Freshwater Biology

Freshwater Biology (2012) **57,** 1579–1591 doi:10.1111/j.1365-2427.2012.02819.x

Nitrogen cycling and dynamics in a macrophyte-rich stream as determined by a $^{15}N\text{-}NH_4^+$ release

T. RIIS*, W. K. DODDS†, P. B. KRISTENSEN* AND A. J. BAISNER*

**Department of Bioscience, Aarhus University, Aarhus, Denmark*

†*Divison of Biology, Kansas State University, Manhattan, KS, U.S.A.*

SUMMARY

1. A $^{15}N\text{-}NH_4^+$ tracer release study was conducted in a macrophyte-rich stream, the River Lilleaa in Denmark. The objectives of the study were to compare uptake rates per unit area of NH_4^+ by primary producers and consumers in macrophyte and non-macrophyte habitats, estimate whole-stream uptake rates of NH_4^+ and compare this to other stream types, and identify the pathways and estimate the rate at which NH_4^+ enters the food web in macrophyte and non-macrophyte habitats.
2. Macrophyte habitats had four times higher primary uptake rates and an equal uptake rate by primary consumers per unit habitat area as compared to non-macrophyte habitats. These rates represent the lower limit of potential macrophyte effects because the rates will be highly dependent on macrophyte bed height and mean bed height in the River Lilleaa was low compared to typical bed heights in many lowland streams. Epiphytes accounted for 30% of NH_4^+ primary uptake in macrophyte habitats, illustrating a strong indirect effect of macrophytes as habitat for epiphytes. N flux per unit habitat area from primary uptake compartments to primary consumers was four times lower in macrophyte habitats compared to non-macrophyte habitats, reflecting much greater biomass accrual in macrophyte habitats. Thus, we did not find higher N flux from macrophyte habitats to primary consumers compared to non-macrophyte habitats.
3. Whole-stream NH_4^+ uptake rate was 447 mgN m^{-2} day^{-1}. On a habitat-weighted basis, fine benthic organic matter (FBOM) accounted for 72% of the whole-stream uptake rate, and macrophytes and epiphytes accounted for 19 and 8%, respectively.
4. We had expected *a priori* relatively high whole-stream N uptake in our study stream compared to other stream types mainly due to generally high biomass and the macrophyte's role as habitat for autotrophic and heterotrophic organisms, but our results did not confirm this. In comparison with other $^{15}N\text{-}NH_4^+$ release study streams, we conclude that nutrient concentration is the overall controlling factor for N uptake rates across streams, mostly as a result of high biomass of primary uptake compartments in streams with high nutrient concentrations in general and not in macrophyte streams in particular.
5. Our results indicate that macrophytes play an important role in the longer-term retention of N and thus a decrease in net downstream transport during the growing season compared to streams without macrophytes, through direct and indirect effects on the stream reach. Direct effects are high uptake efficiency, low turnover rate (partly due to no direct feeding on macrophytes) and high longevity. An indirect effect is increased sedimentation of FBOM in macrophytes compared to non-macrophyte habitats and streams which possibly also increase denitrification. Increased retention with macrophyte presence would decrease downstream transport during the growing season and thus the N loading on downstream ecosystems.

Keywords: lowland streams, macrophytes, nitrogen cycling, nitrogen dynamics

Correspondence: Tenna Riis, Department of Bioscience, Aarhus University, 8000 Aarhus C, Denmark. E-mail: tenna.riis@biology.au.dk

Introduction

Nitrogen (N) is a dominant pollutant in freshwater and marine systems (Smith, 2003). Agricultural areas are major contributors to N pollution (Dodds & Oakes, 2004) because of the use of fertiliser and manure, and small streams and rivers draining agricultural areas contribute substantially to downstream N loading (Alexander *et al*., 2007). Lowland streams in agricultural catchments are especially affected by N and are expected to contribute considerably to downstream transport of N pollutants.

Food-web dynamics, internal N cycling and N retention have been studied in a variety of streams using ^{15}N releases, including a tundra stream (Peterson *et al*., 1997), forest streams (Hall, Peterson & Meyer, 1998; Mulholland *et al*., 2000; Tank *et al*., 2000; Ashkenas *et al*., 2004) and a prairie stream (Dodds *et al*., 2000). Data from 12 such releases indicate that relatively pristine systems are highly retentive of N (Peterson *et al*., 2001; Webster *et al*., 2003). Less is known about N dynamics in N-rich systems (but see Royer, Tank & David, 2004). While much is known about short-term dynamics of N in small streams influenced by agriculture (Mulholland *et al*., 2008), less is known about food webs once N is taken up. Furthermore, ^{15}N tracer studies in open lowland macrophyte-rich streams are few (but see Simon *et al*., 2007), although this type of stream is dominant in large parts of Europe and other regions around the world. Macrophyte-rich streams are common in low-gradient agricultural areas and are often affected by anthropogenic supply of N to the stream environment. Unique aspects of these streams, including high primary producer biomass and the influence of macrophytes on hydrodynamics raise the question whether N cycling differs substantially from other types of streams.

Mosses and vascular macrophytes can be abundant in some streams and likely contribute to N cycling. Lowland macrophyte-dominated streams are often characterised by a high biomass of aquatic vascular plants, including genera such as *Potamogeton*, *Ranunculus* and *Myriophyllum*. Aquatic bryophytes can be very biologically active in some streams. In a deciduous forest stream (Walker Branch, Tennessee), ammonium uptake by the bryophyte *Porella* represented 41% of total N retention at the end of a 6-week ^{15}N addition experiment (Mulholland *et al*., 2000). Meyer & Likens (1979) recorded the removal of phosphorus when it passed over a bryophyte bed. The role of vascular macrophytes in cycling N in streams is even less studied than the role of bryophytes. Macrophytes reduce water velocity (Sand-Jensen & Mebus, 1996) and create a large surface area that is colonised by epiphytes and invertebrates. Owing to the three-dimensional structure of macrophyte beds, much of the biota in macrophyte streams is in intimate contact with the flowing water. These features most likely influence stream N dynamics. Additionally, there is some controversy about the extent to which rooted stream macrophytes use water column nutrients as opposed to sediment-derived nutrients (Madsen & Cedergreen, 2002). Herbivory on mature stream macrophytes is mostly low (Elger, De Boer & Hanley, 2007), so it is not clear how intimately macrophytes are involved in N retention, N flux through lotic food webs and N uptake in streams where they dominate. However, given their substantial biomass and associated organisms, it is likely that macrophyte habitats do influence N dynamics to some degree. In part we are interested to know whether vascular macrophytes serve as a dead end with respect to N uptake into the food web in a manner similar to bryophytes in some streams (e.g. Dodds *et al*., 2000).

The purpose of this study was to describe N cycling in terms of whole-stream NH_4^+ uptake, retention and flux into the food web of a lowland macrophyte-rich stream and to compare macrophyte and non-macrophyte habitats. We added ^{15}N over 12 days and quantified the uptake and turnover rates of various ecosystem compartments for 80 days following the release. The overall benefit of ^{15}N release studies compared to ^{14}N addition studies is the ability to trace the fluxes of N close to ambient concentrations (Peterson *et al*., 2001). Streams with high N concentrations are particularly difficult to study without isotopic methods as they are saturated with N, and perturbation of instream nutrient concentrations leads to little observable effect on other ecosystem attributes (e.g. traditional measures of uptake length using nutrient additions do not work). Our specific research questions were the following: What is the role of macrophytes in N cycling and longer-term N retention? What is the role of macrophyte habitat versus non-macrophyte habitat? What are whole-stream uptake rates of NH_4^+ in macrophyte-rich streams and how does this compare to other stream types? At what rate and through which pathways does NH_4^+ enter the food web in macrophyte versus non-macrophyte habitats? We hypothesised that macrophyte habitats show higher uptake rates on an areal basis than non-macrophyte habitats because of high macrophyte and associated epiphyte biomass. We also hypothesised that macrophyte-rich streams will show high whole-stream NH_4^+ uptake rates because macrophytes have a high active biomass and macrophyte streams have a generally high biomass of autotrophic compartments because of high nutrient concentration,

macrophytes provide a large biologically active surface area for epiphytic communities on leaves consisting of auto- and heterotrophic microorganisms and the structure of macrophytes creates substantial surface area for water-biota contact. Finally, we hypothesised that N enters the primary consumer food web at a higher rate in macrophyte habitats than in non-macrophyte habitats, because macrophyte beds provide habitat for invertebrates and thus increase the biomass of primary consumers.

