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Land use controls on the delivery, processing, and removal of nitrogen from small watersheds:

Insights from the dual isotopic composition of stream nitrate

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Abstract

Studies have repeatedly shown that agricultural and urban areas export considerably more nitrogen to streams than forested counterparts; yet it is difficult to identify and quantify nitrogen sources to streams due to complications associated with terrestrial and in-stream biogeochemical processes. In this study we used the isotopic composition of nitrate ($\delta^{15}$N-NO$_3^-$ and $\delta^{18}$O-NO$_3^-$) in conjunction with a simple numerical model to examine the spatial and temporal variability of nitrate (NO$_3^-$) export across a land use gradient and how agricultural and urban development affects net removal mechanisms. In an effort to isolate the effects of land use we chose small headwater systems in close proximity to each other; limiting the variation in geology, surficial materials and climate between sites. The $\delta^{15}$N and $\delta^{18}$O of stream NO$_3^-$ varied significantly between urban, agricultural and forested watersheds indicating that nitrogen sources are the primary determinant of the $\delta^{15}$N-NO$_3^-$, while the $\delta^{18}$O-NO$_3^-$ was found to reflect biogeochemical processes. The greatest NO$_3^-$ concentrations corresponded with the highest stream $\delta^{15}$N-NO$_3^-$ values due to the enriched nature of two dominant anthropogenic sources, septic and manure, within the urban and agricultural watersheds, respectively. On average net removal of the available NO$_3^-$ pool within urban and agricultural catchments was estimated at 45%. The variation in the estimated net removal of NO$_3^-$ from developed watersheds was related to both drainage area and the availability of organic carbon. The determination of differentiated isotopic land use signatures and dominant seasonal mechanisms illustrates the usefulness of this approach in examining the sources and processing of excess nitrogen within headwater catchments.

Keywords: nitrogen, nitrate-nitrogen, agricultural, urbanization, watershed, nitrate removal, stable isotopes
Introduction

Human activity has greatly altered the nitrogen cycle worldwide (Galloway et al. 2004) resulting in the increased loading of nitrogen to rivers (Peierls et al. 1991, Goolsby et al. 2000). Despite high anthropogenic loadings, results from nitrogen mass balance models suggest that the majority (>70%) of the nitrogen added to watersheds is not exported to the coast but is removed or retained internally (Howarth et al. 1996, Boyer et al. 2002, Schaefer and Alber 2007). However, the relative importance of removal and retention processes (e.g. assimilation and denitrification) is difficult to measure at the watershed scale due to spatial and temporal variation (Van Breemen et al. 2002, Seitzinger et al. 2006).

Numerous researchers have documented the usefulness of the isotopic composition of nitrate (NO$_3^-$) in elucidating sources and deciphering transformations within both aquatic and terrestrial systems (e.g. Aravena et al. 1993, Mayer et al. 2002, Groffman et al. 2006, Kendall et al. 2007). By compiling data from numerous studies researchers have defined ranges of $\delta^{15}$N and $\delta^{18}$O of NO$_3^-$ for various sources (Kendall et al. 2007); however these ranges are not mutually exclusive due in large part to transformations within the environment which fractionate both $\delta^{15}$N and $\delta^{18}$O-NO$_3^-$. For example, the large range and enrichment with respect to the source for sewage and manure $\delta^{15}$N-NO$_3^-$ is likely due to varying degrees of ammonia volatization (e.g. Kreitler 1979). Microbial denitrification is also capable of substantially altering the isotopic composition of NO$_3^-$, enriching the remaining NO$_3^-$ with $^{15}$N and $^{18}$O (e.g. Mariotti et al. 1988, Böttcher et al. 1990). The fractionation associated with denitrification varies and is influenced by factors such as NO$_3^-$ concentration and temperature (Mariotti et al. 1982). Nitrification, in contrast, results in the preferential incorporation of the lighter isotopes into NO$_3^-$ and often leads to a decrease in $\delta^{15}$N-NO$_3^-$ (e.g. Mariotti et al. 1981, Yoshida 1988).
In acknowledgement of the isotopic enrichment associated with a suite of processes both within the terrestrial and stream ecosystem, we investigated the variation in the isotopic composition of NO$_3^-$ to better understand the controls on nitrogen export across a land use gradient. By sampling streams draining small headwater systems relatively close to one another we limited the variation in geology, surficial materials, and climate between our sites, allowing us to focus on land use differences. Through the examination of the $\delta^{15}$N and $\delta^{18}$O of stream NO$_3^-$ we tried to identify the dominant nitrogen source(s) to small watersheds and determine the relative importance of biogeochemical processing to NO$_3^-$ export across space and time. From an aquatic management perspective it is desirable to be able to determine not only the sources of nitrogen loading within watersheds but also the fate of the anthropogenic inputs. This is especially difficult in watersheds dominated by non-point source inputs, as is the case for the majority of systems draining to the North Atlantic (Howarth et al. 1996).

In this paper we present the $\delta^{15}$N and $\delta^{18}$O-NO$_3^-$ results from 15 headwater systems with dominant land use characteristics within the Connecticut River watershed, which contributes 70% of the freshwater and 9.8% of the total watershed nitrogen load to the eutrophic Long Island Sound (NYDEC and CTDEP 2000). By comparing the concentration and isotopic composition of NO$_3^-$ both spatially and temporally we hope to (1) quantify the spatiotemporal variation of the stream concentration and isotopic composition of NO$_3^-$, (2) estimate how net removal of NO$_3^-$ varies between catchments and within a given catchment over time, and (3) determine the factors which best explain the variation in net nitrogen removal from urban and agricultural watersheds.

Methods

**Spatial Analysis**
Watersheds were delineated using ArcHydro tools in ArcMap 9.1 (ESRI, Redlands, CA) using NHDPlus data which includes hydrography and digital elevation information for the region (USGS and USEPA 2005). Land use and impervious cover (MRLC 2005, available at: http://www.mrlc.gov/mrlc2k_nlcd.asp), surficial materials (Stone et al. 1992), bedrock geology (Rodgers 1985, Robinson and Kapo 2003), and sewage service (CTDEP 1998, available at: www.ct.gov/dep/cwp/view.asp?a=2698&q=322898) data were downloaded from several federal and state agencies’ websites. These data were then analyzed using spatial analysis tools in ArcMap 9.1 to determine land use/land cover, percent impervious surface, surficial materials, bedrock geology, sewage service, and monthly precipitation totals of each watershed.

Sample Collection

Sampling of fifteen stream sites (Figure 1) occurred bimonthly over 14 months, June 2005 to August 2006. Sampling was conducted during the 2nd week of each month irrespective of flow conditions. This resulted in sample collection occurring at various points in the hydrograph; close to half of the samples were collected at or near baseflow conditions and two sets of samples (October 2005 and June 2006) were collected near the peak of storm hydrographs. For more information about flow conditions during sampling please see discussion below.

Streamwater for dissolved nitrogen and isotope analyses was filtered in the field through pre-baked GF/F filters, collected in acid-washed, filtered stream water-rinsed HDPE bottles, and stored on ice until returning to lab. Before freezing, samples reserved for isotopic analysis were brought to pH 11 using 6M NaOH (Böhlke et al. 2004, Casciotti et al. 2007); while samples for dissolved nitrogen analyses were transferred to the freezer immediately upon returning to lab.
At the time of sample collection, stream discharge was estimated by measuring stream velocity and depth using a Marsh-McBirney electromagnetic current meter and wading rod at regular intervals across the stream. In field measurements of conductivity and temperature were made using an YSI Model 85 Dissolved Oxygen and Conductivity Meter (Yellow Springs, OH).

Rain samples were collected on an event basis throughout north-central Connecticut from June 2006 through March 2007 with the help of employees at five wastewater treatment plants located in Manchester, Vernon, Farmington, Canton, and Winsted, CT (Figure 1). Glass acid-washed 4 L beakers were placed in open areas preceding a rainstorm and were collected shortly after it ended; rainwater was immediately transferred to acid washed polycarbonate bottles and frozen until analyses.

Groundwater samples were obtained with the assistance of the Connecticut office of the United States Geological Survey. Three groundwater samples were retrieved from wells within the Hockanum River and Broad Brook watersheds in November of 2006 and two additional samples were obtained from the Broad Brook watershed in April of 2007 (Figure 1). All five samples are from stratified drift aquifers underlying these watersheds which past studies have shown to be relatively homogeneous with respect to NO$_3^-$ (Mullaney and Grady 1997, Mullaney and Zimmerman 1997) and wells were chosen in order to best measure the aquifer’s chemistry with a limited number of samples (J. Mullaney, USGS, personal communication). Samples were collected in acid-washed HDPE 1 L bottles in the field, kept on ice and filtered in lab following the same protocols used for stream samples.

Sample Analysis
Nitrogen ion (NO$_3^-$ and NO$_2^-$) analyses were performed using an Astoria 2 Analyzer with detection limits of 0.005 and 0.002 mg N L$^{-1}$, respectively. Approximately 10% of the samples were run in replicate for quality assurance purposes, on average, standard deviations between replicate samples were within 0.01 and 0.002 mg L$^{-1}$ for NO$_3^-$ and NO$_2^-$, respectively.

