Ecological Applications, 16(1), 2006, pp. 299–312

LAND USE CHANGE AND NITROGEN ENRICHMENT OF A ROCKY MOUNTAIN WATERSHED

Sujay S. Kaushal,[1] William M. Lewis, Jr., and James H. McCutchan, Jr.

Center for Limnology, Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, Colorado 80309-0216 USA

Abstract. Headwater ecosystems may have a limited threshold for retaining and removing nutrients delivered by certain types of land use. Nitrogen enrichment was studied in a Rocky Mountain watershed undergoing rapid expansion of population and residential development. Study sites were located along a 30-km transect from the headwaters of the Blue River to Lake Dillon, a major source of drinking water for Denver, Colorado. Ground water in residential areas with septic systems showed high concentrations of nitrate-N (4.96 $\pm$ 1.22 mg/L, mean $\pm$ SE), and approximately 40% of wells contained nitrate with $\delta^{15}N$ values in the range of wastewater. Concentrations of dissolved inorganic nitrogen (DIN) in tributaries with residential development peaked during spring snowmelt as concentrations of DIN declined to below detection limits in undeveloped tributaries. Annual export of dissolved organic nitrogen (DON) was considerably lower in residential streams, suggesting a change in forms of N with development. The seasonal $\delta^{15}N$ of algae in residential streams was intermediate between baseline values from undeveloped streams and stream algae grown on wastewater. Between 19% and 23% of the annual N export from developed tributaries was derived from septic systems, as estimated from the $\delta^{15}N$ of algae. This range was similar to the amount of N export above background determined independently from mass-balance estimates. From a watershed perspective, total loading of N to the Blue River catchment from septic and municipal wastewater (2 $kg \cdot ha^{-1} \cdot yr^{-1}$) is currently less than the amount from background atmospheric sources (3 $kg \cdot ha^{-1} \cdot yr^{-1}$). Nonetheless, nitrate-N concentrations exceeded limits for safe drinking water in some groundwater wells (10 mg/L), residential streams showed elevated seasonal patterns of nitrate-N concentration and ratios of DIN to total dissolved phosphorus, and seasonal minimum concentrations of nitrate-N in Lake Dillon have increased exponentially to 80 $\mu g/L$ over the last decade from an initial value near zero. Results suggest that isotopic ratios in autotrophs can be used to detect and quantify increases in N enrichment associated with land use change. The biotic capacity of headwater ecosystems to assimilate increases in inorganic N from residential development may be insufficient to prevent nitrogen enrichment over considerable distances and multiple aquatic ecosystems downstream.

Key words: *algae; land use; N enrichment; N isotopes; nitrate; streams; wastewater.*

Introduction

Changes in land use are occurring at an accelerated rate throughout many regions of North America, including headwater ecosystems, which contain some of the fastest growing populations within the United States. Watersheds in these areas often contain critical habitat for biota and are used by humans for recreation and as supplies of drinking water (Jackson et al. 2001, Messerli 2004). The balance between these demands is sometimes controversial and can be of great environmental and economic consequence (e.g., Lewis et al. 1984, Byron and Goldman 1989, Daily and Ellison 2001, Korner 2004).

Because of concerns regarding deterioration of habitat and ecosystem services, much work has examined the effects of nutrient enrichment from atmospheric deposition on headwater areas (Lewis et al. 1984, Baron et al. 1994, Sickman et al. 2003, Likens 2004). The effects of atmospheric N pollution originating from power plants, vehicles, and agricultural and urban sources include stimulation of primary production (Wolfe et al. 2002), alteration of biotic communities (Baron et al. 2000, Wolfe et al. 2001), reduced quality of drinking water (Williams et al. 1996, Williams and Tonnesson 2000), and propagation of increased amounts of inorganic N through aquatic ecosystems (Baron et al. 1994, Williams et al. 1996).

Although less recognized, rapid increases in tourism and residential and suburban development of headwater areas may also lead to considerable nutrient enrichment in the eastern and western United States (Lewis et al. 1984, Byron and Goldman 1989, Daily and Ellison 2001). There can be substantial processing of inorganic

Manuscript received 26 January 2005; revised 24 May 2005; accepted 24 May 2005. Corresponding Editor: K. N. Eshleman.

[1] Present address: University of Maryland Center for Environmental Science, Appalachian Laboratory, 301 Braddock Road, Frostburg, Maryland 21532 USA. E-mail: skaushal@al.umces.edu

N within low order streams (Alexander et al. 2000, Peterson et al. 2001), but there also may be thresholds beyond which uptake and removal of inorganic N can decrease in these systems (Dodds et al. 2002, Mulholland et al. 2002) similar to terrestrial environments (Aber et al. 1989, 1998, Baron et al. 1994). As resident populations increase, it becomes unclear how much development can proceed before the capacity of an ecosystem to retain and remove nutrients diminishes (e.g., Wernick et al. 1998).

Headwater streams experiencing residential development may be particularly susceptible to N enrichment. Changes in land use can be concentrated in riparian areas and valleys, which are particularly important for controlling N transport to aquatic systems. Increasing discharges from septic systems and small wastewater treatment plants can also bypass areas of biological uptake and transformation in soils; fixed N then may directly enter ground water, streams, and lakes during times of year when biological activity is low. Concentrations of N can be considerably greater in headwater streams surrounded by low residential and suburban land use (particularly in areas without sewer systems) than in urban streams, and they may even be comparable to N concentrations in agricultural streams (Groffman et al. 2004). Residential development has the potential to alter proximity, forms, and seasonal patterns of nitrogen sources. Modest levels of N enrichment from development may have disproportionately large effects on the N dynamics of headwater ecosystems, but this has been less explored at the landscape scale.

Quantification of the transport of anthropogenic N from changes in land use has relied primarily on estimates and models derived from mass-balance (Lewis et al. 1984, Howarth 1998, Caraco and Cole 1999). Mass-balance models offer the most direct way to measure the influence of a nutrient source on water quality, but do not allow sources to be discriminated along flow paths. Use of the isotopic signature of nitrogen as a natural tracer has the capability to allow for discrimination between N sources, and therefore has received much attention in studies of N pollution (Kohl 1971, Cabana and Rasmussen 1996, McClelland and Valiela 1998*a*, Wolfe et al. 2003).

Isotopic methods have worked well in identifying contributions of nitrogen derived from wastewater in some aquatic environments (e.g., Cabana and Rasmussen 1996, McClelland et al. 1997, McClelland and Valiela 1998*a*). Nitrate in domestic wastewater typically has ^{15}N values between $+10$ to $+20$ (e.g., Kreitler and Browning 1983, Aravena et al. 1993, McClelland et al. 1997), whereas nitrate in stream water influenced by atmospheric deposition typically has δ^{15}N values ranging from -4 to $+6$ (Burns and Kendall 2002, Campbell et al. 2002). Use of the N isotope approach has been less effective in other environments due to preferential loss of ^{14}N during denitrification and enrichment of δ^{15}N in the remaining pool of nitrate (Brandes et al. 1996, Kellman and Hillaire-Marcell 1998, Panno et al. 2001, Ostrom et al. 2002). Consequently, careful assessment of background transformations (Handley and Scrimgenour 1997, Kellman and Hillaire Marcel 2002, Bedard-Haughn et al. 2003) and baseline δ^{15}N (Vander Zanden and Rasmussen 1999, McCutchan and Lewis 2001) may be necessary before using the isotope approach to delineate sources of N within watersheds.

The possibility of using δ^{15}N of dissolved nitrate and autotrophic biomass as a semiquantitative indicator of N status has been explored (McClelland and Valiela 1998*a*, Burns and Kendall 2002, Stewart et al. 2002). Use of the technique in a quantitative manner is hypothetically possible, but the efficacy of this approach has remained virtually untested (Kendall 1998, Kellman and Hillaire-Marcell 2002). It may be particularly well suited in headwater environments where baseline isotopic transformations during each season can be characterized in undeveloped watersheds (McCutchan and Lewis 2001), and isotopic fractionation between N sources and primary producers may be minimal under natural and moderately disturbed conditions (Wolfe et al. 2001, 2003, Savage and Elmgren 2004).

For the present study, contributions of nitrogen from residential wastewater to a montane river in Colorado were quantified independently by mass balance and by N isotope ratios in stream algae. Although seldom compared, stable isotope and mass balance approaches in combination may be useful in examining the effects of land use change on sources and fates of N from anthropogenic sources. A primary objective was to explore the effects of residential expansion and suburban development on sources, forms, and seasonal patterns of nitrogen across multiple headwater ecosystems (groundwater, streams, and lake). A secondary objective was to test the ability of stream autotrophs to record and quantify contributions of N from changes in land use before increased enrichment could cause substantial changes in biological communities and deterioration of water quality. The isotopic signature of autotrophic biomass may provide a rapid and inexpensive method for identifying and quantifying major sources of N to surface waters used for human consumption, recreation, and habitat. Study sites were located along a transect from the headwaters of the Blue River near the continental divide in Summit County, Colorado, to Lake Dillon, a major source of drinking water for Denver, Colorado (Fig. 1, Plate 1).

