

Introduction

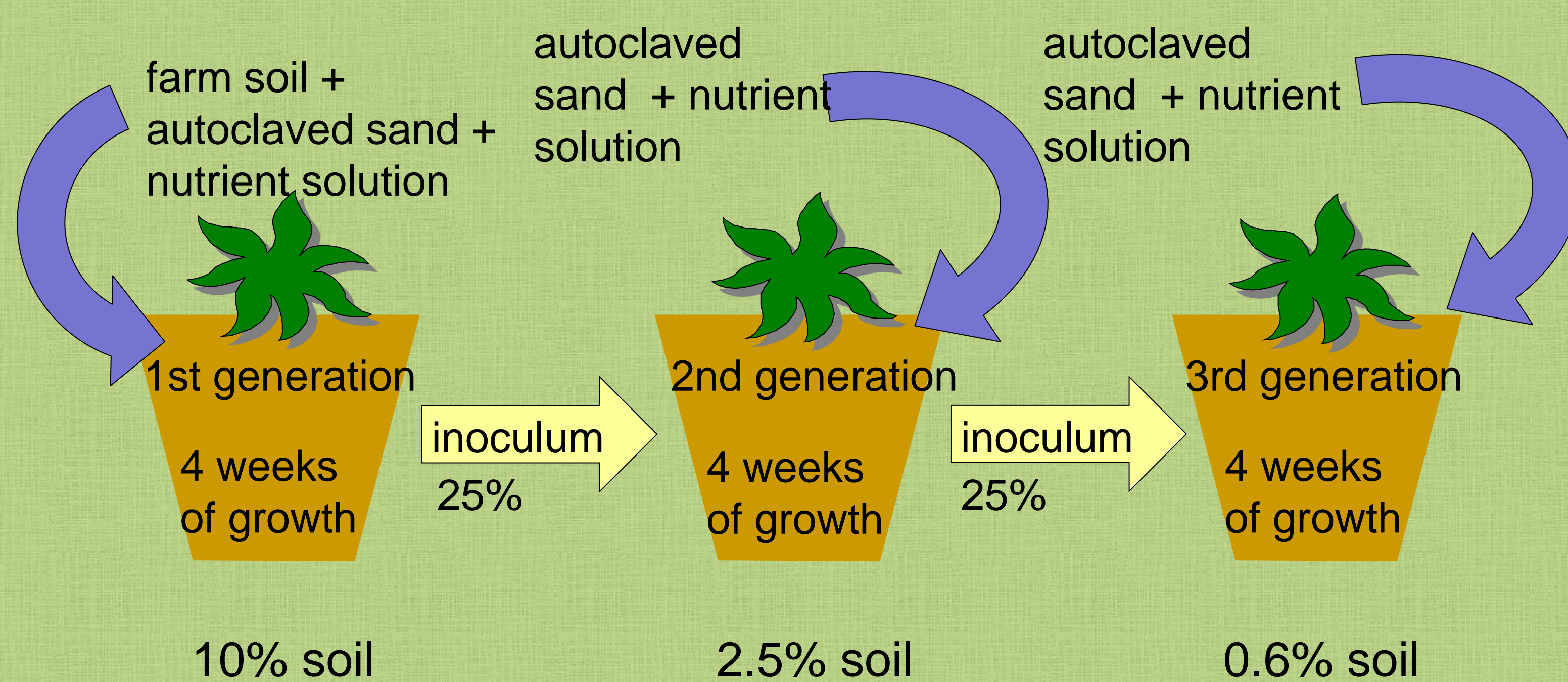
- The rhizosphere is the zone around roots influenced by plant exudates.
- Microbial density and activity is enhanced in the rhizosphere.
- The mutual influence of microbes, plant roots and soil on each other is enormous and our understanding of these relations is still unclear
- Composition of root 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

Methods

Model plants

- *Arabidopsis thaliana* – not colonized by mycorrhiza, dicot
- *Medicago truncatula* – model species for nodulation studies, dicot
- *Brachypodium distachyon* - model species for grasses, monocot
- Unplanted control