The isotopic analysis of NO$_3^-$ was performed using the denitrifier method (Sigman et al. 2001, Casciotti et al. 2002), in which NO$_3^-$ and NO$_2^-$ are quantitatively converted to N$_2$O. The $^{15}$N/$^{14}$N and $^{18}$O/$^{16}$O ratios of the N$_2$O were then analyzed on a Finnigan DeltaPLUS XP isotope ratio mass spectrometer. These analyses were standardized relative to atmospheric N$_2$ and Vienna Standard Mean Ocean Water scales for $\delta^{15}$N-NO$_3^-$ and $\delta^{18}$O-NO$_3^-$, respectively, by parallel analyses of NO$_3^-$ reference materials USGS32, USGS34, and USGS35. Duplicate measurements were made on all samples, with standard deviations falling within the cited reproducibility of 0.3‰ and 0.5‰ (1 standard deviation) for $\delta^{15}$N-NO$_3^-$ and $\delta^{18}$O-NO$_3^-$, respectively (Sigman et al. 2001, Casciotti et al. 2002). In cases where NO$_2^-$ concentrations contributed more than 1% of total NO$_3^-$ and NO$_2^-$ concentration the $\delta^{15}$N- and $\delta^{18}$O-NO$_3^-$ values were corrected using the method discussed in Casciotti et al. (2007) and Casciotti and McIlvin (2007). In summary, the $\delta^{15}$N and $\delta^{18}$O of NO$_2^-$ is measured using the azide method (McIlvin and Altabet 2005) and then subtracted from the $\delta^{15}$N and $\delta^{18}$O results from the denitrifier method (which measures both NO$_3^-$ and NO$_2^-$ together) to yield the $\delta^{15}$N and $\delta^{18}$O of NO$_3^-$.

The $^{15}$N content of atmospheric NO$_3^-$ determined from isotopic measurements of N$_2$O must also be corrected for the contribution of $^{14}$N$^{14}$N$^{17}$O to the mass 45 peak. This correction typically assumes the following mass dependent relationship for the N$_2$O analyte: $\delta^{17}$O $\approx$ 0.52*$\delta^{18}$O (Michalski et al. 2002). However, this relationship is not necessarily true for atmospheric NO$_3^-$ because the $\delta^{17}$O and $\delta^{18}$O of atmospheric NO$_3^-$ are mass independent (e.g.
Michalski et al. 2002, Michalski et al. 2003). Using the average ratio of $\delta^{17}O/\delta^{18}O$ of rain samples collected in Princeton, NJ (Kaiser et al. 2007, Meredith G. Hastings, Brown University, personal communication) the following relationship was assumed ($\delta^{17}O \approx 0.90 \times \delta^{18}O$) to correct the measured $\delta^{15}N$ of $NO_3^-$ in rain for the $^{17}O$ contribution to the $^{15}N/^{14}N$ ratio (see work by Hastings et al. 2004 for a similar correction).

Daily flow estimation and hydrograph separation

Daily flow data for the sampled streams was estimated using the Maintenance of Variance-Extension, type 1 (MOVE.1) method, a record-extension technique (Helsel and Hirsch 1992) utilizing both instantaneous field measurements and daily discharge records from the USGS’s National Water Inventory database (USGS 2007, available at: waterdata.usgs.gov/nwis). Field measurements were compared to at least three nearby USGS gauging station datasets and the correlation coefficient for each gauging station-field data pair was calculated. The pair with the highest correlation coefficient ($R^2 > 0.93$) was chosen to estimate the daily mean flow for each stream using the MOVE.1 equation (eqn 1).

$$Y_i = \bar{Y} + \frac{S_y}{S_x} (X_i - \bar{X})$$

(eqn. 1)

Where $Y_i$ is the estimated daily streamflow on day $i$, $\bar{Y}$ is the mean of the field flow measurements, $S_y$ is the standard deviation of the field flow measurements, $S_x$ is the standard deviation of the daily streamflows at the chosen gauging station, $X_i$ is the mean streamflow on day $i$ for the gauging station, and $\bar{X}$ is the mean of the daily streamflows at the gauging station (corresponding to the days of field flow measurements). This estimation procedure is done on $\log_{10}$ transformed data for linearity purposes. The strength of this procedure lies in the fact that the resulting daily flow estimates are statistically distributed in a similar way to actual stream
flow measurements (Helsel and Hirsch 1992). These estimates were then entered into a web-based hydrograph separation tool (Lim et al. 2005) to determine the approximate flow conditions (i.e. percent baseflow) at the time of sampling.

**Nitrate Removal Model**

In order to use the seasonal signal of the isotopic composition of NO$_3^-$ to estimate the net removal of NO$_3^-$ from each catchment it was necessary to quantify the contributing NO$_3^-$ pools. High [NO$_3^-$] within the shallow aquifers and results from past studies implicate groundwater as the dominant source of NO$_3^-$ to urban and agricultural streams in the region (Grady 1994, Mullaney and Grady 1997, Mullaney 2007). Given that we do not have enough information to quantify individual flow paths the conceptual model groups NO$_3^-$ source pools into two compartments: “old” water (i.e. groundwater, in this case a shallow unconfined aquifer) and “new” water (i.e. precipitation, overland flow, shallow interstitial flow through the vadose zone). Processes affecting NO$_3^-$ occur along several flow paths within both stream and terrestrial ecosystems; in this conceptual model we group the terrestrial and aquatic terms into one, “net removal of NO$_3^-$”. The net removal of NO$_3^-$ (denitrification + biotic uptake – net nitrification) was estimated for each watershed as depicted in Figure 2. We acknowledge that NO$_3^-$ is not a conservative tracer within a watershed, however because the model is physically constrained by the baseflow separation results (see below) and validated by a hydrologic mixing model using $\delta^{18}$O-H$_2$O (see below) we believe the parameter estimates are valid for comparison purposes.

In an effort to quantify the net removal of NO$_3^-$ within each catchment the conceptual model considered each $\delta^{15}$N-NO$_3^-$ stream measurement within the agricultural and urban watersheds as the result of two processes: hydrological mixing and net NO$_3^-$ removal. Our
A conceptual model is derived from a two end-member isotopic mixing model (eqn. 2), a commonly used tool in isotope biogeochemistry. Two end-member mixing models allow one to predict the isotopic and chemical composition of a mixture given known source values, for example if two streams with known flows ($Q_1$ and $Q_2$), known isotopic values ($I_1$ and $I_2$), and concentrations ($C_1$ and $C_2$) merge into one well mixed stream the isotopic value ($I_s$) of the larger stream’s discharge ($Q_s$) can be calculated from eqn. 2, given that $Q_1 + Q_2 = Q_s$.

$$Q_sC_sI_s = Q_1C_1I_1 + Q_2C_2I_2$$  \hspace{1cm} (eqn. 2)

However it has been well established that NO$_3^-$ will likely undergo transformations within a watershed. Therefore an isotopic fractionation term was added to the basic mixing model to account for any biogeochemical processing of the NO$_3^-$ pool, with $\varepsilon$ representing the net isotopic fractionation associated with the loss of NO$_3^-$ from the catchment and $F$ representing the fraction of NO$_3^-$ remaining (eqn. 3).

$$-\varepsilon \times \ln F$$  \hspace{1cm} (eqn. 3)

In order to determine the fraction of NO$_3^-$ remaining ($F$), the initial amount within the watershed needed to be determined. The concentration of the total NO$_3^-$ pool ([NO$_3^-$]$_{\text{MIX}}$) was calculated using a two end-member mixing model (eqn. 4).

$$[\text{NO}_3^-]_{\text{MIX}} = [\text{NO}_3^-]_n \times V_n + [\text{NO}_3^-]_o \times V_o$$  \hspace{1cm} (eqn. 4)

Where $V$ represents the volume fraction of water contributing to [NO$_3^-$]$_{\text{MIX}}$ and the subscripts $n$ and $o$ signify the new and old NO$_3^-$ source pools, respectively. This model assumes that the volume fraction of old water ($V_o$) and volume fraction of new water ($V_n$) must always add to 1, therefore $V_n$ is defined as $1-V_o$. The fraction of NO$_3^-$ remaining is then calculated using eqn. 5.

$$F = \frac{[\text{NO}_3^-]_p}{[\text{NO}_3^-]_{\text{MIX}}}$$  \hspace{1cm} (eqn. 5)
Where $[\text{NO}_3^-]$ represents the $[\text{NO}_3^-]$ in the stream at the time of sampling. Thus, using the above
equations the $\delta^{15}\text{N-NO}_3^-$ in the stream can be explained using eqn. 6.

\[
\delta^{15}\text{N}_o = \delta^{15}\text{N}_n \times \frac{[\text{NO}_3^-]_n}{[\text{NO}_3^-]_m} + \delta^{15}\text{N}_n \times \frac{[\text{NO}_3^-]_m}{[\text{NO}_3^-]_m} - \varepsilon \times \ln P
\]  

(eqn. 6)

The model was only applied to agricultural and urban streams because we did not sample
groundwater within the forested watersheds.

Using the model we solved for $V_n$ by setting $\delta^{15}\text{N}_o$ and $[\text{NO}_3^-]_o$ equal to the average value
of groundwater samples in agricultural and urban areas as appropriate and running 1728
iterations for each stream-month pair. The permutation of isotopic fractionation factors for $\delta^{15}\text{N-}
\text{NO}_3^-$ (0‰ to 18‰, by 3‰), range of values for $\delta^{15}\text{N-NO}_3^-$ (-7.7‰ to +14‰, by 1‰), and $\text{NO}_3^-$
concentrations (0.01 mg l$^{-1}$ to 7 mg l$^{-1}$, by 0.5 mg l$^{-1}$), for $\varepsilon$, $\delta^{15}\text{N}_n$ and $[\text{NO}_3^-]_n$, respectively,
determined the iterations used for each stream-month pair. Each of the 1728 iterations had the
same values entered for stream and groundwater characteristics ($\delta^{15}\text{N}_s$, $[\text{NO}_3^-]_s$, $\delta^{15}\text{N}_o$, $[\text{NO}_3^-]_o$),
with the stream characteristics changing each month to reflect the measured values. This number
of iterations was necessary because we do not have measurements of soil water or other
components of new water, therefore we applied the entire range of $\text{NO}_3^-$ isotopic and
concentration measurements made in streams, rain and groundwater to the new water term.

