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Denitrification by sulfur-oxidizing bacteria in a eutrophic lake

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ABSTRACT: Understanding the mechanistic controls of microbial denitrification is of central importance to both environmental microbiology and ecosystem ecology. Loss of nitrate (NO_3^-) is often attributed to carbon-driven (heterotrophic) denitrification. However, denitrification can also be coupled to sulfur (S) oxidation by chemolithoautotrophic bacteria. In the present study, we used an *in situ* stable isotope ($^{15}NO_3^-$) tracer addition in combination with molecular approaches to understand the contribution of sulfur-oxidizing bacteria to the reduction of NO_3^- in a eutrophic lake. Samples were incubated across a total dissolved sulfide (H_2S) gradient (2 to 95 µM) between the lower epilimnion and the upper hypolimnion. Denitrification rates were low at the top of the chemocline (4.5 m) but increased in the deeper waters (5.0 and 5.5 m), where H_2S was abundant. Concomitant with increased denitrification at depths with high sulfide was the production of sulfate (SO_4^{2-}), suggesting that the added NO_3^- was used to oxidize H_2S to SO_4^{2-}. Alternative nitrate removal pathways, including dissimilatory nitrate reduction to ammonium (DNRA) and anaerobic ammonium oxidation (anammox), did not systematically change with depth and accounted for 1 to 15% of the overall nitrate loss. Quantitative PCR revealed that bacteria of the *Sulfurimonas* genus that are known denitrifiers increased in abundance in response to NO_3^- addition in the treatments with higher H_2S. Stoichiometric estimates suggest that H_2S oxidation accounted for more than half of the denitrification at the depth with the highest sulfide concentration. The present study provides evidence that microbial coupling of S and nitrogen (N) cycling is likely to be important in eutrophic freshwater ecosystems.

KEY WORDS: Denitrification · Nitrate reduction · Sulfur oxidation · Sulfur-driven denitrification · *Sulfurimonas denitrificans* · Sulfide · Wintergreen Lake

INTRODUCTION

Denitrification is an important microbial process with beneficial consequences for water quality. More than 75% of the anthropogenic nitrogen (N) entering watersheds is lost along landscape flow paths before reaching the oceans (Alexander et al. 2000). This 'missing' N is attributed to heterotrophic denitrification, an anaerobic microbial process that couples the oxidation of organic matter with the reduction of nitrate (NO_3^-) to gaseous N_2. Little is known, however, about where and how this N removal takes place (Seitzinger et al. 2006, Burgin & Hamilton 2007). Due to long water residence times and high biological activity, lakes and reservoirs may be important, yet overlooked, sites for N removal (Saunders & Kalff 2001).

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