

Nitrous oxide emission from denitrification in stream and river networks

Jake J. Beaulieu^{a,1,2}, Jennifer L. Tank^a, Stephen K. Hamilton^b, Wilfred M. Wollheim^c, Robert O. Hall, Jr.^d, Patrick J. Mulholland^{e,f}, Bruce J. Peterson^g, Linda R. Ashkenas^h, Lee W. Cooperⁱ, Clifford N. Dahm^j, Walter K. Dodds^k, Nancy B. Grimm^l, Sherri L. Johnson^m, William H. McDowellⁿ, Geoffrey C. Poole^o, H. Maurice Valett^p, Clay P. Arango^q, Melody J. Bernot^r, Amy J. Burgin^s, Chelsea L. Crenshaw^j, Ashley M. Helton^t, Laura T. Johnson^u, Jonathan M. O'Brien^v, Jody D. Potterⁿ, Richard W. Sheibley^{1,3}, Daniel J. Sobota^w, and Suzanne M. Thomas⁹

^aDepartment of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556; ^bKellogg Biological Station, Michigan State University, Hickory Corners, MI 49060; ^cDepartment of Natural Resources and Environment and Complex Systems Research Center, Institute for the Study of Earth, Oceans, and Space, University of New Hampshire, Durham, NH 03824; ^dDepartment of Zoology and Physiology, University of Wyoming, Laramie, WY 82071; ^eEnvironmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831; ^fDepartment of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996; ^gEcosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543; ^hDepartment of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331; ⁱChesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Solomons, MD, 20688; ^jDepartment of Biology, University of New Mexico, Albuquerque, NM 87131; ^kDivision of Biology, Kansas State University, Manhattan, KS 66506; ^lSchool of Life Sciences, Arizona State University, Tempe, AZ 85287; ^mPacific Northwest Research Station, US Forest Service, Corvallis, OR 97331; ⁿDepartment of Natural Resources and the Environment, University of New Hampshire, Durham, NH 03824; ^oDepartment of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT 59717; ^pDepartment of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061; ^qDepartment of Biological Sciences, Central Washington University, Ellensburg, WA 98926; ^rDepartment of Biology, Ball State University, Muncie, IN 47306; ^sDepartment of Earth and Environmental Sciences, Wright State University, Dayton, OH 45435; ^tOdum School of Ecology, University of Georgia, Athens, GA 30602; ^uSchool of Public and Environmental Affairs, Indiana University, Bloomington, IN 47405; ^vSchool of Biological Sciences, University of Canterbury, Christchurch 8014, New Zealand; and ^wSchool of Earth and Environmental Sciences, Washington State University, Vancouver, WA 98686

Edited* by William H. Schlesinger, Cary Institute of Ecosystem Studies, Millbrook, NY, and approved November 11, 2010 (received for review August 4, 2010)

Nitrous oxide (N₂O) 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 N₂O via microbial denitrification that converts N to N₂O and dinitrogen (N₂). The fraction of denitrified N that escapes as N₂O rather than N₂ (i.e., the N₂O yield) is an important determinant of how much N₂O is produced by river networks, but little is known about the N₂O yield in flowing waters. Here, we present the results of whole-stream ¹⁵N-tracer additions conducted in 72 headwater streams draining multiple land-use types across the United States. We found that stream denitrification produces N₂O at rates that increase with stream water nitrate (NO₃⁻) concentrations, but that <1% of denitrified N is converted to N₂O. Unlike some previous studies, we found no relationship between the N₂O yield and stream water NO₃⁻. We suggest that increased stream NO₃⁻ loading stimulates denitrification and concomitant N₂O production, but does not increase the N₂O yield. In our study, most streams were sources of N₂O 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⁻¹ of anthropogenic N inputs to N₂O in river networks, equivalent to 10% of the global anthropogenic N₂O emission rate. This estimate of stream and river N₂O 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 (N₂O), 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 N₂O in soils and aquatic ecosystems is the largest source of anthropogenic N₂O to the atmosphere (2). The production of N₂O 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 N₂O budget (4). However, emissions of anthropogenic N₂O from streams, rivers, and estuaries have received much less attention

and remain a major source of uncertainty in the global anthropogenic N₂O budget.

