Freshwater Biology

Freshwater Biology (2012) **57,** 1113–1125 doi:10.1111/j.1365-2427.2012.02770.x

The fate of assimilated nitrogen in streams: an *in situ* benthic chamber study

JONATHAN M. O'BRIEN*, STEPHEN K. HAMILTON*, LAURA PODZIKOWSKI* AND NATHANIEL OSTROM†

*W.K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI, U.S.A.

†Department of Zoology, Michigan State University, Hickory Corners, MI, U.S.A.

SUMMARY

1. Nitrogen (N) processing in streams has been investigated using whole-stream ^{15}N addition experiments that, in general, have found that a large proportion of added nitrate removed from the water column appears to be assimilated by the stream benthos. The long-term fate of this retained N is unknown, and of particular interest is the possibility that it becomes denitrified through coupled mineralisation–nitrification–denitrification processes (indirect denitrification).
2. We used *in situ* chambers to produce highly ^{15}N-enriched benthic biofilms and removed the chambers to allow biofilms to interact with ambient stream conditions. Nitrogen assimilation and direct denitrification were estimated from the first chamber deployment. Chambers were periodically reinstalled over 4 weeks to measure tracer ^{15}N in ammonium (NH_4^+), nitrate (NO_3^-) and dinitrogen (N_2), from which we estimated subsequent rates of biotic N transformations, including N mineralisation (ammonification), nitrification and indirect denitrification. We also estimated rates of depuration of ^{15}N tracer from benthic biomass compartments.
3. Nitrate uptake was roughly equivalent in the sand and cobble habitats that dominated the stream. Direct denitrification (denitrification of NO_3^- from the water column) was an order of magnitude higher in cobble habitats than in sand habitats, accounting for *c.* 26 and 2% of total nitrate uptake in cobble and sand, respectively.
4. Mean residence times of actively cycling organic N in stream benthos (algae and microbes) were 16 days in cobble habitats and 9 days in sand habitats. The difference between habitat types was driven by the influence of N residence time in epilithic biofilms (18 days) on cobbles.
5. Release of enriched $^{15}NO_3^-$ was the primary flux of remineralised N, while release of enriched $^{15}NH_4^+$ was an order of magnitude less. We detected slight ^{15}N enrichment in dissolved nitrogen gas (N_2) in post-enrichment sampling, indicating that indirect denitrification was taking place. However, indirect denitrification accounted for <0.1% of the assimilated N.
6. These experiments agree with results of whole-stream ^{15}N additions, in that most added N was assimilated rather than directly denitrified. Assimilation was primarily a short-term N retention mechanism in this stream, and indirect denitrification of assimilated N accounted for only a minor proportion of the observed ^{15}N loss over time.
7. Remaining possible fates include export of N as particulate organic matter, which may lead to additional storage of assimilated N in downstream habitats, and consumption by grazers.

Keywords: ammonium, denitrification, nitrate, stable isotopes, uptake

Introduction

Human land use and elevated atmospheric deposition of nitrogen (N) can lead to increased concentrations of anthropogenic N in streams, causing eutrophication in recipient freshwater and coastal ecosystems (Rabalais, 2002; Lewis & Wurtsbaugh, 2008). There has been considerable effort in the past two decades to establish

Correspondence: Jonathan M. O'Brien, School of Biological Sciences, University of Canterbury, Christchurch, Ilam 8041, New Zealand. E-mail: jon.obrien@canterbury.ac.nz

the role that in-stream processes play in retaining anthropogenic N and preventing it from reaching downstream waterbodies. Much of this work has focused on uptake of N from the water column by benthic stream biota (Mulholland & Webster, 2010). An important recent finding is that most of the nitrate (NO_3^-) removed from stream water is assimilated by stream organisms and only a fraction is consumed in direct denitrification (i.e. denitrification of NO_3^- that comes directly from the water column) (Mulholland *et al.*, 2008).

Short-term N uptake experiments provide an incomplete picture of the fate of N that is retained on the stream bottom. Rates of assimilation are such that organic N cannot simply accumulate *in situ* or the equivalent organic matter would soon fill the entire stream channel (e.g. Arango *et al.*, 2008), and therefore, the organic N must be either subsequently transported downstream, consumed by grazers or mineralised and denitrified in place. Far less attention has been paid to the ultimate fate of assimilated N, even though this is a critical issue for our understanding of nutrient retention in streams.

Following assimilation of inorganic N from stream water, N can be cycled in place within microbial communities or biofilms for some length of time. Assimilated N could then be remineralised and released to the water column as ammonium (NH_4^+) or, upon nitrification, as NO_3^-. Prior to being released to the water column, remineralised N may be denitrified through the process of coupled mineralisation–nitrification–denitrification (often referred to as indirect denitrification). Alternatively, assimilated N may be transported some distance downstream as detrital particulate organic matter, dissolved organic N (DON) or living organisms, before eventually becoming remineralised and perhaps denitrified.

Retention time of benthic N is regulated, in part, by the strength of internal nutrient recycling in benthic biofilms. In estuarine sediments, for example, considerable internal recycling of N occurs between benthic algae and sediment bacteria and also within bacterial communities (Cook *et al.*, 2007; Veuger *et al.*, 2007). Recycling of nutrients within a stream biofilm can satisfy a large portion of the nutrient demand in low-nutrient streams (Mulholland *et al.*, 1991) and may be driven by a coupling of autotrophic and heterotrophic activity. Biofilm recycling is greatly influenced by factors such as nutrient availability, light conditions and grazer activity (Mulholland *et al.*, 1991; Steinman, Mulholland & Beauchamp, 1995).

As stream biofilms grow and develop, they incorporate N from the water column while also releasing remineralised N back to the water column as NH_4^+ or NO_3^-. Although both assimilation and remineralisation occur constantly, the balance between net assimilation and net remineralisation depends on biofilm density (accumulated biomass) and growth dynamics (Grimm, 1987; Teissier *et al.*, 2007). Likewise, rates of N transformation in epilithic biofilms, including nitrification and denitrification, are related also to biofilm density (Teissier & Torre, 2002; Teissier *et al.*, 2007). The result is an ontogeny of net N assimilation followed by net remineralisation and transformation as biofilms mature and senesce, which together determine the timing and nature of N retention in a stream or river. In temperate climates, this ontogeny is driven by seasonal changes in temperature, day length, shading by deciduous canopies and flow regimes. In addition to temporal shifts, N transformation may vary spatially within the stream channel owing to heterogeneity in channel habitat.

Sloughing of biofilms by high water flows and grazing activities often result in assimilated N being transported downstream as particulate organic matter. Depending on stream discharge and velocity, particulate matter (and associated N) can be transported to significant distances and is subject to multiple resuspensions before eventually being remineralised (Newbold *et al.*, 2005; Hunken, 2006). Floods and spates scour benthic organic matter from the streambed and increase particle transport, in response to higher discharges and sheer velocities, leading to even further transport downstream. Particulate matter often settles in depositional environments within the channel (e.g. log jams, deep pools), or in hyporheic zones, or in downstream lentic environments (e.g. lakes, beaver ponds or other impoundments) (Hall *et al.*, 2009; O'Brien *et al.*, 2012). Organic matter degradation and nutrient cycling in depositional environments may differ from processes elsewhere in the stream, and anaerobic microbial degradation (including denitrification) may be more prevalent in these habitats.

Indirect denitrification, or coupled mineralisation–nitrification–denitrification, is an important pathway in some aquatic environments (Seitzinger *et al.*, 2006). For instance, indirect denitrification is roughly equal to direct denitrification (denitrification of NO_3^- coming directly from the water column) in unfertilised estuarine sediments (Koop-Jakobsen & Giblin, 2010). The importance of indirect denitrification is less well understood in streams, and it has only been examined in a few studies. Direct denitrification was more important than indirect denitrification in intact cores from an agriculturally impacted stream with high NO_3^- concentrations (Sugar Creek) in Illinois (Smith *et al.*, 2006, 2009). However, indirect denitrification accounted for as much as 66% of total denitrification in the less-impacted Neuse River, North Carolina (Whalen

et al., 2008). Indirect denitrification represents a pathway through which assimilated N is permanently removed from the ecosystem; therefore, the prevalence of indirect denitrification determines whether assimilated N represents a temporary or permanent sink for NO_3^- from stream water.

