

## **Final Report**

### **Land use impacts on nitrogen fixation in Jackson Hole streams**

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Project Duration: 03/01/2005 – 02/28/2007

#### **Abstract:**

Pollution from excess nitrogen (N) threatens many freshwater and marine ecosystems with eutrophication. Rivers and streams play a central role in N cycling at the landscape scale because rivers provide an avenue to transport N from the terrestrial landscape to downstream ecosystems. Rivers are more than conduits and may play a strong role in transforming or storing N. Changing land use in the Western US may alter how streams transform and process N. Currently we are studying how contrasting land use may affect N cycling in streams in Jackson Hole, WY. We are examining how land use alters the removal and fate of nitrate-nitrogen using experimental addition of  $^{15}\text{N}$  tracers. Missing from our studies is an understanding of how N fixation alters stream N budgets and cycling, and how land use may affect N fixation. In these streams, N-fixation (i.e., the creation of biologically available N from atmospheric  $\text{N}_2$ ) may be a dominant pathway for N input. We hypothesize that unimpacted streams will have high rates of N-fixation that drives the stream N budget, while hydrologically impacted streams (e.g., irrigation ditches) and streams with elevated nitrate concentrations will have lower N fixation rates. We will measure N fixation in the context of summer stream N budgets in 9 streams in and around Jackson Hole that we are using as part of our larger study. The 3 land use types are reference (unimpacted; streams in Grand Teton National Park), irrigated cattle pasture (streams on the Snake River Ranch), and suburban (Jackson Hole Golf Club and 2 streams in condominium developments). We will measure N fixation rates in each of these 9 streams using the acetylene reduction method. In 3 streams, we will estimate the importance of N fixation in the context of a stream reach nitrogen budget that considers inputs and outputs of N combined with rates of internal processing.

#### **Background:**

Humans have dramatically increased the global fixation of N into the biosphere, and this N is responsible for the eutrophication of aquatic ecosystems ranging from the Gulf of Mexico to Lake Tahoe. Despite increased N concentrations and associated enrichment of coastal oceans, only a small fraction of N deposited on land reaches oceans via rivers. The fate of this missing N is unknown, and one possibility is that small streams may transform or remove a fraction of this N. Streams play a central role in the transport and fate of anthropogenically fixed N, as they are the primary avenue for transport to downstream ecosystems. Ecologists are now learning that streams are more than just a conduit, however, and can process and retain much of this N (Hall 2003). Streams can attenuate N concentrations from either long-term storage (M. A. Baker and R. O. Hall, unpublished manuscript) or from denitrification, i.e. the conversion of biologically available N to  $\text{N}_2$  gas (Alexander et al. 2003).

The role of streams in transporting and transforming N is likely altered by land use practices that both increase N inputs to streams and alter stream geomorphology (e.g.,

making confined channels in urban streams; Paul and Meyer 2001) yielding lower water residence times and less proportional N removal. High N inputs to streams can also saturate biotic uptake (Royer et al. 2004), thus overwhelming the stream's ability to act as a sink for N. Given a dramatic increase in urbanized land in western Wyoming (Parmenter et al. 2003), there is the potential for alteration of stream N exports from these areas.

Despite the large amount of research examining streams as transformers of N, there is almost no study of a central N transformation: N fixation. N fixation is the conversion of abundant, but mostly inert, dinitrogen ( $N_2$ ) gas to biologically available N. Cyanobacteria are the primary N fixers in streams, and are abundant in many of our study streams. There is much knowledge of terrestrial N fixation, and indeed, terrestrial N fixation has been substantially increased from planting of leguminous crops (Vitousek et al. 1997). Less is known on N fixation in streams. Streams with low N concentrations may actually be net sources of N due to high rates of N fixation. Thus our interpretation of how streams cycle N from the landscape may be incorrect given that much of the N exported downstream may have been fixed in the channel itself, and not imported from the terrestrial component of the ecosystem. If streams do have high rates of N fixation and are net sources, of N stream ecologists will have to refine models of N use by streams, in that they may be next sinks for dissolved N, yet be a net source of new N to downstream ecosystems. N fixation can be an important source of N to certain aquatic ecosystems, dominating the N budget of eutrophic lakes and some wetlands (Howarth et al. 1988). There are very few studies of N fixation in streams (Grimm and Petrone 1997); a review on N fixation by Howarth et al. (1988) did not include any measurements from streams or rivers. However, stream N budgets may be dominated by N fixation despite being open ecosystems, which have high rates of transport of N through the ecosystem. Indeed, N fixation may dominate stream N fluxes more than the well-studied process of denitrification.

