Metagenomic analysis of the influence of the *Medicago* common symbiotic pathway on the rhizosphere microbiome

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Introduction:

Rhizosphere prokaryotic and eukaryotic metagenomic profile has been studied to decipher the nodulation and mycorrhization influence on the microbial community. We have used *Medicago truncatula* A17 *nfp* and *nsp* mutant for the nodulation, *ram1* and *ram2* for the mycorrhization influence and *dmi3* for the combined effect. Rhizosphere DNA has been amplified using prokaryotic and eukaryotic specific primers and sequenced using MiSeq 250bp pair-end technology. Plants were grown in a mix of 10% of grassland soil and 90% sand for 4 weeks in separate 50ml pots and 5mg of KNO_3 was added to each condition. Plants did not nodulate due to limited *rhizobia* abundance in the diluted growth condition. Mycorrhization screen on the wild type plant roots showed that the infection rate was approximately 1% only.

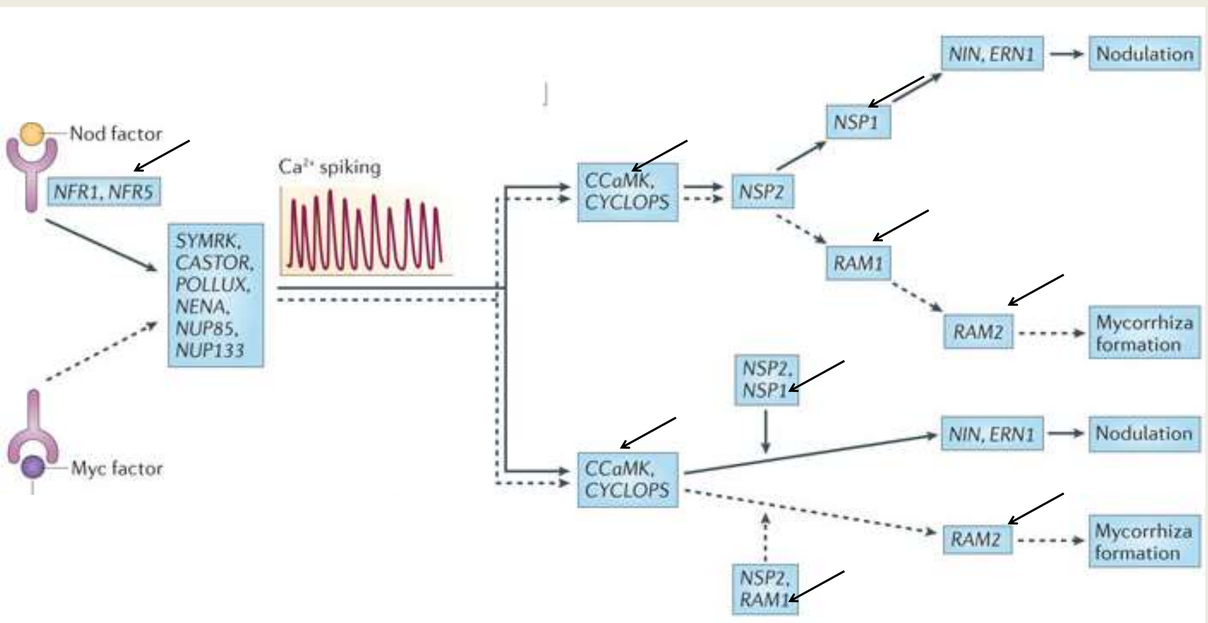
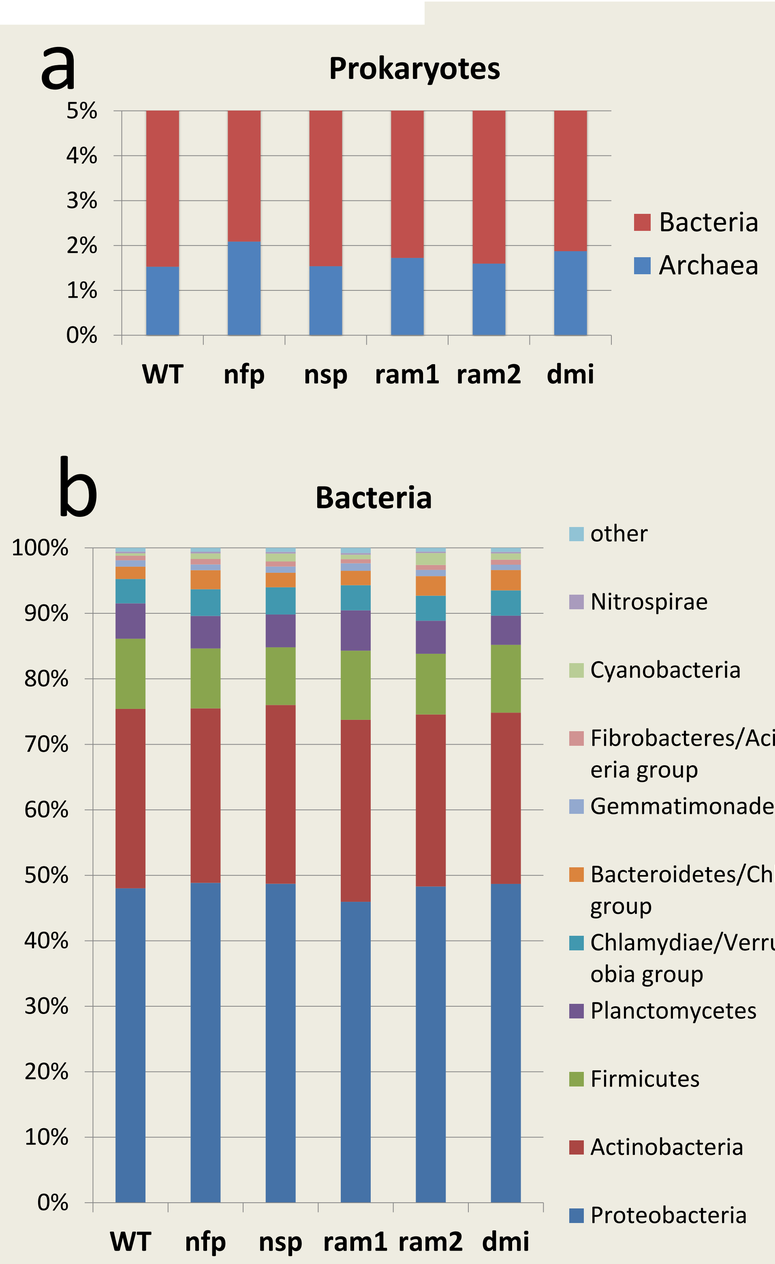
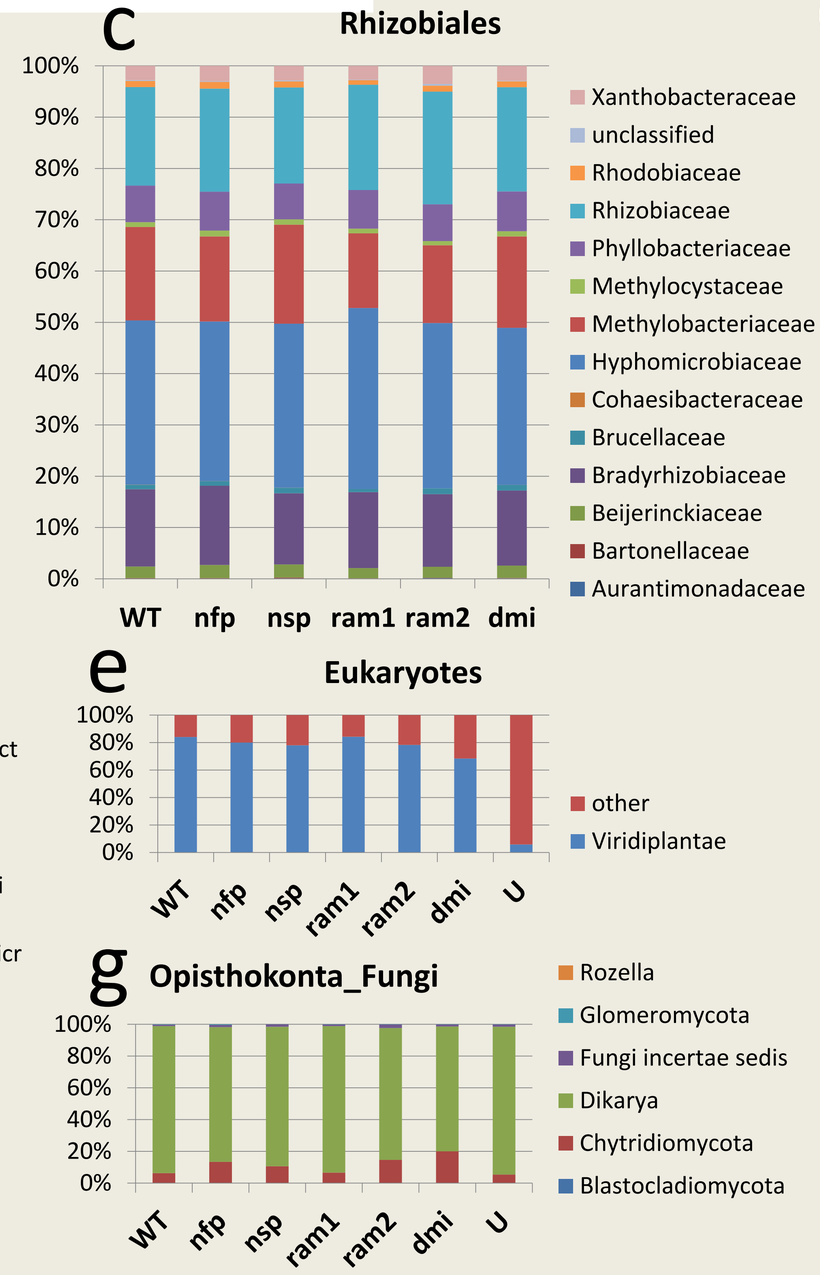