Thus, while it has been well established that assimilation of inorganic N in the benthos is a critical process of N retention time in streams, the relative importance of particular fates of N following assimilation remains unclear. In this study, we used novel *in situ* chamber experiments to directly examine the fate of assimilated N in a stream, focusing on benthic residence time and forms of inorganic N release. We allowed uptake of ^{15}N-enriched NO_3^- to enrich patches of the streambed biofilm and then monitored export of ^{15}N from these patches over the subsequent month. These ^{15}N enrichment experiments were distributed between cobble and sand reaches in the stream to account for differences in these prominent habitat types. We predicted that the assimilated biofilm N would be predominantly remineralised and released to the water column as NH_4^+ and NO_3^-. We also predicted that a significant proportion of assimilated N would be indirectly denitrified within the benthic environment. The goals of this study were to compare rates of biotic N transformations, to assess the relative importance of direct and indirect denitrification and to determine the fate of assimilated N in terms of residence time and form of export.

Methods

Study site

This study was conducted in Augusta Creek at the W.K. Kellogg Experimental Forest (42°36′24″N, 85°35′44″W) in SW Michigan, USA. The stream is 3rd order, has a low gradient and drains a 70-km^2 catchment composed of glacial till with mixed land use (mainly row crops, pasture, forest and wetlands). The stream gains ground water along most of its length, and its water is alkaline. Nitrate concentrations increase along the length of the stream from below the detection limit (<0.001 mmol L^{-1}) in the headwaters to around 0.11 mmol L^{-1} at the study site. Sediment composition in the stream alternates between sand and cobble with variable amounts of detrital organic matter derived mainly from litterfall. In the Kellogg Experimental Forest, the stream is *c.* 5–6 m wide and is bordered by deciduous hardwood forest, but much of the stream channel receives direct sunlight several hours of the day. Our study area is among the sites used to develop the river continuum concept (Vannote *et al.*, 1980), and descriptions of stream chemistry (Manny & Wetzel, 1973; Wetzel & Manny, 1977) and biology (Minshall *et al.*, 1983) have been previously published.

Experimental design

We used *in situ* benthic chambers to label stream biofilms with ^{15}N-enriched NO_3^- over a 24-h period in 10 patches (hereafter called flux patches) along Augusta Creek. The chambers were then removed, and the flux patches were subject to ambient stream conditions. Periodically, we replaced chambers over the patches for short periods of time to monitor ^{15}N release as NO_3^-, NH_4^+ and nitrogen gas (N_2) from biofilms to the water column. We enriched seven additional patches (hereafter called biomass patches) to monitor the decline in ^{15}N remaining in benthic biomass over time (i.e. the ^{15}N depuration). Patches were distributed evenly across the prevalent sand and cobble habitat types.

We constructed open-bottom benthic chambers using clear acrylic (Acrylite®) plastic. Chambers were trapezoidal in cross section with dimensions 0.6 m × 0.3 m × 0.1 m (l × w × h) and had a total volume (including pump and tubing) of 15 L. Water in the chambers was circulated using a submersible AC, magnetic-drive pump (Pondmaster Magdrive; Danner Manufacturing, Islandia, NY, U.S.A.) connected to the front and back of the chamber with vinyl chloride tubing. We fitted chambers with a sampling port to collect water samples and a dissolved oxygen (O_2) sensor (YSI DO200; Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). Chambers were attached to 0.6 m × 0.3 m aluminium frames that had been inserted 10 cm into sediments, flush with the streambed, 7–14 days prior to ^{15}N tracer enrichment. Frames had a 1-cm lip that allowed the chambers to seal with the frame via a foam rubber gasket, and chambers were secured to the frames using elastic cords.

When using open-bottom chambers, a particular concern is ensuring a proper seal between the chamber and substratum. A perfect seal in a flowing environment is difficult, despite best efforts, and some degree of leakage between the chamber and outside stream water is to be expected. To account for any leakage in the chambers, we used low concentrations of fluorescent dye (Rhodamine WT, RWT; Ben Meadows Co, Janesville, WI, U.S.A.) as a visual indicator of leakage in the field, allowing us to make on-site adjustments when necessary. Sodium bromide (Br^-) is a more conservative tracer than RWT and was used as a quantitative measure of exchange during all chamber samplings.

Field and laboratory methods

On the day of isotope enrichment (day 0) at each flux patch, we secured a chamber to the frame and conducted an initial low-level $^{15}NO_3^-$ addition (1.07 atom % ^{15}N, *c.* 2000‰) by injecting 30 mL of tracer solution (0.59 mmol L^{-1} $^{15}NO_3^-$, 15.6 mmol L^{-1} Br^- and RWT) into the chamber to measure uptake and denitrification at ambient conditions. The increase in NO_3^- concentration from this addition was 1.1 μmol L^{-1} or roughly 1% of ambient NO_3^- concentration. We collected samples to monitor NO_3^- and NH_4^+ concentrations every 30 min for 2.5 h and collected pre- and post-^{15}N addition samples for the determination of ^{15}N abundance in NO_3^- and N_2.

After the initial low-level ^{15}N addition, we conducted a high-level $^{15}NO_3^-$ enrichment experiment to label benthic compartments. We injected 30 mL of a more concentrated tracer solution (321 mmol L^{-1} $^{15}NO_3^-$ [60 atom%], 15.6 mmol L^{-1} Br^-, 2.0 mmol L^{-1} PO_4^{3-} and RWT) to the chamber. The isotope enrichment experiments had a target NO_3^- concentration increase of 0.64 mmol L^{-1} (final concentration *c.* 0.75 mmol N L^{-1}) and a target enrichment of *c.* 50 atom% ^{15}N. We added PO_4^{3-} to the isotope enrichment solution to avoid phosphorus limitation in the chamber during the enrichment period. We collected samples for NO_3^- and NH_4^+ concentrations every 30 min for the first 3 h (and at irregular intervals thereafter) with chambers left in place for 22 h. At the end of this period, we removed the chambers and allowed the patch to be exposed to ambient stream conditions.

On days 2, 4, 8, 16 and 24 after isotope addition, we reattached benthic chambers to the frames for 3-h periods during midday. Chambers were open to ambient light for the first 2 h and then covered with a dark plastic sheet for the remaining 1 h. This had the dual effect of enabling us to determine O_2 metabolism in the light and dark and also maintaining O_2 concentrations at approximately in-stream levels. Chambers left uncovered for more than 2 h in direct sun began to exceed normal in-stream dissolved O_2 levels (<110% saturation), especially in cobble habitats. We collected samples to determine ^{15}N abundances of NO_3^-, NH_4^+ and N_2 at the start and end of each 3-h period. Samples for $^{15}NO_3^-$ and $^{15}NH_4^+$ were filtered (using GF/F glass-fibre filters) in the field, chilled immediately and stored frozen prior to analysis. We processed dissolved N_2 samples in the field according to the method of Hamilton & Ostrom (2007), and samples were analysed for ^{15}N : ^{14}N using a Sercon dual-inlet isotope-ratio mass spectrometer.

