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Nitrogen fluxes in Western streams

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Abstract Nitrogen pollution to streams is altering the nitrogen cycling in unknown ways, causing challenges for predicting nitrogen fixation fluxes within aquatic ecosystems. Increasing nitrate pollution decreases the amount of nitrogen fixation occurring in streams. However, the relationship between stream nitrate concentration and the rate of nitrogen fixation is unknown. We predict that lower nitrate streams will have the highest rates of nitrogen fixation. Additionally, there will be much more energy produced in streams with nitrogen fixation compared to the amount required to fix the nitrogen. We estimated whole-stream gross primary production and nitrogen fixation fluxes using the diel change in dissolved nitrogen and oxygen gases compared to the expected dissolved gas saturation. Our whole-stream method is preferable to chamber estimates to understand the relationship between energy requirements for nitrogen fixation and gross primary production, but additional data is needed to distinguish between relationship types and make our measurements generalizable.

Introduction

Since the industrial revolution, anthropogenic addition of nitrogen (N) to the environment has more than doubled the globally available N (Galloway et al., 2008). The increased N increases in stream nitrate (NO_3^-) concentrations, which can alter aquatic autotrophic growth and biogeochemical processes like N cycling (Vitousek et al., 1997). In certain remote areas like many streams in Grand Teton National Park, low N concentration streams still exist that likely have similar biogeochemistry to pre-industrial revolution streams. The low NO_3^- concentration in streams means the aquatic autotrophs need to obtain N by some other means, so many perform N fixation. Nitrogen fixing autotrophic cyanobacteria and diatoms convert N_2 gas into biologically available N. In oligotrophic streams, N fixation can contribute 73% of total N input to the biofilms (Kunza and Hall, 2014). Nitrate pollution provides readily available forms of N that autotrophs can remove from the water column instead of fixing N (Baker et al., 2009). As a result, increased NO_3^- concentrations could decrease the rate of N fixation. The presence of N fixing cyanobacteria is negatively correlated with N fixation rates (Kunza and Hall Jr, 2013; Gillett et al., 2016). However, it is unknown how in situ N_2 fixation responds to varying NO_3^- concentrations.

Nitrate concentrations in streams vary throughout the Greater Yellowstone Area. Many sagebrush steppe streams have extremely low NO_3^- concentrations and high rates of N fixation (e.g. Ditch Creek, Spread Creek Kunza and Hall, 2014). There are also sources of increased NO_3^- concentrations in streams attributed to atmospheric deposition that originates in Utah and Idaho (Nanus et al., 2012; Prenni et al., 2014). Agricultural and urban areas also have increased NO_3^- concentrations compared to lower NO_3^- concentrations in reference streams (Mulholland et al., 2008). As a result, in-stream NO_3^- concentrations vary throughout the Greater Yellowstone Area depending on atmospheric deposition, land use, and local geology in watersheds (Hall et al. unpub-