Furthermore, we do not know the magnitude or nature of nitrogen transformations occurring
within these environments and thus applied a large range of values for the isotopic fractionation
associated with the removal of $\text{NO}_3^-$. The fractionation factors used represent the range of
apparent fractionations attributed to denitrification found in the literature, with 0‰ representing
denitrification limited by diffusion and 18‰ representing riparian denitrification (Sebilo et al.
2003, Lehmann et al. 2004). The isotopic fractionation of $\delta^{15}\text{N-NO}_3^-$ associated with biotic
uptake of $\text{NO}_3^-$ (4-9‰, Granger et al. 2004, Needoba and Harrison 2004, Needoba et al. 2004,
DiFiore et al. 2006) fall within the range defined by denitrification end-members therefore we feel confident that our model accounts for the likely apparent isotopic fractionation associated with NO$_3^-$ uptake. While nitrification will add NO$_3^-$ to the overall system, the majority of this NO$_3^-$ is accounted for in the [NO$_3^-$]$_n$ and [NO$_3^-$]$_o$ terms for two reasons: (1) within the stream NH$_4^+$ makes up a small portion (<10%) of total DIN suggesting the effects of in-stream ammonium oxidation will be small on the overall $\delta^{15}$N-NO$_3^-$ and (2) most inputs to the watershed are in reduced or organic forms and thus must be converted to NO$_3^-$ before adding to the overall NO$_3^-$ pool and thus nitrification has already been accounted for, see discussion below for a more in depth explanation. Finally, the average concentration and isotopic values from the Broad Brook and Hockanum River groundwater samples were used to represent the old water end-member for all agricultural sites and urban sites, respectively. This assumption is based on the simplification that groundwater in unconfined sandy aquifers reflects the overlying land use; with greater NO$_3^-$ concentrations associated with agricultural production (Grady 1994) and higher $\delta^{15}$N-NO$_3^-$ values in developed versus pristine watersheds (Cole et al. 2006).

The equations were solved numerically using Matlab 7.5 (The Mathworks 2007) for $V_n$ and in turn $V_n$ was used to calculate $V_o$, $F$, and [NO$_3^-$]$_{MIX}$. Results from baseflow separation calculations (e.g. Figure 3c-e) were used to hydrologically constrain the model by setting $V_o$ equal to calculated baseflow at the time of sampling (±10%). Iterations where $F$ did not fall between zero and one were discarded. Remaining results were run through a parallel set of equations (eqns. 1-3) for $\delta^{18}$O-NO$_3^-$ using stream and groundwater measurements to define $\delta^{18}$O$_S$ and $\delta^{18}$O$_o$, respectively. This analysis was repeated using the same isotopic fractionation factors used in the $\delta^{15}$N equations and using fractionation factors equal to one-half of the epsilon used in the $\delta^{15}$N equations. Studies have shown that denitrification can enrich $\delta^{15}$N-NO$_3^-$ values
approximately one to two times that of $\delta^{18}$O-NO$_3^-$ in freshwater systems (Aravena and Robertson 1998, Cey et al. 1999, Mengis et al. 1999, Granger et al. 2008), while NO$_3^-$ uptake tends to result in a 1:1 fractionation of the $\delta^{15}$N and $\delta^{18}$O of NO$_3^-$ (Granger et al. 2004) and we wanted to account for this range within our model. Iterations where the predicted $\delta^{18}$O was less than -15‰ or greater than 95‰ were discarded.

Model validation: hydrograph separation using $\delta^{18}$O-H$_2$O

In an effort to provide an independent validation of the results from our conceptual model stream contributions of new and old water were estimated using the $\delta^{18}$O-H$_2$O (e.g. Sklash et al. 1976, Hooper and Shoemaker 1986)

$$Q_s \times I_s = Q_n \times I_n + Q_o \times I_o$$  \hspace{1cm} (eqn. 7)

$$Q_s = Q_n + Q_o$$  \hspace{1cm} (eqn. 8)

where $Q$ is the volume flow rate, $I$ is the $\delta^{18}$O-H$_2$O, and the subscripts denote the water source ($s$ is stream water, $n$ is new water, and $o$ is old water).

The $\delta^{18}$O-H$_2$O of stream samples was measured for all sampling dates except June 2005 and old water’s $\delta^{18}$O-H$_2$O value was defined by the average measured $\delta^{18}$O-H$_2$O of the groundwater samples from the shallow unconfined aquifers in the region. While precipitation samples were measured for $\delta^{18}$O-H$_2$O they were not collected over the full duration of stream sampling efforts and thus monthly values were generated utilizing the Bowen et al. (2005) model. Given our reliance on modeled data, which predicts the long term monthly average $\delta^{18}$O-H$_2$O of precipitation for a given location, we were unable to resolve the mixing model (eqns. 7-8) for all streams in October 2005 and three agricultural streams in April 2006. In the case of the October 2005 sampling event the mismatch in data is due to the fact that sampling occurred
directly following the remnants of Hurricane Tammy, a storm that resulted in 100-yr flood conditions in some of the sampled systems (Glowacki 2005).

Uncertainty for the results of the above mixing models (eqn. 7-8) was calculated using the Gaussian error propagation technique following Genereux (1998):

\[
W_f = \left[ \frac{f_o}{f_n - f_o} W_{1o}^2 + \frac{f_n}{f_n - f_o} W_{1n}^2 + \frac{1}{f_n - f_o} W_{new}^2 \right]^{1/2}
\]  

(eqn. 9)

The calculated uncertainty \(W_f\) is determined using the calculated fractions \(f\) of stream flow attributed to each component. All \(W\) terms on the right hand side of the equation represent uncertainty due to analytical error (0.05) in the case of stream water, spatial variation (0.18) for old water, or range in predicted values (0.07) for new water.

It is important to note that the results from the mixing model using \(\delta^{18}O-H_2O\) are dependent on slightly different definitions of new water in comparison to the nitrate removal model described above. Old water is defined as the water in the shallow unconfined aquifer in both cases, however new water is strictly precipitation in the \(\delta^{18}O-H_2O\) model while it accounts for all event water (i.e. non-groundwater) in the nitrate removal model. Due to these different definitions we would expect the \(\delta^{18}O-H_2O\) model to predict a smaller new water contribution to total stream flow as compared to the nitrate removal model.

**Statistical methods**

A suite of univariate and multivariate statistical approaches were applied to both the measured data and model results. Paired \(t\)-tests were used to determine the statistical seasonal differences between water yield, stream \(NO_3^-\) concentrations, \(\delta^{15}N-NO_3^-\), and \(\delta^{18}O-NO_3^-\) at each sampling location. Comparisons between \(NO_3^-\) concentrations and its isotopic composition in streams draining different watershed types were performed using analysis of variance (ANOVA)
tests with Tukey’s correction for type I errors (family error rate of 5%). The concomitant variation of $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ across land use and sampling date was assessed using multivariate analysis of variance (MANOVA). Descriptive statistics (mean, standard deviation, range) were calculated for each of the predicted parameters in the model. ANOVA was used to assess the variability of each parameter with sampling date, using Tukey’s method of pairwise comparison.

### Study Area

The fifteen headwater catchments are located within the Farmington River, Hockanum River and Broad Brook watersheds within Connecticut and southwestern Massachusetts. All three watersheds are major tributaries to the Connecticut River, which drains to Long Island Sound (Figure 1).

### Land Use

The five forested watersheds are within the Farmington River and Hockanum River watersheds (Figure 1): Charters Brook (CB), Riiska Brook (RB), Sandy Brook (SB), the headwaters of the Still River (HSR), and the headwaters of the West Branch of the Farmington River (HWFR). These watersheds are dominated (80% to 98%) by forests typical of southern New England, mixed deciduous and coniferous stands, and also include a varying amount of wetlands (0% to 11%, Table 1). Agricultural and urban land uses within these watersheds are dominated (>95%) by pasture and open space, respectively.

The agricultural watersheds are located within the Farmington River and Broad Brook watersheds (Figure 1): the headwaters of Broad Brook (HBB), Muddy Brook (MB), Creamery...
Brook (CB), Kendall Brook (KB), and the headwaters of the West Branch of Salmon Brook (HSB). Corn and hay are the principal crops within these watersheds; though alfalfa and dairy production are also present (C. Clark & T. Morris, University of Connecticut, written communication). Agricultural production activities use 32% to 46% of the watershed area in these catchments (Table 1). While pasture and row crops may not be the majority of the land use within the individual watersheds we believe they are the dominant sources of nitrogen to the streams for two reasons: the majority of the remaining area is forested and these forests occupy the headwater portions of the catchments with agricultural activities located directly upstream from the sampling points. Site KB is an exception to this and is not included in the land use statistical analyses because during field work it became apparent that the land use data were not current and much of the agricultural land had been converted to suburban developments.

