Uptake and trophic transfer of nitrogen and carbon in a temperate forested headwater stream

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Abstract

In temperate headwater streams, riparian forests hinder the development of algae by reducing light availability and generate large inputs of detritus. Microbial assemblages associated with this detritus are expected to strongly influence in-stream elemental cycling. However, most research has focused on quantifying nitrogen (N) cycling while we know little about the coupling of N and carbon (C) cycling. We conducted a simultaneous whole-reach tracer addition of ^{15}N-ammonium and ^{13}C-acetate in a forested headwater stream to examine the importance of different primary uptake compartments (e.g. epilithic biofilm, leaves, small wood) on N and C uptake, storage, and transfer to consumers. We predicted high whole-reach uptake of N and C from the water column to satisfy requirements of microbial decomposers. We also predicted a dominant role of the abundant detrital compartments, especially leaf litter, in the uptake, storage and trophic transfer of these labile forms of N and C. Our results show efficient immobilization of both ammonium and acetate along the study reach. Leaf litter showed the highest percentage of contribution among all compartments to whole-reach ammonium and acetate uptake. We also found evidence of rapid transfer of N and C to higher trophic levels, thereby extending the retention time of these elements within the ecosystem. Overall, our study provides relevant insights into the influence of detritus on N and C cycling in headwater streams.

Keywords Primary uptake compartments · Leaf litter · Food web · Stable isotopes · Tracer addition · Nitrogen · Carbon

Introduction

Headwater streams are characterized by strong interactions with the surrounding terrestrial ecosystem, from which they receive large inputs of nutrients, such as nitrogen (N), and organic carbon (C) (Lowe and Likens 2005). Biotic assemblages developed on streambed substrata play a relevant

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role in in-stream N and C cycling because they use these elements as resources to meet their energy and elemental demand (Peterson et al. 2001; Bernhardt and McDowell 2008). These assemblages, hereafter referred to as primary uptake compartments (PUCs; Tank et al. 2018), include primary producers, such as bryophytes, macrophytes or algae, which mostly obtain nutrients from the water column (Peipoch et al. 2014). PUCs also include heterotrophic organisms, mainly bacteria and fungi associated with detrital particulate organic matter (e.g., leaf litter, wood and fine organic particles), which can take up N and organic C from the water column, but also from the substrate where they develop (Gessner et al. 1999; Cheever et al. 2013; Pastor et al. 2014). All these PUCs are also important food resources for higher trophic levels, providing a direct pathway for the transfer of dissolved N and C in food webs, which can delay the export of these elements to downstream reaches (Hall and Meyer 1998; Dodds et al. 2000).

In forested headwater streams, the surrounding riparian vegetation hinders the development of primary producers by reducing light availability in the stream channel (von

Schiller et al. 2007). Nevertheless, riparian vegetation supplies large inputs of detrital particulate organic matter, mostly in the form of leaf litter (Pozo et al. 1997), which promotes the development of heterotrophic organisms that dominate the overall stream metabolism (Vannote et al. 1980; Wallace et al. 1997). In this context, microbial assemblages associated with leaf litter may exert a strong influence on the uptake, storage and trophic transfer of N and dissolved organic C (DOC) in headwater streams, especially in autumn when a large input of detritus occurs. In fact, several studies have indicated that forested streams show higher rates of nutrient uptake during autumn, coinciding with the peak of heterotrophic activity (Mulholland et al. 1985; Webster et al. 2000; Mulholland 2004; Goodale et al. 2009). On the one hand, microbial assemblages growing on leaf litter generally have lower C to N ratios than detritus, and they compensate for this elemental imbalance by relying on N from the water column (Sanzone et al. 2001; Dodds et al. 2004; Pastor et al. 2014). In this line, Tank et al. (2000) reported high uptake of NH_4^+ by the microbial assemblages colonizing leaf litter during autumn, showing the relevance of this compartment for N cycling at whole-reach scale. On the other hand, leaf litter can be a key source of organic C for heterotrophs. In this regard, Hall and Meyer (1998) found that the large abundance of detritus in forested streams during autumn can reduce the uptake of DOC from the water column by bacteria, which is considered the main driver of biological DOC removal in streams (Bott et al. 1984). Nevertheless, some studies have indicated that microbial assemblages on leaf litter can also rely on stream water DOC (Baldy et al. 2007; Pastor et al. 2014), although this uptake pathway seems strongly influenced by the DOC quality and composition (Mineau et al. 2016). Understanding these mechanisms is key to evaluate implications for DOC cycling in streams at broader spatial scales.

Experimental tracer additions of isotopically labelled compounds are a powerful tool for tracking the uptake of N and C from the water column into PUCs and their further transfer to higher trophic levels, without altering the ambient concentration of these elements in the stream water (Newbold et al. 1983; Mulholland et al. 2000a; Glibert et al. 2018). In the case of N, additions have generally used salts of inorganic N compounds, such as nitrate (NO_3^-) and ammonium (NH_4^+), enriched with the ^{15}N stable isotope as tracer (Peterson et al. 2001; Mulholland et al. 2004). Water column NH_4^+ is the preferred form of N for assimilatory uptake by autotrophic and heterotrophic microbial assemblages (Tank et al. 2008), and it can also be transformed into NO_3^- by nitrifying bacteria (Peterson et al. 2001). In forested headwater streams, results from whole-reach ^{15}N-NH_4^+ tracer additions show consistently high uptake efficiencies for NH_4^+ (Tank et al. 2018).

Unlike pathways of in-stream N uptake, pathways of DOC uptake in forested headwater streams have been examined to a lesser extent. Some studies have used additions of ^{13}C-labelled organic compounds as proxies for DOC, to follow the heterotrophic pathway of DOC uptake and storage in streams. The most frequently used DOC sources were either acetate (Hall 1995; Hall et al. 1998; Simon et al. 2003), a monomeric organic molecule representative of the labile fraction of stream DOC, or leachates from tree tissue (Wilcox et al. 2005; Kaplan et al. 2008; Wiegner et al. 2015). These studies focused mainly on DOC uptake by heterotrophic bacteria and its transfer to higher trophic levels, and showed a strong link from stream water DOC to bacteria and macroinvertebrates in headwater streams (Meyer 1994; Hall 1995). Surprisingly, to the best of our knowledge, only one previous study (Collins et al. 2016) has simultaneously examined the coupling between stream water N and DOC uptake using simultaneous stable isotope tracer additions of the two elements. Collins et al. (2016) focused on the trophic link between bacteria and consumers and the influence of light availability on this link; however, their study did not examine and compare the relative contribution of different PUCs to the uptake of N and DOC at the whole-reach scale. Nevertheless, due to the strong linkage between mechanisms involved in N and C cycling in streams (Bernhardt and Likens 2002; Trimmer et al. 2012; Ghosh and Leff 2013) and the observed influence of the C:N ratio on N uptake, important insights can arise from the evaluation of how PUCs simultaneously contribute to N and C dynamics in streams. Different proportions of PUCs could determine different retention and removal rates of these elements at reach-scale and influence their downstream export as well as mineralization and release to the atmosphere.