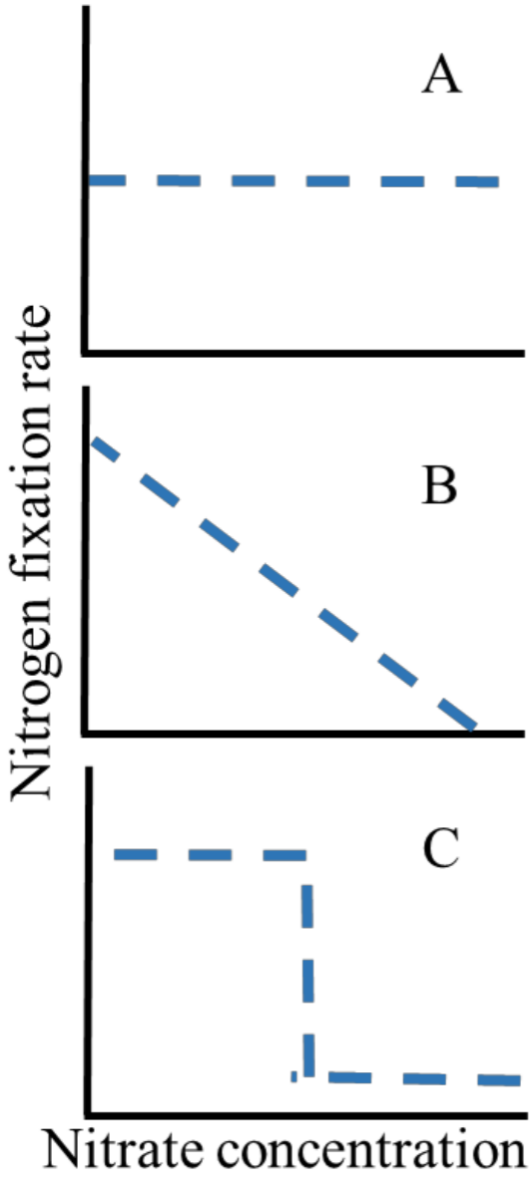

Figure 1. Three predictions of N fixation rates at different NO_3^- concentrations.

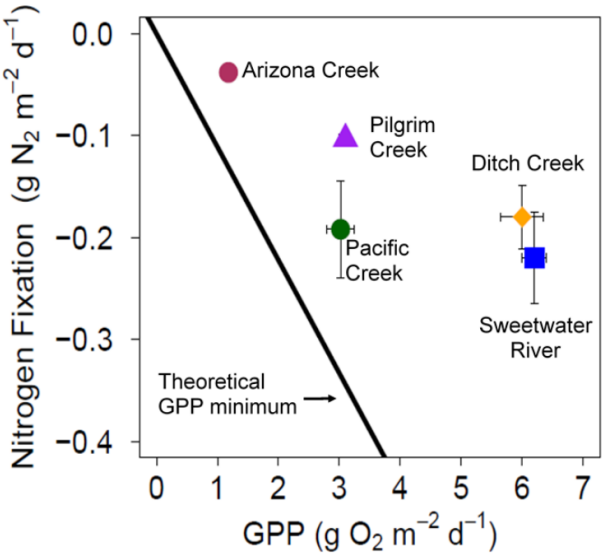

Figure 2. Relationship between gross primary production (GPP) and nitrogen fixation fluxes from previously collected data. All streams were producing more energy than was consumed during nitrogen fixation based on the theoretical GPP minimum line (data fall to the right of the line).

lished research).

Autotrophic photosynthesis rates are high in the Greater Yellowstone Area (Bernot et al., 2010). The LINX II project extensively studied the Greater Yellowstone Area as part of a continental wide N research project to investigate the role of land use N cycling including N uptake, and metabolism (Hall and Tank, 2003; Hall et al., 2009; Bernot et al., 2010). They studied 9 streams and found high rates of stream metabolism, which is the net result of photosynthesis (gross primary production) and the energy used by aquatic organisms (ecosystem respiration). Because stream metabolism requires a source of reactive N, the low NO_3^- concentrations suggest there is high demand for the NO_3^- present. Autotrophic uptake of NO_3^- likely removed much of the NO_3^- in streams because gross primary production and NO_3^- uptake were related (Hall et al., 2009). In the Grand Teton streams, much of the N required for stream metabolism likely came from N fixation.

Nitrogen fixation can contribute a dominant flux of N into aquatic ecosystems in Grand Teton National Park, which requires a large energy input from the aquatic autotrophs (Kunza and Hall, 2014). Nitrogen fixation adds reactive N to the streams in both thermal spring outlets and prairie streams in the Greater Yellowstone Area, but N fixation occurred in streams with comparatively low NO_3^- concentrations (Stewart, 1970; Kunza and Hall, 2014). When aquatic biofilms were grown with added nutrients, additional NO_3^- inhibited growth of N fixing bacteria (Kunza and Hall Jr,

Stream	Latitude	Longitude	Year Sampled
Arizona Creek	43.9742	-110.6443	2015
Ditch Creek	43.6634	-110.632	2015
Gros Ventre River	43.5854	-110.7104	2017
Pacific Creek	43.8656	-110.5047	2015 and 2017
Pilgrim Creek	43.9244	-110.564	2015
Spread Creek	43.7921	-110.5387	2017

Table 1. Sampling locations and years included in the study.