Microbial denitrification is a large source of N₂O emissions in terrestrial and aquatic ecosystems. Most microbial denitrification is a form of anaerobic respiration in which nitrate (NO₃⁻, the dominant form of inorganic N) is converted to dinitrogen (N₂) and N₂O gases (5). The proportion of denitrified NO₃⁻ that is converted to N₂O rather than N₂ (hereafter referred to as the N₂O yield and expressed as the mole ratio) partially controls how much N₂O is produced via denitrification (6), but few studies provide information on the N₂O yield in streams and rivers because of the difficulty of measuring N₂ and N₂O production in these systems. Here we report rates of N₂ and N₂O production via denitrification measured using whole-stream ¹⁵NO₃⁻-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 N₂O yield in streams.

Although N₂O emission rates have been reported for streams and rivers (7, 8), the N₂O 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 N₂O yields are low compared with observations in soils (0–100%) (10), which may be a result of the relatively lower oxygen (O₂) availability in the sediments of lakes and estuaries. However, dissolved O₂ in headwater streams is commonly near atmospheric equilibrium and benthic algal bio-

Author contributions: J.L.T., S.K.H., R.O.H., P.J.M., B.J.P., L.R.A., L.W.C., C.N.D., W.K.D., N.B.G., S.L.J., W.H.M., G.C.P., and H.M.V. designed research; J.J.B., J.L.T., S.K.H., W.M.W., R.O.H., P.J.M., B.J.P., L.R.A., W.K.D., N.B.G., S.L.J., W.H.M., H.M.V., C.P.A., M.J.B., A.B., C.C., A.M.H., L.T.J., J.M.O., J.D.P., R.W.S., D.J.S., and S.M.T. performed research; J.J.B. analyzed data; J.J.B., J.L.T., and S.K.H. wrote the paper; and W.M.W. performed the global river N₂O production modeling.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Freely available online through the PNAS open access option.

¹Present address: US Environmental Protection Agency, Cincinnati, OH 45268.

²To whom correspondence should be addressed. E-mail: beaulieu.jake@epa.gov.

³Present address: US Geological Survey Water Science Center, Tacoma, WA 98402.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1011464108/-DCSupplemental.

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 S1). Denitrification proceeds by sequentially reducing NO_3^- to NO_2^- , nitric oxide (NO), N_2O , and finally N_2 . Each reduction is per-