### **Objectives:**

1. *Measure how land use and associated physical variables control N fixation rates in 9 streams.*

Hypothesis: N fixation rates in these high light streams will be highest in streams with low ambient N concentrations. We measured N-fixation in 9 streams in 3 land-use types during summer 2005 and will repeat the 3 highest streams in 2006. We are currently developing experimental protocols to use  $^{15}N_2$  to calibrate the acetylene reduction method; this method has not been attempted in streams. We will perform an extensive comparison of the  $^{15}N_2$  method with the acetylene reduction method in the 3 streams with the highest N fixation rates.

2. *Measure the degree to which N fixation dominates reach-scale N budgets.*

Hypothesis: In streams with high N fixation, N fixation will constitute a major fraction of the N budget in mid-summer. For 3 of the 9 streams, we created a short-term budget of N at the scale of a 500-m stream reach. In each reach we measured N inputs, outputs, and nitrate and ammonium uptake. Much of the data for the N budget will be collected as part of our ongoing LINX project. We have the associated N fixation data for these streams (Fig. 1), but we are currently analyzing the associated budget data collected as part of the LINX experiment.

3. *Examine controls of nitrogen and phosphorus on N fixation rates.*

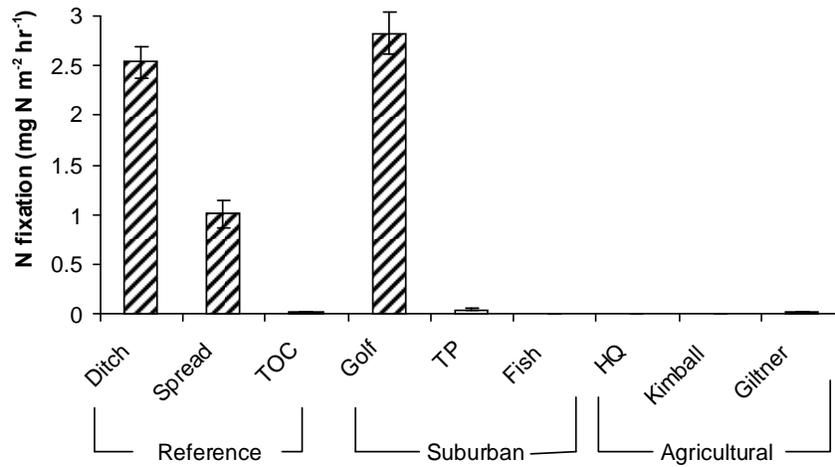
As a mechanistic test of the effects of high nitrate we performed the nutrient addition experiment described below in two streams with high rates of N fixation.