In parallel with the enrichment of the flux patches described above, the natural abundance (background) and enriched ^{15}N content of fine benthic organic matter (FBOM) and epilithon were measured at 7 of the biomass patches. We enriched each biomass patch using the same ^{15}N enrichment protocol and at the same time as was done in the flux patches. We collected samples for FBOM from each biomass patch using a 10-cm pipe corer at the same time as chamber sampling. The pipe was inserted into the stream substratum at a randomly chosen location in each patch, and surface FBOM samples were collected by gently stirring the water in the core and collecting suspended FBOM. We then flushed the water from the core and collected deeper FBOM by vigorously stirring the water and sediment to a depth of 5 cm, again collecting suspended material. We collected epilithon samples by collecting 3 rocks from randomly chosen locations within each patch and scraping the biofilm from the rock with a blunt knife. Subsamples of benthic material were filtered, dried and ashed at 500 °C for 3 h to determine ash-free dry mass (AFDM). Separate subsamples were freeze-dried, ground with mortar and pestle and analysed for ^{15}N abundance as described above.

We determined ^{15}N content of NO_3^- using a modified version of the NH_3 diffusion method (Sigman *et al.*, 1997). Water samples containing *c.* 75 μgNO_3^--N were concentrated and NH_4^+ volatilised (removed) by boiling with 3.0 g MgO and 5.0 g NaCl. Samples were then transferred to 250-mL media bottles to which an additional 0.5 g MgO, 0.5 g Devarda's alloy and a Teflon filter packet were added. We constructed Teflon filter packets by sealing a 10-mm Whatman GF/D glass-fibre filter, acidified with 25 μL of 2.0 M $KHSO_4$, within a packet made of Teflon plumber's tape. Sample NO_3^- was reduced to NH_4^+ by reacting at 60 °C for 48 h with Devarda's alloy. Sealed bottles were then placed on a shaker for 7 days to allow for ammonia to diffuse into the headspace and onto the acidified filter. We then removed the filter from the media bottle, dried it and analysed for ^{15}N abundance using an isotope-ratio mass spectrometer. We used a similar method but without Devarda's alloy to determine ^{15}N content of NH_4^+. Filtered water samples were transferred to 1-L Nalgene polypropylene bottles to which 3.0 g MgO, 50 g NaCl and a Teflon filter packet were added. Bottles were sealed and placed on a heated shaker for 14 days at 40 °C to allow for diffusion of NH_3 onto the acidified filters.

We analysed water samples for NH_4^+ concentration using the phenol hypochlorite method (Aminot, Kirkwood & Kerouel, 1997). We measured NO_3^- and Br^- concentrations in water samples using membrane-suppression ion chromatography with conductivity detection (APHA, 2006, method 4110).

Calculation of N transformations

We calculated rates of N transformation in each chamber run from changes in concentration and isotopic enrichment of NH_4^+, NO_3^- and N_2 and an isotope mixing model (Glibert *et al.*, 1982). We calculated rates of NO_3^- uptake and direct denitrification during the initial tracer $^{15}NO_3^-$ addition on day 0. We calculated ammonium uptake and mineralisation from changes in NH_4^+ concentration. Rates of nitrification and denitrification were then calculated from the appearance of tracer $^{15}NO_3^-$ and $^{15}N_2$ in the chamber using an isotope mixing model.

We calculated NO_3^- uptake rates as the ln-transformed decline in the concentration of $^{15}NO_3^-$ as a function of time (k_n). We calculated direct denitrification from the increase in the abundance of tracer ^{15}N in dissolved N_2 with time in the chamber during the initial low-level $^{15}NO_3^-$ enrichment.

We determined rates of NH_4^+ uptake and mineralisation by analysing changes in NH_4^+ concentration in the chambers using an updated version of a model used by O'Brien & Dodds (2008), modified to correct for chamber leakage. For the purpose of this method, we define mineralisation specifically as the production of N as NH_4^+. Briefly, the model uses variations in NH_4^+ concentration (C) to estimate NH_4^+ uptake and mineralisation (M, $\mu g\ L^{-1}\ h^{-1}$) in a closed chamber, assuming 1st-order NH_4^+ uptake kinetics (k_u*C) and constant mineralisation rates (eqn 1). C_o is the steady-state concentration where uptake equals M.

$$dC/dt = -k_u * C + M + k_L * (C_o - C) \quad (1)$$

The rate of chamber leakage (k_L, h^{-1}) was determined by changes in Br^- concentration during each run. In this study, we did not specifically manipulate NH_4^+ and had to rely on the ambient starting NH_4^+ concentrations from runs on different dates to inform the model. In using this approach, we assumed that uptake coefficients and mineralisation rates were constant at each patch over the length of the study.

We used an isotope mixing model to calculate nitrification and indirect denitrification rates from the observed ^{15}N enrichments in water-column NH_4^+, NO_3^- and N_2 above background at the end of the 3-h chamber run. We modelled the rate of $^{15}NH_4^+$ enrichment by simply inserting the appropriate isotopic abundances into equation 1 (i.e. yielding eqn 4) and solving for the ^{15}N atomic fraction (A') of the 'active' N pool (i.e. the pool of organic N that is actively being mineralised). Once we determined A', we used similar models of enrichment in $^{15}NO_3^-$ and $^{15}N_2$ abundances to estimate nitrification (N) and indirect denitrification (D_n), respectively (eqns 3 & 4).

$$d^{15}NH_4^+dt = A_tC_t - C_{t-1}A_{t-1} = A'M - (k_uA_tC_t) + k_L(A_oC_o - A_tC_t) \quad (2)$$

$$d^{15}NO_3^-/dt = A'N - (k_nA_tC_t) + k_L(A_oC_o - A_tC_t) \quad (3)$$

$$d^{15}N_2/dt = A'D_n + k_L(A_oC_o - A_tC_t) \quad (4)$$

where A_t is the atomic fraction [i.e. $^{15}N/(^{14}N + ^{15}N)$] in the sample at time t and A_o is the background atomic fraction.

Turnover rates of the active N in benthic compartments (i.e. benthic N residence times) were calculated by linear regression of ln-transformed tracer ^{15}N content over time, and residence times of N in benthic compartments were calculated as the inverse of k, the slope of the regression line.

We calculated benthic metabolism during each chamber run. Community respiration (CR) was estimated from the linear decline in O_2 in covered (dark) chambers. Gross primary production (GPP) was calculated from the linear net increase in O_2 concentration in the light (NPP) corrected for CR (GPP = NPP + CR) (Bott *et al.*, 1985). Heterotrophic respiration was calculated (HR = CR – α*GPP) with an α value of 0.2, assuming a partly grazed biofilm (McIntire *et al.*, 1996; Young & Huryn, 1999). All metabolism measurements were converted into rates per unit area based on the chamber volume (15 L) and benthic surface area (0.18 m^2).

To complete a mass balance of added ^{15}N isotope in the plots, we calculated the total assimilation of tracer ^{15}N during the enrichment. Total assimilation was defined as the disappearance of $^{15}NO_3^-$ from water within the chamber, corrected for chamber leakage and denitrification, over the 22-h enrichment period. By elevating the NO_3^- concentration more than 10% during the ^{15}N enrichment period, we elevated both the rates of NO_3^- uptake (U_t) and potentially the rate of direct denitrification of NO_3^- above background levels. We did not measure denitrification during the high-level ^{15}N enrichment but assume that the stimulation of rates of denitrification and assimilation by the biofilm were proportional (Mulholland *et al.*, 2008). We calculated cumulative production of ^{15}N as NH_4^+, NO_3^- and N_2 from the patches by integrating the rates of ^{15}N remineralisation over the 24 days of the study.

