Quantifying the production of dissolved organic nitrogen in headwater streams using ^{15}N tracer additions

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Abstract

Most nitrogen (N) assimilation in lake and marine ecosystems is often subsequently released via autochthonous dissolved organic nitrogen (DON) production, but autochthonous DON production has yet to be quantified in flowing waters. We measured in-stream DON production following 24 h ^{15}N-nitrate (NO_3^-) tracer additions in 36 headwater streams, a subset of sites from the second Lotic Intersite Nitrogen eXperiment. Streams were located in five North American ecoregions and drained basins dominated by native vegetation, agriculture, or urban land use. Using a two-compartment model, we could quantify DON production in 15 streams as a function of DO[15]N derived from ^{15}N tracer in biomass compartments. The streams with detectable DON production had higher % modified land use (agriculture + urban) in their basins than did streams with undetectable DON production. Median DON production represented 8% of total NO_3^- uptake when we used N biomass estimates based on N assimilated over 1 d (measured directly from the ^{15}N additions). Median DON production was 17% of total NO_3^- uptake when we used N assimilated over 42 d (extrapolated from previous ^{15}N tracer studies). Variation in DON production was positively correlated with ecosystem respiration, indicating that stream heterotrophy may influence DON production. In-stream DON production was similar in magnitude to stream denitrification and nitrification, indicating that the production of autochthonous DON can represent a substantial transformation of stream N. Our results confirm that headwater streams can quickly convert inorganic N into organic forms, although the ultimate fate of DON remains unclear.

Dissolved organic nitrogen (DON) generally represents a large fraction of the total dissolved nitrogen (N) pool in relatively unpolluted streams (Lewis et al. 1999; Perakis and Hedin 2002), and total organic N (including particulate N) dominates N flux in large rivers across North America (Scott et al. 2007). Agriculture and urban land uses often increase DON concentrations and bioavailability in streams across the United States (Pellerin et al. 2006), although this increase is generally lower than the drastic increases in inorganic N (Stanley and Maxted 2008). It is typically assumed that the majority of DON in streams originates from allochthonous sources such as riparian soils and that the resulting DON pool comprises mainly refractory humic compounds (Aitkenhead-Peterson et al. 2003).

Most studies examining stream DON have focused on measuring DON fluxes in stream water or describing allochthonous sources of DON. Because production of dissolved organic carbon (DOC) by in-stream processes can be significant (Kaplan and Bott 1989), DON production may also be significant in streams. Production of autochthonous dissolved organic matter (DOM, a portion of which is DON) by aquatic biota can occur through several mechanisms, including the release of cell constituents via death, senescence, viral lysis, or herbivory (Baines and Pace 1991; Bertilsson and Jones 2003), and these processes occur in both autotrophs (e.g., algae, macrophytes) and heterotrophs (i.e., bacteria, fungi). Additionally, DOM release from aquatic primary producers can occur via passive leakage across cell membranes or active exudation, although the physiological explanation behind this release is not well known (Bertilsson and Jones 2003). Autotrophic DOM production may also vary among phytoplankton taxa (Hellebust 1965), decrease with cell size (Malinsky-Rushansky and Legrand 1996), or increase in actively growing phytoplankton (Baines and Pace 1991). Aquatic DON production is usually attributed to extracellular release via autotrophs in both marine and lake ecosystems, and we predict that this mechanism will apply in streams as well.

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It has been challenging to quantify the proportion of in-stream DON production relative to total DON flux because of the lability and rapid turnover rates of this N source, which may include a diversity of amino acids, peptides, and proteins (Kirchman 2003). Kaplan and Newbold (2003) suggested that monomeric forms of DON, which are compounds likely resulting from autotrophic DON production, constitute only a small proportion of the total DON pool in streams. Yet monomeric forms of DON are rapidly taken up in stream both by benthic biofilms (Johnson et al. 2009) and via uptake in hyporheic zones (Brookshire et al. 2005). Results from ^{15}N ammonium (NH_4^+) tracer additions in relatively pristine headwater streams measured in the first Lotic Intersite Nitrogen eXperiment (LINX I) found that DO^{15}N export could be detected in only four of 10 streams, and in those four streams just 4–10% of added ^{15}N was exported as DO^{15}N (reviewed by Ashkenas et al. [2004]), indicating either that in-stream DON production was minimal or that autochthonous production of DO^{15}N was rapidly re-assimilated. In contrast to headwater streams, Bronk et al. (1994) found 25–41% of the N assimilated by phytoplankton was released as DON in oceanic, coastal, and estuarine environments.

As part of the LINX II project, we quantified in-stream DON production and estimated the proportion of DON production relative to total N uptake across a wide range of streams flowing through varying land uses from multiple regions in North America. We measured DO^{15}N following 24 h ^{15}N-nitrate ($^{15}NO_3^-$) additions to 36 streams located in five different regions, which varied in both their rates of gross primary production (GPP) and in water column nutrient availability. Based on results from lake and marine ecosystems, we predicted that in-stream DON production would be influenced by human land use and would vary by region because both of these factors influence GPP and nutrient concentrations. Specifically, GPP is higher in streams with open canopies (e.g., prairie, desert, and tundra streams) and higher incident light (Mulholland et al. 2001; Bernot et al. 2010). Additionally, human land use (e.g., agriculture, urbanization) can reduce stream canopy cover and increase light availability (Allan 2004) and also increase the availability of both inorganic N and phosphorus (P) via fertilizer and sewage inputs (Kemp and Dodds 2001; Paul and Meyer 2001), which in combination can stimulate GPP (Allan 2004; Bernot et al. 2010). We predicted that high rates of GPP, whether associated with naturally open canopies in reference streams (e.g., deserts and prairies) or as a result of human modification (e.g., agriculture and urbanization), would result in higher in-stream DON production because of increased autotrophic extracellular release of DON.

Methods

Site description—We measured DO^{15}N in a subset of headwater streams ($n = 36$) from the LINX II. Streams were located in five regions—three streams from Massachusetts (MA), six streams from southwest Michigan (MI), and nine streams each from Puerto Rico (PR), western

North Carolina and north Georgia (NC), and northwest Wyoming (WY)—and ranged in discharge from 2 to 268 L s^{-1} during the study period (Table 1). We categorized the streams as reference (i.e., dominated by native vegetation), agricultural, or suburban and urban based on land use adjacent to the stream and immediately upstream in the basin. The percentages of each land-use type were measured for each stream using the U.S. Geological Survey (USGS) National Elevation Data Set and the 2001 USGS National Land Cover Datasets, except in the case of PR, for which we used the 1991–1992 Landsat Thematic Mapper imagery, as derived by Helmer et al. (2002). Across all five regions (MA, MI, PR, NC, and WY) we selected streams to encompass a wide range of conditions rather than to fit distinct land-use categories; a more detailed description was published by Mulholland et al. (2008). In general, native vegetation was primarily forest, ranging from tropical to temperate deciduous, except in WY, where native vegetation was shrub-steppe with little forest canopy cover. Agricultural land use varied regionally and included intensive row crops, cattle grazing, and irrigated pasture. Suburban and urban land use varied in intensity, including dense city centers, town parks, golf courses, and residential development.

$^{15}NO_3^-$ tracer additions—Here we briefly describe the methods for the 24 h $^{15}NO_3^-$ tracer additions to each of the 36 streams conducted in 2003–2006; detailed methods were published by Mulholland et al. (2008). At each site, we added ≥ 98% K$^{15}NO_3$ into the stream for 24 h, along with a conservative tracer (either NaCl or NaBr), at a constant rate to reach a target δ^{15}N enrichment of 20,000‰; this addition resulted in a < 7.5% increase in background NO_3^- concentrations at all sites. All $^{15}NO_3^-$ tracer additions were conducted during baseflow conditions during spring and summer for MA, MI, NC, and WY and during the dry season in PR. In each stream, experimental reach lengths ranged from 260 to 1832 m and varied depending on discharge and background NO_3^- concentrations. We collected background samples for $^{15}NO_3^-$, ^{15}N-ammonium ($^{15}NH_4^+$), DO^{15}N, and ^{15}N content in biomass compartments (*see* description below) at six stations downstream from the ^{15}N release point several hours prior to the start of the $^{15}NO_3^-$ tracer addition to estimate natural isotopic abundance. Then we collected $^{15}NO_3^-$ samples at all stations 12 h and 23 h after starting the ^{15}N tracer addition to calculate $^{15}NO_3^-$ uptake. Finally, we collected samples of DO^{15}N, $^{15}NH_4^+$, $^{15}NO_3^-$, and ^{15}N in biomass compartments 24 h after the tracer addition ended to quantify DO^{15}N production. We collected additional water samples concurrently with isotope sampling to analyze for concentrations of NO_3^-, NH_4^+, and total dissolved nitrogen (TDN); all water samples were filtered within 2 h of collection (precombusted Whatman GF/F, 0.7 μm nominal pore size).

