Nitrogen partitioning and transport through a subalpine lake measured with an isotope tracer

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Abstract

We used a stable isotope tracer to measure nitrogen (N) assimilation and transfer through Bull Trout Lake, a 0.3 km^2 mountain lake in Idaho; specifically to explore the relative importance of pelagic and benthic producers. $^{15}NO_3^-$ was added into the inflow stream above the lake during spring runoff and the resulting mass of tracer was measured within the various ecosystem compartments including the outflow stream. Although a portion of the $^{15}NO_3^-$ moved through the lake quickly due to a low hydraulic residence time during the addition, the tracer was also assimilated rapidly by seston in the water column and at a slower rate by benthic primary producers. By the end of the ten-day injection 10% of the tracer had left via outflow, 21% was within seston, and 17% was in epiphytes and macrophytes. However, 70 days after the termination of the injection, only ~1% of the tracer remained within seston while 10% was within the benthic primary production compartment as nitrogen was recycled within the benthic zone. Quantitative transfer of ^{15}N to invertebrate and fish consumers was low, but turnover in these compartments was slow. A conservative water mass tracer (bromide) indicated that the turnover rate for lake water was 1.8% d^{-1} whereas ^{15}N turnover for the whole lake was only 0.7% d^{-1} demonstrating how lakes exert drag on nutrients as they move through the watershed. Due to uptake and storage of nutrients, Bull Trout Lake strongly influenced the timing and magnitude of nutrient export from its watershed.

Introduction

In exploring nutrient transformations in lakes, limnologists have historically overlooked the littoral zone and have largely considered pelagic photoautotrophs to be the foundation of the lake food web, ushering nutrients into the food web (Reynolds 2008). This reflects a 'pelagic-centric' view of lakes that has traditionally dominated the field of limnology (Vadeboncoeur et al. 2002; Vander Zanden et al. 2006). Lakes have also been viewed primarily with regard to processes influencing the vertical structure of the ecosystem, with an emphasis on transport processes between the epilimnion and deep hypolimnion and profundal sediments. Under this perspective, vertical fluxes (sedimentation, eddy diffusion, fall turnover) are considered largely responsible for controlling nutrient concentrations and therefore plankton production (Horne and Goldman 1994). This attitude reflects a general depiction of lakes as large, deep bodies of water.

However, the majority of lakes worldwide are relatively small and shallow, with extensive littoral zones (Wetzel 1990; Schindler and Scheurell 2002; Downing et al. 2006) that can be important for nutrient uptake and recycling. Other than in a few studies (Wetzel and Allen 1972; Wetzel and Hough 1973; Fee 1979) the role of the littoral zone in nutrient uptake has historically been neglected; however, in recent years the importance of the littoral zone has been re-emphasized (Axler and Reuter 1996; Vadeboncoeur et al. 2003; Vander Zanden et al. 2006, 2011). The relative importance of the littoral zone likely depends on the proportion of littoral to pelagic habitats in a given

72 lake (Vadeboncoeur et al. 2001) and the pathways by which nutrients are delivered into
73 the lake.

74 Assimilation of nutrients by littoral and pelagic microbes may also influence
75 nutrient transport at the watershed scale. Even during spring runoff when hydraulic
76 residence times are at their annual low in most temperate mountain lakes (Wurtsbaugh et
77 al. 1994; Arp et al. 2006; Flanagan et al. 2009), the transport of watershed-derived
78 nutrients may be reduced by assimilation and transformation by biota in the benthic zone.
79 Depending on the turnover time of different pools, nutrients may become stored in biota
80 or they may be quickly recycled for re-uptake, sedimented out and subject to
81 resuspension, or exported out of the system. An extensive littoral zone may facilitate
82 faster nutrient cycling as nutrients regenerated within littoral sediments are already within
83 the warmer, epilimnetic photic zone (Carpenter and Lodge 1986). However in deeper
84 lakes, nutrients that sediment out of the water column may be largely lost to the
85 hypolimnion, as nutrients recycled at the sediment interface may not be mixed back into
86 the photic zone. Additional important pathways by which nutrients are recycled and
87 transported include excretion of dissolved nutrients or fecal pellets by zooplankton and
88 other consumers (Vanni 2002) and hydrolysis of organic material in the water column.

89 Submerged macrophytes growing in littoral sediments can also be critical
90 transport mechanisms for nutrients buried within the sediments and the water column
91 (Barko et al.1991). Macrophytes are capable of mining buried nutrients that may
92 otherwise be lost from the lake food web. Additionally, macrophytes and other benthic
93 primary producers may be particularly important in recycling nutrients from multiple
94 sources including the water column, other benthic plants, and the sediments (Dong et al.

2000; Tobias et al. 2003). While the greatest flux of external nutrient inputs to streams and lakes in snowmelt-dominated systems is delivered in the spring (Wurtsbaugh et al. 1994; Boyer et al. 1997; Pellerin et al. 2012) nutrients available in the sediments and sediment pore water may be available to support benthic primary production throughout the year.

In this study we used a stable isotope experiment to explore the transport and removal of watershed-derived inorganic nitrogen (N) through a mountain lake ecosystem. Our study design avoided the limitations of mesocosms or laboratory experiments and aimed to examine the natural pathways of nitrogen uptake and transfer. Additionally, our approach explored the competition between pelagic and benthic primary producers for nutrients and how the assimilation by these pools may influence nitrogen transport through the lake. We hypothesized that due to the oligotrophic nature of our study lake, assimilation by benthic and pelagic producers in the lake would decrease N transport relative to water transport during the snowmelt flush. We expected that both pools would contribute to nitrogen retention, but that nitrogen assimilated by the benthic compartment would be retained longer than in the pelagic compartment.

Methods

Study site

Bull Trout Lake is a 0.30 km^2 subalpine lake located adjacent to the Sawtooth National Recreation Area within the Boise National Forest of central Idaho (Fig. 1a). The lake is at an elevation of 2118 m and is part of the headwaters of the South Fork of the

117 Payette River (44º 17’ 58” N, 115º 15’ 16” W). The watershed is relatively pristine with
118 limited recreational land use and low atmospheric N-deposition (~100 kg km^{-2} yr^{-1};
119 NADP 2010). Bull Trout Lake’s watershed is 99.9% vegetated (Goodman et al. 2011)
120 with upland areas dominated by lodgepole pine (*Pinus contorta*) and stream riparian
121 areas dominated by willows (*Salix* sp.), sedges (*Carex* sp.), and grasses (Arp et al. 2006).
122 The watershed above Bull Trout Lake drains an 11.7 km^2 area of biotite-granodiorite, and
123 glacial deposits (Kiilsgaard et al. 2003) with a maximum elevation of 2550 m. The inflow
124 and outflow stream hydrographs are dominated by spring snowmelt (Arp et al. 2006), and
125 the area is typically snow covered from mid-November to late-May or early-June. The
126 outflow, Warm Springs Creek, is a small, slow-moving stream that originates as a
127 shallow marsh connecting to the epilimnetic shelf at the north end of the lake.
128 Bull Trout Lake is dimictic and the epilimnion thickness varies from
129 approximately 2 m in early June to 11 m in September (Fig. 2a). The lake is oligotrophic
130 with an average epilimnetic summer chlorophyll *a* (Chl *a*) concentration of 1.1 $\mu g\ L^{-1}$ and
131 a maximum around 4 $\mu g\ L^{-1}$, found as a deep chlorophyll layer in the metalimnion (Fig.
132 2b). Primary production in lakes in this region are generally co-limited by N and
133 phosphorus (P) availability (Wurtsbaugh et al. 1997), although phosphorus limitation
134 becomes increasingly important later in the summer (Sawatzky et al. 2006). Summer
135 epilimnetic total phosphorous (TP) and total nitrogen (TN) concentrations average 4.3
136 and 85 $\mu g\ L^{-1}$, respectively, and epilimnetic NO_3-N concentrations range from near 20-30
137 $\mu g\ L^{-1}$ during spring runoff to <3 $\mu g\ L^{-1}$ by late June (M. Baker unpubl. data). Nitrite-N
138 concentrations in the lake are low and were assumed to be negligible in this study.