2013). Because the streams in the Greater Yellowstone Area have variable NO_3^- concentrations and high rates of N fixation they are a useful location to investigate the controls of N fixation and the energy requirements of N fixation.

Because N fixation occurs in low NO_3^- concentration streams, N fixation is likely declining globally in prevalence due to increasing N pollution, yet we do not know the relationship between N fixation fluxes and NO_3^- concentration (Kunza and Hall, 2014). Nitrogen fixation and NO_3^- concentration could be unrelated because N fixation is not measured in high NO_3^- streams. Additionally, if NO_3^- is related to N fixation, N fixation could decline with increasing NO_3^- or it may have a threshold NO_3^- concentration where N fixation is no longer energetically favorable. We intend to measure the rates of N fixation in low NO_3^- concentrations that likely resemble aquatic communities from before the introduction of NO_3^- pollution.

Because N fixation is energetically expensive for organisms, N fixers use the energy produced during photosynthesis to fuel N fixation. Nitrogen fixation requires about 78 g of glucose to fix one g of N (Gutschick, 1981). By accounting for the energy produced in the stream from gross primary production, we can estimate how much of the total energy produced by aquatic autotrophs goes toward N fixation. We sampled N fixation fluxes in different stream NO_3^- concentrations to understand the relationship between ambient NO_3^- concentrations and autotroph production of reactive N using energy made during photosynthesis.

This work aims to address two main questions: How do N fixation fluxes vary in ecosystems with different NO_3^- concentrations? What is the relationship between the energetic requirements of N fixation and gross primary production in streams?

We hypothesize that autotrophs will use the least energetically expensive way to obtain biologically available N and therefore N fixation rates will increase in low NO_3^- concentration streams. N fixation may remain constant in streams as NO_3^- concentrations increase because N fixers continue to fix N rather than removing NO_3^- from the water column (Figure 1A). Additionally, N fixation could hypothetically decrease as NO_3^- concentrations increase because autotrophs remove proportionally more NO_3^- from the water column as concentrations increase (Figure 1B). Finally, there could be a threshold NO_3^- concentration where N fixation stops (Figure 1C). Adding additional sites to sample NO_3^- concentrations and N fixation fluxes in the Greater Yellowstone Area allows us to discriminate between the proposed hypotheses to address the research question about how N fixation relates to NO_3^- concentrations of streams. We also place N fixation within the context of coupled biogeochemical processes by calculating the relationship between N fixation energetic requirements and the gross primary production of the whole-stream ecosystem. We predict that N fixation only occurs when gross primary production provides much more energy than the energetic requirement for N fixation.

