Understanding Effects of Anthropogenic Activities on Element Cycling in Temperate Forest Watersheds

by

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University Program in Ecology
Duke University

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James S. Clark

Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University Program in Ecology in the Graduate School of Duke University

2011
ABSTRACT

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Abstract

Human activities are increasingly altering the ways in which energy and elements cycle within and move between ecosystems. Through fossil fuel combustion and the use of synthetic fertilizers we continue to expose much of the biosphere to new rates and ratios of essential element supply. We are also shifting climate patterns on local, regional and global scales in ways that affect reaction rates and residence times of elements within ecosystems. Even the simplest ecosystems are usually too complex to predict many of the potential consequences that human activities will have on their sustained functioning. Because of this, we often monitor ecosystems as integrated wholes, looking to explain processes that account for important patterns observed across space and time. This dissertation consists of three data chapters, all of which use the small watershed ecosystem as the principal unit of study for understanding how human activities have altered element cycling in temperate forests in the southern Appalachian Mountains.

Following an introductory Chapter 1, in Chapter 2 we present results from repeated synoptic surveys of streamwater chemistry for ~30 watersheds spanning one of the largest nitrogen (N) deposition gradients in North America, located within the Great Smoky Mountains National Park. We primarily focus on patterns in dissolved organic matter (DOM) concentrations and composition across the N gradient, with particular
attention given to dissolved organic nitrogen (DON). DON dominates the global flux of 
N between terrestrial and aquatic systems, yet we have little understanding of how this 
prevailing N form responds to human N pollution. We found that DON concentrations 
often declined significantly with increasing catchment N loading and, through 
laboratory bioavailability assays, found that when N limitation is alleviated increased 
microbial demand for labile carbon (C) may drive this pattern. We use these findings to 
propose a new hypothesis for the potential responses of DON to anthropogenic N 
pollution, which states that DON concentrations in forested watersheds may 
systematically decline with increasing N loading due to increased heterotrophic demand 
for labile reduced C.

Chapter 3 is an extension of Chapter 2, in which we attempt to assess the role of 
DON as either a C or N source within an entire stream reach through a series of 
independent manipulations of labile C and inorganic N availabilities. In the second 
order reach of Walker Branch, a well-studied stream in eastern Tennessee, we performed 
a series of progressive (i.e., sequentially increasing concentrations), kinetic (i.e., very 
short duration), enrichments of acetate and nitrate on two successive days during April 
of 2009 before the tree canopy emerged and when in-stream algal production was high. 
In this system and on these short timescales, we were unable to elicit the same responses 
observed at sites across the chronic N deposition gradient in Chapter 2. We did, 
however, observe that DOM processing and composition was significantly altered.
Using fluorescence characterization of DOM, we found that adding acetate displaced heterotrophic demand for terrestrially derived DOM. Conversely, nitrate additions stimulated production of highly bioavailable autochthonous DOM within the stream channel, which resulted in an indirect displacement of demand for terrestrially derived DOM. Understanding DOM dynamics in streams has long been a priority for stream ecologists because it represents an important energy and nutrient source fueling stream metabolism. Our results not only provide new insight into the processes controlling DOM concentrations and composition in Walker Branch, but also demonstrate the potential of this method for future investigations of DOM in stream ecosystems.

In Chapter 4 we assess the role of climate change on long-term (1989-2009) streamwater concentrations and fluxes from the West Fork of the Walker Branch watershed. At this site, mean annual air temperatures have increased by ~2°C, while mean annual precipitation and runoff have declined by ~20% and >40%, respectively, since 1989. We use weekly streamwater samples to assess trends in concentrations and fluxes for 9 different solutes over this period and, using wet deposition data, also evaluate changes in approximate watershed input-output budgets. The observed change in runoff was accompanied by a change in the proportional contributions of different soil flowpaths to streamflow generation through time, with deep groundwater playing an increasingly important role in recent years. Solutes that increase in concentration deeper in the soil-saprolite profile exhibited significant increases in
streamwater concentrations through time, while solutes with higher concentrations in soil solution in the upper profile decreased in concentration. Solutes that exhibit much less variation across soil flowpaths typically display large seasonal patterns in streamwater concentrations that are driven by in-stream biological uptake. However, most nutrient solutes exhibited little or no trend in concentrations through time, indicating that the biological controls on these solutes have remained relatively unaltered by the observed changes in climate over the 20-year period. On shorter timescales, changes in the frequency or severity of multi-year droughts, as well as changes in the frequency or intensity of storms that disrupt in-stream uptake, can have large impacts on watershed input-output budgets of nutrient solutes even if the effects are not linear through time. Our results demonstrate the important role that changing climates can have on watershed element cycles, illustrating that climate effects can manifest through either changes in hydrologic regime or through changing biogeochemical process rates.

Taken together, these chapters illustrate that human activities are indirectly but substantially changing biogeochemical cycles in temperate forests throughout the Southern Appalachians. Ecosystem structure and function depend on the ways in which energy and elements move within and between ecosystems. We rely on the sustained integrity of ecosystems for their many services and, because of this, it is essential that we understand ecosystem responses to current and future human impacts.
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1. Introduction

1.1 Global Change and Element Cycles: The Role of Human Activities

Human activities have large impacts on the major biogeochemical cycles, altering the ways in which elements move within and between ecosystems. We have more than doubled the rate in which reactive nitrogen (N), the element most commonly limiting forest production globally [Lebauer and Treseder 2008], is supplied to the biosphere [Galloway et al. 2004, Galloway et al. 2008]. We have also more than doubled the concentration of carbon dioxide in the atmosphere in the past century, fertilizing aboveground vegetation with inorganic carbon (C) [Schimel 1995] and altering the amount of solar energy retained within the atmosphere [Houghton 2005]. As a result we are changing regional and global climates, altering temperature and precipitation regimes [Murphy et al. 2004] that, in turn, can alter both biological [Brown et al. 2004] and geochemical [White and Blum 1995] reaction rates governing element cycles. The resulting changes in element cycling can affect vegetation growth dynamics [Melillo et al. 1993], plant-soil interactions [Raich and Schlesinger 1992], organic matter quality and decomposition rates [Davidson and Janssens 2006], and a host of additional processes contributing to the structure and function of ecosystems.

We depend on the sustained integrity of ecosystems for the many services they provide. There are numerous examples of how human activities have had indirect and unintended consequences that have compromised important ecosystem services on a
variety of scales. In industrialized regions, release of strong acid anions to the atmosphere from fossil fuel combustion has led to decreased soil fertility through soil base cation depletion, tree mortality, and the acidification of lakes and streams [Likens et al. 1996, Stoddard et al. 1999]. Nitrogen deposition has altered forest growth dynamics [Vitousek et al. 1997], with excess N leaching from catchment soils and cascading through downstream aquatic ecosystems [Galloway et al. 2003] ultimately contributing to large regions of coastal eutrophication and hypoxia [Howarth and Marino 2006, Diaz and Rosenberg 2008]. The introduction of pests or disease [Swank et al. 1981] and non-native species [Strayer et al. 2006] can disrupt or alter vegetation dynamics and element demands. Increases in temperature can alter soil organic matter decomposition rates, providing a potential positive feedback on greenhouse gas emissions and continued global warming [Davidson and Janssens 2006]. These are just a few examples of the many unintended and often unforeseen negative consequences that human activities have had on ecosystem processes. The extent of human impact is now global, and the effects continue to intensify as population and per capita rates of resource consumption increase [Arrow et al. 1996]. We can anticipate further changes in ecosystems, as well as in the essential services they provide, in response to current and future human activities. Because of this, continued assessment of ecosystems is necessary.
1.2 Assessing Ecosystem Responses: The Watershed Approach

Ecosystems represent a network of myriad both abiotic and biotic objects and processes interacting to form a complex whole that is often impossible to understand from a solely reductionist approach. If we were to set off with the intention of tracing the effects of byproducts of human activity through the natural world we would, in nearly every case, result in an immensely inaccurate understanding. The limitations of this bottom-up approach are complimented by a top-down approach in which we monitor ecosystems as single entities, defining boundaries across which we can measure energy and element fluxes into and out of the system, acknowledging that differences between inputs and outputs are the integrated result of all internal ecosystem processes. Watersheds are often ideal for this because the watershed divide typically demarcates the hydrologic boundary of the system [Likens and Bormann 1995]. We can then readily measure atmospheric inputs and streamwater losses, with the difference between the two representing the sum of combined internal terrestrial and aquatic processes that affect the gains, losses and storage of water and solutes. While this top-down approach is not a substitute for understanding the important processes that control element cycling within ecosystems, it does provide a necessary constraint within which our understanding of cumulative process rates must exist. All of the processes governing element cycles within the ecosystem must sum to the ecosystem-scale value.
1.3 Research Outline

My dissertation is focused on understanding indirect human impacts on biogeochemical cycles at the ecosystem scale, using the watershed as my principal unit of study. In Chapter 2, I report findings from repeated synoptic surveys of streamwater chemistry for several watersheds spanning one of the largest N deposition gradients in North America within the Great Smoky Mountains National Park. We found non-intuitive patterns in dissolved organic matter concentrations and elemental composition across this gradient that suggested N deposition rates alter organic matter processing in these catchments. Through a series of laboratory experiments, we demonstrate support for a potential mechanism that gives rise to the patterns we observed, and we propose a hypothesis that outlines the conditions and processes by which anthropogenic N-loading may give rise to these changes.

In Chapter 3, we assess the interactions between organic matter processing and N availability within the stream channel through a series of reach-scale manipulations of nitrogen and labile carbon availabilities. Although we were unable to produce similar effects to those observed in catchments over the chronic N deposition gradient in Chapter 2, these short-term manipulations provided insight into how changing element supply ratios can differentially alter the production and uptake dynamics of organic matter that is derived from catchment soils versus fixed within the stream channel.
In Chapter 4, we synthesize data from 20 years of weekly streamwater chemistry sampling in the Walker Branch watershed in eastern Tennessee, USA. The Walker Branch watershed has experienced large changes in climate during the past 20-years that have resulted in significant reductions in catchment runoff through time. These trends in climate and catchment hydrology have resulted in significant changes in streamwater chemistry and solute fluxes, primarily through changes in the relative contributions of different soil flowpaths contributing to streamflow generation through time. In-stream biological processes were found to play an important role in governing streamwater nutrient concentrations, particularly for nitrate and phosphate. Nitrate concentrations exhibited an important response to drought conditions that was not observed in other solutes. Overall, however, this chapter illustrates how changing climates can alter both hydrologic and biogeochemical processes controlling watershed element cycles.
2. Examining the coupling of carbon and nitrogen cycles in Appalachian streams under elevated anthropogenic N loading

2.1 Introduction

Humans have doubled the amount of reactive N supplied to the biosphere annually through both fossil fuel combustion and agricultural practices [Vitousek et al. 1997, Galloway et al. 2008]. Where anthropogenic N loading exceeds biological N demand, the capacity of the ecosystem to retain additional N will decline and excess N may be exported to downstream aquatic ecosystems [Stoddard 1994, Aber et al. 1998]. Because NO$_3^-$ is highly mobile in many soils [Vitousek et al. 1982], increased NO$_3^-$ export is the primary symptom of ecosystem N saturation [Stoddard 1994]. The excess NO$_3^-$ lost to receiving aquatic ecosystems from N saturated terrestrial ecosystems has been a leading cause of impairment of freshwater ecosystems [Carpenter et al. 1998] and contributes to coastal eutrophication and hypoxia [Howarth and Marino 2006, Diaz and Rosenberg 2008].

Because NO$_3^-$ is an important indicator of ecosystem N saturation, most research has focused on dissolved inorganic N (DIN) losses from terrestrial to aquatic systems. Dissolved organic nitrogen (DON), however, can represent a major loss of N from many temperate watersheds [Hedin et al. 1995, Campbell et al. 2000, Kroeger et al. 2006, Pellerin et al. 2006, Scott et al. 2007]. While anthropogenic N loading often results in declines in the DON:DIN ratio of watershed losses [Perakis and Hedin 2002], organic N
forms continue to dominate hydrologic losses of N from most terrestrial landscapes [Meybeck 1982]. Even in regions of the United States where anthropogenic N loading is high, DON can constitute a large fraction of total N export [Scott et al. 2007].

Relative to inorganic N forms, our understanding of the processes that control DON fluxes from the landscape is poor. Most investigations of watershed DON losses have occurred in headwater streams of ecosystems with minimal human impact [e.g., Hedin et al. 1995, Brookshire et al. 2005, Mcgroddy et al. 2008] where N limitation is common [Vitousek and Howarth 1991, Lebauer and Treseder 2008]. Under such circumstances, DON often dominates total N export and, due to the perceived demand for biologically available N forms, this DON is thought to consist of N-containing organic molecules largely outside of biotic control [Hedin et al. 1995]. This is supported by positive correlations between DOC and DON losses in pristine watersheds [Goodale et al. 2000, Perakis and Hedin 2002], suggesting that DOM lost from sites that are N-limited is similar in composition. Where anthropogenic N loading is high and these assumptions may not hold, there is little information available on stream water DON concentrations and loss. In contrast to NO$_3^-$ export which is highly correlated with watershed N loading [Howarth et al. 1996], Perakis and Hedin [2002] found that DON concentrations change little between pristine and N-polluted sites. These findings suggest that variation in stream water DON concentrations should be independent of N availability, and that DON concentrations should be positively correlated with dissolved
organic carbon (DOC) concentrations. This pair of assumptions has been collectively termed the Passive Carbon Vehicle Hypothesis [Brookshire et al. 2007] (H1).

While anthropogenic N represents a doubling of the flux of N that enters the biosphere each year, the vast majority of fixed N is contained in plant organic matter and stored in soils, and most annual land plant production is supported by the in situ mineralization of organic N [Schlesinger 1997]. Increasing inorganic N availability may ‘break the cycle’ of organic N turnover [Neff et al. 2003] and total losses of DON from watersheds (and hence stream DON concentrations) may also increase across gradients of N loading [Kroeger et al. 2006]. There is some evidence to suggest that both DON, and the bioavailable subcomponent (hereafter bDON), may increase in watersheds with high N availability [Yano et al. 2000, Kaushal and Lewis 2005, Brookshire et al. 2007]. As watersheds become increasingly saturated with DIN [Aber et al. 1998], we might predict that both the concentration and lability of DON export would increase [Brookshire et al. 2007] – so that DON and DIN concentrations should be positively correlated across sites of varying degrees of N saturation. We term this the DON Release Hypothesis (H2).

We evaluated these two hypotheses by comparing stream water DON, DOC and DIN concentrations across forested watersheds in the Great Smoky Mountains National Park (hereafter GSMNP) during 4 synoptic survey campaigns. Watersheds within GSMNP span one of the largest N deposition gradients in North America [Weathers et al. 2006] and stream water NO3− concentrations increase significantly with increased
mean watershed N deposition rates. We used stream NO₃ concentrations as a proxy for N status (i.e., degree of N saturation) and predicted that DON concentrations will either correlate with DOC concentrations (H₁) or NO₃ concentrations (H₂) across sites. Because the underlying processes on which these predictions are based focus on the bioavailable subcomponent of DON (that it is constantly recalcitrant, H₁; or that bioavailable DON increases with N saturation, H₂) we conducted laboratory bioavailability assays on samples retrieved during 2 of our synoptic sampling campaigns to further evaluate the two hypotheses.

2.2 Methods

2.2.1 Site Description

During each survey we sampled 24 to 32 streams throughout GSMNP, USA (83°25'42"W, 35°36'44"N). The area consists of Precambrian to Cambrian metasedimentary rock, mostly quartzite and phyllite, which is relatively resistant to weathering and provides little neutralization capacity [King et al. 1968]. Sampling sites ranged in elevation from 450 to 1700m. All sampled watersheds were fully forested, with no human habitation and minimal current human impacts (hiking trails and picnic shelters only). Watersheds were delineated using ESRI’s ArcMap 9.2. We used Hawth’s Analysis Tools 3.27 (www.spatalecology.com/htools) to calculate mean watershed characteristics, including percent broadleaf and conifer cover, dominant soil type and
parent geology (defined as being more than 50% of the total watershed area), mean elevation, and mean N deposition. All coverage data for GSMNP were obtained from the National Park Service data clearinghouse (http://www.nps.gov/gis/data_info/) or from park personnel. Mean catchment N deposition rates were calculated from an N deposition map developed for GSMNP [Weathers et al. 2006]. The highest elevation watersheds near Noland Divide have mean deposition rates in excess of 18 kg N ha\(^{-1}\) yr\(^{-1}\), with decreasing N deposition at lower elevations.

Mean catchment N deposition explained 38-58% of the variation in stream water NO\(_x\) concentrations in our surveys (Appendix A: Fig. A1). However, other factors that can potentially influence the residual variation in NO\(_x\) concentrations, as well as DOC and/or DON concentrations, also change systematically with elevation, notably forest cover, parent material and soil type [e.g., Lovett et al. 2002, Mcgroddy et al. 2008]. Attempting to account for these potential confounding factors, we surveyed first through fifth order streams with watershed areas ranging from 21.1 to 11,618.7 ha to avoid comparing only first-order watersheds with little variation in landscape features.

Vegetation in GSMNP is dominated by oaks (Quercus spp.), maple (Acer spp.), and poplar (Liriodendron tulipifera L.) at lower elevations and mature stands of red spruce (Picea rubens Sarg.) and fraser fir (Abies fraseri (Pursh) Poir.) at high elevation, with several species of perennial rhododendron (Ericaceae spp.) shading streams year round [Whittaker 1956]. Few of our watersheds, however, were dominated by any one
vegetation type with average watershed cover of 64.7±19.3% and 15.0±14.7% (mean±sd) for broadleaf and conifer species, respectively. In no survey was any additional variation in stream water NO₃⁻ concentrations explained by the amount of broadleaf or coniferous forest coverage within watersheds (Appendix A: Table A1). On two survey dates, DOM concentrations were significantly correlated with the proportion of broadleaf trees in the watershed, but forest cover explained less than 22% of the variation in either instance (Appendix A: Table A1).

A single parent geology or soil type dominated many of our study watersheds. While stream water DOM concentrations and quality can be sensitive to catchment geochemistry [Mcgroddy et al. 2008], DOC concentrations showed minimal differences in catchments with differing dominant soil type or geology (Appendix A: Table A2). The single exception to this was that 3 watersheds dominated by the Anakeesta formation [an acidic slate, high in iron sulfide, with interbeds of sandstone; King et al. 1968], had significantly higher DOC concentrations than all other watersheds. One of these same watersheds was dominated by a soil of the Luftee-Anakeesta complex (Lr), a sandy-skeletal, isotic, frigid Typic Humaquept, and had higher DOC concentrations than watersheds dominated by other soil types (Appendix A: Table A2). DON concentrations were not significantly different between watersheds dominated by different soil types or geologies, or in those watersheds that had no dominant classification (Appendix A: Table A2). Residuals from the NO₃⁻ by N deposition
regressions were also not significantly different across soil types or geologies (data not shown).

Since approximately half of the variation in $\text{NO}_x$ can be explained by N deposition alone, and because no other watershed characteristics correlate with the residual variation, we use stream water $\text{NO}_x$ as a proxy for watershed N status. Further, comparing $\text{NO}_x$ and DON concentrations is essentially equivalent to comparing DIN and DON concentrations since $\text{NO}_x$ comprises >98% of DIN in any sample (See Results).

2.2.2 Field Sampling Methods

Stream water samples were collected during four sampling campaigns (before litterfall: September 13-14, 2004; before leaf-out: April 25-26, 2005; after leaf-out: May 17-18, 2007; before litterfall October 3-4, 2008). In each survey samples were field filtered at time of collection through syringe mounted Whatman GF/F (0.7$\mu$m) filters. Samples for DOC/DON analysis were acidified in the field with 0.1% 2N HCl. All samples were immediately placed on ice and stored at 4°C prior to analysis. All sample analyses were completed within two weeks of sample collection. All surveys were conducted when streams were near baseflow, however, remnants of Hurricane Frances passed over GSMNP approximately 7d prior to our first sampling effort in September 2004. During this single storm event >35cm of precipitation fell over most of the study area (http://www.nhc.noaa.gov/2004frances.shtml).
2.2.3 Bioavailability Assays (May 2007 and October 2008 Surveys)

Microbial bioavailability assays were conducted on eight 40mL filtered samples from each site collected during the May 2007 and October 2008 surveys; bioavailability assays were not conducted during the first two surveys (September 2004 and April 2005). Within 24hr of collection, all samples were inoculated with 1mL of a standardized microbial solution. Due to permitting restrictions by GSMNP, we could not remove stream sediments from the original sites and, therefore, inocula were prepared by agitating stream sediments collected from a small stream on the Duke campus and filtering the solution through a Gelman AE 1μm filter. Two vials per stream received labile C (target enrichment of 0.3mgC/L as dextrose), N (target enrichment of 0.7 mgN/L as NaNO₃), or deionized water (DI) in a full factorial design (Treatments: C, N, CN, DI), and one vial in each pair was immediately acidified with 0.1% 2N HCl by volume (T₀). The unacidified samples were capped with Parafilm and incubated on a shaker table in the dark at 22°C and 150rpm for 14d. After the two-week incubation each sample was acidified with 0.1% 2N HCl by volume (T₁). Two controls substituting 40mL deionized water in place of stream water were conducted for each treatment. The bioavailability of DOC (bDOC) was calculated as the difference in DOC concentration (T₁-T₀) over the incubation. DON was both consumed and produced during these incubations, and thus positive values represent net production of DON during the incubations while negative values represent net consumption. We conduct
all statistical analyses on the changes in DON and DOC concentrations during the incubations and not on percent changes relative to the starting concentrations (i.e., \([T - T_0]/T_0 \times 100\%\)), as is common. Due to low initial DON concentration in many samples (see Results), there is a high degree of error propagation introduced by denominator effects when calculating percent changes as we approach analytical detection limits.

