

**Consequences of nitrogen fertilization and soil acidification
from acid rain on dissolved carbon and nitrogen stability in the
unglaciated Appalachian Mountains**

Philip Graham Taylor

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H.M. Valett, Chair
R.H. Jones
J.A. Burger

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Abstract

The expansion and proliferation of reactive nitrogen (N) sources, predominantly fertilizer application and fossil fuel combustion, has enriched the earth with N and acidified ecosystems. Acid rain is a primary vector of both N fertilization and acidification, initiating a cascade of consequences that alter biogeochemical cycling and global biological structure and function. Studies on N and acid influences are however rarely linked despite their common source. We used a wide, chronic gradient of N deposition ($5.5 - 31 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) to explore patterns in carbon (C) and N cycling in light of recognized biogeochemical responses to acidic deposition. Specifically, we examined the response of key controls on dissolved C and N stability because soluble pools are involved in decomposition and nutrient recycling, the formation of soil organic matter (SOM), and the translation of elements through the biogeochemical continuum from atmospheric to soil to water. Results suggest that N deposition led to reduced organic matter C/N, enhanced net nitrification, and greater DON generation; and, these patterns were associated with changes in C composition. Conversely, physiochemical processes in the mineral soil seemed to control organic matter dynamics, with effects on N processing. Moreover, pH dependent controls on DOC stability were evidenced by changes in DOC concentration, chemical complexity and recalcitrance. These horizon-specific, differential responses to acid rain indicate that changes in the forest floor N economy were responsible for increased surface water $\text{NO}_3\text{-N}$ concentrations, whereas enhanced organomineral stability of DOC caused a significant increase in DOM concentrations in export.

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Abstract

The expansion and proliferation of reactive nitrogen (N) sources, predominantly fertilizer application and fossil fuel combustion, has enriched the earth with N and acidified ecosystems. Acid rain is a primary vector of both N fertilization and acidification, initiating a cascade of consequences that alter biogeochemical cycling and global biological structure and function. Studies on N and acid influences are however rarely linked despite their common source. We used a wide, chronic gradient of N deposition ($5.5 - 31 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) to explore patterns in carbon (C) and N cycling in light of recognized biogeochemical responses to acidic deposition. Specifically, we examined the response of key controls on dissolved C and N stability because soluble pools are involved in decomposition and nutrient recycling, the formation of soil organic matter (SOM), and the translation of elements through the biogeochemical continuum from atmospheric to soil to water. Results suggest that N deposition led to reduced organic matter C/N, enhanced net nitrification, and greater DON generation; and, these patterns were associated with changes in C composition. Conversely, physiochemical processes in the mineral soil seemed to control organic matter dynamics, with effects on N processing. Moreover, pH dependent controls on DOC stability were evidenced by changes in DOC concentration, chemical complexity and recalcitrance. These horizon-specific, differential responses to acid rain indicate that changes in the forest floor N economy were responsible for increased surface water $\text{NO}_3\text{-N}$ concentrations, whereas enhanced organomineral stability of DOC caused a significant increase in DOM concentrations in export.

Introduction

Humans have altered the nitrogen (N) cycle more than any other biogeochemical element (Holland et al. 1999) through the rapid expansion of industrial, vehicular, and agricultural activity. Despite the momentous benefit of reactive N for human food demands (Smil 1990), the globalization of atmospheric N deposition has caused significant and potentially negative changes in other biogeochemical cycles and ecological dynamics (Vitousek et al. 1997, Matson et al. 2002). Detrimental outcomes include the loss of biological diversity (Schindler et al. 1994), enhanced exotic species

invasion, the advent of N saturation (Peterjohn et al. 1996) and N export (Agren and Bosatta 1988), contributing to eutrophication (e.g., Howarth et al. 1996), and soil (Johnson and Ruess 1991) and water (Fenn et al. 1998) acidification. Understanding these cascading consequences of N fertilization has been a major focus of theoretical and experimental research of biogeochemical cycles (Aber et al. 1989, Galloway et al. 1995, Vitousek et al. 1997) and has illustrated how greatly humans can reorganize environmental drivers (Matson et al. 2002).

Despite much research, the effects of N deposition on carbon (C) and N cycling remain largely unknown and controversial, and even though they are key to predicting global ecological change. For instance, despite well-documented association between N deposition and reduced C/N ratios of soil organic matter (SOM) (Tietema and Beier 1995, Dise et al. 1998, Gunderson et al. 1998, Lovett and Rueth 1999, Goodale and Aber 2001, Ollinger et al. 2002), the mechanism and relative role of biological (Townsend et al. 1996, Nadelhoffer et al. 1999) and abiotic (Dail et al. 2001) N immobilization remains a mystery. Regardless, widespread observation of concomitantly enhanced nitrification and greater nitrate-N ($\text{NO}_3\text{-N}$) loss with reduced C/N has provided a general framework to predict the N status of temperate forested ecosystems (Lovett et al. 2002, Aber et al. 2003).

Despite the apparent effect of N deposition on SOM chemistry and N loss, the cascading effects of N richness on DOM dynamics are just now being considered. Understanding the sensitivity of DOM dynamics to environmental change is of great import because processes that govern soluble C and N cycling regulate the turnover of nutrients (Schimel and Bennett 2004), SOM formation (McDowell and Likens 1988), and long-term soil C storage (Freeman et al. 2001, Waldrop et al. 2004), and maintain N limitation of temperate ecosystems (Hedin et al. 1995, Vitousek et al. 2002, Neff et al. 2002). There is growing evidence that N fertilization increases DOC (e.g., Pregitzer et al. 2004) and DON (e.g., McDowell et al. 2004) generation and loss (Freeman et al. 2004, Brookshire et al. 2007), yet mechanisms remain elusive. Furthermore, the response and fate of DOM to N fertilization appear very different between the forest floor and mineral soil (Aber et al. 2003, Waldrop and Zak 2006). This differential response may reflect differences in biological processing, yet may also reflect differences between forest floor

and mineral soil due to their organic and mineralogical nature, respectively (Currie et al. 1999). Furthermore, coincidental soil acidification via N leaching may be an organizing force, but it is typically not addressed despite the profound influence of soil acidification on biogeochemical cycles, particularly in the mineral soil. For instance, a rich literature documents the pH dependence of physiochemical controls on DOM stability reflecting organomineral interactions (reviewed in Lutzow et al. 2006). A lack of simultaneous assessment of N fertilization and soil acidification may reflect the confined spatial and temporal scale of mechanistic studies addressing these interactions. However, uncertainties associated with soil-specific responses and the concurrent driver of acidification highlights a need to explore the dual consequence of acidic deposition, both N-fertilization and soil acidification, on dissolved C and N dynamics.

Broadly, we sought to examine changes in dissolved carbon and nitrogen stability in forested ecosystems that have received acidic deposition for greater than 100 years. We use the term stability as a comprehensive term for processes that enhance the nutrient cycling efficiency or prolong the turnover time of dissolved C and N in soil by enhancing recalcitrance, organomineral interaction and/or resource unavailability. We were interested in confirming key, well-documented responses of forest floor C and N cycling, as well as exploring changes in DOM stability as a consequence of soil acidification. Specifically, we anticipated concomitant and strongly associated declines in SOM C/N and increases in net nitrification to be associated with greater N deposition (e.g., Ollinger et al. 2002). Furthermore, we expected to find greater DON concentrations (e.g., McDowell et al. 2004) with higher N deposition and an associated reduction in DOC:DON, as observed by others (e.g., Currie et al. 1996). In the mineral soil, we did not expect mineral SOM properties to change with N deposition (Aber et al. 2003), yet we expected that net nitrification in mineral soils would track soil C/N (Aber et al. 2003). Moreover, we expected that enhanced soil acidification (reduction in pH) would influence the strength of well-known physiochemical/mineralogical controls on DOM stability in the mineral soil (Baldock and Skjemstad 2000), reflected by an increase in DOC concentration and recalcitrance. Lastly, if indeed these relationships hold true and different horizons are responsible for different biogeochemical controls, then higher surface water $\text{NO}_3\text{-N}$ concentrations should reflect enhanced forest floor $\text{NO}_3\text{-N}$

production, whereas greater surface water DOM concentrations should reflect patterns found in the mineral soil, mirroring the expected chemical stability, accumulation, and export of DOM due to enhanced soil acidity.

Methods

Conceptual Framework

Ecosystems included in our survey have been receiving acid deposition since the Industrial Revolution, which was in full swing by the 1860's in the southern and middle Appalachian Mountains. We chose sites that have remained undisturbed by fire, logging or farming for > 100 years or were never disturbed because our goal was to understand controls on C and N dynamics in ecosystems with stable microbe-soil-plant complexes developed under the long-term influence of acid deposition (Table 1; McNulty et al. 1991, Pregitzer et al. 1992, Lovett and Rueth 1999). We chose these sites to assess how biogeochemical interactions have reorganized as a result of anthropogenic forcing by N fertilization and soil acidification.

We were also interested in looking for differential consequences of N fertilization and soil acidification between sites receiving acid deposition below and above the widely recognized critical N loading rate of $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Lovett et al. 2002, Aber et al. 2003, Brookshire et al. 2007). Accordingly, we divided sites into categories and designated high ($n = 10$) and low ($n = 6$) deposition sites *a priori* in order to test whether significant differences existed for biogeochemical patterns across this purported breakpoint.

Choice of Forested Ecosystems

Sixteen watersheds were chosen to form a gradient of *natural*, chronic acidic deposition ($5.5\text{--}30.9 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) in the south and middle Appalachian Mountains (Fig. 1). Sites were selected based on: 1) close proximity to acidic deposition monitoring stations (NADP, CASTNET) with > 15-year record of continuous acid deposition monitoring, or 2) numerical estimates based on spatially explicit deposition models (Weathers et al. 2006, Boggs et al. 2004), 3) non-calcareous parent material, 4) unglaciated regolith, and 5) predominance of northern hardwood, spruce-fir forest types, or a mixture of the two.

To estimate acidic deposition for each site, we averaged values over past 15 years (1990—2005). We used N deposition rate as a proxy for acidic deposition because sulfuric acid (H_2SO_4) deposition has declined dramatically under the Clean Air Act since 1990, while N deposition has remained legislatively unregulated; thus, we considered long-term N inputs more representative of chronic and continuing effects of acidic deposition.

Soil sampling and analysis

Design—To obtain representative measurements of C and N parameters for entire watersheds, we followed the natural contour of watershed topography as a transect, where 8 soil samples were collected 150 m apart along two “U”-shape transects across the breadth of the watershed near the top of the catchments. These two transects were separated by roughly 50 m of elevation. A total of 16 samples were collected and combined for a composite sample. At each sample point we harvested forest floor material within the area ($1,256 \text{ cm}^2$) of a steel circular frame after removing the Oi horizon. Mineral soil was sampled to a depth of 15cm with a bulk density sampler adjacent ($<0.25 \text{ m}$) to the location of forest floor material collection. Generally, mineral soil was clearly discernable from forest floor material, so little O horizon was included in the mineral soil composite. Forest floor and mineral soils were sieved (5mm and 2mm screens, respectively), and stored at 4°C until analysis, unless otherwise noted.

Soil chemical and functional analyses—We used sub-samples of forest floor and mineral soil to assess chemical and functional properties of the soil environment, including total C and N content, noncrystalline and humus-complexed Al/Fe and C, soil pH, exchangeable cations (used in summation of effective cation exchange capacity [ECEC]) and total exchangeable acidity; soluble, potentially-soluble, and insoluble DOC (DOC_s , DOC_{ps} , and DOC_{in} , respectively); and, surface water DOM (DOC and DON) and DIN ($\text{NO}_3\text{-N}$ and ammonium-n ($\text{NH}_4\text{-N}$); water extractable DOM bioavailability assays and chemical characterization of DOM via humification and fluorescence indices. We did not assess DOC_s , DOC_{ps} or mineralogical properties of the forest floor because we assumed these would be unimportant predictor variables of forest floor C and N dynamics in organic soils composed of non-mineralogical material.

