The organic nature and atmosphere-climate dependency of nitrogen loss from forest watershed ecosystems

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Abstract

In this dissertation I describe how coupled internal cycling and external forcing from the atmosphere and climate can regulate the dynamics of nitrogen (N) loss from forest watersheds. I address three major gaps in our understanding of the global N cycle: 1) the role of dissolved organic N (DON) in internal N cycling in low-N ecosystems; 2) The influence of atmospheric pollution on DON production and loss from forests; and 3) the inherent climate sensitivity of forest N cycling and loss. In chapter 2, I present the results of a study of DON spiraling that showed enormous capacity for stream microorganisms to immobilize and transform organic nutrients. Although most DON in surface waters is highly refractory products of SOM dissolution, this study revealed very tight internal cycling of DON at the sediment interface and suggested significant production of DON in the hyporheic zone. Most remarkably, this DON was not expressed in stream waters, supporting the idea that watershed DON losses would have been higher in the absence of pronounced benthic demand. The experiments also suggested that coupled dynamics between DOC and DON spiraling may be altered under conditions of elevated N supply.

Chapter 3 challenges the idea that soil organic matter (SOM) and its dissolved products are stoichiometrically static as N pools accumulate. Using a broad geographic survey of forest
streams, I show that DON losses increase as a consequence of N pollution and that this occurs through a disproportionate enrichment of N on dissolved organic matter rather than alteration of soil and dissolved carbon dynamics. These results have implications for N limitation in forests and aquatic systems. In particular, DOC: DON ratios of DOM draining N-saturated forests were strikingly low suggesting possible increases in DOM bioavailability with increasing N supply.

Chapter 4 provides insight into how local forest nutrient cycles may be organized by synchronous global-scale climate-atmosphere dynamics. This study of long term (30 yr) hydro-chemistry from reference forest watersheds provides an integrated example of the overall climate sensitivity of N cycling and underscores the importance of complex synergies between simultaneous vectors of global change. Results from this study argue that the combined influence of N pollution and warming are likely to have pronounced long-term effects on ecosystems globally.
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Chapter 1: General introduction

From the early origins of the living primordial Earth the vast majority of the global nitrogen (N) pool has been locked in the atmosphere as dinitrogen gas (N$_2$). Because of its fundamental properties, N, along with the other light elements, is central to biosynthesis and life in general. Consequently, given its low availability, N has been limiting to biotic activity and thus has strongly contributed to broad scale organization of ecosystem development over the course of plant-microbe coevolution. The evolution of bacteria capable of transforming N$_2$ to plant available forms, and their symbiotic association with major plant lineages, was a central step in the provision of N to N-limited ecosystems, yet there are numerous thermodynamic, hydrologic, and biogeographical constraints to N fixation globally which in turn limit the long-term accumulation of N that could alleviate N limitation.

A fundamental constraint to the long-term accumulation of N in ecosystems is the ubiquity of hydrologic N loss from ecosystem boundaries. Classical models of N cycling have focused on the role of ammonium (NH$_4^+$) and nitrate (NO$_3^-$) uptake, transformation, and loss as these are the readily available forms of N for most plants and can be shown to limit growth. Yet, in most ecosystems, the vast majority of N pools and fluxes occur in organic forms. While some organic N occurs in monomeric biologically available forms such as amino acids, most of organic N is tightly covalently linked with carbon (C) in stereochemically complex polymeric forms that render it largely unavailable to microorganisms and plants. The preponderance of such forms can dramatically constrain N supply and cycling rates by slowing microbial N access and transformation, and can allow organic N to be exported via hydrologic pathways. In this way, stable and dynamic organic N pools can largely dictate the relative openness of N cycling. That is, under natural conditions of low N supply, relative openness (ratio of input-output flux to
internal cycling) is constrained by recycling and loss of complex forms. While the relative openness of ecosystems is dictated to a large degree by water residence time, depolymerization, mineralization (organic N to ammonium), and subsequent nitrification (ammonium to nitrate) of organic N function as critical rate limiting process in N availability and loss.

It is under these conditions that the observable broad geographic patterns of Earth’s ecosystems emerged. Over the last century, however, human activities have more than doubled the supply of available N over much of Earth. This has largely been due to the advent of industrial scale N fertilizer application and atmospheric pollution derived from fossil fuel combustion. Empirical and theoretical studies confirm that this has had, and continues to have, pronounced and persistent effects on ecosystem composition, productivity, and water quality. Moreover, the global acceleration of available N fluxes have fundamentally altered the N cycle in remote natural ecosystems far removed from direct human influence. Many temperate forest ecosystems in the northern hemisphere receive N inputs from atmospheric pollution 2 to 20 times pre-industrial levels. This has resulted in a suite of shifts in N cycling collectively referred to as ‘nitrogen saturation’, which describes cases in which N supply exceeds biotic demand, resulting in accelerated cycling and leakiness of N.

In particular, polluted forests worldwide exhibit high rates of nitrification and nitrate loss. These losses have large implications for the functioning and diversity of terrestrial and aquatic ecosystems alike. In contrast, studies show that forest N losses form unpolluted regions are dominated by dissolved organic N (DON) and that long-term N deposition may drive a shift from organic and ammonium- to nitrate-based N economies. Yet despite increasing understanding of inorganic N cycling and the role of simple DON (amino acids) in terrestrial plant nutrition, there is little known about DON cycling in N-limited systems where DON use is
expected to be highest, particularly how it is coupled to C and inorganic N supply. Further, current theory regards DON as a biologically unavailable form of N that is readily and passively transported from ecosystems. However, recent experiments suggest that this may not be the case under conditions of chronically high levels of N supply. While many insights have been gained into how ecosystems process novel levels of N supply from atmospheric deposition, this is but one external vector expected to affect internal N cycling and loss. The N cycle in particular is thought to be highly sensitive to shifts in moisture and temperature, factors predicted to change with global warming. The synchronous influences of competing vectors of global change on N cycling remain elusive owing to their synergistic and dynamic complexity, yet understanding these interactions represents a critical challenge to understanding the shifting role of N cycling in the organization of nature in general.

Here I address three major gaps in our understanding of the global N cycle: 1) the role of DON in internal N cycling in low-N ecosystems; 2) The influence of atmospheric pollution on DON production and loss from forests; and 3) the inherent climate sensitivity of forest N cycling and loss. In Chapter 2, I investigate the coupled cycling of DON and DOC in a low-N forest stream. Through spiraling experiments I show the linked nature of N and C internal cycling and its relation to inorganic N in a highly open ecosystem and discuss the implications for watershed N loss. In Chapter 3, I investigate the influence of atmospheric N deposition on the stoichiometry and flux of dissolved organic matter in forests spanning a broad range of N loading and show that chronic N supply alters DON loss. In chapter 4, I investigate the dual influences of N deposition and climate variability on long-term patterns of N loss from forests. I use long-term (30 year) hydro-chemical records to show tight coupling of a major atmosphere-climate mode, local temperature, and the dynamics of N loss from forests.
Chapter 2: Coupled cycling of dissolved organic nitrogen and carbon in a forest stream

Abstract

Dissolved organic nitrogen (DON) is an abundant but poorly understood pool of N in many ecosystems. We assessed DON cycling in a N-limited headwater forest stream via whole-ecosystem additions of dissolved inorganic nitrogen (DIN) and labile dissolved organic matter (DOM), hydrologic transport and biogeochemical modeling, and laboratory experiments with native sediments. We sampled surface and subsurface waters to understand how interaction among hydrologic exchange, DIN, DON, and dissolved organic carbon (DOC) influence stream N losses at summer baseflow. Added DON was taken up rapidly from the water column at rates exceeding DOC and DIN. A significant fraction of this DON was mineralized and nitrified. Combined DON and NO$_3$-N uptake lengths resulted in spiraling lengths of ~ 210 m, suggesting the potential for multiple transformations of labile N loads within catchment boundaries.

Simultaneous addition of DIN increased DOM uptake, but more so for C, resulting in an upward shift in the C: N ratio of uptake. Sediment incubations also showed a strong biotic influence on DOC and DON dynamics. Despite efficient uptake of added DOM, background DON and high molecular weight DOC concentrations increased downstream, resulting in higher DOM loads than could be accounted for by groundwater discharge and suggesting net release of less bioavailable forms from the channel/hyporheic zone. At the same time, subsurface DOM was characterized by very low C: N ratios and a disproportionately large DON pool despite rapid hydrologic mixing with dilute and high C: N ratio surface waters. Analysis of expected DON loads from conservative hyporheic fluxes indicated that watershed losses of DON would have been 7 times greater in the absence of apparent benthic demand, suggesting tight internal cycling
of subsurface DON. Our study further demonstrates the potential for significant transformation of N in headwater streams before export to downstream ecosystems.

Introduction

Nitrogen (N) availability can control biological activity from cells to ecosystems. For stream ecosystems, the balance between biotic demand and hydrologic transport determines the relative retention of N. Within small watershed ecosystems (sensu Likens and Bormann 1995), streams are the last step in the hydrologic continuum extending from the atmosphere through terrestrial and aquatic subsystems, and thus the final opportunity for biotic regulation of N before transport to downstream ecosystems. Headwater streams are biogeochemically responsive to N, exhibiting rapid uptake of dissolved inorganic N (DIN; Peterson et al. 2001) and may also act as DIN transformers by affecting the relative abundance of N forms comprising total dissolved nitrogen (TDN).

Nitrogen demand in forest streams, as in forest soils (Currie 1999), is strongly linked to microbial cycling of N contained in organic matter (Triska et al. 1984). In streams, dissolved organic matter (DOM) often constitutes the largest component of the organic matter pool (McKnight et al. 2003) and its flux from rivers often dominates organic loading to estuaries (Seitzinger and Sanders 1997). Dissolved organic N (DON) represents a major pool of dissolved N in many unpolluted headwater catchments (Triska et al. 1984, Perakis and Hedin 2002, Lewis 2002) but it is unclear to what extent N demand in streams is satisfied through accessing the N bound in DOM. Much of the DON in streams is believed to originate from soil as refractory humic and fulvic acids (Qualls and Haines 1991, 1992) and thus may behave conservatively along catchment flow paths (Hedin et al 1995). Alternatively, forest streams are known to be
major generators of dissolved organic carbon (DOC) via microbially mediated leaching of terrestrial detritus (Meyer et al. 1998). Production of DON may be high in forest streams as reflected in large differences in microbial and detrital C: N stoichiometry (Caraco and Cole 2003). Internally produced DON may constitute a relatively fast-turnover compartment of N relative to terrestrial sources and thus an important part of the N economy of forest streams.

The importance of DON to biotic N uptake should vary with the degree of N limitation and DOM quality (Kaushal and Lewis 2003, Neff et al. 2003). Amino acids and other monomers are a small proportion of DOM in surface waters (McKnight et al. 2003), but are the most rapidly consumed forms of DOM in stream sediments (Fiebig 1997, Findlay and Sinsabaugh 2003). They thus represent a labile end member capable of playing a disproportionate role in ecosystem metabolism and transformation of N.

In streams, the downstream advection of water imparts a spatial dimension to nutrient cycling. This dimension has been described in terms of spiraling length ($S$): the distance over which a nutrient transforms from inorganic to organic and back to inorganic form (Webster and Patten 1979, Newbold et al. 1981). Diffusive and capillary forces dictate flow and organize nutrient cycling in unsaturated soils (Wagener et al. 1998) such that residence times are long and transport distances are minimized. In contrast, streams are dominated by advective transport, though zones of relatively slow water movement can have strong influences on system biogeochemistry (Triska et al. 1989, Thomas et al. 2003).

In this study, we examined DON dynamics in surface and subsurface zones of a low DOC, N-limited forest stream via experimental additions of DIN and labile DOM at summer baseflow. Our central objective was to assess how the coupled spiraling of C and N translates into stream ecosystem N fluxes. We hypothesized that in N-limited streams, benthic biota
should be capable of significant uptake and transformation of DON but demand should be mediated by DIN and DOC availability and DOM quality. We tested these hypotheses with experiments addressing how hydrologic exchange, N availability, and DOM quality interact to affect whole-stream N cycling and dissolved N losses.

