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Nitrogen Transport, Transformation and Cycling through a Mountain lake, Bull Trout Lake, Idaho, USA

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NITROGEN TRANSPORT, TRANSFORMATION AND CYCLING THROUGH A MOUNTAIN LAKE, BULL TROUT LAKE, IDAHO, USA

by

Ryan S. Lockwood

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

Ecology

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ABSTRACT

Nitrogen Transport, Transformation, and Cycling Through a Mountain Lake,

Bull Trout Lake, Idaho, USA

by

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Utah State University, 2009

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The effects of a mountain lake on nitrogen dynamics in a sub-alpine watershed were examined via watershed monitoring, mesocosm experiments, microcosm experiments, and enzymatic assays during spring and summer of a single year. Our study addressed the questions: (1) How does hydrologic transport through the lake affect the net fluxes of dissolved nitrogen (N) species? (2) What are the net effects of the littoral zone biota on dissolved N fluxes? (3) What are the seston and benthic uptake rates of nitrate? (4) What is the magnitude of N retention in littoral zone sediments? (5) What role does microbial hydrolysis of amino-groups from organic matter play in the uptake of dissolved nitrogen, relative to rates of nitrate uptake? Our study found a net positive flux of total dissolved N and dissolved organic N (DON), and a net negative flux of nitrate through the lake. During snowmelt, when the majority of nutrients are transported in this watershed, DON was retained in the lake. Several experiments were run to more closely examine the mechanisms behind these observations. Experiments in 2.1 m^3 mesocosms

in June and July measured rates of DON flux from the littoral zone sediments into the water column that were similar to increments measured in the lake. ^{15}N-nitrate mesocosm and microcosm tracer experiments quantified benthic and pelagic nitrate uptake and retention of that nitrate in the benthic sediments. Areal nitrate uptake was 65-times greater in the sediments than in the water column seston and the turnover rate (half life) of the newly input nitrate pool in the sediments was 33-64 days. Finally, the prevalence of DON relative to dissolved inorganic N (DIN) and high measured rates of enzymatic amino acid hydrolysis suggest the importance of DON as a source of N for this aquatic system.

(50 pages)

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Ryan S. Lockwood

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INTRODUCTION

Nitrogen (N) availability has been recognized as an important regulator of ecosystem productivity across a wide array of aquatic systems (Rabalais 2002; Elser et al. 2007), and landscape features such as lakes can be important regulators of the transport and transformation of N (Swanson et al. 1988; Kling et al. 2000). Observed patterns of N import and export in Bull Trout Lake, located in the Sawtooth Mountains of Idaho, USA and other lakes suggest the importance of lakes as sites of N retention and processing (Kaste et al. 2003; Arp and Baker 2007; Brown et al. 2008). N import to lakes via inflowing water often occurs in temporally short, relatively high flux spikes triggered by events such as snowmelt or precipitation that bring in pulses of dissolved inorganic nitrogen (DIN) and dissolved organic nitrogen (DON). N export via outflowing water is then potentially temporally attenuated due to heterotrophic and autotrophic uptake, storage and release. Furthermore, proportionally more of the total dissolved N pool is often exported as DON versus DIN when comparing the outflow and inflow of many lakes (Kling et al. 2000; Fairchild and Velinsky 2006; Brown et al. 2008). This N transformation by the lake biota will likely affect productivity and nutrient cycling in the surface water network downstream of the lake.

In lakes the site of N uptake and storage can be coarsely partitioned into two compartments: benthic and pelagic. Consideration of both of these compartments is critical in lake studies (Vadeboncoeur et al. 2002). It was traditionally thought that nitrate inputs were predominately incorporated into the phytoplankton community of the water column because phytoplankton have higher N affinities and uptake rates than the

benthic community (Goldman and Horne 1983), because the benthic community can be constrained by boundary layer kinetics (Reuter and Axler 1992), and because the phytoplankton often show a greater relative response in growth or atomic enrichment to nutrient or ^{15}N additions relative to the benthic community (K. R. Nydick and W. A. Wurtsbaugh unpublished data). However, whole-lake and mesocosm experiments have demonstrated the importance of the benthic zone as a site of N uptake, processing and denitrification (Axler and Reuter 1996; Vadeboncoeur et al. 2003; K. R. Nydick and W. A. Wurtsbaugh unpublished data). When examining lake N cycling the importance of the littoral zone (defined here as the area of a lake that is in contact with both sediments and the epilimnion) becomes apparent once the biotic processing of the benthic zone is taken into account. Littoral zone sediments in particular are areas of high biomass and biologic activity because: (1) They underlie epilimnetic water and are therefore exposed to higher temperatures than sediments deeper in the lake; (2) They are commonly exposed to quantities of light that allow for the growth of epiphytes, and; (3) Nutrient levels in the sediment are far higher than in the overlying water column. Studies of lakes have shown a positive relationship between extent of the littoral area and productivity of the phytoplankton (Fee 1979), suggesting that the epilimnetic sediments may cycle nutrients back into the water column.

Although the majority of studies in lakes and streams have focused on inorganic N processing, recent workers have recognized that organic matter is an important pool and source of N in aquatic systems (Perakis and Hedin 2002; Hood et al. 2003; Judd et al. 2006). Many DON studies have focused on pools such as urea and dissolved free and combined amino acids (DFAA and DCAA) due to their utility as the most labile

constituents of the naturally occurring DON pool (e.g. Findlay and Sinsabaugh 2003; Brookshire et al. 2005). However DFAA and DCAA usually comprise a minor portion of the total DON pool in aquatic environments (Berman and Bronk 2003; McKnight et al. 2003). Most naturally occurring DON occurs in molecules that are too large for direct uptake. For instance, proteinaceous matter greater than 5 to 7 amino acids long (~650 Daltons) is too large for cross-membrane transport (Law 1980; Payne 1980) and requires extracellular breakdown before it is available to other organisms. Microbial uptake of most DON is therefore regulated by extracellular enzymatic activity.

Much of the bio-available organic N resides in large, heterogeneous molecules that defy classification due to their complex and non-repetitive structures. Consequently, studying this portion of DON presents the difficulty of designing an assay that will be applicable across variations in DON quality, microbial community composition and environmental conditions. Quantifying enzymatic activity is a useful alternative because unlike the heterogeneous structure of naturally occurring dissolved organic matter, an enzymatic pathway operates in the same manner across microbial communities.

Two enzymatic pathways appear to be the most relevant in microbial processing of dissolved organic matter: oxidative and hydrolytic. Oxidative pathways break apart large organic ring structures and other more recalcitrant molecules. These smaller organic molecules are then available for hydrolytic pathways. Hydrolysis cleaves specific functional groups from organic molecules and the functional group is subsequently transported across the membrane into the cell (Chróst 1991; Münster 1991). Extracellular hydrolysis is often the rate-limiting step in aquatic microbial nutrient uptake (Chróst 1991; Münster 1991). Most DON cleavage occurs via a hydrolytic pathway

using an enzyme such as an aminopeptidase. Leucine aminopeptidase (L-AMP) is a common hydrolytic enzyme that is an important regulator of microbial DON uptake (Münster and De Haan 1998). The microbial community produces L-AMP in proportion to the size and lability of the DON pool, in proportion to the community's demand for N, and in inverse proportion to the availability of other more labile forms of N (Chróst 1991; Münster 1991; Francoeur and Wetzel 2003). Because of these three factors, L-AMP activity should change in proportion to the actual rate of DON hydrolysis that is occurring in the environment. Despite many detailed studies on both DON and DIN utilization, little work has been done to compare DIN and DON utilization in natural lakes.

This study examines how the presence of a lake in a small alpine watershed affects N cycling in regard to the following topics: rates of nitrate uptake by seston (suspended organic matter, including phytoplankton) and the benthic zone, relative partitioning of nitrate between the two pools, and rates of DON production in the littoral zone. Planktonic DON hydrolysis was also quantified in a manner that allowed for comparison of this rate and the rate of nitrate uptake by seston. In summary, the goals of this study were to examine and quantify (1) net lake and littoral zone N chemistry changes, (2) benthic and pelagic nitrate uptake, and (3) DON cycling.

STUDY SITE

Bull Trout Lake watershed is located in the Sawtooth Mountains of central Idaho, USA (44º 17' 58" N, 115º 15' 16" W). Watershed lithology is biotite granodiorite from the Idaho Batholith (Kiilsgaard et al. 2003). The physical hydrology of the watershed has been studied extensively (see: Arp et al. 2006). The watershed above Bull Trout Lake drains an area of 11.7 km^2 with a maximum elevation of 2550 m. During midsummer the lake has a single inflow and a single outflow. The inflow, Spring Creek, is a second order gravel-bedded creek that flows from South to North. The outflow, Warm Springs Creek, flows to the South Fork Payette River.

