Linking denitrification with ecosystem respiration in mountain streams

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Scientific Significance Statement
Denitrification in aquatic ecosystems can remove some fraction of nitrate (NO$_3^-$) from ecosystems. However, because directly measuring denitrification, especially at ecosystem levels, is difficult, it is unclear how much denitrification occurs at stream reach scales. We combined microcosm sediment respiration and denitrification fluxes with reach-scale respiration to isolate denitrification’s contribution to respiration and the fraction of NO$_3^-$ removal occurring by denitrification in montane streams. By reconsidering denitrification’s role in ecosystems as NO$_3^-$ respiration, we were able to relate NO$_3^-$ concentrations to NO$_3^-$ and oxygen respiration. Available organic carbon may be mechanistically linking denitrification with oxygen respiration. Coupled biogeochemistry contributes to understanding NO$_3^-$ concentrations.

Abstract
Rivers denitrify a portion of their nitrate (NO$_3^-$) load, but estimates are difficult using microcosm or reach-scale measurements that require specific biogeochemical and hydrologic conditions. Measuring reach-scale oxygen (O$_2$) respiration fluxes is easier than nitrogen (N$_2$) fluxes, thus we paired microcosm estimates of denitrification by N$_2$ production with estimates of aerobic respiration. The median molar ratio of $\Delta$N$_2$: $\Delta$O$_2$ from 13 streams was 0.011 (95% credible interval 0.0002–0.027 mol:mol). We then measured diel O$_2$ concentrations from 11 streams and converted to ecosystem respiration (ER) using a multiday oxygen model. Given reach-scale ER of $\sim$160 mmol O$_2$ m$^{-2}$ d$^{-1}$, the estimated median denitrification was 1.5 mmol N$_2$ m$^{-2}$ d$^{-1}$ (credible interval (CI): 0.18–4.21) across our streams. Our estimates of denitrification constituted 19% of gross NO$_3^-$ uptake (CI: 0–51%). In streams, $\Delta$N$_2$: $\Delta$O$_2$ was lower than in estuarine and marine ecosystems. Despite multiple sources of error, this approach estimates reach-scale denitrification and variation with NO$_3^-$ concentrations.

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Alterations to ambient nitrogen (N) concentrations within aquatic ecosystems is changing N cycling in both known and unknown ways, causing challenges for estimating denitrification (Galloway et al. 2008). Denitrification and other nitrate (NO$_3^-$) reduction processes produce gaseous N products such as nitric oxide (NO), nitrous oxide (N$_2$O), and N$_2$; here, we refer to all N$_2$ production as denitrification (Supporting Information). During denitrification, NO$_3^-$ is removed from both the water column and sediments in aquatic ecosystems, especially where there are high NO$_3^-$ and organic carbon (C) concentrations (Arango et al. 2007; Barnes et al. 2012). Denitrification lowers NO$_3^-$ concentration and downstream export, potentially mitigating pollution of downstream ecosystems. Estimating reach-scale denitrification is difficult, which limits our ability to inform models of N cycling and transport and to remediate high NO$_3^-$ concentrations.

Method improvements for estimating denitrification have increased our understanding of the process; however, not all methods are feasible in all streams. Measuring diel supersaturation of N$_2$ relative to argon (Ar) gas using membrane inlet mass spectrometry (MIMS) can estimate reach-scale denitrification (Kana et al. 1994; Baulch et al. 2010; Reisinger et al. 2016). However, detecting small biological changes to ambient stream water N$_2$ via reach-scale measurements requires low gradient streams with low groundwater infiltration (Baulch et al. 2010; Gardner et al. 2016; Reisinger et al. 2016). Many studies also have investigated denitrification using stable isotopes; however, adding stable isotopes to larger streams or rivers is expensive (Mulholland et al. 2008, 2009). Furthermore, scaling sediment core samples to reach-scale fluxes can be challenging because of sediment and microbial heterogeneity (Groffman et al. 2009; Baxter et al. 2013; Sawyer 2015).

One way to scale denitrification to stream reaches is via estimating respiratory demand for O$_2$. Oxygen respiration covaries with denitrification (Mulholland et al. 2009), albeit by an unknown mechanism. One possible mechanism linking denitrification and O$_2$-based ecosystem respiration (ER) is the presence of heterotrophic labile C, which serves as the electron donor for both O$_2$-based ER and denitrification. Increased labile C stimulates both denitrification and ER (Barnes et al. 2012). Additionally, oxygen respiration can decrease O$_2$ concentrations and form anoxic microsites, facilitating denitrification (Arango et al. 2007; Sawyer 2015). This second scenario posits that oxygen respiration indirectly drives denitrification. Both mechanisms and a variety of methods lead to the same prediction, i.e., ER and denitrification will positively covary (Fennel et al. 2009; Mulholland et al. 2009). Using a ratio of $\Delta$N$_2$:O$_2$ derived from N$_2$:Ar and O$_2$:Ar measured during microcosm incubations, we can estimate reach-scale denitrification based on the more easily measured reach-scale ER (Odum 1956; Appling et al. 2018a), while propagating uncertainties from the technique’s error at each step.