Methods

Site description

The Blue River watershed is located approximately 70 miles west of Denver, Colorado, in Summit County, which is among the fastest growing counties in the United States (data from the U.S. Census Bureau in

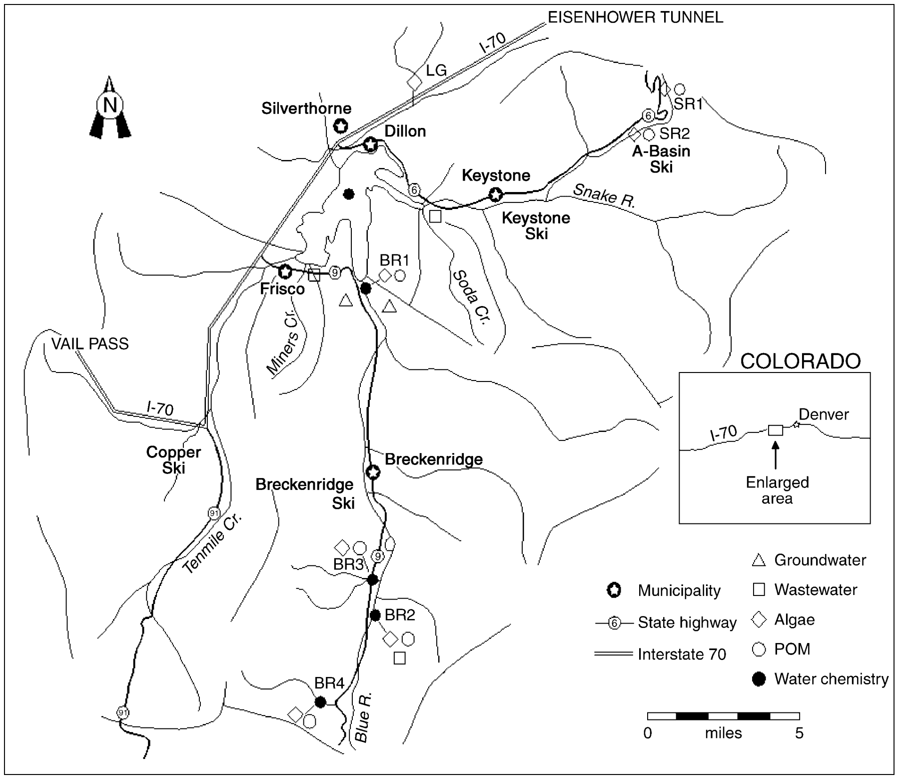

FIG. 1. Locations of sampling sites in Colorado, USA. SI conversion: 1 mile = 1.609 km.

2002). Private land comprises approximately 21% of Summit County by area (113 014 ha), and is mostly concentrated near streams along the bottom of valleys (data from the Northwest Colorado Council of Governments in 2002). The population of Summit County increased by 83% from 12 881 to 23 548 permanent residents between 1990 and 2000 (Summit County Planning Department), and peak ski population was approximately 122 000 in 2001. Wastewater generated by residents and visitors in the Blue River watershed is released from dispersed sources (septic systems) or by municipal wastewater treatment facilities located on the Blue River before its entry into Lake Dillon.

Bedrock in the Blue River watershed is composed primarily of igneous and metamorphic rock of Precambrian age on mountain slopes. Glacial deposits are prevalent near valley bottoms (Lewis et al. 1984). Soils are cryoboralfs in forested areas and are gravelly loams in valleys (data from the Summit County Soil Survey in 1980). Vegetation below tree line consists of pine–spruce–fir communities on slopes and willow and aspen groves in riparian areas.

The hydrographs of small streams in Summit County are strongly controlled by snowmelt (e.g., Brooks et al. 1999, McKnight et al. 2002, Kaushal and Lewis 2003), which produces a peak of discharge in June. The watersheds have a brief growing season (April through September); water temperature and flow decline to their lowest levels during winter months. Nitrate-N is the dominant form of inorganic N (Lewis et al. 1984), and dissolved organic nitrogen (DON) is the most abundant form of N in minimally disturbed streams of Summit County (Kaushal and Lewis 2003).

Sampling design

Sampling design involved a combination of approaches using both synoptic and comparative sampling of watersheds. Flow of nitrogen across multiple aquatic systems was characterized by use of a 30-km transect of the Blue River including groundwater, streams, and Lake Dillon. All sites along the Blue River were located in the forested, subalpine zone with sites having similar soils and vegetation but differing in elevation. Three replicate watersheds located at subalpine and alpine elevations throughout Summit County also were used for comparative purposes.

Two residential sites of the Blue River were chosen for measurements of N export and isotopic ratios in algae (Table 1). Groundwater and wastewater also were taken from areas near residential sites for characterization of N isotope ratios (Fig. 1). The residential sites are forested except in some areas that have been cleared for development. Many homes in the drainage are used primarily during winter and spring months, when population increases from 8833 to 59 819 residents (J. Vest, *personal communication*). Towns are sewered, but residential developments outside of towns use septic systems. The first residential site, BR1, drains an area with

TABLE 1. Information on locations and dates of sampling at sites in Colorado, USA.

Site	Code	Land use	Elevation(m)	Drainage area (ha)	Location	Analyses	Year
Frisco Terrace, Wilborg Park, Ten Mile Vista, Lakeview Meadows	GW	residential	2750		~39°34′00″ N, ~106°02′56″ W	nitrate ^{15}N of nitrate	1999 1999
Blue River at Lake Dillon	BR1	residential	2750	32 020	39°34′00″ N, 106°02′56″ W	N fractions ^{15}N of algae ^{15}N of POM†	1999–2000 1998–1999, 2002 1999–2000
Blue River above Goose Pasture tarn	BR2	residential	3200	5165	39°25′30″ N, 106°02′45″ W	N fractions ^{15}N of algae ^{15}N of POM†	1999–2000 1998–1999 1999–2000
Spruce Creek	BR4	forested (control)	3400	1585	39°26′30″ N, 106°03′00″ W	N fractions ^{15}N of algae ^{15}N of POM†	1999–2000, 2002 2002 1999–2000
McCullough Gulch	BR3	forested (control)	3400	1295	39°24′15″ N, 106°03′30″ W	N fractions ^{15}N of algae ^{15}N of POM†	1999–2000, 2002 2002 1999–2000
Snake River at Loveland Pass	SR1	alpine (control)	3480	683	39°39′28″ N, 105°52′20″ W	^{15}N of algae ^{15}N of POM†	1994–1995 1999–2000
Snake River at Arapahoe Basin	SR2	forested (control)	3120	4499	39°38′03″ N, 105°53′37″ W	^{15}N of algae ^{15}N of POM†	1994–1995 1999–2000
Laskey Gulch	LG	forested (control)	3100	1008	39°38′45″ N, 106°02′00″ W	^{15}N of algae	1998–1999
Lake Dillon	DI00	reservoir	2750	86 765	39°37′00″ N, 106°04′00″ W	nitrate	1990–2001

Note: BR3, BR4, SR1, SR2, AB, and LG are control sites representing background conditions. GW refers to groundwater.
† Particulate organic matter.

1456 septic systems, and the second residential site, BR2, drains an area with 408 septic systems (Summit County Department of Health, *unpublished data*). In addition to septic discharge, BR1 may have received some effluent from a small wastewater treatment plant that went into operation after the first year of the study, but hydrology downstream of the treatment plant involves subsurface flow paths and some blockage of hydrologic connectivity with the main channel due to historical mining in the region. Most of the wastewater from Breckenridge, Colorado (3 million gallons per day or 11.35×10^6 L/d), however, is treated and discharged through a diversion channel directly to the lake below the study sites (Breckenridge Sanitation District, *unpublished data*). BR2 has no municipal wastewater treatment plants located above it, and receives wastewater only from septic systems.

Two unpopulated watersheds in the upper Blue River drainage were chosen for measurement of background N exports as determined by mass balance and seasonal N isotopic ratios. Three additional undeveloped watersheds spread throughout the Lake Dillon watershed were also chosen to characterize background N isotopic ratios for both subalpine and alpine locations. Concentrations of nitrate-N were measured in surface water at the center of Lake Dillon (Fig. 1).

N loading

Annual wastewater contributions of N were compared for residents on septic systems and for all residents in the watershed of the upper Blue River. The mean annual rate of use was assumed to be 1.79 people per septic system (similar to Valiela et al. 1997); this rate reflects use on weekends or during one season of the year, which is typical of ski areas. A compilation of literature values indicates that individuals release a mean of 4.8 kg N/yr (Valiela et al. 1997). Contributions of nitrogen from the septic systems to BR1 and BR2 were calculated from the number of septic systems in each segment, the rate of use, and the amount of nitrogen produced per person each year. In a similar manner, the total amounts of nitrogen from domestic waste (now including both septic and treated effluent) within the watershed were estimated from population data for permanent residents and residents of second homes (data from the Summit County Planning Department). Residents of second homes were assumed to contribute waste over a three-month period. Seasonal change in skier activity was represented by changes in the wastewater outflow obtained from data collected by a local ski resort located on Copper Mountain, Colorado (Copper Mountain Resort, *unpublished data*). Although estimates of total generation of domestic waste based on population data is common (Valiela et al. 1997, Caraco and Cole 1999), some uncertainty in our analysis of N loading may exist due to differences in relative usage rates by residents.

