

# Nitrous oxide emission from denitrification in stream and river networks

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Nitrous oxide (N<sub>2</sub>O) 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<sub>2</sub>O via microbial denitrification that converts N to N<sub>2</sub>O and dinitrogen (N<sub>2</sub>). The fraction of denitrified N that escapes as N<sub>2</sub>O rather than N<sub>2</sub> (i.e., the N<sub>2</sub>O yield) is an important determinant of how much N<sub>2</sub>O is produced by river networks, but little is known about the N<sub>2</sub>O yield in flowing waters. Here, we present the results of whole-stream <sup>15</sup>N-tracer additions conducted in 72 headwater streams draining multiple land-use types across the United States. We found that stream denitrification produces N<sub>2</sub>O at rates that increase with stream water nitrate (NO<sub>3</sub><sup>-</sup>) concentrations, but that <1% of denitrified N is converted to N<sub>2</sub>O. Unlike some previous studies, we found no relationship between the N<sub>2</sub>O yield and stream water NO<sub>3</sub><sup>-</sup>. We suggest that increased stream NO<sub>3</sub><sup>-</sup> loading stimulates denitrification and concomitant N<sub>2</sub>O production, but does not increase the N<sub>2</sub>O yield. In our study, most streams were sources of N<sub>2</sub>O 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·y<sup>-1</sup> of anthropogenic N inputs to N<sub>2</sub>O in river networks, equivalent to 10% of the global anthropogenic N<sub>2</sub>O emission rate. This estimate of stream and river N<sub>2</sub>O 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<sub>2</sub>O), 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<sub>2</sub>O in soils and aquatic ecosystems is the largest source of anthropogenic N<sub>2</sub>O to the atmosphere (2). The production of N<sub>2</sub>O 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<sub>2</sub>O budget (4). However, emissions of anthropogenic N<sub>2</sub>O from streams, rivers, and estuaries have received much less attention

and remain a major source of uncertainty in the global anthropogenic N<sub>2</sub>O budget.

Microbial denitrification is a large source of N<sub>2</sub>O emissions in terrestrial and aquatic ecosystems. Most microbial denitrification is a form of anaerobic respiration in which nitrate (NO<sub>3</sub><sup>-</sup>, the dominant form of inorganic N) is converted to dinitrogen (N<sub>2</sub>) and N<sub>2</sub>O gases (5). The proportion of denitrified NO<sub>3</sub><sup>-</sup> that is converted to N<sub>2</sub>O rather than N<sub>2</sub> (hereafter referred to as the N<sub>2</sub>O yield and expressed as the mole ratio) partially controls how much N<sub>2</sub>O is produced via denitrification (6), but few studies provide information on the N<sub>2</sub>O yield in streams and rivers because of the difficulty of measuring N<sub>2</sub> and N<sub>2</sub>O production in these systems. Here we report rates of N<sub>2</sub> and N<sub>2</sub>O production via denitrification measured using whole-stream <sup>15</sup>NO<sub>3</sub><sup>-</sup>-tracer experiments in 72 headwater streams draining different land-use types across the United States. This project, known as the second Lotie Intersite Nitrogen eXperiment (LINX II), provides unique whole-system measurements of the N<sub>2</sub>O yield in streams.

Although N<sub>2</sub>O emission rates have been reported for streams and rivers (7, 8), the N<sub>2</sub>O 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<sub>2</sub>O yields are low compared with observations in soils (0–100%) (10), which may be a result of the relatively lower oxygen (O<sub>2</sub>) availability in the sediments of lakes and estuaries. However, dissolved O<sub>2</sub> 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 bio-reactive benthic surface area to water volume (12).

