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A Stable Isotope Tracer Study of the Influences of Adjacent Land Use and Riparian Condition on Fates of Nitrate in Streams

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Abstract

The influence of land use on potential fates of nitrate (NO_3^-) in stream ecosystems, ranging from denitrification to storage in organic matter, has not been documented extensively. Here, we describe the Pacific Northwest component of Lotic Intersite Nitrogen eXperiment, phase II (LINX II) to examine how land-use setting influences fates of NO_3^- in streams. We used 24 h releases of a stable isotope tracer ($^{15}NO_3$-N) in nine streams flowing through forest, agricultural, and urban land uses to quantify NO_3^- uptake processes. NO_3^- uptake lengths varied two orders of magnitude (24–4247 m), with uptake rates (6.5–158.1 mg NO_3-N m^{-2} day^{-1}) and uptake velocities (0.1–2.3 mm min^{-1}) falling within the ranges measured in other LINX II regions. Denitrification removed 0–7% of added tracer from our streams. In forest streams, 60.4 to 77.0% of the isotope tracer was exported downstream as NO_3^-, with 8.0 to 14.8% stored in wood biofilms, epilithon, fine benthic organic matter, and bryophytes. Agricultural and urban streams with streamside forest buffers displayed hydrologic export and organic matter storage of tracer similar to those measured in forest streams. In agricultural and urban streams with a partial or no riparian buffer, less than 1 to 75% of the tracer was exported downstream; much of the remainder was taken up and stored in autotrophic organic matter components with short N turnover times. Our findings suggest restoration and maintenance of riparian forests can help re-establish the natural range of NO_3^- uptake processes in human-altered streams.

Key words: land use; streams; nitrate; nitrogen; spiraling; denitrification; organic matter storage; N-15; isotope tracer; Oregon.

Introduction

The availability of nitrogen (N) can strongly influence the structure and function of ecosystems (Grimm 1987). Naturally, N is in scarce supply for most terrestrial and aquatic ecosystems (Galloway and others 2004). However, anthropogenic activities associated with food and energy production

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currently provide as much N to the Earth's land surface as from natural sources alone (Galloway and others 2004). Increased anthropogenic N input to aquatic ecosystems, mostly in the form of nitrate (NO_3^-), has resulted in widespread negative impacts to the productivity and biological diversity in rivers, lakes, estuaries, and near-shore marine ecosystems (Mallin and others 2006; Howarth 2008).

Uptake of NO_3^- in headwater streams is increasingly recognized as a potentially important process for reducing the delivery of N to downstream ecosystems (Alexander and others 2000; Mulholland and others 2008). Less recognized is the diversity of processes involved in stream NO_3^- uptake. Dissolved NO_3^- can be transformed via denitrification and via storage in organic matter. Denitrification is an anaerobic microbial process that converts NO_3^- to N_2 and N_2O gases. Organic matter storage involves uptake of NO_3-N into multiple organic matter types with widely varying N turnover rates. Both denitrification and organic matter storage can reduce export of NO_3^- to downstream ecosystems over short time scales (Mulholland and others 2009; Hall and others 2009a). However, distinguishing organic matter storage from denitrification is important for understanding long-term stream N dynamics and effects of elevated N loading to streams. Denitrification completely removes nitrate-nitrogen (NO_3-N) from downstream transport and, for nutrient-enriched river systems, can be viewed a desirable fate for NO_3^- (Mulholland and others 2008). Organic matter storage, on the other hand, represents temporary retention of NO_3-N, meaning stored NO_3-N can continue to affect stream ecosystem structure and function upon remobilization as organic or inorganic N.

Examination of individual NO_3^- uptake processes and fates in streams was not possible until recent advances in ^{15}N stable isotope tracer methods (Mulholland and others 2004; Böhlke and others 2004). Stable isotope tracer experiments allow measurement of multiple processes of NO_3^- uptake simultaneously and without significant alteration to ambient NO_3^- concentration (Mulholland and others 2009). Most isotope tracer studies have focused on total stream NO_3^- uptake and denitrification (Mulholland and others 2004; Böhlke and others 2004; Earl and others 2006; O'Brien and others 2007; Valett and others 2008; Hall and others 2009b; Mulholland and others 2009; Potter and others 2010). These studies have shown that concurrent alterations associated with land-use activities at different locations and scales in surrounding catchments can affect total NO_3^- uptake and denitrification (Earl and others 2006; O'Brien and others 2007; Hall and others 2009b; Mulholland and others 2009; Von Schiller and others 2009). At the catchment scale, chronically high NO_3^- concentrations associated with N loading from agricultural and urban land uses can lead to less efficient uptake and denitrification of NO_3^- in streams compared to streams surrounded by native forest or grassland with concomitant low NO_3^- concentrations (Earl and others 2006; Hall and others 2009b; Mulholland and others 2009). Simultaneously, reach-scale reduction or removal of riparian vegetation in agricultural and urban settings can increase total NO_3^- uptake rate and denitrification via stimulation of primary production and increased abundance of autotrophically derived carbon (Hall and others 2009a; Mulholland and others 2009; von Schiller and others 2009).

Isotope tracer experiments can also provide important insight on NO_3-N storage in individual organic matter components relative to denitrification. However, few studies have presented information at the level of detail needed for such an analysis (but see Von Schiller and others 2009). In this study, we examined influences of adjacent forest, agriculture, and urban land uses on stream NO_3^- uptake processes and fates in the Pacific Northwest (PNW) component of the Lotic Intersite Nitrogen eXperiment, phase II (LINX II). LINX II was a cross-biome study of stream NO_3^- dynamics that employed stable isotope tracer additions of NO_3^- in 72 North American streams (Mulholland and others 2008). We used short-term (24 h) stable isotope ($^{15}NO_3$-N) tracer experiments to address two questions: (1) does adjacent land use influence NO_3^- uptake processes in streams? (2) what are the fates of NO_3^- and how do they vary among land uses? We predicted multiple alterations, notably light input and NO_3^- concentration, associated with land-use setting would influence total NO_3^- uptake and fate in streams. We also predicted NO_3-N stored in organic matter components would be proportional to the abundance of organic matter types across land uses. Storage in detrital organic matter should be the largest fate in forest streams and storage in autotrophs should be the largest fate in agricultural and urban streams due to increased light availability associated with modified riparian zones. Lastly, we expected denitrification rate and the contribution of denitrification to total NO_3^- uptake to be positively related to NO_3^- concentration (Seitzinger and others 2006).

Methods

Site Descriptions

This study included nine second- to fourth-order stream reaches (hereafter streams) in the Willamette River Basin (WRB) in western Oregon, USA (Table 1; Figure 1). Stream flow in the WRB varies seasonally, with high flow from late autumn to spring and low flow from summer to early autumn. Isotope tracer experiments were conducted during summer at low flow when discharge is stable. Streams were selected to have a range of forest, agriculture, and urban land uses adjacent to the stream. Urban streams were located in urban growth boundaries and agricultural streams were located in agricultural management areas. Land-use types in the WRB coincide with catchment position: forest streams were in mountains or foothills, agricultural streams were in foothills and valleys, and urban streams were in valleys. One stream (Mack Creek) was in a catchment with an old growth (~500 years) coniferous forest. The other two forest streams were in younger, second-growth mixed deciduous–coniferous forests. Two agricultural streams had forested headwaters and narrow riparian gallery forests adjacent to pastures. One agricultural stream (Courtney Creek) had low shrubs and small trees between the channel and plowed fields. Two urban streams (Amazon and Periwinkle) had patchy riparian vegetation and impervious surfaces nearby. The other urban stream (Oak-U) had a continuous riparian gallery forest with adjacent parking lots. Oak-F, Oak-A, and Oak-U were individual reaches located on the same stream. For these reaches, stable isotope experiments were performed in sequence from downstream (Oak-U) to upstream (Oak-A) to avoid isotopic contamination. Reach lengths were determined using water velocity, avoidance of tributaries, and accessibility.