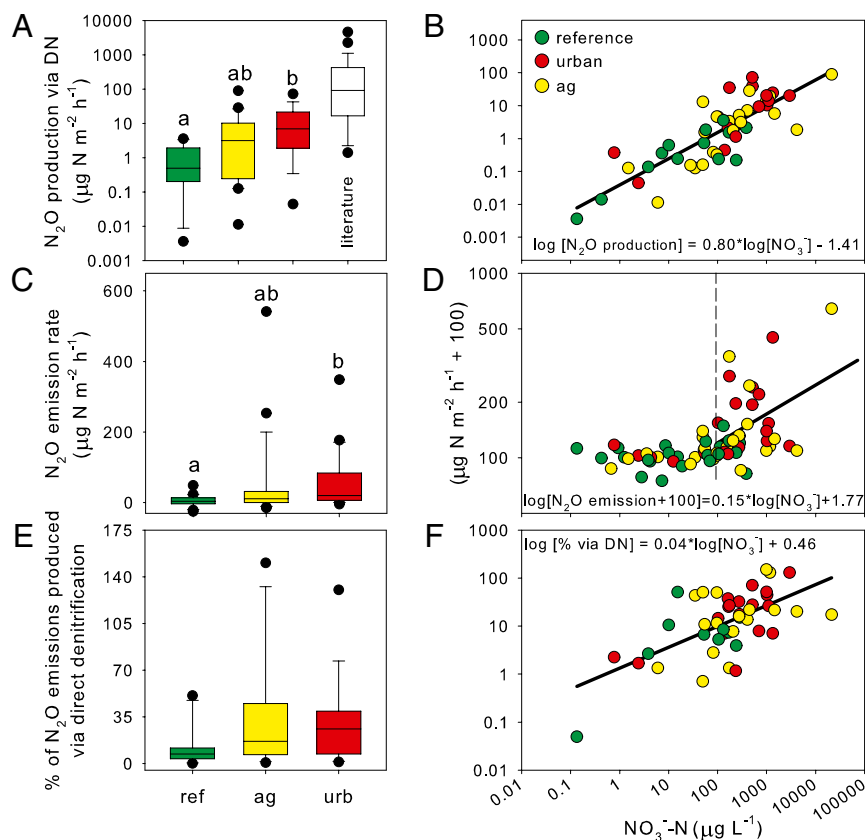


Fig. 1. (A) Box plots of stream N_2O production rates via denitrification of water column NO_3^- by catchment land use (reference, agricultural and urban). Benthic N_2O production rates reported in other studies are also shown. Significant differences between land-use types were determined with a one-way 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 ($95 \mu g N \cdot L^{-1}$) 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 ($r^2 = 0.32$, $P < 0.001$).

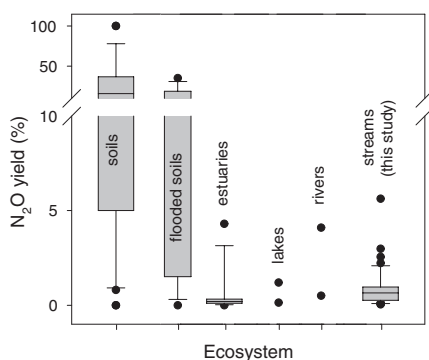


Fig. 2. Denitrification N₂O yields (percentage of denitrified N released as N₂O) measured in this study in comparison with other ecosystems. Data are displayed in box plots unless there were fewer than nine observations, in which case each observation is represented by a solid circle. See *SI Materials and Methods* for references.

formed by a different enzyme and the N₂O yield is determined by the relative activities of the N₂O-producing and reducing enzymes. There is a positive relationship between the N₂O yield and NO₃⁻ concentration in soils (15, 16) and estuarine sediments (9), possibly because higher NO₃⁻ availability suppresses nitrous oxide reductase (*nos*), the enzyme that reduces N₂O to N₂ (16). However, we did not find a significant relationship between stream water NO₃⁻ concentration and the N₂O yield ($P = 0.09$), despite NO₃⁻ concentrations spanning five orders of magnitude. Our findings suggest increased NO₃⁻ loading to streams stimulates overall denitrification rates and concomitant N₂O production, but does not increase the N₂O yield.

The N₂O yield in soils is related to the relative availability of oxidants (NO₃⁻) and reductants (organic carbon). When the availability of NO₃⁻ greatly exceeds that of organic carbon, NO₃⁻ is preferred over N₂O as a terminal electron acceptor and N₂O accumulates (5, 17–19). The N₂O yield was not related to the ratio of stream water NO₃⁻ 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 N₂O yield.

Our data suggest that denitrification in aquatic ecosystems produces lower and less variable N₂O yields than in terrestrial ecosystems (Fig. 2). This finding may be explained by differences in oxygen (O₂) availability and molecular diffusion rates between aquatic sediments and the partially water-filled pore spaces of soils. Because *nos* is the most O₂ sensitive denitrifying enzyme (20), minor amounts of O₂ can suppress the reduction of N₂O without inhibiting its production, resulting in an elevated N₂O yield. Nitrous oxide is produced as a free intermediate that can escape reduction to N₂ by diffusing away from the denitrification zone (16). Partially wet soils may present air-filled routes through which the N₂O 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 N₂O by *nos* before it can diffuse to the overlying water column. Overall, lower O₂ availability and gas diffusion rates in aquatic sediments compared with soils may account for the low aquatic N₂O 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 N₂O emission (23). Our data demonstrate that the N₂O 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 N₂O to streams include in-stream nitrification and upwelling groundwater. The sum of all these N₂O sources determines the total amount of N₂O emitted by a stream. We investigated the potential for these additional sources to contribute to total N₂O emissions by estimating N₂O emission rates from the dissolved N₂O concentration and the air–water gas exchange rates in each of the 72 streams. The majority of streams were net sources of N₂O 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 N₂O budgets (cf. ref. 24). Stream N₂O 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 NO₃⁻ concentrations predicted N₂O emission rates when NO₃⁻-N exceeded 95 μg·L⁻¹ ($P = 0.01$, $r^2 = 0.16$), but below this concentration N₂O emission rates were uniformly low and unrelated to NO₃⁻ concentration (Fig. 1D). This finding suggests that stream N₂O emission rates are not solely controlled by direct denitrification within the stream, but are likely enhanced by other sources including inputs of dissolved N₂O from groundwater.

