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Balancing watershed nitrogen budgets: accounting for biogenic gases in streams

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Abstract Denitrification is critical for removal of reactive nitrogen (Nr) from ecosystems. However, measuring realistic, scalable rates and understanding the role of denitrification and other dissimilatory processes in watershed nitrogen (N) budgets remains a significant challenge in biogeochemistry. In this study, we focused on the stream reach and network scale in three Mid-Atlantic coastal plain watersheds. We applied open channel methods to measure biogenic N_2 and N_2O gas fluxes derived from both in-stream and terrestrial nitrogen processing. A large portion of biogenic N_2 flux through streams (33–100 %, mean = 74 %) was a result of groundwater delivery of biogenic N_2 with the remaining portion due to in-stream N_2 production. In contrast, N_2O was largely produced in-stream, with groundwater delivery contributing on average 12 % of the total biogenic N_2O flux. We scaled these measurements across one stream network and compared them to hydrologic Nr export and net anthropogenic N inputs (NANI) to a 4.8 km^2 watershed. The N budget revealed that, during the study period, the biogenic N_2 flux through streams was comparable to the difference between NANI and hydrologic Nr export (i.e. the "missing" N). This study provides a methodological and conceptual framework for incorporating terrestrial and in-stream derived biogenic N gas fluxes into watershed N budgets and supports the hypothesis that denitrification is the primary fate of NANI that is not exported in streamflow.

Keywords Denitrification · Greenhouse gases · Headwater streams · Nitrogen · Radon · Watershed budget

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Introduction

Denitrification is an essential process for removing reactive nitrogen (Nr) from ecosystems, yet it remains the least constrained transformation in the nitrogen (N) cycle (Groffman et al. 2006; Kulkarni et al. 2008; Seitzinger et al. 2006). Denitrification is carried out by microbes that require low oxygen and an energy

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source (e.g., organic carbon). It converts nitrate (NO_3^-) ultimately to N_2 gas, through intermediates of nitrite (NO_2^-), nitric oxide gas (NO), and nitrous oxide gas (N_2O). The specific controls of denitrification are well known largely through laboratory studies (Garcia-Ruiz et al. 1998; Knowles 1982; Seitzinger 1988), but it remains challenging to obtain in situ measurements in the field, to scale measurements over space and time, and to evaluate its significance to ecosystems (Groffman et al. 2006; Kulkarni et al. 2008; Seitzinger et al. 2006).

It is important to understand denitrification and the fate of anthropogenic N because water quality and global climate change are both affected by human alterations of the N cycle. Anthropogenic N loading (i.e. fertilizer, sewage) to aquatic systems, such as the Chesapeake Bay that drains the watersheds in this study, induces eutrophication and subsequent oxygen depletion with cascading ecological consequences (Diaz 2001; Diaz and Rosenberg 2008; Kemp et al. 2005; Nixon 1995). The prevalence of oxygen-depleted "dead zones" is increasing (Diaz and Rosenberg 2008), and eutrophication continues to be a significant management challenge worldwide.

As denitrification removes Nr, it also produces N_2O, the dominant ozone depletor (Ravishankara et al. 2009) and a greenhouse gas with nearly 300 times the global warming potential of carbon dioxide (Shine et al. 2005). The International Panel on Climate Change (IPCC) estimated that 35 % of anthropogenic N_2O emissions are from groundwater, streams, and rivers as a result of N cycling. These are known as indirect emissions as opposed to direct emissions from soil surfaces or point sources (Foster et al. 2007; Mosier et al. 1998). However, N_2O fluxes through streams have received less attention compared to terrestrial systems despite growing evidence from modeling (Seitzinger and Kroeze 1998) and empirical studies (Baulch et al. 2011; Beaulieu et al. 2011) that suggest N-enriched lotic systems could be significant sources.

Denitrification may account for the difference between anthropogenic N inputs and stream Nr export from watersheds, known as the "missing" N. Hydrologic Nr export from watersheds commonly accounts for less than 30 % of the net anthropogenic N inputs (NANI) to a watershed (Howarth et al. 1996; Jordan et al. 1997; Van Breemen et al. 2002). The impacts of anthropogenic N cannot be fully understood without knowing the fate of the unaccounted for missing N. The missing N could be accumulating within the watershed in the form of biomass, organic matter, and Nr in groundwater, but the prevailing hypothesis is that most of the missing N is denitrified and evades to the atmosphere as N_2 (Fox et al. 2014; Van Breemen et al. 2002). However, denitrification is rarely measured and scaled to a watershed to test the hypothesized link between denitrification and the missing N (Duncan et al. 2013).

Stream networks may provide a pathway for biogenic N gas loss to the atmosphere that is significant at the scale of watershed N budgets. However, both in-stream processes as well as watershed connectivity through groundwater flow paths must be considered. It has been demonstrated that streams can be hotspots for denitrification and N removal (Duff and Triska 1990; McClain et al. 2003). Among aquatic ecosystems, streams have denitrification rates that are high per unit area, but variable over time and space (Piña-Ochoa and Álvarez-Cobelas 2006). What has received less attention is the fact that streams collect groundwater inputs carrying biogenic N gases (N_2 and N_2O) produced elsewhere in the watershed through denitrification, nitrification, or perhaps other microbial processes. Thus, gaining streams may be concentrating biogenic N gases from the entire watershed that subsequently evade into the atmosphere, potentially accounting for some of the missing N. This concept was described by Fox et al. (2014) and is analogous to streams being described as "chimneys" venting terrestrially derived CO_2 (Hotchkiss et al. 2015).

To assess the importance of streams to the venting of biogenic N gases, a reach-scale, in situ method that estimates both in-stream and watershed-derived biogenic N gas fluxes is needed for measurements at spatial scales relevant to watershed management and modeling. The open channel method (Laursen and Seitzinger 2002; McCutchan et al. 2003) is one such method used to measure in-stream denitrification at the reach scale (10^1–10^4 meters) and has been applied in a variety of riverine systems (Harrison et al. 2005; Pribyl et al. 2005; Smith et al. 2008; Yan et al. 2004). Laursen and Seitzinger (2002) developed a multi-station method that applies a Lagrangian sampling design to estimate denitrification within a conceptual moving parcel of water while accounting for atmospheric exchange. McCutchan et al. (2003) presented a one-station approach that directly calculates in-stream

denitrification, correcting for atmospheric exchange and groundwater inputs. Both methods are mathematically similar and involve a whole stream N_2 mass balance to estimate water column fluxes of N_2 that are a result of biological processes. Here we present a modification of the open channel method that estimates both in-stream production and groundwater delivery of biogenic N gases. We recognize that biogeochemical processes other than denitrification could contribute to production of N_2 and N_2O in streams and watersheds (Burgin and Hamilton 2007); therefore, we will use the term "biogenic" N_2 and N_2O from in-stream production or groundwater delivery instead of referring to denitrification.

Open channel methods require measurement or estimation of the gas transfer velocity (Laursen and Seitzinger 2005; Marzolf et al. 1994). The gas transfer velocity (k, m s^{-1}) can be measured using injections of conservative tracer gases (propane and SF_6) or modeled as function of current and/or wind velocity. Use of tracer gases can be laborious and imprecise. Alternative methods are needed for measuring k in streams, for example using dissolved oxygen curves and inverse Bayesian modeling (Holtgrieve et al. 2010).

We utilized two naturally occurring noble gases, Argon (Ar) and Radon (^{222}Rn) to estimate gas transfer velocity and compared different calculation methods. ^{222}Rn has been used as a tracer for groundwater (Ellins et al. 1990; Genereux et al. 1993) and to estimate k in the open ocean in combination with radium isotopes (Peng et al. 1979; Smethie et al. 1985). Yet there are few examples of deriving k directly from ^{222}Rn in streams (Wanninkhof et al. 1990) despite possessing promising tracer properties for this application: inert, radioactive, naturally high concentrations in groundwater, and negligible atmospheric background.

This study builds upon previous work on open-channel methods and N budgeting by incorporating groundwater delivery of biogenic N gases and scaling up measurements in a watershed N budget. Our specific objectives were to (1) assess methods for estimating gas transfer velocity from two natural tracers, Ar and ^{222}Rn, (2) present a modified, single-station open channel method to quantify biogenic N_2 and N_2O fluxes through streams due to in-stream and groundwater processes, and (3) compare N gas fluxes to NANI and hydrologic Nr export within a watershed N budget.

We hypothesized that (1) headwater streams are hotspots for fluxes of biogenic N_2 and N_2O due to in-stream production and groundwater delivery of terrestrially derived N gases, and (2) N gas fluxes through streams account for a large portion of the missing N in the study watershed.

Methods

Sites

The study sites are located in the Choptank River and Nanticoke River Basins, which drain into the Chesapeake Bay from the Delmarva Peninsula within the Atlantic coastal plain. The topography is flat (<30 m above sea level), and the hydrogeomorphology ranges from poorly drained uplands with shallow streams to well-drained, sandy soils with incised stream channels (Hamilton et al. 1993). Land use in the Choptank Basin is dominated by cropland (62 %), followed by forest (26 %), and a small urban component (5 %) (Fisher et al. 2006; Norton and Fisher 2000). The climate is humid temperate, with an average annual rainfall of 112 cm $year^{-1}$ evenly distributed throughout the year. Stream flow is highly variable, with an annual average of 43 cm $year^{-1}$ and strong seasonal variations due to temperature-related evapotranspiration (Fisher et al. 2010).

This study focused on the 4.8 km^2 Baltimore Corner (BC) watershed, which is located within the upper Choptank Basin and includes forest and cropland (Fig. 1, Table 1) The stream network has been channelized to drain adjacent lands in production under a corn-wheat-soybean rotation. Stream sediments are sandy and soils are well drained sandy loams in the lower watershed with hydric soils occupying the uplands. BC1, BC2 and BC3 are the three main branches in the BC watershed with BC2 and BC3 flowing into BC1. These reaches are similar in channel morphology and chemistry. We included two additional sites to explore effects of different land use patterns on stream N dynamics: Marshy Hope (MH), a forested watershed with very low N and P concentrations; and South Forge (SF), with a greater percentage of cropland than the main study site BC (Fig. 1; Table 1).

