic mechanisms of N immobilization in forest soils has important implications for understanding current and future carbon budgets. Abiotic immobilization of nitrate is particularly perplexing because the thermodynamics of nitrate reduction in soils are not generally favorable under oxic conditions. Here we present preliminary evidence for a testable hypothesis that explains abiotic immobilization of nitrate in forest soils. Because iron (and perhaps manganese) plays a key role as a catalyst, with Fe(II) reducing nitrate and reduced forms of carbon then regenerating Fe(II), we call this 'the ferrous wheel hypothesis'. After nitrate is reduced to nitrite, we hypothesize that nitrite reacts with dissolved organic matter through nitration and nitrosation of aromatic ring structures, thus producing dissolved organic nitrogen (DON). In addition to ignorance about mechanisms of DON production, little is known about DON dynamics in soil and its fate within ecosystems. Evidence from leaching and watershed studies suggests that DON production and consumption may be largely uncoupled from seasonal biological processes, although biological processes ultimately produce the DOC and reducing power that affect DON formation and the entire N cycle. The ferrous wheel hypothesis includes both biological and abiological processes, but the reducing power of plant-derived organic matter may build up over seasons and years while the abiotic reduction of nitrate and reaction of organic matter with nitrite may occur in a matter of seconds after nitrate enters the soil solution.

Keywords: dissolved organic nitrogen (DON), Harvard Forest, iron, nitrogen, soil

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Introduction

Atmospheric deposition of nitrogen to temperate forests has increased in recent decades primarily as a result of increased combustion of fossil fuels (Galloway *et al.*, 1995). Because N often limits rates of plant growth, increased N inputs could affect several ecosystem processes, including carbon sequestration by forests. However, recent evidence from N fertilization studies in

temperate forests indicates that soils, rather than plants, are the dominant long-term sink for applied N and, by inference, for N from atmospheric deposition (Gundersen *et al.*, 1998; Nadelhoffer *et al.*, 1999a). Sequestration of N in soils promotes less C storage than would plant uptake of N and attendant enhanced growth of woody biomass (Houghton *et al.*, 1998). The global implication of this finding is that elevated atmospheric deposition of N derived from fossil fuel combustion may not be contributing significantly to an enhanced sink of carbon in forest biomass (Nadelhoffer *et al.*, 1999b).

The explanation for N retention in forest soils is likely related to the mechanisms of immobilization, which

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remain poorly understood. Despite decades of research in ecology, soil science, agronomy and forestry on nitrogen transformations in soils, our understanding of the mechanisms by which inorganic nitrogen is immobilized in soils remains inadequate.

Recent evidence that abiotic immobilization of N may be an important process (Berntson & Aber, 2000; Johnson et al., 2000; Dail et al., 2001; Perakis & Hedin, 2001) challenges a previously widely held view that microbial processes are the dominant pathways for N immobilization in soil (Jones & Richards, 1977; Rice & Tiedje, 1989; Schimel et al., 1989; Zak et al., 1990; Davidson et al., 1990, 1992; Stark & Hart, 1997). These studies employed ^{15}N as a tracer and in pool-dilution calculations (Kirkham & Bartholomew, 1954; Jansson, 1971), yielding rich insight into the dynamic nature of gross rates relative to net rates of microbial N mineralization and immobilization in many soils. However, Davidson et al. (1991) recognized that not all of the applied ^{15}N label could be recovered immediately after addition to the soil (so-called 'T0' recovery) in both live and sterile soils. It was shown that rates of microbial transformations during subsequent 24 h incubations of the pool-dilution methodology would be overestimated if a correction were not made for T0 recovery. The incomplete T0 recovery of label in sterilized soil samples was speculatively attributed to abiotic clay fixation in the case of $^{15}NH_4^+$ application and was not explained in the case of $^{15}NO_3^-$ application. Until now, this incomplete T0 recovery of label was viewed as a source of experimental error or 'noise' for pool-dilution studies of microbial processes that warranted appropriate corrections (Hart et al., 1994). With increasing evidence for the potential quantitative importance of abiotic immobilization of inorganic-N in soils, however, we believe that this 'noise' may, instead, be the 'signal' that we should be studying to understand better the processes of N sequestration in many soils.