We compared N₂O_{DN} (Fig. 1A) to N₂O emission rates (Fig. 1C) and found that the direct denitrification of stream water NO₃⁻ accounted for an average of 26% of N₂O emissions (Fig. 1E). This is a conservative estimate of in-stream N₂O production via denitrification because our method does not detect N₂O produced from the denitrification of NO₃⁻ regenerated within sediments and biofilms (e.g., indirect denitrification following organic N mineralization and nitrification), which can be the dominant source of NO₃⁻ supporting denitrification when stream water NO₃⁻ concentration is low (<140 μg N·L⁻¹) (25). The relative importance of N₂O_{DN} as a source of N₂O was positively related to stream water NO₃⁻ concentrations (Fig. 1F), reflecting the greater importance of direct denitrification with increasing stream water NO₃⁻ concentrations.

Nitrification is a potentially large source of N₂O emissions, but we know of no published measurements of N₂O 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 N₂O yield (i.e., the fraction of nitrified N escaping as N₂O) are sparse, but it appears to be within the same range as the denitrification N₂O yield (9). Therefore, the IPCC assumes that nitrification produces twice as much N₂O emission as denitrification in streams and rivers. Given that N₂O_{DN} produced within the stream contributes an average of 26% of the N₂O 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 N₂O 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 N₂O to the atmosphere, but also active sites of in situ N₂O production, particularly where NO₃⁻ concentrations are elevated by anthropogenic N loading.

The IPCC and others have estimated global anthropogenic N₂O emissions from streams and rivers by assuming all anthropogenic N that enters a river network is nitrified to NO₃⁻ and half of this NO₃⁻ is then denitrified; the N₂O 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

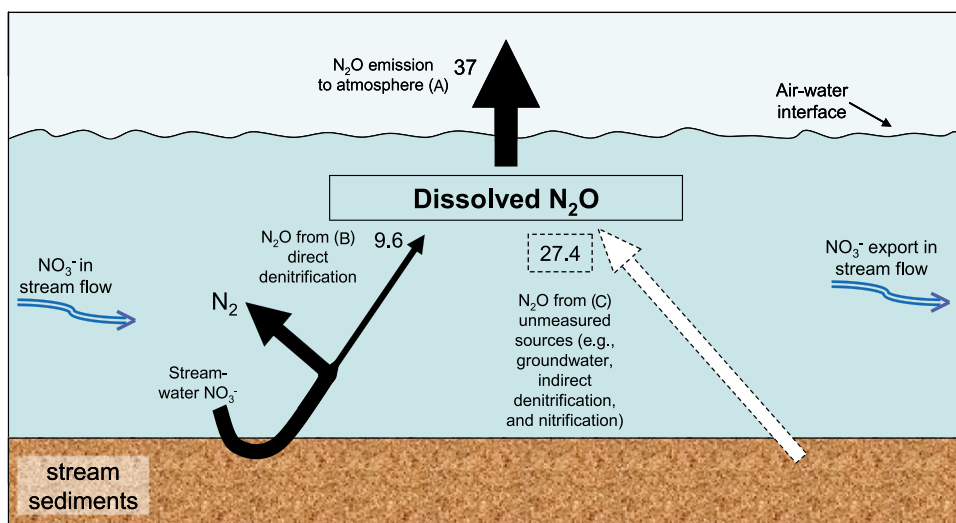


Fig. 3. Average N_2O fluxes estimated in this study (all units are $\mu\text{g N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$). Black arrows represent fluxes that were directly measured and the white arrow with dashed boundaries represents fluxes that were estimated by mass balance. (A) N_2O produced in the 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 $0.68 \text{ Tg}\cdot\text{y}^{-1}$ of anthropogenic DIN inputs to N_2O globally, equivalent to 10% of the global anthropogenic N_2O emissions of

$6.7 \text{ Tg N}\cdot\text{y}^{-1}$ (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-