**Results:**

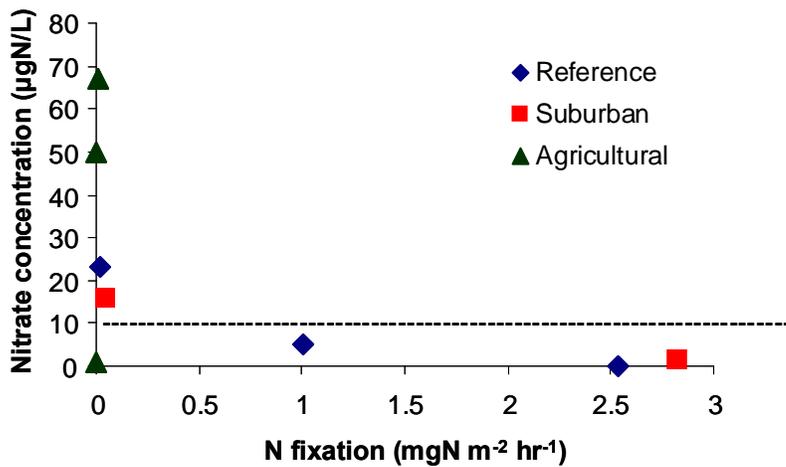
Summer 2005, we measured nitrogen fixation rates using the acetylene reduction method in nine streams in three different land-use types in Grand Teton National Park and Jackson, WY (Reference-relatively unimpacted streams, Suburban-in residential areas, and Agricultural-irrigation return flow streams located on Snake River Ranch, Wilson, WY) (Figure 1). Reference and urban streams were not different, but this may be highly skewed due to high N-fixation occurring in the Golf course stream. The agricultural streams may not be low due to N concentrations, but rather the alteration of stream flow and fine sediments. Intermittent flow patterns and fine sediments may not be suitable habitat for N-fixing assemblages. The higher rates we measured are comparable to Sycamore Creek, Arizona (Grimm and Petrone 1997) and tropical systems (A.J. Ulseth and A.S. Flecker, unpublished) which are much higher than most lake, marine and estuarine systems (Howarth et al. 1988). Ambient nitrate concentrations varied among the 9 streams (Figure 2). Ambient stream nitrate concentrations exceeding  $10\mu\text{gN/L}$  inhibited nitrogen fixation. Our results show that excessive N inputs could reduce nitrogen fixation occurring in these streams and suggest that N fixation is a process that only occurs in streams with ultra low rates of N fixation.

We measured denitrification, that is the conversion of biologically available nitrogen (as nitrate) back to  $\text{N}_2$  gas (i.e. the biological opposite of N-fixation) in 9 stream in Jackson Hole, and 40 other streams across the country as part of the LINX2 experiment. Rates of N fixation equaled or greatly exceeded denitrification rates in all streams except Kimball Spring, on the Snake River Ranch. In streams with high N fixation (Ditch, Golf, Spread), denitrification was at least 100 times lower than N fixation. Thus these streams are a net source of N rather than a net sink, as is commonly believed for streams in highly polluted areas (Alexander et al. 2000). Our highest rate of nitrogen fixation ( $4.2\text{ mg N m}^{-2}\text{ h}^{-1}$ ) was higher than denitrification in all but 4 of the 49 streams measured across the United States, showing the rate of N fixation in Ditch Creek can exceed losses of N in streams highly polluted by nitrate with elevated rates denitrification.

To experimentally examine how nutrients regulate on N-fixing rates we incubated nutrient releasing substrates in Ditch Creek and Spread Creek during Summer 2006. Following Tank and Dodds (2003) we made nutrient diffusing substrates that are  $64\text{ cm}^2$  porous terracotta tiles embedded in agar medium containing high concentrations of nitrate ( $0.5\text{M KNO}_3$ ), phosphate ( $0.5\text{M NaH}_2\text{PO}_4$ ), both, or unamended controls. Five sites in each stream (Ditch and Spread) contained nutrient releasing substrates for each of the four treatments. The tiles were incubated in the stream for 6 weeks (the agar is replaced after 3 weeks) because N fixers are slow-growing. After 6 weeks we measured N fixation rates on each set of tiles using the acetylene reduction method. Nutrient effects are analyzed using 2 way analysis of variance following Tank and Dodds (2003).