Methods

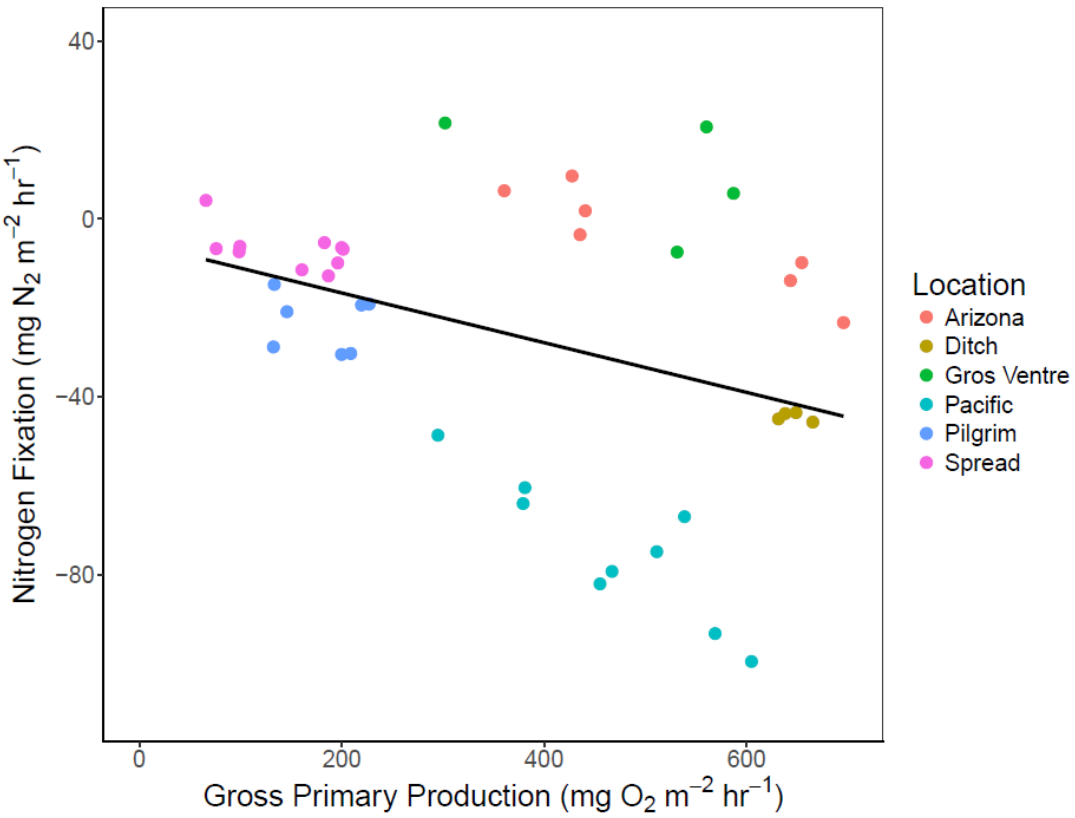

Figure 3. Nitrogen fixation fluxes increased with sample GPP. Samples from Spread and Gros Ventre creeks (2017) and Arizona creek (2015) had low levels of nitrogen fixation.

Approach

Our method of measuring whole-stream N fixation is a new method we developed to estimate whole-stream dissolved N fixation without having to scale N fixation fluxes from individual rocks up to the whole-stream scale. We collect hourly dissolved gas samples for 24 h and analyze them on a membrane inlet mass spectrometer to measure the concentrations of dissolved nitrogen (N_2), oxygen (O_2), and argon (Ar) gases. We also calculate the expected dissolved gas concentration of the streams using the water temperature and barometric pressure. Gross primary production, ecosystem respiration, N fixation, and denitrification underlie the difference between the measured dissolved gas concentration and the expected saturation concentration. We estimate gross primary production and ecosystem respiration using the measured changes in O_2 concentration and we estimate N fixation and denitrification rates using the measured changes in N_2 gases relative to Ar concentrations. We estimate model parameters using an adaptation of the Bayesian model from Hotchkiss and Hall (2014).

To confirm that we are measuring N fixation fluxes appropriately, we also incubate rocks with stream-grown biofilm in chambers to measure metabolism and N fixation. We similarly measure the change in the dissolved gas concentrations of the chambers to estimate metabolism and N fixation compared to control chambers and scale the results of the chamber incubations to the whole-stream using the chlorophyll a concentration of the incubated biofilms. We previously estimated N fixation along a gradient of NO_3^- concentrations in Wyoming and Colorado. Whole stream N fixation rate ranged from <0.04 to 0.3 $gN_2m^{-2}d^{-1}$. Comparing the gross primary production and N fixation fluxes of whole streams, the streams previously sampled all produced enough en-

ergy during gross primary production to fuel the energy used during N fixation (Figure 2). Additionally, chamber incubations at each site had lower but positively related N fixation fluxes compared to whole-stream estimates.

