Nitrous oxide emission from denitrification in stream and river networks

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Nitrous oxide (N_2O) is a potent greenhouse gas that contributes to climate change and stratospheric ozone destruction. Anthropogenic nitrogen (N) loading to river networks is a potentially important source of N_2O via microbial denitrification that converts N to N_2O and dinitrogen (N_2). The fraction of denitrified N that escapes as N_2O rather than N_2 (i.e., the N_2O yield) is an important determinant of how much N_2O is produced by river networks, but little is known about the N_2O yield in flowing waters. Here, we present the results of whole-stream ^{15}N-tracer additions conducted in 72 headwater streams draining multiple land-use types across the United States. We found that stream denitrification produces N_2O at rates that increase with stream water nitrate (NO_3^-) concentrations, but that <1% of denitrified N is converted to N_2O. Unlike some previous studies, we found no relationship between the N_2O yield and stream water NO_3^-. We suggest that increased stream NO_3^- loading stimulates denitrification and concomitant N_2O production, but does not increase the N_2O yield. In our study, most streams were sources of N_2O to the atmosphere and the highest emission rates were observed in streams draining urban basins. Using a global river network model, we estimate that microbial N transformations (e.g., denitrification and nitrification) convert at least 0.68 $Tg \cdot y^{-1}$ of anthropogenic N inputs to N_2O in river networks, equivalent to 10% of the global anthropogenic N_2O emission rate. This estimate of stream and river N_2O emissions is three times greater than estimated by the Intergovernmental Panel on Climate Change.

Humans have more than doubled the availability of fixed nitrogen (N) in the biosphere, particularly through the production of N fertilizers and the cultivation of N-fixing crops (1). Increasing N availability is producing unintended environmental consequences including enhanced emissions of nitrous oxide (N_2O), a potent greenhouse gas (2) and an important cause of stratospheric ozone destruction (3). The Intergovernmental Panel on Climate Change (IPCC) estimates that the microbial conversion of agriculturally derived N to N_2O in soils and aquatic ecosystems is the largest source of anthropogenic N_2O to the atmosphere (2). The production of N_2O in agricultural soils has been the focus of intense investigation (i.e., >1,000 published studies) and is a relatively well constrained component of the N_2O budget (4). However, emissions of anthropogenic N_2O from streams, rivers, and estuaries have received much less attention and remain a major source of uncertainty in the global anthropogenic N_2O budget.

Microbial denitrification is a large source of N_2O emissions in terrestrial and aquatic ecosystems. Most microbial denitrification is a form of anaerobic respiration in which nitrate (NO_3^-, the dominant form of inorganic N) is converted to dinitrogen (N_2) and N_2O gases (5). The proportion of denitrified NO_3^- that is converted to N_2O rather than N_2 (hereafter referred to as the N_2O yield and expressed as the mole ratio) partially controls how much N_2O is produced via denitrification (6), but few studies provide information on the N_2O yield in streams and rivers because of the difficulty of measuring N_2 and N_2O production in these systems. Here we report rates of N_2 and N_2O production via denitrification measured using whole-stream $^{15}NO_3^-$-tracer experiments in 72 headwater streams draining different land-use types across the United States. This project, known as the second Lotic Intersite Nitrogen eXperiment (LINX II), provides unique whole-system measurements of the N_2O yield in streams.

Although N_2O emission rates have been reported for streams and rivers (7, 8), the N_2O yield has been studied mostly in lentic freshwater and marine ecosystems, where it generally ranges between 0.1 and 1.0%, although yields as high as 6% have been observed (9). These N_2O yields are low compared with observations in soils (0–100%) (10), which may be a result of the relatively lower oxygen (O_2) availability in the sediments of lakes and estuaries. However, dissolved O_2 in headwater streams is commonly near atmospheric equilibrium and benthic algal bio-

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films can produce O_2 at the sediment–water interface, resulting in strong redox gradients more akin to those in partially wetted soils. Thus, streams may have variable and often high N_2O yields, similar to those in soils (11). The N_2O yield in headwater streams is of particular interest because much of the NO_3^- input to rivers is derived from groundwater upwelling into headwater streams. Furthermore, headwater streams compose the majority of stream length within a drainage network and have high ratios of bioreactive benthic surface area to water volume (12).