To estimate denitrification at the scale of stream reaches where it is otherwise difficult to measure, we linked the respiratory processes of denitrification with reach-scale oxygen respiration. Our objectives were to:

1. Link nitrate respiration and oxygen respiration in sediment microcosm incubations with reach-scale ER flux to estimate reach-scale denitrification fluxes.

2. Estimate how NO$_3^-$ availability influences the form of respiration in streams, measured as the ratio of nitrate respiration to oxygen respiration.

We hypothesized that higher ER indicates reoxidation conditions or C availability that will stimulate denitrification fluxes. Either mechanism leads to the same prediction that O$_2$ demand will positively covary with denitrification. Given that NO$_3^-$ availability also controls denitrification rates, among site variability in NO$_3^-$ will control the form of respiration, i.e., the ratio of anaerobic NO$_3^-$ respiration to oxygen respiration. We tested this latter prediction by combining data from this study with those from LINX2 (Mulholland et al. 2009) and other studies.

Methods
Field sites
We estimated denitrification and metabolism in 11 high gradient, rocky bottomed, subalpine streams in Rocky Mountain National Park, CO and Medicine Bow National Forest, WY (Table 1; Supporting Information Table A1) (Livers and Wohl 2016). All measurements occurred during stream baseflow after snowmelt concluded; i.e., late July and August.

Sediment denitrification and oxygen respiration
To estimate the link between denitrification and oxygen respiration, we measured the production of N$_2$ and removal of O$_2$ from montane stream sediments compared to stream water controls. We collected 40–100 mL of the upper 2.5 cm of stream sediment from pools with areas of coarse and fine organic deposition. We transferred sediment into microcosms made of 300 mL opaque plastic biological oxygen demand (BOD) bottles (Supporting Information). Treatment BOD microcosms with sediment were filled with stream water and capped without headspace. Additionally, we filled and capped eight control BOD microcosms with only stream water. All treatment and control BOD microcosms were kept at in situ stream temperature for incubation. We randomly sampled four treatment and four control BOD microcosms for dissolved gas concentrations immediately. The remaining treatment and control BOD microcosms incubated for 2–6 h. All dissolved gas samples were preserved with 0.1 mL saturated ZnCl$_2$ and analyzed using MIMS with a two-point calibration of atmospherically equilibrated water and standard methods (Kana et al. 1994).

We analyzed the relationship between net change in N$_2$:Ar where a positive ratio was sediment denitrification and net
change in O$_2$:Ar where a negative ratio was sediment respiration. These ratios come from paired measurements of N$_2$ and Ar or O$_2$ and Ar for each microcosm. Using O$_2$:Ar and N$_2$:Ar, we calculated sediment oxygen consumption corrected by controls $\Delta$(O$_2$: Ar) and sediment nitrogen consumption corrected by controls $\Delta$(N$_2$: Ar) using time zero controls and incubated samples where

$$r = \Delta(O_2: Ar) = \left(\left[\frac{O_2}{Ar}\right]_{t_0} - \left[\frac{O_2}{Ar}\right]_{t_1}\right)_{sed} - \left(\left[\frac{O_2}{Ar}\right]_{t_0} - \left[\frac{O_2}{Ar}\right]_{t_1}\right)_{control}$$

$$d = \Delta(N_2: Ar) = \left(\left[\frac{N_2}{Ar}\right]_{t_0} - \left[\frac{N_2}{Ar}\right]_{t_1}\right)_{sed} - \left(\left[\frac{N_2}{Ar}\right]_{t_0} - \left[\frac{N_2}{Ar}\right]_{t_1}\right)_{control}$$

sed indicates samples collected from microcosms filled with sediment and stream water while control indicates the mean of all filled with only stream water at each stream. Incubation lengths were either 2–6 h in the stream ($t_0$) or immediately following experiment initiation ($t_1$). Each ratio is unitless and has the same incubation length. Incubated microcosms remained oxic with an average 5.6 mL O$_2$ L$^{-1}$.

We estimated a regression slope between $\Delta$(O$_2$: Ar) (called $r$ for ease of notation) and $\Delta$(N$_2$: Ar) ($d$) at each stream. This slope represents the unitless $\Delta$N$_2$:−$\Delta$O$_2$ based on the ratio of paired $r$ and $d$. This slope has no time units because time cancels out of the calculation such that $\Delta$N$_2$:−$\Delta$O$_2$ = $\Delta$(N$_2$: Ar)/$\Delta$(O$_2$: Ar). Time enters at Eq. 7 when the $\Delta$N$_2$:−$\Delta$O$_2$ slope is multiplied by reach-scale ER (mmol O$_2$ m$^{-2}$ d$^{-1}$).

