Non-canonical N_2O production pathways under low oxygen

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Aquatic systems are a key source of the greenhouse gas nitrous oxide (N_2O), generating up to 35% of global N_2O emissions. Yet, little is known about the microorganisms and the partitioning of pathways that mediate N_2O production across oxygen and nutrient gradients. We dissected the contributions of denitrification and nitrification to N_2O production in the oxygen minimum zone of the Eastern Tropical North Pacific (ETNP) using both ^{15}N-labeled substrates and $^{18}O_2$. Production of $^{15}N_2O$ from ^{15}N-nitrate ($^{15}NO_3^-$) additions, but not from ^{15}N-ammonium ($^{15}NH_4^+$) additions, suggested denitrification as the source of N_2O within both the oxycline and the deep chlorophyll maximum. However, the data suggested that microbes reduced the $^{15}NO_3^-$ via $^{15}NO_2^-$ to $^{15}N_2O$ in an atypical "closed" pathway without freely exchangeable intermediates. Surprisingly, no variability in the N_2O production rate via this pathway was observed over a manipulated oxygen range of 0.1 to 15 μM. This stands in contrast to previous work that observed inhibition of N_2O production via canonical denitrification at nanomolar oxygen concentrations. Interestingly, experiments labelled with $^{18}O_2$ showed incorporation of ^{18}O into N_2O. Such incorporation would be expected in N_2O from hydroxylamine oxidation by ammonium oxidizers, but the lack of ^{15}N incorporation from $^{15}NH_4^+$ in parallel incubations does not support this, which suggests that an alternate mode of N_2O production is at play. Multiple pathways of N_2O production were also indicated by metatranscriptomic data showing transcripts encoding proteins of both aerobic and anaerobic N_2O production. In summary, the apparent patterns observed in the ETNP did not match those predicted based on current hypotheses of how N_2O is produced in low-oxygen environments. Further elucidation of these non-canonical pathways and the organisms involved is important for understanding the factors controlling N_2O emissions from aquatic systems.