Deacidification, Dissolved Organic Carbon, and Nitrate Export:

Identifying the Connections

Final Report

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Deacidification, Dissolved Organic Carbon, and Nitrate Export: Identifying the Connections

Final Report

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Abstract

Nitrate (NO_3^-) concentrations in surface waters decreased across the Northeast from the 1970s and 1980s to the 1990s and early 2000s. Many hypotheses have been proposed to explain this decrease, but the cause has remained unclear. Policy and management decisions regulating anthropogenic nitrogen oxide (NO_x) emissions require improved understanding of the controls on the decadal-scale patterns of nitrogen retention and loss, patterns that cannot be fully explained at present. One control may be associated with increasing abundance of dissolved organic carbon (DOC), which in turn may be an unanticipated result of soil recovery from acidification.

A series of soil core leaching experiments was conducted to examine the role of acidification and recovery in driving the net production of DOC and NO_3^- from soils. Over 56 weeks, soil cores from an Adirondack site were leached every 1-2 weeks with simulated rainfall solutions of varying pH (3.4 to 7.0) from additions of H_2SO_4, $CaCO_3$, and NaOH. All cores leached large amounts of NO_3^- and showed acidification attributable to nitrification. The experimental treatments subsequently did induce small shifts in leachate pH (4.4 to 4.9 versus controls of 4.6). DOC had small but variable shifts in quantity and large changes in quality, in that acidified cores had a higher proportion of bioavailable DOC with isotopic composition ($\delta^{13}C_{DOC}$) indicating a decrease in contributions from lignin and similar compounds. Direct extractions of homogenized material from the cores at the experiment's conclusion showed clear decreases in DOC concentration and aromaticity with decreasing pH. These results show that realistic alterations of leachate pH can induce ecologically meaningful changes in solution DOC quantity and quality from surface soils. However, results also demonstrated that even refrigerated, intact soil cores cannot be used to simulate soil nitrogen retention dynamics because disruption of plant nitrogen uptake allows rapid nitrification and acidification.

Compared across catchments, increases in stream DOC concentration are associated with a sharp, nonlinear decrease in NO_3^-, although the mechanism for this pattern has not been explained. This study examined whether this "DOC-NO_3^- curve" results from spatial variation in forest floor C stocks and their production of DOC and immobilization of nitrogen. It also examined whether DOC and NO_3^- concentrations were inversely coupled at the scale of forest floor extracts as well as across catchments. Comparison of extract and surface water chemistry across nine catchments in the Catskill and Adirondack Mountains showed that variation in forest floor carbon stock controlled variation in DOC production and N immobilization, but did not suffice to generate the "DOC-NO_3^- curve" observed across streams. Rather, the curve was best explained by the stoichiometric constraints of labile carbon limitation on both

microbial nitrogen immobilization and denitrification. A proposed explanation for these observations is that surface soil carbon to nitrogen ratio drives DOC and NO_3^- production, sometimes concurrently, and that soil solutions must typically encounter low-oxygen conditions suitable to denitrification deeper in the hydrologic profile, such that NO_3^- is removed unless labile carbon supply limits denitrification, which serves as the master process producing the sharp increase in stream NO_3^- at low carbon levels.

Keywords

^{15}N, ^{18}O, acidification, Adirondacks, C:N ratio, Catskills, carbon-use efficiency, denitrification, dissolved organic carbon (DOC), nitrification, nitrate leaching, stoichiometry, soil carbon

Acknowledgements

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Part A: Changes in Acidity Alter DOC Quality and Quantity

A: Executive Summary

Over the last several decades, concentrations of dissolved organic carbon (DOC) have increased in surface waters across much of the Northeastern U.S. Multiple explanations for this change have been put forward, with increasing recognition of a likely role for decreased sulfate (SO_4^{2-}) loading and ecosystem recovery from acidification. An increase in DOC could potentially also stimulate uptake of nitrogen (N) by microbial immobilization or denitrification, reducing catchment nitrate (NO_3^-) losses.

A laboratory pH-manipulation experiment was conducted to examine the response of intact cores from an Adirondack soil to leaching every 1-2 weeks with solutions of simulated rainfall and H_2SO_4, $CaCO_3$, and NaOH additions of varying pH (3.4 to 7.0). All cores showed acidification attributable to large amounts of within-core nitrification, which prevented study of whether changes in DOC availability might alter NO_3^- consumption, and illustrated that even intact soil core soil manipulation studies may often include unrecognized artifacts from increased nitrogen availability or pH shifts. The applied pH-manipulation treatments induced small shifts in leachate pH (4.4 to 4.9 versus controls of 4.6), with small and variable corresponding responses of DOC concentration and large changes in the quality of leached DOC. Acidified cores produced a much larger proportion of bioavailable DOC than de-acidified cores. At the experiment's conclusion, extraction of homogenized material from the cores demonstrated clear and consistent decreases in extract DOC concentration and aromaticity with decreasing pH, in both organic and mineral soil materials. These measurements support the interpretation that observed increases in surface water DOC over the last several decades in the Adirondack Mountains and elsewhere may be attributable to soil recovery from acidification, yielding larger fluxes of DOC to deeper soils and streamwater that are less rapidly consumed by microbial processes than under acidified conditions.

1   A: Introduction

Long-term observations of surface water chemistry have revealed widespread increases in the concentrations of DOC over the last several decades across the Northeast U.S. and western Europe (Stoddard et al. 2003, Monteith et al. 2007), including in the Catskill and Adirondack Mountains of New York State (Driscoll et al. 2003, Burns et al. 2006, Lawrence et al. 2011, Strock et al. 2014), as well as in Maine (SanClements et al. 2012), eastern Canada (Couture et al. 2011), the U.K. (Evans et al., 2005), Scandinavia (Sjkelkvåle et al. 2005, de Wit et al. 2007), and the Czech Republic (Kopacek et al. 2005). Multiple competing hypotheses have been proposed to explain these trends, including climate warming (Freeman et al. 2001), rising atmospheric CO_2 (Freeman et al. 2004), and increased precipitation (Zhang et al. 2010). All of these mechanisms can affect DOC production or loss in some circumstances.

Nonetheless, there is mounting evidence for a connection between rising DOC concentrations and declining rates of atmospheric SO_4^{2-} deposition and corresponding ecosystem recovery from acidification. This evidence includes long-term records from hundreds of surface waters across the northeast US, southeast Canada, the U.K., and Scandinavia, that show strong spatiotemporal correspondence between increasing surface water DOC concentrations and declining acid inputs of SO_4^{2-} from deposition (Driscoll et al. 2003, Evans et al. 2006a, Monteith et al. 2007, SanClements et al. 2012). Field-scale experimental manipulations of soil pH have also induced changes in DOC loss to soil leachate or streamwater. Acidifying amendments of $AlCl_3$ to a Norwegian forest (Mulder et al. 2001) and H_2SO_4 to a Swedish forest (Ekström et al. 2011) produced decreases in DOC loss. Lime ($CaCO_3$) additions to forests in the Adirondacks (Driscoll et al. 1996) and in Sweden (Nilsson et al. 2001) increased DOC loss. A review of the DOC response at 12 long-term nitrogen-addition studies found correlation with the acidification response of the N additions (acidifying or deacidifying) rather than N application alone (Evans et al. 2008), and a direct comparison of experimental de-acidifying (NaOH and $MgCl_2$) and acidifying (H_2SO_4) additions to peaty soils in the U.K. produced corresponding changes in DOC loss from surface organic horizons (Evans et al. 2012).

The proposed explanation for these changes in DOC loss typically focus on the geochemical mechanism of altered DOC solubility (Evans et al. 2006a, Monteith et al. 2007), expecting an increase in solubility with increased soil pH (e.g., Krug and Frink 1983, Kalbitz et al. 2000, Ekström et al. 2011). Kopacek et al. (2013) also proposed two additional mechanisms: first, that plants could increase their carbon allocation belowground to roots, mycorrhizae, and root exudates of DOC as they recovery from acidification and as N deposition decreases (e.g., Treseder 2004); and second, that decreased availability of SO_4^{2-} and NO_3^- to serve as electron acceptors for decomposers under low-O_2 conditions could lower DOC consumption by sulfate reduction or denitrification.

The Adirondack Mountains of New York State have received elevated rates of SO_4^{2-} deposition for at least half a century. However, rates of SO_4^{2-} deposition have been declining for the last several decades, and surface water SO_4^{2-} concentrations have decreased, with concurrent increase in surface water pH and DOC concentrations (Driscoll et al. 2003, Lawrence et al. 2011, Strock et al. 2014). These increases in DOC loss may have a range of ecosystem consequences, including potential effects on soil formation, aquatic food chains, light penetration into lakes, and need for water treatment. Past work proposed that increased DOC availability could also potentially impact NO_3^- loss to surface waters if the increased supply of DOC fuels microbial N immobilization or provides substrate to carbon (C)-limited denitrifiers (Goodale et al. 2005).

To examine the role of soil acidification and de-acidification in driving changes in solution DOC quality and quantity for an Adirondack forest soil, and to examine whether these changes in DOC correspond with changes in soil NO_3^- loss, we conducted a laboratory pH-manipulation experiment using intact soil cores collected from the western Adirondack region. We expected that cores leached with acidifying solutions simulating historic and experimentally elevated acid deposition would respond with increased loss of SO_4^{2-} and base cations, and that the corresponding pH suppression would depress leaching of DOC. By contrast, we expected that leaching with de-acidifying solutions should increase DOC loss. Further, we expected the aromatic (humic) fraction of DOC to be particularly sensitive in responding to pH increases, such that a pH decrease should increase the bio-availability of the leached DOC. If these changes in DOC supply stimulated NO_3^- immobilization or denitrification, the acidified cores should have increased NO_3^- losses and the de-acidified cores should have reduced NO_3^- loss.

2 A: Methods

2.1 Site Description and Field Methods

Soil cores were collected from the catchment of Woods Lake (43° 52' N, 74° 57' W, 605 m elevation), in the western portion of New York's Adirondack Park. Parts of the Woods Lake catchment received an experimental lime ($CaCO_3$) addition in 1989 (Driscoll et al. 1996, Melvin et al. 2013), but the soil cores for this experiment were collected from the lower portion of an unlimed control sub-catchment (C2 from Melvin et al. 2013 or V in Driscoll et al. 1996). Forest composition consisted of American beech (*Fagus grandifolia*), red maple (*Acer rubrum*), and yellow birch (*Betula alleghaniensis*), with minor contributions of red spruce (*Picea rubens*), sugar maple (*Acer saccharum*), and striped maple (*Acer pensylvanicum*) (Smallidge and Leopold 1994, Melvin et al. 2013). Soils were highly organic Orthod Spodosols (Smallidge and Leopold 1994) developed in sandy glacial till deposited over hornblende granitic gneiss bedrock (April and Newton 1985). The National Atmospheric Deposition Program (NADP) program reports deposition rates of 4.7 – 7.6 kg S ha^{-1} yr^{-1} and 4.3 – 8.6 kg N ha^{-1} yr^{-1}, and 134 – 142 cm yr^{-1} of precipitation for the western Adirondacks during 2004-2008 (Table 1).

In October, 2009, 25 intact soil cores were collected in five blocks (A-E) of five adjacent soil cores. Pipes 7.3 cm in diameter and 30 cm long were pounded into the soil with a rubber mallet until flush with the top of the forest floor. The surrounding soil was then excavated to extricate whole cores intact. Cores were transported on freezer packs to Ithaca, NY and then refrigerated at 4 ^{o}C.

2.2 Laboratory Experiment

From November 2009 until December 2010 (13 months), soil cores were leached weekly with 120 mL of various solutions for 34 weeks, then biweekly with 240 mL of solution for another 18 weeks, amounting to 149 cm of solution input per year, similar to annual precipitation input (Table 1). For the first three weeks, all 25 cores received a common simulated rain solution during a pretreatment period. Experimental acid-manipulation treatments were imposed from week 4 until week 38, by which time relatively stable pH shifts had developed. A range of additional measurements of DOC quality, described below, were conducted on extracts collected during weeks 34 to 38. From week 40 to 56, all cores again received the simulated rain solution to examine solution responses during a recovery period. At each leaching event, treatment solutions were poured onto the top of each core and allowed to drain by gravity into glass beakers. Between leaching events, the cores were covered with air-permeable plastic to minimize water loss, and refrigerated at roughly 4 ^{o}C to minimize microbial activity.

Table 1. Five-year (2004-2008) mean precipitation volume, pH, and solute chemistry (μmol/L) for Adirondack National Acid Deposition Program (NADP) monitoring sites near Woods Lake, NY

	Latitude, Longitude	Precip. (cm/yr)	pH	SO_4^{2-}	Cl^-	NO_3^-	NH_4^+	Ca^{2+}	Mg^{2+}	Na^+	K^+
Moss Lake	43° 47'N, 74° 51'W	134	4.6	11.2	1.7	14.4	10.1	2.0	0.4	1.1	0.3
Bennett Bridge	43° 37'N, 75° 57'W	142	4.5	15.9	2.8	21.8	15.8	3.0	0.8	2.1	2.1

http://nadp.sws.uiuc.edu/sites/sitemap.asp?state=ny

For all five blocks of five cores, one core was randomly assigned to each of five treatments: simulated rain (control), low H_2SO_4 (low S), high H_2SO_4 (high S), NaOH, and $CaCO_3$ (Table 2), yielding five replicate cores of each treatment. The simulated rain was designed to resemble mean precipitation chemistry measured by the closest National Atmospheric Deposition Program (NADP) monitoring site at Moss Lake (NY29) for 2004-08 (Table 1). The low H_2SO_4 treatment (31 kg S ha^{-1} yr^{-1}) simulated a rough doubling of the S deposition received in the western Adirondack region in the early 1970s, estimated by back-extrapolating observed trends in SO_4^{2-} concentration measured at Bennett Bridge (NY52) for its period of record of 1980 to 2008 (-1.2 μmol L^{-1} yr^{-1}). At Hubbard Brook, NH – one of the very few sites with measurements prior to the 1980s – precipitation SO_4^{2-} concentrations declined roughly linearly from the mid-1960s to the mid-1990s (Likens et al. 1996). The high sulfur (S) treatment represented an experimentally accelerated S treatment (107 kg S ha^{-1} yr^{-1}), and is similar to S deposition rates in the U.K. in the 1970s (Evans et al. 2012). Two treatments (NaOH, $CaCO_3$) intended to increase soil pH provided equimolar changes from background conditions as the high S addition (Table 2). For context, the $CaCO_3$ treatment echoed the whole-catchment liming field experiment at Woods Lake in 1989 but at only 4% of its application rate (~300 versus 6,900 kg/ha). The NaOH treatment provided a similar direction of pH forcing as the $CaCO_3$ treatment but was half its ionic strength and lacked any direct effects of calcium (Ca).

At the conclusion of the experiment, the soil cores were separated into organic and mineral horizons measured for C, N, and S content and δ^{13}C, and a last batch extraction was conducted (see next section).

Table 2. Prepared solution characteristics for the laboratory core leaching experiment (µmol/L)

	pH	SO_4^{2-}	Cl^-	NO_3^-	NH_4^+	Ca^{2+}	Mg^{2+}	Na^+	K^+
Simulated Rain	4.7	11.1	30	13	8.7	1.5	0.8	3.5	0.3
Low H_2SO_4	4.0	65 (+54)	30	13	8.7	1.5	0.8	3.5	0.3
High H_2SO_4	3.4	215 (+204)	30	13	8.7	1.5	0.8	3.5	0.3
$CaCO_3$	7.0	11.1	30	13	8.7	205 (+204)	0.8	3.5	0.3
NaOH	7.0	11.1	30	13	8.7	1.5	0.8	207 (+204)	0.3

2.3   Analytical Methods

Leachate solutions were filtered on collection with glass fiber filters to 0.7 µm (Whatman GF/F), or to 2.7 µm (Whatman GF/D) for assays of DOC bio-availability. Extracts were immediately analyzed for pH with an Accumet basic AB15 pH meter with a flushable junction soil probe. Refrigerated samples were analyzed within three days for DOC and total dissolved N (TDN) on a Shimadzu TOC-V$_{CPN}$. Leachate subsamples were frozen for later cation and anion analysis run separately with a Dionex ICS-2000 ion chromatograph.