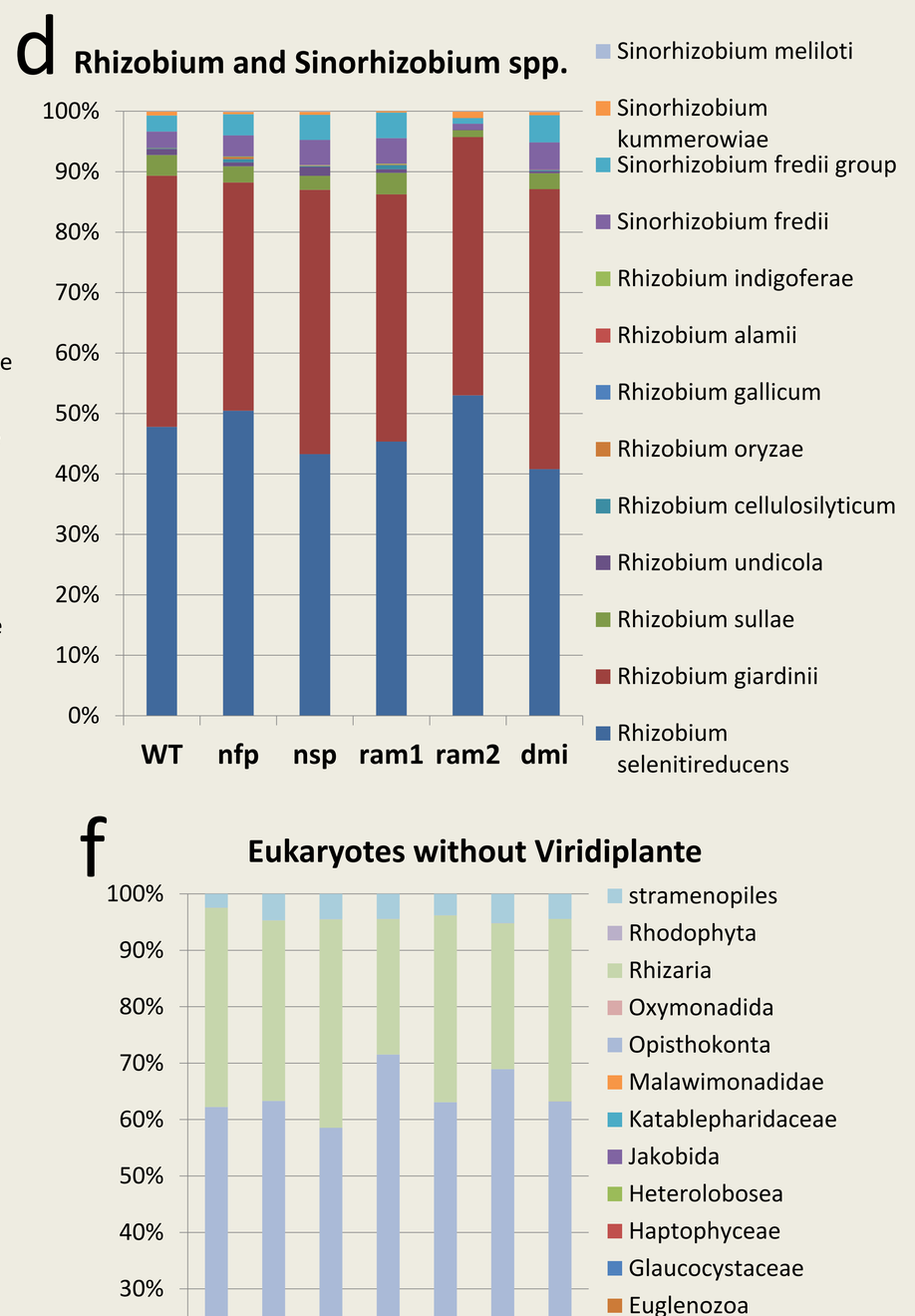
Figure 1. Common symbiotic pathway with annotated genes for which mutant have been screened for the rhizosphere metagenomic profile. NFR5 corresponds to NFP and CCaMK to DMI3 in case of *Medicago.* Figure adapted from the review by Oldroyd GE., ***Nat. Rev. Microbiol***, 2013

