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# Carbon and nitrogen stoichiometry and nitrogen cycling rates in streams

**Abstract** Stoichiometric analyses can be used to investigate the linkages between N and C cycles and how these linkages influence biogeochemistry at many scales, from components of individual ecosystems up to the biosphere. N-specific $NH_4^+$ uptake rates were measured in eight streams using short-term $^{15}N$ tracer additions, and C to N ratios (C:N) were determined from living and non-living organic matter collected from ten streams. These data were also compared to previously published data compiled from studies of lakes, ponds, wetlands, forests, and tundra. There was a significant negative relationship between C:N and N-specific uptake rate; C:N could account for 41% of the variance in N-specific uptake rate across all streams, and the relationship held in five of eight streams. Most of the variation in N-specific uptake rate was contributed by detrital and primary producer compartments with large values of C:N and small values for N-specific uptake rate. In streams, particulate materials are not as likely to move downstream as dissolved N, so if N is cycling in a particulate compartment, N retention is likely to be greater. Together, these data suggest that N retention may depend in part on C:N of living and non-living organic matter in streams. Factors that alter C:N of stream ecosystem compartments, such as removal of riparian vegetation or N fertilization, may influence the amount of retention attributed to these ecosystem compartments by causing shifts in stoichiometry. Our analysis suggests that C:N of ecosystem compartments can be used to link N-cycling models across streams.

**Keywords** Carbon · Carbon:Nitrogen ratio · Nitrogen · Stoichiometry · Streams

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# Introduction

N is a key element limiting primary production in many terrestrial, marine, and freshwater ecosystems (Grimm and Fisher 1986; Elser et al. 1990; Vitousek and Howarth 1991; Dodds et al. 2002). Humans have effectively doubled the global rate of N fixation (Vitousek et al. 1997) and the ultimate fate of this N once it enters terrestrial and aquatic ecosystems depends on the relative rates of influx, biological uptake, denitrification, storage, and export. Anthropogenic increases in N influx produce changes in N availability and potentially alter the C:N stoichiometry of primary uptake compartments (plants and microbes that directly use dissolved N). Altered C:N stoichiometry in plants and microbes could in turn influence N storage in living and nonliving organic matter, the lability of detritus, the movement of N through ecosystems, and the flow of energy through food webs. The aim of this paper is to explore how changes in stoichiometry of individual ecosystem compartments (specifically C:N) may influence rates of N uptake and release in streams.

The amount of N transported by the world's rivers into the oceans has roughly doubled and rates of N transport from developed areas have increased ten- to 50-fold as a result of human activities since the Industrial Revolution (Meybeck 1982). Increased N runoff can cause environmental problems in receiving waters, for example, the zone of decreased dissolved $O_2$ that develops seasonally in the Gulf of Mexico (Rabalais et al. 1998) and toxic algal blooms in lakes and coastal zones (Burkholder and Glasgow 1997). Because nutrient cycling in headwater streams is particularly important in controlling N transport to downstream, recipient aquatic systems (Alexander et al.

2000; Peterson et al. 2001), improved understanding of the fundamental aspects of N cycling in small streams will facilitate efforts to mitigate N loading effects on downstream ecosystems.

The N cycle comprises fluxes among an interacting group of compartments in which N resides (e.g., inorganic N, free organic N, detritus, microbial assemblages, animals, plants). Identifying which components most strongly influence these fluxes is key to understanding N retention (the average length of time a molecule of N spends in a specific ecosystem) by streams and other ecosystems, and the value of this approach is reflected by the ubiquitous box and arrow diagrams published for all types of ecosystems. One way to simplify the compartment-flux approach is to assume that compartments are close to equilibrium or "steady state" (i.e., not losing or gaining a significant amount of mass), so uptake into a compartment is approximately equal to loss from that compartment (e.g., Wollheim et al. 1999). If compartments are assumed to be close to equilibrium, then quantifying N uptake or loss will allow for calculation of an average compartment-wide turnover of N that provides an estimate of how long N resides within that compartment.

N movement through stream ecosystems can be viewed conceptually as nutrient spiraling between the water column and benthic environments (Webster and Patten 1979; Newbold et al. 1981). Short spiral lengths are associated with high biotic N demand and retention (i.e., N retained in biomass or sediment compartments) and decreased rates of hydrological transport. Short-term $^{15}$N tracer experiments have been used to study whole-stream N retention and have revealed that small streams effectively retain inorganic N (Peterson et al. 2001). The partitioning of this retention into various stream compart-