**Methods**

**Study site**

We conducted this research in Hugh White Creek (HWC), a second-order stream draining a 61-ha catchment in the Coweeta Hydrologic Laboratory in the Appalachian Mountains of southwestern North Carolina, USA. Climate is humid marine with moderate mean annual temperature (13°C) and >90% annual precipitation (187 cm) occurring as rain. Catchment vegetation is dominated by mixed oak, hickory, and tulip poplar forest, with birch and hemlock along streamsides (Webster et al. 1997). A dense understory of rhododendron shades many sections of the stream, resulting in <5% incident light. Low gross primary production (~6 g m⁻² yr⁻¹) and high input of terrestrial detritus (~600 g m⁻² yr⁻¹) result in a highly heterotrophic ecosystem (Webster et al. 1997). Stream water is characterized by low concentrations of DOC (<1 mg/L) and inorganic N (<20 µg/L) and phosphorus (<2 µg/L) (Munn and Meyer 1990, Swank and Vose 1997). Channel sediments are dominated by gravels and cobbles, interspersed with sand and bedrock outcrops. Catchment soils are underlain by deep saprolite, which is thought to be the primary source of baseflow (Velbel 1988). HWC has remained undisturbed since 1924 and is a reference site for long-term hydrologic and chemical monitoring (Swank and Vose 1997).
Experimental design and instrumentation

We combined three complimentary approaches to study DON cycling in HWC: (1) whole-stream additions of nitrate (NO$_3$-N) and/or labile DOM, (2) hydrological and biogeochemical modeling, and (3) laboratory experiments with native stream sediments amended with labile DOM. The study reach on the main channel of HWC extended ~ 700 m upstream of a weir at the base of the watershed. In 1999, we established well transects for sampling subsurface water chemistry located at 69, 95, 110, and 159 m below the head of the reach. Transects were composed of 3 to 4 wells distributed across the wetted channel width. Wells ($n = 15$) consisted of 5-cm PVC casings (closed bottom) with 15-cm length slotted screens (254-µm slot) inserted ~25 cm beneath the channel bottom.

Additions of NO$_3$-N and DOM

Nutrient enrichment experiments were conducted from 19 – 21 July 2003 and consisted of sequential individual additions of (1) NaNO$_3$, (2) urea, (3) glutamic acid, and (4) glutamic acid + NaNO$_3$. We used glutamic acid and urea because they differ in C: N mass ratio and molecular weight (L-glutamic acid, Sigma-Aldrich G2128, C: N = 4.3, MW = 147; urea, Sigma-Aldrich U0631, C: N = 0.43, MW = 60), and thus potentially in bioavailability. The glutamic acid + NaNO$_3$ addition was conducted to assess effects of elevated DIN on DON and DOC uptake.

All solutes were dissolved in deionized water with NaCl as a conservative tracer added to elevate Cl$^-$ concentration 3 mg/L above background (~ 0.55 mg/L) and increase electrical conductivity ~ 12 µS. Background NO$_3$-N varied from 3 to 20 µg/L and a NaNO$_3$ solution was used to elevate N concentration by 50 µg/L. DOM was added to elevate stream DON by 300 µg
N/L above background (15 – 80 µg N/L), an enrichment that allowed quantification of N and C above background variability for both DOM forms. Nutrient additions temporarily elevated N: P ratios above that characteristic of baseflow conditions. Additions resulted in a DOC enrichment of 1290 µg C/L for glutamic acid and 129 µg C/L for urea.

Prior to the initial addition, we placed data logger mini- sondes (HydroLab™, Austin, TX) at 200 (immediately upstream of a small tributary), 300, and 650 m downstream of the addition site. Sondes recorded specific electrical conductance every 4 min. Background stream water samples (n = 4) were collected prior to each nutrient release at eight longitudinally distributed stations: –5 m (upstream reference site), 30, 60, 90, 150, 220, 300, and 650 m downstream of the addition site. Hyporheic water was sampled in all wells (n = 15) prior to the initial NaNO₃ release and at 1 well from each of 4 transects prior to subsequent releases using screened vinyl tubing and a syringe. Samples were filtered (Whatman ®GF/F, pore size = 0.7 µm) into acid-washed 125-mL polyethylene bottles and frozen until analyzed for NO₃-N, NH₄-N, DON, and DOC. Specific conductance, temperature, and dissolved oxygen (DO) were recorded at each station and in each well using YSI conductivity and DO meters (YSI Inc, Yellow Springs, OH).

Nutrients were pumped from individual carboys using a fluid metering pump (FMI Inc., Syosset, NY) and delivered to a well-mixed location in the stream at 25 mL/ min for ~ 5 h. Additions were conducted on different days, separated by > 18 h to ensure adequate hydrologic flushing. During each release, we tracked Cl⁻ by monitoring specific conductance at the 300-m station until the signal stabilized (~ 3.5 h). We then sampled during the ‘plateau’ as described for background sampling. After sampling during the glutamic acid release (day 3), the addition was allowed to continue while a second pump simultaneously introduced NaNO₃ to the stream. We
sampled wells synoptically with stream sampling during NaNO$_3$ and glutamic acid + NaNO$_3$ ($n = 4$ wells) and urea and glutamic acid additions ($n = 10$ wells).

*Hydrology and transient storage modeling*

Stream discharge ($Q = \text{L/s}$) was determined using dilution gauging techniques (Webster and Ehrman 1996). Stream depth ($z$) and width ($w$) were measured at regular intervals along the 650 m reach. We developed a standard curve relating specific conductance ($\mu$S/cm) to Cl$_-$ concentrations ($\mu$g/L) in the stream ($\text{Cl}^- = 278.8 \mu\text{S} - 2408.7, r^2 = 0.99$). Lateral inflow ($Q_L, \text{L m}^{-1} \text{s}^{-1}$) was determined from longitudinal patterns of Cl$_-$ dilution. Hydrologic variables were determined by comparing numerical solutions of a one-dimensional advection-dispersion transport model that includes transient storage and lateral inflow (Bencala and Walters 1983) with observed Cl$_-$ concentrations using a model fit program similar to and in conjunction with OTIS-P (Runkel 1998).

Modeled variables included stream discharge ($Q$), lateral inflow ($Q_L, \text{L/s}$), the coefficient of exchange between the stream channel and the transient storage zone ($\alpha, \text{s}^{-1}$), and the cross-sectional areas of the open channel and the theoretical transient storage zones ($A$ and $A_s, \text{m}^2$). Using model results, we calculated mean water velocity ($u = Q/A$), uptake length for water ($L_s = u/\alpha$), mean storage zone water residence time ($T_s = A_s/\alpha A$), and the transient storage exchange flux ($q_s = \alpha A$) as described by Harvey and Wagner (2000). The upper 300 m reach of stream was used for hydrologic modeling because it included all well locations and most surface water transects. Percent surface water in hyporheic wells was determined with a mixing model that compares change in Cl$_-$ concentrations in surface versus well waters (Triska et al. 1989).
In February 2004 (during a period of similar flow as July 2003), we independently assessed the residence time of hyporheic water by injecting ~2 mL of NaCl solution (1 g Cl/L) into a subset of individual wells (n = 8, two per transect) and tracking Cl clearance curves via exponential decay in specific conductance (k_c, h⁻¹) over time (~2 h). Mean water residence time (h) was calculated as 1/k_c.

Nutrient uptake, production, and transformation

We estimated nutrient uptake lengths (S_w; the average distance traveled by a reactive solute) as 1/k_L, where k_L is the longitudinal loss rate (m⁻¹) determined with linear regressions of the natural ln-transformed background-corrected plateau N: Cl or C: Cl ratios against distance downstream (Newbold et al. 1981). Uptake velocity (v_f = Q/wS_w, mm min⁻¹) and rate (U = v_f C, mg m⁻² d⁻¹) were calculated to determine mass transfer and areal influxes of nutrients, where w is mean stream width and C is background concentration (Stream Solute Workshop 1990).

Mineralization of DON was estimated by fitting a two-compartment mass balance model to the longitudinal profile of added DON and NO_3-N loads to produce best-fit estimates of simultaneous DON consumption (k_{DON}) and NO_3-N production (k_{NIT}) and uptake (k_{NO3}) following Mulholland et al. (2000). The preliminary step of ammonification of DON to NH_4-N was ignored because we were unable to detect a NH_4-N response in surface waters to DON additions. Nitrification fluxes were calculated as k_{NIT}/k_{DON} * U_{DON} using the geometric mean of added longitudinal DON concentrations. We were unable to calculate nitrate uptake or nitrification for the glutamic acid + nitrate experiment because of source ambiguity of elevated NO_3-N. We assessed net consumption or production in the hyporheic zone by comparing observed background-corrected plateau N and C concentrations in wells versus expected values.
derived from Cl− concentrations assuming conservative transport from the nearest upstream surface transect (sensu Triska et al. 1989 and Valett et al. 1996).

We calculated ‘realized’ production rates for chemicals that increased in concentration downstream (i.e., increases in load were greater than increases in discharge) by multiplying the up-to-downstream concentration difference by downstream Q and dividing the resultant load by total benthic surface area (Meyer et al. 1998). We also calculated ‘expected’ loads based on conservative subsurface-to-surface nutrient fluxes using $q_sC_H + Q_L C_S$ adjusted for stream width, where $C_H$ is the mean background hyporheic nutrient concentration for all well samples and $C_S$ is the mean background stream nutrient concentration at the transect above the addition point. Expected stream loads were calculated by adding resultant hyporheic fluxes to stream loads derived from upstream surface water concentrations and $Q_L$ (i.e., nutrient loads conservatively tracking water inputs).

*Laboratory microcosms*

We assessed the relative importance of biotic and abiotic uptake of DOM by conducting short-term batch incubation experiments. After field experiments, ~ 2 kg native sediments were collected from a tributary entering HWC at 200 m and were kept in the laboratory at 4°C until initiating microcosm assays (3 days). Sediment was first reacclimated to ambient stream temperatures (15°C) for 24 h, sieved (1-cm mesh), and homogenized. To eliminate biotic activity, half of the sediments were saturated with a solution of HgCl$_2$ (100 mg/L; Dahm 1981). We then added 50 cc (~ 83 g, ~ 1% OM) of well drained live or dead sediments into 500 mL flasks across three treatments: (1) DI water (200 mL), (2) DI water + urea, and (3) DI water + L-glutamic acid, with four replicates each ($n = 24$). We added 60-µL of concentrated DOM to
enrich water by 300 µg DON/L. Water and water + HgCl₂ controls were used to correct for background release of C and N from sediments. Flasks were shaken at 125 rpm and 15°C for at total of 4 h. At 0.5 and 4 h, ~ 30 mL of supernatant was drawn off for analysis. Samples were filtered (GF/F), stored in polyethylene bottles, and frozen. Uptake was analyzed as change in nutrient mass per gram of sediment.

Analytical chemistry

Nitrate-N was determined colorometrically following the acidic diazo method after cadmium reduction (Wood et al. 1967) on a Technicon Auto Analyzer II. Ammonium-N was analyzed colorometrically following an automated phenate method (Soloranzo 1969). Total dissolved N (TDN) was determined by high temperature potassium persulfate oxidation followed by analysis of nitrate. DON was calculated by difference as TDN – (NO₃-N + NH₄-N) using L-glutamic acid as a reference standard (D’Elia et al. 1977, APHA 1998). DOC was determined by sodium persulfate oxidation using a Model 700 Total Organic Carbon Analyzer (Oceanographic International Model 700; Menzel and Vacarro 1964). A subset of background and plateau samples were analyzed for Cl⁻ using a Dionex 500 Ion Chromatograph equipped with an AS14 anion detection column (APHA 1998). To assess large molecular weight humic substances, background samples were analyzed for specific ultraviolet absorbance (SUVA) at 254 nm using a 10-cm path cell in a Shimadzu spectrophotometer (Chin et al. 1994).

Statistical Analysis

Spatial patterns in background nutrients were analyzed with linear regressions of concentrations and fluxes with distance downstream. We tested for differences among uptake
slopes \((k)\) of added nutrients by assessing the interaction term between distance downstream and added nutrient type with multiple linear regression (Zar 1984). We tested for differences between stream and hyporheic concentrations, expected and observed plateau hyporheic concentrations, and differences between live vs. dead sediment treatments using paired t-tests. We compared observed versus expected increases in nutrient loads using chi-squared analysis. All statistical tests were performed using SAS (version 6.12 SAS Institute Inc., Cary, North Carolina, USA).

Results

Hydrologic dynamics

Discharge increased from 8.7 to 20.2 L/s over the first 300 m of study reach, representing a lateral inflow rate of 0.016 L s\(^{-1}\) m\(^{-1}\). At the same time, results from transient storage modeling (Appendix 1) showed that the transient storage exchange flux \((q_s)\) was 0.052 L s\(^{-1}\) m\(^{-1}\), suggesting that > 3 times the volume of water was exchanged with transient storage than was discharged from groundwater per length of stream channel. This relationship was mirrored by mixing within the hyporheic zone, where percent surface water averaged 72.2 ± 9.5 % (mean ± 1 SE) in sampling wells. The uptake length of water \((L_s)\) was 417 m and 71% of all surface water was exchanged with the storage zone over the 300 m reach. Water residence time in transient storage \((T_s)\) was 1.1 h, a value very similar to that derived for hyporheic wells \((1.9 ± 0.4 \text{ h}; n = 8)\). The relative size of the transient storage zone \((A_s/A)\) was 0.8.
Stream water was well oxygenated and characterized by low concentrations of NO$_3$-N (11 ± 0.1 µg/L) and NH$_4$-N (2 ± 0.3 µg/L; Fig. 1). Stream water DON concentrations were significantly ($P < 0.01$) higher than DIN and represented ~ 65% of stream TDN (Fig. 1). Low DOC and high atomic C: N ratios (range = 25 – 96; mean ± 1 SE = 50 ± 4) characterized stream DOM. In contrast to stream water, hyporheic well water was significantly ($P < 0.01$) elevated in DIN (NO$_3$-N = 33 ± 7 µg/L; NH$_4$-N = 8 ± 1 µg/L), DON, and DOC, and depleted in DO (Fig. 1). DON also dominated (~ 89%) TDN in the hyporheic zone. High DON and only moderately elevated DOC resulted in very low hyporheic DOM C: N ratios (1.5 –14.3; 6 ± 1) compared to stream DOM. SUVA analysis of well samples ($n = 4$) indicated overlap between subsurface and surface values (1.9 ± 0.2 vs. 2.0 ± 0.1 L mg C$^{-1}$ m$^{-1}$). Although NO$_3$-N concentrations were highest at the downstream most transect (19.2 ± 0.1 µg/L), the regression with distance downstream was not significant ($P = 0.56$) without this point. In contrast, stream concentrations of DON and DOC and SUVA values increased significantly ($P < 0.01$) with distance downstream at both scales (Fig. 2). These results indicate greater increases in dissolved organic C and N loads than could be accounted for by increases in discharge, indicating an important channel/hyporheic source of nutrients other than groundwater.