Results

Although high discharge events occurred in Augusta Creek prior to and immediately following the study

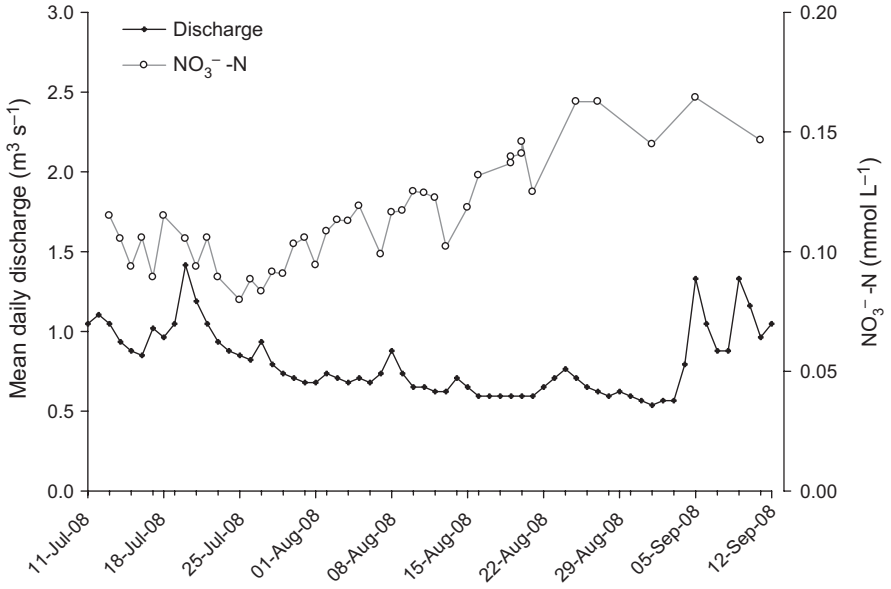

Fig. 1 Discharge and NO_3^- concentrations at Augusta Creek during the study period (13 July to 11 September 2008). Discharge was measured by the United States Geological Survey.

period, there were no major discharge events during the study. A discharge of about 0.8 m^3 s^{-1} was maintained during the study period, with flow gradually decreasing into late August and September (Fig. 1). Background concentrations of NO_3^- rose from 80 to 178 μmol L^{-1} as discharge decreased. Discharge and NO_3^- concentrations are closely, but inversely, coupled in Augusta Creek because of the high NO_3^- concentration in local ground water, which represents a greater fraction of discharge at low flow. Water-column concentrations of NH_4^+ (<0.75 μmol L^{-1}) and soluble reactive phosphorus (<5 μg L^{-1}) both remained relatively low throughout the study period.

Sand and cobble habitats in Augusta Creek had comparable standing stocks of benthic organic matter (measured as AFDM) and benthic N near the surface (Table 1). Midday primary production in the cobble habitats showed nearly 4-fold higher rates than sand habitats ($P < 0.01$, *t*-test) (Table 1). CR rates in cobble habitats were double the rates of sand habitats ($P < 0.01$, *t*-test). The difference in CR between cobble and sand habitats was primarily due to higher respiration by autotrophs (i.e. epilithic algae) in the cobbles, and heterotrophic respiration was not significantly different between the two habitats ($P > 0.05$, *t*-test).

In the sand habitats, the primary biomass compartment was deep FBOM, which accounted for 85% of benthic ^{15}N tracer initially found on the stream bottom (Table 2). In the cobble habitats, epilithon was the primary biomass compartment accounting for 82% of benthic ^{15}N tracer, while deep FBOM accounted for a majority of the remaining tracer assimilation. Observed ^{15}N tracer stocks are only a small fraction (<10% of total assimilated ^{15}N) of what we expected to find, based on $^{15}NO_3^-$ uptake during

Table 1 Ecosystem metabolism and mean standing stocks (±SE) of ash-free dry mass (AFDM), total benthic N and abundance of ^{15}N in benthic compartments of Augusta Creek

Habitat	Benthic compartment	AFDM g m^{-2}	Total benthic N g m^{-2}	GPP mmol C m^2 h^{-1}	CR mmol C m^2 h^{-1}	HR mmol C m^2 h^{-1}
Sand	Surface FBOM	97 (58)	2.8 (1.9)	2.2 (1.2)	1.8 (0.4)	1.1 (0.4)
	Deep FBOM	734 (342)	12.7 (8.9)			
Cobble	Surface FBOM	74 (108)	1.1 (1.6)	8.2 (1.9)	3.9 (0.5)	1.4 (0.3)
	Deep FBOM	117 (108)	3.3 (3.5)			
	Epilithon	140 (72)	5.1 (2.6)			

FBOM, fine benthic organic matter.
Metabolism measurements include gross primary production (GPP), community respiration (CR) and heterotrophic respiration (HR).

Table 2 Abundance of ^{15}N (±SE) and mean turnover times (±95% C.I.) in benthic compartments of Augusta Creek

Habitat	Benthic compartment	Compartment ^{15}N mmol m^{-2}	Turnover time days	Total benthic ^{15}N mmol m^{-2}
Sand	Surface FBOM	0.03 (0.02)	10 (6–31)	0.22 (0.13)
	Deep FBOM	0.19 (0.13)	9 (6–17)	
Cobble	Surface FBOM	0.02 (0.03)	12 (4–>100)	0.82 (0.37)
	Deep FBOM	0.13 (0.13)	11 (8–21)	
	Epilithon	0.67 (0.34)	18 (10–78)	

FBOM, fine benthic organic matter.

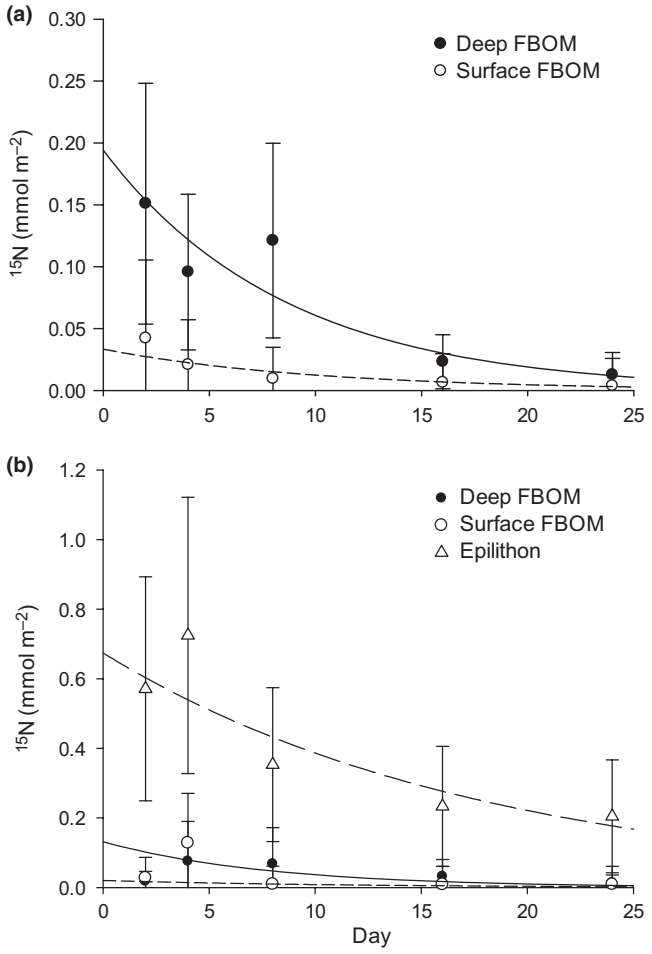

Fig. 2 Atom percentage excess of ^{15}N abundances (i.e. abundances corrected for background) in benthic biomass compartments over time in cobble (a) and sand (b) habitats in Augusta Creek.

the enrichment period. However, these benthic tracer stock data are poorly constrained as seen in the large standard errors surrounding the estimates in Table 2.

Benthic ^{15}N tracer stocks declined exponentially over the 24 days following enrichment (Fig. 2). Mean residence times of benthic N, the average amount of time an atom of ^{15}N tracer spends in the active benthic compartment, were similar in deep FBOM (9 days) and surface FBOM (10 days) within the sand habitats (Table 2). Residence times in FBOM in the cobble habitats were similar to those in sand: 11 and 12 days for deep and surface FBOM, respectively. Residence time in epilithon was longer (18 days) than the FBOM, possibly due to greater recycling between algae and bacteria. Mass-weighted residence times for benthic ^{15}N tracer were 9 days for the sand habitats and 16 days for cobble habitats.