**Table 1** Study sites for the $^{15}$N isotope tracer experiments and additional C:N determinations. Compartments with N-specific uptake rates are: grazer (*GRZ*), shredder (*SHR*), collector (*COL*), filter feeder (*FILT*), invertebrate predator (*IPR*), vertebrate predator (*VPR*), fine benthic organic material (*FBOM*), leaves (*LEV*), wood (*WOO*), epilithon (*EPI*), filamentous algae (*FIL*), bryophytes (*BRY*), suspended particulate organic N (*SPON*)

| Site (abbreviation) | Biome | N-specific uptake rates | Reference |
|---|---|---|---|
| Upper Ball Creek, North Carolina (UBNC) | Deciduous forest | BRY, EPI, FBOM, FILT, GRZ, LEV, SHR | Tank et al. (2000) |
| Walker Branch, Tennessee (WBTN) | Deciduous forest | BRY, COL, EPI, FBOM, GRZ, LEV, SHR, VPR, WOO | Mulholland et al. (2000) |
| Sycamore Creek, Arizona (SCAZ) | Desert | C:N only | |
| Kings Creek, Kansas (KCKS) | Prairie | BRY, COL, EPI, FBOM, FIL, GRZ, LEV, VPR, WOO | Dodds et al. (2000) |
| Bear Brook, New Hampshire (BBNH) | Deciduous forest | BRY, EPI, FBOM, LEV, SHR, WOO | |
| Gallina Creek, New Mexico (GCNM) | Montane coniferous forest | C:N only | |
| Quebrada Bisley, Puerto Rico (QBPR) | Tropical forest | COL, EPI, FBOM, GRZ, IPR, LEV, SHR, SPON | Merriam et al. (2002) |
| Eagle Creek, Michigan (ECMI) | Deciduous forest | COL, EPI, FBOM, FILT, GRZ, LEV, SHR, WOO | Hamilton et al. (2001) |
| Mack Creek, Oregon (MCOR) | Montane coniferous forest | BRY, EPI, FBOM, GRZ, WOO | |
| Kuparuk River, Alaska (E1AK) | Tundra | BRY, COL, EPI, FIL, FILT, GRZ, VPR | Wollheim et al. (1999) |

ments has been documented for a number of individual streams (see list of references in Table 1), but cross-site comparisons of N retention within various compartments are needed.

The N cycle is coupled with other nutrient cycles, particularly C and P. The stoichiometric approach explicitly recognizes that relative abundances of elements can control rates of nutrient cycling and energy flow within and across ecosystem compartments. Ecological stoichiometry has been used as a framework to understand control of nutrient cycling since Redfield (1958) argued that elemental composition of phytoplankton controlled ratios of inorganic nutrients in the ocean. Ecological stoichiometry theory has developed rapidly in recent years (Sterner 1995; Sterner and Elser 2002) to integrate energy flow and nutrient cycling concepts, including intensive analyses of the grazer–producer interface in pelagic systems, particularly lakes (Elser and Urabe 1999).

The relationships between C and N cycling are fundamental biogeochemical links in the biosphere (Redfield 1958; Turner 2002). For example, increased atmospheric $CO_2$ can lead to greater rates of C fixation (photosynthesis) and higher C:N in terrestrial primary producers. The C:N of terrestrial detritus can in turn alter rates of mineralization (Aber and Melillo 1980; McClaugherty et al. 1985; Bosatta and Ågren 1991) and thus availability for uptake. Understanding the links between N and C cycles at the ecosystem scale is essential for predicting the effects of global change on large-scale N and C fluxes (Schimel 1995). Yet, our understanding of how such changes influence the N cycle in aquatic ecosystems is limited, and if streams are indeed important to large-scale fluxes as suggested above, then it is essential to pay attention to coupled C and N cycles in streams.

Research on N cycling in a prairie stream using a $^{15}$N tracer addition (Dodds et al. 2000) revealed a negative correlation between C:N of ecosystem compartments and N-specific uptake rate (rate of N uptake/N in biomass) by those compartments (compartments included wood, leaves, sediments, epiphytes, bryophytes and animals). N-specific uptake is an important component of N turnover, and thus may be related to the amount of N retention that occurs in a system. If this negative correlation is a general feature of ecosystems, it could allow for cross-system prediction of N cycling rates. These results from the prairie stream led us to explore how widespread this pattern is, and what factors produce it. The generality of the C:N-uptake relationship was tested by comparing values for C:N and N-specific uptake rate from a variety of streams where similar methods were used to estimate N-specific uptake rate and N standing stock. We hypothesized that N cycling would be influenced by the degree of N deficiency as established with nutrient enrichment bioassays. We extended our analysis by comparing our stream results to those published from research on a variety of aquatic and terrestrial habitats.