The urbanized sites are located with the Farmington River and Hockanum River watersheds (Figure 1), with residential, industrial and commercial uses making up between 43% and 83% of the watershed area: Bigelow Brook (BB), Ogen Brook (OB), Folly Brook (FB), Thompson Brook (TB), and Chudsey Brook (CBu) (Table 1). The urbanized sites encompass a range of development densities with light to medium density residential development dominating TB and CBu and denser residential and commercial development characterizing BB. The percent of each watershed area served by municipal sewer lines varies greatly between systems with OB being almost entirely sewered (99.2%) and TB and CBu having little sewer service, 2.6% and 3.5%, respectively (Table 1). There are no wastewater treatment plant discharges within the small watersheds. The mean percent impervious cover was highest in the urban watersheds and followed a pattern that was closely correlated with development density, ranging from 9.6% to 36.4% (Table 1).
Bedrock & Surficial Geology

The bedrock mineralogy varied little between watersheds (see Barnes and Raymond (2009) for more detailed description of geology). Glacial till is the dominant surface material in 11 of the 15 watersheds (Table 1). The majority of Broad Brook and Hockanum River watersheds as well as eastern portions of the Farmington River watershed are underlain by highly transmissive stratified drift aquifers, dominated by sand and gravel (Grady 1994). According to Grady (1994) residence times in these aquifers can range from less than a year to greater than 20 years.

Precipitation

Precipitation in the region is distributed evenly throughout the year with snow making up a minor component (9-10%) of the average annual budget of 1140 mm. Northwestern Connecticut receives slightly more snow on average than the central portion of the state, approximately 1524 mm, contributing 13% to the annual budget (Miller et al. 2002). Daily precipitation records for the duration of the study were obtained from the National Climatic Data Center for the weather station at Bradley International Airport (NCDC 2007).

Results

Dissolved nitrogen

Over the duration of sampling, flow weighted mean nitrate-nitrogen (NO$_3^-$) concentrations for streams draining agricultural, urban and forested watersheds were 3.47 mg L$^{-1}$, 1.93 mg L$^{-1}$, and 0.01 mg L$^{-1}$, respectively. For each sampling effort, the NO$_3^-$ concentrations
were significantly different \((p< 0.005)\) between land use types (Figure 3a). Nitrate made up the vast majority (>90%) of dissolved inorganic nitrogen \((\text{DIN}=\text{NO}_3^-+\text{NH}_4^+)\) in both the urban and agricultural streams. In contrast, due to \(\text{NH}_4^+\) dominating the DIN pool in forested streams during high flow periods \(\text{NO}_3^-\) made up an average of 44% of the DIN exported from these systems. Nitrate concentrations fluctuated throughout the sampling period in all watersheds with the greatest temporal variation seen in the agricultural streams \((\text{sd}= 0.71 \text{ mg L}^{-1})\) with urban and forested streams displaying smaller ranges in \(\text{NO}_3^-\) concentrations, 0.38 mg L\(^{-1}\) and 0.08 mg L\(^{-1}\), respectively (Figure 3a). The highest \(\text{NO}_3^-\) concentrations were observed in August of 2005 and 2006 and the lowest concentrations were seen during the large storm event in October 2005 (Figure 3a).

The average \(\text{NO}_3^-\) concentration of atmospheric wet deposition collected across the five sites in northern CT was 0.61 mg L\(^{-1}\). Groundwater \(\text{NO}_3^-\) concentrations in the aquifers were approximately twice that of the streams they fed. The Hockanum aquifer \(\text{NO}_3^-\) concentrations averaged 3.89 mg L\(^{-1}\) as compared to the flow weighted average of 1.83 mg L\(^{-1}\) for its streams. A similar relationship is seen within the Broad Brook basin, with groundwater \(\text{NO}_3^-\) concentrations of 7.10 mg L\(^{-1}\) compared to a flow weighted stream \(\text{NO}_3^-\) average concentration of 3.67 mg L\(^{-1}\).

**Isotopic composition of \(\text{NO}_3^-\)**

Streams draining agricultural watersheds have the most enriched and variable \(\delta^{15}\text{N}-\text{NO}_3^-\) \((\text{avg}= 10.01\%\text{, sd}=1.9\%\)) urban systems were the least variable \(\delta^{15}\text{N}-\text{NO}_3^-\) \((\text{avg}= 6.68\%\text{, sd}=0.6\%\)) and forested streams had the most depleted flux-weighted mean \(\delta^{15}\text{N}-\text{NO}_3^-\) \((2.52\%\text{, sd}=0.86\%)\), Figure 4, 5). In contrast, the means of \(\delta^{18}\text{O}-\text{NO}_3^-\) were similar \((3.22\%\text{, 2.20\%})\), and
0.87‰ for agricultural, urban and forested sites, respectively, Figures 4 and 5). Forested sites displayed a greater variability in $\delta^{18}O$-NO$_3^-$ throughout the year (sd=5.02‰) as compared to agricultural (sd=1.26‰) and urban sites (sd=0.89‰) (Figure 5). The $\delta^{15}N$-NO$_3^-$ and $\delta^{18}O$-NO$_3^-$ were positively correlated in streams draining agricultural ($\delta^{18}O=0.54+0.26*\delta^{15}N$, $R^2=19\%$, $p=0.014$) and urban catchments ($\delta^{18}O=-0.03+0.34*\delta^{15}N$, $R^2=13\%$, $p=0.019$), though not in streams draining forested regions (Figure 4). A MANOVA analysis, using both land use and month as simultaneous predictors for $\delta^{15}N$-NO$_3^-$ and $\delta^{18}O$-NO$_3^-$ indicates that land use is a significant predictor of $\delta^{15}N$-NO$_3^-$, while land use, sampling month and their interaction were significant predictors for stream $\delta^{18}O$-NO$_3^-$. The isotopic composition of atmospheric deposition was considerably different from all stream samples with an average $\delta^{15}N$-NO$_3^-$ of -2.25‰ and $\delta^{18}O$-NO$_3^-$ of +70.94‰ (Figure 4). The two groundwater samples obtained from the Broad Brook aquifer had an average value of 7.1‰ for $\delta^{15}N$-NO$_3^-$ (sd=0.88‰) and 2.2‰ for $\delta^{18}O$-NO$_3^-$ (sd=0.02‰). Streams receiving this groundwater input had $\delta^{15}N$-NO$_3^-$ values higher than those measured in either of the groundwater samples. This pattern was also true within the Hockanum Basin; groundwater had an average $\delta^{15}N$-NO$_3^-$ =5.4‰ (sd=0.51‰, n=3) and $\delta^{18}O$-NO$_3^-$ =1.2‰ (sd=0.9‰, n=3), while streams fed by this aquifer had average values of 6.45‰ and 2.56‰ for $\delta^{15}N$-NO$_3^-$ and $\delta^{18}O$-NO$_3^-$, respectively. 

**Hydrograph Separation**

Across the sampling region average baseflow contributions, as determined by graphical hydrograph separation, were similar. On average, groundwater contributed 86%, 85%, and 83% of the stream discharge in the small tributaries within the Farmington, Hockanum, and Broad Brook watersheds, respectively, at the time of sampling. Sampling occurred at or near baseflow
conditions (baseflow >95%) 43% of the time (Figure 3c-e). The October 2005 and June 2006 sampling events captured a large amount of storm flow; with average new water contributions amounting to 45% and 30%, respectively, of total stream discharge across all sites (Figure 3c-e). In addition, the majority (10 of 15 sites) were running above baseflow conditions during the June 2005 and April 2006 sampling efforts, with new water making up 14 and 13% of stream discharge, respectively.

Model validation using $\delta^{18}O-H_2O$ hydrograph separation

Comparison of the percent of new water contribution to stream flow from the $\delta^{18}O-H_2O$ model and the nitrate removal model shows overlap in 41 out of 49 stream-month combinations (Table 2). It should be noted that comparisons could not be made for any of the sites in June 2005 and October 2005 and three sites in April 2006 due to data and model limitations as discussed above. In the eight cases where there was no overlap between model predictions, results from the nitrate removal model that did not meet the constraint applied by the graphical hydrograph separation were analyzed. Uncertainty of the $\delta^{18}O-H_2O$ predictions was greatest when the isotopic composition of the new and old water were not distinguishable. In general the $\delta^{18}O-H_2O$ model predicted that a smaller fraction of stream flow was derived from new water as compared to the results from the nitrate removal model. This is to be expected given the differences in new water definitions as discussed above and does not necessarily mean that the nitrate removal model predictions are incorrect. However we have chosen to only report the predictions that are validated by the $\delta^{18}O-H_2O$ model results as they are likely the most robust. Analysis of results suggests that by limiting the results of the nitrate removal model to those that are validated by the $\delta^{18}O-H_2O$ model there was a systematic increase in predictions of the
amount of nitrate removed (1-F) and a decrease in the contribution of new water (Vn). Predicted
attributes of the nitrate concentration and isotopic composition of the new water did not change
systematically.

*Nitrate Removal Model*

Results of all model run iterations were averaged for each stream-month permutation. Estimates of the amount of NO$_3^-$ remaining within the system (F) and the total NO$_3^-$ pool ([NO$_3$])$_{MIX}$ were relatively robust across the eighteen per mill range, on average varying only 7% and 11% across the range of fractionation factors, respectively. However the predicted isotopic composition of new water ($\delta^{15}$N$_n$ and $\delta^{18}$O$_n$) showed considerable variation with regards to the choice of fractionation factor, varying 76% and 78%, respectively. Despite this variability in parameter estimates, the relative seasonal patterns in the amount of NO$_3^-$ remaining, the total NO$_3^-$ pool, and the $\delta^{15}$N- and $\delta^{18}$O-NO$_3^-$ of new water were not dependent on the choice of fractionation factor.

