Potential role of copper availability in nitrous oxide accumulation in a temperate lake

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ABSTRACT: Denitrifying bacteria require copper for the synthesis of nitrous oxide reductase. In the absence of sufficient bioavailable Cu, nitrous oxide (N_2O) may accumulate in natural waters during denitrification. Cultures of *Paracoccus denitrificans* and natural bacterial assemblages collected from a mesotrophic lake (Linsley Pond) were grown at varying Cu concentrations to determine the Cu speciation that results in elevated N_2O accumulation. *P. denitrificans* experienced Cu limitation beginning at inorganic Cu concentrations of 0.6 fmol L^{-1} (-log of inorganic Cu, pCu', = 15.2). The natural community did not show an effect until pCu' was reduced to 23.7 with 8-hydroxyquinoline, resulting in an approximate 10-fold increase in N_2O concentrations during denitrification. Additions of ethylenediaminetetraacetic acid alone to natural communities did not affect N_2O concentrations. Natural copper-binding ligands detected with competitive ligand exchange-cathodic stripping voltammetry occurred at concentrations of 6 to 25 nmol L21 with conditional stability constants ($K_{Cu^{2+},L}$) between $10^{14.4}$ and $10^{15.1}$. Although more than 99% of total Cu in Linsley Pond was bound to these ligands, inorganic Cu concentrations remained 10 orders of magnitude above those found to increase N_2O accumulation during denitrification incubations. Measurements of nitrogen species, dissolved oxygen, and sulfide in the water column of Linsley Pond over the spring growing season revealed that N_2O was produced by assimilatory nitrate reduction and nitrification in addition to denitrification, with nitrification generating most of the N_2O found in the surface waters of the lake. The results suggest that inorganic Cu concentrations in Linsley Pond are sufficient to support denitrification. Moreover, some denitrifying bacteria may be able to access organically bound Cu, reducing the potential for this metal to affect N_2O production in other aquatic environments.