A large portion of the water column and benthic sediments are in the photic zone (>1% light), which varies from 9 m depth in spring to 12 m in late summer (W. Wurtsbaugh unpubl. data). The maximum lake depth is 15 m and the mean depth is 4.3 m (Fig. 1b). We estimate that the littoral zone accounts for approximately 63% of the lake's benthic area and is dominated by submerged macrophytes, which cover ~80% of the littoral zone. Although there was considerable overlap, the three main macrophyte species occupy different depths with *Potamogeton* spp. at the shallowest depths, *Elodea* spp. at mid-depths, and *Chara* spp. in the deepest water. Flocculent sediments are present at depths < 1.5 m and > 9 m, and are also interspersed between patches of macrophytes at shallower depths. There is essentially no rocky substrate and little sand in the lake bed.

During the study, the zooplankton community was dominated by rotifers with mean densities of 7.0 L^{-1}. Crustacean zooplankton, dominated by *Bosmina* sp., averaged only 0.2 L^{-1} throughout most of the summer, but rose to 4.2 L^{-1} in mid-September (D. Lamarra and W. Wurtsbaugh unpubl. data). Bull Trout Lake supports a population of brook trout (*Salvelinus fontinalis*) and is stocked through the summer with catchable (15-20 cm) rainbow trout (*Oncorhynchus mykiss*). A low number of kokanee salmon (*O. nerka*) are also present in the lake.

Tracer additions - To measure nitrogen dynamics in a linked, stream-lake ecosystem, two tracer additions were done simultaneously in Spring Creek; one at the top of the watershed and another approximately 50 m above the junction of Spring Creek and Bull Trout Lake. Preliminary experimentation in the headwaters indicated that a large portion of nitrogen is removed before it reaches the lake (Covino et al. 2010), and therefore the

second addition 50 m above the lake was done to ensure adequate tracer was delivered to the lake, which is the focus of the study reported here. The tracers were added for 10 days from 21 – 30 June 2008, during the descending limb of peak spring flows (Fig. 3). This period was chosen because in these cold-climate, high gradient watersheds, most nutrients are transported during spring runoff (Boyer et al. 1997). Over the course of the 10-day injection we added 198 g of $^{15}NO_3$-N (99% atom enriched) along with 65 kg of sodium bromide (NaBr) to the inflow stream 50 m above the lake, and this entire mass was assumed to enter the lake. The injection was done over 10 days mainly to provide a realistic measure of processes occurring in the lake during the spring rather than if we had arbitrarily chosen a single day to add all the tracers. To estimate flux of ^{15}N and Br^- transported to the lake from the upper stream injection we used stream discharge estimates and measured concentrations of Br^- and ^{15}N collected above the lake injection point every 10-60 minutes on days 0 and 9, in addition to daily sampling throughout the injection period and periodic sampling throughout the study duration. By the end of the season we estimate that 95 g of ^{15}N as NO_3^- and 13 g as seston (particulate organic matter) entered into the lake from the upper watershed injection, for a total of 306 g ^{15}N tracer added to the lake. The mass of tracer added to the lake represented 1-2% of the dissolved NO_3^--N pool in the water column. We did not detect a change in lake NO_3^--N concentration. Laboratory measurement protocols for Br^- and ^{15}N analyses are described below. Results concerning ^{15}N storage and transport in the stream will be reported elsewhere.

184 *Lake N pools* - Isotope samples were taken prior to (to obtain background ^{15}N values),
185 during, and subsequent to the tracer injection approximately -5, 0, 3, 8, 15, 30, 51, and
186 115 days after the start of the injection (day 0) for each of the ecosystem compartments.
187 The different pools measured for tracer content included dissolved $^{15}NO_3^-$, seston
188 (phytoplankton + bacteria + detritus in the water column), zooplankton, epiphytes
189 (attached algae growing on macrophyte hosts), macrophytes, sediments, fish (*Salvelinus*
190 *fontinalis*), and benthic invertebrates (Ephemeroptera, Odonata, Amphipoda). We did not
191 measure transformation of ^{15}N via denitrification because previous studies indicated low
192 rates that would not be detectable in the ^{15}N gas pool given the levels of $^{15}NO_3^-$
193 enrichment we would expect from our study design (K. Nydick unpubl. data; Hall et al.
194 2009; Washbourne et al. 2011). Measured fluxes of ^{15}N included the sedimentation rate
195 and the outflow rates (via the outflow stream) for dissolved and seston ^{15}N. All samples
196 were promptly stored on ice while being transported to the lab, then were dried at 60°C
197 and encapsulated in the laboratory in preparation for analysis at the University of
198 California Davis Stable Isotope Facility. Isotopic enrichments and N mass were measured
199 with a PDZ Europa (Sercon) Automated Nitrogen & Carbon Analysis for Gases, Solids
200 and Liquids elemental analyzer linked to a Europa 20-20 mass spectrometer (Sercon).
201 Seston and bromide were sampled with a peristaltic pump at 0.5, 3, 6, 9, and 12 m
202 depths and at four different stations (Fig. 1b) through vinyl tubing. The maximum depths
203 at each station were 15 m (Sta. 1 and 2), 10.5 m (Sta. 3), and 4.5 m (Sta. 4). Sample
204 bottles were acid washed and triple rinsed with sample water before they were filled and
205 subsequently stored on ice. Within 5 hours of collection measured volumes of the sample
206 water were filtered in the laboratory through 80-μm mesh to remove zooplankton and

207 onto 25-mm Gelman A/E filters (1.0 μm pore size) until clogged. The filters were then
208 dried at 60°C before encapsulation for isotope analysis. Filtered water from each seston
209 sample was frozen in a plastic vial for Br^- analysis using ion chromatography. Water
210 samples for $^{15}NH_4$ and $^{15}NO_3^-$ were analyzed using methods from Sigman et al. (1997)
211 and Mulholland et al. (2004). Because of the low nitrate concentrations, 1-L water
212 samples were spiked with NO_3^--N and concentrated to 0.1 L by boiling before Devarda's
213 alloy catalyzed conversion of NO_3^- to NH_4^+ during a 48-h incubation. Bromide, $^{15}NO_3^-$
214 and seston were sampled in the outflow stream at the same frequency as lake water by
215 dipping sample containers into the thalweg. $^{15}NH_4$ samples became contaminated in the
216 laboratory and were not usable in this analysis. Samples were processed and analyzed
217 with the same protocols as lake samples. On the dates we sampled ^{15}N in the seston,
218 temperature and oxygen profiles were measured at the deepest station (1) with a Yellow
219 Springs Instrument Company Model 58 thermistor and Clark polarographic oxygen
220 sensor. Water transparency was measured with a 20-cm diameter disk with black and
221 white quadrants.

222 Zooplankton were sampled during the day in vertical tows with a 24-cm diameter,
223 80-μm mesh net at each of the four stations. Quantitative tows were made at each station
224 from 1 m above the lake bottom to the surface. The sample volume was recorded and a
225 subsample was filtered onto a 25-mm Gelman GF/D filter (2.5 μm pore size), and oven-
226 dried for subsequent isotopic analysis.

227 Gross and net sedimentation out of the water column were measured seven times
228 over the season at the four sampling stations with traps that were 60-cm long, 3.8-cm
229 diameter polyvinyl chloride pipes capped on the bottom. These sediment traps were fitted

with floatation collars and were positioned with the entrance 1.5 m above the bottom of the lake. Traps were deployed at depths of 13.5 (Sta. 1 and 2), 9 (Sta. 3), and 3 m (Sta. 4; Fig. 1b). Traps measuring gross sedimentation were first filled with chilled, non-chlorinated tap water (to limit the entry of lake water with seston), and then 50 mL of high-density formalin preservative (2% formaldehyde and 5 g L^{-1} NaCl) was injected with a long tube to the bottom of the traps to stop organic particle decomposition. Traps measuring net sedimentation were also filled with chilled, non-chlorinated tap water before being deployed but the preservative was not added. The traps were tied to cement blocks and lowered to the bottom. After a 2-day deployment, the sediment traps were slowly raised to the surface where the contents were transferred into storage bottles prior to filtration of subsamples onto 25-mm Gelman A/E glass fiber filters until clogged.

Epiphyte, macrophyte and sediment core samples were taken along four different transects from the 'corners' of the lake into the center (Fig. 1b). These transects provided four spatial replicates of ^{15}N in these benthic pools at each sampling depth. Epiphytes were sampled at 3, 6, and 9 m along each transect by SCUBA divers who engulfed entire plants of designated species in a 41-cm tall, 11-cm diameter cylindrical plastic sample container. To minimize turbulence and the loss of loosely attached materials on the plants, the top cap was modified with 323-μm mesh to allow water through as the container was placed over the plant. A solid cap was screwed onto the bottom of the container once the macrophyte was cut at the sediment surface. After the diver delivered the sample to the boat the mesh cap was replaced with a solid lid, and the sample was vigorously shaken for one minute to dislodge attached epiphytic algae from the macrophyte host. Macrophytes were then removed from the sample, the volume of the

253 epiphyte solution was measured and the samples were stored on ice until laboratory
254 processing. In the laboratory, macrophytes were dried to constant weight and measured
255 volumes of the epiphyte solutions were filtered onto 25-mm Gelman A/E filters until
256 clogged.

