

Assimilatory uptake rather than nitrification and denitrification determines nitrogen removal patterns in streams of varying land use

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Abstract

Agricultural and urban land use increase nitrogen (N) concentrations in streams, which can saturate biotic demand by plants, algae, and bacteria via assimilative uptake, and by nitrification and denitrification. We studied six streams per year in each of three land-use categories (agricultural, urban, and forested) for 3 yr ($n = 18$ streams), and we compared whole-stream N uptake and microbial N transformation rates during spring, summer, and autumn. We measured whole-stream removal of added ammonium (NH_4^+) and nitrate (NO_3^-) in the field and quantified sediment nitrification and denitrification rates in laboratory assays. Relative demand for NH_4^+ (as uptake velocity, V_f) was highest in spring and in streams with open canopies, implying a link with aquatic autotrophy. In agricultural and urban streams, whole-stream removal (as areal uptake, U) of NH_4^+ and NO_3^- , nitrification, and denitrification rates approached saturation at higher inorganic N concentrations. Nitrification and denitrification rates measured in redox-optimized laboratory assays were roughly equivalent, suggesting that in situ redox conditions will determine whether stream sediments are a net source or sink of NO_3^- . Though nitrification and denitrification rates were measured under ideal redox conditions, they were always more than an order of magnitude lower than whole-stream NO_3^- uptake, demonstrating their limited influence on whole-stream NO_3^- dynamics. Assimilatory processes, which temporarily store N removed from the water column, dominated whole-stream N demand and controlled downstream N flux. The ultimate fate of assimilated N remains unknown; in-channel storage cannot account for it, and thus a key question is what fraction may eventually be stored in downstream depositional zones or denitrified upon remineralization.

Biological uptake and transformation of nutrients in headwater streams can regulate nutrient export to downstream ecosystems (Peterson et al. 2001), and this biological activity can be measured at the whole-stream level using nutrient spiraling techniques (Newbold et al. 1981; Stream Solute Workshop 1990). Nutrient spiraling couples nutrient uptake and release with downstream transport, and most studies of stream nutrient spiraling have been performed either in forested biomes (e.g., Tank et al. 2000; Ashkenas et al. 2004) or in stream systems minimally altered by human activities (e.g., Grimm and Fisher 1986; Dodds et al. 2000). Only recently have nutrient-spiraling studies investigated streams dominated by agricultural (Niyogi et

al. 2004; Bernot et al. 2006) or urban (Grimm et al. 2005; Meyer et al. 2005) land uses, even though human-induced changes in land use influence most running waters in the United States (Meyer and Turner 1994). Agricultural and urban land uses typically increase dissolved inorganic nitrogen (DIN) concentrations in streams (Carpenter et al. 1998) and can also reduce riparian vegetation and increase light availability for aquatic autotrophs (Allan 2004). Therefore, knowledge of how land use mediates nutrient uptake and transformation in stream ecosystems is critical for understanding how streams regulate nutrient flux to downstream water bodies.

Although land use can influence stream nutrient cycling by affecting DIN concentrations, seasonality is also an important driver of whole-stream nutrient uptake in midlatitude regions. For example, in temperate, forested streams, nutrient demand shows both a spring peak, when autotrophic activity increases concurrently with increasing light levels and temperature prior to deciduous forest leaf-out, and an autumn peak, when heterotrophs actively colonize and decompose allochthonous leaf litter inputs (Mulholland 1992, 2004). These seasonal peaks in nutrient demand have been confirmed in studies that examine nutrient uptake over an annual cycle (Simon et al. 2005; Hoellein et al. 2007; Roberts and Mulholland 2007), so spring and autumn may be considered biologically important periods for whole-stream nutrient demand in temperate streams. However, seasonality in nutrient uptake has rarely been studied in the context of streams that are

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modified by agricultural and urban land use (Niyogi et al. 2004), particularly in temperate regions where seasonality plays a strong role.

Studies that have used radioisotopes or stable isotopes of nutrients to trace the fate of water-column uptake (e.g., Mulholland et al. 1985; Tank et al. 2000) have shown that assimilatory demand constitutes the majority of ammonium (NH_4^+) (Webster et al. 2003), and nitrate (NO_3^-) uptake (Mulholland et al. 2004) whereas nitrification and denitrification constitute a smaller role (Webster et al. 2003; Mulholland et al. 2004). Assimilatory processes do not represent permanent removal; rather, they slow downstream transport of DIN, which may ultimately be exported from the reach in organic form, remineralized, and removed via coupled nitrification/denitrification (Seitzinger et al. 2006), or may be exported from the reach in inorganic form. Despite a smaller relative role in total N uptake, N remineralization, nitrification, and denitrification have been shown to be coupled in more pristine, low-N systems (Kemp and Dodds 2002), whereby nitrification converts remineralized NH_4^+ to NO_3^- and denitrification permanently removes N from the stream by converting NO_3^- to gaseous N. Therefore, a complete understanding of DIN spiraling requires insight into whole-stream N removal rates and the relative rates of nitrification and denitrification, which can be influenced by seasonal changes in dissolved oxygen and sediment redox conditions (Christensen et al. 1990; Nielsen et al. 1990).

We studied how land use, season, and microbial N transformations influence whole-stream demand for NH_4^+ and NO_3^- . We identified three categorical land-use types based on dominant land cover (agricultural, forested, and urban), and we incorporated seasonal dynamics by sampling during three periods over the 3-yr study when we expected high biological activity: (1) prior to leaf emergence in spring (late April), when we predicted high autotrophic activity in our study streams, (2) during base flow in summer (late August), when we expected high stream temperatures to maximize biological activity, and (3) after leaf-fall in autumn (late November), when we expected high heterotrophic activity during microbial colonization and decomposition of leaf litter. To complement short-term nutrient additions conducted in the field, we measured nitrification and denitrification rates using laboratory assays under optimum redox conditions to identify an upper bound on their contribution to whole-stream N uptake rates. To minimize day-to-day variability in seasonal uptake, we performed field work on sunny days in spring and summer and on overcast days in autumn, which represented commonly occurring light conditions in this region in those seasons.

We made three general predictions based on our study design. First, we predicted that relative nutrient demand (V_f , defined in Methods) would show less seasonality in agricultural and urban streams because of year-round open canopies and higher light levels compared to forested streams, and that indirect measures of autotrophic and heterotrophic activity would be significantly related to relative nutrient demand. Second, we predicted that higher nutrient concentrations in agricultural and urban streams

would saturate areal uptake rates (U , defined in Methods) and decrease V_f . Third, we predicted that microbial N transformations would account for a relatively small fraction of total areal N uptake, but that nitrification would be more important in the spring because of benthic algal production's increasing oxygenation of surficial sediments, whereas denitrification would be more important in the autumn when microbial respiration of decomposing leaf litter would draw down oxygen levels in the sediments and within decaying leaf material.

Methods

Land-use classification—We studied 18 low-gradient headwater streams in the Kalamazoo River catchment of southwest Michigan (Fig. 1), a deciduous forest biome where much of the land supports row crop agriculture. Using ArcGIS 8.2 (ESRI) to analyze land cover data downloaded from the National Land Cover Database (reclassified Landsat Thematic Mapper imagery from 1992; Vogelmann et al. 2001), we grouped streams as agricultural, urban, or forested depending on dominant land cover. We selected six streams from each of the three categories (Table 1) after ground-truthing the geographic information system (GIS) analysis. Study subbasins ranged from 0.79 to 36.39 km², and average annual NH_4^+ concentrations (4–115 $\mu\text{g N L}^{-1}$) were much lower than concentrations of NO_3^- (14–17,496 $\mu\text{g N L}^{-1}$) (Table 1). Urban streams represented a suburban–urban gradient, and some streams had more riparian vegetation than others, so the study streams represented land cover variability among headwater subbasins of the Kalamazoo River catchment.

Nutrient releases—We measured whole-stream uptake of NH_4^+ and NO_3^- using short-term (<1 h) additions of a reactive solute with a conservative tracer (Webster and Ehrman 1996) in stream reaches with a 20–30-min travel time. We sampled background solute concentrations at 10 evenly distributed stations along the study reach (50–250 m, depending on travel time) before we pumped a solution of NH_4Cl or NaNO_3 with a conservative tracer (Br^- as NaBr , or Cl^- as NaCl , or Rhodamine-WT) at a constant rate into the stream. We added NH_4^+ and either Rhodamine or Cl^- first and allowed time for those solutes to leave the study reach before adding NO_3^- with Br^- . The short-term nutrient enrichment method can saturate demand and overestimate uptake length (S_w). For NH_4^+ we minimized this effect by targeting an increase of 10–20 $\mu\text{g NH}_4^+ \text{-N L}^{-1}$, but when NO_3^- concentrations were high (>1 mg L^{-1}), we increased NO_3^- up to 200 $\mu\text{g N L}^{-1}$ so we could reliably measure a downstream decline. For conservative tracers, we targeted an enrichment of 10 $\mu\text{g Rhodamine-WT L}^{-1}$, 50 $\mu\text{g Br}^- \text{L}^{-1}$, or 20 $\mu\text{S cm}^{-1}$ for Cl^- . When the conservative tracer reached constant concentration throughout the reach (i.e., plateau concentration), we took stream-water samples at the 10 sampling stations.