C and N content, mineralogy, soil acidity, and cations—Total C and N content was analyzed on a Variomax CN analyzer (Elementar Vario EL, Hanau, Germany), after soils were ground with mortar and pedestal. Acid oxalate-extractable Fe (Fe_o) and Al (Al_o) were measured on mineral soil samples extracted at a soil:solution ratio of 1:1 (w/v) in 0.2 M ammonium oxalate solution at pH 3 in the dark. This extraction dissolves poorly crystalline aluminosilicates, ferrihydrite, and organic-complexed Fe and Al. Organic-complexed C, Fe and Al was estimate by pyrophosphate-extractable C (C_{py}), Fe (Fe_{py}) and Al (Al_{py}), which was extracted at a 1:1 w/v ratio in a 0.1 M sodium oxalate ($Na_4P_2O_7 \cdot 10H_2O$) solution. $MgSO_4$ (50mM) was added to extracts to encourage flocculation during centrifugation; extracts were centrifuged at 10,000 x g for 10 min. Non-crystalline Fe (Fe_{nc}) and Al (Al_{nc}) was calculated by difference as $Fe_o - Fe_p$ and $Al_o - Al_p$, respectively. Soil pH was measured on 1:1 soil:water v/v ratio using distilled water. Using the 1:1 v/v soil-water mix, Melich pH was measured for estimation of total exchangeable acidity. Base cations (Mg^{2+} , Ca^{2+} , K^+) were extracted using a solution of 0.05M HCl in 0.05M H_2SO_4 , and summed with total exchangeable acidity to ECEC.

Extractable DOC—We extracted soil solution DOC, DON and DIN on four replicate, unsieved samples within 72 hours of collection by shaking 40g soil in 200 ml of distilled water (1:5 w/v ratio) for 1 h at 20°C on a rotary shaker at 200 rpm following Jones et al. (2005). All extracts were filtered through a 0.7 μm (nominal pore-size) Whatman GFF.

Soluble, Potentially Soluble and Insoluble DOC—We used a simple leaching experiment to estimate DOC_s , DOC_{ps} and DOC_{ins} for mineral soil using an approach adapted from Johnson and Todd (1981) for sulfate adsorption analysis. Triplicate, 10-g soil samples were placed in separate filter funnels, equipped with a 0.7 μm Whatman GFF. Each sample was leached sequentially with 0.2 L of distilled water (pH = 6.5; soluble DOC) and 0.05 L 0.016M NaH_2PO_4 (potentially soluble DOC). Additionally, insoluble DOC was estimated by extracting separate triplicate 10-g soil samples with 0.05 L of 2.34 mM Na_2SO_4 using a rotary shaker at 200rpm for 1 h at 20°C.

Net N processing—Net mineralization was measured according to Lovett et al. (1999). Six forest floor and mineral soil sub-samples were allotted to plastic specimen cups. Three were extracted immediately with 2 M KCl. Three were incubated at 20°C for 28 days then extracted with 2 M KCl. Extracts were filtered through 0.7 μm GFF filters, and

immediately analyzed on a flow injection analyzer using analytical methods described below. Potential net ammonification, nitrification, and mineralization were calculated as the difference in extractable NH₄-N, NO₃-N, and DIN (NH₄-N + NO₃-N) between initial and incubated samples, respectively. These indices of net N processing were calculated as µg N per day, and presented as µg N g C⁻¹ or µg N g N⁻¹.

DOC bioavailability— DOC bioavailability was assayed following McDowell et al. (2006). Four replicate, 30-ml soil extracts (method described above) were incubated for 52 days, and cumulative CO₂ evolution was measured 6 times (1,5,12,22,36, and 52 days). In order to maximize C degradation, we added 1) a 1 X 1 cm² GFF to provide surface area for microbial growth, 2) a small nutrient inoculum (1.82mM N, 2.43 mM P, 0.07 mM S, and 2.42 mM K) to alleviate potential nutrient deficiency, and 3) 100µl of microbial inoculum, prepared by shaking 5-g forest floor soil (from Beech site) in 50 ml of deionized water, and filtering the supernatant with a 0.7µm GFF. Samples were incubated in 43-ml EPA TOC vials fitted with septa caps, which allowed direct analysis of accumulative CO₂ in the headspace using the OI-1010 TOC Analyzer (IO Corporation, College Station, Texas, USA). Vials were continually shaken at 25 rpm and 20⁰C on a rotary shaker to achieve equilibrium between the liquid and gaseous phase. Following each CO₂ headspace analysis, vials were left uncapped for 12 hours to avoid anoxia.

Labile and refractory DOC pool size and mineralization rates were estimated with a two-pool exponential decay model (Equation 1; Alvarez and Alvarez 2000) fitted to measured data for DOC mineralized over a 56-day period, which was parameterized using nonlinear curvefitting techniques in JMP 5.0 (SAS Institute 2006).

Equation (1)

$$\text{Cumulative mineralized DOC (\% of initial DOC)} = a(1 - e^{-k_1 t}) + (100 - a)(1 - e^{-k_2 t})$$

where: a is the percent DOC readily mineralized, $100-a$ is the percent DOC slowly mineralized, t is time; k_1 is the decay rate constant for the labile DOC fraction (day⁻¹), k_2 is the decay rate constant for the recalcitrant DOC (day⁻¹). Turnover time of each DOC pool was calculated by $1/k_1$ and $1/k_2$ for labile (T_{DOC-L}) and refractory (T_{DOC-R}) DOC, respectively.

Chemical Complexity of DOC_{ws}—Emission scan fluorescence spectra were collected on soil DOM extracts on a Fluoromax-3 to calculate fluorescence (FI) and humification index (HIX). All 2-D emission scans were corrected for inner-filter effect, lamp spectral properties, emission bias, and Raman-scattering; then FI was calculated as emission intensity at 470 nm divided by 520 nm for an excitation wavelength 370 (Cory and McKnight, 2005), whereas HIX was calculated by dividing the emission intensity in the 435-480 nm region by the emission intensity from the 300-345 nm region (Zsolnay, 1999). We used Suwannee River fulvic acid as a standard. These two indices have been extensively used to examine the chemical complexity and degree of condensation of DOM in aquatic and terrestrial ecosystems. Low FI (1.1-1.3) and high HIX (14-30) values indicate DOM is composed of aromatics (i.e. fulvic and humic acids), whereas high FI (1.7-2.2) and low HIX (5-14) values correspond to DOM composed of polysaccharides and microbial products.

Water sampling and analysis

At each site, surface and ground water sampling was undertaken directly down slope of soil sampling sites. Three samples were longitudinally segregated 15-35m apart to account for heterogeneity in stream and ground water chemistry. Groundwater was sampled using a portable piezometer, which was driven into the streambed to a depth of 30-50 cm. The piezometer was given 15 minutes minimum to equilibrate with the surrounding environment, and then up to 1 L of solution was extracted with a peristaltic pump over the course of up to 1 hour. Samples were collected for analysis of DOC, DON, and DIN, filtered on-site, and analyzed using aforementioned methods. DOC bioavailability was measured using technique previously described to assess bioavailability in soil extractable DOC; however, due to the paucity of DOC mineralization, a one-time measurement of accumulative CO₂ (i.e., after 17 days incubation) was used to estimate percentage of labile and recalcitrant DOC fractions.

Chemical Analyses—C_{py} and DOC were analyzed using sodium persulfate oxidation on an OI-1010 TOC Analyzer. Potassium persulfate and UV bombardment were used to oxidize TDN on a flow injection analyzer followed with subsequent analysis of NO₃-N. Both NO₃-N and NH₄-N concentrations were determined colorimetrically on a Lachat flow injection analyzer. DIN was calculated as the sum of NO₃-N and NH₄-N, and DON

estimated by difference as TDN-DIN. Total soil magnesium (Mg), calcium (Ca), potassium (K), and Fe, Fe_o, Al_o, Fe_p, and Al_p were measured on an inductively coupled argon plasma atomic emission spectrometry (Thermo Elemental ICAP 61E) at Virginia Tech's Soil Testing Laboratory.

Statistical analysis

We analyzed responses of key soil and surface water C and N variables to the effects of N fertility and soil acidity. We used a multivariate approach similar to Hobbie et al. (2007), where predictor variables were initially divided into subsets (Table 2), then used in backward, stepwise multiple linear regression to optimize an initial model for each predictor subset. Next, parameters retained for each subset were included in a final model that included predictor variables from all subsets. We used Akaike's Information Criterion (AIC) to select model terms in both the initial and final model development. AIC is a general criterion that rewards good model fit, yet penalizes over-parameterization. We chose parameters to include in the initial and final model terms by eliminating terms that greatly reduced AIC in order of greatest to smallest effect. This process proceeded until no further reduction in AIC was observed by elimination of predictor variables. While this approach reduces the likelihood of collinearity among potentially correlated variables, we used pairwise correlation to check against collinearity by examining the relationships between model terms included in the initial and final models. If parameters were highly correlated, we eliminated the parameter that reduced AIC the greatest. Additionally, variables that exhibited curvilinear behavior were log-transformed to allow for possible inclusion of these terms in regression models. We considered the interaction of C and N dynamics by using the DOC characteristics subset and avoided using this subset in the development of predictive models for DOC dynamics. At the same time we included net N processing in the development of models for N dynamics and watershed outputs, which were otherwise used as response variables. For prediction of chemical patterns in mineral soil, forest floor predictor subsets were included because the forest floor represents a source pool for mineral soil. Furthermore, we included C and N dynamics as predictor variables for watershed outputs.

We focused on single predictor variables of interest using Reduced Major Axis (RMA) regression in order to explore "best fit" relationships among endogenous

properties of the soil environment and watershed outputs. We used Model II regression because OLS Model I regression assumes no error associated with variable X. Nonlinear regression was used to examine curvilinear relationships among soil properties and watershed outputs when necessary. Significant differences in soil and surface water chemistry between high and low deposition sites, and between deciduous, mixed and coniferous forest types, were inspected using a t-test and ANOVA, respectively, followed by Tukey-Kramer test ($P \leq 0.05$) for assessment of significant differences.

Results

Exogenous Drivers

Natural climatic factors were highly correlated with topography in our survey. Mean annual temperature (MAT) was negatively correlated with elevation ($r = -0.71$, $p \leq 0.001$), whereas mean annual precipitation (MAP) and elevation were positively correlated ($r = 0.78$, $p \leq 0.001$). N deposition varied independently from forest type ($p > 0.05$) even though purely coniferous or mixed forest types were prevalent at greater elevations ($p < 0.007$).

C and N Dynamics in the Forest Floor

Nitrogen Dynamics—N deposition was a strong driver of forest floor C/N, which declined as N deposition increased from 5.5 – 30.9 kg N ha⁻¹ yr⁻¹ (Table 3; Fig. 2A). The increase in relative N abundance in SOM C/N was driven by a decrease in C concentration ($r = 0.57$, $p \leq 0.021$), than by an increase in N concentration ($r = -0.38$, $p > 0.05$). Neither forest floor N concentration nor total N content increased linearly with greater N deposition (Table 3). Instead, forest floor N concentration was moderately correlated to C concentration ($r = 0.52$, $p \leq 0.001$); C concentration increased with total soil C content (11-51 Mg/ha; $r = 0.53$, $p \leq 0.001$). Coniferous forests had greater total soil C content ($p < 0.02$) than deciduous and mixed forest types, which were not significantly different ($p > 0.05$). Neither C ($r = 0.11$, $p > 0.05$) or N ($r = 0.26$, $p > 0.05$) concentration increased with elevation, although total soil C content, which accounted for forest floor depth, showed a trend toward increase with elevation ($r = 0.44$, $p < 0.089$).

N deposition was a significant exogenous driver of net nitrification (Fig. 2B) and net nitrification increased with soil N concentration ($r^2 = 0.40$, $p < 0.008$) and reduced C/N (Fig. 3). Forest floor NO₃-N concentration ranged from 1.2 to 45 mg/kg and was

significantly predicted by N deposition and net nitrification in the initial MLR model (results not shown), whereas elevation and $T_{\text{DOC-R}}$ explained % 60 of the variance in the final model using MLR (Table 3).