Methods

Study site and experimental design

We conducted the ^{15}N release study in a 300-m reach in the River Lilleaa situated in an agriculturally dominated catchment in eastern Jutland of Denmark (10°03′46.96″E, 56°15′00.83″N). The catchment has mostly moraine soil and the run-off area is 72 km^2, of which 74% is in agricultural production. The reach was primarily unshaded with approximately a 20-m zone with overhanging trees. The remaining riparian areas were dominated by cropland and cattle grazing with a 15- to 30-m setback of weedy riparian vegetation in the area of study. The stream reach had been channelised in the past and is more or less straight without meanders. Our study design, methods and data analyses generally follow Mulholland *et al.* (2000) and Dodds *et al.* (2000). The study was designed to measure ^{15}N tracer movement through biotic and abiotic components in the stream during and after ^{15}N release. Prior to 15N addition, we measured stream physical and chemical properties, and biomass of major biological compartments. ^{15}N was released into the stream for 12 days. Before, during and after ^{15}N release, samples from each major compartment were collected for ^{15}N analyses. Another measurement of biomass of major ecosystem compartments was performed one month after commencing the ^{15}N release to account for changes of biomass across the experimental period.

Stream characteristics

Stream width was measured in transects every 10 m along the 300-m experimental reach. In each transect, we measured water depth and inorganic substrate type at five points across the stream. A conservative solute (NaCl) release was performed to calculate mean travel time, mean water velocity, discharge and transient storage parameters (Webster & Valett, 2006). A release of unlabelled NH_4^+ that doubled the stream NH_4^+ concentration was performed a week before ^{15}N release. No statistically significant downstream change in NH_4^+ concentration was observed (data not shown), reinforcing the need for isotopic tracer methods in this stream.

Stream water samples were analysed for total N on a Shimadzu TOC-Vcph including TNM-1 for N degradation. Samples were analysed by spectrometer for NH_4^+ by the phenol hypochlorite method (Greenberg, Clesceri & Eaton, 1992). Analyses for NO_3^- were conducted using flow injection analysis with cadmium columns (LACHAT Instruments). Abundance of macrophytes in the study reach was determined by measuring length, width and height of all macrophyte beds in the stream reach.

Biomass estimation of stream compartments

Biomass of all compartments was determined at five stations located at equally spaced positions along the experimental reach on 24 July (pre-sampling) and again on the 25 August (day 30 after ^{15}N release started). All N biomass values were subsequently habitat weighted by surface area of inorganic substrate type (mud/clay, sand, gravel/cobble) or macrophyte cover and volume (see Data analysis).

We measured the concentration of suspended particulate organic N (SPON) by filtering 0.5–1 L of stream water at six locations each sample day during the experiment through pre-combusted Whatman GF/C filters, which were then dried and weighed. Samples were taken from downstream to upstream to avoid disturbance of sediments and contamination.

We measured biomass of coarse benthic organic N (CBON; >1 mm in size) and fine benthic organic N (FBON; <1 mm in size) at five locations in the reach. An open-ended PVC cylinder (16 cm in diameter; 201 cm^2 area) was pushed into the sediment. CBON was first removed by hand and consisted typically of wood, leaves and parts of emergent plants. The sediments were then lightly disturbed by hand down to about 1 cm depth, and the water in the cylinder was mixed. This sample represented surface FBON. A water sample of 200 mL was taken from the mixed cylinder water, brought to the laboratory, filtered onto a pre-combusted Whatman GF/C filter, dried and weighed. Deep FBON was taken in a similar way but after raising the cylinder slightly to allow the shallow FBON to be taken away by the current, then re-inserting the cylinder at the same location and creating a new hand disturbance down to about 5 cm depth.

Epilithon biomass was sampled at five locations along the stream reach by scrubbing and washing all gravel and stone material within the PVC core area. The collected

material was filtered onto a pre-combusted Whatman GF/C filter, dried and weighed.

Biomass of epiphytes and the two dominant macrophyte species (*Ranunculus aquatilis* Linn. and *Callitriche* sp.) were estimated based on macrophyte biomass taken using box samples (inner dimensions: 17 cm × 11 cm × 12 cm). The box sampler was split horizontally along the centre allowing us to grab a sample in the macrophyte bed equivalent to the box sample volume. The volumetric measures were later converted to area-specific mass as described in the data analysis section. We attempted to sample within macrophyte beds without bias with respect to depth or upstream/downstream location, but always sampled at least 5 cm from the stream bed to avoid N associated with sediments. After capturing a set volume of macrophyte, we used scissors to cut all the macrophyte materials away around the perimeter of the box sampler. The sample included macrophytes, biofilm/sediment on the macrophytes and invertebrates living within the macrophyte bed. Replicate box samples were taken from representative places in the upper part of macrophyte beds distributed along the experimental reach. One box sample was taken in each of five macrophyte beds of each of the two dominant species (total of 10 samples).

The sample from each box was placed in a plastic bag in the field and kept cool (4 °C) until samples were processed (within 24 h following collection). In the laboratory, we separated macrophytes, epiphytes and invertebrates. First, epiphytes were washed off the macrophytes by gently squeezing in 2–3 batches of clean stream water, and all macrophyte material was removed from the wash water. Then, the epiphyte biomass in the wash water was filtered onto a pre-combusted Whatman GF/C filter after removal of all macroinvertebrates, dried and weighed. The clean macrophyte materials were dried in paper bags and weighed.

Invertebrates from the box sampler represent the biomass of invertebrates associated with macrophyte beds. Quantitative benthic macroinvertebrate samples were collected with a small Surber sampler (22.1 cm^2). Three samples were pooled from each of the five sites. For each sample, sediments were disturbed to a depth of at least 5 cm, and invertebrates were collected in a 250-μm net from the suspension made by the disturbance. Samples were preserved in 96% ethanol until identification.

In the laboratory, invertebrate specimens were identified to the lowest possible taxon. Number of individuals was counted for each taxon in each sample, dry mass was measured and a mean dry mass per individual was estimated. Invertebrate taxa were then separated into feeding functional groups, and dry mass of each group was determined based on taxa biomass.

Fish biomass was estimated based on two electrofishing catches according to Seber & Le Cren (1967). Fish biomass was only measured once near the end of the experiment (15 August 2010). Dry mass values were determined after at least 24 h in 60 °C dry oven.

Metabolism

Whole-stream metabolism in terms of gross primary production (GPP) and community respiration (CR) was determined 2 days before commencing the ^{15}N release, using the diurnal upstream-downstream dissolved oxygen exchange technique (Marzolf, Mulholland & Steinman, 1994; Young & Huryn, 1998) modelled with the techniques described by Riley (2011). Concentrations of dissolved oxygen were measured at 10-min intervals at two locations on the reach (70 and 210 m from ^{15}N release) using YSI sondes (6600V2; Yellow Springs Instruments, Yellow Springs, OH, USA). Reaeration rate was determined using a propane method (Marzolf *et al.*, 1994) coupled with salt (NaCl) release to ensure a plateau was reached and to correct for any dilution. Photosynthetic active radiation (PAR) was measured using a Li-Cor quantum sensor and data logger (LI-COR 1400, LiCor Biosciences, Lincoln, NE, USA).