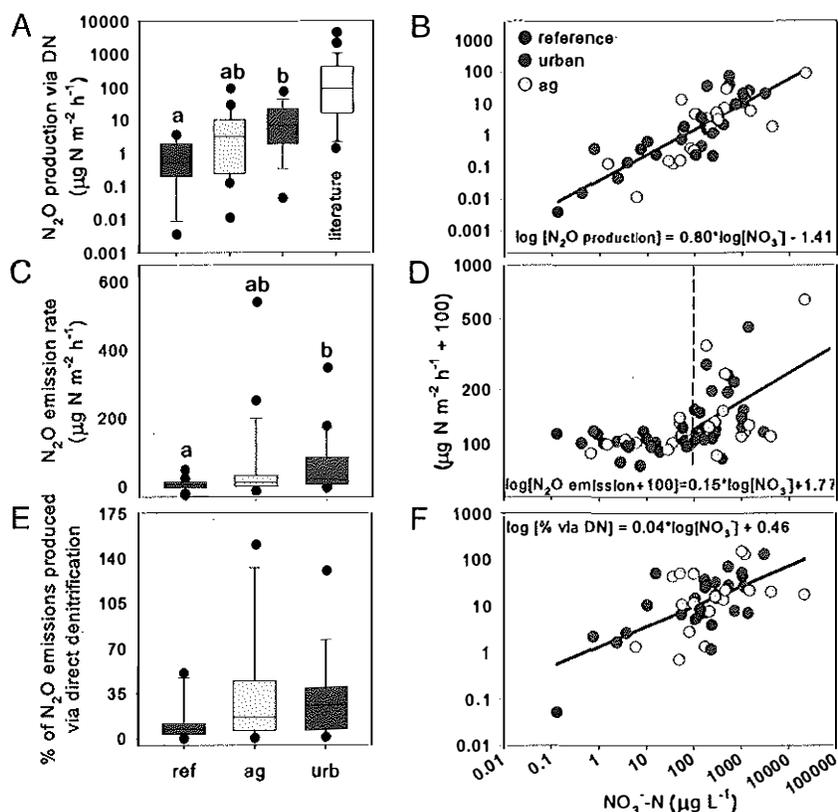
## Results and Discussion

The  $^{15}N-NO_3^-$  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  $^{15}N-N_2O$ . It is unlikely that nitrification was an important source of labeled  $^{15}N-N_2O$  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. 1A). A positive relationship between  $N_2O_{DN}$  and stream water  $NO_3^-$  concentration (Fig. 1B) 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. 1A), 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  $^{15}N-NO_3^-$  tracer was detected in both the dissolved  $N_2$  and  $N_2O$  pools in 40 of the 72 study sites and we assume all  $^{15}N-N_2$  was produced via direct denitrification. The only other potential source of  $^{15}N-N_2$  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 SI). 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 ( $95 \mu g N L^{-1}$ ) 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 ( $r^2 = 0.32$ ,  $P < 0.001$ ).

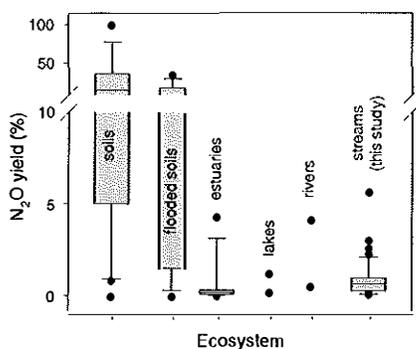


Fig. 2. Denitrification  $N_2O$  yields (percentage of denitrified N released as  $N_2O$ ) measured in this study in comparison with other ecosystems. Data are displayed in box plots unless there were fewer than nine observations. In which case each observation is represented by a solid circle. See *SI 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. 1C). Stream  $NO_3^-$  concentrations predicted  $N_2O$  emission rates when  $NO_3^-$ -N exceeded  $95 \mu\text{g}\cdot\text{L}^{-1}$  ( $P = 0.01$ ,  $r^2 = 0.16$ ), but below this concentration  $N_2O$  emission rates were uniformly low and unrelated to  $NO_3^-$  concentration (Fig. 1D). 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. 1A) to  $N_2O$  emission rates (Fig. 1C) and found that the direct denitrification of stream water  $NO_3^-$  accounted for an average of 26% of  $N_2O$  emissions (Fig. 1E). 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 ( $<140 \mu\text{g}\cdot\text{N}\cdot\text{L}^{-1}$ ) (25). The relative importance of  $N_2O_{DN}$  as a source of  $N_2O$  was positively related to stream water  $NO_3^-$  concentrations (Fig. 1F), 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. 1E), 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

