N₂O and N₂ emissions from pasture soils differing in pH – does the linkage between the gas fluxes, denitrifying activity and size of the denitrifier community exist?

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Denitrification is of environmental concern because, together with nitrification, it is the main biological process responsible for N₂O emissions. N₂O is a potent greenhouse gas and after some reactions in the stratosphere it can also cause the destruction of stratospheric ozone. Both, the amount of denitrification end products (N₂O and N₂) evolved, and the N₂O/(N₂O+N₂) ratio, are important in understanding, predicting and mitigating N₂O fluxes from soils. Soil pH is one of the most important factors influencing both denitrification and N₂O production. In general, denitrification rate increases with increasing pH values, while the N₂O/(N₂O+N₂) ratio decreases. This relationship has already been well characterized in laboratory experiments, but not verified in the field because of methodological limitations for in situ measurement of N₂ emissions. Soil pH is also an important factor influencing denitrifier community composition, which can be an important driver of denitrification activity and N₂O emissions. The objective of the present study was to explore the effect of changes in soil pH on in situ N₂O and N₂ emissions and on denitrifying enzyme activity. In addition, we also investigated whether differences in the N-gas fluxes could be related to the size of the microbial community possessing different denitrification genes.

We established a field experiment situated in a grassland area in South Bohemia, Czech Republic, where we manipulated the soil pH. The field experiment consisted of three treatments which were repeatedly amended with KOH solution (alkaline soil), H₂SO₄ solution (acidic soil) or with water (pH-natural soil) over 10 months. At the site we determined field N₂O and N₂ emissions using ¹⁵N gas-flux method. Soil samples were collected for determination of denitrifying enzyme activity (DEA) and for quantification of the size of the denitrifying community by quantitative PCR of the narG, napA, nirS, nirK, nosZ denitrification genes. The total bacterial community was quantified using 16S rRNA as molecular marker.

Manipulation of soil pH via the application of acid or alkaline solutions resulted in a significant changes in the soil reaction: pH 5.5, 6.8 and 7.7 for the acidic, pH-natural and alkaline soils, respectively. DEA and N₂ fluxes in situ were highest in the alkaline soil and lowest in the acidic soil, but we did not find any differences in N₂O production or emissions between the pH treatments. On the other hand, the N₂O/(N₂O+N₂) molar ratio was the highest in the acidic soil and the lowest in the alkaline soil. The total N-fluxes in situ significantly correlated to DEA and the N₂O/(N₂O+N₂) ratio was significantly correlated to the N₂O/(N₂O+N₂) ratio calculated from the DEA assay. For all denitrification genes and the 16S rRNA gene, the highest gene copy numbers were observed in the pH-natural soil. However, the abundance of none of the denitrification genes was correlated to total N-fluxes in situ and only the abundance of the nirK gene was correlated to DEA. The N₂O/(N₂O/(N₂O+N₂) ratio was negatively correlated to the abundance of the nirS, napA and narG genes and also to the nirS/16S rRNA, narG/16S rRNA and napA/16S rRNA ratios. We found a positive correlation between the nirS/16S rRNA ratio and soil pH. However, we did not find any negative correlation between the proportion of denitrifiers possessing the nosZ gene and the N₂O/(N₂O+N₂) ratio.

To conclude, our results indicate that manipulation of soil pH affected the N₂O/(N₂O+N₂) ratio, which increased with decreasing pH due to changes in total denitrification activity but not in N₂O production. We also showed that denitrification activity and N₂O production measured under laboratory conditions were correlated with N-fluxes in situ and therefore could reflect treatment differences in the field. The size of denitrifying community was uncoupled to in situ N-fluxes but the denitrifying enzyme activity was significantly correlated to the number of NirS-denitrifiers. We also found a relationship between the narG, napA and nirS gene copy numbers and the N₂O/(N₂O+N₂) ratio, which remains to be explored. However, in this study, the proportion of denitrifiers capable to reduce the N₂O did not seem to have a role in determining the N₂O/(N₂O+N₂) ratio. It is crucial in future studies to continue to bridge the gap between studies of denitrifier ecology and of N-fluxes for a comprehensive understanding of the role of denitrifier community ecology in determining not only total denitrification rates but also the nature of the denitrification end products. This work was supported by the research grants AV0Z60660521, MSM 6007665801, LC 06066 and IAA600660605, and by the Barrande Programme 2-07-26.

Keywords: denitrification; pH; soil; N₂O emissions; ¹⁵N; denitrifying enzyme activity; qPCR; narG, napA; nirS; nirK; nosZ.