Of course, both biological and abiological processes may be significant simultaneously or one process may dominate, depending on the soil conditions. It is important to understand the circumstances under which each occurs, the relative contributions of biotic and abiotic pathways of N immobilization, and the differences that these pathways may have on the fate of the organically bound N that they produce. Microbial immobilization of N requires utilization of mostly labile carbon resources by the soil microorganisms. In contrast, abiotic mechanisms of N immobilization in soils and forest litter layers may involve reactions of N with C-containing compounds that are less available to microorganisms and that are more abundant. Hence, the extent to which the soil N sink becomes saturated by N inputs over time (sensu Aber et al., 1998) may depend on the forms of carbon required by the reactive mechanism. Indeed,

Currie et al. (1999) found it necessary to uncouple biological C cycling from N processes in their model in order to explain observed distributions of ^{15}N in various plant and soil pools at the Harvard Forest in Massachusetts. Johnson et al. (2000) found that microbial immobilization of ammonium was correlated inversely with soil N concentrations, but that abiotic immobilization was independent of soil N status.

Furthermore, the fate of the immobilized N may also depend on the mechanisms of immobilization. Microbial immobilization followed by turnover of the microbial biomass releases some fraction of the immobilized N into a form that can be taken up by plants and used to sequester more C in plant tissues (Aber et al., 1998; Houghton et al., 1998). Abiotic N immobilization, on the other hand, is so poorly understood that we do not know if the products are eventually remineralized into a plant-available form.

Abiotic immobilization of nitrate is particularly perplexing because the thermodynamics of nitrate reduction in soils are not generally favorable under oxic conditions. Nevertheless, rapid nitrate immobilization into a form of dissolved organic nitrogen (DON) has been demonstrated recently for organic horizons of the Harvard Forest in central Massachusetts, USA (Dail et al., 2001) and for organic horizons of a temperate forest in Chile (Perakis & Hedin, 2001). Dail et al. (2001) used two sterilization treatments to demonstrate that the conversion of $^{15}NO_3^-$ to DO[15]N within minutes of addition to sterilized organic horizons was abiotic (Table 1). Although Perakis & Hedin (2001) did not use sterilization treatments, the rapidity (0.1 days) with which they observed conversion of $^{15}NO_3^-$ to DO[15]N, suggests that an abiotic mechanism might have been responsible. A similar conclusion was reached by Berntson & Aber (2000) based on rapid (< 15 min) disappearance of over half of their applied $^{15}NO_3^-$ to Harvard Forest soil. These results call into question the common assumption that recovery of ^{15}N in a DON fraction necessarily demonstrates that inorganic-^{15}N had to have been microbially assimilated to produce the organically bound [15]N (Seely & Lajtha, 1997). The objective of this paper is to present plausible and testable hypotheses of how nitrate might, instead, be immobilized by abiotic processes in temperate forest soils.

The ferrous wheel hypothesis

Soil microorganisms are known to assimilate nitrate (Zak et al., 1990; Davidson et al., 1992; Stark & Hart, 1997), but no known mechanism exists for abiotic binding of nitrate to soil organic matter. The reduction of nitrate appears to require a catalyst, which, to date, has been assumed to be enzymatic, and therefore, biological.

Table 1 Effects of sterilization on properties of O_a horizon samples from the Harvard Forest, Massachusetts. Means ($\pm$ SD) of three replicates are given. CFU is colony-forming units. Inorganic-N was extracted in 0.5 M K_2SO_4 and measured colorimetrically. Extractable nitrate was below detection limits prior to adding the label. Total soil-N was determined by combustion. Dissolved organic carbon (DOC) was measured on soil solutions (purged of dissolved carbonate) by high temperature combustion in a CHN analyzer, followed by infrared detection of evolved CO_2 (Shimadzu TOC 5000A). DON was determined by persulphate digest. All are expressed on a dry weight soil basis. Recovery of label occasionally slightly exceeded 100%, which may have been due either to errors in applying the target addition rates or analytical errors. Recoveries as NO_3^- and DON are shown for 15 min after addition of 5 µg NO_3^-–N g^{-1} soil that was 99% enriched in ^{15}N. Recoveries of label as extractable NH_4^+ and as insoluble organic-N were negligible. See Dail *et al.* (2001) for details

