SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

Selection of study streams. We selected streams in order to include a wide range of biomes and land use types. In each of 8 regions representing different biomes (Supplementary Figure 1) we chose three headwater streams (1st and 2nd order) in each of three land-use categories (reference, agricultural, urban), for a total of 72 streams (Supplementary Table 1). Streams were assigned to land-use categories based on visual observation of the dominant land use adjacent to the study reach. Reference streams were bordered by native vegetation according to biome and included forests, grassland, and desert/shrub vegetation. Agricultural streams included a wide variety of cultivated lands, open range grazing, and pastures. Urban streams included those bordered by housing developments, golf courses, urban commercial areas with a few cement-lined channels. Determination of land cover in the catchments of each stream (using the USGS National Elevation Data Set, available at http://seamless.usgs.gov, and 2001 USGS National Land Cover Datasets, available online at http://seamless.usgs.gov, or for the PR streams from 1991-1992 Landsat TM imagery as derived by Helmer et al.[33] showed that reference streams drained catchments with >85% native vegetation (except for 2 streams with 50 and 65% native vegetation), whereas agricultural and urban streams drained catchments ranging from <1 to 100% agricultural and urban land cover types.

Measurement of NO_3^- uptake and denitrification rates. We measured NO_3^- uptake rates for entire stream reaches based on the nutrient spiraling approach[16, 28, 29]. Total NO_3^- uptake was determined from the downstream rate of decline in tracer $^{15}NO_3^-$ mass flux using the model:

$$d^{15}NO_3^-/dx = -k_{tot} * {}^{15}NO_3^- \quad (1)$$

where $^{15}NO_3^-$ is the tracer $^{15}NO_3^-$ flux (μg ^{15}N s^{-1}), x is the stream channel distance from the tracer addition (m), and k_{tot} is the distance-specific NO_3^- uptake rate (m^{-1}). We were able to determine k_{tot} for 69 of the 72 streams studied.

Denitrification rates (calculated separately for production of N_2 and N_2O) were estimated by fitting a model of N gas production to the measured fluxes of tracer ^{15}N as N_2 and N_2O over the study reach as follows:

$$d^{15}NO_3^-/dx = -(k_{den}+k_U)^{15}NO_3^-$$
(2)

$$d^{15}N_{GAS}/dx = k_{den}{}^{15}NO_3^- - k_2{}^{15}N_{GAS} \quad (3)$$

where $^{15}NO_3^-$ is the tracer ^{15}N flux in NO_3^- (μg ^{15}N s^{-1}) and $^{15}N_{GAS}$ is the tracer ^{15}N flux in N_2 or N_2O (ng ^{15}N s^{-1}), k_{den} is the distance-specific N_2 or N_2O production rate (m^{-1}), k_U is the assimilative uptake rate of NO_3^- (m^{-1}), and k_2 is the air-water exchange of N_2 or N_2O. Values of k_2 for N_2 and N_2O were calculated from the measured rates of evasion of propane or SF_6 and the relative values of their Schmidt numbers[34]. Because the total

uptake rate of NO_3^- (k_{tot}) is the sum of denitrification and assimilatory uptake (i.e., $k_{den}+k_U$), the equations above were solved only for k_{den} using the optimization tool "Solver" in Microsoft Office Excel.

Denitrification rates (k_{den}) were measured by model fitting only when there was significant tracer ^{15}N in N_2 or N_2O (defined as $\delta^{15}N$ values greater than the upper 97.5% confidence interval of background values measured prior to the isotope addition) at 3 or more stations along the stream reach. We then calculated a confidence range in the parameters using Maximum Likelihood Estimate (MLE)[35] to each of the model fits to ensure k_{den} values were significantly greater than 0 (i.e., 95% confidence interval of k_{den} did not include 0). We also applied MLE to ^{15}N flux data from streams that did not meet the model fitting criterion above (3 stations with significant tracer ^{15}N in N_2 or N_2O) to determine if k_{den} was sufficiently constrained to assign a non-zero value. This procedure resulted in the determination of k_{den} for 49 and 53 streams for N_2 and N_2O production, respectively. N_2 production rates far exceeded N_2O production rates in all streams, with median N_2 production rate being 99.4% of the sum of N_2 and N_2O production rate (range of 94.3 to 99.9%). Total k_{den} was then calculated as the sum of the k_{den} values for N_2 and N_2O for the 49 streams with measurable N_2 production rates.

Total uptake and denitrification rates reported for each stream are the average of the rates measured for the two sampling periods. On average, k_{tot} was 55% higher for the second sampling (near noon) than the first sampling (near midnight), and this difference was significant ($P < 0.01$, t-test, SAS Proc Means, SAS®, Version 9.1 for Windows, SAS Institute, Inc., Cary, NC, USA). Differences in k_{den} for both N_2 and N_2O production between sampling periods were not statistically different, however.