2.2.4 Chemical Analyses

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations were measured on a Shimadzu TOC-VCPH total carbon analyzer with a TNM-1 nitrogen module (MDL 0.05 mg C L\(^{-1}\), 0.005 mg N L\(^{-1}\); Shimadzu Scientific Instruments, Columbia, MD). Solution concentrations of NO\(_3^–\) were determined by ion chromatography (MDL 0.001 mg NO\(_3^–\) N L\(^{-1}\), Dionex ICS-2000 with eluent generation on an AS18 column, Dionex Corporation, Sunnyvale, CA). Ammonium concentrations in the 2004 and 2005 surveys were determined by automated phenate colorimetry (Seal Analytical Model AA3 autoanalyzer) and in the 2007 and 2008 surveys ammonium concentrations were determined using fluorescence detection following reaction with orthophthalaldialdehyde [Holmes et al. 1999]. The MDL for both NH\(_4^+\) methods is 0.001 mg N L\(^{-1}\). Concentrations of DON were determined by difference [DON = TDN − (NO\(_3^–\) N + NH\(_4^+\) N)].
2.2.5 Statistical Analyses

All statistical analyses were performed in JMP 8.0 (SAS Institute; Cary, NC, USA). We used Pearson’s correlation to examine relationships between stream water DON, bDOC, ΔDON, and DOM C:N molar ratios with other measured variables. Single factor analysis of variance (ANOVA) was used to: (1) compare stream water chemistry across surveys, and (2) compare changes in DON and DOC concentrations and DOM C:N ratios across treatments in the bioavailability assays. Tukey HSD (α=0.05) was used to determine differences among groups when ANOVA results were significant (p<0.05).

2.3 Results

2.3.1 Survey Patterns

During all surveys, NO₃⁻ dominated the inorganic N fraction, and NH₄⁺ concentrations were often near detection limits (Table 1). DOC concentrations were low in all surveys, with mean DOC concentration across sites never exceeding 0.85mg/L (Table 1). DON concentrations ranged widely, and could constitute a substantial portion of the total dissolved N measured at some sites (Table 1). Stream water concentrations of DON were significantly higher and the DOM molar C:N ratio was significantly lower during the September 2004 survey than in the 3 subsequent surveys (Table 1). In this initial survey, DON was positively correlated (r=0.89; p=0.01) and the DOM molar C:N ratio was negatively correlated (r=0.57, p<0.001) to NO₃⁻ concentrations (Fig. 1A). In contrast, during the latter 3 surveys, DON concentrations were negatively
Table 1. Summary of stream water chemistry for all synoptic surveys conducted at GSMNP (mean ± S.E.). Ranges are indicated in parentheses below the mean values. Superscripts denote significant differences in mean values based on one-way ANOVAs, blocked by site, using Tukey’s HSD (p<0.05).

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<tr>
<td>DOC (mg/L)</td>
<td>0.85 ±0.06&lt;sup&gt;B&lt;/sup&gt; (0.42-1.68)</td>
<td>0.53 ±0.05&lt;sup&gt;A&lt;/sup&gt; (0.19-1.48)</td>
<td>0.76 ±0.07&lt;sup&gt;AB&lt;/sup&gt; (0.41-1.56)</td>
<td>0.83 ±0.06&lt;sup&gt;B&lt;/sup&gt; (0.42-1.68)</td>
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<tr>
<td>NO3-N (µg/L)</td>
<td>257 ±36&lt;sup&gt;A&lt;/sup&gt; (11-550)</td>
<td>243 ±37&lt;sup&gt;A&lt;/sup&gt; (10-660)</td>
<td>413 ±41&lt;sup&gt;B&lt;/sup&gt; (17-836)</td>
<td>243 ±40&lt;sup&gt;A&lt;/sup&gt; (41-679)</td>
</tr>
<tr>
<td>NH4-N (µg/L)</td>
<td>4 ±0.5 (1-13)</td>
<td>4 ±0.5 (2-14)</td>
<td>5 ±1 (3-8)</td>
<td>6 ±1 (3-12)</td>
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<tr>
<td>DON (µg/L)</td>
<td>56 ±3&lt;sup&gt;B&lt;/sup&gt; (31-105)</td>
<td>15 ±3&lt;sup&gt;A&lt;/sup&gt; (1-83)</td>
<td>17 ±3&lt;sup&gt;A&lt;/sup&gt; (5-43)</td>
<td>19 ±3&lt;sup&gt;A&lt;/sup&gt; (5-51)</td>
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<tr>
<td>DOM C:N</td>
<td>13.7 ±0.9&lt;sup&gt;B&lt;/sup&gt; (6.6-30.3)</td>
<td>76.6 ±21.6&lt;sup&gt;A&lt;/sup&gt; (7.3-196.5)</td>
<td>62.7 ±7.9&lt;sup&gt;A&lt;/sup&gt; (14.5-150.4)</td>
<td>54.7 ±7.0&lt;sup&gt;A&lt;/sup&gt; (22.1-154.0)</td>
</tr>
<tr>
<td>DON:TDN</td>
<td>30.7 ±2.8&lt;sup&gt;B&lt;/sup&gt; (12.2-68.2%)</td>
<td>13.9 ±2.9&lt;sup&gt;A&lt;/sup&gt; (0.2-61.1%)</td>
<td>6.7 ±1.4&lt;sup&gt;A&lt;/sup&gt; (0.6-25%)</td>
<td>12.7 ±2.3&lt;sup&gt;A&lt;/sup&gt; (0.8-40.0%)</td>
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Figure 1: Relationships between stream water [DON] and the molar C:N ratio of DOM against variation in stream water [NO₃⁻] for all synoptic surveys conducted at GSMNP (sampling date denoted at top of each column of panels). The values for the y-axes are held constant across horizontal rows of panels. Lines represent significant relationships; r and p-values based on Pearson’s correlation.
correlated and DOM molar C:N ratios were positively correlated to NO$_3^-$ concentrations (Fig. 1, B-D). DOM C:N ratios were very high at high NO$_3^-$ sites in the latter 3 surveys, often having values in excess of 100 (Fig. 1, B-D). DON concentrations were only positively correlated to DOC concentrations during the Oct 2008 survey ($r=0.71$, $p<0.001$; Fig. 1E). For the two surveys in which bioavailability assays were conducted (May 2007 and October 2008), there was no relationship across streams between bDON and stream water NO$_3^-$ concentrations, or between bDOC and bDON concentrations, in either survey.

2.3.2 Experimental Enrichment Effects on In-Stream DOM Dynamics

The standardized microbial inocula for the 2 bioavailability assays, while made with identical methods and from stream sediment slurries collected from the same site but at different points in time, were chemically similar except for the amount of bDOC (Fig. 2, C&D; Appendix A: Table A1). While the May 2007 inoculum contained no measureable bDOC, the inoculum made for October 2008 assay unintentionally elevated bDOC concentrations by 0.322 mg/L when added to the samples (Appendix A: Table A1). This is similar to the target enrichment concentrations for the acetate additions (0.3 mg/L bDOC) and, therefore, the DI and N treatments in the October 2008 assay had similar starting concentrations ($T_0$) of bDOC to the C and CN treatments in the May 2007 assay, respectively (Fig. 2, C&D). The C and CN treatments in the October 2008 assay had more than twice the amount of bDOC at $T_0$ compared to the C and CN treatments in
Figure 2: Results of bioavailability assays conducted on samples retrieved during May 2007 and October 2008 synoptic surveys. *Top panels* indicate changes in DON (mean ± S.E.) concentrations during two-week incubations (calculated as T1-T0; positive values=production, negative values=consumption) across treatments. *Middle panels* show initial (T0) concentrations of bDOC; October 2008 concentrations are much higher due to bDOC contamination from microbial inoculum (see Results). *Lower panels* indicate changes in the molar DOM C:N (mean ± S.E.) during two-week incubations. Letters indicate significant differences between treatment effects as determined by Tukey HSD (alpha=0.05).
the May 2007 assay (Fig. 2, C&D). NO₃, DON, and bDON concentrations were not significantly elevated by the addition of the microbial inocula in either bioassay (Appendix A: Table A3).

Carbon and N additions had no significant effect on the amount of bDOC consumed between treatments for both the May 2007 and October 2008 bioavailability assays, with 6.2±1.4% of the DOC in an average sample being bioavailable over these 2wk incubations (data not shown). Across treatments bDOC concentrations were significantly higher ($F_{1,189}=8.06$, p<0.005) in the October 2008 bioassay (0.080±0.013 mg/L) than in the May 2007 bioassay (0.042±0.007 mg/L). When added, nearly all of the acetate in the C treatments was consumed (90.4±10.2%, across all treatments).

During the May 2007 bioassay significant DON consumption was observed when samples were not given a labile C source (both N and DI treatments, Fig. 2A). Most of the measureable DON was consumed in the N treatment (70.0±60.0%; Fig. 4), and much of the DON (39.6±67.0%) was consumed in the DI treatment (Fig. 2A). When labile C was added, DON consumption ceased and small amounts of DON production were observed (Fig. 2A). During the October 2008 bioassay, more DON was produced when N was added (CN and N Treatments) than when it was not (C and DI Treatments), although this difference had a probability (P-value) of 0.104 (Fig. 2B).

The effects of the C and N treatments on the amount of DON produced or consumed during the incubations differed between high- and low- N sites (Fig. 3).
Samples were split into similar sized groups (n=11-13) based on NO₃ concentrations, with samples having greater than 250 μg N L⁻¹ considered high-N and samples having less than this considered low-N. For the May 2008 bioassay, low=N sites showed significant DON consumption when N was added and significant DON production when N and C were added together relative to the control (DI Treatment; Fig. 3A). For the high-N sites, adding labile C (C and CN treatments) resulted in significant DON production relative to the control (DI treatment), while adding N alone had no effect (Fig. 3C). For the October 2008 bioassay, there was significant DON production at low-N sites when N was added (CN and N treatments) and no significant effect when C was added alone (Fig. 3B). For the high-N sites, there were no significant effects from any of the treatments relative to the control (Fig. 3D).

When we were able to stimulate the greatest amount of DON consumption in the bioassays (N treatment, May 2007; Fig. 2 A), only low-N sites had DON that was not consumed during the incubation; there was no DON remaining in any samples from high-N sites (Fig. 4).

During the May 2007 incubation, the consumption of much of the DON led to large increases in the DOM C:N ratio for both N and DI treatments (Fig. 2E)—indicating N-rich DOM molecules were preferentially removed from the bulk pool. Decreases in the DOM C:N ratio were observed for the C and CN treatments in which DON was
produced (Fig. 2E). For the October 2008 incubations, the DOM C:N showed little change across treatments (Fig. 2F).

Figure 3: ANOVA results for DON changes bioavailability assays separated by low- and high-NO₃ sites. For May 2007 bioassay, high NO₃ (>250µg NO₃-N/L) sites only show response to labile DOC, suggesting C-limitation. Low NO₃ (<250µg NO₃-N/L) sites show increased DON consumption with added NO₃ and increased DON production when both NO₃ and dextrose are added together. For October 2008 bioassay, high NO₃ sites show no response to treatments and low NO₃ sites only show response to N additions.
2.4 Discussion

Our results show little support for the coupling of DOC and DON concentrations in forest watersheds subject to high rates of anthropogenic N loading, as is predicted by the Passive Carbon Vehicle Hypothesis (H1). DON and DOC concentrations were positively correlated in only one survey (October 2008, Fig. 1A) and DOM C:N ratios...
changed systematically with increasing NO$_3^-$ concentrations in all surveys (Fig. 1, I-L). Changes in the DOM C:N ratio were largely driven by changes in DON concentrations, which correlated with NO$_3^-$ concentrations (Fig. 1, A-D). However, in only one survey was the relationship between DON and NO$_3^-$ concentrations positive (Fig. 1A), as predicted by the DON Release Hypothesis (H$_2$, Fig. 5). In the other 3 surveys we find negative correlations (Fig. 1, B-D), a relationship that was not predicted by either of the prevailing hypotheses.

While DON only increased with NO$_3^-$ concentrations in a single survey (September 2004), it was our strongest statistical relationship (Fig. 1A). Though we could not replicate this result, others have reported similar patterns. While some have reported increasing DON concentrations with increasing NO$_3^-$ in large rivers [Kroeger et al. 2006, Pellerin et al. 2006, Scott et al. 2007], these patterns have been attributed to high anthropogenic DON loading from wastewaters in densely populated watersheds—a mechanism that does not apply to these minimally disturbed forested watersheds.

Our initial survey occurred one week following Hurricane Francis, an extreme storm which resulted in >35cm of precipitation in a single event. Multiple USGS gauging stations in western North Carolina, many with records extending back nearly a century, reported record heights during this storm (http://waterdata.usgs.gov/nc/nwis). While storm events have been shown to have a major impact on watershed DOM losses [e.g., Hinton et al. 1997, Inamdar et al. 2004], changing subsurface soil flowpaths and
potentially having differential impacts on DOC and DON concentrations [Buffam et al. 2001, Inamdar and Mitchell 2007], we did not sample until our study streams returned to near base flow levels. Therefore, we do not believe typical storm effects (i.e., differing hydrologic flowpaths) caused these patterns. However, due to the intensity and duration of this particular event, it is possible that some other factor associated with this storm resulted in the observed stream water chemistry patterns. For example, with increasing elevation and hillslope steepness, overland flow may have resulted in greater amounts of forest litter deposited in the stream channels, and DOM leaching from this material may have dominated stream water chemistry at the time of our sampling.

Ultimately, we cannot be certain of the cause for the high DON concentrations in our watersheds with high N deposition during this first survey. The DON present in stream water is a mixture of inputs of terrestrial soil DON, the leaching of DON from terrestrial particulate organic matter retained in the stream, and the production of DON by stream biota. How these sources of stream water DON concentrations change seasonally or in response to storm events is largely unknown [but see Inamdar et al. 2004, Inamdar and Mitchell 2007]. Much of our current knowledge about watershed DON export is derived from single synoptic surveys of pristine catchments [Hedin et al. 1995, Mcgroddy et al. 2008] or intermittent sampling by national water quality monitoring networks [Scott et al. 2007], with very few long-term research sites monitoring DON regularly or under high N-loading conditions [Pellerin et al. 2006].
Our data demonstrate that patterns of DON export across landscapes may change significantly over seasons and storm hydrographs and suggest that caution be used in extrapolating the results of a few sampling dates to understanding variation through time or annual patterns.

The negative correlations between DON and NO\textsubscript{3} concentrations in our latter 3 surveys were unexpected yet replicable. Others have also found this pattern in forested watersheds with varying N-deposition throughout the Northeast United States [Lovett et al. 2000]. If biological processes largely drive these negative correlations, it is unlikely that DON is functioning primarily as a source of N for biota since there is high inorganic N availability in the soils and streams that are exporting the least DON.

The findings of our last 3 surveys are inconsistent with the conclusions of Brookshire et al. [2007], who reported that forested watersheds in the southern Appalachians with high rates of N deposition export more DON than watersheds with low rates of deposition. The discrepancy between their results and those of our latter 3 surveys is due in large part to the reliance of the authors on data from 3 watersheds within GSMNP where DON concentrations were found to be high (~200 µg/L). All of the sites included in the Brookshire et al. (2007) paper were included in each of our 4 synoptic surveys, yet only during our first survey do we find DON concentrations on the same order of magnitude as they report. We sampled both early (May 2007) and late (September 2004 and October 2008) in the growing season, while Brookshire et al. [2007]
sampled in the middle of the growing season (July 2002 and July 2004). Resolving the discrepancy between our results will require higher temporal resolution sampling of these streams. However, the large variation in DON measured in our study suggests that extrapolation from a few dates to annual export patterns is inappropriate and that the mechanisms controlling DON export may shift across years, seasons or in response to antecedent conditions.

In retrospect, we realized that our initial hypotheses about DON dynamics in these watersheds treated DON as being functionally equivalent with inorganic N forms (Fig. 5A), with DON losses from N-limited forests presumed to constitute poor N sources [Hedin et al. 1995] and N-demand driving the uptake of bioavailable DON in forest streams [Brookshire et al. 2005, Kaushal and Lewis 2005]. However, unlike inorganic N, the dynamics of N contained within organic molecules is controlled not only by direct biological demand for N, but also by heterotrophic demand for the reduced C to which it is attached.

To explain the negative relationship between stream water DON and NO\textsubscript{3}\textsuperscript{-} concentrations, we propose an additional hypothesis, which we term the Indirect Carbon Control Hypothesis (H\textsubscript{3}, Fig. 5B), whereby alleviating N limitation can result in increased DON uptake as a labile source of reduced C for heterotrophic metabolism. While N-containing DOM molecules exported in stream water under low-N conditions may represent a recalcitrant source of N for biota, this DOM may function as a labile source
of C for heterotrophic metabolism under high-N availability. In general, DOM molecules that are N-rich (e.g., amino acids, proteins) have been found to be more bioavailable than N-poor molecules in soils and streams [e.g., Hopkinson et al. 1998, Hunt et al. 2000]. Even though aboveground primary productivity in many forests is N-limited [Lebauer and Treseder 2008] and N is efficiently retained under these conditions [Vitousek and Reiners 1975], microbial activity in soils below the litter layer is often strongly C-limited [Bosatta and Agren 1991]. Likewise, these forest streams receive little

![Diagram A](image1.png)

**Figure 5:** Contrasting hypotheses for DON losses under elevated N loading. When N loading is low, DON losses are assumed to be recalcitrant sources of N for biota (Panel A, Low N Loading). When placed within the context of the N cycle alone, DON losses may increase as demand for labile N forms attenuates (Panel A, High N loading; DON Release Hypothesis). Though it represents a recalcitrant form of nitrogen at low N loading, the N-containing DOM may be a labile form of C (Panel B). Thus, alleviating heterotrophic N-limitation may result in a decrease in DON export (Indirect Carbon Control Hypothesis). These hypotheses are not mutually exclusive, but represent the potentially contrasting roles of DON within the C and N cycles.
light much of the year due to riparian shading from both the extensive understory and
tree canopy, and can be highly net heterotrophic [P:R<<1; Vannote et al. 1980]. In all of
our surveys, the mean concentration of DOC across these sites (≤0.85 mg/L, Table 1) was
very low in comparison to stream water concentrations typically reported in the
literature [Hope et al. 1994], and only around 6% of this DOC was bioavailable —
suggesting that the majority of labile OM is consumed by heterotrophs within
watershed soils and streams, and that microbes in receiving streams are likely to be
strongly C limited. When demand for bioavailable DOM is high in these systems, we
would expect labile organic matter to be consumed regardless of its N content.

The results of our bioavailability assays are consistent with the Indirect Carbon
Control Hypothesis (H1). For the May 2007 bioassay, we find that adding NO3 can
increase DON consumption and adding labile C can increase DON production. That we
find support for both N- and C-limitation of stream microbial activity is a consequence
of having sites that span an N deposition gradient. When we split our dataset in half we
see that microbial activity in stream water from watersheds with high background NO3
availability (>250 μg NO3-N/L) is strictly C-limited—with net DON production when C
is added, and net DON consumption when it is not (Fig. 3B). In contrast, microbial
activity in stream water from low-N watersheds (<250 μg NO3-N/L) is responsive to N
addition, with higher DON consumption in N amended treatments compared to the
control treatment (Fig. 3A). Only when labile C and NO$_3^-$ are added together to stream
water from the low DIN watersheds is significant DON production observed (Fig. 3A).
Thus, at high-N sites we need only add C and at low-N sites we need to add both C and
N in order to observe DON accumulation—indicating that either C limitation or co-
limitation by both C and N can result in high DON consumption.

Not only was DON consumed when either C or N was limiting in our
bioavailability assays, but N-rich DOM molecules were consumed preferentially from the
bulk DOM pool—resulting in an increase in DOM C:N ratios during the incubations
(Fig. 2E). Moreover, the increases in the DOM C:N ratios were large (~100, Fig. 2E). In
the latter 3 synoptic surveys, the DOM C:N ratios at high-N sites were also extremely
high (>100, Fig. 1,B-D) compared to values reported in the literature [e.g. Hedin et al.
1995, Campbell et al. 2000, Goodale et al. 2000]. Because these values are both similar in
magnitude and atypically high, it is possible that the same processes in the
bioavailability assay (i.e., selective removal of N-rich DOM as a labile C source when
NO$_3^-$ is high) are contributing to the negative relationship between DON and NO$_3^-$
concentrations observed in the field.

Despite contamination with high concentrations of bDOC from the microbial
inoculum, the results from the October 2008 bioavailability assays are consistent with
the May 2007 bioavailability assay and further support the Indirect Carbon Control
Hypothesis (H$_3$). When all sites are combined, there is somewhat higher DON
production when N is added (N and CN treatments) than when it is not (C and DI treatments) although these differences are not significant (Fig. 2B). When we split the sites between high- and low- NO$_3^-$ concentrations, however, DON production increases significantly in the low-N sites when NO$_3^-$ is added (Fig. 3C). Due to the high [bDOC] unintentionally introduced in the microbial inoculum, these low-N sites can be considered strictly N-limited. When NO$_3^-$ is added so that neither N nor C is limiting, we observed significant DON accumulation (N and CN Treatments, Fig. 3C) — similar to the May 2007 assay (CN Treatment, Fig. 3A). For the high-N sites, no treatment effects are observed (Fig. 3D) because neither N nor C is likely limiting in any treatment due to both high background NO$_3^-$ concentrations and the bDOC contamination from the added inoculum.

It is interesting that when neither C nor N is limiting (e.g. CN treatments) there is more net DON production at low-N sites than high-N sites for both the May 2007 and October 2008 bioassays (Fig. 3). However, the DON produced during these assays is a product of microbial metabolism and, therefore, some element other than N or C (since these are added in excess) may be depleted more readily — limiting microbial activity sooner — in high-N sites than in low-N sites. This represents the scenario in which we would expect the DON Release Hypothesis (H$_2$) may be effective in predicting DON losses from watersheds — when limitation of heterotrophic activity by some element other than C results from alleviating N limitation.
That there was not a consistent relationship between DOC and DON concentrations across sites in our synoptic surveys is inconsistent with the Passive Carbon Vehicle hypothesis (H₁). However, our bioavailability assays demonstrate that DON concentrations in these stream waters can exhibit greater relative changes in response to microbial C and N demands than bulk DOC, and supports our finding that DOC and DON concentrations can behave independently across these sites that vary so greatly in N-availability. There have been few other studies of which we are aware that have investigated watershed DON losses across an N deposition gradient of this magnitude, and the decoupling of DOC and DON concentrations may only occur at sites where stream water concentrations of labile C are low and N-pollution is similarly high.