Open channel studies were based on methods presented in McCutchan et al. (2003) with minor

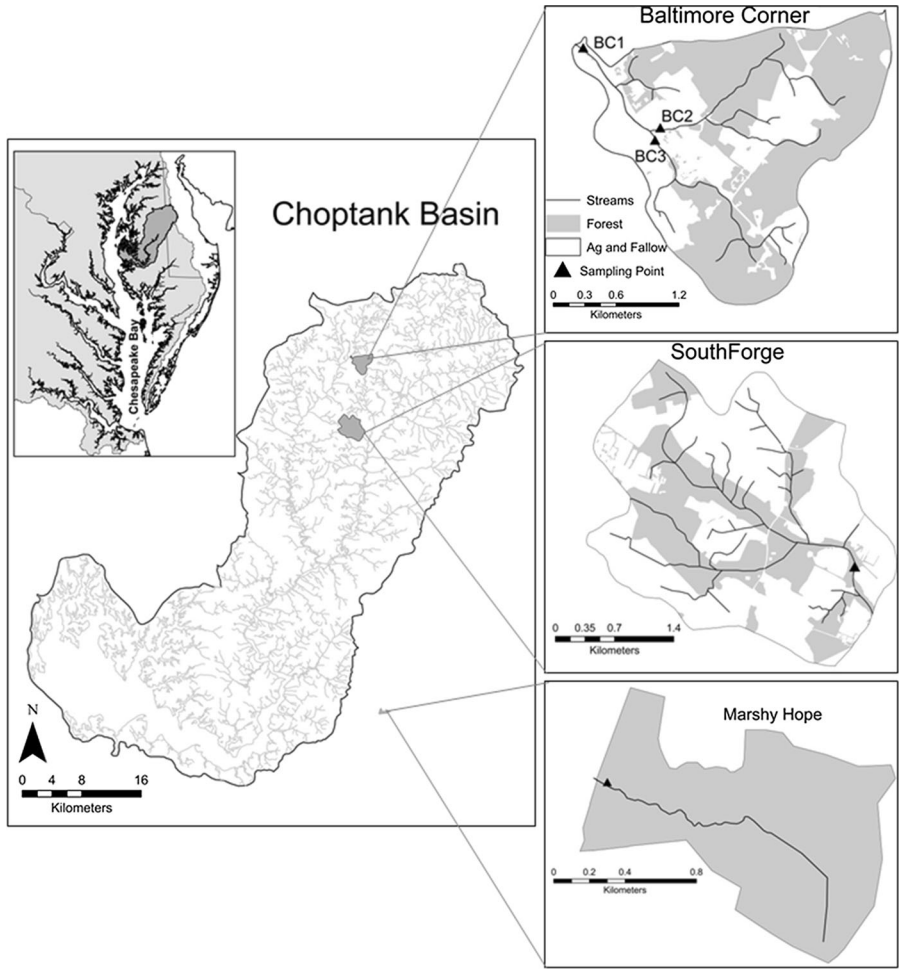

Fig. 1 Map of Choptank Basin and study sites, Baltimore Corner (BC), South Forge (SF), and Marshy Hope (MH)

Table 1 Area, land use, soil properties, and mean (and standard error in parentheses) baseflow nitrate, total nitrogen and total phosphorus concentrations over the 2012–2013 water year for the Marshy Hope (MH), Baltimore Corner (BC), and South Forge (SF) watersheds

Watershed	Area (km^2)	% Crop land	% Forest	% Hydric soils	NO_3^-	TN	TP
MH	1.36	1.0	99	55	1.11 (0.47)	33.3 (3.1)	0.41 (0.07)
BC	4.84	26	60	69	199 (21)	256 (23)	1.15 (0.14)
SF	8.49	66	28	35	325 (17)	354 (18)	1.32 (0.18)

modifications including reducing sampling time to 6–8 daylight hours. By reducing the sampling time from the typical 12–24 h (McCutchan et al. 2003; Laursen and Seitzinger 2002) to 6 h, we were able to achieve greater spatial and temporal coverage within a watershed. Studies were repeated seasonally from September 2012 to July 2013 in the three perennial reaches (BC1, BC2, BC3) of the BC watershed, resulting in 11 sets of measurements. In addition, one study in each of the MF and SF watersheds was conducted in fall 2012.

Field measurements

Streamflow was determined by the area velocity method (measuring cross sectional area and velocity)

and by continuous injection of a conservative tracer, sodium bromide (NaBr, with [Br] = 200 g/L), at a rate of 23 mL min^{-1} with a peristaltic pump at least 50 meters above the upstream sampling point. Stream water was sampled in acid-rinsed 60 mL plastic bottles every 15 min for ion analysis (Br^-, Cl^-, F^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-}). Stream flow (Q, $m^3 s^{-1}$) was calculated according to Eq. 1:

$$Q = \mathrm{Br}^-_{stock} r / \left(\mathrm{Br}^-_{post} - \mathrm{Br}^-_{pre} \right) \quad (1)$$

where r is the injection rate ($m^3\ s^{-1}$), Br^-_{stock} is the bromide concentration that was injected (mg m^{-3}), Br^-_{post} is the steady state concentration, and Br^-_{pre} represents the pre-injection background concentration. In addition to monitoring during field studies, temperature and stage were continuously measured at 30 min intervals using Solinst Leveloggers (Model LT F15/M5) placed at the downstream point of each reach and watershed outlet. Rating curves have been developed to convert stage to discharge at all sites (Fisher et al. 2010).

Distances between the up and downstream sampling points defined the study reach over which groundwater discharge and chemistry was measured. These reach lengths were 364, 109, 227, 140, and 95 meters for BC1, BC2, BC3, SF, and MH respectively. Groundwater discharge into this reach (Q_{gw}, $m^3\ s^{-1}$) was estimated by the difference in streamflow between upstream and downstream sampling points (Q_{us} and Q_{ds}, respectively, $m^3 s^{-1}$) as measured by tracer dilution. The groundwater piston velocity (V_{gw}, m s^{-1}) was then calculated by dividing groundwater discharge by the stream surface area (SA, m^2) estimated as the average of 10-12 stream width measurements multiplied by the reach length.

$$V_{\mathrm{gw}} = (Q_{\mathrm{ds}} - Q_{\mathrm{us}})/SA \quad (2)$$

Groundwater chemistry was sampled during each study from 3 to 5 different in-stream polyvinyl chloride (PVC) piezometers. These had dimensions of 5 cm inner diameter, with a 20 cm slotted length at a depth of 40–60 cm below the streambed and were spaced 25–100 m apart uniformly through the study reach. Hydraulic head was measured using a water level detector (Model 101 M, Solinst, Canada) or meter stick. Piezometers were pumped dry with a peristaltic pump (Solinst model 410, Georgetown, Canada) and allowed to recharge immediately prior to sampling for dissolved gases. A small submersible pump (Model GP1352, Whale Water Systems Inc., Manchester Center, VT) with positive pressure was used for sampling to reduce potential stripping of dissolved gases by negative pressure while pumping. Dissolved gas sample tubes were overflowed with several volumes prior to covering with septa and caps. N_2, O_2, and Ar were sampled in quadruplicate 27 mL glass tubes, N_2O in duplicate, ^{222}Rn in triplicate 250-mL glass RAD-H_2O sampling bottles (Durridge, Billerica, MA), and one additional sample was taken for anion analysis. Dissolved organic carbon (DOC) was sampled during summer 2013 from each piezometer and stream.

Surface water chemistry was sampled at the downstream point. A YSI multiprobe (Model 556 MPS, Xylem Inc., Yellow Springs, OH) was placed at mid-depth in the thalweg to monitor stream temperature, pH, conductivity, and dissolved oxygen (DO) concentration every minute. Samples for N_2, O_2, Ar, and N_2O were collected every 2 h. To collect samples, flexible vinyl tubing (2 mm inner diameter) was inserted to vent air from the inverted glass tube as it was submerged upside-down into the thalweg of the stream. Venting the air minimized water turbulence that might have altered dissolved gas concentrations. When the air was completely vented, the vent tube was removed and the glass tube was closed underwater with a Teflon or silicon septum held on by a plastic screw cap.

^{222}Rn was measured as a tracer for gas transfer velocity every 10 min at the same sampling site. We used continuous pumping of stream water with a submersible pump (Model GP1352, Whale Water Systems Inc., Manchester Center, VT) through a RAD-AQUA attachment connected to a RAD7 radon-in-air monitor (Durridge, Billerica, MA). Barometric pressure was measured on-site to calculate gas solubility every 10 min or less using a pressure transducer (Model 3001 Gold Levelogger, Solinst, Canada).

Laboratory analyses

Ground and surface water samples were stored on ice or in a refrigerator (4 °C) until analysis, except for groundwater ^{222}Rn samples which were kept at ambient temperature and analyzed within 24 h. ^{222}Rn should be measured within a week after collection, but uncertainty due to radioactive decay

is minimized by prompt analysis. Samples for dissolved N_2, O_2, and Ar were generally analyzed within 48 h of collection using a quadrupole mass spectrometer with a membrane inlet (MIMS; Kana et al. 1994). One standard per run was prepared with deionized water in a glass flask and allowed to equilibrate overnight in a water bath under constant stirring. MIMS was calibrated to the mean stream temperature over the day in which the samples were collected. Standards were measured initially and every 40 samples. Ion currents from the standards bracketing each set of ~40 samples were used to correct for instrument drift. Corrections for N_2 and Ar due to O_2 scavenging were also applied based on empirical relationships between O_2 ion currents and the magnitude of scavenging (Fisher et al. 2010; Fox et al. 2014). Equilibrium concentrations for N_2 and Ar were estimated using temperature, barometric pressure, and solubility curves provided in Hamme and Emerson (2004) and for O_2 in Garcia and Gordon (1992).

Dissolved N_2O was measured within 24 h of collection using a Shimadzu Gas Chromatograph-14B (Shimadzu Corp, Kyoto, Japan) equipped with an electron capture detector. Seven mL of water was injected into N_2-purged 12 mL Exetainers® (High Wycombe, UK) through the septum with a vent to maintain atmospheric pressure. Exetainers® were shaken vigorously for 4 min and allowed to equilibrate at room temperature for at least 30 min prior to analysis. The dissolved concentration in water was calculated using water sample and headspace volumes as well as solubility data for the measured room temperature and pressure (Weiss and Price 1980). DOC was measured using a Shimadzu TOC-5000A analyzer (Shimadzu Corp, Kyoto, Japan).

Anion samples were filtered through 45 µM pore-size membranes, and Br^-, Cl^-, F^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-} were measured using a Dionex ion chromatograph fitted with a KOH eluent generator, a conductivity detector, and an AS18 separatory column (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Groundwater ^{222}Rn grab samples were analyzed using a RAD7 with a RAD-H_2O radon-in-water attachment (Durridge, Billerica, MA).

Gas transfer velocity

Gas reaeration coefficients (s^{-1}, gas transfer velocity divided by stream depth) were estimated using conservative gases, Ar and ^{222}Rn. Conversion to gas transfer velocity normalized to a common Schmidt number of 600 was utilized later for comparing values when testing three different equations that can be applied to both tracers: an equation derived from McCutchan et al. (2003), a modified version of the McCutchan et al. equation, and a simplified "unit reach" mass balance.