Bull Trout Lake is dimictic and completely ices over from approximately November to May. The lake is oligotrophic with a maximum pelagic chlorophyll *a* concentration recorded for any stratum of 6.6 $\mu g\ L^{-1}$ and the average epilimnetic summer concentration of 1.1 $\mu g\ L^{-1}$ (2007 data). Epilimnetic total phosphorous (TP) and total nitrogen (TN) concentrations in the summer average 0.14 and 6.1 $\mu mol\ L^{-1}$ respectively. The lake has an average surface area of 0.30 km^2 and lies at an elevation of 2118 m. Maximum lake depth is 15.0 m with an average depth of 4.3 m (Fig. 1). The lake has extensive littoral zone shelves and 17% of the lake's surface area is <1 m deep (Arp et al. 2006). In Bull Trout Lake's littoral zone there are limited peripheral areas of emergent macrophyte growth; however flocculent sediments with negligible macrophyte growth are dominant to a depth of 3 m. Below this depth submerged macrophytes become abundant to 9 m. We suspect that ice scour during spring prevents more extensive macrophyte development in water < 1.5 m deep.

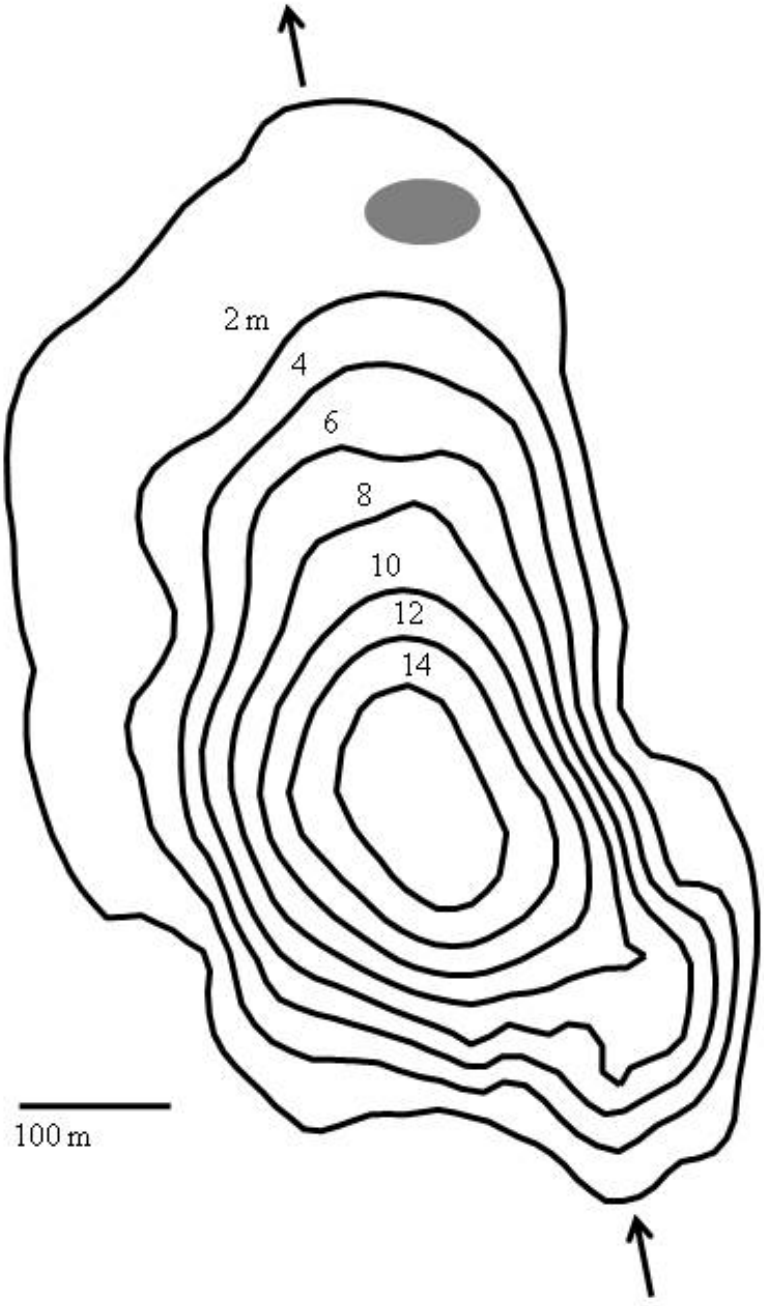

Figure 1. Bull Trout lake morphometry. Contour labels are depth increments in meters. The arrows show the location of the inflow and outflow. The shaded oval shows the locations of the mesocosm experiments.

METHODS

General field sampling and nutrient analyses–In all sampling scenarios water was obtained either via a grab-sample for surface waters, or with a hand driven or battery operated peristaltic pump for deeper waters. Samples for analysis of dissolved fractions were filtered (Whatman GF/F, nominal pore size of 0.7 µm) either in the field or at the laboratory during the same day as collection. Samples for nutrient analysis were stored in acid-washed high-density polyurethane bottles and frozen within 24 h of sampling. Samples for seston chlorophyll *a* analysis were filtered (Whatman GF/F) in the field and immediately placed into 95% ethanol for extraction. Chlorophyll *a* samples were extracted for 24 h in the dark before reading on a Turner 10AU field fluorometer using a non-acidification method (Welschmeyer 1994).

Nitrate (+ nitrite) concentrations were measured using a cadmium reduction method (Nydahl 1976) using an Astoria flow injection autoanalyzer (Astoria-Pacific Int. 2004, method A173). Total N was measured using the same method after the samples were subjected to a recrystallized potassium persulfate digestion that oxidized all organic N to nitrate (Ameel et al. 1993). DON was calculated as nitrate concentration subtracted from total dissolved N (TDN). Past work has shown that ammonium concentrations in the lake are almost always below detection (< 0.05 µmol L^{-1}) and consequently ammonium concentrations were not measured and were not included in the calculations of DON concentrations.

Sediments were sampled by pushing a 3.75 cm diameter section of polyvinyl chloride pipe into the soft littoral zone sediments to a depth of ~25 cm, plugging the top

of the pipe with a rubber stopper to create a vacuum and then removing and plugging the bottom of the pipe with another stopper as the pipe was removed from the water. The cores were then immediately transported to the laboratory for extrusion. Each core was extruded and sectioned into 0-2, 2-4 and 4-8 cm horizons. Once in the sample cup, the sections were shaken to homogenize the sample. For measurement of benthic chlorophyll *a*, a 0.5 ml subsample of the slurry was taken from the 0-2 cm horizon and extracted in 10 ml of 95% ethanol for 24 h before reading on a Turner 10 AU field fluorometer using a non-acidification method (Welschmeyer 1994). The rest of the sample was dried to constant weight at 70ºC and weighed.

Net lake and littoral zone N chemistry changes: Lake and stream monitoring– From June to October of 2007 routine monitoring of several physical, chemical and hydrologic variables in Bull Trout Lake watershed was used to measure patterns of downstream and through-lake N transport and transformation. At 1-3 week intervals from ice-out through summer and into the fall, discharge was measured and water samples were taken for determination of TDN and nitrate concentrations at the inflow and outflow of Bull Trout Lake. Measurement of hydrologic flux and chemical constituency at the lake's inflow and sole outflow allows for a calculation of the net change in chemical flux that results from the presence of the lake along the watershed's flow path. Net flux was calculated as:

$$Flux_{sp} = Q_{out} \times ([Sp]_{out} - [Sp]_{in})$$

Where $flux_{sp}$ is the net flux of a given chemical species or class in µmol s^{-1}, Q_{out} is the hydrologic discharge of the outflow in L s^{-1}, $[Sp]_{out}$ is the chemical activity of the species at the outflow and $[Sp]_{in}$ is the chemical activity of the species at the inflow in µmol L^{-1}. Discharge from only the outflow was used because it forms a more singular flow path, whereas the inflow site can be braided during snowmelt and storms and has significant flow occurring in the hyporheic zone (Arp et al. 2006). This calculation assumes that the unaccounted inflow water has the same chemistry as the water in the inflow channel. This assumption will affect the calculation of $flux_{sp}$ if it does not hold. If the calculated $flux_{sp}$ is positive and the unaccounted for inflow water has a higher $[Sp]_{in}$ than channel inflow water or if the calculated $flux_{sp}$ is negative and the unaccounted inflow water has a lower $[Sp]_{in}$ than channel inflow water, then $flux_{sp}$ will be overestimated. If the calculated $flux_{sp}$ is positive and the unaccounted for inflow water has a lower $[Sp]_{in}$ than channel inflow water or if the calculated $flux_{sp}$ is negative and the unaccounted inflow water has a higher $[Sp]_{in}$ than channel inflow water, then $flux_{sp}$ will be underestimated. Stream discharge was measured with a Marsh-McBirney flow meter (Model 2000) 36 times in the inflow and 35 times in the outflow. Stream stage was continuously monitored with TruTrack® WT-HR water height data loggers. Rating curves were developed to relate stage and discharge for interpolation of discharge data.