Selection experiment



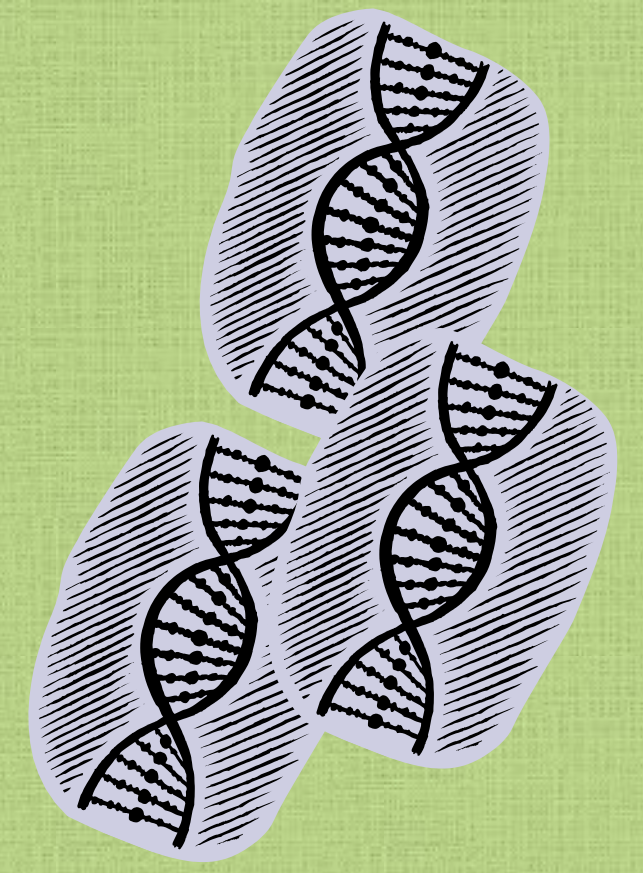
Microbial community was assessed using two methods:

1. ARISA

Fingerprinting method. Analyzes the sizes of 16S-23S rDNA intergenic region. Done for 288 samples in total

2. 454 pyrosequencing

Done on V1-V2 variable region of 16S rRNA. Replicates pooled together into 36 unique barcoded 454 samples