We calculated nutrient uptake length (S_w) using the linear form of the exponential model:

$$\ln N_x = \ln N_0 - ax \quad (1)$$

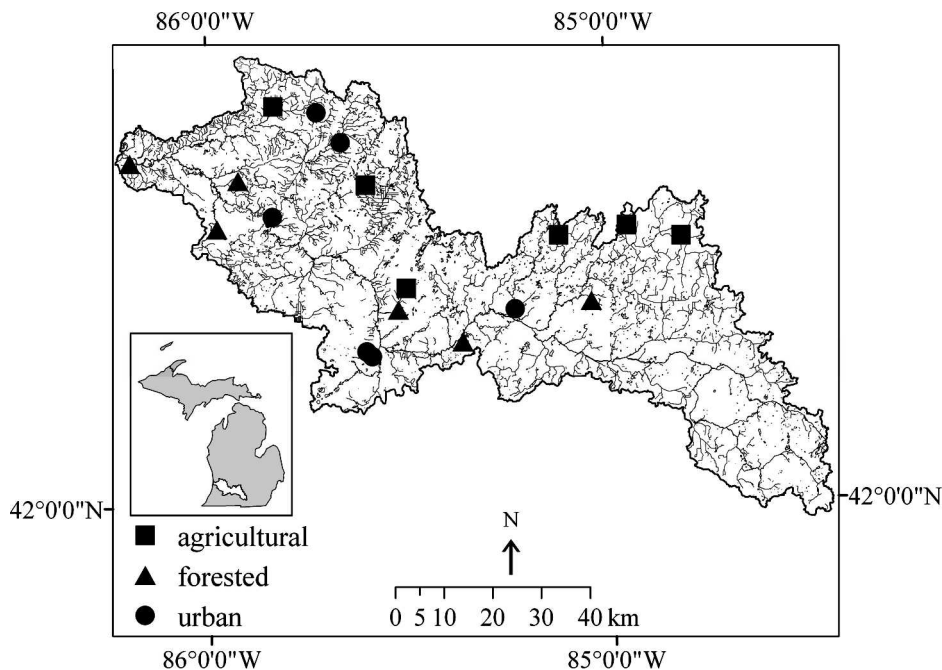


Fig. 1. Study sites in the Kalamazoo River catchment, southwest Michigan.

where N_0 is nutrient concentration at the injection site, N_x is nutrient concentration at x m downstream, and a is the m^{-1} uptake constant (Newbold et al. 1981). We used the conservative tracer to correct plateau nutrient concentrations for dilution and estimated the parameter a in Eq. 1 by plotting dilution-corrected nutrient concentrations vs. distance from the injection site. We calculated S_w as $-a^{-1}$ (Newbold et al. 1981), which represents the average distance (m) traveled by a nutrient molecule before uptake. However, S_w is sensitive to variations in stream size, which can confound inter-site or temporal comparisons of S_w (Davis and Minshall 1999). Therefore, we calculated uptake velocity (V_f) as

$$V_f = Qa/w \quad (2)$$

where Q is stream discharge ($\text{m}^3 \text{min}^{-1}$) and w is wetted width (m) (Stream Solute Workshop 1990). We calculated Q by balancing the mass of conservative tracer released (Webster and Ehrman 1996) and measured wetted width at 10 transects in the study reach. Uptake velocity (m min^{-1}), the velocity at which a nutrient is drawn from the water column toward the benthos, can be compared among streams because it is normalized for stream size. Areal uptake rate (U , $\text{mg m}^{-2} \text{min}^{-1}$) is calculated as

$$U = V_f N_b \quad (3)$$

where N_b is background nutrient concentration, and U is the areal flux of nutrients into the streambed. Hereafter, we use “relative nutrient demand” to refer to V_f , which we use for comparisons among streams and season, and we use “whole-stream nutrient uptake” to refer to U , which we use in Michaelis-Menten (MM) models to investigate uptake saturation. Because stream size varied among sites and

seasons, we do not use uptake length (S_w) for comparisons (Davis and Minshall 1999).

Nitrification assays—We estimated sediment nitrification rates using nitrapyrin inhibition (Hall 1984; Kemp and Dodds 2001; Strauss et al. 2004). Within 1 week of the nutrient releases, we collected sediment samples that reflected the different proportions of inorganic and organic streambed materials by taking 25 cores ($30 \text{ cm}^2 \times 2 \text{ cm}$ deep) from the study reach and pooling them into five separate samples ($\sim 300 \text{ mL}$ each). We stored samples on ice until we returned to the laboratory and immediately began the assays. Each set of paired flasks (5 pairs stream $^{-1}$) came from the same pooled sediment sample, and we made 75-mL slurries by adding 25 mL of sediment and 50 mL of unfiltered site water. One flask in the pair (the production flask) received 10 μL of a 10% solution of nitrapyrin dissolved in dimethyl sulfoxide (DMSO), which blocks the conversion of NH_4^+ to NO_3^- . The other flask (the control flask) received 10 μL of DMSO, and nitrification was not blocked with nitrapyrin. We incubated the assays on a rotary shaker at 150 rpm for 24–48 h, and we terminated the assay by adding 25 mL of 2 mol L^{-1} KCl and shaking for an additional 10 min to flush NH_4^+ from binding sites. We centrifuged the entire slurry and froze the filtered supernatant for future NH_4^+ analysis (methods described below). We calculated nitrification rate (average of five paired flasks stream $^{-1}$) as the difference in NH_4^+ between the production and control flasks, which we scaled by the dry mass (DM) of sediment and the assay length ($\mu\text{g N g}^{-1} \text{ DM h}^{-1}$). We multiplied nitrification rates by sediment standing stocks (g DM m^{-2}) to calculate an areal nitrification rate ($\text{mg N m}^{-2} \text{ h}^{-1}$) for comparison to whole-stream N uptake (U). Using Eq. 3, we calculated relative

Table 1. Physical characteristics and nutrient concentrations (± 1 SE, $n = 3$ dates) of the study streams.

Stream	Subbasin area (km ²)	Land use category (year of study)	Subbasin			100-m buffer			NH ₄ ⁺ ($\mu\text{g N L}^{-1}$)	NO ₃ ⁻ ($\mu\text{g N L}^{-1}$)
			Forest + wetland (%)	Agricultural (%)	Urban (%)	Forest + wetland (%)	Agricultural (%)	Urban (%)		
Burnips	2.98	Agricultural (1)	2	98	0	5	95	0	65 (39)	8235 (941)
Spicerville	6.80	Agricultural (1)	30	70	0	65	35	0	29 (9)	1000 (92)
Richland	13.12	Agricultural (2)	26	73	0	44	56	0	13 (3)	1684 (198)
Shelbyville	1.54	Agricultural (2)	3	97	0	9	91	1	9 (<1)	17,496 (1327)
Ellis	3.66	Agricultural (3)	22	78	0	68	32	0	11 (2)	271 (67)
Sherman	2.60	Agricultural (3)	5	95	0	1	99	0	24 (16)	1647 (1607)
Bellevue	5.28	Forested (1)	44	55	0	75	25	0	13 (2)	77 (49)
Swan	8.96	Forested (1)	71	27	0	86	9	0	29 (17)	225 (37)
Bullet	3.59	Forested (2)	64	28	5	85	15	0	9 (1)	338 (27)
Springbrook	3.58	Forested (2)	49	49	0	92	8	0	8 (2)	448 (11)
Silver Creek	0.79	Forested (3)	97	3	<1	92	7	1	4 (1)	47 (10)
Weber	1.28	Forested (3)	48	52	0	71	29	0	92 (70)	14 (1)
Dorr	2.33	Urban (1)	13	73	14	24	73	3	62 (20)	1113 (31)
Wayland	2.01	Urban (1)	31	20	49	49	21	31	115 (39)	551 (78)
Allegan	1.27	Urban (2)	20	72	7	48	47	5	17 (4)	1055 (359)
Arcadia	36.39	Urban (2)	44	40	15	17	14	67	27 (8)	960 (50)
Axtell	4.35	Urban (3)	39	10	48	19	35	46	69 (12)	186 (30)
Urbandale	18.24	Urban (3)	48	45	4	73	22	3	62 (34)	213 (50)

demand for NH_4^+ via nitrification ($V_{f-\text{NIT}}$) (Royer et al. 2004).

We recognize that nitrification rates measured using this method overestimate ambient rates because we removed redox limitation by measuring them in oxygenated slurries (Strauss et al. 2004). However, they do not represent maximum potential rates because we did not amend incubations with NH_4^+ , but instead used ambient NH_4^+ contained in stream water and sediment at the time of sediment collection.

