



## Denitrification nitrogen gas formation and gene expression in alpine grassland soil as affected by climate change conditions

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Due to methodological problems, reliable data on soil dinitrogen (N<sub>2</sub>) emission by denitrification are extremely scarce, and the impacts of climate change on nitrogen (N) gas formation by denitrification and N gas product ratios as well as the underlying microbial drivers remain unclear. We combined the helium-gas-flow-soil-core technique for simultaneously quantification of nitrous oxide (N<sub>2</sub>O) and N<sub>2</sub> emission with the reverse transcript qPCR technology. Our goals were to characterize denitrification dynamics and N gas product ratios in alpine grassland soil as affected by climate change conditions and to evaluate relationships between denitrification gene expression and N gas emission. We used soils from the pre-alpine grassland Terrestrial Environmental Observatory (TERENO), exposed to ambient temperature and precipitation (control treatment), or three years of simulated climate change conditions (increased temperature, reduction of summer precipitation and reduced snow cover). Soils were amended with glucose and nitrate and incubated subsequently at 1) 5°C and 20% oxygen; 2) 5°C and 0% oxygen; 3) 20°C and 0% oxygen until stabilization of N gas emissions in each incubation step.

After switching incubation conditions to 0% oxygen and 20°C, N<sub>2</sub>O emission peaked immediately and declined again, followed by a delayed peak in N<sub>2</sub> emission. The dynamics of *cnoR* gene expression, encoding the reduction of nitric oxide (NO) to N<sub>2</sub>O, followed the N<sub>2</sub>O emission pattern, while *nosZ* gene expression, encoding N<sub>2</sub>O reduction to N<sub>2</sub> followed the course of N<sub>2</sub> emission. The mean N<sub>2</sub>O:N<sub>2</sub> ratios were 1.31 + 0.10 and 1.56 + 0.16 for control and climate change treatment respectively, but the denitrification potential was overall lower in climate change treatment. Hence, simulated climate change promoted N<sub>2</sub>O but lessened N<sub>2</sub> emission. This stimulation of N<sub>2</sub>O was in accordance with increased *cnoR* gene expression in soil of the climate change treatment. N mass balance calculations revealed that denitrification N gas formation accounted for 21%, dissimilatory nitrate reduction to ammonium for 8%, and microbial immobilization for 73% of nitrate consumption.

Overall, our study shows that changes in climate exert feedback on denitrification N gas formation and N gas product ratios via changes in microbial activity at the level of single denitrification steps. The close relationships found between denitrification N gas formation, N gas product ratios and denitrification gene expression suggests a large potential of molecular methods to predict denitrification dynamics in soil.