The predicted $\delta^{15}$N of new water NO$_3^-$ was enriched within agricultural watersheds relative to the urban systems. The predicted average $\delta^{15}$N and $\delta^{18}$O of NO$_3^-$ in new water for all watersheds ranged from -4.3‰ to 6.5‰ and -4.9‰ to 15.6‰, respectively (Table 3).

The model predicted that old water contributed between 81-94% of the total NO$_3^-$ pool and that on average the urban and agricultural watersheds and streams removed between 22% and 61% of the combined pool of NO$_3^-$ (Table 3). Urban streams had greater contributions of new water than agricultural streams, 12% versus 8%, respectively; and on average urban watersheds removed a greater percentage (47% versus 41%, $p=0.05$), however the largest agricultural watershed, HBB, on average removed only 22% of the NO$_3^-$ pool and is thus an
outlier, removing this point results in urban and agricultural watersheds removing the same percentage of NO₃⁻ on average.

Discussion

Nitrogen sources and the isotopic composition of NO₃⁻

Significant differences in stream δ¹⁵N-NO₃⁻ between land uses (Figure 4) often reflects variations in nitrogen sources (e.g. Durka et al. 1994, Silva et al. 2002, Kellman and Hillaire-Marcel 2003). In this study, a strong positive relationship between δ¹⁵N-NO₃⁻ and NO₃⁻ concentrations (R²=0.62, p<0.0001) across all systems indicates that streams exporting more NO₃⁻ have a higher proportion of δ¹⁵N enriched sources, such as septic and manure waste.

Agricultural sites, had both the highest NO₃⁻ concentration (3.47 mg L⁻¹, Figure 3a) and δ¹⁵N-NO₃⁻ (avg: 9.23‰, Figure 4) reflecting activities associated with dairy and corn silage production, the dominant agricultural activities of the sampled watersheds. On average over 150 kg N ha⁻¹ yr⁻¹ is added via manure and inorganic fertilizers to agricultural land within these watersheds (Clark and Morris, University of Connecticut, written communication, Table 4); the manure inputs were positively correlated with δ¹⁵N-NO₃⁻ (p=0.001), consistent with other studies that have found distinct δ¹⁵N ratios in manure versus inorganic fertilized systems (Cabana and Rasmussen 1996, Chang et al. 2002).

Urban streams exported NO₃⁻ with an average δ¹⁵N that fell between the typical ranges of NO₃⁻ sources found in the literature (Figure 4); implying that no one nitrogen source dominates these watersheds. This reflects the addition of septic, a source enriched in δ¹⁵N but also sources relatively depleted in δ¹⁵N such as inorganic fertilizers and atmospheric deposition (-6‰ to +5‰, e.g. Kendall et al. 2007). Average estimates for septic and leaky sewer lines nitrogen
inputs were 5.3 to 13.7 kg N ha\(^{-1}\) yr\(^{-1}\) based on 2000 Census data (USDOC 2000), per person
estimates of nitrogen loads from the literature (4.8 kg N person\(^{-1}\) yr\(^{-1}\), Valiela et al. 1997, 1.85 kg N person\(^{-1}\) yr\(^{-1}\), Caraco et al. 2003), and a 10% rate of leakage from sewer lines (Groffman et al. 2004, Rose 2007). Literature estimates for inorganic fertilizer application to urban greenspaces are highly varied (Law et al. 2004), therefore based on the recommended rates of application (97.5-195 kg N ha\(^{-1}\) yr\(^{-1}\), Center for Watershed Protection 2000) urban watersheds received at most 0.95 kg N ha\(^{-1}\) yr\(^{-1}\), an input significantly smaller than both atmospheric deposition (~8 kg N ha\(^{-1}\) yr\(^{-1}\), Barnes et al. 2008) and human waste. Between system variations in \(\delta^{15}\)N-NO\(_3^-\) reflected differences in waste disposal systems. With increasing development density a greater portion of the watershed is served by municipal sewage systems, decreasing the number of homes reliant on septic systems and thus the amount of enriched \(\delta^{15}\)N entering the watershed, as illustrated by the \(\delta^{15}\)N enrichment in septic reliant areas relative to sewered watersheds in Tacoma, Washington (Silva et al. 2002) and the negative relationship between sewer coverage and \(\delta^{15}\)N-NO\(_3^-\) in urban watersheds sampled within this study (\(\delta^{15}\)N-NO\(_3^-\)=7.07-0.80*% sewered, \(R^2=13\%\), \(p=0.012\)).

In contrast to the developed watersheds, the NO\(_3^-\) exported by streams draining forested watersheds did not reflect the isotopic composition of the dominant anthropogenic nitrogen source to the systems. The forested streams consistently exported NO\(_3^-\) with the lowest \(\delta^{15}\)N (Figure 4, 5a) and both the \(\delta^{15}\)N- and \(\delta^{18}\)O-NO\(_3^-\) in stream water were significantly (\(p<0.001\)) different from the isotopic composition of atmospheric deposition, the dominant external nitrogen input (average \(\delta^{15}\)N-NO\(_3^-\): -2.3‰, \(\delta^{18}\)O-NO\(_3^-\): +70.9‰, Figure 4). The relatively depleted \(\delta^{15}\)N- and \(\delta^{18}\)O-NO\(_3^-\) signal within the forested streams reflects soil nitrogen and microbial nitrification sources implying that the majority of NO\(_3^-\) entering the watersheds via
atmospheric deposition has been reprocessed within the watershed prior to export (as discussed in detail in Barnes et al. 2008)

The small but statistical difference between the $\delta^{18}$O-NO$_3^-$ values in streams draining different land use types cannot be attributed to a systematic variation in nitrogen sources. The majority of nitrogen additions to watersheds are in organic or reduced forms of nitrogen (e.g. urea or NH$_4^+$) and therefore do not inherently have different $\delta^{18}$O-NO$_3^-$ values. Exceptions to this are NO$_3^-$ delivered via atmospheric deposition ($\delta^{18}$O-NO$_3^-$: +23 to +90‰, e.g. Ohte et al. 2004, Kendall et al. 2007) and NO$_3^-$ fertilizers ($\delta^{18}$O-NO$_3^-$: +18 to +23‰, Amberger and Schmidt 1987). Both of these sources are likely minor relative to manure and septic wastes within the developed watersheds and thus their enriched $\delta^{18}$O signal is not observed in the exported NO$_3^-$. Nitrate fertilizers are not commonly used in the region, with homeowners and farmers preferring NH$_4^+$ and urea based fertilizers (Terry and Kirby 1999). While the study region does receive a moderate amount of NO$_3^-$ via atmospheric deposition, between 3.2 and 4.6 kg NO$_3^-\cdot$N ha$^{-1}$ yr$^{-1}$ (approximately 50% of total nitrogen deposition), this is only one component of the total anthropogenic nitrogen added to the developed watersheds as indicated by the manure and septic estimates discussed above. Thus, within urban and agricultural systems the majority of nitrogen inputs are assimilated and/or mineralized within the watershed before conversion to NO$_3^-$ via microbial nitrification.

Given the dominance of nitrification as a source of NO$_3^-$ to these systems it is important to determine the likely $\delta^{18}$O value of this NO$_3^-$. The oxygen within microbially produced NO$_3^-$ is derived from ambient H$_2$O and O$_2$ in a two to one ratio, respectively (Andersson and Hooper 1983, Kumar et al. 1983, Hollocher 1984). Accordingly, the calculated range of $\delta^{18}$O-NO$_3^-$ values for microbial nitrification in the region is -3.9 to +7.78‰ (Barnes et al. 2008). This range
encompasses the majority of streamwater δ¹⁸O-NO₃⁻ values in this study, providing further
evidence that the majority of NO₃⁻ exported by these watersheds is the product of internal
watershed and/or in-stream nitrification. Unlike other studies which have found the δ¹⁸O-NO₃⁻ to
be very valuable at deciphering nitrogen sources within watersheds (e.g. Burns and Kendall
2002, Silva et al. 2002, Ohte et al. 2004) our data suggests that δ¹⁵N-NO₃⁻ is a better reflection of
likely source pools. The successful application of δ¹⁵N-NO₃⁻ to distinguish land use types, as
opposed to δ¹⁸O-NO₃⁻ within this study is likely due to the significant differences in source pools
between watersheds as well as the similarities (e.g. surficial materials, geology) and close
proximity among watersheds of the same land use type. Furthermore with the exception of
forested systems, enriched δ¹⁸O-NO₃⁻ sources do not make up the majority of nitrogen inputs;
rather most nitrogen inputs must enter the NO₃⁻ pool via nitrification, as discussed above,
effectively eliminating the δ¹⁸O as a useful source determinant.