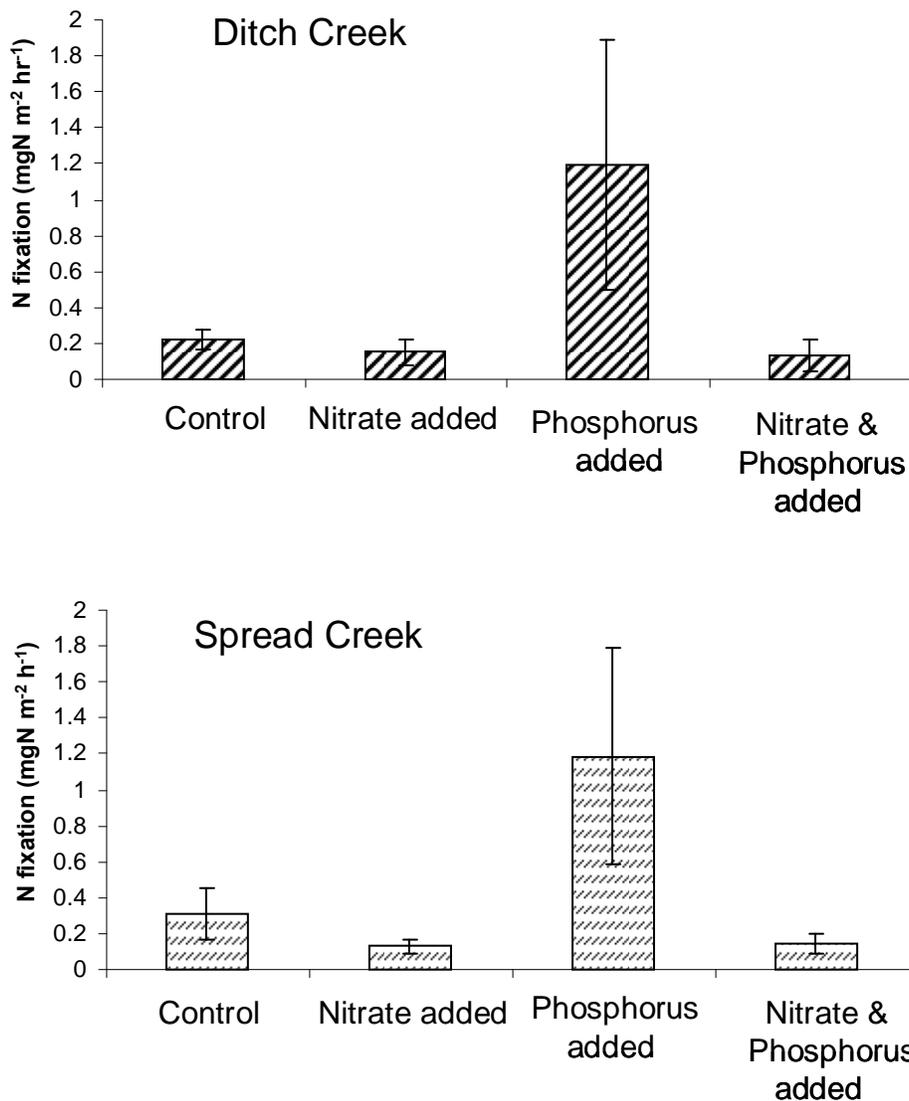


**Figure 1.** Mean stream nitrogen fixation rates ( $n \geq 18$ ). The results from summer 2005 nitrogen fixation measured using the acetylene reduction technique on 9 streams. Error bars represent standard error. TOC= Two Ocean Creek; TP= Teton Pines Waterway; HQ= Headquarters.