Nutrient uptake and transformation

HWC exhibited strong and differential uptake of added DOM and DIN. DON was taken up at a significantly ($P < 0.05$) higher rate than either NO$_3$-N alone or DOC within the DOM pool (Table 1). After correction for dilution, only ~ 6% of added DON remained 300 m downstream of the addition site during the experimental additions. Longitudinal loss rates ($k_L$)
for DOC and DON were similar \( (P > 0.05) \) between urea and glutamic acid additions.

Differential uptake of C and N during urea and glutamic acid additions resulted in mass ratios of C and N uptake \((k_{\text{DOC}}: k_{\text{DON}})\) of 0.8 and 0.65 and areal C and N uptake \((U_{\text{DOC}}: U_{\text{DON}})\) of 0.9 and 3.6, respectively. Addition of NO\(_3\)-N during the glutamic acid experiment substantially increased uptake of DOC and DON (Table 1) but more so for C, thus shifting the stoichiometry of DOM uptake \((k_{\text{DOC}}: k_{\text{DON}} = 1.2 \text{ and } U_{\text{DOC}}: U_{\text{DON}} = 6.5)\).

Rapid nitrification of added DON resulted in significant \((P < 0.01)\) increases in surface water NO\(_3\)-N concentrations (Fig. 3). Although most DON was taken up within the first 300 m, elevated NO\(_3\)-N concentrations were evident at the base of the watershed (650 m). Extremely rapid and transitory ammonification was suggested by stable and low NH\(_4\)-N concentrations in surface and subsurface waters during DOM releases. Nitrification rates accounted for 14 and 23\% of urea-DON and glutamic acid-DON uptake, respectively (Fig. 3).

The mass balance model produced estimates of nitrate uptake lengths 3 times shorter than those calculated from the NO\(_3\)-N addition (Fig. 3, Table 1). Adding DON and produced NO\(_3\)-N uptake lengths resulted in a total N spiraling length \((S)\) of 209 and 210 m. Combining all \(S_{w-\text{NO3-N}}\) estimates indicated that background net uptake could have varied from 9 to 31 mg N m\(^{-2}\) day\(^{-1}\). By comparison, net nitrification calculated using background and added DON levels varied from 7.4 to 46.9 mg N m\(^{-2}\) day\(^{-1}\).

Although we found rapid uptake of added DON in surface waters, analysis of hyporheic concentrations suggested that DON was transported conservatively to the hyporheic zone where observed and expected concentrations were not significantly different \((P = 0.92, \text{ paired t-tests; Fig 4A})\). Concentrations of NH\(_4\)-N did not differ between background and plateau samples \((P > 0.2)\). During the same period, 19 out of 24 samples showed net production of NO\(_3\)-N \((P < 0.001;\)
Fig. 4B). In contrast, we found that the hyporheic zone was a net consumer of added DOC ($P = 0.047$) in 18 of the 24 samples (Fig. 4C).

**Sediment microcosms**

Dissolved organic matter was released over time from killed (DOM C: N = 16) and live (DOM C: N = 25) control sediments. Background release of DON was greater ($P < 0.05$) in killed (3.1 ± 0.5 μg N/g sediment) versus live (1.4 ± 0.3 μg N/g sediment) controls (Appendix 2). Release of DOC was also greater ($P < 0.05$) in killed versus live sediments but concentrations decreased in live sediments and accumulated in killed sediments over time. All live treatments tended toward net consumption of added DOC and DON compared with net production in killed treatments but differences were significant only for glutamic acid and control DOC treatments (Appendix 3). Nitrification in live sediments was indicated by increases ($P < 0.01$) in NO$_3$-N but constant NH$_4$-N levels. In contrast, NH$_4$-N accumulated in killed treatments (Appendix 2).

**Stream fluxes and watershed losses**

Downstream increases in concentration (Fig. 2) translated to net production of DOC (79.4 mg m$^{-2}$ d$^{-1}$) and DON (2.1 mg m$^{-2}$ d$^{-1}$). However, observed increases in DON load were significantly lower ($\chi^2 = 24.3, P < 0.001$) than expected based on conservative hyporheic fluxes (Fig. 5). Export of DON from the study reach would have been 7 times greater than the observed 50 g/day had hyporheic DON been transported conservatively to surface waters and downstream (i.e., 350 g/day). This translates to uptake of hyporheic DON of 478.2 mg m$^{-2}$ d$^{-1}$ along the hyporheic-stream flow path, similar to the mean observed uptake of experimentally added DON.
\( U_{\text{DON}} = 317.5 \text{ mg m}^{-2} \text{ d}^{-1} \). In contrast to apparent removal of DON, this analysis indicated subsurface DOC fluxes would result in loads equivalent \( (\chi^2 = 0.4, P > 0.25) \) to realized loads.

**Discussion**

Our study provides evidence for whole-stream uptake and transformation of labile DON and indicates multiple potential interactions of DOC and DON in forest streams. Despite rapid uptake of added DOM, we found that the stream was a net producer of DOC and DON, resulting in watershed losses greater than expected from groundwater alone. Differential patterns of C and N uptake and production indicate that hydrologic exchange and biotic demand in forest streams can significantly influence watershed N losses.

Coweeta streams are strongly N-limited in part due to extremely low hydrologic N losses from surrounding forest ecosystems (Swank and Vose 1997). Our study showed pronounced uptake of monomeric DON and DOC in HWC, suggesting strong biotic demand for DOM in the face of low ambient N and C availability. Uptake lengths of DOC and DON were similar to that reported for a sucrose addition to HWC (Munn and Meyer 1990) but were longer than those observed for \(^{13}\text{C}-\text{acetate} \) additions (~10 m) in other Coweeta streams (Hall and Meyer 1998). Further, addition of NO\(_3\)N during the glutamic acid experiment showed that uptake of labile DOM, especially DOC, is highly responsive to DIN availability. Together, these observations suggest that DON uptake was mediated by demand for DOC and in turn C demand was mediated by DIN. The shift in the C: N stoichiometry of uptake resulting from addition of DIN illustrates enhanced C uptake at times of high N availability. Accordingly, it also suggests higher labile DON demand in the face of lower DIN availability. These results support the idea of a strong link between inorganic N dynamics and DOC availability (Bernhardt and Likens 2002) and tight
coupling of DOC, DIN, and DON spiraling in forest streams. Combined uptake lengths for DON and NO$_3$-N indicate that multiple complete N transformations may occur along the stream reach. Our results support the notion that low abundance of labile DOM in surface waters may in part be a function of internal biotic demand by microbial biofilms (Kaplan and Newbold 2002, Battin et al. 2003) and are consistent with the idea that much of aquatic metabolism is supported by rapid turnover of low molecular weight DOM (Kirchman 2003).

In small streams, efficient biotic uptake of dissolved nutrients is associated with longer water residence times. Modeling and field measurements demonstrated strong surface-subsurface water exchange in HWC. Hydrologic exchange with longer residence time compartments is a key driver of DIN dynamics in many stream ecosystems (Triska et al. 1989, Valett et al. 1996, Thomas et al. 2003). We found the relationship between the transient storage exchange flux ($q_s$) and lateral inflow ($Q_L$) to be particularly useful for studying DON dynamics. Similar potential for DON transformation likely exists in other streams given that hydrologic metrics calculated for HWC are in the middle of published values for a variety of streams (Runkel 2002).

Large subsurface N pools exist despite rapid hydrologic mixing with dilute surface waters, suggesting internal production. Indeed, DON concentrations are generally higher in subsurface vs. surface waters across a broad range of headwater forest streams in the southern Appalachian Mountains (unpublished data). Concentrations of DOC and DON decline with depth in mineral soils via rapid adsorption (Qualls and Haines 1991), resulting in near-stream groundwater DOM similar to stream water (Meyer and Tate 1983, Qualls and Haines 1991, Golladay et al. 1992), implying that groundwater inflow could not have produced elevated hyporheic DON concentrations. Further, conservative three-compartment (surface, hyporheic,
and groundwater) mixing analysis (sensu Mulholland and Hill 1997) indicated that, given the high level of surface-subsurface mixing compared to lateral inputs, groundwater DON would have had to be unrealistically high ($\geq 1310 \mu g/L$) to produce observed hyporheic concentrations (unpublished data). At the same time, longitudinal increases in stream DON concentrations indicate net DON release. Several possible mechanisms may explain these patterns. Most DOC and DON in soils and stream waters of Coweeta are found in refractory humic and fulvic acids (Qualls and Haines 1991, 1992), consistent with the high C: N ratio and elevated SUVA values (McKnight et al. 2002) of surface water DOM found in our study. Previous studies in HWC also found longitudinal increases in DOC and high molecular weight DOM (Meyer and Tate 1983). Further, work in Coweeta has identified channel-stored terrestrial particulate organic matter (POM) as the primary source of the observed DOC (Meyer et al. 1998). This is consistent with our findings that expected DOC loads from hyporheic fluxes were indistinguishable from realized loads. Similar longitudinal increases in DON observed in our study and large benthic pools of particulate organic N (12.6 g/m$^2$; Webster et al. 1997, Tank et al. 2000) suggest DON production may also be linked to in-stream detrital-N, presumably via hydrolysis and leaching.

Although longitudinal increases in DON suggest net release of less bioavailable forms, convergence of our experimentally determined labile DON uptake with that required to balance conservative hyporheic fluxes argues for very high consumption of internally produced DON, otherwise watershed losses would have been much higher. The low C: N ratios of hyporheic DOM observed in HWC were similar to microbial biomass (Tank et al. 2000), suggesting a microbial origin of hyporheic DON. Production and uptake of amino acids by heterotrophic bacteria is common and constitutes a major internal flow of energy and nutrients in lake and marine ecosystems (Kirchman 2003). Similarly, Fiebig (1997) demonstrated tight microbial
recycling of amino acid-C in hyporheic sediments and Findlay and Sinsabaugh (2003) found comparable uptake of DIN and amino acid-N by hyporheic biofilms and widespread capacity for DON uptake across bacterial taxa. Our sediment experiments also showed a strong biotic influence on amino acid N uptake and revealed a large pool of DON in stream sediments.

Given close coupling of particulate and dissolved organic forms (sensu Meyer et al. 1998), increasing N content of POM associated with decomposition and N immobilization could also influence low C: N ratios of DOM. Tank et al. (2000) found minimal labeling of surface water DON during a \(^{15}\text{N}-\text{NH}_4\) addition to a nearby Coweeta stream, implying slow turnover of most surface water DON relative to transport. However, mass balance of \(^{15}\text{N}-\text{NH}_4\) additions and others conducted with \(^{15}\text{N}-\text{NO}_3\) in Coweeta (H.M. Valett unpublished data) generally yield ~50% or less isotopic recovery, suggesting that some of missing N may have gone to subsurface pools. Differential gaseous losses of C and N could also reduce C: N ratios. Metabolic losses of CO\(_2\) are large in HWC (Webster et al. 1997) while N losses via denitrification are presumably much smaller. Increases in subsurface NO\(_3\)-N and decreases in DOC during DOM experiments and only moderately elevated background DOC relative to surface water concentrations were also consistent with decreasing N limitation and increasing C limitation with depth, similar to hyporheic zones of other N-limited forest streams (Triska et al. 1989).

The large DIN pool in the hyporheic zone of HWC and rapid nitrification of added DON points to mineralization and subsequent benthic uptake as a potential fate of consumed DON. While we are aware that subsurface redox conditions and related N transformations can be very spatially heterogeneous (Baker et al. 2000), subsurface N accumulation and strong depletion of DO suggest that nitrification was mostly restricted to the benthic interface and deeper subsurface concentrations of NO\(_3\)-N were due to conservative transport of nitrate-rich infiltrating water.
Microbial demand and regeneration appear to be equivalent, resulting in lack of longitudinal gradients in NH$_4$-N and NO$_3$-N concentrations in this stream. Further, the lack of an ammonification signal to DON additions is consistent with very rapid uptake of NH$_4$-N in Coweeta streams (Hall et al. 1998, Tank et al. 2000). Conditions generated by our experiments may have restricted NH$_4$-N cycling to the scale of the biofilm itself. Denitrification and downstream loss or deep burial as FPON, perhaps of biofilm origin, are also potential but poorly understood fates of dissolved N.