The aim of this study was to examine the relative contribution of microbial assemblages developed on the different PUCs to in-stream N and DOC cycling. We performed a simultaneous whole-reach tracer addition of ^{15}N-NH_4^+ and ^{13}C-acetate—both labile forms of N and C- in a headwater stream, and we evaluated the relative role of the different PUCs on N and C uptake, storage, and transfer to consumers. We predicted high whole-reach uptake of N and C from the water column to satisfy requirements of microbial decomposers in this headwater stream. Among PUCs, we expected a dominant role of detrital PUCs, especially leaf litter, in the uptake, and storage of these labile forms of N and C. Finally, we predicted that transfer of N and C from PUCs to higher trophic levels would vary among consumers depending on their feeding strategy and the uptake patterns of these elements among PUCs.

Methods

Study site

The Riera de Castellar is a calcareous second-order stream within the Fluvià River watershed (NE Iberian Peninsula). The climate in this area is Mediterranean, characterized by dry, warm summers and scarce precipitation occurring primarily in the spring and autumn. This stream is characterized by an intermittent flow regime and surface water flow usually ceases during summer. We selected a 77-m reach (42°14′57.043″N and 2°29′21.738″E) located at 415 m above sea level and without observed lateral outflows and inflows. Land cover in the watershed upstream of the selected reach (1.55 km^2) consists primarily of mixed forest (96%), with some agricultural (3%) and urban (0.1%) areas (Land Cover Map of Catalonia 2009, CREAF). The selected reach was characterized by alternating riffles and pools (73% and 27% of the total reach length, respectively). Streambed substrata were a mixture of sand (28%), gravel (28%), cobbles (21%) and bedrock (23%). The reach was flanked by a dense riparian forest dominated by *Quercus ilex*, *Corylus avellana*, *Salix* spp. and *Populus nigra*.

Whole-reach addition of ^{15}N and ^{13}C tracers

One week prior to the whole-reach tracer addition, a short-term constant rate addition of a solution including a conservative tracer (i.e., Cl^-, as NaCl) and a regular salt of NH_4^+ (i.e., NH_4Cl) was conducted in the selected reach to estimate the appropriate reach length, establish sampling stations, determine stream discharge and detect possible groundwater and lateral inflows and outflows. The selected site for the tracer addition was in a turbulent zone to allow fast mixing of the added solution with stream water. The first sampling station was established where this mixing was complete, based on cross-section measurements of stream conductivity. We established six sampling stations, which were situated at 17, 27, 37, 47, 57 and 77 m (1st to 6th station) downstream of the addition site. An additional sampling station was established 10 m upstream from the addition site (Up station) and served as a reference site of ambient stable isotope conditions.

The whole-reach ^{15}N-NH_4^+ and ^{13}C-acetate addition was conducted from 9 to 16 December 2013, just after autumn rainfalls and peak leaf fall, to ensure stable hydrological conditions and a well-developed microbial assemblage on leaf litter. The tracer addition was performed following procedures adapted from Hall and Meyer (1998), Mulholland et al. (2000b) and Tank et al. (2000).

At the addition site, we simultaneously added 267 mg of ^{15}N-NH_4Cl (^{15}N-NH_4^+; 72.7 mg ^{15}N; 99% enriched) and 16.5 g of ^{13}C-1-sodium acetate (^{13}C-acetate; 4.8 g ^{13}C; 99% enriched) to the stream. The solute addition, containing filtered stream water (300 μm mesh-size sieve) and the stable isotope tracers, was released from a 110-L carboy at a constant rate (10 mL min^{-1}) using a battery-powered peristaltic pump. Solute injection rate and battery status were checked daily during the addition period.

We collected water samples for nutrient chemistry and isotopic ^{15}N and ^{13}C signals at each sampling station just before the tracer addition (i.e., background sampling), 24 h and 7 days after the beginning of the tracer addition (i.e., plateau 24 h and plateau 7 days, respectively), and 4 h and 7 days after the end of the addition (i.e., post 24 h and post 7 days, respectively). All water samples were immediately filtered through pre-ashed glass fiber filters (Whatman GF/F, Whatman International, Maidstone, UK). Filtered samples for determination of nutrient chemistry (15 mL, two replicates per station) and ^{15}N-NO_3 (1 L, one replicate per station) were stored on ice in the field and then at −20 °C in the laboratory until analysis. Samples for DOC concentration (30 mL, one replicate per station) and ^{13}C-DOC (30 mL, one replicate per station) were acidified with 6 drops of 10% HCl and stored at 4 °C until analysis. Samples for ^{15}N-NH_4 (3 L, one replicate per station) were immediately processed as described in the laboratory methods section.

During the addition, stream water level was monitored every 10 min at the 6th station using a pressure transducer (Solinst Levelogger Junior Model 3001 and Solinst Barologger Gold Model 3001, Solinst Ltd, Georgetown, Canada). Short-term constant rate additions of Cl^- (as NaCl) were also conducted each time water samples were collected to determine stream discharge and to estimate tracer dilution at each station (Gordon et al. 1992). With these data, we developed an empirical relationship between stream water level and discharge that was used to estimate continuous values of stream discharge over the course of the addition. Water temperature, conductivity, dissolved oxygen concentration (DO) and % saturation were recorded continuously at the Up station and the 6th station with optical oxygen probes (YSI 6150 connected to YSI 600 OMS; YSI Corporation, Yellow Springs, OH, USA).

Sampling of PUCs and macroinvertebrates

One day after the end of the addition, we determined wetted width, channel depth, percent coverage of the different substrate types (bedrocks, gravel, sand and cobbles), and the coverage of PUCs and stream habitats (riffle or pool) using cross-sectional point transects located every 5 m along the selected reach. Sampled PUCs consisted of epilithic biofilm (hereafter 'epilithon'), submerged bryophytes, fine

benthic organic matter (FBOM, < 1 mm) and coarse benthic organic matter (CBOM, > 1 mm) sorted into leaf litter and small wood. Reach-weighted standing stocks of biomass, expressed as ash free dry mass (g AFDM m^{-2} reach), and N and C (g N m^{-2} reach and g C m^{-2} reach, respectively) for each PUC were estimated using the percent reach coverage and the average patch-specific estimates of AFDM and N and C content (as % dry mass) along the reach. Samples to measure the patch-specific biomass for epilithon were obtained by scraping the surface of three randomly collected cobbles at each station and filtering the slurries onto pre-ashed glass fiber filters. The cobble surface was estimated by covering it with aluminium foil and applying a weight to area relation. Samples for CBOM biomass were collected from three corers (400 cm^2) placed into the sediment at each station. CBOM samples were sorted into small wood and leaves. Bryophytes, when present at the sampling stations, were sampled by scraping the material found within a 225 cm^2 corer. Samples for FBOM were collected using a 346 cm^2 corer placed over the sediment surface at each sampling station. The water depth in the cylinder was measured, and after sediments were vigorously agitated, aliquots (~ 40 mL) of the total volume were filtered onto pre-ashed glass fiber filters. Additionally, concentration of suspended particulate organic matter (SPOM) in stream water was determined by filtering a known volume of water from each station onto pre-ashed glass fiber filters. For all PUCs and SPOM, we collected 3–5 samples per sampling station and combined them to make one composite sample for analysis of elemental content and ^{15}N and ^{13}C isotopes. These samples were collected at plateau 7 days, and at 24 h, 7 days, and 14 days after the end of the addition (post 24 h, 7 days and 14 days) following the same methods as with the standing-stock sampling. Samples from the Up station were used as the ambient values for ^{15}N and ^{13}C isotopic signal for each PUC on each sampling date. All samples were dried at 60 °C until reaching a constant weight and further processed as described in the laboratory methods section.