Denitrification assays—We estimated denitrification rates in the laboratory using the chloramphenicol-amended acetylene-block method (Smith and Tiedje 1979; Royer et al. 2004; Arango et al. 2007). Acetylene (C_2H_2) blocks N_2 production by denitrifiers, allowing N_2O to accumulate, and the antibiotic chloramphenicol limits the microbial response to ideal redox conditions by preventing new enzyme production (Brock 1961; Smith and Tiedje 1979; Royer et al. 2004). We made slurries from the same sediment pools as described for nitrification assays, except that we amended slurries with chloramphenicol (final concentration 0.3 mmol L^{-1}) before incubating them in bottles with septum caps for headspace gas sampling. We purged each headspace with ultra-high-purity helium (He) for 5 min, shaking periodically to induce anoxia. Then we returned the bottles to ambient atmospheric pressure and injected 15 mL of C_2H_2 to create a 10% atmosphere of C_2H_2 . Before taking each of five headspace samples for N_2O analysis throughout the 4.25-h incubation, we shook the bottles to equilibrate dissolved gases between the headspace and water, then took 5 mL from the headspace and injected 4 mL into a pre-evacuated glass vial. We maintained constant pressure in the assays by replacing each subsample with 5 mL of 10% C_2H_2 in He balance.

We measured N_2O concentrations by manually injecting 100 μL into a Varian Star 3600 gas chromatograph with a Porapak Q column and electron capture detector (injector temperature = 120°C , column temperature = 40°C , detector temperature = 320°C , with a 5% CH_4 :95% Ar carrier gas at 30 mL min^{-1}). We used Bunsen coefficients to calculate total N_2O produced in each bottle, plotted N_2O production vs. time, and calculated N_2O production rate as the slope of the line of best fit ($r^2 > 0.92$, indicating linear N_2O production rates). We divided the N_2O production rate by sediment DM in the assay bottle and length of the assay to calculate sediment denitrification rates ($\mu\text{g N g}^{-1} \text{ DM h}^{-1}$), which we multiplied by sediment standing stock in each reach (g DM m^{-2}) to calculate an areal denitrification rate for comparing to NO_3^- uptake ($U_{-\text{NO}_3}$). Using Eq. 3, we calculated relative NO_3^- demand via denitrification ($V_{f-\text{DEN}}$) (Royer et al. 2004).

Again, we acknowledge that denitrification rates we report may be higher than ambient because we removed redox limitation by inducing anoxia in the slurries (Groffman et al. 2006), but they do not represent maximum potential rates because we did not amend incubations with NO_3^- or organic C. Instead, assays were conducted at ambient stream-water NO_3^- or organic C levels at the time of sediment collection (e.g., Royer et al. 2004; Inwood et al. 2005).

Organic matter standing stocks and substratum distribution—We used stratified random sampling to quantify organic matter standing stocks of sand, fine benthic organic matter (FBOM), leaves, and seasonal macrophytes and benthic algae. Sand and FBOM were the most common habitats, composing $80\% \pm 3\%$ (SE) of the benthic area. From 10 locations within each reach, we selected a benthic area with 100% cover of each substratum and we sampled FBOM, leaves, and macrophytes with a 475-cm^2 core, and sand and benthic algae with a 30-cm^2 core. We dried subsamples of organic matter to constant weight at 60°C to measure DM, and then we combusted ground samples for 3 h at 550°C , rewetted and redried them to constant weight at 60°C to measure ash mass. We calculated organic matter content as the difference between DM and ash-free DM. We scaled substratum standing stocks to the stream reach by weighting each substratum by its proportional abundance, which we estimated by recording benthic cover at 10-cm intervals along 10 transects equally spaced throughout each study reach (Hoellein et al. 2007). We estimated reach canopy closure in every season as the average of canopy closures measured with a hemispherical densiometer held approximately 1 m above the stream surface at each of the 10 sampling stations. Additionally, we extracted chlorophyll *a* (Chl *a*) from sand and FBOM using the hot ethanol method (Sartory and Grobbelaar 1984) and a Turner Designs TD-700 fluorometer (Sunnyvale, California) at 436-nm excitation and 680-nm emission wavelength.

Water chemistry—We collected filtered (1- μm nominal pore size; Pall A/E) background and plateau water samples in acid-washed high-density polyethylene (HDPE) bottles triple-rinsed with filtered stream water. We measured NH_4^+ concentrations (from short-term nutrient releases and nitrification assays) on a Shimadzu UV-1601 spectrophotometer at 630 nm using the phenate method (APHA 1995), and we quantified SRP at 885 nm using the molybdate method (APHA 1995). Concentrations of NO_3^- and Br^- (USEPA 1993) were measured simultaneously using a Dionex 600 ion chromatograph with AS14A analytical and guard columns and an ED50 electrochemical detector. Rhodamine-WT was quantified in the lab using a Turner Designs TD-700 fluorometer at 530 nm excitation and 555 nm emission wavelengths. Finally, we measured background and plateau specific conductance (YSI EC-300 conductivity probe) in the field when we used NaCl as a conservative tracer.

Statistical analyses—We normalized data that did not meet the assumptions of parametric statistics using log or log followed by power transformation. For categorical analyses, we blocked by year to partition interannual variability not associated with seasonality or land use. We used a one-way analysis of variance (ANOVA) to identify significant differences in canopy closure among study streams (SYSTAT 11) and repeated measures ANOVA (rmANOVA) (SAS 9.1; SAS Institute) to detect seasonal and land-use differences in Chl *a* biomass, leaf standing stocks, and V_f among the study streams. We identified significant differences between $V_{f-\text{NH}_4}$ and $V_{f-\text{NO}_3}$ using a

paired *t*-test. We used simple and multiple linear regressions (SYSTAT 11) to identify the independent variables that were related to organic matter standing stocks and nutrient demand, and we used nonlinear regressions (SigmaPlot 10.0) to fit MM uptake models to the measured nutrient uptake rates.

Results

Seasonal and land-use controls on stream predictors—We predicted that the riparian zone would control seasonal variations in light and organic matter, which in turn would drive variability in N uptake, so we analyzed how summer canopy closure and seasonal patterns of benthic Chl *a* and leaf litter varied by land use. Forested streams had greater summer canopy closure than urban streams (one-way ANOVA, $F_{2,18} = 3.6$, $p = 0.05$; Fig. 2A), and agricultural streams had intermediate canopy closure. However, we may have underestimated shading in agricultural streams because of heavy grass cover, which was not quantified in our densiometer measurements. Despite significant differences in canopy closure among land uses, we found no land-use differences in Chl *a* (rmANOVA, $F_{2,13} = 0.4$, $p = 0.69$; Fig. 2B) or leaf litter standing stocks (rmANOVA, $F_{2,13} = 0.2$, $p = 0.79$; Fig. 2C) when we pooled data across seasons. However, among streams we did find higher Chl *a* in spring (rmANOVA, $F_{2,29.5} = 31.3$, $p < 0.0001$) and higher leaf litter standing stocks in autumn (rmANOVA, $F_{2,29.5} = 12.7$, $p = 0.0003$), indicating that seasonality had stronger control than land use over benthic cover and organic matter in these headwater streams.

Seasonal and land-use influence on relative nutrient demand (V_f)—We observed highest relative NH_4^+ demand ($V_{f-\text{NH}_4}$) in the spring (rmANOVA, $F_{2,29.5} = 4.8$, $p = 0.016$), and we found that urban streams had higher demand than forested streams (rmANOVA, $F_{2,13} = 3.9$, $p = 0.047$; Fig. 3A). Contrary to our expectations, urban and forested streams had similar seasonal patterns, with highest $V_{f-\text{NH}_4}$ in the spring and lowest in autumn, whereas agricultural streams had lowest $V_{f-\text{NH}_4}$ in the summer. We found no seasonal (rmANOVA, $F_{2,29.5} = 2.6$, $p = 0.10$) or land use (rmANOVA, $F_{2,13} = 0.3$, $p = 0.77$) differences in relative NO_3^- demand ($V_{f-\text{NO}_3}$) (Fig. 3B), but in this case, the agricultural and urban streams had similar patterns compared to the forested streams. Combining data for all streams and seasons, $V_{f-\text{NH}_4}$ exceeded $V_{f-\text{NO}_3}$ (paired *t*-test, $t = 4.2$, $df = 49$, $p = 0.0001$; data not shown), indicating higher relative demand for added NH_4^+ compared to NO_3^- , not surprising given that NO_3^- concentrations were consistently higher than NH_4^+ , sometimes by orders of magnitude (Table 1).