Lake profiles of temperature, chlorophyll *a*, TDN and nitrate were recorded at two established monitoring sites at depths of 0.5, 1, 3, 5.5, 8, 10.5 and 13 m. One site was located at maximum depth toward the south end of the lake, and the other towards the north end of the lake at 10 m depth. Sampling at the shallower station did not include the 10.5 and 13 m depths. Temperature was measured using a submersible probe.

Net lake and littoral zone N chemistry changes: Mesocosm experiments–To examine the relative influence of the littoral zone in affecting the observed watershed-scale patterns, triplicate reinforced polyethylene mesocosms (Aquatic Research Instruments®, Hope, Idaho, USA) that isolated a 1.5-m diameter column of water from the water surface to a depth of 30 cm below the substrate surface were installed at 1.2 m water column depth. A flotation collar kept the tops of the mesocosms 30 cm above the lake level to reduce or eliminate water exchange and the bottoms were anchored to a 30 cm high steel sediment ring. The mesocosms were placed 100 m from the outlet of the lake in a representative section of the littoral zone overlying soft, flocculent sediments with negligible macrophyte growth. Samples for water column chemistry were taken from the mid-section of the water column (0.5-0.6 m depth) in all of the mesocosm experiments. Seston chlorophyll *a* concentration was monitored and water samples for TDN and nitrate analysis were taken every 48 h for 6 d. Chemistry changes in the mesocosms illustrated the net effects of the littoral zone specifically and decoupled the zone from changes observed in the lake as a whole. This experiment was repeated twice, once in June and once in July, 2007.

Benthic and pelagic N cycling–The mesocosm experiments described above were continued to assess nitrate uptake by the benthic and pelagic communities. At the conclusion of each of the chemistry flux experiments, the mesocosm walls were lowered and the water column was mixed with ambient lake water for 48 h. The walls were then raised and a stable isotope tracer, 0.122 g of 98 atom% ^{15}N as $NaNO_3$, was added at a target initial water column enrichment of $\delta^{15}N = 50{,}000‰$ in order to track nitrate uptake in the mesocosm. Following the addition the water column was mixed with an oar. The

target peak isotopic enrichment of the seston was $\delta^{15}N$ = 1,000‰ and surficial sediments was $\delta^{15}N$ = 100‰. Seston and benthic samples for initial N content and background ^{15}N mole fraction (MF) were taken immediately before the addition.

Sample water for seston ^{15}N content analysis was transported to the laboratory and filtered (Gelman A/E, nominal pore size of 1.0 µm) on the same day as sampling. The filters were immediately dried at 70ºC in an oven for 24 h. After drying they were sealed in scintillation vials until encapsulation into 9 x 12 mm tin capsules (Elementar Americas, Inc.). Samples for sediment ^{15}N content analysis were taken as described above. After drying and weighing, the sample was ground with a mortar and pestle. A subsample of known mass was taken from each sample and encapsulated into 9 x 12 mm tin capsules (Elementar Americas, Inc.) for isotope ratio mass spectrometry ^{15}N analysis at the University of California at Davis Stable Isotope Facility.

Seston was sampled at 0.5 m depth from each replicate mesocosm for N and ^{15}N content every 3 h for 15 h, then again at 24 and 48 h, then every 48 h until 6 d after the addition. The sediments were sampled for N and ^{15}N content every 48 h until 6 d after the addition. Three subsample cores were taken from each experimental replicate and pooled after extrusion and sectioning. The activity of coring the sediments may have enhanced benthic nitrate uptake by facilitating transport of the label into the sediments. Benthic nitrate uptake in this case would be overestimated relative to the actual rate of benthic nitrate uptake. Chemical parameters were measured at the same depths and time intervals as in the initial mesocosm experiments.

Seston nitrate uptake was also measured in separate ^{15}N-NO_3 addition experiments done in 20-L translucent polyethylene Cubitainers® using waters from 0.5 m

and 5.5 m depth that was sampled on 8 Aug 2007. Seston total N content and ^{15}N AR was measured every 3 h for 12 h. These assays allowed for measurement of seston nitrate uptake across a broader range of the water column (82% of lake water volume lies at or above 6 m depth in Bull Trout Lake). Another purpose of these assays was to separate seston nitrate uptake measurements from any competition from simultaneous benthic nitrate uptake that was possible in the mesocosm experiments. As benthic nitrate uptake reduced the pool size of nitrate in the water column during the mesocosm experiment, the rate of sestonic nitrate uptake may not have been as enhanced as in the microcosm experiments. The cubitainer experiments mimicked the conditions of the enzymatic hydrolysis assays (see below) and allowed for a comparison of seston rates of nitrate uptake and DON hydrolysis.

Seston nitrate uptake at a given time point was calculated as:

$$\text{Uptake}_t = N_t \times (MF_t - MF_i) \times (MF_{iw})^{-1}$$

where N_t is the size of the total N seston pool at time t, MF_t is the mole fraction of seston ^{15}N at time t, MF_i is the initial mole fraction of seston ^{15}N and MF_{iw} is the initial mole fraction of $^{15}N\text{-}NO_3$ of the source water. Source water mole fraction was calculated by measuring the background concentration of nitrate, assuming a value for background nitrate MF (0.367 atom %) and adding a known amount of 98 atom % $^{15}N\text{-}NO_3$. This addition increased the total nitrate-N 0.67 µmol L^{-1} in each case which likely saturated the uptake of most phytoplankton. The calculation of nitrate uptake is robust to errors in the calculation of MF_{iw} because all additions were greater than 20 atom %. Interpreting

the data using this formula corrects for the amount of experimental enrichment to yield actual nitrate uptake rather than ^{15}N uptake. Dilution of the ^{15}N-NO_3 pool due to nitrification would have caused uptake rates to be underestimated but significant nitrification is unlikely to occur in this system due to the consistently low concentrations of both ammonium and nitrate.

Temporal nitrate uptake trends of the seston were evaluated with repeated measures analysis of variance (ANOVA) modeling using PROC MIXED in SAS V. 9 (SAS Institute, Cary, NC). The ANOVA tested the null hypothesis that the slope of the trend in nitrate uptake was equal to zero. Variables were transformed as necessary in order to meet the assumptions of normality and homoscedasticity.

Benthic nitrate uptake for a given time point was calculated differently from that of seston nitrate uptake. Due to inherent variability in the sampling of the sediments, there were large and inconsistent temporal variations in the measurement of the size of the total benthic N pool at each horizon. Since the benthic N pool is far larger and has a longer residence time than the seston N pool, we assume a steady state for this model in order to remove the sampling variability. Benthic nitrate uptake at a given time point was consequently calculated as:

$$Uptake_t = N_{ave} \times (MF_t - MF_i) \times (MF_{iw})^{-1}$$

Where N_{ave} is the average of all of the measured values for N content at a given sediment horizon, MF_t is the mole fraction of benthic ^{15}N at time t, MF_i is the initial mole fraction of benthic ^{15}N and MF_{iw} is the initial mole fraction of ^{15}N-NO_3 of the source water. As

with the interpretation of the seston data, the calculation of nitrate uptake is robust to errors in the calculation of MF_{iw} because all additions were greater than 20 atom % and interpreting the data using this formula also yields actual nitrate uptake rather than ^{15}N uptake.

Loss of the added nitrate from the sediments was monitored following each of the two nitrate uptake mesocosm experiments. Following each 6 d nitrate labeling period, the mesocosm walls were lowered to allow for water column mixing with ambient lake water. Loss of the ^{15}N label from the sediments was then monitored at 2-5 d sampling intervals for 9 d in the first experiment and at 2-24 d intervals for 52 d in the second experiment.

Enzymatic assays–The potential enzymatic activity of L-AMP for the whole water community was assayed using waters from the surface (0.5 m) and from a deeper stratum (5.5 m) of Bull Trout Lake and also from the inflow and outflow of the lake. Lake surface water was assayed on 16 July 2007 and 13 August 2007. Lake water from 5.5 m was assayed on 13 August 2007. Inflow and outflow water was assayed on 20 August 2007. In all experiments source water was obtained on the same day that the experiment took place. The water was transported to the laboratory in an insulated container and temperature was monitored during the assay. The assays run on 16 July were held at 12ºC, the assays run on 13 August were held at 14ºC and the assays run on 20 August were held at 10ºC.

All assays were run in triplicate 12 cm glass cuvettes that were acid washed, rinsed with deionized water, and then heat sterilized prior to inoculation. For each assay, 4.4 ml of source water and 0.6 ml of an L-leucine 7-amido-4-methyl coumarin

hydrochloride (L-AMC, Sigma) stock solution was added to each cuvette, resulting in a 200 μmol L^{-1} final L-AMC concentration for the incubation. A preliminary L-AMP assay was run using 200, 500 and 1250 μmol L^{-1} incubation concentrations of L-AMC to test for substrate saturation, an important assumption of the assay. Enzymatic activity was similar at all three concentrations so 200 μmol L^{-1} was used for future assays. Furthermore, Chróst (1991) reported half saturation constant (K_m) values for lake waters of 12-130 μmol L^{-1}.

