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A novel ^{15}N tracer approach for the quantification of N_2 and N_2O emissions from soil incubations in a completely automated laboratory set up

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The microbial mediated production of nitrous oxide (N_2O) and its reduction to dinitrogen (N_2) via denitrification represents a loss of nitrogen (N) from fertilised agro-ecosystems to the atmosphere. Although denitrification has received great interest by biogeochemists in the last decades, the magnitude of N_2lossesand related N_2:N_2O ratios from soils still are largely unknown due to methodical constraints. We present a novel ^{15}N tracer approach, based on a previous developed tracer method to study denitrification in pure bacterial cultures which was modified for the use on soil incubations in a completely automated laboratory set up. The method uses a background air in the incubation vessels that is replaced with a helium–oxygen gas mixture with a 50-fold reduced N_2 background (2 % v/v). This method allows for a direct and sensitive quantification of the N_2 and N_2O emissions from the soil with isotope-ratio mass spectrometry after ^{15}N labelling of denitrification N substrates and minimises the sensitivity to the intrusion of atmospheric N_2 at the same time. The incubation set up was used to determine the influence of different soil moisture levels on N_2 and N_2O emissions from a sub-tropical pasture soil in Queensland/Australia. The soil was labelled with an equivalent of 50 μg-N per gram dry soil by broadcast application of KNO_3solution (4 at.% ^{15}N) and incubated for 3 days at 80% and 100% water filled pore space (WFPS), respectively. The headspace of the incubation vessel was sampled automatically over 12hrs each day and 3 samples (0, 6, and 12 hrs after incubation start) of headspace gas analysed for N_2 and N_2O with an isotope-ratio mass spectrometer (DELTA V Plus, Thermo Fisher Scientific, Bremen, Germany). In addition, the soil was analysed for ^{15}N NO_3^- and NH_4^+ using the ^{15}N diffusion method, which enabled us to obtain a complete N balance. The method proved to be highly sensitive for N_2 and N_2O emissions detecting N_2O emissions ranging from 20 to 627 μN $kg^{-1}soil^{-1}hr^{-1}$and N_2 emissions ranging from 4.2 to 43 μN $kg^{-1}soil^{-1}hr^{-1}$for the different treatments. The main end-product of denitrification was N_2O for both water contents with N_2 accounting for 9% and 13% of the total denitrification losses at 80% and 100%WFPS, respectively. Between 95-100% of the added ^{15}N fertiliser could be recovered. Gross nitrification over the 3 days amounted to 8.6 μN g^{-1} $soil^{-1}$ and 4.7 μN g^{-1} $soil^{-1}$, denitrification to 4.1 μN g^{-1} $soil^{-1}$ and 11.8 μN g^{-1} $soil^{-1}$at 80% and 100%WFPS, respectively. The results confirm that the tested method allows for a direct and highly sensitive detection of N_2 and N_2O fluxes from soils and hence offers a sensitive tool to study denitrification and N turnover in terrestrial agro-ecosystems.