^{15}N release, sampling and analysis

A total of 50 g 98% enriched $^{15}NH_4Cl$ (Batch no. 2181; Campro Scientific, Berlin, Germany) were added over the 12-day release period commencing on 26 July 2010 and ending at 7 August 2010. A total of 13.761 g ^{15}N were added to the stream. To accomplish this, a solution of 1.16 mM $^{15}NH_4^+$ was released into the stream at an average rate of 6.9 mL min^{-1}. This resulted in an average concentration of 0.038 μg ^{15}N L^{-1} in the stream water with an average discharge 63 L s^{-1}. Background NH_4^+ concentration was 64.1 μg N L^{-1}; thus, the addition of $^{15}NH_4^+$ was <1% of total NH_4^+ concentration.

Samples of water, organisms and detritus were collected from an upstream station, and six stations located 30, 73, 94, 134, 214 and 300 m downstream from the ^{15}N release. Sampling of all biological compartments was carried out the day before release, during release (days 1, 3, 6, 11) and after the release period (days 13, 15, 18, 23, 46, 92).

Qualitative samples for ^{15}N analyses of epilithon, epiphytes, detritus, fine benthic organic matter (FBOM) and macrophytes were taken in the same way as described for biomass but in small amounts, working a

few metres upstream on each subsequent sampling date to avoid re-sampling disturbed areas. Samples of fish and each major functional feeding group of macroinvertebrates were taken at each station by netting and hand picking. All solid samples were returned to the laboratory on ice, dried at 60 °C and ground before analysis. Invertebrate and fish samples were freeze-dried. Macroinvertebrate samples were left at 4 °C in water for 12 h so they would empty their guts before they were dried and ground for analysis.

The water was sampled and analysed for $^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}NO_3^-$ 1 day after the release began to estimate uptake of NH_4^+ with minimal interference from mineralisation and to measure nitrification rates. The $^{15}NH_4^+$ was also sampled one day after the release was stopped to calculate the rate of regeneration of ^{15}N back to the water. $^{15}N\text{-}NH_4^+$ in the water was isolated for analysis using an ammonium diffusion method suggested by Sørensen & Jensen (1991) and Holmes *et al.* (1998) and described in detail in Mulholland *et al.* (2000). $^{15}N\text{-}NO_3^-$ was isolated and analysed as described previously (Mulholland *et al.*, 2000; Peterson *et al.*, 2001).

Solid samples were dried and finely ground before analysis for $^{15}N/^{14}N$ ratios by the Stable Isotope Mass Spectrometry Laboratory at Kansas State University on a Thermo Finnigan Delta Plus mass spectrometer. The standard deviation range for ^{15}N was 0.03–0.18‰ based on standards every 12th sample. Analysis of un-enriched replicate samples from this study indicated low analytical variation (<3% coefficient of variation). The $^{15}N\text{-}NH_4^+$ was analysed following an alkaline diffusion procedure (Holmes *et al.*, 1998) on the same mass spectrometer as described above. Additional samples were processed for analysis of $^{15}N\text{-}NO_3^-$ by boiling under basic conditions to concentrate NO_3^- and drive off all NH_4^+ as NH_3. Then, $^{15}N\text{-}NO_3^- + NO_2^-$ was converted to ammonia with De Varda's Alloy, concentrated by diffusion, and analysed as above (Sigman *et al.*, 1997).

Data analysis

Biomass of benthic compartments was calculated as habitat-weighted values. Mass in the reach of each macrophyte species was determined using percentage cover and mass per unit area values. Mass of invertebrates associated with macrophytes was based on mass in volume of macrophytes because of the importance of the three-dimensional structure of this habitat. Volume-based masses were then multiplied by mean height of macrophyte beds in the reach (0.13 m) to convert to mass per m^2 stream reach.

Metabolism was calculated with a model that accounted for light, travel time, aeration and temperature effects. This model minimised sums of squares of observed and calculated dissolved oxygen concentrations through an iterative fitting function (Riley, 2011). Transient storage was calculated using OTIS solute transport model software (Runkel, 1998).

Calculations of $\delta^{15}N$ in samples, uptake lengths and rates, nitrification, regeneration and retention followed the procedures described by Mulholland *et al.* (2000), Dodds *et al.* (2000) and Ashkenas *et al.* (2004). Interconversion among areal uptake rates, turnover rates, turn-over lengths and uptake velocity were calculated according to the equations published by the Stream Solute Workshop (1990). Whole-stream spiral length was calculated from exponential decline in $^{15}N\text{-}NH_4^+$ downstream from the release point on day 1. Uptake rates of NH_4^+-N by each primary uptake compartments were calculated from ^{15}N values on day 3 at station 2–4 and the tracer $^{15}N/^{14}N$ ratios in stream water NH_4^+ at those stations on day 0, following Mulholland *et al.* (2000) and Tank *et al.* (2000). The values were corrected for turnover loss of ^{15}N from each compartment during the first three days (Mulholland *et al.*, 2000). We also calculated uptake rates per unit habitat area in macrophyte habitats and non-macrophyte habitats adjusting to the different compartments present in macrophyte and non-macrophyte habitats, respectively. Turnover rates of N in primary uptake compartments were calculated as the exponential decline of ^{15}N (depuration) from 1 to up to 49 days after ^{15}N drip stop. Nitrogen retention was calculated as mass balance of the total ^{15}N added and retained over the experimental period. Retention of each compartment within the 300 m reach was based on downstream decline in component-specific ^{15}N biomass on day 11, the last day of isotope release. If the slope of the regression of ^{15}N biomass as a function of distance was not significant ($P < 0.05$), we used the mean ^{15}N biomass for the reach.

Uptake and turnover rates of consumers were estimated using a box model that can account for changing label in a food source over time (Dodds *et al.*, 2000). This approach concentrates on a single station over time and uses trends in measured label in food sources, and biomass of the uptake compartment, to calculate a rate of label uptake into the consumer compartment based on an uptake rate. Food sources for each functional feeding group were determined using Wallace & Webster (1996) and Skirver (1982), and proportion of each food source was based on prior experience. The uptake rate is varied to minimise the sum of square of error between observed and calculated consumer isotopic contents. In some cases, the consumer

compartment was more labelled than the putative food sources. In this case, the pattern of labelling in the food compartment was preserved but a multiplier was used to optimise the fit. The multiplier accounts for the selective nature of animal feeding. This additional multiplier was fitted simultaneously with the uptake rate in these cases. The fitting was performed using the Solver option in Microsoft Excel. Values given are based on one best or mean of two best stations.

We included a meta-analysis to compare our macrophyte stream to other stream types. We collated existing data on nutrient dynamic parameters from ^{15}N release studies (Webster *et al.*, 2003; Simon *et al.*, 2007) and related them by linear regression to NH_4^+ concentration in the streams to compare NH_4^+ uptake parameters in our macrophyte-rich stream with other stream types. We also determined the linear relationship between NH_4^+ in stream water and biomass of primary uptake compartments, and the linear relationship between biomass of primary uptake compartments and uptake rate. Strong relationships between NH_4^+ uptake parameters and NH_4^+ concentration were previously found within streams (Wollheim *et al.*, 2001; Dodds *et al.*, 2002) and among a range of streams (Webster *et al.*, 2003).

Results

Environmental properties

Physical characteristics in the stream were typical for summer conditions in small low-gradient macrophyte-rich streams (Table 1). We did not detect any significant groundwater input along the reach, based on NaCl releases, thus simplifying most calculations. Bottom substrata areas were dominated by sand and gravel. Macrophyte cover was 11% in the experimental reach and dominated by *R. aquatilis* L. and *Callitriche* sp. (Table 1). Mean travel time of water through the reach was 46 min, and relative transient storage area (storage area as proportion of profile area; A_s/A, $m^2\ m^{-2}$) was 0.02. Both N and phosphorus content in the water were relatively high (Table 1). Gross primary production was one-third the diurnal respiration, indicating that the stream is net heterotrophic despite the high macrophyte cover.