After the treatments had induced pH shifts and appeared to stabilize, DOC quality was assessed with three sets of additional measurements on extracts from weeks 34-38 (Marschner and Kalbitz 2003). First, bioavailable DOC (bDOC) was quantified for solutions from week 34 as the decrease in DOC concentration in coarsely filtered (2.7 µm) extracts over 7 and 33 days of incubation in amber glass bottles stored at room temperature in the dark (McDowell et al. 2006). Sixty mL subsamples were removed on each date, then filtered to 0.7 µm and analyzed for DOC. Second, specific UV absorbance (SUVA$_{254}$), an index of DOC aromaticity (Weishaar et al. 2003), was quantified for solutions from week 38 using a Beckman Coulter DU 640 spectrometer with a 1-cm diameter cuvette to measure solution absorbance at 254 nm wavelength, then dividing by DOC concentration (as mg/L). Last, DOC isotopic composition ($\delta^{13}C_{DOC}$) was measured for solutions collected on week 36. Lignin and lipids tend to be isotopically lighter (more ^{13}C depleted) than cellulose and amino acids (Kaiser et al.2001). The $^{13}C_{DOC}$ analyses were conducted at the University of California at Davis' stable isotope facility, which used off-line acidification and sparging, then combustion (O.I. Analytical Model 1030 TOC analyzer, College Station, TX) and an interface (GD-100 Gas Trap; Graden Instruments) to an isotope ratio mass spectrometer (IRMS; PCZ Europe 20-20 IRMS; Sercon Ltd., Cheshire, UK), with overall precision of 0.4‰.

At the conclusion of the leaching experiment (week 56), the soil cores were separated into organic and mineral horizons. Soils were sieved to pass a 5.6-mm (organic) or 2-mm (mineral) mesh, weighed, and subsampled for moisture content. Additional subsamples were dried and pulverized using a Retsch Mixer ball mill type MM200, then re-dried and analyzed for total C, N, and S content via combustion with an Elementar vario-EL-III (Hanau, Germany) elemental analyzer at Cornell University. Subsamples of the pulverized soils were sent to the Cornell Stable Isotope Lab (COIL) for analysis of soil ^{13}C composition using combustion (Carlo Erba NC 2500) and an IRMS (Finnigan MAT Delta Plus). All ^{13}C measurements were expressed using customary delta (δ) notation, as the ratio of ^{13}C to ^{12}C in the sample (R_{sample}) relative to the internationally accepted standard ($R_{standard}$) for carbon (PeeDee Belemnite), divided by $R_{standard}$ and multiplied by 1,000 to yield units of per mil (‰).

2.4   Batch Experiment

A final batch extraction was conducted on material from the organic and mineral horizons from the five cores that had received only simulated rainfall for the duration of the experiment. For each of the five cores, 20 g subsamples of field-moist material from both horizons were extracted with 200 mL of each of the treatment solutions (Table 2). This approach is similar to the 1:10 batch extraction approach used by Clark et al. (2011) to examine the DOC response to SO_4^{2-} and salt amendments to peaty soils from the U.K. These extracts were filtered through ashed 0.7-μm glass fiber filters (Whatman GF/F), then analyzed for pH, DOC, TDN, major ions, and $SUVA_{254}$ as previously described. Solution concentrations were computed per unit dry soil mass, and changes in solution chemistry due to the pH manipulations were expressed as the difference from the control (simulated precipitation) extractions.

3 A: Results

3.1 Soil Characteristics

On average, the soil cores were carbon-rich, containing a large forest floor (7.4 kg C m^{-2}) with a relatively low C:N ratio (20.2) (Table 3). As observed previously (Melvin et al. 2013), soils at this site showed the unusual pattern of a higher C:N ratio in the mineral soil (24.4) than the forest floor. Soil C stocks and C:N ratios varied by block but did not vary by assigned treatment, except that the cores randomly assigned to the CaCO$_3$ amendments had slightly higher C:N ratio in the mineral soil (26.2) than the other treatments.

Table 3. Mean (± standard error) element concentrations and stocks of the study soil cores

	%C	%N	%S	C:N Ratio	$\delta^{13}C$ (‰)	N Stock (kg/m^2)	C Stock (kg/m^2)
Organic	34.6 ±2.3	1.71 ±0.11	0.21 ±0.02	20.2 ±0.3	-26.6 ±0.1	0.36 ±0.03	7.41 ±0.74
Mineral	9.9 ±0.6	0.41 ±0.03	0.06 ±0.004	24.4 ±0.5	-26.0 ±0.1	0.22 ±0.02	5.47 ±0.54

3.2 Intact Core Leachate Response

During the three weeks prior to the initiation of the pH-manipulation treatments, leachate pH decreased sharply for all of the intact cores from an average of 5.2 to 4.6, with a corresponding increase in hydrogen ion concentration of 20-30 μmol/L (Figure 1). The pH decline continued through week 8-10, even as some of the treatments received de-acidifying pH manipulation solutions. Mean NO_3^- concentrations rose during this period by roughly 150 μmol/L from an already-high average of 390 μmol/L up to 540 μmol/L. If these high NO_3^- concentrations were produced by nitrification within the cores, this acidifying process more than sufficed to produce the observed pH drop (H^+ increase), as the stoichiometry of nitrification yields two H^+ for every molecule of NO_3^- produced (cf. van Breemen et al. 1982).

Treatment-induced divergence in leachate pH occurred between weeks 15 and 20, and stabilized around week 30, with relatively small pH effects (Figure 1). Both of the H$_2$SO$_4$ treatments yielded leachate with mean pH values of 4.4 compared to 4.6 in the controls and 4.9 of the CaCO$_3$ treatment. The NaOH treatment was slower to respond, rising to a pH of 4.8 only in the last month of pH-manipulation. Leachate SO_4^{2-} concentrations in the two H$_2$SO$_4$ treatments rose sharply in response to treatment, and stabilized at 227 and 82 μmol/L, or roughly similar or slightly higher concentrations as received in their

leachate solutions, by week 20-25. Leachate sodium concentrations rose more slowly in the NaOH treatment, but stabilized near application concentration within the last month of application. By contrast, calcium concentrations did not increase in response to the $CaCO_3$ additions. Both calcium and magnesium concentrations increased in response to the two H_2SO_4 treatments, with both cations rising by 40-50 μmol/L in the low-S and 80 μmol/L in the high-S treatment (Figure 1).

DOC concentrations varied considerably across replicate cores from the start of the experiment, and the cores randomly assigned to receive the $CaCO_3$ treatment had higher DOC concentrations than the rest of the cores (Figure 1). Overall, across all treatments, DOC concentrations generally decreased steeply over the first 5-10 weeks of the study, then continued to decrease slowly over the duration of the experiment, such that DOC concentrations at the end of the experiment averaged roughly 60% those at the start. Because of these long-term trends and core-to-core heterogeneity, clear treatment effects on DOC were difficult to discern. DOC concentrations in cores receiving the $CaCO_3$ treatment showed a trend toward a rise over weeks 10 and 20, then decreased over time (Figure 1). Other treatment-induced changes in DOC concentration were difficult to discern when examined over time (Figure 1), but emerged when comparing DOC concentrations versus pH for the first 38 weeks of study, averaged by treatment, and normalized relative to core carbon content and for DOC levels during weeks one and two, prior to the pH-manipulation treatments (Figure 2). These normalizations partly corrected for variation in background DOC levels. No attempt was made to correct for the general decrease in DOC yield over time, which contributes to variation in the response of DOC to pH that nonetheless indicated greater DOC loss with increased leachate pH and a slight suppression of DOC loss in the acidification treatments (Figure 2).

During the "recovery" phase (weeks 40-56), when all cores were leached with simulated precipitation, leachate SO_4^{2-} concentrations dropped steeply in the cores that had received the H_2SO_4 treatments, reaching control levels by week 52, with leachate calcium and magnesium concentrations that fell correspondingly (Figure.1). In the NaOH treatments, leachate sodium concentrations dropped abruptly, but remained elevated above control values through the end of the study. Leachate pH values in all of the pH-manipulation treatments converged toward control values, but trended in the direction of their pH treatment through week 56 at the end of the study. DOC continued to decline slightly throughout the recovery phase, obscuring any pH-driven changes in DOC solubility. Nitrate concentrations remained high through the end of the experiment, especially in the low-H_2SO_4 and NaOH cores (Figure 1).

The pH-manipulation treatments induced some shifts in DOC quality by week 34-38 (Figure 3 and Figure 4). DOC bioavailability varied significantly by treatment in both 7- and 33-day DOC incubations (Figure 3), DOC bioavailability decreased strongly with increasing pH (Figure 4). $SUVA_{254}$ trended toward an increase (Figure 4B) in that the most-acidified high-H_2SO_4 samples were the most bioavailable and had the lowest $SUVA_{254}$ values, while the NaOH and $CaCO_3$ treatments were the least bioavailable with somewhat higher $SUVA_{254}$ values. There was little direct relationship between $\delta^{13}C_{DOC}$ and pH, but when compared across similar DOC concentration, the de-acidification treatments contained isotopically lighter $\delta^{13}C_{DOC}$ (Figure 4C and Figure 4D) which can indicate a larger proportion of lignin or waxes in these samples. Across all cores, mean $\delta^{13}C_{DOC}$ values (-29.7‰, Figure 4D) were considerably lighter than the forest floor or mineral soil $\delta^{13}C$ they were leached from (-26.5 and -26.0‰, Table 4).

The batch extraction experiment (Figure 5) used the various pH-manipulation solutions (Table 2) to extract homogenized replicate subsamples of the five cores that had received only simulated precipitation for the duration of the study, with separate extractions for forest floor and mineral soil material. These extractions showed that the two H_2SO_4 treatments suppressed extract DOC concentrations by up to 30% for forest floor material (Figure 5A and Figure 5B) and by nearly 50% for mineral soil (Figure 5C and Figure 5D). The de-acidification treatments had divergent responses, as NaOH substantially increased extractable DOC concentrations with small increases in pH, while $CaCO_3$ showed little change in extractable DOC concentrations with modest pH increases. Differences in extractable NO_3^- between the pH-manipulations and controls did not correspond to these observed differences in DOC (forest floor $R^2 = 0.09$; mineral soil $R^2 < 0.01$; not shown). Measurements of $SUVA_{254}$ indicated that the quality of DOC extracted from forest floor material varied with pH, with less aromatic material extracted at lower pH (Figure 5E and Figure 5F).

Figure 1. Time series of leachate chemistry

Time series of leachate chemistry (mean + standard error, 5 cores per treatment), after leaching with a common simulated rainfall for 3 weeks, imposing a range of pH manipulation treatments (Table 2) during weeks 4 to 38 (grey box) then again leaching with the simulated rainfall.
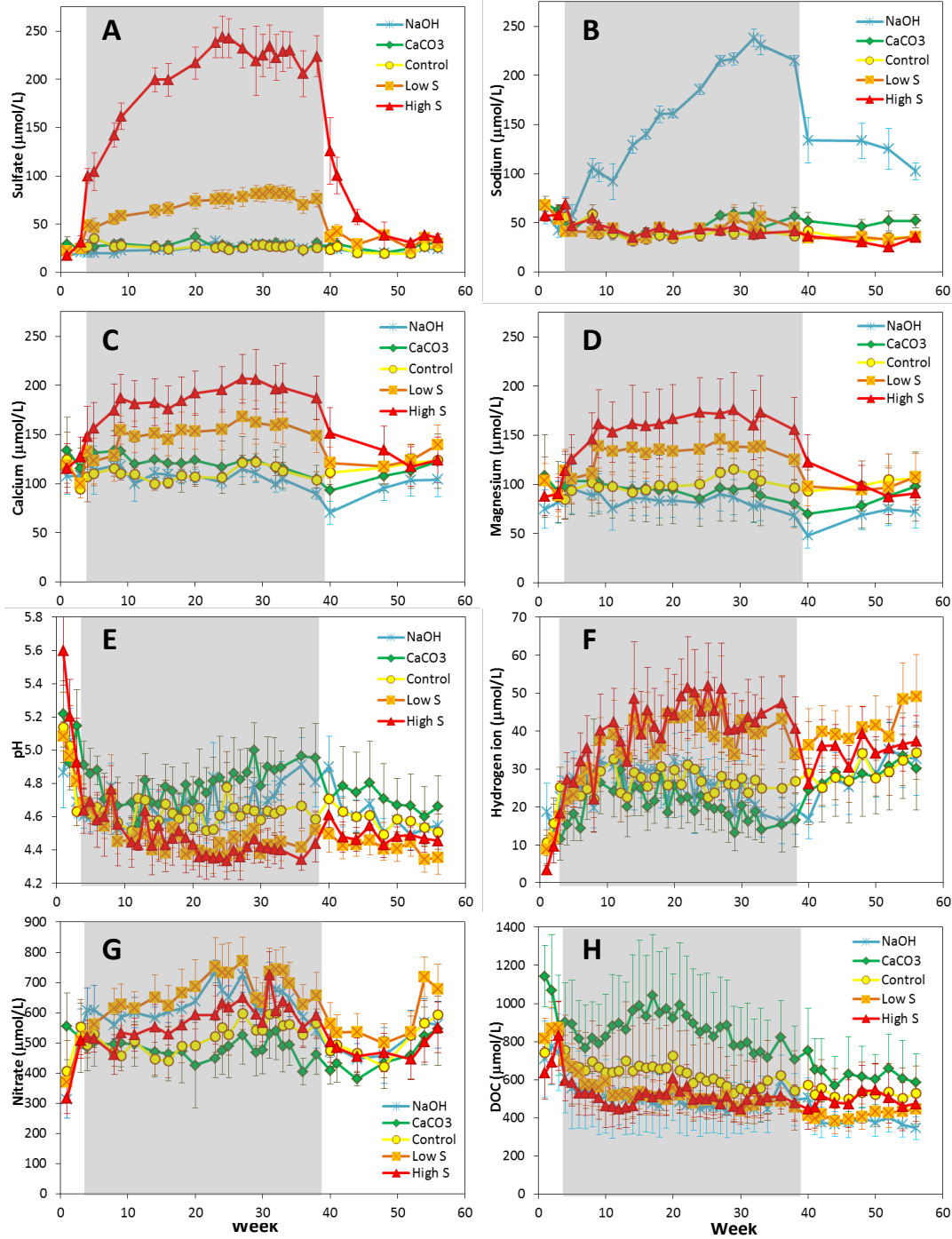
Figure 2. DOC versus leachate pH over weeks 1-38