Relationship between N concentrations and whole-stream U and V_f —Agricultural and urban land uses frequently increase nutrient concentrations in streams. A linear relationship between areal uptake rates (*U*) and background nutrient concentrations indicates that nutrient uptake is proportional to concentration whereas a hyperbolic relationship (i.e., MM) indicates that uptake saturates

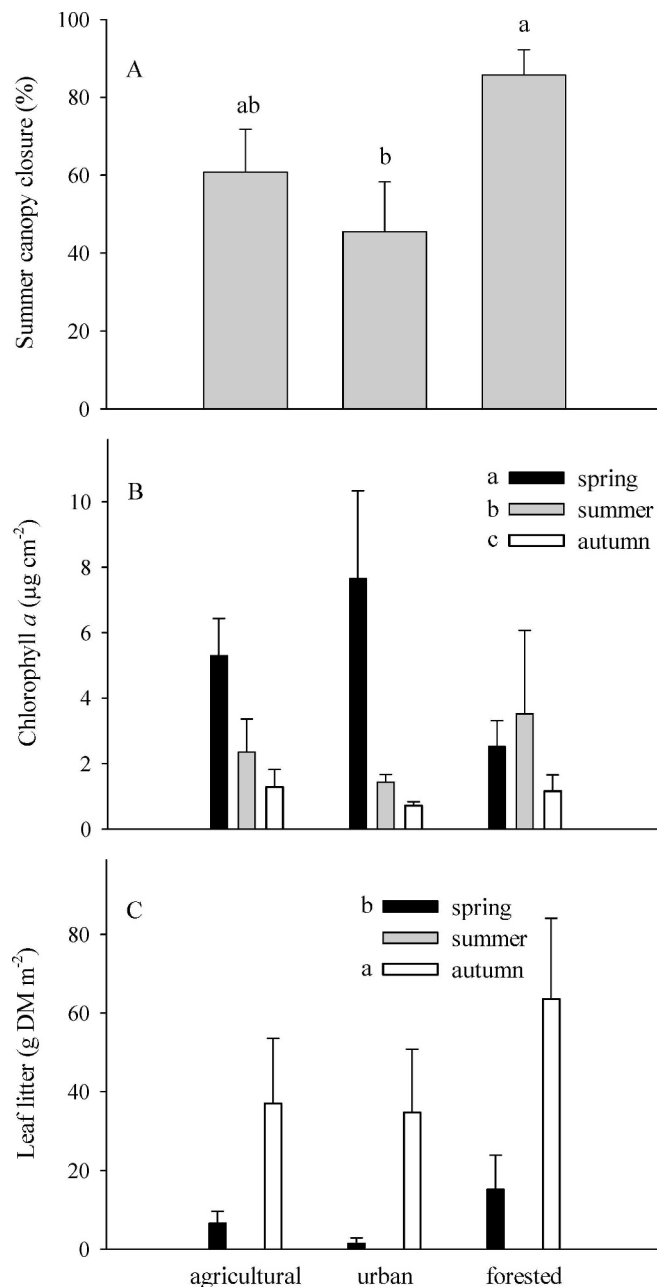


Fig. 2. Summer shading and standing stocks of Chl *a* and leaf litter (± 1 SE) in the study streams. (A) Forested streams had more riparian shading than urban streams (one-way ANOVA). (B) Among streams, Chl *a* standing stocks were highest in spring and lowest in autumn (rmANOVA). Letters preceding the season legend indicate significant differences identified in Tukey's multiple comparison tests. (C) Leaf litter was highest in autumn, and lowest in summer and spring (rmANOVA).

with increasing concentration (Dodds et al. 2002). For areal uptake of NH_4^+ ($U_{-\text{NH}_4}$) and NO_3^- ($U_{-\text{NO}_3}$), analyzing all data together, linear and MM models were both significant, but MM models had better fits (Table 2) suggesting saturation of uptake at higher concentrations (Fig. 4A,B). However, the MM models only explained 33–43% of the variability, indicating that other factors also contributed to

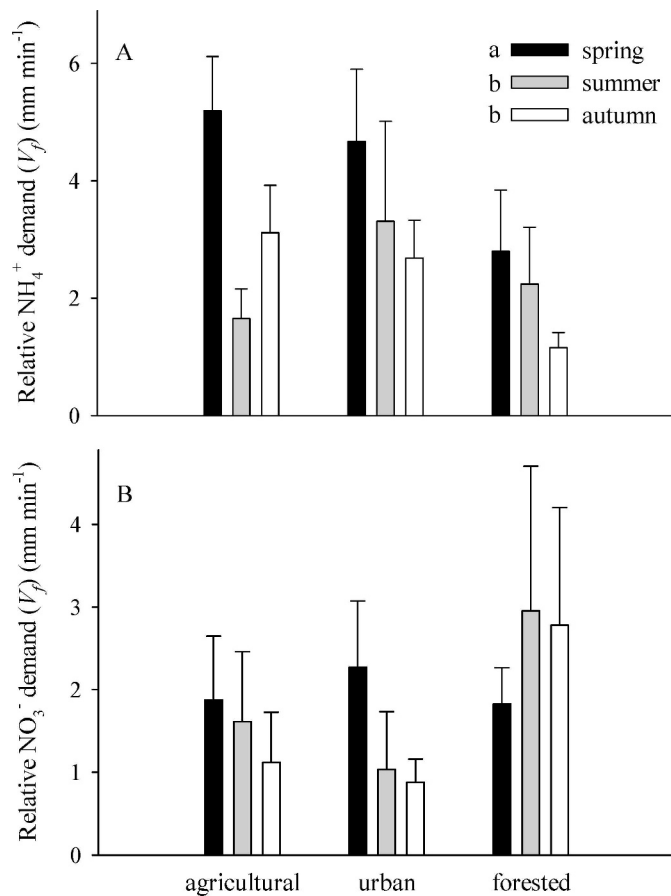


Fig. 3. Seasonal patterns in relative nutrient demand (V_f) (± 1 SE) among streams of different land use. (A) Considered across land uses, relative NH_4^+ demand ($V_{f-\text{NH}_4}$) was highest in spring and in urban streams (rmANOVA). Letters preceding the season legend indicate significant differences identified in Tukey's multiple comparison tests. (B) Relative NO_3^- demand ($V_{f-\text{NO}_3}$) did not differ among seasons or land uses.

the spatiotemporal variation in whole-stream uptake. At the higher NH_4^+ concentrations in urban streams (Table 1) and higher NO_3^- concentrations in agricultural streams (Table 1), $U_{-\text{NH}_4}$ and $U_{-\text{NO}_3}$ displayed saturation kinetics,

indicating that land use affected areal uptake indirectly through its influence on inorganic N concentrations (Fig. 4A,B).

We confirmed uptake saturation kinetics by plotting V_f vs. nutrient concentration, which showed negative relationships with relative demand for NH_4^+ ($V_{f-\text{NH}_4}$) and NO_3^- ($V_{f-\text{NO}_3}$) and indicated decreased uptake efficiency at higher concentrations (Fig. 4C,D). The linear relationship between $V_{f-\text{NH}_4}$ and NH_4^+ concentration is marginally significant ($r^2 = 0.07$, $p = 0.053$, $n = 53$). Given the importance of abiotic sorption dynamics for NH_4^+ , a linear model is expected rather than an exponential decay function (Table 2) (Davis and Minshall 1999), but even the linear model had low explanatory power. Although the relationship between $V_{f-\text{NO}_3}$ and NO_3^- concentration appears to follow a negative exponential model, which would be expected given that NO_3^- is unaffected by abiotic sorption processes (Davis and Minshall 1999), we could not fit an exponential decay function to these data ($r^2 = 0.02$, $p = 0.27$, $n = 51$; Table 2). Our NO_3^- concentrations include values higher than previously reported in the literature for streams, and clearly $V_{f-\text{NO}_3}$ declines with increasing NO_3^- availability.

Relationships between N concentrations and nitrification and denitrification rates—The relationship between relative NH_4^+ demand via nitrification ($V_{f-\text{NIT}}$) and NH_4^+ concentration declined exponentially ($r^2 = 0.23$, $p = 0.0003$, $n = 54$; Fig. 5A, Table 2), suggesting saturation of $V_{f-\text{NIT}}$ with increasing NH_4^+ concentrations, which were generally higher in urban streams. The relationship between $V_{f-\text{NIT}}$ and NH_4^+ concentrations became even stronger when we considered just the highly modified agricultural and urban streams ($r^2 = 0.68$, $p < 0.0001$, $n = 36$). Because nitrification rates ($U_{-\text{NIT}}$) can contribute NO_3^- to the water column, we plotted them vs. NO_3^- concentration but did not find a significant positive relationship (Fig. 5B), probably because catchment NO_3^- loading related to land-use activity had stronger control over NO_3^- concentrations.

High water-column NO_3^- concentrations can increase denitrification rates, and we found that uptake of NO_3^- via

Table 2. Summary of model fits for uptake parameters. Bolded values represent significant fits, and — indicates analysis was not run.

Parameter	Linear		Michaelis-Menten		Exponential decay	
	r^2	p	r^2	p	r^2	p
Regressed against NH_4^+ concentration						
Whole-stream NH_4^+ ($U_{-\text{NH}_4}$)	0.16	0.002	0.33	<0.0001	—	—
Relative NH_4^+ demand ($V_{f-\text{NH}_4}$)	0.07	0.053	—	—	0.05	0.10
NH_4^+ uptake via nitrification ($U_{-\text{NIT}}$)	0.01	0.50	0.04	0.35	—	—
Relative NH_4^+ demand via nitrification ($V_{f-\text{NIT}}$)	0.10	0.02	—	—	0.23	0.003
Regressed against NO_3^- concentration						
Whole-stream NO_3^- ($U_{-\text{NO}_3}$)	0.25	0.000	0.43	<0.0001	—	—
Relative NO_3^- demand ($V_{f-\text{NO}_3}$)	0.03	0.25	—	—	0.02	0.27
NO_3^- uptake via denitrification ($U_{-\text{NO}_3}$)	0.40	<0.0001	0.62	<0.0001	—	—
Relative NO_3^- demand via denitrification ($V_{f-\text{DEN}}$)	0.04	0.13	—	—	0.52	<0.0001

