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## Towards enhanced sensitivity of the $^{15}\text{N}$ Gas Flux method for quantifying denitrification in soil

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Denitrification is one of the major pathways of nitrogen (N) output from soil. In this process, soil nitrate ( $\text{NO}_3^-$ ) is chemically reduced into dinitrogen ( $\text{N}_2$ ) through microbial respiration. Incomplete denitrification leads to the emission of nitrous oxide ( $\text{N}_2\text{O}$ ), a greenhouse gas 300 times more potent in inducing global warming than carbon dioxide ( $\text{CO}_2$ ). Denitrification is highly variable in space and time, which makes it one of the most unconstrained processes in the global N cycle.

Measuring denitrification is challenging because it emits small amounts of  $\text{N}_2$ , hardly distinguishable from the high  $\text{N}_2$  atmospheric background (78% in volume). The aim of this study was to increase the sensitivity of the  $^{15}\text{N}$  Gas Flux method ( $^{15}\text{NGF}$ ), which is considered today, the only suitable method for in situ measurement of denitrification. The  $^{15}\text{NGF}$  consists of injecting a stable isotopic tracer ( $^{15}\text{NO}_3^-$ ) in a pre-determined area of soil and quantifying  $\text{N}_2$  production via its isotopic composition over time under an enclosed chamber. In order to increase the sensitivity of this method, we aimed to optimize two parameters: the quantity of tracer injected and the  $\text{N}_2$  background concentration. Increasing the amount of available nitrate represents a risk of stimulating microbes. Reducing the atmospheric  $\text{N}_2$  background in situ can be challenging because of leaks and diffusion issues.

Our study focused on three different types of agricultural land uses: Arable, Herbal-Rich ley and Grass Clover ley. All three land uses were part of the same experimental field and the leys were in a 3-year rotation with the Arable. We first incubated homogenised soil under lab conditions and under different treatments of added tracer in order to increase sensitivity and observe if a microbial stimulation occurred. Gravimetric moisture was raised to 45% (on a dry mass basis) to simulate a rainfall event and increase the magnitude of denitrification. First experiments showed no detectable amount of evolved  $\text{N}_2$  and thus, a custom-made gas mix had to be used. This gas mix contained 20% of dioxygen ( $\text{O}_2$ ), 5% of  $\text{N}_2$  and 75% of Helium (He) and was used to replace the native atmosphere in the incubation chambers.

First results showed no significant difference in denitrified N for the ley soils treated with different amounts of tracer. The Arable soil however seemed to have been stimulated when using greater quantities of tracer but further results are expected to confirm this. The Arable treatment also had the highest potential of denitrification in the lab with a mean value of  $6.26 \times 10^{-1} \mu\text{gN/kg/h}$  of emitted  $\text{N}_2$ , compared to the leys who both emitted  $1.65 \times 10^{-1} \mu\text{gN/kg/h}$ . The theoretical sensitivity is increased 24 times for the detection  $^{29}\text{N}_2$  and 97 times for the detection of  $^{30}\text{N}_2$  when using the gas mix and a 50% tracer enrichment, compared to a 20% enrichment under atmospheric conditions.

Finally, we measured denitrification directly in-situ using higher quantities of tracer and the custom-made gas mix. This was done using either modified greenhouse gas chambers or sealed plastic liners.