Calibration of the fluorometer based on a deionized water blank and a 7-amino-4-methylcoumarin (AMC, Sigma) standard allowed for quantification of the assay based on a mole of leucine hydrolyzed per unit time basis. Immediately following inoculation, initial AMC fluorescence (380 nm excitation and 440 nm emission wavelengths) was measured on a Turner 10AU field fluorometer. Readings were taken every 30 min for a minimum of 2 h.

Enzymatic activity was interpreted as a linear trend in the change in fluorescence over the time of the incubation. The rate was then corrected for the dilution of the source water that occurs from the addition of the L-AMC stock solution. The rate of increase of AMC concentration corresponds, on a mole/mole basis, to the rate of enzymatic hydrolysis of a leucine molecule from an L-AMC molecule.

Temporal trends in L-AMP activities were evaluated with repeated measures ANOVA modeling using PROC MIXED in SAS V. 9 (SAS Institute, Cary, NC). The ANOVA tested the null hypothesis that the slope of the trend in hydrolysis was equal to zero. Separate analyses were run to evaluate the significance of differences in L-AMP activity between different dates for Bull Trout Lake surface water and between different

sites on a given date. Bull Trout Lake surface waters (0.5 m and outflow) were considered to be the same site because the outflow sampling site was on the lake perimeter.

RESULTS

Net lake and littoral zone N chemistry changes: Lake and stream monitoring–Bull Trout Lake showed an overall pattern of nitrate retention and DON production during the period of ice-out through fall (Table 1, Fig. 2). However, 10 May was the only sampling date on which the lake was a sink of DON. This sampling date was during spring snowmelt and had the highest inflow discharge and one of the highest outflow discharges. This suggests an interaction between hydrologic discharge and DON dynamics through the lake. As the season progressed nitrate concentrations dropped to low levels (< 1 µmol L^{-1}) both in the stream and lake water and the lake acted as a net sink of nitrate and a net source of DON. Throughout the study, dissolved N transport occurred primarily as DON rather than nitrate (Table 1).

Net lake and littoral zone N chemistry changes: Mesocosm experiments–DON increased in both mesocosm experiments examining isolated littoral zone chemistry changes (Fig. 3). During the June mesocosm experiments, epilimnetic temperature averaged 12.8ºC, mean pelagic chlorophyll *a* concentration was 1.01 µg L^{-1} and mean nitrate concentration was 0.12 µmol L^{-1}. During the experimental period from 8 to 14 June 2007, DON increased at a rate of 0.332 µmol L^{-1} d^{-1} or 398 µmol m^{-2} d^{-1} (r^2 = 0.625, p = 0.020). During the July mesocosm experiments, epilimnetic temperature was 18.5ºC, mean chlorophyll *a* concentration was 0.75 µg L^{-1} and mean nitrate concentration was 0.038 µmol L^{-1}. During the experimental period from 10 to 16 July 2007, DON increased at a rate of 0.301 µmol L^{-1} d^{-1} or 361 µmol m^{-2} d^{-1} (r^2 = 0.983, p = 0.019). These patterns

Table 1. Sampling dates, chemistry and discharges for Bull Trout Lake inflow and outflow during 2007. The net flux error terms represent 95% confidence intervals from the rating curve of the outflow stream stage from each date. Sampling and analytical error is unaccounted for.

Date	Inflow DON (µmol L^{-1})	Outflow DON (µmol L^{-1})	Inflow NO_3 (µmol L^{-1})	Outflow NO_3 (µmol L^{-1})	Inflow discharge (L s^{-1})	Outflow discharge (L s^{-1})	NO_3 net flux (µmol s^{-1})	DON net flux (µmol s^{-1})
7 April	2.64	2.92	1.01	1.04	100	160	4.57 ± 0.29	45.0 ± 2.84
10 May	4.46	3.90	1.15	0.50	631	613	-398 ± 27.0	-343 ± 23.3
26 May	3.62	4.34	0.57	0.02	505	668	-363 ± 27.9	483 ± 37.1
4 June	2.90	4.96	0.29	0.07	434	640	-137 ± 9.92	1315 ± 95.1
13 June	1.87	4.97	0.34	0.48	362	515	71.7 ± 3.58	1597 ± 79.7
20 June	2.00	5.10	0.26	0.02	281	362	-85.3 ± 1.23	1121 ± 16.2
14 August	1.37	7.50	0.07	0.09	107	88	1.26 ± 0.15	540 ± 62.7

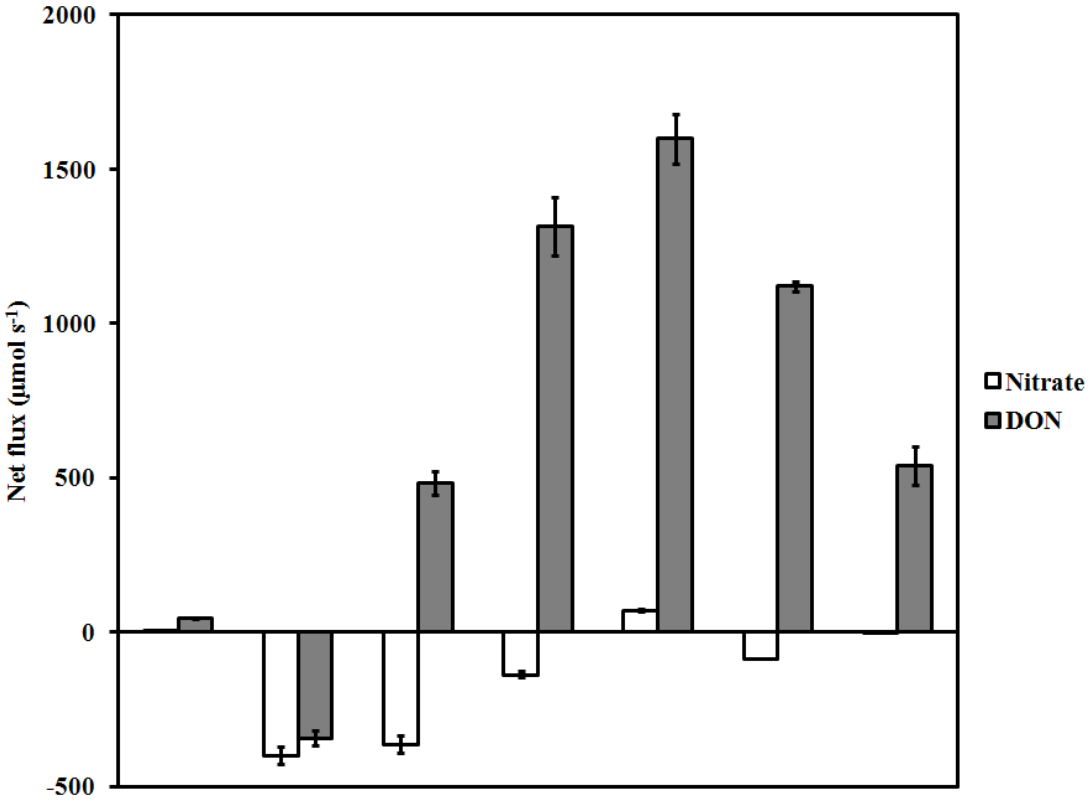

Figure 2. Net flux of dissolved N constituents between the inflow and outflow of Bull Trout Lake during seven sampling dates during spring and summer. Positive bars indicate that Bull Trout Lake is acting as a source of the constituent, negative bars indicate that it is acting as a sink. The sampling dates are displayed in chronological order and were 7 April, 10 May, 26 May, 4 June, 13 June, 21 June and 15 August. The error bars represent 95% confidence intervals from the rating curve of the outflow stream stage from each date. Sampling and analytical error is unaccounted for.