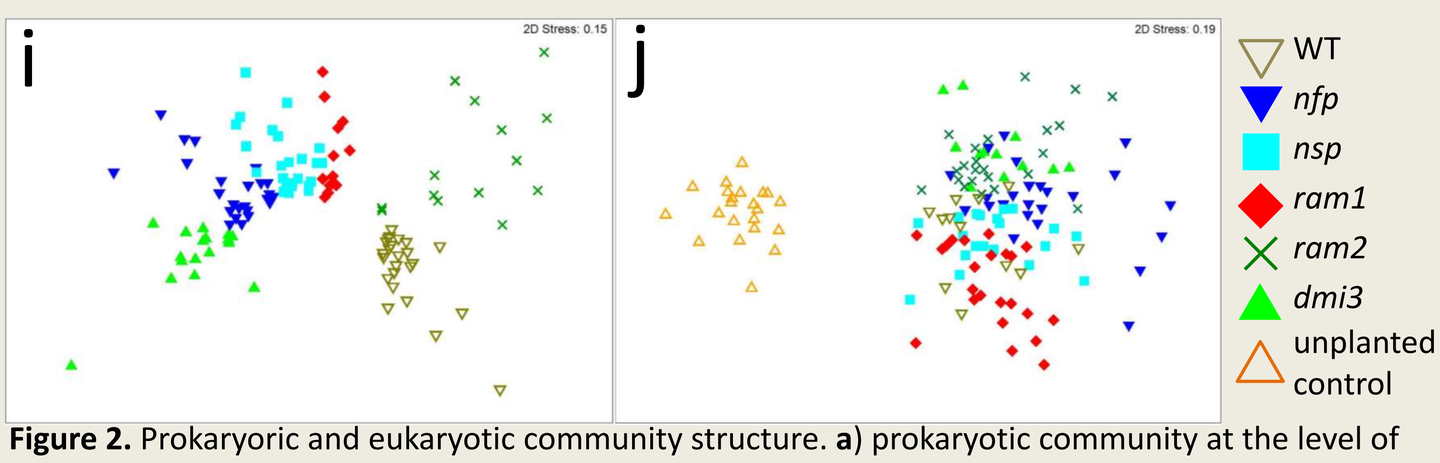
Results:

MiSeq produced 12.7M pair-end reads, of which 8.1M was further analyzed. Below we present a semi-automatic output from a metagenomic software MEGAN. Statistical analysis is based on ANOVA for bacterial community only. Multidimensional Scalling plots are constracted for the total prokaryotic and eukaryotic (excluding Viridiplantae) community.









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NODULATION	MYCORRHIZATION	BOTH
Roseiflexus ~	Acidimicrobiaceae ↓	*Hydrocarboniphaga effusa* ↓
Erysipelothrix inopinata ~	*Listeria* ↓	Sinobacteraceae ↓
Chitinophagaceae ↑	Flavobacteriales ~	*Actinoplanes brasiliensis* ↓
Ralstonia pickettii ↑	Pseudomonadaceae ↑	*Solirubrobacter soli* ~
Mucilaginibacter oryzae ↑	*Mucilaginibacter* ↑	Acidimicrobineae ~
Methylobacter ↑	Cyanobacteria ↑	Bacteroidetes ↑
Candidatus Liberibacter ↑	Chroococcales ↑	Sphingobacteriaceae ↑
Enhydrobacter aerosaccus ↑	*Synechococcus_elongatus* ↑	*Mucilaginibacter paludis* ↑
in total 8 but of 5270	in total 53 but of 5270	in total 15 but of 5270

Figure 2. Prokaryoric and eukaryotic community structure. **a**) prokaryotic community at the level of kingdom, **b**) Bacteria structure at the phylum level, **c**) Rhiobiales structure, **d**) *Rhizobium* and *Sinorhizobium spp.* **e**) Eukaryotic community structure, **f**) Eukaryotic structure excluding plant DNA contamination, **g**) Fungi structure, **h**) 8 common bacterial taxa significantly more ↑ and less ↓ abundant in the mutant rhizospheres (both) than WT based on ANOVA, ~ if different in one mutant but not the other **i**) Bacterial total community structure presented on MDS plot, **j**) Eukaryotic community structure (excluding Viridiplantae) presented on MDS plot.

Conclusions: *Medicago* nod$^-$ and myc$^-$ mutants exert a weak, but significant effect on the rhizosphere microbial community. A semi-automated analysis pipeline revealed some bacterial taxa abundance significantly different in the mutant rhizospheres comparing to the wild type. Approximately 80% of the eukaryotic DNA in the rhizosphere comes from the plant roots (slough-off cells, root hairs), while only 5% was detected in the unplanted control. Probably due to the rhizosphere sampling procedure tightly attached fungal species, for example Glomeromycota were not detected in high abundance.
Future studies will focus on the comparison between rhizospheres of plants that were or were not inoculated with *S.medicae* WSM419 and wild type will be compared against *dmi3* mutant in two different growth conditions.

Acknowledgement: special thanks to Giles Oldroyd and Jeremy Murray (JIC) for the *Medicago* mutant lines and Jitender Cheema for the help with data analysis