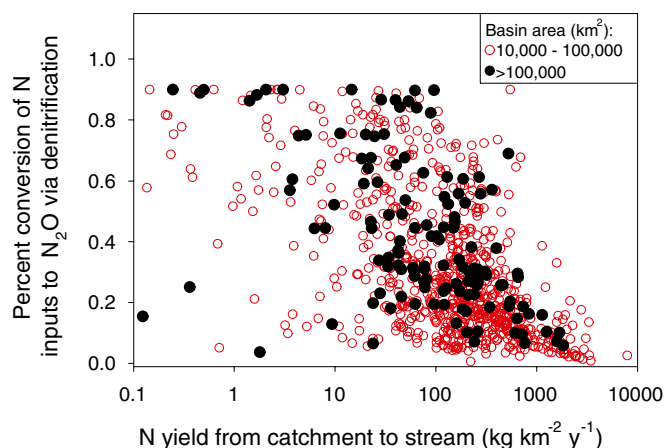


Fig. 4. Relationship between the percentage of dissolved inorganic nitrogen (DIN) inputs to river networks that are converted to N_2O via denitrification and the amount of DIN delivered to the river network from the catchment. Data are from 866 river networks included in a global river network model.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

comitant N₂O emissions than the enhancement of a comparable amount of denitrification in soils. Unfortunately, river networks have a limited capacity to remove NO₃⁻ 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 N₂O production may be smaller in streams than in soils, the best way to reduce N export to coastal waters without enhancing N₂O emissions is to reduce N inputs to watersheds.

Materials and Methods

LINX II consisted of ¹⁵NO₃⁻ additions to 72 small streams distributed across three land-use categories and eight regions to provide in situ measurements of N₂ and N₂O 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 measurable amount of in-stream N processing (105–1,830 m).

Isotope Addition and Sampling. The production of N₂ and N₂O via denitrification was measured by continuously adding a solution of sodium bromide (NaBr, conservative tracer) and ¹⁵N-enriched potassium nitrate (K¹⁵NO₃⁻, 98+% ¹⁵N) 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 δ¹⁵NO₃⁻ by 20,000‰ and the Br⁻ concentration by 100 μg 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 K¹⁵NO₃⁻ addition resulted in a relatively small (~7.5%) increase in stream water NO₃⁻ concentration.

Ten sampling stations were selected along the study reach and water samples were taken for NO₃⁻ (concentration and δ¹⁵N), N₂ (δ¹⁵N), and N₂O (concentration and δ¹⁵N) several hours before, 12 h after, and 23 h after the K¹⁵NO₃⁻ 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 N₂ and N₂O 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 N₂ and N₂O.

Air–Water Gas Exchange Rate Measurement. Within 1 d of the ¹⁵NO₃⁻ 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 SF₆ 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. ¹⁵N-NO₃⁻ was determined on filtered water samples following Sigman et al. (40). Samples were analyzed for ¹⁵N 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 δ¹⁵N₂, δ¹⁵N₂O, and N₂O 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 N₂O concentration was calculated using temperature-corrected Bunsen solubility coefficients, a mass balance of the gas–liquid system in the sample syringe, and an atmospheric N₂O partial pressure of 315 ppbv (41). Propane and SF₆ concentrations were measured using gas chromatography with flame ionization and electron capture detectors, respectively.

¹⁵N content of all samples was reported in δ¹⁵N notation, where δ¹⁵N = [(R_{SA}/R_{ST}) - 1] × 1,000, R = ¹⁵N/¹⁴N, and the results are expressed as per mil deviation of the sample (SA) from the standard (ST), N₂ in air (δ¹⁵N = 0‰). All δ¹⁵N values were converted to ¹⁵N mole fractions (MF = ¹⁵N/¹⁴N + ¹⁵N), and tracer ¹⁵N fluxes were calculated for each sample by multiplying the ¹⁵N MF, corrected for natural abundances of ¹⁵N by subtracting the average ¹⁵N MF for samples collected before the ¹⁵N addition, by the concentrations of NO₃⁻, N₂, or N₂O in stream water (concentrations of NO₃⁻ and N₂O were measured, whereas N₂ 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 N₂ and N₂O production were calculated as best-fit model parameters from a two-compartment model of denitrification linking ¹⁵N₂, ¹⁵N₂O, and ¹⁵NO₃⁻ over the study reach described in *SI Materials and Methods*. Nitrous oxide emission rates via diffusive evasion (*F*, μg N₂O-N-m⁻²-h⁻¹) were calculated as

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

where *h* is the stream depth, N₂O_{obs} is the measured concentration of dissolved N₂O in the water (average across all sampling stations), and N₂O_{equil} is the N₂O concentration expected if the stream were in equilibrium with the atmosphere.