Nitrogen compartments

Nitrogen stocks were dominated by FBON that constituted 94% of the total N biomass (Table 2). Macrophyte biomass supported high epiphyte biomass, which three times higher than epilithon biomass. *Gammarus pulex* (L.)

Table 1 Ecosystem properties in the experimental stream reach in the River Lilleaa during the experiment. Data given as mean ± SD where possible

Ecosystem property (unit)	Value
Reach length (m)	300
Average width (m)	2.6 ± 0.2
Average depth (m)	0.23 ± 0.08
Total surface area (m^2)	709
Gradient (m km^{-1})	1.2
% run	94.3
% riffle	5.7
% mud and clay	18.8
% sand	46.9
% gravel/cobble	34.4
% total macrophyte cover	10.7
Mean macrophyte height (cm)	14
% total macrophyte volume	8.0
% cover *Ranunculus aquatilis*	8.2
% cover *Callitriche* sp.	2.0
% cover *Berula erecta*	0.5
Mean discharge (L s^{-1})	63.2
Mean water velocity (m s^{-1})	0.10
Mean travel time (min)	46
Transient storage A_s/A	0.02
Trans. stor. exc. coef. (α;K_1)	4.50×10^{-5}
Mean water temperature (°C)	12.4 ± 1.2
NH_4^+ (μgN L^{-1})	64.1 ± 6.2
NO_3^- (μgN L^{-1})	1433.3 ± 70.3
Total N (mgN L^{-1})	2.1 ± 0.04
PO_4^{3-} (μgP L^{-1})	63.3 ± 9.7
Total P (μgP L^{-1})	88.5 ± 18.0
TOC (mgC L^{-1})	3.0 ± 0.1
pH	7.90
Alkalinity (mEq L^{-1})	2.87 ± 0.34
Gross primary production ($gO_2\ m^2\ day^{-1}$)	1.65
Respiration (diel O_2 change method, $gO_2\ m^2\ day^{-1}$)	5.29
P/R ratio	0.21

Table 2 Habitat-weighted N biomass of ecosystem compartments ordered by trophic level. Average values based on two measurements at 24 July and 23 August, respectively

Compartment	Biomass (mg N m^{-2})	%N	C:N (molar)
SPON	48	3.3	11.6
Epiphytes	634	11.5	10.6
Epilithon	208	6.2	8.8
Macrophytes	1475	3.7	11.9
FBON surface	22 701	6.0	13.0
FBON deep	23 872	4.8	13.6
Detritus	375	1.2	38.3
Grazers	39	8.5	5.4
Collectors	89	9.9	5.0
Gammarus pulex	115	7.4	6.0
Shredders	72	7.4	7.9
Predators	32	9.6	6.2
Fish	2808	12.6	4.3

was the dominant invertebrate on the reach and constituted 36% of the primary consumer N biomass (Table 2). Biomass of invertebrates in macrophyte habitats was about one-third higher than non-macrophyte habitats (Table 3). Macrophyte habitats were especially important for Simulidae, shredders (dominated by Limnephilidae) and *G. pulex*.

Nitrogen uptake rates

Macrophyte habitats in the River Lilleaa showed fivefold higher NH_4^+ uptake rates than non-macrophyte habitats (Fig. 1). Epiphytes were responsible for one-third of the uptake in the macrophyte habitat. In the non-macrophyte habitat, FBOM was responsible for 98% NH_4^+ uptake. Whole-stream spiral length was 303 m, resulting in a uptake velocity (v_f) of 0.08 mm s^{-1}. Whole-stream uptake rate expressed as uptake rate of water $^{15}NH_4^+$ was 447 mg N m^{-2} day^{-1}. The measured compartment-specific NH_4^+ uptake rates based on incorporation of $^{15}NH_4^+$ from the water column indicated FBON was responsible for 72% of the daily uptake rates (Table 4). Macrophytes and epiphytes were only responsible for 19% and 8% of whole-stream NH_4^+ uptake, respectively (Table 4). Macrophytes were the most highly labelled at day 11 of release of all primary uptake compartments and about 2.5 times higher than epilithon (Fig. 2a,b). When we summed the compartment-specific NH_4^+ uptake rate, we could account for 50% of whole reach NH_4^+ uptake (measured by decline of $^{15}NH_4^+$ in the water column). Biomass-specific uptake rates show the efficiency of the different compartments to take up NH_4^+. Macrophytes and their associated epiphytes have threefold higher biomass-specific uptake rates than epilithon and 10 times higher than FBON (Table 4). There was no trend in ^{15}N-NO_3^- in water down through the experimental reach at day 12 and thus nitrification was not detectable, although rates must be less than the total rate of ^{15}N-NH_4^+ uptake.

Table 3 Comparison of N biomass (gN m^{-2}) in macrophyte habitats (mixed *Ranunculus aquatilis* and *Callitriche* sp.) and non-macrophyte habitats in the River Lilleaa. Values are averages of samples from July and August 2010. The values show the importance of macrophytes as habitats for invertebrates and especially Simulidae, *Gammarus pulex* and shredders

FFG	Macrophyte habitat Lilleaa (mgN m^{-2})	Non-macrophyte habitats (mgN m^{-2})
Grazers	5.1	42.4
Collectors comp	15.2	96.7
Simulidae	110.1	55.1
Shredders	148.1	62.8
G. pulex	247.5	99.2
Predators	6.6	34.3
Total	532.7	390.4

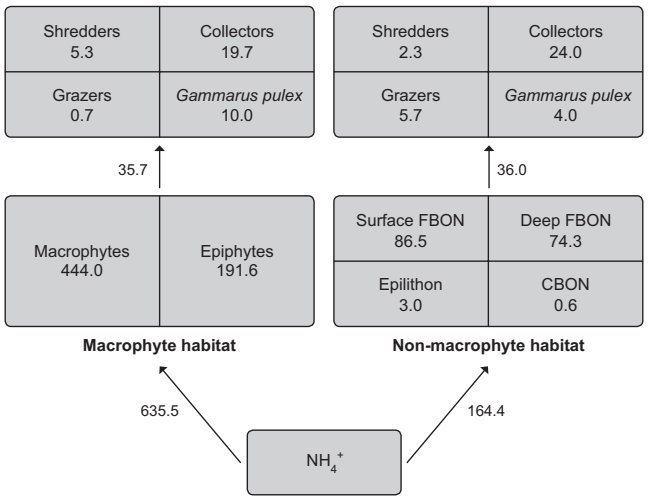

Fig. 1 Diagram of uptake rates per unit habitat area (mg N m^{-2} day^{-1}) in macrophyte and non-macrophyte habitats in the River Lilleaa. Uptake rates of NH_4^+-N by each primary uptake compartments were calculated from ^{15}N values on day 3 at station 2–4 and the tracer $^{15}N/^{14}N$ ratios in stream water NH_4^+ at those stations on day 0, following Mulholland *et al.* (2000) and Tank *et al.* (2000). Uptake rates for primary consumers are from a box model described in Riley (2011).