Results and Discussion

The $^{15}N\text{-}NO_3^-$ tracer was detected in the dissolved N_2O pool in 53 of 72 streams and we assume that direct denitrification of stream water NO_3^- to N_2O (N_2O_{DN}) was the source of this $^{15}N\text{-}N_2O$. It is unlikely that nitrification was an important source of labeled $^{15}N\text{-}N_2O$ because the 24-h duration of the experiments was too short for the tracer to be assimilated by stream biota, mineralized, and subsequently nitrified. Rates of N_2O_{DN} varied by land use with the highest rates observed in high NO_3^- urban streams and the lowest in reference streams (i.e., those with little land conversion in their watersheds) (Fig. 1*A*). A positive relationship between N_2O_{DN} and stream water NO_3^- concentration (Fig. 1*B*) suggests anthropogenic N loading to streams stimulates denitrification and concomitant N_2O production. The N_2O_{DN} rates reported here are lower than most published reports (Fig. 1*A*), possibly because our in situ measurements are not affected by the experimental artifacts and scaling problems associated with sediment slurries, cores, and chambers used in most previously published estimates (13).

The $^{15}N\text{-}NO_3^-$ tracer was detected in both the dissolved N_2 and N_2O pools in 40 of the 72 study sites and we assume all $^{15}N\text{-}N_2$ was produced via direct denitrification. The only other potential source of $^{15}N\text{-}N_2$ production is anammox, a process by which chemolithoautotrophic bacteria convert ammonium (NH_4^+) and nitrite (NO_2^-) to N_2, but available evidence suggests that anammox is unimportant relative to denitrification in streams and rivers (14). Furthermore, any N_2 produced via anammox during the ^{15}N tracer additions would have contained little ^{15}N tracer because stream water NH_4^+ was minimally labeled with the ^{15}N tracer.

The N_2O yield ranged from 0.04% to 5.6% across the 53 streams; however, the interquartile range (0.3–1.0%) was well constrained despite substantial variation in NO_3^- availability, dissolved O_2, primary productivity, sediment organic matter, and stream geomorphology across our study sites (Fig. 2, Table S1). Denitrification proceeds by sequentially reducing NO_3^- to NO_2^-, nitric oxide (NO), N_2O, and finally N_2. Each reduction is per-