## Materials and methods

We measured N uptake rates and C:N of individual compartments in streams in a variety of biomes using the same methods across all sites (Table 1). Site abbreviations used in this paper can be found in Table 1. These sites have been characterized for microbial biomass (Findlay et al. 2002), whole-stream metabolism (Mulholland et al. 2001), and inorganic N uptake (Dodds et al. 2002; Mulholland et al. 2002; Webster et al. 2003). Detailed analyses of N cycling at individual sites have also been published (Table 1). The specifics of the $^{15}$N tracer method and the study sites are well documented in the above publications. Briefly, $^{15}$N-NH$_4^+$ was released into each stream at tracer levels (i.e., there was no significant fertilization) for a period of 42 days (35 days at Kings Creek, Kansas). Samples were collected before, during, and after the $^{15}$N-NH$_4^+$ releases from stream ecosystem compartments [dissolved NH$_4^+$, suspended particulate organic N (SPON), fine particulate organic N (FPON), leaves, small wood fragments, epilithon, filamentous green algae, bryophytes, invertebrates (including separate samples of grazers, collector-gatherers, filter feeders, scrapers, and predators), and vertebrate predators]. NH$_4^+$ was chemically separated from other N forms by trapping on an acid filter (Mulholland et al. 2000). All samples were analyzed for $^{15}$N content by mass spectrometry, and biomass and detrital organic material were analyzed for C and N content using a Carlo Erba elemental analyzer.

We used N-specific uptake rates (mass N taken up by the compartment per unit time/mass N in compartment) as an estimate of compartment-specific N cycling rates. The N uptake rate of each ecosystem compartment was computed as a function of $^{15}$N change in the compartment and the $^{15}$N content of the compartment that the N was derived from. Since the $^{15}$N label was changing over time in both source and uptake compartments, many of the compartments were modeled to establish uptake rates. These box-models are described extensively elsewhere (Hall et al. 1998; Wollheim et al. 1999; Dodds et al. 2000; Mulholland et al. 2000) and have proved adequate to deal with potential errors associated with averaging changes in the $^{15}$N of source compartments (Laws 1984). At some of the sites the models were used to estimate NO$_3^-$ uptake for each primary uptake compartment, whereas at others NO$_3^-$ uptake was calculated only for the entire stream. In the cases where NO$_3^-$ uptake was calculated only for the entire study reach, the uptake of NH$_4^+$ for each compartment was used to calculate total inorganic N uptake using the ratio of NH$_4^+$/NO$_3^-$ uptake for the entire stream (i.e., it was assumed that the NH$_4^+$/NO$_3^-$ uptake ratio in each primary uptake compartment was the same as for the entire stream).

Data from nutrient-diffusing substrata (Tank and Dodds 2003) were used to calculate an index of N deficiency for each stream. In these experiments, nutrient-diffusing substrata (60-ml, nutrient-enriched, agar-filled cups containing 0.5 M KNO$_3$, 0.5 M NaH$_2$PO$_4$, both, or neither, covered with Whatman GF/F glass fiber filters or 1-mm-thick oak veneer) were placed in the stream for 21 days and analyzed for accumulation of algal chlorophyll-$a$ on wood and glass fiber filters, and ergosterol (as an indicator of fungal biomass) on the wood substrata. Chlorophyll was analyzed by standard fluorometric or spectrophotometric techniques with correction for phaeophytin, and ergosterol was analyzed by HPLC.

The index of N deficiency was calculated from the nutrient enrichment bioassays using the following relationship:

$$\text{N deficiency} = (B_{NP} - B_P)/B_C \quad (1)$$

where $B_{NP}$=mean biomass in the N+P diffusion treatments, $B_P$=mean biomass in the P diffusion treatments, and $B_C$=mean biomass in control treatments. This index reflects N deficiency without the confounding effects of P-deficiency, and assumes that even secondary limitation by N can alter uptake and retention of N. Different components of a microbial or periphyton community may be limited by different nutrients, and individual cells may express dual limitation, even under very homogeneous conditions (Egli 1991), which calls for an approach that assesses N deficiency without regard to the degree of limitation by P.

**Table 2** Literature data sources for N cycling rates

| Habitat | Method | Reference |
|---|---|---|
| Arctic tundra | Standard unlabeled N flux measurements | Chapin et al. (1980) |
| Temperate mixed hardwood/pine forest | $^{15}$N tracers | Magill et al. (1997) and Nadelhoffer et al. (1999) |
| Wetland | Standard unlabeled N flux measurements | Francez (1995) |
| Pond | Standard unlabeled N flux measurements | Dodds and Castenholz (1988) |
| Eutrophic lake | $^{15}$N uptake | Takamura and Iwakuma (1991) |
| Oligotrophic lake | $^{15}$N uptake | Dodds et al. (1991) |

For cross-ecosystem comparison, data were synthesized from the experimental studies described in this paper (Table 1) and from studies of lentic and terrestrial ecosystems reported in the literature (Table 2). Aquatic compartments were classified as consumer (grazers, collectors, filter feeders, scrapers, invertebrate predators, and vertebrate predators), aquatic primary producer (epilithon, filamentous green algae, and bryophytes), or aquatic detritus (SPON, FBON, leaves, and wood). Terrestrial compartments were similarly classified as consumer, producer, or detritus for analysis.