N export

Samples of stream water were collected from the residential sites on septic (BR1 and BR2) and background reaches (BR3 and BR4) weekly (May to August) or biweekly (September to April) beginning in June 1999 and ending June 2000. Water also was collected at the same intervals during the winter and spring of 2002. Samples were stored in a dark cooler pending transport to the laboratory where they were filtered within 12 h of collection (using Whatman GF/F filters; nominal pore size 0.7 μm), and frozen until analysis.

Ammonium was determined colorimetrically by a modified Solorzano method involving the production of indophenol blue (Grashoff 1976). Nitrate was measured with an ion chromatograph, and TDN was measured by chemical oxidation with potassium persulfate (modified from Valderrama 1981) and subsequent determination of NO_3^- by ion chromatography (Davi et al. 1993). DON was calculated as the difference between TDN and dissolved inorganic N. Total dissolved P (TDP) was determined by modification of oxidation methods described by Lagler and Hendrix (1982) and Valderrama (1981).

Annual transport of nitrogen was estimated from data on concentration and discharge in 1999–2000. Discharge at BR1 was obtained from USGS gage data. Discharge was estimated for BR2, BR3, and BR4 on the basis of current velocity as measured with a flow meter weekly (May–August) and biweekly (September–April) (Kaushal and Lewis 2003). Export was calculated according to Kaushal and Lewis (2003) and expressed per unit of watershed area ($kg \cdot ha^{-1} \cdot yr^{-1}$).

Export of nitrogen in undeveloped watersheds is strongly related to water yield per unit area (Lewis et al. 1999, Lewis 2002). Water yield per unit area (specific runoff) decreases with decreasing elevation on the Blue River because smaller watersheds at higher elevations receive more precipitation per unit area than larger watersheds (Lewis et al. 1984, Smith et al. 2003). Therefore, differences in water yield between watersheds were standardized by dividing solute yield by water yield to allow comparison of N exports between the study sites, which differed in elevation and precipitation. Standardization is necessary for comparing N concentrations and yields across watersheds because concentrations and yields are strongly influenced by amount of water yield for a given watershed (Lewis et al. 1999), and large variation in N export can result over short distances in many regions due to elevation-related variation in runoff (Smith et al. 2003).

Characterization of $\delta^{15}N$ for autotrophs, groundwater, and wastewater

Forty samples of algae were collected from the two residential segments and a total of 34 samples of algae were collected from the five streams draining undeveloped watersheds (Fig. 1). Dates and locations of collection are presented in Table 1. At least one week was allowed to elapse between collections at a given site. The isotopic signature of wastewater was characterized by growth of five cultures of stream-derived algae on effluent obtained from septic tanks and wastewater facilities on the Blue River.

A filamentous alga, *Ulothrix zonata*, was the most common species collected from the sampling sites. Algal filaments were rinsed with water to remove fine particulate material and were picked clean under magnification (McCutchan and Lewis 2001, 2002). At certain times of the year when *Ulothrix* was not present in the streams, epilithic material was scrubbed from the upper surfaces of rocks. Algal cells were isolated from other components of the epilithon by centrifugation through colloidal silica and were collected on glass fiber filters (Hamilton and Lewis 1992, Hamilton et al. 2001, McCutchan and Lewis 2002). All samples of algae were lyophilized and stored in a desiccator until they were analyzed.

Four-liter samples of groundwater were collected from 19 wells during the spring of 1999 for isotopic characterization of nitrate. Groundwater wells were of shallow depth (<100 m) and were located in yards adjacent to residences. All of the sampled wells served as the domestic water supply for households. For each sample of groundwater, isotopic enrichment due to denitrification was estimated. As nitrate is converted to dinitrogen through denitrification, isotopic discrimination against ^{15}N results in enrichment of the remaining nitrate pool, according to Rayleigh fractionation as follows: $\delta t = \delta_{t=0} + \varepsilon(\ln[C_t/C_{t=0}])$ (Kellman and Hillaire-Marcel 2002), where δt and $\delta_{t=0}$ represent the isotopic composition of the nitrate pool at time t and time $t = 0$, C_t and $C_{t=0}$ are the concentrations of NO_3^- at time t and time $t = 0$, and ε is the isotopic enrichment factor for denitrification, which is related to the isotopic fractionation factor (α), by $\varepsilon = (\alpha - 1) \times 10^3$. A conservative discrimination factor of $\alpha = 1.04$ was taken from existing literature (Yoshinari and Koike 1994, Bedard-Haughn 2003), which may have underestimated the fraction of nitrogen derived from wastewater.

Fifty-liter samples of stream water were collected along with algae on five dates so that $\delta^{15}N$ of algal cells could be compared with $\delta^{15}N$ of nitrate in stream water. The $\delta^{15}N$ of nitrate in stream water and ground water was analyzed after extraction of nitrate with anion exchange resins and further concentration of this nitrate by lyophilization (Wassenaar 1995, Kellman and Hillaire-Marcel 1998, Silva et al. 2000). Isotope ratios of algae were determined with a continuous-flow, isotope ratio mass spectrometer and an elemental analyzer that converted organic N into N_2 gas. Isotope ratios of nitrate in ground water and stream water were determined similarly.

Nitrogen isotope ratios of algae were used to infer the proportion of wastewater N in residential streams by use of a mixing model (Debruyn and Rasmussen 2002):

$$\begin{aligned}\delta X_{\text{residential stream}} &= P_{\text{wastewater}}(\delta X_{\text{wastewater}} + F_{\text{wastewater}}) \\ &\quad + (1 - P_{\text{wastewater}})(\delta X_{\text{background}} + F_{\text{background}})\end{aligned}$$

where δX is the isotopic ratio of algae (in residential

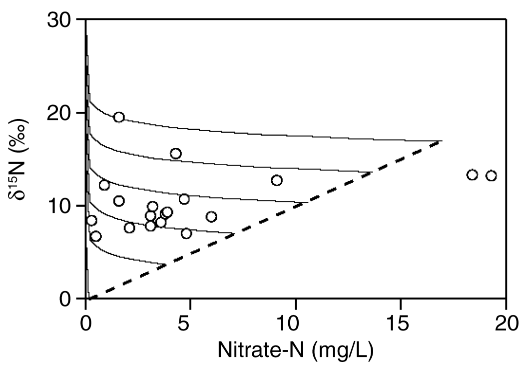

FIG. 2. Concentration and $\delta^{15}N$ of NO_3^--N in groundwater taken from wells in residential areas. The dashed line indicates a mixing line based on estimated end points for background (0.05 mg/L NO_3^--N and $\delta^{15}N$ of +0.5) and wastewater sources (17 mg/L of NO_3^--N and $\delta^{15}N$ of +17). Curved lines represent the expected isotopic values of N if denitrification were occurring based on Rayleigh fractionation.

streams, grown on wastewater, or taken from background streams); $P_{wastewater}$ is the proportion of wastewater N assimilated by the algae; and F is the isotopic shift between inorganic N and algal biomass (grown on wastewater or taken from streams under background conditions). The isotopic shift for algae was determined by comparing the $\delta^{15}N$ of algae with $\delta^{15}N$ of nitrate (which makes up about 90% of DIN) dissolved in stream water and wastewater under field conditions. In addition to inorganic N, algae may also assimilate certain forms of organic N (Antia et al. 1991) leading to some error in characterizing the $\delta^{15}N$ of nitrate, but the magnitude of organic N uptake by stream autotrophs is currently not well known.

Annual transport of DIN derived from wastewater was determined by methods similar to those described earlier using proportions of N from wastewater on sampling dates, concentrations of nitrate-N in stream water, and measurements of discharge.

RESULTS

Wells in the residential areas showed high concentrations of nitrate-N (4.96 ± 1.22 mg/L [mean ± SE]; Fig. 2). Concentrations of nitrate-N in some wells were greater than 10 mg/L, the state limit for drinking water. Approximately 40% of the wells had $\delta^{15}N$ values within the range commonly reported for sewage, +10 to +20, suggesting significant contributions of N from wastewater. Concentrations of NO_3^--N in most ground water samples deviated from a mixing line based on NO_3^- concentration and $\delta^{15}N$, as would be expected if denitrification occurs along flow paths between septic systems and wells. Estimates based on Rayleigh kinetics suggest that denitrification substantially affected concentrations of nitrate in many groundwater wells but isotopic enrichment of nitrate in groundwater was minimal ($\delta^{15}N$ probably <2‰; Fig. 2).