To propagate uncertainty within and among streams, we used a varying slope, varying intercept linear model to estimate a group-level, pooled slope in a hierarchical Bayesian framework. Bayesian analysis allows us to flexibly incorporate error in the model and to incorporate prior information (Gelman and Hill 2007). Given data from microcosms ($i$) within streams ($j$),

$$d_{i,j} \sim N\left(\alpha_i + \beta_j r_{i,j}, \sigma_{sample}\right)$$

where $\alpha_i$ is the $y$-intercept and $\beta_j$ is the slope, and thus represents the net O$_2$ and N$_2$ ratio ($\Delta$N$_2$:−$\Delta$O$_2$) for any one stream, $j$. The data-level residual errors were normally distributed with a mean of 0 and standard deviation, $\sigma_{sample}$, within stream variance. We partially pooled the both slopes and intercepts across streams such that the prior probabilities for $\beta_j$ and $\alpha_i$ were

$$\beta_j \sim N(\beta_{mean}, \sigma_\beta)$$

$$\alpha_i \sim N(\alpha_{mean}, \sigma_\alpha)$$

where $\beta_{mean}$ is the group-level slope and $\alpha_{mean}$ is the group-level intercept. Prior probability of $\beta_{mean}$ was $\beta_{mean} \sim N(-0.016, 0.028)$; the mean value of $-0.016$ was based on the ratio of mean denitrification and mean respiration rates from the LIXN2 project (Mulholland et al. 2009; Bernot et al. 2010), and a wide standard deviation of 0.028. Therefore, the slope of $d$ vs. $r$ can vary within a range of denitrification contributions to respiration. $\sigma_\beta$ was distributed as a half normal $\sigma_{\beta} \sim N(0, 0.14)$. For each stream $y$-intercept, we expected that $d$ was small while $r$ was 0 so we used a prior of $\alpha_{mean} \sim N(0, 0.05)$, where the prior had a mean of 0 and a group-level standard deviation of 0.05. Prior for $\sigma_\alpha$ was distributed as half normal $\sigma_{\alpha} \sim N(0, 0.14)$ in N$_2$:Ar and represents the stream to stream variation in intercepts.

We simulated the posterior probability distributions of the parameters using Markov chain Monte Carlo (MCMC) methods in the program Stan (Stan Development Team 2016) from the rstan package in program R (R Core Team 2018) using the data collected in this study (Madinger and Hall Jr.)
2019). For each parameter, we ran four chains with 1500 steps burn in and 1500 for sampling, visually checked all chains for convergence, and $\hat{R} < 1.1$. We evaluated this model using posterior predictive checks, which operate under the premise that a good-fitting model will generate “data” that resemble the distribution of actual data. Plots for these are in the Supporting Information and available in the archived code and data (Madinger and Hall Jr 2019).

We investigated relationships between microcosm N$_2$ production and stream conditions including temperature and NO$_3^-$ concentration. Average temperature for incubations was calculated as the average of start and end microcosm temperatures. We measured stream NO$_3^-$ concentration in $\mu$mol L$^{-1}$ using a Submersible Ultraviolet Nitrater Analyzer (SUNA V2; 0.3 $\mu$mol L$^{-1}$ precision, 2 $\mu$mol L$^{-1}$ accuracy).

**Reach-scale metabolism**

At each site, we measured reach-scale metabolism using diel O$_2$ data. We deployed a PME miniDOT dissolved O$_2$ sensor in each stream to record 2–5 weeks of continuous diel temperature and dissolved O$_2$ data. We also collected water samples for dissolved gases at deployment and retrieval of miniDOTs to measure using MIMS in order to calibrate miniDOTs.

We estimated metabolism using open channel diel O$_2$ change (Odum 1956). We fit a model of O$_2$ dynamics to O$_2$ data to estimate the parameters gross primary production (GPP), ER, and $K_{600}$ using inverse fitting and Bayesian inference. We estimated these parameters for each day using the streamMetabolizer R package (Appling et al. 2017, 2018a). A brief overview of these methods follows.

Model structure was

$$\frac{\Delta m_{O_2,i,d}}{\Delta t} = \left(\frac{GPP_d}{Z_{i,d}} \times \frac{PPFD_{i,d}}{\sum PPFD_d} \right) + \left(\frac{ER_d}{Z_{i,d}} \right) + K_{t,i,d} \left(1.05 \times O_{\text{sat},i,d} - m_{O_2,i,d}\right)$$

(6)

where $m_{O_2,i,d}$ is modeled O$_2$ on day $d$ at time step index $i$; $\Delta t$ is the length of each time step; GPP$_d$ and ER$_d$ are daily average rates of gross primary productivity and ER, respectively (g O$_2$ m$^{-2}$ d$^{-1}$); $Z_{i,d}$ is the stream depth (m) averaged over the width and length of the upstream reach; and PPFD$_{i,d}$ is the photosynthetic photon flux density at time $i$ and $\sum$PPFD$_d$ is the daily total solar insolation. We integrated Eq. 6 using the trapezoid rule (Appling et al. 2018a) to produce a time series of modeled O$_2$ concentrations to fit to the observed values. We used a state space time series model for O$_2$, so that we could incorporate both observation and process errors. Thus, $O_{t,d} \sim N(m_{O_2,i,d}, \sigma_{\text{obs}})$. This method provides low bias and more accurate estimates of parameter error than assuming either process error or observation error alone (Appling et al. 2018a).