Results

1st generation ARISA

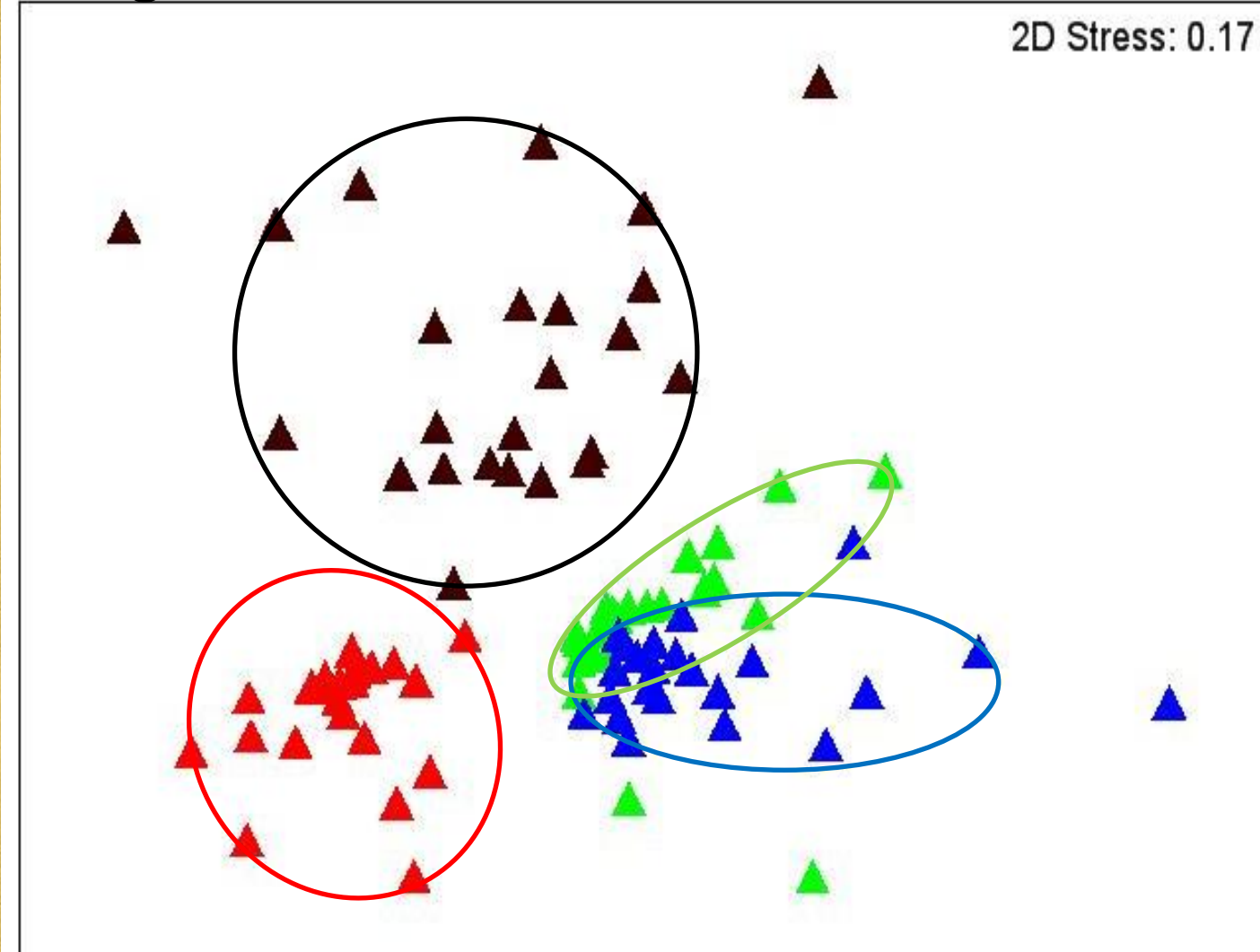


Fig. 1

All generations ARISA (3 data points for 24 plants)