In streams of the residential areas, seasonal patterns in concentrations of inorganic nitrogen were different than in streams under background conditions. Tourist activity peaked in February, and was reflected in the volume of wastewater generated by a nearby ski resort (Fig. 3A). Concentrations of DIN in streams of residential areas upstream of ski resorts followed a similar pattern (Fig. 3A, B); they were highest during winter months, peaked at the end of the tourist season, and remained elevated throughout the growing season. DIN in background streams showed no peaks during spring and sustained very low concentrations (sometimes <5 μg/L) throughout the growing season. During the growing season, the N:P ratio in streams of residential areas increased rapidly because of high DIN. The N:P ratio of streams in undisturbed watersheds declined below the Redfield Ratio (Fig. 3C), suggesting potential nutrient limitation by inorganic N. Concentrations of total dissolved phosphorus in all streams were typically very low (<10 μg/L) and showed little seasonal variation.

The $\delta^{15}N$ of epilithic algae was strongly related to the $\delta^{15}N$ of nitrate in stream water and appeared to follow a relationship similar to data from Harrington et al. 1998 (Fig. 4A). The slope of this relationship was not significantly different from 1, suggesting that the fractionation factor appeared to be very low under field conditions. The baseline $\delta^{15}N$ of algae in control streams showed a small but statistically significant decrease during the growing season (t test, $P = 0.036$). Mean ^{15}N during the growing season was 1.05 (SE = 0.33) and mean $\delta^{15}N$ during the dormant season was 2.08 (SE = 0.33). The $\delta^{15}N$ of algae at the residential sites was consistently above baseline values but below the mean value of 17.1 (SE = 3.06) for algae grown on wastewater (Fig. 4B and C). The $\delta^{15}N$ of algae in streams of residential areas peaked during the tourist season, as did DIN, and remained high throughout the growing season.

Isotopic estimates of nitrogen derived from wastewater varied seasonally in streams of residential areas (Fig. 5). The percentage of wastewater N was greatest during the tourist season and declined during snowmelt. Peak values were 25% for BR1 and 56% for BR2 during winter months of 1999. Much of the mass transport of wastewater N occurred during spring snowmelt. Daily maxima were greater in 1999 than in 2002, which was an especially dry year. During 1998–1999, 19% of the nitrate-N export was derived from residential wastewater at BR1 and 23% of nitrate-N export was derived from residential wastewater at BR2.

Estimates of N contributions from isotope studies in 1999 were compared with mass-balance data for 2000, which showed similar hydrologic conditions. Organic nitrogen was the most abundant form of nitrogen exported from both residential and undeveloped sites (Table 2), but DIN comprised a greater proportion of the total N export from residential sites. Annual export of total N was approximately 7% greater than background

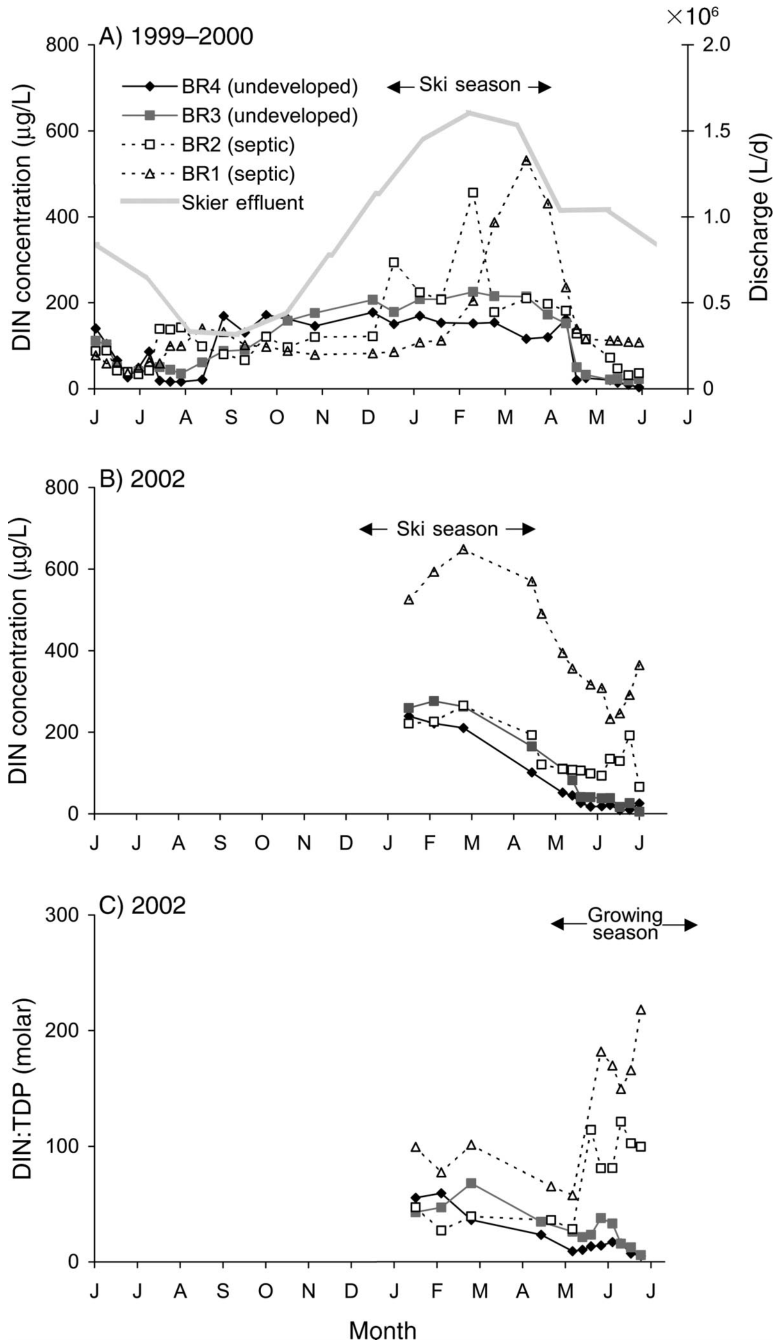

FIG. 3. (A) Concentration of dissolved inorganic N (DIN) in stream water at residential and background sites during 1999–2000. The thick gray line represents fluctuations in the volume of wastewater generated by a nearby ski resort. Discharge is in millions of liters per day. (B) Concentration of DIN in stream water at residential and background sites during 2002. (C) DIN:TDP (total dissolved phosphorus) ratio in stream water at residential and background sites during 2002.

at BR2 and within the range of background at BR1, after adjustment for differences in runoff between sites. Annual export of DIN was approximately 32% greater than background at BR2 and 24% greater than background at BR1, after adjustment for differences in water yield. Estimates from isotopic measurements were between the two bounds for inorganic N and total N determined independently from mass-balance estimates of export.

The population of the upper Blue River watershed is projected to increase by 2010, and peak population in this area is strongly influenced by the number of residents living in second homes (J. Vest, *personal communication*; Fig. 6A). Estimates of nitrogen gen-

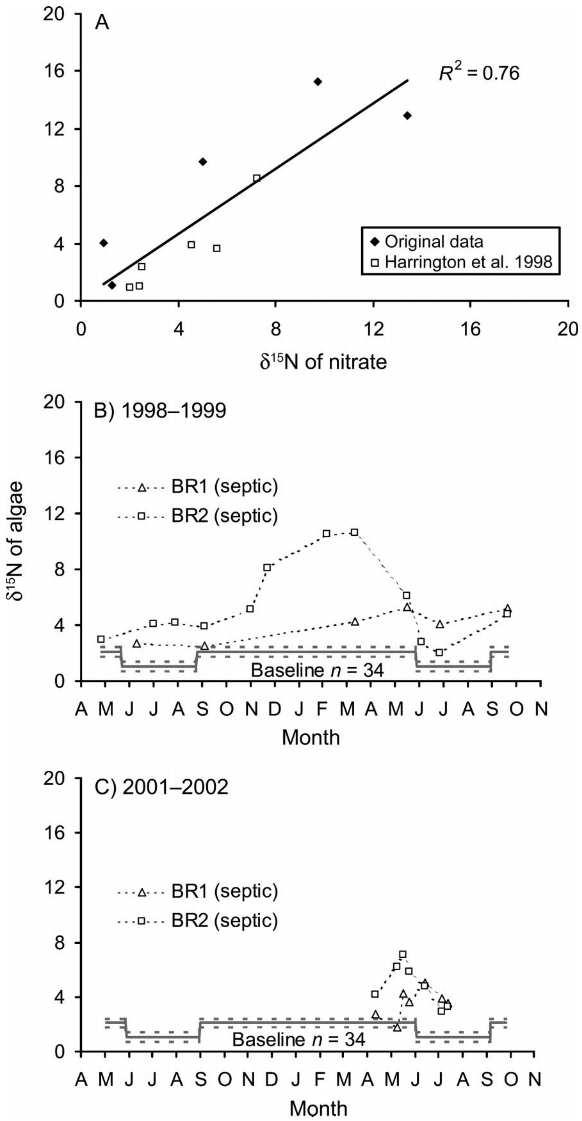

FIG. 4. (A) The relationship between δ^{15}N of nitrate in stream water and δ^{15}N of algae from our study and from Harrington et al. (1998). (B) Seasonal pattern in δ^{15}N of algae taken from residential streams during 1998–1999. (C) Seasonal pattern in δ^{15}N of algae taken from streams in residential areas during 2001–2002.

erated by domestic waste delivered from both septic systems and treatment plants are also increasing (Fig. 6B). On an areal basis, nitrogen loading associated with total domestic waste (2 kg·ha^{-1}·yr^{-1}) is approaching the contribution of nitrogen from background atmospheric sources (3 kg·ha^{-1}·yr^{-1}); if short-term skiers and vacationers are considered, the differences between these sources would be smaller. By 2010, N loading from domestic waste is expected to exceed to atmospheric N loading. In a similar manner, concentrations of nitrate-N in Lake Dillon have been steadily and significantly ($P < 0.05$) increasing over the last decade despite yearly variability in hydrologic conditions (Fig. 6C). Minimum concentrations during the growing season have increased exponentially from a previous baseline below the detection limit to values approaching those of the Blue River and ground water in upper segments of the watershed (Fig. 6D).