Our study was conducted during summer baseflow when nutrient uptake is likely higher than during periods of high flow and colder temperatures. DOC concentrations and fluxes increase during storms relative to baseflow (Meyer and Tate 1983) and the same may apply to DON. Also, DIN uptake in Coweeta is highest after leaf-fall (H.M. Valett, *unpublished data*) reflecting increases in microbial demand associated with decomposition. Given the observed relationship between DON and DIN uptake, labile DON uptake may also increase during fall. However, stream NO$_3$-N concentrations and fluxes (Swank and Vose 1997) and subsurface DON concentrations (*unpublished data*) in Coweeta are highest during summer, suggesting a potentially important role of summer N cycling to annual watershed N losses.

Our results support the idea of an important role for DON production in forest streams (Caraco and Cole 2003). We propose two major pools of DON in HWC: 1) refractory DON derived primarily from catchment soils and secondarily from in-stream detrital leaching; and 2) labile internally recycled DON. This framework is consistent with fast and slow turnover pools described for stream DOM (Kaplan and Newbold 2003) and terrestrial DON (Neff et al. 2003). Our results suggest extremely short spiraling lengths for monomeric DON and much longer (i.e., at the scale of river networks) spiraling of complex, predominantly humic, terrestrial DON.
Large increases in global N availability via human activities (Vitousek et al. 1997) require better understanding of biotic controls on cycling of N at ecosystem scales. The results presented here argue for a much more influential role of stream biota in modulation of watershed DON losses than is commonly assumed. Our estimates of DIN and DON spiraling suggest that a significant portion of the dissolved N pool is transformed as it moves through headwater streams. Further, this study provides support for the idea that even small increases in DIN availability may alter ecosystem DOC and DON dynamics.
Literature cited


Table 1. Spiraling metrics for experimental nutrient releases. Included are longitudinal uptake coefficients ($k_L$) nutrient uptake lengths ($S_w$), uptake velocities ($v_f$), and areal uptake ($U$).

<table>
<thead>
<tr>
<th></th>
<th>Nitrate</th>
<th>Urea</th>
<th>Glutamic acid</th>
<th>Glutamic + Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>DOC</td>
<td>DON</td>
<td>DOC</td>
</tr>
<tr>
<td>$k_L$ (m$^{-1}$)†</td>
<td>0.0028$^a$</td>
<td>0.0077$^b$</td>
<td>0.0096$^{bc}$</td>
<td>0.0065$^b$</td>
</tr>
<tr>
<td>$S_w$ (m)</td>
<td>357.1</td>
<td>129.9</td>
<td>104.2</td>
<td>153.8</td>
</tr>
<tr>
<td>$v_f$ (mm/min)</td>
<td>0.60</td>
<td>1.65</td>
<td>2.06</td>
<td>1.39</td>
</tr>
<tr>
<td>$U$ (mg m$^{-2}$ day$^{-1}$)‡‡</td>
<td>9.3</td>
<td>295.0</td>
<td>311.0</td>
<td>1155.0</td>
</tr>
</tbody>
</table>

†Different superscripted letters indicate significant ($P < 0.05$) differences among uptake coefficients ($k_L$).

‡‡Areal uptake is based on background concentrations for NO$_3$-N and the geometric mean of added concentrations for DOC and DON.
Figure 1. Comparison of mean (± 1 SE) background stream and hyporheic water chemistry. Means were significantly ($P < 0.01$) different as determined by paired t-tests.
Figure 2. Background longitudinal patterns in ambient stream (A) DON, (B) DOC, and (C) SUVA values in HWC. Data are means (± 1 SE, n = 4 per location). Statistics are from regressions of all samples against distance downstream. Regressions remained significant (P < 0.01) following removal of data for 650 m.
Figure 3. Nitrate production curves during urea and glutamic acid additions. Values are mean (± 1 SE) background-corrected nitrate loads. Included are results from the mass balance model for the longitudinal nitrification rate ($k_{\text{NIT}}$), the proportion of DON uptake nitrified ($k_{\text{NIT}}/k_{\text{DON}}$), and simultaneous nitrate uptake lengths ($S_{w-\text{NO3-N}}$). The arrow indicates the nutrient addition site.
Figure 4. Subsurface responses to both urea and glutamic acid additions (filled circles) and glutamic + NO$_3$-N (open circles) showing (A) DON, (B) production of NO$_3$-N, and (C) consumption of DOC. Values above the 1:1 observed-expected line indicate net production while values below the line indicate net consumption. $P$ values represent results of paired t-tests.
Figure 5. Increase in DOC and DON load versus increase in water load. Values represent the proportional increase in nutrient load at downstream transects relative to loads at the upstream most transect. The solid line represents the proportional increases in discharge and expected nutrients if nutrient loads tracked groundwater inputs (1:1 line). Dashed lines represent expected DOC and DON loads based on conservative transport of hyporheic concentrations.
Appendix 1. Results of transient storage modeling for the upper 300-m study reach of Hugh White Creek stream, Coweeta Hydrologic Laboratory, NC. Definitions of hydrologic variables are provided in Runkel (2002).

<table>
<thead>
<tr>
<th>Hydrologic variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (m²)</td>
<td>0.27</td>
</tr>
<tr>
<td>A_s (m²)</td>
<td>0.216</td>
</tr>
<tr>
<td>A_s/ A</td>
<td>0.8</td>
</tr>
<tr>
<td>α (s⁻¹)</td>
<td>0.00019</td>
</tr>
<tr>
<td>Q_L (L m⁻¹ s⁻¹)</td>
<td>0.016</td>
</tr>
<tr>
<td>q_s (L m⁻¹ s⁻¹)</td>
<td>0.052</td>
</tr>
<tr>
<td>T_str (min)</td>
<td>86.8</td>
</tr>
<tr>
<td>T_sto (min)</td>
<td>69.4</td>
</tr>
<tr>
<td>L_s (m)</td>
<td>417</td>
</tr>
<tr>
<td>F_{med}^{300} (%)</td>
<td>23</td>
</tr>
</tbody>
</table>
Appendix 2. Mean (± 1 SE, n = 4) nutrient concentrations for the laboratory microcosm experiment. C = live controls, Hg = killed (HgCl$_2$) controls, G = glutamic acid additions, U = urea additions, G + Hg = glutamic acid additions to killed sediments, and U + Hg = urea additions to killed sediments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (hr)</th>
<th>NO$_3$-N (µg/L)</th>
<th>NH$_4$-N (µg/L)</th>
<th>DON (µg/L)</th>
<th>DOC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.5</td>
<td>19.3 (2.0)</td>
<td>67.9 (5.4)</td>
<td>100.5 (16.4)</td>
<td>2.3 (0.1)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>65.2 (21.3)</td>
<td>97.2 (20.2)</td>
<td>138.0 (66.4)</td>
<td>1.5 (0.1)</td>
</tr>
<tr>
<td>Hg</td>
<td>0.5</td>
<td>19.6 (2.8)</td>
<td>81.4 (10.9)</td>
<td>241.3 (74.3)</td>
<td>2.6 (0.3)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10.7 (1.7)</td>
<td>152.5 (11.6)</td>
<td>274.8 (37.0)</td>
<td>3.6 (0.1)</td>
</tr>
<tr>
<td>G</td>
<td>0.5</td>
<td>14.1 (1.6)</td>
<td>124.5 (8.0)</td>
<td>308.7 (29.6)</td>
<td>2.9 (0.2)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>39.6 (2.1)</td>
<td>119.5 (7.6)</td>
<td>237.1 (14.9)</td>
<td>2.3 (0.2)</td>
</tr>
<tr>
<td>U</td>
<td>0.5</td>
<td>14.2 (2.6)</td>
<td>51.3 (9.0)</td>
<td>355.9 (23.2)</td>
<td>1.9 (0.1)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>45.3 (3.9)</td>
<td>111.9 (11.3)</td>
<td>267.3 (25.9)</td>
<td>1.7 (0.1)</td>
</tr>
<tr>
<td>G + Hg</td>
<td>0.5</td>
<td>30.0 (6.8)</td>
<td>155.4 (42.6)</td>
<td>291.1 (14.9)</td>
<td>3.7 (0.2)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>19.7 (6.7)</td>
<td>152.8 (5.3)</td>
<td>579.4 (123.4)</td>
<td>4.8 (0.2)</td>
</tr>
<tr>
<td>U + Hg</td>
<td>0.5</td>
<td>46.8 (9.2)</td>
<td>58.9 (3.2)</td>
<td>396.9 (22.8)</td>
<td>2.4 (0.1)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>75.8 (49.8)</td>
<td>141.0 (14.7)</td>
<td>420.8 (100.5)</td>
<td>3.5 (0.0)</td>
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</table>
Appendix 3. Mean (± 1 SE, n = 4) production (above 0 line) and consumption (below 0 line) of (A) DON, and (B) DOC during sediment incubation experiments. Asterisks indicate significant (P < 0.05) differences between live and dead treatments as determined by paired t-tests.
Chapter 3: Atmospheric N deposition increases organic N loss from temperate forests

Abstract

Atmospheric deposition of nitrogen (N) resulting from fossil fuel combustion has increased N inputs to temperate forests worldwide with large consequences for forest productivity and water quality. Recent work has illustrated that dissolved organic N (DON) often dominates N loss from unpolluted forests and that the relative magnitude of dissolved inorganic (DIN) loss increases with atmospheric loading. In contrast to DIN, DON loss is thought to be controlled by soil dynamics that operate independent of N supply and demand and thus should track dissolved organic carbon (DOC) following strict stoichiometric constraints. Conversely, DON loss may shift with N supply if soil organic matter (SOM) is stoichiometrically altered. Here, we assess these two explanations of DON loss, which we refer to as the Passive Carbon Vehicle and the Stoichiometric Enrichment hypotheses, by analyzing patterns in soil and stream C and N in forest watersheds spanning a broad gradient in atmospheric N loading (5 to 45 kg N ha\(^{-1}\) yr\(^{-1}\)). We show that soil N and DON losses are not static but rather increase asymptotically with N loading whereas soil C and DOC do not, resulting in enrichment of organic N expressed as decreased soil C: N and stream DOC: DON ratios. DON losses from unpolluted sites are consistent with conservative dissolution of refractory SOM. As N supply increases, however, N enrichment of organic losses is greater than expected from simple dissolution of enriched soils, suggesting activation of novel pathways of DON production or shifting dynamics as forests approach N saturation.
**Introduction**

Nitrogen supply regulates biotic structure and function in many ecosystems (Vitousek and others 2002). For millennia, N limitation in most temperate forests has been maintained, in part, by low inputs from atmospheric deposition (i.e., < 4 kg ha\(^{-1}\) yr\(^{-1}\); Holland and others 1999). In combination with low supply from biological fixation, high demand by plants and microbes and sequestration of N into soil organic pools can retard N losses from forest ecosystems over long time scales (Vitousek and others 2002). Under such conditions, dissolved organic N (DON) often dominates hydrologic N losses from soils and watersheds (Sollins and McCorrision 1981; Perakis and Hedin 2002). Now, however, many temperate forests in the northern hemisphere receive N inputs from atmospheric deposition far above pre-industrial levels (Holland and others 1999). While elevated N loading has caused shifts in forest N cycling globally (Aber and others 1998) and consequent increases in dissolved inorganic N (DIN) losses to stream waters (Perakis and Hedin 2002), most forest watersheds remain highly retentive of N. Much of this additional N is suspected to be sequestered into soil organic matter (SOM; Van Breeman and others 2002), but we know little about the long-term consequences of N enrichment on N transfers between biota and SOM, and ultimately dissolved losses from watersheds.

The “N saturation” concept describes cases in which N supply exceeds biotic demand, resulting in the alleviation of N limitation and distinctly non-linear increases in DIN fluxes from soils to stream waters (Ågren and Bosatta 1988; Aber and others 1989). In addition to decreases in biotic uptake, long term N pollution can further exacerbate N losses by collapsing the C: N ratios of organic substrates that regulate rates of immobilization and mineralization, thus increasing nitrification rates and losses of nitrate-N (NO\(_3\)-N) from watersheds. Results from both theoretical and empirical studies have identified two broadly applicable thresholds that
when breached cause abrupt increases in NO$_3$-N leaching from temperate forests: [1] chronic deposition greater than 7 to 11 kg N ha$^{-1}$ yr$^{-1}$ and [2] soil C : N ratios less than 19 to 24 (Aber and others 1998, Gundersen and others 1998).

In contrast to DIN, the alteration of DON dynamics with N loading is a topic of active debate. Some research has suggested that DON losses from watersheds remain consistent across broad geographic areas that vary considerably in N loading rates (Perakis and Hedin 2002) while others have observed large and persistent increases in soil solution DON flux in long-term forest fertilization experiments (McDowell and others 2004; Pregitzer and others 2004). These contrasting observations lead us to propose 2 alternate hypotheses for explaining DON losses from temperate forests, the Passive Carbon Vehicle Hypothesis and the Stoichiometric Enrichment Hypothesis.