C to N data for streams and other ecosystems (cross-system data) were analyzed using two-way ANOVA with habitat (terrestrial or aquatic) and trophic category as factors. Data were tested for normality prior to analysis. Regression analysis was used to compare slopes of the C:N – N-specific uptake relationships for the stream and cross-system data (Proc Mixed, SAS). When regressing $\log_{10}$ of the N-specific uptake data against $\log_{10}$ C:N (because regression gave the best fit), we expect a relationship with a slope between 0 and 1. The reason for this expectation is that N-specific uptake is calculated as N uptake per unit area divided by N content per unit area, and C:N by C content per unit area divided by N content per unit area; the value of the slope of this form of plot yields an expected relationship with a slope between 0 and 1 depending upon the coefficient of variation of each variable (Dr E. Rastetter, Woods Hole Oceanographic Institute, personal communication). We modeled the expected relationship between $\log_{10}$ N-specific uptake and $\log_{10}$ C:N, using the observed means and standard deviations of N uptake, N content and C content to generate a randomly selected set of values for each variable, and the expected slope was ~0.5. For our most conservative tests we compared negative slopes to an expected slope of 0. We also tested for a bivariate distribution of the relationship between C:N and N-specific uptake rates using a non-parametric, 2-dimensional Kolmogorov–Smirnov test (Garvey et al. 1998) and with piecewise linear regression using Statistica 6.1 (Statsoft, Tulsa, Oklahoma).

## Results

### C:N and N deficiency

There was a significant influence of both stream location (site) and trophic category on C:N values of compartments from our experimental stream studies (Fig. 1a, Table 3). Since there was a statistically significant interaction between site and trophic category, direct pairwise comparison of values by site or trophic category was not appropriate. However, the trend at all but one site was for the greatest C:N ratios to occur in detritus, with intermediate ratios in primary producers, and the smallest ratios in consumers. In all cases, mean consumer C:N was less than for producers or detritus.
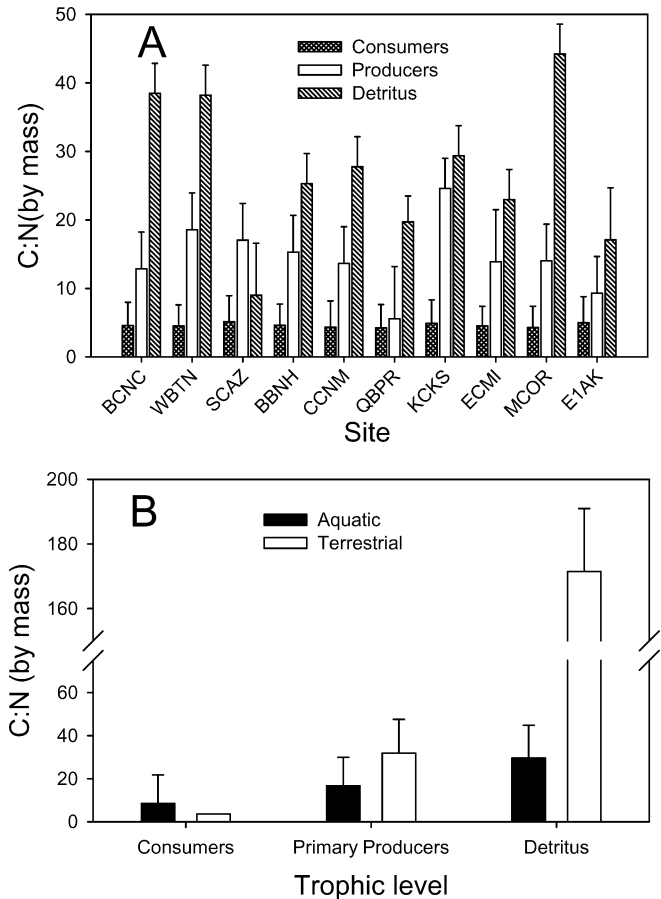


**Fig. 1** C:N by trophic category at each experimental stream site (**a**) and across habitats (**b**). See Table 1 for site abbreviations, and Tables 2 and 3 for ANOVA results. *Error bars* indicate 1 SE

The cross-habitat analysis of C:N indicated an effect of trophic category and habitat (aquatic or terrestrial) as well as a significant interaction between these two (Fig. 1b, Table 3). Again, pairwise comparison of individual means was not appropriate because of the significant interaction, but the trend of C:N being greatest in detritus, intermediate in primary producers, and least in consumers was even more striking in the terrestrial systems than in the streams. The mean value for C:N of detritus in terrestrial systems was >4 times greater than that of aquatic detritus.