Macroinvertebrates were collected at plateau 7 d. For biomass estimations, organisms were sampled in riffles using a Surber net (three replicates, 0.06 m^2, 250 μm mesh) at each sampling station. Samples were preserved in a 70% alcohol solution, and all individuals were sorted from the debris upon arrival to the laboratory. All taxa were identified and counted. The invertebrate biomass was estimated by a length/dry mass conversion, based on the application of a regression function for each specific taxon (Meyer 1989; Burgherr and Meyer 1997). Abundances and biomass were converted to units of streambed surface (m^2) based on the percentage of riffle habitat in the reach. The most abundant macroinvertebrate species (Table S1) that were present at all stations, including the Up station, were selected for the elemental and ^{15}N and ^{13}C analyses. These invertebrates were hand-picked directly in the field at the sampling stations and stored in plastic vials with a small amount of stream water, where they were kept for 24 h to allow them to clear their gut contents. After this period, we removed the snails from their shells, and all individuals were dried at 60 °C until constant weight and stored in a desiccator until further processing. Organisms from shredders and collector-filterer feeding groups could not be used for isotopic analyses because in the former, density and size were too small for reliable isotopic analyses, and in the latter, there were no collector-filterers at the Up station, so we were unable to calculate isotopic enrichment.

Laboratory methods

Water samples were analysed for NH_4^+, NO_3^-, and soluble reactive phosphorus (SRP) following standard colorimetric methods (American Public Health Association 1995) on an Automatic Continuous Flow Futura–Alliance Analyser (Alliance Instruments, France). The concentration of acetate was analysed by ion exclusion chromatography with an HPX-87H organic acid column (300 × 7.8 mm; Aminex, Bio-Rad, UK) and a mobile phase of 0.01 M H_2SO_4 at a flow rate of 0.8 mL min^{-1} at 65 °C. The isotopic signals of ^{15}N-NH_4^+ and ^{15}N-NO_3^- were determined following the ammonia-diffusing technique adapted from Holmes et al. (1998) and Sigman et al. (1997), respectively. For the ^{15}N-NO_3^- analyses, we amended each water sample with 3 g of MgO and 5 g of NaCl, and then, we boiled it to concentrate the water volume and remove the dissolved NH_4^+. Water samples were then amended with 0.5 mg MgO and 0.5 mg Devarda's alloy (to reduce NO_3^- to NH_4^+) and then processed as described for ^{15}N-NH_4^+ estimates. The ^{15}N-NH_4^+ samples were amended with 3 g L^{-1} of MgO, 50 g L^{-1} of NaCl and a Teflon filter pack containing a pre-ashed Whatman GF/D glass fiber filter (1 cm Ø) acidified with 25 mL of 2.5 M $KHSO_4$ and incubated on a shaker at 40 °C for 4 weeks. Due to the low NH_4^+ concentration in the stream water, the ^{15}N-NH_4^+ samples were spiked with 20 μg of unlabelled NH_4^+ to reach the detection limit needed for the N mass spectrometer analyses. To determine the influence of the NH_4^+ spike on the ^{15}N signal, we added 20 μg of unlabelled NH_4^+ to 3 L deionized water and processed these samples identically to the ^{15}N-NH_4^+ water samples. At the end of the incubation, we removed the filter packs, dried them in desiccators for 4 days, encapsulated the filters in tins and stored them until the ^{15}N analysis (see below). The DOC concentration was determined on a Shimadzu TOC-V CSH analyser (Shimadzu Corporation, Kyoto, Japan). Water samples of the ^{13}C-DOC were bubbled for 6 min with compressed nitrogen gas (N_2) prior to the isotopic analyses to remove gaseous inorganic C retained after acidification. After that, the samples were analysed by FIA-IRMS (Flow

Injection Analysis–Isotope Ratio Mass Spectrometry) using a system composed of an LC unit (Surveyor MS-pump with autosampler) coupled to a DeltaV-Advantage IRMS via an LC-IsoLink interface (Thermo Fisher Scientific, Germany) to determine their $^{13}C:^{12}C$ ratio.

Samples from all PUCs were dried at 60 °C for several days and weighed to the nearest 0.1 mg to determine the dry mass. A subsample of the dried material was combusted at 500 °C for 4 h to calculate the ash-free dry mass (AFDM) for biomass standing-stock estimations. For the elemental and isotopic analyses of N and C, another subsample of the dried material of bryophytes, leaf litter and small wood was ground to a fine powder, weighed to the nearest 0.001 mg, encapsulated in tins and stored until analysis. For the epilithon, FBOM and SPOM samples, discs of a known surface area were cut out from the previously oven-dried filters, weighed to the nearest 0.001 mg, encapsulated in tins and processed as the rest of samples. For macroinvertebrates, the dried organisms were ground, weighed to the nearest 0.001 mg, encapsulated in tins and stored until analysis. While the largest macroinvertebrates were analysed individually, for taxa of smaller size we used a composite sample of 2–5 individuals for each station. Whole-body samples were analysed for all taxa except for odonates, from which we used only muscle tissue.

Encapsulated samples of ^{15}N-NO_3^-, ^{15}N-NH_4^+, SPOM, PUCs and macroinvertebrates were analysed for N and C content (as a percentage of the total dry mass) and the ^{15}N and ^{13}C isotopic signal by the EA-IRMS technique (Elemental Analysis—Isotope Ratio Mass Spectrometry) using a Carlo-Erba Flash 1112 series elemental analyser (Carlo-Erba, Italy) interfaced with a Finnigan Delta C isotope ratio mass spectrometer (IRMS) via a Finnigan Conflo III interface (Thermo Fisher Scientific, Germany). Measurement precision was 0.1‰ and 0.3 ‰ for $^{13}C:^{12}C$ and $^{15}N:^{14}N$, respectively.

Parameter calculation

Data for ^{13}C and ^{15}N were expressed as δ values in per mil units (‰) using international reference standards Vienna Pee Dee Belemnite and air, respectively. The $δ^{13}C$ and $δ^{15}N$ values from the isotope tracer addition were corrected for ambient isotope values measured at the Up station to estimate the isotopic enrichment during the addition.

We estimated the uptake length (S_w, m; i.e., the average distance travelled by a molecule before being removed from the water column) and the uptake velocity (V_f, mm min^{-1}; i.e., the velocity at which a nutrient molecule is removed from the water column) for NH_4^+ and acetate at plateau 24 h and 7 days based on Mulholland et al. (2000b). The S_w was calculated as the inverse of the slope of the linear regression between the natural logarithm of the tracer ^{15}N-NH_4^+

or ^{13}C-DOC flux in the water column at each sampling station and the distance downstream. The V_f was calculated by dividing specific discharge (i.e., discharge/wetted width) by the S_w of ^{15}N-NH_4^+ and ^{13}C-acetate. Tracer ^{15}N-NH_4^+ or ^{13}C-DOC fluxes (µg ^{15}N s^{-1}; mg ^{13}C s^{-1}) were estimated using ambient-corrected $δ^{15}N$-NH_4^+ or $δ^{13}C$-DOC, the NH_4^+ or DOC concentrations, and dilution-corrected values of stream discharge at each sampling station. We estimated whole-reach ^{15}N-NH_4^+ areal uptake from the water column ($U_{NH4water}$; mg N m^{-2} day^{-1}) by multiplying V_f by the mean NH_4^+ concentration. We calculated the whole-reach areal nitrification (U_{NIT}; mg N m^{-2} day^{-1}) using the background-corrected $δ^{15}N$-NO_3^- at plateau 24 h following procedures by Mulholland et al. (2000b) and Hamilton et al. (2001). Even though we used ^{13}C-acetate as a tracer, we estimated $δ^{13}C$-DOC in water because the acetate concentration was below the detection limit (0.5 mg L^{-1}). We estimated S_w and V_f for acetate from longitudinal changes in $δ^{13}C$-DOC; however, we were not able to calculate areal uptake for the whole reach because we would need to assume that all stream water DOC was acting as a labile fraction of C (i.e., acetate); and thus, this would probably overestimate areal uptake of DOC.

