Insights into mechanisms of nitrous oxide generation from measurement of nine N_2O isotopologues

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Nitrous oxide (N_2O), a greenhouse gas and ozone-depleting molecule, is currently accumulating in the atmosphere. This accumulation is thought to be driven by biological nitrification and denitrification, but the exact balance of biological contributions of N_2O in the environment is unclear. We are assessing whether distinct isotopic signatures are associated with different biological sources of N_2O, using new analytical methods of high resolution mass spectrometry that enable quantification of: $\delta^{15}N$, $\delta^{18}O$, $\Delta^{17}O$, ^{15}N site preference, and the clumped isotopologues $^{14}N^{15}N^{18}O$, $^{15}N^{14}N^{18}O$, $^{15}N_2{}^{16}O$, and the sum of $^{14}N^{15}N^{17}O+^{15}N^{14}N^{17}O$. Each of these parameters records different aspects of the substrates and bond-making and bond-breaking reactions that involve N_2O and its precursors, including both equilibrium and kinetic effects.

We have applied these techniques to measure the isotopologues of N_2O associated with a number of environmentally relevant processes, including bacterial and fungal denitrification, bacterial ammonia oxidation, and abiotic nitrite reduction by ferrous iron. The bulk isotopic compostion and ^{15}N site preference for each sample match the expected mechanism of formation and previous pure culture measurements. No sample has position-specific and clumped isotope compositions consistant with an equilibrated final product. Most processes produce a $\Delta(^{14}N^{15}N^{18}O+^{15}N^{14}N^{18}O)$ value, which represents the abundance of these two isotopologues relative to a random distribution of ^{15}N and ^{18}O among all isotopologues, between 0 and 1‰. But for some samples that come from organisms with a copper-type nitrite reductase, including both ammonia oxidizing bacteria and denitrifiers, this parameter can be less than zero. One possibility is that these values can be inherited from from precursors to N_2O, like nitrite. Finally, the site preference for ^{15}N in ^{18}O-containing isotopomers proves to be useful to distinguish N_2O from fungal denitrifiers and the hydroxlamine oxidation pathway of bacterial nitrifiers, which overlap in both $\Delta(^{14}N^{15}N^{18}O+^{15}N^{14}N^{18}O)$ and conventional ^{15}N site preference. These results suggest that all measured isotopologues are useful for distinguishing among various mechanisms of nitrous oxide production.