Physical, chemical, and biological variables were measured in each stream during or within 1 day of the ^{15}N experiment to determine potential predictors of total NO_3^- uptake and denitrification rates. Detailed sampling and analysis methods are available at the LINX project website (http://www.biol.vt.edu/faculty/webster/linx/). Average stream width (w) was determined from measurements of wetted width at 5-10 m intervals along the experimental reach. Average discharge was measured by dilution of the conservative solute tracer. Average water velocity was measured by the time of travel of the rising limb of the conservative tracer profile and average depth was determined by discharge/(width × velocity). An advection-dispersion model with transient storage was applied to the conservative tracer data to determine hydraulic characteristics related to transient storage zones[36]. We measured concentrations of NO_3^- (either by ion chromatography or by azo dye colorimetry after Cu-Cd reduction), NH_4^+(by phenate colorimetry or fluorometry), total soluble N (TSN, high temperature combustion, Shimadzu TOC-V with total Nitrogen Module), soluble reactive phosphorus (SRP, ascorbic acid-molybdenum blue), and dissolved organic carbon (DOC, high temperature combustion, Shimadzu TOC-V). Concentrations of dissolved organic N (DON) were estimated as the difference between TSN and the sum of NH_4^+ and NO_3^-. Abundance of several benthic organic matter components (coarse and fine benthic organic matter, epilithon, bryophytes, filamentous algae, vascular plants) was determined by collecting materials from known areas of the stream bottom at 5-10 locations within the study reach and measurement of dry mass (60°C) and ash-free dry mass (after combustion at 500°C) of the material collected. Reach-scale rates of metabolism (gross primary production,

GPP, and ecosystem respiration, ER) were measured using the diel dissolved oxygen method[37].

Calculation of nutrient spiraling metrics. We calculated several additional spiraling metrics for NO_3^- uptake from k_{tot} and k_{den} (ref [16]). Nitrate uptake length (S_W) was calculated from the rate of decline of tracer [^{15}N] NO_3^- flux (μg ^{15}N s^{-1}) over distance downstream from the addition site. Tracer [^{15}N] NO_3^- flux at each sampling station was determined from the $\delta^{15}N$, NO_3^- concentration, and discharge at each station, corrected for background values based on measurements just prior to the experiment. S_W was determined from the negative inverse of the slope (k_{tot}) of natural log-transformed tracer [^{15}N] NO_3^- fluxes versus distance [$S_W = -1/k_{tot}$, where k_{tot} is the distance-specific uptake rate (m^{-1})]. Values of k_{tot} were determined for the total removal of NO_3^- from water as well as for the NO_3^- removal by denitrification (k_{den}) as indicated by $^{15}N_2$ and $^{15}N_2O$ gas fluxes. To compare biotic nutrient removal among streams, we calculated uptake velocity because, unlike uptake rates per unit distance (k_{tot}, k_{den}), uptake velocity is independent of the effects of water transport rate[16]. Uptake velocity (υ_f) was calculated as: $\upsilon_f = (v \times h)/S_W$, = Q/(10 × w × S_W), where v = water velocity (cm s^{-1}), h = water depth (m), S_W = uptake length (m), Q = discharge (L s^{-1}), and w = average stream wetted width (m). Areal uptake rate (U, μg m^{-2} h^{-1}) was calculated as: $U = (\upsilon_f \times C) \times 36000$, where C is the ambient NO_3^- concentration (μg N L^{-1}) and 36000 is a conversion factor. Uptake length, uptake velocity, and aerial uptake rate for denitrification (S_{Wden}, υ_{fden}, and U_{den}, respectively) were determined in the same way, except that k_{den} was used rather than k in the calculations.

Stream network model structure. We developed a simulation model of NO_3^- loading, transport, and biotic uptake within streams. The model routes NO_3^- and water from the landscape and through a stream network from the headwaters to the outlet, and biological uptake removes NO_3^- from the water column in each stream reach (Supplementary Figure 2).

The model calculates the mass of NO_3^- and discharge (Q) with a simple steady-state mass-balance approach in which incoming fluxes are subtracted from outgoing fluxes for each stream reach. Discharge for each reach is calculated by subtracting outgoing water fluxes from incoming water fluxes according to the following equation (terms defined in Supplementary Table 2):

$$Q_p = \overset{\text{inflows}}{(\Sigma Q_{p\text{-}1_i} + Q_L)} - \overset{\text{outflows}}{(Q_w + Q_{p+1_i})} \quad (4)$$

$$\text{where } Q_L = A_p \cdot Y_p \quad (5)$$

Nitrate for each reach is calculated similarly by subtracting outgoing nitrate from incoming nitrate fluxes as follows (terms defined in Supplementary Table 2):

$$NO_3^-{}_p = (\overset{\text{inflows}}{\Sigma\, NO_3^-{}_{p\text{-}1_i} + NO_3^-{}_L}) - (\overset{\text{outflows}}{NO_3^-{}_R + NO_3^-{}_{p+1_i}}) \quad (6)$$