^{15}N sample analysis—We extracted $^{15}NO_3^-$ using the alkaline headspace diffusion method (Sigman et al. 1997), which required that we add 3 g of MgO and 5 g of NaCl to each water sample to drive off NH_4^+ as NH_3 while boiling

Table 1. Stream regions; land-use classifications (class.); and mean physical, chemical, and biological characteristics. Adjacent land use to the stream includes reference streams with native vegetation (REF), agricultural (AG), and urban (URB). Mean stream discharge, temperature (Temp), nitrate (NO_3^-), ammonium (NH_4^+), dissolved organic nitrogen (DON) and soluble reactive phosphorus (SRP) concentrations as well as gross primary production (GPP) and ecosystem respiration (ER) are reported. Modeled in-stream DON production rates ($k_{DON_{prod}}$) are shown, where detected. Parameters below detection are indicated by nd.

Region	Stream	Land-use class	Discharge (L s⁻¹)	Temp (°C)	NO_3^- (μg N L⁻¹)	NH_4^+ (μg N L⁻¹)	DON (μg N L⁻¹)	SRP (μg P L⁻¹)	GPP (g O₂ m⁻² d⁻¹)	ER (g O₂ m⁻² d⁻¹)	$k_{DON_{prod}}$ (h⁻¹)
MA	Cart Creek	REF	5	17.9	15	293	502	2	0.2	3.8	0.0040
MA	IS_104	URB	2	17.1	1336	121	60	2	0.7	9.1	0.0014
MI	Bellingham	AG	23	11.8	1453	28	310	2	0.9	4.1	0.0013
MI	Arcadia	URB	110	20.0	274	32	194	11	0.8	14.1	0.0070
NC	Jerry Branch	AG	26	18.0	406	108	450	18	0.5	4.5	0.0220
PR	Grande	AG	12	23.0	276	11	70	13	5.2	7.6	0.0107
PR	Maizales	AG	25	23.0	206	7	78	12	7.3	5.3	0.0315
PR	Bisley	REF	13	21.3	171	3	10	22	0.05	2.4	0.0056
PR	Pared	REF	5	22.1	105	3	45	7	0.4	0.4	0.0013
PR	Vaca	AG	112	23.0	446	3	163	9	3.1	15.7	0.0593
PR	Petunia	URB	5	24.3	997	15	53	26	0.3	4.6	0.0010
PR	Ceiba	URB	49	25.3	512	50	483	22	9.3	11.7	0.0121
WY	Giltner	AG	158	12.0	50	3	92	3	16.2	11.4	0.0010
WY	Spread	REF	268	14.3	3	2	93	3	3.2	9.8	0.0009
WY	Teton Pines	URB	9	10.9	152	1	26	3	2.7	1.5	0.0003
MA	Gravelly Brook	REF	2	21.4	112	435	802	80	0.2	11.3	nd
MI	Buskirk	AG	6	17.9	82	21	468	11	0.1	5.6	nd
MI	Bullet	REF	6	12.6	385	11	114	2	1.6	15.6	nd
MI	Honeysuckle	REF	99	22.2	4	21	325	4	0.1	7.9	nd
MI	Wayland	URB	12	17.8	695	74	562	5	1.8	4.1	nd
NC	Blacks Branch	AG	189	16.1	173	9	56	7	0.5	8.7	nd
NC	Hoglot Branch	AG	53	17.6	155	17	100	3	0.3	1.6	nd
NC	Big Hurricane Branch	REF	12	14.7	241	6	86	3	0.1	3.4	nd
NC	Cunningham Creek	REF	49	12.7	10	3	2	2	0.05	5.2	nd
NC	Hugh White Creek	REF	19	12.7	7	3	9	3	0.1	2.2	nd
NC	Crawford Branch	URB	45	17.3	103	15	32	4	3	6.5	nd
NC	Mud Creek	URB	52	16.7	140	6	89	2	0.1	7.8	nd
NC	Sugarloaf Creek	URB	80	13.8	54	3	17	3	0.1	17.9	nd
PR	Rit	REF	20	19.0	131	7	23	<1	0.5	4.5	nd
PR	Mtrib	URB	23	20.8	174	2204	847	311	7.1	7.4	nd
WY	Headquarters	AG	131	16.0	1.0	3	141	15	3.3	7.1	nd
WY	Kimball	AG	154	11.2	28	1	70	4	13.6	12	nd
WY	Ditch	REF	56	16.8	<1	2	110	2	2.8	4	nd
WY	Two Oceans	REF	65	12.8	19	4	85	10	2.9	12.6	nd
WY	Fish	URB	103	9.9	235	4	202	6	7.3	nd	nd
WY	Golf	URB	110	18.6	1	1	103	2	4.2	10	nd

to reduce sample volumes to $\sim$ 100 mL. Initial water volumes varied across streams from 0.1 to 2 liters, to obtain at least 20 μg of N for analysis via mass spectrometry. Samples collected during the ^{15}N tracer addition were spiked with ^{14}N to reduce the δ^{15}N by a fivefold measure for analytical purposes. After boiling, we added 0.5 g of Devarda's alloy to each sample to reduce NO_3^- to NH_3 and immediately sealed the samples in polyethylene bottles containing a suspended, acidified (with 25 μL of 2.5 mol L^{-1} KHSO$_4$) Teflon filter pack to trap NH_3 from the headspace. Bottles were then incubated at 60°C for 48 h followed by 1 week at room temperature on a shaker table to ensure complete diffusion of NH_3 into the headspace and onto the acidified filter. After incubation, filters were dried in an acidified dessicator, sealed in tin capsules, and analyzed via mass spectrometry.

We processed water samples for $^{15}NH_4^+$ using a similar alkaline headspace diffusion method (Holmes et al. 1998) by adding 3 g of MgO and 50 g of NaCl per liter of sample; suspending an acidified filter pack in the bottle headspace; and incubating the samples on a shaker table at 40°C for 2 weeks to allow for full diffusion of NH_3 into the headspace to be captured on the acidified filter. Again, sample volumes varied across streams based on background NH_4^+ concentrations and ranged from 0.5 to 4 liters. We spiked $^{15}NH_4^+$ samples with $^{14}NH_4^+$ when concentrations were below 2 μg N L^{-1} to raise the mass of N to 20 μg N to allow detection by mass spectrometry. Following incubation, filters were dried and analyzed using the same procedure as for $^{15}NO_3^-$ samples.

We calculated DO^{15}N by measuring TD^{15}N and subtracting $^{15}NO_3^-$ and $^{15}NH_4^+$. We analyzed TD^{15}N samples using a persulfate digestion, which oxidized all N forms to NO_3^- (Valderamma 1981; Ameel et al. 1993). The persulfate digestion reagent was added to the samples at a ratio of 2 mL reagent to 15 mL sample and was then autoclaved at 120°C for 1 h. After cooling, samples were boiled and incubated as described above for the $^{15}NO_3^-$ samples, ensuring a pH > 10 prior to incubation for optimal diffusion of NH_3. Recovery of N on the filters relative to the expected mass of N calculated from measured TDN concentrations varied across streams and regions. In streams where we were able to fit a model of DON production to measured DO^{15}N flux (*see* description below), mean recovery by region was as follows: MI = 92%, WY = 26%, PR = 45% (range 12–97%), NC = 59%, and MA = 85%. In addition to dissolved N pools, we analyzed the ^{15}N content in various benthic biomass compartments in each stream. Distinct patches of filamentous green algae, epilithon, epipsammon, epixylon, bryophytes, macrophytes, grass, algal mats, sand, microbial biofilm, decomposing leaves and wood, fine benthic organic matter, roots, and rarely invertebrates were sampled at each downstream station throughout the reach using methods described by Tank et al. (2000) and Mulholland et al. (2000). Samples from biomass compartments were dried and ground to fine powder, sealed in tin capsules, and analyzed via mass spectrometry.