Ecosystem Characteristics

We measured wetted width, depths, inorganic substrate diameters, and riparian cover (using a concave spherical densiometer) at 15 transects on each stream before the isotope tracer experiment. Photosynthetically active radiation (PAR) was measured at a representative location on the stream bank using a quantum sensor during the isotope tracer experiment (LiCor 190SA, LiCor Biosciences, Lincoln, NE, USA).

Ammonium (NH_4^+), total dissolved N (TDN), and soluble reactive phosphorus (SRP) concentrations were analyzed in water samples from six sites per

Table 1. Location and Catchment Characteristics of the Study Streams in Western Oregon, USA

Stream name	Geographic coordinates	Land use[a]	Catchment area (ha)	Reach length (m)	Stream order (1:24,000)	Catchment land use/land cover (%)			
						Forest	Agriculture	Urban	Impervious[b]
Mack	44°13′N; 122°10′W	FOR	531	404	3	100	0	0	0
Oak-F	44°37′N; 123°20′W	FOR	617	423	2	97	1	2	0
Potts	44°16′N; 122°29′W	FOR	349	590	2	100	0	0	0
Camp	44°07′N; 122°49′W	AGR	2681	462	4	98	2	0	0
Oak-A	44°34′N; 123°18′W	AGR	3051	350	3	74	18	8	1
Courtney	44°22′N; 123°58′W	AGR	4169	352	2	75	25	0	0
Oak-U	44°34′N; 123°17′W	URB	3221	223	3	71	18	11	2
Periwinkle	44°37′N; 123°05′W	URB	2179	119	3	0	70	30	22
Amazon	44°03′N; 123°06′W	URB	1026	546	3	31	0	69	20

[a] *Urban stream reaches were located in urban growth boundaries; agricultural streams were located in agricultural management areas.*
[b] *Included as a subset of urban land use/land cover.*

Figure 1. Study streams in western Oregon, USA. Photographs taken by Sherri Johnson.

stream, collected immediately before the isotope tracer experiment. Average NO_3-N concentration was calculated from 12 sites per stream during the isotope tracer experiment. Water samples were filtered through pre-combusted (500°C) glass fiber filters (Whatman GF/F, pore size = 0.7 μm; Florham Park, NJ, USA). NO_3-N was measured colormetrically as NO_3-N + NO_2-N on a Technicon Autoanalyzer II (Technicon, Emeryville, California, USA) following reduction in a copperized cadmium column. Ammonium-nitrogen (NH_4-N) was measured colorimetrically on a Technicon Autoanlyzer II following additions of alkaline phenol and hypochlorite. TDN was measured by performing a persulfate oxidation to convert all dissolved N species to NO_3-N + NO_2-N and following the NO_3-N protocol described above. SRP was measured colorimetrically on a Technicon Autoanalyzer II following addition of ammonium molybdate and antimony potassium tartrate. Detection limits for chemical species were as follows: NO_3-N = 1 μg l^{-1}; NH_4-N = 10 μg l^{-1}; TDN = 10 μg l^{-1}; and SRP = 1 μg l^{-1}. Measurements below detection were set to zero for statistical analyses. Chemical analyses were performed by the Cooperative Chemical Analytical Laboratory (http://www.ccal.oregonstate.edu/).

Leaves, needles, fine benthic organic matter (FBOM; >0.7 μm and <1 mm in diameter), epilithon, wood biofilm, filamentous green algae (FGA), and other plants (bryophytes, vascular macrophytes, and non-attached fine algal material) were sampled quantitatively from a known area at ten sites per stream in pool and riffle habitats (Ashkenas and others 2004). Samples were dried (60°C) to a constant weight and combusted (500°C) to calculate ash free dry mass (AFDM). AFDM standing stocks were calculated by weighting by the area of pools and riffles. Wood standing stocks were quantified separately using the linear transect method (Wallace and Benke 1984). Wood volume was converted to organic matter by assuming a density of 0.4 g organic matter per cm^{-3} (Harmon and others 1986). Reach-scale wood biofilm standing stocks were calculated using surface area of submerged wood. Carbon (C) and N content of all organic matter types were measured on a Heraeus CHN elemental analyzer (Hanau, Germany). At Oak Creek reaches, C was estimated as 45% of AFDM due to lack of direct measurements (Simon and others 2004).

Whole-system gross primary production (GPP) and ecosystem respiration (ER) were measured at six streams using the two-station open stream method (Young and Huryn 1999). At three streams,

the one-station method (Young and Huryn 1999) was used due to instrument failure. Dissolved oxygen (DO) concentration and water temperature were measured at 5 min intervals during the tracer experiment using field-calibrated Clark cells attached to Hydrolab 4A Minisondes (Hach USA, Loveland, CO, USA). Barometric pressure was recorded with a handheld meter at 2-h intervals. The Atmospheric exchange rate of DO was determined using coefficients calculated from a steady-state release of SF_6 and a conservative solute tracer (Hall and Tank 2003).

Isotope Tracer Experiments

We conducted a 24-h steady-state addition of $^{15}NO_3$-N isotope tracer to quantify NO_3^- uptake processes in each stream. The $\delta^{15}N$ of NO_3-N was elevated to approximately 20,000‰ while increasing NO_3^- concentration by no more than 7.5% to minimize effects of N fertilization (Mulholland and others 2008). The total amount added and rate of addition were determined from measurements of discharge and NO_3^- concentration collected the week preceding the tracer addition. A conservative tracer (Cl^- or Br^-) was used to calculate discharge, specific discharge (discharge/wetted width), and water retention time (stream velocity/reach length) during the addition (Hall and others 2009b). The conservative tracer was also used to determine isotope tracer uptake by correcting for dilution by groundwater (Stream Solute Workshop 1990). Conservative solute tracers were analyzed on a Dionex DX500 Ion Chromatograph (Dionex, Sunnyvale, CA, USA).

A solution of 98% pure ^{15}N as KNO_3 (Cambridge Isotope Laboratories, Andover, Massachusetts, USA) and the conservative solute tracer was added to each stream from a single release site. Six sampling sites for tracer in dissolved N and organic matter components were established downstream from this addition point to the lower end of the reach (Table 1). A seventh site for background ^{15}N was located upstream of the release site. Dissolved gases (N_2 and N_2O) were sampled at these and at four additional sites distributed between the first and fifth sampling sites. Duplicate samples for background ^{15}N in NO_3^-, NH_4^+, DON, particulate N (PN), N_2, and N_2O were sampled from the sites immediately before the midday (1300 h) start of tracer experiment. After 11 h (0000 h; hereafter night), we collected water samples for $^{15}NO_3$-N analysis at the seven sites and ^{15}N in dissolved gases (N_2 and N_2O) at the 11 sites. Sampling was repeated 12 h later (1200 h; hereafter day) after which we stopped the tracer addition. Twenty-four hours later, we collected samples for tracer in NH_4^+, DON, PN, and organic matter components at the seven sites.