The passive carbon vehicle hypothesis argues that DON losses from watersheds are largely bio-unavailable compounds leaching from slow-turnover SOM in strict stoichiometric proportion with dissolved organic carbon (DOC; Hedin and others 1995; Rastetter and others 2005). Therefore, DON losses are under the control of soil organic matter dissolution rather than specific nitrogen cycling processes. Under low N supply, dominance of hydrologic N losses by DON is also expected if N supply limits organic matter decomposition (Vitousek and others 2002, Aber and others 1998), resulting in C losses as DOC rather than CO$_2$, and DON accompanies the DOC loss. Further, DON losses are maintained by the prevalence of DON in refractory humic substances (Thurman 1985; Qualls and Haines 1991) that render DON largely unavailable to higher plants and microorganisms (Qualls 2000). The power of this hypothesis is that it predicts both the higher than expected loss of DON under low N availability as well as lower than expected DON losses (as compared to DIN) in regions of high N deposition (Perakis
and Hedin 2002). Under high N loading rates, DON losses remain stable simply because dissolved organic matter (DOM) export is dominated by refractory compounds whose production is not directly tied to nitrogen cycling. Observed correlation between DOC and DON fluxes in soil and stream waters (Perakis and Hedin 2002; Michalzik and others 2001) support this hypothesis.

The passive carbon vehicle hypothesis is based on the prediction that DOM is stoichiometrically static. More recently, several researchers have documented increasing DON in soil solution when N loading has been experimentally increased (McDowell and others 2004; Pregitzer and others 2004). Furthermore, these studies observed decreases in DOC: DON suggesting that DOM leaching from soils may not be as stoichiometrically fixed as predicted by the passive carbon vehicle model. The stoichiometric enrichment hypothesis predicts that the stoichiometric alteration of the SOM pool, which has been observed with N loading (Aber and others 1989, Gundersen and others 1998), will be expressed in the DOM leaching from that pool and a disproportionate increase in DON export, relative to DOC, from watersheds exposed to chronic N deposition.

While mechanisms for increased DON production remain unclear, its widespread occurrence in temperate forests suggests that dominant processes controlling transfers of DIN to DON, and ultimately ecosystem N loss, may shift under conditions of chronically elevated N supply. This potential for increased DON production and loss with enhanced N loading has important implications for coupled C and N cycling and long-term N-limitation of productivity in both terrestrial and aquatic ecosystems. Given the non-linear nature of ecosystem responses to N supply, it is possible that shifts in DON quantity or quality may only be evident over relatively long experimental time scales or across broad gradients of N loading. Alternatively, shifts in
DON may simply be a transitory biogeochemical response to disturbance (N loading) observed until systems reach a new steady state. Here we employ the ‘small-watershed’ approach (Bormann and Likens 1967) to analyze atmosphere-soil influences on DON losses from forests spanning a broad N loading gradient (5 to 45 kg N ha\(^{-1}\) yr\(^{-1}\)) in the Appalachian Mts., USA. Our objective was to determine which of the models described above, the passive carbon vehicle model or the stoichiometric enrichment model, is most consistent with watershed scale patterns in DON loss in response to increasing N supply to forest ecosystems.

**Methods**

*Study sites*

We restricted our sites to systems for which atmospheric deposition is known or has been modeled based on local data. Most sites are long term research watersheds with extensive records of water and inorganic N fluxes and include well documented cases of N saturation. All sites (n = 16) are unglaciated forested watersheds receiving 1450 to 2300 mm/yr precipitation (<10 – 14% as snow) but differ widely in forest composition and age (Table 1). Half of the sites were historically logged but were never under agriculture use and all have low abundances of the dominant N fixer *Robinia*. Soils at all sites are inceptisols or ultisols derived from sandstones.

The Coweeta Hydrologic Laboratory is a 2185-ha area located in the Natahala Mountain Range of western North Carolina. Bedrock in the basin is composed of metasandstone, coarse grained diorite gneiss, and interlayered muscovite. Soils consist of dystrochrepts and hapludults. Vegetation is dominated by aggrading (~ 80 years old) mixed oak, hickory, and tulip poplar forest. Deposition to Coweeta is low for the eastern USA and stream DIN concentrations are
often near analytical detection limits, but N inputs generally increase with elevation (Swank and Vose 1997).

The Noland Divide study area is a 17-ha, high elevation (1650 – 1910 m) watershed within the Great Smoky Mountains National Park (GSMNP). Bedrock geology is dominated by feldspathic sandstone; soils consist of dystrochrepts and haplumbrepts. Vegetation is dominated by old-growth red spruce and understory balsam fir and birch. This site is believed to be N-saturated, indicated by high NO$_3$-N concentrations in stream water (Van Miegroet and others 2001). In 2002 and 2004, we sampled additional high elevation GSMNP streams located within 6 km of Noland Divide.

The Joyce Kilmer Memorial Forest is a 1540-ha old-growth forest located in the Unicoi Mountains ~ 60 km northwest of Coweeta and ~ 20 km southwest of the GSMNP. This forest is similar in composition to Coweeta but has never been logged. Precipitation and temperature are also similar to Coweeta (K. Elliot USFS, personal communication). Geology consists of metasandstone and soils are classified as dystrochrepts and hapludults (Daniels and others 1987).

The Fernow Experimental Forest is a 1902 ha area located in the Allegheny Mountains, in northern West Virginia. Bedrock consists of acidic sandstone and soils are classified as dystrochrepts (Adams and others 1997). Overstory vegetation is dominated by aggrading sugar maple, cherry, beech, and oak. Symptoms of N saturation in this forest include high and non-seasonal stream NO$_3$-N concentrations (Peterjohn and others 1996). We focused sampling in WS 4 and WS 3 which are the subjects of a long-term paired-watershed (WS 4 is the control) experiment; WS 3 has been helicopter-fertilized with ammonium sulfate since 1989 (Gilliam and others 1996). In July 2004 and April 2005, we sampled additional streams in the Fernow area.
Atmospheric N deposition and water fluxes

We used data on total (wet + dry) inorganic N (NO$_3$-N + ammonium-N [NH$_4$-N]) deposition in all analyses. Data on organic N deposition were not available for most sites. Long-term water and N fluxes for Coweeta watersheds are from Swank and Vose (1997) and considered representative of recent deposition (J. Vose, USFS Coweeta Hydrologic Laboratory, personal communication). Total N deposition to the Joyce Kilmer forest was estimated using a simple model based on long-term data from Coweeta and Smokies deposition, local precipitation, and contribution from cloud deposition (K. Elliot, USFS Coweeta Hydrologic Laboratory, personal communication). This estimate was applied to all Joyce Kilmer watersheds. Long-term water and N deposition fluxes to the Noland Divide watershed are from Van Miegroet and others (2001) and applied to the other high-elevation GSMNP watersheds. Long-term mean deposition to Fernow WS 3 and WS 4 was provided by M.B. Adams (USFS Northeastern Forest Experiment Station, personal communication), and water fluxes are from Adams and others (1993). Watershed 3 represented our highest N loading watershed (45 kg N ha$^{-1}$ yr$^{-1}$; supplied in part by experimental additions). The deposition rate in WS 4 was applied to other watersheds sampled in the Fernow area.

Soil sampling and analysis

Soils were collected in July over 2002 to 2004 in Coweeta watersheds and Fernow WS 4. Duplicate cores (5 cm diameter) consisting of forest floor (Oe + Oa) and mineral soils (0 -10 cm) were collected from 14 ~ 1-m$^2$ areas distributed in a stratified random manner in near-stream (0.5 – 2 m from the stream edge) and hillslope (~ 20 - 40 m upslope from stream edge) watershed positions. In the laboratory soils were dried to a constant weight at 60°C for determination of
bulk density. Homogenized root- and rock-free sub samples of soil horizons from combined duplicate cores were ground using a mortar and pestle and analyzed for C and N content using a Variomax CNS analyzer (Elementar, Germany). Carbon and N pools were calculated by multiplying element concentrations by bulk density.

We supplemented our soil analyses with soil core data for the Noland Divide watershed collected by H. Van Miegroet ($n = 50$, unpublished data), WS 3 in Fernow with data from M B. Adams ($n = 35$, unpublished data), and Joyce Kilmer using information from Daniels and others (1987). Soil data for the Joyce Kilmer forest were not available for individual watersheds in which stream water was collected. Instead, we used mean soil characteristics calculated from eight large sites distributed throughout the forest. We used mineral soil data in analyses of watershed N losses because we lacked data for organic soils for some sites and because differences in horizon classification and sampling across studies confounded calculation of organic pools. Further, we assumed that although organic layers may dominate N transformation and DOM production, mineral soils effectively integrate long term retention and leaching dynamics relevant to DON fluxes occurring along subsurface flowpaths to streams (Qualls 2000; Aitkenhead-Peterson and others 2003).

Stream water chemistry

Stream water samples ($n = 4 – 10$/ date) were collected from study watersheds in July from 2002 to 2004. Over this same period, Coweeta reference streams were intensively sampled ($n = 170$) to assess temporal variation in DON. Samples were filtered (Whatman ®GF/F, pore size = 0.7 µm) in the field into acid-washed polyethylene bottles and frozen until chemical analysis. Nitrate-N was determined colorometrically following the acidic diazo method after
cadmium reduction (Wood and others 1967) on a Technicon Auto Analyzer II (Technicon, Emeryville, California, USA). Ammonium-N was analyzed colorometrically following an automated phenate method (Solorzano 1969). Total dissolved N (TDN) was determined by high temperature potassium persulfate oxidation followed by analysis of nitrate. DON was calculated by difference as TDN – (NO$_3$-N + NH$_4$-N) using L-glutamic acid as a reference standard (APHA 1998). Beginning in 2003, all water samples were analyzed for DOC by sodium persulfate oxidation using an Oceanographic International (OI) Model 700 Total Organic Carbon Analyzer (Menzel 1964; Oceanographic International, College Station, USA). We used an OI Model 1010 for 2004 samples. Analyses of external standards run on both instruments yielded equivalent results. Method detection limits were: NO$_3$-N, 3 µg/ L; NH$_4$-N, 3 µg/ L; DON, 15 µg/ L; and DOC 0.1 mg/L. We assigned a value half the detection limit for samples below these detection limits.

**Data analysis**

We used regression analysis to examine relationships between N loading, soil C: N, soil pools, and stream chemistry and to assess relationships among DOC, DIN, and DON. We addressed the influence of N deposition on DON after accounting for the concomitant influences of forest type and age, elevation, and precipitation using stepwise multiple regression analysis. For analyses of watershed exports, we only used sites for which there were long-term water and N flux data. We calculated hydrologic export by multiplying the long-term mean annual discharge by average N concentrations. We tested for differences in water chemistry between Fernow fertilized and control watersheds using two-way (treatment, season) analysis of variance of stream nutrient concentrations.
Results

*Soil C: N ratios and pools*

Organic and mineral soil C: N ratios declined steeply (~ 30 to 17) with N loading from 5.1 to 10 kg N ha\(^{-1}\) yr\(^{-1}\), followed by little change up to 45 kg N ha\(^{-1}\) yr\(^{-1}\) (Fig. 1a). This initial decline in C: N corresponded to strong linear increases in total (organic + mineral horizon) soil N pools \((r^2 = 0.98, P = 0.0015)\) with N loading. Across all sites, mineral soil C: N ratios were inversely related to N pool size \((r^2 = 0.57; P = 0.019)\) resulting from strong logarithmic increases in mineral soil N (Fig. 1b). In contrast, mineral soil C varied from 24 to 36 Mg/ha but did not change \((P > 0.2)\) with N supply.

*Stream chemistry and watershed export*

Concentrations of DON in stream water increased significantly and asymptotically over the N-loading gradient, shifting abruptly at loading rates ≥10 kg N ha\(^{-1}\) yr\(^{-1}\) (Fig. 2a). This increase was also significant \((r^2 = 0.33, P = 0.0079)\) but linear across the smaller and more geographically constrained 5.1 to 7.7 kg N ha\(^{-1}\) yr\(^{-1}\) gradient. Across all sites, deposition was the only significant variable explaining stream DON in a stepwise multiple regression model \((F_{1, 14} = 38.63, R^2 = 0.75, P < 0.0001)\). In contrast, N deposition \((P < 0.0001)\), elevation \((P = 0.0254)\), precipitation \((P = 0.0006)\), and forest type \((P = 0.0001)\) were strong predictors of stream water NO\(_3\)-N \((F_{4, 14} = 112.27, R^2 = 0.98, P < 0.0001)\). Concentrations of NO\(_3\)-N increased more rapidly with N loading than DON (Fig. 2a), resulting in a shift in DIN: DON ratios from < 1 to >1 in watersheds receiving >7 kg N ha\(^{-1}\) yr\(^{-1}\). Ammonium-N was universally low (< 4 µg/L), driving a > 500-fold increase in NO\(_3\)-N: NH\(_4\)-N ratios (2 to 1074) over the N-loading gradient.
Concentrations of DOC were consistently low (<3 mg/L) and decreased slightly with N loading \( (F_{1, 13} = 5.01, R^2 = 0.29, P = 0.045) \), though this pattern was driven largely by differences in sampling intensity (high N sites were not sampled in the fall when DOC is higher). Across the entire N loading gradient, DON was strongly associated with NO\(_3\)-N \( (r^2 = 0.77, P < 0.0001) \) but not DOC \( (r^2 = 0.04, P = 0.13) \). This pattern also held for the low-N Coweeta watersheds where DON was aseasonal \( \text{(unpublished manuscript)} \) and more closely associated with NO\(_3\)-N \( (r^2 = 0.30, P < 0.0001) \) than DOC \( (r^2 = 0.11, P < 0.0001) \).