DISCUSSION

The results of this study suggest that considerable portions of the Blue River watershed are enriched with N derived from domestic waste. Over both years of study, concentrations of inorganic N in stream water of residential areas were high throughout growing seasons (50–400 μg/L), which can be an indicator of N availability exceeding biological demand (Aber et al. 1989, 1998, Williams et al. 1996). In contrast, concentrations of DIN in streams of undeveloped watersheds declined below detection (<5 μg/L) during the growing season. In the absence of disturbance, concentrations of nitrate in streams of this region of Colorado are typically near or below detection limits during the growing season due to low rates of atmospheric N deposition (Lewis et al. 1984, Stottlemeyer et al. 1997, Heuer et al. 1999, Kaushal and Lewis 2003).

During winter base flow, isotopic estimates indicated that approximately 15–60% of nitrate-N in stream water was derived from dispersed sources of wastewater. Mass transport of wastewater N increased markedly during spring runoff and remained high throughout the

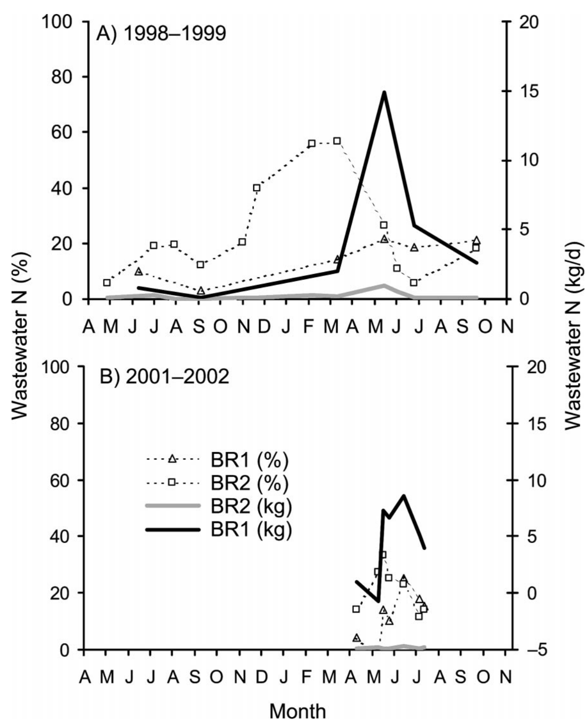

FIG. 5. (A) Isotopic estimate of N derived from wastewater transported in residential streams during 1998–1999. (B) Isotopic estimate of N derived from wastewater transported in residential streams during 2002.

TABLE 2. Characteristics of segments of the upper Blue River, Colorado, USA, during 1999.

Site	No. septic systems	Watershed area (ha)	Septic N loading ($kg \cdot ha^{-1} \cdot yr^{-1}$)	Water yield (mm)	Yield ($kg \cdot ha^{-1} \cdot yr^{-1}$)†				DIN yield (%)	Specific N yield (kg/mm)	
					DON	ON	NO_3^-	DIN		DIN	TN
BR4	0	1295	0	600	0.70	0.83	0.41	0.45	35	0.00075	0.0021
BR3	0	1585	0	491	0.60	0.72	0.33	0.36	33	0.00073	0.0022
BR2	408	5165	0.68	387	0.40	0.50	0.35	0.38	43	0.00098	0.0023
BR1	1456	32 020	0.39	303	0.30	0.36	0.26	0.28	44	0.00092	0.0021

Note: Specific N yield allows standardization for differences in water yield between watersheds.
† Abbreviations: DON, dissolved organic nitrogen; ON, organic nitrogen; DIN, dissolved inorganic nitrogen; TN, total nitrogen.

growing season. This is to be expected because N released by septic systems is carried to streams in proportion to the amount of water percolating through the soil (Lewis et al. 1984). Estimates of wastewater contributions based on export and on isotope data were similar and were within the range previously reported by Lewis et al. (1984) for other watersheds of this drainage, which vary in annual runoff and density of residents.

From the perspective of mass transport, there was only a small increase in annual DIN export from residential watersheds after standardizing for elevational differences in runoff. This may be expected because the rate of loading compared to background atmospheric sources was relatively low and there is potential for in-stream processing and retention across drainage networks (Howarth 1998, Alexander et al. 2000, Peterson et al. 2001). We observed strong localized effects of land use change, however, and they appeared to be most pronounced during base flow conditions (when less mass transport occurs). Concentrations of nitrate-N exceeded limits for safe drinking water (10 mg/L) in some wells; also, residential streams showed elevated concentrations of nitrate-N and DIN:TDP ratios, $\delta^{15}N$ was elevated in ground water and stream autotrophs, and there have been significant increases in nitrate-N concentrations during growing seasons in Lake Dillon over the past decade. The balance between retention and export of excess N may be influenced by the proximity of nutrient sources and timing of N delivery associated with changes in land use (Alexander et al. 2000). Geographic concentration of relatively low amounts of N enrichment to valleys near streams may be important in influencing the N status of surface waters, particularly when the capacity for in-stream or near-stream processes in headwaters to take up or remove N are exceeded during certain times of year (Baron et al. 1994). In this way, aquatic systems may develop conditions similar to the ones proposed by the concept of N saturation, which has been widely applied to understanding how human activities have altered N

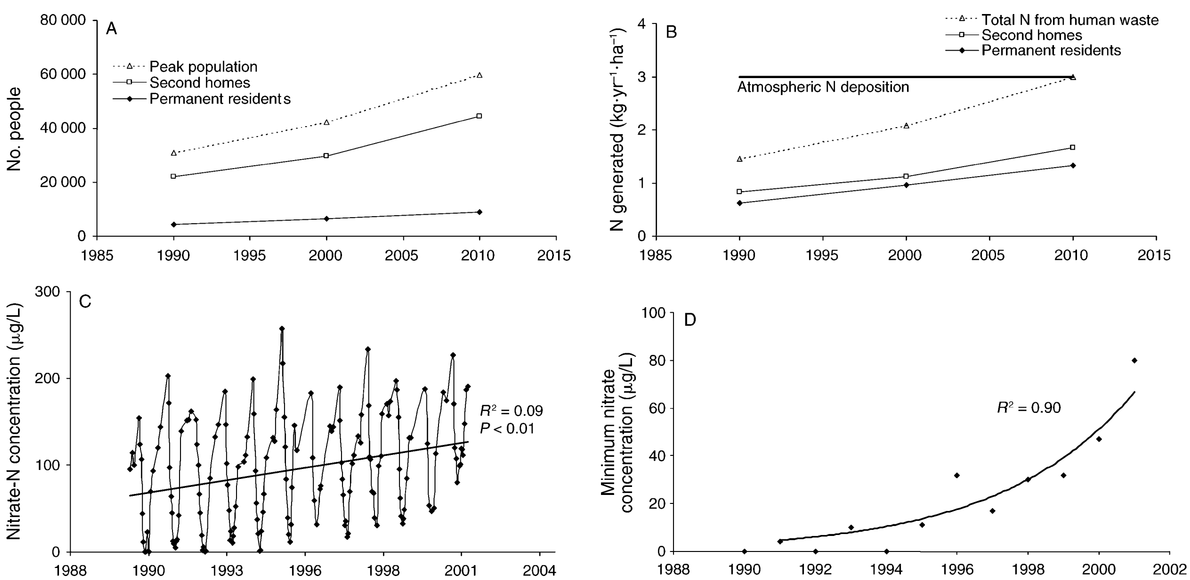

FIG. 6. (A) Number of residents in the upper Blue River watershed in past years and projected for 2010. (B) Mass of N generated from human waste per unit area in past years and projected for 2010. (C) Seasonal concentrations of nitrate-N in the center of Lake Dillon over the last decade. (D) Rate of increase in minimum concentrations of nitrate-N in the center of Lake Dillon during growing seasons over the last decade.