The reach-weighted masses of isotopic enrichment (in mg ^{15}N m^{-2} reach and mg ^{13}C m^{-2} reach) measured for each PUC and macroinvertebrate taxon were calculated as the product of the fraction of ^{15}N or ^{13}C, obtained using the ambient-corrected $δ^{15}N$ and $δ^{13}C$, and the estimated standing stock of N and C at the reach scale (mg N m^{-2} reach; mg C m^{-2} reach). Using samples from plateau 7 d, compartment-specific areal uptake for NH_4^+ (U_{NH4}; mg N m^{-2} d^{-1}) and acetate ($U_{acetate}$; mg C m^{-2} d^{-1}) were calculated as the reach-weighted mass of tracer ^{15}N or ^{13}C in each compartment divided by the time of addition (7 days) and the fraction of ^{15}N or ^{13}C present in the water column at the same station and sampling time (Hamilton et al. 2001). These calculations were performed only with samples from the upper three stations (1st to 3rd station), since they were the least affected by the isotopic signal from N and C regeneration, and average values for these three stations were used as the compartment-specific areal uptake of ^{15}N-NH_4^+ and ^{13}C-acetate at the reach scale. The turnover rates of N and C (d^{-1}) for each benthic biomass compartment were estimated from the decay in the ^{15}N and ^{13}C signal at the 1st station over the first 14 days after the end of the addition (Mulholland et al. 2000b; Tank et al. 2000). We assumed first-order dynamics [i.e., slope of the linear relationship between ln ($^{15}Nbiomass$) or ln ($^{13}Cbiomass$) and time]. This approach assumes no reuptake of ^{15}N released to water upstream, a reasonable assumption for the upper sampling station, but may result in slight underestimates of turnover rate. Turnover times were calculated as the inverse of turnover rates.

We also used ^{15}N and ^{13}C labelling to estimate reach-scale N and C storage in PUCs and selected macroinvertebrate

taxa at the end of the addition, expressed as a percentage of total ^{15}N and ^{13}C added that was retained along the reach. For each compartment, we used the integration of the downstream decline in their tracer ^{15}N or ^{13}C mass along the reach, considering a reach length of five times S_w for each tracer (Mulholland et al. 2000a, b). In cases where the slope of the regression of tracer ^{15}N or ^{13}C mass vs. distance was not significant ($p > 0.05$), we used the mean tracer ^{15}N or ^{13}C mass for the entire reach (Hamilton et al. 2001; Ashkenas et al. 2004). The total mass export of ^{15}N (mg ^{15}N) and ^{13}C (mg ^{13}C) tracers from the study reach as NH_4^+, NO_3^-, DOC and SPOM during the tracer addition was calculated from the product of the fluxes of tracer ^{15}N (mg ^{15}N s^{-1}) or ^{13}C (mg ^{13}C s^{-1}) at the 6th station at plateau 7 days for each form, the discharge, and the total addition time (Tank et al. 2018).

Data analysis

The profiles of ^{15}N-NH_4^+ and ^{13}C-DOC fluxes along the reach were compared between tracers and sampling times (plateau 24 h and plateau 7 days) by one-way analyses of covariance (ANCOVA) using distance as a co-variable for all tests.

To evaluate the feeding relation between macroinvertebrates and PUCs as potential benthic food resources, we calculated the ratio of $\delta^{15}N$ or $\delta^{13}C$ signals between macroinvertebrates and their potential food resources (i.e., PUCs; Mulholland et al. 2000a; Simon et al. 2003). To determine these pairs, we used the most common food resources and feeding strategies described in the species traits list of Tachet et al. (2000). We used tracer ambient-corrected $\delta^{15}N$ or $\delta^{13}C$ to remove the effect of trophic fractionation. Ratios were calculated for each sampling station only when both the macroinvertebrate and the food resource were labelled. Ratios for each station were averaged to obtain a representative consumer-food resource ratio at the reach scale. We also performed simple linear regressions between each consumer-food pair to illustrate these relations.

All statistical analyses were conducted using R version 3.2.2, with a significance level set at a $p < 0.05$.

Results

Environmental conditions

Environmental conditions in the reach remained relatively constant over the course of the 7-day tracer addition. The stream was shallow (mean depth $\pm$ SE = 6 $\pm$ 1 cm) and relatively narrow (mean width $\pm$ SE = 1.9 $\pm$ 0.2 m, range = 0.9–2.9 m), and discharge was low ranging between 3.0 and 3.8 L s^{-1} (mean $\pm$ SE = 3.33 $\pm$ 0.01 L

s^{-1}). The water temperature was low, ranging from 2.3 °C to 4.4 °C (mean $\pm$ SE = 3.3 $\pm$ 0.01 °C). The stream water was highly oxygenated (mean $\pm$ SE = 13.0 $\pm$ 0.01 mg O$_2$ L^{-1}, range = 13.5–12.6 mg O$_2$ L^{-1}), and DO saturation approached 100% (mean $\pm$ SE = 97.6 $\pm$ 0.03%, range = 95.7–100.0%). Conductivity was relatively high (mean $\pm$ SE = 513 $\pm$ 0.1 μS cm^{-1}, range = 518–508 μS cm^{-1}), which is common in calcareous streams. In general, the concentration of dissolved inorganic nutrients was low, especially in the case of SRP (mean $\pm$ SE = 5 $\pm$ 0.2 μg P L^{-1}) and NH_4^+ (mean $\pm$ SE = 1 $\pm$ 0.1 μg N L^{-1}). The concentration of NO_3^- averaged 102 $\pm$ 4 μg N L^{-1}. The concentration of DOC was also low, averaging 1.30 $\pm$ 0.03 mg C L^{-1}, and the concentration of acetate was below the analytical detection limit of 0.5 mg L^{-1}. The tracer addition resulted in an increase of 5% and 0.2% of the ambient NH_4^+ and DOC stream concentrations, respectively.

Whole-reach ^{15}N-NH_4^+ and ^{13}C-acetate uptake

Fluxes of tracer ^{15}N-NH_4^+ and ^{13}C-DOC at plateau decreased along the reach, and the decrease was similar between samplings done at plateau 24 h and at plateau 7 days (Sampling time × Distance: $F_{1,8} = 1.2$, $p = 0.3$ for ^{15}N-NH_4^+; and Sampling time × Distance: $F_{1,8} = 1.8$, $p = 0.20$ for ^{13}C-DOC). In addition, the fluxes of the two tracers showed a similar decrease along the reach (Tracer type × Distance, $F_{1,8} = 0.5$, $p = 0.50$; Fig. 1). The S_w was 35 m for ^{15}N-NH_4^+ and 44 m for ^{13}C-acetate, and the V_f was 3.0 mm min^{-1} for ^{15}N-NH_4^+ and 2.4 mm min^{-1} for ^{13}C-acetate. The total $U_{NH4water}$ was 4.2 mg N m^{-2} day^{-1}, and nitrification only accounted for 9% of this uptake. At post 24 h and post 7 d samplings, the $\delta^{15}N$-NH_4^+ and $\delta^{13}C$-DOC returned to background levels, and tracer fluxes of ^{15}N-NH_4^+ and ^{13}C-DOC were practically undetectable.