These patterns of N loss were further supported by significantly higher DON \( (F_{5, 34} = 28.03, R^2 = 0.83, P < 0.0001; \text{treatment } P = 0.0059) \) and NO\(_3\)-N \( (F_{5, 34} = 411.37, R^2 = 0.99, P < 0.0001; \text{treatment } P < 0.0001) \) in fertilized vs. control watersheds in Fernow. In these watersheds DON was lower and NO\(_3\)-N was higher \( (P < 0.001) \) in winter vs. summer samples. There was also a greater response in NO\(_3\)-N (i.e., 0.23 kg N ha\(^{-1}\) yr\(^{-1}\) increase in loss per kg ha\(^{-1}\) yr\(^{-1}\) N added) than DON (0.021 kg N ha\(^{-1}\) yr\(^{-1}\) increase in loss) to long-term N fertilization.

Exports of DON and NO\(_3\)-N increased logarithmically with atmospheric N loading (Fig. 2b) and more abruptly for NO\(_3\)-N (0.09 to 12.1 kg N ha\(^{-1}\) yr\(^{-1}\)) than DON (0.3 to 4.2 kg N ha\(^{-1}\) yr\(^{-1}\)). Our DON export estimate for Noland Divide (4.2 kg N ha\(^{-1}\) yr\(^{-1}\)) was lower than that based on volume-weighted means (5.5 kg N ha\(^{-1}\) yr\(^{-1}\), H. Van Miegroet, \textit{unpublished data}) but this difference did not affect overall patterns relating DON export and N loading. Losses of NH\(_4\)-N were low (0.02 to 0.07 kg N ha\(^{-1}\) yr\(^{-1}\)) and did not vary with N loading. Despite increasing losses, annual hydrologic losses of N were 2 to 12 times lower than atmospheric input rates across all watersheds.
Stream DON (Fig. 3a) and NO$_3$-N concentrations increased asymptotically with decreasing C: N ratios in both mineral ($P < 0.001$) and organic ($P < 0.001$) soils across the N loading gradient. This pattern was not attributable to changes in soil C, but rather strong increases in DON ($r^2 = 0.69, P = 0.0002$) and NO$_3$-N ($r^2 = 0.45, P = 0.009$) with increasing soil N pool size. Concentrations of DOC did not vary with soil C: N ratios or C pools ($P > 0.2$).

Comparison of DOC: DON ratios in stream water and mineral soil C: N revealed a rapid decline in stream water DOC: DON as soil C: N decreases (Fig. 3b). Stream water DOC: DON drops substantially below the 1:1 line relating bulk soils (assumed source) and stream solution as soil C: N declines, suggesting a proportionally greater N enrichment in dissolved compared to solid organic pools.

**Discussion**

*Chronic N loading and patterns of DON formation and loss*

Our study revealed distinctly asymptotic patterns in DON loss, characterized by steep initial increases followed by little change with additional N loading, consistent with previous observations of ecosystem responses to N saturation (Aber and others 1998). Patterns in stream water N in our study paralleled those from other studies showing strong dominance of DON over NO$_3$-N and NH$_4$-N in unpolluted temperate forest watersheds (Sollins and McCorison 1981; Hedin and others 1995) and reversal of this pattern as a consequence of chronic N pollution (Perakis and Hedin 2002). While we recognize that numerous other factors (e.g., climate, biome, soil mineralogy, ecosystem type) influence soluble soil N pools and DON fluxes (Qualls 2000, Michalzik and others 2001), we contend that stoichiometric enrichment of DOM resulting from
increased N supply is the most likely explanation for observed increases in DON losses from temperate forests.

Although processes underlying the shift toward DIN losses with increasing N inputs are becoming better understood (Perakis and others 2005), changes in the relative losses of DOC and DON in the face of increasing N deposition depend on long-term feedbacks among elevated N, immobilization, and sequestration into soils that remain largely unknown. If mechanisms controlling the solubility of DOM remain constant (i.e., constant leak rates from slow-turnover SOM despite changing deposition), and flow paths mix in a simple conservative manner, the low DOC: DON ratios observed in high N deposition watersheds in this and other studies require a substantial shift in SOM stoichiometry or contributions of N-rich DOM from unknown sources. This prediction is borne out by comparison of DON vs. DOC relationships from studies of temperate forest streams spanning a wide range of N loading (Fig. 4).

By definition, organic N is inextricably linked to the ‘carbon vehicles’ in which it is transported and thus our two hypotheses represent domains in a gradient of control by static vs. dynamic stoichiometry as N supply increases (Fig. 5). If DON simply tracks DOC following strict stoichiometric constraints, DON loss should increase proportionally with soil C pool size and, assuming static soil stoichiometry, C: N ratios (Rastetter and others 2005). Strong positive correlation between river DOC fluxes, soil C pools, and C: N ratios across biomes have been observed (Aitkenhead and McDowell 2000). In addition, tight correlations between DOC and DON and wide (humic-like) DOC: DON ratios in temperate soil (Michalzik and others 2001) and stream waters (Goodale and others 2000; Perakis and Hedin 2002) have also been reported. Together, these studies support the passive carbon vehicle model of DON losses. Yet, concluding that soil C: N ratio controls DOC loss can lead to the counterintuitive prediction that
DON losses should decrease with N pool size (Rastetter and others 2005). While fixed stoichiometric coupling of DON and DOC may provide an adequate model for understanding DON loss from low-N ecosystems, our results indicate that an alternative model is required to capture the dynamics of DON flux as N availability increases. Indeed, increasing fluxes of DON have been observed when soil N pools increase due to soil age (Neff and others 2000) and abundance of N-fixing trees (Compton and others 2003) and this may also help explain generally higher DON losses from tropical vs. temperate forests (Lewis and others 1999). As soil N pools grow, SOM C: N should decrease and there should be a concomitant increase in DON losses per unit loss of carbon, assuming that SOM represents the dominant sink for N and that chronic levels of N input do not contribute substantially to ecosystem C sequestration (Nadelhoffer and others 2004). We did not observe consistent shifts in C pools with N loading but did observe changes in DON flux that were tightly associated with soil N pools, DIN losses, and soil C: N.

Our results are consistent with widely reported declines in soil C: N ratios and associated increases in nitrification and NO\textsubscript{3}-N fluxes from watersheds receiving high N deposition (Aber and others 1998; Gundersen and others 1998). Strong negative association between NO\textsubscript{3}-N and DOC: DON in stream waters (Goodale and others 2000; Campbell and others 2000; Lovett and others 2000) has also been interpreted as an integrator of watershed soil C: N ratios and N status. Our results complement these findings and are consistent with studies indicating that C: N ratios of DOM are generally similar to or higher than those of bulk SOM (Goodale and others 2000). In contrast, DOC: DON ratios fall below SOM values at chronically N-polluted sites, implying that sources of additional DON are not fully captured by bulk soil characteristics.

Most N in forest soils is thought to be bound in highly stable humic materials derived from condensation reactions with polyphenols and quinones (Stevenson 1994; Qualls 2000).
Because steady state N dynamics respond to inputs as a function of pool residence time, increased deposition should not increase DON losses if N pools are large (relative to inputs) and turn over slowly. Observed increases in DON losses thus suggest activation of novel pathways of organic N production as ecosystems approach critical states of N supply. Declines in the DOC: DON ratio with N loading observed in our study and plot-scale fertilization experiments (McDowell and others 2004; Pregitzer and others 2004) indicate a shift in the stoichiometric constraints on DOM composition imposed by hydrolysis and leaching of slow-turnover SOM as N becomes more available. Plot-scale N fertilization experiments have produced mixed results with regard to DOC production, with some reporting increases in DOC (Pregitzer and others 2004) and others showing no change in DOC fluxes with N enrichment (McDowell and others 2004). Decreased DOC: DON ratios in soil solution and increased DON fluxes with N addition have been linked to higher levels of hydrophilic compounds (McDowell and others 2004) possibly resulting from increased mycorrhizal exudation of amine-rich DOM (Aitkenhead-Peterson and others 2003). Given the large capacity for mineral soil to retain DOM (Qualls 2000), increased losses to stream water are surprising and thus imply saturation of sorption sites or shifts in the composition of DOM toward smaller, more neutral molecules (e.g., hydrophilic compounds) that sorb poorly to mineral soils (Kaiser and Zech 2000). Further, because plant demand for amino acid N should decrease in high N systems (Neff and others 2003; Schimel and Bennett 2004), increases in DON may reflect greater export of bioavailable forms as N demand saturates. Alternatively, increased DON could result from decreased mineralization via depressed microbial activity (Pregitzer and others 2004), acid release of amine groups from humic compounds (Stevenson 1994), or abiotic N sorption to humic DOM (Aber and others 1998; Kaizer and Zech 2000).
**DON, N saturation, and implications for watershed ecosystems**

Our results suggest that increases in DON loss are asymptotic and that once a threshold N input level has been exceeded little change in DON losses are observed. This relationship is indicative of fundamental limitations on DON losses from soils. This is best illustrated by the Fernow and Noland Divide watersheds; both have similar DON concentrations despite a 3- to 5-fold difference in N loading. In contrast, losses as highly mobile NO$_3$-N would ultimately be expected to approach unity with inputs (Fig. 5) as abiotic and biotic compartments saturate as a result of chronic N exposure (Gundersen and others 1998). The potential implications of increased DON losses for ecosystem N limitation depend to a large degree on whether increases are compensatory (i.e., constancy in TDN loss rates but shifts in forms) vs. additive (constancy in DIN loss rates and increases in TDN losses). Transformation of NO$_3$-N to DON may enhance watershed N retention even under chronically polluted conditions because of the much greater affinity of soil sorption sites for DOM versus NO$_3$-N. Conversely, additive formation and loss of DON may dampen N saturation and further prolong N limitation (Hedin and others 1995) even as N supply grows. As reported for other systems (Goodale and others 2000), watersheds in our study remained highly retentive of N after inclusion of DON losses even in systems receiving > 15 times pre-industrial deposition rates and under old-growth conditions, consistent with sequestration into SOM (Nadelhoffer and others 2004). Assuming that gaseous N losses represent a small contribution to apparent retention relative to hydrologic losses (Van Breeman and others 2002), inclusion of DON losses in our study failed to balance N inputs (DIN input >> TDN output), resulting in net ecosystem retention of ~ 4 to 30 kg N ha$^{-1}$ yr$^{-1}$ or 50 to 92% of inputs.
In this investigation we have used patterns in stream water chemistry to understand watershed scale responses to chronic N loading. This approach integrates production, uptake, and retention processes occurring in all terrestrial and aquatic watershed subsystems. While much of our analysis points to atmosphere-soil control over DOM losses, we recognize that DIN, DOC, and DON can undergo substantial uptake and transformation in surface and subsurface components of streams (Brookshire and others 2005). Therefore, patterns presented in this study represent net watershed processing. Indeed, there is potential for N-induced shifts in DOM to affect aquatic metabolism and N cycling. Given the low bioavailability of DOM (Qualls and Haines 1991) at low N sites, however, we suspect that stream transformation of DOM is dominated by internal cycling of labile forms (Brookshire and others 2005). Although C and N contained in amino acids can be tightly cycled in terrestrial and aquatic ecosystems (Neff and others 2003), free amino acids generally make up < 1% of the DOM pool (Thurman 1985). Decreased DOC: DON ratios at N polluted sites suggest increases in DOM lability, and thus potentially increases in aquatic C demand. However, because analogous patterns of saturation of N uptake occur in streams (Payn and others 2005), we expect that higher loads of DON in N saturated watersheds may be exported farther downstream, with subsequent potential for mineralization and eutrophication of previously enriched rivers and estuaries (Seitzinger and Sanders 1997). While many questions remain regarding the production and fate of DON in temperate forest watersheds, our results suggest that current theory regarding ecosystem N retention and loss needs to encompass an understanding of the role of DON under conditions of high levels of long-term N deposition.
Literature cited


Rastetter EB, Perakis SS, Shaver GR, Ågren GI. Terrestrial C sequestration at elevated CO$_2$ and temperature: The role of dissolved organic nitrogen loss. Ecol Appl 15: 71-86.


Table 1. Characteristics of study watersheds.