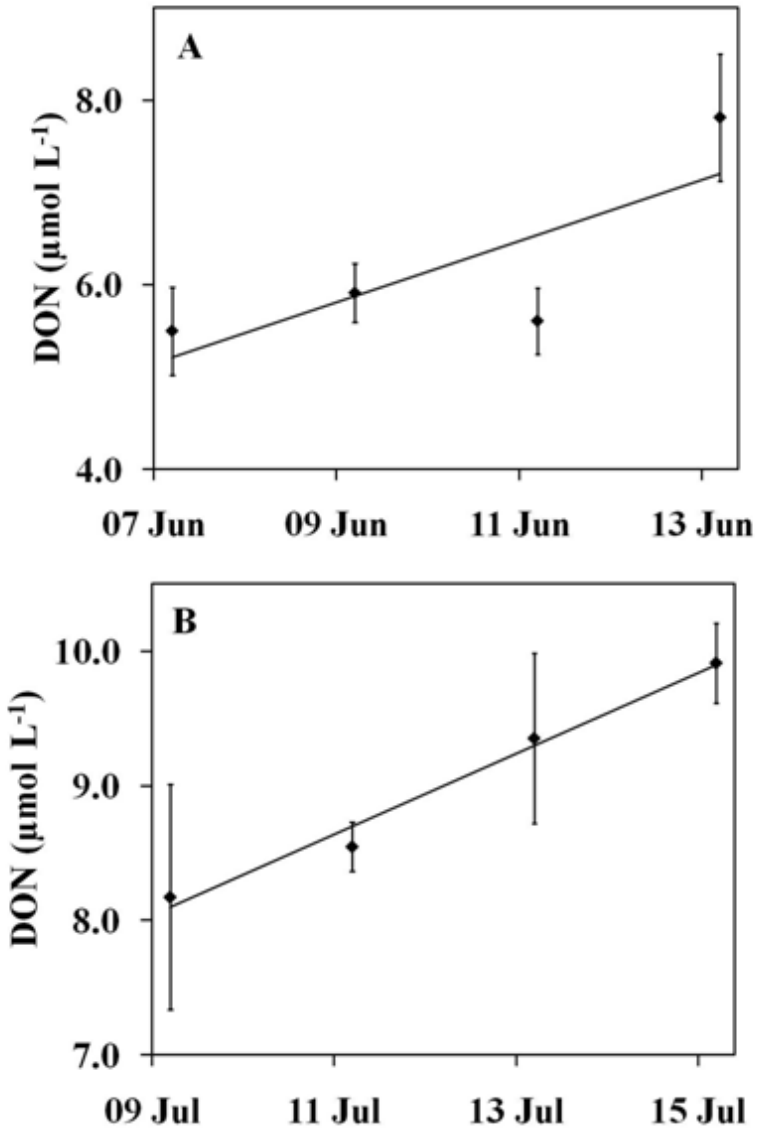

Figure 3. DON production during the (A) June and (B) July mesocosm experiments to examine littoral zone N fluxes. The rate of DON increase was 0.332 µmol L^{-1} d^{-1} in June and 0.301 µmol L^{-1} d^{-1} in July. Data points are mean +/- standard error (n=3). The linear regressions displayed are statistically significant ($p < 0.05$).

demonstrate that the littoral zone produces and releases DON on a sustained basis during the summer.

We calculated total littoral zone DON production based on lake morphometrics and the release rates measured in the mesocosms. Our temperature profiles indicated that the epilimnion resided at a depth of 0-6 m from the lake surface during the June and July experimental periods. If we define the littoral zone as the area of the epilimnion that is in contact with sediments then Bull Trout Lake's littoral zone had an area of 227,253 m^2. Allowing the assumption that the littoral zone acts similarly regardless of depth we can extrapolate our mesocosm experiments to estimate total littoral zone net DON fluxes of 90.5 mol d^{-1} or 1050 µmol s^{-1} during mid-June and 82.0 mol d^{-1} or 949 µmol s^{-1} during mid-July. These estimated whole-lake littoral zone DON production rates fall within a factor of 2 of the observed net DON fluxes from the lake (Table 1, Fig. 2) suggesting that the mesocosm experiments provided a reasonable estimate of littoral zone DON production rates. In June, the mesocosm experiment underestimated DON production rate by roughly 40% when compared to the lake-scale positive net flux. The unaccounted lake DON production could come from pelagic DON production or the lake-scale positive net flux could be overestimated if the unaccounted inflow water has a higher DON concentration than the in-channel inflow water. In July, the mesocosm experiment estimated a DON production rate that falls within the interpolated lake-scale positive net flux.

Pelagic nitrate uptake–Seston nitrate uptake in the June tracer mesocosm experiment was low relative to the net change in DON concentration that was observed during the June net N chemistry change experiment. Nitrate uptake by seston was 0.121

nmol L^{-1} hr^{-1} or 2.90 nmol L^{-1} d^{-1} (r^2 = 0.979, p = 0.032) at 0.5 m depth (Fig. 4). Although the seston was sampled for N and ^{15}N content for 6 d in the June mesocosm experiment, only data from the first 12 h of the experiment was used for calculation of nitrate uptake rates because uptake during this time was nearly exactly linear and exclusion of the later data eliminates the need for correction of uptake rate due to N turnover (Stark 2000). We could not calculate seston nitrate uptake for the July mesocosm experiment due to the loss of several samples during the analysis.

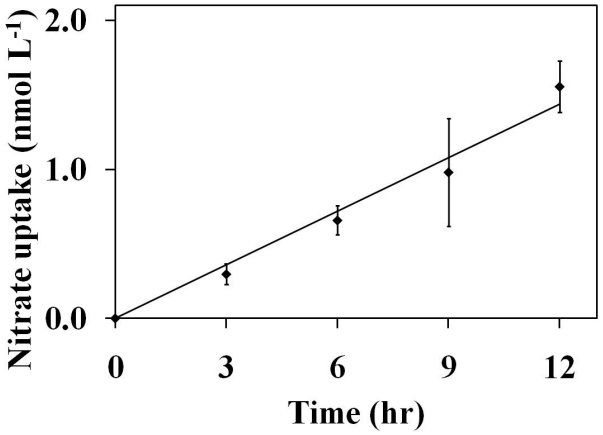

Figure 4. Seston nitrate uptake during the June mesocosm experiment. Nitrate uptake was 0.120 nmol L^{-1} hr^{-1} at 0.5 m depth. Data points are mean +/- standard error (n=2, 3 or 6). The linear regression displayed is statistically significant ($p < 0.05$).

In the August microcosm assays (Fig. 5) the estimated seston nitrate uptake was 2.54 nmol L^{-1} hr^{-1} or 61.0 nmol L^{-1} d^{-1} (r^2 = 0.978, $p < 0.01$) and 3.81 nmol L^{-1} hr^{-1} or 91.4 nmol L^{-1} d^{-1} (r^2 = 0.987, $p < 0.01$) at 0.5 and 5.5 m depth, respectively. Average standard error was 5% of the measured value. Nitrate uptake at 5.5 m was significantly higher than nitrate uptake at 0.5 m ($p < 0.01$). Seston nitrate uptake rate at 0.5 m depth

was 21 times greater in the August assay versus the June assay despite there being little change in seston biomass as measured by chlorophyll *a* (Table 2). In all three experiments the addition of the tracer resulted in a large increase in the concentration of nitrate (+ 0.67 µmol L^{-1}) and the nitrate uptake rates presented therefore more closely approximate potential uptake rates than actual rates.

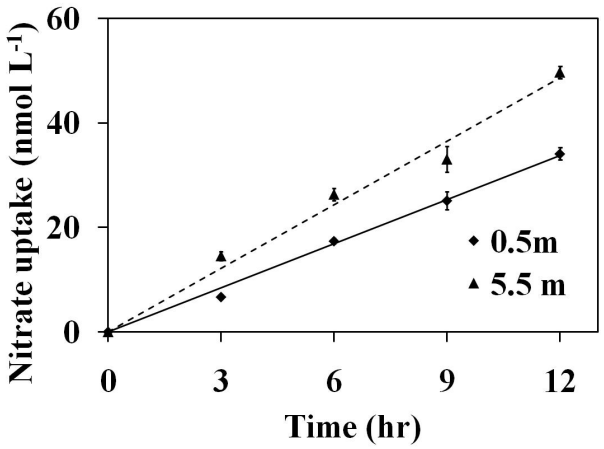

Figure 5. Seston nitrate uptake during the microcosm experiment on 8 August 2007. Nitrate uptake is 2.54 and 3.81 nmol L^{-1} hr^{-1} at 0.5 and 5.5 m depth respectively. Data points are mean +/- standard error (n=2). The linear regressions displayed are statistically significant ($p < 0.05$).

Benthic nitrate uptake and loss in mesocosm experiments–Benthic nitrate uptake rates from 0-8 cm were 85 ($r^2 = 0.953$, $p = 0.023$) and 133 ($r^2 = 0.888$, $p = 0.042$) µmol m^{-2} d^{-1} in the June and July mesocosm experiments respectively (Table 3, Fig. 6). If labeled N moved into sediments deeper than 8 cm then total benthic uptake rates are underestimated.

Table 2. Sample date, site, biomass, nutrient abundance and potential rates of nitrate uptake and amino acid hydrolysis for Bull Trout Lake's microbial and seston communities measured in mesocosm and microcosm experiments during 2007. N concentrations are pre-addition concentrations. Additions in the nitrate uptake assays resulted in a + 0.67 µmol L-1 increase in pool size. Additions in the enzymatic assays resulted in a + 200 µmol L-1 increase in pool size. "nd" denotes "no data".