Contribution of PUCs to whole-reach ^{15}N-NH_4^+ and ^{13}C-acetate uptake

The total biomass standing stock of the sampled PUCs in the study reach was 125.1 g AFDM m^{-2}. Detrital compartments (leaf litter, small wood and FBOM) accounted for 96% of this stock, with leaf litter being the dominant compartment in the reach (49% of total biomass standing stock). Epilithon and bryophytes only accounted for 4% in the total biomass standing stock. The N and C standing stocks accounted for the sampled PUCs in the reach were 1.62 g N m^{-2} and 74.8 g C m^{-2}, respectively (Table 1). Detrital compartments accounted for 93% and 95% of the total standing stocks of N and C, respectively, with leaf litter being the dominant compartment (55% for N; 46% for C). Epilithon (6% for N; 4% for C) and bryophytes (1% for N; 1% for C) accounted for a small proportion of the total standing stocks of N and

Fig. 1 Linear regressions for the natural logarithm of tracer ^{15}N-NH_4^+ flux (continuous line) and tracer ^{13}C-DOC flux (dashed line) in the water column over reach distance at plateau 7 days. The inverse of each regression slope is the uptake length (S_w) of each tracer. The result from a one-way ANCOVA with tracer type (^{15}N-NH_4^+ or ^{13}C-DOC) as factor and distance as a co-variable is shown
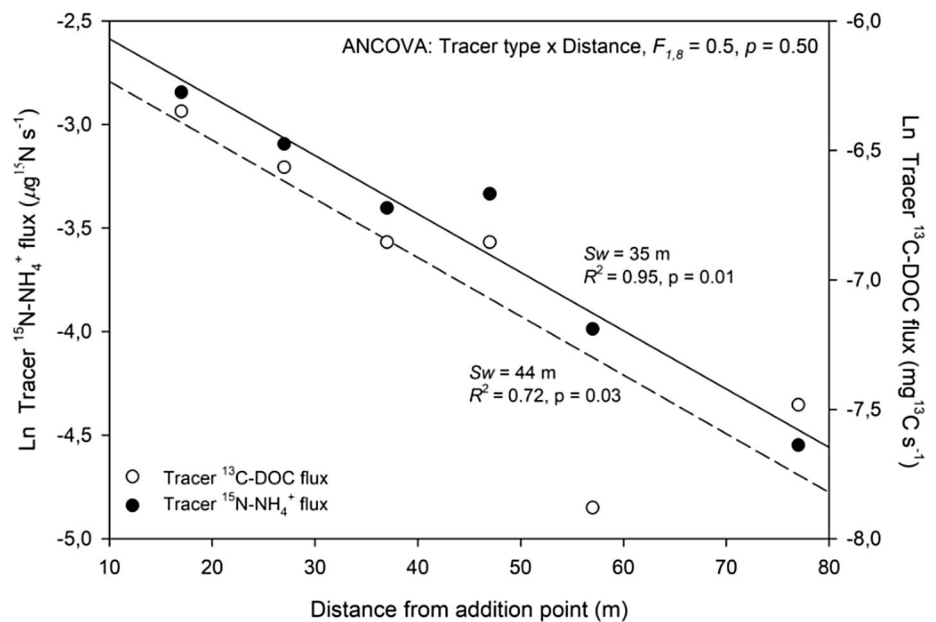

Table 1 Standing stocks of C (g C m^{-2} reach) and N (g N m^{-2} reach), C to N molar ratio, areal uptake of acetate ($U_{acetate}$, mg C m^{-2} day^{-1}) and NH_4^+ (U_{NH4}, mg N m^{-2} day^{-1}), and the molar ratio of these uptakes ($U_{acetate}$: U_{NH4}) for each compartment and for the entire reach based on data from plateau at 7 days

Compartment	C stock g C m^{-2}	N stock g N m^{-2}	C:N Molar ratio	$U_{acetate}$ mg C m^{-2} day^{-1}	$U_{acetate}^a$ day^{-1}	U_{NH4} mg N m^{-2} day^{-1}	U_{NH4}^a day^{-1}	$U_{acetate}$:U_{NH4} Molar ratio
Epilithon	2.9	0.10	33.2	107.4	0.040	0.30	0.0001	411.6
Bryophytes	0.7	0.02	40.5	6.7	0.010	0.11	0.0001	71.9
FBOM	10.3	0.24	50.7	152.6	0.015	0.18	0.0174	1000.3
Leaf litter	34.4	0.89	45.0	255.7	0.007	0.72	<0.0001	417.3
Small wood	26.5	0.37	83.4	93.6	0.003	0.04	<0.0001	2503.6
Reach total	74.8	1.62		616.0		1.35		

FBOM fine benthic organic matter

^{a}Uptake normalized by the standing stocks

C. The C:N molar ratio varied among PUCs, ranging from 33.2 in epilithon to 83.4 in small wood (Table 1). The total assimilatory uptake determined from the sum of all compartment-specific uptakes was 1.35 mg N m^{-2} day^{-1} for NH_4^+ and 616.0 mg C m^{-2} day^{-1} for acetate (Table 1). Leaf litter accounted for the largest proportion of this assimilatory uptake for the two elements (53% for N and 42% for C), followed by epilithon for N uptake (22%) and FBOM for C uptake (24%). Small wood (3%) and bryophytes (1%) accounted for the lowest proportion of total assimilatory N and C uptake, respectively. The molar ratios between the compartment-specific $U_{acetate}$ and U_{NH4} were > 1, indicating a higher uptake for labile C than for N from the water column for all PUCs (Table 1). However, this ratio differed widely across PUCs, ranging from 71.9 in bryophytes to 2503.6 in small wood (Table 1).

At the end of the 7-day tracer addition, we accounted for 76% and 14% of the total ^{15}N and ^{13}C added, respectively (Table 2). In this sense, PUCs contributed to the storage of 65% and 7% of the total ^{15}N and ^{13}C added, respectively (Table 2). Leaf litter contributed to the largest fraction of this storage (39.7% of ^{15}N added; 2.9% of ^{13}C added) and epilithon also contributed to a relevant fraction of it (14.7% of ^{15}N added; 2.3% of ^{13}C added).

Over the first 14 days after the addition ended, leaf litter showed a significant decline in the $\delta^{15}N$ and $\delta^{13}C$ values. Based on this decline, the turnover rates of N and C for leaf litter were 0.088 day^{-1} (i.e., a turnover time of 11.4 days) and 0.047 day^{-1} (i.e., a turnover time of 21.3 days), respectively. Small wood also showed a significant decline in $\delta^{13}C$ over time, with a turnover rate of 0.036 day^{-1} (i.e.,

Table 2 Percentage of the total ^{15}N and ^{13}C tracer stored in PUCs and macroinvertebrates or exported at the end of the addition

	Tracer ^{13}C added	Tracer ^{15}N added
	%	%
Stored		
Epilithon	2.3	14.7
Bryophytes	0.2	1.9
FBOM	1.0	6.3
Leaf litter	2.9	39.7
Small wood	0.5	1.4
Habroleptoides sp.	0.113	0.56
Oulimnius sp.	0.001	0.01
Radix sp.	0.105	0.04
Boyeria irene	0.003	0.03
Total stored	7.2	64.8
Exported		
SPOM	0.04	0.2
NH_4^+	–	8.6
NO_3^-	–	2.5
DOC	6.8	–
Total exported	6.8	11.2
Reach total	14.0	76.0

FBOM fine benthic organic matter

a turnover time of 27.8 days). For the remaining PUCs, the declines in the δ^{15}N and δ^{13}C values were not significant.

