Unchanged nitrate and nitrite isotope fractionation during heterotrophic and Fe(II)-mixotrophic denitrification suggest a non-enzymatic link between denitrification and Fe(II) oxidation

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Natural-abundance measurements of nitrate and nitrite (NOx) isotope ratios (delta N-15 and delta O-18) can be a valuable tool to study the biogeochemical fate of NOx species in the environment. A prerequisite for using NOx isotopes in this regard is an understanding of the mechanistic details of isotope fractionation ((15)epsilon, (18)epsilon) associated with the biotic and abiotic NOx transformation processes involved (e.g., denitrification). However, possible impacts on isotope fractionation resulting from changing growth conditions during denitrification, different carbon substrates, or simply the presence of compounds that may be involved in NOx reduction as co-substrates [e.g., Fe(II)] remain uncertain. Here we investigated whether the type of organic substrate, i.e., short-chained organic acids, and the presence/absence of Fe(II) (mixotrophic vs. heterotrophic growth conditions) affect N and O isotope fractionation dynamics during nitrate (NO3-) and nitrite (NO2-) reduction in laboratory experiments with three strains of putative nitrate-dependent Fe(II)-oxidizing bacteria and one canonical denitrifier. Our results revealed that (15)epsilon and (18)epsilon values obtained for heterotrophic ((15)epsilon-NO3-: 17.6 +/- 2.8 parts per thousand, (18)epsilon-NO3-:18.1 +/- 2.5 parts per thousand; (15)epsilon-NO2-: 14.4 +/- 3.2 parts per thousand) vs. mixotrophic ((15)epsilon-NO3-: 20.2 +/- 1.4 parts per thousand, (18)epsilon-NO3-: 19.5 +/- 1.5 parts per thousand; (15)epsilon-NO2-: 16.1 +/- 1.4 parts per thousand) growth conditions are very similar and fall within the range previously reported for classical heterotrophic denitrification. Moreover, availability of different short-chain organic acids (succinate vs. acetate), while slightly affecting the NOx reduction dynamics, did not produce distinct differences in N and O isotope effects. N isotope fractionation in abiotic controls, although exhibiting fluctuating results, even expressed transient inverse isotope dynamics ((15)epsilon-NO2-: -12.4 +/- 1.3 parts per thousand). These findings imply that neither the mechanisms ordaining cellular uptake of short-chain organic acids nor the presence of Fe(II) seem to systematically impact the overall N and O isotope effect during NOx reduction. The similar isotope effects detected during mixotrophic and heterotrophic NOx reduction, as well as the results obtained from the abiotic controls, may not only imply that the enzymatic control of NOx reduction in putative NDFeOx bacteria is decoupled from Fe(II) oxidation, but also that Fe(II) oxidation is indirectly driven by biologically (i.e., via organic compounds) or abiotically (catalysis via reactive surfaces) mediated processes co-occurring during heterotrophic denitrification.

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