Sterilization treatment	Plate counts CFU g^{-1}	pH	NH_4^+ mg N kg^{-1}	Total soil-N g N kg^{-1}	DOC g C kg^{-1}	^{15}N recovered as NO_3^- (%)	^{15}N recovered as DON (%)
Control	3.2×10^5	3.9	16 ± 1	10.8 ± 0.4	0.16 ± 0.02	67 ± 5	28 ± 6
γ-irradiated	No growth	4.0	95 ± 5	11.4 ± 0.3	1.58 ± 0.02	59 ± 9	43 ± 7
Autoclaved	No growth	3.8	119 ± 7	12.0 ± 1.0	5.38 ± 0.45	56 ± 2	51 ± 5

Nitrite (NO_2^-), on the other hand, is known to react readily and abiotically with soil organic matter (Smith & Chalk, 1980; Thorn & Mikita, 2000). Nitrate could follow a similar fate if it is first reduced to nitrite. The study by Dail *et al.* (2001) on Harvard Forest soils yielded a clue in support of a role for dissolved organic-C (DOC) in the formation of DON – namely that abiotic N immobilization was positively correlated with DOC concentrations. First, DON production from nitrate was not observed in mineral soils, where organic-C contents are lower than in the humus layer. Second, the amount of nitrate immobilized in the O_a horizon varied among the three treatments in the same order as the amount of measured DOC (autoclaved > gamma irradiated > control; Table 1). Hence, one of the artifacts of the sterilization techniques – an increase in DOC – may shed light on the importance of DOC for the abiotic N immobilization mechanism. Sterilization increased both DOC concentrations and nitrate immobilization rates, although nitrate immobilization was also substantial and rapid in the live humus samples. The forest soils (O_a horizons) studied by Perakis & Hedin (2001) in Chile are also C-rich.

Given the known reactivity of nitrite, we hypothesize that abiotic immobilization of nitrate might be accomplished if nitrate is first reduced to nitrite or a reactive intermediate, such as radical $^\bullet NO_2$, prior to binding to soluble organic matter. We hypothesize a reaction pathway whereby Fe(II) or Mn(II) (the latter being less important under most relevant soil conditions) reduces NO_3^- to NO_2^- that then reacts quickly with soluble organic matter (Fig. 1). The ultimate source of reducing power is plant-derived reduced carbon that is incorporated into the forest floor and/or mineral soil and that regenerates the reduced form of the metals needed for catalyzing nitrate reduction. The O_a horizon contains the three conditions necessary for the hypothesized reaction
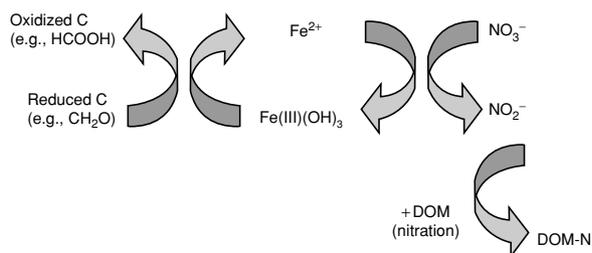

Fig. 1 The 'ferrous wheel hypothesis' for abiotic nitrate immobilization is that carbon compounds derived from photosynthate reduce Fe(III) in soil minerals, producing reactive Fe(II) species that reduce nitrate to nitrite, and nitrite subsequently reacts with receptive moieties in dissolved organic matter (DOM) to produce dissolved organic-N (DON).

sequence to occur: (1) some Fe or Mn-containing minerals mixed in from the mineral soil below that can reduce nitrate; (2) anaerobic microsites resulting from microbial and root respiration and high water holding capacity of organic matter that would promote favorable redox conditions for nitrate reduction; and (3) abundant DOC that can react with nitrite to produce DON.

Three lines of preliminary evidence lend support to the hypothesis outlined in Fig. 1. First, we have observed oxidation of Harvard Forest DOM coupled to reduction of solid phase Fe(III) hydroxide in aqueous suspensions under oxic conditions (Chorover & Amistadi, 2001), which would provide a source of Fe(II). Second, the reaction of Fe(II) with NO_3^- to form Fe(III) and NO_2^- is thermodynamically favorable, and our lab and others (Buresh & Moraghan, 1976; Ottley *et al.*, 1997; Senn & Hemond, 2002) have observed nitrate reduction by aqueous phase Fe(II) at relevant time scales. Third, phenolic compounds common to soil DOM solutions react with nitrite to form nitrophenols. Evidence for each of these three statements is briefly presented below.