We also recognize that microbial metabolism is just one factor among many that can give rise to landscape patterns of DON loss. Forests subject to chronic N pollution are likely to have altered vegetation dynamics, litter qualities, and soil organic matter dynamics [Aber et al. 1989, Aber et al. 1998]—all of which may influence stream water DON concentrations. Both the Passive Carbon Vehicle Hypothesis (H₁) and the DON Release Hypothesis (H₂) suggest that watersheds should lose some amount of recalcitrant DON that is beyond biotic control, with the latter also positing an additional release of more labile DON at high-N sites. However, we found no recalcitrant DON at our high-N sites (Fig. 4). Yet at our low-N sites, even when we were able to stimulate the greatest amounts of DON consumption (N Treatment - May 2007 bioassay), we find
many sites having DON that was not consumed (Fig. 4). Thus, we hypothesize that the composition and overall quality of DON transported to these streams may change with watershed N-status, and that shifts in microbial metabolism that increase DON consumption as a C-source may only be partially responsible for the patterns we observed in both our surveys and bioavailability assays.

2.4.1 Conclusions

Although we draw contrasts between these hypotheses for watershed DON dynamics (Fig. 5), we do not believe that they are competing or mutually exclusive. Instead, we view them as end-members that describe the potential influences of how C and N demands may affect watershed DON export. Globally, riverine fluxes of N from the landscape are dominated by DON (11 Tg yr⁻¹), with NO₃⁻ losses equal to only about one third of this (3.7 Tg yr⁻¹) [Meybeck 1982]. While the Passive Carbon Vehicle Hypothesis (H₃) has been important for helping to explain high DON losses from pristine, N-limited forests, it is unlikely that DON losses will remain unchanged as humans continue to double the rate in which N is supplied to the biosphere.

While investigations of DON losses from ecosystems with high-N pollution are only recently arising in the literature, efforts to understand changes in DOM composition in response to increased N availability are sorely lacking. Although we found little support for the DON Release Hypothesis (H₂) in our synoptic surveys, our bioavailability assays did indicate that DON production—and, therefore, stream DON
concentrations and loss—could increase when both labile C and DIN are increasingly available. At the same time, our bioavailability assays indicate microbial C demands can exert strong control over DON concentrations when either C or N is limiting microbial metabolism. Because of this, and because heterotrophic demand for labile DOM is often high in catchment soils and forested streams, we believe the Indirect Carbon Control Hypothesis (H3)—and explicit consideration of the dual role of DON in both C and N cycles—is necessary for predicting changes in DON losses from N-polluted landscapes.
3. Distinguishing terrestrial and autochthonous organic matter dynamics in a forested stream

3.1 Introduction

Dissolved organic matter (DOM) represents an important source of energy in stream ecosystems [Fisher and Likens 1973, Cummins 1974], fueling heterotrophic microbial production and establishing the foundation on which stream trophic structure and ecosystem function relies [Minshall et al. 1983, Webster and Meyer 1997]. DOM, however, is a single term applied to an extremely complex pool of organic molecules, with independent subcomponents that can exhibit distinct dynamics. Treating DOM as a single bulk pool has likely lead to an incomplete and potentially erroneous understanding of the processes controlling the production and consumption of DOM, and therefore the role that DOM plays in governing stream ecosystem dynamics.

Determining the source of DOM is a useful distinction that can be made to distill the heterogeneity of the bulk pool into subcomponents exhibiting important functional differences. Streams are highly open systems that can receive the majority of their DOM inputs from the hydrologic flux of terrestrial materials supplied from catchment soils [Fisher and Likens 1973]. The in situ fixation of inorganic C by autotrophs (autochthonous production) in the water column or attached to the benthos can also be an important source of DOM [Minshall 1978]. The relative contribution of each of these DOM sources varies both spatially (across sites) and temporally (across seasons within a site) [Minshall 1978, Kaushal and Lewis 2005]. Further, these two sources of DOM
typically exhibit important and generalizable differences in their chemical composition that affect their function within stream ecosystems [Mcknight et al. 1994, Sun et al. 1997, Mcknight et al. 2001].

Terrestrial DOM is largely derived from vegetation consisting of structural compounds that are highly aromatic and relatively resistant to decomposition (i.e., slowly degrading) [Malcolm 1990, Kalbitz et al. 2003]. Subsequent humification can further decrease the bioavailability of these complex molecules as they pass through soil profiles, resulting in highly processed and typically recalcitrant DOM entering the stream channel [Qualls and Haines 1991, 1992]. Conversely, autochthonous DOM fixed by algae in the stream channel lack extensive aromatic structural molecules [Mcknight et al. 1994], often contain a higher proportion of essential nutrient elements in addition to carbon (C) [Sun et al. 1997], and are typically much more bioavailable [Sun et al. 1997, Findlay and Sinsabaugh 1999].

While investigators increasingly appreciate the chemical heterogeneity of DOM in streams, we currently lack for methods to distinguish between different DOM subcomponents [Hedges et al. 2000]. The heterogeneity of the native DOM in streams restricts our ability to manipulate the DOM pool in an experimental context because we are unable to synthesize the native DOM pool in its entirety. Because of this Nutrient Spiraling Theory [NST; Newbold et al. 1981, Workshop 1990], which has been a powerful tool for investigating solute biogeochemistry in streams, cannot be directly
applied to DOM. While several studies have used NST with manipulations of specific subcomponents of the DOM pool, such as litter leachates [Mcdowell 1985, Wiegner et al. 2005, Bernhardt and Mcdowell 2008] or simple organic monomers [Bernhardt and Likens 2002, Brookshire et al. 2005], these experiments share a common limitation in that they focus on limited fractions of the DOM pool that are often highly dissimilar from the DOM cycling under background conditions.

Recognizing that we cannot add the diversity of native DOM molecules to a stream and measure differential rates of uptake of each terrestrial or autochthonous form, we instead attempted to displace demand for—and observe the release of—native DOM by independently altering the relative rates of production and consumption of terrestrial and autochthonous DOM. By increasing the availability of a highly labile source of non-native reduced C substrate, we hypothesized that we would displace heterotrophic demand for native DOC (Fig. 6B). Due to its lower relative bioavailability we expected that terrestrial DOM should be displaced more readily (H1a) than autochthonous DOM (H5a).

In addition to providing an alternative source of labile reduced C to displace heterotrophic demand for native DOC, we hypothesized that increasing inorganic nutrient and/or light availability would increase autotrophic production of autochthonous DOM (Fig. 6C). We hypothesized that an increase in in-stream production of DOC would result in a direct increase in autochthonous DOM loss
Figure 6: Conceptual model for DOM sourcing, uptake, and loss within a stream channel. DOM is derived from two principal sources (terrestrial material or autochthonous production) and has two principal fates (heterotrophic uptake or loss to the water column) (panel A). Adding a source of highly bioavailable reduced carbon to the stream can displace heterotrophic demand for both terrestrial DOM ($H_{1A}$) and autochthonous DOM ($H_{1B}$) (panel B). Increases in light availability or inorganic nutrients limiting in stream production can result in an increase in autochthonous DOM lost to the water column ($H_{2A}$) or, because autochthonous DOM is often highly bioavailable, the indirect displacement of terrestrially derived DOM ($H_{2B}$).
to the water column ($H_{2A}$), or, because autochthonous DOM is typically more bioavailable than terrestrially-derived DOM [Sun et al. 1997], ($H_{2A}$) an indirect displacement of heterotrophic demand for terrestrial DOM as microbes take greater advantage of the added labile C source (Fig. 6C).

The objectives of this study were to evaluate these hypotheses for the relative roles of terrestrial versus autochthonous DOM, in a well-studied stream, Walker Branch (Tennessee, USA), during the spring prior to tree canopy closure when autotrophic production and heterotrophic demands are both commonly high [Rosemond 1994, Mulholland 2004, Roberts and Mulholland 2007]. Autochthonous production in this system has been shown to exhibit some degree of N limitation [Rosemond 1994, Roberts and Mulholland 2007], with increased algal growth often leading to a drawdown of streamwater nitrate concentrations [Mulholland 1992, Roberts and Mulholland 2007]. Through a series of back-to-back (i.e., sequential) solute additions at increasing levels of concentration (i.e., progressive) that are sustained for very short periods of time (i.e., kinetic), we alter the supplies of both a highly labile C source (acetate) and inorganic N (potassium nitrate) to differentially alter heterotrophic uptake and autotrophic production rates.

We assessed changes in the native DOM pool in response to these sequential acetate and nitrate enrichments by measuring changes in both concentrations of streamwater DOM, as well as changes in DOM fluorescence. Rapidly advancing
modeling approaches of three-dimensional excitation-emission matrices (EEMs) of organic matter fluorescence have demonstrated considerable utility for differentiating autochthonous and terrestrial sources of DOM forms hereby providing information on the biogeochemical processes involved in DOM cycling [e.g., Cory and Mcknight 2005, Stedmon and Markager 2005, Murphy et al. 2008].

3.1.1 Study Site

We conducted this experiment in the second-order reach of Walker Branch, located in Eastern Tennessee on the US Department of Energy’s Oak Ridge National Environmental Research Park (latitude 35°58’N, longitude 84°17’W). The stream drains an aggrading temperate forest, largely of oak-hickory composition [Johnson 1989]. The site is typical of the humid southern Appalachians, with a mean annual temperature of 14.5°C and mean annual precipitation of 1400mm [Mulholland 2004]. The head of the 250 m study reach was located approximately 50 m downstream of two weirs draining separate upstream subcatchments (East and West Forks). The stream is moderately oligotrophic, with inorganic N (NO₃-N + NH₄-N), inorganic P (SRP), and DOC concentrations rarely exceeding ~40 µg N L⁻¹, ~4 µg P L⁻¹, and ~1.5 mg C L⁻¹, respectively, during this time of year (Pat Mulholland, unpublished data). For more information regarding site location and characteristics, see Johnson and Van Hook [1989].
3.2 Methods

3.2.1 Solute Injections and Sampling

We conducted this experiment over two days (April 8-9) in the spring of 2009, immediately before leaf canopy closure. Previous studies at this site have shown that in-stream production during this time of year can be an important source of organic matter to the system [Mulholland 1992], and that the stream is often net autotrophic [production:respiration >1, Roberts et al. 2007] with in-stream production commonly limited by N availability [Rosemond et al. 1993].

On each day we performed a sequential enrichment experiment in which we elevated either bioavailable DOC (bDOC, Day 1) or nitrate concentrations (Day 2) by approximately 2, 4, 8, and 16 times their background concentrations. At each enrichment level plateau we collected streamwater samples from the thalweg at 7 stations distributed longitudinally along the study reach (-5m, 50m, 75m, 110m, 155m, 200m, 250m). The time to reach enrichment plateau was determined when the conductivity from a co-injected conservative tracer (Br⁻) stabilized.

We estimated discharge at the mid-point of the study reach at ~30 L sec⁻¹ on the day prior to the first kinetic enrichments (approximately 12hr beforehand) using a slug injection of NaCl [Gordon et al. 1992]. From long-term weekly streamwater chemistry monitoring at an upstream site, we estimated nitrate concentrations would be ~15 µg N L⁻¹ and DOC concentrations would be ~0.5 mg C L⁻¹. We used these estimates of
discharge and background concentrations to determine the rates of additions necessary to reach each of our target enrichment levels.

On each date all additions were performed using a single batch of nutrient solution. We increased the pump rate for each enrichment level in order to achieve target streamwater concentrations rather than making multiple solutions. The stock N solution was a 10 L volume of 82 g NO$_3$-N L$^{-1}$ as KNO$_3$, and contained 281 g Br L$^{-1}$ as KBr. The C-solution was a 10 L volume of 270 g C L$^{-1}$ as CH$_3$CO$_2$K with 281 g Br L$^{-1}$ as KBr.

Additions were begun at approximately 0900hr on both mornings. A peristaltic metering pump with a pump range from 5 – 60 mL min$^{-1}$ (Watson-Marlow 520N pump; Watson-Marlow Inc., Wilmington, MA, USA) was used for the additions. The pump addition rates were verified several times during each addition in the field using graduated cylinders to ensure accuracy and no drift through time. Approximately 1 hr following the initiation of each addition, we commenced sampling at the top of the reach and worked in a downstream direction. Not having to wait for the furthest downstream station to reach plateau allowed us to minimize the addition durations, and care was taken to avoid entering the stream or disturbing sediments as sampling occurred. The short addition durations were a priority to avoid potential changes in the stream benthic community associated with changes in the resource supply resulting from the enrichments.
Samples were field filtered through a Whatman GF/F 0.7 μm inline filter (Whatman Inc., Piscataway, NJ, USA) connected with Tygon R-1000 tubing (Saint-Gobain Ceramics & Plastics, Inc., Northboro, MA, USA) through a peristaltic pump driven by a cordless electric hand drill to minimize sampling time [Woessner 2007]. Samples were collected into two new HDPE bottles 250 mL and one 40 mL I-Chem vial (Thermo Fisher Scientific, Rockwood, TN, USA) at each station. Samples were acidified in the field within 30 min of collection with 1% 2M HCl by volume and kept on ice until returned to the laboratory (<12 hr), where they were stored at 4°C until analysis.

3.2.2 Reach Metabolism

Two sondes with conductivity and O₂ sensors (YSI Model 6920 equipped with YSI model 6562 DO probes; YSI Inc., Yellow Springs, OH, USA) were positioned in the middle (155 m) and lower end (250 m) of the study reach and were set to record measurements every 15 min. These sondes were deployed in well-mixed locations along the reach the day prior to the first additions and were recording data across the diel cycle throughout both days of additions. Immediately prior to deployment sondes were calibrated in water-saturated air. Reaeration rates (k_o2) were determined in the upper 100 m of the study reach, above the first sonde location, through co-injections of propane gas and concentrated NaCl solution. Background conductivity was recorded both above and below the study reach before the additions using a YSI Model 55 Conductivity Meter. Specific conductance was monitored two downstream stations from the injection
site at 1 min intervals until reaching plateau. Travel time was calculated as the distance between monitoring sites divided by the amount of time it took between observations of the maximum rate of change in conductivity at each site. Three replicate samples from each the upstream and downstream stations were collected for propane analysis. A 7 ml volume of streamwater was placed into 10 ml evacuated vacutainers and air-equilibrated headspace gas from each vacutainer was analyzed on a Hewlett Packard Model 5890 Series II gas chromatograph equipped with an Agilent HP-PLOT Al203 column and a flame ionization detector. Propane reaeration rates were estimated as the difference between the natural log values of the upstream and downstream propane concentrations divided by reach length, and O₂ reaeration rates were estimated to by multiplying the propane value by 1.39 [Roberts et al. 2007].

Metabolism was calculated as the change in DO concentrations over 15 min intervals [Roberts et al. 2007]. Areal estimates per day were calculated by summing the 15 min increment values of each GPP and ER and dividing by the mean stream depth. For more information regarding reach oxygen measurements or metabolism calculations, see Roberts et al. [2007].

### 3.2.3 Chemical Analysis

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations were measured on a Shimadzu TOC-VCPH total carbon analyzer with a TNM-1 nitrogen module (Shimadzu Scientific Instruments, Columbia, MD, USA). The
method detection limits (MDLs) for DOC and TDN were <0.01 mg C L⁻¹ and <0.003 mg N L⁻¹, respectively. These low detection limits were achieved by analyzing large (2ml) sample volumes. Solution concentrations of NO₃⁻, Br⁻, and acetate (CH₃CO₂⁻) were determined by ion chromatography (MDL 0.001 mg NO₃⁻N L⁻¹; Dionex ICS-2000 with eluent generation on an AS18 column, Dionex Corporation, Sunnyvale, CA).

Ammonium concentrations were determined using fluorescence detection following reaction with orthophthalaldialdehyde [Holmes et al. 1999]. The MDL for ammonium detection is 0.001 mg N L⁻¹. Concentrations of DON were determined by difference [DON = TDN – (NO₃⁻-N + NH₄⁺-N)]. Concentrations of non-acetate DOC (DOCₙ₋ₐ) during the acetate additions were also determined by difference [DOCₙ₋ₐ = DOC – CH₃⁺CO₂⁻]. Hereafter we always use ‘DOC’ to indicate the non-acetate, or native, DOC concentration and we report no DOC concentrations from the acetate treatments where the added acetate has not been subtracted from the total DOC measurement.

3.2.4 Organic Matter Fluorescence and Modeling

Fluorescence excitation-emission matrices (EEMs) were collected for each sample within 4 weeks of returning the samples to the lab. All EEMs were measured on a Fluoromax-3 fluorometer (Jovin Yvon Horiba, Edison, NJ, USA) at excitation wavelengths ranging between 240-450nm (at 10nm increments) and emission wavelengths ranging between 300-600nm (at 2nm increments) using methods outlined in Cory and McKnight [2005]. No samples had an absorbance greater than 0.04 at 300
nm in a 1-cm cuvette and, because of this, no efforts to correct for potential inner filter effects were made [Stedmon and Bro 2008]. All EEMs were corrected for instrument bias and normalized to the area under the Raman peak at 350nm. The complete dataset consisted of 70 EEMs. Parallel Factor (PARAFAC) Analysis was conducted in MATLAB (Mathworks, Natick, MA, USA) using the DOMFluor toolbox as outlined by Stedmon et al. [Stedmon and Bro 2008]. The PARAFAC model was validated via a 4-way split analysis according to Stedmon and Bro (2008)(Fig. 7).

In order to assure that the elevated nitrate or acetate concentrations did not alter the modeled fluorescence component values, both deionized water and streamwater samples were spiked with each two levels of KNO₃ (0.05 and 0.3 mg N L⁻¹) and two levels of CH₃CO₂K (0.5 and 3.5 mg C L⁻¹). EEMs were collected to ensure that adding these solutions did not produce fluorescence signals within the deionized water, or alter the fluorescence patterns in streamwater relative to the unamended samples.

### 3.2.5 C and N uptake calculations

Uptake lengths (Sₘ) for acetate and nitrate were calculated for enrichment levels as the inverse of the slope value of the ln-transformed equation describing the longitudinal decline in solute concentration per unit length (m) downstream of the addition point:

\[ \ln N_x = \ln N_0 - k_x \]  
\[ \text{Eq. 1} \]
where \( N_0 \) is the solute concentration above the addition point and \( N_x \) is the concentration \( x \) meters downstream, with \( k \) representing the per-meter rate of decline [Newbold et al. 1981]. Concentrations were corrected for groundwater dilution by dividing by the concentration of the conservative tracer, Br [Stream Solute Workshop 1990]. The vertical mass transfer coefficients were calculated as:

\[
V_i = \frac{(Q \cdot w)}{S_w} \quad \text{Eq. 2}
\]

where \( Q \) is discharge (L s\(^{-1}\)) and \( w \) is mean stream width (m). Areal uptake rates (U) were calculated as:

\[
U = V_i \cdot C \quad \text{Eq. 3}
\]

where \( C \) is the background solute concentration at the head of the reach.

### 3.2.6 Statistical Analyses

Data for the station upstream of the addition point were analyzed separately from downstream stations in order to isolate treatment effects associated with the enrichments. For the upstream station data, simple linear regression (SLR) was used to assess potential changes in either streamwater concentrations or DOM fluorescence throughout the day based on sampling time, since each level of addition occurred sequentially within a day. Where upstream values exhibited a significant change, all downstream values were centered (via subtraction) to the value of the upstream station for each enrichment level to isolate diurnal effects not associated with the solute manipulations. SLR was used to assess longitudinal trends in solute concentrations and
DOM fluorescence. Where no significant longitudinal trends were observed at any level of enrichment, mean downstream concentrations or fluorescence component signals across enrichment levels were assessed using a one-way analysis of variance (ANOVA) with enrichment level as the factor. Tukey’s honestly significant difference (HSD) post-hoc test was used to determine differences among enrichment levels when significant differences existed.

3.3 Results

3.3.1 PARAFAC Model: Fluorescence Components

PARAFAC modeling revealed 3 distinct fluorescent components. Component 1, which accounted for the greatest amount of variation in EEMs across samples, had a dominant peak with maximum excitation to emission values (Exmax/Emmax) of 260/480 nm and a second smaller peak at Exmax/Emmax = 350/480 nm (Fig. 8A). Component 2 also has two distinct peaks, though both peaks (peak 1: Exmax/Emmax 250/420 nm; peak 2: Exmax/Emmax = 320/420 nm) were blue shifted (i.e., located at lower wavelengths) relative to Component 1 (Fig. 8B). The final component, which captured the least amount of variation across EEMs, had a peak at Exmax/Emmax = 270/355 nm (Fig. 8C). We term and will further refer to Components 1, 2, and 3 as being ‘Terrestrial-like’, ‘Autochthonous-like’, and ‘Amino Acid-like’, respectively, based on the similarity of these components to previously characterized components in earlier studies (Table 2)(See Discussion). It is
Figure 7: Excitation-emission plots (left) of the 3 fluorescence components identified in this study. Line plots (right) represent the split-half validations of the PARAFAC model.
Table 2: Characteristics of the fluorescence components identified within the PARAFAC model, as well as a brief description of each component as they have been described by others.

<table>
<thead>
<tr>
<th>PARAFAC Component</th>
<th>Primary Peak</th>
<th>Secondary Peak</th>
<th>Description</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EX_{max} (nm)</td>
<td>EM_{max} (nm)</td>
<td>EX_{max} (nm)</td>
<td>EM_{max} (nm)</td>
</tr>
<tr>
<td>Component 1</td>
<td>260</td>
<td>480</td>
<td>350</td>
<td>480</td>
</tr>
<tr>
<td>Component 2</td>
<td>250</td>
<td>420</td>
<td>320</td>
<td>420</td>
</tr>
<tr>
<td>Component 3</td>
<td>270</td>
<td>355</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Important to note that component scores indicate the relative concentration of the fraction of fluorescent DOM represented by each component. Because the components cannot be attributed to specific organic compounds with known quantum efficiencies, component scores cannot be converted to actual concentrations [Stedmon et al. 2003].