As noted in Laursen and Seitzinger (2004), when the measured Ar concentration in surface water deviates from the equilibrium concentration, the reaeration coefficient can be estimated by the rate of re-equilibration needed to predict observed Ar concentrations. Equation (6) from McCutchan et al. (2003) was solved for the reaeration coefficient K_{Tr} (s^{-1}) and applied to both tracers,

$$K_{Tr} = \frac{\frac{C_t - C_o}{\Delta T} Z - V_{gw}(C_{gw} - C_t)}{Z(CE_{t-} C_t)} \quad (3)$$

where C_t is the concentration of the tracer (either Ar in mmol m^{-3} or ^{222}Rn in Bq m^{-3}) at the end of the measurement interval, C_o is the concentration of the tracer at the beginning of the measurement interval, ΔT is the length of the time interval (s), V_{gw} is the groundwater piston velocity (discharge per unit area, m ΔT^{-1}), C_{gw} is the average groundwater tracer concentration measured from 3 to 5 piezometers spaced uniformly across the entire reach length (either Ar in mmol m^{-3} or Rn in Bq m^{-3}), Z is the average stream water depth (measured from cross sections), and CE is the concentration of the tracer in equilibrium with the atmosphere ($CE = 0$ for ^{222}Rn).

The modified McCutchan et al. equation given below (Eq. 4) is identical to Eq. 3 except that terms varying over time represent the average over ΔT instead of the final concentration and parameters are in units of per ΔT. The average between data points is used instead of the final concentration over the time interval to prevent biasing the final gas transfer velocity when ΔT is large and/or the total time of data collection is short. For Ar, ΔT was equal to 2 h and for ^{222}Rn 10 min.

$$K_{Tr} = \frac{\frac{C_t - C_o}{\Delta T} Z - V_{gw}\left(C_{gw} - \frac{C_t + C_o}{2}\right)}{Z\left(\frac{CE_t + CE_o}{2} - \frac{C_t + C_o}{2}\right)} \quad (4)$$

Finally, we simplified a "unit reach" mass balance adapted from a ^{222}Rn mass balance (Eq. 8) in Wanninkhof et al. (1990). Conceptually, this is a one meter

long unit reach with a measured width where the tracer concentration in surface water does not change spatially in transit across this one meter. For Ar, we can then reduce Eq. 4 to:

$$K_{Tr} = \frac{V_{gw}(C_{gw} - C)}{Z(C - CE)} \quad (5)$$

For ^{222}Rn, the equilibrium concentration (CE) is equal to 0, simplifying further to,

$$K_{Tr} = V_{gw}\left(\frac{C_{gw}}{C} - 1\right)\frac{1}{Z} \quad (6)$$

where V_{gw} is the groundwater piston velocity presented in Eq. 2 (m s^{-1}), C is the ^{222}Rn concentration in stream water (Bq m^{-3}), and C_{gw} is the ^{222}Rn concentration in groundwater (Bq m^{-3}). This also assumes constant V_{gw} and C_{gw} over the measurement period. Radioactive decay of ^{222}Rn was negligible since its residence time in the water column was orders of magnitude less than its half-life (3.8 days).

Reaeration coefficients (K, s^{-1}) were converted between K_{Tr}, K_{N2}, K_{N2O}, and $k600$ based on Schmidt numbers derived in Raymond et al. (2012) using the following equation (Wanninkhof 1992),

$$K_N = \left(\frac{Sc_N}{Sc_{Tr}}\right)^{-2/3} \times K_{Tr} \quad (7)$$

where Sc is the Schimdt number and the subscripts Tr and N refer to the tracer (Ar or ^{222}Rn) and any other gas of interest (N_2, N_2O, or the Schmidt number of 600) respectively. The exponent −2/3 (Jähne et al. 1987) was used as there was little surface turbulence in these low-gradient, coastal plain streams that were also protected from wind by riparian trees or high banks from channelization. Reaeration coefficients (K, s^{-1}) were multiplied by Z and converted to a common Schmidt number of 600 ($k600$, m s^{-1}), and then averaged to produce a daily mean $k600$ in order to compare k values from both Ar and ^{222}Rn across studies. When calculating flux over time for a given study, Eq. 7 was used to adjust K for temperature variability and convert to the gas of interest, K_{N_2} or K_{N_2O}.

Estimation of N_2 and N_2O production

To measure biogenic N gas flux we needed to distinguish it from non-biogenic N gas flux. Water infiltrating into the soil contains some atmospherically-derived N_2 and N_2O that can be delivered to groundwater. We estimated atmospherically-derived N gases in groundwater by using Ar as a conservative tracer indicating the temperature at the time of groundwater recharge. N gases in excess of atmospheric equilibrium at the temperature during recharge were presumed to have been added to the groundwater by biotic processes. We recognize that atmospheric N_2 and N_2O may ultimately be biogenic, but for our purposes we need to measure the biogenic N gases added to the water in transit through the watershed. Using published solubility data (Colt 1984; Böhlke and Denver 1995; Hamme and Emerson 2004; Fisher et al. 2010), we derived an equation to estimate recharge temperature ($T_{recharge}$, °C) from Ar concentration ([Ar], mmol m^{-3}).

$$T_{recharge} = 0.0099[Ar]^3 + 0.651[Ar]^2 - 16.086[Ar] + 144.62 \quad (8)$$

Note that Eq. 8 assumes a water density of (1000 kg m^{-3}) and should only be used in freshwater; see citations above to develop curves accounting for salinity. After calculating recharge temperature, the recharge concentration for any gas can be estimated using temperature dependent solubility curves. The difference between measured and recharge N_2 or N_2O concentrations were assumed to be biogenic N gas (Wilson et al. 1990).

Total flux (in-stream + groundwater) of biogenic N_2 and N_2O (F_T, mmol N m^{-2} h^{-1}) was calculated using Eq. 9. Conceptually, the total flux of biogenic N_2 or N_2O is equal to the change in stream N gas inventory—non biogenic groundwater N gas inputs—N gas loss to atmosphere (Eq. 9):

$$F_T = Z\frac{C_t - C_o}{\Delta T} - V_{gw}\left(C_{rec} - \frac{C_t + C_o}{2}\right) - Z\left(\frac{KN2_t + KN2_o}{2}\right)\left(\frac{CE_t + CE_o}{2} - \frac{C_t + C_o}{2}\right) \quad (9)$$

where C_{rec} equals the gas concentration during groundwater recharge (mmol m^{-3}). Other variables are as described in Eq. 4 and C_{rec}, V_{gw}, and Z are assumed constant. This calculation is similar to that of McCutchan et al. (2003) except that the concentration of N gases at recharge was used instead of total

measured N gases in groundwater, and the concentrations and K were averaged over the 2 h intervals between our measurements. With a ΔT of 2 h, using concentrations at time t biases the calculations during the day when gas concentrations are rapidly changing due to temperature; thus, averaging becomes necessary. With a shorter ΔT or longer study duration, averaging may not be required. Our calculated rates for each 2 h interval were averaged to find the mean daily rate.

N_2 and N_2O from groundwater delivery to the stream (F_{gw}, mmol N m^{-2} h^{-1}) was calculated as follows,

$$F_{gw} = \left(C_{gw} - C_{rec}\right) V_{gw} \quad (10)$$

where C_{gw} is the total measured N_2 or N_2O concentration in emerging groundwater (mmol m^{-3}), C_{rec} is the recharge concentration. F_{gw} is a flux of biogenic N gas across the groundwater-surface water interface.

In-stream biogenic N gas production (F_{st}, mmol N m^{-2} h^{-1}) was found by subtracting biogenic groundwater delivery (F_{gw}) from the total biogenic flux (F_T).

$$F_{st} = F_T - F_{gw} \quad (11)$$

Note that Eq. 9 is typically used to directly solve for in-stream production by substituting the measured concentration in groundwater (C_{gw}) for the recharge concentration (C_{rec}). This is mathematically equivalent to subtracting groundwater delivery from total flux as in Eq. 11. Any two of the three terms (F_T, F_{gw}, F_{st}) can be directly calculated, and the third found by addition or subtraction.

To assess the scalability of the one station approach to the study reach and beyond, the following equation (Chapra and Di Toro 1991) was used to estimate the travel distance (m) over which stream water will exchange 95 % of its dissolved gases:

$$Distance = {}^{3V}/_{K} \quad (12)$$

where V is velocity (m s^{-1}), and K is the stream reaeration coefficient (s^{-1}). This has been used to estimate the upstream distance that influences dissolved gas measurements taken at a downstream point (Baulch et al. 2011; Beaulieu et al. 2007). For all but one measurement, the study reach was shorter than 3 V/K (Table 2). This, together with the fact that the reaches in the primary study watershed (BC) were geomorphically uniform as a result of channelization, suggests that our measurements of gas production could be extrapolated over each of the three stream segments upstream of the one-station measurement locations within the BC watershed.

Uncertainty analysis

The uncertainty in gas transfer velocity and production of N_2 and N_2O for all 13 studies was evaluated using a Monte Carlo approach. For gas transfer velocity ($k600$) all terms in Eqs. 3–6 were randomly sampled 1000 times from normal distributions described by empirical means and empirical or literature-derived standard deviations. The same approach was applied to Eqs. 9–11 for biogenic N gas fluxes. Error in measured N_2 and Ar was assumed to be due to limits of precision and error in equilibrium concentrations due to measurement error of temperature. Error in groundwater inputs was assumed to be 10 % of the measured value (McCutchan et al. 2003), and depth and surface area error were set at 2.5 % which lies in between values reported in McCutchan et al. (2003) and Smith et al. (2008). Variance in groundwater concentrations of N_2, Ar, N_2O, and ^{222}Rn was assumed to be due to spatial variation along the reach; therefore, the standard deviation of the 3–5 piezometers was used (Table 3). Output from the Monte Carlo analysis provided a 95 % confidence interval for each method of calculating $k600$ and as well as rates of total flux, in-stream production, and groundwater delivery of N_2 and N_2O. Analyses were performed in R (R Core Development Team 2014) and SigmaPlot 12.5 (Systat Software Inc. 2013).

N_2O Emissions

To place our measurements of N_2O in a broader context, we estimated N_2O emission factors, assessed different calculation methods, and compared our estimates to emission factors used by the Intergovernmental Panel on Climate Change (IPCC) to scale global N_2O emissions. Indirect emission factors, or the proportion of N inputs that escape to the atmosphere as N_2O through hydrologic pathways, are applied to rivers (EF5-r), estuaries (EF5-e), and streams and groundwater (EF5-g). A value of 0.25 % is currently used for all indirect emission factors: EF5-r, EF5-e, and EF5-g (IPCC 2006). The EF5 was originally derived from several empirical studies on the ratio of dissolved N_2O–N to NO_3^-.