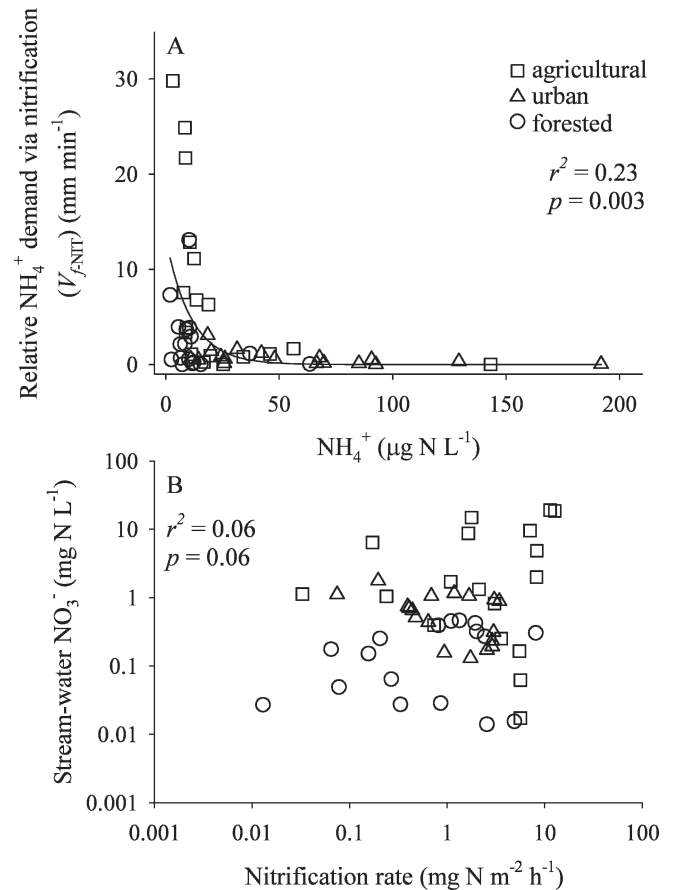
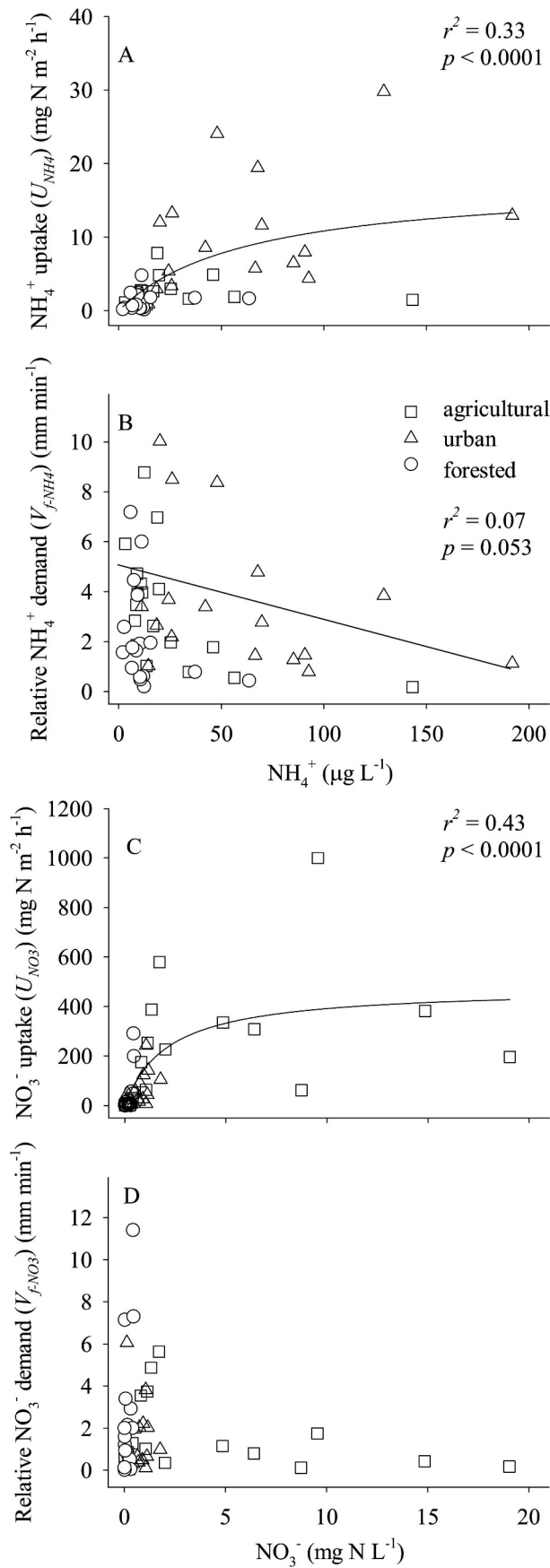


Fig. 5. (A) Relative NH_4^+ demand via nitrification ($V_{f\text{NIT}}$) vs. NH_4^+ concentration. Relative NH_4^+ demand via nitrification declined exponentially, but the relationship becomes stronger when we evaluated only the highly altered streams ($r^2 = 0.68$, $p < 0.0001$). (B) NO_3^- concentrations vs. nitrification rate (U_{NIT}). Stream-water NO_3^- concentrations were not related to production of NO_3^- via nitrification (U_{NIT}).

denitrification (U_{DEN}) saturated as NO_3^- concentration increased ($r^2 = 0.61$, $p < 0.0001$, $n = 54$; Fig. 6A, Table 2), with agricultural streams more likely to be saturated than urban or forested streams because of their higher NO_3^- concentrations. We also found that relative NO_3^- demand via denitrification ($V_{f\text{DEN}}$) declined exponentially with increasing NO_3^- concentrations ($r^2 = 0.52$, $p < 0.001$, $n = 54$; Fig. 6B, Table 2), confirming saturation of $V_{f\text{DEN}}$.

←

Fig. 4. Relationship between nutrient concentrations and areal uptake (U) and relative nutrient demand (V_f). (A) Areal NH_4^+ uptake (U_{NH_4}) plateaued at $17.7 \text{ mg N m}^{-2} \text{h}^{-1}$ with a half-saturation constant (K_s) of $64 \mu\text{g NH}_4^+\text{-N L}^{-1}$. (B) Areal NO_3^- uptake (U_{NO_3}) plateaued at $475.7 \text{ mg N m}^{-2} \text{h}^{-1}$ with $K_s = 2.2 \text{ mg NO}_3^-\text{-N L}^{-1}$. (C) Relative NH_4^+ demand ($V_{f\text{NH}_4}$) varied widely at low concentration, and a linear model fit better than a negative exponential model ($r^2 = 0.05$, $p = 0.10$). (D) Although relative NO_3^- demand ($V_{f\text{NO}_3}$) appeared to decline exponentially, a negative exponential model was not significant ($r^2 = 0.02$, $p = 0.27$).

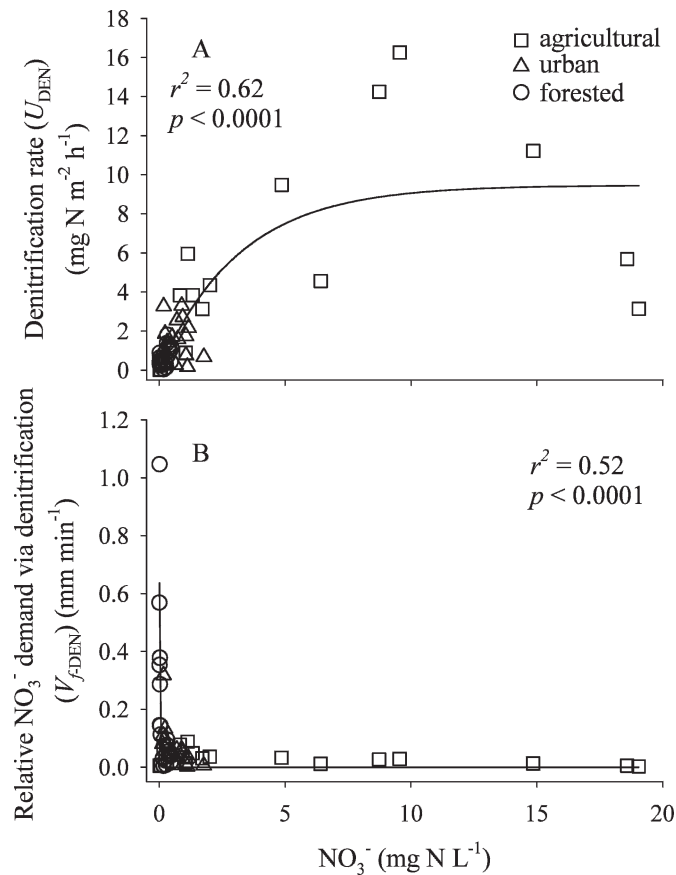


Fig. 6. Relationship between NO_3^- concentrations and whole-stream uptake (U) and relative nutrient demand (V_f) via denitrification. (A) Denitrification rate (U_{DEN}) plateaued at $11.4 \text{ mg N m}^{-2} \text{ h}^{-1}$ with $K_s = 3.2 \text{ mg NO}_3^- \text{ N L}^{-1}$. (B) Relative NO_3^- demand via denitrification ($V_{f\text{-DEN}}$) declined exponentially.