A potential problem with metabolism models that estimate K as well as GPP and ER is equifinality in the parameters. We attempted to solve this problem by solving for gas exchange $K_{600}$ hierarchically and enabling partial pooling of $K_{600}$ across all days in the data set (Gelman and Hill 2007). Thus, the prior probability was $K_{600} \sim \text{lognormal}(K_{600\text{mean}}, \sigma_K)$. Discharge variation was low in our forested, mountain stream at baseflow, thus we expected low among day variation in $K_{600}$. Hence, we specified a half-normal prior, $\sigma_K \sim N(0, 0.05)$. This prior encourages low day to day variation in $K_{600}$.

Scaling from microcosms to stream reaches

We scaled $\Delta$N$_2$: $\Delta$O$_2$ with reach-scale ER. We resampled reach-scale ER measurements to estimate average ER and error from our population of streams. To do so, we sampled the 11 time-averaged ER estimates with replacement, making a distribution of 6000 bootstrapped estimates of daily ER ($\text{ER}_{\text{boot}}$).

Next, we scaled sediment estimates of $\Delta$N$_2$: $\Delta$O$_2$ ($\beta_{\text{mean}}$) to reach-scale ER estimates. We treated each stream reach as one unit (data-level) and estimated the mean denitrification flux of the sample of streams ($\beta_{\text{mean}}$; group-level). The posterior distribution of $\beta_{\text{mean}}$ from Eq. 4 was the ratio between sediment respiration and denitrification. We solved for the estimated reach-scale denitrification ($D$) distribution using

$$D = \beta_{\text{mean}} \times |\text{ER}_{\text{boot}}|$$

(7)

where $\beta_{\text{mean}}$ is a vector of 6000 samples from the posterior probability distribution of $\beta_{\text{mean}}$ ($\Delta$N$_2$: $\Delta$O$_2$). This equation does not include an intercept because in our oxic sediment samples (mean O$_2$ concentration of 5.6 mg L$^{-1}$), there will also be no denitrification when there is no respiration. We multiplied $\beta_{\text{mean}}$ by 6000 iterations of $\text{ER}_{\text{boot}}$ estimates. The resulting distribution of $D$ was a distribution for estimated reach-scale denitrification with credible intervals. We reported $D$ in units of mmol N$_2$-N m$^{-2}$ d$^{-1}$.
We compared our estimates of reach-scale denitrification to measurements of NO$_3^-$ uptake in streams from the same region and reported in another study (Day 2015). Day (2015) allowed comparing the magnitude of our denitrification fluxes relative to direct uptake of NO$_3^-$ estimated from a pulse addition of NO$_3^-$ and a conservative tracer, NaCl (Tank et al. 2008). We used a SUNA to measure NO$_3^-$ and a HOBO conductivity sensor to measure specific conductivity. The NO$_3^-$ and NaCl measurements were used to estimate NO$_3^-$ uptake via mass balance. In total, we have 42 estimates of NO$_3^-$ uptake in the same population of streams we used to estimate reach-scale denitrification (Day 2015).

We then sampled with replacement 42 estimates of the distribution of NO$_3^-$ 6000 times to estimate the sampling distribution of the original 42 NO$_3^-$ uptake measures. We compared the central tendency of NO$_3^-$ uptake with denitrification fluxes as N$_2$ mmol m$^{-2}$ d$^{-1}$. All data used in these methods are available online through DataCorral Repository (Madinger and Hall 2019).

**Results**

Microcosm estimates of denitrification increased as sediment respiration increased in mountain headwater streams. We calculated the group-level slope between sediment denitrification and respiration ($\beta_{mean}$) across 13 sampling events and 191 microcosm incubations because of large among site variation. In headwater mountain streams, median $\beta_{mean}$ was $-0.0106$ ΔN$_2$-ΔO$_2$ mol:mol ($-1.1\%$) with a 95% credible interval of $-0.029$ to $-0.0002$ (CI: 0.02–2.9%) (Fig. 1). This slope indicates that nitrate respiration contributed 1.1% of total oxygen respiration occurring in our 11 headwater streams. O$_2$ consumption increased with N$_2$ production. Some samples had O$_2$ generation and N$_2$ loss, which were likely due to measurement error.

The slope between denitrification and respiration varied at each site, as calculated by the stream-level regression coefficients, $\beta_i$. Site-specific ΔN$_2$–ΔO$_2$ ranged from 0.0014 to $-0.020$ mol:mol. NO$_3^-$ concentration and temperature did not strongly control variation in slopes or intercepts for the denitrification to respiration slope among our sites (Supporting Information Fig. A3).