257 In addition to sampling epiphytes, SCUBA divers estimated percent cover of each
258 macrophyte genus (and bare sediments) at 1.5-m depth increments along each of the four
259 transects. A rectangular quadrant (divided into a grid) was used by two different divers to
260 visually estimate percent cover of each macrophyte species and bare sediments along the
261 lake bed. At each depth the divers randomly selected a square to estimate percent of each
262 cover type. The mean coefficient of variation between the two divers for these
263 observations was 22%, indicating moderate error in our estimates. One section (4%) of
264 the quadrant was harvested entirely, dried, and weighed to obtain a standard weight of
265 plant material per area at each transect and depth. These estimates were used to estimate
266 whole-lake macrophyte and epiphyte biomass.

267 In an effort to measure the ^{15}N uptake by bare sediments (not covered by
268 macrophytes), cores were taken with a Wildco® 4.8-cm diameter gravity corer at lake
269 depths of 0.5, 1, 3, 6, 9, and 12 m along each of the four transects. In the field, the first 4
270 cm of the upper part of the core were sectioned into two separate 2 cm thick slices, placed
271 into sample cups and dried in the lab at 60°C until the weight was constant. Once dried,
272 these samples were homogenized with a mortar and pestle prior to encapsulation for
273 isotopic analysis.

274 Muscle plugs from the dorsal region of brook trout (18 – 23.5 cm fork length)
275 were collected from anglers, dried and then ground into a powder before encapsulation.

We estimated fish biomass as 220 kg km^{-2} based on a mean summer Chl *a* concentration of 1.0 μg L^{-1} in Bull Trout Lake, and from chlorophyll-fish biomass relationships ($y=2.2x^{1.3}$; $r^2=0.63$) of similar Idaho ecosystems (Reiman 1992, Gross et al. 1998). Aquatic insects were sampled at three different stations along the western edge of the littoral zone with dip nets. Insects of the same order were dried and ground into a powder for encapsulation. We assumed a biomass estimate of 1.0 g dry wt m^{-2} based on a range of 0.4 – 1 g dry wt m^{-2} reported for alpine lakes and oligotrophic temperate lakes by Le Cren and Lowe-McConnell (1980).

Tracer mass balance - A mass-balance was constructed for ^{15}N in Bull Trout Lake for the extent of our sampling program (summer 2008). The ^{15}N mass-balance summarizes ^{15}N uptake, storage and transfer from the $^{15}NO_3^-$ delivered by the inflow (Spring Creek) to bacteria and primary producers, primary and secondary consumers, sedimentation out of the water column, and/or export via the stream outflow. Uptake of the ^{15}N tracer was assessed by change in the atom ratio of samples above background, which is depicted by $\delta^{15}N$ and calculated by:

$$\delta^{15}N = [((R_{sample} - R_{background})/R_{standard}) - 1] \times 1000 \quad (1)$$

where $\delta^{15}N$ is expressed per thousand (‰) , R_{sample}, $R_{background}$ and $R_{standard}$ are the ^{15}N: ^{14}N ratios of the sample, background samples and standard (‰ =0), respectively.

The ^{15}N content in each sample was determined by the isotopic enrichment of the sample, the isotopic enrichment of samples taken prior to the tracer addition, and the mass of N in the sample according to:

$$N_x = N_i \times AP_{sample} - AP_{background} \quad (2)$$

where N_x is the mass of tracer ^{15}N in the sample, N_i is the mass of nitrogen in the sample, AP_{sample} is the atom percent of the sample ((^{15}N / ^{14}N+^{15}N) x 100), and $AP_{background}$ is the atom percent of the background taken prior to the ^{15}N injection. The total mass of tracer ^{15}N in each ecosystem compartment was calculated from the product of the mass of ^{15}N tracer in the samples and the total volume (or area) of the given compartment within a depth strata A hypsographic curve for Bull Trout Lake was used to estimate volumes and areas of different strata. The total mass estimates from samples at each transect or station were averaged to generate one whole lake ^{15}N estimate (± SD) based on four independent replicates.

Estimates of ^{15}N tracer in benthic invertebrates and fish were done by multiplying ^{15}N atom ratio values for each taxon by the biomass estimates from the literature (g m^{-2}), and by the total lake area to obtain whole-lake ^{15}N estimates. For the mass balance analysis and estimates of uptake and turnover, all insect taxa were grouped together to generate one estimate for the compartment. Although this approach yielded less accurate and detailed information than for other compartments, the pools of isotope in the invertebrates and fish were small; thus these estimates had less of an effect on the overall isotope budget (*see* below).

Tracer uptake and turnover rates were calculated for each of the lake ecosystem compartments. Uptake rates for ecosystem compartments were estimated as the slope of the line fit to the natural log of delta ^{15}N vs. time during the injection period (21-30 June). Net turnover rates (day^{-1}) were estimated from the exponential decline of delta ^{15}N values in each ecosystem compartment over time (days since the end of the injection; Dodds et

al. 2000). The same method of turnover rate estimation was also done for all ecosystem compartments using Br^- and mass ^{15}N data.

Results

Hydrodynamics - Complete ice-out on Bull Trout Lake occurred on 30 May 2008, and by mid-June the lake was weakly stratified (Fig. 2). However, epilimnetic temperatures did not reach 15°C until mid-July. Heterograde oxygen profiles were recorded on most dates, with peak concentrations in the metalimnion (deep chlorophyll layer). Oxygen was generally above 5 mg L^{-1} in the hypolimnion, but by mid-August it declined to concentrations near 2 mg L^{-1} within 1 m of the deepest sediments.

The cold, dense water from Spring Creek inserted into the epilimnion and metalimnion of Bull Trout Lake and, with the exception of samples collected 1 day after the tracer addition began, the highest concentrations of Br^- (24 - 32 mg m^{-3}) were between 3 and 6 m (Fig. 4). Br^- tracer concentrations were lowest at 12 m, indicating that there was limited underflow and/or mixing into the hypolimnion until mid-August. Epilimnetic and metalimnetic concentrations of Br^- decreased rapidly following the termination of the injection when discharges were still high (Fig. 3), and then slowly over the rest of the summer. By late July, the average concentration throughout the entire water column was ~12 mg m^{-3}. Hypolimnetic Br^- concentrations peaked at about 10 mg m^{-3} near the end of the injection and these elevated (above background) concentrations were sustained or increased slightly in the late summer when deep mixing occurred (Fig. 4).

343 *Nitrogen dynamics* - Tracer ^{15}N was quickly transformed from inorganic ($^{15}NO_3^-$) to
344 particulate forms as it was incorporated into the lake food web upon delivery from Spring
345 Creek (Fig. 5). The highest and most rapid enrichment of the biota was in the seston
346 (peak value 485‰), within the first few days of the injection. However, after the injection
347 ended on 30 June, the tracer moved out of this compartment in an exponential decline
348 (0.038 day^{-1}; Table 1), indicating short-term storage of the tracer and rapid turnover.
349 Seston in the epilimnion became much more enriched than the hypolimnion with the
350 highest delta values sampled at 3 and 0.5 m (Fig. 6a). There was a short time lag before
351 hypolimnetic seston became enriched and peak enrichment was only 238‰ on 21 July (9
352 m). While the tracer rapidly moved into and out of the epilimnetic seston (uptake 0.48
353 day^{-1}, turnover 0.038 day^{-1}), the rates of uptake and turnover were slower within the
354 hypolimnion (uptake 0.36 day^{-1}, turnover 0.029 day^{-1}; Table 1).
355 Concentrations of tracer ^{15}N in seston (Fig. 6b) followed a different distribution
356 from delta values (Fig. 6a) due to the presence of relatively high seston biomass in the
357 deep chlorophyll layer (Fig. 2b). Although ^{15}N was most concentrated in the epilimnion
358 shortly after the injection, particulate ^{15}N was rapidly lost from that layer via
359 sedimentation and export to the outflow, so that by late July and early August the highest
360 concentrations of seston ^{15}N were found at 9 and 12 m. The specific uptake rate of the
361 whole-lake seston pool from 20 June to 29 June was 0.50 day^{-1} (Table 1).
362 Zooplankton ^{15}N enrichment peaked shortly after that of the seston and reached
363 similarly-elevated values to the seston. Mean $\delta^{15}N$ values of zooplankton peaked at
364 483‰ (Fig. 5) and were highest at Sta. 3 and 4 (mean = 523‰) where there were only
365 epilimnetic and metalimnetic organisms due to the shallower depths of the stations. Delta

366 values for zooplankton increased rapidly and as a compartment had a specific uptake rate
367 of 0.41 day^{-1}. Additionally, the tracer was lost quickly as the zooplankton exhibited a
368 turnover rate of 0.034 day^{-1} (Table 1).