Relationship between nitrification, denitrification, and whole-stream DIN uptake—We tested our prediction that autotrophic and heterotrophic parameters would be related to N uptake using multiple linear regressions (MLRs) to identify relationships between whole-stream relative nutrient demand (V_f) and benthic organic matter standing stocks, land use in the subbasin and in riparian buffers, and nitrification and denitrification rates, expressed as U and V_f . Relative NH_4^+ demand ($V_{f\text{-NH}_4}$) was positively related to relative NH_4^+ demand via nitrification ($V_{f\text{-NIT}}$) and negatively related to canopy cover as an index of autotrophic activity (MLR, $R^2 = 0.33$, $p < 0.0001$, $n = 51$). The relationships identified in the MLR, in conjunction with the highest Chl a standing stocks (Fig. 2B) and highest $V_{f\text{-NH}_4}$ we observed in the spring (Fig. 3A), suggest that autotrophy influences $V_{f\text{-NH}_4}$ directly through assimilatory demand and indirectly by influencing nitrification. Relative NO_3^- demand ($V_{f\text{-NO}_3}$) was negatively related only to nitrification rates (U_{NIT} , $r^2 = 0.13$, $p = 0.01$, $n = 51$), suggesting that NO_3^- produced in the sediments via nitrification may reduce relative NO_3^- demand from the water column. The lack of a relationship between $V_{f\text{-NO}_3}$ and denitrification suggests that denitrification is not a significant component of whole-stream NO_3^- dynamics.

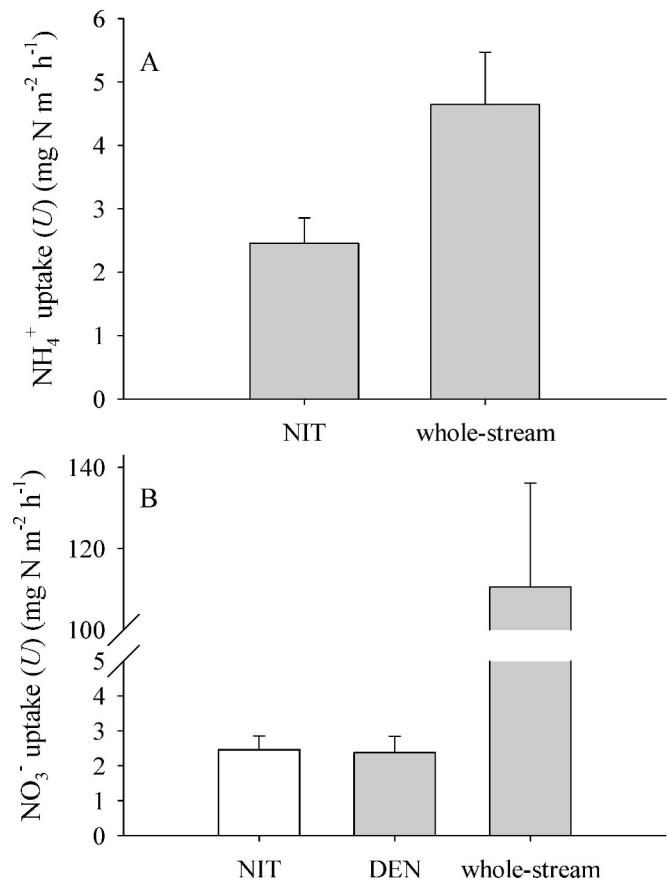


Fig. 7. Nitrification and denitrification rates compared to whole-stream uptake (± 1 SE). (A) Areal NH_4^+ uptake (U_{NH_4}) exceeded NH_4^+ uptake via nitrification (U_{NIT}). (B) Total NO_3^- removal (U_{NO_3}) exceeded NO_3^- removal via denitrification (U_{DEN}) by nearly two orders of magnitude among streams and seasons (± 1 SE). Nitrification (open bar) produces NO_3^- , but it is included to emphasize the balance between nitrification and denitrification under the optimum redox conditions of the lab assays.

Neither $V_{f\text{-NH}_4}$ nor $V_{f\text{-NO}_3}$ were related to total organic matter standing stocks, organic matter compartments (i.e., FBOM or leaves), or any metric of land use in the subbasin or the riparian buffer.

We compared nitrification rates (U_{NIT}) to whole-stream NH_4^+ uptake (U_{NH_4}) to identify the relative magnitudes of these processes. Nitrification rates accounted for $53\% \pm 11\%$ (SE) of whole-stream NH_4^+ uptake (Fig. 7A). Because nitrification converts NH_4^+ to NO_3^- , nitrification rates (U_{NIT}) are equivalent to NO_3^- production rates, so we compared U_{NIT} to NO_3^- consumption via denitrification (U_{DEN}) to understand the contribution of coupled nitrification–denitrification to whole-stream NO_3^- uptake (U_{NO_3}). Nitrification rates sometimes exceeded denitrification rates and vice versa, but the rates were of the same magnitude and roughly balanced overall (Fig. 7B). Contrary to our predictions, rates of nitrification (U_{NIT}) and denitrification (U_{DEN}) did not differ among seasons, but whole-stream U_{NO_3} always exceeded U_{NIT} and U_{DEN} by at least an order of magnitude. Even under optimum redox conditions (i.e., oxygenated sediment

slurries), nitrification produced NO_3^- equivalent to only $3.3\% \pm 0.5\%$ (SE) of whole-stream NO_3^- uptake whereas denitrification in deoxygenated slurries permanently removed NO_3^- equivalent to only $2.5\% \pm 0.3\%$ (SE) of whole-stream NO_3^- uptake. Therefore, coupled nitrification-denitrification did not contribute substantially to whole-stream NO_3^- dynamics, which were evidently dominated by assimilatory demand across streams and seasons.

Discussion

Land-use-mediated, in-stream autotrophy as a determinant of inorganic N demand—Higher relative NH_4^+ demand ($V_{f\text{-NH}_4}$) in the spring (Fig. 3A) implied stimulated N demand by autotrophs prior to shading by riparian canopies. Primary producers are important determinants of NH_4^+ demand in streams with high light levels such as in desert (Webster et al. 2003), prairie (Dodds et al. 2000), and tundra (Peterson et al. 1997) environments, and in forested streams with logged riparian zones (Sabater et al. 2000). However, autotrophy can also be important in the spring in temperate forested streams prior to leaf emergence, when high light levels cause a peak in assimilatory N demand by primary producers (Simon et al. 2005; Hoellein et al. 2007; Roberts and Mulholland 2007). The unstable, fine sediments (i.e., sand and FBOM) that dominated our study streams did not provide enough stability for filamentous algae or macrophytes, which have previously been identified as important determinants of $V_{f\text{-NH}_4}$ (Bernot et al. 2006). However, sand and FBOM had highest Chl *a* content in the spring (Fig. 2B), indicating that diatoms colonizing fine particles were an important component of the algal assemblage that probably contributed to higher $V_{f\text{-NH}_4}$ in the spring. In regression analysis that pooled our data points across seasons and land uses, $V_{f\text{-NH}_4}$ was not related to indirect indicators of heterotrophic activity such as organic matter standing stocks, whether expressed at the reach scale or as individual compartments (e.g., FBOM or leaf standing stock). In contrast, we found higher $V_{f\text{-NH}_4}$ associated with more open canopies, suggesting that riparian dynamics played an important role in mediating NH_4^+ demand. An open riparian canopy in the spring, when Chl *a* was highest, probably stimulated autotrophic N demand by increased light availability, and an open riparian canopy in the autumn after leaf fall could have been associated with higher heterotrophic N demand during decomposition.

Urban streams had higher $V_{f\text{-NH}_4}$ compared to forested streams (Fig. 3A). In other urban streams, open riparian canopies positively influence autotrophic activity (Grimm et al. 2005; Meyer et al. 2005), and our urban streams had highest Chl *a* in the spring associated with high $V_{f\text{-NH}_4}$. However, increased impervious surface cover in urban streams can also increase scouring flows that can remove attached algae (Grimm et al. 2005) and FBOM (Meyer et al. 2005), resulting in lower $V_{f\text{-NH}_4}$ compared to more pristine streams with unmodified hydrology. We observed similar scouring flows because of summer thunderstorms in our urban streams, and we also measured relatively low Chl

a in summer and autumn despite significantly less riparian cover compared to other land uses. The relatively high $V_{f\text{-NH}_4}$ found in urban streams in the summer and autumn associated with relatively low Chl *a* indicates that heterotrophic activity is also an important driver of $V_{f\text{-NH}_4}$ in these systems.

The seasonal pattern in relative NO_3^- demand ($V_{f\text{-NO}_3}$) in urban streams suggested an autotrophic influence because of higher $V_{f\text{-NO}_3}$ in the spring (Fig. 3B), but we found no seasonal differences because forested streams had high variability and a seasonal pattern that diverged from the other streams. Recent studies indicate the importance of NO_3^- as a whole-stream N source when autotrophic activity is high (Hall and Tank 2003; Fellows et al. 2006; Mulholland et al. 2006). However, substratum-specific (Munn and Meyer 1990; Kemp and Dodds 2002) and whole-stream (Martí and Sabater 1996) studies frequently show that NH_4^+ is the first choice as an N source. In our study streams, high N demand by autotrophic production in the spring probably cannot be met solely by the low concentrations of NH_4^+ , so NO_3^- supplements autotrophic demand. The lack of a strong seasonal signature in $V_{f\text{-NO}_3}$ among our study streams probably reflects the consistently high NO_3^- concentrations ($>2 \text{ mg N L}^{-1}$ averaged across all streams) and the preference for using a moderate supply of NH_4^+ (overall average $37 \mu\text{g N L}^{-1}$) as a primary N source, which would both mask patterns in NO_3^- demand.