Reach-scale ER was high in our headwater, mountain streams. Mean reach-scale ER was $-160$ mmol O$_2$ m$^{-2}$ d$^{-1}$ (CI: $-210$ to $-106$ mmol O$_2$ m$^{-2}$ d$^{-1}$) (Fig. 2). GPP was a smaller flux than ER with a mean of 18 mmol O$_2$ m$^{-2}$ d$^{-1}$ (CI: 10–30 mmol O$_2$ m$^{-2}$ d$^{-1}$)

![Fig. 1. Nitrogen production from denitrification (Δ(N$_2$:Ar) mol:mol; Eq. 2) increased as respiration (Δ(O$_2$:Ar) mol:mol; Eq. 1) increased in sediment incubations. At each of our 13 sampling events, the range of sediment respiration and denitrification change varied with slopes ranging from $-0.004$ to $-0.022$ mol:mol. The group-level slope was $-0.011$ mol:mol (CI: $-0.0002$ to $-0.027$; bottom right plot).](attachment:nitrogen_production.png)
ER ranged from $-280$ to $-67$ mmol O$_2$ m$^{-2}$ d$^{-1}$ at each site, in part because of difficulty estimating $K$. Despite partial pooling of rates of $K_{600}$, plots of ER and $K$ showed strong covariation indicating equifinality in ER and K estimates. Thus, we did not analyze variation among days, but rather took the mean of ER and GPP to obtain a single estimate for each stream reach.

We scaled the group-level sediment respiration and denitrification slope ($\beta_{\text{mean}}$) with reach-scale ER to estimate the median reach-scale denitrification flux ($D$) of 1.5 mmol N$_2$ m$^{-2}$ d$^{-1}$ (Fig. 3). The 95% credible interval for this flux was 0.18–4.21 mmol N$_2$ m$^{-2}$ d$^{-1}$.

NO$_3^-$ uptake in the same sample of streams in Day (2015) was 12.7 mmol N$_2$ m$^{-2}$ d$^{-1}$ across 42 measurements (Fig. 3). NO$_3^-$ uptake varied strongly with a wide uncertainty interval of 7.3–20 mmol N$_2$ m$^{-2}$ d$^{-1}$. The wide variation NO$_3^-$ uptake and denitrification created a broad range in the estimate of contribution of denitrification to whole stream NO$_3^-$ uptake. On average, the denitrification flux was 19% (CI: 0–51%) of the stream NO$_3^-$ uptake flux.

**Discussion**

We estimated reach-scale denitrification using measurements of microcosm $\Delta$N$_2$: $\Delta$O$_2$ fluxes scaled with reach-scale measurements of ER. Across 13 sampling events on 11 streams,
Denitriﬁcation contributed 1.1% of total respiration (Fig. 1). Based on NO$_3^-$ uptake measurements, denitriﬁcation contributed 19% (CI: 0–51%) of total N uptake. The scaling approach used here to estimate reach-scale denitriﬁcation is useful for estimating denitriﬁcation at larger scales and in a coupled biogeochemical framework. Additionally, we identiﬁed the proportion of denitriﬁcation to gross NO$_3^-$ uptake in headwater mountain streams. Denitriﬁcation contributed a small, but measurable amount of total stream respiration in addition to contributing to stream N uptake. These contributions to ecological understanding of streams highlight the importance of coupled biogeochemical pathways rather than studying processes like oxygen respiration or denitriﬁcation in isolation.

Common proposed controls on denitriﬁcation rates did not predict stream-speciﬁc, data-level denitriﬁcation ﬂuxes in our study. Denitriﬁcation rates can covary with NO$_3^-$ concentrations, O$_2$ demand, organic matter, and temperature (Kemp and Dodds 2002; Böhlke et al. 2009; Mulholland et al. 2009). Regulation of denitriﬁcation varies among studies, and complex interactions among ambient conditions make it difﬁcult to identify underlying controls for denitriﬁcation (Groffman et al. 2009). Variation in either NO$_3^-$ or temperature was not high enough in our streams to enable linking their variation with denitriﬁcation (Supporting Information Fig. A3).

ΔN$_2$:ΔO$_2$ varied across ecosystems, where freshwater streams similar to our study site have lower denitriﬁcation to respiration ratios than marine ecosystems. While we found ΔN$_2$:ΔO$_2$ was a ratio of 1.1% (mol:mol), a comparison of sediments from the North Atlantic continental shelf found a ΔN$_2$:ΔO$_2$ of 5.8% mol:mol based the ratio of denitriﬁcation ﬂux to sediment oxygen consumption (Seitzinger and Giblin 1996). Continental shelves had smaller denitriﬁcation and respiration ﬂuxes than we measured in mountain streams, but the proportion of respiration originating from denitriﬁcation was higher. Additionally, Fennel et al. (2009) analyzed 609 incubation estimates of denitriﬁcation and respiration from across aquatic ecosystems and found ΔN$_2$:ΔO$_2$ was 4.3%. These among ecosystem comparisons indicate that the proportion of respiration in denitriﬁcation coming from our streams was smaller than that of estuarine and marine ecosystems, although the median rates of both denitriﬁcation and respiration were about 10 times higher in streams.

We can compare our estimates of ΔN$_2$:ΔO$_2$ with denitriﬁcation and respiration measurements from streams across the U.S. The LINX2 project used $^{15}$NO$_3^-$ additions to measure reach-scale denitriﬁcation and O$_2$ sensors to measure two-station metabolism and therefore ER at 48 total sites, as well as reporting stream NO$_3^-$ concentration (Mulholland et al. 2009; Bernot et al. 2010). Median ΔN$_2$:ΔO$_2$ (mol:mol) of LINX2 data was 0.7% compared to the 1.1% found in our study, again lower than estuarine and marine ratios (Fig. 4).