There was a significant positive correlation ($P<0.05$) between C:N content in epilithon and the N deficiency

**Fig. 2** Relationships among C:N of various ecosystem compartments and N deficiency indices. Site abbreviations are in Table 1. See Materials and methods for details of N deficiency index. Statistically significant (*P*<0.05) relationships as determined by linear regression occurred in (**a**) and (**c**), even without data from site KCKS, and not in (**b**) and (**c**)
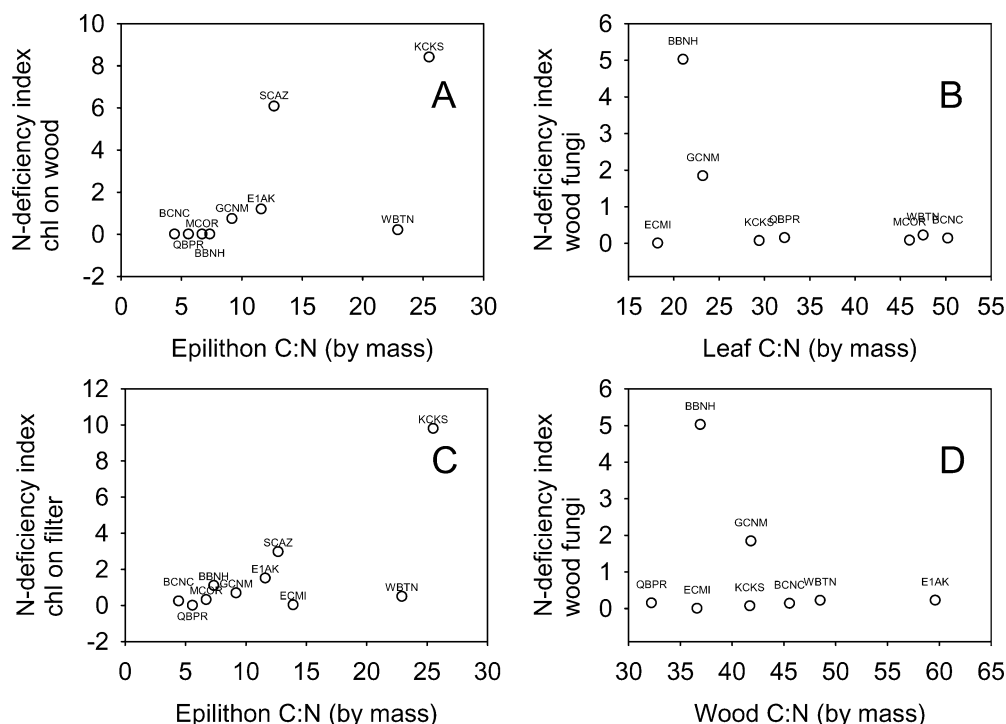


**Table 3** Two-way ANOVA of all stream C:N data by experimental site and trophic level (consumer, producer, detritus), and for combined aquatic and terrestrial data (all sites). See Fig. 1 for data. Log$_{10}$ transformation of C:N values did not substantially alter the results

|  | SS | *df* | MS | *F* | *P* |
|---|---|---|---|---|---|
| Experimental streams |  |  |  |  |  |
| Intercept | 17,270 | 1 | 17,270 | 299.38 | <0.0001 |
| Trophic category | 7,885 | 2 | 3,943 | 68.35 | 0.000000 |
| Site | 1,174 | 9 | 130 | 2.26 | 0.028 |
| Trophic category×site | 1,950 | 18 | 108 | 1.87 | 0.033 |
| Error | 3,923 | 68 | 58 |  |  |
| All sites |  |  |  |  |  |
| Intercept | 53,183 | 1 | 53,183 | 12.69 | <0.0001 |
| Habitat | 17,946 | 1 | 17,946 | 4.28 | 0.041 |
| Trophic category | 102,945 | 2 | 51,472 | 12.28 | 0.000019 |
| Habitat×trophic category | 69,533 | 2 | 334,767 | 8.29643 | 0.0005 |
| Error | 332,958 | 89 | 4,191 |  |  |

index generated from nutrient enrichment bioassays using algal chlorophyll on both wood and glass fiber substrates (Fig. 2). In contrast, C:N of wood or leaves in the streams was not significantly correlated with the N deficiency index assessed by fungal biomass (ergosterol content). Gallina Creek (New Mexico.) and Bear Brook (New Hampshire) had strong N deficiency as estimated by fungal biomass, but relatively small values for C:N in wood and leaves collected from those sites.

C:N and N-specific uptake

A significant negative correlation existed between log$_{10}$ C:N and log$_{10}$ N-specific uptake rates (Fig. 3a). Regression of all our stream sites together yielded the equation:

$$\text{Log}_{10}\,(\text{N} - \text{specific uptake}) = \\ -0.126 - 1.492 \cdot \text{Log}_{10}\,\text{C:N} \quad r^2 = 0.41 \tag{2}$$

N-specific uptake rates are in units of day$^{-1}$ and C:N is the mass ratio of C to N. This relationship is highly statistically significant; the null model using random numbers for N-specific uptake and C:N yields a positive slope ≈0.5.

We used regression analysis to explore if the slope of N-specific uptake versus C:N within individual sites was equal to zero (i.e., if the C:N effect is site specific or the same across streams), and this hypothesis was rejected (*P*<0.001). Slopes were determined from C:N and N-specific turnover for all compartments present at each site (five to nine compartments per site) and we tested which slopes within sites were significantly different from zero. All sites but Quebrada Bisley (Puerto Rico), Upper Ball Creek (North Carolina), and Walker Branch (Tennessee) had negative slopes that were significantly different from zero (*P*<0.02). When the relationship between log$_{10}$ C:N and log$_{10}$ (N-specific uptake) was run without the three sites where slope was not significantly less than zero, the $r^2$ of the regression increased to 0.60.