DOM Dynamics— DOC_{we} and DON concentrations ranged from 243-560 and 26-78 mg/kg, respectively. DOC_{we} and DON were strongly correlated, especially when separated by N loading rate (Fig. 4). DON concentrations were greater for high deposition sites ($p \leq 0.001$), while DOC concentrations were not significantly different ($p > 0.05$) between groups. Greater DON concentrations for high deposition sites caused a significant reduction in DOC:DON ($p \leq 0.001$) for high deposition sites. Furthermore, this decline in DOC:DON mirrored reduced SOM C/N for high deposition sites (Fig. 5A); however, the opposite pattern emerged for the six low deposition sites where forest floor DOC:DON increased with decreasing SOM C/N (Fig. 5A). The later pattern was clearly evident in mineral soils (Fig. 5B), but there was no significant difference in DOC:DON ($p > 0.05$) between N loading groups.

On average, 15% (6-39%) of total incubated DOC was mineralized over the 52-day course of bioavailability assays. Nonlinear fitting of a two-pool exponential decay model showed that labile DOC_p (range, 1.6-16%) represented half of the total C mineralized. $T_{\text{DOC-L}}$ was positively correlated to the size (%) of the labile DOC pool ($r = 0.64$, $p \leq 0.001$), which ranged from 1.7-16.3% of DOC_{we} . MLR also revealed that $T_{\text{DOC-R}}$ was related neither to $T_{\text{DOC-L}}$ nor any other DOM parameters; however, was negatively predicted by pH and ECEC in the final model (Table 2).

Concentrations of DON were negatively related to $T_{\text{DOC-L}}$ (Table 3), and stronger patterns were revealed when DON was regressed against $T_{\text{DOC-L}}$ (Fig. 6A) when sites were separated into N loading groups.. Specifically, for a given $T_{\text{DOC-L}}$ or SOM C/N, DON concentrations were ~20 mg/kg greater for high deposition sites. In an opposite pattern to DON, water-extractable $\text{NO}_3\text{-N}$ increased with greater $T_{\text{DOC-L}}$, and this relationship also strengthened when sites separated by N loading groups (Fig. 6B). Moreover, the same pattern emerged when DON concentration was regressed against SOM C/N and sites separated by loading group (Fig. 6C).

Soil Acidity—N deposition was the only significant exogenous variable predicting forest floor pH (Table 3). Also, forest floor pH was not significantly different among the three

forest types ($p > 0.05$). Forest floor soil acidity played a relatively minor role in forest floor C and N dynamics. No parameters reflecting changes in soil acidity (i.e., pH, exchangeable acidity, ECEC, base cation status) emerged as significant drivers of patterns in SOM or DOM C and N (Table 2), except for $T_{\text{DOC-R}}$.

C and N Dynamics in the Mineral Soil

Nitrogen Dynamics – Unlike the forest floor, mineral soil C/N and N concentration were unrelated to N deposition (Table 3). Instead, SOM C/N decreased with greater elevation (Table 3). The decrease in C/N with increased elevation was driven by a relative enrichment of SOM with N while both C and N concentration increased with elevation. Furthermore, N was strongly correlated with C concentration ($r = 0.92$, $p \leq 0.001$), and showed no relationship with N deposition or forest floor SOM N using MLR or RMA regression. Net nitrification in the mineral soils was positively related to mineral soil N concentration ($r^2 = 0.65$, $p < 0.001$) and negatively correlated with C/N (Fig. 3).

DOM Dynamics—In comparison to the forest floor where acidity played a minor role, changes in DOM dynamics were strongly related to soil acidity. Specifically, DOC_{ps} (Table 2, Fig 7A) and DON (Fig. 7B) concentration, as well as $T_{\text{DOC-R}}$ (Fig. 7C), increased as soil acidity intensified (Table 3).

Enhanced organomineral interaction with greater acidic deposition is evidenced in the strong association of $\text{C}_{\text{py}}/\text{Al}_{\text{py}}$ with N deposition (Fig. 8A) and pH (Fig. 8B). Pyrophosphate (organically bound) and oxalate-extractable (amorphous noncrystalline) Al and Fe concentration did not change with acidic deposition (results not shown); however, the portion of soil C affiliated with active Al (Al_{py}), which increased with greater soil acidification (Fig. 8) strongly predicted an increase in $T_{\text{DOC-R}}$ (Fig. 9A) and DOC_{ps} (Fig. 9B).

A strong negative relationship between $T_{\text{DOC-R}}$ (Fig. 9C) and DOC_{ps} (Fig. 9D) with FI (Table 3) suggests that longer turnover time representing increased DOM chemical complexity was evident in the mineral soil. Increased $T_{\text{DOC-R}}$ may reflect greater DOC pool size, indicated by the significant prediction of $T_{\text{DOC-R}}$ by DOC_{we} in the final model (Table 3). Potentially soluble DOC (DOC_{ps}) predicted 52% of the variation in DOC_{we} ($p \leq 0.002$). Moreover, DOC_{we} and DOC_{ps} concentration were predicted by similar variables, and appear to be controlled by changes in soil acidity (Fig. 8D,E).

Several patterns in C and N dynamics in the forest floor translated to the mineral soil. Particularly, mineral soil $P_{\text{DOC-L}}$ was positively correlated with forest floor $P_{\text{DOC-L}}$ ($r = 0.55$, $p = 0.013$), and forest floor DOC_{we} and DON predicted %52 and %44 of the variation in mineral soil DOC_{we} ($p \leq 0.001$) and DON ($p \leq 0.005$). Also, forest floor DOC:DON predicted % 52 of the variation in mineral soil DOC:DON ($p \leq 0.002$). As previously mentioned, mineral soil DOC:DON also mirrored changes in mineral soil SOM C/N (Fig. 4B). Interestingly, despite close association of forest floor and mineral soil C and N patterns, forest floor and mineral soil $T_{\text{DOC-R}}$ were not correlated ($r = 0.20$, $p > 0.05$), even though forest floor $T_{\text{DOC-R}}$ similarly increased with reduced pH.

Soil Acidity—N deposition was not a significant predictor of mineral soil acidity in final regression models because internal controls were strongly predictive of changes in pH and exchangeable acidity (Table 3). However, using single-factor linear regression, increased N deposition significantly predicted reduced pH ($r^2 = 0.47$, $p < 0.003$) and increased exchangeable acidity ($r^2 = 0.42$, $p < 0.007$). Furthermore, forest floor pH and exchangeable acidity, which were related to N deposition (Table 2), were strong predictors of mineral soil pH ($r^2 = 0.67$, $p < 0.001$) and exchangeable acidity ($r^2 = 0.83$, $p < 0.001$), respectively.

Watershed Outputs

Horizon-specific responses to acidic deposition translated to distinct elemental loss patterns observed in the surface water draining these ecosystems. Stream water concentrations of $\text{NO}_3\text{-N}$ increased with N deposition ($r^2 = 0.41$, $p < 0.008$), which was mediated by declined forest floor C/N (Table 2, Fig. 10A) and increased net nitrification (Fig. 10B). Additionally, the slope describing the predictive relationship between forest floor C/N and surface $\text{NO}_3\text{-N}$ steepened for high deposition sites when separated by N loading rate.

Forest floor and mineral soil C/N were significant predictors of surface water DOC and DON (Fig. 11 A-D). Even though high deposition sites had greater concentrations of DON in the forest floor, surface DON concentration were generally negatively related with forest floor DON concentration because forest floor DON concentration generally decreased with N fertility when sites were separated by N loading rate (See Fig. 6A and B). Surface water DON concentration was predicted by one soil

parameter using MLR, mineral soil $T_{\text{DOC-R}}$ (Table 3). However, while surface water DON concentration was predicted by surface water DOC concentration ($r^2 = 0.42$, $p < 0.006$), increased surface water $\text{NO}_3\text{-N}$ concentration was the strongest predictor of DON concentration (Fig. 12). Conversely, surface water refractory DOC concentration increased in strong association with enhanced stability of DOM in the mineral soil (Fig. 13), specifically, greater mineral soil DOM $T_{\text{DOC-R}}$ (Fig. 13A), acidity (Fig. 13B), and $\text{C}_{\text{py}}/\text{Al}_{\text{py}}$ ratio (Fig. 13C).

Surface water DOC:DON was positively related with forest floor ($r^2 = 0.52$, $p \leq 0.002$) and mineral soil ($r^2 = 0.37$, $p \leq 0.012$) SOM C/N, as well as forest floor ($r^2 = 0.42$, $p \leq 0.007$) and mineral soil ($r^2 = 0.36$, $p \leq 0.014$) DOM C/N. Similar to the mineral soil, DON concentrations for high deposition sites were not significantly greater than low N loading sites ($p > 0.05$); however, surface water DOC:DON declined with greater N deposition ($r^2 = 0.46$, $p \leq 0.004$). Surface water DOC:DON ($p < 0.001$) and $\text{NO}_3\text{-N}$ concentration ($p < 0.001$) was also significantly lower and greater for high deposition sites, respectively.

Discussion

Regression analysis revealed that N fertilization and soil acidification differentially influence forest floor and mineral soil C and N cycling. Characteristic differences in the organic and mineralogical nature of the forest floor and mineral soil mediate the response of biogeochemical cycling to acid rain and N deposition. This is evidenced by the progressive enrichment of forest floor organic matter with N associated with greater acidic (N) deposition, causing enhanced net nitrification and increased soil $\text{NO}_3\text{-N}$ concentrations in the forest floor. Conversely, the influence of acidification in the mineral soil changed the concentration, chemical complexity, and recalcitrance of DOC, and potentially DON, by changing pH dependent physiochemical controls on DOM stability. These horizon-specific changes led to greater surface water concentrations of $\text{NO}_3\text{-N}$ and DOM with $\text{NO}_3\text{-N}$ and DOC loss originating from the forest floor and mineral soil, respectively. It is evident that pathways of DON generation are different between soil horizons; however, controls on DON loss remain unclear.

Controls on SOM Composition and Net $\text{NO}_3\text{-N}$ Production

Biological (Townsend et al. 1996, Nadelhoffer et al. 1999) and abiotic (Dail et al. 2001, Davidson et al. 2003) pathways sequester roughly 75% (Lovett et al. , Brookshire et al. 2007) of atmospherically deposited N into SOM, which is eventually recycled during decomposition. N immobilization into SOM may be observed as an inverse relationship between N deposition and C/N, which has been reported widely (McNulty et al. 1991, Van Miegroet et al. 1992, Lovett and Rueth 1999) and observed in our study. The N enrichment of SOM expressed as forest floor C/N has emerged as a broad, integrative predictor of ecosystem export of NO₃-N (Gunderson et al. 1998, Lovett et al. 2002). In combination with observations of depressed C/N and enhanced net nitrification (Dise et al. 1998, Lovett and Rueth 1999, Goodale and Aber 2001, Ollinger et al. 2002, Aber et al. 2003, Fig. 3), a growing body of evidence suggests C/N may reflect a critical balance of C and N availability for microbial metabolism. Eventually, a shift in the forest floor N economy, from DON and NH₄-N to NO₃-N based (Corre et al. 2003), amplifies N loss from ecosystems due to the anionic, mobile nature of NO₃-N, which is exacerbated when biological demand for N is saturated (Stoddard 1994, Aber et al. 1992).

Despite these widely observed patterns, the mechanisms responsible for the reduction of C/N (Aber et al. 1989, Dail et al. 2001) and the simultaneous stimulation of nitrification (*sensu* Chapin, Matson and Mooney 2002) are unknown. For instance, several important factors also known to correspond with nitrification rate were imbedded in this survey and may also contribute to decreased forest floor C/N. Higher elevation (Table 2) emerged as fellow exogenous drivers of reduced C/N, likely reflecting interplay between climate, soil, and vegetation that determines forest productivity and nutrient cycling (Nadelhoffer et al. 1983, Pastor et al. 1984, Reich et al. 1997, Scott and Binkley 1997, Lovett et al. 1999, 2004, Bohlen et al. 2001). Similar observed decreases in mineral soil C/N with increased elevation may reflect a comparable interplay of state factor controls. Also, forest successional status may be a spatially segregated factor that indirectly governs soil C/N through plant C allocation patterns (Vitousek et al. 1988, Goodale and Aber 2001, Ollinger et al. 2002) and microbial resource demand (*sensu* Chapin, Matson and Mooney 2002) that changes characteristically from young to old growth forest. As Goodale and Aber (2001) posit, greater N deposition will only exacerbate nitrification and N loss because reduced C inputs and diminished demand on

external N sources in old growth forests—both more prevalent at higher elevations—drive forest floor stoichiometry toward the critical C/N ratio where the N cycle becomes relaxed

According to expectations, we observed no relationship mineral soil C/N and N deposition, confirming results from a regional analysis by Aber et al. (2003), which also declines in forest floor C/N with greater N deposition as we observed in our study. In the same survey, Aber et al. (2003) showed that mineral soil C/N and mineral soil net nitrification were closely associated, suggesting that something other than N deposition controlled SOM composition in the mineral soil and indirectly regulated mineral soil net nitrification.

