# The production and emission of nitrous oxide from headwater streams in the Midwestern United States

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## Abstract

The emission of nitrous oxide ($N_2O$) from streams draining agricultural landscapes is estimated by the Intergovernmental Panel on Climate Change (IPCC) to constitute a globally significant source of this gas to the atmosphere, although there is considerable uncertainty in the magnitude of this source. We measured $N_2O$ emission rates and potential controlling variables in 12 headwater streams draining a predominantly agricultural basin on glacial terrain in southwestern Michigan. The study sites were nearly always supersaturated with $N_2O$ and emission rates ranged from $-8.9$ to $266.8\,\mu g\,N_2O\text{-}N\,m^{-2}\,h^{-1}$ with an overall mean of $35.2\,\mu g\,N_2O\text{-}N\,m^{-2}\,h^{-1}$. Stream water $NO_3^-$ concentrations best-predicted $N_2O$ emission rates. Although streams and agricultural soils in the basin had similar areal emission rates, emissions from streams were equivalent to 6% of the anthropogenic emissions from soils because of the vastly greater surface area of soils. We found that the default value of the $N_2O$ emission factor for streams and groundwater as defined by the IPCC (EF5-g) was similar to the value observed in this study lending support to the recent downward revision to EF5-g. However, the EF5-g spanned four orders of magnitude across our study sites suggesting that the IPCC's methodology of applying one emission factor to all streams may be inappropriate.

## Introduction

Nitrous oxide ($N_2O$) is a potent greenhouse gas that also contributes to the destruction of stratospheric ozone (Hahn & Crutzen, 1982; Khalil & Rasmussen, 1983). The concentration of $N_2O$ in the atmosphere is increasing at a rate of $0.2$–$0.3\%\,yr^{-1}$ and the biological conversion of agricultural fertilizer nitrogen (N) to $N_2O$ is the largest anthropogenic source (Mosier *et al.*, 1998). Direct emissions of $N_2O$ from agricultural soils and animal feedlots are fairly well studied (Bouwman, 1996; Beauchamp, 1997; Kasimir-Klemedtsson *et al.*, 1997), but less is known about the indirect emissions of $N_2O$ from groundwater, streams, and rivers receiving N-rich drai-

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nage water from agricultural soils. Seitzinger & Kroeze (1998) modeled $N_2O$ emissions from rivers and concluded that these systems are responsible for 20% of global anthropogenic $N_2O$ emissions, but they did not explicitly include headwater streams in the analysis. The Intergovernmental Panel on Climate Change (IPCC) estimated that $N_2O$ emissions from rivers, streams, and groundwater total $1.6\,Tg\,N\,yr^{-1}$ or 35% of global anthropogenic $N_2O$ emissions (Mosier *et al.*, 1998). However, there is considerable uncertainty in the magnitude of indirect emissions with the IPCC estimates ranging from $0.13$ to $7.7\,Tg\,N\,yr^{-1}$. The broad range of estimates results partly from uncertainties in the controls on the conversion of N to $N_2O$ via several microbially mediated N transformations.

$N_2O$ is produced as a byproduct of microbially mediated denitrification and nitrification. Nitrification is an aerobic, chemolithoautotrophic process in which bacteria oxidize ammonium ($NH_4^+$) to nitrate ($NO_3^-$) and $N_2O$, and nitrification rates are controlled by $NH_4^+$, organic carbon (C) concentrations and quality, pH, and temperature (Bianchi *et al.*, 1999; Strauss *et al.*, 2002,

2004). Denitrification is an anaerobic process in which bacteria sequentially reduce $NO_3^-$ to $N_2O$ and dinitrogen ($N_2$) with small amounts of $N_2O$ 'leaking' out in the process. Most attention has been paid to respiratory denitrification although other microbial processes such as sulfur oxidation can also entail denitrification (Burgin & Hamilton, 2007). Respiratory denitrification rates are primarily controlled by the availability of $NO_3^-$, organic C, and oxygen (Knowles, 1982; Baker & Vervier, 2004; Inwood *et al.*, 2005). While the controls on denitrification and nitrification rates have been well studied, the relative importance of these two processes as sources of $N_2O$ in streams and rivers is not well known.

The IPCC methodology for estimating aquatic $N_2O$ emissions is based on the idea that as N moves through the landscape from groundwater to streams, rivers, and then oceans, a fraction of that N is converted to $N_2O$. This fraction is estimated as an emission factor, which is derived separately for streams and groundwater (EF5-g), rivers (EF5-r), and estuaries (EF5-e). The rationale for treating streams and rivers differently is based on the assumption that groundwater derived $N_2O$ is the dominant source of $N_2O$ in streams while *in situ* production makes up the dominant $N_2O$ source in rivers. The value of EF5-g is based on observed ratios of $N_2O$ to $NO_3^-$ concentrations in streams and groundwater and was originally designated as 1.5% based on six studies published before 1998 (Mosier *et al.*, 1998). Since 1998, new studies have become available from Japan and the United Kingdom indicating that 1.5% is too large (Nevison, 2000; Hack & Kaupenjohann, 2002; Harrison & Matson, 2003; Reay *et al.*, 2005; Sawamoto *et al.*, 2005) and in 2006 the IPCC revised EF5-g to 0.25% (IPCC,

2006). Our study evaluates this recent revision to the IPCC methodology by monitoring EF5-g across a broader spatial and temporal scale than encompassed by most previous studies.

In this study, we used a combination of laboratory and field methods to examine the controls on $N_2O$ emission rates from 12 streams in the agricultural Midwest USA. We measured denitrification, nitrification, and $N_2O$ production rates to examine the relationship between N-processing rates and whole-stream $N_2O$ emission rates. We also measured stream water and sediment chemical characteristics to further elucidate controls on $N_2O$ emission rates. From these data, we calculated the EF5-g for each stream, scaled up our results to determine total $N_2O$ emissions from the stream network, and compared total emissions from streams to estimated emissions from terrestrial soils in their drainage basins.

## Methods

### Site description and sampling schedule

This research was conducted in the Kalamazoo River basin in southwestern Michigan, where the main stem of the Kalamazoo River flows largely from east to west for 280 km before discharging to Lake Michigan (Fig. 1). Overall, the dominant land use within the 523 177 ha basin is agriculture (58%, mainly corn and soybeans), but there are sub-basins dominated by urban development and native vegetation (mainly deciduous forest). Mean annual air temperature in the basin is 9 °C and average precipitation is 89 cm (Wesley, 2005).
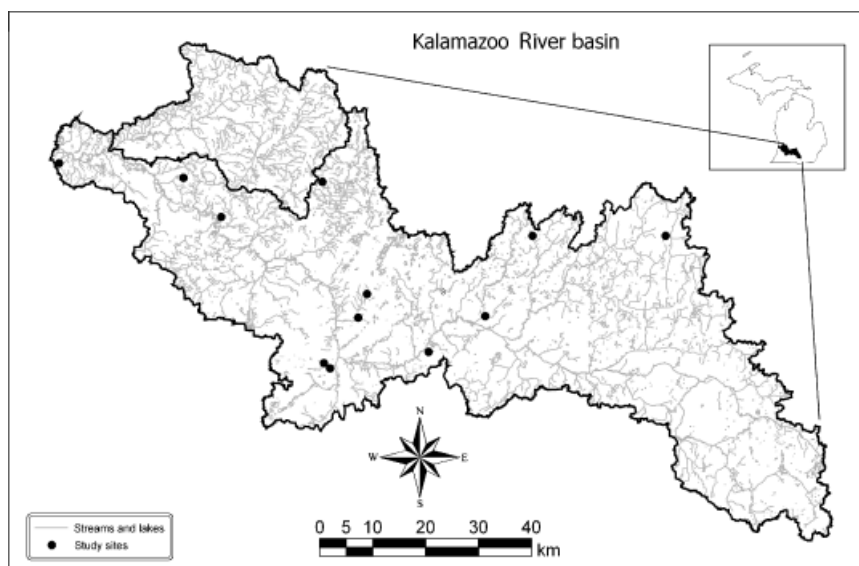


**Fig. 1** Study sites were located in the Kalamazoo River basin in southwest Michigan, USA. Individual study sites are indicated with black dots and the Rabbit River sub-basin is outlined in black in the northwestern Kalamazoo River basin.

The Kalamazoo River system is an ideal place to conduct this research because stream water $NO_3^-$ and $NH_4^+$ concentrations span a broad range depending largely on land use (Inwood *et al.*, 2005; Moerke & Lamberti, 2006). We defined six land-use categories representing various degrees of urban, agricultural, and forested land use, and selected two study sites within each land-use category ($n = 12$ streams). We ranked the study sites by the percentage of agriculture within the basin and will, hereafter, refer to the sites as A1–A12 (Table 1).