Table 4 Habitat-weighted uptake rates of NH_4^+, biomass-specific uptake rates, turnover rates and % ^{15}N retained of total retention for major ecosystem compartments in Lilleaa. Uptake rates for NO_3^- are estimated as the difference between total N uptake and NH_4^+ uptake (see text for further explanation). Biomass-specific uptake rate is calculated as uptake rate (mgN m^{-2} day^{-1})/compartment biomass (mgN m^{-2})

Ecosystem compartment	NH_4^+ uptake rate (mg N m^{-2} day^{-1})	Biomass-specific NH_4^+ uptake rate (day^{-1})	Turnover rate (day^{-1})	NO_3^- uptake rate (mg N m^{-2} day^{-1})	%^{15}N retained of total retention
Epiphytes	18	0.032	0.042	12	7.6
Epilithon	2.3	0.011	0.081	14	0.2
Macrophytes	42	0.027	0.027	0	3.0
FBON surface	86	0.004	0.017	–	50.8
FBON deep	74	0.003	0.102	–	31.9
Detritus	0.6	0.002	0.015	5	0.7
Grazers	5.3	0.136	0.135	–	0.1
Collectors	14	0.157	0.158	–	0.7
Gammarus pulex	4.7	0.041	0.041	–	0.2
Shredders	2.6	0.036	0.036	–	0.1
Predators	0.5	0.016	0.014	–	0.03
Fish	22	0.008	0.008	–	4.8

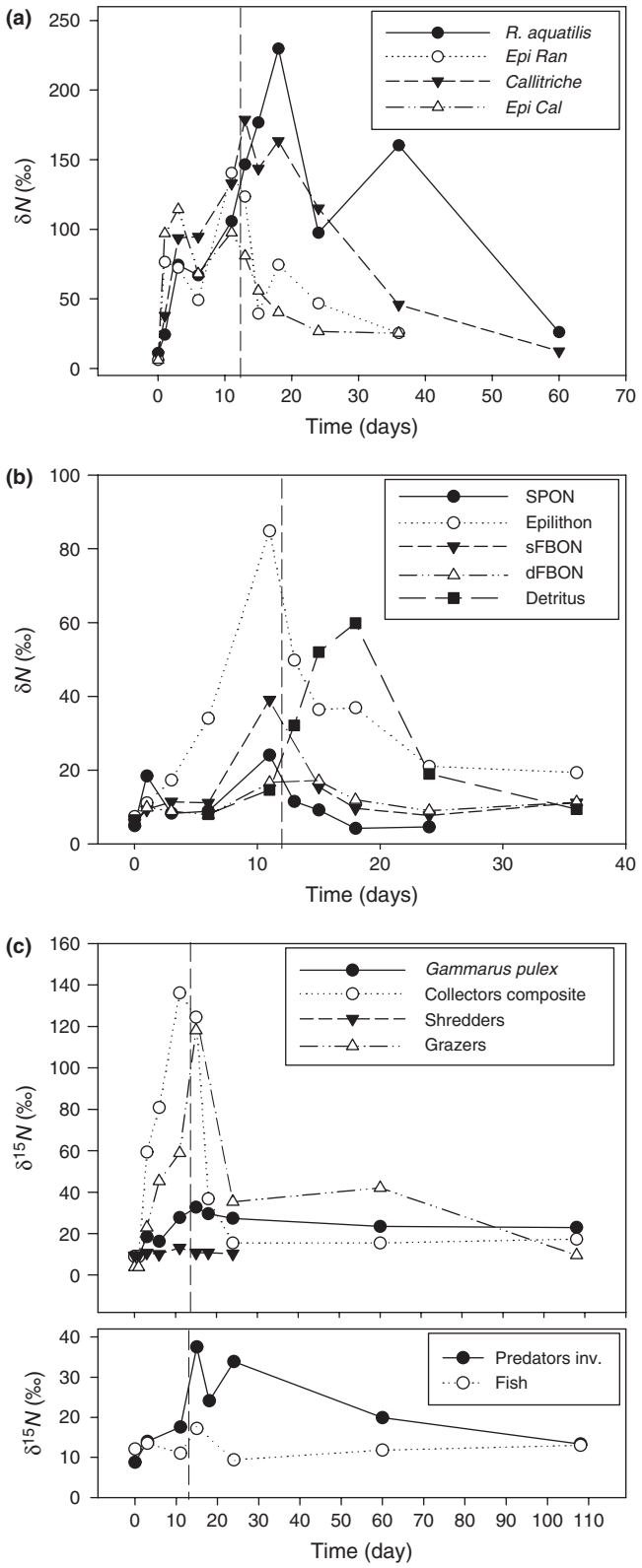

Fig. 2 Time series of observed $\delta^{15}N$ at station 3 (94 m from release site) for (a) macrophytes and epiphytes, (b) other primary uptake compartments, and (c) primary and secondary consumers. The broken line marks the time (day 12) when $^{15}NH_4^+$ addition was stopped.

Table 5 Statistical parameters for three models tested on the relationship between areal uptake rate (U) and NH_4^+ concentration (data shown in Fig. 4). Models are the linear model, the efficiency loss model and the Michaelis–Menten saturation model as described in O'Brien *et al.* (2007)

Model	Equation	R^2	P
Linear: $y = ax + b$	$U = 0.007x + 0.033$	0.79	0.000
Efficiency loss: $y = ax^b$	$U = 0.002^* x^{0.774}$	0.77	0.049
Michaelis–Menten: $y = ax/(b + x)$	$U = 1.389x/(136.9 + x)$	0.75	0.054

Turnover rates of ^{15}N were within the same range for all autotrophic compartments although macrophyte turnover time was three times slower than epilithon (Table 4). The level of ^{15}N enrichment in all primary uptake compartments except macrophytes declined quickly after ^{15}N-NH_4^+ addition was stopped (Fig. 2a,b). Enrichment of ^{15}N in macrophytes peaked after the addition stopped, stayed high in the plants for longer than most other primary uptake compartments and was almost back to background ^{15}N values 48 days after the isotope release ended (Fig. 2a). The same occurred for detritus (Fig. 2b). The ^{15}N retention in primary uptake compartments was also reflected in the primary consumers that were still ^{15}N enriched after 96 days after drip stop (Fig. 2c). Turnover rate of NH_4^+ in the water was 31 day^{-1}, and SPON and collectors had the highest turnover rate of any compartment other than the NH_4^+, at 0.19 day^{-1} and 0.16 day^{-1}, respectively. *Gammarus pulex*, shredders and detritus had low turnover rates, and predators and fish had the lowest.

If we assume that biomass does not change, then total loss rate of N (mineralisation) should equal total uptake. Total uptake, assuming organic N uptake is minimal, is the sum of NO_3^- and NH_4^+ uptake. Thus, we can calculate compartment-specific total N uptake, NH_4^+ uptake, and the difference should represent the compartment-specific NO_3^- uptake. We are only comfortable using this calculation for compartments that are in intimate contact with the water column because the NH_4^+ uptake calculation depends on knowing the $^{15}NH_4^+$ content of the water. Estimations suggest that NO_3^- uptake was considerable and in most cases as important as or more important than NH_4^+ uptake (Table 4). The estimations showed that no NO_3^- was taken up by macrophytes.

At the end of the ^{15}N release at day 11, a total of 26% of the added tracer was retained in the stream reach. Most of the retained ^{15}N was retained in FBON (83%), and macrophytes and epiphytes were responsible for 8% of the retained ^{15}N (Table 4). The significant but not complete retention of added ^{15}N is consistent with the 303 m calculated uptake length for NH_4^+.

Uptake rates of ^{15}N by primary consumers in Lilleaa were similar in macrophyte and non-macrophyte habitats (Fig. 1). In macrophyte habitats and non-macrophyte habitats, respectively, 6 and 22% of the primary uptake was taken up by primary consumers. On a whole-stream basis, total N flux into primary consumers constituted 16% of the primary producer's uptake in Lilleaa (total uptake rate of primary consumers as proportion of total uptake rate by primary uptake compartments; Table 4).