Tracer in NO_3^-, NH_4^+, and TDN from filtered samples was collected on acidified filters (precombusted Whatman GF/D) enclosed in Teflon tape using the modified headspace diffusion technique (Sigman and others 1997; Holmes and others 1998; O'Brien and others 2007). Filters were sent for analysis at the Marine Biological Laboratory (MBL; Woods Hole, MA). For $TD^{15}N$, samples underwent persulfate digestion before the headspace diffusion was performed. Tracer ^{15}N in DON was calculated as $TD^{15}N$–$^{15}NO_3$-N–$^{15}NH_4$-N. Tracer ^{15}N in PN (material retained on GF/F filters) and organic matter components were analyzed by drying, grinding to a fine (<500 μm) powder, and sending the material to MBL. All ^{15}N samples were analyzed on a PDZ Europa 20-20 mass spectrometer.

Tracer ^{15}N in N_2 and N_2O gases was sampled by diffusing gases from unfiltered 40 ml water samples into a 20 ml headspace of 99% pure helium (Mulholland and others 2009). A 14-ml subsample was injected into an evacuated 10-ml exetainer for storage until analysis. Samples for ^{15}N gases were analyzed on a Europa Hydra Model 20-20 mass spectrometer (Stable Isotope Laboratory at the University of California-Davis), a ThermoFinnigan Delta-Plus mass spectrometer (Stable Isotope Laboratory at Kansas State University), or a VG Instruments Prism Series II mass spectrometer (Biogeochemistry Laboratory, Department of Zoology, Michigan State University).

Total Uptake and Denitrification Calculations

We calculated uptake length (S_w), uptake velocity (v_f) and uptake rate (U) of NO_3^- from downstream flux of $^{15}NO_3$-N tracer (Stream Solute Workshop 1990). We calculated uptake length of tracer for night and day separately as the inverse of the slope from the log-linear regression of tracer flux versus downstream distance (Stream Solute Workshop 1990). Uptake velocity and rate were calculated using the following equations:

$$v_f = \frac{Q}{wS_w} \quad (1)$$

$$U = v_f C \quad (2)$$

where Q is average discharge for the reach ($m^3\ s^{-1}$), w is average wetted width (m), and C is average NO_3-N concentration ($\mu g\ l^{-1}$).

Uptake length, velocity, and rate for denitrification were determined from the model described in Mulholland and others (2004; Appendix A in supplementary material). Denitrification was reported only where ^{15}N abundance in gases was equal to or greater than the upper 97.5% confidence limit of background ^{15}N for three or more samples (Mulholland and others 2009).

Organic Matter Uptake

Uptake rate of total NO_3-N in an individual organic matter component at each sampling site was calculated by dividing the background-corrected ^{15}N standing stock (mg ^{15}N m^{-2}) in the organic matter component by the hydrologic fluxes of $^{15}NO_3$-N tracer (mg $^{15}NO_3$-N day^{-1}) and total NO_3-N (mg NO_3-N day^{-1}) at the sampling site. Average organic matter specific uptake rate was calculated from sampling sites where the organic matter component was present. Turnover time of NO_3-N in organic matter components was calculated as specific uptake (mg N m^{-2} day^{-1}) divided by the N standing stock of the specific component (mg N m^{-2}; Dodds and others 2004).

Mass Balance

We calculated mass balances by comparing the combined total of tracer exported downstream, denitrified, and stored in organic matter with the amount of isotope tracer released. Hydrologic export of tracer as NO_3^- was calculated by subtracting whole-stream tracer uptake (using the night-day average of uptake length) from tracer released (O'Brien and others 2007). Hydrologic exports of tracer as NH_4^+, DON, and PN were calculated as in Mulholland and others (2000; Appendix B in supplementary material). Denitrification of tracer was calculated using average denitrification uptake length (Appendix A in supplementary material; O'Brien and others 2007).

We calculated uptake lengths for individual organic matter components by regressing tracer recovered in an organic matter component (natural log-transformed mg ^{15}N m^{-2}) versus distance downstream of the isotope release point (Hamilton and others 2001). For significant regression models ($P < 0.05$), we used the individual organic matter uptake length to calculate the amount of tracer stored in the organic matter component for the stream (Hamilton and others 2001). If the regression was not significant ($P > 0.05$), organic matter tracer storage was quantified as the standing stock of ^{15}N in the organic matter type multiplied by area of the study stream (Hamilton and others 2001).

Statistics

We used linear and nonlinear regression models ($\alpha = 0.05$) to compare total uptake metrics with measured characteristics of the stream and riparian zone. Due to the small number of streams ($n = 9$), we only described regression models for which removal of one or two visible outliers did not change model significance. One-way analysis of variance (ANOVA) was used to determine if differences of total uptake metrics (uptake lengths, velocities, and rates), denitrification, and organic matter storage of $^{15}NO_3$-N tracer were attributable to adjacent land use. A Bonferroni-adjusted $\alpha = 0.017$ indicated a significant difference among land uses and a Bonferroni-adjusted $\alpha = 0.033$ indicated a significant difference among organic matter storage components regardless of land use. We also tested for night and day differences in tracer uptake metrics two-way ANOVAs with interaction terms (land use × sampling period). Variables were log transformed when inspection of scatter plots showed a positive skew. ANOVA models were constructed in R v.2.6.0 (R Development Core Team 2009) and regression models were constructed in SigmaPlot 10.0 (Systat, Software, Inc., San Jose, CA).

Results

Ecosystem Characteristics

Discharge varied by two orders of magnitude among streams (2.7–113.4 l s^{-1}) whereas specific discharge varied by one order of magnitude (0.002 to 0.012 m^2 s^{-1}; Table 2). Average water residence time ranged from 1.2 to 9.8 h (Table 2). Median inorganic substrate diameter (D_{50}) ranged from 8 to 128 mm (Table 2). Riparian cover and PAR were inversely related across streams (Table 2).

Mean NO_3-N concentration was consistently low and ranged from 2 to 160 μg l^{-1} across streams (Table 2). NH_4^+, DON, and PN concentrations were less than or equal to NO_3-N concentration for all except two urban streams (Periwinkle and Amazon), where DON and PN exceeded NO_3^- by two orders of magnitude (Table 2). Mean SRP concentrations were generally high (Table 2). DIN/SRP was low in all except two agricultural streams (Table 2).

GPP and ER both varied by two orders of magnitude among streams (Table 2). All streams were net heterotrophic (GPP/ER < 1). Average dissolved oxygen concentration ranged from 6.02 to 9.11 mg l^{-1} and average temperature ranged from 13 to 23°C (Table 2).