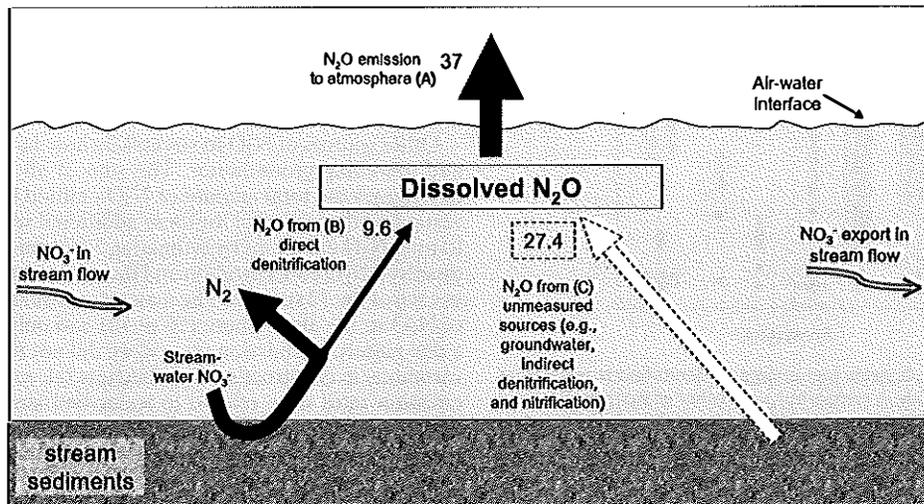


Fig. 3. Average  $\text{N}_2\text{O}$  fluxes estimated in this study (all units are  $\mu\text{g N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ). Black arrows represent fluxes that were directly measured and the white arrow with dashed boundaries represents fluxes that were estimated by mass balance. (A)  $\text{N}_2\text{O}$  produced in the stream, or imported to the stream via groundwater, temporarily resides in a pool of dissolved  $\text{N}_2\text{O}$  before being emitted to the atmosphere. (B) Direct denitrification is the conversion of stream water nitrate to  $\text{N}_2$  and  $\text{N}_2\text{O}$ . Less than 1% of stream water nitrate subject to direct denitrification is converted to  $\text{N}_2\text{O}$ , but this is the source of 26% of the  $\text{N}_2\text{O}$  emitted by the stream. (C) The balance of  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$ -supersaturated groundwater.

of 15% of global anthropogenic emissions, but this estimate is poorly constrained due to uncertainty in the  $\text{N}_2\text{O}$  yield and proportion of anthropogenic N inputs denitrified in river networks. We improved the estimate of global anthropogenic  $\text{N}_2\text{O}$  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  $\text{NO}_3^-$  concentrations based on the LINX II  $^{15}\text{N}$  tracer studies (22), and the mean  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  emissions (Fig. 3).

The percentage of DIN inputs converted to  $\text{N}_2\text{O}$  via direct denitrification of water column  $\text{NO}_3^-$  in river networks across the globe ranges from 0% to 0.9% (Fig. 4). The percentage of N inputs converted to  $\text{N}_2\text{O}$  decreases with increasing N inputs because denitrification becomes less efficient as a  $\text{NO}_3^-$  sink at higher  $\text{NO}_3^-$  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  $\text{N}_2\text{O}$  via direct denitrification and nitrification, threefold greater than the IPCC's estimate. This  $\text{N}_2\text{O}$  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  $0.68 \text{ Tg}\cdot\text{y}^{-1}$  of anthropogenic DIN inputs to  $\text{N}_2\text{O}$  globally, equivalent to 10% of the global anthropogenic  $\text{N}_2\text{O}$  emissions of

$6.7 \text{ Tg N}\cdot\text{y}^{-1}$  (2) (for calculation details see *Global  $\text{N}_2\text{O}$  Budget in SI Materials and Methods*). This estimate of anthropogenic  $\text{N}_2\text{O}$  emissions from river networks is conservative because our model does not include several potentially large sources of  $\text{N}_2\text{O}$  (e.g., indirect denitrification and groundwater inputs). We also caution that our estimate of  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  emissions from river networks, even though <1% of denitrified  $\text{NO}_3^-$  was converted to  $\text{N}_2\text{O}$ , 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-

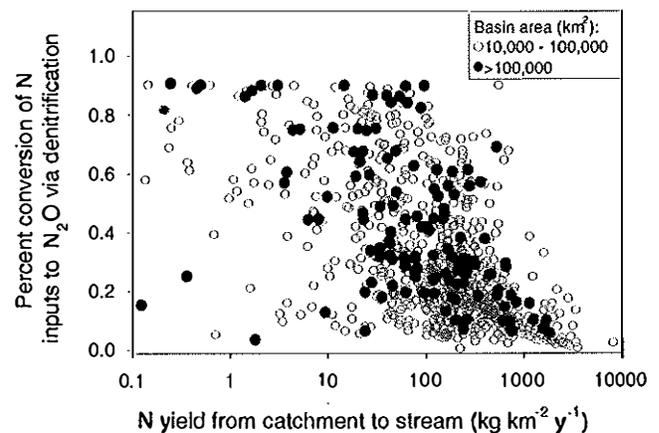


Fig. 4. Relationship between the percentage of dissolved inorganic nitrogen (DIN) inputs to river networks that are converted to  $\text{N}_2\text{O}$  via denitrification and the amount of DIN delivered to the river network from the catchment. Data are from 866 river networks included in a global river network model. Data points are split into rivers draining basins  $> 100,000 \text{ km}^2$  (solid black circles) and those draining between  $10,000$  and  $100,000 \text{ km}^2$  (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  $L s^{-1}$ ) 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  $\approx 13:00$  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  $\mu g L^{-1}$ . 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 ( $\sim 7.5\%$ ) 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- $\mu m$  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.**  $^{15}N-NO_3^-$  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/sims/>).

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 ( $F$ ,  $\mu g N_2O-N m^{-2} h^{-1}$ ) were calculated as

$$F = k_2 \times h \times (N_2O_{obs} - N_2O_{equl}),$$

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  $N_2O_{equl}$  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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