

# Root nodule symbiosis in *Lotus japonicus* drives the establishment of distinctive rhizosphere, root, and nodule bacterial communities.

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Root nodule symbiosis is a very specific mutualistic interaction in which the host selects compatible nitrogen fixing soil bacterium for infection. Legumes are able to engage in symbiotic relationships with nitrogen-fixing bacteria and as a result they are able to colonise marginal soils and improve their nutritional status, making it available for other crops. Previously it was not known whether the beneficial impact of legumes on the soil status was strictly correlated with the increase in nitrogen availability, or whether the legume root-associated microbes have an impact. Furthermore, an understanding of the mechanisms behind the symbiotic interactions in a community context and how this impacts on the microbial community has been lacking. In order to understand the evolution of symbiosis and use rhizobia inoculum effectively it is important to combine these very specific interactions into an ecological community context. On this basis we investigated how symbiotic nitrogen fixation and the nodulation pathway impacts on the structure of root associated bacterial microbiota in *L. japonicus*. We carried out community profiling experiments using wild type (WT) plants and symbiotic mutants (*nfr5-2*, *nfr5-3*, *nin-2*, and *lhk1-1*) that had been impaired at different stages of symbiosis.

We found that the bacterial communities in nodules differed from those in the roots and rhizosphere. We confirmed that nodules represent a very selective bacterial niche and that the nodule-enriched microbiota in the root and rhizosphere also had similar abundances. These findings suggest that a parallel selection of bacteria from the rhizosphere and root compartments is responsible for the establishment of distinctive nodule and root-inhabiting communities.

Our community analyses showed that the four symbiosis mutant plant types had similar communities associated with their roots and rhizospheres and that these were significantly different from those associated with WT plants. We further characterized the bacterial shifts by looking at microbiota that were specifically enriched in different compartments. The results support the idea that the symbiotic pathway plays a key role in the selection of specific soil-derived microbiota and the creation of differentiated microbiota in the root and rhizosphere compartments.

We further investigated the bacterial community shifts by looking specifically at the taxonomy of the microbiota. The results from this showed that the differentiation at the root and rhizosphere, which is driven by the symbiosis pathway, occurs across multiple bacterial orders.

To find out if the different community structures we had observed in *Lotus* mutant roots and the rhizosphere were due to potential differences in nitrogen status we carried out community analyses on plants grown in nitrogen-supplemented soil. We found that the plants grown in supplemented soil still displayed the same differential phenotypes as those grown in non-supplemented soil, suggesting that the symbiosis pathway is key for promoting plant growth regardless of whether there are functional nodules. Next, we looked at the bacterial communities in WT and *nfr5-2* and *nfr5-3* mutants and found that the mutants maintained their different community structure in nitrogen-supplemented soil. This suggests that it is the disabled symbiosis pathway itself that impacts on the structure of the root-associated community, as opposed to an indirect effect from the removal of symbiotic nitrogen fixation.

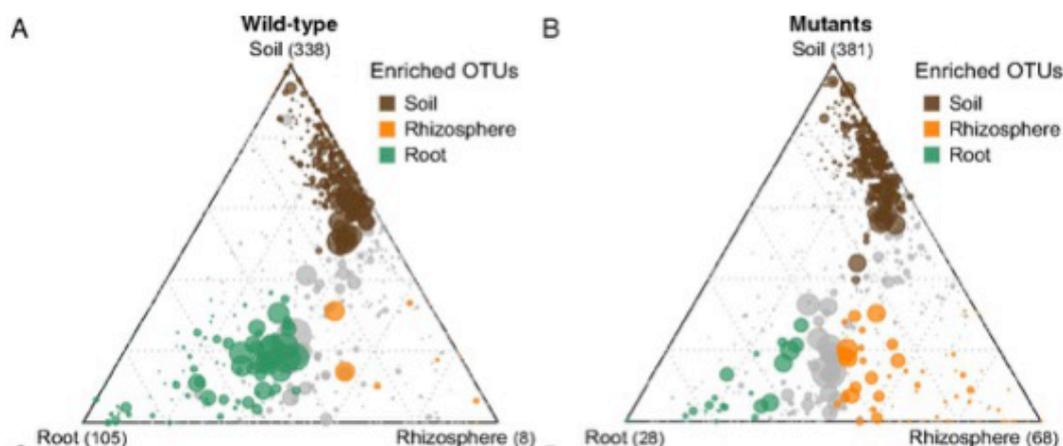


Figure 1 Ternary plots depicting compartment relative abundances of all operational taxonomic units (OTUs) for WT (A) and mutant samples (B)