Rates of N transformations were surprisingly similar between sand and cobble habitats (Table 3). NO_3^- uptake rates accounted for the largest proportion of N transformation with an average of 350 ± 46 μmol m^{-2} h^{-1} (mean ± SE) in sand habitats and 400 ± 33 μmol m^{-2} h^{-1} in cobble habitats and did not significantly differ. Nitrification rates also were similar between habitats and were much lower than NO_3^- uptake, leading to net declines in NO_3^- concentration over time in the chambers. Ammonium turnover rates (NH_4^+ uptake and mineralisation) were much smaller than rates of NO_3^- uptake and nitrification. Denitrification rates were 12 ± 5.3 μmol m^{-2} h^{-1} in sand and 83 ± 37 μmol m^{-2} h^{-1} in cobble, resulting in a higher proportion of NO_3^- uptake ascribed to direct denitrification in cobble than in sand habitats.

Based only on the N transformation rates in Table 3, the combined inorganic N uptake (NO_3^- and NH_4^+), corrected for denitrification, exceeded the combined rates of inorganic N regeneration (mineralisation and nitrification). This suggests either that N was accruing in the benthic biofilm pool or that N was being exported in another form. Benthic N pools did not greatly increase over the course of the study, suggesting that N was exported, probably as dissolved and/or particulate organic N, or consumed by grazers. By difference, this flux appears to be on the order of 220 ± 60 μmol m^{-2} h^{-1}, which accounts

Table 3 Rates of N transformation (±SE) in sand and cobble habitats of Augusta Creek

Habitat	NH_4^+ uptake μmol m^{-2} h^{-1}	NH_4^+ mineralisation μmol m^{-2} h^{-1}	Nitrification μmol m^{-2} h^{-1}	NO_3^- uptake μmol m^{-2} h^{-1}	Denitrification μmol m^{-2} h^{-1}
Sand	20 (5.5)	4.6 (0.7)	141 (45)	350 (46)	12 (5.3)
Cobble	13 (4.2)	4.3 (1.4)	100 (33)	400 (33)	83 (37)

for more than 50% of total N assimilation for both habitat types.

Remineralisation fluxes of tracer ^{15}N from benthic biofilms as $^{15}NH_4^+$ and $^{15}NO_3^-$ were relatively steady over the 24 days following enrichment and did not significantly differ between sand and cobble habitats. The ^{15}N atom percentage excess of remineralised N followed a similar temporal pattern to the ^{15}N benthic stock but was considerably more enriched in ^{15}N. This is because it represents remineralisation of the highly ^{15}N-enriched 'active N' pool, rather than the total benthic stock, which incorporates both 'active N' and 'dead N' pools. Remineralisation fluxes of $^{15}NH_4^+$ ranged 0.04–3.2 μmol m^{-2} h^{-1} from the sand habitat and 0.03–2.8 μmol m^{-2} h^{-1} from the cobble habitat (Fig. 3a). Remineralisation fluxes of $^{15}NO_3^-$ ranged 0.4–1.9 μmol m^{-2} h^{-1} from the sand habitat and 0.5–1.5 μmol m^{-2} h^{-1} from the cobble habitat (Fig. 3b). We were able to detect significant increases in $^{15}N_2$ abundance above background only on days 2 and 4 following the period of ^{15}N enrichment (Fig. 3c).

We estimated that $^{15}NO_3^-$ assimilation was 12.2 mmol m^{-2} in sand and 10.4 mmol m^{-2} in cobble. Over the 24-day period, we observed approximately constant rates of ^{15}N export owing to remineralisation and nitrification between sand and cobble habitats. Cumulative remineralisation of $^{15}NH_4^+$ was 0.037 mmol m^{-2} (±0.007 SE) from sand and 0.035 mmol m^{-2} (±0.007 SE) from cobbles. We measured a similar magnitude of cumulative $^{15}NO_3^-$ release to the water over the same period, with 0.65 mmol m^{-2} (±0.04 SE) from sand and 0.57 mmol m^{-2} (±0.04 SE) from cobble. Cumulative release of $^{15}N_2$ accounted for only 0.01 mmol m^{-2} at both habitats. In total, we observed a cumulative inorganic ^{15}N release (NH_4^+ + NO_3^- + N_2) of 0.70 mmol m^{-2} (±0.09 SE) for sand and 0.63 mmol m^{-2} (±0.10 SE) for cobbles, which only accounted for a small fraction of the assimilated $^{15}NO_3^-$ (Fig. 4). Based on the rates of benthic N turnover, a majority of the enriched ^{15}N isotope was lost from the plots by the end of the 24 days (93% for sand and 80% for cobble), but the observed flux of tracer ^{15}N leaving the plots only accounts for a small proportion of the total ^{15}N that was evidently taken up and then later lost from the benthos.

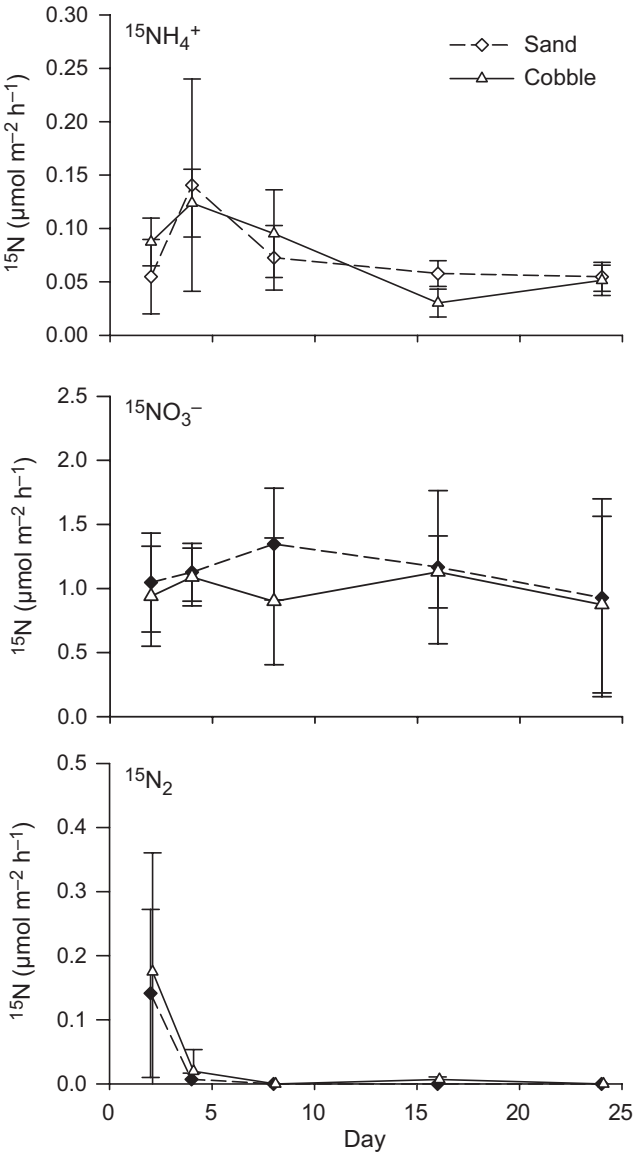

Fig. 3 Remineralisation fluxes of tracer ^{15}N from benthic biofilms as NH_4^+, NO_3^- and N_2 in sand and cobble habitats over the 24 days following enrichment. Mean release rates did not differ significantly between the two habitats, although there was considerable variation in release rates within habitats (i.e. in replicate plots) and over time.