We did not detect background fluorescence of either acetate or nitrate when added at low (0.05 mg N L^{-1} and 0.5 mg C L^{-1}) or high (0.3 mg N L^{-1} and 3.5 mg C L^{-1}) concentrations to deionized water, within the excitation-emission space quantified by our three components. Slight fluorescence was measured in the Ex/Em 240/350 nm region (data not shown) for the highest enrichment levels (0.3 mg N L^{-1} and 3.5 mg C L^{-1}) likely due to fluorescence from impurities in these stock solutions. We did not observe any detectable shifts in component concentrations or distributions to streamwater EEMs upon addition of acetate or nitrate.
Table 3: Nitrate, acetate, and non-acetate DOC (DOCN) concentrations, as measured at the first downstream station from the addition point, for each level of addition for both acetate and nitrate additions. Spiraling metrics are listed for each acetate and nitrate, at each level of enrichment.

<table>
<thead>
<tr>
<th>Enrichment Level</th>
<th>NO3-N (μg/L)a</th>
<th>CH3CO2- (mg/L)a</th>
<th>DOCN (mg/L)a,b</th>
<th>kL (m⁻¹)</th>
<th>Sw (m)</th>
<th>Vf (mm/min)</th>
<th>U (mg/m² min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate Additions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0x</td>
<td>10.4</td>
<td>0</td>
<td>0.402</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2x</td>
<td>13.8</td>
<td>0.431</td>
<td>0.365</td>
<td>-0.0031</td>
<td>322.6</td>
<td>1.5</td>
<td>0.469</td>
</tr>
<tr>
<td>4x</td>
<td>10.7</td>
<td>0.982</td>
<td>0.307</td>
<td>-0.0013</td>
<td>769.2</td>
<td>0.6</td>
<td>0.450</td>
</tr>
<tr>
<td>8x</td>
<td>9.8</td>
<td>2.118</td>
<td>0.229</td>
<td>-0.0006</td>
<td>1666.7</td>
<td>0.3</td>
<td>0.491</td>
</tr>
<tr>
<td>16x</td>
<td>11.1</td>
<td>4.030</td>
<td>0.365</td>
<td>-0.0001</td>
<td>10000.0</td>
<td>&lt;0.1</td>
<td>0.162c</td>
</tr>
<tr>
<td>Nitrate Additions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0x</td>
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<td>0.359</td>
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<td>-</td>
</tr>
<tr>
<td>2x</td>
<td>39.4</td>
<td>-</td>
<td>0.423</td>
<td>-0.0027</td>
<td>370.4</td>
<td>1.3</td>
<td>0.033</td>
</tr>
<tr>
<td>4x</td>
<td>77.9</td>
<td>-</td>
<td>0.393</td>
<td>-0.0021</td>
<td>476.2</td>
<td>1.0</td>
<td>0.052</td>
</tr>
<tr>
<td>8x</td>
<td>143.3</td>
<td>-</td>
<td>0.440</td>
<td>-0.0012</td>
<td>833.3</td>
<td>0.6</td>
<td>0.066</td>
</tr>
<tr>
<td>16x</td>
<td>318.1</td>
<td>-</td>
<td>0.421</td>
<td>-0.0004</td>
<td>2500.0</td>
<td>0.2</td>
<td>0.047</td>
</tr>
</tbody>
</table>

a = Concentrations as measured at first downstream station (50m below point of addition).
b = DOC concentrations reported for acetate additions are for non-acetate DOC (see Methods: Chemical Analysis)
c = This value was excluded from any results reported in the text due to dissimilarity (probable inaccuracy due to enrichment level).
3.3.2 Pre-Enrichment and Upstream Station Data

Background concentrations of DOC (~0.375 mg C L\(^{-1}\)) and nitrate (~10.5 µg N L\(^{-1}\)) (Table 3) were similar to the values we expected based on the historical long-term data [Mulholland 2004]. DOC concentrations at the upstream station increased significantly during both days of additions by ~0.100 mg C L\(^{-1}\) in each case (Fig. 8A,B), representing an increase of ~25% throughout the day. DON concentrations did not exhibit a significant diurnal pattern at the upstream station on the first day when acetate was added (Fig. 8C). However, DON concentrations at the upstream station declined significantly on day two, though this decline was modest (~0.01 mg N L\(^{-1}\)) (Fig. 8D).

Similar to DOC concentrations, at the upstream sampling station (5 m above addition site) all fluorescence components exhibited an increase in concentration over the course of each day (Fig. 9). This increase was significant for both the autochthonous and terrestrial-like components, but only marginally significant (0.05 < \(P\) < 0.15) for the amino acid-like component. There were no significant longitudinal patterns in any of the fluorescence components at the downstream stations under background conditions (i.e., before either acetate or nitrate was added) (Fig. 10).

The metabolism data exhibit a clear diel pattern with in stream O\(_2\) production peaking at approximately 1400 hr. GPP rates were 2.29 and 2.21 g O\(_2\) m\(^{-2}\) d\(^{-1}\) during the acetate and nitrate additions, respectively. While significant amounts of C were being
Figure 8: Concentration data as measured at the sampling station above the point of addition (black squares) and averaged across downstream sampling stations (gray circles). Where present, lines indicate significant trend in concentration data at the upstream station between addition levels based on simple linear regression. For downstream station data, error bars indicate ± 1 S.E. and letters indicate significant differences between addition levels based on Tukey’s HSD (alpha = 0.05).
fixed within the stream channel each day, the reach was net heterotrophic (ER > GPP) with ER rates of -5.98 and -6.47 g O₂ m⁻² d⁻¹.

3.3.3 Acetate Enrichment Effects

During the enrichment, acetate concentrations, as measured at the first downstream station from the addition point, were very close to targeted enrichment concentrations (Table 3). The longitudinal decay rate (ki) declined with increasing rates of acetate addition and demand appeared to be saturated (ki=0) at the highest level of acetate addition (Table 3). The vertical mass transfer rate (Vi) declined with increasing enrichment concentrations and areal rates of acetate uptake ranged between 0.450 - 0.491 mg C m⁻² min⁻¹ (Table 3).

Acetate enrichment did not result in any measurable changes in the concentrations of non-acetate DOC or DON concentrations within the treatment reach. Further, when averaged across all downstream stations, mean DOC concentrations did not change significantly across enrichment levels, but had increasing variation (increase in SE) with increasing enrichment level (Fig. 8A). DON concentrations averaged across downstream stations did not show any significant patterns across acetate enrichment levels (Fig. 8C).

In contrast to the bulk DOM pool, acetate additions led to longitudinal increases in the amount of the terrestrial-like fluorescence component (Fig. 10, A-E). The autochthonous-like component did not exhibit a longitudinal pattern during any of the
Figure 9: (A-F) There were significant increases (indicated by solid lines), based on simple linear regression, in the fluorescence intensities of each the terrestrial- and autochthonous-like DOM components throughout the day during both the acetate and nitrate additions at the upstream station. Increases in the amino acid-like component were marginally significant (0.05 < P-value < 0.15; indicated by dashed lines). (G-H) Net metabolic fluxes for each day of solute additions. Gray shaded areas indicate approximate time periods during which additions were performed.
Figure 10: Longitudinal trends in fluorescence components in response to the acetate and nitrate additions. Upstream values (open diamonds) are always equal to zero because all data were corrected for the diurnal effect (Fig. 4) by subtracting the initial upstream values. Lines do not always represent significant trend, but are included to aid in visualization of longitudinal patterns. Where regressions are significant, slope values and P-values are indicated on each plot.
acetate additions, with downstream values at all enrichment levels being similar to the upstream station (Fig. 10, F-J). The amino acid-like component showed no clear longitudinal pattern in response to acetate additions and, while most downstream stations had a greater concentration of this component relative to the upstream station, there was large variation in values (Fig. 10, K-O).

3.3.4 Nitrate Enrichment Effects

During the nitrate enrichment experiment, nitrate concentrations at the first downstream station were close to each of our targeted values. At the highest level of enrichment nitrate uptake appeared to be saturated ($k_r$-0) (Table 3). The vertical mass transfer rate ($V_f$) declined sharply with each level of addition, and areal uptake of nitrate ranged between 0.033-0.066 mg N m$^{-2}$ min$^{-1}$ (Table 3).

There were no significant longitudinal trends in either DOC or DON across downstream stations during any level of nitrate enrichment. However, when averaged across all downstream stations, mean DOC concentrations increased significantly throughout the day with each level of enrichment, with this increase being very similar to the increase observed in DOC concentrations at the upstream station (Fig. 8B). DON concentrations, when averaged across all downstream stations, also exhibited a significant increase with each level of enrichment, which was not observed in the data from the upstream sampling station (Fig. 8D).
Both the terrestrial-like and autochthonous-like fluorescence components exhibited an abrupt increase at the first downstream station during the nitrate additions that was not observed during the acetate additions (Fig. 10, A-J). Further, this effect was similar at all levels of nitrate enrichments. Unlike the terrestrial-like component, which showed no clear longitudinal pattern following the initial increase, the autochthonous-like component showed a small longitudinal decline downstream during each addition (Fig. 10, F-J). The amino acid-like component was highly variable across the downstream stations though observed values were typically greater relative to the upstream station, as well as during the acetate additions (Fig. 10, K-O).

3.4 Discussion

3.4.1 PARAFAC Modeling: Fluorescence Components

The fluorescence components identified in the PARAFAC model have been ascribed to different DOM sources or functional forms by a large number of studies which have investigated the optical properties of DOM throughout a variety of freshwater and marine ecosystems. The terrestrial-like component (Component 1; Table 2, Fig. 6A), having lower excitation energies (i.e., longer excitation wavelengths), is characteristic of molecules having a high proportion of aromatic carbon that is largely exclusive to lignin and other terrestrial plant derived precursor material [Coble 1996, Cory and Mcknight 2005]. The autochthonous-like component (Component 2; Table 2,
Fig. 6B) has a clear shift in fluorescence peaks towards lower excitation and emission wavelengths. This shift is attributed to lower aromatic carbon content and is indicative of the less-conjugated structure of recently synthesized DOM from autotrophic production [Coble 1996]. This component has historically been referred to as marine-like because it was first identified in open ocean waters [Coble 1996], but has since been found in a variety of marine and freshwater ecosystems where autochthonous production is an important source of DOM [Murphy et al. 2008]. The amino acid-like component (Component 3; Table 2, Fig. 6C) is easily distinguishable from the first two components and has been closely associated with the fluorescence properties of tyrosine and tryptophan [Cory and Mcknight 2005].

3.4.2 Diurnal Patterns

Previous work at this site has demonstrated that autochthonous production in Walker Branch can be relatively high during the spring prior to tree canopy closure. Continuous metabolism measures have indicated that the rate of carbon fixation (gross primary production) often exceeds community respiration (i.e., production : respiration > 1) before leaf-out [Roberts et al. 2007], and that the standing crop of benthic algae can be high during this time or year [Hill et al. 2001]. Our finding that DOC concentrations increase during daylight hours by approximately 0.1 mg C L⁻¹ is consistent with previously reported values [Mulholland 1992] and represents a ~25% increase over a 6 hr period. This diurnal increase in DOC concentrations, caused by increases in primary
production within the stream channel, suggests that the chemical composition of DOM, and the ratio of autochthonous to terrestrial DOM sources, is also likely to increase during the daytime. Other studies in temperate forest ecosystems in this region have similarly found that spring algal production can lead to diurnal increases in DOC prior to tree canopy closure [Kaplan and Bott 1982]. That we did not observe a commensurate diurnal increase in DON concentrations with the increase in DOC concentrations is not unexpected. Given the magnitude of the DOC increase, even if the DOM released by the increased algal production had a low molar C:N ratio (i.e., was N-rich), say C:N = 15, the expected increase in DON concentrations would be less than 0.006 mg N L⁻¹, which is near our analytical detection limits.

The experiments reported here provide new insights into these diurnal patterns in DOM through measuring changes in DOM fluorescence signatures. The increase in the autochthonous-like component during the course of each day (Fig. 7, B-C) is consistent with the metabolism data and the diurnal increase in DOC concentrations (Fig. 8, A-B). Likewise, other studies have observed increases in amino acid-like fluorescence with increases in autochthonous production [Stedmon and Markager 2005] and the marginally significant increase in the amino acid-like component during the daytime (Fig. 9, D-E) is also likely associated with diurnal changes in the rate of algal production. The diurnal increase in the terrestrial-like component (Fig. 9, A-B), however, is not immediately intuitive. Certainly increasing light availability is unlikely
to alter the rate of hydrologic transport of terrestrial DOM to the stream channel. Photo-
chemically DOM with these fluorescence characteristics have been shown to readily
photo-degrade [Opsahl and Benner 1998, Stedmon and Markager 2005] and, based on
light patterns alone, we would predict a decrease in this terrestrial-like component
during the daytime. Thus, because we cannot hypothesize a potential abiotic
mechanism that would result in this diurnal increase in the terrestrial-like component, it
is possible that this increase is biotically driven. Since terrestrial material is necessarily
derived from outside of the stream channel, an increase in this component suggests the
potential for decreased in-stream demand (i.e., net release) for terrestrial DOM during
the day. Given the response of the terrestrial-like component is similar to the diurnal
increase observed in the autochthonous-like component, displacement of demand for
terrestrial DOM by increased autochthonous DOM availability is one possible
explanation. We find further support for such close coupling of autochthonous and
terrestrial DOM dynamics in the results from the acetate and nitrate manipulations (See
below).

3.4.3 Effects of Acetate and Nitrate Enrichments

Despite nearly saturating the reach with acetate and nitrate (i.e., $k$ values at
highest additions close to zero, Table 3), DOC and DON concentrations remained
remarkably little affected by the enrichments. During the acetate additions, DOC
concentrations showed no clear response to enrichment other than greater variation at
higher levels of addition (Fig. 8A). This lack of trend could, in part, have resulted from needing to subtract the acetate concentrations from these downstream stations to estimate the concentration of non-acetate DOC (See Methods: Chemical Analysis). With each increased level of enrichment, as acetate begins to dominate the total organic carbon pool, the percent error for each the total organic carbon and acetate measurements becomes larger relative to the absolute concentration of DOC. These limitations of estimating the concentration of a solute by difference from multiple independent measures have been detailed elsewhere [e.g., Lee and Westerhoff 2005] and likely contribute to the increasing variation we observed at higher enrichment levels.

During the nitrate additions, DOC concentrations increased at the downstream stations (Fig. 8B). This increase, however, is highly similar to the increase observed at the upstream station (Fig. 8B) and, because of this, is more likely the result of the diurnal effect related to in-stream production rates than a nitrate enrichment effect. DON concentrations also increased across downstream stations with each increase in nitrate enrichment level and, because this effect was not observed upstream or during the acetate additions (Fig. 8D), likely represents an increase in DON production resulting from greater nitrate availability. This indicates that the stream is, to some degree, limited by N availability and is consistent with previous findings that spring algal production removes inorganic N from the water column [Mulholland 1992, 2004,
Roberts and Mulholland 2007] and further suggests that a portion of this DIN uptake is rapidly converted to DON exports.

The fluorescence data are much more sensitive to changes in the DOM pool than are bulk concentration measurements. While longitudinal trends downstream of the addition point could not be resolved in DOC or DON concentrations, the terrestrial-like and autochthonous-like DOM components exhibited clear responses (Fig. 10, A-J). Thus, systematic changes in the chemical composition of the DOM pool occurred despite lack of measurable changes in the bulk DOM pool.

The longitudinal increase in terrestrial-like fluorescence during the acetate additions indicates that we were able to displace some ecosystem demand for terrestrial DOM through the addition of a more labile C source (H1A, Fig. 6B) (Fig. 8A). Autochthonous DOM fluorescence did not change as a result of the acetate additions (H1B, Fig. 6B). The lack of change in the autochthonous-like DOM fluorescence during the acetate additions (Fig. 10, F-J) suggests that any algal-derived DOM contributing to heterotrophic metabolism may be more bioavailable, or in some way serves as a more attractive substrate for microbial uptake, than acetate. This is not surprising as algal biomass and exudates are commonly considered to be highly bioavailable [Sun et al. 1997].

In contrast to the acetate additions, when we added nitrate we observed an abrupt increase in the fluorescence intensities of both the terrestrial-like and
autochthonous-like components immediately downstream of the addition point (Fig. 10, A-J). The increase in the autochthonous-like component was expected since algae are commonly limited by N-availability during this time of year (H2a, Fig. 6C) [Mulholland and Rosemond 1992, Rosemond 1994]. It is also interesting to note that we observe no additional effect of increased enrichment beyond the first doubling of nitrate concentrations (Fig 10, F-J). Walker Branch is highly oligotrophic and algae are very near to co-limitation by both N and P [Rosemond 1994], and the first doubling of N availability may have alleviated N-limitation of algal growth.

While increasing nitrate availability may also affect heterotrophic metabolism, especially if N concentrations limit heterotrophic activity under background conditions, the similarity in the response of the terrestrial-like component to that of the autochthonous-like component is striking (Fig. 10, A-J). Both of these components exhibit an abrupt increase immediately downstream of the addition point, and we observed few differences in response between the lowest and highest levels of nitrate enrichment. These findings are consistent with the ambient diurnal patterns observed at the upstream site in which the diurnal increases in GPP and the autochthonous-like components were mirrored by unexpected increases in the terrestrial-like component. Taken together, these results suggest a very tight coupling of these two distinct DOM sources such that terrestrial DOM consumption is measurably reduced during times of high autochthonous DOM production (H2b, Fig. 6C).
3.4.4 Differentiating DOM Subcomponents: Implications

Our understanding of the ecological role of DOM within a given stream ecosystem depends on determining why one portion of the DOM pool is consumed, while another resists uptake and is transported downstream. Unlike other solutes, which are typically single element ions or specific molecules, the dynamics of the DOM pool cannot be expressed solely as a function of relative availability, or concentration, but must also take into consideration quality, or DOM chemical composition. Our data demonstrate that we can elicit changes in DOM composition independently of changes in concentration by altering the relative availabilities of labile C and inorganic N.

As opposed to other studies of DOM biogeochemistry in stream ecosystems, which have typically relied on adding some form of DOM and measuring its rate of uptake or removal from the water column, our focus was on assessing the response of native DOM to labile C or inorganic N availability rather than the fate of either the acetate or nitrate being supplied. While fluorescence measures have limited quantitative information since DOM fluorescence intensities cannot be directly translated into concentration values [Stedmon and Bro 2008], they provide insight into the structuring of the interactions between different native DOM subcomponents that cannot be gained by observing uptake rates of specific DOM molecules. While we are currently limited in our abilities to quantify qualitative changes in the native DOM pool, both fluorescence
data [Stedmon et al. 2003], as well as a broad variety of alternative analytical approaches [e.g., Seitzinger et al. 2005, Abdulla et al. 2010], are rapidly closing this gap.

3.5 Conclusions

The importance of DOM to stream ecosystem trophic structure and function has been well demonstrated through decades of research and is widely appreciated. The chemical heterogeneity of the DOM pool, however, has made it an elusive entity to study and the majority of research has been limited to treating DOM as a single bulk pool, with little ability to resolve differences in contributing subcomponents that exhibit distinct dynamics. Through a series of sequential, progressive, kinetic enrichments of acetate and nitrate we have shown that we could differentially alter the processes controlling the release of terrestrial and autochthonous DOM to the stream water column, and that we can assess the responses of each of these different DOM subcomponents through the use of high-sensitivity measures of DOM fluorescence. For Walker Branch, terrestrial-like DOM is under direct control of heterotrophic metabolism, while autotrophic production (and the release of autochthonous DOM to the water column) increases with increasing light and N availability. Our results imply a tight coupling between autochthonous and terrestrial DOM cycling, with increases in in-stream production displacing heterotrophic demand for terrestrial DOM. The ability to alter the native DOM pool by changing resource supply ratios provides insight into the
different processes controlling distinct DOM subcomponents, and may be a useful approach for future experimental investigations of ecosystem DOM dynamics.
4. Long Term Biogeochemistry of a Forested Stream: Walker Branch, Tennessee

4.1 Introduction

Stream chemistry patterns and input-output budgets have long been used to infer watershed biogeochemical dynamics and ecological trends. Understanding how changes in temperature and precipitation patterns will affect biogeochemical cycles and the movement of elements within and between ecosystems is a topic of increasing importance [White and Blum 1995, Mitchell et al. 1996, Mulholland et al. 1997, Murdoch et al. 2000, Davidson and Janssens 2006]. While changes in climate patterns may have a variety of manifestations [Vorosmarty et al. 2000, Arnell 2004], the effects on watershed biogeochemical cycles fall within two basic domains: (1) changes in element cycling process rates (i.e., changes in temperature or moisture regimes that alter biological or geochemical reaction rates) and (2) changes in the volume or dynamics of water flux through catchments.

While the flux of solutes cannot occur independently from the flux of water, changes in hydrologic and biogeochemical processes can have separate effects on watershed input-output budgets. For example, increases in temperature can increase mineralization rates of soil carbon [Kirschbaum 1995, Davidson and Janssens 2006] and organically bound nutrients [Rustad et al. 2001, Knoepp and Swank 2002], as well as alter rates of geochemical weathering of parent materials [White and Blum 1995]—all of which can occur independently of changes in catchment hydrology. Likewise, changes
in the annual distribution, frequency, and intensity [e.g., Boyer et al. 1997, Creed and Band 1998, Inamdar et al. 2006] of precipitation can alter watershed input-output budgets even if there are not significant changes in either biogeochemical process rates or element supply and demand. Rising temperatures in many regions of the world will lead to altered precipitation patterns and altered biogeochemical processes, which will affect biological activity within watersheds as well as the rates and pathways by which water moves through watersheds.

Here we present approximately 20 years (1989 - 2008) of weekly streamwater chemistry data for several major dissolved solutes in the West Fork of the Walker Branch watershed (hereafter Walker Branch) in eastern Tennessee, USA. Walker Branch is distinct from many other long-term watershed monitoring sites in that it has a highly weathered and exceptionally deep soil profile [-30 m; Johnson 1989]. Catchments with deep soils generally have large groundwater volumes and may have different hydrologic responses to climate change than those with shallow soils [Porporato et al. 2004]. While Walker Branch is distinct among other small watershed monitoring sites, it is not distinct from large regions of the landscape—similar areas of low-relief Ultisols are common throughout the southeastern US, as well as many other locations globally (Brady and Weil 2002). Thus, data from Walker Branch have the potential to extend our understanding of climate effects on watershed biogeochemistry to these areas where
results from other small watershed monitoring programs may not apply. Our objectives were:

1. To identify long-term changes in climate variables (temperature and precipitation), as well as catchment hydrology (runoff, evapotranspiration, and base- versus storm-flow contributions to runoff).
2. To identify and, where possible, explain potential causes for seasonal (within year) and long-term (inter-annual) patterns in streamwater concentration and flux for major solutes and nutrients, as well as long-term changes in annual watershed input-output budgets.
3. To assess the relative importance of changes in biogeochemical process rates versus changes in catchment hydrology that arise from changing climate patterns for this site.
4. To contrast results for Walker Branch with other long-term watershed records.