Transfer of ^{15}N and ^{13}C tracers to macroinvertebrates

The biomass of the taxa of benthic macroinvertebrates considered in the tracer addition (81% of total abundance found in the reach) was 0.2 g DM m^{-2}. This biomass was dominated by grazers (39%; primarily *Habroleptoides* sp.) and predators (34%; primarily *Boyeria irene*), followed by collector-filterers (15%; primarily *Hydropsyche* sp.), collector-gatherers (11%; primarily *Radix* sp.) and a negligible presence of shredders (1%; primarily *Nemoura* sp.). Macroinvertebrate feeding groups considered in parameter calculations (grazers, collector-gatherers and predators) accounted for 84% of the total biomass of selected consumers. The total N and C standing stocks associated with the selected macroinvertebrates were 16.2 mg N m^{-2} and 69.9 mg C m^{-2} (Table 3), which represented 1% of the total N (1.64 g N m^{-2}) and 0.1% of the total C (74.9 g C m^{-2}) standing stock measured at the reach scale. The N and C standing stocks of the macroinvertebrates were dominated by *Habroleptoides* (37% of N standing stock; 41% of C standing stock) and *Boyeria irene* (49% of N standing stock; 44% of C standing stock). The C:N molar ratios were similar

Table 3 Standing stocks of C (mg C m^{-2}) and N (mg N m^{-2}) and molar C to N ratio (C:N) for each sampled macroinvertebrate across the study reach

Macroinvertebrate	C stock mg C m^{-2}	N stock mg N m^{-2}	C:N Molar ratio
Grazer—*Habroleptoides* sp.	28.3	6.0	5.4
Grazer—*Oulimnius* sp.	2.5	0.5	5.5
Collector gatherer—*Radix* sp.	8.2	1.8	5.3
Predator—*Boyeria irene*	30.9	7.9	4.6
Total	69.9	16.2	

among macroinvertebrates, with values close to 5 for all of them (Table 3).

All sampled macroinvertebrates were labelled with ^{15}N and ^{13}C by the end of the 7-day tracer addition. *Habroleptoides* showed the highest isotopic tracer labelling, with δ^{15}N and δ^{13}C tracer values following those of epilithon, its potential food source, along the reach. In fact, the δ^{15}N ratio between *Habroleptoides* and epilithon averaged 1.1 ± 0.2 along the reach (mean $\pm$ SE; Fig. 2, a1). In contrast, the δ^{13}C ratio between *Habroleptoides* and epilithon averaged 17.0 ± 8.0 (mean $\pm$ SE; Fig. 2, a2). *Oulimnius*, another grazer that presumably feeds on epilithon, was less isotopically enriched than epilithon and showed a relatively constant isotopic signature along the reach for the two tracers (Fig. 2, b1 and b2). In relation to the collector-gatherer *Radix*, the δ^{15}N values closely followed those of the δ^{15}N in FBOM along the reach, and the isotopic ratio between them was 1.1 ± 0.2 (mean $\pm$ SE; Fig. 2, c1). In contrast, the δ^{13}C ratio between *Radix* and FBOM averaged 4.3 ± 2.2 (mean $\pm$ SE; Fig. 2, c2). The predator *Boyeria irene* was less isotopically enriched than any of the other macroinvertebrate taxa sampled. The ratios between the average isotopic signal from all sampled primary consumers and the isotopic signal for this predator were 0.2 ± 0.05 for δ^{15}N and 0.1 ± 0.04 for δ^{13}C. At the end of the 7-day tracer addition, storage of N and C in sampled macroinvertebrates accounted for 0.6% and 0.2% of the total ^{15}N and ^{13}C tracer added, respectively (Table 2).

Discussion

Whole-reach N and C uptake

Our results indicated that NH_4^+ and acetate uptake in the study reach were high (i.e., short S_w and high V_f for both elements) as was anticipated in our initial prediction. Stream water NH_4^+ was efficiently removed from the water column, exhibiting a V_f of 3.0 mm min^{-1}, that is within the range of values found in other headwater streams (NH_4^+ V_f range: 0.2–10.6 mm min^{-1}, Hall et al. 2002; von Schiller et al.
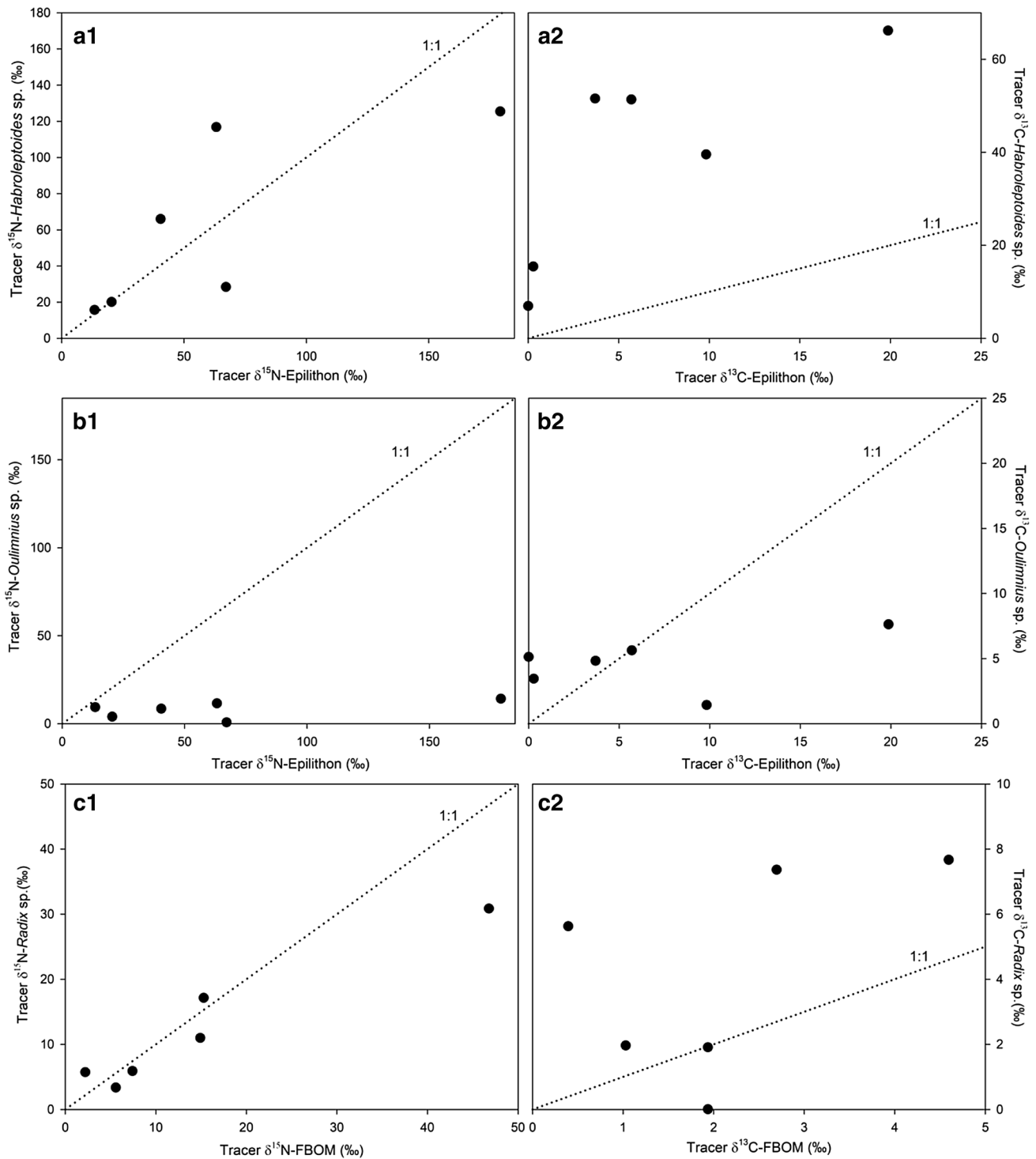
Fig. 2 Relationship between the isotopic signal of each macroinvertebrate taxon (**a–c**) and that of its potential food resource for the ^{15}N tracer (1) and the ^{13}C tracer (2). Values are from samples collected at plateau 7 days. The 1:1 relation is indicated on each panel with a pointed line

