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Visualizing carbon and nitrogen transfer in the tripartite symbiosis of Fagus sylvatica, ectomycorrhizal fungi and soil microorganisms using NanoSIMS

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Translocation of recently photoassimilated plant carbon (C) into soil via root exudates or mycorrhizal fungi is key to understand global carbon cycling. Plants support symbiotic fungi and soil microorganisms with recent photosynthates to get access to essential elements, such as nitrogen (N) and phosphorus. While a 'reciprocal reward strategy' (plants trade C in exchange for nutrients from the fungus) has been shown for certain types of mycorrhizal associations, only little is known about the mechanisms of C and N exchange between mycorrhizal fungal hyphae and soil bacteria. Our understanding of the underlying mechanisms is hampered by the fact that C and N transfer between plants, mycorrhizal fungi and soil bacteria takes place at the micrometer scale, which makes it difficult to explore at the macro scale.

In this project we intended to analyse carbon and nitrogen flows between roots of beech trees (Fagus sylvatica), their associated ectomycorrhizal fungi and bacterial community. In order to visualize this nutrient flow at a single cell level, we used a stable isotope double labelling (13C and 15N) approach. Young mycorrhizal beech trees were transferred from a forest to split-root boxes, consisting of two compartments separated by a membrane (35 μm mesh size) which was penetrable for hyphae but not for plant roots. After trees and mycorrhizal fungi were allowed to grow for one year in these boxes, 15N-labelled nitrogen solution was added only to the root-free compartment to allow labelled nitrogen supply only through the fungal network. 13C- labelled carbon was applied by exposing the plants to a $13CO_2$ gas atmosphere for 8 hours. Spatial distribution of the isotopic label was visualised at the microscale in cross sections of mycorrhizal root-tips (the plant/mycorrhizal fungi interface) and within and on the surface of external mycorrhizal hyphae (the fungi/soil bacteria interface) using nanoscale secondary ion mass spectrometry (NanoSIMS). Corresponding morphological structures were established using light microscopy and scanning electron microscopy. In addition, isotopic signals in plant tissue as well as in fungal and soil microbial communities were traced by EA-IRMS and GC-C-IRMS of 13C phospholipid fatty acid, respectively.

Our NanoSIMS images demonstrate a rapid transfer of photoassimilated plant C from the root's central cylinder to 1) ectomycorrhizal fungal cells in the Hartig net in the root cortex, and 2) to external ectomycorrhizal hyphae residing in the root-free compartment. In the cross-section of the mycorrhizal root, 13C enrichment was spatially correlated to 15N enrichment indicating a strongly controlled exchange of C and N between plant and fungus.

Overall, our study shows the potential of NanoSIMS imaging as a tool for getting insight into mechanisms of plant-soil interactions by visualizing in situ C and N flows between plants, fungi and soil microbes at the microscale.