Land use within each sub-basin was determined with a geographical information system (ARCGIS version 8.0, Environmental Systems Research Institute, Redland, CA, USA) using data derived from the USGS 1992 Land Cover Dataset. Hydrography and digital elevation layers were obtained from the Michigan Department of Natural Resources Geographic Data Library (http://www.mcgi.state.mi.us/mgdl/).

*Sample collection*

A summary of the analyses and methods can found in Table 2. The study was conducted for 18 months; half of the streams were sampled for 6 consecutive months while the remaining six streams were sampled for 12 consecutive months. During each sampling visit (one per month), 25 sediment cores (6 cm diameter) were collected to a depth of 1.5 cm along a 100 m reach to be used for denitrification, nitrification, and $N_2O$ production assays. Sediment cores were taken from the dominant substrates in the reach [fine benthic organic matter (FBOM) and sand] in proportion to their relative abundance based on a visual assessment. The cores were equally distributed into five 500 mL HDPE bottles, providing five replicate sediment samples from each stream. Sediment samples were stored on ice during transport and laboratory assays were conducted within 24 h of sample collection.

Six water samples for dissolved $N_2O$ analysis were collected from the thalweg of the stream at evenly spaced intervals along the 100 m reach. A 140 mL syringe (Becton-Dickson, Franklin Lakes, NJ, USA) was used to collect 120 mL of stream water from approximately 3 cm below the water surface, and 20 mL of Ultra High Purity helium (He) was immediately added to the syringe. The syringe was shaken for 5 min and the headspace gas was transferred to a 12 mL Exetainer vial (Labco Ltd, High Wycombe, UK) and stored underwater until analysis. All gas transfers were done underwater to minimize the potential for contamination from atmospheric $N_2O$. The Exetainers were pre-evacuated to <10 mTorr before use and tests showed that $N_2O$ partial pressures in the vials did not change with up to 6 months of storage.

During each sampling visit discharge ($Q$) was estimated from channel profiles of width ($w$), water velocity ($\upsilon$), and depth ($h$). Water temperature, specific conductivity, pH, and dissolved oxygen were measured with a calibrated Hydrolab Quanta sonde (Loveland, CO, USA). Water samples for nutrient chemistry were filtered through glass fiber filters (Pall A/E, 1 μm nominal pore size) into acid washed and rinsed HDPE bottles, stored on ice during transport to the lab, and frozen until analysis.

*Air–water gas exchange measurement*

The $N_2O$ emission rate ($F$) is a function of both the degree of stream water $N_2O$ supersaturation relative to the atmosphere and the $N_2O$ air–water gas exchange rate ($K_{N_2O}$):

$$F = K_{N_2O} \times h \times (N_2O_{obs} - N_2O_{exp}), \qquad (1)$$

where $h$ is the stream depth, $N_2O_{obs}$ is the measured concentration of dissolved $N_2O$ in the water and $N_2O_{exp}$ is the $N_2O$ concentration expected if the stream were in equilibrium with the atmosphere (assuming an atmospheric $N_2O$ partial pressure of 315 ppbv). The air–water gas exchange rate $K$ (units of time$^{-1}$) for each study site was estimated using the energy dissipation model (Tsivoglou & Neal, 1976), which conceptualizes gas-exchange as a function of stream slope, $\upsilon$, and physical properties of the channel such as bed roughness and channel shape

$$K_{N_2O\text{-pred}} = C_{esc}\left(\frac{\Delta h \upsilon}{l}\right), \qquad (2)$$

where $K_{N_2O\text{-pred}}$ is the air–water gas exchange rate for $N_2O$ predicted from the model, $\Delta h$ is the change in elevation across the reach (m), $l$ is the reach length (m), and $C_{esc}$ is the 'escape coefficient' (m$^{-1}$), which reflects the effect of the physical properties of the channel on the gas exchange rate. The escape coefficient was empirically determined by measuring $K$ six times in one stream (site A8) across a range of hydrologic conditions ($Q$ ranged from 15 to 86 L s$^{-1}$). The coefficient, $C_{esc}$, estimated by regression of $K$ on $(\Delta h \upsilon)/l$, was used to calculate $K_{N_2O\text{-pred}}$ for all study sites and sampling periods based on measured $\Delta h$ and estimates of $\upsilon$ and $h$ (Ortiz-Zayas *et al.*, 2005). The 12 streams in this study had similar physical characteristics of low slope (<1.3%) and low bed roughness with substrata dominated by sand and FBOM, hence we assumed that $C_{esc}$ was similar among these study streams. The change in elevation across each stream reach was measured with a self-leveling rotary laser (Lasermark LMH, Watseka, IL, USA), and $\upsilon$ and $h$ at the time of sampling were determined from empirical relationships among $\upsilon$, $h$,

**Table 1** Mean and (SE) of physical and chemical characteristics of study sites

| | % land use | | | $K_{N_2O\text{-pred}}$ (day$^{-1}$)* | Q (L s$^{-1}$)† | Depth (cm) | $\upsilon$ (m min$^{-1}$)‡ | Groundwater discharge (% Q increase 100 m$^{-1}$)§ | NO$_3^-$-N (mg L$^{-1}$) | NH$_4^+$-N (µg L$^{-1}$) | DOC (mg L$^{-1}$) | N$_2$O-N (µg L$^{-1}$) | Sediment (%C) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Agricultural | Forested | Urban | | | | | | | | | | |
| A1 | 10 | 48 | 36 | 19.3 (0.1) | 32.6 (2.3) | 7.3 (0.2) | 6.8 (0.2) | 0.80 (0.45) | 0.18 (0.02) | 83 (8) | 0.9 (0.1) | 0.42 (0.03) | 1.12 (0.11) |
| A2 | 17 | 2 | 69 | 18.4 (0.6) | 8.7 (3.5) | 12.6 (2.4) | 1.6 (0.4) | nd | 0.13 (0.03) | 72 (19) | 5.1 (0.46) | 0.52 (0.04) | 0.30 (0.04) |
| A3 | 28 | 5 | 64 | 34.0 (5.0) | 4.0 (1.7) | 6.0 (0.5) | 4.0 (1.14) | 0 | 0.38 (0.05) | 5 (1) | 0.8 (0.2) | 0.73 (0.12) | 1.62 (0.33) |
| A4 | 40 | 15 | 42 | 31.1 (0.4) | 62.7 (6.5) | 10.6 (0.8) | 9.7 (0.2) | 0.10 (0.03) | 1.08 (0.12) | 46 (9) | 0.9 (0.1) | 0.91 (0.12) | 1.21 (0.04) |
| A5 | 45 | 4 | 42 | 22.4 (0.7) | 37.9 (5.3) | 14.2 (0.2) | 8.6 (1.0) | 0.15 (0.03) | 0.33 (0.05) | 47 (8) | 3.3 (0.3) | 0.45 (0.02) | 0.82 (0.13) |
| A6 | 49 | 0.4 | 48 | 33.6 (0.1) | 16.9 (0.7) | 9.1 (0.7) | 7.8 (0.02) | 0.13 (0.07) | 0.69 (0.01) | 7 (2) | 0.8 (0.1) | 0.67 (0.10) | 0.49 (0.20) |
| A7 | 52 | 0.0 | 45 | 28.1 (2.5) | 6.8 (2.2) | 5.5 (0.7) | 6.0 (1.2) | 0.35 (0.11) | 0.03 (0.01) | 95 (33) | 5.0 (0.5) | 1.22 (0.14) | 0.32 (0.08) |
| A8 | 72 | 7 | 20 | 25.5 (0.7) | 21.9 (5.5) | 9.1 (0.6) | 6.1 (0.5) | 0.27 (0.27) | 1.65 (0.39) | 19 (5) | 2.5 (0.3) | 0.75 (0.11) | 0.21 (0.04) |
| A9 | 73 | 0.3 | 24 | 19.4 (0.09) | 48.7 (3.7) | 15.9 (1.3) | 6.8 (0.1) | 0.05 (0.03) | 1.78 (0.12) | 11 (4) | 1.1 (0.3) | 0.82 (0.09) | 1.33 (0.26) |
| A10 | 78 | 0.0 | 20 | 19.7 (0.3) | 20.1 (2.1) | 14.4 (0.7) | 5.4 (0.4) | 0.05 (0.03) | 0.51 (0.13) | 29 (4) | 2.1 (0.2) | 0.56 (0.13) | 5.28 (1.27) |
| A11 | 95 | 0.0 | 5 | 16.8 (0.2) | 3.0 (1.0) | 11.3 (0.5) | 1.2 (0.2) | 1.12 (0.15) | 2.46 (1.06) | 39 (9) | 2.2 (0.6) | 0.89 (0.17) | 4.82 (0.31) |
| A12 | 97 | 0.2 | 3 | 28.0 (0.6) | 7.8 (1.7) | 8.9 (1.1) | 5.1 (0.3) | 0.20 (0.02) | 21.76 (1.44) | 9 (2) | 2.3 (0.2) | 2.17 (0.34) | 2.08 (0.34) |

*$K_{N_2O\text{-pred}}$ is the air–water gas exchange rate derived from the energy dissipation model and is reported in terms of N$_2$O at 20 °C. To facilitate comparison with the literature $K_{N_2O\text{-pred}}$ can be expressed in terms of O$_2$ at 20 °C by multiplying by 0.935.
†Q, stream discharge.
‡$\upsilon$, water velocity.
§Groundwater discharge rates (L s$^{-1}$ 100 m$^{-1}$) were divided by surface water discharge (L s$^{-1}$) to provide an index of the relative importance of groundwater inputs (% Q increase 100 m$^{-1}$).