DOC concentrations are expressed per gram total carbon content in each core and normalized relative to starting values prior to the pH-manipulations. Values are averaged by treatment type (n = 5 per treatment).
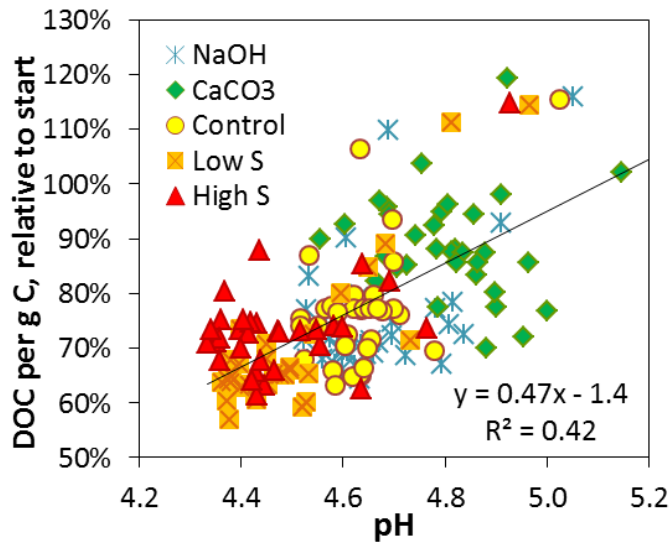
Figure 3. Proportion of leachate DOC lost over a 7-day (left) or 33-day (right) incubation to assess bioavailable DOC (bDOC), with means compared by analysis of variance (n = 5 per treatment)
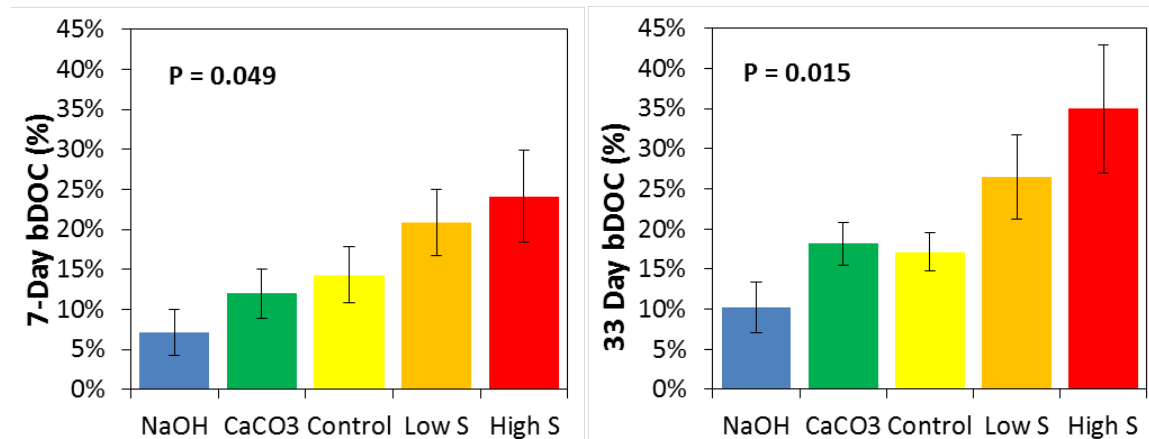
Figure 4. Effects of pH manipulation on DOC quality at 34-36 weeks, measured as (A) % of DOC lost over 33 days (bDOC), (B) SUVA$_{254}$, and (C and D) $\delta^{13}C_{DOC}$
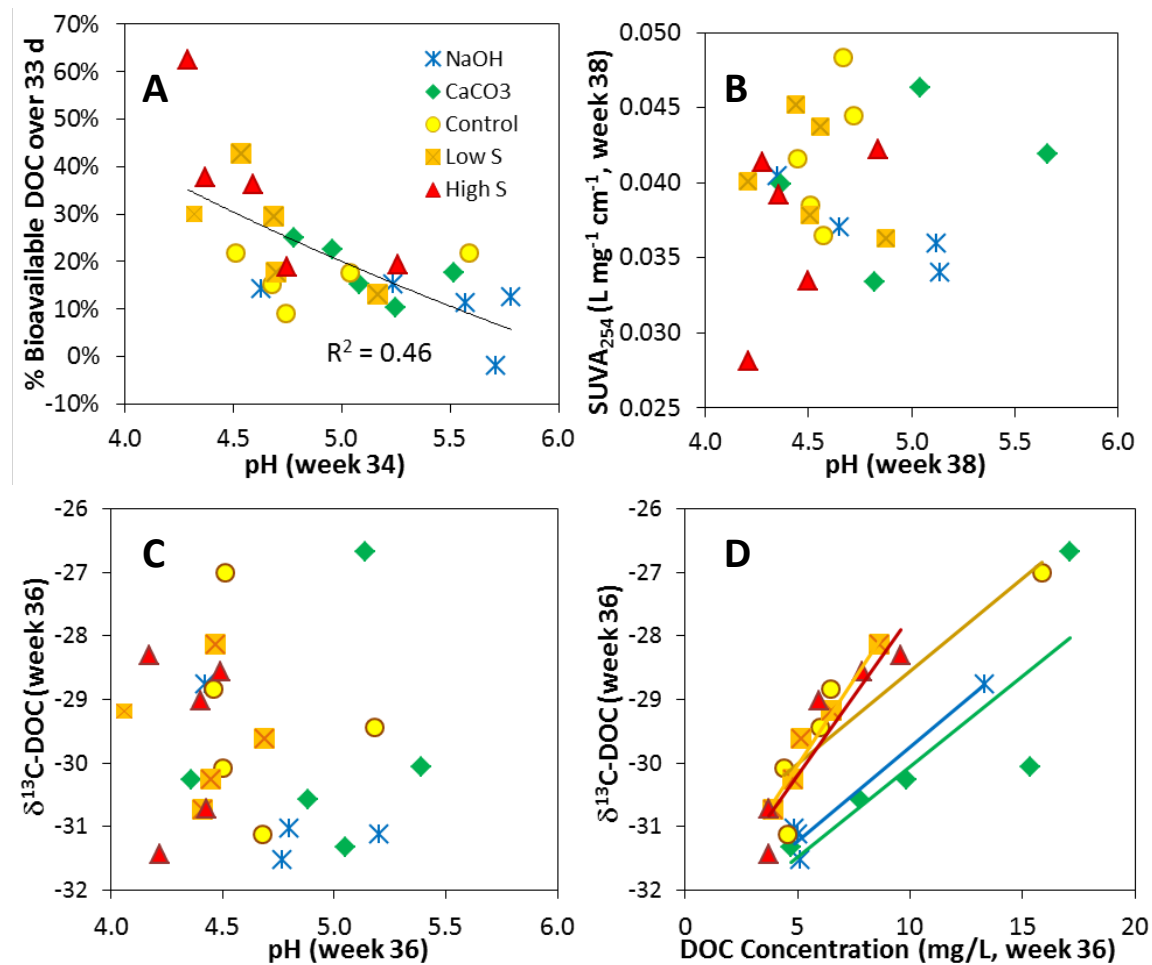
Figure 5. Response of DOC quantity (A-D) and quality (E, F) to extractions with varying solutions (Table 2), expressed as both absolute measurements (A, C, F) and relative to controls (B, D, F)
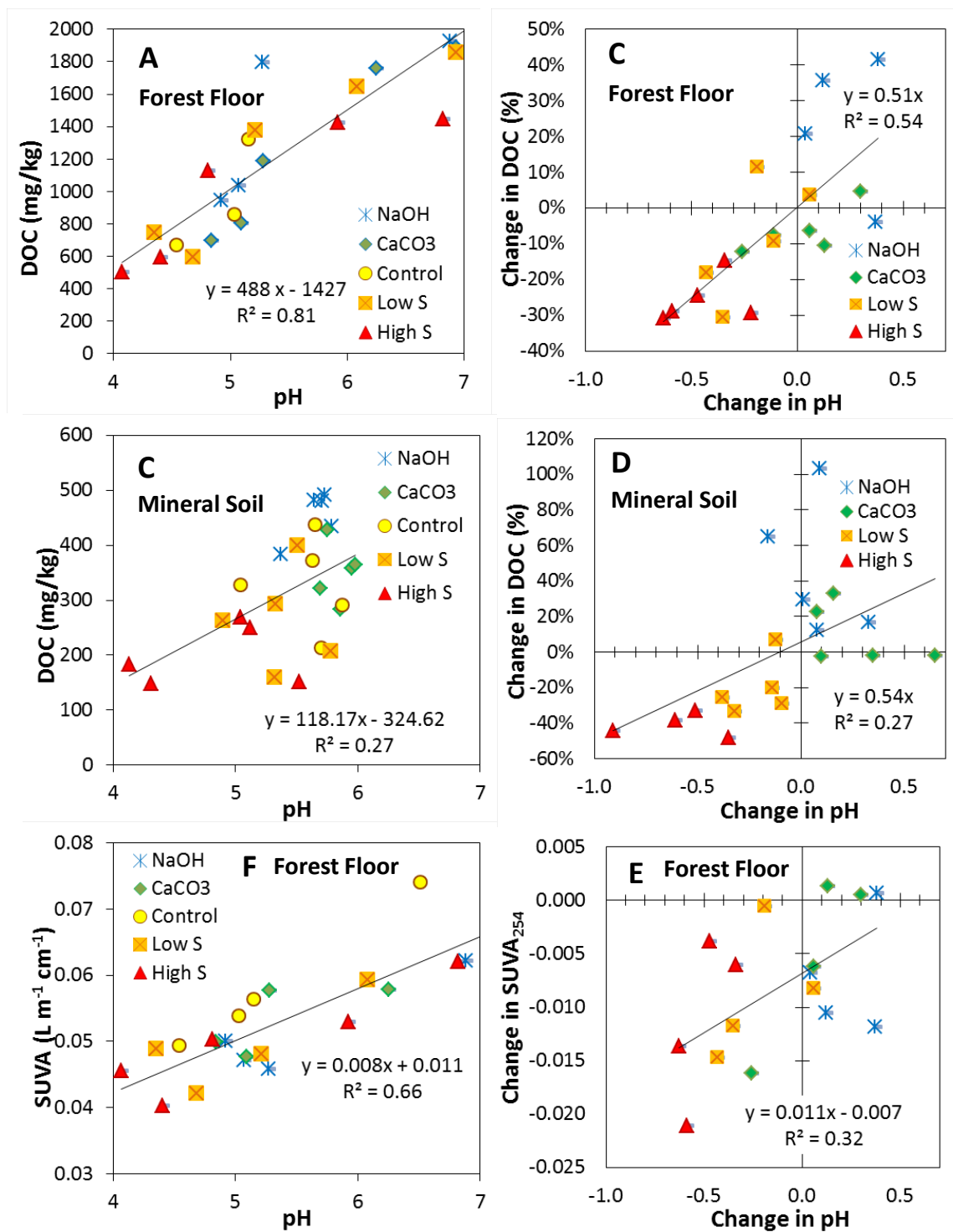
4 A: Discussion

Large reductions in SO_4^{2-} deposition to the Northeast U.S. and much of northern Europe has led to decreases in surface water SO_4^{2-} concentrations, often accompanied by decreases in base cation concentrations and increases in pH and DOC concentration (Driscoll et al. 2003, Evans et al. 2006a, Monteith et al. 2007, Strock et al. 2014). These increases in surface water DOC are now often attributed to changes in DOC solubility resulting from increased soil pH, although a range of alternative hypotheses have been proposed (Evans et al. 2006a, 2012, Monteith et al. 2007, SanClements et al. 2012). In addition, although DOC can form an important substrate for microbial decomposers, relatively few studies to date have examined how changes in ecosystem SO_4^{2-} input and acidification status have affected DOC quality or how these changes in DOC quantity or quality might impact soil N retention.

4.1 Acidification Effects on DOC Quality and Quantity

These soil core leaching and extraction experiments demonstrated that small changes in leachate pH can correspond with ecological meaningful changes in leachate DOC quantity and large changes in DOC quality. In the long-term leaching experiment, acidity-induced changes in leachate DOC concentration were difficult to discern because of core-to-core heterogeneity in background DOC leaching levels, and because all cores showed decreases in leachable DOC over time. Although cores were refrigerated between leaching events, some microbial activity continued and DOC supplies may have been gradually depleted throughout the duration of the experiment. Yet, the batch extractions of five sets of homogenized forest floor and mineral soil materials greatly reduced problems of pre-treatment heterogeneity, and demonstrated large reductions in extractable DOC with decreasing pH even after a year of treatment. Both the forest floor and mineral soil showed ~50% changes in extractable DOC concentration for every 1.0 pH unit shift (Figure 4B and Figure 4D). These extracts also showed large decreases in SUVA$_{254}$ values with decreasing pH, indicating that the DOC extracted at low pH contained fewer aromatic compounds. These results are consistent with a similar batch-extraction experiment using peat and organic horizon material from the U.K., which found large decreases in extract DOC concentration with decreasing pH, but that the magnitude of these responses varied by sample (Clark et al. 2011). Acidification also drove decreased SUVA$_{254}$ values in the U.K. study with relative patterns nearly identical to those observed here (Figure 5F), and were interpreted as evidence that the humic aromatic fraction of DOC is especially responsive to pH change (Clark et al. 2011).

Two recent field acidification studies demonstrated similar responses. In the U.K., three years of H_2SO_4, NaOH, and $MgCl_2$ amendments to four U.K. peat or peaty podzol sites induced changes in DOC concentration in shallow lysimeter leachate that corresponded directly with induced changes in pH (Evans et al. 2012). In a small-plot field H_2SO_4-addition experiment in a Swedish forest, acidification reduced the concentrations of DOC leached from the forest floor, and produced DOC that was less colored, less aromatic ($SUVA_{260}$), less hydrophobic, and of lower molecular weight than in low-acid plots (Ekström et al. 2011). Conversely, fluorescence spectroscopy indices of DOC quality in Maine lakes recovering from acidification show that lakes with increasing DOC concentrations contain increasing proportional contributions of DOC from litter and soils rather than of microbial origin (SanClements et al. 2012). Similar changes in lake DOC quality were detected using fluorescence measurements of archive samples from a whole-lake acidification experiment in Ontario (Donahue et al. 1998). This suite of measurements support interpretations that ecosystem acidification particularly affects the solubility of aromatic and hydrophobic fractions of DOC, which are expected to be more resistant to both decomposition and photochemical degradation (Donahue et al. 1998, Marschner and Kalbitz 2003, Ekström et al. 2011, SanClements et al. 2012).

The DOC incubations showed that small decreases in leachate pH produced large increases in the bioavailability of the DOC that was leached (Figure 4). On average, 35% of the DOC leached from the most acidic cores was bioavailable over 33 days, roughly twice the proportion lost from control cores (17%), while only 10% of the DOC leached from the NaOH-amended cores was bioavailable (Figure 3). These large changes in DOC bioavailability mean that DOC leached to soil solutions in acidified ecosystems is likely to be more completely consumed by the time these solutions reach deep soils or streamwater. These shifts could contribute to C-limitation of biogeochemical processes in these receiving ecosystems.

4.2   Connections to Nitrate Leaching

Previous work hypothesized that increasing supplies of DOC through de-acidification might fuel increased uptake of NO_3^- by microbial immobilization or denitrification (Goodale et al. 2005). Thus, NO_3^- core leachate concentrations were expected to decline with CaCO3 or NaOH driven de-acidification during the long-term core incubation. However, these relatively small potential changes in DOC-driven NO_3^- consumption could not be examined because all of the cores appear to have experienced massive increases in NO_3^- supply. From the start of the leaching treatments, one month after the soil cores were collected, NO_3^- concentrations rose from 400 to over 600 μmol/L, exceeding 800 μmol/L in some samples (Figure 1). By contrast, field measurements of NO_3^- in soil water at the Woods Lake sub-catchment where

these soils were collected average < 100 μmol/L (Geary and Driscoll 1996). The NO_3^- was likely supplied by nitrification within the intact cores, as nitrifiers used NH_4^+ produced by continuation of microbial N mineralization concurrent with the cessation of plant uptake when the cores were collected. Even though these cores were kept intact and refrigerated between leaching events, their low C:N ratio (averaging 20.2 in the forest floor) may have made them especially vulnerable to these nitrification responses. Net nitrification in northeastern forest soils typically increases sharply as C:N ratios decrease below ~20-23 (Aber et al. 2003). Classic field experiments show that NO_3^- losses increase tremendously after experimental cessation of plant N uptake by harvest and herbicide or root-trenching (Bormann and Likens 1979, Vitousek et al. 1979). However, this well-known field response is rarely considered in the vast number of studies that use soil cores in the lab for a wide range of experimental purposes. Many of the results from these lab studies might be called into question due to potential interference from greatly increased N availability, and from the acidification that comes along with nitrification.

Nitrification within the intact cores not only prevented examination of DOC effects on NO_3^- consumption, it also complicated the application of the pH treatments in the long-term core leaching study. That is, the process of nitrification generates 2 mol H^+ per 1 mol of NO_3^- produced ($NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$) (van Breeman et al. 1982). Thus, the roughly 200-400 μmol/L increase in leachate NO_3^- concentrations in the cores over background conditions could have produced 400-800 μmol/L of acidity. This acidification more than sufficed to explain the pH drop in all cores prior to the initiation of the pH-manipulation treatments (Figure 1). The magnitude of this potential acidification effect rivaled that imposed by the high-H_2SO_4 treatment, and varying amounts of NO_3^- production in different cores further complicated detection of effects of the various pH- manipulation treatments. Cores receiving the NaOH and low-H_2SO_4 treatments both had especially large increases in NO_3^- over the course of the experiment (Figure 1), which could have perhaps accelerated the intended acidification in the low H_2SO_4 cores to nearly match the high-H_2SO_4 treatment and suppressed the intended pH response to NaOH.