Table 2 Average conditions during each 6–8 h study period including discharge (Q), stream depth (Z), stream width (W), distance corresponding to 95 % turnover of dissolved gases (3 V/K), stream temperature, nitrate concentration (NO_3^-), percent saturation of nitrogen gas (N_2), nitrous oxide (N_2O), and oxygen (O_2), and dissolved organic carbon (DOC)

Date	Site	Q (L s^{-1})	Z (m)	W (m)	3 V/K (m)	Stream Temp (°C)	NO_3^- (mmol m^{-3})	N_2 (% sat)	N_2O (% sat)	O_2 (% sat)	DOC (mmol m^{-3})
9/25/2012	BC1	4.49	0.12	1.9	581	15.6	371	104	2463	81	–
11/20/2012	BC1	46	0.27	2.3	2609	11.2	199	106	2045	85.5	–
2/18/2013	BC1	68.3	0.3	2.4	2910	4.44	173	105	978	98.8	–
4/15/2013	BC1	167	0.33	2.5	5393	14.1	110	104	1124	80.5	–
7/17/2013	BC1	82.5	0.3	2.4	1369	24	171	103	2585	73.7	680
2/25/2013	BC2	49.8	0.24	3.4	3121	6.45	46	105	344	100	–
5/14/2013	BC2	61.8	0.29	4.2	1113	17.4	21	104	1077	88.2	–
7/9/2013	BC2	42	0.22	3.3	192	24.6	41	102	1982	51.4	2044
3/4/2013	BC3	24.5	0.33	2.6	2216	7.13	247	107	3111	93.6	–
5/6/2013	BC3	19.2	0.27	2.1	3603	13.7	230	104	5004	82.3	–
6/25/2013	BC3	28.4	0.21	2.3	1830	23	209	106	6681	63.9	531
12/13/2012	SF	81.3	0.23	3.5	8369	7.74	317	103	616	83.4	
11/15/2012	MH	9.71	0.2	1.9	2649	10.3	0.59	100	89.2	66.7	

We compared emission factors derived from the simple ratio of dissolved N_2O–N to NO_3^- (IPCC, 2006) to modified ratios for streams and groundwater. For streams, a modified ratio was proposed by Beaulieu et al. (2007) using the N_2O–N concentration in excess of atmospheric equilibrium (*xs* N_2O–N) divided by NO_3^-. For groundwater, Well et al. (2005) included excess N gases in the denominator to account for transformation of NO_3^- along groundwater flow paths.

$$EF5g = \frac{xsN_2O \cdot N}{xsN_2O \cdot N + xsN_2 \cdot N + NO_3} \quad (13)$$

Watershed N budget

A watershed nitrogen budget for the BC site was developed over the 2013 water year to test our primary hypothesis linking denitrification and the missing N. We included net anthropogenic nitrogen inputs (NANI) and outputs of measured hydrologic Nr export and extrapolated biogenic N gas fluxes through streams. We estimated NANI to the BC watershed based on inputs to cropland and other land types in Queen Anne's County adjacent to the BC watershed. NANI to non-cropland is 12 kg N ha^{-1} $year^{-1}$ and is the result of wet plus dry atmospheric deposition of NO_x, assuming that dry deposition equals wet (Jordan and Weller 1996). Sources of NANI to agricultural land include atmospheric deposition, fertilizer, livestock waste, and N_2 fixation by crops. N removed from harvest of crops is subtracted from inputs to calculate the net input to agricultural land, which ranges from 77

Table 3 Error in measured variables used in uncertainty analysis of gas transfer velocity and N gas flux rates

Variable	Coefficient of Variation
Z	0.025
SA	0.025
V_{gw}	0.10
K	0.034*
$^{222}Rn_{sw}$	0.12*
$^{222}Rn_{gw}$	0.26*
Ar_{sw}	0.001*
Ar_{gw}	0.058*
N_2-sw	0.0012*
N_2-gw	0.05*
recharge N_2-gw	0.053*
N_2O_{sw}	0.047*
N_2O_{gw}	1.24*
recharge N_2O_{gw}	0.092*

* Varied depending on measurements from each study. Shown here are average error terms across all dates

to 90 kg N ha^{-1} $year^{-1}$, depending on assumptions about net loss of NH_3 to the atmosphere from livestock waste (Jordan and Weller 1996).

Given that the BC watershed is 25.6 % cropland, the NANI to the watershed would be 28.6–32.0 kg ha^{-1} $year^{-1}$, based on the NANI estimates (Jordan and Weller 1996). Hydrologic Nr export from the BC watershed was 20 kg N ha^{-1} $year^{-1}$ (66 % of NANI) over the 2013 water year based on measurements of N concentration during base flow and storm flow and continuous measurement of stream discharge collected in a related study (Gardner 2014).

Loss of biogenic N gases through the BC stream network was estimated by empirical modeling of daily N_2 and N_2O flux rates and stream surface area for the three streams in the BC network over the 2013 water year. N gas flux rates were multiplied by surface area for each of the three streams and summed to estimate total N gas losses from the entire network. The length of each stream segment was measured in ArcMap (version 9.3) and assumed constant over time (i.e. ephemeral channels were not considered). Daily variability in stream area was estimated by varying stream width as a function of measured discharge according to hydraulic geometry equations developed for each site (Leopold and Maddock 1953). The first order streams, BC2 and BC3, were assumed to taper to zero width while the second order channel (BC1) had a uniform channel width.

Daily groundwater delivery and in-stream production of N_2 and N_2O were scaled up independently based on empirical relationships presented in results. For groundwater delivery, we estimated variables in Eq. 10, excess N gas and groundwater piston velocity. Relationships between groundwater piston velocity (V_{gw}) and antecedent stream depth (ASD), where ASD is defined as the mean stream depth at the downstream end during the week prior to a given gas flux measurement, were used to predict daily V_{gw} from continuous stream depth gauging. Excess N gas concentrations in groundwater were linearly interpolated between measurements at a daily time step. For N_2, this is justified by the uniform seasonal pattern in excess N_2 observed in all piezometers presented in results. In-stream N_2 and N_2O production were directly estimated from regressions with average daily stream temperature.

This scaling of measurements to annual fluxes of biogenic N gases does not account for diel variability. We assumed that our measured daytime rates were representative of a full 24 cycle, and therefore may have overestimated in-stream biogenic N gas production. Other studies have documented higher in-stream N_2 production during daylight (Harrison et al. 2005; Laursen and Seitzinger 2004), but a related diel study suggested there was little difference between day and nighttime in-stream N_2 fluxes at our sites (Knee et al., unpublished data).

Results

Groundwater

Consistent dilution of the Br^- tracer in streams and positive hydraulic heads in piezometers confirmed that the study reaches were receiving groundwater inputs. This enabled us to use ^{222}Rn from groundwater inputs as a tracer for calculating K. Across all sites (BC, MH, and SF) groundwater piston velocity ranged from 0.04 to 0.72 m day^{-1}, while hydraulic head ranged from 0 to 0.36 m, with a mean hydraulic head of 0.11 m in the primary stream network, BC. Groundwater discharge per meter of stream length estimated from the Br^- dilution (0.25–1.75 m^3 day^{-1}) was comparable to the estimated average groundwater flux in the upstream network of the BC watershed found by dividing stream discharge by upstream length (0.08–1.70 m^3 day^{-1}). This comparison of measured with estimated groundwater discharge for each stream segment suggested that the measured groundwater fluxes were representative of the BC stream network.

Groundwater recharge temperature was critical for separating biogenic from non-biogenic N gases in groundwater. Estimated temperatures (range = 8.52–22.09 °C, mean = 15.02 °C) were comparable with a previous study where recharge temperatures ranged from 9 to 14 °C across the entire Delmarva peninsula based on similar methods (Dunkle et al. 1993). Recharge temperatures greater than 20 °C are uncommon (Fisher et al. 2010) and may indicate gas stripping by ebullition, which decreases Ar concentrations (Cey et al. 2009). We excluded data from one piezometer sampled summer 2013 in BC2 with a recharge temperature of 30 °C.

Dissolved gases in emerging groundwater were spatially variable and typically deviated considerably from equilibrium concentrations. Excess N_2–N, the

difference between measured (C_{gw}) and equilibrium concentrations at a given recharge temperature (C_{rec}), was detected in all samples, with a mean of 292 mmol m^{-3} (124 % saturation) and a range of 33-591 mmol m^{-3} (102–150 % saturation). Oxygen concentrations in groundwater were below equilibrium (3.0–212 mmol m^{-3} or 0.03–60.9 % saturation) with a mean of 39.3 mmol m^{-3} (11 % saturation). N_2O–N concentrations were highly variable, ranging from 0.001 to 2.1 mmol m^{-3} with a mean of 0.39 mmol m^{-3}, and 46 % of measurements were undersaturated. Despite the variable O_2 concentrations, there was a significant difference (two sample *t* test, $P < 0.05$) in mean O_2 between groups of samples partitioned by the 50 mmol m^{-3} threshold NO_3^- concentration below which denitrification is N limited (Golterman 2004; Seitzinger 1988). The mean O_2 for the high-NO_3^- group was 61 mmol m^{-3} and 27 mmol m^{-3} for the low- NO_3^- group.

Consistent temporal patterns in excess N_2–N were observed in emerging groundwater (Fig. 2a). Excess N_2–N followed a seasonal curve, with peaks in February to March and lows in the summer. NO_3^- was more variable, ranging from 0 to 1313 mmol m^{-3} with a mean of 200 mmol m^{-3} (Fig. 2b). These patterns suggested consistent and distinctive groundwater flow paths were sampled by each piezometer.

Radon proved to be an appropriate tracer for groundwater, justifying its utility as a tracer for gas transfer velocity in gaining streams. Within the BC watershed, reach averaged groundwater ^{222}Rn concentration was positively related to total groundwater flux ($r^2 = 0.70$, $P < 0.01$, Table 4). Additionally, aggregating all individual piezometer measurements in the BC watershed, groundwater ^{222}Rn activity was positively correlated with hydraulic head ($r^2 = 0.32$, $P < 0.05$, Table 4). These relationships both suggest ^{222}Rn concentrations in emerging groundwater are linked to local hydrologic conditions within the stream and the surrounding hyporheic zone.

