



Effect of saturation and hotspot architecture on denitrification in hierarchical porous media

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Soil-borne greenhouse gas emissions from soil exhibit large spatial and temporal variability, which is commonly attributed to hotspots and hot moments of microbial activity in the soil matrix. Yet there is only limited information about the biophysical processes that regulate the production of GHGs on microscopic scales. In this study, we introduce a framework of incubation experiments with simplified porous media that avoids some of the complexities occurring in natural soil while physical constraints on microbial activity are fully accounted for.

Porous aggregates were inoculated with two different strains of denitrifying bacteria, *Agrobacterium tumefaciens* (final product N₂O) and *Paracoccus denitrificans* (final product N₂) and placed inside a sterile, sandy matrix in different spatial configurations. The differently inoculated pellets were either placed in distinct layers apart from each other, or randomly with equal distance to each other. The sandy matrix was adjusted to a water saturation of 30%, 60% or 90%. Kinetics of CO₂, N₂O, NO, N₂ and O₂ exchange above the surface of the sand was studied by high-resolution, automated gas chromatography in closed vessels.

Our results show a clear effect of hotspots architecture with fastest growth, fastest CO₂ production and earliest onset of N-gas emission in the random architecture due to less local competition for oxygen. Overall, a more abrupt onset of anoxia led to larger N-gas emissions from denitrification. The contribution from both denitrifier strains to total N-gas emissions could be separated on the basis of their distinct regulatory phenotypes. Differences in timing between layered architectures (*A. tumefaciens* above *P. denitrificans* and vice versa) revealed diffusion constraints and potential N₂O sinks along the way to the headspace. Aggregate architecture and water saturation resulted in distinct diffusion constraints with consequences for N-gas emissions. The experiments provide useful data for spatially explicit modelling of denitrification activity in aggregated soil.