4.3   Conclusions

Nitrification greatly complicated the implementation and interpretation of the intact-core pH-manipulation study, and prevented examination of whether increased DOC supply might have stimulated NO_3^- consumption processes. These complications likely apply to many if not most soil core-based studies of N cycling or acidification processes, as well as studies of microbial processes that may depend on these conditions, thus calling these prior studies into question and highlighting the need for field-based studies in intact ecosystems. Yet, above and beyond these complications, the soil core pH-manipulation experiment and the subsequent batch extraction experiment demonstrated that solubility of DOC from Adirondack soils decreased with decreasing pH, and that the DOC that remained in solution was composed of highly bioavailable forms of carbon. As these Adirondack ecosystems recover from historic acidification, these patterns should reverse, with increased soil production of DOC containing a higher proportion of more aromatic material that may persist as these solutions flow into deeper soils or surface water where this DOC could contribute to processes such as podsolization, light attenuation and stratification in lakes, and eventually as a possible low-grade source of C to otherwise C-limited microbes.

Part B: The Stoichiometry of Nitrification and Denitrification Drive Inverse DOC-Nitrate Loss Patterns in Forested Catchments

B: Executive Summary

Nitrate loss from forested catchments varies greatly across sites and over time, with few reliable correlates. One of the few recurring patterns, however, is the negative nonlinear relationship that occurs regularly between surface water nitrate (NO_3^-) and dissolved organic carbon (DOC) concentrations: that is, NO_3^- declines sharply as DOC concentrations increase, and high NO_3^- levels occur only at low DOC concentrations.

Several hypotheses have been proposed to explain this pattern, but its cause has remained speculative. It is broadly attributed to probable carbon or nitrogen limitation of biological processes such as microbial immobilization or denitrification, but the identity of which biological process or the main landscape position of their activity is not known. This study examined whether DOC and NO_3^- loss are both driven by surface soil C content, at scales of both forest floor blocks and across catchments, by measuring forest floor extract and surface water chemistry across nine catchments selected from long-term monitoring networks in the Catskill and Adirondack Mountains.

Forest floor C and N content, and water-extractable NO_3^- and DOC were measured to examine whether spatial variation in stocks of forest floor C partly controls DOC and NO_3^- loss from forested catchments. A subset of extract and streamwater samples were also measured for DOC quality ($SUVA_{254}$, bioavailability, $\delta^{13}C$-DOC) and NO_3^- isotopic composition ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$). These measurements showed that forest floor C stock drives DOC production and that its C:N ratio controls NO_3^- production, reflecting microbial stoichiometry and shifting carbon-use efficiency. Isotopic measurements supported the interpretation that extract and stream NO_3^- derived primarily from nitrification, with moderate $\delta^{15}N$ fractionation effects that produced isotopically light NO_3^-, especially in the forest floor. Yet, these

processes of nitrification and DOC generation did not suffice to produce the inverse DOC-NO_3^- curve for extracts, as was observed at the catchment scale. Rather, catchment-scale NO_3^- loss appears to occur only when the stoichiometric constraints of denitrification indicate of limitation bioavailable DOC to this NO_3^- removal process.

Persistent enrichment in $\delta^{15}N_{NO3}$ between the forest floor and streamwater may support the inference of partial consumption of NO_3^- by denitrification, although the lack of a corresponding signal of denitrification in stream $\delta^{18}O_{NO3}$ requires further investigation. Overall, stoichiometric and isotopic constraints indicate that catchment-scale DOC-NO_3^- patterns are likely governed by N consumption in high-C:N soils and NO_3^- production as heterotrophic microbes become C-limited, with NO_3^- consumption by denitrification as allowed by the supply of bioavailable DOC occurring deeper in the soil profile.

5 B: Introduction

The Adirondack and Catskill Mountains of New York State receive some of the highest rates of atmospheric deposition of nitrogen in the United States. Excess N deposition can stimulate soil nitrification and increase NO_3^- loss to surface waters (Aber et al. 2003), processes that have a range of adverse consequences. Both nitrification and NO_3^- loss contribute to acidification of soils and surface waters, and increased N availability to microbes can stimulate the production of a powerful greenhouse gas, nitrous oxide (N_2O; Galloway et al. 2003).

When leached, exported NO_3^- can trigger eutrophication in downstream estuaries. Cross-site comparisons show that stream NO_3^- export tends to increase with increasing rates of N deposition; however, catchments receiving similar amounts of N deposition often vary tremendously in the amount of N that they leach (e.g., Aber et al. 2003, MacDonald et al. 2002), and long-term trends have defied ready explanation. For example, despite relatively constant rates of N deposition from the mid-1980s to early 2000s, stream NO_3^- concentrations in surface waters decreased across the Northeast U.S. (Stoddard et al. 2003, Driscoll et al. 2003, Burns et al. 2006), and stream NO_3^- in the White Mountains of New Hampshire have dropped sharply from a peak in the 1970s (e.g., Aber et al. 2002, Goodale et al. 2003, Bernal et al. 2012).

The reasons for this NO_3^- decline remain unresolved, although many hypotheses have been suggested, with roles proposed for climate variation and rising atmospheric CO_2 (Aber et al. 2002, Hong et al. 2005), recovery from insect outbreaks (Eshleman et al. 1998), successional changes in wood inputs to streams and in-stream uptake (Bernhardt et al. 2005) and other successional factors (Huntington 2005, Bernal et al. 2012). Different factors may be important in different catchments, but only a regional driver such as changes in climate or atmospheric chemistry can readily explain the NO_3^- decline observed across a range of sites (Goodale et al. 2003 and 2005). Climate-driven biogeochemistry models can simulate some aspects of temporal patterns of NO_3^- loss, yet they completely fail to explain the 1990s decreases in stream NO_3^- (e.g., Aber et al. 2002, Bernhardt et al. 2005, Hong et al. 2005, Wu and Driscoll 2009). One hypothesis with little testing to date is that the NO_3^- decline could be associated in some way with observations of increasing dissolved organic carbon (DOC) in streamwater (Goodale et al. 2005).

Long-term monitoring of surface water chemistry has enabled detection of a substantial increases in DOC concentrations across the Northeast U.S. and Europe (Stoddard et al. 2003, Monteith et al. 2007), including in the Catskill and Adirondack Mountains of New York State (Driscoll et al. 2003b, Burns et al. 2006, Lawrence et al. 2011, Strock et al. 2014). Many mechanisms have been proposed to explain this trend, with mounting evidence for a connection between rising DOC concentrations and declining rates of atmospheric SO_4^{2-} deposition and corresponding ecosystem recovery from acidification, as observed in both long-term observations of surface water chemistry (Evans et al. 2006a, Monteith et al. 2007, SanClements et al. 2012), and in field-scale experimental manipulations of soil pH (Evans et al. 2012). The proposed explanation for changes in DOC loss with acidification status (Evans et al. 2006a, Monteith et al. 2007) typically focuses on the geochemical mechanism of increased DOC solubility increases with increased soil pH (e.g., Krug and Frink 1983, Kalbitz et al. 2000, Ekström et al. 2011). Additional mechanisms include increased DOC production by plant roots and mycorrhizae as S and N deposition decline, and decreased DOC consumption by decomposers under low-O_2 conditions due to lower supplies of of SO_4^{2-} and NO_3^- to serve as electron acceptors. Even without a complete understanding of the mechanisms driving the DOC increase, it is worth examining how changes in DOC could affect NO_3^- loss.

Compared across sites, a recurrent pattern has emerged connecting stream DOC and NO_3^- (Goodale et al. 2005, Evans et al., 2006b, Taylor and Townsend 2010, Kopacek et al. 2013). For the Northeastern U.S., surface water NO_3^- concentrations decrease sharply with increasing DOC concentrations, down to trace levels of NO_3^- when DOC concentrations exceed 1-3 mg/L (Goodale et al. 2005). High concentrations of stream NO_3^- only occurred in sites with low DOC concentrations, even in regions receiving moderately elevated rates of N deposition. A similar DOC-NO_3^- pattern – hereafter, "the DOC-NO_3^- curve" – has now been reported across sites in the U.K. (Evans et al., 2006b) and the Czech Republic (Kopacek et al. 2013), and for a broad compilation of measurements from soil solutions, streams, rivers, lakes, estuaries, and major ocean basins that showed that the pattern recurs, with varying DOC thresholds (Taylor and Townsend 2010). Nonetheless, it is unclear what processes drive this pattern. Evans et al. (2006b) attributed the U.K. DOC-NO_3^- curve to variations in soil C stocks and their role in both producing DOC and immobilizing N.

Goodale et al. (2005) and Taylor and Townsend (2010) speculated that the curve may result from C versus N limitation to some microbial processes, particularly to (a) C-driven microbial N immobilization (assimilation) or (b) denitrification. Yet, it is not apparent whether one of these processes dominates, or in which part of the landscape either has the greatest effect – e.g., in surface or deep soils, riparian zones,

sediments, or surface waters. Whereas the biotic capacity for N immobilization can saturate and lead to increased N export (Lovett and Goodale 2011), denitrification permanently removes N from drainage waters but concurrently produces N_2O. Thus, identifying what process and landscape position controls this pattern is important both for understanding catchment biogeochemical processes and for discerning the fate and management of N pollution.

This study tested the primary hypothesis that the DOC-NO_3^- curve observed across catchments results from variation in surface soil C stocks, which could control the net production of both DOC and NO_3^- (Evans et al. 2006b). That is, the hypothesis was that DOC production should increase with the size of the forest floor C pool, and that this C pool should control the overall demand for N immobilization by heterotrophic microbes. Alternatively, it was considered whether the DOC-NO_3^- curve could be driven by the process of denitrification, which consumes both organic C and NO_3^- and could be limited by the supply of either NO_3^- or bioavailable DOC. These hypotheses were tested with measurements of a range of C and N properties of the forest floor, forest floor extract solutions, and streamwater across nine small New York catchments. Measurements included estimates of DOC lability and examination of the isotopic composition of NO_3^- ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) used to infer NO_3^- sources, production, and consumption by denitrification.

First, a classic understanding of ecosystem C-N stoichiometry was used to generate quantitative expectations of how ecosystem NO_3^- concentrations correspond to labile C availability (Figure 6). Long-standing textbook explanations of microbial N mineralization-immobilization dynamics (e.g., Rosswall 1982, Chapin et al. 2011) hold that immobilization of N by heterotrophic microbes should occur as long as the C:N ratio of the organic matter substrate exceeds the C:N ratio of microbial biomass after considering microbial respiratory losses of C to CO_2. These factors can be combined to estimate the critical C:N ratio of organic matter substrate for net N mineralization, as the C:N ratio of microbial biomass divided by microbes' carbon-use efficiency (CUE; Equation 1).

Equation 1   **C:N ratio $_{N\,min.}$ = C:N ratio $_{microbe}$ / CUE $_{microbe}$**

The C:N ratio of microbial biomass is often approximated at roughly 10 (Chapin et al. 2011, Paul and Clark 1996), but a global literature review of microbial stoichiometry found a mean mass-based C:N ratio of 7.4 (molar ratio of 8.6; Cleveland and Liptzin 2007). Microbial CUE varies (Manzoni et al. 2008) and microbes may use C more efficiently as C becomes limiting (Schimel and Weintraub 2003). Yet CUE values for surface soil decomposers are expected to fall in the range of 30-40% (Chapin et al. 2011, Manzoni et al. 2008). Together, these values produce threshold C:N ratios for net N mineralization from organic matter C:N of 18.4 (40% CUE) to 25 (30% CUE). Net microbial N immobilization should occur at higher available C:N ratios (Figure 6, zone A) and net N mineralization should occur at lower values. Organic matter C:N ratio does not directly affect NO_3^- production by autotrophic nitrifers because, by definition, these aerobic bacteria use NH_4^+ or NO_2^- as their energy source rather than organic C (Equation 2). Yet, organic matter C:N ratio indirectly affects net NO_3^- production because immobilization of NH_4^+ by heterotrophic microbes in high C:N soils should strongly limit the supply of NH_4^+ to poorly competitive autotrophic nitrifiers (Verhagen et al. 1992), and because heterotrophic microbes should immobilize NO_3^- as well as NH_4^+ when C:N ratios exceed 18-25. Together, these factors should yield a tight, if indirect, association between net NO_3^- production and the critical C:N ratio for mineralization, governed ultimately by the stoichiometry and CUE of the heterotrophic microbes. Under aerobic conditions, NO_3^- should occur in soil solutions when the labile C:N ratio falls below 18-25 (Figure 6, zone B). If these solutions encounter low-O_2 conditions, denitrifying microbes should consume NO_3^- as long the labile C supply meets or exceeds the stoichiometric demand for this process (Figure 6, zone B; Equation 3). Carbon limitation to denitrification should occur at a labile C:N molar ratio less than 5:4 (mass ratio = 1.07), at which point NO_3^- should remain in solution even in low-O_2 conditions (Figure 6, Zone C).

Equation 2   **Nitrification (aerobic, autotrophic): $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$**

Equation 3   Denitrification (low-O_2, heterotrophic): $5CH_2O + 4NO_3^- + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O$

This framework in Figure 6 was used to assist interpretation of processes affecting solution chemistry in both forest floor extracts and in streams.

Figure 6. Proposed stoichiometric constraints on solution NO_3^- relative to labile C concentrations (units of relative mass)

In zone A, abundant C drives microbial net N immobilization; in zone B, NO_3^- is produced by net nitrification in high O_2 conditions or subsequently removed by denitrification in low O_2 conditions; in zone C, denitrification is limited by C availability.
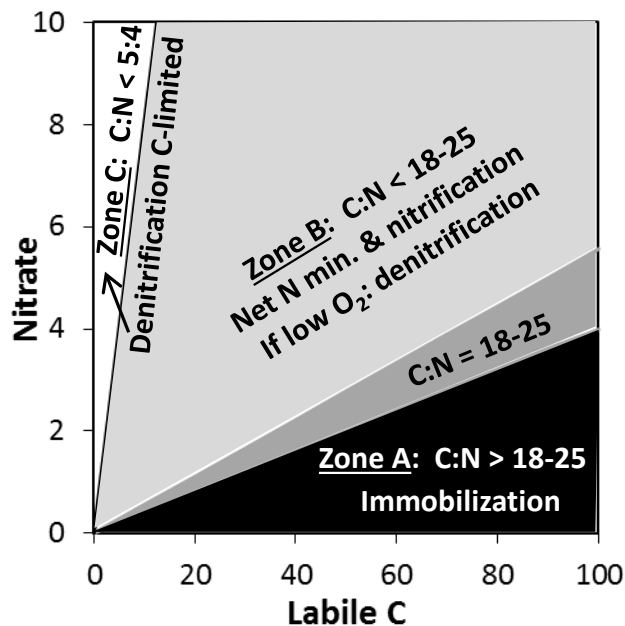
6 B: Methods

6.1 Site Description

Nine headwater catchments were selected (Table 4) to span a range of baseline stream DOC and NO_3^- concentrations. Six streams were in the Catskill Mountains and were sampled previously by Lovett et al. (2000 and 2002). The other three streams were in the Adirondack Mountains, and formed part of the U.S. EPA's Episodic Response and Long-term Monitoring Networks (Driscoll et al. 2003, Lawrence et al. 2004 and 2011). Climate conditions are broadly similar across sites, if slightly cooler in the Adirondacks. Annual mean temperature at Slide Mountain in the Catskills (808 m) averages 4.3 $^{\circ}$C with 153 cm yr^{-1} of precipitation (Lovett et al. 2000). In the western Adirondacks at lower elevations, annual precipitation averages 115-125 cm yr^{-1} and temperatures average 4.4 $^{\circ}$C (Ito et al. 2002). Wet deposition averaged 5-7 kg N ha^{-1} yr^{-1} during the late 1990s and early 2000s (Lovett et al. 2000, Ito et al. 2002, Lawrence et al. 2008).

Forests cover all catchments, dominated by northern hardwoods (*Betula alleghaniensis, Fagus grandifolia, Acer saccharum, A. rubrum,* and *Fraxinus americana*) with occasional red oak (*Quercus rubra)* or black cherry (*Prunus serotina*) in the Catskills. Evergreen conifers *(Abies balsamea, Pinus strobus, Tsuga canadensis,* or *Picea rubens* in the Adirondacks) occur on a few peaks or streamsides. Forests in both regions were mostly second-growth following varying intensities of logging during the late 19th and early 20th century with a few localized fires (McMartin 1994, Lovett et al. 2002). Acidic, sandy, and stony soils developed in glacial till in both regions, forming Spodosols over granite and gneiss in the Adirondacks (Lawrence et al. 2004) and forming Inceptisols over sandstone, shale, and conglomerates in the Catskills (Lovett et al. 2000).