4.1.1 Study Site

The West Fork of Walker Branch is a forested watershed drained by a first-order stream on the U.S. Department of Energy’s Oak Ridge National Environmental Research Park (latitude 35°58’N, longitude 84°17’W)(Fig. 11). The climate is typical of the humid southern Appalachian region, with mean annual temperature of 14.5°C and mean annual precipitation of ~135cm, distributed relatively evenly throughout the year.
[Johnson 1989]. The West Fork of Walker Branch drains a 38.4 ha catchment underlain by siliceous dolomite that has weathered to form deep soils with abundant chert [Mcmaster 1963]. Soils are acidic (pH 4.2 – 5.0) and low in exchangeable bases, nitrogen, and phosphorus [Johnson 1989]. Surface infiltration rates are high resulting in little overland flow even during the most intense storms [Luxmoore 1983]. Although surface soils have high hydraulic conductivity due to high macroporosity, hydraulic conductivity declines sharply in the B horizon and zones of perched saturation develop in the surface soil layers producing rapid lateral subsurface flow during larger storms [Wilson et al. 1991, Mulholland 1993]. Watershed vegetation consists of a second-growth deciduous forest dominated by chestnut oak (Quercus prinus), tulip poplar (Liriodendron tulipifera), red maple (Acer rubrum), and white oak (Quercus alba) [Kardol et al. 2010].

The 1st-order stream draining the West Fork Watershed flows primarily over bedrock with shallow accumulations of cobble, gravel, and fine organic sediments filling bedrock depressions. The stream is perennial and fed by several springs with relatively constant discharge and chemical composition [Mulholland 1993]. Discharge is monitored at a site approximately 300 m downstream from the headwaters at a 120° v-notch weir with 15-minute stage recordings. Precipitation in the catchment is recorded hourly at two locations using weighing bucket collectors (prior to 1999) and electronic tipping bucket collectors thereafter. Air temperature data were acquired from the
climate station operated by NOAA’s Atmospheric Turbulence and Diffusion Laboratory at a site approximately 5 km from Walker Branch (http://www.atdd.noaa.gov/?q=node/20).

![Map of Walker Branch watershed.](image)

Previous hydrologic studies of Walker Branch indicate that there is groundwater transfer from the East to the West Fork watershed, primarily during base flow. Conservative tracers added to the East Fork stream have appeared in springs in the West Fork catchment within a few days, empirically demonstrating transfer from the East
Fork to West Fork (P. J. Mulholland, unpublished data). Using streamflow data and a catchment hydrologic model that included evapotranspiration, Luxmoore and Huff [1989] concluded that ~ 30% of the baseflow originating in the East Fork is annually transferred to the West Fork. Wilson et al. [2001] compared annual evapotranspiration rates determined from eddy covariance measurements with those determined from catchment water balance measurements (the difference between annual precipitation and annual runoff) and found good agreement for the West and East Fork catchments combined. However, comparison of annual runoff rates from the two catchments indicates that West Fork runoff is about 25% higher as a result of interbasin transfer. Because of this, solute exports measured in the West Fork are higher than would occur without interbasin water transfer. The two watersheds are very similar in vegetation, geology, and soils.

The water year for this site, or that successive 12-month period in which there exists the strongest correlation between discharge and precipitation in the long-term hydrologic record [Likens and Bormann 1995], begins May 1 and ends April 30. All data presented will be on a water year basis unless otherwise noted, with reference to each water year coinciding with the calendar year in which it began (e.g., the 1989 water year begins May 1, 1989 and ends April 30, 1990).
4.2 Methods

4.2.1 Sample Collection and Analytical Chemistry

Streamwater samples were collected weekly beginning in January 1989 about 60 m upstream from the weir. Samples were collected on Mondays between 1000 and 1200 hours through 31 Mar 1992 and on Tuesdays between 1000 and 1200 thereafter. Measurements of water temperature and specific conductance (Orion model 122) were made in situ. Samples for chemical analysis were collected in well-rinsed polyethylene bottles, returned to the laboratory and filtered (Nuclepore polycarbonate filters, 0.4-μm pore size) within 3 hours of collection. Measurements of pH were made by electrode on unfiltered samples prior to atmospheric equilibration. Alkalinity was determined by fixed endpoint titration to pH of 4.5.

Samples for major cations were acidified after filtration (0.5% HNO₃) and concentrations of Ca²⁺ and Mg²⁺ were determined by inductively coupled plasma emission spectrometry. Samples for SO₄²⁻ were refrigerated after filtration and analyzed by ion chromatography. Samples for N and P were frozen after filtration until analysis could be performed (usually within 3 months). Concentrations of NH₄⁺ were determined by phenate colorimetry [American Public Health Association, APHA 1992] and NO₂⁻ + NO₃⁻ by Cu-Cd reduction followed by azo dye colorimetry [Apha 1992], both using an autoanalyzer (Bran Lubbe TRAACS Model 800 or Seal Analytical Model AA3). Because stream water was always relatively high in dissolved oxygen concentration (> 6 mg/L) and because spot checks revealed minimal NO₂⁻ concentrations (< 2 μg N/L),
hereafter we refer to measurements of NO$\text{}_2$ + NO$\text{}_3$ as NO$\text{}_x$. Concentrations of total soluble nitrogen (TSN) were determined by UV/persulfate oxidation followed by NO$\text{}_x$ analysis as described above prior to 1999 and by high temperature combustion (Shimadzu TNM-1) thereafter. Concentrations of soluble reactive phosphorus (SRP) were determined by the ascorbic acid-molybdenum blue method [APHA 1992] using a 10-cm spectrophotometer cell to achieve low detection limits. Concentrations of total soluble phosphorus (TSP) were determined by persulfate digestion followed by SRP analysis.

There were 15 dates over the 20-year period for which weekly samples were not collected, including a 5-week period in Sep-Oct 1995. There was a nearly 14-month interruption in collection of samples for analysis of major anions and cations between November 1989 and 21 Dec 1990. There was a nearly 6-year interruption in collection of DOC samples from January 1997 to October 2002. The TSN record does not begin until May 1996.

Atmospheric wet deposition is measured in Walker Branch Watershed as part of the National Atmospheric Deposition Program (NADP) National Trends Network (NTN) (http://nadp.sws.uiuc.edu/sites/siteinfo.asp?id=TN00&net=NTN).

### 4.2.2 Trends in Temperature, Precipitation, Runoff and Evapotranspiration

Simple linear regression was used to assess trends in climate and hydrologic variables on both an annual (water year) and seasonal (January-March) basis. Because
collection of temperature, precipitation and runoff data began in 1969, while the weekly streamwater chemistry record we present did not start until 1989, trends in climate and hydrology variables were assessed over both the full record (1969-2009), as well as the portion of the record overlapping with streamwater chemistry measurements (1989-2009). Runoff was separated into base- and quick-flow components using the smoothed minima technique [Gustard et al. 1992], which involves estimating baseflow contributions as a simple linear interpolation between the minima of each 5-day non-overlapping segment of the record. Evapotranspiration rates for the 41-year period were estimated as the difference between precipitation and runoff for the combined East and West Forks due to the known interbasin water transfer between the two subcatchments [Wilson et al. 2001].

4.2.3 Modeling Solute Concentrations and Flux Estimation

We use multiple regression models fitted to the weekly chemistry data to estimate solute fluxes on a daily basis using the continuous runoff record with 15 min resolution. For hydrochemical solutes, or those solutes with concentrations that are predominantly controlled by discharge (Ca$^{2+}$, Mg$^{2+}$, SO$_4^{2-}$), we include terms that account for long-term linear trend, contemporaneous and antecedent streamflow conditions, and sin/cos terms to capture any seasonality that might not be accounted for in the hydrologic terms alone:
\[ \ln C_i = \beta_0 + \beta_1 T_i + \beta_2 \ln(Q) + \beta_3 \ln(Q^2) + \beta_4 Q_{ANT} + \beta_5 \sin(2\pi T_i) + \beta_6 \cos(2\pi T_i) + \epsilon_i \]  
Eq. 4

where \(\ln\) is the natural logarithm, \(C_i\) is the instantaneous solute concentration for the \(i^{th}\) observation, \(T_i\) is decimal time in years, \(Q\) is the instantaneous streamflow value, \(Q^2\) is the square of the instantaneous streamflow value, and \(Q_{ANT}\) is the mean of the \(Q\) values for the 3 years preceding and inclusive of the current observation subtracted from the mean \(Q\) value for the 20-year record, the sin and cos functions of decimal time describe seasonal variation according to a single annual cycle, \(\epsilon_i\) is the model error assumed to be independent and normally distributed about a mean of zero, and \(\beta_0, \beta_1, \beta_2, \ldots \beta_6\) are estimated regression coefficients. This approach is generally similar to that used by Alexander and Smith [2006] in their analysis of long-term trends in N and P concentrations for US rivers.

For the nutrient solutes (NH\(_4^+\), NO\(_3^-\), TSN, SRP, TSP, and DOC) two additional sin/cos functions with one-half year periods were added to the model to account for asymmetrical and semi-annual in-stream biological processes associated with autumn leaf input (which peaks in early November) and decomposition and spring algal blooms (which usually peaks in early April) shown to be important as controls on concentrations in previous studies [Mulholland 2004, Roberts and Mulholland 2007]. Therefore, the nutrient solute model is as follows:

\[ \ln C_i = \beta_0 + \beta_1 T_i + \beta_2 \ln(Q) + \beta_3 \ln(Q^2) + \beta_4 Q_{ANT} + \beta_5 \sin(2\pi T_i) + \beta_6 \cos(2\pi T_i) + \beta_7 \sin(4\pi T_i) + \beta_8 \cos(4\pi T_i) + \epsilon_i \]  
Eq. 5
Although the sin and cos terms include seasonal phenomena, they represent phenological events (spring algal bloom prior to leaf emergence, summer period of low energy inputs in the form of organic matter and light, and fall input of leaves).

We further explored a variety of terms in an attempt to capture any temperature effect on solute concentrations independent of the seasonal and hydrologic effects already included in the models. We only assessed potential temperature effects within the models for NO₃ and SRP because we expect biological processes to exhibit greater temperature dependence than geochemical processes, and N and P are the two most biologically limiting nutrient solutes. We assessed the significance of 3 candidate temperature terms: (1) a one-week antecedent air temperature anomaly, (2) a one-month antecedent air temperature anomaly, and (3) the instantaneous stream temperature anomaly. The air temperature terms were calculated as the mean of the average daily air temperature values for the period (one week or one month) immediately prior to sampling, divided by the mean of the same period (based on Julian day) over the 20-year record. The stream temperature term was calculated as the recorded stream temperature at the time of sampling divided by the mean streamwater temperature for that Julian day over the 20-year record. We do not include these terms in equation 2 because none of these temperature terms were found to explain a significant portion of the remaining variation in stream NO₃ or SRP concentrations and were not included in the final analyses.
Prior to fitting the MLR models we removed observations based on results from outlier (t-tests with Bonferroni correction) and leverage (Cook’s Distance) tests [Fox 1997]. This resulted in deletion of 1 to 8 individual values, depending on the solute. The model was then fitted to the data record for individual solutes using multiple linear regression (MLR) with stepwise AIC using the software package R (R Foundation for Statistical Computing, Vienna Austria, 2010, ISBN: 3-900051-07-0, http://www.R-project.org) and a significance threshold of $P < 0.15$ for inclusion of terms.

4.2.4 Analysis of Trends: Solute Concentrations and Fluxes

For both precipitation and streamwater chemistry samples, intra- and inter-annual patterns in solute concentrations were assessed based on volume-weighted mean monthly and volume-weighted mean annual values, respectively. Significant trends across the 20-year record were assessed using simple linear regression. For the streamwater data, a significant trend in volume-weighted annual concentrations through time differs from observing a significant time parameter ($\beta_t$) in the MLR models, which only represents changes in concentrations that are not accounted for by changes in discharge through time. We are interested in both changes in the observed streamwater concentrations through time, as well as changes that cannot be accounted for by changes in watershed runoff.

Solute fluxes in precipitation were calculated as volume weighted fluxes, derived by multiplying the mean monthly volume-weighted solute concentration in rainwater
for a given month by the total water volume entering the catchment that month. For annual precipitation flux estimates, monthly values were summed on a water-year basis. Streamwater fluxes were estimated by multiplying predicted daily concentrations from the MLR models by recorded daily runoff values, and summing on both monthly and annual timescales. For each solute, the watershed budget (input – output) was calculated using wet deposition solute input values from the NADP data less the estimated export values for streamwater. Of the solutes measured in streamwater, only Ca$^{2+}$, Mg$^{2+}$, SO$_4^{2-}$, and NO$_3$ are also measured in precipitation under NADP protocol and, therefore, can be used in budget analyses. Trends over the 20-year period in the annual budgets for these solutes were assessed using Simple Linear Regression.

Dry deposition and interbasin transfer of water are not accounted for in the reported budget values, both of which have the potential to significantly alter estimates. We do not attempt to account for these known inaccuracies by adjusting budget values. Instead we use historical and intermittent data on dry deposition rates from the site, as well as dry deposition data from other nearby locations within the region, throughout the discussion to inform our understanding of how dry deposition data may alter our reported values.
4.3 Results

4.3.1 Climate and Hydrology: Trends and Variation

Both mean annual temperatures (MAT) and mean winter temperatures (MWT; January-March) have increased significantly over the past 40 years, with the increase in MWT (+0.068°C yr⁻¹) being significantly greater than the increase in MAT (+0.047°C yr⁻¹)(Fig. 12). Over the entire record since monitoring at Walker Branch Watershed was established (1969-2009), these rates of change translate to a +1.88°C and +2.72°C increase in MAT and MWT, respectively. While there is more variation in MWT than MAT, the amount of inter-annual variation for both of these measures is small (Fig. 12). While significant over the 40-year period, the increasing trends in MAT and MWT are not statistically significant over the most recent 20 years of record that coincide with stream solute chemical records (P>0.05).

Relative to temperature data, there is much greater variation in inter-annual values of mean annual precipitation (MAP) and mean winter precipitation (MWP) (Fig. 12). While inter-annual variation in precipitation is large, there is little intra-annual (i.e., seasonal) variation in precipitation (Fig. 13). There is a marginally significant decline (P=0.12) in MAP (-1.34 cm yr⁻¹) over the last 20 years of record, overlapping with the stream solute chemical records (Fig. 12). However, there is no clear trend in MWP over this period.

Runoff, or that portion of precipitation which leaves the watershed via the stream channel, shows large variation on both annual and winter timescales (Fig. 12).
Figure 12: Mean annual and mean winter (Jan-Mar) air temperatures (A), precipitation (B), and runoff (C) for Walker Brach from 1969 through 2008. Annual runoff was separated into baseflow and quickflow components using the smoothed minima technique (D; Gustard et al. 1992). Annual evapotranspiration losses (E) were calculated as the difference between precipitation and runoff for the combined East and West Forks of Walker Branch to avoid complications associated with known inter-basin groundwater transfer between the two subcatchments (See Introduction: Site Description). Simple linear regression was used to assess changes through time for each variable for both the entire 40-year period, as well as over the most recent 20-year period in which streamwater chemistry records are available. Regression lines indicate a significant relationship existed ($P < 0.05$) and are truncated to indicate the period over which the relationship holds. All annual values are based on the water year (May-Apr).
Figure 13. Mean monthly precipitation (A) and runoff (B) values (± S.E.) separated by the first- and last-10 years of record (1989-2008). There is little seasonal variation in precipitation patterns and no systematic differences between the two periods of record are apparent. Runoff exhibits a seasonal maximum in late winter and minimum in late summer. The winter months exhibit large declines in runoff during the second half of the record.
There is also large seasonal variation in runoff, with greater runoff occurring during the winter dormant season (Fig. 13). Annual runoff over the most recent 20 years of record exhibits a significant annual rate of decline (-2.33 cm yr\(^{-1}\))(Fig. 12). Because annual runoff declined more precipitously than did precipitation (-1.34 cm yr\(^{-1}\)) indicates that the loss of water from the catchment as evapotranspiration (ET) increased (0.85 cm yr\(^{-1}\)) significantly over this period (Fig. 12). The total decline in MAR over the 20-year period (-46.6 cm) represents a ~33% reduction from the runoff that was observed during the first year of streamwater chemistry sampling (1989; 138.8 cm). There is also a significant decline in mean winter runoff (-1.31 cm yr\(^{-1}\); Fig. 12) during this period, with the decline during the 3 winter months accounting for more than 50% of the total annual decline. Because the decline in winter runoff over the 20-year record is disproportionately large compared to the other months of the year, a reduction in the seasonality in runoff is observed in recent years (Fig. 13).

Despite the large and seasonally asymmetric declines observed in runoff, quickflow (i.e., storm event flow) and baseflow decline significantly and at similar rates (-1.18 cm yr\(^{-1}\) and -1.07 cm yr\(^{-1}\), respectively; Fig. 12). Baseflow typically accounts for the majority of annual runoff from Walker Branch (mean = 62.8%, sd = 7.7%) (Fig. 12). Because baseflow and quickflow decline at similar absolute rates, and because baseflow is the dominant contributor to annual runoff, the ratio of baseflow to quickflow losses increases over the 20-year period.
While the total volumes of quickflow and baseflow decline similarly through time, there are trends in the frequency and intensity of both precipitation events and quickflow runoff through time (Fig. 14). The number of days in which there is measurable precipitation inputs has increased significantly over the past 40 years (+1.15 d yr$^{-1}$; Fig 4) while the median intensity, or median value of precipitation falling within a one-day period, has declined significantly (-0.013 cm d$^{-1}$ yr$^{-1}$; Fig 4). Despite an increase in the number of days having measurable precipitation, the decrease in the intensity of precipitation events results in an overall decrease in the number of days in which quickflow is measured in stream runoff (Fig. 14), leading to a significant decline in median values of stream quickflow losses (-0.001 cm d$^{-1}$ yr$^{-1}$) over the past 20 years (Fig. 14).

The directional trends in precipitation, runoff, and ET data over the last 20 years are distinct from the first 20 years of data from Walker Branch, so that no significant trends are observed over the entire 40-year record (Fig. 12). The trends observed in the last 20 years of record are similar in direction and range to the first 20 years of record—suggesting a potential for an inter-decadal periodicity in these hydrology parameters.

### 4.3.2 Solute Concentrations in Precipitation and Streamwater

Precipitation entering Walker Branch is very acidic with a mean pH of 4.40 (Table 4) with H$^+$ as the dominant cation on an equivalents basis (39.81 μEq L$^{-1}$; Table 4). NH$_4^+$ is the second most abundant cation in precipitation on both a mass (0.161 mg N L$^{-1}$)
Figure 14: Changes in the frequency and intensity of precipitation (A) and quickflow runoff (B) through time. The frequencies are indicated by the number of days within a given year in which any amount of precipitation was measured or in which there was estimated quickflow runoff (See Methods: Trends in Temperature, Precipitation, Runoff and Evapotranspiration for discussion of quickflow estimation). The number of precipitation days increases significantly over the 40-year period, while the decline in median daily precipitation amount suggests a significant decline in precipitation intensities. The number of days with quickflow runoff declines over the 40-year period, but the intensity of quickflow runoff only exhibits a significant decline over the most recent 20 years. Slope values are indicated to the right of each regression line.
and equivalent basis (11.49 μEq L⁻¹) (Table 4). The base cations Ca²⁺ and Mg²⁺ are at low concentrations in rainfall and combined account for a small portion of the cation charge (7.42 μEq L⁻¹) (Table 4). The strong acid anions SO₄²⁻ (41.52 μEq L⁻¹) and NO₃⁻ (17.28 μEq L⁻¹) account for nearly all of the anionic charge in precipitation (Table 4).

Despite the high acidity of precipitation, the streamwater of Walker Branch is alkaline (pH 8.01, Table 4) due to weathering of the underlying dolomite bedrock. Calcium and Mg²⁺ concentrations are very high in streamwater (25.3 and 14.2 mg L⁻¹, respectively; Table 4) and constitute nearly all of the cation charge in streamwater (Table 4). Carbonate alkalinity accounts for the vast majority of the total anion charge (Table 4). Sulfate concentrations are slightly higher in streamwater (2.3 mg L⁻¹) than in precipitation (1.994 mg L⁻¹; Table 4). Concentrations of both nitrogen ions (NO₃⁻ and NH₄⁺) are significantly lower in streamwater than in precipitation (Table 4). TSN concentrations, which include the inorganic N ions, as well as any N contained in organic molecules, averaged 0.051 mg L⁻¹ for the 12 years (1996-2008) when TSN measurements were made (Table 4). DOC concentrations are, on average, low (0.7 mg L⁻¹) and highly variable (SD = 0.4 mg L⁻¹) through time (Table 4).
Table 4: Summary statistics for precipitation and streamwater chemical compositions from the period between 1989 and 2008. All values are calculated on a water year basis. For precipitation, values are based on reported monthly volume weighted concentrations from the Walker Branch NADP site. For streamwater, values reported are based on the weekly streamwater chemistry measurements. A dash (-) indicates that values were not measured.