PLATE 1. (Left) Mountain stream draining a minimally disturbed watershed in Summit County, Colorado, and (right) increasing low-density residential development in headwater areas of the Colorado Rocky Mountains. Photo credits: J. McCutchan.

cycling in terrestrial ecosystems (Aber et al. 1989, Baron et al. 1994, Williams et al. 1996).

In Summit County, the rate of loading and timing of N release from residential development and suburban growth have been sufficient to cause elevated concentrations of DIN in streams and Lake Dillon. This pattern differs from related work in undeveloped mountain watersheds demonstrating shifts in export of predominantly DIN in higher elevation headwaters to DON at lower elevations (Hood et al. 2003). N enrichment can lead to increases in the DIN:DON ratio in some streams due to the diminished capacity of organisms to assimilate N and increases in the release of inorganic N from developed watersheds (Caraco and Cole 1999, Williams et al. 2001). Higher concentrations of inorganic N relative to organic N also have been observed in other headwater streams experiencing dispersed residential and suburban development (Groffman et al. 2004). DON was still relatively abundant in developed streams of the present study, but these streams, which were not fully serviced by sewers, may show increased propagation of inorganic N relative to organic N in surface waters due to contributions of DIN from deeper ground water and decreased processing and transformation of DIN in streams and riparian zones that are incised or channelized (Groffman et al. 2002). Work in minimally disturbed watersheds has shown that streams may act as transformers of DIN to DON on a seasonal basis (Kaushal and Lewis 2005), but the effects of land use change on the balance between production and consumption of organic N in surface waters deserves further attention.

Changes in the proximity, timing, and form of N entering aquatic systems due to residential development may be expected to affect ecological responses of biotic communities. Previous work has shown that some stream organisms preferentially assimilate nutrients derived from sewage (DeBruyn and Rasmussen 2002), and nitrogen from wastewater can be cycled at different rates through aquatic food webs due to shifts in primary producers (McClelland and Valiela 1998*b*). Other work at higher trophic levels has described changes in the community composition and diversity of secondary consumers in response to N enrichment (Tate and Heiny 1995). In mountain watersheds, changes in the duration of N delivery may cause changes in the duration of peaks in biotic activity. Such changes may be particularly important in aquatic systems of mountain regions where primary and bacterial production has been limited by inorganic nitrogen during parts of the growing season (e.g., Goldman et al. 1993, Morris and Lewis 1988, 1992, Interlandi and Kilham 1998). Dissolved organic N is an important biotic source of N in some minimally disturbed streams of the Colorado Rockies, where rates of N enrichment from anthropogenic sources are low (Kaushal and Lewis 2005). A switch in the absolute and relative abundance of inorganic versus organic sources of N due to changes in land use may influence the overall lability of N pools transported in surface waters, the metabolic activity of microbial communities, and the structure of food webs (Baron et al. 2000, Pace and Cole 2000, Kaushal and Lewis 2005).

Typical delivery of septic wastewater in mountain watersheds may involve minimal contact with soil, where N can be assimilated (Fisk et al. 1998), lost through denitrification (Groffman et al. 2002), or adsorbed onto soil particles. Septic systems in montane environments deliver nitrogen to thin soils and shallow groundwater, and much of the N enrichment occurs at a time when biotic activity is low. Aquatic ecosystems of these areas may be less effective at removing excess nitrogen due to low temperatures, rapid hydrologic flushing, and low rates of hydrologic exchange with sediments (e.g., Baron et al. 1994). Hydrologic connectivity also may effectively facilitate delivery and redistribution of nitrogen from anthropogenic sources over large spatial scales (Pringle 2001).

Elevated concentrations of nitrate-N in the center of Lake Dillon demonstrate that N from wastewater may travel over considerable distances and across multiple aquatic ecosystems without being assimilated or removed. Concentration of nitrate-N in the last few years of the water chemistry record may have been influenced by severe drought in Colorado, although other work has shown that concentrations of nitrate-N in the streams of Summit County did not change during this time period (Kaushal and Lewis 2005). The exponential increase in seasonal nitrate-N minima of Lake Dillon over the entire decade suggest that the watershed and its streams have little capacity to reduce increases in N loading. Further work will be necessary, however, to determine which types of headwater and mountain ecosystems are most susceptible to development based on terrain and land cover (Baron et al. 1994, Clow and Sueker 2000, Groffman et al. 2004) and interactions between climate change, land use, and water quality.

The contribution of atmospheric deposition to N enrichment has received considerable attention in the Colorado Rockies (Baron et al. 1994, Williams et al. 1996, Baron and Campbell 1997) and in other mountain watersheds (Goodale et al. 2000, Lovett et al. 2000, Sickman et al. 2002, Likens 2004). N enrichment from atmospheric sources can lead to elevated concentrations of nitrate-N in surface waters (Baron et al. 1994, Williams et al. 1996) and biological changes in autotrophic and heterotrophic communities (Baron et al. 2000, Wolfe et al. 2001, 2002). The expected ecological effects of increasing N enrichment from domestic waste in headwater ecosystems may be similar, but changes in ecosystem level processes and the composition of biological communities due to widespread residential and suburban development are currently less known. Nitrate is a biotically available component of wastewater, and it is assimilated until a saturation point is reached. Other unregulated compounds in wastewater such as caffeine, cholesterol, and detergents may be transported along with nitrate, but their fate in surface waters is currently unknown (Kolpin et al. 2002). Identification of N sources may be useful in indicating the influence of wastewater on major supplies of drinking water in rapidly developing watersheds.

N from point sources is relatively simple to detect, but detection of N from dispersed, nonpoint sources is much more difficult. Nonpoint sources have received increased attention from federal regulators (U.S. Environmental Protection Agency 2000). In the present study, rates of denitrification may have altered concentrations of nitrate-N in ground water but appeared to have little effect on isotopic composition of N based on Rayleigh fractionation. It is possible that there was error in our analysis based on limited number of samples from septic systems, which may show both temporal and spatial variability in concentration of NO_3^--N and $\delta^{15}N$. Our measured values for $\delta^{15}N$ of waste water, however, appeared to be within the range reported from previous studies (Cabanna and Rasmussen 1996, McClelland et al. 1997, McClelland and Valiela 1998*a*).

Results from the present study suggest that N isotope signatures in stream algae have the potential to support quantification of N from nonpoint sources of wastewater. The isotopic signature of algal $\delta^{15}N$ from residential streams was higher than background $\delta^{15}N$ of algae from undeveloped, forested sites of this study and other Colorado streams (McCutchan and Lewis 2001, 2002). Use of isotope techniques may work well in mountain watersheds, especially when there is a large separation in isotopic signatures (15‰ in the present study) between the pollution source and background sources. Seasonal oscillations in background $\delta^{15}N$ due to denitrification and uptake may be quantified by sampling streams in undeveloped watersheds of headwater ecosystems. Seasonal changes in $\delta^{15}N$ are relatively small, but involve a decline in $\delta^{15}N$ during snowmelt (McCutchan and Lewis 2001, Campbell et al. 2002). Previous work has indicated that isotope ratios in marine (McClelland and Valiela 1998*a*, Savage and Elmgren 2004) and terrestrial autotrophs (Stewart et al. 2002) also reflect signals from pollution sources integrated over time. There may be minimal fractionation between primary producers and in situ sources of N in aquatic environments (Harrington et al. 1998, Wolfe et al. 2003, Savage and Elmgren 2004) under natural conditions of growth (Goericke et al. 1994). Few data exist on simultaneous measurement of the $\delta^{15}N$ of nitrate and autotrophic biomass in small streams (but see Harrington et al. 1998), and although the fractionation factor appears to be low using combined data from the present study and Harrington et al. (1998), it is likely not zero. The importance of isotopic fractionation of N by autotrophs may increase in streams with warmer temperatures, greater light availability, and higher concentrations of DIN. In practice, it may be best to use a combination of mass balance and isotope approaches (Kellman and Hillaire-Marcel 2002, Bedard-Haughn et al. 2003). Isotopic ratios of aquatic autotrophs can be useful in identifying changes in sources of N due to wastewater (McClelland and Valiela 1998*a*, Savage and Elmgren 2004, Steffy and Kilham 2004). Stream algae may also allow quantitative use of the isotope technique, but this approach should be tested in other watersheds with varying physical characteristics and nutrient status.

Much of the world's population relies on water originating from mountain areas for human consumption and agriculture (Food and Agriculture Organization of the United Nations 2003, Messerli 2004). The level of development and land use change is increasing in headwater ecosystems around the world (Lewis et al. 1984, Daily and Ellison 2001, Korner 2004), and relatively modest levels of nutrient enrichment may lead to cascading effects on large supplies of water further downstream, particularly on a seasonal basis. Isotope ratios in stream algae may provide a rapid and effective means of directly quantifying N from pollution sources at low levels before increased enrichment can cause substantial changes in biological communities and deterioration of water quality. The present study suggests that the biotic capacity of headwater ecosystems (ground water, streams, and lakes) to attenuate inorganic N from dispersed sources and residential development and suburban growth may be minimal.