Abiotic reduction of Fe (hydr)oxides by DOM

In oxic soils, oxidation state of Fe(III) (ferric) is favored; it forms a wide variety of oxides and hydroxides that are sparingly soluble in natural waters and that limit biological iron availability (Cornell & Schwertmann, 1996). It has been demonstrated that the presence of Fe(II) in soil solution or on particle surfaces (a prerequisite for the proposed pathway) is promoted by redox reactions of Fe(III) (hydr)oxides with dissolved organic matter (DOM) in extracts of Spodosols (Buerge & Hug, 1998), waters of a eutrophic Swiss lake (Emmenegger et al., 1998), and humic substances (Lovley et al., 1998). Plant and microbe-derived soluble organic constituents (fulvic acid, low molecular weight organic acids [LMWOAs], carboxylated phenolic compounds), can also form complexes with Fe(III), increasing its total concentration in solution (Hider, 1984; Tam & McColl, 1991; Stevenson, 1994; Fox, 1995; Goodell et al., 1997), promoting Fe hydroxide dissolution (Stumm & Morgan, 1996) and, in some cases, transferring electrons to bound Fe(III) to form Fe(II) (Sulzberger & Laubsher, 1995). Formic, acetic, citric and malonic acids (Krzyszowska et al., 1996) are usually found in micromolar concentrations in soil solutions, and oxalic acid can exceed 1 mM (Fox & Comerford, 1990; Krzyszowska et al., 1996).

We compared the affinity and reactivity of minerals for natural organic matter in forest floor leachates from hardwood and pine stands at the Harvard Forest (Chorover & Amistadi, 2001). Leachates were ultrafiltered (< 10 kDa) to remove microbial cells and macromolecular enzymes and were acidic (pH 4), with ionic strengths of 1.4 mM (hardwood) and 1.1 mM (pine). The leachates were diluted to provide a range of initial dissolved organic carbon (DOC) concentrations (0–140 g C m^{-3}). All inorganic ions were adjusted to match undiluted concentrations. These oxic suspensions (and corresponding blanks) were reacted in the dark with goethite (α-FeOOH), birnessite (δ-MnO$_2$) and smectite (Wyoming montmorillonite, Na$_{0.56}$[Si$_{7.98}$Al$_{0.02}$]Al$_{3.01}$Fe$_{0.41}$Mg$_{0.54}$Ti$_{0.02}$O$_{20}$(OH)$_4$). The results indicate significant abiotic oxidative transformation of natural organic matter to organic acids upon interaction with goethite (Fe[III]) and birnessite (Mn[IV]) solid phases, which resulted in a net production of formic and acetic acids. This oxidation was most likely coupled to the production of Fe(II) and Mn(II), which were found to increase in solution concentration (Mn) or were readsorbed and reoxidized (Fe) under oxic conditions. In these birnessite and goethite suspensions, acetic acid formation was correlated with initial DOC concentration (Fig. 2). In contrast, we were unable to detect any measurable formic or acetic acid in the mineral-free controls or in smectite suspensions, clearly indicating the greater
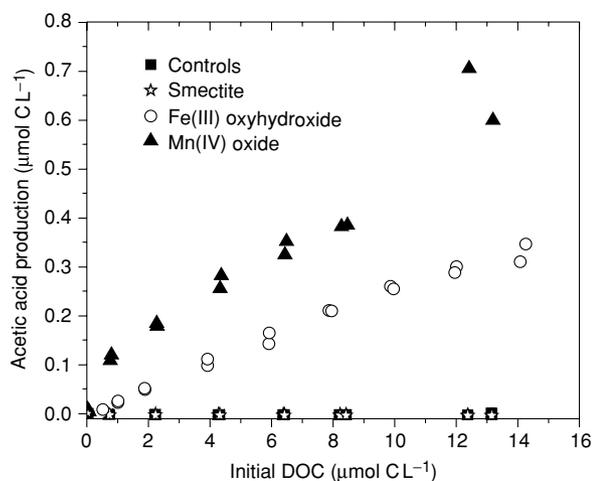