Table 4. Sampling locations and catchment characteristics

Catchment	Region	Latitude (N)	Longitude (W)	Max. Elev. (m)	Min. Elev. (m)	Stream Length (km)	Catchment Area (km^2)
BWS6	CAT	42° 05' 55"	74° 51' 20"	754	512	2.0	1.4
Halcott Brook	CAT	42° 11' 03"	74° 24' 55"	1039	555	1.6	1.1
Hollow Tree	CAT	42° 09' 21"	74° 15' 40"	1137	622	1.6	1.1
Kittle	CAT	42° 04' 18"	74° 40' 44"	957	567	1.8	0.6
Mill Brook	CAT	42° 03' 54"	74° 35' 14"	1137	674	2.9	2.8
Myrtle	CAT	42° 08' 01"	74° 13' 52"	1219	475	4.3	4.8
Bald Mtn Brook	ADK	43° 45' 03"	74° 54' 39"	715	570	2.2	2.2
Fly Pond Brook	ADK	43° 45' 05"	74° 54' 34"	710	563	0.8	0.9
Squash Pond Brook	ADK	43° 49' 07"	74° 53' 10"	763	578	1.6	1.1

CAT = Catskill Mountains; ADK = Adirondack Mountains

From: Lovett et al. 2000, 2002, Driscoll et al. 2003, Lawrence et al. 2004, 2011

6.2   Field Methods

Forest floor material and streamwater were collected during the late growing season in 2010 and 2011. In each catchment, twenty 15 × 15 cm intact forest floor blocks were collected from locations recorded by GPS. Sampling generally occurred along three transects forming a wide triangle that spanned catchment conditions and encompassed each stream. In those catchments with hiking trails, 2-10 samples were distributed along and at least 20 m from these trails, with the rest of the samples distributed along linear transects spanning other portions of the catchment. Each forest floor block was collected using a serrated knife to slice within a 15 × 15 cm wooden template, clipping roots and removing rocks when necessary. Samples contained the whole forest floor, and included Oi (litter), Oe (fragmented), and Oa (humified) layers, collected by visual separation in the field from the top of the mineral soil.

Streamwater samples were collected from the base of each catchment and at 1-2 additional locations upstream on the same day as the forest floor sampling. Stream samples were field-filtered through ashed 0.7-μm glass fiber filters (Whatman GF/F) using polyethylene syringes (BD Luer-lok Tip), and collected in thrice-rinsed 1-L HDPE bottles. Soil and water samples were stored on freezer packs in a cooler during transport back to the lab. Additional streamwater samples from the six Catskill streams were collected monthly from July through December 2010 (C. Johnson, personal communication).

6.3   Laboratory Analyses

On return to the laboratory, forest floor samples were weighed and sieved through a 5.6-mm mesh. Gravimetric soil moisture was measured on 10-g subsamples of field-moist material dried at 60 $^{\circ}$C for one week. Additional subsamples were dried and ground to a fine powder using a ball mill (Retsch mixer mill MM200), weighed, and stored in an 80 $^{\circ}$C drying oven until measurement of C, N, and S concentration using an Elementar vario-EL-III elemental analyzer. Forest floor solutions were obtained through water extraction by adding 20 g of field-moist forest floor material to 200 mL of deionized water, shaking on a shaker table for 24 hours, and then sequentially filtering through 2.7 μm (Whatman GF/D) and 0.7 μm glass fiber filters (Whatman GF/F). For the Catskill samples, subsamples of the 2.7 μm filtrate were reserved for assays of bioavailable DOC (bDOC). For the Adirondack samples, fine- and coarsely filtered extracts were produced from separate extractions created in parallel.

Filtered streamwater and forest floor extracts were refrigerated and analyzed for pH, DOC, and total dissolved N (TDN) concentrations within 14 days of collection or extraction. Solution pH was measured using an Accumet AB15 pH meter. DOC and TDN concentrations were measured following acidification and sparging to remove dissolved inorganic C using platinum-catalyzed oxidative combustion followed by infrared (DOC) or chemiluminescent (TDN) detection (Shimadzu TOC-N). Anion (Cl^-, SO_4^{2-}, NO_3^-, PO_4^{3-}) and cation (NH_4^+, Ca^{2+}, Mg^{2+}, Na^+, K^+) concentrations were measured on frozen and thawed subsamples using ion chromatography (Dionex ICS-2000; Dionex Corp.). Dissolved organic N (DON) concentrations were computed by difference ($DON = TDN - NO_3^- - NH_4^+$).

Extract concentrations were expressed per mass of dry forest floor material (mg/kg) and extrapolated to stocks per unit area (kg/ha) by multiplying by forest floor dry mass (t ha^{-1}). A metric of "excess" forest floor C was computed as the stock of C exceeding the measured N stock (t N ha^{-1}) times a mid-range threshold C:N ratio for net N mineralization of 18.4 (Equation 4).

Equation 4   Excess C stock (t C ha-1) = C stock (t C ha-1) − [N stock (t N ha-1)] × C:N ratio $_{N\ min}$

Only a portion of DOC is labile. To quantify labile DOC, several metrics of DOC quality were measured including $SUVA_{254}$, $\delta^{13}C_{DOC}$, and bioavailable DOC (bDOC). Specific UV absorbance ($SUVA_{254,}$ L mg^{-1} cm^{-1}), an index of DOC aromaticity (Weishaar et al. 2003), was quantified for all samples by measuring absorbance at 254 nm using a Beckman Coulter DU 640 spectrometer, and dividing by DOC concentration (mg/L). DOC bioavailability (bDOC) and isotopic composition ($\delta^{13}C_{DOC}$) were measured for all Catskill forest floor samples and for a systematic 20% subset of those from the Adirondacks. Hydrophilic compounds such as cellulose and hemicellulose often have relatively heavy $\delta^{13}C$ values (~ -26‰) whereas more hydrophobic compounds such as lignin are often isotopically light (-30‰) (Kaiser et al. 2001). Subsamples for $^{13}C_{DOC}$ analysis were sent to the University of California at Davis stable isotope facility, which used off-line acidification and sparging, and then combustion (O.I. Analytical Model 1030 TOC analyzer, College Station, TX) and an interface (GD-100 Gas Trap; Graden Instruments) to an isotope ratio mass spectrometer (IRMS) (PCZ Europe 20-20 IRMS; Sercon Ltd., Cheshire, UK), with overall precision of 0.4‰. Bioavailable DOC was quantified as the decrease in DOC concentration in coarsely filtered (2.7 μm) solutions during incubation in amber glass bottles stored at room temperature in the dark, with glass fiber filters supplied as a surface for microbial growth (McDowell et al. 2006). Sixty mL subsamples were removed at the start and after one week and one month of incubation, then filtered to 0.7 μm and analyzed for DOC as previously described. Low DOC concentrations prevented measurements of streamwater bDOC.

Best-fit empirical relationships among forest floor C or solution DOC metrics were used to approximate bDOC (%) for streamwater and for the subset of Adirondack forest floor samples where this property was not measured directly (Equation 5 and Equation 6). All streamwater samples were analyzed for $SUVA_{254}$ and $\delta^{13}C_{DOC}$, and so the relationship between bDOC (%) and these two properties in soil extracts was used to estimate stream %bDOC (Equation 5). Similarly, %bDOC for Adirondack extracts that lacked this direct measurement were estimated from $SUVA_{254}$ and forest floor C stocks (Equation 6). Quantities of bDOC (mg/L, mg/kg or kg/ha) were computed by multiplying measured DOC concentration or pool by measured or estimated %bDOC.

Equation 5

$$bDOC_{stream} (\%) = -5.99 \text{ } SUVA_{254} \text{ (L mg}^{-1} \text{ cm}^{-1}) - 0.0575 \text{ } \delta^{13}C_{DOC} (\text{‰}); R^2 = 0.45, P < 0.0001$$

Equation 6

$$bDOC_{extract} (\%) = -4.06 \text{ } SUVA_{254} \text{ (L mg}^{-1} \text{ cm}^{-1}) - 0.0019 \text{ C stock (t ha}^{-1}); R^2 = 0.53, P < 0.0001$$

Nitrate isotopic composition was measured on all streamwater samples and 20% of the forest floor extracts from all sites. Subsamples were sent to the stable isotope facility at the University of California at Davis, which used the denitrifier sample preparation method (Casciotti et al. 2002) followed by trace gas pre-concentration (ThermoFinnigan GasBench and PreCon) and analysis by IRMS (Thermo Scientific Delta V Plus) for an overall precision of 0.4‰ for $\delta^{15}N_{NO3}$ and 0.5‰ for $\delta^{18}O_{NO3}$. Corresponding samples of forest floor material were analyzed for ^{15}N and ^{13}C composition at the Cornell Stable Isotope Laboratory using combustion (Carlo Erba NC 2500) and an IRMS (Finnigan MAT Delta Plus). Corresponding streamwater samples were analyzed for $\delta^{18}O_{H2O}$ at the Cornell Stable Isotope Lab using a GFL 1086 water equilibrator unit interfaced to an IRMS (Finnigan MAT Delta Plus).

6.4   Stable Isotopic Calculations and Expectations

All stable isotopic measurements were expressed using standing delta (δ) notation (Equation 7), as the ratio of heavy to light isotope in the sample (R_{sample}) relative to an internationally accepted standard ($R_{standard}$) of atmospheric N_2 for ^{15}N, Pee Dee Belemnite for ^{13}C, and Vienna Standard Mean Ocean Water for ^{18}O, multiplied by 1,000 to yield per mil units (‰).

Equation 7 $\delta X (\text{‰}) = (R_{sample} / R_{standard} - 1) \times 1000$

Dual isotopic composition of NO_3^- ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) can provide information on NO_3^- sources (atmospheric deposition versus microbial nitrification) and consumption by denitrification (e.g., Kendall et al. 1998). Precipitation typically contains heavy $\delta^{18}O_{NO3}$ relative to NO_3^- from microbial production (e.g., Kendall et al. 2007). Measurements of precipitation $\delta^{18}O_{NO3}$ for Upstate New York using the same methods as this study average $+78 \pm 8$‰ (Burns et al. 2009, Goodale et al. 2009). Nitrate produced by nitrification should have $\delta^{18}O_{NO3}$ values of roughly -10 to +10‰ and $\delta^{15}N$ values of roughly -10 to +5‰ (Kendall et al. 2007; see next section and Discussion). A simple theoretical model (Equation 8; Anderson and Hooper 1983) established that NO_3^- contains one oxygen atom from atmospheric O_2 ($\delta^{18}O = +23.5$‰; Kroopnick and Craig 1972) and two from local water ($\delta^{18}O_{H2O}$).

Equation 8 $\delta^{18}O_{NO3} = 1/3 \text{ } \delta^{18}O_{O2} + 2/3 \text{ } \delta^{18}O_{H2O}$

As a caveat, lower $\delta^{18}O_{NO3}$ values than predicted by Equation 8 can result from both kinetic fractionation against ^{18}O during nitrification and from exchange of oxygen by NO_2^- with water, which occurs rapidly at low pH (Casciotti et al. 2010, Snider et al. 2010, Fang et al. 2012). Elevated $\delta^{18}O_{NO3}$ values can result from mixing with precipitation NO_3^- and from denitrification (Kendall et al. 1998, Mayer et al. 2001). Whereas NO_3^- from precipitation and nitrification typically have similar $\delta^{15}N_{NO3}$ values, the process of denitrification fractionates against both $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ and leads to enrichment in both isotopes in the residual NO_3^- (e.g., Mariotti et al. 1981, Kendall et al. 2007, Granger et al. 2008). The $\delta^{15}N_{NO3}$ values in NO_3^- produced by nitrification should reflect the $\delta^{15}N$ content of the organic N mineralized and subsequently nitrified, along with any fractionation that occurs during either process. Mineralization is thought to fractionate little if at all, but nitrification can fractionate against ^{15}N, especially when the rate of NH_4^+ supply exceeds the rate of nitrification (Högberg 1997, Kendall et al. 2007). Pure cultures of nitrifiers can produce isotope enrichment factors for nitrification (ε_{nitrif}) of -14 to -38‰ (Mariotti et al. 1981, Högberg 1997, Casciotti et al. 2003). These isotope enrichment factors can be computed for closed, well-mixed systems using the modified Rayleigh equation for an accumulated product (Mariotti et al. 1981, Casciotti et al. 2003; Equation 9 and Equation 10), where f_{NH4} is the fraction of the initial NH_4^+ pool consumed by nitrification.

Equation 9 $f_{NH4} = [NH_4^+] / [NH_4^+]_{initial}$

Equation 10 $\delta^{15}N_{NO3,\ cumulative} = \delta^{15}N_{NH4,\ initial} + \varepsilon_{nitrif} \times f_{NH4} \times \ln(f_{NH4}) / (1 - f_{NH4})$

ε_{nitrif} was estimated for the mixed field conditions in this study as the slope of the relationship between measured extract or stream $\delta^{15}N_{NO3}$ and the term $f \ln(f)/(1-f)$, where f was estimated as the ratio of the measured concentration of NH_4^+ relative to the concentration of dissolved inorganic N (DIN = NO_3^- + NH_4^+), assuming that solution NO_3^- was produced from the initial NH_4^+ pool. This approach approximates the net isotope effect of all steps in the nitrification processes under varied field conditions, and where various competitive interactions may constrain the supply of NH_4^+ or consume NO_3^-.

Denitrification also fractionates against ^{15}N, and isotope enrichment factors for denitrification (ε_{denit}) can be computed using a modified Rayleigh equation for a consumed substrate (Mariotti et al. 1981; Equation 11 and Equation 12). Field-based estimates of ε_{denit} for ^{15}N range from -6 to -23‰ (Kendall et al. 2007, Granger et al. 2008, Houlton and Bai 2009), with a value of -13‰ estimated recently for northern hardwood forests in New Hampshire (Wexler et al. 2014).

Equation 11 $\quad f_{NO3} = [NO_3^-] / [NO_3^-]_{initial}$

Equation 12 $\quad \delta^{15}N_{NO3} = \delta^{15}N_{NO3,\ initial} + \varepsilon_{denit} \times \ln(f_{NO3})$

For the forest floor extract samples that exhibited dual isotopic evidence of denitrification in this study, the expected initial NO_3^- concentration of these samples prior to denitrification was estimated by rearranging Equation 11 and 12 to solve for $[NO_3^-]_{initial}$, and using observed measurements of NO_3^- concentration and $\delta^{15}N_{NO3}$, and assuming that $\varepsilon_{denit} = -13‰$ and that initial $\delta^{15}N_{NO3}$ values were those predicted from an observed relationship between $\delta^{15}N_{NO3}$ and forest floor $\delta^{15}N$ for non-denitrified extracts.

7   B: Results

7.1   DOC-Nitrate Patterns

Measurements of stream DOC and NO_3^- concentrations from the catchments in this study broadly conformed to patterns observed previously from streams spanning the Northeastern U.S. (Goodale et al. 2005). That is, high NO_3^- concentrations occurred only in streams with low DOC concentrations, and streams with high DOC concentrations had very low NO_3^- concentrations (Figure 7A). As not all DOC is labile, comparison of stream NO_3^- against estimated concentrations of bioavailable DOC for this study showed that all stream samples with high NO_3^- had less than 1.0 mg/L bDOC, while the few stream samples with higher bDOC concentrations contained little if any NO_3^- (Figure7B). Streams with elevated NO_3^- concentrations all had molar bDOC:NO_3^--N ratios less than or equal to 5:4, the threshold expected for C limitation to denitrification (Figure 7B; Zone C from Figure 6).