Global River Network N₂O Emission Model. Global anthropogenic N₂O 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*.

ACKNOWLEDGMENTS. We are grateful to N. E. Ostrom for assistance with stable isotope measurements of N₂ and N₂O and G. P. Robertson for comments on the manuscript. We thank the US Forest Service, National Park Service, and many private landowners for permission to conduct experiments on their lands. We also acknowledge the many workers who helped with the Lotic Intersite Nitrogen experiments. Funding for this research was provided by the National Science Foundation (DEB-0111410). The National Science Foundation's Long Term Ecological Research (NSF-LTER) network hosted many of the study sites included in this research and partially supported several of the authors during the project. We specifically acknowledge Andrews, Central Arizona-Phoenix, Coweeta, Kellogg Biological Station, Konza, Luquillo, Plum Island, and Sevilleta NSF-LTERs for support.

1. Robertson GP, Vitousek PM (2009) Nitrogen in Agriculture: Balancing the cost of an essential resource. *Annu Rev Environ Resour* 34:97–125.
2. Forster P, et al. (2007) Changes in atmospheric constituents and in radiative forcing. *Climate Change 2007: The Physical Science Basis. Contributions of Working Group I to*

- the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, eds Solomon S, et al. (Cambridge Univ Press, New York).
3. Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous oxide (N₂O): The dominant ozone-depleting substance emitted in the 21st century. *Science* 326:123–125.