Fig. 2 Abiotic reaction of Harvard Forest DOM at Fe(III) oxyhydroxide (goethite) and Mn(IV) oxide (birnessite) surfaces results in oxidative transformation of DOM that is coupled to release of Fe(II) and Mn(II), respectively. One major product of DOM oxidation is acetic acid; its concentration correlates with initial DOC concentration in aqueous suspensions. No accumulation of low molecular weight acid products was detected in smectite suspensions or in mineral-free controls. From Chorover & Amistadi (2001).

redox reactivity of the Fe- and Mn-dominated mineral surfaces (Chorover & Amistadi, 2001).

Lovley et al. (1996) have shown that humic substances in marine sediments have high electron accepting capacities – attributed to electron-deficient quinone groups – which may be reduced during suboxic respiration to electron-donating phenols. Humic material is thought to act as an 'electron shuttle' during dissimilatory Fe reduction (Scott et al., 1998): quinone groups accept electrons directly from respiring microbes and the 'reduced' (hydroquinone or phenol) form of NOM then transfers those electrons abiotically to Fe(III) surfaces, renewing the electron deficient quinone groups for further microbial reduction. Such a reaction may accelerate the ferrous wheel mechanism of abiotic nitrate immobilization since microbial respiration of quinones increases the concentration of reduced and reactive NOM (phenols) that favor Fe(III) reduction.

Thermodynamic favorability and kinetics

The full redox reaction, whereby nitrate reduction to nitrite is coupled to the oxidation of dissolved Fe(II) to form Fe(III) solid (e.g. goethite or ferrihydrite) may be written as (standard potential data from McBride, 1994):

$$2\,Fe^{2+} + NO_3^- + 3\,H_2O \rightarrow$$
$$2Fe(III)OOH(s) + NO_2^- + 4H^+ \quad E^0 = -0.324\,V \quad (1)$$
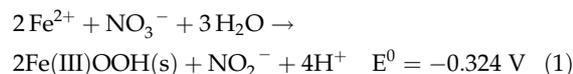
Applying the Nernst equation to appropriate conditions (assuming values of 10^{-4} for activity of Fe(II) and unit activity for H_2O and solids) gives the requirement for the reaction to be favorable thermodynamically:

$$\log\left[\frac{(NO_3^-)}{(NO_2^-)}\right] > 18.97 - 4\,pH \qquad (2)$$

For equimolar concentrations of NO_3^- and NO_2^-, the reaction proceeds (forming goethite in this case) at pH > 4.7. As the ratio of NO_3^- to NO_2^- in solution increases, the reaction proceeds at increasingly acidic pH (< 4.7). This assessment is conservative because Fe(II) becomes a stronger reducing agent (increasing the range of conditions under which the reaction is favorable) in the presence of Fe(III) stabilizing ligands and Fe(II) sorbing surfaces that are common in soils (Stumm & Morgan, 1996; Buerge & Hug, 1998; Struyk & Sposito, 2001).

Despite the thermodynamic favorability, Ottley *et al.* (1997) found that nitrate reduction by dissolved Fe(II) under acidic conditions was very slow ($t_{1/2} > 200$ days at pH 4), which is insufficient to explain the rapid abiotic incorporation observed by Dail *et al.* (2001) and Perakis & Hedin (2001). However, the data of Ottley *et al.* (1997) and preliminary results from our lab show that rates increase dramatically (> two orders of magnitude) in the presence of hydroxide surfaces such as $Cu(OH)_2$ (Fig. 3). Stumm & Sulzberger (1992) and Liger *et al.* (1999) have also

reported that the Fe(II) adsorbed to iron oxide surfaces is a much stronger reducing agent than is the dissolved form. The higher reactivity of the adsorbed form may be due to donation of electron density from surface OH groups. Surface-mediated Fe(II) reduction of nitrate is likely to be important in soil environments, where solid-solution interfacial area is extremely high.