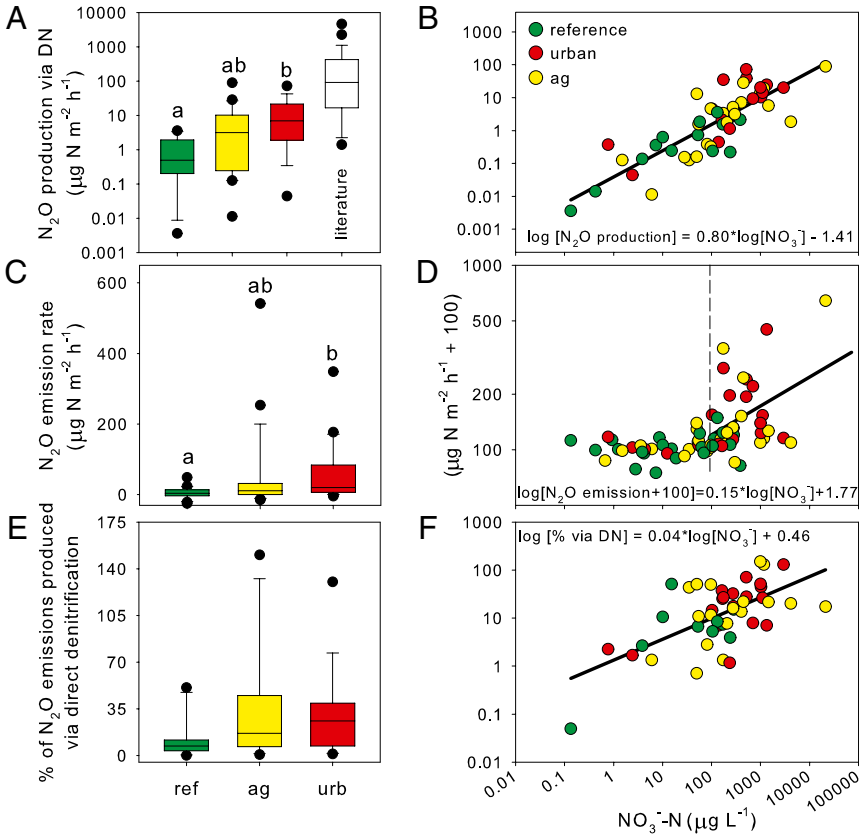

Fig. 1. (*A*) Box plots of stream N_2O production rates via denitrification of water column NO_3^- by catchment land use (reference, agricultural and urban). Benthic N_2O production rates reported in other studies are also shown. Significant differences between land-use types were determined with a one-way ANOVA followed by Tukey's post hoc test ($P = 0.004$) and are displayed as different lowercase letters above the box plots. See *SI Materials and Methods* for references. (*B*) Relationship between stream water NO_3^- and rates of N_2O production via denitrification ($r^2 = 0.68$, $P < 0.001$). (*C*) Nitrous oxide emission rates from streams. Significant differences between land use types were determined with a one-way ANOVA ($P = 0.002$) followed by Tukey's post hoc test and are displayed as different lowercase letters above the box plots. (*D*) Relationship between stream water NO_3^- concentrations and N_2O emission rates. The vertical dashed line represents a NO_3^- threshold (95 µg N·L^{-1}) below which N_2O emission rates are unrelated to NO_3^- (two-dimensional Kolmogorov–Smirnov test). Above the threshold N_2O emission rates are positively related to NO_3^- concentrations as represented by the least-squares best-fit line (solid black). (*E*) Percentages of stream N_2O emissions attributed to direct denitrification. Values >100% indicate N_2O was accumulating in the water column. There was no effect of land use ($P = 0.13$). (*F*) Variation in the percentage of stream N_2O emissions attributed to direct denitrification is partially explained by stream water NO_3^- concentration ($r^2 = 0.32$, $P < 0.001$).

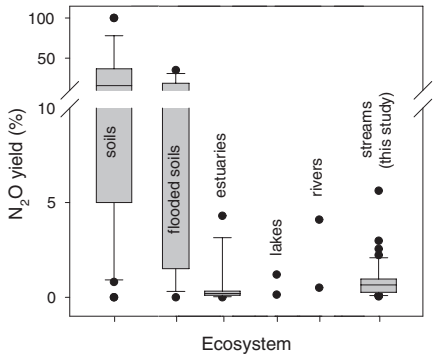

Fig. 2. Denitrification N_2O yields (percentage of denitrified N released as N_2O) measured in this study in comparison with other ecosystems. Data are displayed in box plots unless there were fewer than nine observations, in which case each observation is represented by a solid circle. See *SI Materials and Methods* for references.

formed by a different enzyme and the N_2O yield is determined by the relative activities of the N_2O-producing and reducing enzymes. There is a positive relationship between the N_2O yield and NO_3^- concentration in soils (15, 16) and estuarine sediments (9), possibly because higher NO_3^- availability suppresses nitrous oxide reductase (*nos*), the enzyme that reduces N_2O to N_2 (16). However, we did not find a significant relationship between stream water NO_3^- concentration and the N_2O yield ($P = 0.09$), despite NO_3^- concentrations spanning five orders of magnitude. Our findings suggest increased NO_3^- loading to streams stimulates overall denitrification rates and concomitant N_2O production, but does not increase the N_2O yield.

