

The impacts of microbial physiology on the stable isotopic biosignature of nitrate reduction

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The global nitrogen cycle has been severely altered, with human interference resulting in more than double the amount of bioavailable nitrogen being released into ecosystems^[1]. Introducing excess nitrogen into ecosystems has many negative environmental impacts including widespread eutrophication, ozone depletion, and acidification^[1]. To predict ecosystem impacts and develop mitigation strategies to nitrogen pollution, a detailed understanding of the nitrogen cycle is required. Denitrification, or the conversion of bioavailable nitrate into inert N₂ gas, is a key release lever in the nitrogen cycle. The first step of this process, nitrate reduction to nitrite, produces an isotopic signature that researchers use to identify and quantify nitrogen removal rates in ecosystems. However, significant variation in the magnitude of the isotope fractionation for nitrate reduction (¹⁵ε_{NR}) has been observed in both natural and laboratory settings^[2]. It has been previously shown that microbial growth rates can impact ¹⁵ε_{NR}, with increasing growth rates correlating to increased magnitude of ¹⁵ε_{NR}^[2]. However, this research has only been conducted in bacteria that use the membrane bound NarG enzyme to reduce nitrate. Recent work has indicated the potential importance of NapA (located in the periplasm of the cell) mediated nitrate reduction in ecosystems^[3], and so there is a need to characterize its isotopic fractionation under different growth rates as well. Therefore, we grew *Shewanella loihica*, a denitrifier that uses only NapA, at different growth rates in chemostat culture. We collected isotopic and proteomic samples to uncover what changes to microbial physiology may cause changes to ¹⁵ε_{NR}. Our preliminary data suggests NapA mediated nitrate reduction has the opposite trend of what has been previously reported for ¹⁵ε_{NR}. As growth rate is affected by numerous environmental factors, this work underscores the importance of examining the physiologies of the microorganisms controlling the range of isotopic signatures produced to make better inferences about stable isotopic signatures in ecosystems.

References

- [1] Battye, W. *et al. Earths Future* **2017**, 5 (9), 894–904. <https://doi.org/10.1002/2017EF000592>.
- [2] Kritee, K. *et al. Geochim. Cosmochim. Acta* **2012**, 92, 243–259. <https://doi.org/10.1016/j.gca.2012.05.020>.
- [3] Asamoto, C. K. *et al. Environ. Sci. Technol.* **2021**, 55 (8), 5537–5546. <https://doi.org/10.1021/acs.est.0c07816>