Examined on a concentration basis, forest floor extracts (Figures 7C through 7F) did not produce the strongly inverse DOC-NO_3^- relationship typically observed in streamwater (Figure7A and Figure 7B). Forest floors with high levels of extractable DOC often also had high levels of extractable NO_3^- (Figure 7C). Most of these samples with high levels of both DOC and NO_3^- fell in the zone of moderate DOC:NO_3^- ratios where net nitrification is expected (Figure 6 zone "B"), and most came from forest floor material with C:N ratios < 18. Similar patterns emerged when considering extractable NO_3^- relative to bDOC (Figure7D). A pattern closer to streamwater observations emerged in forest floor extract chemistry examined on an areal basis, where extractable NO_3^- pools were low in forest floors with a large pool of extractable DOC (Figure 7E) or bDOC (Figure 7F). In all cases, the highest NO_3^- levels occurred in extracts from forest floor material with C:N ratios less than 18.

In both forest floor extracts (Figure 7C and Figure 7F) and in streamwater (Figure 7B), NO_3^- did not occur in solution if bDOC was present at measureable levels with a bDOC:NO_3^--N ratio > 25:1 (zone A), consistent with an expectation of microbial assimilation of N during consumption of bDOC. When bDOC was present with bDOC:NO_3^--N ratios < 25:1 (zone B), forest floor samples often contained relatively high levels of both NO_3^- and bDOC (Figure 7C, 7F), but streamwater did not (Figure 7B), consistent with expectations of production of NO_3^- by nitrification in oxic forest floors and its potential removal by denitrification by the time water reaches the stream – unless levels of bDOC are too low to support denitrification (zone C).

Figure 7. Nitrate versus DOC (left panels) or bioavailable DOC (right panels) in stream water (A, B) or forest floor extracts on a concentration (C, D) or areal (E, F) basis

Values from this study (A) are compared against prior measurements from the Northeast U.S. (Goodale et al. 2005). Stream %bDOC (B) was not measured directly but was estimated from other DOC properties (Equation 5). Extracts (C-F) were grouped by values of corresponding forest floor C:N ratio. Zones A, B, and C and corresponding C:N threshold lines follow Figure 6.
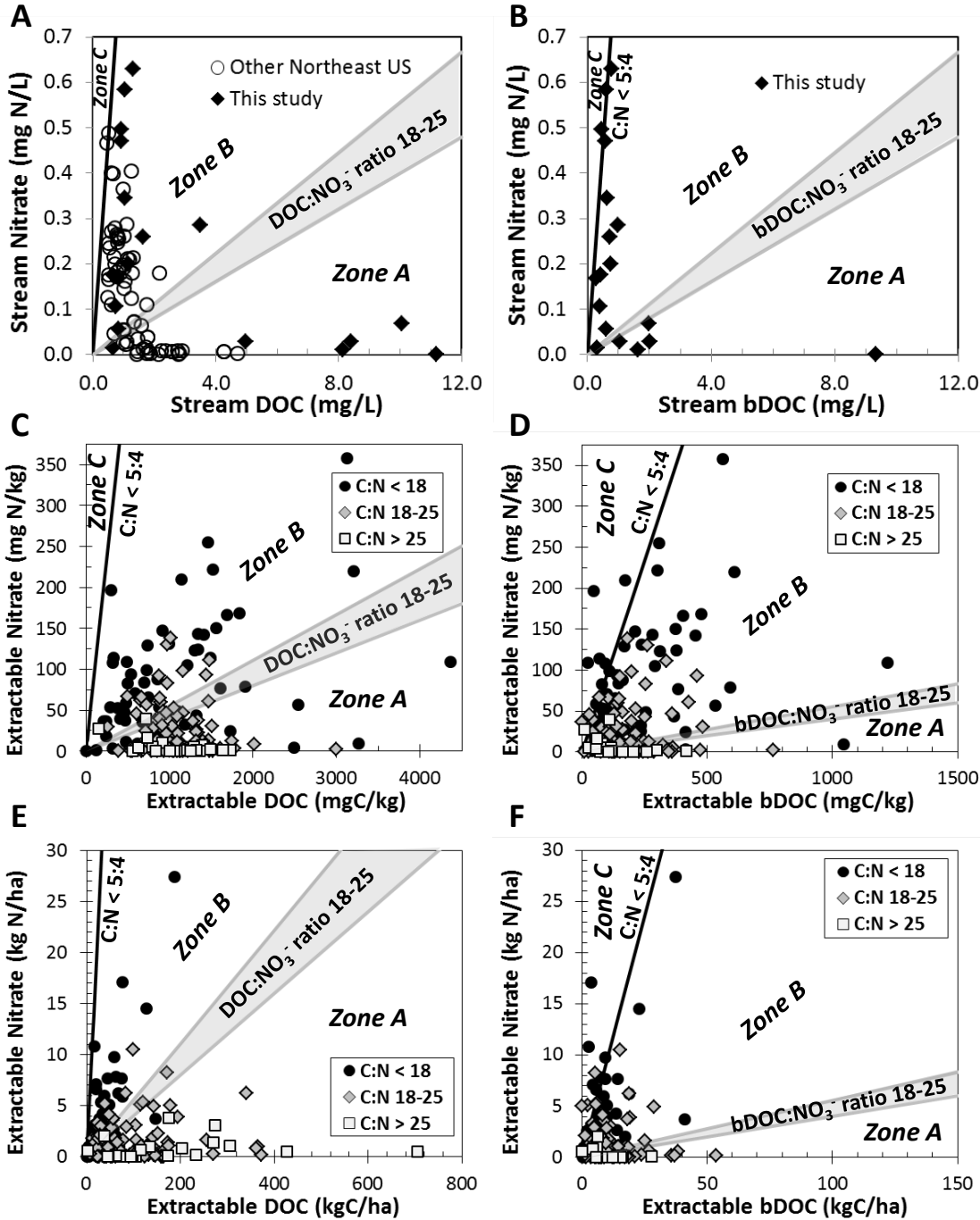
7.1.1 Forest Floor Carbon Stocks and Extract Chemistry

As predicted, the extractable DOC pool increased directly with forest floor C content, whether considered on a concentration (Figure 8A) or areal (Figure 8B) basis. Calculations of forest floor C stock and extractable DOC stock both include measurements of forest floor mass, compromising their direct comparison (Figure 8B) which yet illustrates useful catchment-scale measurements of both properties. Extractable DOC content on average amounted to 0.25% of the total forest floor C stock (Figure 8B). DOC bio-availability decreased as forest floor C stock increased. Roughly 25% of extracted DOC was consumed over the one-month incubation at Catskill sites with small forest floors, whereas almost none of the DOC was bioavailable in some of the Adirondack sites with large forest floors (Figure 8C). $SUVA_{254}$ increased with forest floor C stock, although the relationship was noisy ($R^2 = 0.23$; Figure 8D).

Figure 8. Relationships among measurements of extractable DOC, forest floor C (% and stock), and two measurements of DOC quality: bioavailable DOC (%bDOC) and $SUVA_{254}$ (L mg C^{-1} cm^{-1})

35
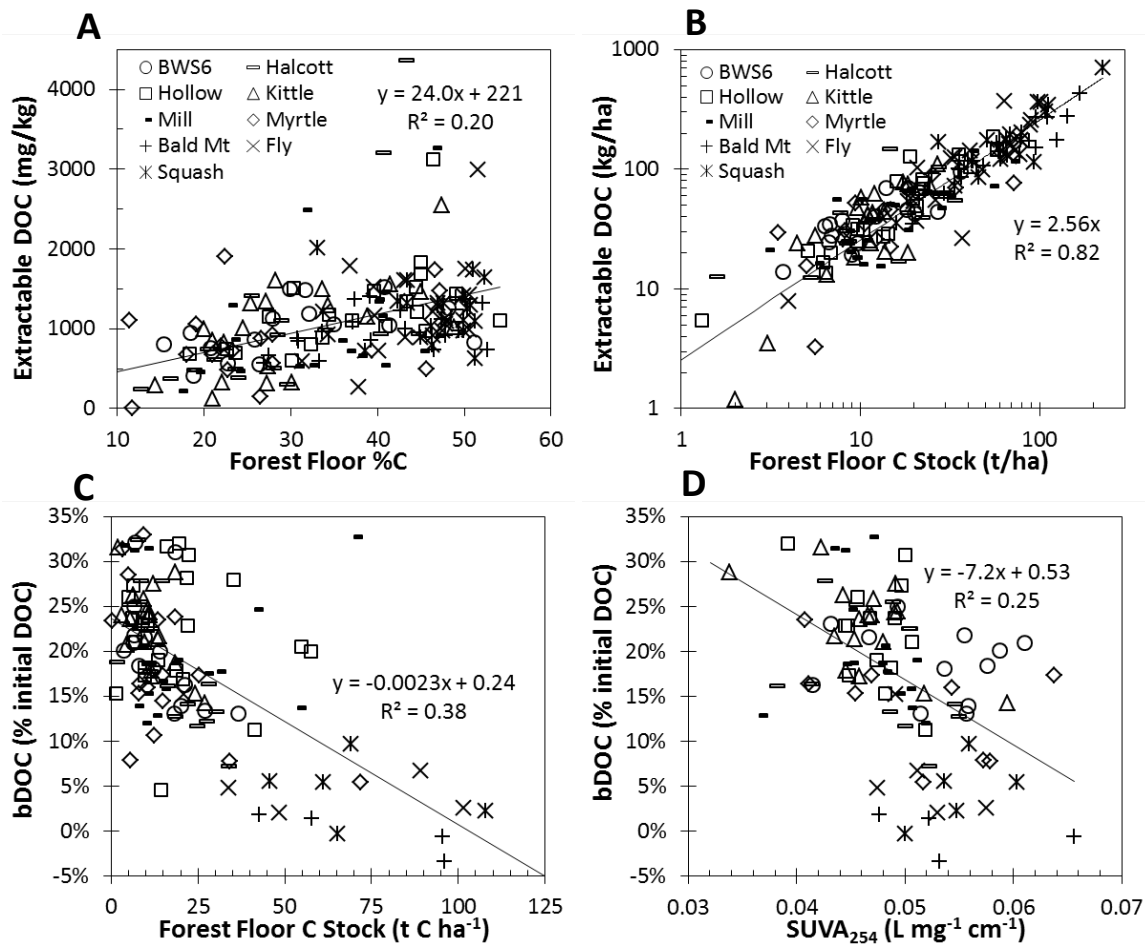
Contrary to initial hypotheses, extractable NO_3^- and NH_4^+ did not decrease as forest floor C content increased. Rather, some of the highest concentrations of forest floor inorganic N occurred at sites with the highest forest floor C concentrations (Figure 9A), and the largest pools of extractable NO_3^- occurred in the largest forest floors (Figure 9B). Extractable inorganic N concentrations instead corresponded to forest floor C:N ratio, increasing as forest floor C:N ratio decreased below 25 (microbial biomass with 30% carbon-use efficiency) then rose sharply as forest floor C:N decreased below 18 (microbial biomass with 40% CUE; Figure 9C). Considered on an areal basis, the stock of extractable NO_3^- was high only when the "excess" forest floor C stock was negative (< 0) and decreased to negligible levels as estimates of the "excess" C stock in the forest floor became positive (> 0) (Figure 9D).

Figure 9. Water-extractable NO_3^- and NH_4^+ versus forest floor carbon or C-N status

Values are expressed on the basis of both concentration (left panels) and stocks (right panels).Critical C:N ratios for N mineralization (C) are shown for microbial carbon-use efficiency values of 30, 35, and 40% assuming that the C:N ratio of microbial biomass = 7.4 (Equation 1). The "excess" forest floor C stock (D) considers the C stock exceeding the measured N stock multiplied by a critical C:N$_{Nmin}$ ratio of 18.4 (Equation 4).
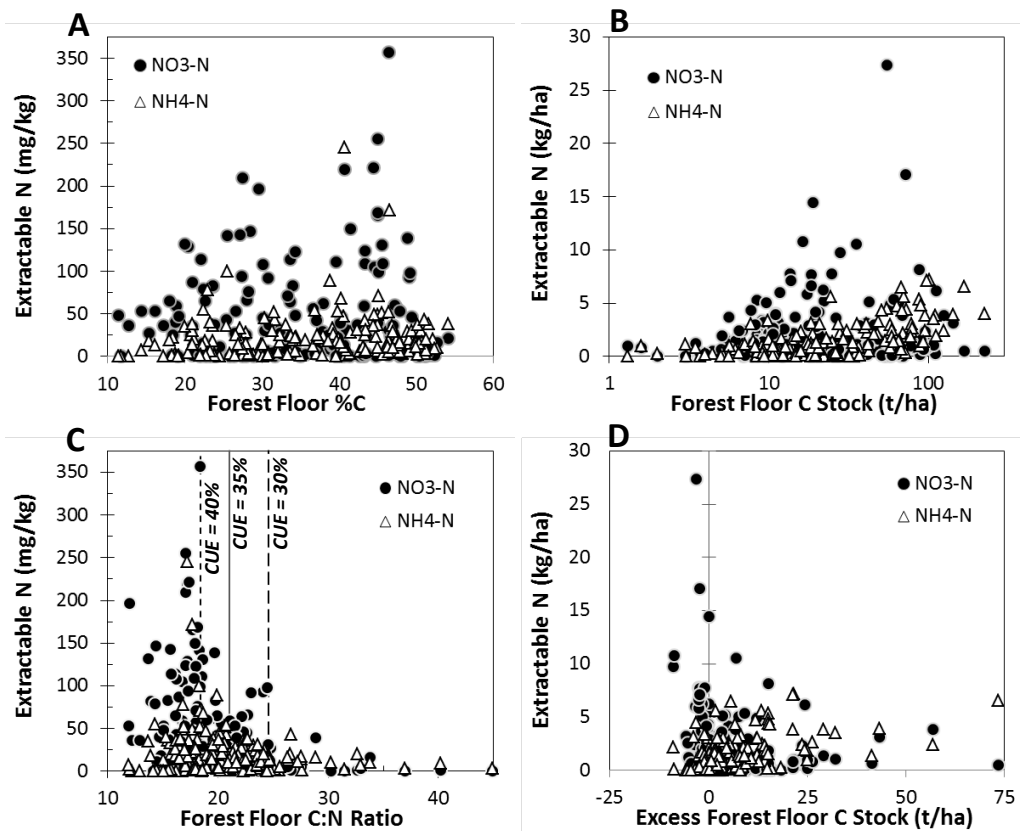
7.1.2 Relationships between Stream and Soil Extract Chemistry

Across catchments, patterns of forest floor C and N stocks partly explain stream DOC and NO_3^- loss. Correspondence between stream NO_3^- concentrations and forest floor NO_3^- was poor ($R^2 = 0.09$; Figure 10A) but was strong for DOC ($R^2 = 0.91$, Figure 10C), although the large regional difference between Catskill and Adirondack catchments drove much of this relationship. The catchments with the most enriched $^{15}N_{NO3}$ in streamwater tended to have the most depleted $^{15}N_{NO3}$ in their forest floor extracts ($R^2 = 0.29$, Figure 10B), potentially reflecting especially large transformations of NO_3^- between the forest floor and stream sampling points in these catchments. Forest floor C content partly controlled stream NO_3^-, in that the catchments containing "excess" forest floor C had little to no NO_3^- in streamwater, while those lacking "excess" C had variable and often high streamwater NO_3^- concentration (Figure 10C).