**Table 2** Methods employed in this study

| Analyses | Method | Reference |
|---|---|---|
| Denitrification rates | Chloramphenicol-amended acetylene-inhibition | Smith & Tjiedje (1979) as modified by Royer *et al*. (2004) |
| $N_2O$ production rates | Chloramphenicol-amended direct flux | As above without acetylene |
| Nitrification rates | Nitrapyrin inhibition | Hall (1984) |
| $K_{SF_6}$ | Steady state gas tracer injection | Modified from Genereux & Hemond (1992) |
| $K_{N_2O\text{-pred}}$ | Energy dissipation model | Tsivoglou & Neal (1976) |
| $NO_3^-$ | Ion chromatography | Dionex application note 133 |
| $NH_4^+$ | Phenol-hypochlorite | Solorzano (1969) |
| SRP | Ascorbic acid | Eaton *et al*. (1995) |
| DOC | 680 °C combustion with FDIR detection | – |
| $N_2O$ and $SF_6$ | Gas chromatography, ECD detector | Crill *et al*. (1995) |
| Sediment organic matter | Mass loss upon ignition | Benfield (2006) |
| Sediment organic C and N | Acid fuming pretreatment followed by analysis on an elemental analyzer | Hedges & Stern (1984) |

$K_{SF_6}$, air–water gas exchange rate measured using $SF_6$ tracer; $K_{N_2O\text{-pred}}$, air–water gas exchange rate for $N_2O$ predicted from energy dissipation model; SRP, soluble reactive phosphorus; DOC, dissolved organic carbon.

and $Q$ that were derived from three to six conservative solute tracer releases per stream (described below).

To calibrate the energy dissipation model, the air–water gas exchange rate $K$ was measured six times in stream A8 across a range of discharge conditions using the steady state tracer gas method (Wanninkhof *et al*., 1990; Genereux & Hemond, 1992). Sulfur hexafluoride ($SF_6$, Mitler gas) and sodium chloride (NaCl) were added to the stream at a constant rate. Specific conductivity was recorded with a Hydrolab sonde at five stations ranging from 110 to 535 m downstream from the addition point to quantify transport and dilution of the added salt. Once the tracer concentrations reached steady state throughout the reach as indicated by conductivity, 10 replicate $SF_6$ samples were taken at each sampling station by collecting 2.5 mL of stream water in a 5 mL syringe and injecting the water into a pre-evacuated (<10 mTorr) 5 mL Vacutainer. $SF_6$ was analyzed by injecting 10 μL of the Vacutainer headspace into the Varian Star 3600 gas chromatograph (GC) (Walnut Creek, CA, USA) equipped as described below and relative area counts were used to calculate $K$ (Marzolf *et al*., 1994).

The air–water gas exchange rate for $SF_6$ ($K_{SF_6}$) was calculated using the model

$$\ln[SF_6]_x = \ln[SF_6]_0 - (a * x), \qquad (3)$$

where $[SF_6]_x$ is the $SF_6$ concentration $x$ m downstream from the addition site and at the most upstream station ($[SF_6]_0$), and $a$ is the rate constant for $SF_6$ loss per meter of stream channel, which was converted to $K_{SF_6}$ by multiplying by $v$ (m day$^{-1}$) (Hall & Tank, 2003). Tracer gas concentrations were corrected for dilution by lateral

inflows based on the conservative tracer data collected at each station. All $K_{SF_6}$ measurements were converted to $K_{N_2O}$ using the ratio of their Schmidt numbers (1.258) (Wanninkhof *et al*., 1993) and corrected to 20 °C for use in the energy dissipation model with

$$K_{20} = K_T/(1.0241)^{T-20}, \qquad (4)$$

where $K_{20}$ is $K$ at 20 °C and $K_T$ is $K$ measured at temperature $T$ (Elmore & West, 1961).

In a companion study (Arango *et al*., in review) at least three conservative tracer additions were performed as described above at all of the study sites except A2. From these data, we estimated discharge from a mass balance of the added tracer, $v$ from the time required for the tracer to travel the length of the study reach, and $h$ was calculated as

$$h = \frac{Q}{wv}, \qquad (5)$$

where $w$ was measured at 10 locations across the reach. We developed an empirical relationship between $Q$, $v$, and $h$ for each stream to estimate $v$ and $h$ from $Q$ measured at the time of dissolved $N_2O$ sampling, except in stream A2 where $v$ was measured in at least five locations at one cross-section with an electromagnetic flow sensor (Marsh McBirney, Flo Mate, Frederick, MD, USA) and $h$ was estimated using Eqn (5). Velocity was used in Eqn (2) to estimate reaeration rates and $h$ was used in Eqn (1) to estimate $N_2O$ emission rates. Groundwater inflow rates (L s$^{-1}$ 100 m$^{-1}$) were estimated from dilution of the conservative tracer across the reach and were normalized to surface water dis-

charge to provide an index of the relative importance of groundwater inputs in each site (% $Q$ increase 100 m$^{-1}$).

### Microbial N transformation rate measurements

Denitrification rates were measured using the chloramphenicol-amended acetylene inhibition technique (Brock, 1961; Smith & Tiedje, 1979) as modified by Royer *et al.* (2004). Chloramphenicol inhibits the *de novo* synthesis of denitrifying enzymes during the assay, which reduces bottle effects and gives better estimates of *in situ* rates (Royer *et al.*, 2004; Inwood *et al.*, 2005; Wall *et al.*, 2005). The assay was conducted by adding 25 mL of sediment and 50 mL of unfiltered stream water amended with 0.3 mM chloramphenicol to a 175 mL media bottle, sealing the bottle with a gray butyl septum, and purging the bottle headspace with He to create anoxic conditions. The bottle headspace was amended with acetylene to a final concentration of 0.1 atm to inhibit the reduction of $N_2O$ to $N_2$. Gas samples (5 mL) were collected from the headspace every hour for 4 h and stored in 3.5 mL Vacutainers until analysis. Denitrification rates were calculated from a linear regression fit to the $N_2O$ accumulation rate in the bottle headspace, and the rates represent the total amount of $N_2$ and $N_2O$ produced via denitrification. To measure the direct production of $N_2O$ via denitrification we conducted assays as above, with the exception that no acetylene was added and thus $N_2O$ production in the bottles is an estimate of ambient rates of $N_2O$ production via denitrification (Weier *et al.*, 1993; García-Ruiz *et al.*, 1998; Rudaz *et al.*, 1999). Hereafter, these assays will be referred to as '$N_2O$ production' assays. On one sample date, we conducted nutrient amendment experiments to determine if $NO_3^-$ or dissolved organic C (DOC) limited $N_2O$ production. The assays were conducted as described above, except that the stream water was amended with $NO_3^-$ (as $KNO_3^-$) to 6 mg N L$^{-1}$ above ambient concentrations, with DOC as glucose to 30 mg C L$^{-1}$ above ambient concentrations, or with both DOC and $NO_3^-$.

Nitrification was measured using the nitrapyrin-inhibition method (Hall, 1984). The assay was conducted by placing 25 mL of sediment and 50 mL of unfiltered water in paired Erlenmeyer flasks and adding nitrapyrin to a final concentration of 10 mg L$^{-1}$ to one flask and dimethyl sulfoxide (DMSO) to both flasks. Nitrapyrin inhibits nitrification allowing $NH_4^+$ to accumulate in the sediment slurry while DMSO is a solvent that allows nitrapyrin to cross the cellular membranes of the nitrifiers. After a 48 h incubation the slurry was collected from the flasks, 2 M potassium chloride (KCl) was added to extract $NH_4^+$ from cation-binding sites, and each bottle was shaken for 10 min, centrifuged, and the supernatant filtered and frozen for later analysis of $NH_4^+$. Nitrification rates were calculated as the difference in exchangeable $NH_4^+$ between the nitrapyrin amended and control flasks.

### Analytical methods

Filtered stream water samples were analyzed for $NH_4^+$ with the phenol-hypochlorite method (Solorzano, 1969), and soluble reactive phosphorus (SRP) was determined with the molybdate method (Murphy & Riley, 1962; APHA, 1995); absorbances were measured on a 10 cm pathlength in a Shimadzu spectrophotometer (Columbia, MD, USA). Nitrate was determined using ion chromatography (Dionex DX640, IonPac AG14A guard column, AS14A analytical column; method derived from Dionex application note 133) and DOC was measured on a total organic C analyzer with high-temperature Pt-catalyzed combustion and NDIR detection (Shimadzu TOC-V).