Processing and the isotopic composition of NO₃⁻

Seasonal differences in δ¹⁸O-NO₃⁻ provide evidence of temporal variation in
biogeochemical processing across all land use types. In ecosystems which are likely NO₃⁻ limited
(e.g. forests) increased processing of NO₃⁻ inputs results in overprinting the enriched δ¹⁸O-NO₃⁻
atmospheric signal with the more depleted nitrification signal. In contrast, urban and agricultural
watersheds are likely not nitrogen limited and thus partial uptake and removal results in the
isotopic enrichment of the remaining NO₃⁻ pool. Specifically, within the watershed the NO₃⁻ pool
can be reprocessed via assimilation and denitrification; resulting in progressive enrichment of
the remaining NO₃⁻ with heavier isotopes (¹⁵N and ¹⁸O) due to isotopic fractionation (Kendall et
al. 2007). Conversely, the addition of NO₃⁻ via subsequent nitrification of NH₄⁺ results in the
addition of isotopically lighter NO$_3^-$ to the total pool. Flow weighted NO$_3^-$ concentrations suggest
that the NO$_3^-$ pool was 458 and 255 times larger in agricultural and urban systems than forested
watersheds, respectively. The average $\delta^{18}$O-NO$_3^-$ values for the summer months, the period with
the greatest biotic N demand and thus internal processing, were statistically different (-2.2‰, 
2.2‰, and 3.3‰ in forested, urban, and agricultural streams, respectively), despite the lack of
inherent differences in the $\delta^{18}$O of nitrogen sources to the systems. Thus, our data suggest that
with increasing NO$_3^-$ availability there was greater enrichment of $\delta^{18}$O due to the larger
likelihood of incomplete turnover of the substrate pool and therefore expression of associated
isotopic enrichment (Kendall et al. 2007). It should be noted that there are other possible reasons
for the observed differences in $\delta^{18}$O-NO$_3^-$. Variability in $\delta^{18}$O-NO$_3^-$ across land uses could be
due to differences in the $\delta^{18}$O of O$_2$ in response to varying rates of soil respiration,
photosynthesis, evaporation, or diffusion from the atmosphere and/or differences in the $\delta^{18}$O-
H$_2$O used by microbes (Kendall et al. 2007 and references therein). Finally there could be
variability in the ratio of H$_2$O and O$_2$ used by the microbes which has been shown to differ from
the 2:1 ratio (e.g. Aravena et al. 1993, Casciotti et al. 2002). Our data does not allow us to rule
these possibilities out, however given the similarity in surficial materials and close proximity of
sites to one another it seems unlikely that there were systematic variation in the $\delta^{18}$O of
nitrification substrates between land use types.

The pattern of isotopic enrichment with NO$_3^-$ pool size also pertains to the variation in
$\delta^{15}$N-NO$_3^-$ between land use types. While differential source pools are the probable driver in the
overall variation of $\delta^{15}$N-NO$_3^-$ values between forested, urban, and agricultural streams;
increased expression of isotopic fractionation due to differences in the relative extent of nitrogen
transformations are possibly compounding these differences. Furthermore, inherent differences
in denitrification, uptake, and nitrification rates through time and between land use types could also result in differing isotopic compositions of NO$_3^-$.

Model results suggest that the average annual net removal of NO$_3^-$ does not differ significantly between urban and agricultural systems; however, net NO$_3^-$ removal varied significantly through time in all systems and it is likely that specific process rates (e.g. denitrification, nitrification, plant uptake) also varied between sites and through time.

### Seasonality of the isotopic composition of stream NO$_3^-$

The isotopic composition of NO$_3^-$ exported from streams sampled within this study showed distinct seasonal patterns (Figure 5). Temporal variation in the $\delta^{15}$N-NO$_3^-$ and $\delta^{18}$O-NO$_3^-$ can be attributed to three driving mechanisms: (1) shifts in the amount of uptake, retention, and removal of NO$_3^-$, (2) changes in the mixing ratios between sources and (3) seasonal variation in the isotopic composition of NO$_3^-$ sources.

Nitrate exported from streams draining forested watersheds had distinct seasonal variation in the $\delta^{18}$O-NO$_3^-$, with an average $\delta^{18}$O-NO$_3^-$ increase of $\sim$10‰ between summer and winter/spring stream samples (Figure 5b). The higher values reflect a greater proportion of unprocessed atmospherically derived NO$_3^-$ making it to the stream during the winter months, likely due to shorter watershed residence times attributed to higher recharge rates and reduced plant uptake in the winter (Barnes et al. 2008).

The isotopic composition of NO$_3^-$ exported by the four agricultural and five urban watersheds exhibit similar temporal patterns (Figure 5). The temporal variation in site standardized isotopic values (standard isotopic value= monthly value-annual mean) is similar between $\delta^{15}$N and $\delta^{18}$O of NO$_3^-$ in both urban and agricultural watersheds and is strongly
correlated in eight of the developed streams \((p<0.005)\). The highest \(\delta^{15}\text{N}-\) and \(\delta^{18}\text{O-NO}_3^-\) values were seen in the summer months and thus were positively related to water temperature (Figure 6). The direct relationships between \(\delta^{15}\text{N-NO}_3^-\) and \(\delta^{18}\text{O-NO}_3^-\) and temperature suggest that biotic processing plays an important role in NO\(_3^-\) dynamics within both urban and agricultural watersheds (Ostrom et al. 1998, Karr et al. 2003). Note that the same is not true for forested watersheds; in contrast a negative relationship between temperature and \(\delta^{18}\text{O-NO}_3^-\) was observed \((p<0.0001)\) due to less reprocessing of atmospherically derived NO\(_3^-\) during the winter months (Barnes et al. 2008).

Other possible explanations for the seasonality in the isotopic composition of NO\(_3^-\) are variations in the mixing and/or composition of inputs. Model results indicate that the sources of NO\(_3^-\) to new water do differ among watersheds and vary significantly between months in 5 of the 9 developed watersheds, as indicated by the estimated \(\delta^{15}\text{N and } \delta^{18}\text{O of NO}_3^-\) (Table 3). For example, agricultural watershed HBB had the highest predicted \(\delta^{15}\text{N of new water}\) during the spring and summer months following manure application. Increases in stream \(\delta^{15}\text{N-NO}_3^-\) and decreases in \(\delta^{18}\text{O-NO}_3^-\) correlate with decreased discharge in urban systems. This pattern suggests that new water is diluting the old water NO\(_3^-\) given that the \(\delta^{15}\text{N-NO}_3^-\) in old water \((5.4\%\) \(\)) is greater than in new water \((-2.7\%\), Table 3\) with the opposite pattern true for \(\delta^{18}\text{O-NO}_3^-\) \((2.2\%\) versus \(1.2\%\) in new and old water, respectively) within urban watersheds. These patterns are likely driven by the fact that shallow aquifers contribute a large amount of NO\(_3^-\) to the streams in the area (Mullaney and Grady 1997). Studies in other suburban watersheds have shown similar results with stormflow diluting stream NO\(_3^-\) concentrations (Burns et al. 2009 and references therein). Given the dominance of old water as a NO\(_3^-\) source within the urban and agricultural watersheds, the significant variation in new water contribution (Table 3), and the
relationships between new water characteristics and NO$_3^-$ concentrations and isotopic compositions, it seems likely that the relative mixing of old and new water is an important driver of the seasonal variability of the isotopic composition and amount of NO$_3^-$ exported from each watershed.

**Watershed NO$_3^-$ removal and retention**

Removal and retention estimates (22-61%, on average 47%) across the developed watersheds (Table 3) and the temporal variation of the isotopic composition of NO$_3^-$ indicate that there is significant biotic uptake and/or denitrification consuming NO$_3^-$ within these systems. These uptake estimates for urban and agricultural systems are significantly lower than estimates for nearby forested watersheds which retain or remove over 98% of incoming NO$_3^-$ (Barnes et al. 2008). However it should be noted that in this study removal and retention estimates were made based on an estimated nitrate input from both new and old waters (i.e. [NO$_3^-$]$_{\text{MIX}}$, eqn. 4) not by calculating total inputs and outputs from the watershed. As follows, the removal and retention estimates within this study are likely conservative, not fully accounting for all transformations and losses within the watershed. A study comparing nitrogen budgets in forested watersheds within the northeastern U.S. found a range of retention estimates (24% to 99%) for inorganic nitrogen and attributed differences to variety of factors including soil and forest type, land use history, and hydrology (Campbell et al. 2004). Studies of developed watersheds also indicate a range in nitrogen retention. For example, Groffman et al. (2004) found 75% and 77% of nitrogen inputs removed within suburban and agricultural watersheds near Baltimore, MD and Jordan et al. (1997a) calculated greater than 50% retention within agricultural watersheds of the Piedmont region of the Chesapeake Bay. Similarly, Wollheim et al. (2005) found that urban areas within
the Ipswich watershed in Massachusetts were able to retain between 65-85% of their total nitrogen inputs. Our estimates for agricultural and urban streams fall at the bottom of this range, though it should be noted that the Groffman et al. (2004), Jordan et al. (1997a), and Wollheim et al. (2005) studies used a watershed budget approach, examining total nitrogen inputs and outputs while this study compared modeled NO$_3^-$ inputs and measured outputs. Despite this difference we used these studies as a point of comparison because they were also conducted in small watersheds within a similar geographic region (i.e. northeastern U.S.).