Controls on DON and DOM Composition

Pristine temperate ecosystems unpolluted with reactive N exhibit efficient N cycling because high resource demand by the microbe-soil-plant complex limits the availability of labile C and N (Hedin et al. 1995, Schimel and Chapin 1996, Vitousek et al. 2002). In addition to biological competition, chemical complexity and a suite of abiotic factors constrain C and N bioavailability by protecting organic matter from degradation (Kaiser and Guggenberger 2003). Elucidating controls on DON production and availability are becoming central to understanding the forest N cycle because processes that mediate DON supply are gatekeepers to an active N cycle (Chapin et al. 2001, Neff et al. 2003, Schimel and Bennett 2004), whereas mechanisms that create unavailable DON, in the face of high N demand, maintain N limitation over the course of ecosystem development (Hedin et al. 1995, Vitousek et al. 2002). Mounting evidence indicates that N deposition changes DON production and consumption pathways (McDowell et al. 2004, Brookshire et al. 2007), illustrated by increased DON loss from soils (Park et al. 2002, Magill et al. 2000, McDowell et al. 2004, Corre et al. 2004, Pregitzer et al. 2004) and watersheds (Pellerin et al. 2006, Brookshire et al. 2007, E. Bernhardt *personal communication*) experimental and survey studies. Whether DON production and turnover are related (Goodale et al. 2000, Neff et al. 2000, Qualls 2000, Pregitzer et al. 2004) or unrelated (Chapin et al. 2001, Lovett et al. 2004, McDowell et al. 2004, Brookshire et al. 2007) to processes that control the fate DOC remains largely unknown.

We observed distinct relationships between forest floor DOC and DON concentration that differed when sites were separated at a depositional rate of $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. These results suggest that changes in DON are coupled to changes in DOC. At the same time, DON in high deposition sites was significantly greater than in low deposition sites suggesting that N deposition enhances the production of DON relative to DOC illustrating autonomous behavior of this dissolved organic constituent. The significantly greater forest floor DON concentrations characteristic of high deposition sites could be ascribed to 1) physical dissolution of SOM, which becomes progressively N enriched with greater N deposition; 2) abiotic incorporation of N into organic matter (Dail et al. 2001); 3) preferential sorption of N-poor DOM (Kaiser et al. 2000, Kaiser and Zech 2000) or desorption of DOM due to changes in soil acidity, 4) residual differences in DOM stoichiometry among forest types (Magill and Aber 2000, Smolander and Kitunen 2002), 5) increased microbial DON production pathways (Bronk and Ward 1999, Mulholland et al. 2000, Park et al. 2002, McDowell et al. 2004, Brookshire et al. 2005); and/or 6) decreased DON consumption due to a shift in microbial N resource preference for inorganic rather than organic N (Carreiro et al. 2000, Kaushal and Lewis 2005) or an increase in chemical recalcitrance (Fog 1988).

Although it is unclear which of these processes caused the increase in DON concentration, we suggest that our data do not support the physical mechanisms listed above. The influence of dissolution reactions may be suggested by close association between forest floor SOM C/N and DOC:DON (Hedin et al. 1995, Rastetter et al. 2005). In our study, the relationship between forest floor DOM C/N and SOM C/N differed dramatically when sites were segregated by N loading rate. While the cause of this divergence is uncertain, several patterns provide evidence against passive solubilization. First, although forest floor DOC:DON and C/N concomitantly decline when N deposition rate is high, variation in DOM stoichiometry was driven solely by differences in DON concentration, whereas variable C concentrations were responsible for differing SOM C/N. These data suggest that soluble and insoluble OM is likely distinct in formation and composition (Neff et al. 2000). The disproportionate increase in forest-floor DON concentration relative to DOC when deposition is high also suggests pH-induced

dissolution was not responsible since that mechanism should result in a simultaneous increase in DOC (Qualls et al. 2002).

While C/N of DOM was lower when N deposition was high, in nearly every case DOC:DON ratios were greater than associated SOM C/N. This pattern suggests an additional source of DOM (Qualls and Haines 1991, Currie et al. 1996), which may be microbial (Northup et al. 1995, Seely and Lajtha 1997, TrasarCepeda et al. 2000, McDowell et al. 2004), or may indicate that DOM is not directly enriched with N independently of SOM. While these patterns may reflect preferential sorption of N-poor DOM (Kaiser et al. 2000, Kaiser and Zech 2000) or abiotic immobilization of $\text{NO}_3\text{-N}$ into DOM, the distinct declines in DON concentrations when regressed against $T_{\text{DOC-L}}$ and SOM C/N, when sites were separated by loading rate likely indicate that any possible expression of abiotic immobilization of N is dominated by other pathways.

Turnover times for labile DOC (i.e., $T_{\text{DOC-L}}$) ranged from 2.5-7 days, values appropriate for labile OM (Kalbitz et al. 2003). Hence, we considered an increase in $T_{\text{DOC-L}}$ to illustrate an increase in DOC bioavailability since $T_{\text{DOC-L}}$ was correlated with effective labile DOC pool size, $P_{\text{DOC-L}}$. The observed decline in DON concentration association with greater $T_{\text{DOC-L}}$ occurred for both high and low N deposition groups and fits empirical observations of lower leaching rates of DON with greater labile C sources (Park et al. 2002, Corre et al. 2003), potentially indicating greater biological demand for DON with enhanced DOC bioavailability. Conversely, when $\text{NO}_3\text{-N}$ is available suppressed microbial exoenzyme expression may reduce depolymerization (Waldrop et al. 2004) or increase recalcitrance (Fog 1988, Zech et al. 1994) and be an alternative explanation for lower DON concentration if DON generation via these pathways is reduced as a result of enhanced N availability. However, different mechanisms hinge on inorganic N availability. For example, increased DON production occurs with greater inorganic N availability (Park et al. 2002, Corre et al. 2003), either by assimilation of DIN and release of DON (Seely and Lajtha 1997, McDowell et al. 2004) or preferential use of DIN to meet microbial N demand (Kaushal and Lewis 2005) allowing for DON accumulation.

Despite uncertainties concerning mechanisms, these natural patterns support experimental responses to N fertilization, as well as highlight the complexity of controls

on DON dynamics. It is clear that more explicit experimental work is needed to parse the role of disparate consumption and production pathways that govern the fate of DON in ecosystems.

Soil Acidification and Mineral Soil DOM Stability

Compared to the forest floor, different environmental drivers control mineral soil DOM. It is well known that mineral soil stabilizes DOC that originates from the partial decomposition of organic matter in the forest floor (Guggenberger and Zech 1994, Hagedorn et al. 2001, 2003, Qualls et al. 2002, Willett et al. 2004). Unique properties encourage stabilization by promoting chemical recalcitrance, organomineral interactions, and physical inaccessibility (Kaiser et al. 1996, Kaiser and Guggenberger 2003). Because we were interested apparent role of soil acidification in altering DOM stability, we focus on a subset of mechanisms that govern DOM solubility and complexation known to be pH dependent.

Negative relationships between pH and DOM concentrations in the mineral soil suggest a strong association between acid-base chemistry and DOC mobility. This general relationship is well represented in soil solution models (Tipping et al. 1994, Milne et al. 2001), and likely represents a pH driven stabilization of DOM either by a “salt effect”, which forces DOM sorption by reducing colloidal repulsion via increased ionic strength (Stumm et al. 1996), or protonation of carboxylic functional groups, which reduces DOM solubility by decreasing humic charge density (Tipping et al. 1991, Kalbitz et al. 2000, Weng et al. 2002). Differentiating between these mechanisms is impossible to discern herein because these processes occur in concert with greater acidic deposition, and are not mutually exclusive. Others have shown increased DOC associated with greater pH (Evans et al. 2006, de Wit et al. 2007, Monteith et al. 2007) due to a decrease in the occupation of binding sites with protons (Tipping et al. 1991, Kalbitz et al. 2000). In our study, the potentially soluble DOC pool (DOC_{ps}) increased with enhanced acidity and it is reasonable to expect that higher DOC_{we} should reflect a greater potentially soluble pool, DOC_{ps} , an observation supported by laboratory (Kaiser et al. 1996) and field (McDowell and Likens 1988, Michalzik and Matzner 1999) studies. While shifts in pH likely determine DOM solubility and explain changes in DOM concentration, co-

occurring processes of organomineral complexation are likely responsible for increased chemical complexity and recalcitrance

Enhanced aluminum mobility and reactivity is one of the most pronounced soil biogeochemical responses to acid deposition (Mulder et al. 1989). Moreover, a growing body of literature documents the role of organic complexation reactions in controlling aluminum solubility in acid soils, which are partly regulated by pH (Mulder et al. 1989, Berggren and Mulder 1995, Kalbitz et al. 2000, Mulder et al. 2001, Nierop et al. 2002, de Wit et al. 2007, Scheel et al. 2007). Such complexation also effectively decreases DOC solubility (de Wit et al. 1999) as well as bioavailability (Boudot et al. 1986, Klemmedson and Blaser 1988, Mulder et al. 2001, Scheel et al. 2007) by neutralizing electrical charge (Weng et al. 2004) and altering structural conformation (Ares and Ziechmann, 1988), particularly when aluminum is implicated (Stevenson 1994). A coincidental effect is the protection of organic matter from decomposers (Mulder et al. 2001, Oades and Waters 1991, Hobbie et al. 2007). Patterns observed in our study support widespread evidence that decreased pH enhances physical processes that increase DOM concentration, chemical complexity and recalcitrance. Organic complexation with reactive aluminum is suggested by the strong association between mineral soil DOM properties and the degree of SOM and DOM affiliated with organically bound aluminum; both C_{py}/Al_{py} increased strongly with reduced pH and increased chemical complexity, DOC turnover time, and acidic deposition. At the same time, complexation did not vary with labile DOC (T_{DOC-L} or P_{DOC-L}) dynamics, patterns similar to those observed by Schwesig et al. (2003).

Dissolved C and N Loss

Surface water NO_3-N and DOM concentrations increased along the gradient of acidic deposition. Strong association between NO_3-N export and forest floor N dynamics as well as between DOM output and mineral soil physiochemical properties suggests elemental loss was determined by distinct processes associated with distinct soil horizons. Furthermore, elevated surface water elemental concentrations reflected differential consequences of acidic deposition, N fertilization and soil acidification, in forest floor and mineral soil, respectively.

Surface water NO_3-N concentrations increased dramatically with higher forest floor net nitrification and reduced C/N likely enhanced with greater N deposition. To the

extent that greater $\text{NO}_3\text{-N}$ loss is controlled by SOM composition, greater $\text{NO}_3\text{-N}$ concentration for a given C/N comparing low and high deposition groups supports Evans et al. (2006) model that soil C status determines in part the susceptibility of N leaching. Enhanced $\text{NO}_3\text{-N}$ production changes the forest floor N economy implicating enhanced N input as a cause of increased leakiness of $\text{NO}_3\text{-N}$. Greater net $\text{NO}_3\text{-N}$ production with reduced C/N is widely observed (Table 2; McNulty et al. 1991, Van Miegroet et al. 1992, Lovett and Rueth 1999, Goodale and Aber 2001, Ollinger et al. 2002), and C/N has emerged as strong predictor of surface water $\text{NO}_3\text{-N}$ concentrations (Gunderson et al. 1998, Lovett et al. 2002). This study confirms this general relationship in a new region, the unglaciated Appalachian Mountains, and highlights the need for explicit investigation into the mechanism that produces the relationship between C/N and net N processing and $\text{NO}_3\text{-N}$ loss.