Discussion

Uptake of N from the water column has long been viewed as a key indicator of N retention in streams. The fraction of N uptake that is directly denitrified is permanently removed, but the fraction that is assimilated may represent only a temporary sink. Therefore, as commonly stated, 'N retention in streams' can include direct denitrification, assimilated N that is temporarily detained (i.e. eventually remineralised to become available again), assimilated N that is converted to organic N and exported in a less available form (e.g. as particulate organic N or DON or grazer biomass) and/or assimilated N that is later removed entirely via indirect denitrification. In Augusta Creek, assimilated ^{15}N tracer was retained within the patches where enrichment was conducted for an average of 2–3 weeks before disappearing. Remineralisation and subsequent release of inorganic N was a significant fate of assimilated N, whereas conversion to N_2 gas via indirect

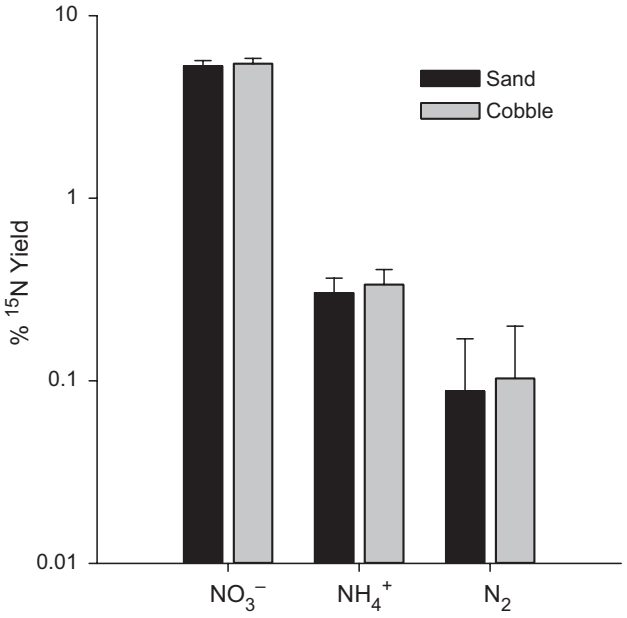

Fig. 4 Cumulative release of ^{15}N tracer from the plots over the 24 day study as a proportion of the initial tracer mass (i.e. % ^{15}N yield) shows that DIN release accounted for a small portion of total ^{15}N tracer uptake, with NO_3^- as the primary product of benthic N remineralisation and N_2 as a minor product.

denitrification accounted for very minor proportion of assimilated N (*c.* 1% of the cumulative remineralisation). A mass balance of the tracer ^{15}N suggests that remineralisation at the site of assimilation in general can account for up to 10% of the tracer $^{15}NO_3^-$ that was initially assimilated by the stream biofilms (Fig. 5). A comparison of N transformation rates similarly suggests an imbalance between uptake and remineralisation. We hypothesise that the remainder of the tracer ^{15}N was lost via organic forms (perhaps released as dissolved and particulate organic nitrogen or consumed by grazers) that we were unable to quantify during this study.

We found that ^{15}N tracer rapidly cycled through the benthic compartments with benthic N retention times between 8 and 18 days. The results from Augusta Creek are in line with retention times reported in previous studies. Similar N retention times were found in nearby Eagle Creek (MI, U.S.A.) for epilithon (14 days) and FBOM (7 days) (Hamilton *et al.*, 2001). Kings Creek, an open prairie stream, had much faster turnover of N in epilithon (1.5 days), but slower N turnover in the FBOM (20 days) (Dodds *et al.*, 2000). Much longer turnover times were reported for FBOM from Upper Ball Creek (53 days, Tank *et al.*, 2000) and Walker Branch (>100 days, Mulholland *et al.*, 2000), but these were both in forested catchments with very low nutrients, and the FBOM may have been more refractory than the FBOM in Augusta Creek. The higher NO_3^- concentration in Augusta Creek may have also influenced the turnover times of FBOM in our study.

Direct denitrification accounted for 3 and 24% of the NO_3^- removed from the water column at ambient NO_3^-

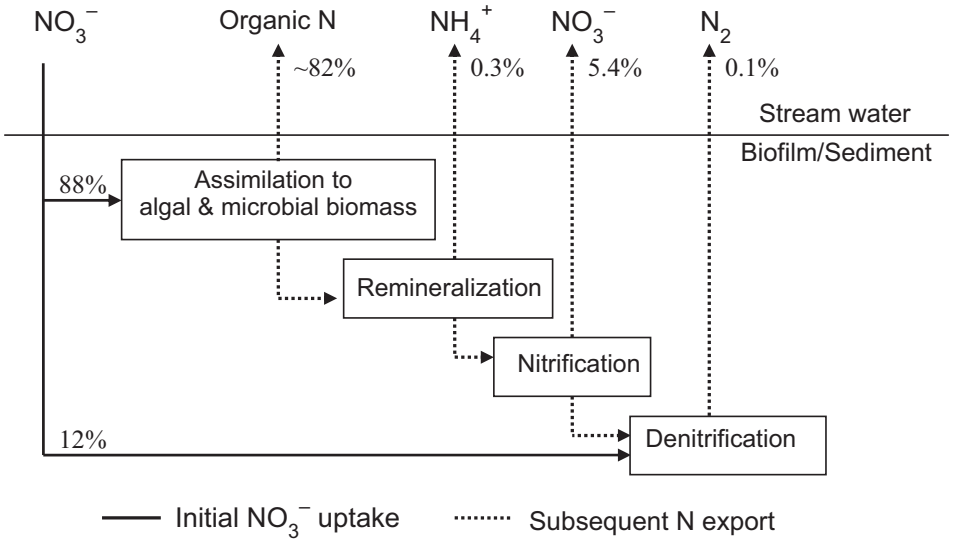

Fig. 5 Fate of NO_3^- following uptake in Augusta Creek (generalised between sand and cobble habitats) based on a mass balance of tracer ^{15}N. A majority of NO_3^- uptake from the water column was attributable to assimilation (88%), while a smaller proportion (12%) was consumed by direct denitrification. Most of the assimilated N was apparently exported from the point of uptake as organic N, while NO_3^- was the main form of export following remineralisation. Organic N exported from the point of uptake may eventually be mineralised as it travels downstream or, if consumed by grazers, would eventually be remineralised.

concentrations in the sand and cobble habitats, respectively, which resembles the median percentage of 15% reported for a large set of whole-stream ^{15}N addition experiments by Mulholland *et al.* (2008). Of the assimilated tracer ^{15}N, only a very small portion was eventually subject to indirect denitrification (<0.01%), and thus, direct denitrification was much more important than indirect denitrification in this stream. This result is not surprising, given the high concentration of NO_3^- in the stream water. The balance between direct and indirect denitrification in other aquatic systems is heavily influenced by N availability (Jenkins & Kemp, 1984; Koop-Jakobsen & Giblin, 2010). Water-column NO_3^- in Augusta Creek was well above the 30–60 μmol L^{-1} suggested by Seitzinger *et al.* (2006) as the threshold below which indirect denitrification exceeds direct denitrification. The implication is that direct denitrification is the primary mechanism of N removal for streams with elevated NO_3^- concentrations, and assimilatory uptake of NO_3^- is primarily a temporary sink for N.

Scaling up to the ecosystem

The ^{15}N labelling and tracking strategy used in this study required us to employ benthic chambers, rather than an ecosystem-level assay. The chamber approach allowed us to measure slow processes such as ^{15}N release without the problem of tracer dilution that would have been the case in an open-channel approach. This was especially advantageous for indirect denitrification, which would have been impossible to detect in an open-channel setting owing to the effects of both dilution and reaeration on dissolved N_2. It would have been prohibitively expensive to sufficiently label a reach of Augusta Creek with tracer ^{15}N to be able to measure the $^{15}N_2$ production. By allowing the sediments to be exposed to ambient flow, light, temperature and grazer conditions for most of the study period, the chamber approach we employed also provided an additional level of realism. Despite this, the results may still suffer many of the same limitations common to all benthic chamber studies in streams.