<table>
<thead>
<tr>
<th>Parameter</th>
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<th></th>
<th></th>
<th></th>
<th>Streamwater</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Mean (mg/L)</td>
<td>Mean (µEq/L)</td>
<td>Range (mg/L)</td>
<td>S.D. (mg/L)</td>
<td>n</td>
<td>Mean (mg/L)</td>
<td>Mean (µEq/L)</td>
<td>Range (mg/L)</td>
<td>S.D. (mg/L)</td>
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<tr>
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<td>39.81</td>
<td>0.011-0.145</td>
<td>0.0169</td>
<td>238</td>
<td>1.24 x 10⁻⁸</td>
<td>1.23 x 10⁻⁵</td>
<td>1.32 x 10⁻⁷</td>
<td>1.32 x 10⁻⁸</td>
</tr>
<tr>
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<td>1262.5</td>
<td>4 – 38.7</td>
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<td>0.114-0.187</td>
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<td>-</td>
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<td>-</td>
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4.3.3 Controls on Streamwater Solute Concentrations: Multiple Regression Models

Based on the multiple regression models, solutes can be broadly classified into two groups: (1) Hydrochemical Solute, which have the majority of their variation in concentration explained by discharge-related parameters (Ca$^{2+}$, Mg$^{2+}$, and SO$_4^{2-}$), and (2) Nutrient Solute, which have the majority of their variation in concentration explained by the seasonal phenology terms related to biological activity (NH$_4^+$, NO$_3^-$, TSN, SRP, and TSP) (Table 5, Fig. 15). While DOC concentrations, as measured in the early (DOC1; Fig. 15, Table 5) or later (DOC2; Fig. 15, Table 5) parts of the record, do not clearly fit into either of these categories, DOC will be discussed with nutrient solutes from here on.

The parameter estimates for the discharge term ($\beta_5$) for both Ca$^{2+}$ (-0.4669) and Mg$^{2+}$ (-0.4377) are large and negative (Table 5)—indicating a strong dilution of both these base cations during high-flow events (Fig. 16A&B). The parameter coefficient estimate for Ca$^{2+}$ is significantly larger than the estimate for Mg$^{2+}$, indicating that there is a 6.67% greater dilution of Ca$^{2+}$ than Mg$^{2+}$ with increasing discharge. Though significant, the seasonal phenology parameters ($\beta_5$: sin2Pi; $\beta_6$: cos2Pi) explain a relatively small portion (<25%) of the total explained variation in Ca$^{2+}$ and Mg$^{2+}$ concentrations (Fig. 15), representing very small increases in concentrations during the summer baseflow period (Fig. 17). The antecedent flow term ($\beta_4$) is significant and positive for both for both Ca$^{2+}$ and Mg$^{2+}$ (Table 5); however, in both cases it explains little of the overall variation in
Figure 15. Relative parameter importances for each MLR model, calculated using the lm method with the R package relaimpo (Gromping 2006). Y-axis values indicate the proportion of the total variation explained by the model that is captured within each parameter (values are normalized to sum 100%). Error bars denote upper 95% CI based on 500 bootstrap replicates. Bars are shaded to indicate difference between parameter types (white = time term, gray = discharge parameters, black = seasonal phenology parameters). More variation in hydrochemical solutes (Ca^{2+}, Mg^{2+} and SO_{4}^{2-}) was explained than in nutrient solutes (NH_{4}^{+}, NO_{3}^{-}, TSN, SRP, and TSP). Variation in discharge explained more of the variation in hydrochemical solute concentrations, while the seasonal phenology parameters explained the majority of the variation in nutrient solutes.
concentrations (<5%, Fig. 15). The time term (\(\beta_t\)) is significant, positive, and small in magnitude (0.0053) for Ca\(^{2+}\) (Table 5), and accounts for very little of the explained variation in the model (Fig. 15A); the time term is not significant for Mg\(^{2+}\). Overall, the vast majority of the variation in Ca\(^{2+}\) (\(R^2 = 92.7\%\)) and Mg\(^{2+}\) (\(R^2 = 93.1\%\)) concentrations is explained by their respective models.

In contrast to the base cations, SO\(_4^{2-}\) concentrations exhibit a marked increase in concentration with increasing discharge [Johnson et al. 1982] (Fig. 16). Similar to Ca\(^{2+}\) and Mg\(^{2+}\), there was a minor but significant seasonal phenology effect (Table 5) that accounted for a small amount of the explained variation in SO\(_4^{2-}\) concentrations (Fig. 15); however, the direction of the effect was opposite, with a slight decrease in summer SO\(_4^{2-}\) concentrations (Fig. 17). Both the time (\(\beta_t\)) and antecedent flow terms (\(\beta_i\)) were significant (Table 5) but accounted for extremely little (<5%) of the total explained variation (Fig. 15). Overall, the model explained 81.2% of the variation in SO\(_4^{2-}\) concentrations (Table 5).

While we were able to explain 75% or more of the variation in hydrochemical solute concentrations, we were only able to explain 30-55% of the variation in any of the nutrient solute concentrations, with the exception of NH\(_4^+\) where we were only able to explain 4.7% of the variation (Table 5). The seasonal phenology terms, as opposed to the discharge terms, had a much larger relative effect (Fig. 17) and explained more of the
total variation in nutrient solute concentrations than in hydrochemical solute concentrations (Fig. 15).

Figure 16. Discharge by concentration relationships for all solutes. Values are Ln-transformed. Lines indicate significant relationship as determined by simple linear regression. Where significant, regression summaries are listed on each panel.
Table 5. Best model fits to concentration data. Parameter values (SE in parentheses) are listed with significance level as follows: + (P<0.15), ++ (P<0.10), * (P<0.05), ** (P<0.01), and *** (P<0.001). Adjusted R² values, F-statistics and df (in parentheses) are listed in last column. All models had overall significance level <0.001. Because of the near 6-year break in DOC data, separate models for the period 1989-1996 and 2003-2008 are given for DOC (indicated by 1 and 2, respectively).

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<th>Solute</th>
<th>Intercept</th>
<th>β₁ (Time)</th>
<th>β₂ (lnQ)</th>
<th>β₃ (lnQ²)</th>
<th>β₄ (Ant. Q)</th>
<th>β₅ (sin2Pi)</th>
<th>β₆ (cos2Pi)</th>
<th>β₇ (sin4Pi)</th>
<th>β₈ (cos4Pi)</th>
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<td>-0.4377</td>
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Figure 17: Fitted seasonal pattern for each solute (based on sin/cos functions). Y-axis values indicate the percent deviation for each part of the year from the annual mean concentration.

At any point in time, NH₄⁺ concentrations are near detection limits (Table 4) and, because of this, there is little variation to explain. The model performs as well as can be expected given the lack of variation in NH₄⁺ concentrations. We include the model results for NH₄⁺ for the sake of consistency, but we will not give further consideration to the NH₄⁺ parameter estimates due to the high degree of uncertainty around their estimated values (Fig. 15).
As opposed to NH₄⁺, NO₃⁻ concentrations are almost always above detection limits (Table 4) and, of all the nutrient solutes we were able to explain the greatest amount of variation in NO₃⁻ concentrations (54.2%, Table 5). Nitrate exhibits very distinct seasonality, with an annual maximum in the summer months and annual minimum during fall (Fig. 17). Additionally, there is a local maximum in the winter and a local minimum in the spring that was captured by the second pair of sin and cos terms (sin4Pi and cos4Pi, Table 5, Fig. 17). In addition to the seasonal phenology parameters, the antecedent flow term (β₄) explained a substantial amount of the total variation in NO₃⁻ concentrations (~25%, Fig. 15). Less than 10% of the explained variation in NO₃⁻ concentrations could be accounted for by variation in discharge (Fig. 15), and the time term was not significant (Table 5).

TSN exhibited a similar, but dampened, seasonal pattern to NO₃⁻ (Fig. 17). This similarity likely arises because NO₃⁻ accounts for, on average, 55% of the TSN pool (Table 4) and, because of this, the NO₃⁻ dynamic strongly influences TSN dynamics. Similarly, the antecedent flow term is significant but also reduced in importance for TSN in comparison to NO₃⁻ (Table 5, Fig. 15). Unlike NO₃⁻, the time term is significant for TSN and is positive (β₄ = 0.0281, Table 5), suggesting a 2.81% yr⁻¹ increase in TSN concentrations between 1996 and 2008. Overall, however, we were only able to explain
31.1% of the variation in TSN concentrations (Table 5)—which is little more than half the amount of variation we could explain in NO₃.

SRP concentrations exhibited a similar seasonal pattern to NO₃, except were lacking a clearly defined local minima in the spring and local maxima in the winter (Fig. 17). For SRP, concentrations were much more dependent on the contemporaneous flow rate (Fig. 16) than on the antecedent flow term (Table 5, Fig. 15), which was opposite what was observed for NO₃ concentrations. However, the model explained 50.2% of the variation in SRP concentrations (Table 5), which was very similar to the amount of variation accounted for by the NO₃ model.

TSP concentrations showed a similar, though slightly muted, seasonal pattern as SRP (Fig. 17). Further, TSP concentrations were not as responsive to changes in discharge (Fig. 16, Fig. 15). The most notable discrepancy between SRP and TSP concentrations, however, was in the time term (βₖ) which was highly significant and positive (+0.0208, Table 5) for TSP, indicating a 2.08% yr⁻¹ increase in TSP concentrations over the 20-year record. The overall model explained 34.1% of the variation in TSP concentrations, which was much lower than the amount of variation explained in SRP concentrations (Table 5).

A similar amount of the variation in DOC1 (R² = 35.5%; the first 8 years of DOC record, 1989-1997) and DOC2 (R² = 36.2%; the last 3 years of DOC record, 2003-2008) was
explained by their respective models. However, the parameters that were found to have significant effects, as well as their fitted values, differed considerably between the two periods of record. The only term that was highly significant in both models was the time parameter ($\beta_1$) and, while this term accounted for similar amounts of the explained variation in each model (Fig. 15), it was opposite in sign between models (Table 5). Thus, the only characteristic of DOC concentrations that can be confidently claimed is that they are highly non-stationary through time and that the direction of their change can vary.

4.3.4 Trends in Solute Concentrations and Fluxes

The multiple regression models discussed above were used to gain insight into the potential factors controlling stream solute concentrations (parameter estimate values), as well as to provide flux estimates by extrapolating the weekly sampling data to match the continuous discharge record. When we assess trends in concentrations, however, we are identifying trends in the volume-weighted concentration data (i.e., the concentrations observed through time within the stream). In this section all trends in concentration data presented refer to regression analyses of monthly or annual volume-weighted concentration data (See Methods: Analysis of Trends: Solute Concentrations and Fluxes).
The concentrations of Ca\(^{2+}\) and Mg\(^{2+}\) in both precipitation and streamwater increased significantly over the 20-year period (Fig. 18). For precipitation, the increases in concentrations were offset by the decline in precipitation volume (Fig. 12) so that the flux of Ca\(^{2+}\) and Mg\(^{2+}\) into the watershed did not exhibit a significant trend through time (Fig. 19). For streamwater, however, the large declines in runoff over the record (Fig. 12) resulted in declines in Ca\(^{2+}\) and Mg\(^{2+}\) fluxes, despite increases in volume-weighted concentrations (Fig. 18). Because streamwater losses of Ca\(^{2+}\) and Mg\(^{2+}\) are typically two orders of magnitude greater than precipitation input rates (Fig. 19), the decline in streamwater fluxes through time resulted in significant declines in net watershed Ca\(^{2+}\) and Mg\(^{2+}\) loss (Fig. 19).

Sulfate concentrations declined sharply in both precipitation (-1.6% yr\(^{-1}\)) and streamwater (-1.3% yr\(^{-1}\)) over the 20-year period (Fig. 18). Likewise, SO\(_4^{2-}\) fluxes also declined significantly, though the rate of decline was slightly higher for streamwater exports (-1.05 kg ha\(^{-1}\) yr\(^{-1}\)) than for precipitation inputs (-0.71 kg ha\(^{-1}\) yr\(^{-1}\); Fig. 19). Precipitation and streamwater fluxes, however, are similar in magnitude decline at similar rates (Fig. 19). Thus, there is no significant trend in the long-term watershed net SO\(_4^{2-}\) budget, and catchment wet deposition rates often balance streamwater SO\(_4^{2-}\) export rates (i.e., budget values close to zero; Fig. 19).
Figure 18: Trends in the Ln-transformed concentrations for each of the hydrochemical solutes over the 20-year period in precipitation (left-column) and streamwater (right-column). Results of simple linear regression are listed on each panel.
Figure 19: Input-output budgets (left-column) and significant changes in flux through time (right-column) for each hydrochemical solute. For the barplots, y-axis values for Ca\(^{2+}\) and Mg\(^{2+}\) inputs were increased by a factor of 10 to be viewable. Regressions on right indicate assess changes in inputs (In), outputs (Out), or the difference between these (Out-In = Net). Where significant, regression lines are present and regression summaries are printed on each panel. The dashed gray line on the Net SO\(_4^{2-}\) panel is equal to zero to indicate when the watershed is losing (+ Net loss) versus retaining (- Net loss) SO\(_4^{2-}\).
There was a significant decline in NO$_x$ concentrations in precipitation at an average rate of -1.3% over the 20-year period (Fig. 20). This, coupled with the decline in precipitation over the same period (Fig. 12), resulted in a decrease in NO$_x$ precipitation fluxes of ~40% from the beginning (~3.8 kg ha$^{-1}$ yr$^{-1}$) to the end (~2.2 kg ha$^{-1}$ yr$^{-1}$) of the record (Fig. 20). Conversely, streamwater NO$_x$ concentrations showed no significant trend through time (Fig. 20). While there were no significant trends in streamwater NO$_x$ concentrations, the first and last years had exceptionally low values relative to other years (Fig. 20). These both were years in which precipitation and runoff rebounded from the two of the most severe, multi-year droughts in the 40-year record (Fig. 12). Despite the lack of trend in streamwater NO$_x$ concentrations, the strong decline in annual streamwater runoff over this period (Fig. 12) led to a significant decline in NO$_x$ fluxes (Fig. 10). Over the course of the record, the decline in precipitation flux (-0.075 kg ha$^{-1}$ yr$^{-1}$) was an order of magnitude greater than the decline in streamwater flux (-0.006 kg ha$^{-1}$ yr$^{-1}$) leading to an overall decrease in the amount of NO$_x$ that enters and is retained within the watershed (Fig. 20). However, precipitation inputs remain more than an order of magnitude higher than streamwater outputs, indicating the watershed is still highly retentive of NO$_x$ inputs.

TSP and SRP are not measured in precipitation and thus we cannot report patterns of input fluxes or watershed input-output budgets. In streamwater, however,
Figure 20. Ln-transformed NO$_3$ concentrations in precipitation (upper-left) and streamwater (upper-right) through time, as well as NO$_3$ fluxes (lower-left) and changes in input-output budgets through time (lower-right). For input-output regressions, In = Precipitation Inputs, Out = Streamwater Outputs, and Net = Out-In. Negative values for Net indicate watershed retention, and the reduction in the magnitude of the Net value through time indicates that the watershed is retaining less NO$_3$ through time. However, this is driven by the large decrease in precipitation inputs. Where present, regression lines indicate a significant trend and regression summaries are printed on each panel where appropriate.
both TSP and SRP concentrations increased significantly through time, though the percent increase in TSP concentrations was at a rate nearly twice (+2.5% yr⁻¹) that of SRP (+1.4% yr⁻¹) (Fig. 21). Further, because TSP concentrations are, on average, more than two-fold greater than SRP concentrations (Table 4), the absolute rate of increase in TSP concentrations over the last two decades (0.025 mg L⁻¹ yr⁻¹) is far greater than for SRP (0.014 mg L⁻¹ yr⁻¹) (Fig. 21). The decline in precipitation offsets these increases in concentration over the 20-year period, so that stream exports of SRP decline significantly (-0.004 kg ha⁻¹ yr⁻¹, Fig. 21) over the last two decades. In contrast, the larger increase in TSP concentrations is sufficient to offset declines in runoff so that no significant trend in streamwater TSP flux is observed (Fig. 21).

![Figure 21](image_url)

**Figure 21.** Changes in Ln-transformed streamwater SRP and TSP concentrations (A) and fluxes (B) through time. Where significant trends exits (P<0.05) regression lines are shown and regression summaries are printed on each panel.
4.4 Discussion

4.4.1 Past and Projected Changes in Climate and Hydrology

4.4.1.1 Climate

Climates in the Southeast United States have changed significantly during the past several decades [reviewed in U.S. Global Change Research Program 2009]. Regional temperatures have increased significantly since 1970 by -1°C, with winter months exhibiting the greatest increases [U.S. Global Change Research Program 2009]. Similarly, mean annual and mean winter temperatures over the 40-year period at Walker Branch have increased by 1.88°C and 2.72°C, respectively (Fig. 12). Future temperatures are projected to continue increasing significantly throughout the region [Christensen et al. 2007]. Anticipated changes by 2100 range from -3.0°C to -7.5°C, based on uncertainty in human greenhouse gas emissions in coming decades [Christensen et al. 2007].

Precipitation is more variable than temperature throughout the Southeast US, and precipitation at Walker Branch is also highly variable across years. Since 1970 mean annual precipitation in the region has decreased by 7.7% [U.S. Global Change Research Program 2009]. At Walker Branch there has not been a statistically significant decline over the 40-year record (since 1969), but precipitation has declined in the most recent 20-years (Fig. 12). Unlike other regions of the conterminous US, which have precipitation
patterns and trends highly correlated with various sea surface temperature patterns [e.g., Schubert et al. 2004, Seager et al. 2005], precipitation patterns in the Southeast US are often weakly or not correlated with these exogenous drivers [Seager et al. 2009]. Because of this, we are limited in our ability to project probable changes in precipitation patterns in coming decades [Ruiz-Barradas and Nigam 2006, Dale et al. 2010].

4.4.1.2 Variation and Trends in Catchment Hydrology

Though there is large inter-annual variability in catchment runoff, the ~40% decline in mean annual runoff over the previous 20-year period is remarkable (Fig. 12). This is much larger than the decline in precipitation over the same period (Fig. 12), and increased evapotranspiration (ET) rates (Fig. 12) account for approximately half of the runoff decline. Evapotranspiration rates can be influenced by both forest dynamics and changes in climate [Stoy et al. 2006]. Much of the forest within the Walker Branch watershed is second-growth hardwood and is strongly aggrading [Johnson 1989], and increases in forest biomass may, in part, be driving increases in catchment ET. Evapotranspiration is also likely to increase with surface air temperatures [Linsley et al. 1949], and the long-term trend in mean annual temperatures has likely increased ET fluxes over the past several decades. Future projections of ET in the region are largely based on anticipated increases in temperature because changes in precipitation patterns, and therefore changes in humidity and soil moisture [other dominant controls on ET
rates; Linsley et al. 1949], are difficult to predict. Neilson and Marks [1994] report that, regardless of potential changes in precipitation, the Southeast US will likely see future reductions in stream and river runoff driven by higher temperatures and increased ET rates.

While increased ET rates have contributed to the long-term (inter-annual) decline in runoff, the largest seasonal (intra-annual) declines in runoff occurred during winter months (Fig. 12). Changes in ET rates cannot directly explain this change in seasonality because it occurs during a period of the year when deciduous trees have shed their leaves and forest transpiration rates are low. In addition, changes in the seasonality of precipitation cannot account for this decline in winter runoff because there was no significant decline in winter precipitation through time (Fig. 12). The deep soils and water storage capacity within the watershed generate substantial lag times, such that higher summer ET may lead to greater retention of winter precipitation within drier soils (i.e., soil water storage replenishment) in recent years. Mean water residence times within the soil profile are suspected to be on the order of several months to a couple of years [Wilson et al. 1991, Mulholland 1993], though these investigations have not been comprehensive.

The highly weathered and deep soil profiles of Walker Branch generate strong vertical element profiles [Mulholland et al. 1990, Mulholland 1993, 2004], so that changes
in the total water flux from the catchment may be less important than changes in the route which water masses take as they pass through the catchment. Three principal flowpaths have been identified in Walker Branch [Wilson et al. 1991, Genereux et al. 1993a, b, Mulholland 1993, Wilson et al. 1993]. These include fracture flow through bedrock, saturated soil flow immediately above the bedrock, and shallow subsurface flow through upper soil horizons [Genereux et al. 1993b, Mulholland 1993]. Shallow subsurface flow only occurs during storm events when a perched water table develops above the well-defined saprolite layer in the upper B horizon that has highly reduced water permeability relative to A horizon soils [Wilson et al. 1989, Jardine et al. 1990, Mulholland 1993, Wilson et al. 1993]. Baseflow is comprised of both fracture flow from bedrock, as well as saturated soil flow immediately above bedrock [Mulholland 1993], and the ratio of these two source contributions to baseflow runoff is likely to differ based on drought or other changes in soil water storage. Given the 20-year decline in runoff (Fig. 12), as well as a decrease in the median intensity of precipitation events (Fig. 13), the relative contribution of these different flowpaths to streamflow generation has changed through time. These changes in the hydrologic connectivity of the different soil compartments to the stream channel drive many of the observed changes in streamwater concentrations and fluxes over the 20-year period.
4.4.2 Variation and Trends in Solute Concentrations and Fluxes

4.4.2.1 Calcium and Magnesium

For both Ca$^{2+}$ and Mg$^{2+}$, precipitation concentrations have increased significantly over the past 20 years (Fig. 18), while precipitation fluxes have remained fairly constant (Fig. 19). The constant flux suggests that the increase in concentrations may simply result from lower precipitation volumes. Transport distances for Ca$^{2+}$ and Mg$^{2+}$ are short, originating from nearby sources, including plant matter, exposed soil surfaces, and roadbeds [Luxmoore and Huff 1989]. Because the watershed sits within the 14,000 ha protected Oak Ridge Reservation, which has changed little in land use since 1942 [Hughes et al. 2009], we did not expect significant changes in precipitation fluxes of Ca$^{2+}$ and Mg$^{2+}$ through time. In addition to wet deposition, historical data for the site indicate that dry deposition rates often dominate total fluxes of Ca$^{2+}$ and Mg$^{2+}$ [Luxmoore and Huff 1989]. While there are limited data available for Mg$^{2+}$, dry deposition of Ca$^{2+}$ often exceeds wet deposition by a factor of 2 to 3 [Luxmoore and Huff 1989]. Regardless, if we had dry deposition data for this period, input fluxes for both Ca$^{2+}$ and Mg$^{2+}$ would remain more than an order of magnitude below streamwater export (Fig. 19).