Field sites

We sampled three sites in Grand Teton National Park during the summer of 2017 to augment data collected from other streams beginning in 2015 (Table 1, Figure 2). *Nostoc* and *Epithemia* nitrogen fixers are present in similar regional streams (Kunza and Hall Jr, 2013; Kunza and Hall, 2014). At each site, we measured stream biofilm GPP using chamber incubations and nitrogen fixation rate using chamber incubations and accounting for the denitrification and ER of sediments.

Nitrogen fixation

We measured the change in dissolved gases due to biological activity from aquatic biofilms on a rock inside enclosed chambers at each stream. We placed two to four haphazardly collected stream rocks in a single layer in five treatment chambers at each site. Control chambers were filled with scrubbed terrestrial rocks that were left in a water tight bag overnight to come to stream temperature. Chambers were gently filled with stream water and lidded underwater with no enclosed bubble. All chambers were incubated underwater at stream temperature with approximately 2 cm of stream water above the chamber. Following a 3 h incubation, we sampled the dissolved gas concentration. We gently mixed the sealed chambers and then siphoned water out of treatment and control chambers into exetainers. Duplicate vials were overfilled from the bottom three times and preserved with 0.1 mL 50% $ZnCl_2$. Immediately following the dissolved gas sample collection, we measured chamber water temperature using a reference Thermapen and recorded the barometric pressure in mmHg from an Extech SD700 barometer. This sampling method was performed once at midday and once at night when there was no light to power GPP. After both incubations, We removed all biofilm on the rocks and measured the chlorophyll a concentration for each sample using fluorometric analysis before and after acidification (Nusch, 1980). Then we measured the planer surface area of all the rocks.

Denitrification

We estimated the change in dissolved gases due to sediment respiration and denitrification. We placed approximately 50 mL of stream sediment into darkened bottles, filled the bottles with ambient stream water, and capped them with no enclosed air bubble to make samples. Time zero samples were immediately sampled for dissolved gas by removing a water sample and measuring the temperature as described for the chamber method above. The remaining bottles filled with sediment were incubated for up to 3 h. We also made controls by filling darkened bottles with only ambient water and sampled them immediately and after incubation. All water samples were analyzed for dissolved gas concentration.

Analyses

We measured the dissolved gas concentration of samples from chamber and bottle incubations using membrane inlet mass spectrometry. We measured the dissolved oxygen to argon ratio (O_2:Ar) and nitrogen to argon ratio (N_2:Ar) where Ar is a naturally occurring tracer gas (Kana et al., 1994). To calibrate the MIMS, we used a two-point calibration where water baths were slightly warmer and cooler than ambient stream temperature. Water baths were equilibrated to atmospheric gas concentrations at known temperature and barometric pressure during each calibration (Garcia and Gordon, 1992; Hamme and Emerson, 2004). We ran five to ten samples between calibrations.

We measured the relationship between chamber GPP and nitrogen fixation. We were interested in the production of O_2:Ar (GPP) compared to the removal of N_2:Ar (nitrogen fixation). We calculated the dissolved gas flux at each site as

$$F = ((R - \overline{R})_{day} - (R - \overline{R})_{night}) \times t \times v \times s \quad (1)$$

where the flux (F) in $mgm^{-2}h^{-1}$ of O_2 and N_2 are calculated as the dissolved gas ratio (R) of the sample