Benthic chambers only capture a portion of the spatial heterogeneity of biological processes that occur in a stream, which may limit the applicability of results to the whole ecosystem (Marzolf, Mulholland & Steinman, 1994). Heterogeneity in biotic activity and biomass leads to large variations in process rates throughout the channel. Spatial heterogeneity led to large variation in estimates of ^{15}N fluxes and process rates in Augusta Creek. The effect of spatial heterogeneity is especially prevalent for denitrification, which can vary greatly even across the width of a channel (Groffman, Dorsey & Mayer, 2005; O'Brien & Williard, 2006; Knapp *et al.*, 2009). Additionally, the use of chambers may miss important hotspots such as hyporheic flow paths (Hall *et al.*, 2009). Despite these issues, chamber measurements of N cycling have been shown in some instances to closely approximate measurements taken at the whole-stream level (O'Brien & Dodds, 2008; Smith *et al.*, 2009).

Based on the rates of N transformation, it appears that chambers in this study were able to capture enough of the channel heterogeneity to be comparable to ecosystem-level measurements. The average rates of NO_3^- uptake and denitrification fall in the ranges previously published for agriculturally influenced streams (Mulholland *et al.*, 2008, 2009). Average NO_3^- uptake rate and uptake velocity (V_f = 0.0001 cm s^{-1}) from our August Creek chamber measurements fell close to the values of streams with similar NO_3^- concentrations in the Lotic Intersite Nitrogen Study (Mulholland *et al.*, 2008).

In Augusta Creek, the two main habitat types (sand and cobble) were quite similar in terms of NO_3^- uptake and the eventual fate of assimilated N. This similarity between habitats makes it easier to scale the data to the stream reach, without having to account for the relative abundance of habitats. Based on the overall mean NO_3^- uptake rate, at the mean discharge and NO_3^- concentrations during the study, the uptake length of NO_3^- in Augusta Creek was around 146 km. This uptake length is considerably longer than reported for many headwater streams, implying export of most of the NO_3^- to downstream parts of the fluvial network, and is due both to the size of the stream ($Q \sim 800$ L s^{-1}) and to its elevated NO_3^- concentration (Helton *et al.*, 2011).

The rates of N transformations studying Augusta Creek were consistent with those reported for other agriculturally influenced Midwestern streams. Published rates of N remineralisation for streams are rare in the literature, but the rates in Augusta Creek are on the same order of magnitude as rates reported in tall-grass prairie streams (O'Brien & Dodds, 2008). Nitrification rates were similar to those reported for other low-order streams in the Kalamazoo River catchment (Arango *et al.*, 2008), but denitrification rates in Augusta Creek were lower than those reported for nearby streams. A recent study by Smith *et al.* (2009) employed a similar chamber approach to measuring N transformations at Sugar Creek in Illinois. Denitrification rates in Augusta Creek were on the lower end of the range reported by Smith *et al.* (2009) (34–212 μmol N m^{-2} h^{-1}), although NO_3^- concentrations at Sugar Creek were much higher than in our study. Nitrification rates in Sugar Creek (3.5 μmol N m^{-2} h^{-1})

were much lower than the rates measured in our study (Smith *et al*., 2009).

Effects of elevated NO_3^- loading on N retention

Increased NO_3^- concentrations result in decreased efficiency of uptake and stimulate greater direct denitrification in streams (O'Brien *et al*., 2007; Mulholland *et al*., 2008), but what effect will increased NO_3^- concentrations have on the fate of N following uptake? Retention times of biofilm N may decrease with elevated N availability as internal cycling and coupling between autotrophic–heterotrophic production and carbon exchange decrease (Scott *et al*., 2008; Lyon & Ziegler, 2009). Biofilm standing stocks increase with nutrient availability, but biofilm density may increase to the point where net N remineralisation occurs (Teissier *et al*., 2007). The relative importance of indirect denitrification decreases as NO_3^- concentrations increase (Seitzinger *et al*., 2006). Particulate organic matter export increases owing to greater biofilm sloughing from productive biofilms and can also be increased by enhanced flood intensity in human-dominated landscapes (e.g. Murdock, Roelke & Gelwick, 2004).

Based on results of this and previous studies, we predict that the fate of N in streams with lower available N concentrations than Augusta Creek will be characterised by longer retention times (more internal recycling), increased relative importance of indirect denitrification and lower rates of remineralisation to the water column, all resulting in longer nutrient retention. In high-nutrient streams like Augusta Creek, the fate of assimilated N will be characterised by rapid cycling through biofilms followed by remineralisation to the water column and particulate organic N export. These predictions need to be tested across a range of streams, and the chamber approach pioneered in this study will provide a valuable tool in such efforts.

Acknowledgments

We thank Yi Kuang (Bruce) Wang and David Weed for field and laboratory assistance. We also thank Walter Dodds, Robert Hall, Jay Lennon, Patrick Mulholland, Jennifer Tank and Merritt Turetsky for advice in the design and analysis of the project. Two anonymous reviewers provided helpful comments on the manuscript. This project was funded through a post-doctoral fellowship grant from the Michigan State University Center for Water Sciences and grants from the U.S. National Science Foundation (grants DEB 0423627 and DEB 0743402).

References

American Public Health Association (APHA) (2006) *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

Aminot A., Kirkwood D.S. & Kerouel R. (1997) Determination of ammonia in seawater by the indophenol-blue method: evaluation of the ICES NUTS I/C 5 questionnaire. *Marine Chemistry*, **56**, 59–75.

Arango C.P., Tank J.L., Johnson L.T. & Hamilton S.K. (2008) Assimilatory uptake rather than nitrification and denitrification determines nitrogen removal patterns in streams of varying land use. *Limnology and Oceanography*, **53**, 2558–2572.

Bott T.L., Brock J.T., Dunn C.S., Naiman R.J., Ovink R.W. & Petersen R.C. (1985) Benthic community metabolism in four temperate stream systems – an inter-biome comparison and evaluation of the river continuum concept. *Hydrobiologia*, **123**, 3–45.

Cook P.L.M., Veuger B., Boer S. & Middelburg J.J. (2007) Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment. *Aquatic Microbial Ecology*, **49**, 165–180.

Dodds W.K., Evans-White M.A., Gerlanc N., Gray L., Gudder D.A., Kemp M.J. *et al.* (2000) Quantification of the nitrogen cycle in a prairie stream. *Ecosystems*, **3**, 574–589.

Glibert P.M., Lipschultz F., McCarthy J.J. & Altabet M.A. (1982) Isotope dilution models of uptake and remineralization of ammonium by marine plankton. *Limnology and Oceanography*, **27**, 639–650.

Grimm N.B. (1987) Nitrogen dynamics during succession in a desert stream. *Ecology*, **69**, 1157–1170.

Groffman P.M., Dorsey A.M. & Mayer P.M. (2005) N processing within geomorphic structures in urban streams. *Journal of the North American Benthological Society*, **24**, 613–625.

Hall R.O., Baker M.A., Arp C.D. & Koch B.J. (2009) Hydrologic control of nitrogen removal, storage, and export in a mountain stream. *Limnology and Oceanography*, **54**, 2128–2142.

Hamilton S.K. & Ostrom N.E. (2007) Measurement of the stable isotope ratio of dissolved N_2 in ^{15}N tracer experiments. *Limnology and Oceanography, Methods*, **5**, 233–240.

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

Helton A.M., Poole G.C., Meyer J.L., Wollheim W.M., Peterson B.J., Mulholland P.J. *et al.* (2011) Thinking outside the channel: modeling nitrogen cycling in networked river ecosystems. *Frontiers in Ecology and the Environment*, **4**, 229–238.

Hunken A. (2006) Resuspension of particulate organic matter in sand-bed lowland streams. *Archiv für Hydrobiologie*, **166**, 169–183.