Furthermore, the LINX2 ΔN$_2$:ΔO$_2$ were lower than Midwestern and Florida streams (Heffernan and Cohen 2010; Reisinger et al. 2016). It is possible that LINX2 underestimated denitriﬁcation because they measured denitriﬁcation of NO$_3^-$ in the water column, which did not include coupled denitriﬁcation/nitriﬁcation (Mulholland et al. 2008). Across all streams, with 0.03–1500 μmol NO$_3^-$ L$^{-1}$, ΔN$_2$:ΔO$_2$ increased as a function of NO$_3^-$ concentration with a scaling exponent of 0.60 (CI: 0.44–0.75) (Fig. 4). The value of |ΔN$_2$:ΔO$_2$| increased at 60% of the rate of NO$_3^-$ concentration showing that denitriﬁcation increased with increasing NO$_3^-$, a central ﬁnding of (Mulholland et al. 2008). The span of NO$_3^-$ concentrations enabled quantifying a relationship with NO$_3^-$ that we could not observe in our data where NO$_3^-$ concentrations ranged 11.6 μmol NO$_3^-$ L$^{-1}$ in our streams (Table 1). Furthermore, we included data from Midwestern rivers where NO$_3^-$ concentrations were higher than many of the streams we sampled (Reisinger et al. 2016). Across these spatially diverse ΔN$_2$:ΔO$_2$ data, increasing NO$_3^-$ concentrations were linked to increasing contribution of denitriﬁcation to respiration.

Our estimate of reach-scale denitriﬁcation (1.5 mmol N$_2$ m$^{-2}$ d$^{-1}$, CI: 0.18–4.2 mmol N$_2$ m$^{-2}$ d$^{-1}$) was similar to rates found using
other techniques to estimate denitrification. A meta-analysis of freshwater denitrification produced average site denitrification ranging from 0 to 27 mmol N2 m$^{-2}$ d$^{-1}$, with a mean of 3.4 mmol N2 m$^{-2}$ d$^{-1}$ across streams (Reisinger et al. 2016). Piña Ochoa and Alvarez-Cobelas (2006) also performed a meta-analysis and found a median annual river denitrification of 5.8 mmol N2 m$^{-2}$ d$^{-1}$ while measurements conducted during the warmest month of the year were $6.9 \pm 6.0$ mmol N2 m$^{-2}$ d$^{-1}$. Given that we measured changes in $N_2$ (and not other denitrification products, e.g., NO or N2O), our estimates are likely conservative, but also fall within literature reported ranges. Additionally, we could not address how much denitrification end products change at different NO$_3^-$ concentrations. Furthermore, our estimates of denitrification and oxygen respiration occurred in microcosms, which disrupt biochemical processes in stream sediments. We sampled unconsolidated sediments with high flow through the pore spaces where $O_2$ and redox layers are weaker, although anoxic microsites are present but cannot be estimated using our methods. The sealed microcosms also prevented water flow through including advective hyporheic exchanges. Despite possible underestimates, the across methods patterns we found suggest that our scaling approach is a viable method for estimating reach-scale denitrification rates.

Reach-scale denitrification was 20% (0–40%) of reach-scale NO$_3^-$ uptake, which agreed with the range from the LINX2 study (median 16%, range: 0.5–100%) (Mulholland et al. 2009). We sampled relatively NO$_3^-$ enriched streams in Rocky Mountain headwaters where there was abundant NO$_3^-$ for denitrification (Baron et al. 2009). In these NO$_3^-$ enriched streams, other factors such as availability of organic C may have limited rates of denitrification (Barnes et al. 2012). The remaining 80% of NO$_3^-$ uptake likely derived from assimilatory uptake (Mulholland et al. 2008). As a test of this amount of assimilatory uptake, we estimated the amount of assimilatory uptake of NO$_3^-$ due to GPP based on a 20:1 C:N for uptake and mean GPP of 18 mmol C m$^{-2}$ d$^{-1}$ (Hall and Tank 2003). Approximately 0.9 mmol N m$^{-2}$ d$^{-1}$ of NO$_3^-$ uptake was likely due to GPP assimilatory uptake. This value was only ~10% of gross NO$_3^-$ uptake and smaller than denitrification, suggesting and unmeasured sink for NO$_3^-$, possibly heterotrophic assimilatory demand.