Acknowledgments

This work was primarily supported by grant NA17RJ1229 from NOAA. Additional funding was provided by the National Science Foundation (DEB 9318205), the Summit County Water Quality Committee, and the University of Colorado (Department of Ecology and Evolutionary Biology and the Cooperative Institute for Research in Environmental Sciences). Carol Kendall provided guidance with methods used in isotopic characterization of nitrate during initiation of the project. Blake Audsley, Todd Avant, Wyatt Cross, and Jeremy King assisted with field work and laboratory analyses. Jim Saunders provided logistical support. Peter Groffman, Winsor Lowe, and anonymous reviewers provided comments that improved this manuscript.

Literature Cited

Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems—hypotheses revisited. BioScience **48**:921–934.

Aber, J. D., K. J. Nadelhoffer, P. Steudler, and J. M. Melillo. 1989. Nitrogen saturation in northern forest ecosystems. BioScience **39**:378–386.

Alexander, R. B., R. A. Smith, and G. E. Schwarz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature **403**:758–761.

Antia, N. J., P. J. Harrison, and L. Oliveira. 1991. The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia **30**:1–89.

Aravena, R. M., M. L. Evans, and J. A. Cherry. 1993. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. Ground Water **31**:180–186.

Baron, J. S., and D. H. Campbell. 1997. Nitrogen fluxes in a high-elevation Colorado Rocky Mountain Basin. Hydrological Processes **11**:783–799.

Baron, J. S., D. S. Ojima, E. A. Holland, and W. J. Parton. 1994. Analysis of nitrogen saturation potential in Rocky Mountain tundra and forest: implications for aquatic systems. Biogeochemistry **27**:61–82.

Baron, J. S., H. M. Rueth, A. M. Wolfe, K. R. Nydick, E. J. Allstott, J. T. Minear, and B. Moraska. 2000. Ecosystem responses to nitrogen deposition in the Colorado Front Range. Ecosystems **3**:352–368.

Bedard-Haughn, A., J. W. van Groenigen, and C. van Kessel. 2003. Tracing ^{15}N through landscapes: potential uses and precautions. Journal of Hydrology **272**:175–190.

Brandes, J. A., M. E. McClain, and T. P. Pimental. 1996. N-15 evidence for the origin and cycling of inorganic nitrogen in a small Amazonian catchment. Biogeochemistry **34**:45–56.

Brooks, P. D., D. H. Campbell, K. A. Tonnessen, and K. Heuer. 1999. Natural variability in N export from headwater catchments: snow cover controls on ecosystem N retention. Hydrological Processes **13**:2191–2201.

Burns, D. A., and C. Kendall. 2002. Analysis of δ N-15 and δ O-18 to differentiate NO_3^- sources in runoff at two watersheds in the Catskill Mountains of New York. Water Resources Research **38**:1051.

Byron, E. R., and C. R. Goldman. 1989. Land-use and water-quality in tributary streams of Lake Tahoe, California–Nevada. Journal of Environmental Quality **18**:84–88.

Cabana, G., and J. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the Natural Academy of Sciences (USA) **93**:10844–10847.

Campbell, D. H., C. Kendall, C. C. Y. Chang, S. R. Silva, and K. A. Tonnessen. 2002. Pathways for nitrate release from an alpine watershed: determination using delta N-15 and delta O-18. Water Resources Research **38**:1052.

Caraco, N. F., and J. J. Cole. 1999. Human impact on nitrate export: an analysis using major world rivers. Ambio **28**:167–170.

Clow, D. W., and J. K. Sueker. 2000. Relations between basin characteristics and stream water chemistry in alpine/subalpine basins in Rocky Mountain National Park, Colorado. Water Resources Research **36**:49–61.

Daily, G. C., and K. Ellison, editors. 2001. The new economy of nature: the quest to make conservation profitable. Island Press, Washington, D.C., USA.

Davi, M. L., S. Bignami, C. Milan, M. Liboni, and M. G. Malfatto. 1993. Determination of nitrate in surface waters by ion-exchange chromatography after oxidation of total organic nitrogen to nitrate. Journal of Chromatography **644**:345–348.

DeBruyn, A. M. H., and J. B. Rasmussen. 2002. Quantifying assimilation of sewage-derived organic matter by riverine benthos. Ecological Applications **12**:511–520.

Dodds, W. K., et al. 2002. N uptake as a function of concentration in streams. Journal of the North American Benthological Society **21**:206–220.

Fisk, M. C., S. K. Schmidt, and T. R. Seastedt. 1998. Topographic patterns of above- and belowground production and nitrogen cycling in Alpine tundra. Ecology **79**:2253–2266.

Food and Agriculture Organization of the United Nations (FAO). 2003. Unlocking the water potential of agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy.

Goericke, R., J. P. Montoya, and B. Fry. 1994. Physiology of isotopic fractionation in algae and cyanobacteria. Pages 187–221 *in* K. Lajtha and R. H. Michener, editors. Stable isotopes in ecology and environmental science. Blackwell Scientific Publications, London, UK.

Goldman, C. R., A. D. Jassby, and S. H. Hackley. 1993. Decadal, interannual, and seasonal variability in enrichment bioassays at Lake Tahoe, California–Nevada, USA. Canadian Journal of Fisheries and Aquatic Sciences **50**:1489–1496.

Goodale, C. L., J. D. Aber, and W. H. McDowell. 2000. The long-term effects of disturbance on organic and inorganic nitrogen export in the White Mountains, New Hampshire. Ecosystems **3**:433–450.

Grashoff, K. 1976. Determination of dissolved oxygen and nutrients. Pages 126–137 *in* K. Grashoff, M. Ehrhardt, and K. Kremling, editors. Methods of seawater analysis. Verlag Chimie, Weinheim, Denmark.

Groffman, P. M., N. J. Boulware, W. C. Zipperer, R. V. Pouyat, L. E. Band, and M. F. Colosimo. 2002. Soil nitrogen cycle processes in urban Riparian zones. Environmental Science and Technology **36**:4547–4552.

Groffman, P. M., N. L. Law, K. T. Belt, L. E. Band, and G. T. Fisher. 2004. Nitrogen fluxes and retention in urban watershed ecosystems. Ecosystems **7**:393–403.

Hamilton, S. K., and W. M. Lewis, Jr. 1992. Stable carbon and nitrogen isotopes in algae and detritus from the Orinoco River floodplain, Venezuela. Geochimica Cosmochimica Acta **56**:4237–4246.

Hamilton, S. K., J. L. Tank, D. F. Raikow, W. M. Wollheim, B. J. Peterson, and J. R. Webster. 2001. Nitrogen uptake and transformation in a midwestern U.S. stream: a stable isotope enrichment study. Biogeochemistry **54**:297–340.

Handley, L. L., and C. M. Scrimgeour. 1997. Terrestrial plant ecology and ^{15}N natural abundance: the present limits to interpretation for uncultivated systems with original data from a Scottish old field. Advances in Ecological Research **27**:133–212.

Harrington, R. R., B. P. Kennedy, C. P. Chamberlain, J. D. Blum, and C. L. Folt. 1998. ^{15}N enrichment in agricultural catchments: field patterns and applications to tracking Atlantic salmon (*Salmo salar*). Chemical Geology **147**:281–294.

Heuer, K., P. D. Brooks, and K. A. Tonnesson. 1999. Nitrogen dynamics in two high elevation catchments during spring snowmelt 1996, Rocky Mountains, Colorado. Hydrological Processes **13**:2203–2214.

Hood, E. W., M. W. Williams, and N. Caine. 2003. Landscape controls on organic and inorganic nitrogen leaching across an alpine/subalpine ecotone, Green Lakes Valley, Colorado Front Range. Ecosystems **6**:31–45.

Howarth, R. W. 1998. An assessment of human influences on fluxes of nitrogen from the terrestrial landscape to the estuaries and continental shelves of the North Atlantic Ocean. Nutrient Cycling in Agroecosystems **52**:213–223.

Interlandi, S. J., and S. S. Kilham. 1998. Assessing the effects of nitrogen deposition on mountain waters: a study of phytoplankton community dynamics. Water Science and Technology **38**:139–146.

Jackson, R. B., S. R. Carpenter, and C. N. Dahm. 2001. Water in a changing world. Ecological Applications **11**:1027–1045.

Kaushal, S. S., and W. M. Lewis, Jr. 2003. Patterns in the chemical fractionation of organic nitrogen in Rocky Mountain streams. Ecosystems **6**:483–492.

Kaushal, S. S., and W. M. Lewis, Jr. 2005. Fate and transport of organic nitrogen in minimally disturbed montane streams of Colorado, USA. Biogeochemistry, **74**:303–321.

Kellman, L., and C. Hillaire-Marcel. 1998. Nitrate cycling in streams: using natural abundances of NO_3^--^{15}N to measure in situ denitrification. Biogeochemistry **43**:273–292.

Kellman, L. M., and C. Hillaire-Marcel. 2002. Evaluation of nitrogen isotopes as indicators of nitrate contamination sources in an agricultural watershed. Agriculture, Ecosystems and Environment **95**:87–102.