The N_2O yield in soils is related to the relative availability of oxidants (NO_3^-) and reductants (organic carbon). When the availability of NO_3^- greatly exceeds that of organic carbon, NO_3^- is preferred over N_2O as a terminal electron acceptor and N_2O accumulates (5, 17–19). The N_2O yield was not related to the ratio of stream water NO_3^- concentration to dissolved or particulate organic carbon concentration ($P \geq 0.17$), but was negatively related to stream ecosystem respiration ($P = 0.04$, $r^2 = 0.11$), suggesting factors promoting aerobic respiration (e.g., labile carbon availability) may decrease the N_2O yield.

Our data suggest that denitrification in aquatic ecosystems produces lower and less variable N_2O yields than in terrestrial ecosystems (Fig. 2). This finding may be explained by differences in oxygen (O_2) availability and molecular diffusion rates between aquatic sediments and the partially water-filled pore spaces of soils. Because *nos* is the most O_2 sensitive denitrifying enzyme (20), minor amounts of O_2 can suppress the reduction of N_2O without inhibiting its production, resulting in an elevated N_2O yield. Nitrous oxide is produced as a free intermediate that can escape reduction to N_2 by diffusing away from the denitrification zone (16). Partially wet soils may present air-filled routes through which the N_2O could more readily evade from soil solutions and ultimately escape to the atmosphere, whereas in aquatic sediments there may be a much greater likelihood of interception of the dissolved N_2O by *nos* before it can diffuse to the overlying water column. Overall, lower O_2 availability and gas diffusion rates in aquatic sediments compared with soils may account for the low aquatic N_2O yield.

Resource managers have used stream restoration to reduce watershed N export to estuaries and coastal oceans where it can contribute to eutrophication (21). This approach has been criticized on the grounds that stream denitrification alone cannot alleviate watershed N pollution (22) and that enhanced stream denitrification may lead to increased N_2O emission (23). Our data demonstrate that the N_2O yield in headwater streams is no larger than in other aquatic ecosystems and much lower than in soils (Fig. 2), indicating that measures to promote stream denitrification may have a relatively lower impact on climate change than the promotion of an equivalent amount of denitrification in terrestrial environments.

Other sources of N_2O to streams include in-stream nitrification and upwelling groundwater. The sum of all these N_2O sources determines the total amount of N_2O emitted by a stream. We investigated the potential for these additional sources to contribute to total N_2O emissions by estimating N_2O emission rates from the dissolved N_2O concentration and the air–water gas exchange rates in each of the 72 streams. The majority of streams were net sources of N_2O to the atmosphere (55 of 72) and only 2 streams showed a diel pattern in emission rates so we did not further consider diel variations in constructing N_2O budgets (cf. ref. 24). Stream N_2O emission rates were related to watershed land use, with highest emission rates in urban streams, intermediate rates in agricultural streams, and lowest rates in reference streams (Fig. 1*C*). Stream NO_3^- concentrations predicted N_2O emission rates when NO_3^--N exceeded 95 $\mu g \cdot L^{-1}$ ($P = 0.01$, $r^2 = 0.16$), but below this concentration N_2O emission rates were uniformly low and unrelated to NO_3^- concentration (Fig. 1*D*). This finding suggests that stream N_2O emission rates are not solely controlled by direct denitrification within the stream, but are likely enhanced by other sources including inputs of dissolved N_2O from groundwater.

We compared N_2O_{DN} (Fig. 1*A*) to N_2O emission rates (Fig. 1*C*) and found that the direct denitrification of stream water NO_3^- accounted for an average of 26% of N_2O emissions (Fig. 1*E*). This is a conservative estimate of in-stream N_2O production via denitrification because our method does not detect N_2O produced from the denitrification of NO_3^- regenerated within sediments and biofilms (e.g., indirect denitrification following organic N mineralization and nitrification), which can be the dominant source of NO_3^- supporting denitrification when stream water NO_3^- concentration is low (<140 $\mu g\ N \cdot L^{-1}$) (25). The relative importance of N_2O_{DN} as a source of N_2O was positively related to stream water NO_3^- concentrations (Fig. 1*F*), reflecting the greater importance of direct denitrification with increasing stream water NO_3^- concentrations.

