A Cross-Ecoregion Evaluation of Nitrogen Fixation and Denitrification in Streams and Rivers of the United States of America

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Abstract

It is typically assumed that dinitrogen (N₂) fixation and denitrification are mutually exclusive processes in riverine ecosystems because N₂ fixation is favored in high light, low nitrogen (N) environments but denitrification is favored under anoxic, high N conditions. Yet recent work in marine and lake ecosystems has demonstrated that N₂ fixation can happen under high N conditions and in sediments, challenging this assumption. We conducted a cross-ecoregion study to test the hypothesis that N₂ fixation and denitrification would co-occur in streams and rivers across a range of reactive N concentrations. Between 2017 and 2019, we sampled 30 streams in 13 ecoregions, using chambers to quantify N_2 flux using membrane inlet mass spectrometry, N₂ fixation using acetylene reduction, denitrification using acetylene block, and microbial diversity using 16S gene sequencing. 25 of the study streams were part of the National Ecological Observatory Network or the StreamPULSE network, which provided data on water temperature, light, nutrients, discharge and metabolism. We found that N_2 fixation rates were detectable in half of the streams surveyed, and were most frequently detected on rock, wood, and/or macrophyte substrates. Denitrification potential was detected in all streams, with rates 1-2 orders of magnitude higher than N₂ fixation rates and the highest rates measured in sediments. Substrate heterogeneity, and associated variation in environmental conditions, appeared to facilitate the coexistence of N_2 fixation and denitrification in the study streams. Rates of denitrification were significantly positively related to streamwater nitrate concentrations ($r^2 = 0.35$), but N₂ fixation rates were not, despite the common simplifying assumption that denitrification dominates the N₂ flux in streams under high N and N₂ fixation only occurs under low N conditions. Additional analyses are exploring reach to watershed characteristics, and metabolic regimes as drivers of cross-ecoregion patterns in processes.

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Objective: Conduct a cross-ecoregion study to test the hypothesis that N₂ fixation and denitrification would co-occur in streams and rivers across a range of reactive N concentrations.

Background

Typically assumed that nitrogen (N_2) fixation and denitrification do not co-occur in streams and rivers because N_2 fixation is favored in high light, low N environments but denitrification is favored under anoxic, high N conditions.

Recent work in marine and lake ecosystems has demonstrated that N₂ fixation can happen under high N conditions and in sediments, challenging this assumption.



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Figure 2. (a) Comparison of rates measured on all substrates, across all streams. (b) Denitrification and (c gen (DIN) concentration measured in the stream on that sampling date.



Figure 3. (a) Denitrification [regression $R^2 = 0.254$, p < 0.001, log(y + 1) = -0.2844 + 0.2833 log(x + 1)] and (b) N₂ fixation rates measured during this study (CAREER) vs. literature review (Marcarelli et al. 2008) https://doi.org/10.1899/07-027.1) and LINX II (Mulholland et al. 2008 https://doi.org/10.1038/nature06686)





Figure 4. A taxa plot of the % proportion of the microbial community determined using 16S sequencing, represented by specific Class for each study stream. Streams are organized from West to East along the xaxis. Classes that represented < 2% of the overall proportion were grouped together.

Next steps: Scale substrate-specific rates using benthic cover estimates to compare process co-occurrence and relative contributions at the reach scale.

Evaluate microbial diversity and composition relative to process rates across streams and biomes.

Explore interrelationships between N process rates, stream energy budgets and environmental controls across streams and biomes.

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