369 Due to sedimentation of organic material out of the water column onto submerged
370 macrophytes, the epiphyte compartment we measured included both epiphytic algae
371 growing on macrophyte hosts and sedimented material that collected on leaves and stems.
372 Enrichment of epiphyte samples was moderate (peak of ~62‰) compared to that of
373 seston and there was a time lag (24 days) between the end of the injection and peak
374 enrichment of epiphytes (sestonic peak enrichment was reached by the end of the
375 injection; Fig. 5). Difference in epiphyte enrichment among depths were limited (range
376 64-96‰), although samples collected at 6 m had the highest enrichment. While there
377 were potential differences in enrichment of epiphytic algae on different macrophyte
378 hosts, such an effect would likely be confounded by the fact that the macrophyte species
379 distribution was largely determined by depth. The specific uptake rate (0.18 day^{-1}) and
380 turnover rate (0.018 day^{-1}; Table 1) were much lower than that of the seston and
381 zooplankton indicating longer-term storage within the epiphyte compartment.

382 Enrichment of submerged macrophytes (*Potamogeton* spp*., Elodea* spp*.,* and
383 *Chara* spp.) was much lower than that of the epiphytes. The highest delta ^{15}N value for
384 any macrophyte sample was 18.5‰ (Fig. 5), and average values for each depth and
385 station were never greater than 9.5‰. This value may have also represented some
386 residual epiphytes that were not dislodged by our sampling procedure. Individual
387 macrophytes took up minimal ^{15}N tracer; however, due to the large mass of macrophytes
388 (~ 30,000 kg dry weight) the total uptake in this compartment was significant (*see*

389 below). The specific uptake rate for macrophytes was 0.13 day^{-1} (Table 1) but the
390 turnover time could not be calculated due to sustained or increased enrichment through
391 the end of the season.

392 Sedimentation rates out of the water column varied substantially throughout the
393 lake. Rates of sedimentation were the greatest at Sta. 4 (4.5 m depth, near the outflow)
394 followed by Sta. 3 (10.5 m depth). Therefore, rates of sedimentation reaching the benthos
395 were the lowest in the deepest portion of the lake. Delta values of sedimented materials
396 peaked at 394‰, and followed the same trend as those of the seston (Fig. 5). Peak gross
397 sedimentation rates ranged from 0.02 to 1.1 g ^{15}N day^{-1} (± 0.5 SD) among stations and
398 net sedimentation was on average 76% (± 27 SD) of gross sedimentation.

399 Enrichment of Bull Trout Lake sediments with ^{15}N was not observed, as delta
400 values of sediment samples did not increase above background following the injection.
401 While we expect that a portion of the tracer was taken up and stored within the
402 sediments, we were unable to directly estimate this mass (*see* Discussion).

403 Enrichment of fish and insects was low compared to other lake compartments;
404 nevertheless we were able to quantify tracer uptake into these compartments and then
405 extrapolate them to whole-lake estimates based on regional biomass models (see
406 Methods; Fig. 5). Of the insect and fish taxa sampled, damselflies (Odonata-Zygoptera:
407 50‰) and then mayflies (Ephemeroptera: 43‰) labeled the highest, while amphipods
408 (*Gammarus* sp.: 16.6‰) and brook trout (3.6‰) became enriched to a lesser extent. The
409 specific uptake rate of the insect compartment (average for all taxa combined) was low:
410 0.082 day^{-1} and the turnover rate was only 0.003 day^{-1} (Table 1). Delta values in fish
411 increased from 8‰ (background) to a maximum of 12‰ in mid-August. Uptake and

412 turnover rates of the fish were not determined because mass of tracer within the
413 compartment was still increasing when sampling terminated in the fall.
414 Tracer (^{15}N) enrichment of seston in Warm Springs Creek (outflow) peaked at
415 399‰ following the termination of the tracer addition (Fig. 5). Concentrations of ^{15}N
416 tracer in seston also peaked near the end of the injection at 39 μg m^{-3} (Fig. 6b) and
417 declined immediately after the injection. The flux of ^{15}N tracer moving out of the lake as
418 seston followed the same trend and peaked at 2.8 g ^{15}N day^{-1} near the end of the injection.
419 From exponential models fit to the decline of the ^{15}N and Br^- tracer from
420 maximum concentrations, we found the most rapid turnover was in the seston (0.038),
421 followed by Br^- from the water column (0.018), the epiphyte compartment (0.013), and
422 the slowest turnover was for total ^{15}N in the whole lake (0.009). All three models were
423 found to be statistically significantly different from one another (whole lake and seston
424 (p<0.0001) and whole lake and Br^- (p=0.0114); analysis of covariance, Statistical
425 Analysis System Institute 2011).
426
427 *Tracer mass-balance* -
428 The ^{15}N tracer mass-balance on the last day of the ten-day injection (30 June
429 2008) is summarized in Fig. 7 and Table 2. Although a large portion of the tracer
430 remained as NO_3^- within the water column (36%), the greatest mass in any biological
431 compartment was found in seston (21%). Due to high stream discharge and low lake
432 residence time, a portion of the tracer had passed through the lake and into the outflow
433 rather quickly in the form of NO_3^- (4%) and seston (6%). The benthic primary production
434 compartment (epiphytes and macrophytes combined) contained 17% of the tracer and 2%

had sedimented out of the water column. Only about 1% of the tracer was found in the higher trophic levels of the ecosystem (zooplankton, benthic invertebrates and fish). A portion of the tracer (13%) had an 'unknown' fate on this date (Table 2).

After the end of the injection ^{15}N tracer shifted from the pelagic zone into benthic compartments (Fig. 8). As shown previously, seston quickly assimilated the most ^{15}N (62 g on 30 June) of any biological compartment, but after the termination of the injection, tracer within this pool sedimented out of the water column (50 g cumulative sedimentation by 12 September) or was exported from the lake via the outflow stream (cumulative 42 g). By the end of the season only 1% of the total added tracer remained in seston and only 3% remained as NO_3^-. In contrast, 10% of the tracer assimilated by the epiphyte and macrophyte complex remained at the end of the season. The majority of the total flux of nitrate and seston out of the lake was exported via Warm Springs Creek by mid-July. Additional export of tracer via Warm Springs Creek in the late summer was minimal due to low discharges at this time. At the end of our sampling program 36% of the tracer was unaccounted for in the compartments that we were able to measure.

Discussion

Our stable isotope experiment showed that watershed-derived inorganic nitrogen is assimilated rapidly within Bull Trout Lake, transferred throughout the ecosystem, and slowly released downstream. We discovered both benthic and pelagic primary producers to be important in assimilating watershed-derived inorganic nitrogen into the lake food web. While this nitrogen may enter the food web fastest via the seston (pelagic primary producers), it is retained longer within the benthic zone in part because in addition to

458 benthic assimilation from the water column, seston is transported to the benthic zone via
459 sedimentation. While we were able to follow the flow of nitrogen tracer into zooplankton,
460 benthic invertebrates and fish, the total mass assimilated into the higher trophic levels in
461 the water column was small in comparison to primary producers. A portion of the
462 nitrogen delivered in the spring by the inflow stream passes quickly though Bull Trout
463 Lake; however, the majority moves slowly as it is assimilated and transferred within the
464 lake ecosystem.

465 While ^{15}N and Br^- were both delivered to the lake by the inflow stream, the
466 movement of ^{15}N through the lake differed distinctly from the inflow water measured
467 with the Br^- tracer. Bromide moved quickly through the system and after three weeks the
468 high concentrations in the epilimnion had been lost via the outflow. In contrast to the
469 water, little $^{15}NO_3^-$ was initially exported from the lake due to rapid uptake by lake biota.
470 Rapid tracer uptake by the seston and its subsequent sedimentation to deeper strata
471 retarded ^{15}N loss to the outflow. Additionally, moderately rapid uptake by benthic
472 epiphytes retarded ^{15}N loss to the outflow, thus representing a 'drag' on nutrient flux
473 through the watershed. This drag is conceptually identical to the idea of nutrient
474 retardation used by engineers in continuously stirred tank reactors, where the movement
475 is slowed due to the non-conservative nature of the ^{15}N tracer relative to the Br^- tracer
476 (Chapra 1997).