Very rapid nitrate reduction by Fe(II) is also observed in systems comprising mixed valence Fe-bearing solids, particularly 'green rust' compounds (e.g. $FeII_4$ $FeIII_2(OH)_{12}SO_4 \cdot yH_2O$). Green rusts (GR) occur as metastable solids in redox fluctuating forest soils (Cornell & Schwertmann, 1996; Trollard *et al.*, 1997). We have observed that GR is the first solid to form during reaction of Fe(II) and NO_3^- in aqueous solution and that it rapidly (minutes) transforms to magnetite (Fe_3O_4) by further oxidation. Hansen *et al.* (1996, 2001) showed that the kinetics of nitrate reduction by GR compounds can exceed microbially induced rates.

Formation of nitrophenols in the presence of DOM and nitrite

Once formed, nitrite is highly reactive with DOM constituents that are commonly found in forest soil solutions. Phenolic compounds, such as extracellular soluble benzene derivatives identified from filtrates of heterotrophic fungi (Goodell *et al.*, 1997), also are present in soil solutions and can promote Fe(III) reduction as well
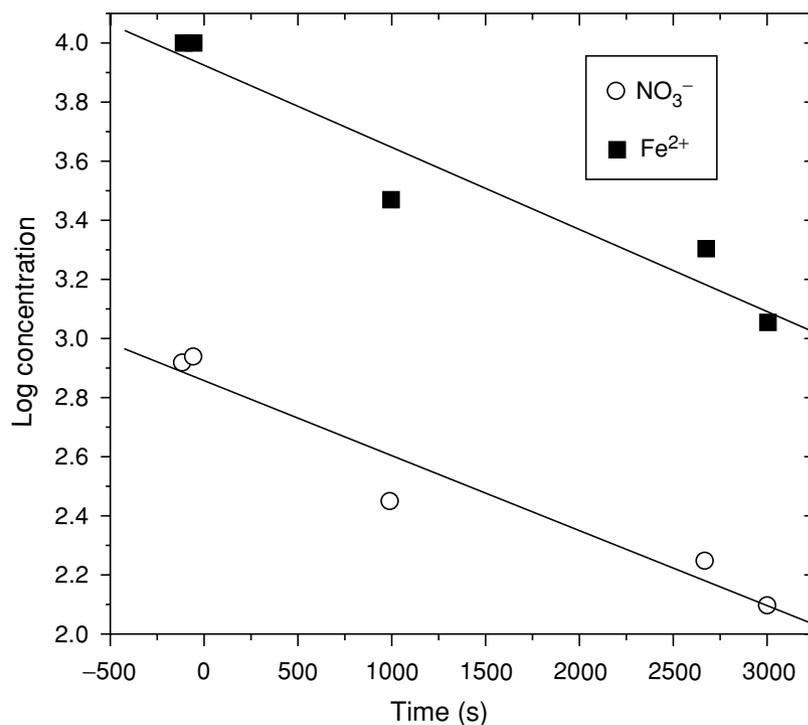

Fig. 3 Iron(II)-mediated reduction of nitrate in aqueous anoxic solution. Experiments were conducted in a He-purged vessel and glove box. The nitrate reduction was coupled to the formation of mixed valence iron oxides; Fe(II) was oxidized first to green rust and ultimately to magnetite (Fe_2O_3), a mixed valence Fe oxide. $Cu(OH)_2$ was added as a catalyst.

as nitration (addition of $-NO_2$) and nitrosation (addition of $-NO$) on aromatic structures (Azhar *et al.*, 1986). We recently confirmed that abiotic reaction of nitrite with numerous organic molecules, dominantly phenolic compounds and lignin derivatives, occurs via nitration and nitrosation of aromatic rings under conditions that are representative of acidic forest soils (Fig. 4). These experiments were carried out at pH 4.0 in 0.01 M LiCl solution. Nitrite reactivity was investigated in kinetic experiments with equimolar additions of nitrite and selected organic acids (1-naphthol, 2,3-dihydroxynaphthalene, catechol, syringic acid, vanillic acid and Harvard Forest O horizon organic matter). Time-dependent consumption of NO_2^- was monitored using flow injection analysis and transformation of model aromatic compounds was measured using high pressure liquid chromatography (HPLC). Products were further analyzed by HPLC-mass spectrometry and UV-Vis spectroscopy. Nitrite was found to be highly reactive toward phenolic compounds in soil solutions, and rates of transformation were found to be compound specific. The greatest conversion to products occurred within the first 24 h with highest rates during the first 2 h. The products formed are dominantly ortho- and para-substituted nitro- and nitroso-phenols (mass spec data not shown).