When data were analyzed across all terrestrial and aquatic habitats, a significant negative relationship occurred between C:N and N-specific uptake rates (data from Fig. 3a, b). This relationship was described by the
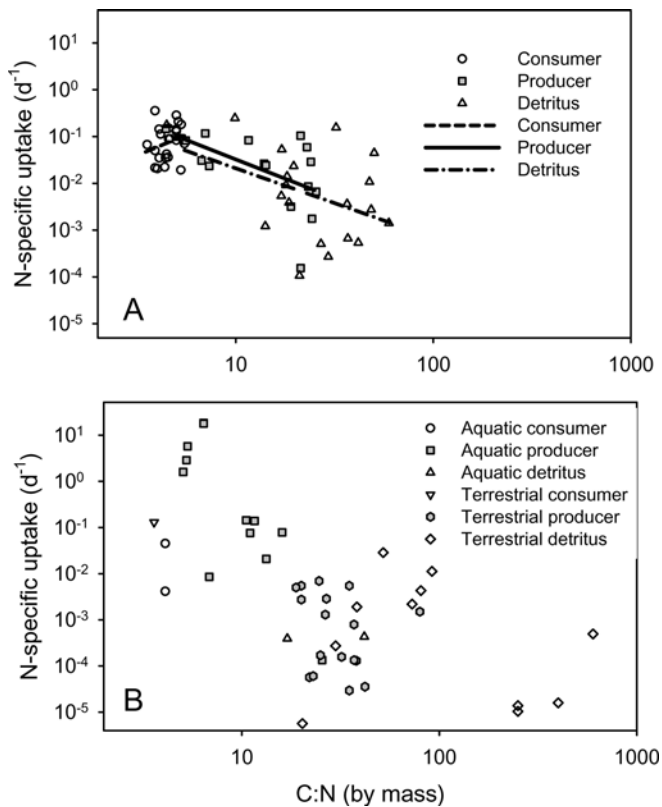
Fig. 3 Relationships between C:N and N-specific uptake rates for study streams (**a**) and additional aquatic and terrestrial habitats (**b**)

equation:

$$\text{Log}_{10}\,(\text{N} - \text{specific uptake}) = \\ -0.88 - 1.12 \cdot \text{Log}_{10}\,\text{C}:\text{N} \quad r^2 = 0.42 \tag{3}$$

In looking at the distribution of data for the above relationship, we hypothesized a threshold C:N above which the rate of N-specific uptake was less. We tested for a breakpoint in the variance using a non-parametric, two-dimensional Kolmogorov–Smirnov test and found evidence for a statistically significant breakpoint for our experimental stream data considered alone or together with literature data from other ecosystem types (Table 4). Piecewise linear regression was used as an independent method to confirm the Kolmogorov–Smirnov test, and this indicated a breakpoint at a C:N of 10.5 for the stream sites. This breakpoint is probably related to consumer compartments with high N-specific uptake that varies little with consumer C:N. When our stream data were analyzed

Table 4 Results of a non-parametric two-dimensional Kolmogorov–Smirnov test for bivariance in the relationship between C:N and N turnover

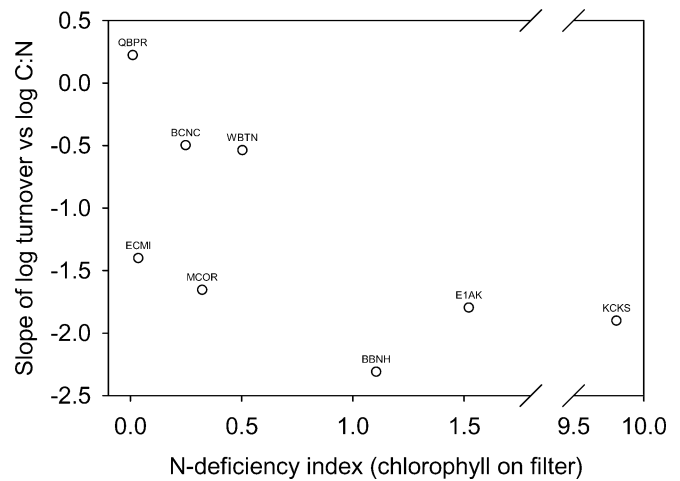| Data set | $P$ | C:N breakpoint | N turnover breakpoint |
|---|---|---|---|
| Streams alone | 0.0002 | 13.9 | 0.026 |
| All data | 0.0002 | 18.7 | 0.014 |



Fig. 4 Relationship between N deficiency as determined with nutrient-diffusion bioassays of chlorophyll on filters and the slope of the relationship between C:N and N-specific uptake rates

separately by trophic category using linear regression (Fig. 3), there was a significant negative relationship between $\log_{10}$ C:N and $\log_{10}$ N-specific uptake among all primary producers ($P<0.03$), a marginally significant relationship among detritus categories ($P<0.07$), and an insignificant positive relationship across consumer categories ($P>0.23$). These regression results indicate a breakpoint that falls roughly along trophic lines (i.e., C:N has little relationship to N-specific uptake in consumers, but does in producer and detrital compartments).