In general, we anticipated that land use would mediate seasonal patterns in the relative demand for inorganic N (as V_f), with agricultural and urban streams exhibiting less seasonality than forested streams because of open canopies year-round, resulting in greater autotrophic influence through the year. Land use did influence seasonal patterns, but not in the way we expected. For example, $V_{f\text{-NH}_4}$ in forested and urban streams paralleled the seasonal pattern in Chl *a*, which was highest in spring and lowest in autumn, but agricultural streams had lowest $V_{f\text{-NH}_4}$ in summer. In the upper midwestern U.S., most low-order agricultural streams are shallow and have low water velocity at summer base flow. In combination with the high light levels in these streams, channel geometry and hydrology encourage the growth of tall grasses such as *Phalaris arundinacea* on channel edges, which may ultimately restrict light availability during summer. Also, high turbidity often associated with agricultural streams may further reduce light reaching the stream bottom (Harding et al. 1999).

A growing body of evidence indicates the importance of ecosystem metabolism (gross primary production and ecosystem respiration) for determining rates of nutrient uptake in streams (Hall and Tank 2003; Hoellein et al. 2007; Roberts and Mulholland 2007). We used indirect indicators of autotrophy (e.g., Chl *a*, canopy closure, and macrophyte and algal biomass) and heterotrophy (e.g., standing stocks of detrital organic matter) rather than direct measures of metabolism, but we still found evidence that autotrophy played a significant role in relative NH_4^+ demand ($V_{f\text{-NH}_4}$) in the spring. Even so, demand for NH_4^+ and NO_3^- was relatively high throughout the year and in times with low Chl *a* indicating the importance of heterotrophic uptake of DIN throughout the year. Our

bulk measures of heterotrophy, which included biomass in various stages of decomposition, did not add explanatory power, even in the summer when we expected higher temperatures to stimulate heterotrophic activity. If we had measured ecosystem respiration, we might have identified a greater role for heterotrophic demand among streams and seasons, but our results are consistent with recent evidence that warm temperatures may not be enough to stimulate nutrient uptake from the water column (Roberts and Mulholland 2007).

Land-use influence on saturation of whole-stream uptake—Because agricultural and urban land uses frequently increase nutrient concentrations in streams (Carpenter et al. 1998), we predicted that agricultural and urban streams would show saturation of inorganic N uptake more than forested streams, and we tested our prediction by regressing U and V_f against water-column N concentrations. Although models of uptake kinetics are more appropriately used to examine the uptake response to different nutrient concentrations in one stream (Dodds et al. 2002), comparing across streams and time periods has also been used to investigate saturation kinetics from a regional or seasonal perspective (Simon et al. 2005; Newbold et al. 2006; Hoellein et al. 2007). Our data set is bolstered by the additional component of varying land use, which expands the concentration range further than has been examined previously. Among streams and seasons, areal NH_4^+ uptake ($U_{\text{-NH}_4}$) and NO_3^- uptake ($U_{\text{-NO}_3}$) approached saturation with increasing concentrations. The degree to which saturation was approached varied by land use, with higher NH_4^+ concentrations in urban streams saturating $U_{\text{-NH}_4}$ and higher NO_3^- concentrations in agricultural streams saturating $U_{\text{-NO}_3}$.

Relationships between U and nutrient concentration were explained better by MM models than by linear models, yet the variability explained by MM models was relatively low (Fig. 4A,B). This is not surprising, because the total variability in areal N uptake among streams and seasons is a combination of nutrient concentration and relative nutrient demand (V_f ; see Eq. 3), which varies throughout the year (Newbold et al. 2006). Therefore the unexplained variability in $U_{\text{-NH}_4}$ and $U_{\text{-NO}_3}$ was probably caused by the influences of autotrophy on V_f as well as additional ecosystem metrics we did not measure such as ecosystem respiration.

Saturation of biological uptake is indicated when streams exhibit MM dynamics, but decreased uptake efficiency at higher nutrient concentrations (i.e., a decline in V_f with increasing concentration) confirms saturation of biological demand (Dodds et al. 2002). Additionally, when abiotic sorption removes solutes from the water column (e.g., NH_4^+ or PO_4^{3-}), V_f should decline linearly with increasing nutrient concentration, particularly at low concentrations. When abiotic sorption does not influence a solute (e.g., NO_3^-), V_f declines exponentially with increasing nutrient concentration (Davis and Minshall 1999). We observed a marginally significant linear decline in $V_{f\text{-NH}_4}$ (Fig. 4C), and although we observed lower $V_{f\text{-NO}_3}$ at higher NO_3^- concentrations (Fig. 4D), the

apparent exponential decline could not be described with a negative exponential model. Across the concentration range found in our study streams, biological demand for NH_4^+ increased linearly at low concentrations but reached a plateau at high concentrations, so we were able to calculate the half-saturation constant (K_s) for NH_4^+ at $\sim 64 \mu\text{g NH}_4^+\text{-N L}^{-1}$, which is higher than that estimated by Simon et al. (2005) for various low-nutrient New Zealand streams ($0.5\text{--}19.0 \mu\text{g NH}_4^+\text{-N L}^{-1}$). The higher half-saturation constants we report probably reflect the influence of higher NH_4^+ concentrations in our mixed land-use study streams compared to the pristine grassland streams studied by Simon et al. (2005).

Areal NO_3^- uptake ($U_{\text{-NO}_3}$) reached a significant plateau (Fig. 4B), but we did not see an associated statistically significant decline in relative NO_3^- demand ($V_{f\text{-NO}_3}$) at higher concentrations (Fig. 4D) that would allow us to attribute the plateau in $U_{\text{-NO}_3}$ to lower relative nutrient demand. However, the half-saturation constant ($K_s = 2.2 \text{ mg NO}_3^-\text{-N L}^{-1}$) in our streams was orders of magnitude above that reported by Simon et al. (2005; $K_s = 1.4 \mu\text{g N L}^{-1}$) for New Zealand streams with very low NO_3^- concentrations. Thus, the K_s values we report are much higher than those found in the literature, suggesting that the capacity for whole-stream N uptake can increase considerably, presumably through increased biological demand in response to nutrient loading and light availability. This uptake becomes less efficient at higher concentrations, however, as indicated by V_f .

Land-use influence on saturation of nitrification and denitrification—Water-column concentrations of NH_4^+ and NO_3^- influence rates of benthic nitrification and denitrification (e.g., Kemp and Dodds 2002), so we analyzed patterns in relative NH_4^+ demand via nitrification ($V_{f\text{-NIT}}$). As NH_4^+ concentration increased, relative NH_4^+ demand via nitrification decreased exponentially (Fig. 5A), consistent with saturation of biological demand. The relationship became stronger when we considered the agricultural and urban streams without the forested streams, indicating that anthropogenic N loading in streams can saturate nitrification. Abiotic sorption typically causes NH_4^+ demand to decline linearly with increasing concentration (Davis and Minshall 1999), so the exponential decay relationship may indicate saturation of sediment binding sites at high pore-water NH_4^+ concentrations. If anthropogenic N loading saturated sediment binding sites, relative NH_4^+ demand ($V_{f\text{-NH}_4}$) would behave nonlinearly with respect to concentrations because biological processes would drive uptake.

The lack of a significant relationship between NO_3^- produced via nitrification ($U_{\text{-NIT}}$) and water-column NO_3^- concentrations (Fig. 5B) may be explained by land-use practices that increased NO_3^- loading to the streams (Inwood et al. 2005). Because denitrification requires NO_3^- , increasing concentrations typically increase denitrification rates in streams (Bernot and Dodds 2005). We observed saturation of denitrification rates ($U_{\text{-DEN}}$) at high NO_3^- concentrations (Fig. 6A), as have other studies in waters with relatively high NO_3^- concentrations (Seitzinger 1988; García-Ruiz et al. 1998), and we

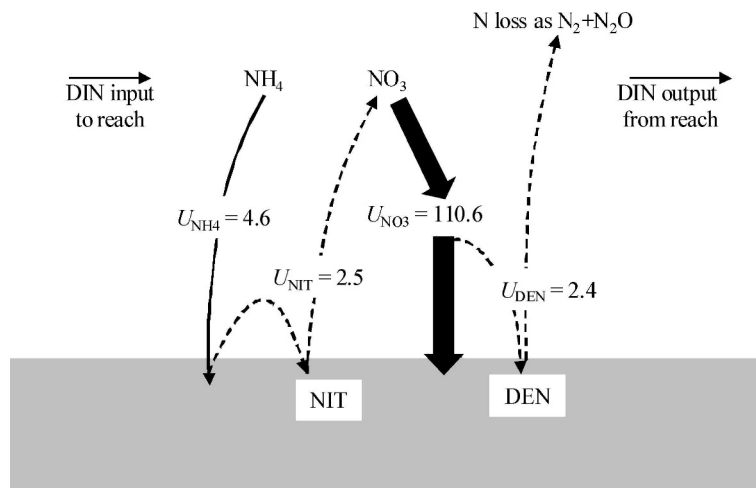


Fig. 8. Whole-stream nitrogen (N) uptake processes. Numbers represent rates ($\text{mg N m}^{-2} \text{ h}^{-1}$) of whole-stream uptake (U_{NH_4} and U_{NO_3}) and removal via nitrification (U_{NIT}) and denitrification (U_{DEN}), averaged among land uses and seasons. Assimilatory demand dominated uptake of NH_4^+ and NO_3^- , and U_{NIT} and U_{DEN} approximately balanced. The size of the arrows approximates the relative magnitudes of N uptake and transformation rates.