These three preliminary findings – (1) that forest floor organic matter reduces Fe(III) and Mn(IV) (hydr)oxides, releasing Fe(II) and Mn(II), (2) that Fe(II) reduces NO_3^- to NO_2^- in solution, and (3) that NO_2^- reacts with phenolic compounds present in soil solution to produce soluble nitro- and nitroso-aromatics – provides the basis for the hypothesized tight coupling of the redox cycles of nitrate/nitrite, Fe(III)/Fe(II), and organic matter (Fig. 1). We hypothesize that NO_3^- is reduced to NO_2^- in anaerobic microsites of the humus layer, that NO_2^- reacts with DOC to form DON, and that oxidized forms of Fe and Mn in soil minerals are eventually reduced again by organic acids and phenolic compounds derived from plants and microorganisms. The microsite concentrations of NO_2^- are probably very low and extremely dynamic, which may affect the reactions that are favored and the differences in products (soluble vs. insoluble organic-N) when NO_3^- and NO_2^- are added directly to the soil (Dail *et al.*, 2001). An interesting consequence of the postulated mechanism is that it can be saturated if nutrient imbalances due to high N inputs cause a decline in primary productivity, and hence a decline in the input of organic acids needed to reduce metals contained in soil minerals.

The fate of DON formed from abiotic immobilization of nitrate

Although it has been known for some time that much of the N in throughfall and soil solutions of temperate forest
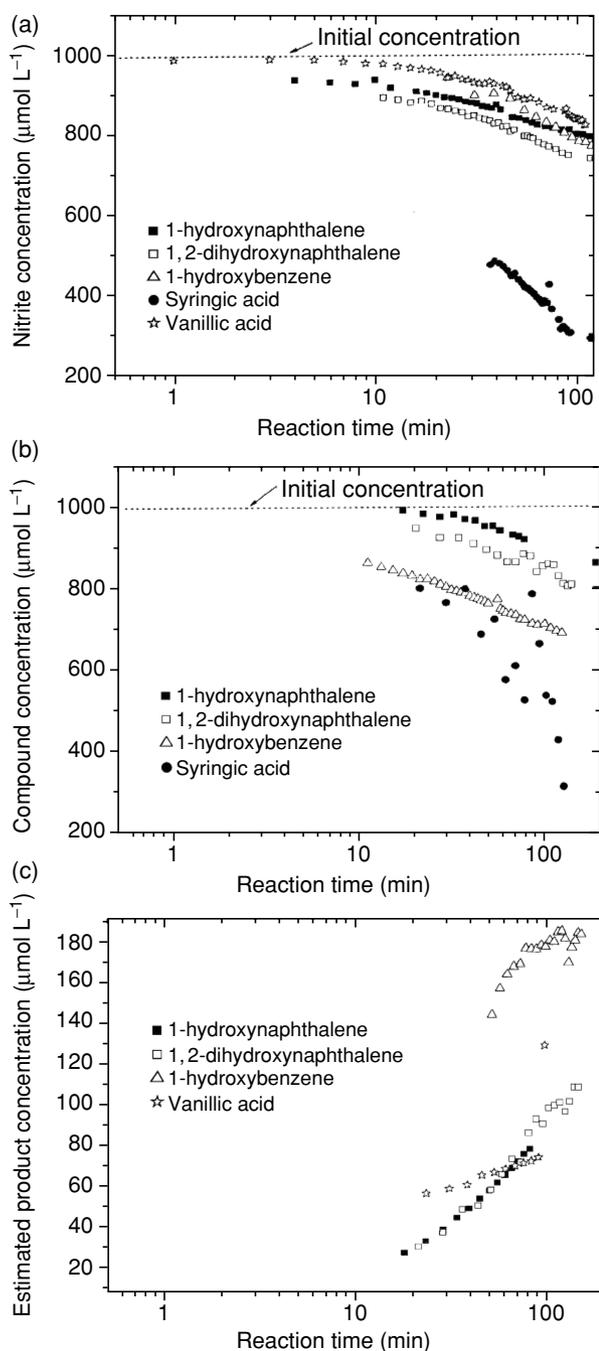

