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Model plants select different microbial communities in the rhizosphere

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Introduction

- The rhizosphere is the zone around roots influenced by plant exudates.
- Microbial density and activity is enhanced in the rhizosphere.
- The mutual influence of microbes, plant roots and soil on each other is enormous and our understanding of these relations is still unclear
- Composition of root exudates varies between different plants species and this can affect microbial community structure.
- Only about 1% of rhizosphere bacteria can be cultured.
- Aim of this work is to test microbial succession over plant generations and screen for possible plant growth promoting rhizobacteria (PGPR)

Methods

Model plants

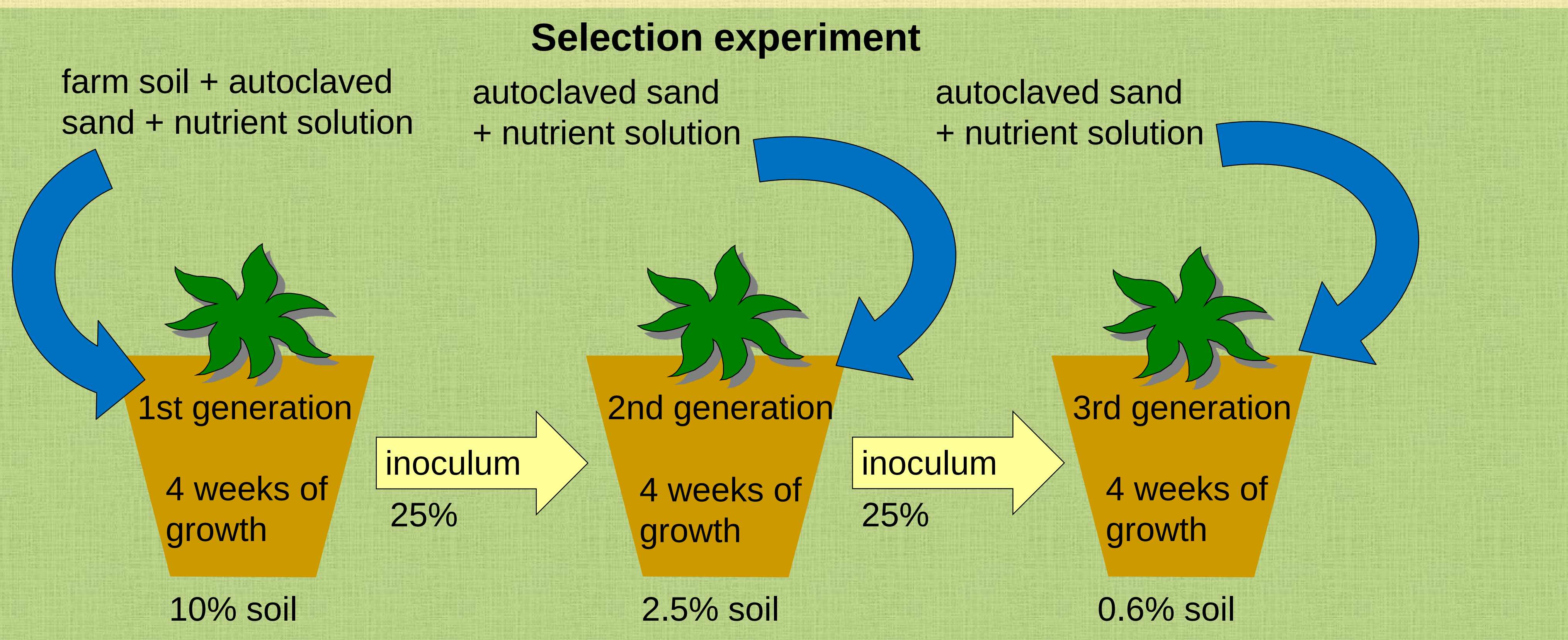
- *Arabidopsis thaliana* **(A)**– not colonized by mycorrhiza, dicot
- *Medicago truncatula* **(M)**– model species for nodulation studies, dicot
- *Brachypodium distachyon* **(B)** - model species for grasses, monocot
- unplanted control **(U)**