477 The importance of benthic retention of nitrogen is highlighted when we compare
478 exponential loss rates of the Br^- (water mass tracer) and ^{15}N tracer. Losses of the ^{15}N
479 tracer from the seston pool included export via the outflow stream (0.008 day^{-1}), but
480 consumption by zooplankton and sedimentation out of the water column also contribute

481 to the seston turnover rate. Therefore, even though the turnover of Br^- appeared to be
482 slower than ^{15}N in seston, the lost Br^- moved out of the lake only via the outflow stream
483 while ^{15}N from seston was in part transferred to other pools within the lake. Increased
484 hydraulic residence time by mid-summer contributed to the slow turnover rate for the
485 tracers out of the lake. At a seasonal scale, when we consider the loss of total ^{15}N (not
486 just seston) from the system, the loss rate of Br^- was actually faster (0.018 day^{-1}) than
487 total ^{15}N (0.007 day^{-1}) demonstrating the drag on nutrients as they move through the lake.
488 By 12 September in-lake compartments accounted for over 40% of the total mass of ^{15}N
489 tracer accounted for at the end of the injection while only 26% of the Br- tracer remained
490 in the lake.

491 Nitrogen inputs to Bull Trout Lake delivered during spring runoff are taken up
492 quickly by organisms within the lake, indicating strong reliance on inorganic nitrogen
493 delivered from the upper watershed. Pelagic organisms may have first chance at
494 assimilating inorganic nitrogen delivered by the inflow due to the hydrodynamics of the
495 lake-inflow stream interaction. Overall, we found benthic and pelagic primary producers
496 to have assimilated a similar mass of ^{15}N, with around 20% of the ^{15}N tracer taken up in
497 both the pelagic and benthic-littoral zones. These findings are different from those of
498 Axler and Reuter (1996) who estimated that periphyton activity accounted for >70% of
499 inorganic nitrogen depletion in Castle Lake (CA) and 56% of nitrate disappearance in a
500 related mesocosm experiment. The discrepancy may be related to how tracers were
501 added. Axler and Reuter manually distributed ^{15}N tracer throughout the epilimnion of the
502 whole lake and mesocosms located on a shallow littoral shelf. However, in Castle Lake
503 most primary production of phytoplankton occurs in a deep chlorophyll layer (Priscu and

504 Goldman 1983) that did not receive the ^{15}N tracer. In contrast, in our experiment the
505 inflow stream naturally delivered ^{15}N to directly into the pelagic zone and deep
506 chlorophyll layer of Bull Trout Lake (Fig. 1b). Thus a portion of the watershed-derived
507 nutrients did not reach the littoral zone before passing through the pelagic portion of the
508 food web.

509 The benthic portion of the Bull Trout Lake food web may not have access to
510 nutrients delivered by the inflow stream until they arrive at the water-sediment interface
511 either via lateral mixing or sedimentation out of the seston. During mid-summer,
512 hypolimnetic ^{15}N concentrations in the seston increased where Br^- concentrations did not,
513 indicating transfer of ^{15}N via sedimentation and not mixing (c.f. Figs. 4, 6). However, all
514 of the sedimenting N may not reach the lake bed, as organic matter is hydrolyzed within
515 the water column, potentially at the fastest rates in the epilimnion (Ohle 1962). Lower
516 enrichment of sedimented material compared to the epilimnetic seston indicated dilution
517 by material moving out of deeper depths and possibly resuspension of epiphytes and
518 benthic material. The highest rates of sedimentation were measured at the shallowest
519 station (4), where there was less time for N to be hydrolyzed before it reached the
520 benthos, compared to Sta. 1 and 2 where sedimentation took longer, thus allowing for
521 hydrolysis of organic material during settling.

522 In many lakes, estuaries, and streams there is a greater biomass of benthic primary
523 producers than pelagic primary producers (Vadeboncoeur et al. 2002; Tobias et al. 2003)
524 and therefore they can serve as a large sink for nitrogen. The dominance of benthic
525 nutrient uptake and production is most pronounced in streams due to the high surface area
526 to volume ratios: this ratio is lower in estuaries and lowest in lakes. For example, Hall et

al. (2009) found that essentially all of $^{15}NO_3^-$ tracer uptake in the inflow to Bull Trout Lake (Spring Creek) was in the benthic compartment; however, the majority of exported tracer was in the form of seston. Similarly, in an estuarine tracer study, Tobias et al. (2003) found that benthic processing was almost two orders of magnitude more important than pelagic sinks for ^{15}N tracer.

Axler and Reuter (1996), Vadeboncoeur et al. (2001), and Liboriussen and Jeppesen (2003) have shown that benthic primary production may be comparable to, or even greater than pelagic primary production in oligotrophic lakes. However, the benthic primary producers may obtain some of the needed N (and other nutrients) from pelagic organisms that sediment out of the water column and are mineralized. Additionally, the complex of benthic primary producers may retain nitrogen longer than pelagic organisms due to cycling within the benthic complex (Carpenter and Lodge 1986; Dong et al. 2000; Tobias et al. 2003). Therefore pelagic primary producers must rely on new inputs of nutrients from the littoral zone, the inflow stream, or other sources to continually fuel production whereas the benthic zone may hold onto and recycle nutrients (Saunders and Kalff 2001). In Bull Trout Lake both the pelagic and benthic zones appear to be important for nutrient uptake and transport through the lake food web.

From a mass-balance perspective, higher trophic levels such as zooplankton play a small role in the Bull Trout Lake nutrient budget. However, zooplankton can play an important role in the food web by influencing the persistence of the deep chlorophyll maximum (Pilati and Wurtsbaugh 2003) and regenerating nutrients that may fuel primary production (Hambright et al. 2007). Aquatic insects and fish also represented a small pool of recovered ^{15}N, and their nitrogen turnover rate was much slower than that of the seston

550 and zooplankton, which is consistent with what we know about body size and turnover
551 rates (Brown et al. 2004). While the ^{15}N pool in insects and fish was small, the nitrogen
552 in these pools remained there for a long time as it is turned over extremely slowly. Due to
553 this slow turnover it is possible that additional tracer accumulated in these compartment
554 in the months following the termination of our study.
555 At the end of the tracer addition we were unable to account for approximately
556 13% of the ^{15}N tracer we added, but we expect that a substantial portion was taken up by
557 the epipelic sediments and a small portion could have been lost via denitrification.
558 Results from other studies suggest that a significant portion of the ^{15}N tracer can enter
559 epipelic sediments (Axler and Reuter 1996; Nydick et al. 2004; Lockwood 2009). We
560 were not, however, able to detect the actual mass of tracer in this pool, probably because
561 the large ^{14}N nitrogen pool there overwhelmed our relatively small tracer addition.
562 Lockwood (2009), using much higher ^{15}N enrichment in a littoral zone mesocosm
563 experiment in Bull Trout Lake found that a substantial portion of nitrate was taken up by
564 the epipelic microbial complex. If we extrapolate Lockwood's results to the bare
565 sediments in our tracer experiment using our measured enrichment of overlying water,
566 we estimate that the sediments would have taken up 30 g of ^{15}N tracer by the end of our
567 10-day addition, almost accounting for the total mass of unknown tracer at the end of the
568 injection. While we cannot be sure nitrogen uptake in the sediments behaved the same as
569 it did in Lockwood's mesocosm experiment, we can be quite confident that a portion of
570 the unknown tracer ended up within the sediments. We did not measure denitrification or
571 dissimilatory nitrate reduction to ammonium in this study because potential rates of both
572 processes are so low they were not likely significant sinks for our ^{15}N tracer, at least

during the spring-summer period (Washbourne et al. 2011). Nevertheless, some loss of tracer via denitrification could be expected and this could account for a small portion of the missing ^{15}N in the budget.