We compared the N deficiency index, derived from bioassays using relative chlorophyll accumulation on glass fiber filters, with the relationship between N-specific uptake and C:N ratio across the eight stream sites for which these data were available. The N deficiency index was negatively correlated with the slope of the relationship between C:N and N-specific uptake rates (Fig. 4). Kings Creek (Kansas) was an obvious (and statistically significant) outlier in this relationship with a very steep slope and a high value for the N deficiency index. However, non-parametric correlation analysis, which is not sensitive to outliers, also indicated a significant ($P<0.05$) negative correlation (Kendall $\tau=-0.67$) between N deficiency and slope. A similar relationship occurred when using the N deficiency data derived from the chlorophyll response to nutrient enrichment measured on wood veneer substrata (data not shown).

## Discussion

Variation in C:N of ecosystem compartments

The C:N of ecosystem compartments varied over 20-fold in the streams, and over 100-fold when considering all the terrestrial and aquatic data. Consumers had small and fairly narrowly constrained values for C:N, regardless of habitat. Primary producers had intermediate C:N and detritus had widely variable, but generally the highest

values. The relative proportion of C-rich, structural biopolymers such as cellulose in the producer and detritus compartments probably dictated these patterns, and this in turn apparently influenced N-specific uptake rates.

Primary producers had slightly greater C:N in terrestrial than in aquatic environments, and the variance in primary producer C:N was also greater in terrestrial environments. The data for primary producers in aquatic habitats (both from our study and literature values) were primarily for phytoplankton and microphytobenthos (periphyton), which are composed of a relatively high proportion of metabolically active cells compared to vascular plants commonly sampled in terrestrial habitats. There were a few data points for bryophytes in streams. The data for terrestrial plants were for foliage and roots of higher plants that contain more structural material (such as wood). Primary producers in aquatic and terrestrial environments have more environmentally induced phenotypic variation in C:N than consumers because of their ability to vary structural C; even single-celled primary producers can alter their C:N widely in response to nutrient limitation, whereas metazoans are more narrowly constrained (Elser et al. 2000). Suspended matter in large lakes and oceans, which tends to consist mainly of algae and detritus derived from algae, can vary in C:N (mass ratio) from 5.5–17 (Hecky et al. 1993).

Much aquatic detritus is derived from terrestrial plant material, but C:N of detritus is on average 4 times higher in terrestrial than aquatic habitats (e.g., Fig. 1). There are three potential explanations for this higher terrestrial C:N:

1. Aquatic detritus may be derived in part from aquatic producers with a lower C:N than terrestrial detritus.
2. In aquatic ecosystems the detritus, whether of aquatic or terrestrial origin, is rapidly colonized by microbial decomposers that can sequester N from the water column to supplement their needs.
3. Organic N could be concentrated by sorption to organic detritus (Aufdenkampe et al. 2001).

In open-canopied, small streams such as Sycamore Creek (Arizona) and Kings Creek (Kansas), aquatic primary producers could provide a substantial proportion of the detrital material in the stream channel and in such systems, explanation 1 could hold, and is supported for Sycamore Creek by the higher C:N of primary producers than of detritus. Most forested small streams have substantial terrestrial litter input, and the C:N of leaves decreases with time after the material falls into streams (Tate and Gurtz 1986), owing to colonization by microorganisms that assimilate N from the water. In these closed-canopy systems, explanations 2 or 3 would be most important.

## C:N and N-specific uptake

The ratio of C to N has been viewed as a central controlling factor of terrestrial N cycling (Currie 1999; Zheng et al. 1999), but such an approach has not been used as commonly in aquatic ecosystems, although microbial ecologists studying marine and lacustrine sediments have recognized its importance (reviewed by Fenchel et al. 1998). Terrestrial environments with abundant production of woody detrital material would be expected to have a larger range of C:N in organic matter fueling heterotrophic food webs (Aber and Melillo 2001), and C:N has proven to be a useful predictor of organic matter decomposition in forests and in forest streams. In contrast, marine and lacustrine ecosystems often have less variable C:N. This is perhaps why previous studies have not been able to demonstrate that C:N consistently explains N cycling rates across diverse aquatic and terrestrial habitats, even though the habitats are linked and act in concert to determine total N output of most ecosystems.

Our approach considers C:N of all ecosystem compartments and allows establishment of relationships across habitats. C:N is related to N cycling rates because it controls the relative assimilation versus release of N in organic matter that is degraded by bacteria and fungi, both in terrestrial soils and aquatic sediments (Fenchel et al. 1998). For consumers at higher trophic levels, the relationship between C:N in food and their relative demands for energy and N potentially exert similar control over feeding rates as well as excretion of N, in the same way as C:P has been shown to influence the elemental balance of consumers in P-limited freshwater lakes (Elser and Urabe 1999).