Microbial community was assessed using two methods:

1. ARISA
Fingerprinting method. Analyzes the sizes of 16S-23S rDNA intergenic region **(ITR)**.
Done for 288 samples in total

2. 454 pyrosequencing
Done on V1-V2 variable region of 16S rRNA. Reads binned into Operational Taxonomic Units **(OTUs)** based on 95% sequence similarity. OTUs were annotated using SILVA database

Selection experiment



farm soil + autoclaved sand + nutrient solution

autoclaved sand + nutrient solution

autoclaved sand + nutrient solution

1st generation
4 weeks of growth
10% soil

inoculum
25%

2nd generation
4 weeks of growth
2.5% soil

inoculum
25%

3rd generation
4 weeks of growth
0.6% soil

Samples for DNA isolation have been taken from each generation (24 biological replicates for each plant species / unplanted)

Results

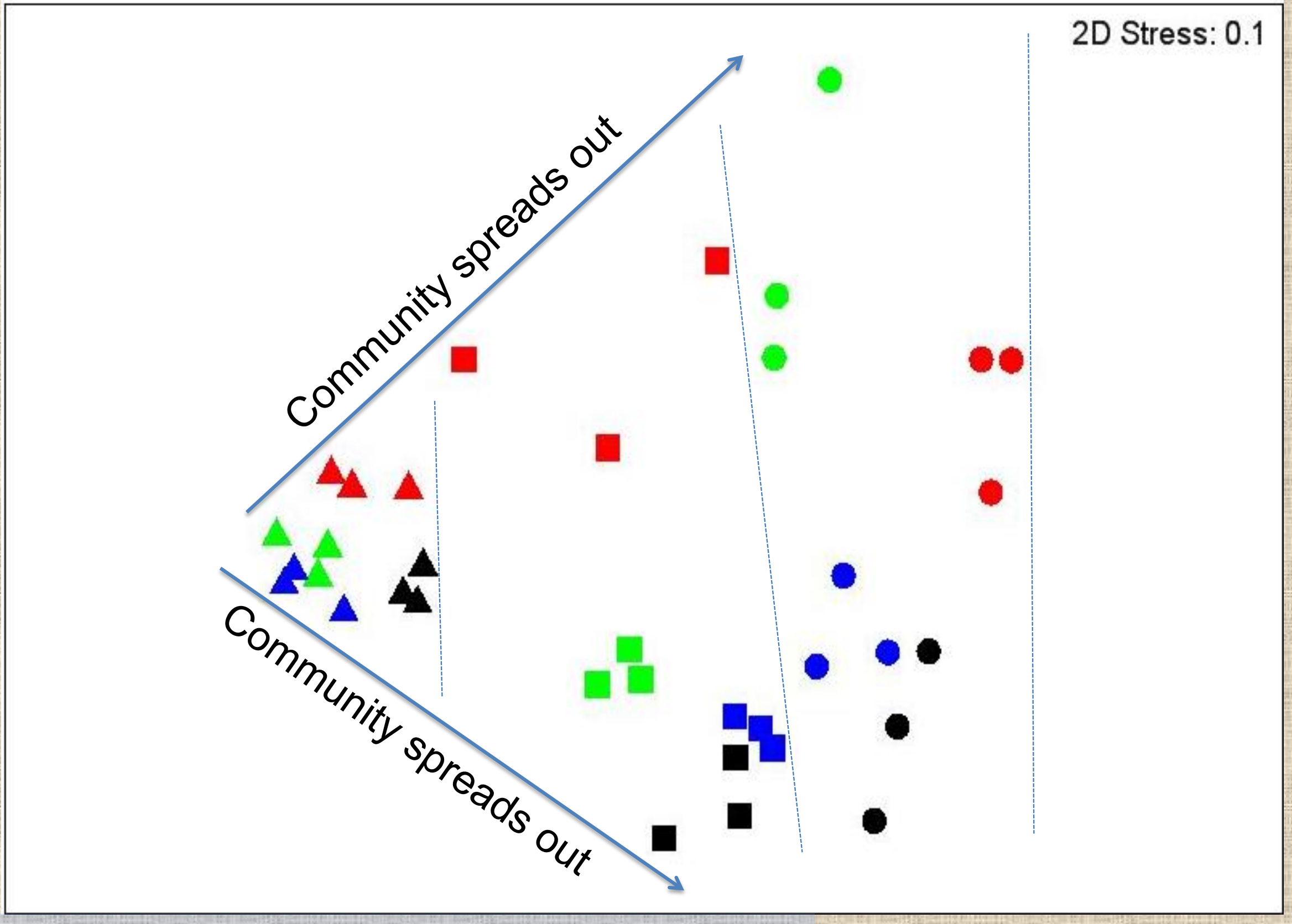
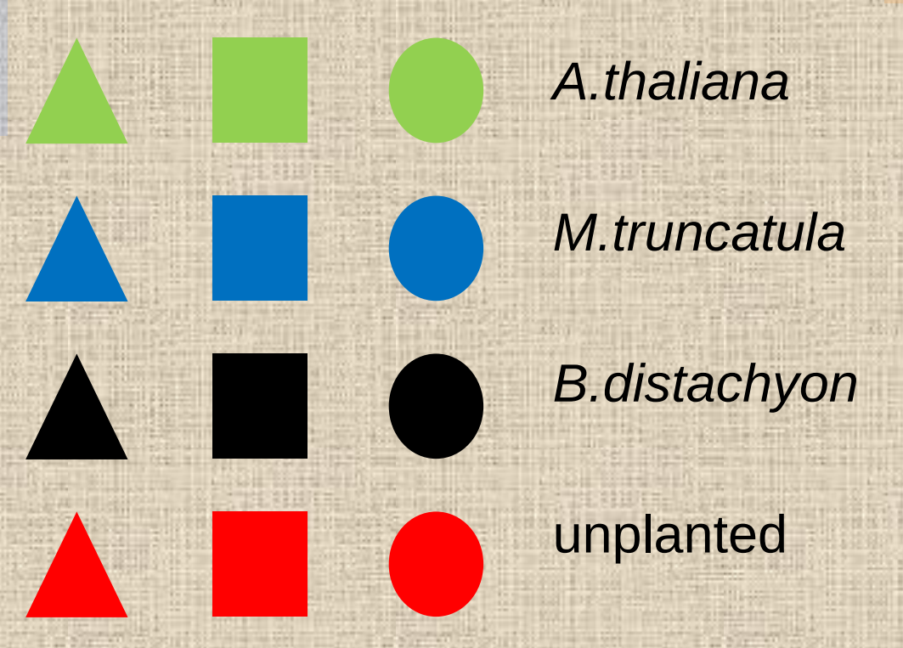


Fig. 1 MDS showing similarity between ARISA samples for all 3 generations (3 data points for each 24 ARISA samples



Multidimensional Scaling plot **(MDS)** shows the similarity between samples. The closer they are plotted to each other the more similar they are (based on 100 highest ITRs)

