



## **Effect of N-fertilization on N<sub>2</sub> and N<sub>2</sub>O flux rates in relation to the structure of the denitrifying bacterial community in fen soil**

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Drained fen peatlands of north-east Europe managed by different agricultural regimes constitute an important source for emissions of nitrous oxide (N<sub>2</sub>O) into the atmosphere. The strength of N<sub>2</sub>O fluxes showed a high variability in time and space influenced by complex interactions of formation and transfer processes with environmental parameters which makes prediction of emissions uncertain. This applies particularly to the denitrification. This is one of the microbial processes with special interest for N<sub>2</sub>O production in the context of frequently varying soil-moisture content of fen soils that favouring anaerobic conditions. Moreover, denitrification may result in the production of both N<sub>2</sub>O and N<sub>2</sub> in varying proportions. However, not only the knowledge about the effect of soil chemical and physical properties, climate, vegetation, and management on the actual N<sub>2</sub>O and N<sub>2</sub> fluxes is very incomplete at fen soils. There are also no information about the role of structure and activity of denitrifying microbial communities in this context either. The experiments presented in this study aim at addressing this subject matter. Relatively undisturbed soil columns (250 cm<sup>3</sup>) from a long-term N-fertilization experiment on a fen grassland (over a period of 45 years) were used for measurement of the current N<sub>2</sub> and N<sub>2</sub>O fluxes followed by processing the soil sample for microbiological analysis immediately to investigate the influence of N-fertilization on these parameters.

The direct measurement of N<sub>2</sub> and N<sub>2</sub>O fluxes was performed by a helium atmosphere incubation technique in the lab. Results showed a strong influence of N-fertilization and soil moisture on the emission of N<sub>2</sub> and N<sub>2</sub>O. Shifts in the total microbial community were estimated by phospholipid fatty acid analysis, a fingerprint focused on active organisms. Results showed that seasonal effects interfered the influence of N-fertilization on shifts of the total microbial community composition. To characterise the denitrifying bacterial community, a combination of cultivation (screening and isolation) and cultivation-independent methods targeting different enzymes of the denitrification process were used. In addition to selective cultivation the abundance of denitrifying bacteria was studied by a real-time PCR approach quantifying the nitrite reductase (*nirK*) and the nitrous oxide reductase (*nosZ*) genes. Results of both methods demonstrated only a marginal effect on the abundance of this functional group. The analysis of the community structure of denitrifying bacteria was performed by cultivation under selective conditions, physiological tests of the isolates and their phylogenetic assignment. In parallel, the community structure was studied by a *nirK* clone library. In contrast to the abundance of the *nirK* and *nosZ* genes, both the composition of the isolates as well as the cloning approach revealed a long-term effect of N-fertilization.

The results show clearly that the combination of different experimental approaches can actually lead to a better understanding of the N turnover and loss processes of fen peatlands.