Table 2. Average Values of Physical, Chemical, and Biological Characteristics for Study Streams in Western Oregon, USA

Stream name	Width (m)	Depth (cm)	Discharge ($l\ s^{-1}$)	Q/w[a] ($m^2\ s^{-1}$)	Residence time (h)	D_{50}[b] (mm)	Temp[c] (°C)	Riparian cover (%)	PAR[d] ($mol\ m^{-2}\ day^{-1}$)
Mack	6.7	5	30.7	0.005	1.2	128	13	56	1.97
Oak-F	2.1	8	7.5	0.004	2.6	64	15	92	1.45
Potts	2.9	8	19.0	0.007	2.0	45	14	88	3.96
Camp	5.9	25	113.4	0.019	1.7	45	18	69	21.87
Oak-A	2.7	19	5.5	0.002	9.1	23	17	75	2.66
Courtney	3.3	21	38.8	0.012	1.7	32	16	27	26.40
Oak-U	3.9	32	7.9	0.002	9.8	16	21	82	2.57
Periwinkle	3.4	19	2.7	0.001	7.9	64[e]	23	1	37.60
Amazon	6.5	7	25.0	0.004	2.8	8[e]	21	49	39.37

Stream	NO_3-N ($\mu g\ l^{-1}$)	NH_4-N ($\mu g\ l^{-1}$)	DON ($\mu g\ l^{-1}$)	PN ($\mu g\ l^{-1}$)	SRP ($\mu g\ l^{-1}$)	DIN/SRP[f]	DO ($mg\ O_2\ l^{-1}$)	GPP[g] ($g\ O_2\ m^{-2}\ d^{-1}$)	ER[h] ($g\ O_2\ m^{-2}\ day^{-1}$)	$^{15}NO_3$-N released (g)
Mack	63	bdl[i]	38	11	13	12	8.20	0.21	−4.78	16.0
Oak-F	71	bdl[i]	79	63	35	5	7.74	0.09	−0.99	4.3
Potts	69	bdl[i]	169	21	25	7	8.23	0.28	−14.33	9.6
Camp	54	bdl[i]	51	47	5	25	7.94	0.32	−4.89	21.0
Oak-A	96	bdl[i]	89	31	48	5	6.57	0.20	−0.98	5.8
Courtney	103	11	100	20	5	49	9.11	3.03	−4.03	35.2
Oak-U	160	19	115	39	45	9	6.02	0.83	−6.92	6.3
Periwinkle	8	bdl[i]	347	130	209	<1	4.90	2.35	−9.85	0.1
Amazon	2	bdl[i]	321	90	18	<1	8.96	1.95	−4.87	4.0

[a] *Specific discharge (discharge/width).*
[b] *Median diameter of inorganic substrates in the reach.*
[c] *Water temperature.*
[d] *Photosynthetically active radiation.*
[e] *Portions of the reach lined with concrete or packed clay.*
[f] *Atomic fraction (mol/mol), where NH_4-N was below the detection limit, only NO_3-N was used.*
[g] *Gross primary production.*
[h] *Ecosystem respiration.*
[i] *Below detection limit (10 $\mu g\ NH_4$-N L^{-1}).*

Oak Creek-U had the highest standing stock of total organic matter (7,143 g m^{-2}) among streams and Amazon Creek had the lowest (74 g m^{-2}; Table 3). FBOM was present in all of the streams; all other components were absent at least once (Table 3). In-stream wood was not present at two urban streams (Periwinkle and Amazon), but it made up the largest fraction of total organic matter in four of the remaining streams (Table 3). Wood biofilms and epilithon had comparable standing stocks and distributions among streams. FGA was found in five of the streams and made up the largest fraction of total organic matter in one urban reach (Periwinkle). Aquatic bryophytes were found in two of the forest streams. Vascular macrophytes and non-attached fine algal material were present only once (Table 3).

Total Uptake and Denitrification

Tracer added to the streams ranged from 0.1 to 35.2 g of $^{15}NO_3$-N (Table 2). Uptake of tracer was measured on all streams except during the day at Camp Creek, where isotopic contamination of samples occurred. Uptake length ranged from 24 to 4247 m, uptake rate ranged from 6.48 to 158.11 mg $^{15}NO_3$-N m^{-2} day^{-1}, and uptake velocity ranged from 0.07 to 2.28 mm min^{-1} (Figure 2). There were no significant differences of total uptake metrics between night and day, among land uses, or for the interaction of sampling period and land use ($P > 0.07$). Five streams had shorter uptake lengths and higher uptake rates and velocities during the day than night (Figure 2).

Log-transformed night uptake length was significantly correlated with log-transformed values of discharge, specific discharge, and DIN/SRP (Table 4; Figure 3A). Night uptake rate had a significant positive linear correlation with NO_3^- concentration (Table 4; Figure 3B). Regression models for day uptake length and rate were either non-significant (Figure 3C, D) or model significance was controlled by one or two streams. Day uptake velocity was significantly and linearly correlated with riparian cover, PAR, and GPP (Figure 4D–F). Night uptake velocity was only significantly correlated with PAR; but this correlation was not significant when two unshaded urban streams (Amazon and Periwinkle) were held out of the analysis (Figure 4B).

Denitrification of tracer was above detection limits in the three agricultural streams and one urban stream (Figures 2B, C). Between 2 and 27% of total (night–day average) tracer uptake consisted of denitrification in these streams. N_2O accounted for less than 2% of tracer recovered in N gases among streams.

Table 3. Habitat-weighted Standing Stocks of Stream Organic Matter Components (Ash-free Dry Mass (g) per m^2) for Study Streams in Western Oregon, USA

Stream	FBOM	Leaves/ needles	Wood	Wood biofilms	Epilithon	FGA[a]	Algal fines	Other plants	
								Macrophytes	Bryophytes
Mack	64 (29:1)	<1 (70:1)	4911 (194:1)	2 (26:1)	4 (20:1)	0	0	0	38 (18:1)
Oak-F	182 (22:1)	6 (25)	2322 (45:1)	12 (30:1)	3 (8:1)	0	0	0	0
Potts	94 (21:1)	32 (19:1)	2074 (63:1)	1 (26:1)	2 (9:1)	0	0	0	8 (14:1)
Camp	120 (26:1)	14 (29:1)	426 (48:1)	4 (49:1)	8 (8:1)	0	0	0	0
Oak-A	845 (22:1)	21 (26:1)	724 (26:1)	1 (37:1)	1 (9:1)	20 (10:1)	0	0	0
Courtney	166 (12:1)	5 (26:1)	113 (39:1)	1 (14:1)	4 (4:1)	<1 (26:1)	0	0	0
Oak-U	3257 (21:1)	9 (30:1)	3883 (100:1)	5 (43:1)	21 (20:1)	10 (28:1)	0	0	0
Periwinkle	100 (8:1)	<1 (53:1)	0	0	0	195 (26:1)	0	52 (11:1)	0
Amazon	58 (13:1)	0	0	0	3 (14:1)	13 (20:1)	1 (44:1)	0	0

[a]*Filamentous green algae.*
Atomic ratios of carbon-to-nitrogen are in parentheses. Zeros indicate not found.