2008; Martí et al. 2009). Despite the fact that our study reach had a standing stock of leaf litter similar to that described by Tank et al. (2000), their reported values of $NH_4^+ V_f$ were 10 times higher than our measured values. This may be explained by the fact that cold water temperatures limited microbial activity in our stream during the addition period (Fabre and Chauvet 1998; Brown et al. 2004). Indeed, measurements of ecosystem respiration in the study reach during

the following autumn (ER = 4.5 g O_2 m^{-2} day^{-1}; Pastor et al. 2017) were 7 times lower than those found by Tank et al. (2000). Previous studies have indicated that NH_4^+ V_f is positively related to ecosystem respiration (Hall and Tank 2003) and negatively related to stream water temperature at low discharges (Butturini and Sabater 1998), which supports the observed interpretation.

The rapid V_f estimated for acetate in our reach (2.4 mm min^{-1}) was similar to those previously reported for in-stream uptake of labile DOC forms in streams (V_f range: 0.1–28.7 mm min^{-1}, Kaplan et al. 2008; Martí et al. 2009; Johnson et al. 2009; Mineau et al. 2016). Despite the large amounts of detritus on the streambed in the sampling period, our results support that labile forms of DOC are at least as strongly retained as NH_4^+ in streams (Wiegner et al. 2005; Martí et al. 2009; Johnson et al. 2009). Given the stream acetate concentration maintained during the addition, we assumed that only bacteria were able to take up the tracer ^{13}C-acetate (Wright and Hobbie 1966; Newell 1984; Hall and Meyer 1998). We acknowledge that labile DOC, such as acetate, comprises a small fraction of the bulk DOC, but it represents the in-stream dynamics of a readily available form of C (Mineau et al. 2016). Moreover, our results support the idea that labile DOC can be an important energy source for heterotrophic bacteria developed on streambed substrata (Kaplan and Newbold 2003; Wiegner et al. 2015; Catalán et al. 2018; Seybold and McGlynn 2018).

Relative contribution of PUCs to N and C uptake

The microbial assemblages established on leaf litter showed the highest contribution to whole-reach uptake of NH_4^+ and acetate in our stream. The importance of leaf litter to whole-reach NH_4^+ uptake was previously described in forested streams (Mulholland et al. 2000b; Tank et al. 2000). However, the few previous ^{13}C-acetate addition studies (Hall and Meyer 1998; Simon et al. 2003; Collins et al. 2016) showed low or even non-detectable isotopic enrichment in leaf litter. Labelling by itself does not quantify the role of leaf litter in the uptake of DOC because the large amount of unlabelled detrital C in detritus diluted the ^{13}C tracer taken up by the active microbes (Hall and Meyer 1998; Collins et al. 2016). In contrast, our estimation of the specific compartment areal uptake at the whole-reach scale indicated that, despite a low ^{13}C enrichment, the relative contribution of heterotrophic bacteria developed on leaf litter was important to DOC uptake at whole-reach scale.

Our results showed differences in the relative contribution of different detrital compartments to whole-reach NH_4^+ and acetate uptake. Small wood exhibited a moderate contribution to whole-reach acetate uptake despite its large standing stock, indicating a low use of water column DOC by the bacteria colonizing this substrate. This pattern does not

necessarily indicate a reliance of microbial assemblages on the substrate C, but most likely it suggests a low bacterial decomposer activity on small wood. Wood is considered poor-quality organic matter litter due to its high C:N ratio and low surface-to-volume ratio (Allan and Castillo 2007; Arroita et al. 2012). These characteristics limit the development of microbial assemblages on wood, which results in low decomposition rates (Meentemeyer 1978; Melillo et al. 1982). FBOM was the second compartment after leaf litter contributing to whole-reach acetate uptake. Two similar previous ^{13}C-acetate tracer additions reported FBOM as the most-labelled detrital compartment (Hall and Meyer 1998; Simon et al. 2003). FBOM is considered more refractory than leaf litter (Yoshimura et al. 2008), but in contrast to leaf litter, its microbial biomass is mainly formed by bacteria rather than by fungi (Weyers and Suberkropp 1996; Hamilton et al. 2001; Sanzone et al. 2001). Therefore, FBOM may contain a dominant bacterial biomass that would be more dependent on DOC than the bacteria colonizing the leaf litter. In this sense, if areal acetate uptake values obtained in our study are normalized by the compartment C standing stock, they are higher in FBOM (0.015 day^{-1}; Table 1) than in leaf litter (0.007 day^{-1}; Table 1). The higher relative contribution of leaf litter to whole-reach acetate uptake than that of FBOM was likely the result of the larger standing stock of leaf litter. Regarding NH_4^+ uptake, leaf litter had 4 and 18 times greater areal N uptake than FBOM and small wood, respectively. As in previous ^{15}N studies (Mulholland et al. 2000b; Tank et al. 2000), our results indicate that microbial assemblages in leaf litter were taking up more N than those assemblages in FBOM or small wood. This microbial biomass has large amounts of C available in leaf litter, but the leaf N content is low relative to the stoichiometric requirements for microbial growth. Our results indicate that to overcome the stoichiometric constraints caused by this large elemental imbalance, microbes rely upon dissolved N (Sterner and Elser 2002; Cheever et al. 2013; Pastor et al. 2014).

Epilithon contributed significantly to whole-reach areal NH_4^+ and acetate uptake despite its low standing stock. In addition, epilithon had a similar $U_{acetate}:U_{NH4}$ molar ratio compared to leaf litter. These results suggest an important reliance on N by epilithon (auto- and heterotrophs), together with an important reliance on DOC by epilithic heterotrophic bacteria, thereby denoting a certain heterotrophic character of the epilithon. By contrast, as we expected, bryophytes showed a limited contribution to whole-reach NH_4^+ uptake. Additionally, we observed C uptake in bryophytes, although at low rates, which is likely the result of the microbial biofilms commonly associated with them (Suren, 1988). The $U_{acetate}:U_{NH4}$ ratio in bryophytes was low, reflecting their autotrophic character and its dependence on N from the water column.