Stream water

Patterns in dissolved gases in surface water were comparable across all studies in agriculturally impacted streams, but strongly contrasted with the stream in the MH forested watershed. As stream temperature increased over the day (Fig. 3e), dissolved N_2 concentrations tracked equilibrium but were always supersaturated (Fig. 3a), except in the forested MH reach where N_2 varied around equilibrium (Fig. 4a). The N_2:Ar ratio should be affected by changes in solubility due to diel temperature changes, but varied minimally during each study and generally did not follow patterns predicted by equilibrium N_2:Ar ratio (Figs. 3d, 4d). N_2O was highly supersaturated (340–6700 %) in the BC reaches with little temporal variability (Fig. 3b), but was under-saturated in the forested MH reach (average of 89 %, Fig. 4b). In BC and SF, stream O_2 was undersaturated during all studies and peaked in the afternoon, suggesting photosynthetic production (Fig. 3e). In contrast, O_2 saturation decreased over the day at the forested MH site (Fig. 4e). ^{222}Rn concentrations in stream water were variable, but often exhibited a decreasing trend from morning to afternoon (except results in Fig. 4f), consistent with the fact that gas transfer velocity increases with temperature, thus more ^{222}Rn is lost as the stream warms during the day (Fig. 3f).

Combining all sites in the BC watershed, NO_3^- and N_2O showed consistent relationships. There was a significant, positive relationship between NO_3^- and

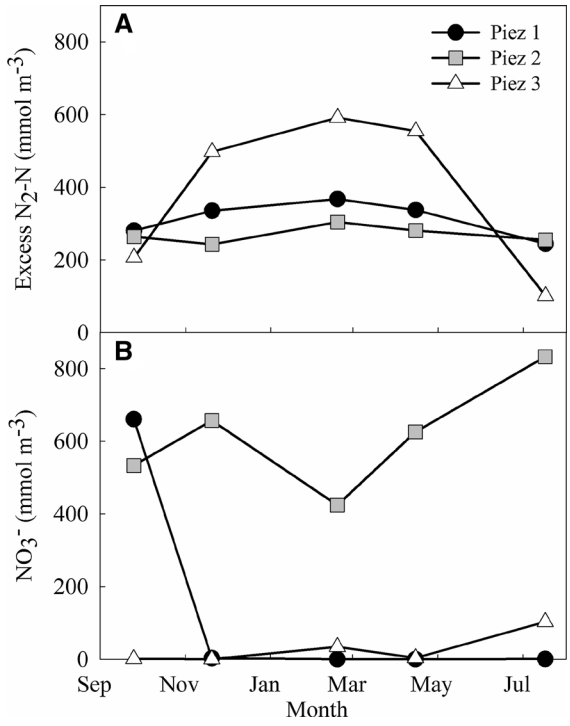

Fig. 2 Temporal patterns of NO_3^- and excess N_2–N from individual in-stream piezometers within the main stem of the BC watershed (BC1 reach)

Table 4 Details of linear and non-linear regressions including X and Y variables, R^2, P-value and sample size, N

Sites	X	Y	Equation	R^2	P	N
BC	ASD (cm)	Groundwater piston velocity (m s^{-1})	$y = 10^{-7}x + 8^{-7}$	0.73	<0.001*	11
BC	Reach averaged ^{222}Rn in groundwater (Bq m^{-3})	Groundwater flux per reach (m^3 h^{-1})	$y = 0.0028x - 8.2$	0.70	0.001*	11
BC	Hydraulic head (cm)	^{222}Rn in groundwater (Bq m^{-3})	$y = 197x + 4361$	0.32	<0.001*	40
BC	Groundwater NO_3^- (μmol L^{-1})	Groundwater N_2O (μmol L^{-1})	$y = 0.0013x + 0.094$	0.58	<0.001*	40
BC	Stream NO_3^- (μmol L^{-1})	Stream N_2O (μmol L^{-1})	$y = 0.0012x + 0.19$	0.60	0.003*	11
BC	Groundwater NO_3^- (μmol L^{-1})	Stream NO_3^- (μmol L^{-1})	$y = 0.73x + 2.93$	0.70	0.001*	11
BC	Stream temp (°C)	In-stream N_2 (mmol m^{-2} h^{-1})	$y = 0.23x - 1.3$	0.32	0.06	11
BC	Stream temp (°C)	In-stream N_2O (μmol m^{-2} h^{-1})	$y = e^{0.21x}$	0.83	<0.001*	11
BC	Stream temp (°C)	N_2O emissions (μmol m^{-2} h^{-1})	$y = e^{0.21x}$	0.83	<0.001*	11
BC, MH, SF	N_2:Ar	Total biogenic N_2 (mmol m^{-2} h^{-1})	$y = 4.9x - 187$	0.51	0.006*	13
BC	ASD (cm)	Total biogenic N_2 (mmol m^{-2} h^{-1})	$y = 0.22x - 0.5$	0.78	<0.001*	11

An * in the P column denotes a significant relationship at $\alpha = 0.05$

N_2O concentration in stream water ($r^2 = 0.64$, $P < 0.01$, Fig. 5a) as well as NO_3^- and N_2O in groundwater ($r^2 = 0.58$, $P < 0.001$, Fig. 5b). The slopes of these regressions were the same, but the tighter fit around the surface water data indicates the integrating effect of streams on heterogeneous groundwater flow paths. Stream water NO_3^- concentration was also positively correlated with the average groundwater NO_3^- concentration ($r^2 = 0.70$, $P < 0.01$) indicating that groundwater was the dominant NO_3^- source in BC stream water. The slope of this regression suggests 73 % (SE of slope = 16 %) of the nitrate in emerging groundwater appeared in surface water, demonstrating in-stream nitrate removal by denitrification or assimilation.

Gas transfer velocity

Gas transfer velocities estimated by ^{222}Rn were more consistent compared to those from Ar regardless of the calculation method (Fig. 6). In fact, the *k*600 values calculated from ^{222}Rn data and the modified McCutchan and simple unit reach equations were not significantly different when comparing the means of the distributions from the Monte Carlo analysis within all studies (pairwise t-test, $P > 0.05$). However, the modified McCutchan et al. (2003) equation applied to ^{222}Rn estimated significantly higher values than the other two ^{222}Rn approaches. Ar as a tracer for *k* often estimated unreasonable values in magnitude and uncertainty. The mean 95 % confidence interval across all studies for Ar was ±0.54 (mean = 1.8), ±37.0 (mean = 3.7), and ±0.5 (mean = 4.2) m day^{-1} for the McCutchan, modified McCutchan et al. (2003), and simple unit reach equations respectively. However, for ^{222}Rn, the mean 95 % confidence interval across studies was ±0.085 (mean = 3.6), ± 0.066 (mean = 3.3), and ±0.065 (mean = 3.4) m day^{-1} for the McCutchan, modified McCutchan et al. (2003), and simple unit reach equations respectively. Among the Ar methods, the McCutchan et al. (2003) approach performed best, and is thus displayed in Fig. 6 to compare with the ^{222}Rn results. Based on these results, and to be consistent with biogenic N gas

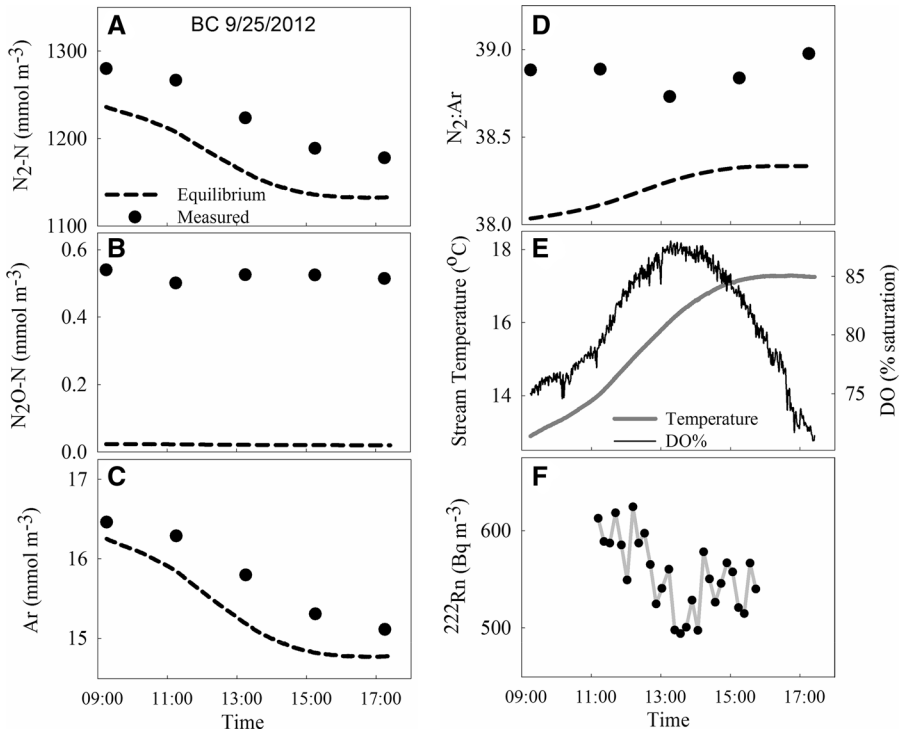

Fig. 3 Example of dissolved gas data in surface water from a typical study in the BC watershed (BC1 9/25/2012) including **a** N_2–N, **b** N_2O–N, **c** Ar, **d** N_2: Ar, **e** dissolved oxygen and temperature, and **f** ^{222}Rn activity

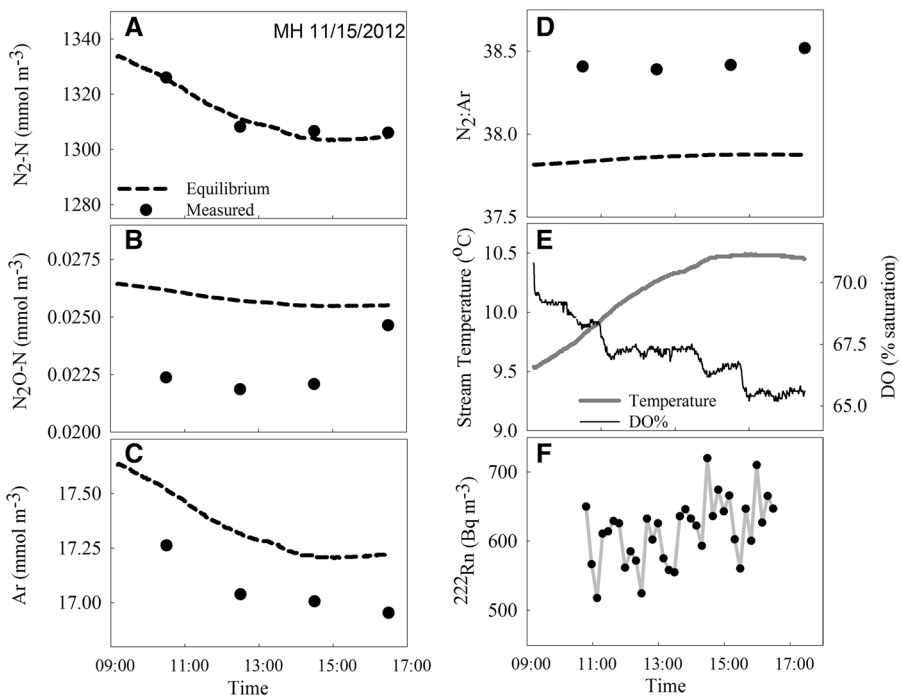

Fig. 4 As in Fig. 3, dissolved gas concentrations in surface water but in the MH forested site on 11/15/2012