Denitrification contributed 1.1% by moles of reach-scale ER in mountain headwater streams (Fig. 1). This fraction was small, but measurable, showing that in these high $O_2$ streams, a small amount of overall respiratory activity used NO$_3^-$ as an electron acceptor. In ecosystems with large fractions of anoxic habitats and higher concentrations of NO$_3^-$, we might expect higher relative contributions of denitrification to overall ER. Understanding the proportional role of denitrification among respiration processes can more generally inform the role of alternative electron acceptors within ecosystems. Coupled N, C, and $O$ cycling has implications for management and restoration efforts like ameliorating NO$_3^-$ enrichment (Schlesinger et al. 2011), and the simple model we present here represents a step forward in linking element cycles as a means to scale cycling rates of one element (O) to another, in this case N. Current efforts to identify large scale patterns and drivers of ER could further consider including coupled N cycling estimates via scaling exercises such as what we presented here to understand how N cycling interacts with ecosystem metabolism and respiration (Sadro et al. 2014; Bourgeois et al. 2017; Bernhardt et al. 2018). With the advent of large-scale metabolism syntheses (Appling et al. 2018b) comes the opportunity to combine metabolism estimates with NO$_3^-$ data (Fig. 4) to estimate denitrification at large spatial scales and through time.

References

Appling, A. P., R. O. Hall, M. Arroita, and C. B. Yackulic. 2017. streamMetabolizer: Models for estimating aquatic photosynthesis and respiration; [accessed 2019 July 09]. Available from https://github.com/usgs-r/streammetabolizer/tree/v0.10.1

Appling, A. P., R. O. Hall, C. B. Yackulic, and M. Arroita. 2018a. Overcoming equifinality: Leveraging long time series for stream metabolism estimation. J. Geophys. Res. Biogeo. 123: 624–645. doi:10.1002/2017JG004140

Appling, A. P., J. S. Read, L. A. Winslow, M. Arroita, E. S. Bernhardt, N. A. Griffiths, R. O. Hall, J. W. Harvey, J. B. Heffernan, E. H. Stanley, E. G. Stets, and C. B. Yackulic. 2018b. The metabolic regimes of 356 rivers in the United States. Sci. Data 5: 180292. doi:10.5066/F70864KX

Arango, C. P., J. L. Tank, J. L. Schaller, T. V. Royer, M. J. Bernot, and M. B. David. 2007. Benthic organic carbon influences denitrification in streams with high nitrate concentration. Freshw. Biol. 52: 1210–1222. doi:10.1111/j.1365-2427.2007.01758.x

Barnes, R. T., R. L. Smith, and G. R. Aiiken. 2012. Linkages between denitrification and dissolved organic matter quality, Boulder Creek watershed, Colorado. J. Geophys. Res. Biogeo. 117: G01014. doi:10.1029/2011JG001749

Baron, J. S., T. M. Schmidt, and M. D. Hartman. 2007. Climate-induced changes in high elevation stream nitrate dynamics. Glob. Chang. Biol. 15: 1777–1789. doi:10.1111/j.1365-2486.2009.01847.x

Baulch, H., J. Venkiteswaran, P. Dillon, and R. Mananger. 2010. Revisiting the application of open-channel estimates of denitrification. Limnol. Oceanogr.: Methods 8: 202–215. doi:10.4319/lom.2010.8.202

Baxter, A. M., L. Johnson, T. Royer, and L. G. Leff. 2013. Spatial differences in denitrification and bacterial community structure of streams: Relationships with environmental conditions. Aquat. Sci. 75: 275–284. doi:10.1007/s00204-012-0472-5

Bernhardt, E. S., and others. 2018. The metabolic regimes of flowing waters. Limnol. Oceanogr. 63: S99–S118. doi:10.1002/ino.10726

Bernot, M. J., and others. 2010. Inter-regional comparison of land-use effects on stream metabolism. Freshw. Biol. 55: 1874–1890. doi:10.1111/j.1365-2427.2010.02422.x
Böhlke, J. K., R. C. Antweiler, J. W. Harvey, A. E. Laursen, L. K. Smith, R. L. Smith, and M. A. Voytek. 2009. Multi-scale measurements and modeling of denitrification in streams with varying flow and nitrate concentration in the upper Mississippi River basin, USA. Biogeochemistry 93: 117–141. doi:10.1007/s10533-008-9282-8

Bourgeois, S., P. Archambault, and U. Witte. 2017. Organic matter remineralization in marine sediments: A Pan-Arctic synthesis. Global Biogeochem. Cycles 31: 190–213. doi:10.1002/2016GB005378

Day, N. K. 2015. Nitrogen cycling in headwater streams. M.S. thesis. Univ. of Wyoming.

Fennel, K., D. Brady, D. DiToro, R. W. Fulweiler, W. S. Gardner, A. Giblin, M. J. McCarthy, A. Rao, S. Seitzinger, M. Thouvenot-Korppoo, and C. Tobias. 2009. Modeling denitrification in aquatic sediments. Biogeochemistry 93: 159–178. doi:10.1007/s10533-008-9270-z

Galloway, J. N., A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger, and M. A. Sutton. 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320: 888–892. doi:10.1126/science.1163674

Gardner, J. R., T. R. Fisher, T. E. Jordan, and K. L. Klee. 2016. Balancing watershed nitrogen budgets: Accounting for biogenic gases in streams. Biogeochemistry 127: 231–253. doi:10.1007/s10533-015-0177-1

Gelman, A., and J. Hill. 2007, Data analysis using regression and multilevel/hierarchical models. Analytical methods for social research. Cambridge Univ. Press.