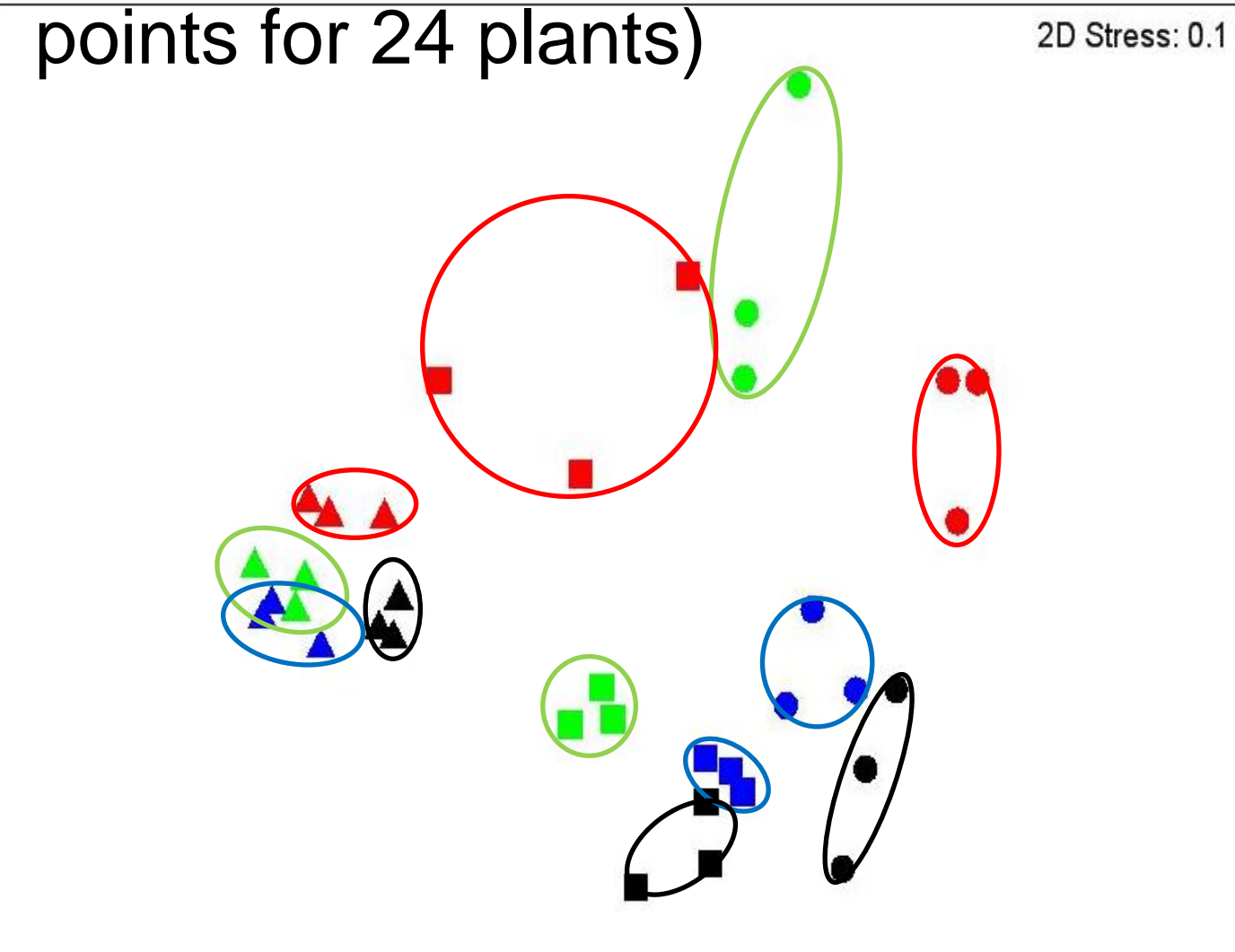


Fig. 2

All generations 454 data 16S rDNA OTUs

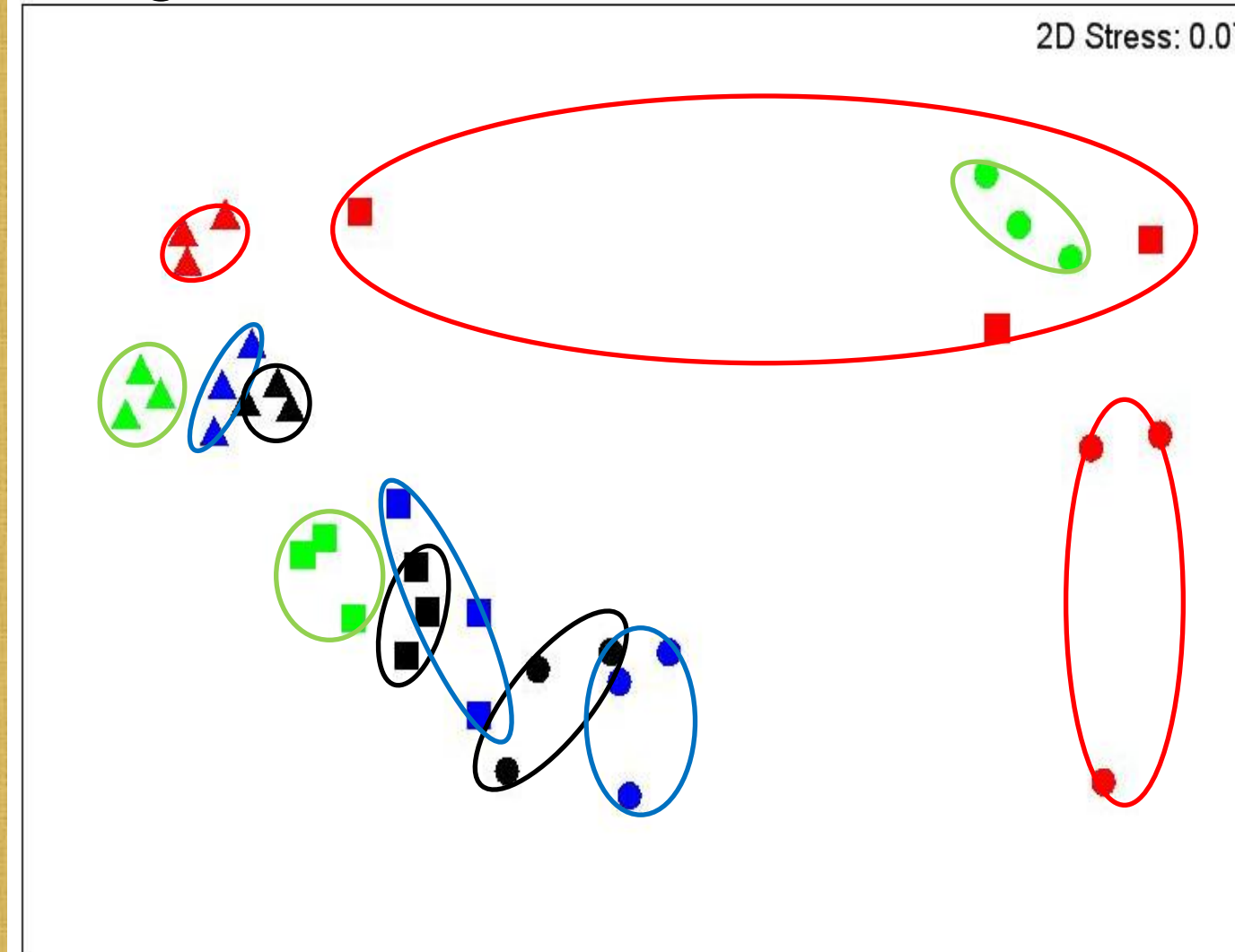
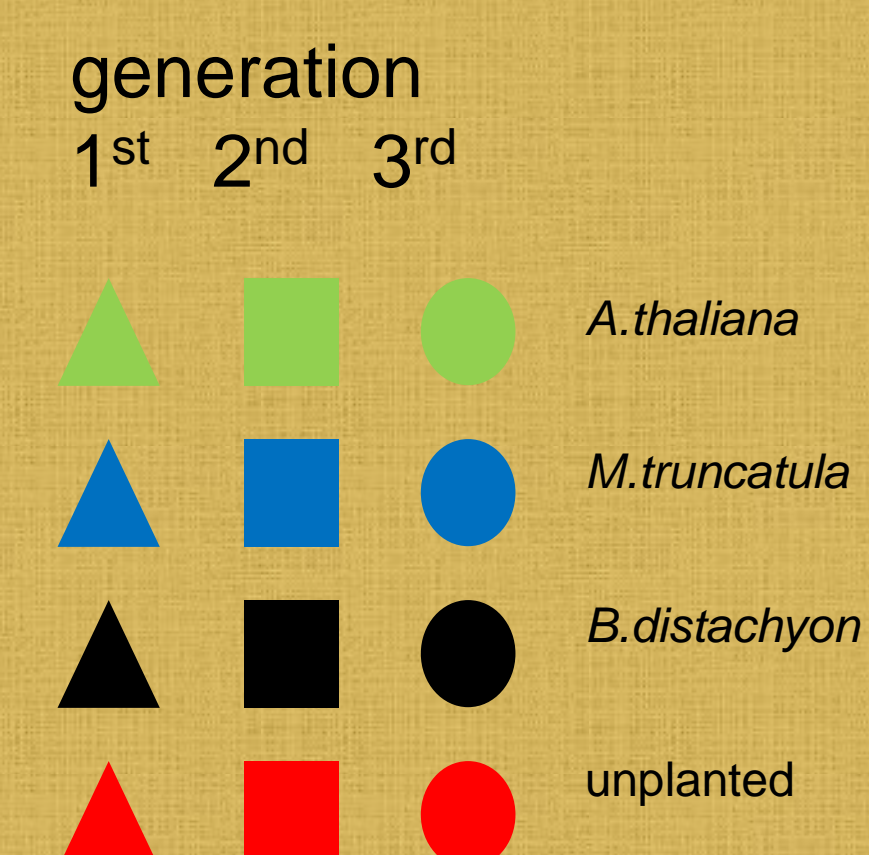


