Insights into the effect of growth rate and temperature on nitrogen isotope fractionation during denitrification

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Isotopic effects of microbially mediated processes are often considered immutable parameters of biogeochemical cycles, yet few microbial fractionation factors are truly constant. Decades of work on key isotope effects in the sulfur and carbon cycle have revealed that a number of physiological constraints, such as electron acceptor availability, temperature and nutrient limitations, can lead to distinct isotopic effects. In the case of the nitrogen cycle, previous culture-based evidence revealed significant variability in the expressed isotope effect of denitrification in response to environmental constraints that more closely reflect natural growth conditions. This highlights the current limits of our understanding of how environmental growth conditions modulate the isotopic effects of denitrification.

Here, we applied continuous culture (chemostat) techniques to precisely control the biophysical environment, nutrient availability and growth rate of the model organism *Paracoccus denitrificans*. Under the tested experimental conditions, we observed expressed nitrogen isotope effects ranging from 5‰ to 25‰. Temperature, cell-specific nitrate reduction rate, and electron donor vs. acceptor limitation all correlate strongly with the observed fractionation, while other physiological constraints such as pH do not appear to influence the expressed isotope effect. Oxygen isotope measurements further suggest minimal reversibility in the nitrate reduction step even at the lowest growth rates explored here (~1 week doubling times) and confirmed the previously observed ~1:1 relationship of oxygen and nitrogen isotope effects ($^{15}\varepsilon$:$^{18}\varepsilon$) in pure denitrification systems, regardless of the magnitude of the expressed isotope effects.

These results suggest that the expressed isotope fractionation during denitrification can vary significantely within a single organism. The physiological constraints explored provide insight into the potential mechanisms underlying isotopic variation, and highlight the large range of isotopic effects that may contribute to observed nitrogen isotope patterns in denitrification environments in nature.