Through this tracer study we described the incorporation of NO_3^- nitrogen into the Bull Trout Lake ecosystem. Both pelagic and benthic primary producers proved to be important, ushering this nitrogen into the lake ecosystem through the spring and summer. While uptake occurred in the epilimnion, nitrogen was quickly passed down to the hypolimnion and on to the surfaces of macrophytes in the littoral zone via sedimentation and direct uptake. Even though the lake is dominated by littoral habitats, the hydrodynamics and hypsometry of Spring Creek entering into Bull Trout Lake may influence the proportion of nitrogen that enters into pelagic and benthic compartments. While a large portion of the ^{15}N tracer was incorporated into the epiphyte compartment, this uptake occurred more slowly than that into the seston and may have been partially due to sedimentation out of the epilimnion. This experiment demonstrates the influence that lakes may have on nutrient transport, creating drag on nutrients as they move through watersheds.

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Table 1. Uptake (day^{-1}) and turnover (day^{-1}) rates for the biological compartments in Bull Trout Lake generated from regression models fit to delta ^{15}N data. Uptake rates were estimated from the slope of the linear fit to the natural log of delta ^{15}N during the injection period. Turnover rates were estimated from an exponential fit to the decline of the delta ^{15}N data following the end of the injection; however, the turnover rate for the macrophyte compartment was not available (na), as enrichment of samples had not peaked by the end of our sampling.

Compartment	Uptake rate (day^{-1})	Turnover rate (day^{-1})
Seston	0.5	0.038
Zooplankton	0.41	0.034
Epiphytes	0.18	0.018
Macrophytes	0.13	na
Benthic invertebrates	0.082	0.003

Table 2. Mass estimates of ^{15}N (grams) ± 95% confidence interval for in-lake biological compartments for Bull Trout Lake on the last day of the injection (30 June 2008). Error estimates for the insect and $^{15}NO_3^-$ compartments were not possible as the mass estimates were made from single samples without replicates.

Date	Seston	Epiphytes	Zooplankton	Macrophytes	Insects	$^{15}NO_3^-$	Unknown
30 Jun	64 ± 15	44 ± 15	3 ± 0.6	3.9 ± 4	0	111	34
06-08 Jul	48 ± 10	38 ± 13	2 ± 1	1.9 ± 3	1	13	114
21-23 Jul	21 ± 6	38 ± 15	3 ± 1.6	4.0 ± 2	3	35	68
11-13 Aug	10 ± 3	27 ± 12	1 ± 0.4	4.7 ± 3	2	8	101
12-13 Sep	4 ± 1	17 ± 4	0.5 ± 0.2	3.5 ± 1.0	2	9	111

Figure Captions

Figure 1. (a) Location of the Bull Trout Lake watershed in Central Idaho. Bull Trout Lake is the large lake in the watershed cutout of the figure. (b) Bathymetric map of Bull Trout Lake showing depth (m), as well as the site of the tracer addition (star), benthic sampling transects (numbered boxes), and pelagic sampling stations (numbered circles). The inflow stream (Spring Creek) and the outflow stream (Warm Springs Creek) are both shown with arrows on the bathymetric map.

Figure 2. (a) Temperature profiles of Bull Trout Lake throughout the spring and summer of 2008. Curves represent stratification of the water columns and horizontal rectangles on each curve represent the measured Secchi depth at the given date. (b) Distribution of chlorophyll *a* in the Bull Trout Lake water column at five different dates during the summer of 2008. During the whole study the maximum chlorophyll concentrations were in the metalimnion and migrated progressively deeper as the summer progressed.

Figure 3. Hydrograph at the outflow stream of Bull Trout Lake (Warm Springs Creek) during the summer of 2008 (solid line). The theoretical average hydraulic residence time for a fully-mixed lake is shown by the dotted line.

Figure 4. Concentrations of bromide tracer in different depth strata of Bull Trout Lake. Horizontal thick black line represents the duration of the bromide and ^{15}N tracer addition. The maximum value for the 0.5 m depth is cut off from the figure but was 45 mg m^{-3} on 01 July 2008.

Figure 5. $\delta^{15}N$ values throughout the summer of 2008 for ecosystem compartments in Bull Trout Lake. Horizontal thick black line represents the duration of the tracer addition. The curve representing the macrophyte compartment (open circles connected by a dotted curve) is just above the x-axis and may be hard to see clearly.

Figure 6. (a) $\delta^{15}N$ values throughout the 2008 season for Bull Trout lake seston in each depth strata sampled. Error bars represent the standard error (SE) of samples. (b) Concentrations of ^{15}N tracer ± SE in the Bull Trout Lake seston in different depth strata.

Figure 7. Mass-balance of ^{15}N tracer within Bull Trout Lake at the end of the 10-day injection (30 June 2008). Individual sections are the percentage of total mass of tracer found within each labeled compartment on day 10 of the injection.

Figure 8. Mass-balance of ^{15}N tracer within Bull Trout Lake from 21 June to 14 September 2008. The black bar on the x-axis shows the tracer injection period. The horizontal thick black line in the lower left portion of the figure represents the duration of the tracer injection. The total height of the figure represents the total mass of tracer (306 g) entering the lake. The thickness of each individual shaded band represents the mass of tracer in the given compartment. Zooplankton and insect + fish are found towards the middle of the figure but are hardly visible due to the small mass. The figure also shows the cumulative amounts of ^{15}N lost from the water column as: epilimnetic seston and nitrate leaving via the lake's outflow, and; gross sedimentation. The 'unknown' compartment represents tracer that was not accounted for in the compartments we sampled; including cumulative errors in other compartment estimates. The large portion of the unknown pool may be dominated by epipelic algal uptake, which we could not measure (*see* Discussion). Day 0 values were actually measured 4-6 days prior to the start of the tracer addition.

Figure 1

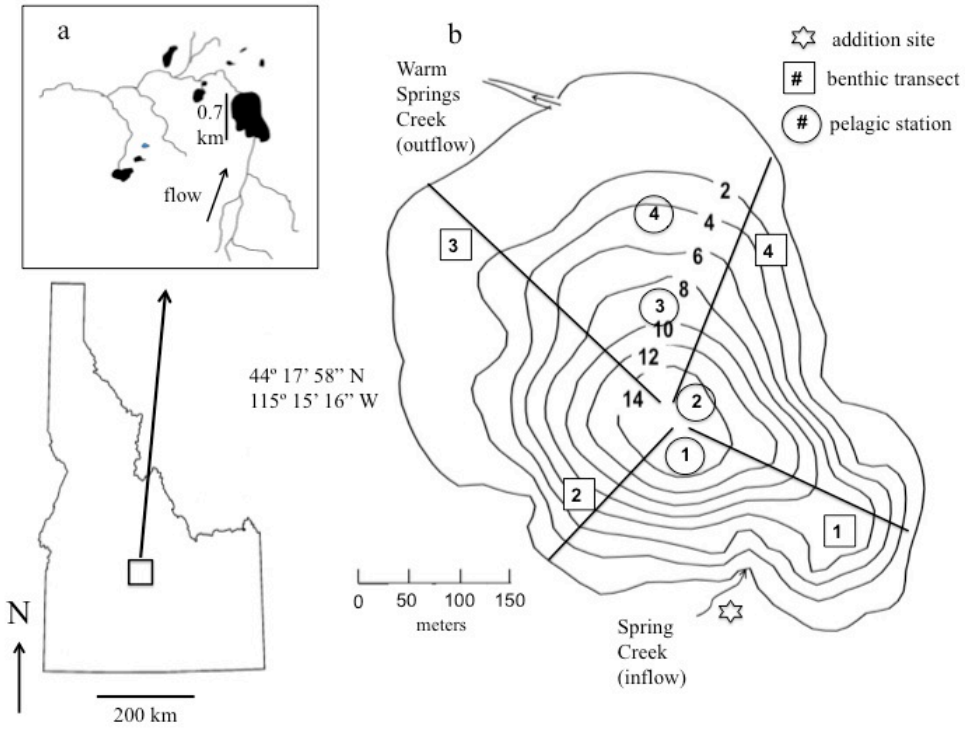

a
0.7 km
flow
N
200 km
44º 17' 58" N
115º 15' 16" W
b
addition site
benthic transect
pelagic station
Warm Springs Creek (outflow)
Spring Creek (inflow)
0 50 100 150
meters
2
4
6
8
10
12
14
3
4
3
2
1
2
1