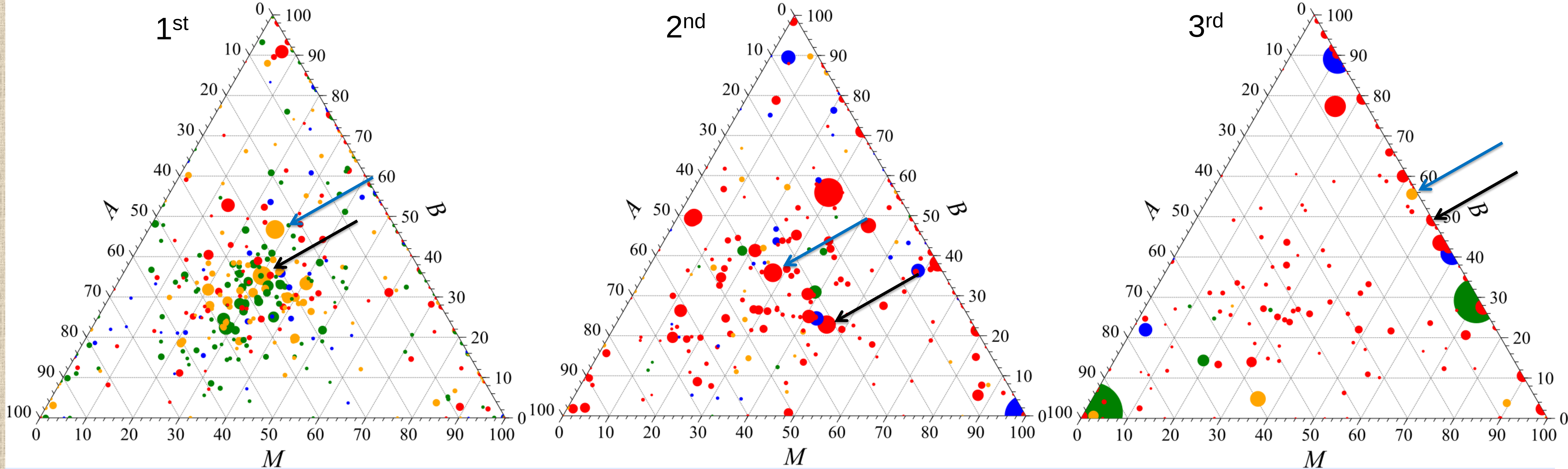


Fig. 2 Triangle plots showing plant species influence on OTUs (only >0.1% abundance) represented as dots with the size corresponding to the total OTU abundance in the generation and colour as a ratio of plant selection against unplanted control