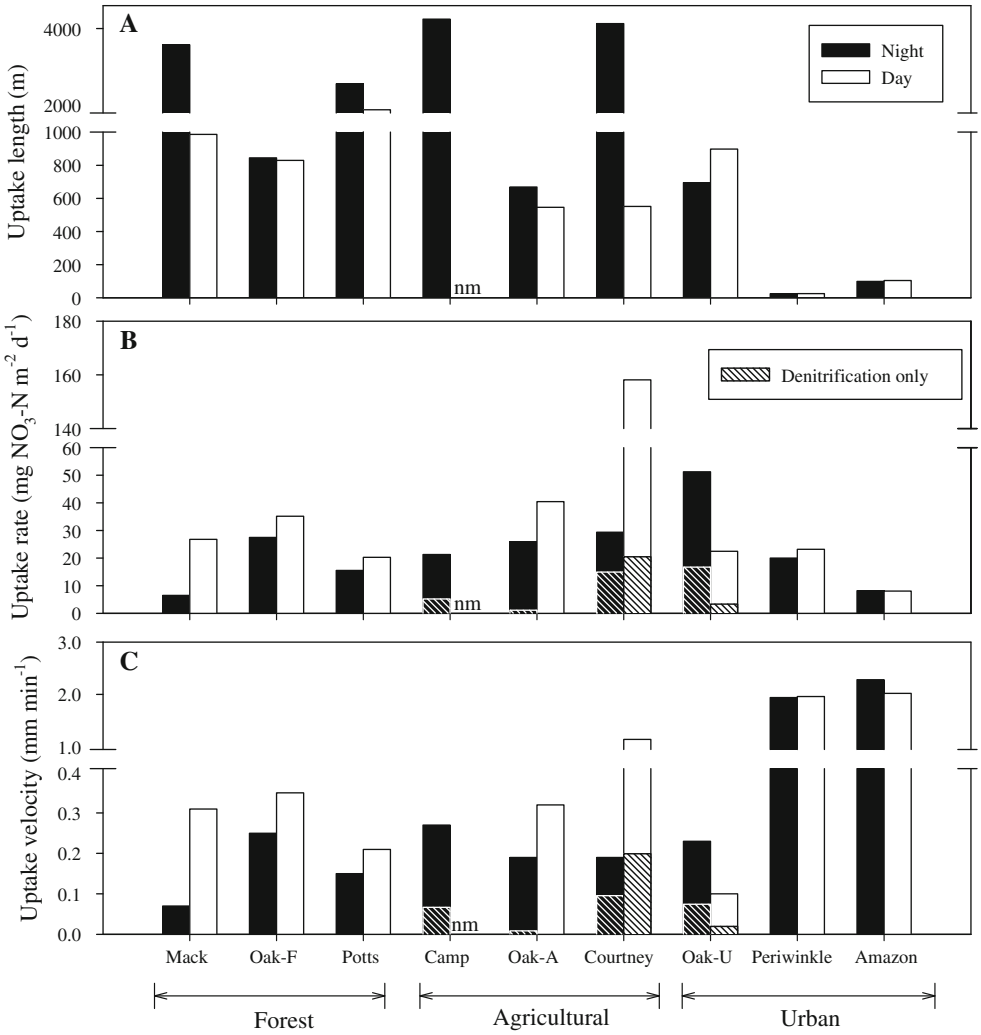

Figure 2. **A** Uptake lengths, **B** uptake rates, and **C** uptake velocities of $^{15}NO_3$-N tracer at night (*black*) and day (*white*) in study streams in western Oregon, USA. *nm* not measured due to isotopic contamination of samples.

Organic Matter Uptake

Organic matter specific NO_3-N uptake rates ranged from 0.004 ± 0.001 mg N m^{-2} day^{-1} for leaves in Amazon Creek to 114 ± 25 mg N m^{-2} day^{-1} for FBOM in Courtney Creek (Figure 5A). Overall, FGA had the highest rate (26 ± 13 mg N m^{-2} day^{-1}) and leaves had the lowest (3 ± 1 mg N m^{-2} day^{-1}). There were no significant differences among uptake rates of organic matter components by land-use type ($P = 0.11$) or total organic matter-weighted uptake rate among land uses ($P = 0.36$).

Turnover time of NO_3-N in organic matter components ranged from 7 days in FGA in Courtney Creek to 2356 d in FBOM in Oak Creek-U (Figure 5B). Turnover time ranged from 31 ± 45 for FGA to 820 ± 653 days for FBOM. N turnover time was significantly longer in FBOM than in epilithon ($P = 0.003$), wood biofilms ($P = 0.001$), and FGA ($P = 0.003$). Biomass-weighted turnover time did not differ among land uses ($P = 0.90$) and there was no significant difference for the interaction of land use and type of organic matter ($P > 0.32$).

Mass Balance

A median of 69.7% (range: 9.5 to 90.0%) of tracer was exported downstream of the study reaches as NO_3^- (right side bars in Figure 6). Hydrologic export of isotope tracer as NH_4^+, DON, and PN together was less than 0.1% for all streams. Denitrification accounted for 1.2 to 6.8% of the isotope tracer in the four streams where it was above the detection limit (right-side bars in Figure 6). A median of 14.8% (6.8–131.1%) of tracer was stored in organic matter (left-side bars in Figure 6). In one agricultural and three urban streams, denitrification, organic matter storage, and hydrologic export of tracer summed to greater than 100%.

Table 4. Correlations between $^{15}NO_3$-N Uptake Metrics and Ecosystem Characteristics for Study Streams in Western Oregon

$^{15}NO_3$-N uptake metric	Model	Adjusted r^2	P
log(Night uptake length)	1.62 + 1.08log(Q)	0.40	0.039
log(Night uptake length)	6.49 + 1.50log(Q/w)	0.59	0.009
log(Night uptake length)	2.76 + 0.54log(DIN/SRP)	0.70	0.003
Night uptake rate	7.18 + 0.22(NO_3-N)	0.58	0.010
Day uptake velocity	1.98 – 0.02(riparian cover)	0.60	0.016
Day uptake velocity	0.13 + 0.05(PAR)	0.97	<0.001
Day uptake velocity	0.19 + 0.55(GPP)	0.57	0.020

See Table 2 for descriptions of explanatory variables in the models.