$$\text{where } NO_3^-{}_L = A_p \cdot L_p \quad (7)$$

For each stream reach, the mass of nitrate removed ($NO_3^-_R$) is equal to the nitrate in the stream reach times the fractional removal of nitrate:

$$NO_3^-_R = R * NO_3^-_p \quad (8)$$

The fractional removal (R) is determined by the following equation recently applied by Wollheim et al.[15] (terms defined in Supplementary Table 2):

$$R = 1 - e^{(-vf/H_L)} \quad (9)$$

$$\text{where } H_L = Q_p / SA_p \quad (10)$$

The surface area (SA, L^2) equals the stream reach length (l) multiplied by average width (w), where the average width of a stream segment is estimated based on the equation of Leopold and Maddock[38] (terms defined in Supplementary Table 2):

$$w = aQ^b \quad (11)$$

Model parameterization. The stream network topology was parameterized using the 5th-order Little Tennessee River network in western North Carolina and northeastern Georgia (Supplementary Figure 3). Digital stream networks were derived by using 30-meter raster digital elevation models (USGS National Elevation Data Set, http://seamless.usgs.gov) and the Hydrologic Modeling extension in ArcView GIS software (Version 3.3, ESRI, Redlands, CA 2002). The stream network derived from the DEM was similar to the 1:24,000- scale stream network derived from USGS quadrangles. The stream network was divided into segments, defined as the length of stream between tributary junctions. Segments were divided into approximately 500-meter reaches, which yielded 1722 stream reaches. Model parameters were derived as described in Supplementary Table 3.

SUPPLEMENTARY DISCUSSION

Nitrate removal over a standardized stream reach. Across our streams, total uptake resulted in 72% removal of nitrate inputs from water over a standardized 1 km stream reach (median value, lower and upper quartile values of 34% and 98%, respectively). Denitrification resulted in 10% nitrate removal (median, lower and upper quartile values of 4% and 22%, respectively). Nitrate removal presented in this way (as a fractional removal rate per unit distance as determined directly from our measurements, k_{tot} and k_{den}, Supplementary table 1) is a function of both hydrology (rate of water transport) and biology (rate of uptake, primarily by benthic organisms). There was no significant effect of land use on the fractional removal rate per unit distance (Kruskal-Wallis test, SAS®, Version 9.1 for Windows, SAS Institute, Inc., Cary, NC, USA) because transport rate (discharge) varied by several orders of magnitude among streams within each land use category resulting in high variability in fractional removal rate within each category. We

did observe significant inverse correlations (Pearson Correlations, SAS®, Version 9.1 for Windows, SAS Institute, Inc., Cary, NC, USA) between k_{tot} and water discharge per unit stream width (Q/w) ($r = -0.453$, $P < 0.0001$, log-transformed values) and between k_{den} and Q/w ($r = -0.308$, $P = 0.031$, log-transformed values) as expected based on spiraling theory[16]. Stream discharge was unrelated to land use category (Kruskal-Wallis test, SAS®, Version 9.1 for Windows, SAS Institute, Inc., Cary, NC, USA); thus, variations in discharge did not bias our analysis of land use category effects.

Study limitations. Although our inter-biome experiments and stream network modeling results add considerable insight to in-stream NO_3^- dynamics, many limitations arise from both field methodologies and model assumptions that affect our ability to extrapolate our results both temporally and spatially. Our experimental approach did not quantify the ultimate fate of $^{15}NO_3^-$ assimilated but not immediately denitrified. This $^{15}NO_3^-$ will not be stored indefinitely on the streambed, but eventually will be denitrified, re-released as some form of dissolved N to the water column, or transported downstream as particulate N. Furthermore, all tracer additions were conducted at or near steady-state hydrologic conditions, and typically at or near annual low flows. High rates of nutrient transport typically occur during infrequent high flows whereas high rates of biological assimilation and storage typically occur during frequent low flows in streams[39], and rates of biological assimilation may be higher in the summer when temperatures are warmer and there is greater biological activity. Therefore, our experimental design, although based on state-of-the-art methods for measuring N processing in streams, prevents extrapolation of our results to annual N processing rates.

We are also limited in our ability to scale empirical results spatially across diverse steam networks. The structure of our stream network model does not include spatial variation in N inputs from the landscape, yet the spatial distribution of N sources in a watershed may drive patterns of N removal[12,14]. The representation of hydrogeomorphology in our stream network model also prevents us from extrapolating our model results to systems in which the channel is extensively hydrologically connected to off-channel (floodplain or wetlands) or subsurface (hyporheic zone or groundwater) systems, which may be important determinants of N removal within streams[40,41,42]. Finally, because our empirical results are based on experiments conducted only in small streams (0.2 – 267 Ls^{-1}; Supplementary Table 1) there is some uncertainty in extrapolating these results to larger systems, even though our model accounts for expected changes channel cross section (which influences the relationship between discharge and stream bed area). The general lack of *in situ* measurements of uptake and denitrification rates in large streams further adds to this uncertainty.

SUPPLEMENTARY NOTES

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