<table>
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<th>Region</th>
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<th>N Input (kg ha(^{-1}) yr(^{-1}))</th>
<th>Elevation max (m)</th>
<th>Watershed area (ha)</th>
<th>Precipitation (mm/yr)</th>
<th>Vegetation(^a)</th>
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\(^a\)Numbers in parentheses refer to names of gauged watersheds

\(^b\)AD = aggrading deciduous; OD = old-growth deciduous; OC = old-growth conifer
Figure 1. Mean organic and mineral (0-10 cm) soil C: N ratios (by mass) (A) and mineral soil N pools (B) as a function of atmospheric N loading. The relationship between organic soil C: N ratios versus N input was explained by the equation: $y = 18.76 + 107.11e^{-0.47x}$; $r^2 = 0.72$, $P = 0.079$. The relationship between mineral soil C: N ratios versus N input was explained by the equation: $y = 17.66 + 290e^{-0.67x}$; $r^2 = 0.58$, $P = 0.076$. The relationship between soil N versus loading was explained by the equation: $y = 1.27 + 0.16 \ln (x - 4.93)$; $r^2 = 0.79$, $P = 0.0097$. 
Figure 2. Mean (± SE) DON and NO₃-N concentrations collected in study streams over 2002-2005 (A) and watershed N exports (B) as a function of atmospheric N loading. Relationships between DON and NO₃-N concentration and load versus N input were explained by the logarithmic equations: DON concentration = -95.97 + 87.27 (x), r² = 0.54, < 0.0001; NO₃-N concentration = -1024.56 + 580.64 * ln (x), r² = 0.63, P < 0.0001; DON load = -1.69 + 1.33 * ln (x); r² = 0.74, P < 0.0001; NO₃-N load = -10.18 + 5.99 * ln (x); r² = 0.99, P < 0.0001). Note log scale on y-axis in panel A. Vertical dashed line in panel A indicates approximate N input value where DON concentration increases but losses switch from DON > DIN to DIN > DON.
Figure 3. Mean DON concentrations and the proportion of TDN as DON (dashed line) in stream water (A) and mean DOC: DON ratios in stream water (B) as a function of mean catchment mineral soil C: N ratios (note log scale on y-axis). The relationship between DON and mineral soil C: N ratio was explained by the equation: 

\[ y = 11.27 \times e^{(14.87/(x - 12.24))} \]  

\[ r^2 = 0.43, P = 0.0063. \]

The relationship between the stream water DOC: DON ratio and mineral soil C: N ratio was explained by the equation: 

\[ y = 11.06 + 13.54 \times \ln (x - 16.93) \]  

\[ r^2 = 0.52, P = 0.0184. \]
Figure 4. DON and DOC in stream water across varying levels of atmospheric N deposition. Symbols represent stream samples from this study, and dotted and dashed lines represent mean DOC: DON ratios from sites receiving greater than or less than 7 kg N ha$^{-1}$ yr$^{-1}$, respectively. Numbered lines are DON vs. DOC regression lines from: 1) temperate South America, ~3 kg N ha$^{-1}$ yr$^{-1}$ (Perakis and Hedin 2002); 2) White Mountains USA, ~9 -11 kg N ha$^{-1}$ yr$^{-1}$ (Goodale et al. 2000); 3) Catskill Mountains USA, ~12 kg N ha$^{-1}$ yr$^{-1}$ (Lovett et al. 2000); and 4) Noland Divide USA, 32.2 kg N ha$^{-1}$ yr$^{-1}$ (H. Van Miegroet unpublished data).
Figure 5. Hypothetical domains of control over forest N loss by fixed versus dynamic organic matter stoichiometry. Under unpolluted conditions (low N supply) losses are dominated by DON whose production and transport follow the stoichiometry of soil organic matter. As N supply increases, losses become dominated by DIN and DON eventually asymptotes due to fundamental limits on DON production (dotted region). In contrast, DIN losses would ultimately be expected to reach unity with inputs.
Chapter 4: Thermal dependency synchronizes forest N losses and the North Atlantic Oscillation

Abstract

Hydrologic losses of nitrogen from forests have provided integrated understanding of ecosystem processes\textsuperscript{1-3} and direct insight into human alteration of Earth systems\textsuperscript{4, 5}. Despite increasing understanding of nitrogen cycling in pristine\textsuperscript{2, 5} and polluted regions\textsuperscript{5-7}, little is known regarding climate-sensitivity of nitrogen cycling at ecosystem scales\textsuperscript{8-10} and how local dynamics mechanistically couple to global meteorological processes\textsuperscript{11}. As human activities simultaneously alter nitrogen availability\textsuperscript{6, 7} and global climate it will become increasingly important to understand how these Earth system processes interact\textsuperscript{8}. Here we report detailed long-term (30 yr) patterns of climate, hydrological variation, and nitrogen input and loss from temperate forests in North Carolina, USA. Our analyses indicate that ammonium and nitrate losses from these forests are strongly associated with patterns in mean annual temperature which is strongly coupled to positive trends and sub- and multi-decadal variation in the North Atlantic Oscillation (NAO)\textsuperscript{12-14}. We suggest that temperature stimulation of soil microbial mineralization and nitrification and increased N loading are responsible for oscillatory patterns and accelerated N losses from these forests. Collectively, our results identify coherent climate-forest synchronicity, recognize critical synergisms between global changes in climate and biogeochemical cycles, and demonstrate the linked nature of Earth systems over vast geographic areas.

Forest nutrient cycles result from complex interactions among organisms, climate, geology, the atmosphere\textsuperscript{1, 2, 11, 16}, and increasingly, human influences on these factors\textsuperscript{5-8}. Perhaps the most dramatic expression of human influence on nutrient cycling is the widely recognized increase in biologically-available nitrogen, an element fundamentally limiting to biological
systems, over large areas of the globe. In remote forest landscapes, increased N loading derives predominantly from fossil fuel-driven atmospheric pollution and can result in pronounced effects on forest productivity, shifts from largely organic and ammonium- to nitrate-based N economies, and declines in water quality. Concurrently, mean air temperatures have increased or are expected to increase by 1 – 3 °C globally over the next century, and precipitation patterns are expected to shift in many places. Interactions between climate variation and N pollution are predicted to have large and persistent influences on ecosystem processes and services, yet they are difficult to identify because of simultaneous and confounding dynamics that are regional to global in origin and vary over annual to millennial time scales.

A fundamental step toward understanding the organization of nutrient cycles is elucidation of intrinsic climate-sensitivity of element transformations and losses at ecosystem scales. Microbially-driven processes, such as those operating in the N cycle, are expected to be particularly sensitive to climate change because of their sensitivity to temperature and redox conditions. Although there is general understanding of temperature and moisture effects on microbial kinetics of N cycling, documenting the effects of climatic variation on forest N economies has remained elusive. Experiments designed to isolate factors of global change (warming, hydrology, N deposition) have suggested critical interaction among drivers influencing nitrogen cycling but have not demonstrated these interactions at higher temporal and spatial scales.

We focused attention on one of the Earth’s major climate teleconnection patterns known to influence much of northern hemisphere, the North Atlantic Oscillation (NAO). The NAO describes north-south oscillation in atmospheric mass between the Icelandic low- and the Azores
high-pressure oceanic centers\textsuperscript{12-14}. Though largely aperiodic\textsuperscript{14}, strong inter-decadal patterns in the NAO are evident\textsuperscript{12} and there has been an unprecedented positive trend in the NAO over the last 30 years\textsuperscript{13}. Currently, there is an active debate on whether this pattern reflects human influence on climate or represents a natural phase of an internally random process\textsuperscript{13,14}. Regardless, it is widely accepted that the positive trend in the NAO is closely associated with regional surface warming over Europe, Asia, and the eastern USA\textsuperscript{13}. Herein, we describe multi-decadal dynamics in the NAO, long-term temperature variability, and human induced shifts in precipitation chemistry and illustrate how these phenomena interact to control and accelerate hydrologic N losses forests of the southeastern United States.

We analyzed long-term (1972 -2003) patterns in precipitation and stream water chemistry from relatively unpolluted forested watersheds in the Coweeta Hydrologic Laboratory located in the southern Appalachian Mountains, NC, USA. Coweeta is one of the longest running biogeochemical monitoring sites globally. Long-term nitrogen dynamics have been described previously for all reference watersheds and were found to be highly similar inter-annually\textsuperscript{15,19}. Thus, we focused our analysis on two altitudinally contrasting small watersheds (WS 14: 849 m, 61 ha; WS 36: 1282 m, 49 ha) that span a range of state factors expected to influence N cycling in the region: mean annual temperature (MAT) and precipitation (MAP), soil depth, and hydrologic responsiveness (i.e., water residence time)\textsuperscript{20,21}. Basin wide, climate is humid marine with < 10\% of precipitation received as snow. The growing season is long, extending from April to November, and mean temperatures range from 3.3 to 21.6 °C. Vegetation is composed of aggrading (85 yr) deciduous forest dominated by \textit{Quercus}, \textit{Carya}, \textit{Liriodendron}, and \textit{Betula}. Increasing atmospheric inputs and hydrologic losses of N have been documented for these forests\textsuperscript{15}, but dissolved inorganic N (DIN) losses remain low\textsuperscript{15,20} with most N lost as dissolved
organic forms (DON). We analyzed long-term relationships between climate and forest N losses by assessing patterns in the NAO index, air temperature, water fluxes and volume-weighted concentrations of Cl⁻, Na⁺ (hydrologic tracers), total dissolved N, NO₃⁻, and NH₄⁺.

We detected a long-term, positive trend in mean annual air temperature (0.0401 °C/yr). Superimposed on the long-term pattern were 6 – 8 yr oscillations of ~1- 2 °C. Similarly, a long-term positive trend and inter-decadal variation in the NAO are also evident (Fig. 1). Using autoregressive moving average (ARMA) smoothed data, we found that the relationship between the NAO and air temperature was highly synchronized at annual and sub-decadal scales (P < 0.001). Further analysis showed coherent tracking of NAO and precipitation chemistry in these forests: precipitation Cl: Na (charge equivalent basis) revealed strong positive divergence from global average marine aerosol ratios (1.16) and periodicity that closely tracked (P < 0.001) variation in the NAO (Fig. 1). Both Cl⁻ and Na⁺ in rainwater derive predominantly from dilute marine aerosols in this area and have declined significantly in precipitation over the last 30 yrs, with the ratio shift resulting from slightly steeper declines in Na⁺. Precipitation Cl: Na is influenced by a complex array of atmospheric reactions involving hydrochloric acid, sulfuric acid, and nitric oxides, variable geologic and industrial sources, and potential shifts in storm tracks. Regardless of the mechanism, the increasing Cl: Na ratio points to an overall deterioration of a dilute marine signal in nutrient inputs to these forests.

NAO and precipitation chemistry diverged during the 1990’s (Fig. 1), which corresponded with large and steady increases in atmospheric loading of NO₃⁻ and NH₄⁺ (Fig. 2) over the study period. Inputs of NH₄⁺ have increased at a higher rate than NO₃⁻ until recent equilibration with NO₃⁻ input levels (flux and weighted charge equivalent basis). Inorganic nitrogen inputs and losses were consistently greater in high (7.1 and 0.3 kg N ha⁻¹ yr⁻¹) vs. low
(6.4 and 0.1 kg N ha\(^{-1}\) yr\(^{-1}\)) altitude watersheds (Fig. 2) reflecting increased water fluxes and N leakiness with altitude. Higher losses with altitude is likely due to lower ANPP (6 vs. 10 mg ha yr\(^{-1}\))\(^{26}\) and shorter water residence times expressed as higher quick-flow (i.e., amount of precipitation rapidly translated to stream flow is 11.3 cm or 30% vs. 3.3 cm or 10%)\(^{20,21}\). Yet, unlike some northern forests which have reached apparent NO\(_3\)-N input-output steady state\(^1\), all Coweeta reference forests remain highly N retentive (> 85 %)\(^{15}\) even after accounting for losses of dissolved organic N (DON)\(^{22}\).

Unlike recent declines in stream NO\(_3\)\(^-\) observed in more northern forests\(^{24}\), NO\(_3\)\(^-\) loss from these forests increased consistently through this study as would be predicted for a forest in the early stages of N saturation\(^7\). This is further supported by steep increases in loss: input ratios for NO\(_3\)\(^-\) (Fig. 2), a pattern consistent with enhanced rates of nitrification and subsequent NO\(_3\)\(^-\) loss despite presumably high demand in these aggrading forests\(^{15}\). Mineralization and nitrification are critical rate limiting processes in plant N availability and use\(^{1,3,25}\) and thus have the potential to affect plant productivity and community structure\(^7\). Like all microbially-driven processes, N transformations are thermally sensitive and expected to follow Arrhenius-type stimulation\(^9\).