Food-web relations

To determine whether macrophytes are directly food source for invertebrates in the River Lilleaa, we analysed the natural abundance of ^{15}N in primary and secondary uptake compartments (Fig. 3). Epiphytes, epilithon, SPOM and detritus showed similar ^{15}N values around 6‰, and FBON was 4–5‰. Macrophytes had greater ^{15}N values than the other primary uptake compartments and greater than the primary consumers, the latter reflecting that there was little direct feeding on the macrophytes. Among the primary consumers, the filterer Simulidae had the lowest natural ^{15}N value. *Gammarus pulex* and predators were close and lower than Baetidae, *Ephemera* and shredders. This probably indicates that the omnivore *G. pulex* to a high degree feeds on FBON and primary producers, and invertebrate predators feed primarily on Simulidae. Grazers had lower natural ^{15}N than their presumed food source (epilithon), most likely reflecting a missing food source in the sampling or physiological discrimination of food source by the grazers.

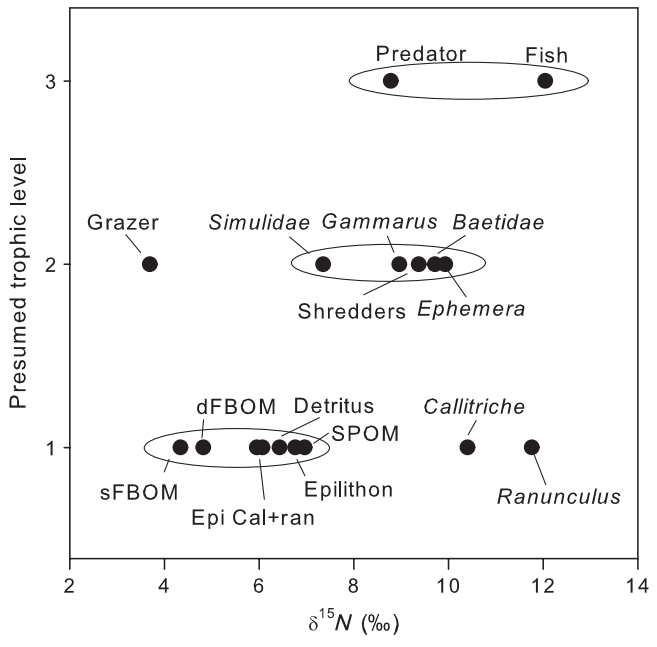

Fig. 3 Natural abundance for main compartments arranged after presumed trophic level. Values are average from background samples.

Meta-analysis of whole-stream uptake parameters

We compared whole-stream uptake parameters for NH_4^+ in our macrophyte stream to other stream types (Fig. 4). The parameters in the River Lilleaa were measured at twice the NH_4^+ concentrations found in other streams we used for comparison. Uptake length in Lilleaa was shorter than some streams with lower NH_4^+ concentration (Fig. 4a), but uptake velocity was similar to other streams with lower NH_4^+ concentrations (Fig. 4b). Areal uptake rates in Lilleaa and two New Zealand macrophyte streams were at the same level as other stream types (Fig. 4c).

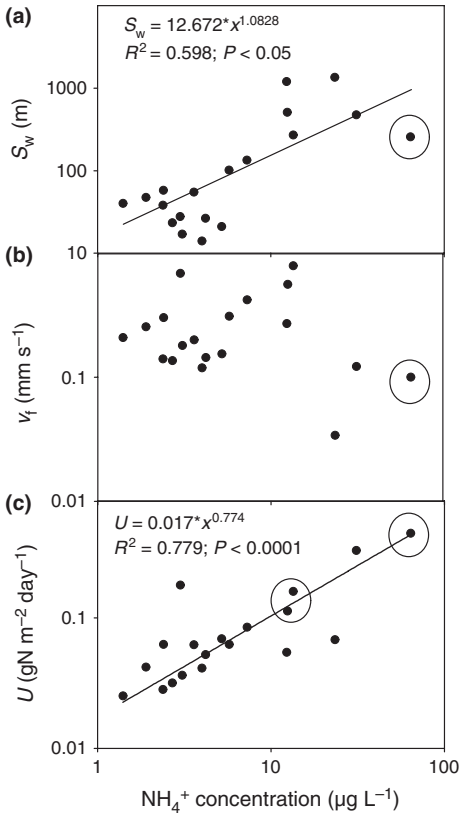

Fig. 4 Relationships of uptake length (S_w; a), uptake velocity (v_f; b) and uptake rate (U; c) as a function of water NH_4^+ concentration in a range of stream types. Data from Lilleaa (a, b and c) and the two macrophyte streams reported in Simon *et al.* (2007; c) are circled. Data from other streams collated from Webster *et al.* (2003) and Simon *et al.* (2007). Note log-log transformation.

Discussion

Macrophyte habitat versus non-macrophyte habitats

Macrophyte habitat showed four times higher NH_4^+ uptake rates in the River Lilleaa than non-macrophyte habitats. This represents a lower level for uptake rate in macrophyte habitats because of the relative low height of macrophyte beds in the study river. In deeper streams, macrophyte beds are often more than 0.5 m (Riis, Sand-Jensen & Larsen, 2001). Provided that uptake is evenly distributed throughout the vertical dimension of macrophyte beds, the uptake rates can therefore easily exceed the uptake rate found in this study. As an example, if the mean height of macrophyte beds in the River Lilleaa were 0.5 m instead of 0.14 m, the primary uptake rate would be about four times higher than the actual value in macrophyte habitats and consequently 14 times higher than in non-macrophyte habitats. Direct uptake by macrophytes was responsible for 70% and epiphytes for 30% of the total uptake in macrophyte habitats, showing a relatively large indirect effect of macrophytes through their role as habitats for epiphytes. Ammonium uptake efficiency expressed as biomass-specific uptake rate of NH_4^+ was higher in both macrophytes and epiphytes compared to the uptake compartments in non-macrophyte habitats.

Biomass-specific uptake rate for macrophytes in the River Lilleaa (0.027 day^{-1}) corresponds to biomass-specific uptake rates measured in macrophytes in two New Zealand (NZ) streams (0.021 and 0.032 day^{-1}), although the dominant species was *Nasturtium officinale* in the NZ streams and *R. aquatilis* and *Callitriche* sp. in Lilleaa. This indicates a relatively stable uptake efficiency among macrophyte species and even across growth forms because *N. officinale* is an amphibious species whereas *R. aquatilis* and *Callitriche* sp. are submerged species (Riis *et al.*, 2001). We did not measure uptake in the sediment under macrophyte beds, but if we assume it was the same as in open habitats, then total N retention in macrophyte habitats will be about 10% higher than estimated here because of the *c.* 10% cover of macrophytes in the reach.

Uptake rates of NH_4^+ in non-macrophyte habitats were highly dominated by FBOM, which is a mix of direct uptake by autotrophic and heterotrophic microalgae and sorption onto sediments. Richey, McDowell & Likens (1985) concluded that sorption of NH_4^+ to sediments was responsible for significant storage during summer and autumn. We were not able to separate abiotic and biotic uptake in FBOM in this study but this is unimportant for estimating total N retention. Uptake rates by FBOM generally increase with increasing agricultural development (Simon *et al.*, 2007), supporting the high FBOM uptake in the highly agriculturally impacted River Lilleaa. In our study, abiotic and biotic NH_4^+ uptake by surface FBOM was released back into the water after about eight weeks, corresponding to findings in Peterson *et al.* (2001). Deep FBOM (3–5 cm sediment depth) had a fivefold faster turnover rate than surface FBOM, possibly due to denitrification in this depth zone (Stelzer *et al.*, 2011).