The ^{15}N content in water samples and biomass compartments was analyzed on either a Finnigan Delta-S or a Europa 20/20 mass spectrometer at the Stable Isotope Laboratory at the Marine Biological Laboratory in Woods Hole, Massachusetts, or on a Europa Integra mass spectrometer at the Stable Isotope Laboratory at the University of California, Davis, California. Results were expressed as δ^{15}N (‰) = ([$R_{sample}/R_{standard}$] − 1) × 1000, where R_{sample} is the ratio of ^{15}N : ^{14}N of the sample and $R_{standard}$ is the ratio of ^{15}N : ^{14}N of the standard, atmospheric N_2 (R = 0.0036765). The δ^{15}N values were converted to mole fraction (MF = ^{15}N/[^{14}N + ^{15}N]) and then corrected for ambient MF or natural abundance by subtracting the MF of pre-addition or samples from the upstream control reach at each site. Tracer ^{15}N fluxes were calculated for each water sample by multiplying the MF excess by stream discharge and the concentrations of N as NO_3^-, NH_4^+, or DON. Tracer ^{15}N (μg ^{15}N m^{-2}) for each biomass compartment was then calculated by multiplying MF excess by N standing stock (μg N m^{-2}) of that compartment in the stream. Biomass ^{15}N (B, μg m^{-1}) per distance of stream length (m) was estimated by multiplying the areal biomass ^{15}N (μg ^{15}N m^{-2}) by wetted width (m).

DO^{15}N production model—The rate of DON production was calculated by fitting a two-compartment mass balance model to the longitudinal profile of DO^{15}N flux (μg ^{15}N s^{-1}) measured at three to seven stations 24 h after the ^{15}N addition ended, based on the method in Mulholland et al. (2000). The model describes the change in biomass ^{15}N (B, μg m^{-1}) and DO^{15}N over distance (x; Fig. 1) using the equations

$$\frac{d\,B}{dx} = k_1 B \tag{1}$$

$$\frac{d\,DO^{15}N}{dx} = k_{DON_{prod}} B - k_2 DO^{15}N \tag{2}$$

where k_1 is the measured rate of decline in biomass ^{15}N with distance (m^{-1}), $k_{DON_{prod}}$ is the DON production rate per unit time (s^{-1}), and k_2 is the DO^{15}N uptake rate per unit distance (m^{-1}). The solution to Eq. 1 is

$$B = B_0 e^{-k_1 x} \tag{3}$$

where B_0 is the biomass ^{15}N (μg m^{-1}) at the point of the $^{15}NO_3^-$ addition. The linearized version of Eq. 3 results in

$$\ln B = -k_1 x + B_0 \tag{4}$$

which we used to calculate k_1 (slope of the decline) and B_0 (y-intercept; where x = 0) via linear regression. When the decline in biomass ^{15}N was not statistically significant (via linear regression analysis, p > 0.05), we used the most upstream values of biomass ^{15}N for B_0 and previously published rates of assimilatory NO_3^- uptake for k_1 (which equals total NO_3^- uptake rate, k_{tot}, minus NO_3^- uptake due to denitrification, k_{den}; Mulholland et al. 2008). Substituting for B into Eq. 2, we solved for DO^{15}N (μg ^{15}N s^{-1}), thus:

$$DO^{15}N = \frac{k_{DON_{prod}} B_0}{k_2 - k_1}(e^{-k_1 x} - e^{-k_2 x}) + DO^{15}N_0 e^{-k_2 x} \tag{5}$$

Because there was no tracer DO^{15}N at the point of the $^{15}NO_3^-$ addition (DO^{15}N$_0$ = 0), we ignored the last term in Eq. 5.
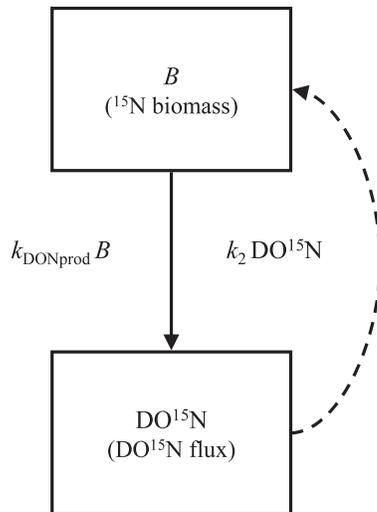
Fig. 1. Conceptual diagram of the two-compartment mass balance model used to calculate DON production rates. This model describes the rate ($k_{DON_{prod}}$; h^{-1}) at which ^{15}N in biomass (B; μg ^{15}N m^{-2}) is released as DO^{15}N (DO^{15}N; μg ^{15}N s^{-1}) and the rate of uptake for released DO^{15}N (k_2; m^{-1}). We found no evidence of DO^{15}N uptake in 14 of 15 streams and solved only for $k_{DON_{prod}}$.

We iteratively solved for values of the two unknowns, $k_{DON_{prod}}$ and k_2, that minimized the negative log-likelihood of the modeled DON flux (Eq. 5) with observed longitudinal DO^{15}N flux (Fig. 2; Hilborn and Mangel 1997). The likelihood function assumed errors were normally distributed. To estimate k_2, or the uptake rate of DON, the longitudinal pattern in the flux of DO^{15}N must exhibit a hump-shaped profile (rather than a linear increase), which would result from an increase in DO^{15}N via production followed by a downstream decrease due to uptake. Because only one stream exhibited a humped-shaped curve (WY urban 2004, data not shown), we generally assumed DO^{15}N uptake was 0 (i.e., $k_2 = 0$) and only solved for one parameter $k_{DON_{prod}}$ that best fit the increase in DO^{15}N flux with distance downstream. We then estimated $k_{DON_{prod}}$ by minimizing the negative log-likelihood of the model in Eq. 5 relative to the data using the *nlm* function in R (R Development Core Team 2011). We graphed the likelihood profile across a broad range of the free parameter, $k_{DON_{prod}}$, for each model fit to ensure that that the solution estimated using the nonlinear minimization was in fact the global minimum of the negative log-likelihood profile. We estimated uncertainty on parameter values via a likelihood ratio test that was equivalent to a 95% confidence interval (Hilborn and Mangel 1997). In this test, values of $k_{DON_{prod}}$, with negative log-likelihood > 1.92 of the minimum were deemed unlikely and outside of the 95% confidence interval (Hilborn and Mangel 1997). If the values of $k_{DON_{prod}}$ within this confidence interval included zero, then $k_{DON_{prod}}$ was considered to be not significantly different than zero.

Constraining estimates of total in-stream DON production—To calculate DON production (mg m^{-2} h^{-1}), we multiplied $k_{DON_{prod}}$ (h^{-1}) by the summed benthic standing stock of N (mg m^{-2}) in each stream that was available to be released as DON. Because we do not know the exact size of the N standing stock that contributed to DON production, we bracketed our estimate of DON production using two estimates of the benthic N standing stock that would actively cycle DON. The first estimate we used was the actively cycling N biomass based on 1 d of N assimilation. This estimate was directly measured as the amount of N assimilated into biomass during the 24 h ^{15}N tracer addition and not immediately mineralized to NH$_4^+$ (mg N m^{-2} h^{-1}, derived from ^{15}NO$_3^-$ tracer uptake during 24 h additions; *see* Mulholland et al. [2008] for reach-scale uptake rates). Assimilatory N uptake corrected for loss via mineralization (U_{assim}) was calculated using the equation

$$U_{assim} = (U_{tot} - U_{den}) \times (1 - \text{fraction mineralized}) \quad (6)$$

where U_{tot} is the total N uptake measured during the ^{15}N tracer addition, U_{den} is the amount of N uptake due to denitrification, and the fraction mineralized is the proportion of U_{assim} released as NH$_4^+$ (L.T. Johnson unpubl.). We multiplied the corrected U_{assim} by 24 h to calculate the amount of N assimilated during the ^{15}N tracer addition. Hereafter, we refer to DON production calculated with this estimate of N biomass as DON production based on N assimilated over 1 d.

Because stream N pools did not reach isotopic equilibrium during our 24 h ^{15}NO$_3^-$ tracer additions, actively cycling N biomass based on 1 d of N assimilation may underestimate the true N biomass available for release as DON. Therefore, we also calculated the actively cycling N biomass based on previous ^{15}N tracer additions conducted for 42 d, in which most biomass compartments reached isotopic equilibrium (Peterson et al. 2001). We call this approach DON production based on N assimilated over 42 d. These estimates of actively cycling N biomass were calculated using previously published measurements from the LINX I experiments of the % actively cycling N of total N biomass from various stream compartments, except for filamentous green algae, which we set at 100% (*see* Table 2 for citations, note that streams differed from LINX II). In each stream, we then multiplied the % of actively cycling N by the total benthic N standing stock for each compartment and summed each compartment within the stream. Compartments that were not included in these earlier studies were categorized into similar compartments (e.g., dead grass was assigned the same % as leaves; Table 2).