Sediment samples were analyzed for %organic matter by measuring mass loss upon ignition (Benfield, 2006) and sediment organic C and N content were measured on an elemental analyzer (Costech Analytical Technologies ECS 4010, Valencia, CA, USA) after carbonates were removed with the acid-fuming method (Hedges & Stern, 1984).

$N_2O$ samples were analyzed on a Varian Star 3600 GC or a Hewlett Packard (HP) 5890 Series II GC (Santa Clara, CA, USA) for the denitrification and stream water samples, respectively. The GCs were equipped with electron capture detectors and Porapak $Q$-packed columns set at 325 and 70 °C, respectively (Crill *et al.*, 1995). Samples were introduced through a 0.5 mL sample loop and an autosampler on the HP instrument. The original stream water $N_2O$ concentration was calculated using temperature-corrected Bunsen solubility coefficients, assuming an atmospheric partial pressure of 315 ppbv (Weiss & Price, 1980).

### Statistics

All statistical analyses were performed using SAS Analyst (version 9.1, SAS Institute, Cary, NC, USA) or SYSTAT (version 11, Systat Software Inc., San Jose, CA, USA). Simple linear regression was used to detect relationships between independent variables and $N_2O$ emission rates. Correlation analysis was used to examine the relationship between the EF5-g and $NO_3^-$ concentrations. The population mean of lognormally distributed N-cycling rates were calculated as $\exp(\bar{x} + (s^2/2))$ where $\bar{x}$ and $s^2$ are the arithmetic mean and variance of $\ln(x)$ (Parkin & Robinson, 1992). Statistically significant differences for variables compared among streams

and months were determined using repeated measures ANOVA followed by the Bonferroni adjusted least-square means test for *post-hoc* multiple contrasts (Zar, 1991). To determine seasonal trends in $N_2O$ emission rates across all streams emission rates were transformed to their $Z$-score to control for differences in means and variances among streams (Gotelli & Ellison, 2004). Months were grouped into seasons based on stream water temperature. All data were tested for normality with the Kolmogorov–Smirnov goodness of fit procedure with a Lillefors distribution and transformed as necessary.

A two-way ANOVA was used to determine whether $N_2O$ production via denitrification was significantly affected by the $NO_3^-$ or C amendments (Tank & Dodds, 2003). $N_2O$ production was determined to be N or C limited if either the N or C amendment elicited a significant response. If both the N and C treatments stimulated $N_2O$ production rates, or if there was a significant interaction between the treatments, $N_2O$ production was deemed to be colimited.

## Results

### Chemical and physical characteristics

Discharge ($Q$) in the streams monitored throughout the year ranged from 0.1 to 92.8 $L s^{-1}$ and one stream (A2) became dry for part of the study. Discharge exhibited a seasonal pattern with lower $Q$ during the summer and fall and higher $Q$ during the winter and spring; however, sites with upstream springs were less variable than other sites. The streams in this basin are largely maintained by groundwater discharge, and seasonal variation in $Q$ tends to reflect fluctuations in the water table (Rheaume, 1990). All study sites except A3 were very slightly gaining reaches with surface water discharge increasing by 0.32% on average across the 100 m reach (0.05–1.12%) (Table 1).

Dissolved $NO_3^-$-N concentrations showed significant variation among streams, ranging from 0.003 to 27.4 $mg L^{-1}$, and were generally lowest in late summer and highest in winter ($P < 0.001$, Fig. 2). $NO_3^-$-N concentrations above 4 $mg L^{-1}$ were observed in streams receiving tile drainage from basins with >95% agricultural land cover (A11 and A12). Nitrate in the stream with the highest level of agricultural development (A12) never dropped below 16 $mg N L^{-1}$. Tile drainage rapidly transports water from agricultural soils directly to headwater streams leading to high in-stream $NO_3^-$ concentrations (David *et al.*, 1997).

Ammonium concentrations also spanned a broad range among streams, ranging from undetectable to 348 $\mu g N L^{-1}$ with an overall mean of 40 $\mu g N L^{-1}$ (Table 1). SRP concentrations were generally low (mean 12 $\mu g L^{-1}$) and the mean DOC concentration was 2.41 $mg C L^{-1}$.

Stream sediments were dominated by sand, FBOM, or a mixture of the two, with very little cobble or gravel. Consequently, sediment organic matter content spanned a broad range from 0.42% of dry mass in a sand dominated stream to 8.73% in a FBOM dominated stream. Sediment organic C content spanned a similarly broad range from 0.10% to 13.7% carbon (Table 1) and sediment N content ranged from 0.005% to 0.98% N (as % of dry mass).

### Air–water gas exchange rates

To calibrate the energy dissipation model, we measured $K_{SF_6}$ in stream A8 on six dates over a broad range of discharge (15–86 $L s^{-1}$). For each measurement, the natural log of the dilution-corrected $SF_6$ concentration was linearly related to distance from the injection site indicating that $K$ was relatively constant throughout the stream reach ($0.97 \leq r^2 \leq 0.99$). $K_{N_2O}$ ranged from 23.9 to 35.6 $day^{-1}$, a surprisingly small range given that discharge varied by a factor of 5.7.

The data fit the energy dissipation model well (Fig. 3, $r^2 = 0.98$) with an escape coefficient ($C_{esc}$) of 0.233 $m^{-1}$ which is lower than Tsivoglou & Neal (1976) suggested for small streams but is within the range reported by Ortiz-Zayas *et al.* (2005) (0.12–0.36 $m^{-1}$) and seems reasonable for a low-gradient sand-bottomed stream.

Our estimates of $N_2O$ emission rates from all of the study sites could be biased if $C_{esc}$ in those sites is not near the value of 0.233 $m^{-1}$ that we measured in stream A8. Without having data demonstrating how $C_{esc}$ varies among these streams it is impossible to evaluate this bias. However, the factors likely to influence $C_{esc}$ (e.g. bed roughness, discharge, and slope) were very similar among the sites and stream A8 had both an intermediate slope and discharge, so we are confident that $C_{esc}$ was not strongly biased high or low when compared to the other 11 streams. $K_{N_2O-pred}$ ranged from 16.3 to 45.5 $day^{-1}$ (Table 1) which is within the range reported for nine other headwater streams in the basin (13.8–283.6 $day^{-1}$) (J. L. Tank, unpublished data) and very similar to the range reported for four low gradient agricultural streams in northwest Indiana (12.8–34.0 $day^{-1}$) (N. Griffiths, unpublished data). Thus, the model predicts values for $K_{N_2O}$ that agree reasonably well with independent estimates in similar kinds of streams.

### $N_2O$ concentration and emission rates

Dissolved $N_2O$ was supersaturated with respect to the atmosphere in 105 of the 108 samples. Dissolved $N_2O$
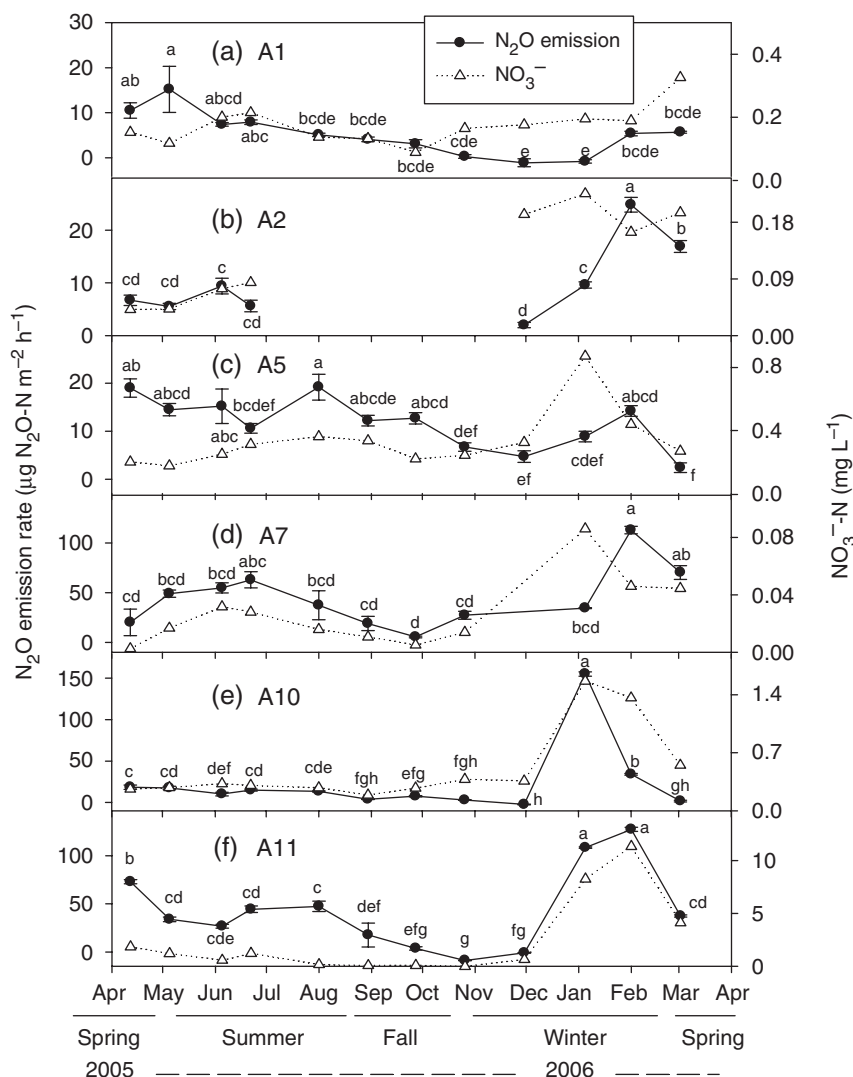
Fig. 2 (a–f) Nitrous oxide ($N_2O$) emission rates and $NO_3^-$ in streams monitored for 12 consecutive months. Different letters indicate significant differences in $N_2O$ emission rates among months. Note the different $Y$-axis scale between panels.

