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Multiple approaches to understand regulation of N2O from denitrification: the case of pH

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Despite many decades of field research, no clear strategy has emerged for reducing soil N_2O emissions associated with food production. To the contrary, atmospheric N_2O continues to rise (0.3% yr^{-1}) as anthropogenic N fixation increases to meet human population and prosperity growth. Any reactive nitrogen added to the biosphere will eventually return to the atmosphere via microbial N transformations that produce N_2O in variable proportions. We believe that it is the stoichiometry of these processes (nitrification and denitrification) that ultimately determines atmospheric N2O. Recent evidence suggests that denitrifying prokaryotes differ widely in their ability to perform complete denitrification in response to anoxia and hence display specific ratios in their denitrification products. The UMB nitrogen group uses high-resolution gas kinetics (O_2, NO, N_2O and N_2) with batch incubations to explore such product ratios in search for distinct 'denitrifier regulatory phenotypes' (DRP). To understand which factors are critical for denitrification regulatory phenotypes, gene transcription analyses (*nir*S, *nir*K, *nos*Z) are run in controlled experiments together with the gas kinetics. Pure culture experiments with denitrifying model organism are used to generate hypotheses regarding the underlying mechanisms of regulation, while extracted complex bacterial communities from soils are used to test the hypotheses. In the present contribution, we report combined functional/molecular approaches seeking to unravel the effect of pH on denitrifier performance, functional taxonomic composition and relative N_2O production. Acidic soils are wide spread and acidification may exacerbate with further intensification of agriculture and increased use of chemical fertilizers. There is abundant circumstantial evidence that acidity increases the denitrification product ratio (N_2O/N_2), but nothing is known about the mechanism of pH control. Moreover, since pH affects virtually all biochemical processes in soil, confounding of direct and indirect control by pH is formidable. To explore direct effects of pH, we designed experiments with the denitrifier model organism *Paracoccus denitrificans* and found high N_2O accumulation at suboptimal pH. Expression studies suggested that pH interferes with the assembly of the N_2O reductase enzyme at the post-transcriptional level. This mechanism was corroborated indirectly in a study with intact soil from a long-term liming experiment and with bacteria extracted from these soils, suggesting pervasive pH control on N_2OR functioning on a cellular level, i.e. a DRP common to all bacterial denitrifiers. In contrast, when running experiments with soil slurries and extracted bacterial consortia from geographically remote organic soils with contrasting pedogenic pH, congruent differences in denitrifier taxonomic composition/abundance and function were found, suggesting that pH selects for specific DRPs on a community level with clear-cut consequences for the propensities to emit N_2O. As proof of concept, we present first results from a field study recently initiated in a long term liming experiment in Norway where we found dramatic differences in fertiliser induced N_2O emissions depending on liming history. Based on the mechanistic understanding of direct and indirect pH control as well as the empirical evidence from field experiments, we hypothesized that liming acid agricultural soils may be an efficient way to reduce N_2O emissions. Moreover, our data call for a refinement of biogeochemical models, many of which do not account for pH control and denitrification regulatory phenotypes.