Stream ecosystem characteristics—As a part of the larger LINX II project, multiple ancillary variables were measured to help identify controls on NO$_3^-$ cycling. Background water chemistry measurements were analyzed using the following methods: NO$_3^-$ was measured using ion chromatography or colorimetry (APHA 1995), NH$_4^+$ using indophenol colorimetry or fluorometry (APHA 1995; Holmes et al. 1999; Taylor et al. 2007), soluble reactive phosphorus (SRP) using molybdate-blue colorimetry (APHA 1995), and DOC and TDN using high-temperature combustion and chemiluminescence (APHA 1995). We calculated DON concentration as TDN minus the sum of
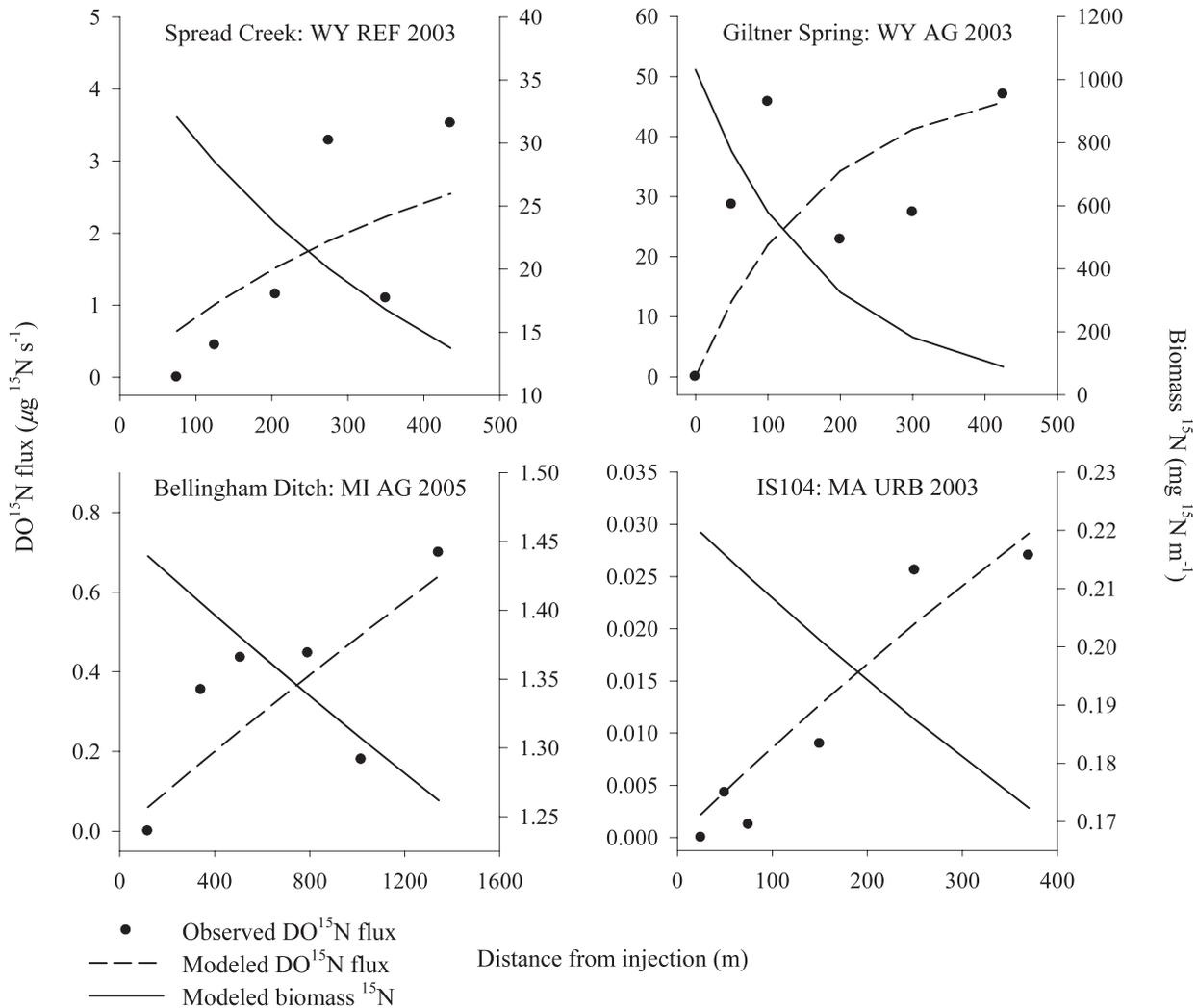
Fig. 2. Examples of the best model fit (dashed line) to observed DO[15]N flux (closed circles) given the decline in biomass [15]N (solid line).

NO_3^- and NH_4^+. We quantified biomass compartment standing stocks within each study reach by measuring area-specific ash-free dry mass at 10 locations within the reach and scaling to the proportionate area of each compartment within the reach (Hoellein et al. 2007). Following the 24 h $^{15}NO_3^-$ tracer additions, we measured GPP and ecosystem respiration (ER) using open-channel diel oxygen budgets (Odum 1956) corrected for reaeration (Marzolf et al. 1994)

and groundwater inputs when necessary (Hall and Tank 2005); these results were reported by Bernot et al. (2010). Metabolism was calculated using the two-station method when possible (in 22 streams) and using the one-station method when factors such as sensor malfunction or drift occurred at one of the stations (in 14 streams; Bernot et al. 2010). We calculated P:R ratios (e.g., GPP:ER) as an index of stream metabolic state across all sites. We also

Table 2. Percentages and references for calculating the % of total benthic nitrogen (N) standing stocks that were actively cycling N based on 42 d of N assimilation.

Compartment	% Actively cycling N	References	Compartments assigned the same %
Leaves	12	Tank et al. 2000; Hamilton et al. 2001; Sanzone et al. 2001	Dead roots and grass
Wood	8	Sanzone et al. 2001	Roots
FBOM	5	Tank et al. 2000; Hamilton et al. 2001; Sanzone et al. 2001	
Epilithon	45	Tank et al. 2000; Hamilton et al. 2001; Sanzone et al. 2001	Macrophytes, bryophytes, grass, sedge
Filamentous green algae	100	Not applicable	Epiphyton

measured stream temperature continuously for 3 d during the tracer additions and calculated discharge from the dilution of a conservative tracer (sodium chloride or bromide; Webster and Valett 2006).

Statistical analysis—To analyze these data, we first examined factors that influenced our ability to detect DO^{15}N production, then we explored how varying land use affected DON production, and finally we identified what factors controlled variation in detectable DON production. We used a logarithmic transformation when necessary to homogenize variances if the assumptions of parametric statistics were not met. We used logistic regression to identify stream characteristics that differed among streams with detectable vs. undetectable DON production. We also used a one-way ANOVA followed by a Tukey's test to categorically examine the effect of land use on DON production. We used Pearson's product moment correlation to relate ancillary variables to in-stream DON production. To assess how the magnitude of DON production compared to other N cycling processes in streams, we compared DON production to denitrification measured within the same streams (from the LINX II project, Mulholland et al. 2008) using a paired *t*-test and to nitrification measured in 11 relatively unaltered streams (LINX I project, Peterson et al. 2001) using a *t*-test. These statistical analyses were performed using SYSTAT 12 (Systat Software), with statistical significance determined at the $\alpha = 0.05$ level.

Results

Stream characteristics—Streams were highly variable in biological and chemical attributes (Table 1). Ambient NO_3^- concentration varied greatly (< 1–1453 μg N L^{-1}) among streams and was higher in urban streams (389 ± 418 standard deviation [SD] μg N L^{-1}) compared to agricultural (297 ± 410 SD μg N L^{-1}) and reference (92 ± 117 SD μg N L^{-1}) streams (ANOVA, $F_{2,35} = 3.45$, $p = 0.043$). Although some agricultural streams had higher NO_3^- concentrations compared to reference streams, they were not statistically distinct (Tukey's test, $p = 0.16$). GPP and ER differed by a 10-fold measure among the 36 streams (GPP 0.1–16.2 g O$_2$ m^{-2} d^{-1}; ER 0.4–17.9 g O$_2$ m^{-2} d^{-1}), resulting in P:R ratios from 0.01 to 1.8 (Bernot et al. 2010). Most streams ($n = 31$) were heterotrophic (i.e., P:R < 1), and five streams were autotrophic on the day of the tracer addition (P:R > 1). Other factors were similarly variable (Table 1); mean stream temperature varied from 9.9°C to 25.3°C, NH_4^+ concentrations varied from 1 to 2204 μg N L^{-1}, and ambient SRP concentrations varied from < 1 to 311 μg P L^{-1}. DON concentrations ranged from 2 to 847 μg N L^{-1}.