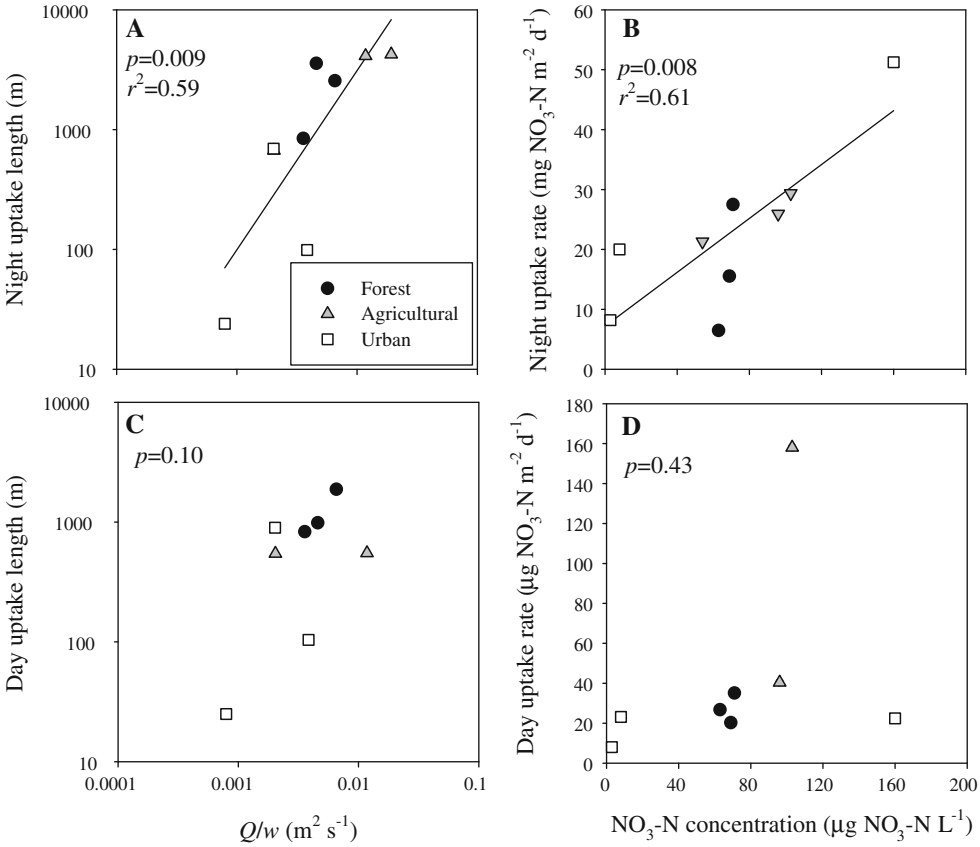

Figure 3. **A** Night uptake length versus specific discharge (Q/w), **B** night uptake rate versus NO_3-N concentration, **C** day uptake length versus specific discharge, and **D** day uptake rate versus NO_3-N concentration for study streams in western Oregon, USA. See Table 4 for descriptions of significant ($P < 0.05$) regression models.

We found no significant difference in hydrologic export of tracer as NO_3^- among land uses (P = 0.14). Likewise, there were no significant differences in combined denitrification and organic matter storage of isotope tracer (P = 0.10). The highest hydrologic export of tracer occurred on Camp Creek (Figure 6) and the highest total uptake of tracer, entirely due to organic matter storage, occurred on Amazon Creek (Figure 6). The only significant land-use effect on an organic matter storage component was for FBOM (P = 0.006). Tracer stored in FBOM was 14 to 57% (95% confidence interval) greater in urban streams than in forest streams. Lastly, there was no significant difference for isotope tracer denitrified among land uses (P = 0.37).

Discussion

Our study presents a detailed account of denitrification and organic matter storage of ^{15}N-labeled NO_3^- tracer in multiple streams from several different land-use settings. Our findings suggest storage in a diverse range of organic matter types is the

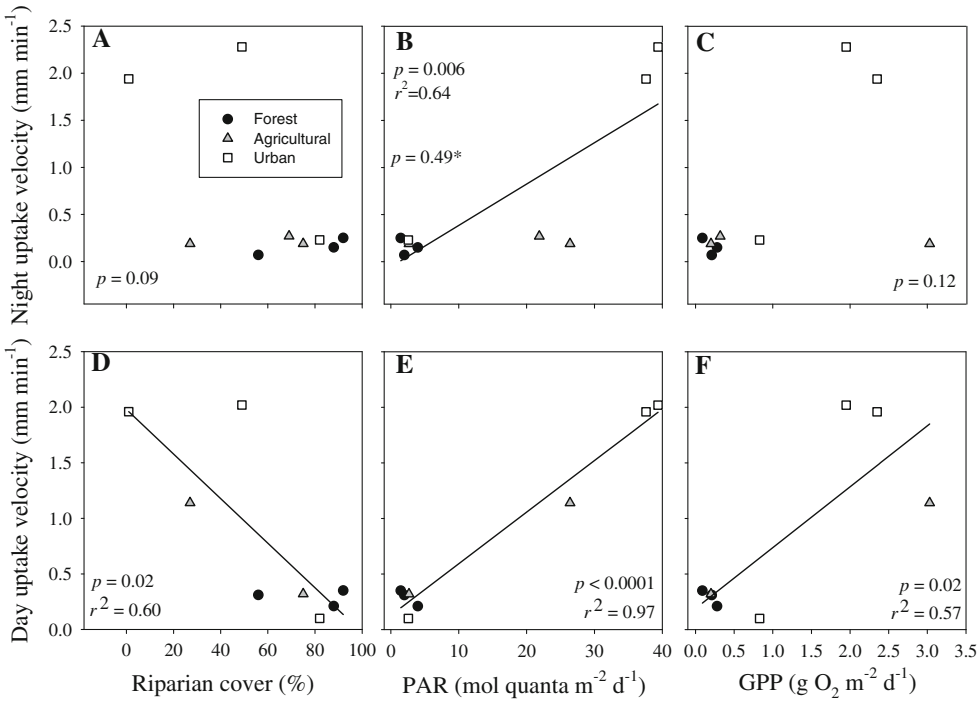

Figure 4. Comparison of night (**A–C**) and day (**D–F**) uptake velocity with (**A** and **D**) riparian cover, (**B** and **E**) photosynthetically active radiation (PAR), and (**C** and **F**) gross primary production (GPP) for study streams in western Oregon, USA. *Significance of the regression model without Periwinkle and Amazon Creeks.

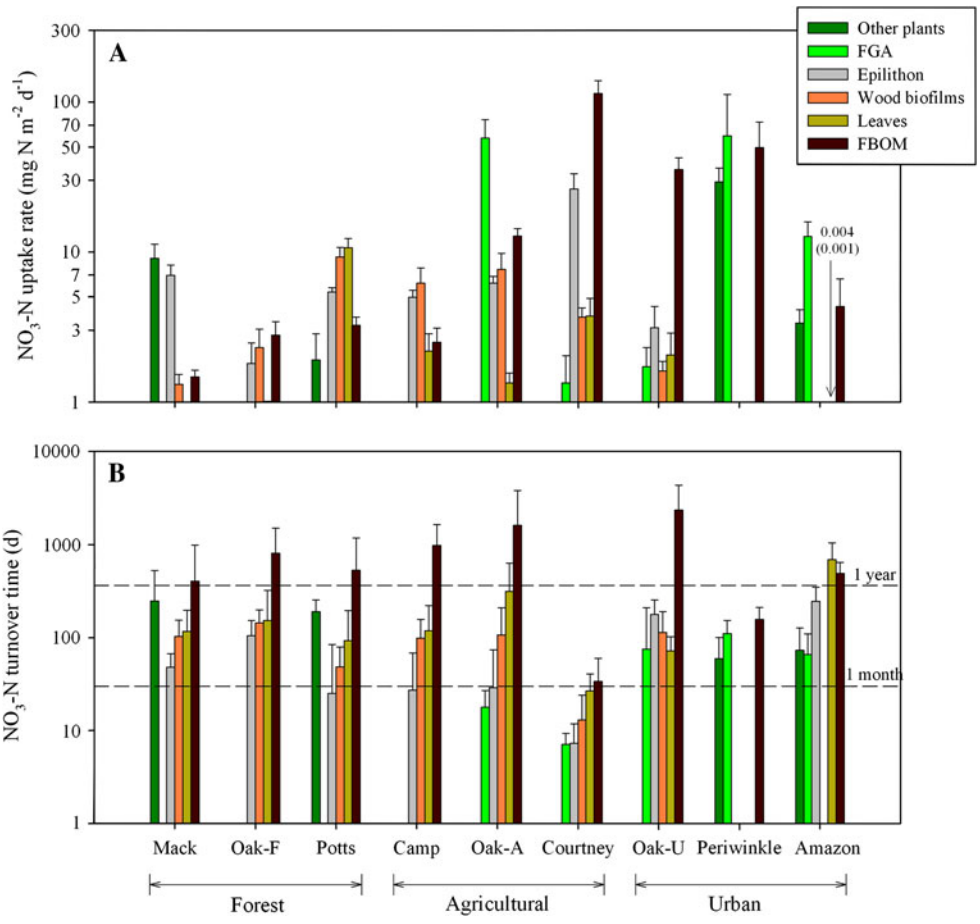

Figure 5. Averages and standard errors of $^{15}NO_3$-N tracer **A** uptake rate and **B** turnover time in stream organic matter components for study streams in western Oregon, USA.