concentrations ranged from 0.15 to 5.13 µg $N_2O$-N $L^{-1}$ (45–1358% of atmospheric equilibrium) and the overall mean $N_2O$ concentration was 0.81 µg $N_2O$-N $L^{-1}$ or 236% saturation (Table 1, Fig. 4a). Emission rates ranged from −8.9 to 266.8 µg $N_2O$-N $m^{-2} h^{-1}$ with an overall mean of 35.2 µg $N_2O$-N $m^{-2} h^{-1}$ (Fig. 4b).

At sites where samples were collected for 12 consecutive months, $N_2O$ emission rates were variable during the spring of 2005 and showed a gradual decrease beginning in mid-summer and extending into the fall (Fig. 2). The lowest emission rates were observed during late summer or fall, and averaged across all streams, the summer season had significantly lower emission rates than the other seasons ($P < 0.001$). By the winter of 2006 $N_2O$ emission rates had increased and then began to decline by early March 2006.

$N_2O$ emission rates were significantly predicted by stream water $NO_3^-$ concentrations ($r^2 = 0.28$, $P < 0.001$), and the fit improved when stream A7 was excluded from the analysis ($r^2 = 0.59$, $P < 0.001$) (Fig. 5, Table 3). Emission rates were weakly predicted by stream water $NH_4^+$ and DOC concentrations ($r^2 = 0.04$, $P = 0.04$ and $r^2 = 0.04$, $P = 0.04$), however there was no relationship between emission rates and sediment N or C content expressed as % organic matter, % organic C, %N, or C : N ($P > 0.36$).
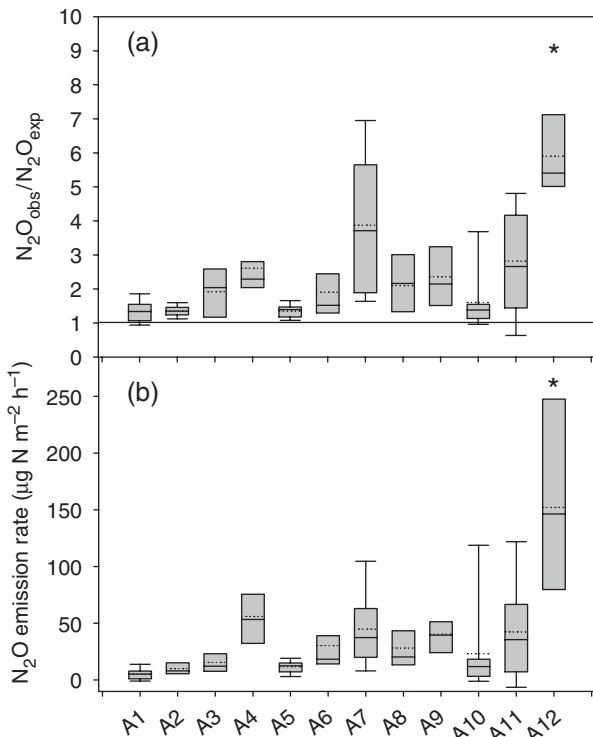
*Rates of microbial N transformations*

Denitrification rates were highly variable among streams (0.01 to 50.3 mg N $m^{-2} h^{-1}$; mean = 3.8 mg N $m^{-2} h^{-1}$) spanning the range of rates reported in
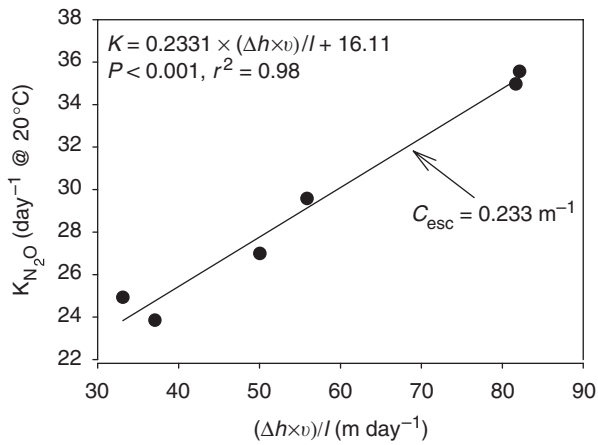
**Fig. 3** Air–water gas exchange rate vs. rate of energy dissipation. $\Delta h$ is the change in elevation between the top and bottom of the reach, $\upsilon$ is water velocity, and $l$ is the reach length.



**Fig. 4** (a) Degree of nitrous oxide ($N_2O$) saturation in each of the 12 study streams, expressed as the concentration of $N_2O$ in the stream that we measured (obs) and that which is expected (exp) if the stream were in equilibrium with the atmosphere. Values above 1 indicate supersaturation and values below 1 indicate undersaturation. (b) $N_2O$ emission rate. The dotted line, solid center line, box extent, and error bars denote the mean, median, 25th and 75th, 10th and 90th percentiles. *streams that are significantly different than all other streams.

the literature for streams and rivers (Pina-Ochoa & Alvarez-Cobelas, 2006) (Table 4). Denitrification rates were predicted by stream water $NO_3^-$ concentrations
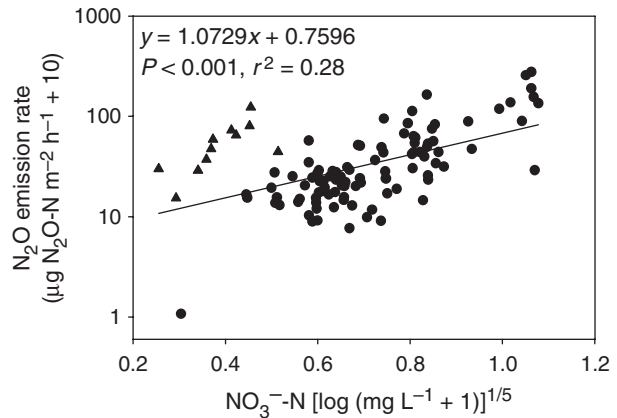


**Fig. 5** Regression analysis of normalized nitrous oxide ($N_2O$) emission rate and normalized $NO_3^-$-N concentration. When stream A7 (▲) is excluded the relationship improves ($r^2 = 0.58$).

**Table 3** Relationship between physicochemical variables and $N_2O$ emission rates*

| Independent variable† | Units | Simple linear regression | |
| --- | --- | --- | --- |
| | | $P$ | $r^2$ |
| Log DOC | $mg\,L^{-1}$ | 0.04 | 0.04 |
| Log $NH_4^+$-N | $\mu g\,L^{-1}$ | 0.04 | 0.04 |
| Log $(NO_3^-\text{-N} + 1)^{\wedge 1/5}$ | $mg\,L^{-1}$ | < 0.001 | 0.28 |
| Log denitrification | $mg\,N\,m^{-2}\,h^{-1}$ | 0.001 | 0.11 |
| Log $N_2O$ production | $\mu g\,N\,m^{-2}\,h^{-1}$ | 0.001 | 0.14 |
| Log nitrification | $mg\,N\,m^{-2}\,h^{-1}$ | 0.72 | – |

*$N_2O$ emission rate was normalized with the log $(x + 10)$ transformation.
†All variables have been transformed to meet the assumption of normality. Normality was determined with the Kolmogorov–Smirnov goodness of fit procedure with a Lillefors distribution and an $\alpha$ of 0.05.
DOC, dissolved organic carbon.

($P < 0.001$, $r^2 = 0.21$) but not by any other water or sediment chemical characteristic.