Detecting in-stream DON production—Across the 36 streams, DO^{15}N flux was measurable in 26 streams but exhibited no downstream pattern and thus resulted in non-significant production rates in 11 streams. In the remaining 15 streams, DO^{15}N flux was measureable and increased throughout the stream reach, resulting in DON production rates that were significant (i.e., the confidence intervals did

not contain zero). Of those streams, DON production rates ($k_{\mathrm{DON_{prod}}}$) ranged 44-fold from 0.0003 to 0.059 h^{-1} (Fig. 3A). Across regions, 11 of the 15 streams with $k_{\mathrm{DON_{prod}}} > 0$ drained agricultural or urban land use, and almost half of these streams were in PR (five streams). We expected to find detectable $k_{\mathrm{DON_{prod}}}$ across all WY streams because they had the highest rates of GPP (> 2.7 g O$_2$ m^{-2} d^{-1}); however, we only detected DON production in three of nine streams in that region. We also expected to find detectable $k_{\mathrm{DON_{prod}}}$ in all agricultural and urban streams with high GPP, but $k_{\mathrm{DON_{prod}}}$ was undetectable in seven of 13 such streams that had GPP between 1.8 and 13.6 g O$_2$ m^{-2} d^{-1} (Table 1).

Streams with detectable DON production ($n = 15$) had a higher % modified land use (agriculture + urban) in the stream basin compared to streams with undetectable DON production ($n = 21$; Fig. 4; logistic regression, $p = 0.01$). Similarly, NO_3^- concentrations in streams with detectable DON production were significantly higher than in streams with undetectable DON production (logistic regression, $p = 0.04$). NO_3^- concentrations correlated positively with % modified land use (Pearson's correlation, $r = 0.56$, $p = 0.03$).

Among streams with detectable DON production, $k_{\mathrm{DON_{prod}}}$ was positively correlated with background SRP concentrations (Fig. 5A; $r = 0.53$, $p = 0.04$, $n = 15$) and mean stream temperature (Fig. 5B; $r = 0.64$, $p = 0.01$, $n = 15$) and weakly correlated with increases in ER (Fig. 5C; $r = 0.44$, $p = 0.10$, $n = 15$). Temperature and SRP concentrations were also strongly correlated with each other ($r = 0.78$, $p > 0.001$, $n = 15$). There were no significant correlations between $k_{\mathrm{DON_{prod}}}$ and GPP, P:R ratios, or forms of dissolved N (NO_3^-, NH_4^+, DON, or TDN; $p > 0.05$, $n = 15$).

DON production—The standing stock of N available for DON production varied among streams and depended upon whether we estimated actively cycling N biomass based on 1 d of N assimilation (measured directly from our $^{15}NO_3^-$ additions) or based on 42 d of N assimilation (from LINX I studies; Table 3). When calculated from 1 d of N assimilation, actively cycling N biomass estimates ranged from 7 to 659 mg N m^{-2}, and, except for three streams, actively cycling N biomass estimates based on 42 d of N assimilation were higher (53–6435 mg N m^{-2}; Table 3). Furthermore, N biomass from the two calculations within a stream were up to 188% different (median 122%), although in one stream (Bellingham), N biomass measures from the two calculations fell within 2% of each other (Table 3).

DON production based on N assimilated over 1 d varied widely across streams and ranged from 0.02 to 6.84 mg N m^{-2} h^{-1} (median = 0.22 mg N m^{-2} h^{-1}). Unlike $k_{\mathrm{DON_{prod}}}$, the lowest DON production was in Spread Creek, a WY reference stream, and the highest was in Maizales, an agricultural stream in PR. DON production was marginally correlated with SRP concentrations (Fig. 5D; $r = 0.50$, $p = 0.06$, $n = 15$), stream temperature (Fig. 5E; $r = 0.50$, $p = 0.06$, $n = 15$), and ER (Fig. 5F; $r = 0.51$, $p = 0.06$, $n = 15$) but was not associated with metrics of stream autotrophy or forms of dissolved N ($p > 0.05$, $n = 15$).
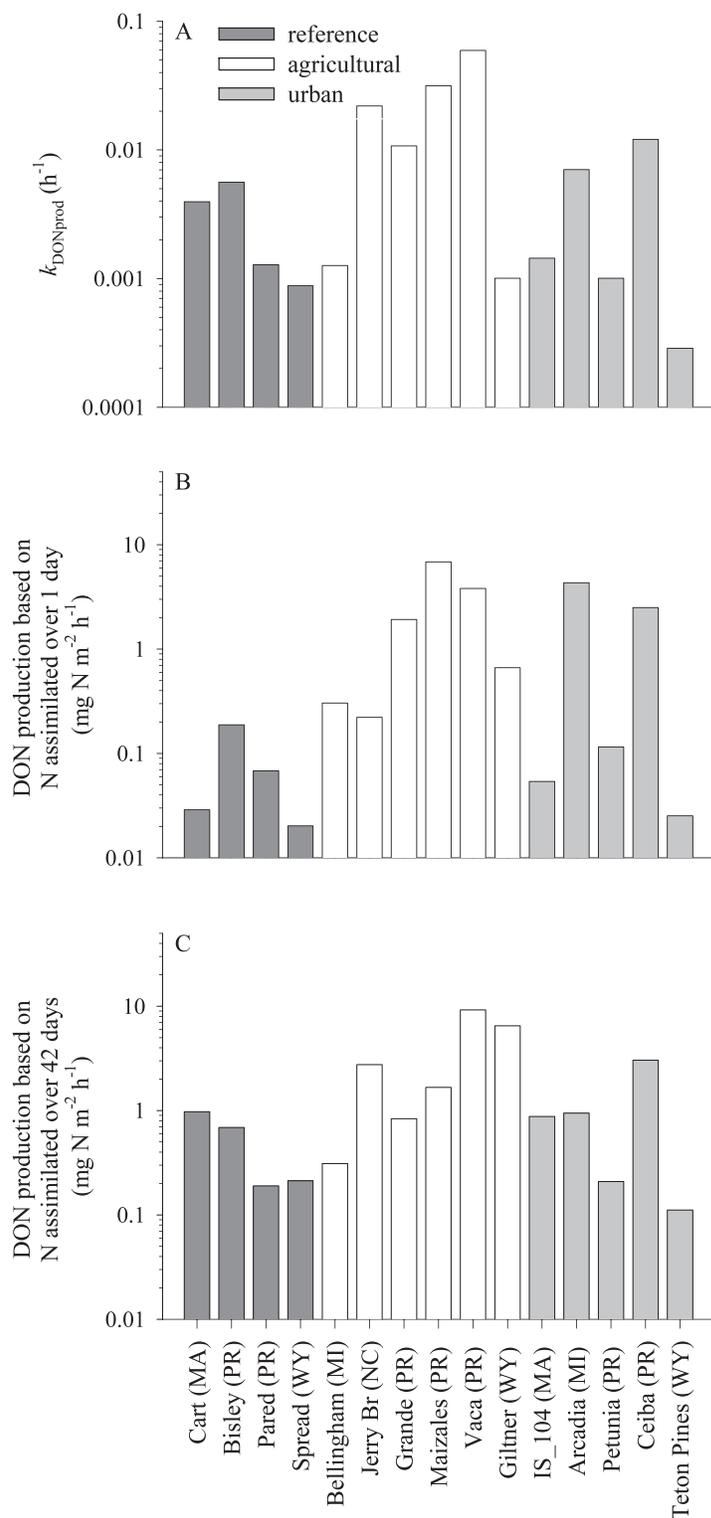
Fig. 3.   DON production across all 15 streams varied by stream, dominant riparian land use (reference = dark gray, agricultural = white, urban = light gray), and year. The best model fit determined from maximum likelihood estimation (MLE) are reported for (A) per-unit time DON production rates ($k_{DON_{prod}}$) and two estimates of areal DON production calculated by multiplying $k_{DON_{prod}}$ by actively cycling N biomass based on (B) 1 d of N assimilation or (C) 42 d of N assimilation.
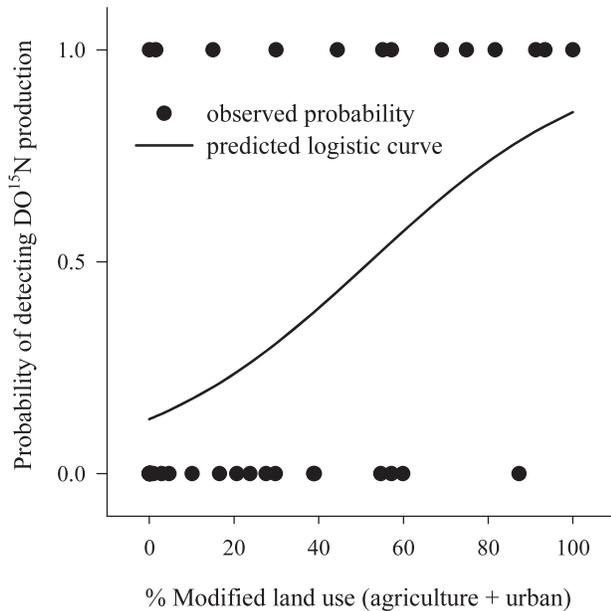
Fig. 4. Streams with detectable DO^{15}N production had higher % modified land use (agriculture + urban) in the stream basin compared to streams undetectable DO^{15}N production ($n_{detectable}$ = 15, $n_{undetectable}$ = 21), as indicated via logistic regression. The logit curves of the probability of detectable DON production are reported (Logit = -1.404 + [0.0311 × arcsine square root % modified land use]; p = 0.013).