Whereas the connection between N deposition and $\text{NO}_3\text{-N}$ production and loss is well established, the effect of deposition on DOM loss is more controversial and less understood (Freeman et al. 2001, Perakis and Hedin 2002, Neff et al. 2002, Evans et al. 2006, Montieth et al. 2007, Brookshire et al. 2007). In line with Aitkenhead-Peterson and McDowell (2000), higher soil C/N resulted in greater DOC export. Although C and N pools may adequately reflect dissolved C and N pools in predominantly organic soils (Currie and Aber 1997, Evans et al. 2006), on local (Qualls and Haines 1991), regional (Michalzik et al. 2001) and global scales (Aitkenhead and McDowell 2000), C and N stocks largely fail to predict DOM concentrations and loss in mineral soils and surface waters. Surface water DOC:DON was moderately related to forest floor and mineral soil DOC:DON and surface water DON concentrations increased with greater mineral soil DOC_{we} and $T_{\text{DOC-R}}$. Collectively these relationships suggest that the bulk of surface DON is linked to terrigenous sources that originate from processes that make DOM relatively unavailable for biological use (Hedin et al. 1995, Vitousek et al. 1998, Neff et al. 2000).

Perhaps the most intriguing finding is the observed increase in DOC concentration that is ostensibly due to greater soil acidification, since it has been convincingly argued that *recovery* from acid rain is responsible for the increase in DOC concentration observed across the northern hemisphere (Evans et al. 2006, Montieth et al. 2007, de Wit et al. 2007). Our results appear to generate a contradiction. How can acidity

increase DOM stability in the mineral soil and yet result in reduction on the scale of the catchment? We cannot answer this using this dataset, yet there is evidence that shallow, unglaciated soils, with fundamentally different mineralogical structure are comparatively unresponsive to triggering mechanisms that quickly change the biogeochemistry of glaciated soils.

It is clear that surface water DOC concentration and bioavailability reflect the nature of DOM in the mineral soil, and correspond strongly to the relative amount of C associated with organically bound, reactive aluminum. Support for strong association between DOC and organically active aluminum in export comes from a large survey (National Survey Water Survey, EPA) also conducted in the unglaciated Appalachian surface waters (Herilhy et al. 1993), which revealed strong correlation between soluble organic Al and DOC, providing further evidence that DOC and aluminum accompany each other in export (Mulder et al. 1991, Vogt and Muniz 1997, Pellerin et al. 2002, Cory et al. 2007, Yavitt et al. 2007) and alter DOM complexity and bioavailability.

Conclusion

We have presented compelling evidence that the inherent nature of the forest floor and mineral soil strongly determine consequences of N fertilization and acid deposition on dissolved C and N cycling, and these horizon-specific changes determine the propensity for and composition of dissolved C and N export. Distinct biogeochemical patterns confirm well-documented experimental responses to N fertilization and soil acidification, and highlight the need to explore fundamental interactions between N fertility, organic matter formation and stabilization, microbial mediated N turnover, and processes that generate labile and recalcitrant forms of DOM. Further investigation into these basic interactions will help us understand the ecological complexity of natural systems, and how the ecological condition and economic value of ecosystems should respond to anthropogenic alterations.

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Table 1. Environmental factors for sampling sites.

Site *	Forest Type	Total N Input (kg N ha ⁻¹ yr ⁻¹)	Mean Sampling Elevation (m)	MAP (mm)	MAT (°C)	Soil Order
Coweeta LTER	Angiosperm	5	992	1870	5.5	Ultisol
GSMNP Cosby	Angiosperm	7.5	1444	1800	9	Ultisol
Mt. Mitchell SP	Gymnosperm	8.5	2031	2000	6	Inceptisol
Mt. Mitchell SP	Mixed	8.5	1915	2000	6	Inceptisol
Mountain Lake BS	Angiosperm	6.9	1180	1500	8	
Laurel Highlands SP	Angiosperm	7.3	851	1069	11.1	Inceptisol
USFS Fernow EF	Angiosperm	11.6	870	1458	8.9	
Mt. Rogers NRA	Mixed	14	1504	1320	7	Inceptisol
Mt. Rogers NRA	Mixed	14	1474	1320	7	Inceptisol
GSMNP Balsam	Gymnosperm	22.5	1700	2270	8.5	Inceptisol
GSMNP Deep Creek	Mixed	22.5	1748	2270	8.5	Inceptisol
GSMNP Beech	Angiosperm	20	1748	2270	8.5	Inceptisol
GSMNP	Angiosperm	22.5	1763	2270	8.5	Inceptisol
Newfound Gap GSMNP Mt. Le Conte	Mixed	27.5	1672	2270	8.5	Inceptisol
GSMNP Mt. Collins	Gymnosperm	22.5	1824	2270	8.5	Inceptisol
GSMNP Clingsman Dome	Gymnosperm	30.9	1870	2270	8.5	Inceptisol

*See Figure 1 for site geographic locations and abbreviations. MAP, Mean Annual Precipitation; MAT, Mean Annual Temperature

Table 2. Predictor variable subsets used for multiple stepwise regressions ($n = 16$)

Subset	Variable	Mean (std. deviation, range)	
External	Elevation (m)	1543 (851-2031)	
Drivers	MAP (mm/yr)	1918 (1069-2270)	
	MAT (°C)	8.5 (6-13)	
	N deposition (kg N ha ⁻¹ yr ⁻¹)	15.6 (5.5-30.9)	
		Forest floor	Mineral soil
Cations/ Soil acidity	Ca (mg/kg)	406.5 (296.1, 146.3-1169.3)	118.2 (53.6, 66-248.7)
	Mg (mg/kg)	60.0 (21.4, 35.3-108.0)	27.0 (3.7, 22.0-34.7)
	Fe (mg/kg)	32.1 (17.0, 13.0-72.9)	101.2 (71.1, 9.4-240.7)
	Exch. Acidity (%)	79.3 (13.5 47.0-91.0)	88.3 (4.9, 76.8-93.9)
	CEC (meq/100g)	13.4 (1.0, 12.1-16.0)	8.5 (1.4, 4.6-10.1)
	pH _{water}	4.2 (0.4, 3.8-5.2)	4.3 (0.3, 3.8-4.9)
Soil C and N	C (%)	33.4 (4.5, 23.6-40.7)	9.4 (2.35, 3.8-12.5)
	N (%)	2.0 (0.2, 1.5-2.3)	0.6 (0.2, 0.2-0.9)
	C/N	17.1 (2.2, 13.5-20.7)	15.7 (2.0, 13.3-19.1)
	NH ₄ -N _{KCl} (mg/kg)	80.7 (62.7, 18.3-265.2)	22.6 (17.5, 7.0-81.0)
	NH ₄ -N (mg/kg)	5.5 (5.7, 0.4-24.3)	3.3 (2.7, 0.6-8.5)
	NO ₃ -N _{KCl} (mg/kg)	74.2 (116.3, 7.8-463.8)	35.2 (25.8, 4.7-97.8)
	NO ₃ -N (mg/kg)	19.9 (13.8, 1.16-45)	9.3 (5.4, 1.5-18.2)
	C _{py} (g/kg)		72.8 (24.9, 32.3-112.9)
	Net ammonification (µg N g C ⁻¹ day ⁻¹)	23.9 (6.9, 15.5-39.5)	6.7 (2.2, 1.5-10.7)
	Net nitrification (µg N g C ⁻¹ day ⁻¹)	7.7 (3.1, 1.8-15.8)	4.3 (3.9, -0.1-13.7)
DOM properties	DOC _{we} (mg/kg)	377.0 (87.0, 243.2-559.7)	211.9 (106.8, 100.6-432.4)
	DON _{we} (mg/kg)	50.6 (13.4, 25.9-77.7)	19.7 (5.0, 11.0-29.9)
	T _{DOC-L} (days)	5.1 (1.3, 2.5-7.2)	6.1 (1.7, 2.9-7.3)
	T _{DOC-R} (days)	1127.8 (297.8, 495.1-1639.3)	1053.1 (1053.1, 414.9-2222.2)
	FI	1.21 (0.04, 1.14-1.26)	1.34 (0.04, 1.25-1.41)
	HI	14.9 (5.4, 5.5-23.7)	9.68 (3.11-31.6)
	DOC:DON	23.3 (4.4, 17.5-31.6)	26.0 (14.8, 8.2-56.0)
	DOC _s (mg/kg)		165.7 (69.9, 53.1-276.7)
	DOC _{ps} (mg/kg)		43.5 (16.2, 16.4-73.9)
	DOC _{in} (mg/kg)		349.0 (73.6, 234.6-493.9)
	∑ Fe _{nc} + Al _{nc} (mg/kg)		36.3 (13.1, 14.9-55.8)
	∑ Fe _{py} + Al _{py} (mg/kg)		54.4 (17.8, 21.9 -73.6)
	C _{py} /∑ Al _{py} (w/w)		3.5 (1.4, 1.0-5.7)

See text for abbreviations and details regarding predictor variables.

Table 3. Results of selected final stepwise regressions using Site mean values as observations ($n = 16$).

Dependent Variable	Forest floor	R^2	Mineral soil	R^2
Nitrogen Dynamics				
C/N	N deposition [*] (-), MAP ^{***} (-)	0.55	Elevation [*] (-); Mineral soil DOC _{we} ^{**} ; Forest floor C/N ^{**}	0.74
N (%)	Forest floor DOC:DON [*] (-)	0.25	MAT ^{**} (-); Mineral soil C ^{***} , DOC:DON [*] (-)	0.97
DOM Dynamics				
DON (mg/kg)	Forest floor NH ₄ -N _{we} ^{**} , labile DOC _t ^{**} (-)	0.63	Mineral soil exchangeable acidity [*] , pH ^{**} (-) C _{py} /Al _{py} ^{***}	0.64
DOC _{we} (mg/kg)	Forest floor C ^{***} (-), N ^{**} , C/N ^{***} , DON ^{***}	0.82	Elevation ^{**} (-); T _{DOC-R} ^{***} , pH [*] (-)	0.79
T _{DOC-R} (days)	Forest floor pH ^{**} (-), ECEC [*] (-)	0.41	Mineral soil DOC _{we} ^{***} , FI ^{***} (-), C _{py} /Al _{py} ^{**}	0.80
T _{DOC-L} (days)	N deposition [*] ; Forest floor DON [*] (-)	0.46	Forest floor T _{DOC-L} ^{**} , Mineral soil DOC:DON ^{**} (-), pH [*]	0.67
DOC _{ps} (mg/kg)			Mineral soil pH [*] (-) C _{py} /Al + Fe _{nc}	0.56
Soil Acidity				
pH	N deposition [*] (-); Forest floor acidity ^{***}	0.92	Mineral soil DOC _{we} ^{***} (-), exchangeable acidity ^{***} (-)	0.85
Watershed Outputs				
NO ₃ -N (μg/L)	Forest floor C/N ^{**} (-), net nitrification [*]	0.60		
DON (μg/L)	Mineral soil refractory DOC _t ^{**} ; Surface water NO ₃ -N ^{**}	0.57		
DOC (mg/L)	Mineral soil DOC ^{***} , FI ^{***} (-) refractory DOC _t	0.89		
Refractory DOC (mg/L)	N deposition [*] ; Mineral soil pH ^{**} (-), C [*] , refractory DOC _t ^{**} , FI ^{**} (-)	0.95		
Labile DOC (mg/L)	N deposition ^{***} (-); Mineral soil exchangeable acidity ^{**} (-); Forest floor NO ₃ -N ^{**} (-), refractory DOC _t ; Surface water DOC [*] , NO ₃ -N [*]	0.97		

See text for abbreviations and details regarding response and predictor variables, and **Table 2** for Predictor Subset delineation. Significant ($P \leq 0.05$) final model predictor variables are reported, and direction of relationship is indicated in parentheses. *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$.