Date	Site	Chl *a* ($\mu g\ L^{-1}$)	TDN ($\mu mol\ L^{-1}$)	NO3 ($\mu mol\ L^{-1}$)	DON ($\mu mol\ L^{-1}$)	NO_3 uptake ($nmol\ L^{-1}\ hr^{-1}$)	L-AMP Vmax ($nmol\ L^{-1}\ hr^{-1}$)
15 June	0.5 m	0.98	5.50	0.06	5.44	0.121 ± 0.024	nd
16 July	0.5 m	0.74	7.04	0.07	6.97	nd	55.6 ± 3.2
8 August	0.5 m	1.11	5.48	0.18	5.30	2.54 ± 0.015	nd
8 August	5.5 m	1.27	5.41	0.54	4.87	3.81 ± 0.120	nd
13 August	0.5 m	0.83	5.48	0.18	5.30	nd	77.7 ± 10.9
13 August	5.5 m	1.29	5.41	0.54	4.87	nd	68.4 ± 8.9
20 August	Inflow	0.10	1.44	0.07	1.37	nd	32.0 ± 2.7
20 August	Outflow	0.97	7.59	0.09	7.50	nd	63.6 ± 6.8

Table 3. Quantity of nitrogen derived from nitrate recovered in different layers during the A. June and B. July mesocosm experiments. Error estimates are standard error (n=3).

A					
Date	Experiment	Nitrogen uptake and loss (mmol m^{-2})			
	day	0-2 cm	2-4 cm	4-8 cm	Total
14 June	0	0	0	0	0
16 June	2	0.191 ± 0.021	0.020 ± 0.006	0.005 ± 0.001	0.216 ± 0.027
18 June	4	0.229 ± 0.032	0.033 ± 0.011	0.016 ± 0.010	0.278 ± 0.033
20 June	6	0.410 ± 0.068	0.100 ± 0.037	0.030 ± 0.007	0.531 ± 0.028
	Mesocosm walls were lowered following sampling on 20 June.				
22 June	2	0.336 ± 0.087	0.156 ± 0.074	0.061 ± 0.033	0.553 ± 0.110
24 June	4	0.241 ± 0.104	0.118 ± 0.049	0.123 ± 0.073	0.482 ± 0.220
29 June	9	0.228 ± 0.010	0.115 ± 0.016	0.039 ± 0.009	0.382 ± 0.003
B					
Date	Experiment	Nitrogen uptake and loss (mmol m^{-2})			
	day	0-2 cm	2-4 cm	4-8 cm	Total
18 July	0	0	0	0	0
20 July	2	0.278 ± 0.083	0.066 ± 0.009	0.049 ± 0.017	0.393 ± 0.078
22 July	4	0.534 ± 0.062	0.057 ± 0.012	0.026 ± 0.002	0.617 ± 0.069
24 July	6	0.449 ± 0.097	0.143 ± 0.053	0.106 ± 0.069	0.698 ± 0.211
	Mesocosm walls were lowered following sampling on 24 July.				
26 July	2	0.302 ± 0.048	0.235 ± 0.116	0.086 ± 0.030	0.623 ± 0.136
28 July	4	0.474 ± 0.080	0.159 ± 0.038	0.108 ± 0.014	0.741 ± 0.088
3 August	10	0.393 ± 0.111	0.110 ± 0.034	0.189 ± 0.131	0.692 ± 0.163
7 August	14	0.318 ± 0.131	0.178 ± 0.098	0.093 ± 0.040	0.589 ± 0.253
21 August	28	0.235 ± 0.079	0.166 ± 0.030	0.102 ± 0.022	0.503 ± 0.098
14 September	52	0.127 ± 0.017	0.112 ± 0.019	0.068 ± 0.018	0.307 ± 0.053

In both the June and July experiments, N derived from labeled nitrate was recovered from the sediments in significant quantities throughout the loss period. At the end of the 9-day loss period of the June experiment, 72% of the N that had moved into the sediments during the labeling period remained in the sediments. Assuming an exponential loss rate, 4.1 ± 0.9% of the newly incorporated nitrogen was lost per day and the estimated half life of the nitrogen for this experiment was 33 days. At the end of the 52-day loss period of the July experiment, 44% of the N remained in the sediments. Making the same assumptions, 1.5 ± 0.2% of the newly incorporated nitrogen was lost per day and the half life of N in this experiment was 64 days. Dividing the amount of N lost by the number of days of the experimental period yields estimated linear loss rates of 17 and 8 μmol m^{-2} d^{-1}, respectively, for the June and July experiments. These loss rates are less than the uptake rates indicating that nitrate moves into littoral zone sediments at a faster rate than the N is subsequently lost due to transport to other compartments such as sediment strata deeper than 8 cm or the water column or lost due to denitrification. The dominant N loss pathway is suspected to be transport rather than denitrification because dissolved dinitrogen gas samples taken in situ during the July uptake period of the experiment showed no isotopic enrichment of the gas (R. Lockwood unpublished data) indicating that the labeled N was not being denitrified.

The overall size of the N pool from 0-8 cm was 3.59 and 3.64 mol m^{-2} during the June and July experiments, respectively. Allowing the assumption that nitrate movement into the sediments is the sole input of N, then dividing the pool size by the rate of nitrate uptake yields N mean residence times of 116 and 75 years. This assumption is flawed because there are other inputs such as sedimentation that are likely to be important

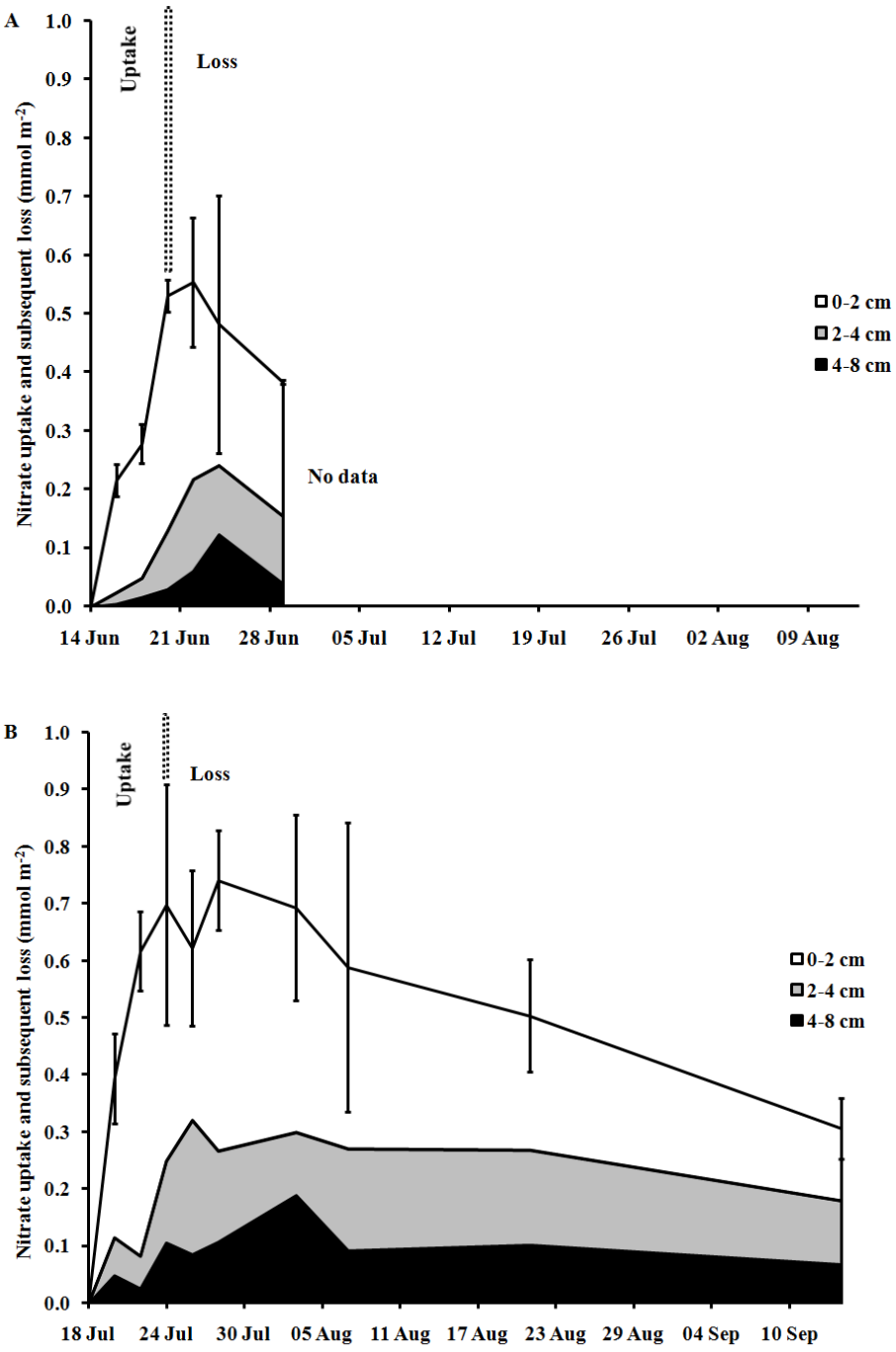

Figure 6. Benthic nitrate uptake and subsequent loss during the (A) June and (B) July ^{15}N tracer mesocosm experiments. The top line in each chart shows the mean +/- standard error (n=3) for the total (0-8 cm) sediment uptake in the three replicate mesocosms. The uptake and loss is expressed for 3 sediment horizons. White is 0-2 cm, grey is 2-4 cm and black is 4-8 cm.