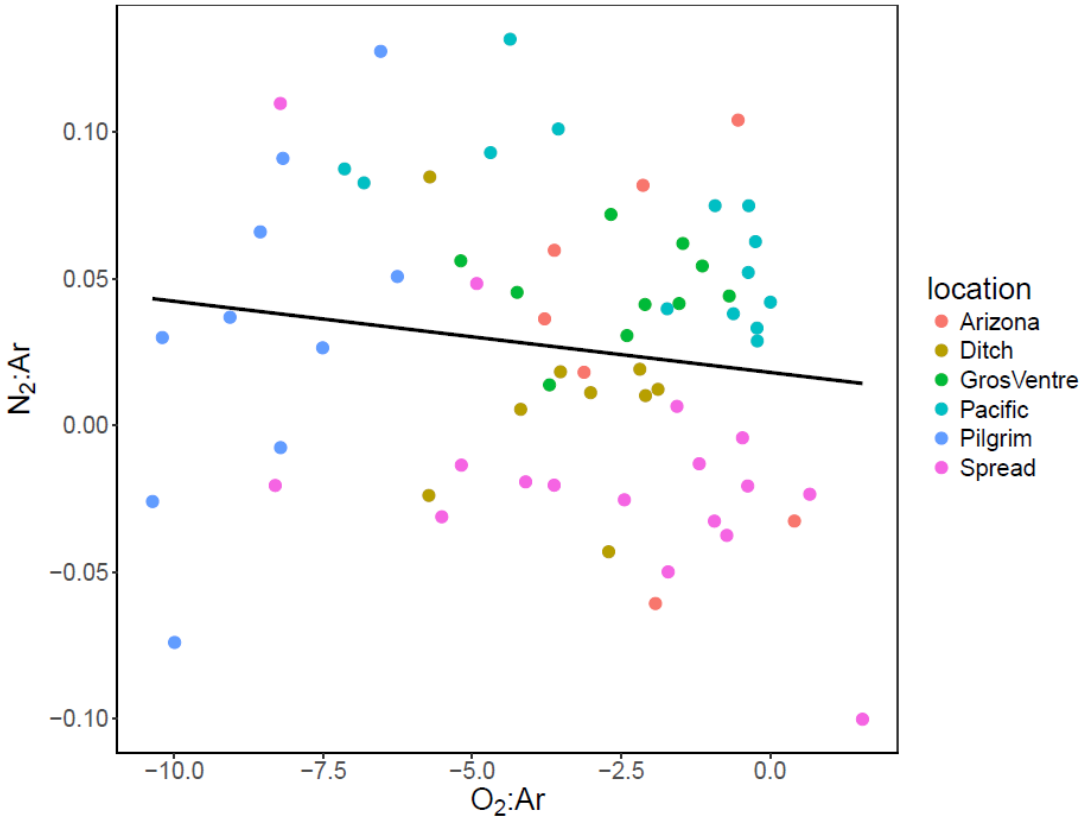

Figure 4. Sediment denitrification (N_2:Ar) and ER (O_2:Ar) were weakly related where low rates of sediment respiration also had low sediment denitrification.

minus the average of all control ratios. The night samples were used for the ER or denitrification correction and subtracted from the same chamber's day values resulting in a GPP or nitrogen fixation estimate. The GPP or nitrogen fixation value was then standardized by correcting by incubation length (t), chamber volume excluding the rocks (v), and the planer rock surface area (s). We regressed nitrogen fixation on GPP to address hypotheses about the dependence of rates of fixation on available energy (Table 1, Figure 2).

We also investigated the relationship between stream sediment denitrification and sediment respiration. We were interested in the removal of O_2:Ar (ER) compared to the production of N_2:Ar (denitrification). We calculated the dissolved gas flux as

$$ER = (O_2 : Ar_{t_2} - \overline{O_2 : Ar_{t0}}) - (O_2 : Ar_{t2} - \overline{O_2 : Ar_{t0}})_{\overline{control}} \quad (2)$$

$$Denitrification = (N_2 : Ar_{t2} - \overline{N_2 : Ar_{t0}}) - (N_2 : Ar_{t2} - \overline{N_2 : Ar_{t0}})_{\overline{control}} \quad (3)$$

where control indicates samples filled with only stream water compared to samples with incubated sediment. Samples incubated in the stream for one to three hours were labeled as t_2 while t_0 indicates samples collected upon experiment initiation. The samples were not corrected to sediment volume, but instead were used to investigate if denitrification and ER processes are related using linear regression.

