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Reach-scale isotopic patterns in riparian groundwater nitrate and nitrous oxide reflect spatial variation in metabolic diversity that consumes agricultural nitrate in central Montana, USA

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Redox conditions in riparian zones counteract nitrate (NO_3^-) loading from agricultural land management, yet interacting processes defy easy quantification of rates and controls. Here, we explore how chronic agricultural nitrate loading (inflows carrying ~20 mg nitrate-N L^{-1}) results in a cascade of redox sensitive metabolisms producing characteristic stable isotope signals of nitrate and nitrous oxide (N_2O) that vary dramatically with extent of nitrate loss and limitation by drought conditions. Across upland groundwater, stream channel, and riparian aquifer field locations over a decade, nitrate-$d^{15}N$ ranged from +5 to +50‰ and nitrate-$d^{18}O$ ranged from -5 to +15‰, with higher values of both in riparian groundwater corresponding to lower concentrations, suggesting fractionating consumption of nitrate. Consistent isotopic patterns in stream channels suggest internal low-$d^{15}N$ (<-10‰) nitrate production in addition to loss, with redox conditions dictating expression of kinetic isotope effects.

In dissolved N_2O, a key intermediate in both production and consumption of nitrate, molecular site preference (difference in ^{15}N content of central versus outer N atoms in the N_2O molecule) was higher (25-55‰) at riparian sites relative to stream channel and upland groundwater (10-20‰) and inversely correlated with a wide range of bulk N_2O-$d^{15}N$ (-40 to +40‰), again suggesting a precursor phase with low $d^{15}N$. At the same time, positive correlation of site preference with elevated bulk N_2O-$d^{18}O$ (+35 to +60‰) suggests oxygen isotopic enrichment of the precursor nitrate pool, implying a contribution of fractionating nitrate loss.

We evaluate these patterns in light of microbial metabolic potential and activity. Diverse clade II (i.e., atypical) and clade III (i.e., lactonase type) N_2O reductases were observed in both metagenomes and metatranscriptomes. These abundant and active N_2O reducers likely play important roles in driving elevated N_2O site preference values in oxygen depleted riparian zones. Our data set also highlights diverse and transcriptionally abundant autotrophic processes dependent on nitrite -- specifically nitrite-dependent anaerobic methane oxidation and anaerobic ammonia oxidation (annamox). Genes and transcripts associated with the latter process were consistently observed within riparian groundwater at nearly all times of year, highlighting a possible role of anammox in controlling nitrogen removal.