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The quest for active microbial players of Biological Nitrogen Fixation in rice cultivation

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The first step of nitrogen cycling in soil is provided through Biological Nitrogen Fixation (BNF), a process performed by a diverse group of microorganisms. These so-called diazotrophs possess the genetic capability to fix atmospheric nitrogen (N) that will eventually be available to other organisms. Major crop plants such as wetland rice are strongly limited by low N availability in soils which is commonly amended by the application of hazardous and costly industrial fertilizers. Although it is known that diazotrophs can alleviate the N-shortage in rice cultivation, and thus carry out an important ecosystem function, major questions regarding the identity of active microbial players, their spatial distribution, and the transfer of N to rice plants remain unanswered.

We have performed mesocosm experiments to investigate the diversity, spatial distribution, and activity of diazotrophs in association with wetland rice. Amplicon sequencing of nifH genes was performed to study the effects of soil type, rice variety, and biocheochemical factors on the diversity and potential activity of diazotrophs in soil-plant microenvironments (bulk soil, rhizosphere, root). Nitrogen fixation activity in these microenvironments was assessed via destructive and whole-plant incubations with 15N2-containing atmospheres as analyzed on community (EA-IRMS) and single-cell level (NanoSIMS). To obtain information on potential hotspots of N-transfer from bacteria to roots we have performed gnotobiotic experiments with a diazotrophic isolate. Fluorescence microscopy was combined with automated image analysis and spatial statistics to explore the distribution of diazotrophic colonization patterns on rice roots. Whole-plant incubations with 15N2 in conjunction with a newly developed Gold-FISH – NanoSIMS approach allowed us to link the visualization of single bacteria on rice roots in situ with the incorporation of stable isotopes of 15N into their cells for the first time.

Overall, we will provide novel insights into Biological Nitrogen Fixation in wetland rice cultivation, ranging from the microbial community scale to the individual players of BNF, single diazotrophic cells.