4. Stehfest E, Bouwman L (2006) N₂O and NO emission from agricultural fields and soils under natural vegetation: Summarizing available measurement data and modeling of global annual emissions. *Nutr Cycl Agroecosyst* 74:207–228.
5. Firestone MK, Davidson EA (1989) Microbiological basis of NO and N₂O production and consumption in soil. *Exchange of Trace Gases Between Terrestrial Ecosystems and the Atmosphere*, eds Andreae MO, Schimel DS (Wiley, Chichester, UK), pp 7–21.
6. Codispoti LA (2010) Oceans. Interesting times for marine N₂O. *Science* 327:1339–1340.
7. Cole JJ, Caraco NF (2001) Emissions of nitrous oxide (N₂O) from a tidal, freshwater river, the Hudson River, New York. *Environ Sci Technol* 35:991–996.
8. Beaulieu JJ, Arango CP, Hamilton SK, Tank JL (2008) The production and emission of nitrous oxide from headwater streams in the midwestern USA. *Glob Chang Biol* 14: 878–894.
9. Seitzinger SP, Kroeze C (1998) Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. *Global Biogeochem Cycles* 12: 93–113.
10. Stevens RJ, Laughlin RJ, Malone JP (1998) Measuring the mole fraction and source of nitrous oxide in the field. *Soil Biol Biochem* 30:541–543.
11. Stevens RJ, Laughlin RJ (1998) Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils. *Nutr Cycl Agroecosyst* 52:131–139.
12. Alexander RB, Boyer EW, Smith RA, Schwarz GE, Moore RB (2007) The role of headwater streams in downstream water quality. *J Am Water Resour Assoc* 43:41–59.
13. Groffman PM, et al. (2006) Methods for measuring denitrification: Diverse approaches to a difficult problem. *Ecol Appl* 16:2091–2122.
14. Burgin A, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Front Ecol Environ* 5: 89–96.
15. Firestone MK, Firestone RB, Tiedje JM (1980) Nitrous oxide from soil denitrification: Factors controlling its biological production. *Science* 208:749–751.
16. Firestone MK, Smith MS, Firestone RB, Tiedje JM (1979) The influence of nitrate, nitrite, and oxygen on the composition of the gaseous products of denitrification in soil. *Soil Sci Soc Am J* 43:1140–1144.
17. Hutchinson GL, Davidson EA (1993) Processes for production and consumption of gaseous nitrous oxide in soil. *Agricultural Ecosystem Effects on Trace Gases and Global Climate Change*, eds Harper LA, Mosier AR, Duxbury JM, Rolston DE (Am Soc Agron, Madison, WI), pp 79–94.
18. Swerts M, Merckx R, Vlassak K (1996) Denitrification, N₂-fixation and fermentation during anaerobic incubation of soils amended with glucose and nitrate. *Biol Fertil Soils* 23:229–235.
19. Miller MN, et al. (2008) Crop residue influence on denitrification, N₂O emissions and denitrifier community abundance in soil. *Soil Biol Biochem* 40:2553–2562.
20. Tiedje JM (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. *Biology of Anaerobic Microorganisms*, ed Zehnder AJB (Wiley, New York), pp 179–244.
21. Craig LS, et al. (2008) Stream restoration strategies for reducing river nitrogen loads. *Front Ecol Environ* 6:529–538.
22. Mulholland PJ, et al. (2008) Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature* 452:202–205.
23. Schlesinger WH, Reckhow KH, Bernhardt ES (2006) Global change: The nitrogen cycle and rivers. *Water Resour Res* 42:W03506.
24. Laursen AE, Seitzinger SP (2004) Diurnal patterns of denitrification, oxygen consumption and nitrous oxide production in rivers measured at the whole-reach scale. *Freshw Biol* 49:1448–1458.
25. Seitzinger S, et al. (2006) Denitrification across landscapes and waterscapes: A synthesis. *Ecol Appl* 16:2064–2090.
26. Holmes RM, Jones JB, Fisher SG, Grimm NB (1996) Denitrification in a nitrogen-limited stream ecosystem. *Biogeochemistry* 33:125–146.
27. Webster JR, et al. (2003) Factors affecting ammonium uptake in streams—an inter-biome perspective. *Freshw Biol* 48:1329–1352.
28. Arango CP, Tank JL (2008) Land use influences the spatiotemporal controls of nitrification and denitrification in headwater streams. *J North Am Benthol Soc* 27: 90–107.
29. Richardson WB, et al. (2004) Denitrification in the Upper Mississippi River: Rates, controls, and contribution to nitrate flux. *Can J Fish Aquat Sci* 61:1102–1112.
30. Mosier A, et al. (1998) Closing the global N₂O budget: Nitrous oxide emissions through the agricultural nitrogen cycle—OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. *Nutr Cycl Agroecosyst* 52:225–248.
31. Seitzinger SP, Kroeze C, Styles RV (2000) Global distribution and N₂O emissions from aquatic systems: Natural emissions and anthropogenic effects. *Chemosphere Glob Chang Sci* 2:267–279.
32. Wollheim WM, et al. (2008) Global N removal by freshwater aquatic systems using a spatially distributed, within-basin approach. *Global Biogeochem Cycles* 22:GB2026.
33. Caraco NF, Cole JJ (1999) Human impact on nitrate export: An analysis using major world rivers. *Ambio* 28:167–170.
34. Bernot MJ, Dodds WK (2005) Nitrogen retention, removal, and saturation in lotic ecosystems. *Ecosystems (N Y)* 8:442–453.
35. Bernot MJ, et al. (2010) Inter-regional comparison of land-use effects on stream metabolism. *Freshw Biol* 55:1874–1890.
36. Mulholland PJ, et al. (2009) Nitrate removal in stream ecosystems measured by ¹⁵N addition experiments: Denitrification. *Limnol Oceanogr* 54:666–680.
37. Hamilton SK, Ostrom NE (2007) Measurement of the stable isotope ratio of dissolved N₂ in ¹⁵N tracer experiments. *Limnol Oceanogr Methods* 5:233–240.
38. Genereux DP, Hemond HF (1992) Determination of gas-exchange rate constants for a small stream on Walker Branch Watershed, Tennessee. *Water Resour Res* 28: 2365–2374.
39. Wanninkhof R, Mulholland PJ, Elwood JW (1990) Gas-exchange rates for a 1st-order stream determined with deliberate and natural tracers. *Water Resour Res* 26:1621–1630.
40. Sigman DM, et al. (1997) Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: An adaptation of the ammonia diffusion method. *Mar Chem* 57:227–242.
41. Weiss RF, Price BA (1980) Nitrous oxide solubility in water and seawater. *Mar Chem* 8: 347–359.