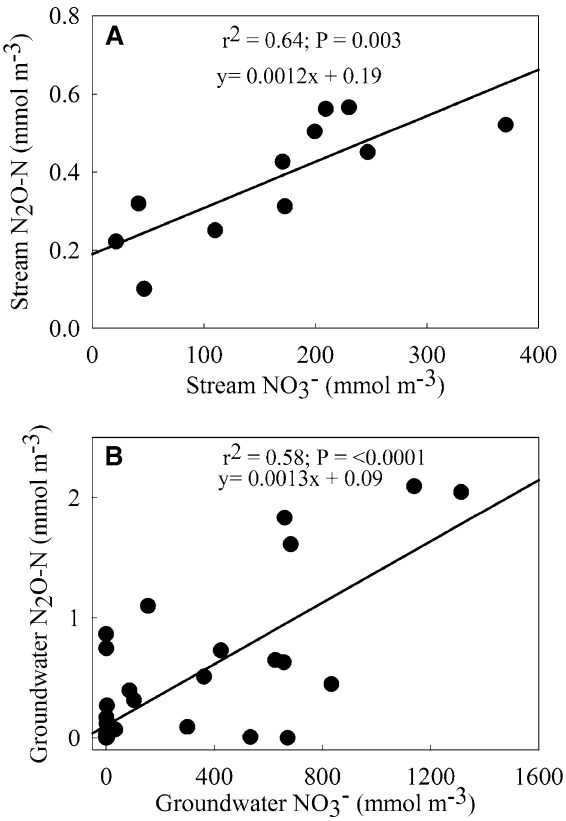

Fig. 5 **a** Linear regressions between NO_3^- and N_2O–N concentrations for groundwater and **b** surface water using data from all BC reaches

flux equations, the method used to calculate gas transfer velocity was the modified McCutchan et al. (2003) equation with ^{222}Rn data.

N_2 and N_2O production

The delivery of biogenic N_2 from groundwater (F_{gw}) was always positive and generally the dominant biogenic N_2 source. Including all sites, total flux of biogenic N_2 (F_T) ranged from −0.24 to 14.6, in-stream production (F_{st}) from −1.5 to 7.7, and groundwater delivery (F_{gw}) from 0.38 to 6.91 mmol N m^{-2} h^{-1} (Table 5). In the BC and SF reaches, biogenic N_2 delivery by groundwater (F_{gw}) accounted for 31-100 % (mean = 73 %) of the total biogenic N_2 flux, assuming when in-stream rates are negative 100 % of the total N_2 is from groundwater. In three of the thirteen studies, negative in-stream production was calculated. This might suggest N fixation, which is unlikely in the two studies from the BC watershed considering the high NO_3^- concentration, but possible in the MH reach where NO_3^- concentrations were low. We interpret negative N_2 production rates in streams with high NO_3^- as below the detection limit of this method.

N_2O was produced mostly in-stream and rates varied seasonally. Including all sites, total N_2O flux ranged from -0.08 at MH in November to 215 μmol N m^{-2} h^{-1} at BC in July (Table 5). In-stream production was detected at all sites, ranging from 0.06 to 204 μmol N m^{-2} h^{-1}, and was responsible for most (66–100 %, not including MH) of the total N_2O flux, with the remaining portion attributable to groundwater delivery (−0.15 at MH to 11.2 μmol N m^{-2} h^{-1} at BC). N_2O emission rates were also calculated for comparison with the literature, but were nearly equivalent to in-stream production, ranging from −0.09 at MH to 202 μmol N m^{-2} h^{-1} at BC (or 74–100 % of total flux at BC). N_2O emissions in the BC stream reaches were among the highest published values from lotic systems which have ranged up to 175 μmol N m^{-2} h^{-1} (Beaulieu et al. 2007; Harrison et al. 2005; Hemond and Duran 1989; Stow et al. 2005), although higher episodic emissions (~4000 μmol N m^{-2} h^{-1}) have been observed in extremely N polluted systems near wastewater treatment facilities (Hasegawa et al. 2000).

In stream production of biogenic N gases generally increased as temperature increased. In-stream production and emissions of N_2O increased exponentially with mean daily stream temperature ($r^2 = 0.83$, $P < 0.01$, Fig. 7b, Table 4) in the BC watershed, but groundwater delivery of N_2 or N_2O was not affected by stream temperature ($P > 0.05$). However, the relationship between in-stream N_2 production and mean daily stream temperature was marginally not significant ($r^2 = 0.32$, $P = 0.06$, Fig. 7a). Total biogenic production of N_2 gas was also significantly related to the mean N_2:Ar ratio during the study across all sties ($r^2 = 0.51$, $P < 0.01$, Fig. 8).

N_2O emissions

Modified ratios for deriving emission factors from streams and groundwater estimated more realistic and constrained values compared to a simple N_2O–N:NO_3^- ratio. In all streams except MH, the simple ratio and modified ratio resulted in a comparable mean

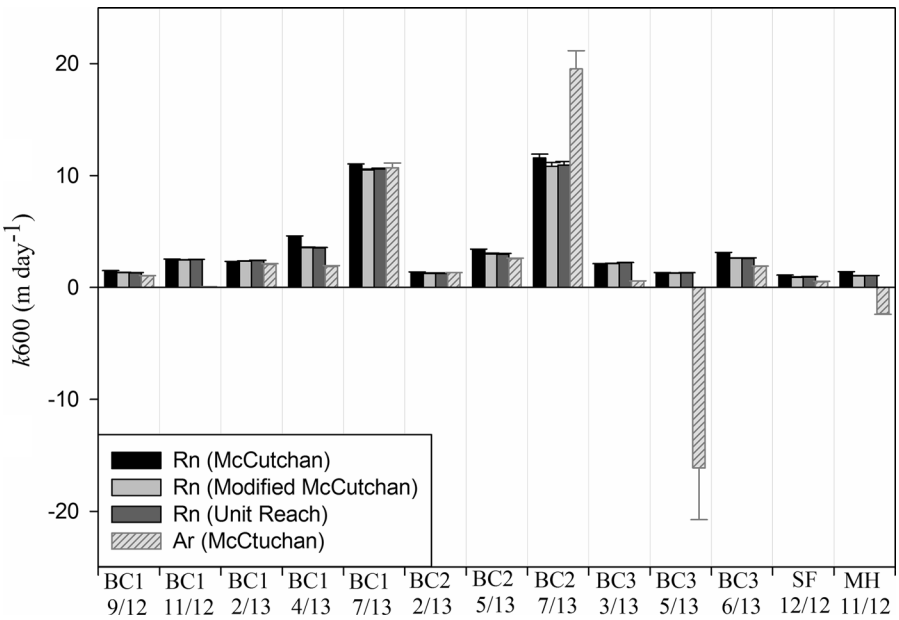

Fig. 6 *k*600 values (m day^{-1}) from all studies using ^{222}Rn data applied to all three equations (McCutchan, Modified McCutchan, Simplified Unit Reach) and Ar data applied to one equation (McCutchan). *Error bars* represent the 95 % confidence interval in only one direction

Table 5 Total, in-stream, and groundwater delivery N_2 and N_2O flux rates as well as N_2O emissions

Date	Site	Total N_2	In-stream N_2	Ground-water N_2	Total N_2O	In-stream N_2O	Ground-water N_2O	N_2O Emissions
9/25/12	BC1	2.3 (0.07)	0.9 (0.07)	1.4 (0.02)	25.8 (0.5)	21.1 (0.6)	4.69 (0.3)	23.1 (0.5)
11/20/12	BC1	7.0 (0.09)	3.0 (0.08)	4.0 (0.08)	41.5 (0.4)	39.3 (0.5)	2.14 (0.2)	34.8 (0.4)
2/18/13	BC1	5.3 (0.08)	1.0 (0.08)	4.3 (0.09)	17.2 (0.2)	14.6 (0.3)	2.55 (0.2)	14.2 (0.2)
4/15/13	BC1	6.7 (0.12)	0.8 (0.11)	5.9 (0.10)	30.1 (0.4)	25.8 (0.5)	4.32 (0.3)	26.8 (0.3)
7/17/13	BC1	14.6 (0.30)	7.7 (0.26)	6.9 (0.26)	215 (2.1)	204 (2.3)	10.8 (0.8)	202 (2.1)
2/25/13	BC2	2.3** (0.14)	−0.4* (0.14)	2.7 (0.03)	2.60 (0.1)	2.59 (0.1)	0.01 (0.01)	2.13 (0.1)
5/14/13	BC2	4.1 (0.18)	0.4 (0.17)	3.7 (0.08)	24.2 (0.6)	23.3 (0.6)	0.90 (0.05)	22.3 (0.6)
7/9/13	BC2	10.1 (0.43)	6.7 (0.43)	3.4 (0.11)	166 (5.0)	164 (5.0)	2.34 (0.3)	160 (5.0)
3/4/13	BC3	7.1 (0.09)	2.3 (0.10)	4.8 (0.07)	26.7 (0.3)	17.9 (1.0)	8.80 (0.9)	21.8 (0.3)
5/6/13	BC3	3.7** (0.06)	−1.5* (0.07)	5.2 (0.05)	30.5 (0.5)	22.8 (1.0)	7.66 (0.9)	22.6 (0.5)
6/25/13	BC3	6.8 (0.13)	1.0 (0.14)	5.7 (0.08)	76.3 (0.9)	65.1 (1.5)	11.2 (1.2)	66.6 (0.9)
12/13/12	SF	1.2 (0.04)	0.8 (0.05)	0.4 (0.03)	3.9 (0.1)	3.88 (0.1)	−0.003 (<0.01)	3.4 (0.1)
11/15/12	MH	−0.2 (0.02)	−0.7 (0.05)	0.5 (0.06)	−0.08 (<0.01)	0.06 (<0.01)	−0.15 (<0.01)	−0.1 (<0.01)

All N_2 rates are in mmol N m^{-2} h^{-1} and all N_2O rates are in μmol N m^{-2} h^{-1} ± the 95 % confidence interval in parenthesis

* Negative in-stream N_2 rates in BC should be interpreted as below detection limit in streams with high NO_3^- concentrations

** If in-stream N_2 is negative, total N_2 should be interpreted as equal to groundwater

EF5-g of 0.32 and 0.29 % respectively; and therefore were generally in agreement with the IPCC EF5-g of 0.25 %. However, the simple ratio varied over a greater range compared to the modified ratio (Fig. 9c, d), and the modified ratio performed better in the MH stream, reducing EF5-g from 3.86 to −0.46 %. This negative EF5-g reflected that MH was a small N_2O sink.