Figure 2

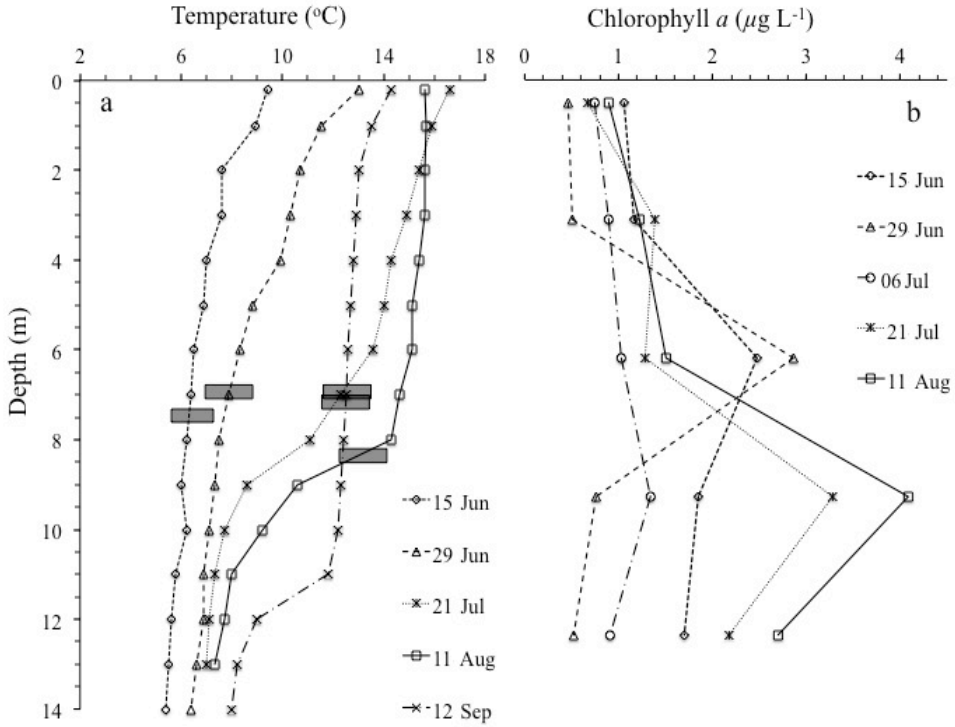

Temperature (°C)
Chlorophyll a (μg L-1)
Depth (m)
a
b
15 Jun
29 Jun
06 Jul
21 Jul
11 Aug
12 Sep

Figure 3.

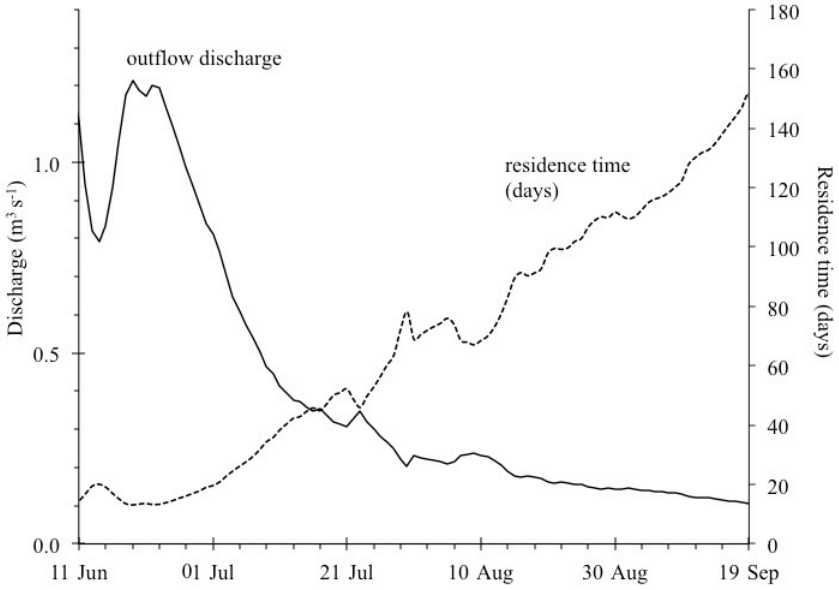

outflow discharge
residence time
(days)
Discharge (m³ s⁻¹)
Residence time (days)
0.0
0.5
1.0
0
20
40
60
80
100
120
140
160
180
11 Jun
01 Jul
21 Jul
10 Aug
30 Aug
19 Sep

Figure 4.

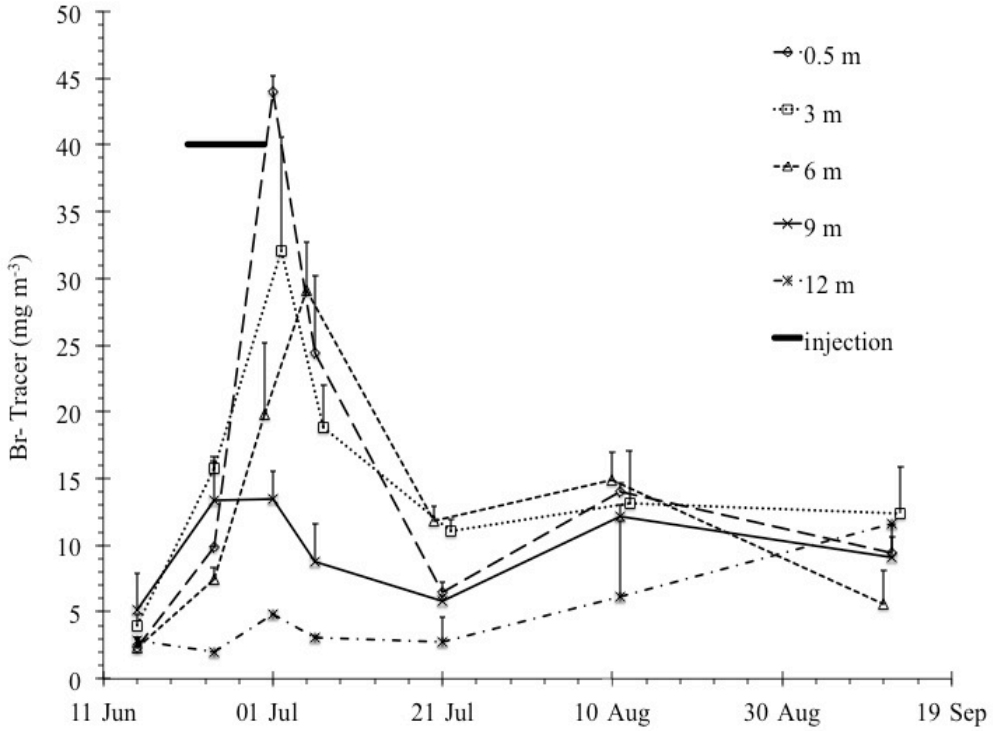

Br- Tracer (mg m-3)
50
45
40
35
30
25
20
15
10
5
0
11 Jun
01 Jul
21 Jul
10 Aug
30 Aug
19 Sep
0.5 m
3 m
6 m
9 m
12 m
injection

Figure 5.

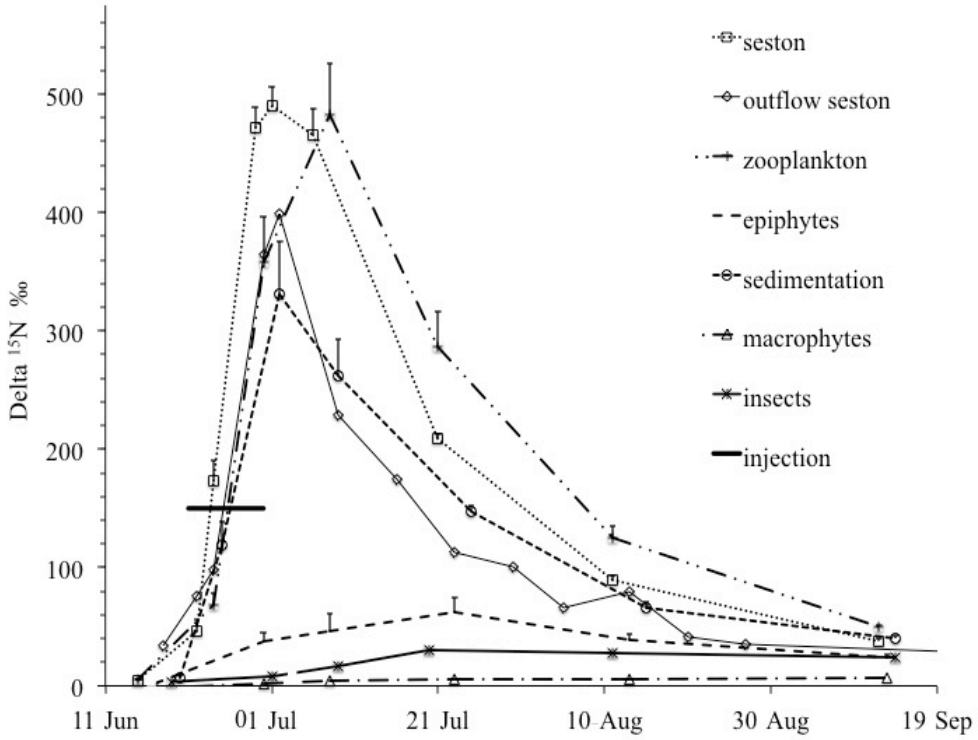

seston
outflow seston
zooplankton
epiphytes
sedimentation
macrophytes
insects
injection
Delta 15N ‰
0
100
200
300
400
500
11 Jun
01 Jul
21 Jul
10 Aug
30 Aug
19 Sep

Figure 6.