Nitrification is a potentially large source of N_2O emissions, but we know of no published measurements of N_2O production via nitrification in streams. Several studies have shown that nitrification rates can be equal to or greater than denitrification rates in streams (26–28) and rivers (29), and the IPCC assumes nitrification rates exceed denitrification by twofold (30). Measurements of the nitrification N_2O yield (i.e., the fraction of nitrified N escaping as N_2O) are sparse, but it appears to be within the same range as the denitrification N_2O yield (9). Therefore, the IPCC assumes that nitrification produces twice as much N_2O emission as denitrification in streams and rivers. Given that N_2O_{DN} produced within the stream contributes an average of 26% of the N_2O emitted by headwater streams (Fig. 1*E*), nitrification could account for as much as an additional 52%, with groundwater inputs and indirect denitrification composing the remainder (Fig. 3). This budget highlights the potential importance of nitrification and indirect denitrification to stream N_2O production, but these processes remain poorly understood and therefore represent critical research gaps. Nevertheless, our research demonstrates that headwater streams are not only conduits for the emission of groundwater-derived N_2O to the atmosphere, but also active sites of in situ N_2O production, particularly where NO_3^- concentrations are elevated by anthropogenic N loading.

The IPCC and others have estimated global anthropogenic N_2O emissions from streams and rivers by assuming all anthropogenic N that enters a river network is nitrified to NO_3^- and half of this NO_3^- is then denitrified; the N_2O yield is assumed to range from 0.3% to 3.0% in each transformation (9, 30, 31). This approach has shown that streams and rivers may be the source

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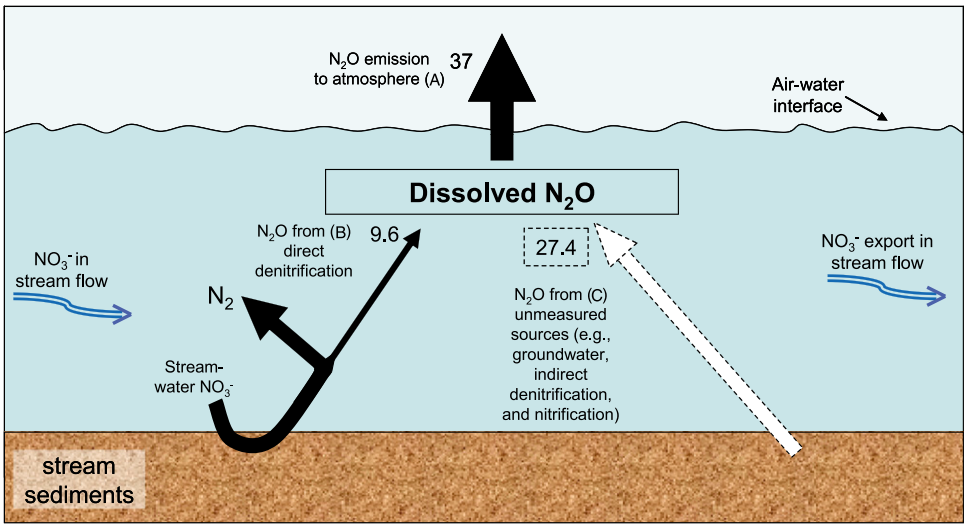

Fig. 3. Average N_2O fluxes estimated in this study (all units are $\mu g\ N \cdot m^{-2} \cdot h^{-1}$). Black arrows represent fluxes that were directly measured and the white arrow with dashed boundaries represents fluxes that were estimated by mass balance. (*A*) N_2O produced in the stream, or imported to the stream via groundwater, temporarily resides in a pool of dissolved N_2O before being emitted to the atmosphere. (*B*) Direct denitrification is the conversion of stream water nitrate to N_2 and N_2O. Less than 1% of stream water nitrate subject to direct denitrification is converted to N_2O, but this is the source of 26% of the N_2O emitted by the stream. (*C*) The balance of N_2O emission in excess of that produced via direct denitrification (e.g., 37 − 9.6 = 27.4) must have entered the stream via another mechanism. Likely mechanisms include indirect denitrification (e.g., the denitrification of nitrate generated within the sediments), nitrification, and inputs of N_2O-supersaturated groundwater.