Differences found in this study in the rate of areal uptake versus uptake corrected for standing stocks for each PUC, highlight the potential consequences of changes in the standing stocks of different benthic compartments on N and C cycling at whole-ecosystem scale. Land use changes (e.g. reduction of riparian forest) or longer periods of dry conditions leading to forest water stress and increased leaf litter on the dry riverbed, may have important implications for in-stream N and C processing and downstream export.

N and C transfer from PUCs to consumers

Macroinvertebrate consumers were labelled with ^{15}N and ^{13}C tracer at the end of the addition period, showing a transfer of N and DOC from the water column throughout PUCs to higher trophic levels. According to our results, each macroinvertebrate taxon seemed to assimilate the two elements from the same resource. *Habroleptoides* sp. reached the strongest labelling for both elements, reflecting the high enrichment in epilithon, its main food resource. Our results support findings from other studies showing the role of epilithon as a valuable pathway to transfer microbial N (Tank et al. 2000; Ashkenas et al. 2004; Norman et al. 2017) and bacterial C (Hall 1995; Hall and Meyer 1998; Simon et al. 2003; Parkyn et al. 2005; Collins et al. 2016) to higher trophic levels, even in forested streams where the epilithon biomass is low. In contrast, *Oulimnius* sp., another grazer that presumably feeds also on epilithon, showed a low enrichment, which was nearly constant among the samples and did not coincide with the pattern followed by the epilithon tracer. One possible explanation for this observation may be related to the morphology of *Oulimnius* sp., which is characterized by a thick, chitinous exoskeleton (Liess and Hillebrand, 2005) that results in a large body mass with slow turnover rates and long times to reach isotopic equilibrium. However, accordingly to the body mass correction proposed by Vander-Zanden et al. (2015), the isotopic incorporation rate for *Oulimnius* sp. was 3 days, which would be within the 7 days tracer addition performed in our experiment. Applying this correction for the other studied invertebrates, we obtained 3.4 days for *Habroleptoides* sp., 6.8 days for *Radix* sp. and 31 days for *Boyeria irene*. Therefore, only *Boyeria irene* showed an isotopic half-life higher than the experimental addition duration, which could explain the low enrichment found for this predator in our study. Unfortunately, we cannot reliably evaluate the transfer of N and C linked to the microbial assemblages established on leaf litter and small wood because the presence of shredders in our reach was extremely low during the study period. Scarcity of leaf-shredding invertebrates under flow intermittency has been previously described (Maamri et al. 1997; Muñoz 2003; Burgazzi et al. 2018). Moreover, travertine precipitation on leaf litter is common in calcareous streams,

impeding invertebrate decomposer activity (Casas and Gessner 1999). The reduced abundance and biomass of this feeding group compared to other feeding groups in the studied stream suggests that the invertebrate-driven leaf processing is less relevant in this stream (Langhans and Tockner 2006; Datry et al. 2011) than other ways of detritus processing. For example, the relevance of the detrital pathway was apparent in the collector *Radix* sp., which obtained N and C from the microbes associated with the FBOM and retained a considerable proportion of ^{15}N and ^{13}C tracer despite its low standing stock. Allochthonous C from detritus might allow consumers to survive when in-stream primary production is low, as during the autumn–winter period. The more recalcitrant but energetically high glucose from terrestrial inputs might be more consumed under nutritional stress for energy (Thorp and Bowes, 2017).

Habroleptoides sp. and *Radix* sp. were consistently more isotopically enriched than their potential food resources for both tracers, but especially for ^{13}C. This label mismatch has been observed in previous ^{15}N (Dodds et al. 2014) and ^{13}C tracer studies (Hall, 1995; Collins et al. 2016), and it was attributed to a selective assimilation by macroinvertebrates of a more biologically active fraction of their resource (e.g., algae, bacteria and fungi). Previous ^{15}N (Mulholland et al. 2000a; Tank et al. 2000) and ^{13}C studies (Hall and Meyer 1998; Collins et al. 2016) showed that bulk detritus is less labelled than the associated microbial biomass. This dilution effect on the isotopic signature could be even more prominent in ^{13}C studies because the amount of detrital C is larger than that of detrital N. However, the wider mismatch for ^{13}C than for ^{15}N label could also indicate a selective assimilation of bacterial C as was reported previously (Edwards and Meyer 1987, 1990; Hall and Meyer 1998; Collins et al. 2016).

Storage of N and C in the study reach

In our experiment, we accounted for 76% of the total ^{15}N tracer added, with most ^{15}N being stored in leaf litter and, to a lesser extent, in epilithon. This amount is within the range observed in other ^{15}N tracer additions performed in forested streams (e.g., Mulholland et al. 2000b; Ashkenas et al. 2004). The remaining 24% of ^{15}N was probably not detected because we may have underestimated the standing stocks. For example, a miscellaneous fraction of CBOM (> 1 mm), not classifiable into leaf litter or wood, accounted for 27.7 g AFDM m^{-2} but was not used for standing stocks or for uptake calculations. Moreover, the macroinvertebrate biomass used for isotopic analyses accounted for 84% of the total macroinvertebrate biomass estimated in the studied reach; and therefore, another 16% of macroinvertebrate biomass was not considered in the mass balance. Apart from standing stocks, some ^{15}N may

have been lost due to abiotic sorption onto stream sediments (Richey et al. 1985; Peterson et al. 2001), involvement in processes that occur in hyporheic waters (Triska et al. 1989) or consumption by meiofauna, and therefore not included in our study.

Although we observed relatively high biological demand for DOC in our reach, we were able to account for only 14% of the total ^{13}C tracer added at the end of the addition. Approximately half of the total ^{13}C tracer that could be accounted for was stored in leaf litter and epilithon, whereas the other half was exported downstream as DOC or mineralized to CO_2. The same explanations offered above for ^{15}N tracer could at least partially explain the missing ^{13}C tracer, including abiotic absorption (McDowell 1985), hyporheic processes (Sobczak and Findlay 2002) and consumption by meiofauna (Suberkropp 1998). However, we must also consider that some C was lost by respiration. In this sense, Simon et al. (2003) calculated that, as in our study, only 0.8–6.3% of the ^{13}C tracer added was stored within the studied reaches, with a large amount of the ^{13}C missing due to respiration. These results could indicate that an important fraction of the labile DOC removed from the water column by bacteria was rapidly mineralized and lost as CO_2 via respiration rather than being stored or incorporated into the food web (McDowell and Fisher 1976).

Conclusions and perspectives

We found that similar to NH_4^+, acetate, a labile form of DOC, is also rapidly taken up from the water column when large amounts of detritus are available. Among PUCs, microbial assemblages established on leaf litter showed the highest contribution to whole-reach NH_4^+ and acetate uptake, indicating the influence of inputs of leaf litter on the immobilization of N and DOC in streams. The predominant role of leaf litter in the whole-reach uptake of NH_4^+ and acetate relative to other compartments was the result of the large standing stock of leaf litter. Changes in the PUC stocks may imply changes in N and C cycling at broader scales. Our results also demonstrated that N and DOC taken up by PUCs can be rapidly transferred to macroinvertebrate consumers thereby extending the retention time of these elements within the ecosystem. Future research should consider analysing only the actively cycling N and C fraction from food resources to reduce the observed label mismatch and improve the interpretation of the results (Dodds et al. 2014). Finally, the small percentage of the total added ^{13}C tracer retained in the study reach at the end of the addition suggested that labile forms of DOC such as acetate could be rapidly mineralized and lost as CO_2 via respiration, indicating that future studies on C cycling should account also for this pathway.

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