Median DON production based on N assimilated over 42 d was 0.88 mg N m^{-2} h^{-1} and was higher than over 1 d in all but three PR streams (Grande, Maizales, and Ceiba). As with $k_{DON_{prod}}$, the lowest DON production was in Teton Pines, a WY urban stream (0.11 mg N m^{-2} h^{-1}), while the highest DON production was in Vaca, a PR agricultural stream (9.22 mg N m^{-2} h^{-1}). DON production was not correlated with SRP concentrations (Fig. 5G; r = 0.19, p = 0.49, n = 15) or stream temperature (Fig. 5H; r = 0.25, p = 0.37, n = 15). However, DON production was significantly correlated with ER, similar to results found with $k_{DON_{prod}}$ (Fig. 5I; r = 0.57, p = 0.03, n = 15). DON production was not correlated to metrics of stream autotrophy (e.g., GPP or P:R ratios) or forms of dissolved N (p > 0.05, n = 15).

DON production was often a substantial proportion of total NO$_3^-$ uptake in streams. DON production based on N assimilated over 42 d was a greater proportion of total NO$_3^-$ uptake (range = 3–317%; median = 17%) than over 1 d (range = 1–59%; median = 8%; Fig. 6). In fact, in two streams, DON production calculated from N assimilated over 42 d was even higher than total NO$_3^-$ uptake (Cart, a MA reference stream [317%] and Jerry Branch, a NC agricultural stream [240%]), which indicates that we overestimated actively cycling N biomass with the 42 d approach in these streams. In comparison to other N cycling fluxes, rates of DON production calculated from N assimilated over either 1 d (median = 0.22 mg N m^{-2} h^{-1}) or 42 d (median = 0.88 mg N m^{-2} h^{-1}) were not significantly different than rates of denitrification measured in the same streams of mixed land use used in the LINX II project (median = 0.58 mg N m^{-2} h^{-1}; 1 d paired t-test, t =

-0.35, df = 14, p = 0.74; 42 d paired t-test, t = 0.44, df = 14, p = 0.67) or nitrification measured in 11 relatively unaltered streams from the LINX I project (median = 0.42 mg N m^{-2} h^{-1}; 1 day t-test, t = -0.67, df = 24, p = 0.51; 42 d t-test, t = 1.11, df = 24, p = 0.28; Fig. 7).

Discussion

Overall, in 26 of 36 headwater streams draining different land-use types across North America, we were able to measure DO^{15}N flux from assimilated ^{15}NO$_3^-$ incorporated into biomass compartments after a 24 h tracer addition indicating DON was produced within each of these streams. Further, DON production was statistically significant in 15 streams, where DO^{15}N flux increased with distance downstream. Thus, we suggest that in some streams, a measurable fraction of assimilated NO$_3^-$ can quickly (< 24 h) be released back into stream water as DON. When it was measurable, in comparison to other N transformation rates, DON production was not statistically distinguishable from denitrification rates quantified in the same streams (Mulholland et al. 2008) or from nitrification rates measured in 11 relatively pristine streams associated with the LINX I project (Peterson et al. 2001; Webster et al. 2003; Fig. 7); all three of these transformation rates vary considerably across headwater streams.

Our results showing that autochthonous DON production can be similar to other transformation rates of N (e.g., nitrification, denitrification) support results from other aquatic ecosystems. Autochthonous DON production is a key component of the N cycle in the open ocean and is a dominant source of the total DON pool (Berman and Bronk 2003). Autochthonous DON production is similarly important in lake ecosystems, although most studies have previously focused on DOC production as a percent of C fixed during photosynthesis (reviewed by Bertilsson and Jones [2003]). Other estimates of autochthonous DON production in streams are limited to results from the ^{15}NH$_4^+$ additions from the LINX I studies (as reviewed by Ashkenas et al. [2004]) and ^{15}NO$_3^-$ additions to Spring Creek in Idaho (Hall et al. 2009a), where incorporation of ^{15}N into DO^{15}N was analyzed via mass balance, rather than direct measurement. From these studies in relatively pristine low-nutrient streams, DO^{15}N production represented no more than 10% of assimilated ^{15}N tracer. In addition, Kaplan and Bott (1989) found that labile constituents of DON (dissolved free amino acids) exhibited diel cycles associated with a vernal algal bloom in a stream draining an agricultural landscape (White Clay Creek, Pennsylvania). Our results from 15 streams indicate that in-stream DON production represented a median of 8–17% of total NO$_3^-$ uptake and was therefore a potentially large transformation of N. By including the study of streams with varying nutrient status, our results refine our understanding of N cycling in headwater streams, where DON inputs have previously been assumed to be dominated by allochthonous sources (Aitkenhead-Peterson et al. 2003).