An important issue in linking N uptake rates to N retention is how well uptake rates characterize whole-compartment turnover (i.e., the average amount of time N is retained in a compartment). At equilibrium, uptake is offset by remineralization. Our approach assumes uptake is roughly equal to remineralization and thus N-specific uptake rates can be used to approximate whole compartment N turnover rates. The case where this assumption is most likely not true is in aquatic detritus; uptake must exceed remineralization in this compartment if N content increases after it enters the stream. However the commonly observed increases in N content of detritus strengthen the arguments based on using N-specific uptake as an indicator of N turnover because in these high C:N compartments, N-specific uptake rates are greater than actual whole-compartment turnover rates (i.e., because these compartments are accumulating N, the N turnover of the whole compartment must be less than N-specific uptake). Thus, extrapolating our results to the ecosystem level is based on conservative estimates of turnover in detrital pools.

The relationship between N-specific uptake rates and C:N may be explained by two factors: (1) detritus and C-rich producer assemblages represent N-deficient organic matter sources, leading to small values for N-specific uptake rates, and/or (2) detrital and high C:N compartments are made up of relatively recalcitrant N that cycles slowly. Given substrates that are rich in C, and if little N is available (e.g., water column concentrations are low), microbial activity will be limited by N availability and N

remineralization rates will be less because microbes will conserve N (Goldman et al. 1987). In this case, N should cycle fairly slowly supporting the former explanation. Furthermore, there will be relatively slow rates of whole-compartment N turnover. In streams with a little N deficiency there was a weaker relationship between N-specific uptake rates and C:N in ecosystem compartments. These changes mostly appeared to be related to shifts in the stoichiometry of the producers and to detritus with more N and greater rates of N-specific uptake in systems with stronger N deficiency. These data support explanation (1). Finally, in N-deficient detrital compartments labile N should be consumed rapidly by microbes, leaving mainly recalcitrant abiotic N pools, thereby supporting the latter explanation (2). The end result is that N-deficient compartments have relatively slow uptake and are made up of a relatively large proportion of recalcitrant N, leading to small values of N-specific uptake.

## The potential links between stoichiometry of ecosystem compartments and whole ecosystem N retention

We speculate that when N is added to ecosystems, C:N of compartments throughout the ecosystem will decrease and eventually the system may become less retentive of N. Saturation of N in ecosystems can occur as a result of atmospheric deposition, anthropogenic eutrophication from point source effluents, and direct application of fertilizers. N yields increase from systems subject to chronic N additions (Loehr 1974; Aber et al. 1998; Fenn et al. 1998) and prediction of the saturation point based on C:N of compartments of a specific ecosystem would be useful in the light of these challenges.

Large-scale disturbance may change C:N, which may partially explain altered amounts of N retention. For example, conversion of grassland to cropland can decrease the C:N in soil (Robles and Burke 1997), and N export from streams draining cropland can be proportionally greater than export from pristine grasslands (Dodds et al. 1996). In such cases, decreased N retention in streams could be due partially to a net decrease in C:N of the nutrient pools left behind (after export), concomitant increases in N-specific uptake rates in biomass and, ultimately, saturation of biotic demand. Removal of tropical forest increased N limitation by decreasing N inputs from terrestrial vegetation and soils (Neill et al. 2001), suggesting that removal of C-rich litter sources does not always decrease N limitation.

Channelization, snag removal, alteration of riparian forest, and scouring, all can eliminate significant portions of woody debris and leaves from river and stream channels. This removal of C-rich material could decrease N retention of the ecosystem on the basis of the C:N versus N-specific uptake rate relationships described here. Exclusion of high C:N leaf litter and subsequent removal of small wood led to increased N transport from an Appalachian stream (Webster et al. 2000). While these results support our hypothesis that the presence of high C:N material leads to greater N retention, the Appalachian results may also have been due to physical effects of increased water transport and a decreased area for microbial colonization. Nonetheless, large particulate organic detritus in these Appalachian streams has an active role in nutrient dynamics in the stream (Tank et al. 2000), and is physically retained in the stream channel for long time periods (Webster et al. 1999). Sewage outfall, being rich in N, could also decrease N retention.

That C:N alone can explain 41% of the variance in N-specific uptake rates in ecosystem compartments across streams from a wide variety of sites suggests that this relationship may provide a powerful way to characterize ecosystem function. Many other factors may influence uptake and retention, such as feedbacks and controls from grazers and higher trophic levels, species-specific N requirements, climate, growing season, and supply and demand of other nutrients. Explaining the additional 59% of the variance in N-specific uptake rates will require study of other factors. Ecosystems that deviate from the relationships described here may thus be particularly deserving of study. The relationship between N deficiency and the slope of the relationship between C:N and N-specific uptake may provide initial avenues for exploration of the relative importance of C and N stoichiometry as related to N cycling rates.

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