$$1 - \frac{U}{\left(\frac{A+M+B}{3}\right) + U} * 100\%$$

● 100-75% ● 75-50% ● 50-25% ● 25-0%

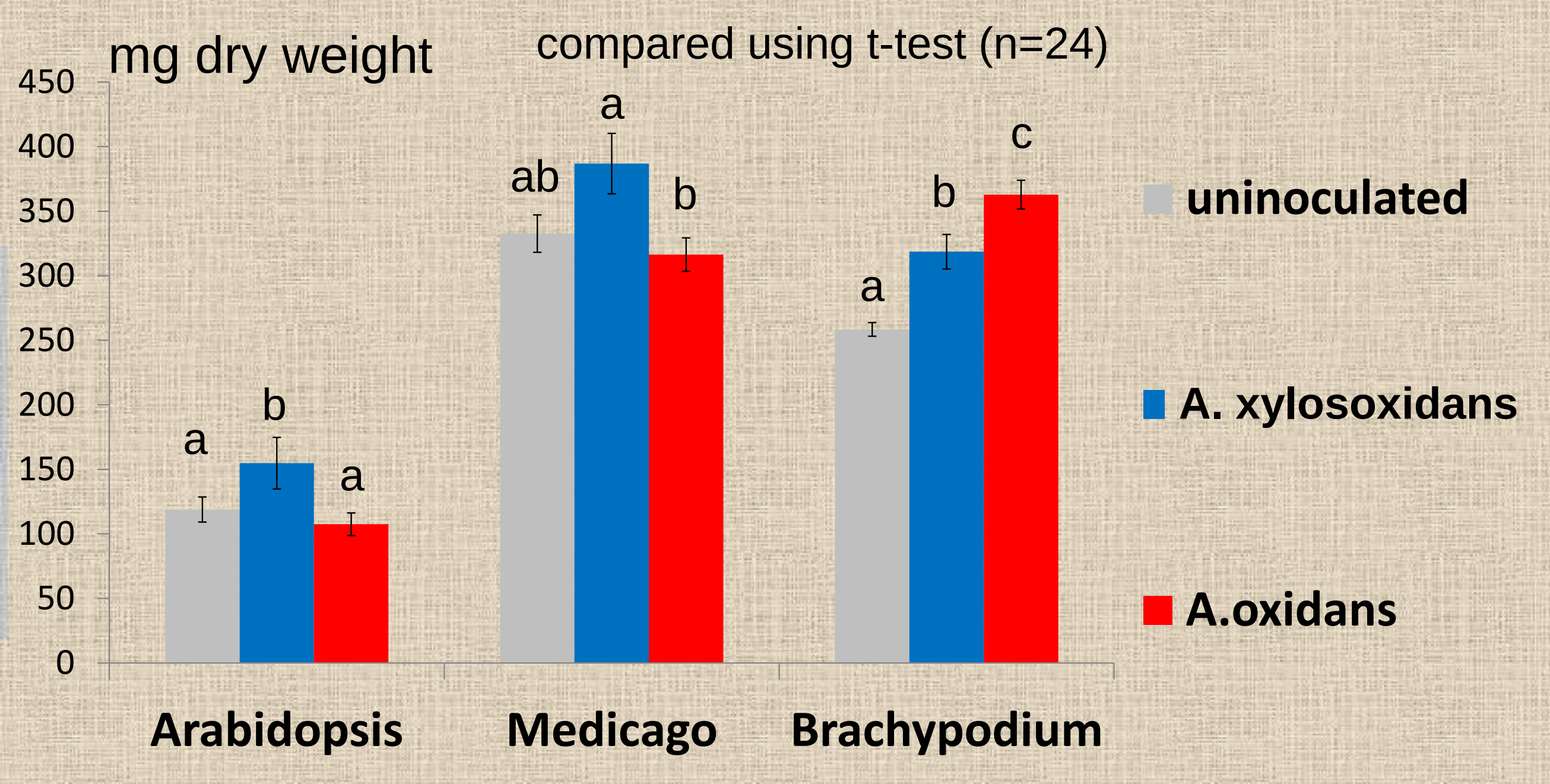


Fig.4 Plant growth assay using isolated strains as potential PGPRs

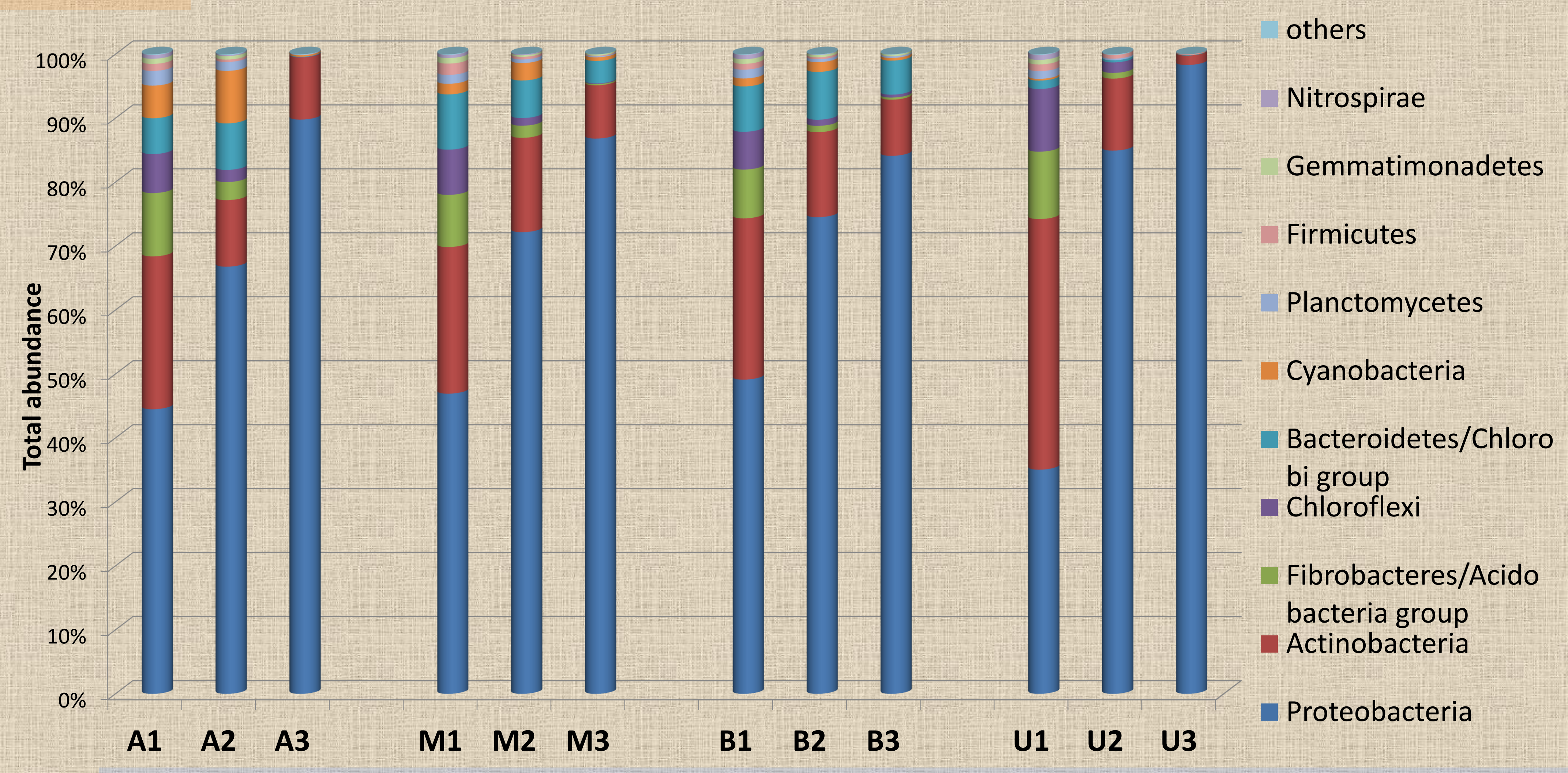


Fig. 3 Bacterial structure represented at the phylum level for each sample for each generation based on SILVA database. Graph plotted in MEGAN using 1% as top percent (1,2,3 – generation number)

- Plants exert different influence on microbial community - statistically significant differences confirmed by MANOVA analyses (fig.1)
- During 2nd and 3rd generations community spreads out. However differences between each 3 data points remain relatively small (fig.1)
- 16S rDNA sequencing confirms the ARISA results (not shown), More and more OTUs are being selected by one or more plants through generations (fig.2)
- *Achromobacter xylosoxidans* (black arrow) *and Arthrobacter oxidans* (blue arrow) (cultured) have been found to be abundant in all generations and are plant-specific, especially in Medicago and Brachypodium rhizosphere (fig.2). Achromobacter influences growth of all plants, while Arthrobacter is Brachypodium-specific (fig.4)
- Proteobacteria are dominant in the rhizosphere and unplanted soil and their abundance is rising over generations (fig.3). Arabidopsis rhizosphere and unplanted contol are relatively similar to each other at the phylum level (fig.3)

Conclusions

Plant species select different rhizosphere communities
There is strong microbial selection over generations of plant species
ARISA method is sufficient for testing the similarity of microbial communities
Poor soil conditions allow soil opportunists to take over the community
Selection experiments give a unique opportunity to isolate bacteria under strong plant selection pressure

Ongoing work

gDNA and mRNA sequencing of isolated strains grown in lab cultures and in the rhizosphere (only for mRNA)
Repeating the experiment using rich compost with stable amount of nutrients in the system using model plants as well as crop plants
Testing the microbial community between selected mutants of model plants