Micro-scale distribution of water around plant roots using neutron tomography

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There is gap of knowledge about the water flow from soil through the soil-root interface and to roots. This is due to the technical difficulties resolving the water distribution in such a small area around the roots. Spatially and temporally resolved data on the water movement from soil to roots is needed in order to improve our understanding of mechanisms controlling water uptake by roots. Neutron tomography is a non-destructive imaging technique enabling us to map water distribution around the roots *in situ*. We grew chick pea, maize, and lupine in cylinders for 10 days at a water potential of -15 hPa. The samples then were tomographed for 4 days over day and night and during a drying period and after rewetting. We observed an increase in water content towards the root surface for all plants. We speculate that this increase is due to specific properties of the soil in immediate vicinity of the roots, i. e. rhizosphere. The shape and the extent of this increase in water differed along the root length and also over day and night. As the soil water was being consumed by the roots over the course of the measurement, the extent of the water-increase zone around the roots decreased slightly and then increased again after rewetting. Using modelling scenarios we showed that the presence of rhizosphere with higher water-holding capacity than the bulk soil has consequences on the flow of water from soil to roots. Most of the present modelling approaches neglect this effect.

Using hydrogen isotopes to assess proton flux during biological hydrogen production: Part 2

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Biological hydrogen (H_2) production is critical to microbial nutrient cycling in many anaerobic communities and may also provide a sustainable, non-polluting energy source. A major impediment to improving our understanding of H_2 metabolism is our inability to adequately define the regulation of and flux through key pathways involved in H_2 production. To fill this need, we are developing the use of H isotopes as a tool for addressing fundamental questions related to hydrogenases and intracellular proton trafficking. We are using isotopic analysis to investigate H_2 production in a model organism, *Shewanella oneidensis*.

S. oneidensis is a faculatative anaerobe that can use a variety of metabolic electron acceptors including iron, manganese, and other metals. In the absence of suitable electron acceptors, it reduces water, forming H_2. *S. oneidensis* encodes two hydrogenases, [Fe-Fe] hydrogenase HydA and [Ni-Fe]-hydrogenase HyaB, which catalyse the reversable reaction of water and electrons to form H_2. The two purified enzymes produce isotopically distinct H_2 from identical substrate water in vitro, with fractionations remarkably similar to those observed in vivo.

The aim of this project is to characterize the flux of protons through the two *S. oneidensis* hydrogenases in vivo, and explore factors affecting those fluxes. Wild-type, hydrogenase mutant, and electron-transfer deficient strains of *S. oneidensis* show different patterns of H_2 production. We are assessing the effects of these mutations on the isotopic content of resulting H_2. We hypothesize that different isotopic fractionations will permit quantification of H_2 production by each hydrogenase and may permit temporal differentatiation of peak hydrogenase activity during *S. oneidensis* growth. Hydrogenase enzymes represent a key bridge between inorganic and organic proton cycling. Understanding the roles of hydrogenases and H_2 production in cellular metabolism is a prerequiste for understanding the H_2 environment within cells as well as within their native communities.