In groundwater, methods for calculating EF5-g produced drastically different results. The simple

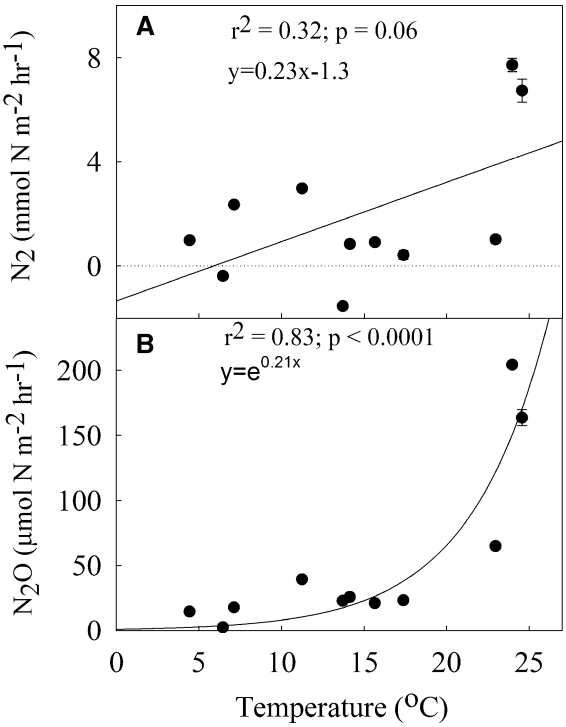

Fig. 7 Mean daily stream temperature relationship with **a** in-stream N_2 production and **b** in-stream N_2O production using data collected in the BC watershed. **a** is a linear fit and **b** is exponential

N_2O–N:NO_3^- ratio estimated unreasonably high and variable values (mean = 4.4 %, range = 0.002–70 %). The modified ratio was lower, more constrained (mean = 0.042 %, range = −0.059 to 0.59 %) (Fig. 9a, b), and comparable with the range (0.043–0.44 %) reported by Weymann et al. (2008) using the same method. Biogenic N_2–N can be the dominant N species in groundwater; therefore, excluding excess N_2–N in this calculation can drastically skew estimates of EF5-g, explaining the discrepancies when comparing these two methods.

Watershed N balance

The watershed N balance showed that biogenic N_2 gases fluxes through the stream network accounted for all of the missing N and more (117 %) over the 2013 water year. The missing N was 10.3 kg N ha^{-1} $year^{-1}$, while total N biogenic N_2 gas flux through streams was 12 kg N ha^{-1} $year^{-1}$ with 8.3 and 3.7 kg N ha^{-1} $year^{-1}$ attributed to groundwater delivery and in-stream production respectively. Total biogenic N_2O flux only added an additional 0.085 kg N ha^{-1} $year^{-1}$ to (0.71 %) to the missing N.

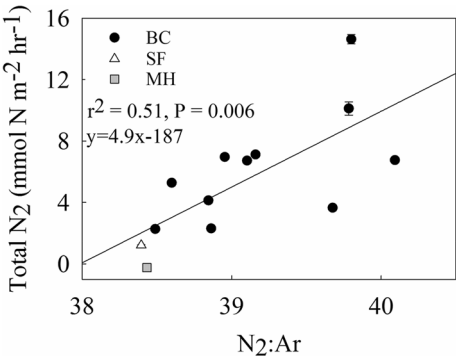

Fig. 8 Total biogenic N_2 production versus the average N_2:Ar ratio in stream water including data from all three sites, BC, MH, and SF

Measurement and modeling of N fluxes suggest that the relative magnitudes of different fluxes changed throughout the year. Hydrologic Nr export and groundwater delivery of biogenic N_2 were the largest fluxes in the annual watershed budget (Table 6) and were the largest fluxes most days of the year (Fig. 10). Groundwater delivery of biogenic N_2 could exceed hydrologic N export during low flow. Watershed N_2 losses due to in-stream production were typically orders of magnitude lower than hydrologic Nr export and groundwater delivery of biogenic N_2, except during summer months where watershed scale N loses from in-stream sources were equivalent to groundwater delivery of biogenic N_2.

Discussion

Nitrogen gas fluxes

The results support our hypothesis that N enriched streams are hotspots for fluxes of biogenic N gases as a result of in-stream and groundwater processes, but the relative importance of these sources differed for N_2 and N_2O. Groundwater delivery was the primary source of biogenic N_2. The rate of delivery of biogenic N_2 from groundwater exceeded in-stream production for all but one study, which occurred in the summer of 2013. The importance of groundwater delivery of N_2 may be enhanced in the BC watershed as a result of channelization that drains groundwater by design. Yet, even in the non-channelized SF reach characterized by

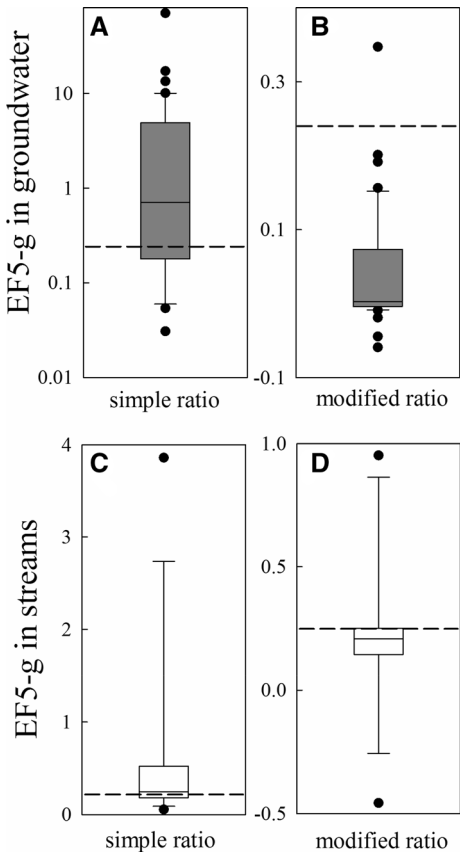

Fig. 9 Emission factors (EF5-g) in streams and groundwater (*rows*) comparing the IPCC and modified methods (*columns*). Note the different scales of the y-axis for each plot. The *dashed line* indicates IPCC's EF5-g equal to 0.25 %

low groundwater discharge and low excess N_2 values, groundwater delivery still accounted for 31 % of total biogenic N_2 flux. It should be noted that in-stream N_2 production rates were not trivial ($\sim$0–7.7 mmol N m^{-2} h^{-1}) and fit within the range of previous open channel studies (0–15.9 mmol N m^{-2} h^{-1}; Baulch et al. 2010).

In contrast to N_2, groundwater delivery was a small source of N_2O compared to in-stream production, accounting for only 0.4–33 % (12 % on average) of the total N_2O flux and 15 % of N_2O emissions in the BC stream network. N_2O concentrations in emerging groundwater were highly variable, characterized by the greatest coefficient of variation compared to other dissolved gases in groundwater. However, to produce the observed total N_2O flux exclusively from a groundwater source would require reach-averaged groundwater N_2O concentrations 1.5–60 fold greater than the measured concentrations, depending on the particular study. This indicates that in-stream production was the dominant source of N_2O in stream water, and ultimately to the atmosphere.

The variability in groundwater chemistry suggests distinct N sources and biogeochemical transformations along flow paths. The positive, linear relationship (Fig. 5b) between NO_3^- and N_2O in groundwater suggests a terrestrial nitrification source of N_2O (Hiscock et al. 2003; Ueda et al. 1993; Vilain et al. 2011; Werner et al. 2010). High NO_3^- and N_2O samples may have traveled along flow paths with oxic conditions and less opportunity for denitrification, whereas low NO_3^- samples likely passed through reducing conditions associated with riparian areas and saturated depressions. The observed relationships in groundwater chemistry could also partially result from groundwater originating from source areas with differing nitrate concentrations. However, the high excess N_2–N concentrations in all samples of emerging groundwater in the BC watershed provide strong evidence of widespread denitrification occurring throughout the watershed.

Controls of N_2 and N_2O Production

Temperature is one potential reach-scale control of biogenic N gas fluxes through streams. The strong non-linear effect of temperature on in stream production of N_2O (Fig. 7) likely reflects the first order control of microbial activity by temperature that has been well demonstrated for denitrification through laboratory studies (Nowicki 1994; Pfenning and McMahon 1997; Stanford et al. 1975). Another field based study of in-stream denitrification also observed a positive relationship between temperature and in-stream denitrification (McCutchan and Lewis 2008).

Hydraulics may also have a strong effect on N gas fluxes through streams. Biogenic N_2 flux increased with antecedent stream depth (ASD), but it is difficult to know how much this reflects the positive correlation of ASD with groundwater piston velocity (Table 4), which is a term in the flux calculation of biogenic N_2. Floods could create ideal hydro-biogeochemical conditions for denitrification. For example, residence time in the riparian zone increases when the hydraulic head gradient between the aquifer and stream surface reverses injecting NO_3^- rich stream water into low-oxygen riparian groundwater rich in labile organic

Table 6 Annual fluxes (Kg N ha^{-1} $year^{-1}$) of biogenic N_2 and N_2O and hydrologic Nr export compared to NANI during the study year (water year 2013) and during conditions of average water discharge

Watershed budget term	Water year 2013	Average discharge
Biogenic N_2 in-stream	3.7	3.7
Biogenic N_2 groundwater	8.3	3.5
Biogenic N_2 total	12	7.2
Biogenic N_2O in-stream	0.076	0.076
Biogenic N_2O groundwater	0.009	0.004
Biogenic N_2O total	0.085	0.08
NANI	30.3	30.3
Hydrologic Nr	20	8.5
Missing N = NANI − Nr	10.3	21.8
Total biogenic N_2 + N_2O	12	7.3
as % of missing N	117 %	33 %

Fluxes of hydrologic Nr and groundwater-delivered biogenic N_2 and N_2O during average discharge are estimated by multiplying by ratio of average water yield to 2013 water yield (36/85 cm). In-stream production of N_2 and N_2O are assumed to be the same regardless of the water yield. NANI is estimated as a long term average