In-stream DON production correlated positively with ER, a measure of both stream heterotrophy and autotrophy,
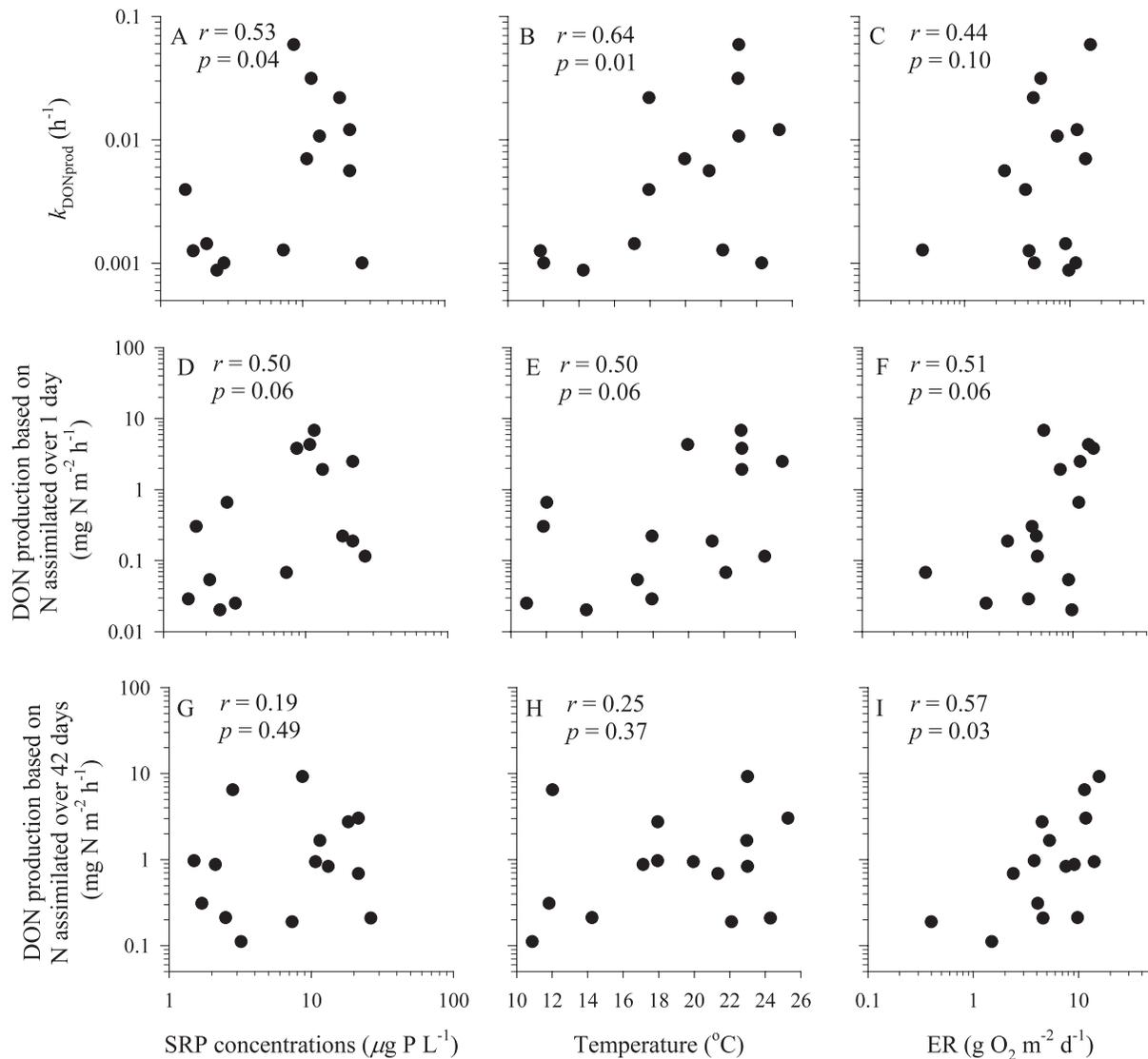
Fig. 5.   Correlations of SRP, temperature, and ER with (A–C) DON production rates ($k_{DON_{prod}}$) and DON production based on N assimilated over (D–F) 1 d and (G–I) over 42 d. Significant associations were estimated using Pearson's product moment correlation ($p <$ 0.05), and all data except for temperature data were log-transformed prior to analysis.

rather than with any metric directly reflecting stream autotrophy (GPP, P:R, light availability), thus implying an influence of stream heterotrophy on DON production. We found this result somewhat surprising given that assimilated N standing stocks were positively correlated to GPP ($r = 0.55$, $p = 0.04$, $n = 15$). The role of heterotrophs in the cycling of DON is often considered to be consumptive, especially because autochthonous DON tends to be more bioavailable than allochthonous DON (Kaplan and New-bold 2003). Further, DON produced in-stream may also be a source of energy (i.e., organic C to support heterotrophic respiration) when inorganic N is elevated, as suggested by Lutz et al. (2011) for streams across an N gradient in the southern Appalachian Mountains. Across all 72 streams in the LINX II data set, ER was influenced by a multitude of parameters, including land-use type, inorganic N availability, benthic organic matter standing stocks, temperature,

and GPP, but ER was primarily driven by fine benthic organic matter standing stocks and was also correlated with GPP (i.e., indicating that part of ER was autotrophic respiration; Bernot et al. 2010). Thus, we think that this correlation with ER results from a combination of production and consumption processes and indicates a potential role of heterotrophic microbes in regulating DON production in streams.

Another possible explanation for this correlation is extracellular enzyme activity. In soils, extracellular proteases are thought to be responsible for much of the production of labile DON (e.g., amino acids; Schimel and Bennett 2004). Proteolysis of soil proteins and peptides is usually higher than net mineralization and can be a large source of free amino acids (Lipson and Näsholm 2001). If stream microbes are behaving similarly to their terrestrial counterparts, streams with high ER may also have high

Table 3.   Actively cycling benthic nitrogen (N) biomass based on 1 d and 42 d of N assimilation compared to total benthic standing stocks for streams with detectable DO^{15}N production. Adjacent land use to the stream includes reference streams with native vegetation (REF), agricultural (AG), and urban (URB).

Region	Stream	Land-use classification	Total benthic N (mg m^{-2})	1 Day actively cycling N (mg m^{-2})	42 Days actively cycling N (mg m^{-2})
MA	Cart	REF	8030	7	246
MA	IS_104	URB	10,800	37	610
MI	Bellingham	AG	10,700	241	245
MI	Arcadia	URB	6970	616	135
NC	Jerry Br	AG	1280	10	125
PR	Bisley	REF	2400	34	123
PR	Pared	REF	2170	53	148
PR	Grande	AG	400	179	78
PR	Maizales	AG	485	217	53
PR	Vaca	AG	1700	64	156
PR	Petunia	URB	2440	115	207
PR	Ceiba	URB	2120	207	252
WY	Spread	REF	744	23	241
WY	Giltner	AG	67,400	659	6440
WY	Teton Pines	URB	1540	88	387

protease activity, resulting in high in-stream DON production. This explanation seems likely given that higher fine benthic organic matter standing stocks are associated with high ER, which could lead to stream conditions more similar to soils than to oceans or lakes. Therefore, heterotrophic metabolism could be a more substantial source of labile DON to stream ecosystems compared to autotrophic release, but more studies are needed to examine the importance of extracellular enzyme activity in stream benthic habitats.

The effect of human land use on in-stream DON production was less clear. Across the LINX II streams a combination of factors affected GPP, particularly land-use
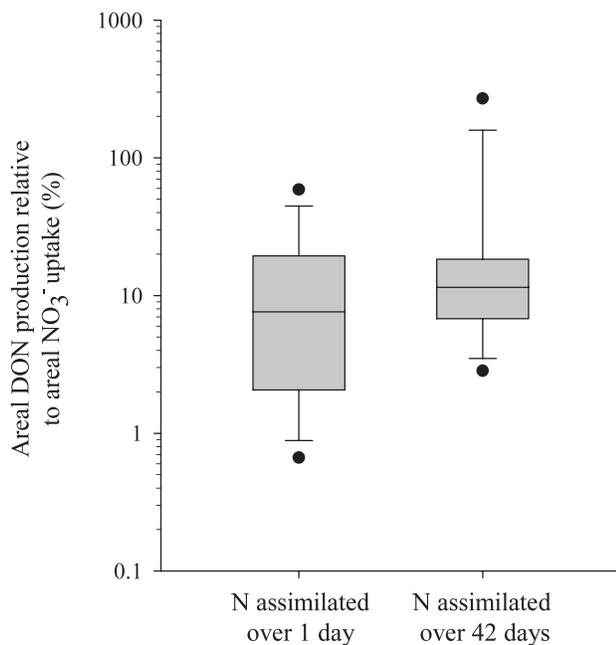

Fig. 6.   DON production as a percentage of total areal NO$_3^-$ uptake was highly variable and slightly higher when calculated from N assimilated over 42 d (median = 14%) compared to over 1 d (median = 3%). Boxes are drawn from the 25th to the 75th percentiles, and the horizontal line within each box is the median. Vertical lines extending above and below the box represent data within the 10th and 90th percentiles, with data lying outside this range represented by circles.
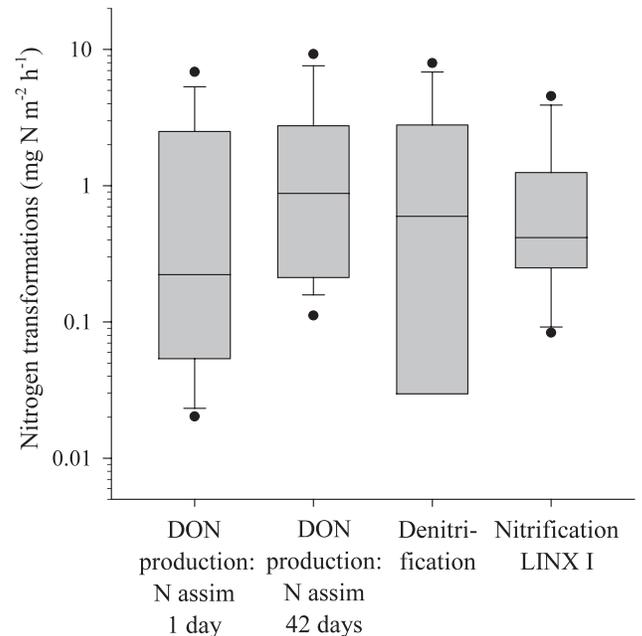