Our second hypothesis that N enters the primary consumer food web at a higher rate in macrophyte habitats than in non-macrophyte habitats, because macrophyte beds provide habitats for invertebrates and thus increase the density of primary consumers, was partly verified. The ^{15}N uptake rate by primary consumers was equal in the macrophyte and non-macrophyte habitats in the River Lilleaa, because invertebrate biomass is almost equal in the two habitats. This was surprising because invertebrate density generally increases with increasing habitat area of macrophytes (e.g. Strayer & Malcolm, 2007). However, the low macrophyte bed height in the River Lilleaa limits the difference in invertebrate density between macrophyte and non-macrophyte habitats. For example, if macrophyte mean height had been 0.5 m there would have been about four times higher invertebrate biomass and consequently four times higher uptake rate by primary consumers in macrophyte habitats compared to non-macrophyte habitats, given equally distributed uptake by primary consumers throughout the macrophyte bed. However, detailed studies on N uptake within macrophyte beds are needed to validate a linear upscaling of invertebrate uptake in macrophyte beds of varying sizes.

The large discrepancy between uptake by primary uptake compartments and flux into primary consumers in macrophyte habitats most likely reflects high biomass accrual by macrophytes and lack of feeding in macrophytes by consumers. In macrophyte habitats, only 6% of the NH_4^+ taken up was passed on to primary consumers, compared to non-macrophyte habitats where 22% moved into primary consumers. Although this could indicate high mineralisation directly from the primary producer's uptake, the turnover rates of macrophytes are similar to other compartments and a substantial part of the discrepancy is most likely due to build-up of biomass in macrophytes. Our study confirms that more ^{15}N persist longer in macrophytes than other primary uptake compartments, reflecting substantial biomass accrual.

Natural ^{15}N abundances in macrophytes were much more enriched than microalgae in our study. The isotope natural abundance could reflect that macrophytes use sediment nutrients that are affected by enriched ground water in addition to stream water nutrients. Thus,

although Madsen & Cedergreen (2002) conclude that vascular stream plants can satisfy their demand for nutrients by leaf uptake alone, our study indicates that even in nutrient-rich stream water macrophytes could be using sediment nutrients. However, we did not sample the various sources and we cannot exclude the possibility of temporal variability.

Whole-stream nitrogen dynamic

We were not able to verify our hypothesis that whole-stream uptake rate was higher in macrophyte streams as compared to other stream types. The hypothesis was driven by an expected greater biologically active surface area with autotrophic and heterotrophic epiphytic communities, relatively higher biota–water contact and high standing stocks of biotic compartments. This hypothesis implies that N uptake length should be shorter and uptake velocity higher in macrophyte streams.

We did find a shorter uptake length in the River Lilleaa compared to other stream types at the same NH_4^+ concentration and this could be due to hydrological effects in terms of low discharge, high biological activity or efficiency loss. A strong relationship between stream discharge and N uptake length has previously been reported (Peterson *et al.*, 2001; Simon & Benfield, 2002). According to the relationship found between stream discharge and NH_4^+ uptake length in a range of streams, including LINX streams (Simon & Benfield, 2002), the uptake length in the River Lilleaa should only be 129 m, which is much less than the 303 m that we measured and counteract that discharge alone can explain the relatively shorter uptake length. Hence, it leaves high biological activity or efficiency loss as the two possible explanations.

If biological activity is controlling the short uptake length, it should be reflected in a relatively high uptake velocity in Lilleaa compared to other stream types. However, we found lower uptake velocities than other streams. In fact the uptake velocity was roughly equal to streams with half the NH_4^+ concentration, indicating saturation in NH_4^+ uptake and consequently efficiency loss at NH_4^+ concentrations in streams above 20 $\mu g\ L^{-1}$.

Efficiency loss of N uptake in NO_3^- enriched streams has previously been reported (O'Brien *et al.*, 2007) but the question whether it also occurs in NH_4^+-rich streams is still open. O'Brien *et al.* (2007) described the saturation effect by an efficiency loss model where uptake rates increase exponentially with NO_3^- concentration with a slope <1. When we analyse uptake rates with increasing water NH_4^+ concentration in our stream data set, the data fit the efficiency loss model. This is a new result for NH_4^+ and is not necessarily expected since we would not expect NH_4^+ uptake to saturate as readily as NO_3^- because nitrification can continue at very high NH_4^+ concentrations (Bernot & Dodds, 2005). The curve fits indicate that linear and exponential functions fit the data equally well. The Michaelis–Menten function was not significant (Table 5) with respect to saturation of uptake. The exponential function had an exponent that was significantly >0, and significantly <1. These data suggest that if Michaelis–Menten saturation is occurring, the half saturation constant is high relative to those published for pure cultures of bacteria or algae. The data are not inconsistent with the efficiency loss hypothesis and, therefore, we cannot rule out the efficiency loss hypothesis.

The meta-analysis did not indicate that the River Lilleaa has higher NH_4^+ uptake rates from that predicted from the water column NH_4^+ concentration. A higher uptake rate in macrophyte streams compared to other stream types should shift the Lilleaa data point as well as the two NZ macrophyte streams reported in Simon *et al.* (2007) above the regression line, but this was not found. A higher uptake rate in macrophyte streams should also diminish the saturation effect of uptake rate at higher NH_4^+ concentration in macrophyte streams compared to other stream types because N is stored in macrophytes, but this issue was hard to elucidate from the data. Data fitting was strongly controlled by the highest NH_4^+ concentration represented by Lilleaa, and thus, more data from streams with high N concentrations are needed to clarify the efficiency loss in macrophyte streams compared to other stream types. On the basis of these results, we conclude that NH_4^+ concentration in stream water is the overall controlling factor for whole-stream NH_4^+ uptake rate across different stream types.

The higher whole-stream uptake rate with increasing water column NH_4^+ is best explained by higher standing stocks and biological activity in streams with high NH_4^+ concentration. Biomass-specific uptake rates for the different primary uptake compartments are in the same range across the streams indicating that the increased uptake rate was controlled by higher biomass rather than process rate. In the River Lilleaa, with the highest uptake rate and the highest water column NH_4^+ concentration, biomass standing stocks were higher than in other stream types (Dodds *et al.*, 2000; Ashkenas *et al.*, 2004; Tank *et al.*, 2000; Mulholland *et al.*, 2000; Simon *et al.*, 2007). The large N biomass was attributed to very high FBON pools, macrophytes themselves and high amounts of auto- and heterotrophic microorganisms because of more surface area provided by the macrophytes. Nitrogen stocks were also dominated by FBON in other streams, but the higher

nutrient concentration and the role of macrophytes in sedimentation (Sand-Jensen & Mebus, 1996) results in tenfold higher FBON pools in Lilleaa.

Longer-term nitrogen retention

Our results strongly indicate that macrophytes play an important role in the longer-term retention of N and thus a decrease in net downstream transport during the growth season compared to streams without macrophytes through direct and indirect effects on a stream reach.

First, macrophytes increase long-term retention through food-web relations. Turnover time for macrophytes measured in Lilleaa (21–36 days) was long and comparable to that found for moss by Ashkenas *et al.* (2004; 38 days). We found that macrophytes were labelled longer (48 days after addition stopped) than the other primary uptake compartments in the stream because of greater longevity and had a relatively high biomass-specific uptake rate. We also found that no primary consumers feed directly on macrophytes, and this also increased the turnover time. However, the prolonged labelling of detritus in the River Lilleaa indicates that a substantial part of the detritus is made up of macrophytes and will eventually enter the food web through decomposition or consumption of detached macrophyte fragments by heterotrophic microorganisms and detritivorous animals. Although herbivory on mature stream macrophytes is mostly low (Elger *et al.*, 2007), our results indicate that macrophytes might not be a total dead end in food-web relations. N retention in macrophytes at day 11 only constituted 3% of the total N retention, but macrophytes continue to grow over the season and little is eaten, and thus a substantial amount of N ends up in macrophyte biomass. Consequently, N retention could be substantial on a time scale of weeks and months during the growth season. The quantitative effect of this N accumulation in macrophyte biomass on whole-stream N retention was not quantified in this study but should be addressed in future studies.