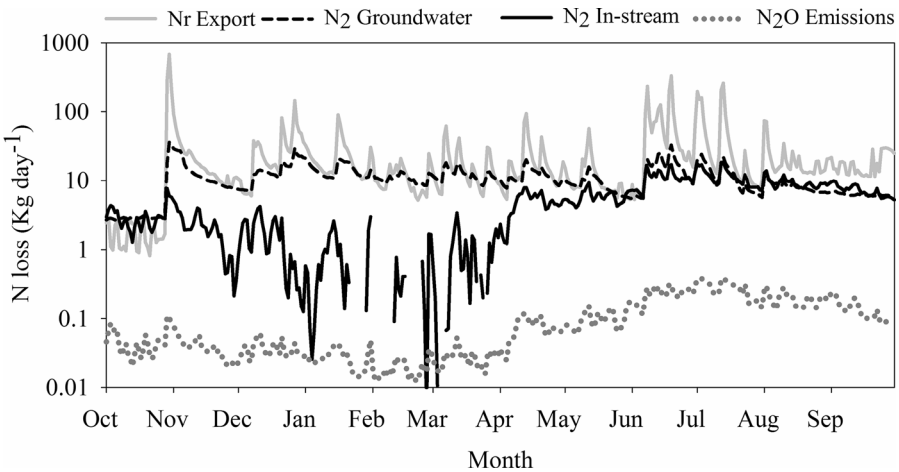

Fig. 10 Total N loss (Kg day^{-1}) from the BC stream network as a result hydrologic Nr export, groundwater delivery of biogenic N_2, in-stream N_2 production, and N_2O emissions. Export was measured and N gas fluxes were empirically modeled

matter. Modeling and field studies have shown stream depth fluctuations can stimulate floodplain, riparian, and in-stream denitrification due to similar mechanisms (Gu et al. 2008, 2012; Ocampo et al. 2006; Roley et al. 2012). Furthermore, loading of biogenic N_2 gas from groundwater to streams may increase during floods as rates of groundwater discharge increase. Future studies could incorporate the effects of hydraulics and temperature to advance our understanding of biogenic N gas fluxes through streams.

The N_2:Ar ratio in stream water is not a control of biogenic N_2 flux but may be a semi-quantitative indicator of watershed scale denitrification detectable in streams. Aggregating data from all sites, the mean N_2:Ar in surface water was positively correlated with total biogenic N_2 flux ($r^2 = 0.51$, $P < 0.01$, Fig. 8). To apply this in other streams, open channel measurements of biogenic N_2 flux would be needed to calibrate the relationship between flux and N_2:Ar. We recognize N_2 concentration is present in the calculation of both variables of this relationship, and suggest that N_2:Ar is a potential indicator of the relative amount of biogenic N_2 fluxing through the stream but not a predictive tool.

Gas transfer velocity

^{222}Rn was more reliable than Ar for estimating gas transfer velocity at our study sites. The analytical error associated with Ar in stream water (0.1 %) as well as the average spatial variation in groundwater Ar concentration (5.8 %) was much lower than ^{222}Rn (analytical = 12 %, spatial = 26 %, Table 2); however, the uncertainty in k from ^{222}Rn was orders of magnitude lower than Ar when comparing the mean 95 % confidence intervals from each method as discussed in results. Estimating k from Ar involves measuring small deviations of Ar from atmospheric equilibrium. This requires high analytical precision and accuracy in measurements of concentration, temperature, and atmospheric pressure as well as relatively homogenous groundwater Ar concentrations. This method is most applicable when Ar concentration is furthest from equilibrium due to large diel temperature swings and/or when groundwater influx is low. The ^{222}Rn method requires less analytical precision given the large difference between stream and groundwater ^{222}Rn activities. Also, the theoretical equilibrium value is zero, thus eliminating temperature and pressure measurement error. Uncertainty is driven by analytical error inherent to the RAD7 and the spatial variability in groundwater ^{222}Rn concentrations, which can be assessed by sampling groundwater at multiple locations. Another advantage is that ^{222}Rn measurements can be obtained in the field and are methodologically simple compared with Ar or artificial tracers such as propane or sulfur hexafluoride (SF_6). Furthermore, the simplified unit reach method (Eq. 6) does not require repeated measurements of surface water ^{222}Rn; therefore, it is feasible to rapidly assess gas transfer velocity in many streams with several surface and groundwater samples as well as groundwater inflow rates. However, a more rigorous calculation framework, such as Bayesian inverse modeling, would be useful in combining parameter and uncertainty estimation to open channel equations for gas transfer velocity as well as biogenic N gas production.

Limitations to open channel methods

One source of uncertainty in open-channel estimates of biogenic N_2 fluxes is the N_2 signal to noise ratio. A low signal to noise ratio makes precise measurements of in-stream N_2 production difficult in the late winter when low temperatures limit in-stream denitrification and groundwater delivery of biogenic and non-biogenic N_2 is at a maximum. Open channel studies often report lower in-stream denitrification rates ranging from 0 to 0.03 mmol m^{-2} h^{-1} during winter months (McCutchan and Lewis 2008; Pribyl et al. 2005). However, previous studies have preferentially selected reaches with less groundwater inputs compared to our study sites.

Another source of error is stripping of dissolved N_2 from groundwater due to formation of bubbles (ebullition) of N_2 or CH_4, which would decrease estimates of N_2 fluxes from groundwater. Fluxes of bubbles composed primarily of N_2 totaled 6–16 % of the diffusive fluxes at two locations on the Platte River (Higgins et al. 2008), but in our study only diffusive fluxes were measured by the open-channel method. Sharp drops in N_2 often coincide with increases in methane in piezometers sampled at our study sites, suggesting that methane ebullition removes N_2 (Fisher et al. 2010; Fortuin and Willemsen 2005; Fox et al. 2014). Even if groundwater N_2 has been lost by ebullition, our measurements still provide a basis for estimating dissolved N_2 input to the stream from emerging groundwater if our piezometers are representative in terms of the amount of ebullition that has occurred.

In contrast to ebullition, dissolution of air bubbles trapped during groundwater recharge ("excess air"; Heaton and Vogel 1981) could cause overestimation of biogenic N_2 in groundwater. Analysis of noble gases in emerging groundwater in the BC streams prior to this study suggested that excess air accounted for a small portion ($\sim$5 %) of the excess N_2 in emerging groundwater (Hamme and Fisher, unpublished data).

N_2O emissions

Contributions of N_2O from groundwater to the atmosphere may be overestimated by the IPCC emission factors. Weymann et al. (2009) demonstrated that groundwater N_2O contributes negligibly to the flux of N_2O to the atmosphere from surface soils. Similarly, we found that groundwater delivery of N_2O makes up a small portion of total biogenic N_2O emissions (15 % on average) from strongly gaining agricultural streams where groundwater would be expected to be an

important source compared to other aquatic systems. N_2O may be consumed in the aquifer and riparian zones prior to emergence in surface water or diffusion through the vadose zone. Additionally, N_2O was largely produced in-stream, yet the IPCC's estimate of global N_2O emissions assumes that groundwater is the dominant N_2O source in small streams and that in-stream production is the dominant N_2O source in large rivers (Mosier et al. 1998). These results suggest that (1) small streams and large rivers could be grouped together, instead of streams and groundwater, under the current EF5-r of 0.25 % (IPCC 2006) and (2) the groundwater EF5-g could be lowered from 0.25 to ~0.04 %, but confirming this would require wider assessment beyond our geographically limited study.

Watershed N budget

Our findings support our hypothesis that N gas fluxes through streams could account for a large portion of the missing N. However, the amount of missing N was probably lower than average during our study due to the high water yield, 85 cm compared to the average water yield of 36–43 cm for nearby watersheds (Fisher et al. 2010; Darling 1962; Jordan et al. 1997). The high water yield likely increases the Nr export by the stream, explaining the unusually high portion of NANI lost through hydrologic Nr export (66 %). In contrast, hydrologic Nr export from nearby watersheds studied by Jordan et al. (1997) was estimated at 15–25 % of NANI during average flow conditions. Hydrologic Nr export is often around 25 % of NANI (Howarth et al. 2012), but the percentage of NANI accounted for by export varies widely and can reach 100 % (McIsaac and Hu 2004).

We explored the potential effect of water yield on the portion of the missing N accounted for by N gas fluxes through streams. If hydrologic Nr export from BC during a year with average water yield is proportionally lower than in our study year, then Nr export would be 8.5 kg N ha^{-1} $year^{-1}$ and would account for 28 % of NANI (Table 6). To estimate fluxes during an average year we multiplied the fluxes of hydrologic Nr, and groundwater-delivered biogenic N_2 and N_2O by 36/85, the ratio of average water yield to the 2013 yield. We assumed that in-stream production of N_2 and N_2O would not be effected by water yield. Under average water yield conditions, biogenic N gas fluxes through the streams would only account for 33 % of the missing N (Table 6). This is still a significant flux approximately equal to hydrologic Nr export, but there remains ~50 % of NANI that cannot be accounted for in this scenario due to N storage or gas flux through soils.

Accounting for 117 % of the missing N in our study year may reflect inter-annual variability of N storage within the watershed (e.g. in soil, groundwater, biota). In this case, there may have been a net loss of stored N via hydrologic Nr export and biogenic N gas fluxes. On the other hand, it could also suggest uncertainty in watershed budget terms. But NANI varies little from year to year, while hydrologic export of Nr and loss of biogenic N gases markedly fluctuate as a function of water yield and other environmental factors. Therefore, N budgets will undoubtedly be unbalanced in a single year. Developing N budgets over several years spanning a range of water yields could provide an informative perspective on long-term watershed N budgets.

Conclusions

This study provides a conceptual and methodological framework for assessing the importance of headwater stream networks in losses of biogenic N gases. Regarding methods, we demonstrated that the one-station open channel method of McCutchan et al. (2003) can be used to quantify biogenic N_2 and N_2O production from in-stream and groundwater sources by using Ar as a tracer for groundwater recharge temperature. We also found that ^{222}Rn can be used as a natural and more precise tracer for measuring gas transfer velocity in gaining streams.

Our findings support our conceptual model (Fox et al. 2014) that biogenic N gases are produced throughout the watershed and that hydrological connections funnel biogenic N gases to streams, which act as chimneys (sensu Hotchkiss et al. 2015) venting biogenic N gases to the atmosphere. Flux of biogenic N_2 from streams was dominated by groundwater sources while flux of biogenic N_2O from streams was dominated by in-stream production. Our measurements of biogenic N_2O flux supported the emission factor of 0.25 % used by the IPCC to scale up N_2O emissions in streams and rivers, but suggested a lower emissions factor for groundwater (~0.04 %).

This conceptual model of N gas flow through streams and watersheds should be explored further in a

variety of systems and incorporated into N biogeochemical accounting. Terrestrially derived N_2 fluxes through streams could account for a large portion of the missing N, but the portion will likely vary with water yield. Integrating biogenic N gas flux measurements with watershed hydro-biogeochemical models across a range of stream and watershed types presents a significant challenge, but is a logical step towards an improved understanding of N biogeochemistry and managing the ultimate fate of anthropogenic N.

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