Fig. 4 Abiotic reaction of nitrite with selected phenolic compounds and low molecular weight organic acids (LMWOAs) representative of dissolved organic constituents in forest floor leachate. Loss of nitrite (a), loss of parent LMWOA (b), and formation of nitro- and nitroso-aromatic products (c) in aqueous solutions at pH 4.0, 0.01 M LiCl background and 25 °C.

ecosystems occurs as DON (Grier *et al.*, 1974; Sollins & McCorison, 1981; Chorover *et al.*, 1994; Fernandez & Adams, 2000), less is known about the fate of DON. If abiotic immobilization of nitrate is also a significant

source of DON, then the need to know more about its fate and dynamics in soils is all the more urgent. Simple amino acids and amino sugars can be directly assimilated by some plants and bacteria (Näsholm *et al.*, 1998) or deaminated to produce plant-available inorganic-N, but DON might also include small cyclic structures that are relatively stable in soils and soil solutions. Furthermore, DON may be adsorbed onto soil surfaces (Moore *et al.*, 1992; Currie *et al.*, 1996; Kaiser *et al.*, 1996) before mineralization and acquisition of the N by plants can occur. The pulse of DO^{15}N observed by Perakis & Hedin (2001) 0.1 days after adding $^{15}NO_3^-$ had mostly disappeared by the time they resampled at 1.0 days. The label may have moved into pools of soil organic matter, fine roots, or microbial biomass, all of which increased in ^{15}N enrichment between 0.1 and 1.0 day sampling points. The dominant long-term fate (1–2 years) of the label was soil organic matter, and its ultimate availability to plants was unknown. Strickland *et al.* (1992) showed that some of the $^{15}NH_4^+$ that they added to soil and that was immobilized into soil organic matter could later be released as DON by sonication, indicating that some soluble organic-N forms are physically protected in soil aggregates, but their bioavailability also remains unknown.

DON may also be leached from the soil profile (Sollins & McCorison, 1981; Qualls *et al.*, 1991; Qualls & Haines, 1992; Currie *et al.*, 1996; Marcus *et al.*, 1998), and DON is often the dominant form of N exported from many temperate forested watersheds (Hedin *et al.*, 1995; Campbell *et al.*, 2000; Goodale *et al.*, 2000; Perakis & Hedin, 2002). However, hydrologic losses of DON are a small fraction of DON production within the canopy, litter layer, and soil, indicating that the DON sink in the soil is very important. Moreover, these watershed studies show that seasonal variation of DON streamwater concentrations are typically small, suggesting a limited role of seasonally-dependent biological processes on DON production and retention and an important proximate role for abiotic processes.

Foci of needed research

Abiotic processes are at least one important pathway of DON formation, and once formed, transport, retention, and loss of DON may be largely uncoupled from proximate controls of biological processes. Ultimately, biological processes produce the DOC and reducing power that affect DON formation and the entire N cycle (Hedin *et al.*, 1995), but these biological and abiological processes may follow different temporal patterns, thus obscuring the complex linkages. The ferrous wheel hypothesis (Fig. 1), for example, includes both biological and abiological processes, but the reducing power of plant-derived organic matter may build up over seasons

and years while the abiotic reduction of nitrate and reaction of organic matter with nitrite may occur in a matter of seconds after nitrate enters the soil solution.

Laboratory and field studies are needed to test these and other hypothesized mechanisms by which NO_3^- is converted to organic-N in litter layers and soils of forests. The evidence that we present here is preliminary and incomplete, but sufficient to formulate plausible hypotheses that are worthy of further testing in numerous environments. Similarly, the fate of DON, which is increasingly recognized as central to the N cycle of many forests, deserves more attention. Whether DON is lost from the ecosystem, retained in soils, or converted to plant available form may depend in part upon how (biotic vs. abiotic) and where (canopy to soil) it is formed. Addressing societal concerns about the fates of C and N emitted into the atmosphere will require an understanding of these basic mechanisms of N transformations in forest ecosystems.

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