



Isotopologue signatures of nitrous oxide produced by nitrate-ammonifying bacteria isolated from soil

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Agricultural soils are the largest single source of anthropogenic N₂O to the atmosphere, primarily driven by microbiological processes such as denitrification and dissimilatory nitrate reduction to ammonium (DNRA). Both processes occur under similar conditions of low oxygen concentration and therefore, source partitioning of emitted N₂O is difficult. Understanding what controls the dynamics and reaction equilibrium of denitrification and DNRA is important and may allow the development of more effective mitigation strategies. ¹⁵N site preference (SP), i.e. the difference between ¹⁵N of the central and peripheral N-position of the asymmetric N₂O molecule, differs depending on processes involved in N₂O formation. Hence investigation of the isotopomer ratios of formed N₂O potentially presents a reliable mean to identify its source.

In this study, bacterial isolates obtained from organic soils were screened for their ability to reduce nitrate/nitrite to ammonium and to release N₂O to the atmosphere. Taxonomic characterisation of the strains revealed that N₂O formation was only detected in ammonifying strains affiliated to several genera of the family *Enterobacteriaceae* and strains belonging to the genus *Bacillus* and *Paenibacillus*. Sampling of N₂O was conducted by incubation of strains under oxic and anoxic conditions. Investigation of the ¹⁵N site preference showed SP values in the range of 39 to 57 ‰. Incubation conditions had no influence on the SP. The lowest values were achieved by a strain of the species *Escherichia coli* which was included in this study as a DNRA reference bacterium harbouring the *NrfA* gene that is coding the nitrite reductase, associated with respiratory nitrite ammonification. Soil isolates showed SP-values higher than 40 ‰. Comparison of these results with SP-values of N₂O produced by denitrifying bacteria in pure cultures (-5 to 0 ‰)^[1,2] revealed significant differences. In contrast, N₂O produced by denitrifying fungi displayed SP-values in a range of 21 to 36 ‰^[3], which are much closer to the values of N₂O from the investigated DNRA bacteria. However, the N₂O formed under denitrifying conditions by organisms investigated so far can be distinguished with respect to its source (DNRA or denitrification) but a broader database is needed which cover a larger spectrum of taxa.

[1] Sutka *et al.* Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundances. *Appl. Env. Microbiol.* **2006**, 72, 638.

[2] Toyoda *et al.* Fractionation of N₂O isotopomers during production by denitrifier. *Soil Biol. Biochem.* **2005**, 37, 1535.

[3] Rohe *et al.* Dual isotope and isotopomer signatures of nitrous oxide from fungal denitrification – a pure culture study. *Rapid Commun. Mass Spectrom.* **2014**, 28, 1893