Progress Towards N_2O Source Apportionment in Biofuel Soils: Understanding Sources of Variation in Isotopic Discrimination During Denitrification

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Project Goals: Maximizing the environmental performance of bioenergy crops is critical to their productivity in marginal lands. Management of nitrogen loss in the form of nitrous oxide (N_2O) emissions, an important greenhouse gas and the leading source of ozone depletion, can improve productivity and mitigate climate effects. Modelling N_2O emissions is a considerable challenge in that soil N_2O emissions are principally caused by microbes through diverse processes. Spatiotemporal variation of the microbial communities, the pathways active in individual species, and environmental factors such as soil structure and vegetation further complicate this challenge. Our goal is to employ a combination of new methodologies including the analysis of N_2O isotope values to disentangle the processes contributing to N_2O formation *in situ*. Knowledge of the active processes can be combined with that of the rhizosphere microbial communities to help identify plant traits that promote rhizosphere N_2O reduction by microbes.

Efforts to mitigate agricultural N-losses as N_2O emissions commonly focus on reducing fertilization rates; however management of the microbial processes directly responsible for N_2O production can also reduce N_2O emissions. To this end, evaluation of stable isotope values observed during production of N_2O has been applied to differentiate between the two predominant microbial processes responsible for its production, nitrification and denitrification. To better understand the factors contributing to isotopic variation during denitrification, we have characterized under a range of carbon-source conditions the $\delta^{15}N$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{18}O$, and site preference (SP; the intramolecular distribution of ^{15}N in N_2O) of N_2O produced during NO_3^- reduction by two denitrifying bacteria lacking nitrous oxide reductase. The evaluation of microbial N_2O sources is challenged because the isotopic discrimination for $\delta^{15}N$ and $\delta^{18}O$ during production, while expected to be constant, can vary markedly over the course of the reaction. Additionally, the multi-step nature of denitrification violates critical assumptions of the traditional Rayleigh approximation used to estimate changes in $\delta^{15}N$ and $\delta^{18}O$ values of a single-step unidirectional reaction. The change in isotope value of a multi-step reaction is more aptly termed a net isotope effect (NIE), because many isotopic fractionations can contribute to the observed isotopic discrimination of the final product. We demonstrate that the NIE varies by as much as 100 ‰ over the course of a single reaction, which clearly limits the use of $\delta^{15}N$ and $\delta^{18}O$ as apportionment tools. Given our observations of changes in the degree of fractionation of $\delta^{15}N$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, and $\delta^{18}O$ across all treatments, we developed a non-linear approach to estimate this changing isotopic discrimination associated with multi-step reactions, such as denitrification. In contrast, SP values for denitrification did not change over the course of the

reaction, although the mean SP of N_2O produced by each species differed. Therefore, SP remains a robust indicator of the origin of N_2O.

To better understand the observed non-linear change in isotope values during denitrification and the potential sources of species-specific variation in SP, we characterized isotopic fractionation during *in vivo* reduction of nitric oxide (NO) to N_2O by *P. aureofaciens* and *P. chlororaphis*. The enzyme cNOR is thought to be the primary enzyme responsible for the reduction of NO to N_2O in soils and is present in both of the aforementioned species. Therefore we also performed *in vitro* reduction of NO by isolated cytochrome *c*-dependent NO reductase (cNOR) from *Paracoccus denitrificans* to determine the intrinsic fractionation associated with this enzyme. Collectively, these data suggest that species-specific factors likely contribute to the fractionation expressed during NO reduction and provide insight into the yet unknown mechanism of N_2O production by cNOR.

Funding statement

This work was funded by the National Science Foundation's Earth Sciences Instrumentation and Facilities program (grant #1456430) and DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494).