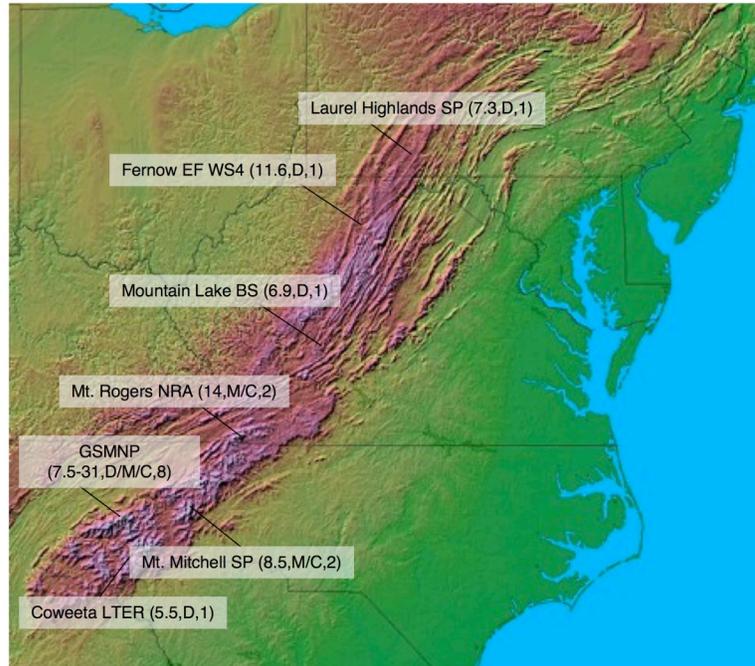


Figure 1. Sixteen sites were sampled within the unglaciated mountains of middle and south Appalachia. Nitrogen deposition ($\text{kg N ha}^{-1} \text{ yr}^{-1}$), forest type (D: deciduous, M: mixed, C: coniferous), and the number of ecosystems sampled within each site is shown in parentheses. See *Methods* for determination of acid deposition at each sampling location. Sites: Laurel Highlands State Park (SP), PA; USFS Fernow Experimental Forest (EF), WV; University of Virginia Mountain Lake Biological Station (BS), VA; Mt. Rogers National Recreation Area (NRA), VA; Mt. Mitchell SP, NC; USFS Coweeta Hydrologic Laboratory and NSF-LTER, NC.

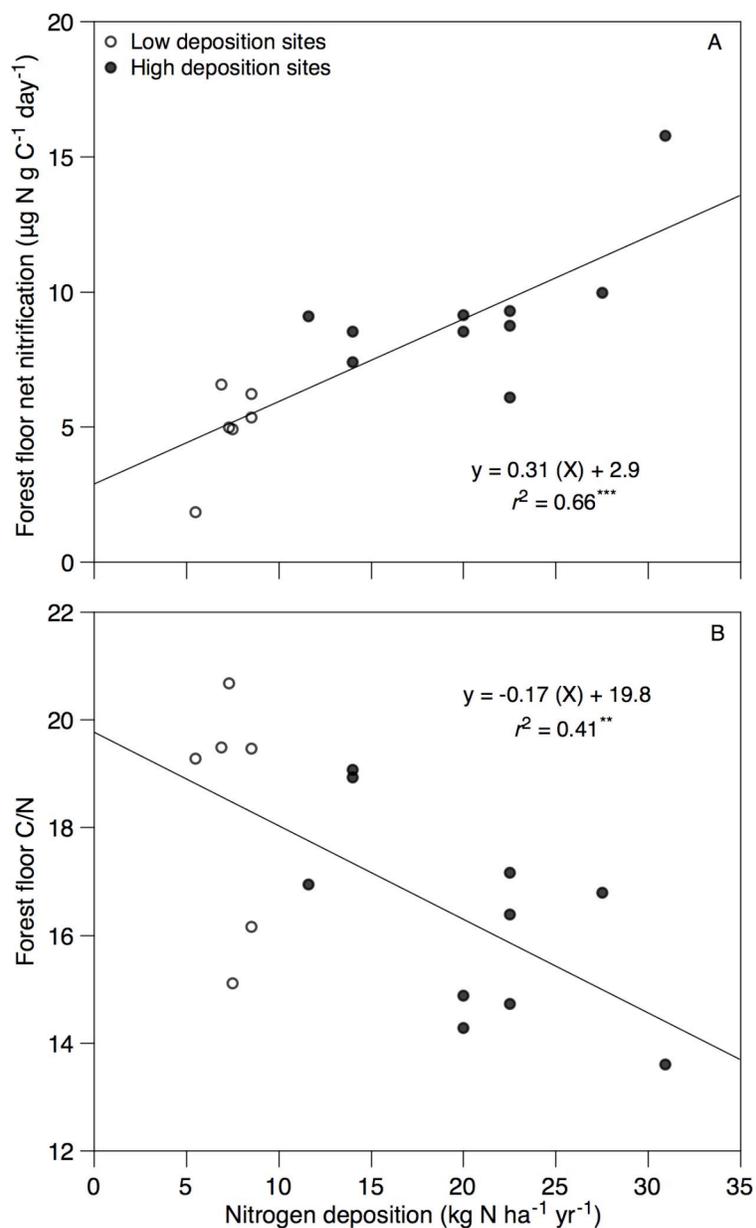


Figure 2. Relationships between N deposition and forest floor net nitrification (A) and C/N (B). *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$. This convention is maintained throughout all figures.

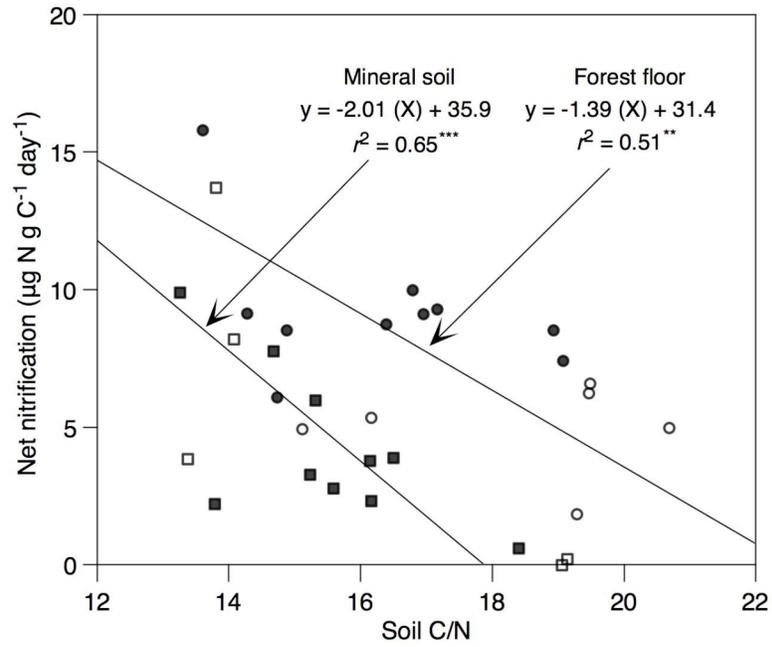


Figure 3. Relationships between SOM C/N and forest floor and mineral soil net nitrification. Circles represent forest floor samples, whereas squares represent mineral soil.

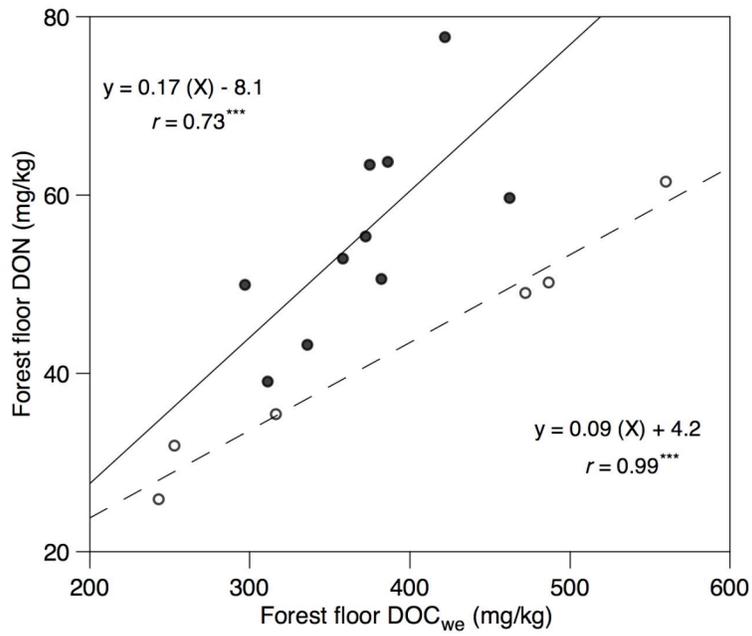


Figure 4. Correlations between forest floor DOC_{we} concentration and DON concentration when sites are separated by N loading at 10 kg N ha⁻¹ yr⁻¹. See *Methods* for reasons behind the separation of sites into N loading groups.

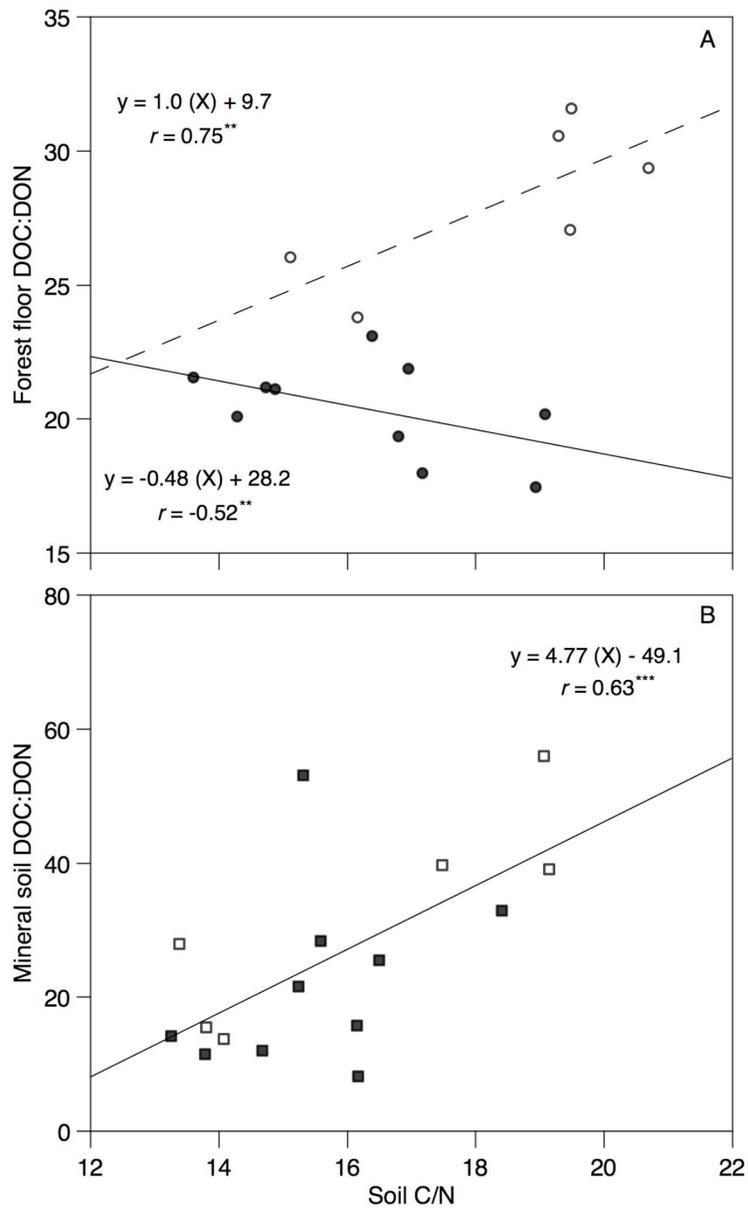


Figure 5. Correlations between forest floor (A) and mineral soil (B) DOC:DON and SOM C/N.

