



## Nitrogen cycling in biological soil crusts; microbial transformation processes and atmospheric nitrous acid and nitric oxide emissions

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Biological soil crusts (abbreviated as biocrusts) are composed of photoautotrophic cyanobacteria, algae, lichens, and bryophytes, growing together with heterotrophic bacteria, archaea and fungi and forming an intimate association with soil particles in the uppermost millimeters of the substrate. They occur globally in drylands, where they cover about 1/3 of the soil surface, corresponding to an area of about  $18 \times 10^6 \text{ km}^2$ . Biocrusts fix atmospheric nitrogen (N), which is needed for physiological processes and the formation of biomass. However, it recently was also shown that similar to bulk soil, N is cycled within biocrusts and major fractions of it are released as nitrous acid (HONO) and nitric oxide (NO) to the atmosphere.

Based on these initial results, we investigated the biologically mediated N-cycling processes in biocrusts as related to wetting and drying events. We investigated the microbial activity at different drying stages by means of transcriptome analysis and related these results to soil nitrite and nitrate concentrations over time. In addition, we utilized catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) to quantify the number of bacteria, archaea, and nitrite oxidizing bacteria in different strata over time.

Our results revealed that within less than 30 minutes after wetting, genes encoding for all relevant N cycling processes, including N fixation, ammonification, nitrification, denitrification, and assimilatory and dissimilatory N reduction were expressed. The most abundant transcriptionally active N-transforming microorganisms belonged to the *Rhodobacteraceae*, *Enterobacteriaceae* and *Pseudomonadaceae* within the *Alpha*- and *Gammaproteobacteria*. The soil nitrite contents increased significantly during the desiccation process, likely serving as a precursor for NO and HONO emissions, which peaked at relatively low water contents of ~20% water holding capacity. This nitrite accumulation was likely caused by a differential expression of nitrite as compared to nitrate reductase encoding genes over the course of desiccation. Additionally, our data suggest

that ammonia-oxidizing organisms may have responded to changing local oxygen conditions during drying. These mechanisms are also supported by process-based modelling, which has been conducted by us. Thus, our results show that the activity of N-cycling microorganisms, as related to the water and oxygen conditions within the substrate, determines the process rates and overall quantity of reactive nitrogen emissions.