Fig. 7.   DON production based on N assimilated (assim) over 1 d or 42 d were not statistically distinguishable from denitrification in the same streams (Mulholland et al. 2008; paired *t*-test, *p* > 0.05) or from nitrification for 11 streams across North America (LINX I, Peterson et al. 2001; *t*-test, *p* > 0.05). Boxes are drawn from the 25th to the 75th percentiles, and the horizontal line within each box is the median. Vertical lines extending above and below the box represent data within the 10th and 90th percentiles, with data lying outside this range represented by circles.

activity, light availability, and DIN concentrations (Bernot et al. 2010). We anticipated that increased GPP resulting from human land-use activities, specifically canopy-clearing combined with increased background nutrient concentrations, would stimulate in-stream DON production. In support of this prediction, streams with detectable DO^{15}N production had a higher proportion of human-modified land use in their drainage basins, and SRP availability was positively correlated with DON production rates ($k_{DON_{prod}}$). This correlation with SRP may indicate an influence of land use because agricultural and urban land-use practices generally increase stream SRP. However, SRP concentrations in our 15 streams with measurable in-stream DON production were not related to % human land use in the basin and only reached a maximum of 26 μg P L^{-1}. In addition, we found no direct relation with GPP and DON production, indicating that autotrophic DON release was not the mechanism behind the influence of land use. Because bacteria can use DON as a source of organic C (Lutz et al. 2011), if human land use resulted in high labile DOC along with elevated inorganic N, DON produced within the stream may have been consumed slowly, allowing us to detect its production.

The most consistent correlate of DON production, ER, was not directly affected by human land use based upon structural equation modeling (SEM; Bernot et al. 2010). In fact, again using SEM, we found no direct effect of human land use on assimilatory $^{15}NO_3^-$ uptake in the larger LINX II study of 72 streams because the effects of human land use were varied and in opposition to each other (Hall et al. 2009b). Such complicated relationships likely hold true for in-stream DON production as well. Overall, human land use may increase in-stream DON production, but further study is required to fully understand the strength and causative nature of this relationship.

Our inability to detect DO^{15}N production in many of our study streams may have been influenced by methodological difficulties associated with the indirect measurement of DO^{15}N using the persulfate digestion method (Valderrama 1981; Ameel et al. 1993). In general, this approach requires highly accurate measurements of $^{15}NH_4^+$, $^{15}NO_3^-$, and TD^{15}N to accurately estimate DO^{15}N by subtraction; analytical errors in any step can result in high variation in DO^{15}N estimates (Bronk et al. 2000). Streams with lower percent recovery ($< 50\%$) often had a confounding matrix of complex DOM in stream water, as exemplified by one of the MI reference streams that drained a wetland (recovery = 44%). In these cases, we suggest that the digestion reagent may interact with the surrounding stream-water matrix, thereby reducing diffusion of $^{15}NH_3$ into the headspace and reducing percent recovery. We are unsure of the effect that low percent recovery following persulfate digestion may have on the δ^{15}N signatures used to calculate DO^{15}N, but prior research in soils indicates that the mole fraction of ^{15}N decreases with increasing percent recovery (Stark and Hart 1996). Therefore, in cases in which the percent recovery of DON was low (e.g., in WY and Pared and Maizales in PR), we may have overestimated DO^{15}N and thus overestimated DO^{15}N production rates. More likely, however, the lower recovery in some streams would result in higher variability among samples, making it less likely that we would be able to detect significant DON production. Nevertheless, streams with low N recovery generally had low DO^{15}N production rates compared to streams with higher percent recovery, but estimates from streams with low percent recovery should be viewed with caution.

Both structural (e.g., biomass) and functional (e.g., activity) attributes of the particulate organic N pool will influence apparent rates of in-stream DON production. The metrics used to represent actively cycling N biomass affected estimates of autochthonous DON production among some of the 15 streams. For example, Giltner Spring, a WY agricultural stream, had the third lowest $k_{DON_{prod}}$ (0.001 h^{-1}), but because it had the highest actively cycling N standing stocks, DON production per unit stream bed area was the second highest we measured. In contrast, Cart Creek, a MA reference stream, had moderate $k_{DON_{prod}}$, but N assimilated into standing stocks was the lowest across all our study streams, resulting in the third lowest DON production rates. Yet in other cases, all components of the DON production calculation were high, such as in Vaca, an agricultural stream in PR, where we found the highest $k_{DON_{prod}}$ as well as the third highest DON production based on N assimilated over 1 d and the highest DON production based on N assimilated over 42 d. Additionally, in most cases, the estimate of DON production based on N assimilated over 42 d was higher than that estimated over 1 d. Although we are confident that N assimilated over 1 d likely underestimates actively cycling N standing stocks, there is some uncertainty in applying estimates of N assimilated over 42 d from the fraction of actively cycling N standing stocks derived from other studies. Therefore, we feel these estimates of DON production bracket actual rates that likely fall somewhere in between. More research quantifying in-stream DON production is needed, with particular emphasis on the identification of the fraction of total benthic N standing stocks that is available for transformation into other forms of N.

In-stream DON production may be an overlooked and underestimated pathway that can alter the form and bioavailability of N in streams. Research on stream DON cycling has focused primarily on allochthonous DON because in many streams it represents most of the DON pool (McDowell and Asbury 1994; Campbell et al. 2000; Perakis and Hedin 2002). Often only a fraction of allochthonous DON is bioavailable (Volk et al. 1997; Kaushal and Lewis 2005), though this fraction may increase with human activity (Seitzinger and Sanders 1997; Pellerin et al. 2006). Therefore, DON produced within streams following assimilation of inorganic N is likely a more bioavailable source of DON and may be a biologically significant source of N for stream organisms compared to allochthonous DON. That said, when we used the two-compartment model to estimate DON uptake, we could only quantify DO^{15}N uptake in one stream. We suggest three potential explanations for this phenomenon: (1) reach lengths were not long enough to detect a significant decline in DO^{15}N 24 h after the end of the $^{15}NO_3^-$ addition, because reach lengths were optimized to

measure NO_3^- uptake; (2) in-stream DON may not have been consumed at a measurable rate in these streams; or (3) bioavailable DON may have been rapidly consumed within stream biofilms (i.e., internal cycling) prior to reaching the water column where $DO^{15}N$ samples were collected. Although we generally could not document DON uptake, we know that DON produced within the stream was exported downstream, where it may serve as a source of bioavailable organic N for downstream systems.

At the watershed scale, in-stream DON production in human-altered streams could influence N removal within a stream network. Across all 72 LINX II streams, most $^{15}NO_3^-$ retained was assimilated into biomass (84% of N retention) rather than denitrified (16%; Mulholland et al. 2008; Hall et al. 2009b). Given that some fraction of assimilated $^{15}NO_3^-$ would be released as DON, it then becomes unavailable for immediate and permanent removal via denitrification. Moreover, for this newly produced DON to be transformed back into NO_3^- and become available for denitrification, it would have to be assimilated by stream biota, mineralized to NH_4^+, and then nitrified to NO_3^-. We also know that as inorganic N concentrations increase in streams (e.g., due to land-use change), the efficiency of denitrification in reducing NO_3^- loads also declines (Royer et al. 2004; Mulholland et al. 2008), and the in-stream DON production pathway may have less of an effect on net stream N retention. It is currently unclear how in-stream DON production and its subsequent assimilation is influenced by high background concentrations of NH_4^+ and NO_3^-, both of which are highly bioavailable in comparison. Yet, once transported by rivers to estuaries, autochthonous DON may stimulate eutrophication, as Seitzinger and Sanders (1997) found in the Delaware and Hudson River estuaries draining mixed land use. Thus, it is imperative that we fully understand the interaction between in-stream DON production and inorganic N cycling, especially in the context of changing human land use.

In summary, the use of 24 h $^{15}NO_3^-$ tracer additions showed that in-stream DON production can represent a substantial portion of NO_3^- uptake in some streams, and DON production rates were similar to other biogeochemical transformations of N (e.g., denitrification and nitrification). The specific mechanisms by which stream heterotrophy and human land use affected autochthonous DON production are less clear, but nonetheless our results indicate that both may regulate DON production either through stream structure (e.g., the standing stock of N available for DON production or function (e.g., the rate of DON production per unit time). More importantly, our data reinforce the conclusion that streams quickly convert inorganic N into organic forms, both particulate and dissolved. Yet the fate of organic N in downstream ecosystems remains uncertain, particularly in the face of anthropogenic N enrichment.

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