of 15% of global anthropogenic emissions, but this estimate is poorly constrained due to uncertainty in the N_2O yield and proportion of anthropogenic N inputs denitrified in river networks. We improved the estimate of global anthropogenic N_2O emissions from lotic systems by modifying an existing global river network model (32) to include spatially explicit N loading in the contemporary era, an empirically derived relationship between denitrification and NO_3^- concentrations based on the LINX II ^{15}N tracer studies (22), and the mean N_2O yield of 0.9% reported here. The model estimates the percentage of dissolved inorganic nitrogen (DIN) delivered to the world's streams and rivers that is converted to N_2O via direct denitrification as water flows through the river network, including lakes and reservoirs. However, the model does not include indirect denitrification or denitrification associated with off-channel features (e.g., floodplains, riparian zones) and therefore provides a conservative estimate of anthropogenic N_2O emissions (Fig. 3).

The percentage of DIN inputs converted to N_2O via direct denitrification of water column NO_3^- in river networks across the globe ranges from 0% to 0.9% (Fig. 4). The percentage of N inputs converted to N_2O decreases with increasing N inputs because denitrification becomes less efficient as a NO_3^- sink at higher NO_3^- concentrations (22). We expected that the longer water-residence time in large rivers would result in a larger percentage of N inputs being denitrified compared with smaller river networks. However, we found no effect of catchment area (a surrogate for river network length), likely because the size of the network is confounded by other factors including variation in the distribution of N inputs, temperature, runoff conditions, and the presence of lakes and reservoirs within river networks (32).

We estimate that at the global scale, 0.75% of DIN inputs to river networks are converted to N_2O via direct denitrification and nitrification, threefold greater than the IPCC's estimate. This N_2O is likely to be emitted to the atmosphere from the turbulent water columns of streams and rivers. Using the IPCC's modeling framework and the results of our work, we estimate that nitrification and denitrification in river networks convert 0.68 $Tg \cdot y^{-1}$ of anthropogenic DIN inputs to N_2O globally, equivalent to 10% of the global anthropogenic N_2O emissions of 6.7 $Tg\ N \cdot y^{-1}$ (2) (for calculation details see *Global N_2O Budget* in *SI Materials and Methods*). This estimate of anthropogenic N_2O emissions from river networks is conservative because our model does not include several potentially large sources of N_2O (e.g., indirect denitrification and groundwater inputs). We also caution that our estimate of N_2O emissions attributed to nitrification is supported by few data (see above and Fig. 3).

We found that the combination of high denitrification rates and large anthropogenic DIN inputs results in substantial anthropogenic N_2O emissions from river networks, even though <1% of denitrified NO_3^- was converted to N_2O, a much lower percentage than has been reported for upland or flooded soils. Management efforts to enhance stream denitrification will reduce the delivery of N to sensitive coastal waters with less con-

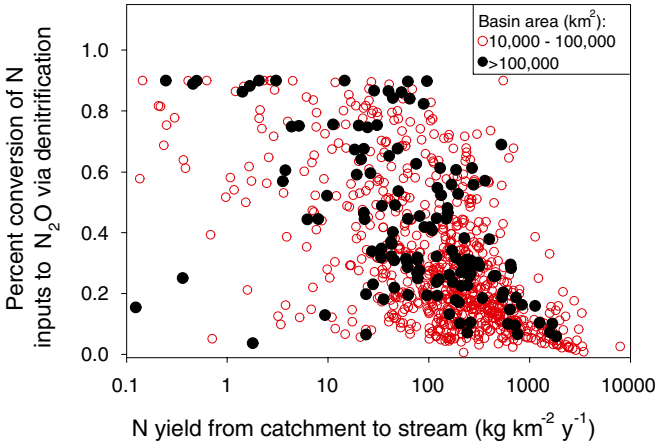

Fig. 4. Relationship between the percentage of dissolved inorganic nitrogen (DIN) inputs to river networks that are converted to N_2O via denitrification and the amount of DIN delivered to the river network from the catchment. Data are from 866 river networks included in a global river network model. Data points are split into rivers draining basins >100,000 km^2 (solid black circles) and those draining between 10,000 and 100,000 km^2 (open red circles).

comitant N_2O emissions than the enhancement of a comparable amount of denitrification in soils. Unfortunately, river networks have a limited capacity to remove NO_3^- from the water column and anthropogenic N inputs have already overwhelmed this capacity in many river systems (33, 34). Whereas the trade-off between desirable N removal and undesirable N_2O production may be smaller in streams than in soils, the best way to reduce N export to coastal waters without enhancing N_2O emissions is to reduce N inputs to watersheds.