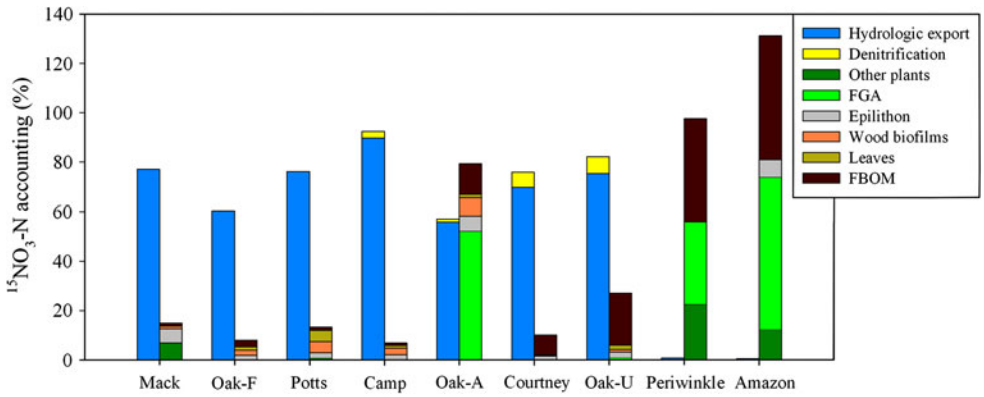

Figure 6. Mass balances of $^{15}NO_3$-N tracer added to study streams in western Oregon, USA. Export of tracer as ammonium, dissolved organic N, and particulate N was less than 0.1% combined in all streams and is not included in the figure.

largest short-term fate of dissolved NO_3^- taken up in streams regardless of adjacent land uses. In addition, the presence of riparian forest buffers along streams in agricultural and urban settings can influence overall efficiency of NO_3^- uptake and specific organic matter uptake fates by regulating the distribution of NO_3-N storage among autotrophs and detrital organic matter components.

Total Uptake

We did not find evidence for saturation of NO_3^- uptake across streams, probably due to low NO_3^- concentrations (2–160 μg NO_3-N l^{-1}) and N limitation (DIN/SRP $<$ 15; Redfield 1958). Despite the low NO_3^- concentrations, the range of NO_3^- uptake rates measured in this study (6.5–158.1 mg NO_3-N m^{-2} day^{-1}) was similar to the range (13–134 mg NO_3-N m^{-2} day^{-1}) measured using isotope and fertilization methods in streams with widely varying NO_3^- concentrations (Ensign and Doyle 2006). Uptake lengths, rates, and velocities measured in this study also spanned the range of values measured in the other LINX II streams (Mulholland and others 2008).

Our results suggest that light input controlled overall NO_3^- uptake in the streams during the isotope additions. Light input, which influences GPP, is frequently observed to limit NO_3^- uptake in other biomes across North America (Hall and Tank 2003; Fellows and others 2006; Mulholland and others 2008). The presence or absence of riparian forests controlled light availability and hence efficiency of NO_3^- uptake in our streams.

Stream nutrient uptake can also be influenced by contact time of a nutrient with the stream substrates (Wollheim and others 2001; Dodds and others 2002). The positive correlations of night uptake length with discharge and specific discharge and of night NO_3^- uptake rate with NO_3^- concentration reflect this influence and are similar to results from other LINX II studies (Hall and others 2009a; Potter and others 2010). However, these correlations were not significant for daytime, suggesting that autotrophic communities took up NO_3^- more efficiently than did heterotrophic communities associated with detrital organic matter.

Denitrification

Denitrification comprised 0 to 27% of total NO_3^- uptake in the study streams (0–7% of tracer added), and was probably limited by NO_3^- availability (Table 2; Findlay and others 2010). In comparison, denitrification is often the major fate of NO_3^- taken up in streams with high NO_3^- concentrations, presumably due to saturation of assimilatory uptake processes (Böhlke and others 2004; O'Brien and others 2007).

In this study, denitrification rates were low and fell within the ranges measured in previous studies of nutrient-poor aquatic ecosystems in Oregon (Dodds and Jones 1987) and elsewhere (Mulholland and others 2004; O'Brien and others 2007; Mulholland and others 2009; Hall and others 2009b). Denitrification was above zero at only four streams, limiting our ability to quantify relationships of denitrification with land use and characteristics of the stream and riparian zones. However, we suggest that in two of the agricultural streams where denitrification was above detection limits, light limitation of GPP (Bernot and others 2010) and high DIN:SRP ratios may have led to excess of NO_3^- beyond the assimilatory needs of stream organisms (Cross and others 2005). In addition, a shaded channel, relatively high NO_3-N concentration, and an abundance of deep, slow-moving pools

may have provided ideal conditions for denitrification on the agricultural and urban reaches of Oak Creek.

Land-Use Setting and Organic Matter Storage

Research on stream NO_3^- uptake has largely focused on denitrification (Seitzinger and others 2006; Mulholland and others 2008). However, organic matter storage is increasingly recognized as an important, and often major, component of stream NO_3^- uptake (Arango and others 2008; Hall and others 2009a, b; von Schiller and others 2009). Our results demonstrate the magnitude of NO_3-N storage in a variety of in-stream organic matter components in different land-use settings.

The distribution of tracer storage in wood biofilms, epilithon, FBOM, and aquatic bryophytes in our forested streams was similar to findings from previous studies of forest stream N dynamics in the Pacific Northwest (Triska and others 1984; Ashkenas and others 2004). The experiment on one of the forest streams, Mack Creek, provides additional insight on organic matter storage of inorganic N. Mack Creek has now been studied using two different stable isotope tracers of N, $^{15}NH_4$-N (Ashkenas and others 2004) and $^{15}NO_3$-N (this study), during low flow (Table 5). The similar retention rate of both ^{15}N tracers in autotrophs (12–13% of added tracer) shows active uptake of inorganic N, regardless of form, by this organic matter type (Table 5). However, the tenfold difference between $^{15}NH_4$-N and $^{15}NO_3$-N storage in detrital organic matter components (20% vs. 2% of added tracer; Table 5) suggests that heterotrophs preferentially took up NH_4^+ over NO_3^- or that abiotic sorption of NH_4^+ was an important component of inorganic N uptake. Although the $^{15}NH_4$-N tracer addition lasted 6 weeks whereas the $^{15}NO_3$-N addition lasted 24 h, both showed similar uptake lengths, rates, and velocities for NO_3^- (Table 5). In both studies, the largest proportion of tracer was downstream export as NO_3^- (43% in the $^{15}NH_4$-N experiment and 77% in the $^{15}NO_3$-N experiment).