Heterogeneity within the environment is one of the primary limitations in our ability to understand NO$_3^-$ removal across and within watersheds (Seitzinger et al. 2006). Thus it is beneficial to examine differences between watersheds for an explanation of lower removal and retention rates. There are several reasons why the developed systems in this study could have lower retention rates as compared to published studies from Maryland and Massachusetts including the high NO$_3^-$ concentrations in groundwater (Burns et al. 1998, Campbell et al. 2004) and differences in surficial materials and riparian vegetation (McClain et al. 2003 and references therein). Nitrate in groundwater is often subject to reduced biotic activity (e.g. Burns et al. 1998) and thus the presence of high NO$_3^-$ concentrations in the groundwater underlying the agricultural and urban watersheds provides a NO$_3^-$ subsidy to the streams year round. Furthermore past studies within the aquifers underlying Broad Brook and the Hockanum River show oxic conditions throughout the system (Mullaney and Grady 1997, Mullaney 2007); providing evidence that denitrification is an unlikely removal mechanism within the groundwater feeding these streams thus resulting in large NO$_3^-$ loading. There was no mention of high NO$_3^-$ loading from groundwater within the Baltimore study (Groffman et al. 2004), however studies within the Chesapeake and Ipswich watersheds (Jordan et al. 1997b, Wollheim et al. 2005) note that
groundwater is an important contributor to stream nitrogen loads. Sand and gravel make up significant portions of the urban and agricultural watersheds (Table 1) and the surficial material maps tell us that the majority of streams in developed areas run through sandy alluvium, a highly porous material. Past studies indicate that porous soils have less capacity to retain nitrogen (Lajtha et al. 1995) and thus these watersheds may be more prone to high NO₃⁻ loading. The nature of soils and surficial materials are not described within the cited studies in Maryland, however Jordan and others (1997a) note that the Coastal Plain watersheds have higher retention rates as compared to similar systems within the Piedmont region due to  the presence of poorly drained riparian forests which several studies have shown to be hotspots for denitrification (McClain et al. 2003 and references therein). Wetland areas have also been identified nitrogen removal landscapes and thus the larger presence of wetlands within the Ipswich watersheds (on average 13.8%) as compared to the watersheds within this study (on average 1.5%) could account for differences in nitrogen retention. In addition to the lack of wetland areas within the studied systems, field observations tell us that in many instances, the riparian zones were often dominated by agricultural fields and lawns and not natural vegetation, once again reducing the potential for nitrogen removal.

Differences in overall inputs (Mulholland et al. 2008) have also been shown to effect nitrogen removal rates. While we were not able to create detailed budgets for our systems, estimates using literature values suggest that on average total nitrogen inputs are similar to the suburban and agricultural systems in the Baltimore, MD study (Groffman et al. 2004); despite these similarities average estimated instantaneous NO₃⁻ yields at the time of sampling are larger, especially in the case of urban and suburban systems. For example, on average our urban and suburban watersheds export ~15.7 kg NO₃⁻-N ha⁻¹yr⁻¹, while similar systems in MD exported an
average of 6.7 kg N ha\(^{-1}\) yr\(^{-1}\) (Groffman et al. 2004). Therefore, while the watersheds within this study are clearly retaining less NO\(_3^-\) than those in the Baltimore, MD study it seems unlikely that this is due to input differences.

Microsite variability within watersheds and streams such as presence of anoxic conditions (Knowles 1982), organic carbon availability (Goodale et al. 2005), ecosystem photosynthesis and respiration rates (Mulholland et al. 2008) can also effect nitrogen removal and retention rates. Predicted net NO\(_3^-\) removal was positively correlated with DOC concentrations in urban ecosystems (\(p<0.005\)) but not within agricultural watersheds; reflecting the possibility that denitrification and biotic uptake are limited by carbon availability within urban systems (Hedin et al. 1998, Goodale et al. 2005). Net NO\(_3^-\) removal was negatively related to watershed size across the entire study region (\(p<0.05\)) likely reflecting that smaller streams remove a greater percentage of nitrogen as compared to larger systems (Peterson et al. 2001) and smaller watersheds will on average have a greater proportion of headwater streams.

**Conclusions**

Past research tells us that headwater catchments are an important control on nitrogen flux, due to higher rates of removal and retention (Peterson et al. 2001) and because they account for the majority of stream length within a watershed (Alexander et al. 2000). Thus by examining small catchments with differing dominant land use we were able to compare the relative importance of sources and processing on variations in stream NO\(_3^-\) flux across a land use gradient. Our data indicate that the systematic across system differences in stream \(\delta^{15}\)N-NO\(_3^-\) illustrates the dominant influence of nitrogen sources, while the variation in \(\delta^{18}\)O-NO\(_3^-\) reflects biogeochemical processing within the watershed. Despite the inherent seasonality of nitrogen
processes within ecosystems, distinct isotopic compositions of stream NO$_3^-$ were observed across the land use gradient. The strong relationships between inputs, land use and $\delta^{15}$N-NO$_3^-$ reflect the dominance of anthropogenic nitrogen inputs within developed watersheds (e.g. Howarth et al. 1996). In contrast, the relative consistency of $\delta^{18}$O-NO$_3^-$ that fall within the range of values associated with microbial nitrification is indicative of the biogeochemical processing that occurs within each watershed, regardless of land use and associated inputs.

Seasonal variability in the isotopic composition of exported NO$_3^-$ was attributed to variation in biogeochemical processes as indicated by positive correlations with temperature within developed watersheds (both $\delta^{15}$N and $\delta^{18}$O-NO$_3^-$) and a negative relationship ($\delta^{18}$O-NO$_3^-$) within forested systems with temperature. Net removal rates within these watersheds were depressed slightly as compared to similar watersheds within Maryland, potentially due to site attributes such as porous sandy surficial materials and the presence of shallow aquifers with high NO$_3^-$ concentrations. Organic carbon concentrations and watershed size were significant predictors of relative removal rates of NO$_3^-$ from developed watersheds reflecting past studies in small watersheds that suggest small streams are more efficient at removing nitrogen (Peterson et al. 2001) and that stream NO$_3^-$ concentrations may be controlled by organic carbon availability (Hedin et al. 1995, Goodale et al. 2005).

The application of a simple numerical model in conjunction with isotopic data allowed us to estimate net removal of NO$_3^-$ from catchments. Greater information about source pool characteristics, in particular interstitial soil waters and other components of new water, combined with the modeling approach presented here would increase our ability to identify and quantify the relative importance of NO$_3^-$ retention mechanisms. A better understanding of the mechanisms
controlling NO₃⁻ export would provide more information for managers to target their non-point source reduction efforts.

Acknowledgements
We thank K. Casciotti for use of her lab for stable isotope analysis and help in developing the numerical model, M. McIlvin for help in processing our isotope samples, J. Saiers for programming assistance, and D. Burns and an anonymous reviewer for providing comments on earlier drafts. Assistance in the field was provided by J. Mullaney, K. Mull, D. Butman, D. Karwan, B. Feingold, M. Bozeman and C. May. Funding for this work was provided by an EPA STAR Fellowship (FP-91637501-1) and a grant from QLF/The Sound Conservancy to RTB.

Literature Cited

Ground Water 36:975-982.


NCDC. 2007. Quality Controlled Local Climatological Data.


Table 1: Watershed characteristics for all sampling locations: the percent of watershed area covered by a given land use, impervious cover, and sewer service. Bedrock types and surficial materials were grouped into classes, metamorphic (M), sedimentary (S), igneous (I) and till (T), sand/gravel (SG), swamps (S), respectively. Only the dominant (>60% of the watershed area) is listed except for the cases where one type did not constitute a majority of the watershed.
<table>
<thead>
<tr>
<th>watershed</th>
<th>size (ha)</th>
<th>type</th>
<th>wetland</th>
<th>forest</th>
<th>urban</th>
<th>ag</th>
<th>impervious</th>
<th>sewered</th>
<th>bedrock</th>
<th>surficial</th>
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<td>M</td>
<td>T</td>
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<tr>
<td>MB</td>
<td>304</td>
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<td>0.6</td>
<td>41.9</td>
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<td>T</td>
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<td>51.8</td>
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<td>0.0</td>
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<td>T</td>
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<td>KB</td>
<td>356</td>
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<td>30.8</td>
<td>22.2</td>
<td>38.1</td>
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<td>1.0</td>
<td>S</td>
<td>T&amp;S&amp;G</td>
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<td>0.0</td>
<td>M &amp; I</td>
<td>T</td>
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<td>79.9</td>
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<td>0.0</td>
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<td>T</td>
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<td>87.8</td>
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<td>0.0</td>
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<td>HSR</td>
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<td>97.8</td>
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<td>0.0</td>
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<td>urban</td>
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<td>31.4</td>
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<td>16.1</td>
<td>47.0</td>
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<td>315</td>
<td>urban</td>
<td>3.1</td>
<td>24.7</td>
<td>62.6</td>
<td>10.6</td>
<td>18.0</td>
<td>99.0</td>
<td>I</td>
<td>T</td>
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</tr>
</tbody>
</table>
Table 2: Model estimates of the percent of new water entering the stream at a given sampling event. The range shown for the δ^{18}O-H_2O model was determined by uncertainty estimates, while the range given for the nitrate removal model is the actual range of prediction values. All months could not be compared due to data limitations as discussed in the text.