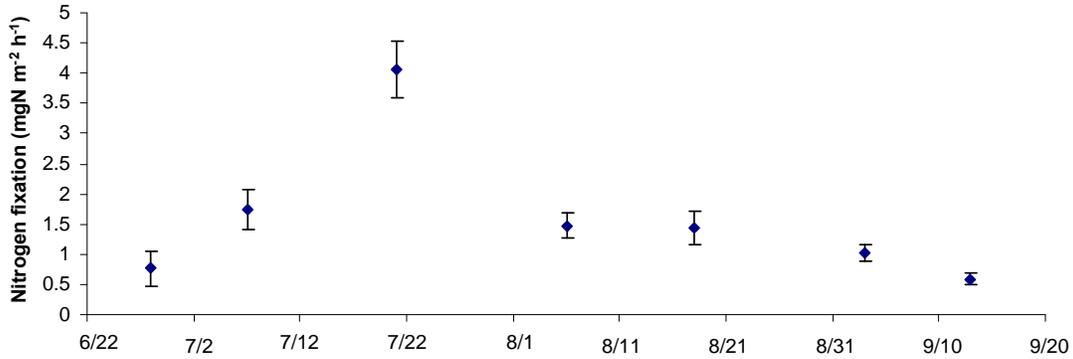
**Figure 2.** Ambient stream nitrate concentrations exceeding  $10 \mu\text{gN/L}$  have low nitrogen fixation rates. The only exception from this study is Headquarters which has  $0.7 \mu\text{gNO}_3\text{-N/L}$  and  $0.002 \text{mg N m}^{-2} \text{h}^{-1}$  nitrogen fixation rate. Headquarters has predominately silt substrate and intermittent flow.

Nitrogen and phosphorus are the two major nutrients necessary for growth of most organisms. Nitrogen fixing bacteria make nitrogen available by fixing gaseous nitrogen from the atmosphere. Nitrogen fixation is an energetically expensive process, so we hypothesize that the N-fixing bacteria do not fix nitrogen when nitrogen is readily available. Also, the N-fixing bacteria will thrive when phosphorus is added because they have access to an abundant supply of N from the atmosphere, thus it is likely that phosphorus is the element in shortest supply. Our experiments show that indeed nitrogen fixation was inhibited by the addition of nitrate (Figure 3), while the phosphorus addition increased nitrogen fixation four-fold. It is interesting to note that adding nitrogen and phosphorus together reduced N-fixation. The stimulatory effect of phosphorus is swamped out by adding extra nitrogen.

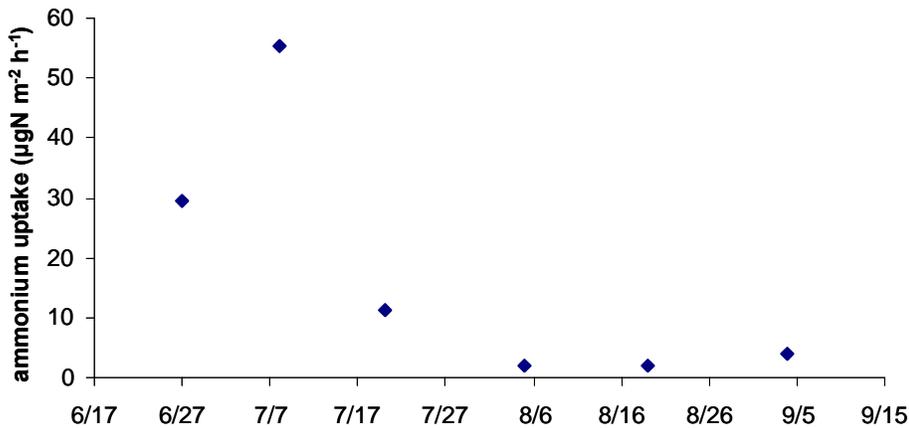


**Figure 3.** Ditch Creek and Spread Creek mean treatment nitrogen fixation rates (n=5). Error bars represent 95% confidence intervals. Addition of nitrogen inhibits N-fixation, while addition of phosphorus stimulates N-fixation.