Preliminary Results

In general, for streams in Grand Teton National Park, as GPP increased, nitrogen fixation increased, but GPP only predicted 17% of the variation in nitrogen fixation (R^2 = 0.17, p<0.01; Figure 3). Ditch and Pacific Creeks had the highest nitrogen fixation rates whereas Arizona Creek, Spread Creek, and Gros

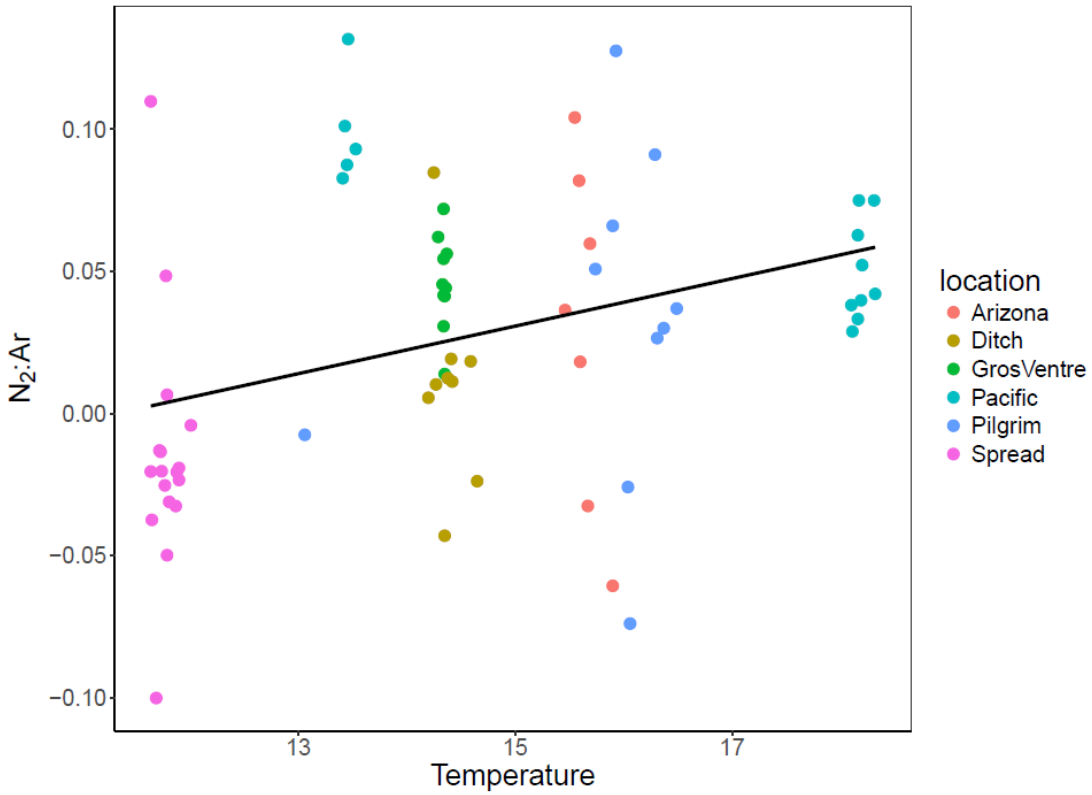

Figure 5. As sediment incubation temperature increased, sediment N_2:Ar flux increased, indicating higher denitrification rates.

Ventre River samples averaged near 0 mg $N_2 m^{-2} h^{-1}$. The low rates of nitrogen fixation in Spread Creek and in the Gros Ventre River could be due to low biofilm levels from high snow runoff levels earlier in the growing season. No streams had high levels of nitrogen fixation without correspondingly high levels of GPP.

Sediment respiration and denitrification were not related in Grand Teton National Park streams. We sampled a range of sediment respiration values, but found no relationship with sediment denitrification (R^2 = 0.02, $p>0.1$; Figure 4). Sediment respiration and denitrification did not have clusters of stream samples either. Sediment denitrification flux was related to stream temperature (R^2 = 4%, $p<0.01$) where warmer stream sediment incubation temperature led to higher denitrification fluxes (Figure 5).

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