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## Integrating microbiological and isotope methods for studying nitrification and denitrification processes in soils of drained and rewetted peatland forest

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Due to the complexity and diversity of nitrogen cycle processes, different methods, e.g., microbiological and isotope analysis, are used to study them. Their combined application helps make the most accurate estimates of the processes occurring, which is essential for the future management of drained peatlands to mitigate soil degradation and negative atmospheric impact. Nitrification and denitrification processes in soil are the main processes behind the harmful greenhouse gas nitrous oxide (N<sub>2</sub>O) emission.

This study aimed to investigate the effect of drainage and rewetting on nitrification and denitrification processes and N<sub>2</sub>O emissions using real-time PCR and isotope methods. In the summer of 2020, the 1 m<sup>2</sup> triangle-shaped mesocosms were established to achieve varying oxygen conditions for flooding and drainage experiment in Estonia's *Oxalis* site-type drained peatland forest. In the experiments, heavy nitrogen tracers of potassium nitrate <sup>15</sup>N 98% atom (Sigma Aldrich) and ammonium chloride <sup>15</sup>N 98% atom (Sigma Aldrich) were applied to soil to amplify and get an insight into N<sub>2</sub>O production mechanisms and on its soil moisture dependence. N<sub>2</sub>O concentration was measured, and soil samples were collected six times from the study sites between October 2020 and January 2021. Besides different physical and chemical parameters measured of soil samples, quantitative real-time PCR was used to measure the abundance of bacterial and archaeal specific 16S rRNA, nitrification (bacterial and archaeal *amoA* genes) and denitrification (*nirK*, *nirS*, *nosZI* and *nosZII* genes) marker genes from the samples. Isotope composition of soil and gas samples were also measured.

This study indicates that different hydrological regimes influence nitrification and denitrification processes. Regarding control of N<sub>2</sub>O fluxes, nitrification played a major role on drained sites, and denitrification was the main process in rewetted sites, which is easily related to the oxygen content in the soils. This is supported by a higher proportion of <sup>15</sup>N-N<sub>2</sub>O in <sup>15</sup>N-NO<sub>3</sub> treatment in rewetted mesocosms. In the case of <sup>15</sup>N-NH<sub>4</sub> treatment, the highest proportion of heavy N was found in the drained mesocosms. Overall, heavy nitrogen proportion in both alpha and beta positions was higher in the N<sub>2</sub>O produced by denitrification, whereas N<sub>2</sub>O contained only one <sup>15</sup>N atom per N<sub>2</sub>O molecule. Abundances of *nosZI* and *nosZII* genes behaved differently in drained and rewetted mesocosms. Both microbiological and isotope methods showed similar results and backed each other very well, which makes either of them a perfect tool for predicting N<sub>2</sub>O emissions.