confirmed saturation of biological demand with a significant exponential decay model (Fig. 6B). Agricultural streams with high NO_3^- concentrations were the only ones in the saturated portion of the curve, and the MM curve predicted maximum denitrification at $11.4 \text{ mg N m}^{-2} \text{ h}^{-1}$, within the range of the theoretical maximum U_{DEN} reported in Bernot and Dodds (2005; $4.2\text{--}20.8 \text{ mg N m}^{-2} \text{ h}^{-1}$). Our data suggest that denitrification capacity in agricultural streams was near the maximum possible level, and was probably limited by organic C to support respiration or overall anoxic habitat in contact with overlying stream water (Arango et al. 2007). Additionally, the high coefficient of determination for U_{DEN} ($r^2 = 0.61$; Fig. 5B) indicates that NO_3^- concentration is a better predictor of denitrification than of whole-stream U_{NO_3} ($r^2 = 0.43$), probably because denitrification requires NO_3^- whereas whole-stream NO_3^- removal could occur through a variety of processes, adding variability to the relationship between NO_3^- concentration and U_{NO_3} .

Contribution of nitrification and denitrification to whole-stream N uptake—Combining all streams and seasons, areal NH_4^+ uptake via nitrification (U_{NIT}) accounted for about half of whole-stream NH_4^+ uptake (U_{NH_4}) (Fig. 7A). Although we interpret our nitrification rates cautiously because we measured them in the laboratory in oxygenated slurries, they compare well with nitrification rates measured in situ using isotopic tracer methods. In an interbiome comparison using $^{15}\text{NH}_4^+$ tracer additions, nitrification accounted for 3–60% of whole-stream NH_4^+ uptake (Peterson et al. 2001), including a Michigan stream in the Kalamazoo River catchment where the proportion was 57% (Hamilton et al. 2001). Nitrification converts NH_4^+ , which can be removed from the water column via sorption processes, to NO_3^- , a mobile ion that travels longer distances in streams before uptake (Webster et al. 2003). Consequently, nitrification, if high enough, can add to chronically high

NO_3^- loads and exacerbate downstream N exports (Bernot and Dodds 2005). In our streams where whole-stream NO_3^- uptake (U_{NO_3}) and NO_3^- uptake via denitrification (U_{DEN}) approach saturation at high N concentrations (Fig. 4B, 6A), any NH_4^+ converted to NO_3^- via nitrification may be exported far downstream prior to uptake.

Areal NO_3^- removal rates (U_{NO_3}) measured using short-term, whole-stream additions reflect the balance between assimilatory uptake (i.e., temporary NO_3^- storage), denitrification (i.e., permanent NO_3^- removal), and nitrification (i.e., NO_3^- production), but few studies have measured these processes simultaneously. We recognize that lab assays of nitrification and denitrification were measured under optimum redox conditions, but note that inorganic N availability represented ambient conditions. Additionally, whole-stream NO_3^- uptake may be underestimated because we used short-term nutrient additions that increased NO_3^- concentrations and thus could have saturated demand (Mulholland et al. 2002). Therefore, comparing these rates represents a maximum estimate of the contribution of nitrification and denitrification to whole-stream NO_3^- dynamics. Under optimum redox conditions, sediment nitrification and denitrification rates approximately balanced each other (Fig. 7B), suggesting that site-specific factors controlling sediment redox potential will determine net production or removal of NO_3^- in the benthos. For example, diel oxygen changes may shift the sediment from net production of NO_3^- in the day, when increasing oxygen stimulates nitrification, to net consumption at night, when increasing respiration stimulates denitrification (Risgaard-Petersen et al. 1994). Additionally, seasonal changes could influence sediment redox potentials, favoring nitrification in the spring when autotrophic activity peaks and denitrification in the autumn when heterotrophic activity peaks (Christensen et al. 1990). Although we predicted seasonal changes in the dominance of nitrification and denitrification, the redox-

optimized N transformation assays we used were probably not sensitive enough to examine this question.

The streams we studied had high NO_3^- concentrations because of land-use activity, so we asked whether the benthos was a net producer of NO_3^- via nitrification or a net consumer of NO_3^- via denitrification. Our data indicate that nitrification and denitrification rates were a small fraction of whole-stream NO_3^- uptake ($U_{-\text{NO}_3}$), which was an order of magnitude less than $U_{-\text{NO}_3}$ on average (Fig. 7B), and they emphasize the importance of assimilatory uptake in the removal of inorganic N from the water column, which dominated $U_{-\text{NO}_3}$ in our study streams (Fig. 8). A whole-stream $^{15}\text{NO}_3^-$ tracer experiment using more accurate but much more expensive methods confirmed that assimilatory demand controlled NO_3^- uptake and that $U_{-\text{DEN}}$ was a relatively small fraction (overall mean of 16% compared to our data showing 2.5%) of whole-stream $U_{-\text{NO}_3}$ (Mulholland et al. 2004), the same result found in 72 $^{15}\text{NO}_3^-$ tracer experiments conducted in eight biomes of varying land use across North America (Mulholland et al. 2008). Although we calculate assimilatory $U_{-\text{NO}_3}$ as the fraction of total NO_3^- removal not accounted for by denitrification, this fraction may not be solely attributable to assimilatory demand. Other processes, such as dissimilatory NO_3^- reduction to NH_4^+ or NO_3^- reduction coupled to iron oxidation, may account for an unknown fraction of $U_{-\text{NO}_3}$, but we know comparatively little about these processes in the context of total NO_3^- uptake (Burgin and Hamilton 2007).

Long-term fate of assimilated N—Although assimilatory N uptake is only temporary, it slows the downstream flux of NO_3^- by removing it from the water column and transforming it into a particulate organic form (Mulholland 2004). Previous studies have rarely considered the ultimate fate of this assimilated N, but a synthesis of denitrification studies indicates the removal of assimilated N following remineralization and coupled nitrification/denitrification when water residence times are long (Seitzinger et al. 2006). Scaling the total assimilatory DIN uptake we measured ($U_{-\text{NH}_4} + U_{-\text{NO}_3} = 115 \text{ mg N m}^{-2} \text{ h}^{-1}$) to an annual rate corresponds to the production of $0.9 \text{ kg organic N m}^{-2} \text{ yr}^{-1}$, which we compared to a detailed analysis of organic matter characteristics done in the same catchment we studied. Given a C:N mass ratio for detrital FBOM of 14.3 (Hamilton et al. 2001) and assuming that detrital organic matter is 45% C, this production equates to $2.5 \text{ kg organic matter m}^{-2} \text{ yr}^{-1}$. Assuming a bulk density of deposited FBOM of 0.018 g cm^{-3} (Hamilton et al. 2001), each month of accumulated FBOM would occupy a depth of 0.13 m, which would have been very noticeable in these shallow streams. We did not observe such accumulations of detrital or live biomass over the year that we studied each of our streams; thus, we assume that organic matter storage within the study reaches cannot account for the assimilated N. Assimilated organic matter is more likely exported downstream in particulate form, or in dissolved form following solubilization, to be buried in depositional zones or mineralized. Previous research has shown that episodic

export of organic matter can dominate downstream material flux in streams (Meyer and Likens 1979; Royer and David 2005). Although we did not measure annual N stocks and fluxes, the lack of organic matter accumulation in our streams suggests that much of the assimilated N we measured was either exported or mineralized on site and removed via subsequent nitrification and denitrification. Future studies that consider both the short- and long-term fate of assimilated N in streams will make valuable contributions to our overall understanding of stream N cycling.

In the mixed-land-use streams we studied, autotrophic uptake exerted strong control over relative nutrient demand (V_f) in the spring, but high uptake associated with low Chl *a* indicated the importance of heterotrophic uptake at other times of year. Although the land-use category of a stream was not a very powerful predictor of V_f , land use did influence uptake rates of NH_4^+ and NO_3^- , because higher nutrient concentrations associated with agriculture and urbanization caused biological demand to approach saturation and thus become less efficient at removing N from overlying water. The half-saturation constants we found were higher than those reported in the literature, reflecting the higher nutrient availability in the streams that we studied and indicating that stream nutrient demand can increase substantially under chronically high nutrient loads. Denitrification rates were saturated and near a theoretical maximum in some agricultural streams with high NO_3^- concentrations (Bernot and Dodds 2005), implying that additional NO_3^- loads could travel far downstream with minimal biological processing. Although nitrification and denitrification approximately balanced each other when measured under ideal redox conditions, both processes made small contributions to whole-stream NO_3^- demand, which evidently was dominated by assimilatory uptake. Our results suggest that assimilatory demand dominates nutrient uptake in headwater streams, ultimately controlling the capacity of streams to reduce downstream N flux, if only temporarily.

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