Parent material weathering releases high concentrations of Ca$^{2+}$ and Mg$^{2+}$ to bedrock-contacting flowpaths. Immediately above the parent material, Ca$^{2+}$ and Mg$^{2+}$ concentrations decline precipitously, and concentrations in soil solutions from surface
horizons are approximately an order of magnitude lower than in groundwater [Mulholland 1993]. Concentrations of \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) are expected to increase when a greater proportion of water entering the stream channel is derived from bedrock-contacting flowpaths; concentrations will be diluted when increases in precipitation result in greater water losses through elevated soil flowpaths (Mulholland 1993). The significant increases observed in \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) concentrations (Fig. 18) indicate a greater proportion of catchment runoff is derived from deep soil flowpaths in recent years. This is supported by the observed changes in baseflow and quickflow contributions to runoff through time. Though baseflow and quickflow losses decline at similar rates over the 20-year period (Fig. 12), baseflow represents a greater portion of the total runoff in most years. Because of this, similar absolute declines in both quickflow and baseflow runoff results in a proportional increase in the baseflow contribution to total annual runoff.

The decline in catchment runoff has ultimately resulted in a decline in streamwater \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) fluxes (Fig. 19), despite the increases in concentrations that were observed. Streamwater losses of \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) remain very high, typically exceeding a combined loss rate of 225 kg ha\(^{-1}\) yr\(^{-1}\) (Fig. 19). The large streamwater fluxes of \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \) derived from bedrock weathering obscure our ability to use streamwater chemistry for assessing either the comparatively small leaching rates of these base cations from the soil profile or changes in the leaching rates of soil
exchangeable pools through time. Long-term monitoring of soil plots has indicated that, between 1982 and 2004, only 58 ± 46 kg ha⁻¹ of exchangeable Ca²⁺ (or ~2.6 kg ha⁻¹ yr⁻¹) was leached from the upper 60 cm of the soil profile [Johnson et al. 2008]. This amount is less than 2% of the >140 kg Ca ha⁻¹ yr⁻¹ exported from the catchment via streamwater runoff (Fig. 19). While this rate of Ca²⁺ leaching from surface soils is an inconsequential fraction of total watershed losses, it may be of considerable importance with respect to forest dynamics and growth. Over the same period (1982-2004), the aggrading vegetation on Walker Branch sequestered 497 ± 138 kg Ca²⁺ ha⁻¹ (or ~22.6 kg ha⁻¹ yr⁻¹) into aboveground biomass [Johnson et al. 2008]. Thus, the rate of leaching of Ca²⁺, while small compared to total watershed losses is appreciable (>10%) compared to aboveground vegetation accrual.

Our data indicate that leaching rates of Ca²⁺ from the upper soil profile may have declined over the past 20 years. The decline in runoff over this period, while leading to an increase in streamwater Ca²⁺ concentrations (Fig. 18), has ultimately resulted in a decrease in watershed flux through time (Fig. 19). The reduction in the intensity of precipitation events (Fig. 13) and the decline in quickflow runoff (Fig. 12) suggest that decreases in leaching losses of base cations from upper soil horizons has likely contributed to this decline. Calcium availability has the potential to limit future forest growth at Walker Branch [Johnson et al. 1988], as well as throughout much of the
southeastern US [Huntington 2000], and a change in surface soil leaching rates, as suggested by our data, may be of consequence. However, we are unable to quantify the magnitude of this potential change using only watershed input-output budgets.

Unlike Ca\(^{2+}\), Mg\(^{2+}\) is found in much lower concentrations in aboveground biomass and is not as essential for forest growth. For Mg\(^{2+}\), only 18 ± 4 kg ha\(^{-1}\) was incorporated into aboveground vegetation between 1982 and 2004, while 118 kg ha\(^{-1}\) (or ~ 5.6 kg ha\(^{-1}\) yr\(^{-1}\)) was lost to leaching [Johnson et al. 2008]. With greater rates of leaching from surface soils than Ca\(^{2+}\), Mg\(^{2+}\) losses account for most of the charge-balance that buffers excess SO\(_4^{2-}\) entering the system via deposition [Johnson et al. 2008]. That more Mg\(^{2+}\) is leached from surface soils is also apparent in the regression models predicting solute concentrations, where the discharge (\(\beta_1\): LnQ) parameter coefficient for Mg\(^{2+}\) (-0.4669 ± 0.0076 SE) is significantly greater than that of Ca\(^{2+}\) (-0.4377 ± 0.0070 SE) (Table 5)—indicating less dilution, or proportionally more Mg\(^{2+}\) being leached relative to Ca\(^{2+}\), when elevated flowpaths are established in the soil profile. However, like Ca\(^{2+}\), leaching of Mg\(^{2+}\) from upper soil horizons represents a very small loss with respect to total watershed flux (Fig. 19). While Mg\(^{2+}\) leaching from upper soil horizons has likely declined over the last 20 years due to the decrease in SO\(_4^{2-}\) deposition over this period (see below), we are unable to detect such a change in the long-term input-output budgets given the high Mg\(^{2+}\) losses from bedrock weathering.
4.4.2.2 Sulfate

Wet deposition rates of SO$_4^{2-}$ at Walker Branch remain high (~20 kg ha$^{-1}$ yr$^{-1}$) despite having declined significantly from values in 1989 (~30 kg ha$^{-1}$ yr$^{-1}$) (Fig. 19). The Walker Branch watershed is located within 22 km of 3 coal-fired power plants [Johnson 1989], and efforts to control emissions from these sources are likely the primary cause for the reduced input fluxes. While the marginally significant decline in precipitation over the past 20 years (Fig. 12) may have also reduced inputs due to reductions in atmospheric washout of SO$_4^{2-}$ from these nearby sources [Hutcheso.Mr and Hall 1974], the inter-annual variation in SO$_4^{2-}$ flux (Fig. 19) is much lower than the variation in precipitation between years (Fig. 12), which suggests precipitation may play only a minor role in the overall reduction in input fluxes. This is further supported in that SO$_4^{2-}$ concentrations in precipitation declined at a much greater rate (~1.6% yr$^{-1}$; Fig. 18) than the decline in precipitation volume (~0.9% yr$^{-1}$; Fig. 12).

Streamwater concentrations of SO$_4^{2-}$ also declined significantly over the 20 years and at a rate (-1.3% yr$^{-1}$) proportionally similar to the rate of decline in precipitation concentrations (-1.6% yr$^{-1}$) (Fig. 18). Taken together with the decline in streamwater runoff, watershed export of SO$_4^{2-}$ declined at a rate of -1.05 kg ha$^{-1}$ yr$^{-1}$ (Fig. 19), which is also similar in magnitude to the decline in precipitation fluxes (-0.71 kg ha$^{-1}$ yr$^{-1}$; Fig. 19). Because input and output fluxes have declined at similar rates, we did not observe a
significant change in the net budget of SO$_2^-$ from the watershed over the 20-year period
(Fig. 19). However, this should not be interpreted to indicate that the decline in
precipitation fluxes of SO$_2^-$ is what caused the decline in streamwater SO$_2^-$ fluxes. This
would require that SO$_2^-$ travel through the watershed is passive, or at least that no
processes internal to the watershed are causing net accretion or depletion of SO$_2^-$ pools.
There are two reasons this is not likely.

First, wet deposition alone significantly underestimates the total mass of SO$_2^-$
entering the catchment each year, and historical data indicate that dry deposition often
equals or exceeds wet deposition rates [Luxmoore and Huff 1989]. Because dry
deposition has not been regularly monitored over the same period as streamwater
chemistry we do not know if it has declined similarly to wet SO$_2^-$ deposition.
Regardless, we expect dry deposition rates to be appreciable and that the watershed is
retaining significant quantities of SO$_2^-$ through time, likely via sorption within the
mineral soil profile [Johnson et al. 1982].

Second, instantaneous discharge explains the vast majority of variation in the
multiple regression model predicting SO$_2^-$ concentrations (Fig. 15). If changes in
precipitation inputs were driving changes in streamwater concentrations the correlation
between discharge and SO$_2^-$ concentrations would not be as strong. Variation not
associated with discharge or seasonal phenology would be captured by the time term
(βi), which accounted for less than 5% of the variation within the current model (Fig. 15).

Mechanistic support for the correlations between streamwater SO4\(^{2-}\) concentrations and runoff in the regression models has been demonstrated through previous studies of soil hydrologic flowpaths [Mulholland 1993]. Similar to Ca\(^{2+}\) and Mg\(^{2+}\), exchangeable SO4\(^{2-}\) in the soil profile exhibits strong vertical variation but in opposite direction. Sulfate concentrations are highest in surface soils, with Fe- and Al- rich deep mineral soil profiles efficiently removing SO4\(^{2-}\) from solution [Johnson et al. 1982]. This, in conjunction with the regression model results, suggests that the decline in watershed SO4\(^{2-}\) fluxes is primarily driven by changes in catchment hydrology, with little support that the decline in wet deposition has had much effect on streamwater losses.

Ultimately, the similar decline in precipitation and streamwater SO4\(^{2-}\) fluxes is likely coincidence rather than resulting from a causal linkage between the two.

Continued efforts to control atmospheric emission from coal-fired power plants are expected to maintain, or further decrease, watershed input fluxes via precipitation. However, if the decline in runoff is not maintained, or if the frequency and intensity of storm events increases, we predict that an increase in streamwater SO4\(^{2-}\) fluxes will result.
4.4.2.3 Nitrate

Nitrate concentrations in precipitation have declined significantly through time (Fig. 20). Similar to SO\textsubscript{2}, much of the NO\textsubscript{3} in precipitation in this region is from local coal-fired power plants [Luxmoore and Huff 1989], and we found a significant correlation between SO\textsubscript{2} and NO\textsubscript{3} concentrations in precipitation over the 20-year period \((r = 0.4, P < 0.001)\). Because precipitation volume and NO\textsubscript{3} concentrations decline significantly through time, there has been a substantial decline in the flux of NO\textsubscript{3} into the watershed in recent years (-0.075 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}; Fig. 20). Historical data on dry deposition of NO\textsubscript{3}, collected from 1976-1983, accounted for 40\% of the total N flux into the system [Luxmoore and Huff 1989], representing an important missing component in our budgets for which we cannot account.

Streamwater NO\textsubscript{3} concentrations did not exhibit a significant trend over the 20-year period (Fig. 20). Unlike the hydrochemical solutes, streamwater NO\textsubscript{3} concentrations are only weakly influenced by discharge (Fig. 16) and, because of this, changing flowpath contributions through time has little effect on the concentration of NO\textsubscript{3} entering the stream channel. Despite this, the decline in catchment runoff resulted in a significant decline in streamwater NO\textsubscript{3} flux over the 20-year period (-0.006 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}; Fig. 20). Streamwater export is typically more than an order of magnitude lower than precipitation input (a conservative value base on wet deposition only), indicating
the Walker Branch forest is strongly N-limited [i.e., retains most N; Luxmoore and Huff 1989]. The much greater decline in precipitation inputs relative to the decline in streamwater losses indicates that the amount of NO$_3^-$ entering the watershed and being retained has declined significantly through time (-0.069 kg N ha$^{-1}$ yr$^{-1}$; Fig. 20). This corresponds to a contemporaneous annual flux value that is approximately 1.4 kg ha$^{-1}$ yr$^{-1}$ less than what was observed in the early years of the record, which may have important implications for forest growth.

While streamwater NO$_3^-$ concentrations did not show a significant linear trend across all 20 years of data, there are important patterns over shorter periods. First, at seasonal time scales, in-stream biological processes (high uptake rates by attached algae in early spring prior to leaf out and by heterotrophic microbes during leaf decomposition after autumn leaf fall), ultimately controlled by phenology of the riparian forest, override hydrologic processes and result in lower nutrient concentrations and fluxes. Second, at multi-year scales, antecedent hydrology (particularly multi-year droughts) has a significant effect on concentrations and flux, particularly during summer months.

The seasonal pattern of streamwater NO$_3^-$ concentrations in Walker Branch diverges from NO$_3^-$ dynamics in many other small watersheds. Seasonality in stream NO$_3^-$ concentrations typically indicate early stages of N saturation in temperate forests,
with increases in higher concentrations during dormant months when biomass
production is suspended and N demands are low [Stoddard 1994]. While there is
seasonality in streamwater NO₃⁻ at Walker Branch, the dynamic is not controlled by
forest N demands but instead by biologic uptake within the stream channel.
Mulholland and Hill [1997] measured groundwater NO₃⁻ concentrations at Walker
Branch ~weekly for 18 months between September 1993 and March 1995 and found that
groundwater NO₃⁻ concentrations were high and relatively constant (mean = 46 μg N L⁻¹;
SD = 3 μg N L⁻¹), exceeding 88% of streamwater NO₃⁻ measures during the same period.
The highest concentrations are observed during summer months when streamwater and
groundwater values are similar, with lower values at other times of the year due to in-
stream uptake [Mulholland and Hill 1997, Mulholland 2004]. The greatest rate of
instream NO₃⁻ uptake occurs in autumn when litterfall drives stream NO₃⁻
concentrations to their annual minimum (Fig. 17)[Roberts and Mulholland 2007].
Nitrate concentrations slowly increase through the winter as the organic matter fueling
stream microbes is depleted, and decline to a seasonal minimum during the spring when
in-stream algal production is high immediately prior to tree canopy closure (Fig.
17)[Roberts and Mulholland 2007].

While the seasonal pattern of streamwater NO₃⁻ concentrations has been
described in previous studies [Mulholland and Hill 1997, Mulholland 2004], the

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synthesis of the 20-year record provides two additional insights: (1) The center of the seasonal pattern for any year (i.e., the annual mean) is not stationary and shows large drift about the long-term (20 year) mean (Fig. 22), and (2) While the seasonal pattern is strong there is large variation in concentrations during fall and spring that our model does not capture (Fig. 22).

The gradual drift in NO$_3^-$ concentrations about the 20-year mean can be seen in Figure 13. The lowest values over the course of the entire record were observed during the first and last two years of record (Fig. 22). Anecdotally, these years correspond to periods in which the watershed was either emerging from or heading into severe, multi-year drought, respectively. For this reason we introduced the 3-year antecedent flow anomaly term into the MLR models (see Modeling Solute Concentrations and Flux Estimation). This term captured the long-wave pattern in NO$_3^-$ data remarkably well (Fig. 22) and, while the seasonal phenology terms explained the majority of the overall variation in NO$_3^-$ concentrations, the antecedent flow effect captured a significant amount (~25%) of the balance (Fig. 15).

We are uncertain of the potential causes for this drought-associated change in streamwater NO$_3^-$ concentrations based on the available data. While groundwater NO$_3^-$ concentrations were shown to be seasonally invariant over an 18 month period [Mulholland and Hill 1997], there has not been long-term monitoring of groundwater
Figure 13. Raw NO$_3^-$ data and fitted seasonal and 3-year drought effect terms for the entire record (A), as well as seasonal variation in fitted NO$_3^-$ concentrations (B) and residual values (C) over the 20-year period. A conceptual model (D) is included to indicate dominant controls on NO$_3^-$ concentrations within an annual cycle. For fitted (Continued on next page)
(Continued from previous page) values (panel B), the dark black line indicates the mean of the fitted NO$_3$ values for all years, with the lighter black lines representing the fitted values for each of the 20 years. For the residuals plot (panel C), the dark black line indicates the mean value of the residuals for all 20 years of data; the dashed black line runs through zero. For panels B and C, the gray shaded area represents the 95% CI for the fitted and residual values, respectively, across all 20-years of data. These confidence intervals were estimated for each Julian day by bootstrapping all data within +/- 4 d for any year within the dataset 1000 times. We describe the mechanisms causing differences in both the explained (fitted) and unexplained (residual) variation in NO$_3$ values across seasons in the conceptual figure (panel D). The large variation in fitted NO$_3$ concentrations during summer months is driven almost exclusively by the 3-year antecedent flow term, with drought years having significantly lower summer NO$_3$ concentrations. The local minima in spring and fall are due to increased algal and microbial NO$_3$ assimilation, respectively. Because these in-stream processes are highly subject to disturbance by stochastic hydrologic variability (i.e., scouring floods), there is large variation that was not captured in the model during these times of year (i.e., large variation in residuals in fall and spring).

NO$_3$ concentrations. It is possible that drought alters groundwater NO$_3$ concentrations on inter-annual timescales. Henderson and Harris [1975] have shown that a dominant pathway of N return to the forest ecosystem is via root mortality and decomposition. A variety of research has demonstrated that, in response to drought, many tree species have the capacity to reduce fine root mortality rates and increase root production to forage for soil water [Joslin et al. 2001]. It has also been suggested that maintenance of the additional root mass following drought may occur (as opposed to immediate mortality) to ensure protection against near-term reversion to drought conditions for already drought stressed forests [Eissenstat et al. 2000]. An experiment in the East Fork of Walker Branch to alter the amount of precipitation reaching large plots of forest has shown increased net root production with decreases in precipitation inputs [Joslin et al.
2001]. Thus, if roots are a primary source of N return to the system, it is plausible that
drought-induced temporal dynamics may reduce root turnover and, as a consequence,
reduce NO$_3^-$ losses to groundwater from below the rooting zone.

Alternatively, it is possible that deep groundwater NO$_3^-$ concentrations are
invariant to drought cycles and that the drought-associated changes in streamwater
NO$_3^-$ concentrations result from changes in in-stream dynamics. In-stream metabolism
rates (both GPP and ER) have been shown to control NO$_3^-$ uptake [Roberts and
Mulholland 2007]. For summer NO$_3^-$ concentrations, because little light reaches the
stream channel below the tree canopy and GPP rates are low [Roberts et al. 2007], inter-
annual variation in summer in-stream NO$_3^-$ uptake would likely be governed by
heterotrophic metabolism and, therefore, the availability of DOC. However, we were
unable to find any correlation between summer DOC and NO$_3^-$ concentrations across
years. Though this limits support for the hypothesis that in-stream processes drive
inter-annual variation in NO$_3^-$ concentrations, it is possible that streamwater DOC
concentrations are not a sufficient proxy for respiration since DOC concentrations alone
cannot indicate bioavailability or DOC uptake rates.

The second finding from the long-term record, that there is high residual
variation in NO$_3^-$ concentrations during non-summer months—particularly in autumn
(October-December)—, likely arises from the frequency and intensity of stochastic
hydrologic events. Unlike the variation that was observed in summer months, where all summer values were either consistently higher or lower than the long-term mean throughout a season, the variation during other months of the year can be characterized by large changes in NO$_3^-$ concentrations occurring over short timescales (i.e., between weekly samples). While there is little discharge-dependency of NO$_3^-$ concentrations (Fig. 16), the mechanisms driving the seasonal phenology patterns are highly hydrologically sensitive. For example, autumn litter inputs occur quickly; when leaves begin to fall there is often a sharp drop in NO$_3^-$ concentrations between the week immediately prior to and the week immediately following the onset of litterfall. After much of the litter has fallen, large storm events can flush much of the litter from the stream channel or disrupt the benthic community, causing NO$_3^-$ concentrations to abruptly rebound. Similar dynamics can occur during the spring period of high in-stream productivity; storm events that scour algal biomass can result in elevated NO$_3^-$ concentrations due to lack of in-stream uptake. While we believe this hydrologic scouring is the likely cause for much of the variation in NO$_3^-$ concentrations, this is a very difficult effect to predict. The response is likely to exhibit threshold behavior, as well as depend on the intensity and frequency of storm events.

While NO$_3^-$ concentrations did not exhibit a long-term trend across the entire 20-year record, both the drought effect and the stochastic hydrologic effect indicate that
changes in climate patterns can have important effects on shorter timescales. Changes in the frequency or severity of multi-year droughts, as well as changes in the frequency or intensity of storms during periods when in-stream uptake rates are high, can have a large impact on watershed input-output budgets of NO$_3^-$ even if they do not manifest as linear trends through time.

4.4.2.4 SRP

Soluble reactive phosphorus is an essential element that can, at certain times of the year, limit in-stream activity [Newbold et al. 1983, Rosemond et al. 1993]. Because of this, SRP exhibits a seasonal pattern similar to NO$_3^-$ (Fig. 15). However, unlike NO$_3^-$, the principal source of P to the watershed is from bedrock weathering [Luxmoore and Huff 1989] and P is distributed within the soil profile similarly to the base cations, with SRP concentrations increasing near bedrock [Mulholland 1992]. Because of this, SRP concentrations also exhibit significant discharge-dependence similar to the hydrochemical solutes, with dilution occurring at higher rates of runoff (Fig. 16). Given the negative relationship between stream SRP concentrations and discharge, the long-term decline in stream runoff resulted in an increase in streamwater SRP concentrations over the 20-year record (Fig. 21) that was not apparent in streamwater NO$_3^-$ concentrations (Fig. 20). Because streamwater NO$_3^-$ concentrations show no trend through time while SRP concentrations increase significantly, the decline in runoff over
the 20-year period has resulted in an increase in the availability of SRP relative to NO$_3$.

Because Walker Branch is oligotrophic in-stream processes are often very near co-
limitation by SRP and NO$_3$ [Mulholland and Rosemond 1992, Rosemond 1994], and this
shift in the SRP:NO$_3$ ratio can have important implications on in-stream processes.

The decrease in water flux led to an overall decrease in SRP flux from the
catchment (Fig. 21) despite the increase in SRP concentrations. This is similar to what
was observed for the base cations and is driven by the change in hydrology over the 20-
year period. We cannot assess changes in watershed input-output budgets because SRP
deposition rates at the NADP station are not recorded. However, historical data indicate
that atmospheric deposition rates are minimal (<0.5 kg ha$^{-1}$ yr$^{-1}$) [Luxmoore and Huff
1989].

4.4.2.5 DOC, TSN, and TSP

In Walker Branch, energy availability and, in particular, the availability of
organic matter plays an important role in governing in-stream biogeochemical
processes. Unfortunately our DOC record is not continuous and, with different
analytical procedures for DOC quantification during the first 8 years of record from
what was used during the last 7 years of record (see Methods: Sampling and Analytical),
we were required to model the two periods of DOC concentration data separately. A
similar amount of variation (~37%) was explained in each case (Fig. 15, Table 5), and for
both DOC records the time parameter accounted for ~60% of the explained variation in each model (Fig. 15). However, while the magnitude of the time terms between the two periods were similar they are opposite in sign (DOC1: -0.0946, DOC2: 0.1066; Table 5), indicating that there was a significant decline in DOC concentrations during the first 8 years of record and a significant increase in DOC concentrations during the last 7 years of record.

In addition to opposing directional changes in DOC concentrations, the two periods of DOC record differed in fitted seasonal patterns (Fig. 17) and neither showed a strong dilution or concentration effect with increasing runoff (Fig. 16). DOC concentrations are highly variable across the entire record (Mean = 0.7 mg C L⁻¹ / SE = 0.4 mg C L⁻¹; Table 4), especially during periods of autumn litterfall and in-stream algal production in the spring. In part this may be the result of stochastic hydrologic effects, as detailed above for NO₃ concentrations, which can lead to rapid scouring and removal of algal standing crops and benthic organic matter deposits.