contributors of N to the sediments and these residence times are therefore probably overestimated. Allowing the assumption that the loss rate of recently input N derived from nitrate accurately reflects the overall output rate of N from the sediments, then dividing the pool size by the rate of N loss yields N mean residence times of 579 and 1250 years. This assumption is also likely flawed because recently input N derived from nitrate is probably more labile than older more recalcitrant N and these residence times are therefore probably underestimated. Overall these assumptions and estimations reveal a great uncertainty in sediment N mean residence time but indicate that it is on the order of tens to hundreds of years. Overall, these estimations yield a conceptual model whereby nitrate moves rapidly into the sediments when it is available. The majority of this N input is then transported to other compartments and therefore leaves the 0-8 cm horizon during the following months. However, some of the N remains, most likely as recalcitrant material, and the remaining sediment N has a mean residence time of tens to thousands of years.

The overall quantity of nitrate uptake was much higher in the sediments than in the seston during the June mesocosm experiment (Fig. 4, 6). Mean peak value for nitrate uptake in the seston community occurred on day six of the experiment and was 8.18 μmol m^{-2}. Mean peak value of nitrate uptake in the benthic community also occurred on day six of the experiment and amounted to 530 μmol m^{-2}. Movement into the sediments accounted for 98.5% of benthic/pelagic nitrate uptake in the June mesocosm experiment. There was also a pronounced and similar difference in the standing stock of chlorophyll *a* in the seston vs. the sediments during the experiment. Chlorophyll *a* in the 1.2 m deep study area of the littoral zone amounted to 1.82 mg m^{-2} in the seston and 86.9 mg m^{-2} in

the 0-2 cm horizon of the sediments amounting to 98% of the littoral zone chlorophyll *a* residing in the sediments. These observations point to benthic dominance over littoral zone N biogeochemistry and metabolism.

Enzymatic assays–While nitrate uptake was measured for seston, particles and cells larger than 0.7 µm, L-AMP activity can also be attributed to dissolved fractions (Münster 1991) so it was measured for the entire planktonic community. Planktonic L-AMP activities ranged from 32.0 to 77.7 nmol L^{-1} hr^{-1} (Table 2, Fig. 7). The L-AMP activity in Bull Trout Lake surface water was higher on 13 Aug compared to 16 July and 20 August ($p < 0.01$) but there was no difference between 16 July and 20 August ($p > 0.05$). This shows that L-AMP activity varied with date but did not have an increasing or decreasing trend during the mid-summer. L-AMP activity did not differ between 0.5 and 5.5 m depth in Bull Trout Lake in the 13 August assay ($p > 0.05$) suggesting that L-AMP activity may be consistent throughout the epilimnion. The L-AMP activity of outflow water was twice that of inflow water during the 20 August assay and was significantly different ($p < 0.01$) showing that the presence of the lake in the flow path did affect enzymatic processing of DON. However, this is less than the relative difference of seston biomass between the inflow and outflow. On the assay date, the seston chlorophyll *a* concentration was 0.10 µg L^{-1} in the inflow and 0.97 µg L^{-1} in the outflow.

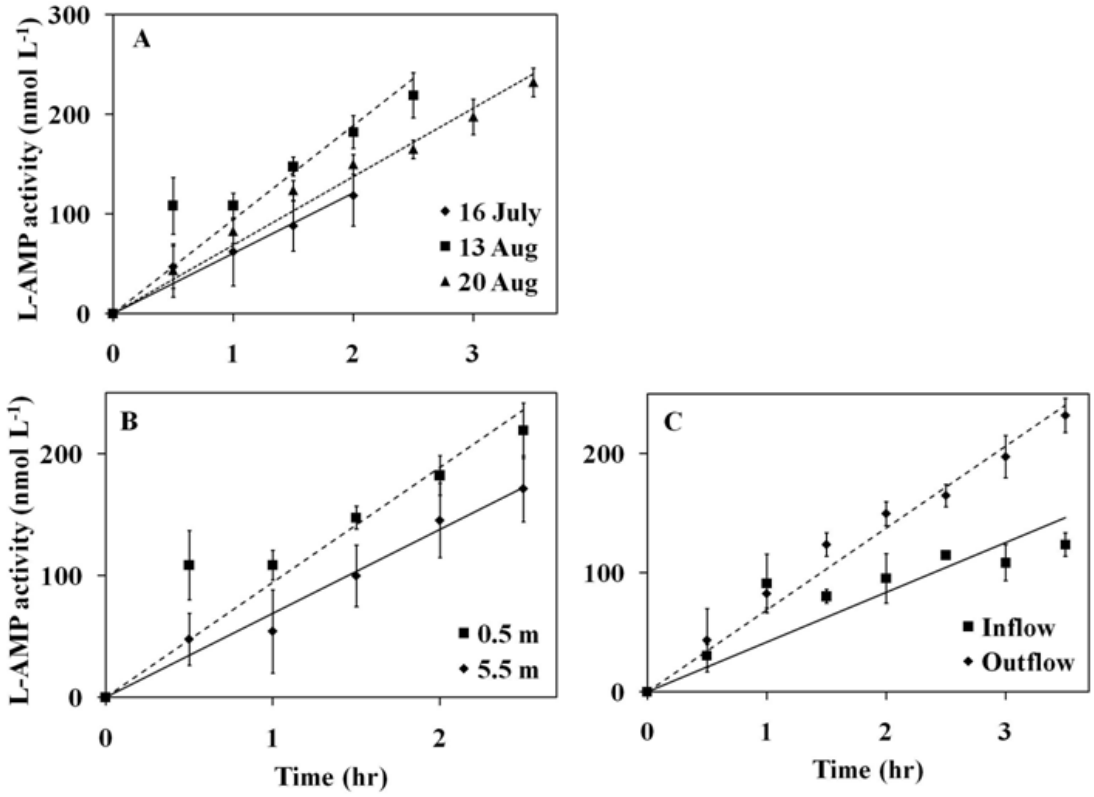

Figure 7. Planktonic L-AMP activities of Bull Trout Lake surface waters at (A) three dates at 0.5 m, (B) two depths on 13 Aug, and (C) the inflow and outflow on 20 Aug. Data points are mean +/- standard error (n=3).

DISCUSSION

The dominance of DON over DIN in our study waters (Table 1) indicates that the ecosystem is relatively pristine with low atmospheric N deposition, consistent with the biogeochemical theory of Hedin et al. (1995) and the results of Perakis and Hedin (2002). The atmospheric N deposition that does occur in the Bull Trout Lake watershed most likely occurs as forms of DIN (Vitousek et al. 1997). That the watershed losses of dissolved N are predominately organic indicates strong biotic control over the processing and transport of N. Furthermore, the net retention of nitrate and net positive flux of DON between the inflow and outflow of Bull Trout Lake (Fig. 2) shows the importance of the lake as a site of biotic processing. We suspect that the main sink of nitrate is biological assimilation and that much of the DON is produced via littoral zone N cycling, particularly by the benthic biota. Bull Trout Lake's shallow mean depth and littoral shelf is likely a key feature of its ability to act as a nitrate sink, consistent with an analysis of 100 northern European lakes that found a negative relationship between mean lake depth and seasonal nitrate depletion from the epilimnion (Weyhenmeyer et al. 2007).

Bull Trout Lake was a net sink of DON on a single sampling date, 10 May, during peak snowmelt flooding (Fig. 2, Table 1). This suggests that there is a positive interaction between DON bioavailability and peak discharge and other researchers have observed similar patterns. Wikner et al. (1999) found that spring flood DOC was of a higher nutritional quality for bacterioplankton than DOC transported during base flow. Stepanauskas et al. (2000) observed high DON bioavailability for bacteria during spring flooding using waters from boreal streams where TDN transport occurs primarily as

DON. It is likely that spring snowmelt delivers a pulse of nitrate and a pulse of more bioavailable DON to Bull Trout Lake and that the downstream delivery of this N is both temporally attenuated and delivered as more refractory forms.