Materials and Methods

LINX II consisted of $^{15}NO_3^-$ additions to 72 small streams distributed across three land-use categories and eight regions to provide in situ measurements of N_2 and N_2O production via denitrification at the whole-stream scale. We used a standardized set of experimental protocols to measure biogeochemical process rates including denitrification, ecosystem respiration, and gross primary production (35). We also measured a broad suite of physicochemical characteristics including organic matter standing stocks, water column nutrient concentrations, effective stream depth, stream width, stream discharge, and water velocity. The experiments were conducted as previously described (22, 35, 36) and as reported online in the project protocols (http://www.biol.vt.edu/faculty/webster/linx/). A detailed description of the experimental protocols, study site locations, and characteristics can be found in *SI Materials and Methods*, Fig. S1, and Table S1.

Site Selection. Study sites were selected to encompass a broad range of conditions across three land-use categories and eight regions. Within each region headwater streams (discharge ranged from 0.2–268 $L \cdot s^{-1}$) were selected draining basins dominated by native vegetation (reference), urban land use, or agricultural land use, with three sites in each land use for a total of nine sites per region (Table S1, Fig. S1). We selected stream reaches that had minimal groundwater or surface water inputs and were long enough to allow for a measureable amount of in-stream N processing (105–1,830 m).

Isotope Addition and Sampling. The production of N_2 and N_2O via denitrification was measured by continuously adding a solution of sodium bromide (NaBr, conservative tracer) and ^{15}N-enriched potassium nitrate ($K^{15}NO_3^-$, 98+% ^{15}N) to each stream for 24 h beginning at ≈13:00 hours using a small pump. The pump rate and injectate concentration were chosen to increase the stream water $\delta^{15}NO_3^-$ by 20,000‰ and the Br^- concentration by 100 $\mu g \cdot L^{-1}$. The conservative tracer was used to account for ground and surface water inputs to the reach and to measure channel hydraulic properties. The $K^{15}NO_3^-$ addition resulted in a relatively small (~7.5%) increase in stream water NO_3^- concentration.

Ten sampling stations were selected along the study reach and water samples were taken for NO_3^- (concentration and $\delta^{15}N$), N_2 ($\delta^{15}N$), and N_2O (concentration and $\delta^{15}N$) several hours before, 12 h after, and 23 h after the $K^{15}NO_3^-$ addition began. Nitrate samples were filtered (GFF; 0.7-μm pore size; Whatman) in the field and stored on ice or frozen before analysis. Our protocol for dissolved gas sampling is described in detail elsewhere (37) and is briefly outlined here. Gas samples were taken by slowly withdrawing 40 or 120 mL of stream water into a 60- or 140-mL polypropylene syringe (BD Falcon and Harvard Apparatus) equipped with a polycarbonate stopcock. Twenty mL of ultrahigh purity helium was then added to the syringes, which were gently shaken for 5 min to equilibrate the dissolved N_2 and N_2O between the aqueous and gas phases. The headspace gas was then transferred to a preevacuated 12-mL Exetainer (Labco) and stored underwater before analysis. All gas transfers were done underwater to minimize contamination from atmospheric N_2 and N_2O.