Our denitrification rate measurements reflect the sum production of both $N_2$ and $N_2O$ via denitrification. We also measured the production of $N_2O$ alone via denitrification. Rates of $N_2O$ production via denitrification ranged from 0.4 to 3236.6 $\mu g\,N\,m^{-2}\,h^{-1}$ (mean = 128.1 $\mu g\,N\,m^{-2}\,h^{-1}$) and were lower than denitrification rates in all but one sample (Table 4). This is consistent with reports that $N_2$ is the dominant denitrification end product in aquatic sediments (Seitzinger & Kroeze, 1998). $N_2O$ production rates were predicted by stream water $NO_3^-$ concentrations ($P < 0.001$, $r^2 = 0.28$) and were not related to any other water or sediment chemical characteristics. Five sites were used to examine N and C limitation of $N_2O$ production via denitrification.

**Table 4** Mean (SE) denitrification, N$_2$O production, and nitrification rates in study sites

| Stream | Population mean (SE)* | | |
|---|---|---|---|
| | Denitrification (mg N m$^{-2}$ h$^{-1}$) | N$_2$O production (µg N m$^{-2}$ h$^{-1}$) | Nitrification (mg N m$^{-2}$ h$^{-1}$) |
| A1 | 1.6 (0.3) | 7.0 (3.7) | 2.5 (0.4) |
| A2 | 0.9 (0.2) | 75.3 (134.2) | 0.8 (0.3) |
| A3 | 1.9 (0.3) | 146.1 (50.9) | 4.4 (1.0) |
| A4 | 2.3 (0.5) | 55.7 (8.1) | 1.5 (0.3) |
| A5 | 2.1 (0.6) | 71.3 (145.1) | 2.6 (0.5) |
| A6 | 0.9 (0.2) | 100.7 (31.0) | 0.9 (0.1) |
| A7 | 0.7 (0.1) | 24.0 (22.0) | 1.7 (0.5) |
| A8 | 0.6 (0.1) | 33.3 (7.1) | 0.4 (0.1) |
| A9 | 4.7 (0.4) | 975.0 (313.5) | 4.9 (1.4) |
| A10 | 2.4 (1.7) | 40.6 (41.1) | 4.9 (0.8) |
| A11 | 11.1 (5.4) | 102.2 (179.8) | 9.0 (1.5) |
| A12 | 5.2 (0.5) | 74.5 (6.0) | 5.4 (2.0) |

*If the data were normally distributed then the arithmetic mean was computed as the population mean. If the data were log normally distributed then the population mean of $\exp(\bar{x} + (s^2/2))$ was computed, where $\bar{x}$ and $s^2$ are the arithmetic mean and the variance of ln(variable). If the data had neither a normal or log normal distribution, then the arithmetic mean was computed as the population mean (Parkin & Robinson, 1992).

The NO$_3^-$ concentrations ranged from 0.01 to 0.33 mg N L$^{-1}$ on the day of the experiments and all sites responded to the NO$_3^-$ amendment with significantly higher N$_2$O production rates than in the control assays ($P < 0.01$). The glucose amendment stimulated N$_2$O production rates in two of the five sites ($P < 0.03$), indicating that these sites were colimited by N and C.

Nitrification rates were generally similar to denitrification rates and ranged from undetectable to 19.7 mg N m$^{-2}$ h$^{-1}$ (mean = 3.9 mg N m$^{-2}$ h$^{-1}$) which is comparable with literature values for streams and rivers (Strauss et al., 2004; Starry et al., 2005) (Table 4). Nitrification rates were positively related to all metrics of sediment N and C content [%C $r^2 = 0.33$, $P < 0.001$; %N $r^2 = 0.31$, $P < 0.001$; organic matter content $r^2 = 0.38$, $P < 0.001$] and varied significantly among streams ($P < 0.001$). Nitrification rates were not related to stream water NH$_4^+$ concentrations ($P = 0.98$). For a detailed discussion of the nitrification and denitrification rates see Arango & Tank (in press).

### Links between in-stream N transformations and N₂O dynamics

Both denitrification and nitrification are known to produce N$_2$O in aquatic sediments; however, we found no relationship between nitrification and N$_2$O emission rates ($P = 0.72$) (Table 3). Emission rates were predicted by both denitrification and N$_2$O production rates ($P = 0.001$, $r^2 = 0.11$ and $P < 0.001$, $r^2 = 0.14$, respectively).

### IPCC emission factor

The emission factor (ratio of N$_2$O to NO$_3^-$) ranged from 0.003 to 25.0%, but all values >3.0% came from one site (A7). The mean value was 1.01%, but when site A7 was excluded the mean value was 0.20%, very close to revised IPCC value of 0.25%.

### Scaling up

To determine if streams are a significant component of the landscape-wide anthropogenic N$_2$O flux to the atmosphere, we estimated the magnitude of terrestrial and lotic N$_2$O emissions from the Rabbit River basin, a fifth order sub-basin of the Kalamazoo River basin (Fig. 1). The total basin area is 69 830 ha and the surface area of the stream network is 361 ha. We applied the mean N$_2$O emission rate we measured (35.2 µg N$_2$O-N m$^{-2}$ h$^{-1}$) to the entire stream network and calculated that 1087 kg N$_2$O-N evade from the stream network every year. This analysis ignores potential systematic differences in emission rates between large and small streams; however, a survey of dissolved N$_2$O concentrations in 50 streams ranging from first to fifth order in this basin demonstrated no significant difference in dissolved N$_2$O concentration across this range of stream order (J. J. Beaulieu, unpublished data).

To estimate terrestrial anthropogenic N$_2$O emissions from the basin we assumed that soils in native vegetation represent a nonanthropogenic background flux and we did not include these areas in our analysis. To estimate anthropogenic emissions from agricultural

soils we used background-corrected emission rates for conventionally managed agricultural systems in the Kalamazoo River basin (8.33 µg $N_2O$-N m$^{-2}$h$^{-1}$) as reported by Robertson et al. (2000). We assumed that all 40 501 ha of agricultural soils in the basin are under conventional management (corn–wheat–soybean rotation with chemical inputs and tillage). Based on these assumptions agricultural soils emit 17 416 kg of anthropogenic $N_2O$-N each year. This is probably an underestimate because we assumed that frozen soils do not produce $N_2O$ and recent evidence suggests that this is not the case (Groffman et al., 2006b; Yashiro et al., 2006). Based on these estimates emissions from streams are equivalent to only 6% of anthropogenic emissions from agricultural soils.

## Discussion

We found that $N_2O$ emission rates showed considerable variation among streams (Fig. 4), yet were on the low end of the range that has been reported for small agricultural streams (Table 5). Reay et al. (2003) reported $N_2O$ emission rates ranging from 100 to 1000 µg $N_2O$-N m$^{-2}$h$^{-1}$ for an agricultural stream in the United Kingdom and Hasegawa et al. (2000) reported a mean $N_2O$ emission rate of 7444 µg $N_2O$-N m$^{-2}$h$^{-1}$ for an agricultural stream in Japan. It is possible that our rates appear low because we missed $N_2O$ emission 'hotspots.' Reay et al. (2003) demonstrated that $N_2O$ emissions can be high below tile drains delivering $N_2O$ supersaturated water to the stream, but decline rapidly as the drain water moves downstream and equilibrates with the atmosphere. Two of our study sites contained tile drains within the 100 m study reach, however, we did not witness elevated stream water $N_2O$ concentrations below these drains. $N_2O$ in tile drainage water is spatially variable and it is likely that a systematic sampling of stream water $N_2O$ below tiles draining into the study sites would reveal localized areas of elevated emission rates (Hack & Kaupenjohann, 2002). $N_2O$ emission rates in our study sites are comparable with larger systems including the Platte River (McMahon & Dennehy, 1999), the Lii River in New Zealand (Clough et al., 2006), and the Neuse River in North Carolina (Stow et al., 2005), among others (Table 5). Very high $N_2O$ emission rates appear to be less common in larger rivers, probably because the larger volume of surface water in the channel acts to dilute tile drainage and groundwater sources of $N_2O$ leading to less spatially variable emission rates. However, emission rates exceeding 1000 µg N m$^{-2}$h$^{-1}$ have been reported for the Platte (McMahon & Dennehy, 1999) and Assabet Rivers (Hemond & Duran, 1989), where measurements were made directly downstream of wastewater treatment plants that may have functioned as large $N_2O$ point sources to the river. Based on our data and a survey of the literature for streams and rivers it appears that $N_2O$ emission rates $>500$ µg $N_2O$-N m$^{-2}$h$^{-1}$ are uncommon and when they do occur are probably spatially limited and linked to a $N_2O$ point source.