Our data indicates that there is more dissolved N exported than imported in Bull Trout Lake, at least during spring through fall when most nutrients are transported. N fixation is a possible explanation that would provide for mass balance of the system; however this pathway has been examined in this watershed and the process cannot fully explain the initial N source of the observed DON production. N fixation was measured for the pelagia and several benthic habitat types seasonally in Bull Trout Lake during 2002 and 2003. The fixation rate was 38 μmol m^{-2} d^{-1} averaged from June to November (Marcarelli and Wurtsbaugh 2009). Scaled to the lake level this corresponds to an average input of 131 μmol s^{-1}. Even if all of the autochthonous fixed N was exported from the lake, this source would account for only 8-32% of the observed dissolved N flux during these months. Annual direct atmospheric deposition of inorganic N to Bull Trout Lake is circa 3,000 mol yr^{-1} or 95 μmol s^{-1} (NADP site ID15 2005). Again, even if all of this input were exported there is still not mass balance for the spring and summer. We suspect allochthonous inputs such as fine and course particulate organic matter to be important sources of N to the system.

Benthic and littoral zone metabolism and biogeochemistry have been under-examined in limnology relative to pelagic processes (Reynolds 2008). Benthic dominance of ecosystem metabolic functioning has been observed in studies of oligotrophic lakes and other aquatic systems. In our study, nitrate movement into the sediments accounted for 98.5% of benthic/pelagic nitrate uptake in the June mesocosm

experiment. Similarly, $^{15}N\text{-}NO_3$ addition studies of oligotrophic Castle Lake in California, USA showed strong dominance of periphyton over phytoplankton assimilation. Periphyton uptake accounted for ~90% of the disappearance of added nitrate in whole-epilimnion enrichments and epipelic periphyton incorporation accounted for 56% of the disappearance of labeled nitrate in a mesocosm experiment (Axler and Reuter 1996). Similarly, periphyton accounted for 80-98% of primary production in a study of 11 shallow oligotrophic lakes in Greenland (Vadeboncoeur et al. 2003). In a $^{15}N\text{-}NO_3$ addition study of a New England estuary, essentially all of the in-site nitrate processing occurred in the sediments (Tobias et al. 2003).

Our observed long-term retention of nitrogen in sediments (44% after 52 d) is less than the retention observed in a tracer study of a tidal freshwater marsh (Gribsholt et al. 2009). One hundred and eighty-two days after that addition, 42-48% of the added label remained in plants, roots and sediment. The dominant retention processes in the marsh study were bacterial immobilization and plant root assimilation. Plant roots were strong long-term sinks and the lack of macrophytes in Bull Trout Lake's shallow littoral zone may explain the shorter measured benthic N turnover time.

The measured quantity of N lost during the benthic N-loss mesocosm experiments is far less than the amount of DON production measured during the initial littoral zone net N chemistry change observation period. The estimated rates of littoral zone DON production were 23 and 45 times greater than the amount of N that was lost from the sediments after labeling. This indicates that littoral zone DON production is supported by N that moved into the sediments over a long period of time. These observations

support the hypothesis that nitrate uptake by littoral zone sediments is rapid and that this N is then gradually released back to the water column as DON.

The pronounced difference in measured rate of seston nitrate uptake between June and August is probably due to two factors. Due to a seasonal decline in epilimnetic nitrate concentration (Table 1) there is likely an increase in nitrate demand for the seston community that explains some of the difference. However, the rate of nitrate uptake measured in June in the mesocosm experiment may also have been underestimated due to immediate post-addition reduction of the pool size due to uptake into other pools such as the benthic community. The August microcosm experiments did not have the potential for benthic uptake and therefore represent more realistic estimates of potential seston nitrate uptake. Other unknown experimental artifacts from the two types of experiments may also have contributed to the observed differences.

It is noteworthy that in the enzymatic assays, particularly in the inflow/outflow assays, there was not a 1:1 correlation between DON concentration and enzymatic activity (Table 2), showing that DON quality, as well as microbial biomass and community composition, are important in determining microbial DON utilization. At the time of the inflow and outflow comparison L-AMP assay, the concentration of DON was 1.37 $\mu mol\ L^{-1}$ in the inflow and 7.50 $\mu mol\ L^{-1}$ in the outflow. With this 5.5-fold increase in DON concentration L-AMP activity only doubled when temperature was held constant. This suggests that while the presence of the lake in the flow path leads to a marked increase in DON concentration, the resultant DON has a lesser per molecule abundance of hydrolysable amino acids. The decrease in per molecule DON quality probably results from repeated microbial utilization leading to a lower abundance of

amine-bearing bioavailable molecules. This repeated microbial cycling in the lake can occur because water mean residence times in the summer range from 15-160 days (W. Wurtsbaugh, unpublished data), whereas those in the inflow are 4-6 hours (M. Baker, unpublished data).

Although the L-AMP activity of the outflow was twice that of the inflow during the 20 August assay (Table 2, Fig. 7), this is likely an underestimate of the actual difference in activity between the two sites because assays for both sites were run at 10ºC; however on this date the temperature of the inflow was 10º C and the temperature of the outflow was 22ºC. Applying a biological Q10 temperature coefficient correction that assumes that the enzymatic rate doubles with a 10ºC increase in accordance with the Arrhenius equation (Leskovac 2003) yields a corrected outflow enzymatic activity of 146.2 nmol L^{-1} hr^{-1} or a 4.6 times increase in the enzymatic potential of the outflow water versus the inflow water. These two calculated differences in L-AMP activity show two aspects of Bull Trout Lake's influence on the flow path's enzymatics. First, the increase in DON concentration and the 10-fold increase in microbial biomass that results from the surface water's residence time in the epilimnion increase the enzymatic potential of the community from 32.0 to 63.6 nmol L^{-1} hr^{-1}. Second, the temperature difference that results from epilimnetic warming further enhances the enzymatic activity of the outflow community from 63.6 to 146.2 nmol L^{-1} hr^{-1}.

Extracellular planktonic L-AMP activities were measured in two river systems in the United Kingdom and the values obtained for V_{max} in those studies correspond to the measurements presented here. The range of L-AMP activities measured in the headwaters of the River Swale was 33 to 201 with a mean of 78 nmol L^{-1} hr^{-1} (Ainsworth

and Goulder 1998) and in the headwaters of the River Tweed was 14 to 481 with a mean of 228 nmol L^{-1} hr^{-1} (Ainsworth and Goulder 2000). The values presented in our headwater system ranged from 32 to 78 nmol L^{-1} hr^{-1}. Both of the UK systems showed patterns of downstream increase similar to the pattern we found between the inflow and outflow of Bull Trout Lake.

The nitrate uptake rates and enzymatic activities presented are both potential rates so they can be directly compared to each other when they were run in parallel. Both parameters were measured for the seston community in the same week for Bull Trout Lake waters at 0.5 m and 5.5 m in August. At both depths the potential for DON hydrolysis was greater than the potential for nitrate uptake on mole/mole of N basis. In the surface waters the hydrolytic potential was 31 times greater than the potential nitrate uptake and at depth the difference was 18 times. This, combined with the greater concentration of DON versus DIN, underscores the importance of the DON pool as a potential N source in this system. These observations also suggest the importance of the microbial community as a means of amino acid hydrolysis. Because the amino acids are hydrolyzed extracellularly they are not necessarily incorporated into the heterotrophic microbial community but rather a portion of the newly freed dissolved amino acids may be incorporated into the primarily photosynthetic seston community. Once incorporated into the seston community, the N will be more available to grazing organisms such as zooplankton and then more available to planktivorous organisms such as larval fish.

This study provides evidence for the importance of DON and microbial DON processing at the landscape level and there is room for more detailed work. A study that both pinpoints the rates of DON hydrolysis and traces the uptake of the hydrolyzed amino

acids into the microbial loop and the higher seston, zooplankton, vertebrate food web would be useful for understanding the role of DON in sustaining aquatic ecosystem production.

CONCLUSION

This study demonstrated the influence of a lake on the N cycling of a surface water network. Between the inflow and outflow of the lake there were pronounced increases in temperature, sestonic chlorophyll *a* concentration, and L-AMP activity. There was also net retention of nitrate and net positive flux of DON that was sustained during the spring and summer. This study quantified some of the mechanisms for the observed patterns. The uptake and release of nitrogen from littoral zone sediments in mesocosm experiments correspond with the lake-scale observations suggesting the importance of littoral sediments as an area of N processing. The magnitude of nitrate uptake by the seston was overshadowed by the magnitude of nitrate uptake by the littoral zone sediments and by the potential for hydrolysis of amine groups from DON. This study supports three main conclusions: (1) The impoundment of water in a lake produces pronounced changes in chemistry and biology; (2) benthic N processing is prevalent over pelagic N processing, and; (3) DON is likely an important N source for the biota both due to the larger pool size and apparent ability of the microbial community to hydrolyze labile functional groups from it.

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