Air–Water Gas Exchange Rate Measurement. Within 1 d of the $^{15}NO_3^-$ addition the air–water gas exchange rate (k_2, units of $time^{-1}$) was measured using the steady-state tracer gas injection method with either propane or SF_6 as tracers (Table S1) (38, 39). The tracer gas and a conservative tracer [e.g., sodium chloride (NaCl) or rhodamine] were added to the stream at a constant rate. Water samples were collected from each of the 10 downstream sampling stations after the tracer concentrations reached a plateau throughout the reach as indicated by conductivity or fluorescence at the most downstream sampling station. Gas tracer samples were collected in 5-mL polypropylene syringes and injected into preevacuated glass storage vials. The air–water gas exchange rate was calculated from the dilution-corrected decline in tracer gas concentration across the experimental reach (Table S1).

Analytical Methods. $^{15}N\text{-}NO_3$ was determined on filtered water samples following Sigman et al. (40). Samples were analyzed for ^{15}N on a Finnigan Delta-S or a Europa 20/20 mass spectrometer in the Mass Spectrometer Laboratory of the Marine Biological Laboratory in Woods Hole, MA (http://ecosystems.mbl.edu/SILAB/about.html); a Europa Integra mass spectrometer in the Stable Isotope Laboratory of the University of California, Davis, CA (http://stableisotopefacility.ucdavis.edu/); or a ThermoFinnigan DeltaPlus mass spectrometer in the Stable Isotope Laboratory at Kansas State University, Manhattan, KS (http://www.k-state.edu/simsl).

Gas samples were analyzed for $\delta^{15}N_2$, $\delta^{15}N_2O$, and N_2O concentration by mass spectrometry using either a Europa Hydra Model 20/20 mass spectrometer at the Stable Isotope Laboratory of the University of California, Davis, CA, or a GV Instruments Prism Series II mass spectrometer in the Biogeochemistry Laboratory, Department of Zoology, Michigan State University, East Lansing, MI. The original stream water N_2O concentration was calculated using temperature-corrected Bunsen solubility coefficients, a mass balance of the gas–liquid system in the sample syringe, and an atmospheric N_2O partial pressure of 315 ppbv (41). Propane and SF_6 concentrations were measured using gas chromatography with flame ionization and electron capture detectors, respectively.

^{15}N content of all samples was reported in $\delta^{15}N$ notation, where $\delta^{15}N = [(R_{SA}/R_{ST}) - 1] \times 1{,}000$, $R = {}^{15}N/{}^{14}N$, and the results are expressed as per mil deviation of the sample (SA) from the standard (ST), N_2 in air ($\delta^{15}N$ = 0‰). All $\delta^{15}N$ values were converted to ^{15}N mole fractions (MF = $^{15}N/^{14}N + {}^{15}N$), and tracer ^{15}N fluxes were calculated for each sample by multiplying the ^{15}N MF, corrected for natural abundances of ^{15}N by subtracting the average ^{15}N MF for samples collected before the ^{15}N addition, by the concentrations of NO_3^-, N_2, or N_2O in stream water (concentrations of NO_3^- and N_2O were measured, whereas N_2 was taken as the concentration in equilibrium with air at the ambient stream temperature) and stream discharge derived from the measured conservative solute tracer concentrations.

Gas Production and Emission Calculations. Rates of N_2 and N_2O production were calculated as best-fit model parameters from a two-compartment model of denitrification linking $^{15}N_2$, $^{15}N_2O$, and $^{15}NO_3^-$ over the study reach described in *SI Materials and Methods*. Nitrous oxide emission rates via diffusive evasion (F, $\mu g\ N_2O\text{-}N \cdot m^{-2} \cdot h^{-1}$) were calculated as

$$F = k_2 \times h \times \left(N_2O_{obs} - N_2O_{equil}\right),$$

where h is the stream depth, N_2O_{obs} is the measured concentration of dissolved N_2O in the water (average across all sampling stations), and N_2O_{equil} is the N_2O concentration expected if the stream were in equilibrium with the atmosphere.

Global River Network N_2O Emission Model. Global anthropogenic N_2O emissions from river networks were estimated using a river network model. The model was run under mean annual conditions and accounts for the spatial distribution of DIN loading, temperature, hydrology, and denitrification efficiency loss. Model details and prediction errors can be found in Fig. S2 and *SI Materials and Methods*.

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