**Table 5** Comparison of $N_2O$ emission rates from streams and rivers

| Site | Emission rate (µg $N_2O$-N m$^{-2}$h$^{-1}$) | | | Method* | Reference |
|---|---|---|---|---|---|
| | Mean | Minimum | Maximum | | |
| Agricultural drains, UK | – | 100 | 1000 | Enclosed tunnel | Reay et al. (2003) |
| Agricultural/urban canals, Mexico | 165.0 | 50.0 | 2450 | Floating dome | Harrison & Matson (2003) |
| Agricultural/urban canals, Mexico | 601.0 | – | – | ? | Harrison et al. (2005) |
| Platte River | 62.0 | 0.38 | 1360 | Fixed dome | McMahon & Dennehy (1999) |
| Changjiang River, China | – | 0.028 | 8.138 | Modeled K | Yan et al. (2004) |
| LII River, New Zealand | 171.0 | 38.00 | 501.0 | Floating dome | Clough et al. (2006) |
| Amazon River | 7.4 | – | – | Modeled K | Richey et al. (1988) |
| Amazon River backwaters 'Varzea' | −0.030 | – | – | Modeled K | Richey et al. (1988) |
| Neuse River, North Carolina | 12.9 | −9.21 | 64.9 | Floating dome | Stow et al. (2005) |
| Hudson River, New York | 6.4 | 2.33 | 22.2 | Modeled K | Cole & Caraco (2001) |
| Agricultural rivers, mid-west USA | – | 5.60 | 840 | Measured K | Laursen & Seitzinger (2004) |
| Humber Estuary, UK | 5042.4 | – | – | Modeled K | Barnes et al. (1998) |
| Agricultural drains, Japan | 7440 | 240 | 56580 | Fixed domes | Hasegawa et al. (2000) |
| Assabet River, MA | 159.1 | 54.10 | 1591 | Measured K | Hemond & Duran (1989) |
| Small streams | 35.2 | −8.9 | 267 | Measured K | This study |

*Emission rates were measured directly with a variation of the floating dome technique, were calculated based on dissolved $N_2O$ concentrations and a modeled air-water gas exchange rate ($K$), or $K$ was measured with a gas tracer technique.

*Controls on $N_2O$ emission rates*

Our results indicate that stream water $NO_3^-$ concentration is the best predictor of $N_2O$ emission rates (Table 3, Fig. 5). Nitrate concentrations are indirectly related to $N_2O$ emission rates by regulating denitrification, a process which produces $N_2O$. This notion is corroborated by the significant positive relationship between stream water $NO_3^-$ concentrations and both denitrification rates and $N_2O$ production rates. Additionally, previous work in headwater streams in this basin has shown that denitrification is $NO_3^-$ limited (Inwood et al., 2005). In fact, $N_2O$ emission rates were positively related to both denitrification and $N_2O$ production rates.

Assuming that $N_2O$ produced in the benthos dissolves in stream water and ultimately evades to the atmosphere we can calculate the importance of in-stream denitrification (as indicated by our assays) to whole stream emissions. The ratio of benthic $N_2O$ production rates to whole stream emissions was highly variable with a tendency for production to exceed emission (median 120%). It is unlikely that benthic $N_2O$ production rates actually exceeded $N_2O$ emission rates in the streams because $N_2O$ was not found to be accumulating in the water column. We suspect that the benthic $N_2O$ production assays overestimate ambient rates because the assays were carried out under anoxic conditions and $NO_3^-$ delivery to the anoxic sediments was accelerated by the use of sediment slurries. These bottle assays are useful for determining controls on $N_2O$ emission rates, but they are not the best method for estimating *in situ* N-processing rates (Groffman et al., 2006a). However, the fact that the $N_2O$ production rates were within the same order of magnitude as $N_2O$ emission rates suggests that these rates were not unreasonable.

Nitrate limitation of in-stream denitrification may be partly responsible for the strong relationship between stream water $NO_3^-$ concentrations and $N_2O$ emission rates, but it is also possible that stream water $NO_3^-$ concentrations are a proxy for denitrification rates in groundwater and riparian soil flow paths, which also contribute $N_2O$ to the stream water. Groundwater in agricultural landscapes is frequently supersaturated with $N_2O$ (Ronen et al., 1988; Muhlherr & Hiscock, 1997; McMahon et al., 2000) and riparian zones can be sources of $N_2O$ via denitrification (Weller et al., 1994). $N_2O$ produced in riparian soils could be transported to streams via subsurface flow. Trace gas emissions from small streams are often driven by metabolic activity in the soils and groundwater in the basin. For example, Jones & Mulholland (1998a) demonstrated that the overwhelming source of $CO_2$ evading from a small woodland stream in Tennessee was soil respiration. Likewise, methane emissions from another small woodland stream in Tennessee were dominated by methanogenesis in groundwater and riparian zones (Jones & Mulholland, 1998b). It is probable that some fraction of the $N_2O$ evading from our study sites originated from microbial N transformations in soils and/or groundwater. Our only indicator of groundwater-derived $N_2O$ in the study sites is the groundwater discharge rate measured by tracer dilution three times per year in each study site. However, there was no relationship between the amount of groundwater entering the stream reach and emission rates. This is probably because the analysis does not account for seasonal or intersite differences in groundwater $N_2O$ concentrations. Spatially detailed measurements of the groundwater discharge rate and $N_2O$ concentrations would be required to accurately quantify the contribution of groundwater-derived $N_2O$ to the stream.

Stream water $NO_3^-$ concentrations may indirectly affect $N_2O$ emission rates by controlling the production of $N_2O$ relative to $N_2$ via denitrification ($N_2O$ yield). Nitrate can directly inhibit the activity of $N_2O$ reductase, the enzyme which reduces $N_2O$ to $N_2$, while not decreasing the activity of the enzymes responsible for converting $NO_3^-$ to $N_2O$ (Knowles, 1982). This effect has been clearly demonstrated in soils (Blackmer & Bremner, 1978; Firestone et al., 1979; Vinther, 1984; Weier et al., 1993; Dunfield et al., 1995) and estuarine sediments (Seitzinger & Nixon, 1985), but strong evidence of this effect in streams and rivers is lacking. Mulholland et al. (2004) increased the denitrification $N_2O$ yield in a small stream from 0.15% to 3.0% by experimentally increasing the stream water $NO_3^-$ concentration from 26 to 380 $\mu g\,N\,L^{-1}$, however this was a short term experiment (hours in duration) and it is unclear if this effect would have been sustained over longer time periods. Resolving the controls on the denitrification $N_2O$ yield in streams and rivers is key to modeling $N_2O$ emissions from lotic systems (Schlesinger et al., 2006).

Nitrification has been shown to be a source of $N_2O$ in lakes (Mengis et al., 1997), estuaries (Law et al., 1992), and oceans (Cohen & Gordon, 1979); however, there are few data demonstrating that nitrification is a significant $N_2O$ source in lotic systems (Harrison & Matson, 2003). The IPCC methodology assumes that riverine nitrification and denitrification produce equal amounts of $N_2O$ per unit N processed, and that nitrification rates are twice as high as denitrification rates (Mosier et al., 1998). Thus, according to the IPCC methodology nitrification is a twofold greater source of $N_2O$ than denitrification in rivers. However, we did not find a significant relationship between nitrification and $N_2O$ emission rates in our study. This is surprising as nitrification rates

were generally similar to denitrification rates (mean = 3.88 and 3.76 mg N m$^{-2}$ h$^{-1}$, respectively) and the nitrification N$_2$O yield is thought to be similar to that of denitrification (Seitzinger & Kroeze, 1998). Our data suggest that in streams, nitrification may not be as important of a N$_2$O source as previously thought. However, if the N$_2$O yield via nitrification varied temporally or seasonally this could obscure the relationship between nitrification and N$_2$O emission rates. Little is known about the nitrification N$_2$O yield in aquatic sediments and most information has been extrapolated from studies using pure cultures (Goreau *et al.*, 1980). Studies of N$_2$O production via nitrification conducted *in situ* are needed to resolve the role of nitrification in N$_2$O emissions from stream ecosystems.

*Upstream influences on dissolved gas concentrations*

It is important to examine the controls on dissolved gas concentrations in streams over an appropriate longitudinal scale. Processes which consume or produce gases at one point in a stream will affect the concentration of that gas for a finite distance downstream, determined by the air–water gas exchange rate (*K*) and $v$. Gas inputs to streams of lower *K* have a longer residence time and are exported further downstream (assuming the same *Q*, $v$, *h*, and *w*). Chapra & Di Toro (1991) proposed that the distance upstream from a sampling point over which a dissolved gas signal is integrated is given by

$$\text{Distance} = \frac{3v}{K} = \frac{3}{a}. \qquad (6)$$

This formula was derived from the first-order relationship between dissolved gas concentrations in excess of atmospheric equilibrium and diffusive evasion rates. Thus, the amount of dissolved gas remaining in the water column decreases exponentially from the addition point, and at a distance equal to 3/*a*, ∼5% of the original gas load remains in solution. The mean rate constant (*a*) for N$_2$O evasion in our study streams was 0.0023 m$^{-1}$, therefore on average our samples reflect the N$_2$O balance up to 1300 m upstream from the study reach. This suggests that unless our 100 m study reaches were representative of the upstream 1200 m, the reaches were too short to capture all of the processes influencing N$_2$O emission rates. Fortunately, our study sites were generally quite homogeneous for some distance upstream of our 100 m reach, with no incoming tributaries within 700 m of the reach in all but one site. Thus, our study reaches were probably representative of the upstream 700 m in most cases which is long enough to account for 80% of the dissolved gas sampled at the bottom of our 100 m study reach. Nonetheless, these estimates illustrate the importance of recognizing that dissolved gases can reflect processes over long upstream distances in streams and rivers.