Riparian forest buffers maintain channel shading and contribute wood and other detrital material to streams (Gregory 1997). The presence of riparian forest buffers along study streams in agricultural and urban settings facilitated a similar distribution of organic matter storage of NO_3-N tracer to that measured in forest streams, particularly in leaves/

Table 5. Comparison of the Fates of ^{15}N Tracer in Mack Creek, Oregon, USA, using $^{15}NH_4$-N (Ashkenas and others 2004) and $^{15}NO_3$-N (This Study)

Distribution of tracer (%)	$^{15}NH_4$-N	$^{15}NO_3$-N
Detritus		
FBOM	11	1
Leaves/needles	<1	<1
Wood biofilms/small wood	9	1
Detrital retention	20	2
Autotrophs		
Epilithon	1	6
Aquatic bryophytes	9	7
Riparian plants	2	nm
Autotrophic retention	12	13
Higher trophic levels	<1	nm
Denitrification	nm	<1
Export as		
NO_3^-	43[a]	77
NH_4^+	2	<1
DON	4	<1
PN	<1	<1
Total	81	92
NO_3^- uptake metrics	Day only	Night–day
Uptake length (m)	1111–1491	3575–987
Uptake rate (mg NO_3-N m^{-2} day^{-1})	31–34	6–27
Uptake velocity (mm min^{-1})	0.35–0.44	0.07–0.31

nm not measured.
[a]*Following nitrification.*

needles, wood biofilms, and epilithon (Figure 6). This highlights the role of riparian forest buffers in agricultural and urban settings in influencing the distribution of NO_3-N storage in organic matter components of streams.

Complex autotrophic communities often develop during stable flow in un-shaded streams and can exert strong influences on stream water chemistry (Dent and Grimm 1999). High light input and the resulting autotrophic community in agricultural and urban streams without forest buffers resulted in nearly complete storage of ^{15}N tracer in FGA, vascular macrophytes, attached and non-attached algae, and FBOM. In particular, FGA was an active component of NO_3^- uptake in our streams, similar to findings from open streams in other regions (Martí and others 1997; O'Brien and others 2007). The strikingly low NO_3^- concentrations in two of the urban streams, Periwinkle and Amazon Creeks, differ from the commonly observed pattern of high NO_3^- concentrations in urban settings (Mulholland and others 2008; Stanley and Maxted 2008). In these two streams, high light and low DIN:SRP ratios (Table 2) probably created conditions where NO_3^- was efficiently stored in autotrophic and FBOM organic matter components and greatly reduced concentrations of inorganic N. In addition, algal production in these streams probably influenced heterotrophic uptake of NO_3^- at night (Figure 4B) via lysis or excretion of labile organic carbon (Von Schiller and others 2009).

Tracer storage in hyporheic zones (Hall and others 2009a), storage in unmeasured stream organic matter components, or uptake by riparian plants (Ashkenas and others 2004) may account for missing isotope tracer in several of our study streams. Mass balances of tracer that summed to greater than 100% probably resulted from the extrapolation of microhabitat measurements to the experimental reach. These types of errors often occur when scaling patchily distributed organic matter components to larger areas (O'Brien and others 2007).

Turnover Times and Temporal Considerations

Our calculated turnover times of N in organic matter components fell within the range measured with both short-term and long-term additions of a ^{15}N tracer (Mulholland and others 2000; Tank and others 2000; Hamilton and others 2001; Ashkenas and others 2004; Hall and others 2009a). Long residence times of NO_3-N in several detrital organic matter components (FBOM, leaves, and wood biofilms) suggest organic matter storage in forested and riparian-buffered streams can have an important role in regulating effects of N enrichment. NO_3-N could remain unavailable for ecosystem cycling over extended periods if NO_3-N is directly incorporated into an organic matter component with a long N turnover time. Thus, uptake and storage of NO_3-N in several types of organic matter might be functionally similar to denitrification (complete removal of NO_3-N) in forested and riparian-buffered N-limited streams. In contrast, fast turnover rates of NO_3-N stored in important autotrophic organic matter components from partially shaded and unshaded human-altered streams (Courtney, Periwinkle, and Amazon Creeks) suggests effects of elevated N loading can propagate downstream quickly during the summer growing season.

We emphasize that turnover time does not fully capture the long-term fate of NO_3-N taken up in stream organic matter components. Specifically, variability in hydrology and N loading can influence the long-term aspects of NO_3-N storage in streams (Hall and others 2009a). In the context of our study, flow and NO_3^- concentrations in PNW streams can be highly seasonal, with NO_3^- concentration increasing during autumn and winter high flows (Poor and McDonnell 2007). In retentive streams, N stored in organic matter can remain even after high flows (Merriam and others 2002; Hall and others 2009b). In contrast, highly modified streams (Courtney, Periwinkle, and Amazon Creeks) lack complex structure and, annually, retain organic matter poorly. Coupled with data on organic matter N turnover times, we suggest that forest streams and riparian-buffered streams store or denitrify (via coupled denitrification; Seitzinger and others 2006) a higher proportion of NO_3^- over longer periods than straightened, non-buffered streams.

Conclusions and Implications

These stable isotope additions document NO_3^- uptake fates in streams with multiple adjacent land uses and riparian conditions. Although the majority of added $^{15}NO_3$-N tracer was transported downstream in forested and riparian-buffered streams, we found that an important fate of $^{15}NO_3$-N was storage in diverse autotrophic and detrital organic matter components with short-to-long N turnover times. Denitrification was a less important fate during our short-term experiments, but could be a relevant fate upon mineralization and nitrification of stored N. Storage of tracer in organic

matter was extremely high in several of the unshaded agricultural and urban streams with high autotrophic organic matter and low concentrations of dissolved inorganic N. However, fast N turnover rates in autotrophic organic matter and the poorly retentive stream channels suggests that over the longer term, influences of biotic storage on elevated N loading in these streams might be smaller than the influences of biotic storage in forest streams and riparian-buffered streams. In addition, organic matter components responsible for NO_3-N storage in partially shaded or unshaded human-altered streams can have undesirable ecosystem effects ranging from noxious algal blooms (Bunn and others 1999) to hypoxia (Mallin and others 2006). We suggest that restoration and maintenance of riparian forests can contribute to re-establishing the natural range of stream NO_3^- uptake processes and reduce impacts of N enrichment in human-altered catchments.

As in other LINX II studies (Hall and others 2009b; Mulholland and others 2009), our findings highlight the need for specific information on effects of adjacent and catchment land uses on stream ecosystem function. We further show that these categories are not sufficient to describe how land use and riparian zone conditions interact to influence the range of NO_3^- uptake fates in streams. Further research is critical to understand how seasonal variations in hydrology and N loading influence long-term aspects of all potential fates of NO_3^- in streams with various riparian conditions and adjacent land uses.

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