Figure 10. Correspondence of stream and forest floor extract

(A) NO_3^-, (B) $^{15}N_{NO3}$, or (C) DOC, as well as (D) stream NO_3^- versus the "excess" C stock, which is the forest floor C above a C:N ratio of 18.4. Values are mean $\pm$ SE, by catchment.
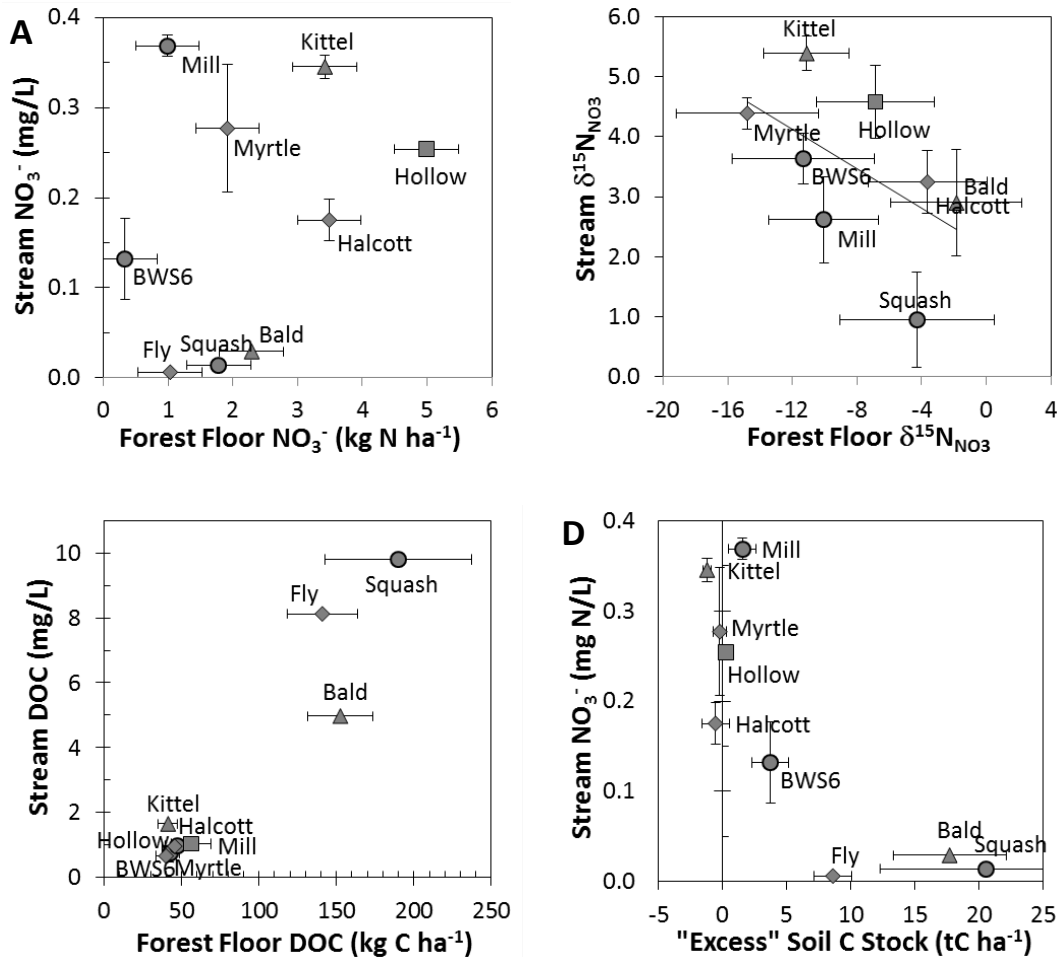
7.2　Stable Isotopic Indicators of Nitrogen Cycling and Loss

Dual isotopic ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) analysis of NO_3^- indicated that most, but not all, forest floor extracts and streamwater contained NO_3^- reflecting a dominant isotopic signal from the process of nitrification (Figure 11). Predicted $\delta^{18}O_{NO3}$ values for nitrification in these catchments (Equation 8) were +0.4 to +2.2‰, if one oxygen atom derived from atmospheric O_2 ($\delta^{18}O$ = +23.5‰) and two were from local water ($\delta^{18}O_{H2O}$), which was measured across 15 stream samples as -11.4 to -8.4‰. Many of the stream $\delta^{18}O_{NO3}$ measurements fell below this theoretically predicted range, while many of the forest floor extract $\delta^{18}O_{NO3}$ measurements fell well above it (Figure 11). Two of the extracts with elevated $\delta^{18}O_{NO3}$ values were collected during rain events (Figure 11) and showed likely enrichment by rain $\delta^{18}O_{NO3}$, and were excluded from further analyses of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ patterns.

Figure 11. Dual isotopic composition of NO_3^- ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) from forest floor extracts (squares) and streams (circles)

Streams were sampled concurrently with the forest floor sampling (filled circles) and from other monthly collections June – Oct. (open circles). The grey "precipitation" box shows measurements for precipitation in New York using the same methods as this study (Burns et al. 2009, Goodale et al. 2009). The open box (solid line) shows the expected isotopic range for NO_3^- produced by nitrification (Kendall et al. 2007), with the filled grey band showing $\delta^{18}O_{NO3}$ values calculated using Equation 8. The dashed box shows a nitrification range extended to encompass the values observed in this study. Dashed arrows show the trajectory of isotopic enrichment expected by denitrification (slopes of 0.5 and 1.0).
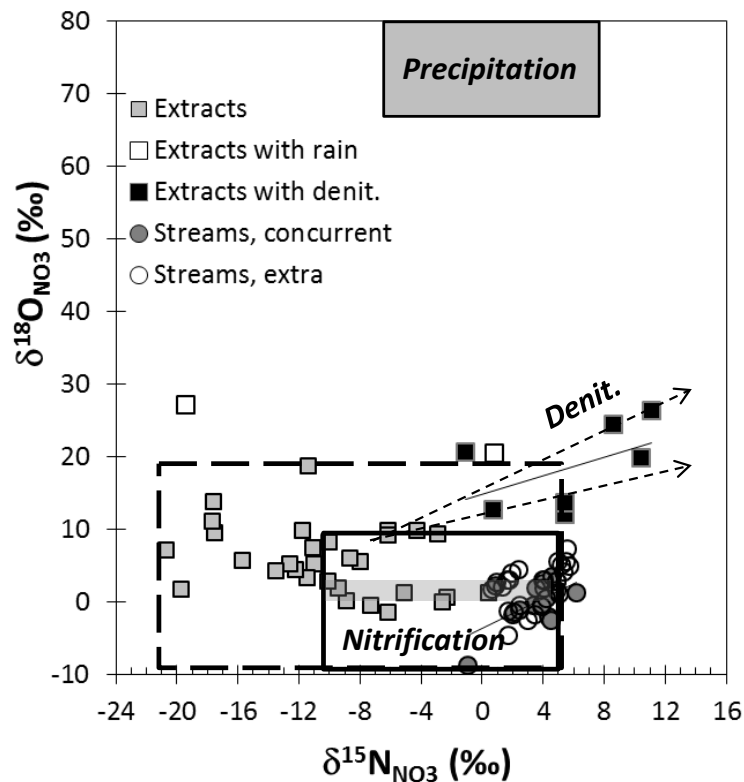
Values of $\delta^{15}N_{NO3}$ in these forest floor extracts spanned an uncommonly broad range, from -20.7 to +11.1‰ (Figure 11 and Figure 12). The three extracts with the most enriched $\delta^{15}N_{NO3}$ values, all from Adirondack sites, also showed concurrent enrichment in $\delta^{18}O_{NO3}$, consistent with the pattern of dual-isotopic enrichment expected from denitrification (Figure 11). Four other extract samples may have included partial denitrification, as they showed marginal dual isotopic enrichment along with higher $\delta^{15}N_{NO3}$ values than expected from forest floor $\delta^{15}N$ and relative NO_3^- : NH_4^+ concentrations (Figure 12A and Figure 13B).

For the remaining extract samples that lacked evidence of denitrification, variation in $\delta^{15}N_{NO3}$ corresponded partly with variation in forest floor $\delta^{15}N$, in that more depleted $\delta^{15}N_{NO3}$ came from forest floors with lighter $\delta^{15}N$ values (Figure 12A; $R^2 = 0.41$). All extracts showed marked depletion in $\delta^{15}N_{NO3}$ relative to the $\delta^{15}N$ of the forest floor material from which they were extracted, averaging 12‰ lighter overall, with more depletion at lower forest floor $\delta^{15}N$ values (Figure 12A). The overall depletion in extract $\delta^{15}N_{NO3}$ values was broadly consistent with the nitrification process, which fractionates against ^{15}N especially when the substrate for nitrification, NH_4^+, is abundant relative to the end product, NO_3^-. The lightest $\delta^{15}N_{NO3}$ occurred in extract samples that had the highest NH_4^+ concentration relative to total DIN (Figure 12B; $R^2 = 0.37$). Stream $\delta^{15}N_{NO3}$ also correlated inversely with NH_4^+:DIN ratio ($R^2 = 0.39$), although stream $\delta^{15}N_{NO3}$ was ~10‰ heavier than forest floor extract $\delta^{15}N_{NO3}$ for the same relative abundance of NH_4^+ (Figure 12B). Streamwater consistently showed heavier $\delta^{15}N_{NO3}$ than the forest floor, with values closer to the partly denitrified extracts than those extracts dominated by nitrification (Figure 12B). The slope of the extract- and stream relationships between $\delta^{15}N_{NO3}$ and $f \times \ln(f)/(1-f)$ provided estimates of the net isotope enrichment factors for nitrification, yielding values of $\varepsilon_{nitrif} = -9.0‰$ for the forest floor extracts and -5.0‰ for streamswater (Figure 12C), indicating less fractionation on conversion of NH_4^+ to NO_3^- in streams than in forest floor extracts. Overall, most forest floor extracts showed isotopic signals dominated by the process of nitrification, and reflecting varied ^{15}N depletion depending on the amount of extractable NH_4^+ relative to DIN.

For the seven extracts with some dual isotopic evidence of denitrification (Figure 11), Equations 11 and 12 were solved for initial (pre-denitrified) NO_3^- concentration by assuming that initial $\delta^{15}N_{NO3}$ values corresponded with forest floor $\delta^{15}N$ as for the other extracts (Figure12A), along with a denitrification enrichment factor ($\varepsilon_{denitrif}$) of -13‰ (Wexler et al. 2014). These calculations indicated that denitrification could have consumed 46-82% of the initial NO_3^- in these samples (Figure 12D). Using this same approach, initial (pre-denitrified) NO_3^- concentrations in streamwater at the base of each catchment were computed by assuming that the $\delta^{15}N_{NO3}$ values prior to denitrification were the average of those measured in the forest floor extracts for each catchment (cf. Figure 10B). These calculations indicated that denitrification could have perhaps consumed 58% (45-72%) of the NO_3^- leached from the forest floor prior to export of NO_3^- in streamwater.

Figure 12. Variation of extract (A-D) and stream (B, C) $\delta^{15}N_{NO3}$ (‰)

Variation in $\delta^{15}N_{NO3}$ (‰) with (A) forest floor $\delta^{15}N$, (B) the fraction of NH_4^+ in DIN, (C) kinetic isotope effects, and (D) extract NO_3^- concentration (mg N/kg), along with estimated "pre-denitrified" extract NO_3^- concentrations and $\delta^{15}N_{NO3}$ values (dotted lines) assuming that initial $\delta^{15}N_{NO3}$ values follow forest floor $\delta^{15}N$ (from panel A) and that ε_{denit} = -13‰ (Equations 11 and 12).Regression lines in all panels exclude samples with dual isotopic evidence (Figure 11) of denitrification (filled squares).
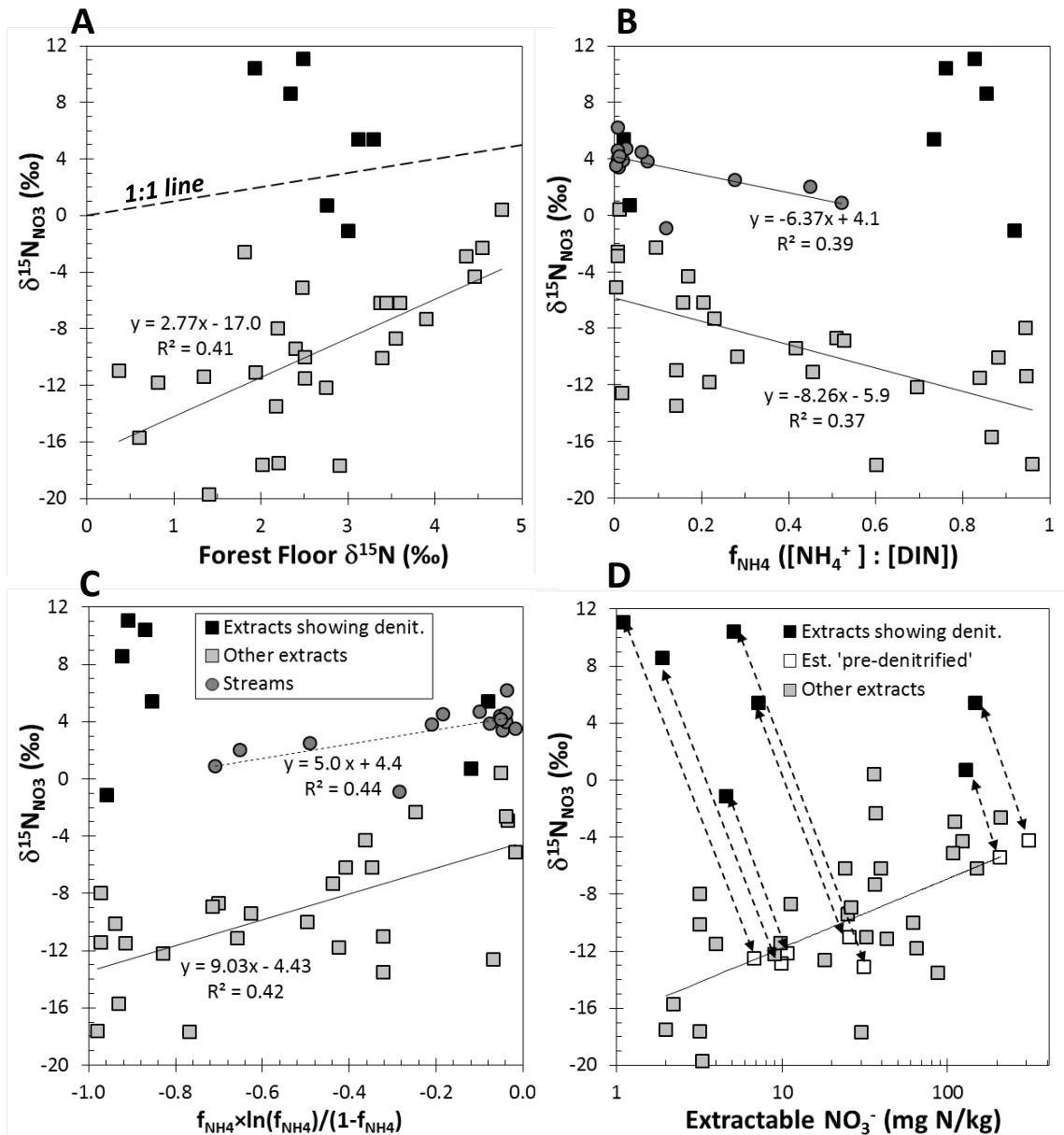
8   B: Discussion

Losses of NO_3^- in surface waters draining forests and other natural ecosystems vary greatly across sites and over time, in ways that remain exceedingly difficult to predict or explain. Yet, policy and management decisions regulating anthropogenic NOx emissions require understanding of the controls on catchment N retention and loss processes. One of the few recurring patterns for NO_3^- loss now observed across many sites and ecosystems is the DOC-NO_3^- curve, in which NO_3^- losses drop sharply as DOC concentrations increase, and high NO_3^- levels occur only at low DOC concentrations (Goodale et al. 2005, Evans et al. 2006b, Taylor and Townsend 2010, Kopacek et al. 2013). Although the pattern appears to be relatively widespread, its exact cause is uncertain; speculation typically points to C or N limitation to some microbial process such as immobilization or denitrification, occurring at an undetermined place in the landscape. Identifying the mechanism and location of NO_3^- retention is important for long-term NO_3^- management and for predicting future NO_3^- losses. Here, measurements from nine New York catchments were used to examine the hypothesis that both DOC and NO_3^- losses correspond with cross-site variation in soil C pools (Evans et al. 2006b), which could govern DOC production and NO_3^- immobilization capacity. As discussed in the following section, DOC production increased with the size of the forest floor C pool (Figure 9), and that this forest floor C pool partly controlled the overall demand for N for immobilization by heterotrophic microbes, when considered relative to the forest floor N pool and microbial carbon-use efficiency. However, variation in soil C stocks and C-driven immobilization explain only part of the DOC-NO_3^- pattern and do not produce the overall curve. The sharp drop in stream NO_3^- as DOC concentrations increase appears best explained by the process of denitrification and its limitation by bioavailable DOC.

