



Quantification of nitrous oxide (N₂O) uptake in boreal forest soils by combining isotopic and microbial approaches

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The amount of nitrous oxide (N₂O) produced during denitrification is highly regulated by the function of the last reductase enzyme (nitrous oxide reductase; nosZ) which is known to be inhibited by oxygen, low pH and low temperature, which are typical characteristics of boreal peatlands and some forest soils. Denitrification can be a sink for N₂O, if the last step of the process is very efficient. Generally, the N₂O sink potential of soils is poorly constrained; while uptake rates were often observed in field studies, the data was rejected as analytical errors or artifacts. This led to the question: when and by which mechanisms does N₂O uptake occur in natural boreal forests?

In order to answer this question, we established a ¹⁵N₂O tracer experiment where the production of ¹⁵N₂ and consumption of ¹⁵N₂O were quantified in aerobic and anaerobic conditions followed by abundance analyses of genes and transcripts. The laboratory incubations were complemented with molecular approaches which linked the N₂O dynamics with individual microbial species and transcriptomics. The abundance of denitrifying functional genes and gene transcripts reducing nitrous oxide (nosZ) were quantified throughout the experiment with sacrificial sampling in order to solve the role of typical and atypical denitrifying populations on N₂O consumption.

For this study, a Finnish boreal spruce forest and peatland were selected where previous field measurements have revealed negative N₂O fluxes (i.e. N₂O uptake). Soil horizons were selected in both the organic layer and uppermost mineral soil layer and in the peat layers 0-10 cm and 10-20 cm, where oxygen is limited and N₂O uptake occurs at the field scale. ¹⁵N-N₂O (99 AT %) was added to an initial N₂O concentration of 1.7 ppm. All soils were flushed with 100% helium prior to the N₂O addition to ensure that the NO₃ stocks were reduced, leaving the added N₂O as the sole activator of N₂O uptake and primary N source.

Aerobic N₂O uptake was quantified in the mineral horizon of the spruce forest soil (20% from initial concentrations) and both layers of the peat (average 35%). Anaerobic N₂O uptake was quantified in both the peat (85- 98%) and spruce forest soils (89-95%) at each layer at a much higher rate than aerobic uptake, indicating that the microbial community responsible for N₂O uptake is present and can be activated within the soil and peat layers. The majority of the N₂O uptake occurred within the first 48 hours after N₂O additions, indicating that this is a quickly occurring reaction when N stocks are limited to only N₂O. Only typical denitrifier transcripts of nosZ were found in the soils layers under the conditions consuming N₂O in both spruce forest soils and boreal peatland, suggesting that typical denitrifiers are primarily responsible for N₂O uptake.