Fig. 3



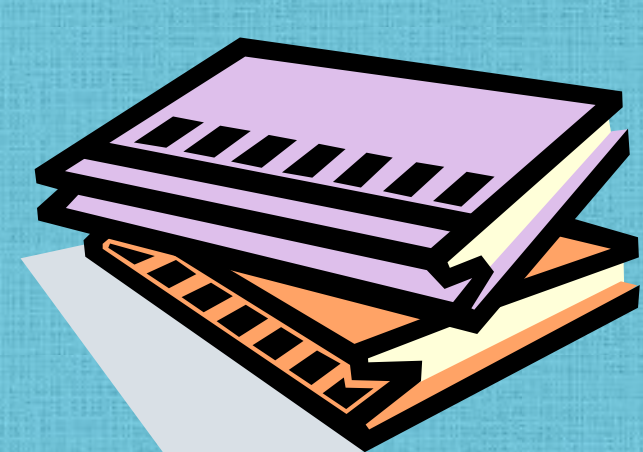
Multidimensional Scaling plot shows the similarity between samples. The closer they are plotted to each other the more similar they are.



- Plants exert different influence on microbial community (Fig.1)
- During 2nd and 3rd generations community spreads out. However differences between each 3 data points remain relatively small (Fig.2)
- 16S rDNA sequencing confirms the ARISA results (Fig.3).
- *Achromobacter xylosoxidans* (cultured) has been found to be very abundant in 1st generation and abundant in the rhizosphere of plants in 2nd and 3rd one (but not in control).
- *Arthrobacter oxidans* (cultured) is abundant in all samples in all generations.

Conclusions

Plant species select different rhizosphere communities
There is strong microbial selection over generations of plant species
Selection experiments give a unique opportunity to isolate bacteria under strong plant selection pressure



Future 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 crop plants
Testing the microbial community between selected mutants of model plants