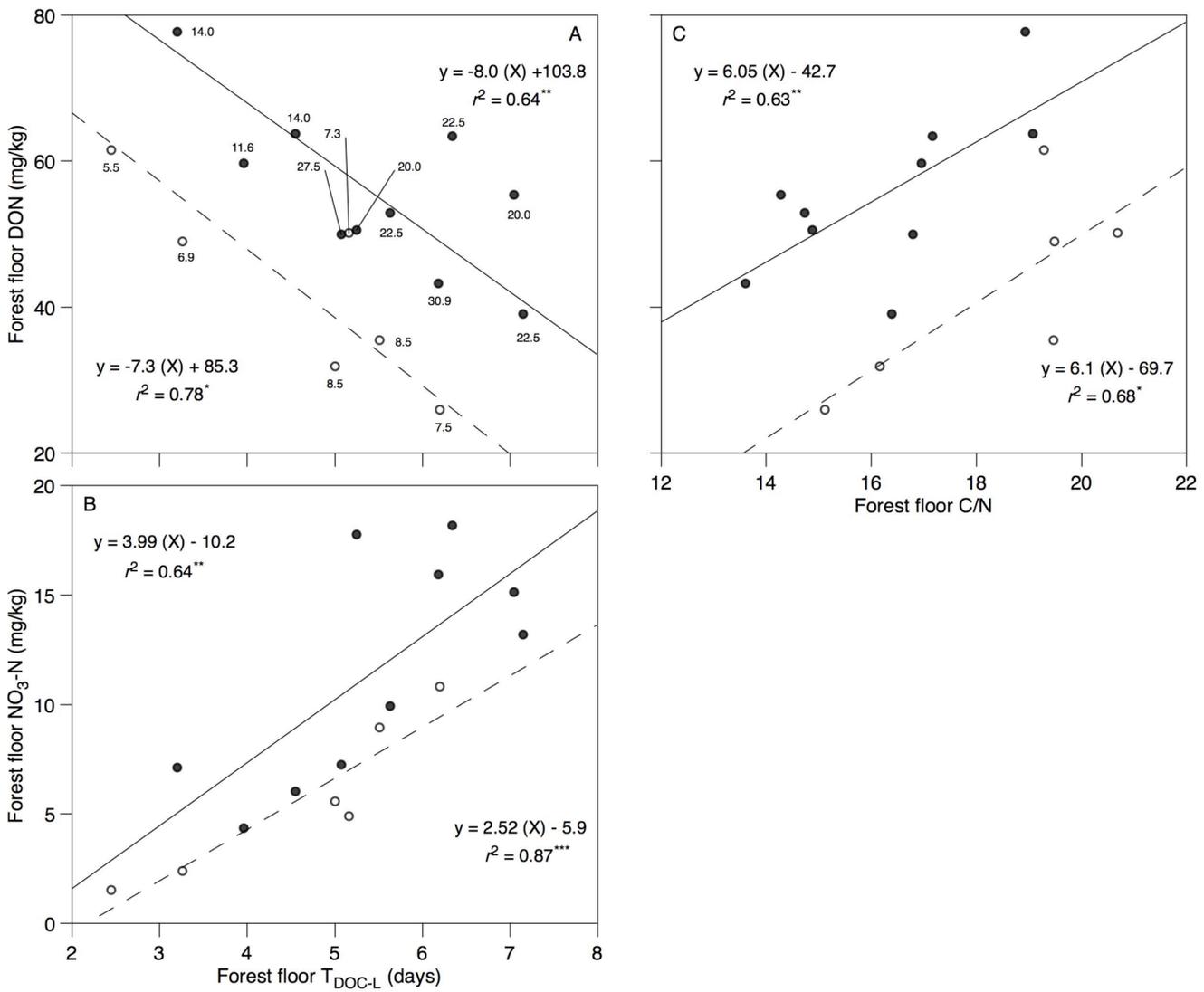


Figure 6. Relationships between forest floor DON (A) and NO₃-N (B) concentration and T_{DOC-L} and SOM C/N (C). N deposition values, in kg N ha⁻¹ yr⁻¹, are shown as a third dimension. DON and NO₃-N concentrations were significantly greater for high deposition sites at $p < 0.05$.

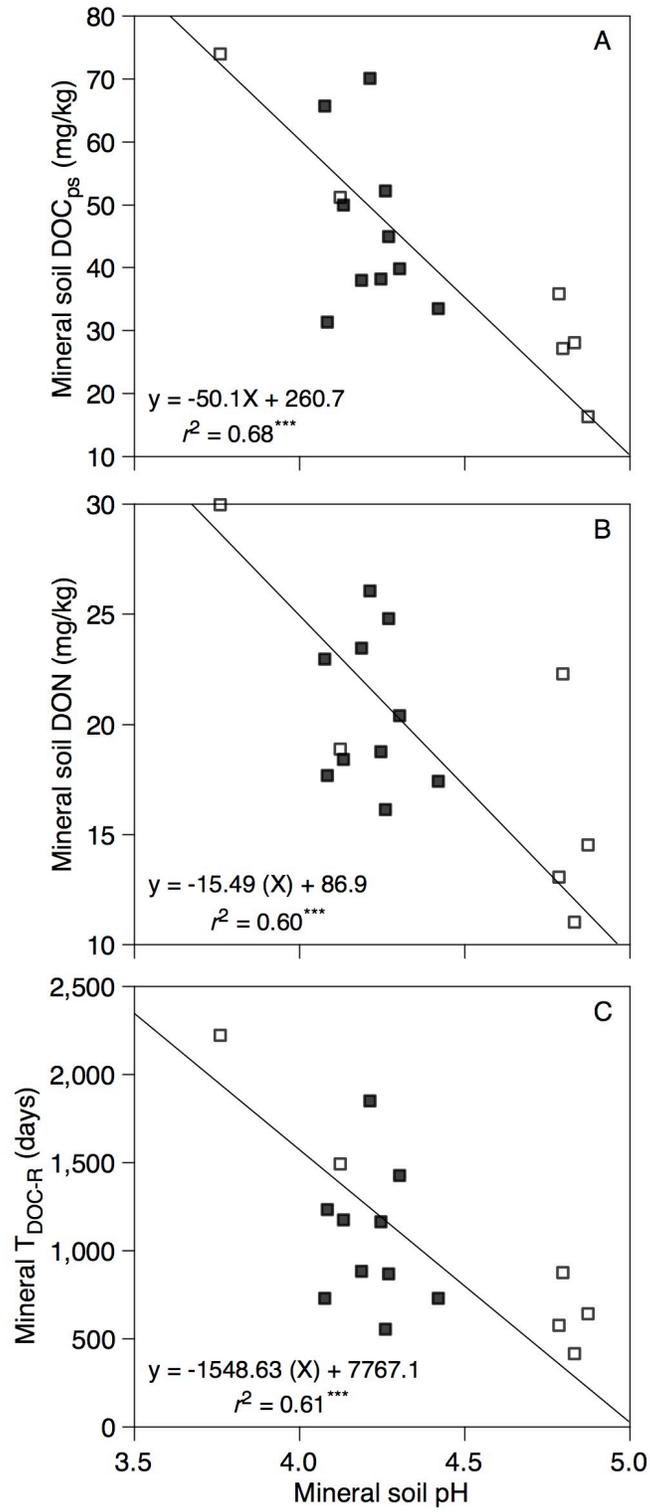


Figure 7. Relationships between DOC_{ps} (A) and DON (B) concentration, and T_{DOC-R} (C), and mineral soil pH.

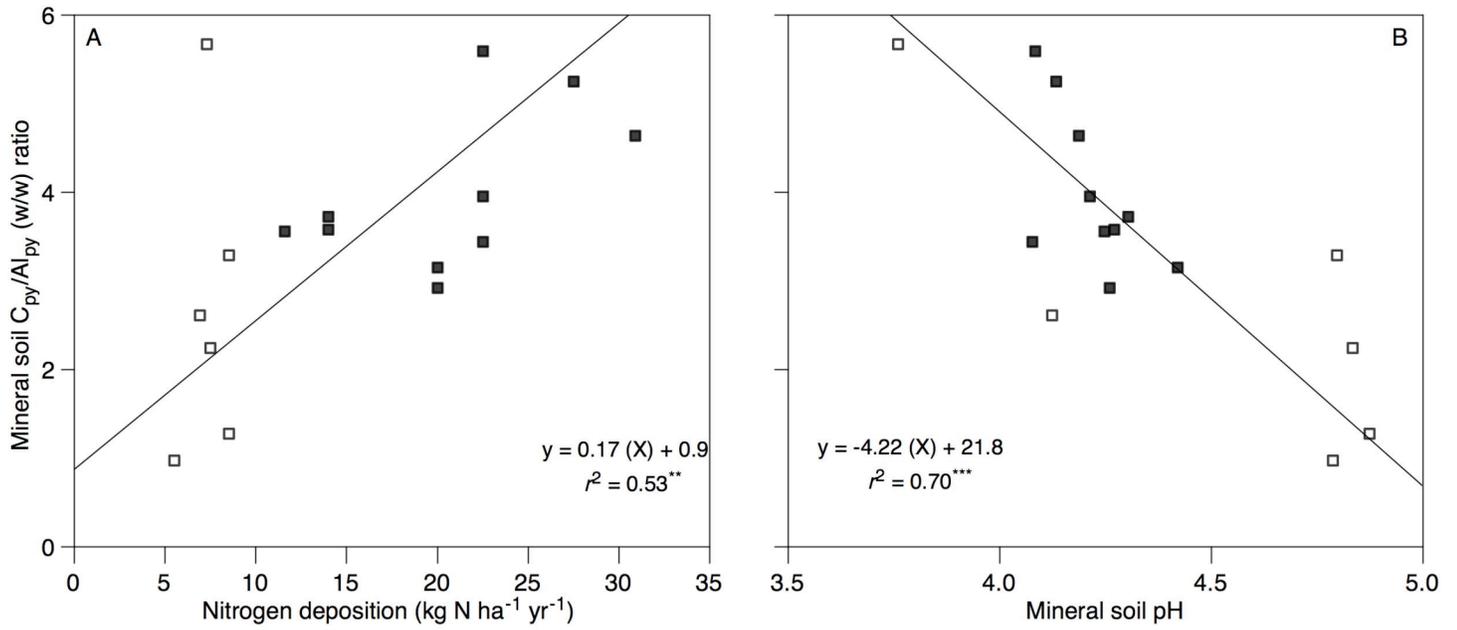


Figure 8. Relationships between mineral soil C_{py}/Al_{py} and N deposition (A) and mineral soil pH (B).

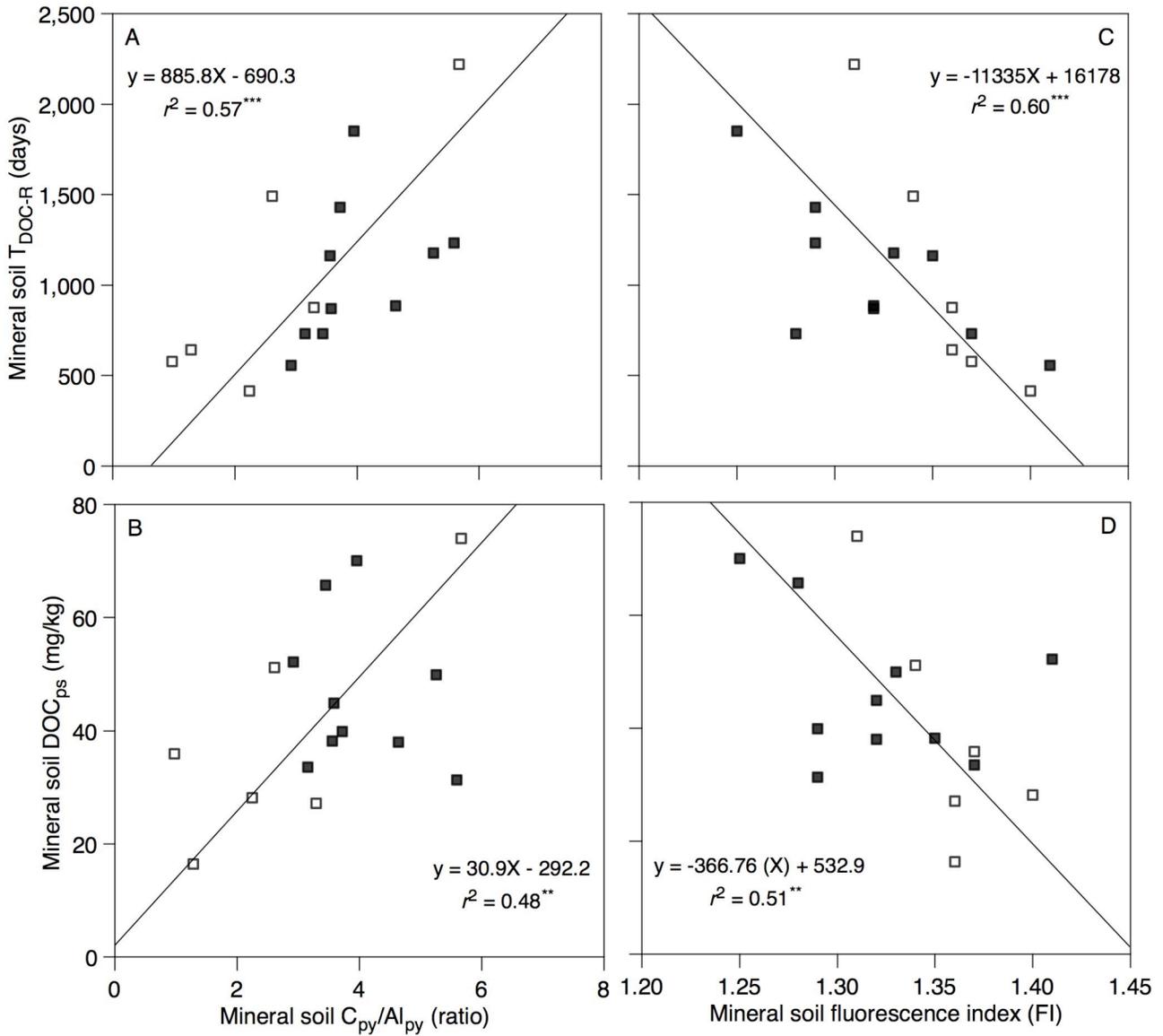


Figure 9. Changes in T_{DOC-R} and DOC_{ps} as a function of C_{py}/Al_{py} (A and B, respectively) and FI (C and D, respectively).