Second, our data indicated that macrophytes have an indirect effect on N retention through higher sedimentation rates of particulate organic matter in macrophyte streams. We found that although N biomass stocks were dominated by FBON in all stream types, it was tenfold higher in our macrophyte stream. While deep FBON has a substantial N content, much of this may not be involved in transport processes, even with high discharge events (T. Riis, personal observations). Consequently, macrophytes could accumulate N within the stream reach making it unavailable for direct use at higher trophic levels and increasing net retention and decreasing net transport during the growth season. Moreover, the effect of macrophytes in sedimentation will also allow for potentially higher denitrification, which is the only removal mechanism of N in streams.

Acknowledgments

We thank The Danish Council for Independent Research and Carlsberg Foundation for financial support. Moreover, we thank the technicians at Plant Biology, Department of Bioscience, Aarhus University, for help in collecting data and analyse samples. We also thank Prof. Colin Townsend and two anonymous reviewers for valuable comments on the manuscript.

References

Alexander R.B., Boyer E.W., Smith R.A., Schwarz G.E. & Moore R.B. (2007) The role of headwater streams in downstream water quality. *Journal of the American Water Resources Association*, **43**, 1–19.

Ashkenas L.R., Johnson S.L., Gregory S.V., Tank J.L. & Wollheim W.M. (2004) A stable isotope tracer study of nitrogen uptake and transformation in an old-growth forest stream. *Ecology*, **85**, 1725–1739.

Bernot M.J. & Dodds W.K. (2005) Nitrogen retention, removal, and saturation in lotic ecosystems. *Ecosystems*, **8**, 442–453.

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

Dodds W.K., Lopez A.J., Bowden W.B., Gregory S., Grimm N.B., Hamilton S.K., *et al.* (2002) N uptake as a function of concentration in streams. *Journal of the North American Benthological Society*, **21**, 206–220.

Dodds W.K. & Oakes R.M. (2004) A technique for establishing reference nutrient concentrations across watersheds affected by humans. *Limnology and Oceanography, Methods*, **2**, 333–341.

Elger A., De Boer T. & Hanley M.E. (2007) Invertebrate herbivory during the regeneration phase: experiments with a freshwater angiosperm. *Journal of Ecology*, **95**, 106–114.

Greenberg A.E., Clesceri L.S. & Eaton A.D. (1992) *Standard Methods for the Examination of Water and Wastewater*, 18th edn. American Public Health Association, Washington, USA.

Hall R.O., Peterson B.J. & Meyer J.L. (1998) Testing a nitrogen cycling model of a forest stream by using nitrogen-15 tracer addition. *Ecosystems*, **1**, 283–298.

Holmes R.M., McClelland J.W., Sigman D.M., Fry B. & Peterson B.J. (1998) Measuring $^{15}N\text{-}NH_4^+$ in marine, estuarine, and fresh waters: an adaptation of the ammonium diffusion method for samples with low ammonium concentrations. *Marine Chemistry*, **60**, 235–243.

Madsen T.V. & Cedergreen N. (2002) Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. *Freshwater Biology*, **47**, 283–291.

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.

Meyer J.L. & Likens G.E. (1979) Transport and transformation of phosphorus in a forest stream ecosystem. *Ecology*, **60**, 1255–1269.

Mulholland P.J., Helton A.M., Poole G.C., Hall R.O., Hamilton S.K., Peterson B.J. *et al.* (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature*, **452**, 202–205.

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 15N tracer addition. *Ecological Monographs*, **70**, 471–493.

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: N-15 experiments across a broad gradient of nitrate concentrations. *Biogeochemistry*, **84**, 31–49.

Peterson B.J., Bahr M. & Kling G.W. (1997) A tracer investigation of nitrogen cycling in a pristine tundra river. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2361–2367.

Peterson B.J., Wollheim W.F., Mulholland P.J., Webster J.R., Meyer J.L., Tank J.L. *et al.* (2001) Control of nitrogen export from watersheds by headwater streams. *Science*, **292**, 86–90.

Richey J.S., McDowell W.H. & Likens G.E. (1985) Nitrogen transformation in a small mountain stream. *Hydrobiologia*, **124**, 129–139.

Riis T., Sand-Jensen K. & Larsen S.E. (2001) Plant distribution and abundance in relation to physical conditions and location within Danish stream systems. *Hydrobiologia*, **448**, 217–228.

Riley A. (2011) *Effects of Riparian Woody Vegetation Encroachment on Prairie Stream Structure and Function with Emphasis on Whole-Stream Metabolism*. PhD Dissertation, Kansas State University, Manhattan, KS, p. 166.

Royer T.V., Tank J.L. & David M.B. (2004) Transport and fate of nitrate in headwater agricultural streams in Illinois. *Journal of Environmental Quality*, **33**, 1296–1304.

Runkel R.L. (1998) One dimensional transport with inflow and storage (OTIS): a solute transport model for streams and rivers. US Geological Survey Water-Resources Investigation Report 98- 4018. US Geological Survey, Denver, Colorado. Available at: http://water.usgs.gov/software/OTIS/.

Sand-Jensen K. & Mebus J.R. (1996) Fine-scale patterns of water velocity within macrophyte patches in streams. *Oikos*, **76**, 169–180.

Seber G.A.F. & Le Cren E.D. (1967) Estimating population parameters from catches large relative to the population. *Journal of Animal Ecology*, **36**, 631–643.

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.

Simon K.S. & Benfield E.F. (2002) Ammonium retention and whole-stream metabolism in cave streams. *Hydrobiologia*, **482**, 31–39.

Simon K.S., Niyogi D.K., Frew R.D. & Townsend C.R. (2007) Nitrogen dynamics in grassland streams along a gradient of agricultural development. *Limnology and Oceanography*, **52**, 1246–1257.

Skirver J. (1982) *Food Resources of* Gammarus pulex (L.). In Danish. Ms Thesis, Freshwater Biological Laboratory, Copenhagen University, Copenhagen.

Smith V.H. (2003) Eutrophication of freshwater and coastal marine ecosystems. A global problem. *Environmental Science and Pollution Research*, **10**, 126–139.

Sørense P. & Jensen E.S. (1991) Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for 15N determination. *Analytical Chimica Acta*, **252**, 201–203.

Stelzer R.S., Bartsch L.A., Richardson W.B. & Strauss E.A. (2011) The dark side of the hyporheic zone: and its processing in stream sediments. *Freshwater Biology*, **56**, 2021–2033.

Strayer D.L. & Malcolm H.M. (2007) Submersed vegetation as habitat for invertebrates in the Hudson River Estuary. *Estuaries and Coasts*, **30**, 253–264.

Stream Solute Workshop. (1990) Concepts and methods for assessing solute dynamics in stream ecosystems. *Journal of the North American Benthological Society*, **9**, 95–119.

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

Wallace J.B. & Webster J.R. (1996) The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology*, **41**, 115–139.

Webster J.R., Mulholland P.J., Tank J.L., Valett M.H., Dodds W.K., Peterson B.J. *et al.* (2003) Factors affecting ammonium uptake in streams – an inter-biome perspective. *Freshwater Biology*, **48**, 1329–1352.

Webster J.R. & Valett H.M. (2006) Solute dynamics. In: *Methods in Stream Ecology* (Eds F.R. Hauer & G.A. Lamberti), pp. 169–187, Academic Press, London, U.K.

Wollheim W.M., Peterson B.J., Deegan L.A., Hobbie J.E., Hooker B., Bowden W.B. *et al.* (2001) Influence of stream size on ammonium and suspended particulate nitrogen processing. *Limnology and Oceanography*, **46**, 1–13.

Young R.G. & Huryn A.D. (1998) Comment: 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*, **55**, 1784–1785.

(Manuscript accepted 2 May 2012)