Groffman, P. M., K. Butterbach-Bahl, R. W. Fulweiler, A. J. Gold, J. L. Morse, E. K. Stander, C. Tague, C. Tonitto, and P. Vidon. 2009. Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. Biogeochemistry 93: 49–77. doi:10.1007/s10533-008-9277-5

Hall, R. O., and J. L. Tank. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. Limnol. Oceanogr. 48: 1120–1128. doi:10.4319/lo.2003.48.3.1120

Hall, R. O., J. L. Tank, M. A. Baker, E. J. Rosi-Marshall, and E. R. Hotchkiss. 2016. Metabolism, gas exchange, and carbon spiraling in rivers. Ecosystems 19: 73–86. doi:10.1007/s10021-015-9918-1

Hamme, R., and S. Emerson. 2004. The solubility of neon, nitrogen and argon in distilled water and seawater. Deep-Sea Res. Part I Oceanogr. Res. Pap. 51: 1517–1528. doi:10.1016/j.dsr.2004.06.009

Heffernan, J. B., and M. J. Cohen. 2010. Direct and indirect coupling of primary production and die nitrate dynamics in a subtropical spring-fed river. Limnol. Oceanogr. 55: 677–688. doi:10.4319/lo.2010.55.2.0677

Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham, G. E. Bennett, and J. C. Cornwell. 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N2, O2, and Ar in environmental water samples. Anal. Chem. 66: 4166–4170. doi:10.1021/ac00095a009

Kemp, M. J., and W. K. Dodds. 2002. The influence of ammonium, nitrate, and dissolved oxygen concentrations on uptake, nitrification, and denitrification rates associated with prairie stream substrata. Limnol. Oceanogr. 47: 1380–1393. doi:10.4319/lo.2002.47.5.1380

Livers, B., and E. Wohl. 2016. Sources and interpretation of channel complexity in forested subalpine streams of the southern rocky mountains. Water Resour. Res. 52: 3910–3929. doi:10.1002/2015WR018306

Madinger, H. L., and R. O. Hall. 2019. Data for “Linking denitrification with ecosystem respiration in mountain streams”. Univ. of Wyoming DataCorral, [accessed 2019 Jan 5]. Available from https://doi.org/10.15786/1PY5-HD11

Mulholland, P. J., A. M. Helton, G. C. Poole, R. O. Hall, S. K. Hamilton, B. J. Peterson, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, S. E. G. Findlay, S. V. Gregory, N. B. Grimm, S. L. Johnson, W. H. McDowell, J. L. Meyer, H. M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, L. T. Johnson, B. R. Niederlehner, J. M. O’Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota, and S. M. Thomas. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452: 202–205. doi:10.1038/nature06686

Mulholland, P. J., and others. 2009. Nitrate removal in stream ecosystems measured by 15N addition experiments: Denitrification. Limnol. Oceanogr. 54: 666–680. doi:10.4319/lo.2009.54.3.0666

Oдум, H. T. 1956. Primary production in flowing waters. Limnol. Oceanogr. 1: 102–117. doi:10.4319/lo.1956.1.2.0102

Piña Ochoa, E., and M. Álvarez-Cobelas. 2006. Denitrification in aquatic environments: A cross-system analysis. Biogeochemistry 81: 111–130. doi:10.1007/s10533-006-9033-7

R Core Team. 2018, R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Reisinger, A. J., J. L. Tank, T. J. Hoellein, and R. O. Hall. 2016. Sediment, water column, and open-channel denitrification in rivers measured using membrane-inlet mass spectrometry. J. Geophys. Res. Biogeosci. 121: 1258–1274. doi:10.1002/2015JG003261

Sadro, S., G. W. Holtgrieve, C. T. Solomon, and G. R. Koch. 2014. Widespread variability in overnight patterns of ecosystem respiration linked to gradients in dissolved organic matter, residence time, and productivity in a global set of lakes. Limnol. Oceanogr. 59: 1666–1678. doi:10.4319/lo.2014.59.5.1666

Sawyer, A. 2015. Enhanced removal of groundwater-borne nitrate in heterogeneous aquatic sediments. Geophys. Res. Lett. 42: 403–410. doi:10.1002/2014GL062234

Schlesinger, W. H., J. J. Cole, A. C. Finzi, and E. A. Holland. 2011. Introduction to coupled biogeochemical cycles. Front. Ecol. Environ. 9: 5–8. doi:10.1890/090235
Seitzinger, S. P., and A. E. Giblin. 1996. Estimating denitrification in North Atlantic continental shelf sediments. Biogeochemistry 35: 235–260. doi:10.1007/BF02179829

Stan Development Team. 2016. Stan modeling language users guide and reference manual; [accessed 2019 July 09]. Available from http://mc-stan.org

Tank, J. L., E. J. Rosi-Marshall, M. A. Baker, and R. O. Hall. 2008. Are rivers just big streams? A pulse method to quantify nitrogen demand in a large river. Ecology 89: 2935–2945. doi:10.1890/07-1315.1

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