Kendall, C. 1998. Tracing nitrogen sources and cycling in catchments. Pages 519–576 *in* C. Kendall and J. J. McDonell, editors. Isotope tracers in catchment hydrology. Elsevier Science B.V., Amsterdam, The Netherlands.

Kohl, D. H., G. B. Shearer, and B. Commoner. 1971. Fertilizer nitrogen: contribution to nitrate in surface water in a corn belt watershed. Science **174**:1331–1334.

Kolpin, D. W., E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber, and H. T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environmental Science and Technology **36**:1202–1211.

Korner, C. 2004. Mountain biodiversity, its causes and function. Ambio **13**:11–17.

Kreitler, C. W., and L. A. Browning. 1983. Nitrogen-isotope analysis of groundwater nitrate in carbonate aquifers: natural sources versus human pollution. Journal of Hydrology **61**:285–301.

Lagler, C. L., and P. F. Hendrix. 1982. Evaluation of persulfate digestion method for particulate nitrogen and phosphorus. Water Research **16**:1451–1454.

Lewis, W. M., Jr. 2002. Yield of nitrogen from minimally disturbed watersheds of the United States. Biogeochemistry **57**:375–385.

Lewis, W. M., J. M. Melack, Jr., W. H. McDowell, M. Mclain, and J. E. Richey. 1999. Nitrogen yields from undisturbed watersheds in the Americas. Biogeochemistry **46**:149–162.

Lewis, W. M., Jr., J. F. Saunders, III, D. W. Crumpacker, Sr., and C. Brendecke. 1984. Eutrophication and land use. Springer-Verlag, New York, New York, USA.

Likens, G. E. 2004. Some perspectives on long-term biogeochemical research from the Hubbard Brook ecosystem study. Ecology **85**:2355–2362.

Lovett, G. M., K. C. Weathers, and W. V. Sobczak. 2000. Nitrogen saturation and retention in forested watersheds of the Catskill Mountains, New York. Ecological Applications **10**:73–84.

McClelland, J. W., and I. Valiela. 1998*a*. Linking nitrogen in estuarine producers to land-derived sources. Limnology and Oceanography **43**:577–585.

McClelland, J. W., and I. Valiela. 1998*b*. Changes in food web structure under the influence of increased anthropogenic nitrogen inputs to estuaries. Marine Ecology Progress Series **168**:259–271.

McClelland, J. W., I. Valiela, and R. H. Michener. 1997. Nitrogen-stable isotope signatures in estuarine food webs: a record of increasing urbanization in coastal watersheds. Limnology and Oceanography **42**:930–937.

McCutchan, J. H., Jr., and W. M. Lewis, Jr. 2001. Seasonal variation in stable isotope ratios of stream algae. Verhandlungen Internationale Vereinigung für theoretische und angewandte Limnologie **27**:3304–3307.

McCutchan, J. H., Jr., and W. M. Lewis, Jr. 2002. Relative importance of carbon sources for macroinvertebrates in a Rocky Mountain stream. Limnology and Oceanography **47**:742–752.

McKnight, D. M., G. M. Hornberger, K. E. Bencala, and E. W. Boyer. 2002. In-stream sorption of fulvic acid in an acidic stream: a stream scale transport experiment. Water Resources Research **38**:1–12.

Messerli, B. 2004. Mountains of the world—vulnerable water taverns for the 21st century. Ambio **7**:29–34.

Morris, D. P., and W. M. Lewis, Jr. 1988. Phytoplankton nutrient limitation in Colorado mountain lakes. Freshwater Biology **20**:315–327.

Morris, D. P., and W. M. Lewis, Jr. 1992. Nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. Limnology and Oceanography **37**:1179–1192.

Mulholland, P. J., et al. 2002. Can uptake length in streams be determined by nutrient addition experiments? Results from an interbiome comparison study. Journal of the North American Benthological Society **21**:544–560.

Ostrom, N. E., L. O. Hedin, J. C. von Fischer, and G. P. Robertson. 2002. Nitrogen transformations and NO_3^- removal at a soil-stream interface: a stable isotope approach. Ecological Applications **12**:1027–1043.

Pace, M. L., and J. J. Cole. 2000. Effects of whole-lake manipulations of nutrient loading and food web structure on planktonic respiration. Canadian Journal of Fisheries and Aquatic Sciences **57**:487–496.

Panno, S. V., K. C. Hackley, H. H. Hwang, and W. R. Kelly. 2001. Determination of the sources of nitrate contamination in karst springs using isotopic and chemical indicators. Chemical Geology **179**:113–128.

Peterson, B. J., et al. 2001. Control of nitrogen export from watersheds by headwater streams. Science **292**:86–90.

Pringle, C. M. 2001. Hydrologic connectivity and the management of biological reserves: a global perspective. Ecological Applications **11**:981–998.

Savage, C., and R. Elmgren. 2004. Macroalgal (*Fucus vesiculosus*) $\delta^{15}N$ values trace decrease in sewage influence. Ecological Applications **14**:517–526.

Sickman, J. O., J. M. Melack, and D. W. Clow. 2003. Evidence for nutrient enrichment of high-elevation lakes in the Sierra Nevada, California. Limnology and Oceanography **48**:1885–1892.

Silva, S. R., C. Kendall, D. H. Wilkinson, A. C. Ziegler, C. C. Y. Chang, and R. J. Avanzino. 2000. A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. Journal of Hydrology **228**:22–36.

Smith, R. A., R. B. Alexander, and G. E. Schwarz. 2003. Natural background concentrations of nutrients in streams and rivers of the conterminous United States. Environmental Science and Technology **37**:3039–3047.

Steffy, L. Y., and S. S. Kilham. 2004. Elevated $\delta^{15}N$ in stream biota in areas with septic tank systems in an urban watershed. Ecological Applications **14**:637–641.

Stewart, G. R., M. P. M. Aidar, C. A. Joly, and S. Schmidt. 2002. Impact of point source pollution on nitrogen isotope signatures (delta N-15) of vegetation in SE Brazil. Oecologia **131**:468–472.

Stottlemeyer, R., C. A. Troendle, and D. Markowitz. 1997. Change in snowpack, soil water, and streamwater chemistry with elevation during 1990, Fraser Experimental Forest, Colorado. Journal of Hydrology **195**:114–136.

Tate, C. M., and J. S. Heiny. 1995. The ordination of benthic invertebrate communities in the South Platte River basin in relation to environmental factors. Freshwater Biology **33**:439–454.

U.S. Environmental Protection Agency. 2000. Final TMDL rule: fulfilling the goals of the Clean Water Act. U.S. Environmental Protection Agency, Washington, D.C., USA.

Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and phosphorus in natural waters. Marine Chemistry **10**:109–122.

Valiela, I., G. Collins, J. Kremer, K. Lajtha, M. Geist, B. Seely, J. Brawley, and C. H. Sham. 1997. Nitrogen loading from coastal watersheds to receiving estuaries: new method and application. Ecological Applications **7**:358–380.

Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology **80**:1395–1404.

Wassenaar, L. I. 1995. Evaluation of the origin and fate of nitrate in the Abbotsford Aquifer using the isotopes of ^{15}N and ^{18}O in NO_3^-. Applied Geochemistry **10**:391–405.

Wernick, B. G., K. E. Cook, and H. Schreier. 1998. Land use and streamwater nitrate-N dynamics in an urban-rural fringe watershed. Journal of the North American Water Resources Association **34**:639–650.

Williams, M. W., J. S. Baron, N. Caine, R. Sommerfeld, and R. Sanford. 1996. Nitrogen saturation in the Rocky Mountains. Environmental Science and Technology **30**:640–646.

Williams, M. W., E. Hood, and N. Caine. 2001. Role of organic nitrogen in the nitrogen cycle of a high-elevation catchment, Colorado Front Range. Water Resources Research **37**:2569–2581.

Wolfe, A. P., J. S. Baron, and R. J. Cornett. 2001. Anthropogenic nitrogen deposition induces rapid ecological changes in alpine lakes of the Colorado Front Range (USA). Journal of Paleolimnology **25**:1–7.

Wolfe, A. P., S. S. Kaushal, J. R. Fulton, and D. M. McKnight. 2002. Spectrofluorescence of sediment humic substances and historical changes of lacustrine organic matter provenance in response to atmospheric nutrient enrichment. Environmental Science and Technology **36**:3217–3223.

Wolfe, A. P., A. C. Van Gorp, and J. S. Baron. 2003. Recent ecological and biogeochemical changes in alpine lakes of Rocky Mountain National Park (Colorado, USA): a response to anthropogenic nitrogen deposition. Geobiology **1**:153–168.

Yoshinari, T., and I. Koike. 1994. The use of stable isotopes for the study of gaseous nitrogen species in marine environments. Pages 114–137 *in* K. Lajtha and R. H. Michener, editors. Stable isotopes in ecology and environmental science. Blackwell Scientific Publications, London, UK.