Using N and O Isotopes to Determine the Source of Microbial N_2O Production

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https://bmb.natsci.msu.edu/faculty/eric-l-hegg/current-research/research-project-4/

Project Goals: The Great Lakes Bioenergy Research Center supports research that generates technology for the conversion of cellulosic biomass to biofuels. However, agricultural production of cellulosic biomass is associated with emissions of greenhouse gases such as N_2O and CO_2. N_2O is particularly problematic because it has a 100-year global warming potential 300 times that of CO_2 and is the leading cause of stratospheric ozone depletion. Currently the relative importance of microbial processes contributing to N_2O flux is poorly understood. Stable isotopes of N and O have emerged as a tool to discriminate between pathways responsible for N_2O flux. The goals of our project are to (1) determine the sources of microbial N_2O production in soil communities using stable isotopes, (2) elucidate the biochemical pathways of N_2O production associated with specific naturally occurring enzymes and (3) develop methodology for real-time source determination via stable isotope analysis using laser spectroscopy.

Site preference (SP), the difference in the isotope ratios between the central and terminal N atoms in nitrous oxide (N_2O), has emerged as a robust discriminator of the microbial origins of N_2O. SP has emerged as a conservative tracer of N_2O production, differentiating N_2O produced bacterial nitrification and denitrification. In contrast, the $\delta^{15}N$ and $\delta^{18}O$ of N_2O are not conservative tracers of N_2O production. A number of variables (e.g. electron donor abundance, nitrogen substrate and growth rate) markedly influence the $\delta^{15}N$ and $\delta^{18}O$ of N_2O. Importantly, the influence of these variates on SP has not been documented. Thus, we investigated the influence of these variates on SP with different bacterial denitrifiers. Specifically, we examined the effect of the electron donor source (citrate or succinate) and concentration (10, 1, 0.1, and 0.01 mM) on SP in *Pseudomonas chlororaphis subsp. chlororaphis* and *Pseudomonas chlororaphis subsp. aureofaciens*. There was no net isotope effect (no shift in SP) observed across the conditions evaluated (ANOVA, $p>0.05$). These data confirm that SP is a conservative tracer of N_2O production. The observation that SP differed slightly between our two denitrifiers suggests that changes in the predominance of different bacterial species may account for some of the variation in SP for denitrifiers reported in the literature.

In addition to evaluating the influence of different factors on SP, we are now beginning to evaluate how differences in enzymatic mechanisms contribute to variation in SP. We are specifically interested in nitric oxide reductases (NORs), which are responsible for converting nitric oxide (NO) to N_2O. We have chosen to examine if shifts in SP occur for bacterial

cytochrome *c*-dependent NOR, bacterial quinol-dependent NOR, and hydroxylamine oxidoreductase.

Trace gas-isotope ratio mass spectrometry (TG-IRMS) is the current the gold standard for isotopic measurements of N_2O; however the sporadic nature of N_2O production, along with the costs and analytical time associated with TG-IRMS create challenges to its large scale application. Isotopic analysis of N_2O *via* laser spectroscopy offers both reduced analytical time, *in situ* analysis, and continuous real-time measurements of SP. We show that SP determinations from the LGR are comparable to those generated by traditional TG-IRMS. Further, we are able to measure SP at atmospheric concentrations without pre-concentration, a first in spectroscopic analysis of SP.

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