Our analysis revealed close coupling between NAO-induced temperature variability and sub-decadal patterns of DIN loss that suggest strong synchronization of climate and soil mineralization and nitrification in these forests. Despite dilute concentrations (<5 µg N/L), we observed oscillations in NH\(_4\)\(^+\) loss that closely tracked temperature fluctuations (\(P < 0.001\)), but with a pronounced ~2 yr lag (Fig. 3). Clear but lagged temperature tracking of NH\(_4\)\(^+\) losses suggests strong ecosystem memory reflecting transport time through soils of this highly immobile cation\(^{3-5,19}\), and pronounced temperature-sensitivity of N mineralization in apparent
excess of plant and microbial immobilization. No consistent long term trend in NH$_4^+$ was observed.

In contrast to NH$_4^+$, long term periodicity of mean annual volume-weighted NO$_3^-$ concentrations varied in tandem with oscillations in mean annual temperature ($P < 0.001$) (Fig. 3), exhibiting little to no response lag over the first 15 years of record. Coherent linkage of temperature and NO$_3^-$ loss appeared to break down in the early 1990’s, whereupon losses correspond to a ammonium-like ~2 year lag, apparently coupled to large increases in NH$_4^+$ inputs (Fig. 2) and the diverging Cl: Na-NAO relationship (Fig. 1) but also to a period of normal rainfall following the most severe drought on record (1984-1988)$^{19}$. Such a hydrological shift would be expected to affect the production, cycling, and transport of highly mobile NO$_3^-$ ions$^3,19$. Coincident with NO$_3^-$ variation, stream pH also varied but in an inverse manner (Appendix 1). Since precipitation sulfate declined over the study period$^{20}$, the pH pattern provides additional support for nitrification (and associated H$^+$ release) as the mechanism responsible for the observed pattern in NO$_3^-$ loss$^1$. Long term pH declines likely reflect acidification from increasing nitric acid inputs, as observed in other forest ecosystems$^1$.

We did not observe a consistent relationship between precipitation and NAO or temperature over the 30-yr period. However, slight declines in stream flow were observed, presumably resulting from a trend toward higher evapotranspiration (ET) rates. Increasing ET is supported by increasing Cl$^{-}_{\text{stream}}$: Cl$^{-}_{\text{precipitation}}$ ratios (1.6 to 3), a pattern known to be caused by evapotranspiratory concentration of this conservative element$^1$. While nitrification is expected to vary inversely with soil moisture owing to aerobic constraints of autotrophic nitrification$^{17}$, we observed no shift in NO$_3^-$ loss during severe drought conditions. However, large losses of NO$_3^-$ were observed following return to normal precipitation (1989) which could not be explained by
thermal patterns. We suggest that this transitory NO$_3^-$ response resulted from enhanced production and insufficient hydrologic transport during drought$^3,19$, consistent with the results of hydrologic models showing strong nitrate memory in Coweeta watersheds$^{19}$.

In contrast, charge balance analysis ($\sum$ cation equivalents – $\sum$ anion equivalents) of stream water suggested decreased organic nutrient losses during the 1984-1988 drought. Assuming organic anions account for charge discrepancy$^{20}$ and applying average charge densities (14)$^2$ and C: N ratios (45)$^{22}$ for stream dissolved organic matter in these forests, we conservatively estimate that DON losses declined during the drought but showed no long term trend, a pattern consistent with observed DOC losses$^{20}$ and expected passive hydrologic control of DON loss in unpolluted forests$^2,5$.

Modeling$^9$ and experimental$^8,10,18$ investigations of temperature effects on mineralization and nitrification across biomes consistently demonstrate that microbial N transformations in soils are highly sensitive to ecosystem perturbation. Mineralization, nitrification, and associated NO$_3^-$ leaching increase with N deposition$^{1,2,7}$, release from tree-demand due to logging$^{1,15}$ and trenching$^3$, drought$^3$, and with increasing temperature in reciprocal transplant$^{27}$ and soil warming experiments$^{10,18}$. Surprisingly, quantitative linkage between natural temporal variation and N loss at the ecosystem scale has remained intractable, until now. Mineralization assays in Coweeta forests show high sensitivity to moisture and temperature changes with altitude and topography$^{28}$ and, similarly, temperature increases $< 2^\circ$ C were found sufficient to stimulate NO$_3^-$ leaching from soils in nearby high-altitude spruce-fir forests$^{29}$.

Soil nitrification rates$^{28}$ and stream nitrate losses$^{19}$ in Coweeta forests are highest during spring and summer and lowest during fall and winter. Analyses of monthly time series indicated that annual patterns in N losses were driven largely by spring-summer fluxes. We also observed
that minimum (winter) temperatures increased at a higher rate (0.0475 °C/yr) than maximum
temperatures (summer, 0.0326 °C/yr) suggesting that milder winter conditions may have
positioned microbial communities for higher N processing and leakiness during the growing
season. The striking pattern of N loss with temperature despite positive forest NPP suggests that
plant growth had little influence on periodicity of N loss and highlights microbial activity as a
key constraint on nutrient availability and loss. Further, clear translation of the original
temperature response through time despite high N demand and key differences in mobility
between N ions argues for dynamic equilibrium between N uptake and production and
subsequent propagation of coherent N transport waves.

Our results show that consistent synchronicity between remote climate behavior and
local-scale losses of N can occur on time intervals that are much shorter than those associated
with recognized linkages between climate and biome-scale ecological patterns\textsuperscript{11,16}. Our analysis
also demonstrates that the coupling of climate and nutrient cycles can be detected under non-
steady state conditions (e.g. increasing N inputs and losses, aggrading forest). Combined, these
patterns underscore the linked nature of Earth systems at broad scales, provide insight into the
temperature-sensitivity of forest ecosystems to early stages of N saturation\textsuperscript{7}, and strongly support
a synergistic influence of temperature and atmospheric deposition on N cycling in temperate
forest ecosystems\textsuperscript{8,11}. It remains to be seen whether this coupling persists as forest N demand
saturates or systems achieve new thermal equilibria. Oscillatory coupling of climate and N loss
from these relatively unpolluted forests may represent early-stage reorganization of N cycling in
response to novel levels of N input and provide a window into the critical transition from
organic- and ammonium-based N economies in temperate forests.
Methods

We analyzed monthly and annual time series of precipitation and stream volumes and chemistry extending from 1972 to 2003 for reference watersheds in the Coweeta Hydrologic Laboratory, NSF long-term ecological research site, managed by the USDA Forest Service. Bulk precipitation and dry deposition inputs are monitored and collected weekly from plastic funnels. Stream discharge is measured continuously using 90° and 120 ° V-notch weirs. Filtered (<0.7 μm) water samples for chemical analysis are collected weekly. Nitrate was determined by automated cadmium reduction until 1990, and thereafter by ion chromatography, and ammonium was determined by the automated phenate method. Cl⁻ was analyzed by ion chromatography, Na⁺ was determined by Atomic Absorption Spectrophotometry, and pH was determined by potentiometry. The Coweeta Hydrologic Laboratory follows strict analytical quality control protocols including routine analysis of external standards and rigorous analytical calibration during instrument switch-over. Mean monthly and annual chemical concentrations in precipitation and stream flow were calculated as flow-proportional levels (sum of concentrations divided by proportional water volume) to account for hydrologic variation. Chemical fluxes were calculated by multiplying untransformed concentrations by water flux.

We used the publicly available (www.cpc.ncep.noaa.gov) index of the NAO describing wintertime differences in sea level pressure (SLP) between Libson, Portugal and Stykkisholmur, Iceland. Positive phases of the NAO are strongly correlated to sea surface temperatures and correspond to a persistent mid latitude warm anomaly centered off Cape Hatteras, NC, USA (refs. 12-14). We analyzed relationships among NAO, air temperature, and watershed chemistry time series by first subjecting synchronous or time-lagged (e.g., NH₄⁺) data to ARMA analysis employing negative exponential smoothing to eliminate white noise and isolate ecologically
relevant trends and seasonality, and then used linear regression of smoothed data to assess statistical association and significance.
Literature cited


22. Brookshire, E.N.J., Valett, H.M., Thomas, S.A., & Webster, J.R. Atmospheric deposition increases dissolved organic nitrogen loss from temperate forests. *In review*


Figure 1. Time series of ARMA-smoothed NAO (grey lines) with: a, ARMA mean annual temperature and; b, ARMA precipitation Cl: Na (dark lines) in Coweeta forests. Dashed line represents the global average charge equivalent Cl: Na ratio for marine aerosols. Regression lines ($P < 0.001$) are for temperature and Cl: Na ratios vs. time. Regressions between ARMA temperature and Cl: Na vs. NAO were significant ($P < 0.001$).
Figure 2. Patterns of hydrologic nitrogen inputs and loss in Coweeta forests. 

**a**, Annual inputs of ammonium-N (open symbols) and nitrate-N (closed symbols) over time. Inputs of both forms to both sites increase ($P < 0.001$) over time.

**b**, Diverging patterns (all regressions significant, $P < 0.001$) in ratios of hydrologic inorganic nitrogen outputs (stream) to inputs (precipitation) over time.
Figure 3. Time series of ARMA smoothed annual volume-weighted hydrologic N losses (dark lines) and temperature (grey lines) in the high elevation Coweeta forest (WS 36). **a,** patterns of ammonium loss are shown with uncorrected smoothed data and a 2 year temperature-response lag (dashed line). **b,** patterns of nitrate closely track temperature until early 1990’s (arrow). Regressions for ARMA ammonium and nitrate loss vs. temperature were significant ($P < 0.001$) for both forests.
Appendix 1. Time series of stream water pH in reference forests of Coweeta.
Chapter 5: Synthesis

Life is, thus, potently and continuously disturbing the chemical inertia on the surface of our planet. It creates the colours and forms of nature, the associations of animals and plants, and the creative labour of civilized humanity, and also becomes a part of the diverse chemical processes of the Earth's crust. There is no substantial chemical equilibrium on the crust in which the influence of life is not evident, and in which chemistry does not display life's work.

Vladimir I. Vernadsky, The Biosphere, 1926

The preceding chapters have flowed from a systems perspective in thinking about nature. The approach implicitly assumes that the emergent properties and behavior resulting from myriad complexities of interaction between biotic and abiotic phenomena can be broadly understood through application of general theoretical principles underlying thermodynamics and evolution and integrative measures of structure and function. The study of biogeochemistry attempts to elucidate the chemical and energetic dynamics underlying patterns in ecosystem properties and behavior across spatial and temporal scales. Of paramount importance is understanding how system level properties emerge, feed-back upon, and self organize over space and time to maintain the coherent and distinct patterns observed in nature. In particular, how pathways of nutrient transformation and transport are influenced by and dictate persistence of ecosystem state factors remains an enduring question in ecology. Moreover, the widespread and unprecedented influence of human activity on global nutrient cycles and biodiversity demands better understanding of the linked nature of earth systems.

The ecological system of interest integrating these chapters is the watershed and the common currency is nitrogen. Watersheds are inherently linked systems characterized by sub-compartment pools and processes that are connected over varying spatial and temporal scales and are characterized by widely varying directionality of information flow and feedback.
strength. In this context, watersheds are composed of distinct but integrated subsystems that differ in their relative openness of material flow and cycling.

The unifying medium of material translation is water. The relative openness (ratio of input-output fluxes to internal cycling) of watershed subsystems (plants, soils, regolith, riparian zones, hyporheic zones, and streams) varies with the average residence time of water, the inherent kinetics of transformation, and the capacity to store. In this way, watersheds are inherently hierarchical in nature in that the slow rates (e.g., SOM dynamics) dictate the behavior of ‘faster’ receiving systems (e.g., baseline nutrient levels in streams) and thus can be organized along a continuum of openness from the plant-soil system to streams. In this dissertation I have described how external forcing from the atmosphere and climate can regulate the dynamics of N in all watershed subsystems.

The study of DON spiraling showed enormous capacity for stream microorganisms to immobilize and transform organic nutrients. Although most DON in surface waters is highly refractory products of SOM dissolution, this study revealed very tight internal cycling of DON at the sediment interface and suggested significant production of DON in the hyporheic zone. Most remarkably, this DON was not expressed in stream waters, supporting the idea that watershed DON losses would have been higher in the absence of pronounced benthic demand. The experiments also suggested that the coupled dynamics between DOC and DON spiraling may be altered under conditions of elevated N supply.

The study of atmosphere-soil controls over broad patterns in forest DON loss challenged the idea that SOM and its dissolved products are stoichiometrically static as N pools accumulate. This survey showed that DON losses increase as a consequence of N pollution and that this occurs through a disproportionate enrichment of N on dissolved organic matter rather than
alteration of soil and dissolved carbon dynamics. These results have implications for N limitation in forests and aquatic systems. In particular, DOC: DON ratios of DOM draining N-saturated forests were strikingly low suggesting possible increases in DOM bioavailability with increasing N supply.

The chapter on atmosphere-climate-forest connectivity provides insight into how local forest nutrient cycles may be organized by synchronous global-scale climate-atmosphere dynamics. In particular, this study of long term hydro-chemistry from reference forest watersheds provides an integrated example of the overall climate sensitivity of N cycling and underscores the importance of complex synergies between competing vectors of global change. Results from this study argue that the combined influence of N pollution and warming are likely to have pronounced long-term effects on ecosystems globally.