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The mechanism of oxygen isotopic fractionation during fungal denitrification – A pure culture study

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Nitrous oxide (N_2O) from soil denitrification originates from bacteria and - to an unknown extent - also from fungi. During fungal denitrification, oxygen (O) exchange takes place between H_2O and intermediates of the denitrification process as in bacterial exchange[1,2]. However, information about enzymes involved in fungal O exchanges and the associated fractionation effects is lacking.

The objectives of this study were to estimate the O fractionation and O exchange during the fungal denitrifying steps using a conceptual model[2] adapted from concepts for bacterial denitrification[3], implementing controls of O exchange proposed by Aerssens, et al.[4] and using fractionation models by Snider et al.[5]

Six different pure fungal cultures (five *Hypocreales*, one *Sordariales*) known to be capable of denitrification were incubated under anaerobic conditions, either with nitrite or nitrate. Gas samples were analyzed for N_2O concentration and its isotopic signatures (SP, average $\delta^{15}N$, $\delta^{18}O$). To investigate O exchange, both treatments were also established with ^{18}O-labelled water as a tracer in the medium.

The *Hypocreales* strains showed O exchange mainly at NO_2^- reductase (Nir) with NO_2^- as electron acceptor and no additional O exchange at NO_3^- reductase (Nar) with NO_3^- as electron acceptor. The only *Hypocreales* species having higher O exchange with NO_3^- than with NO_2^- also showed O exchange at Nar. The *Sordariales* species tested seems capable of O exchange at NO reductase (Nor) additionally to O exchange at Nir with NO_2^-. The data will help to better interpret stable isotope values of N_2O from soils.

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