Jenkins M.C. & Kemp W.M. (1984) The coupling of nitrification and denitrification in two estuarine sediments. *Limnology and Oceanography*, **29**, 609–619.

Knapp C.W., Dodds W.K., Wilson K.C., O'Brien J.M. & Graham D.W. (2009) Spatial heterogeneity of denitrification genes in a highly homogenous urban stream. *Environmental Science & Technology*, **43**, 4273–4279.

Koop-Jakobsen K. & Giblin A.E. (2010) The effect of increased nitrate loading on nitrate reduction via denitrification and DNRA in salt marsh sediments. *Limnology and Oceanography*, **55**, 789–802.

Lewis W.M. & Wurtsbaugh W.A. (2008) Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. *International Review of Hydrobiology*, **93**, 446–465.

Lyon D.R. & Ziegler S.E. (2009) Carbon cycling within epilithic biofilm communities across a nutrient gradient of headwater streams. *Limnology and Oceanography*, **54**, 439–449.

Manny B.A. & Wetzel R.G. (1973) Diurnal changes in dissolved organic and inorganic carbon and nitrogen in a hardwater stream. *Freshwater Biology*, **3**, 31–43.

Marzolf E.R., Mulholland P.J. & Steinman A.D. (1994) Improvements to the diurnal upstream-downstream dissolved-oxygen change technique for determining whole-stream metabolism in small streams. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 1591–1599.

McIntire C.D., Gregory S.V., Steinman A.D. & Lamberti G.A. (1996) Modeling benthic algal communities: an example from stream ecology. In: *Algal Ecology: Freshwater Benthic Ecosystems* (Eds R.J. Stevenson, M.L. Bothwell & R.L. Lowe), pp. 669–704. Academic Press, San Diego, CA.

Minshall G.W., Petersen R.C., Cummins K.W., Bott T.L., Sedell J.R., Cushing C.E. *et al.* (1983) Interbiome comparison of stream ecosystem dynamics. *Ecological Monographs*, **53**, 1–25.

Mulholland P.J., Hall R.O., Sobota D.J., Dodds W.K., Findlay S.E.G., Grimm N.B. *et al.* (2009) Nitrate removal in stream ecosystems measured by N-15 addition experiments: denitrification. *Limnology and Oceanography*, **54**, 666–680.

Mulholland P.J., Helton A.M., Poole G.C., Hall R.O., Hamilton S.K., Peterson B.J. *et al.* (2008) Excess nitrate from agricultural and urban areas reduces denitrification efficiency in streams. *Nature*, **452**, 202–205.

Mulholland P.J., Steinman A.D., Palumbo A.V., Elwood J.W. & Kirschtel D.B. (1991) Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. *Ecology*, **72**, 966–982.

Mulholland P.J., Tank J.L., Sanzone D.M., Wollheim W.M., Peterson B.J., Webster J.R. *et al.* (2000) Nitrogen cycling in a forest stream determined by a N-15 tracer addition. *Ecological Monographs*, **70**, 471–493.

Mulholland P.J. & Webster J.R. (2010) Nutrient dynamics in streams and the role of J-NABS. *Journal of the North American Benthological Society*, **29**, 100–117.

Murdock J.N., Roelke D. & Gelwick F. (2004) Interactions between flow, periphyton, and nutrients in a heavily impacted urban stream: implications for stream restoration effectiveness. *Ecological Engineering*, **22**, 197–207.

Newbold J.D., Thomas S.A., Minshall G.W., Cushing C.E. & Georgian T. (2005) Deposition, benthic residence, and resuspension of fine organic particles in a mountain stream. *Limnology and Oceanography*, **50**, 1571–1580.

O'Brien J.M. & Dodds W.K. (2008) Predicting NH_4^+ uptake and mineralisation in prairie streams by using chamber incubation and short-term nutrient addition experiments. *Freshwater Biology*, **52**, 102–112.

O'Brien J.M., Dodds W.K., Wilson K.C., Murdock J.N. & Eichmiller J. (2007) The saturation of N cycling in Central Plains streams: ^{15}N experiments across a broad gradient of nitrate concentrations. *Biogeochemistry*, **84**, 31–49.

O'Brien J.M., Hamilton S.K., Kinsman L.E., Lennon J.T. & Ostrom N.E. (2012) Nitrogen transformations in a through-flow wetland using whole ecosystem pulsed ^{15}N additions. *Limnology and Oceanography*, **57**, 221–234.

O'Brien J.M. & Williard K.W.J. (2006) Potential denitrification rates in an agricultural stream in southern Illinois. *Journal of Freshwater Ecology*, **21**, 157–162.

Rabalais N.N. (2002) Nitrogen in aquatic ecosystems. *Ambio*, **31**, 102–111.

Scott J.T., Back J.A., Taylor J.M. & King R.S. (2008) Does nutrient enrichment decouple algal-bacterial production in periphyton? *Journal of the North American Benthological Society*, **27**, 332–344.

Seitzinger S., Harrison J.A., Böhlke J.K., Bouwman A.F., Lowrance R., Peterson B.J. *et al.* (2006) Denitrification across landscapes and waterscapes: a synthesis. *Ecological Applications*, **166**, 2064–2090.

Sigman D.M., Altabet M.A., Michener R., McCorkle D.C., Fry B. & Holmes R.M. (1997) Natural abundance-level measurement of nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. *Marine Chemistry*, **57**, 227–242.

Smith L.K., Voytek M.A., Böhlke J.K. & Harvey J.W. (2006) Denitrification in nitrate-rich streams: application of N_2:Ar and ^{15}N-tracer methods in intact cores. *Ecological Applications*, **16**, 2191–2207.

Smith R.L., Böhlke J.K., Repert D.A. & Hart C.P. (2009) Nitrification and denitrification in a midwestern stream containing high nitrate: in situ assessment using tracers in dome-shaped incubation chambers. *Biogeochemistry*, **96**, 189–208.

Steinman A.D., Mulholland P.J. & Beauchamp J.J. (1995) Effects of biomass, light, and grazing on phosphorus

cycling in stream periphyton communities. *Journal of the North American Benthological Society*, **14**, 371–381.

Tank J.L., Meyer J.L., Sanzone D.M., Mulholland P.J., Webster J.R., Peterson B.J. *et al.* (2000) Analysis of nitrogen cycling in a forest stream during autumn using a N-15-tracer addition. *Limnology and Oceanography*, **45**, 1013–1029.

Teissier S. & Torre M. (2002) Simultaneous assessment of nitrification and denitrification on freshwater epilithic biofilms by acetylene block method. *Water Research*, **36**, 3803–3811.

Teissier S., Torre M., Delmas F. & Garabetian F. (2007) Detailing biogeochemical N budgets in riverine epilithic biofilms. *Journal of the North American Benthological Society*, **26**, 178–190.

Vannote R.L., Minshall G.W., Cummins K.W., Sedell J.R. & Cushing C.E. (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130–137.

Veuger B., Eyre B.D., Maher D. & Middelburg J.J. (2007) Nitrogen incorporation and retention by bacteria, algae, and fauna in a subtropical intertidal sediment: an in situ N-15-labeling study. *Limnology and Oceanography*, **52**, 1930–1942.

Wetzel R.G. & Manny B.A. (1977) Seasonal-changes in particulate and dissolved organic-carbon and nitrogen in a hardwater stream. *Archiv für Hydrobiologie*, **80**, 20–39.

Whalen S.C., Alperin M.J., Nie Y. & Fischer E.N. (2008) Denitrification in the mainstem Neuse River and tributaries, USA. *Fundamental and Applied Limnology*, **171**, 249–261.

Young R.G. & Huryn A.D. (1999) Effects of land use on stream metabolism and organic matter turnover. *Ecological Applications*, **9**, 1359–1376.

(Manuscript accepted 19 February 2012)