<table>
<thead>
<tr>
<th>Watershed</th>
<th>Dominant Land Use</th>
<th>δ^{18}O-H_2O Avg Min</th>
<th>δ^{18}O-H_2O Avg Max</th>
<th>Nitrate Removal Avg Min</th>
<th>Nitrate Removal Avg Max</th>
<th>Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBB</td>
<td>Agricultural</td>
<td>1%</td>
<td>36%</td>
<td>7%</td>
<td>19%</td>
<td>4/5</td>
</tr>
<tr>
<td>MB</td>
<td>Agricultural</td>
<td>4%</td>
<td>41%</td>
<td>1%</td>
<td>15%</td>
<td>4/5</td>
</tr>
<tr>
<td>CBa</td>
<td>Agricultural</td>
<td>0%</td>
<td>31%</td>
<td>6%</td>
<td>21%</td>
<td>4/5</td>
</tr>
<tr>
<td>HSB</td>
<td>Agricultural</td>
<td>1%</td>
<td>32%</td>
<td>12%</td>
<td>24%</td>
<td>4/5</td>
</tr>
<tr>
<td>BB</td>
<td>Urban</td>
<td>6%</td>
<td>35%</td>
<td>7%</td>
<td>27%</td>
<td>5/6</td>
</tr>
<tr>
<td>FB</td>
<td>Urban</td>
<td>6%</td>
<td>37%</td>
<td>4%</td>
<td>20%</td>
<td>4/6</td>
</tr>
<tr>
<td>OB</td>
<td>Urban</td>
<td>1%</td>
<td>32%</td>
<td>4%</td>
<td>20%</td>
<td>5/5</td>
</tr>
<tr>
<td>TB</td>
<td>Urban</td>
<td>0%</td>
<td>29%</td>
<td>5%</td>
<td>20%</td>
<td>5/6</td>
</tr>
<tr>
<td>CBu</td>
<td>Urban</td>
<td>0%</td>
<td>32%</td>
<td>2%</td>
<td>15%</td>
<td>6/6</td>
</tr>
</tbody>
</table>
Table 3: The calculated average (based on monthly means) and range (i.e. temporal variation) of each parameter for each watershed are given. Results from ANOVA analysis by sampling date are noted with asterisk, indicating which variables varied significantly with sampling date.

<table>
<thead>
<tr>
<th>Watershed</th>
<th>Dominant Land Use</th>
<th>$\delta^{15}$N-NO\textsubscript{3} new water</th>
<th>$\delta^{18}$O-NO\textsubscript{3} new water</th>
<th>$[\text{NO}_3^-]$ new water</th>
<th>Concentration of NO\textsubscript{3}^- pool</th>
<th>Mixing ratio between new and old water</th>
<th>Fraction of NO\textsubscript{3}^- remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBB</td>
<td>Agricultural</td>
<td>7.0 (3.1, 11)*</td>
<td>-1.6 (-5.3, 2.8)*</td>
<td>3.6 (2.4, 4.5)**</td>
<td>4.1 (3.0, 5.2)**</td>
<td>0.06 (0.01, 0.11)**</td>
<td>0.78 (0.60, 0.98)*****</td>
</tr>
<tr>
<td>MB</td>
<td>Agricultural</td>
<td>2.7 (1.7, 4.4)</td>
<td>-2.6 (-4.6, -1.5)</td>
<td>2.5 (2.3, 2.5)</td>
<td>6.6 (5.9, 7.0)**</td>
<td>0.12 (0.03, 0.28)**</td>
<td>0.70 (0.40, 0.92)*****</td>
</tr>
<tr>
<td>CBA</td>
<td>Agricultural</td>
<td>3.6 (0.6, 6.6)</td>
<td>-2.8 (-3.4, -1.8)</td>
<td>2.4 (1.9, 2.7)</td>
<td>6.8 (6.7, 6.9)*</td>
<td>0.07 (0.04, 0.10)**</td>
<td>0.48 (0.40, 0.54)*****</td>
</tr>
<tr>
<td>HSB</td>
<td>Agricultural</td>
<td>-1.4 (-5.5, 4.7)**</td>
<td>7.0 (-0.4, 23.17)*</td>
<td>2.4 (1.3, 2.9)*</td>
<td>6.8 (6.4, 6.9)**</td>
<td>0.08 (0.04, 0.16)**</td>
<td>0.39 (0.21, 0.56)*****</td>
</tr>
<tr>
<td>BB</td>
<td>Urban</td>
<td>-4.3 (-5.4, -2.0)**</td>
<td>-5.0 (-8.5, -3.0)**</td>
<td>4.0 (1.7, 4.7)**</td>
<td>4.0 (3.9, 4.1)</td>
<td>0.15 (0.02, 0.25)**</td>
<td>0.62 (0.53, 0.72)*****</td>
</tr>
<tr>
<td>FB</td>
<td>Urban</td>
<td>-1.7 (-4.8, 1.5)**</td>
<td>6.8 (-2.3, 32.7)**</td>
<td>3.3 (0.9, 5.0)**</td>
<td>3.7 (3.1, 4.0)**</td>
<td>0.18 (0.02, 0.40)**</td>
<td>0.40 (0.28, 0.50)*****</td>
</tr>
<tr>
<td>OB</td>
<td>Urban</td>
<td>-2.2 (-3.8, 0.6)*</td>
<td>15.6 (5.32, 51.0)**</td>
<td>3.9 (1.7, 4.4)**</td>
<td>3.9 (3.7, 4.0)*</td>
<td>0.10 (0.03, 0.21)**</td>
<td>0.50 (0.38, 0.64)*****</td>
</tr>
<tr>
<td>TB</td>
<td>Urban</td>
<td>-2.5 (-4.0, -1.2)</td>
<td>-3.8 (-5.7, -2.0)</td>
<td>3.9 (2.5, 4.8)**</td>
<td>3.9 (3.8, 4.0)</td>
<td>0.08 (0.02, 0.22)**</td>
<td>0.59 (0.49, 0.75)*****</td>
</tr>
<tr>
<td>CBU</td>
<td>Urban</td>
<td>-3.0 (-4.6, -1.3)</td>
<td>-2.8 (-4.5, -1.6)</td>
<td>4.3 (2.9, 5.3)</td>
<td>3.9 (3.8, 4.0)</td>
<td>0.07 (0.03, 0.12)**</td>
<td>0.56 (0.47, 0.66)*****</td>
</tr>
</tbody>
</table>
* significant at $\alpha = 0.05$, **significant at $\alpha = 0.005$, ***significant at $\alpha = 0.0005$
**Figure 1:** Sampling locations. The urban sites are labeled with squares: Folly Brook (FB), Bigelow Brook (BB), Ogen Brook (OB), Thompson Brook (TB) & Chudsey Brook (CBu); the forested sites are indicated with circles: Charters Brook (CBf), Headwaters of the West Branch Farmington River (HWFR), Riiska Brook (RB), Sandy Brook (SB), & Headwaters of the Still River (HSR); and the agricultural sites are labeled with the diamonds: Headwaters of Broad Brook (HBB), Creamery Brook (CBa), Muddy Brook (MB), Headwaters of the East Branch of Salmon Brook (HSB), & Kendall Brook (KB). Atmospheric deposition collection sites and groundwater sampling locations are indicated with stars and triangles, respectively.

**Figure 2:** This diagram depicts the model described by equations 1-3 in the text. The amount and isotopic composition of stream and groundwater NO$_3^-$ were constrained by our measurements. New water was estimated using a wide range of both isotopic ($-7$‰ to $+14$‰ for $\delta^{15}$N-NO$_3^-$) and concentration values (0.01 to 7 mg NO$_3^-$ L$^{-1}$). A range (0-18) of fractionation factors ($\epsilon$) associated with the net removal (1-F) of NO$_3^-$ from the system was applied; net removal$= \text{denitrification} + \text{uptake} - \text{net nitrification}$. Notice that removal terms are located within the intersection of the dotted box (watershed) and dashed box (stream), indicating that this term includes both watershed and in-stream processes.

**Figure 3:** The average (a) NO$_3^-$ concentration and (b) yield across watersheds and hydrographs of representative (c) urban, (d) agricultural, and (e) forested streams.

**Figure 4:** Dual NO$_3^-$ isotope source plot, reported ranges for various NO$_3^-$ sources are taken from Kendall et al. (2007). Note that the boxes representing soil N, NH$_4^+$ in precipitation and
fertilizer, and manure and septic wastes have δ^{18}O-NO_3^- values ranging from -15‰ to +15‰, the range of values resulting from the nitrification of these three sources.

**Figure 5:** The seasonal variation in the δ^{15}N-NO_3^- (a) and the δ^{18}O-NO_3^- (b) across watersheds of similar land use types. The error bars represent the standard error across watersheds of the same type, i.e. between site variations. The grey triangles, open squares and closed circles represent forested, urban, and agricultural watersheds, respectively.

**Figure 6:** The relationship between the isotopic composition (both δ^{15}N and δ^{18}O) of NO_3^- is related to water temperature in both (a) urban and (b) agricultural systems. The closed circles represent the δ^{15}N-NO_3^- values and the open triangles represent the δ^{18}O-NO_3^- values.
runoff & shallow subsurface $\text{NO}_3^-$

$1-V_0$

$V_0$

Groundwater $\text{NO}_3^-$

$\text{NO}_3^-$ mix

denitrification

$\text{NO}_3^-$ removed & retained

Biotic uptake

$F$

Net nitrification

stream $\text{NO}_3^-$

watershed
The diagram shows the distribution of δ¹⁸O and δ¹⁵N values for nitrate (NO₃⁻) in various environmental sources. The x-axis represents δ¹⁵N-NO₃⁻ (‰), while the y-axis shows δ¹⁸O-NO₃⁻ (‰). Various data points are depicted by different symbols and colors, indicating different sources:

- Diamond: Agricultural streams
- Circle: Forested streams
- Square: Urban streams
- Cross: Precipitation

The diagram includes regions marked by "atmospheric NO₃⁻", "NH₄⁺ in soil & precipitation", "soil NH₄⁺", and "manure & septic", which represent different sources of nitrate and their isotopic compositions. The nitrification process is also indicated on the right side of the diagram.