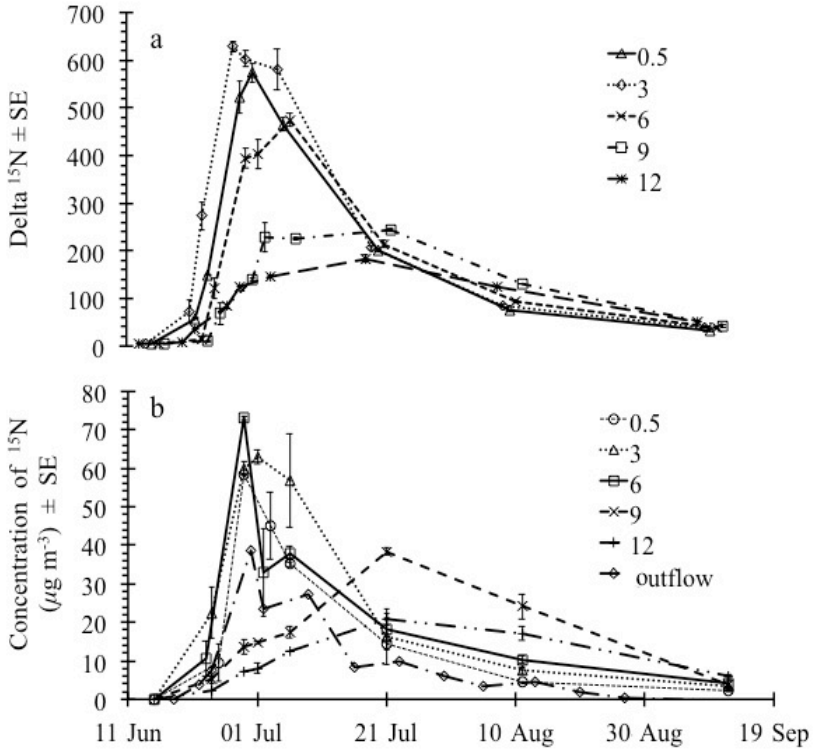

a
b
Delta ^{15}N ± SE
Concentration of ^{15}N (μg m^{-3}) ± SE
0.5
3
6
9
12
outflow
11 Jun
01 Jul
21 Jul
10 Aug
30 Aug
19 Sep

Figure 7.

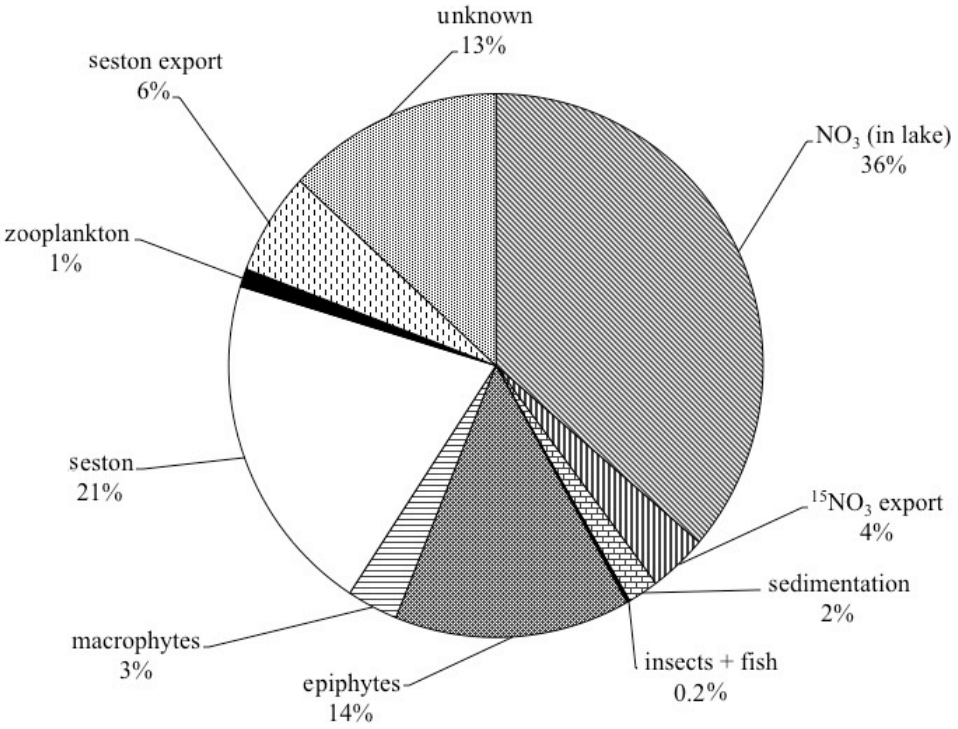
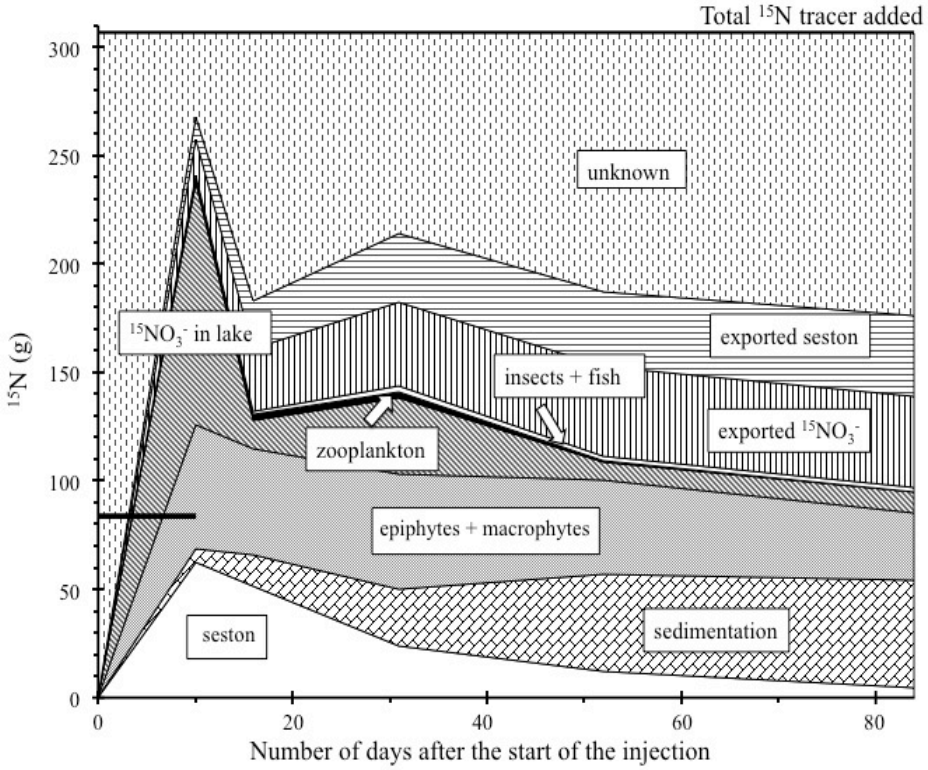

unknown
13%
seston export
6%
NO_3 (in lake)
36%
zooplankton
1%
seston
21%
$^{15}NO_3$ export
4%
sedimentation
2%
macrophytes
3%
epiphytes
14%
insects + fish
0.2%

Figure 8.


Total ^{15}N tracer added
unknown
exported seston
$^{15}NO_3^-$ in lake
insects + fish
exported $^{15}NO_3^-$
zooplankton
epiphytes + macrophytes
sedimentation
seston
^{15}N (g)
300
250
200
150
100
50
0
0
20
40
60
80
Number of days after the start of the injection