We compared N-fixation to ammonium uptake, and nitrate uptake, biweekly from the end of June through September 2006. Nitrogen fixation rates greatly exceeded rates of ammonium cycling in Ditch Creek. This stream has nitrogen fixation rates among the highest in the literature, but like our 2005 data rates were among the highest measured in the literature and peaked during the warmest part of July (Fig. 4). Nitrogen fixation rates throughout the summer season were 10 to 100 times greater than uptake rates. Thus, N-fixation dominated internal cycling of nitrogen.



**Figure 4.** Nitrogen fixation in Ditch Creek measured throughout the 2006 summer season. Error bars represent 95% confidence intervals (n=18).



**Figure 5.** Ammonium uptake throughout the 2006 summer season. These measurements are in smaller units ( $\mu\text{gN m}^{-2} \text{h}^{-1}$ ) than nitrogen fixation ( $\text{mgN m}^{-2} \text{h}^{-1}$ ). Nitrogen fixation rates are at least 10 to 100 times higher than ammonium uptake rates showing that the creation of new nitrogen by fixation great exceeds the rate at which N is cycled in Ditch Creek.

**Student Support:**

Lisa Kunza (Neerhof) was funded for 2 years towards a Ph.D. in the department of Zoology and Physiology. Lisa started graduate school in Fall 2004, and has performed two summers of fieldwork on this project. Undergraduate students were hired to assist with field work and lab work. During summer 2005 we hired Jon Hefner, a junior from Nebraska in the Dept. of Zoology and Physiology. Marci Trana, a University of Wyoming student was hired in Fall 2005 to assist with lab work. Additionally using National Science Foundation funds we hired Leslie Henry, a Research Experience for Undergraduates student who examined how nutrients control N fixation. Leslie is a Biology major at UW from Cody, WY. We hired Marley Vaughn from June through September 2006 to assist with fieldwork. Marley was an undergraduate from the University of Oregon, and she is from Wilson, WY.

**Products:**

Lisa presented a talk in June 2006 at the national meeting of the North American Benthological Society entitled "High Nitrogen Fixation in Wyoming Streams". She will present another talk entitled "The Contribution of Nitrogen Fixation to Nitrogen Cycling in Ditch Creek, WY throughout the Summer Season" at the national meeting of the North American Benthological Society in June 2007. Two manuscripts are currently in preparation. A third manuscript and Lisa's dissertation will be completed by 2008.

**Additional Financial Support:**

This project was conducted in tandem with a National Science Foundation funded project examining mechanisms of nitrate removal from streams (Lotic Intersite Nitrogen eXperiment 2). In addition to this project we received additional funds (\$5000) for a Research Experience for Undergraduate student (Leslie Henry). Lisa Kunza also received funds (\$4000) from the University of Wyoming EPSCoR program to develop a method to calibrate acetylene-derived measures of N fixation using isotopically labeled  $^{15}\text{N}_2$  gas.

**References:**

- Alexander, R. B., R. A. Smith, and G. E. Schwarz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* 403: 758-761.
- Grimm, N.B., and K.C. Petrone. 1997. Nitrogen fixation in a desert stream ecosystem. *Biogeochemistry* 37:33-61.
- Howarth, R.W., R.M. Marino, J. Lane, and J.J. Cole. 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. *Limnology and Oceanography* 33:669-687.
- Parmenter, A. W., A. Hansen, R. E. Kennedy, W. Cohen, U. Langer, R. Lawrence, B. Maxwell, A Gallant, and R. Aspinall. 2003. Land use and land cover change in the greater Yellowstone Ecosystem: 1975-1995. *Ecological Applications* 13:687-703.
- Paul, M.J. and J.L. Meyer. 2001. Streams in the urban landscape. *Annual Review of Ecology and Systematics*. 32:333-365.
- Royer, T. V., J. L. Tank, and M. B. David. 2004. The transport and fate of nitrate in headwater agricultural streams in Illinois. *Journal of Environmental Quality* 33:1296-1304.

- Tank, J.L. and W.K. Dodds. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology* 48:1031-1049.
- Vitousek, P.M. J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and D.G. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications* 7:737-750.