8.1   DOC Production, Bioavailability, and Denitrification

As expected, we found that larger forest floors produced more water-extractable DOC (Figure 9A, 9B), which supports the hypothesis that cross-site variation streamwater DOC may be partly attributed to variation in surface soil C stocks (Evans et al. 2006b), although many other processes can also affect streamwater DOC. DOC is typically produced by partial decomposition in surface organic horizons and upper mineral soils, with subsequent decomposition or adsorption onto mineral surfaces deeper in the soil as leachate percolates down through the ecosystem (McDowell and Likens 1988, Kalbitz et al. 2000, Hagedorn et al. 2012). Measurements of the stocks of water-extractable DOC from the forest floor averaged 47 (1-188) kg C ha^{-1} for the Catskill catchments and 163 (8-705) kg C ha^{-1} for the Adirondacks (Figure 8B and Figure 10C). These DOC pools are both roughly 4-5 times the annual flux of DOC exported in surface waters in these regions, which average 10 kg C ha^{-1} yr^{-1} (range 5-24 kg C ha^{-1} yr^{-1}) for

the Catskills (Lovett et al. 2000) and 37 kg C ha^{-1} yr^{-1} (range: 16 to 100 kg C ha^{-1} yr^{-1}) for the Adirondacks (Ito et al. 2005). Measurements of extractable DOC in the forest floor provide one-time measurements of pools likely to be replenished throughout the year; as an order-of-magnitude estimate, these measurements may capture roughly half of the DOC produced annually by the forest floor. For comparison, lysimeters below the organic horizon in hardwood forests at Hubbard Brook, NH, capture an annual DOC flux of 134-200 kg C ha^{-1} yr^{-1} , or ~6-10 times the site's annual stream export losses of ~20-25 kg C ha^{-1} yr^{-1} (McDowell & Likens 1988, Dittman et al. 2007).

Only a portion of DOC can readily serve as a substrate for microbial growth or for denitrification. Although the Adirondack forest floors had much larger stocks of extractable DOC than the Catskills, a smaller fraction of the Adirondack DOC was degradable over one-month incubations, such that forest floors in both regions contained roughly 6-10 kg ha^{-1} of extractable bDOC. This relatively small pool of bDOC could constrain rates of denitrification at high-NO_3^- sites, especially in deep soils or in streamwater. Stoichiometrically (Equation 3), the bDOC extracted from these New York forest floors should support roughly 5-9 kg N ha^{-1} of N loss to denitrification, an amount that might be increased to the extent that bDOC is replenished throughout the year and reduced by the extent to which other processes consume bDOC. Coincidentally, forested catchments in the northeast U.S. show almost no NO_3^- losses to streamwater until inputs of N from atmospheric N deposition exceed ~7-9 kg N ha^{-1} yr^{-1} (Aber et al. 2003), a threshold of comparable magnitude to the potential denitrification that might be supplied by bDOC from these Catskill and Adirondack forest floors

Experimental additions of labile DOC to streamwater in both Hubbard Brook streams (Bernhardt and Likens 2002) and in oxic mesocosms containing Catskill streamwater (Sobczak et al. 2003) have been shown to stimulate microbial assimilation of NO_3^- with relatively little consumption by denitrification. By contrast, labile DOC additions appear to enhance NO_3^- removal by denitrification when added to a low-DOC aquifer in Florida (Bradley et al. 1992) and to a low-DOC riparian zone in an agricultural catchment in Michigan, yielding a sharp DOC-NO_3^- curve among various well samples (Hedin et al. 1998). Differences in the O_2 levels in these surface and subsurface environments could perhaps explain these differences in responses, and point to groundwater rather than streamwater as a likely location for catchment denitrification.

8.2   Forest Floor Carbon-Use Efficiency and Immobilization

The initial hypothesis was that the forest floor capacity to retain N should increase with its C content, because of increased capacity to support immobilization by heterotrophic microbes. However, we found the opposite, in that the largest pools of extractable N often occurred in soils with the most C (Figures 9A and 9B). More nuanced consideration of controls on heterotrophic N immobilization should consider relative C and N supply rather than C supply alone. Forest floor C:N ratio and estimates of the stock of C in excess of a critical C:N ratio provided threshold indices for extractable NO_3^- (Figures 9C and 9D) and for stream NO_3^- (Figure 10D), illustrating that a large forest floor C stock can prevent NO_3^- loss, but only when considered relative to forest floor N.

Several past analyses have reported threshold forest floor C:N ratio of 20-25 as the best biogeochemical indicator of net nitrification in soils (e.g., Aber et al. 2003, Ross et al. 2009) or for plot- or catchment-scale NO_3^- losses (Gundersen et al. 1998, Dise et al. 1998, Lovett et al. 2002). The results here are broadly consistent with these past analyses, in that results showed a rise in extractable NH_4^+ and NO_3^- as forest floor C:N ratios dropped below 25, with a large increase in extractable inorganic N below a C:N ratio of 18 (Figure 9C). These threshold C:N ratios likely reflect the capacity of heterotrophic microbes to immobilize N during decomposition. Extractable NO_3^- concentrations in solution should increase as C limitation constrains the capacity of heterotrophic microbes to take up both NO_3^- and NH_4^+, which also increases the supply of NH_4^+ to poorly competitive autotrophic nitrifiers.

These C:N thresholds for inorganic N loss may also yield insights about microbial carbon-use efficiency, a term difficult to quantify at the ecosystem scale but of increasing importance for model simulations of soil organic matter processes centered on microbial dynamics (e.g., Allison et al. 2010). Initial assumptions used standard CUE values of roughly 30-40% (Chapin et al. 2011, Manzoni et al. 2008) along with a mass-based average C:N ratio for microbial biomass of 7.4 (Cleveland and Liptzin 2007) to predict critical C:N ratios for N mineralization (Equation 1) in the range of 18-25. Reversing this calculation to solve for CUE from the C:N thresholds observed here for N release to solution (Figure 9C) implies an increase in CUE from 30% in high-C:N (>25) forest floors to near 40% in low C:N (~18) forest floors. The modest increase in extract DIN concentrations between this range of forest floor C:N values could perhaps reflect a gradual increase in the CUE by some microbes as C becomes limiting (Schimel and Weintraub 2003) up to an empirical threshold CUE of ~40% (forest floor C:N of 18) at which point C becomes strongly limiting and extract N levels rise sharply. These empirically derived values of CUE and their shift in response to increased N availability relative to C may prove useful in a next generation of soil C-N models.

8.3   Inferences from Stable Isotopes

Dual isotopic analysis of NO_3^- in the forest floor extract and stream samples largely indicated production of NO_3^- in these samples by nitrification. Excluding a few extracts with dual isotopic evidence of denitrification, extracts contained isotopically light $\delta^{15}N_{NO3}$ averaging -10‰ (-20 to +0.4‰), or roughly 12‰ lighter than the $\delta^{15}N$ of the forest floors from which it was extracted, along with mean $\delta^{18}O_{NO3}$ values much lighter than expected for precipitation NO_3^- (Figures 11 and 12A). These forest floor extracts had markedly lighter $\delta^{15}N_{NO3}$ than typically reported for well- or streamwater at most other small temperate forested catchments (Kendall et al. 2007, Wexler et al. 2014) or in the streams measured here (-0.9 to +6.2‰). The unusually light $\delta^{15}N_{NO3}$ values could reflect nitrification stimulated during sample collection and extraction, following cessation of plant N uptake following extraction of the intact forest floor blocks.

These $\delta^{15}N_{NO3}$ measurements also come from a rarely measured part of the landscape, the forest floor, material which usually has much lighter $\delta^{15}N$ than deeper mineral soils (Nadelhoffer and Fry 1988). The few other studies to examine water-extractable $\delta^{15}N_{NO3}$ from surface soils also report relatively light values. Mayer et al. (2001) report mean $\delta^{15}N_{NO3}$ values of -8 to -21‰ in NO_3^- leached from forest floor material over a 16-week lab experiment. Fang et al. (2012) report $\delta^{15}N_{NO3}$ values of -9 to -4‰ in NO_3^- extracted from surface mineral soils (0-5 cm depth) from Japanese forests, with newly produced NO_3^- averaging 5‰ lighter than the $\delta^{15}N$ of the soil from which it was extracted. Consistent with the interpretation of nitrification as the source of the NO_3^- in the extracts, $\delta^{15}N_{NO3}$ values varied with the proportion of NO_3^- present relative to total extract DIN (Figure 12B), with the lightest $\delta^{15}N_{NO3}$ occurring when NH_4^+ was most abundant relative to NO_3^-. The range of $\delta^{15}N_{NO3}$ values observed in these extracts is interpreted here to reflect nitrifiers' varying completeness of consumption of extract NH_4^+ at the time that the extraction occurred.

In pure culture, nitrifiers can fractionate against ^{15}N with enrichment factors (ε_{nitrif}) of -12 to -36‰ (Mariotti 1981, Högberg 1997, Casciotti et al. 2003). The ε_{nitrif} estimated for the cross-site samples here, of -9‰ for forest floor extracts and -5‰ for streamwater (Figure 7C), may reflect lessened fractionation by nitrification expressed under field conditions constrained by the relative scarcity of NH_4^+ – especially in streamwater, where NH_4^+ concentrations were always low.

Many forest floor extracts had $\delta^{18}O_{NO3}$ values somewhat higher (averaging ~+6‰) than theoretically expected for NO_3^- produced by autotrophic nitrifiers (Equation 3), as composed of two oxygen atoms from local water (here, $\delta^{18}O_{H2O}$ = -8.4 to -11.4‰) and one from atmospheric O_2 (+23.5‰; Kroopnick and Craig 1972) for an expected $\delta^{18}O_{NO3}$ at these New York catchments of +0.2 to +2.2‰ (Andersson and Hooper 1983; Figure 11). Recent work has shown that this 2:1 ratio oversimplifies, as kinetic fractionation against ^{18}O during nitrification and abiotic exchange of oxygen between water and the nitrification intermediate NO_2^- can both yield markedly lighter $\delta^{18}O_{NO3}$ than otherwise expected by Equation 3 (Buchwald and Casciotti 2010, Casciotti et al. 2010, Snider et al. 2010, Fang et al. 2012). However, neither of these processes can explain microbial $\delta^{18}O_{NO3}$ values heavier than the theoretical expectation, as observed here. Mayer et al. (2001) proposed that production of NO_3^- by heterotrophic nitrification in high-carbon soils could perhaps yield higher-than-expected $^{18}O_{NO3}$, but if this process contributed to elevated $\delta^{18}O_{NO3}$ values in the forest floor extracts here, it occurred without correspondence to forest floor C:N ratios ($R^2 < 0.02$, not shown). Snider et al. (2010) has shown that measurements of microbial NO_3^- frequently have $\delta^{18}O_{NO3}$ values higher than predicted, but they concluded that explanations for such enrichment remain elusive.

A few forest floor extracts showed isotopic evidence of denitrification, with dual enrichment in both $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ (Figure 11). Consistent with this interpretation, these same samples had $\delta^{15}N_{NO3}$ values distinctly heavier than otherwise predicted by forest floor $\delta^{15}N$ or by the ratio of NH_4^+:DIN in solution (Figures 12A and 12B). It was not readily apparent why denitrification occurred in these particular samples, as they did not have distinctive forest floor C:N, extract bDOC:NO_3^-, extract pH, or moisture measurements, although they were among the wetter half of the forest floor samples.

Across all catchments, streamwater had markedly heavier $\delta^{15}N_{NO3}$ than forest floor extracts from the same catchments, by 5 to 18‰ (Figures 10B, 11, 12B, and 12C). Fang et al. (2012) observed a similar enrichment, by ~5‰, between surface soil extracts and nearby streamwater in Japanese forests. Part of these differences between forest floor and stream $\delta^{15}N_{NO3}$ could occur if stream NO_3^- derived from NO_3^- produced from deep soil relatively enriched in $\delta^{15}N$, or if NO_3^- incurred partial denitrification somewhere between its production at the soil surface and its leaching loss in streamwater. Deep soil $\delta^{15}N$ rarely exceeds 10‰ even in C horizons (e.g., Nadelhoffer and Fry 1988, Pardo et al. 2007), which means that varying sources of organic N with depth could explain perhaps some (~4 to 9‰) but not all of the 5 to 18‰ $\delta^{15}N_{NO3}$ enrichment we observed between forest floor extract and streamwater. Consumption by denitrification should enrich the residual NO_3^- in both ^{15}N and ^{18}O (Kendall et al. 2007, Granger et al.

2008), and dual isotopic enrichment of NO_3^- has now been observed in shallow groundwater in forested headwaters at Hubbard Brook (Wexler et al. 2014) and in Japan (Osaka et al. 2010), as well as in aquifers impacted by agricultural N inputs. Osaka et al. (2010) noted that streamwater contained low streamwater NO_3^- concentrations and enriched $\delta^{15}N_{NO3}$, while lacking concurrent enrichment in $\delta^{18}O_{NO3}$ that would be expected if NO_3^- were lost to denitrification, similar to patterns observed here (Figure 11). They propose that coupled nitrification and denitrification in shallow groundwater, with dominance by denitrification, could yield these isotopic patterns, especially if oxygen exchange or fractionation during nitrification yielded especially light $\delta^{18}O$ in deeper groundwater. These exchange processes may be especially prominent at low soil pH (Fang et al. 2012). If so, and if these processes occurred in these New York catchments, they could perhaps produce streamwater containing partly denitrified NO_3^- that lacked the concurrent $\delta^{18}O$ signal typically expected for denitrification, and may provide one explanation for why so few streamwater samples show isotopic evidence of denitrification (reviewed in Wexler et al. 2014). If denitrification drove the broad enrichment between forest floor and stream $\delta^{15}N_{NO3}$ in all of the catchments examined here, then denitrification at some point in these catchments could have removed 45-72% of the NO_3^- produced by the forest floor.

8.4   What Controls the DOC-NO_3^- Curve?

The stoichiometric and isotopic analyses in this study provided evidence of roles both for microbial immobilization and for denitrification in generating components of the DOC-NO_3^- curve observed across surface waters of the Northeast U.S. (Figure 7; Goodale et al. 2005), and perhaps elsewhere as well (Evans et al. 2006b, Taylor and Townsend 2010, Kopacek et al. 2013). Distinctive mineralization-immobilization thresholds governed net NO_3^- production and consumption in forest floor extracts, consistent with a shift from N to C limitation for microbial biomass as forest floor C:N ratios fell from 25 to 18 or lower (Figure 10C). Microbial immobilization can explain the absence of NO_3^- in stream- and forest floor extracts with C:N or bDOC:NO_3^--N ratios greater than 25 (Zone A, Figures 11 and 12). However, this immobilization threshold does not suffice to produce the overall DOC-NO_3^- curve, as extracts from forest floors with C:N ratios less than 25 often produce solutions with high levels of both NO_3^- and DOC or bDOC (Zone B, Figure 12).

Isotopic analyses of NO_3^- from these extracts largely indicate a NO_3^- source from internal nitrification to varying degrees of completeness (Figures 11 and 12). Extracts from forest floors, which were presumably largely oxic, frequently contained samples in this zone, but streamwater did not, perhaps indicting that NO_3^- had been removed from solution. When NO_3^- did occur in streamwater, it did so only when labile

C supply was expected to be stoichiometrically limiting to denitrification of NO_3^- (Zone C, Figure 12B), and all streamwater showed enriched in $\delta^{15}N_{NO3}$ relative to the forest floor. Taken together, these observations may indicate that pools of NO_3^- and DOC produced in mostly oxic forest floors likely encounter low-O_2 zones at some point between the forest floor and the stream, where denitrification removes NO_3^- as long as the labile C supply suffices, and that catchments that display the inverse DOC-NO_3^- curve may reflect denitrification in deep soil or shallow groundwater as a master control on stream NO_3^- loss.

Cross-catchment variations in soil C stock may thus partly contribute to cross-site variations in solution DOC and NO_3^- concentrations (Evans et al. 2006b), but they do not suffice to explain the sharp DOC-NO_3^- curve. Removal of NO_3^- by denitrification, and limitation of this process by labile DOC, can better explain the curve's threshold response. If these processes do form primary controls on stream NO_3^- losses, then denitrification could form an underappreciated and widespread regulator of stream NO_3^- loss across a great many sites, or through time. Furthermore, factors that affect the availability of labile DOC to dentrifiers may be far more important regulators of stream NO_3^- than commonly recognized. De-acidification (Evans et al. 2006a, Monteith et al. 2007) and increased DOC flushing in catchments receiving more precipitation with climate change could both drive enhanced removals of NO_3^- to denitrification, and contribute to a decrease in stream NO_3^- over time.

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