*Seasonal patterns*

Monthly sampling for 1 year showed a general pattern of lower N$_2$O emission rates during the summer and fall and higher rates in the winter and spring (Fig. 2). This pattern in N$_2$O emissions may be partly driven by seasonal variation in NO$_3^-$ delivery to the stream channel. All streams showed a peak in NO$_3^-$ concentrations in the January–March period (Fig. 2). During the sampling year, January was a particularly wet month receiving 7.90 cm of precipitation (160% of the monthly average for January based on 59 years of data, www.weatherunderground.com) following a dry summer and fall where precipitation was only 58% of the average for that time of year. Elevation of stream NO$_3^-$ concentrations in winter and spring is common in the agricultural Midwest due to increased leaching and runoff and little biological activity (Royer *et al.*, 2006). Nutrient amendment experiments conducted in late summer 2005 demonstrated that N$_2$O production via denitrification was NO$_3^-$ limited, thus elevated stream water NO$_3^-$ concentrations in the winter and spring may have stimulated in-stream N$_2$O production leading to higher N$_2$O emission rates.

Temporal patterns in N$_2$O emission rates could also result from temporal variation in stream hydrology. Streams receive groundwater discharge from both shallow riparian zone soils and deeper groundwater flow paths (Genereux *et al.*, 1993). Water-saturated riparian soils are likely to be anoxic due to high C availability (Jones *et al.*, 1995) and provide ideal conditions for denitrification and N$_2$O production. By contrast, deeper groundwater generally is lower in C availability and often is oxic in the Kalamazoo basin (Rheaume, 1990). Therefore, groundwater derived from shallow riparian soils likely contains higher concentrations of dissolved N$_2$O than water derived from deeper flow paths and the relative contribution of deep and shallow groundwater to surface flow may partly control N$_2$O emission rates from these streams. The discharge of shallow riparian groundwater to the study sites is lowest in late summer and early fall and is greatest in winter and spring when stream discharge peaks. Therefore, variation in the relative contribution of N$_2$O-rich, riparian groundwater may partly explain seasonal variations in whole stream N$_2$O emission rates. A similar pattern was reported by Jones & Mulholland (1998a) where seasonal patterns in CO$_2$ emissions from a small stream were partly controlled by seasonal variation in the relative contribution

of $CO_2$-rich shallow groundwater. A more thorough understanding of groundwater $N_2O$ dynamics and surface-groundwater interactions could help explain seasonal patterns in $N_2O$ emissions from these streams.

*Test of the IPCC phase II methodology*

The IPCC methodology for calculating $N_2O$ emissions from small streams assumes that the ratio of $N_2O$ to $NO_3^-$ dissolved in the water column (the emission factor, EF5-g) represents the fraction of the N load that has been converted to $N_2O$. When the initial IPCC model was constructed there were few data available to validate EF5-g and it was recognized that the default value of 1.5% was highly uncertain (Nevison, 2000). In 2006 the IPCC revised EF5-g to 0.25% (IPCC, 2006) based on new data from Japan (Sawamoto *et al.*, 2005) and the United Kingdom (Reay *et al.*, 2005). We calculated emission factors spanning four orders of magnitude (0.00003–0.25) in this study (Table 1) suggesting that the current IPCC approach of applying one emission factor to all streams may not be appropriate.

We calculated a mean EF5-g value of 1.01%, however, this value was strongly influenced by the high $N_2O$/$NO_3^-$ ratios observed in one stream (A7) with unusually low $NO_3^-$ concentrations (Table 1). When stream A7 was excluded, the mean EF5-g value was 0.20%, very close to the revised IPCC value of 0.25%. Thus, our data strongly support the recent revision of EF5-g from 1.5% to 0.25%.

Although the revised value of EF5-g agrees well with our data, the emission factor may overestimate $N_2O$ emissions from streams because it does not account for $N_2O$ dissolved in surface waters due to equilibrium with atmospheric $N_2O$. Stream water with $N_2O$ concentrations in equilibrium with the atmosphere is not a net source of $N_2O$ to the atmosphere, yet according to the IPCC methodology these water bodies would have a positive emission factor. Only $N_2O$ concentrations exceeding equilibrium concentrations represent a net flux to the atmosphere. Perhaps a more logical formulation of the emission factor would be

$$\text{EF5-g} = \frac{N_2O^*}{NO_3^-}, \qquad (7)$$

where $N_2O^*$ is the difference between the observed $N_2O$-N concentration and the equilibrium concentration. Using Eqn (7), the mean emission factor across all our streams was 0.20%, but when stream A7 was excluded the mean was only 0.01%, 25 times lower than the current default value of 0.25%.

The current formulation of the EF5-g is based on the assumption that $N_2O$ produced in groundwater accumulates in the water and the ratio of dissolved $N_2O$ to

$NO_3^-$ concentrations represents the fraction of the N load that is converted to $N_2O$. This assumption may be appropriate for groundwater systems where gas exchange with the atmosphere is minimal; however, open stream channels rapidly exchange gases with the atmosphere. Thus, the ratio of dissolved $N_2O$ to $NO_3^-$ concentrations in streams may underestimate the fraction of the N load converted to $N_2O$ because much of the $N_2O$ has already been lost to the atmosphere. A more conceptually consistent emission factor might be based on the ratio of the total $N_2O$ flux to $NO_3^-$ flux through a stream reach (Clough *et al.*, 2006). This calculation requires detailed information on $N_2O$ emission rates throughout the length of a first or second order stream, which are rarely available. An alternative approach would be to restrict the application of the EF5-g to groundwater and water discharging from tile drains, and extend the application of the emission factor for rivers (EF5-r) to streams.

*Scaling up*

The streams in this study have higher $N_2O$ emission rates on an aerial basis (mean = 35.23 μg $N_2O$-N m$^{-2}$ h$^{-1}$) than the anthropogenic component of conventionally managed agricultural soils in the same basin (mean = 8.33 μg $N_2O$-N m$^{-2}$ h$^{-1}$, Robertson *et al.*, 2000), yet they only emit 6% as much $N_2O$ as agricultural soils because the total surface area of streams and rivers is small relative to agricultural soils. Streams and rivers would constitute an even smaller component of the anthropogenic $N_2O$ budget if we knew what fraction of the $N_2O$ evading from the stream network resulted from human activities within the drainage basin. These results appear to contrast with IPCC estimates that emissions associated with 'leaching and runoff' compose about 25% of the total indirect agricultural emissions (Mosier *et al.*, 1998). However, the IPCC estimate of 'leaching and runoff' emissions includes groundwater and estuaries and we have no estimate of groundwater emissions in this basin. Furthermore, we do not know how much of the N exported from this basin may be converted to $N_2O$ during transport through the remainder of the hydrologic system before reaching the ocean. Thus, to accurately quantify emissions associated with N leaching one must look beyond the borders of the study basin and incorporate N transformation processes occurring in downstream ecosystems.

**Conclusion**

We found that $N_2O$ emission rates from headwater streams in southwestern Michigan were similar to most

literature reports for streams, rivers, and agricultural soils. However, we did not find evidence of the very high emission rates ($>1000 \, \mu g\text{-}N \, m^{-2} \, h^{-1}$) reported in the literature for some small agricultural streams. In-stream denitrification rates were found to be significantly related to $N_2O$ emission rates, however nitrification rates were not. We also found that the IPCC approach of using one emission factor for all streams may be inappropriate because emission factors are highly variable across streams. However, our data support the recent downward revision of the emission factor for streams and groundwater (EF5-g). On the scale of the fifth order drainage basin we studied, streams were a significant, but small source of $N_2O$ to the atmosphere relative to agricultural soils because of the relatively small surface area of streams. Given that streams appear to be a small source of $N_2O$ emissions in agricultural basins, we suggest investigating other sources of $N_2O$. For example, the few reports of $N_2O$ emissions from riparian soils demonstrate a potential for very high emission rates yet riparian soils are not distinguished from upland soils in the IPCC budget. Research investigating the production and fate (i.e. subsequent consumption, emission, or transport) of $N_2O$ in riparian soils would make it possible to explicitly model $N_2O$ emissions from these systems.

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