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Autoxidation and acetylene-accelerated oxidation of NO in a 2-phase system; implications for the expression of denitrification in ex situ experiments

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Denitrification allows microorganisms to sustain respiration under anoxic conditions. The typical niche for denitrification is an environment with fluctuating oxygen concentrations such as soils and borders between anoxic and oxic zones of biofilms and sediments. In such environments, the organisms need adequate regulation of denitrification in response to changing oxygen availability to tackle both oxic and anoxic spells. The regulation of denitrification in soils has environmental implications, since it affects the proportions of N_2, N_2O and NO emitted to the atmosphere. The expression of denitrification enzymes is regulated by a complex regulatory network involving one or several positive feedback loops via the intermediate nitrogen oxides. Nitric oxide (NO) is known to induce denitrification in model organisms, but the quantitative effect of NO and its concentration dependency has not been assessed for denitrification in soils. NO is chemically unstable in the presence of oxygen due to autoxidation, and the oxidation of NO is accelerated by acetylene (C_2H_2) which is commonly used as an inhibitor of N_2O reductase in denitrification studies. As a first step to a better understanding of NO's role in soil denitrification, we investigated NO oxidation kinetics for a closed "two phase" system (i.e. liquid phase + headspace) typically used for denitrification experiments with soil slurries, with and without acetylene present. Models were developed to adequately predict autoxidation and acetylene-accelerated oxidation. The minimum oxygen concentration in the headspace ($[O_2]_{min}$, mL L^{-1}) for acetylene-accelerated NO oxidation was found to increase linearly with the NO concentration ([NO], mL L^{-1}); $[O_2]_{min}$= 0.192 + [NO]*0.1 (r^2=0.978). The models for NO oxidation were then used to assess NO-oxidation rates in denitrification experiments with batches of bacterial cells extracted from soil. The batches were exposed to low initial oxygen concentrations in gas tight serum flasks (with and without C_2H_2), and monitored for O_2, NO, N_2O and N_2 production while depleting the oxygen and switching to anoxic respiration. Acetylene effectively scavenged NO from the cultures until oxygen concentration reached below $\sim$0.19 mL L^{-1}, and the estimated rate of acetylene-accelerated NO oxidation was more than sufficient to explain an observed reduction of the N_2O production induced by acetylene. When $[O_2]$ reached below 0.19 mL L^{-1}, the NO concentrations increased and stabilized at the same level as in the treatments without acetylene, but the rate of denitrification was much lower than without acetylene. The results indicate that the early accumulation of 10-20 nM NO during oxygen depletion has a significant effect on the expression of denitrification in soil communities. This warrants a greater interest in NO as a regulator of denitrification in soils and shows that the acetylene inhibition method may be problematic even for intentionally anoxic incubations, unless precautions are taken to secure initial O_2-concentrations below 0.19 mL O_2 L^{-1}.