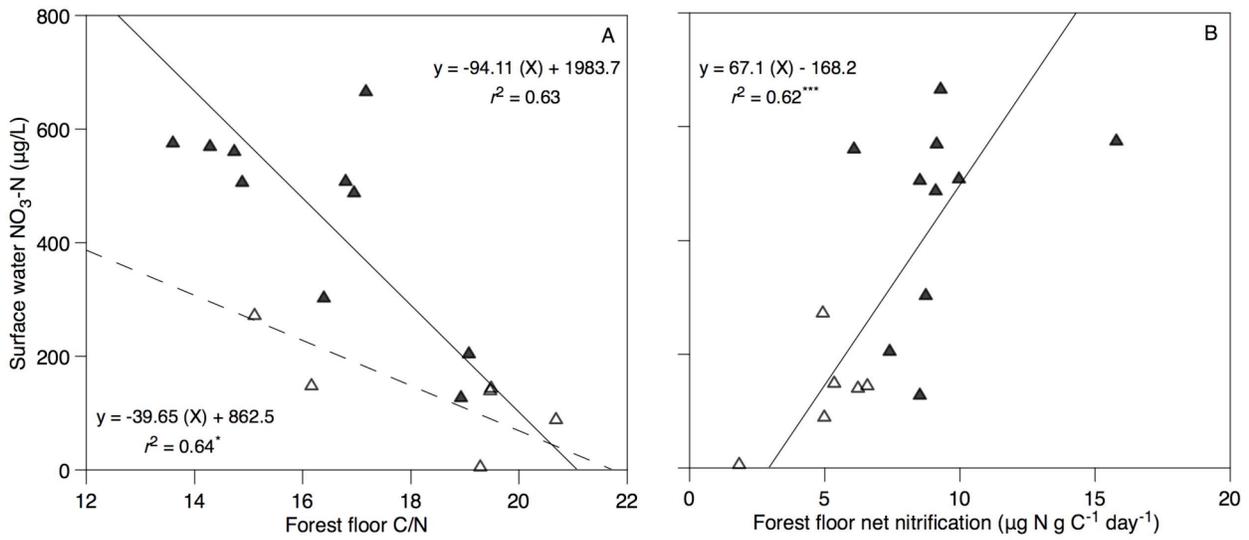


Figure 10. Predictions of surface water $\text{NO}_3\text{-N}$ concentration as a function of forest floor SOM C/N (A) and net nitrification (B), where sites are separated by N loading rate.

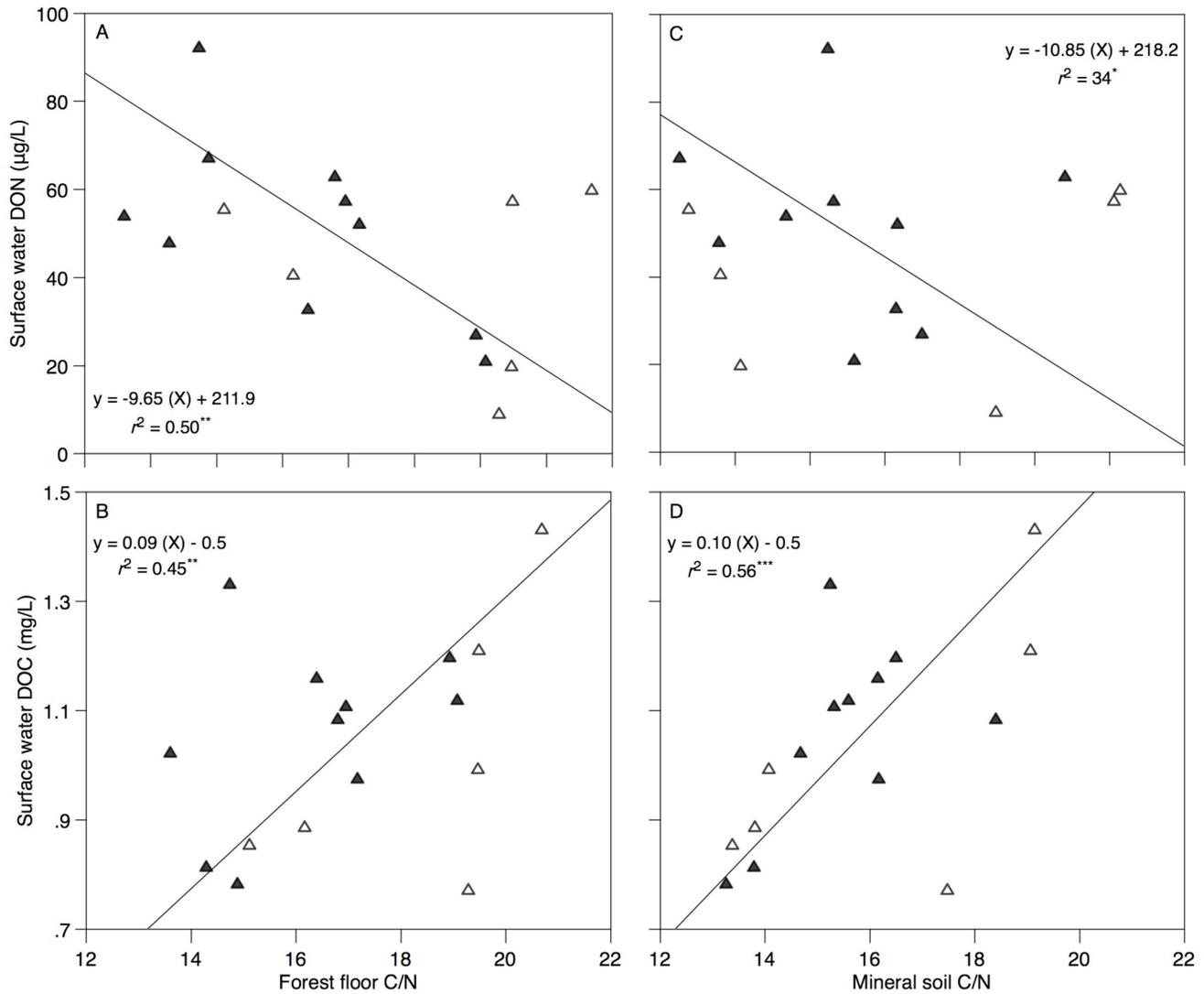


Figure 11. Surface water DON and DOC concentration as a function of forest floor (A and B, respectively) and mineral soil (C and D, respectively) SOM C/N.

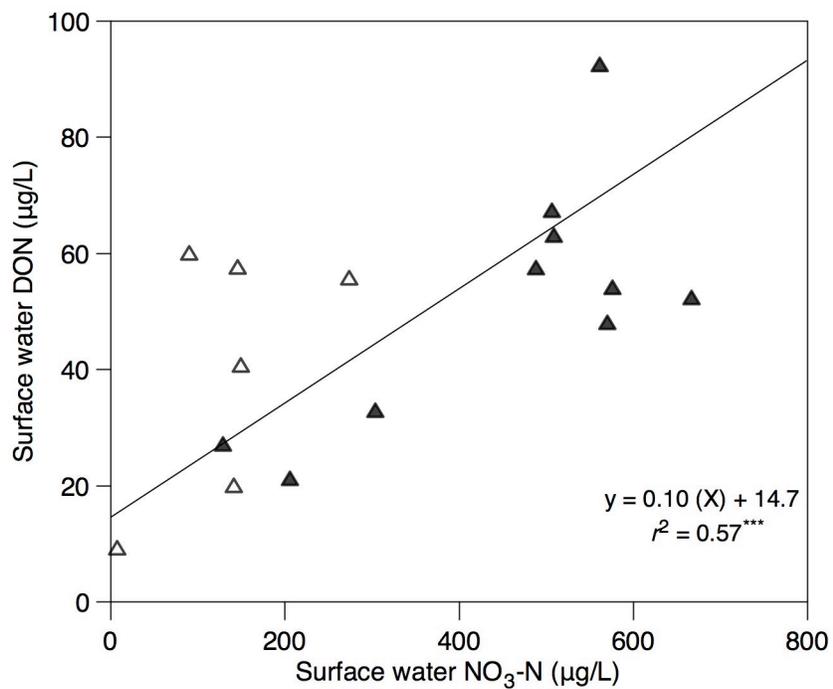


Figure 12. Surface water DON concentration as a function surface water NO₃-N concentration.

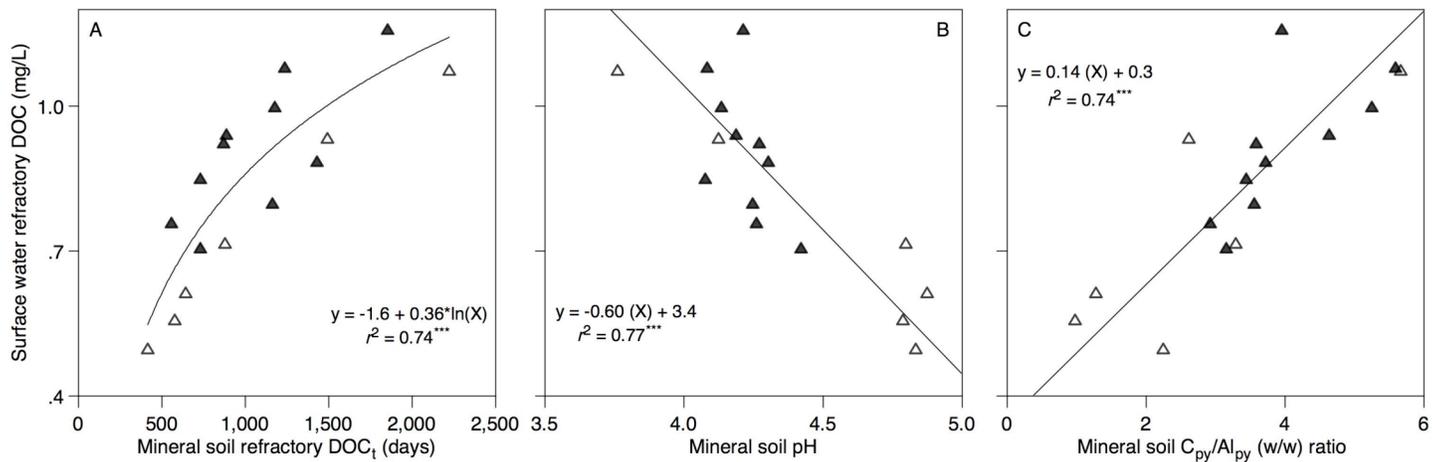


Figure 13. Surface water refractory DOC concentration as a function of mineral soil $T_{\text{DOC-R}}$ (A), pH (B), and $C_{\text{py}}/Al_{\text{py}}$ (C).