While inorganic N and P forms are generally considered to be more bioavailable than N or P contained within organic matter, organic forms of N and P make up the majority of the total N and P export from Walker Branch. Due to high biological demand for N and P within the system, understanding organic N and P dynamics is important because organic nutrient losses can: (1) represent an important loss pathway
for these essential elements that is beyond biological control, and/or (2) represent an actively cycling pool of elements supporting ecosystem function.

We model TSN and TSP concentrations directly, rather than subtract the inorganic subcomponents first, in order to avoid compounding the errors associated with the multiple measures and obscuring our abilities to discern pattern in the data. For TSN and TSP, however, the seasonal phenology terms accounted for the majority of explained variation in concentrations (Fig. 15) and a seasonal pattern emerged for each that was similar, yet somewhat muted, from the seasonal patterns in either NO₃⁻ and SRP concentrations (Fig. 17). An important distinction between the modeled TSN and TSP concentrations from NO₃⁻ and SRP concentrations, however, was the significance of the time parameters. Both TSN and TSP concentrations increased significantly through time (Fig. 15, Table 5), while neither NO₃⁻ nor SRP exhibited a significant trend. Additionally, the amount of explained variation in TSN and TSP concentrations was ~40% and ~30% lower than the amount of explained variation in NO₃⁻ and SRP concentrations, respectively (Table 5).

In comparison to the other solutes, two important results emerge across DOC, TSN, and TSP data: (1) all three solutes have highly variable concentrations and we are unable to explain the majority of that variation, and (2) the concentrations of all three
solutes have exhibited significant changes through time. Future investigations of
organic matter dynamics in Walker Branch represents an area of much needed research.

4.5 Across Solute Patterns

Ultimately, over the 20-year period all solutes exhibited either a significant
change in concentration, a significant change in flux, or both. The significant decline in
runoff during this time period requires that this be true—a flux rate, being the product
of runoff volume and concentration, cannot be maintained as runoff declines if
concentrations do not increase to compensate. Despite the changes we observed
through time, in nearly every case (excepting the DOC, TSN and TSP data) the time
parameter ($\beta$) in the multiple regression models explained little or no variation in solute
concentrations (Fig. 15). This indicates that changes in hydrologic processes were the
predominant factor governing long-term trends in streamwater solute concentrations
and fluxes during the past 20 years.

The hydrochemical solutes (Ca$^{2+}$, Mg$^{2+}$, and SO$^{4}_{2-}$) were clearly distinct in that
instantaneous discharge explained nearly all of the variation in their concentrations over
the entire record (Fig. 15, Table 5). Since the discharge by concentration relationship for
all hydrochemical solutes was robust through time, we are able to infer that the decline
in catchment runoff has been accompanied by changes in the relative contributions of
different soil flowpaths over time. The change in hydrology had much smaller effects
on long-term nutrient concentrations, if significant trends were observed at all.

Compared to the hydrochemical solutes, nutrient concentrations are not highly
discharge dependent (Fig. 16), with in-stream biological controls dominating over
hydrologic controls on shorter timescales (i.e., seasonal).

Despite the assorted long-term trends in solute concentrations, all solutes
exhibited significant long-term declines in flux (with the exception of TSP, which
showed no significant trend; Fig. 21). Over the 20-year period, Ca$^{2+}$, Mg$^{2+}$, and SO$_4^{2-}$
fluxes declined by ~40, ~33, and ~21 kg ha$^{-1}$ (or ~22%, ~25%, and ~44%), respectively.
Streamwater NO$_x$ fluxes declined by ~0.12 kg N ha$^{-1}$ (or ~48%) and SRP fluxes declined
by ~0.008 kg P ha$^{-1}$ (or ~28%). These declines are certainly not trivial and represent the
important effect that declining runoff has had on watershed element budgets.

4.6 Implications for Watershed Biogeochemistry and Climate Change

There is a considerable amount of uncertainty in both future climate projections
[Ruiz-Barradas and Nigam 2006], and to an even greater extent in anticipated ecosystem
responses to climate change [Walther et al. 2002]. We find it remarkable that over the
past 20 years the large changes in streamwater solute concentrations and fluxes in
Walker Branch resulted almost exclusively from altered hydrologic flowpaths and
decreases in catchment runoff. In part, this may be due to the deep soils throughout the
watershed (~30 m; Johnson 1989). In deep soils, there will be greater isolation of organic
matter processing in surface soil horizons from bedrock weathering at depth. Because of this, streamwater originating from different flowpaths is conditioned by these separate processes and will likely have distinct chemical compositions. When flowpaths exhibit large dissimilarities in chemistry, changes in flowpath structure through time would be expected to result in large changes in streamwater concentrations and fluxes, as we observed at Walker Branch. In shallow soils, the biologically active rooting zone may extend to the zone of parent material weathering and differences in the chemical composition of different flowpaths may be less pronounced. Because of this, it may be easier to detect changes in biogeochemical process rates in shallow soils where large effects from changing hydrologic flowpaths do not dominate.

Catchment hydrology and changes in flowpath contributions may not continue to be the dominant driver of changes in solute concentrations and flux in future years. Runoff is not likely to continue to decline indefinitely. Further, unlike changes in hydrology, biotic controls on biogeochemical process rates are more likely to exhibit abrupt changes associated with threshold effects [Scheffer et al. 2001, Scheffer and Carpenter 2003]. Over the past 20 years, despite having observed significant changes in temperature and precipitation patterns, we have not observed any abrupt changes in solute concentrations or fluxes associated with changes in forest dynamics or growth. As we continue to shift the baseline climate condition for this ecosystem, we may be
moving closer to an abrupt response associated with biological reorganization in coming
years. Dale et al. [2010] recently modeled future forest responses to different scenarios
of climate change, predicting that East Tennessee Broadleaf forests similar to that of
Walker Branch are likely to experience major changes in forest stand, structure and
composition in the next several decades, particularly if conditions remain dry.

It is important to note that were we to shift the 20-year record back in time by a
decade (1979-1998) we likely would not have observed linear trends in solute
concentrations and fluxes because there would not have been a linear decline in runoff.
During the first 20 years of the climate record (1969-1988) there was a similar decline in
catchment runoff as was observed in the last 20 years of the record (1989-2008) (Fig. 12).
These two periods were separated by an extremely large, continental-scale drought
from this drought beginning in 1989. It is possible that the previous 20-year decline in
catchment runoff is associated with some inter-decadal periodicity in climate patterns
and that an increase in precipitation and runoff may occur in the near future. Consistent
with this is a large increase in runoff observed during the 2009 water year compared to
2005-2008 (Fig. 12). Such analysis of long-term pattern in the climate data is beyond the
scope of this paper. Nonetheless, despite uncertainty in the causes or persistence of the
decline in runoff, this 20-year record provides valuable insight into the responses of
streamwater solute concentrations and watershed fluxes to large changes in
temperature, precipitation, and runoff.

4.7 Comparisons to Other Watershed Monitoring Programs

Most watershed monitoring programs have been established in the Northeastern
United States, Canada, and Western Europe [Moldan et al. 1994]. In part this has
resulted from the research questions of interest, with the majority of watershed budget
studies focused on effects of atmospheric deposition of SO$_2^-$ and NO$_3^-$ associated with
industrial activities [Moldan et al. 1994]. Additionally, high latitude sites with post-
glacial soils are often selected because the approximate age of soil formation is known
(i.e., time since the last glaciation) and, because of this, it is easier to estimate rates of
geochemical weathering of parent materials [Moldan et al. 1994]. There are far fewer
monitoring sites on soils that have escaped Pleistocene glaciation [Moldan et al. 1994]. In
low-latitude temperate, subtropical and tropical regions where watersheds have been
monitored, research sites are typically located in areas of high relief where down-slope
mass wasting often prohibits the formation of deep soil profiles and streams can be
easily gauged [Moldan et al. 1994].

Given the need to have a balanced water budget for a catchment, sites with
springs or evidence of deep groundwater sources are often intentionally avoided
because the groundwater exiting at springs has the potential to originate from outside of
the watershed boundary [Winter et al. 2003]. While there is a practical justification for avoiding sites with known groundwater sources, there has been increasing awareness that groundwater dynamics can fundamentally alter the ways in which we conceptualize patterns and processes governing watershed element dynamics [Burns et al. 1998, Winter et al. 2003, Mulholland 2004]. Our data indicate that, specifically with respect to changing climates, catchments with groundwater influence may exhibit distinct hydrologic responses resulting from changes in flowpath structure. Because long-term monitoring records from small, forested watersheds are becoming increasingly used for assessing ecosystem-scale impacts of climate change, awareness of such potential biases in watershed site characteristics is necessary as we extrapolate these findings to the broader landscape.

4.8 Conclusions

The changes in climate we have observed at Walker Branch over the past 20 years are large. Mean annual temperatures increased by ~2°C and mean annual precipitation, though highly variable across years, declined by nearly 30 cm since 1989—a ~20% reduction from the long-term mean. The decline in precipitation, together with increases in catchment evapotranspiration, has resulted in a >40% decline in catchment runoff since 1989.
Across solutes the responses to changes catchment hydrology were not
generalizable. Hydrochemical solute concentrations were highly correlated with
instantaneous discharge and these relationships were robust through time. There was a
long-term increase in Ca\(^{2+}\) and Mg\(^{2+}\) concentrations, and a long-term decline in SO\(_{4}^{2-}\)
concentrations, indicating significant changes in contributions of different soil flowpaths
to annual runoff, with a greater proportion of streamwater derived from groundwater
flowpaths in recent years. Nutrient concentrations (NH\(_{4}^{+}\), NO\(_{3}^{-}\), SRP, TSN, TSP and
DOC) exhibited limited correlation to discharge. At seasonal time scales, in-stream
biological processes override hydrologic processes and result in lower nutrient
concentrations and fluxes. At intermediate time scales, antecedent hydrology
(particularly multi-year drought) has a significant effect on solute concentrations and
flux, particularly for NO\(_{3}^{-}\). Despite the different responses across hydrochemical and
nutrient solute concentrations, the large decline in runoff resulted in significant long-
term declines in solute fluxes. Since 1989, Ca\(^{2+}\), Mg\(^{2+}\), and SO\(_{4}^{2-}\) fluxes have declined by
~40, ~33, and ~21 kg ha\(^{-1}\) (or ~22%, ~25%, and ~44%), respectively. Streamwater NO\(_{3}^{-}\)
fluxes declined by ~0.12 kg N ha\(^{-1}\) (or ~48%) and SRP fluxes declined by ~0.008 kg P ha\(^{-1}\)
(or ~28%).

Ecosystem responses to projections of future climate change remain shrouded in
high levels of uncertainty. Where climates have been changing in recent years, long-
term watershed records are only beginning to reveal initial effects on biogeochemical cycles and altered element input-output budgets. Changes in temperature and precipitation regimes have the potential to impact watershed biogeochemistry through either altering catchment hydrology, or through altering the geochemical and biological processes governing element reaction rates. The long-term Walker Branch record illustrates the important role that changes in catchment hydrology can have on streamwater solute concentrations and flux.
5. Summary and Conclusions

Human activities have a broad range of impacts on ecosystem element cycles. In many cases, humans have changed the rate of supply of essential elements entering ecosystems. The N cycle is among the most perturbed of the element cycles, and changing the rate of N supplied to ecosystems can have many important effects because N availability often limits primary production in many terrestrial and aquatic ecosystems. In Chapter 2, we assessed patterns in streamwater chemistry across one of the largest N deposition gradients in North America. We found that dissolved organic nitrogen (DON) concentrations typically decreased with increasing levels of N loading, which was a non-intuitive response and is inconsistent with previously published findings. We also found a surprising lack of correlation between DON and dissolved organic carbon concentrations (DOC). Through laboratory bioavailability assays we observed that artificially elevating inorganic N concentrations in low N samples could stimulate heterotrophic demand for reduced C, and that organic molecules having a greater density of N atoms were preferentially consumed over bulk DOC. This mechanism produced patterns that were consistent with our field observations of entire watersheds. Dissolved organic N represents the dominant pathway of N loss from terrestrial to aquatic ecosystems globally, and the response of DON to human alterations of N availability can have important implications on our understanding of ecosystem N
budgets. We used these findings to propose a new hypothesis that gives explicit consideration to the dual role of DON in both C and N cycles necessary for predicting changes in DON losses from N-polluted landscapes.

In Chapter 3 we scaled the laboratory bioavailability assays we performed in Chapter 2 to the stream, performing whole reach manipulations of labile DOC and inorganic N availabilities. While we were unable to stimulate DON consumption as we had in the laboratory assays, we were able to differentially alter the dynamics of terrestrial and authochthonous DOM forms. Additions of labile DOC displaced demand for terrestrially derived organic matter but did not alter rates of in-stream production. Additions of inorganic nitrogen led to a direct increase in autochthonous production, as well as an indirect displacement of heterotrophic demand for terrestrial DOM due to the increased production of bioavailable algal-derived DOM. Our results demonstrate that autochthonous and terrestrially derived DOM sources exhibit important differences in their dynamics, and that the ability to differentiate between these sources provides insight into the processes controlling DOM production and consumption within the stream channel.

In Chapter 4, we found that changes in climate patterns have altered watershed biogeochemistry in the Walker Branch watershed. The changes in climate we observed at Walker Branch over the past 20 years we large, with a ~2°C increase in mean annual
temperatures, a ~20% reduction mean annual precipitation, and >40% decline in catchment runoff since 1989. Over the 20-year time period, changes in catchment hydrology alone were the single best determinant of long-term trends in solute concentrations and fluxes. At seasonal time scales, in-stream biological processes (high uptake rates by attached algae in early spring prior to leaf out and by heterotrophic microbes during leaf decomposition after autumn leaf fall), which are ultimately controlled by phenology of the riparian forest, override hydrologic processes and result in lower nutrient concentrations and fluxes. At intermediate time scales, antecedent hydrology (particularly multi-year drought) has a significant effect on solute concentrations and flux, particularly for NO$_3^-$. Changing climates have the potential to alter both hydrologic and biogeochemical processes; though in many cases it is unclear how each of these processes will affect watershed element cycles. While there is likely to be greater sensitivity to changes in hydrologic processes in catchments with deep and highly varied soil profiles and complex hydrologic flowpath structure, many small watershed ecosystems with long-term chemical records have shallow soils and well-constrained and often simplified hydrology. In this respect, the Walker Branch record offers insight into the potential effects of climate change for regions of the landscape where results from other small watershed monitoring programs may not apply.
The data presented in this dissertation illustrate that human activities are having significant effects on biogeochemical cycles in temperate forests throughout the Southern Appalachians. Human impacts are likely to increase in extent and intensity in coming decades. Ecosystem structure and function depends on the ways in which energy and elements move within and between ecosystems. We rely on the sustained integrity of ecosystems for their many services and, because of this, it is essential that we continue to refine our understanding of ecosystem responses to current and future human impacts.
Appendix A

Figure A1: Regressions between mean catchment N deposition and stream water NO$_3$ concentrations for each survey [September 2004: NO$_3 = 0.043$ NDep -0.31; April 2005: NO$_3 = 0.0579$ NDep -0.43; May 2007: NO$_3 = 0.071$ NDep -0.46, October 2008: NO$_3 = 0.042$ NDep -0.30]. Mean catchment N deposition rates were calculated from an N deposition map developed for GSMNP (Weathers et al. 2006).
Table A1: Simple linear regression results of: (1) residuals from NO3- by N deposition relationships (from Appendix A: Fig. A1), (2) DON concentrations, and (3) DOC concentrations regressed against each broadleaf and conifer percent coverages of watershed areas. “n.s.” indicates no significant relationship (α=0.05).

<table>
<thead>
<tr>
<th>Model</th>
<th>Significance</th>
<th>Intercept</th>
<th>Slope</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO3 (Residuals of NO3 by N Dep)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resid. NO3 x Broadleaf %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>September 2004</td>
<td>n.s.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>April 2005</td>
<td>n.s.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>May 2007</td>
<td>n.s.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>October 2008</td>
<td>n.s.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| Residuals NO3 x Conifer % | | | | |
| September 2004 | n.s. | - | - | - |
| April 2005 | n.s. | - | - | - |
| May 2007 | n.s. | - | - | - |
| October 2008 | n.s. | - | - | - |

| DON | | | | |
| DON x Broadleaf % | | | | |
| September 2004 | n.s. | - | - | - |
| April 2005 | n.s. | - | - | - |
| May 2007 | n.s. | - | - | - |
| October 2008 | p=0.020 | 0.037 | -0.0003 | 0.17 |

| DON x Conifer % | | | | |
| September 2004 | p=0.006 | 0.047 | 0.0006 | 0.20 |
| April 2005 | n.s. | - | - | - |
| May 2007 | n.s. | - | - | - |
| October 2008 | n.s. | - | - | - |

| DOC | | | | |
| DOC x Broadleaf % | | | | |
| September 2004 | n.s. | - | - | - |
| April 2005 | n.s. | - | - | - |
| May 2007 | n.s. | - | - | - |
| October 2008 | p=0.016 | 1.39 | -0.0088 | 0.19 |

| DOC x Conifer % | | | | |
| September 2004 | p=0.005 | 0.68 | 0.0122 | 0.21 |
| April 2005 | p=0.007 | 0.415 | 0.0081 | 0.22 |
| May 2007 | n.s. | - | - | - |
| October 2008 | n.s. | - | - | - |
Table A2. Mean (±S.E.) DOC and DON concentrations across dominant catchment parent geologies and soil types. Dominant classification is defined as >50% coverage of watershed area. Results from all surveys are lumped.

<table>
<thead>
<tr>
<th>Dominant</th>
<th>Mean DOC (mg/L)</th>
<th>Mean DON (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parent Geology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anakeesta</td>
<td>1.051±0.093A</td>
<td>30±7</td>
</tr>
<tr>
<td>Elkmont</td>
<td>0.822±0.086AB</td>
<td>22±6</td>
</tr>
<tr>
<td>Metcalf</td>
<td>0.809±0.121AB</td>
<td>30±9</td>
</tr>
<tr>
<td>Roaring Fork</td>
<td>0.425±0.228AB</td>
<td>30±17</td>
</tr>
<tr>
<td>Thunderhead</td>
<td>0.663±0.043AB</td>
<td>28±3</td>
</tr>
<tr>
<td>No Dominant</td>
<td>0.756±0.076AB</td>
<td>29±5</td>
</tr>
<tr>
<td><strong>Soil Type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bp</td>
<td>0.754±0.123A</td>
<td>27±9</td>
</tr>
<tr>
<td>Dt</td>
<td>0.858±0.072AB</td>
<td>32±5</td>
</tr>
<tr>
<td>Jt</td>
<td>0.877±0.098AB</td>
<td>27±7</td>
</tr>
<tr>
<td>Lr</td>
<td>1.177±0.162AB</td>
<td>33±12</td>
</tr>
<tr>
<td>So</td>
<td>0.641±0.074B</td>
<td>28±5</td>
</tr>
<tr>
<td>No Dominant</td>
<td>0.675±0.047B</td>
<td>26±3</td>
</tr>
</tbody>
</table>

* No significant differences found in mean DON concentrations across parent geologies or soils.

Table A3. Chemical compositions of microbial inocula for each bioavailability assay. Values represent the concentrations after the inocula were added to 40mL DI water.

<table>
<thead>
<tr>
<th></th>
<th>May 2007</th>
<th>October 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN</td>
<td>24±5</td>
<td>62±6</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>15±1</td>
<td>39±0</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>3±3</td>
<td>10±2</td>
</tr>
<tr>
<td>DON</td>
<td>5±0'</td>
<td>13±0</td>
</tr>
<tr>
<td>DOC</td>
<td>0.221±0.022</td>
<td>0.409±0.018</td>
</tr>
<tr>
<td>bDOC</td>
<td>0.001±0.001</td>
<td>0.322±0.025</td>
</tr>
</tbody>
</table>

*All values were less than detection limit.
References


Biography

Brian D. Lutz was born March 22, 1983, an exact 28 years prior to the defense date of this dissertation. Raised on a family grain farm, Brian developed an interest in nutrient biogeochemistry, and the role that humans play in altering element cycles, from an early age. He graduated from Lordstown High School in 2001 and from The College of Wooster in 2005, receiving a B.A. summa cum laude in 2005 with a major in Biology and minor in Geology. Brian’s undergraduate thesis, titled “Responses of Lythrum salicaria seedbanks to repeated annual treatments of the aboveground vegetation with glyphosate based herbicides,” earned highest honors.

During 2005-2006 Brian worked as a GIS technician at Techni Graphics, Inc. (Wooster, OH) processing classified satellite imagery for the US Department of Defense. He also worked during this time as a GIS programmer for the Office of the Auditor in Trumbull County, OH.

Brian entered graduate school at Virginia Polytechnic and State University in 2006 working with Drs. Maury Valett, Jack Webster, and Fred Benfield. In the summer of 2007 he transferred to Duke University to complete his graduate research with Dr. Emily Bernhardt. Brian has held several fellowships during his graduate career, including a National Science Foundation Graduate Research Fellowship (2007-2010), a joint National Science Foundation and Research Council of Norway Fellowship to study...
in Oslo, Norway for 3 months (Fall 2009), and a Katherine Goodman Stern Dissertation Fellowship through Duke University (2010-2011). In support of his research, he has received the following awards: Budweiser and National Fish and Wildlife Foundation Conservation Scholarship (2004), a President’s Award from the North American Benthological Society (2008), student grants-in-aid of research from the Department of Biology at Duke University (2008) and from Duke Chapter of Sigma Xi (2008). He has also received travel awards from Duke University, the American Society of Limnology and Oceanography, and the National Science Foundation to attend a variety of national and international conferences.

Brian is a member of the North American Benthological Society, the American Society of Limnology and Oceanography, the Ecological Society of America, the American Geophysical Union, Sigma Xi, and the International Humic Substance Society.

Brian lives in Durham, NC with his wife of 5 years, Dory Lutz, and his daughter of 3 years, Gracie Rae Lutz. Brian and Dory are expecting a second daughter in June.

Publications
