Unraveling plant-microbe interactions across space, time, and environmental contexts

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Introduction

Microbes play a key role in plant health and development. They inhabit various plant tissues and are present throughout the plant's life cycle. Over this journey through space and time, the microbial composition changes and adapts—this is also true when the plant encounters challenging conditions. Plants can recruit and modulate beneficial microbes; however, we still do not fully understand the mechanisms behind this complex process. Our group has conducted an extensive ecological study in which *Arabidopsis thaliana* plants from contrasting natural environments were sampled over several years. Phenotypic and transcriptomic profiling of the plants were performed. In the present study, we randomly selected 96 plants from different collection years (2021 to 2023) and locations (Spiekeroog and Brachwitz, both in Germany) to assess the microbial diversity through 16S ribosomal RNA amplicon sequencing. We then integrated the microbiome with their respective plant transcriptome datasets in an attempt to understand how plants modulate and/or are affected by microbes in the field.

Sphingomonas (almost) dominates *Arabidopsis* microbiome

16S rRNA amplicon sequencing was processed on DADA2: filtered out chloroplast and mitochondria reads + rarefied.

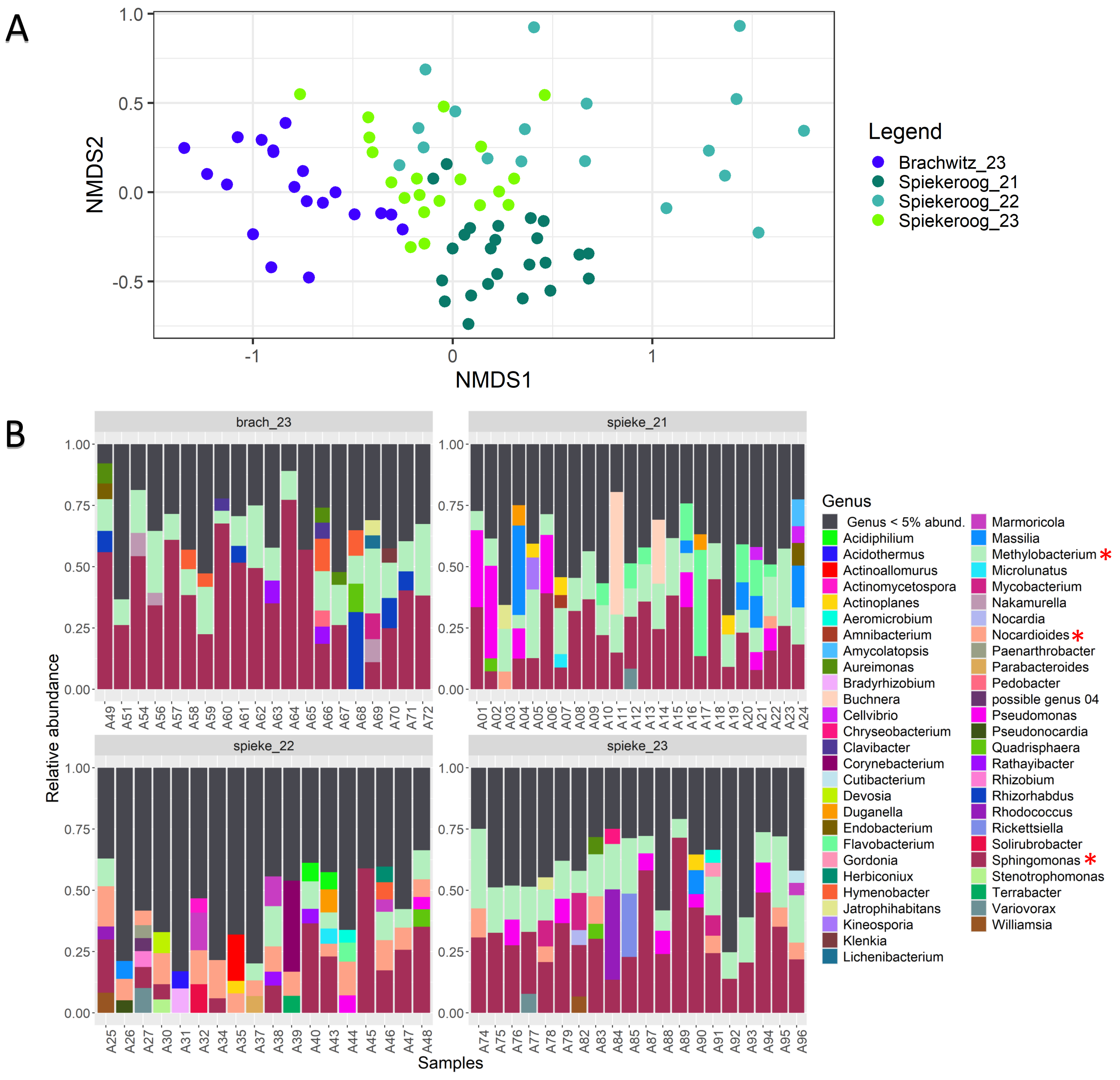

Figure 1 – Bacterial community analysis of *A. thaliana* plants. **(A)** Non-metric multi-dimensional scaling (NMDS) ordination based on Bray-Curtis distance and **(B)** bacterial community composition.

Data reduction for integration

16S rRNA amplicon sequencing:
> 0.01% sum of abundance: 837 amplicon sequence variants (ASVs).

RNA sequencing: nf-core/rnaseq + batch correction + DESeq2 normalization (variance stabilizing transformations) + filtered variance > 2.5: 1000 transcripts

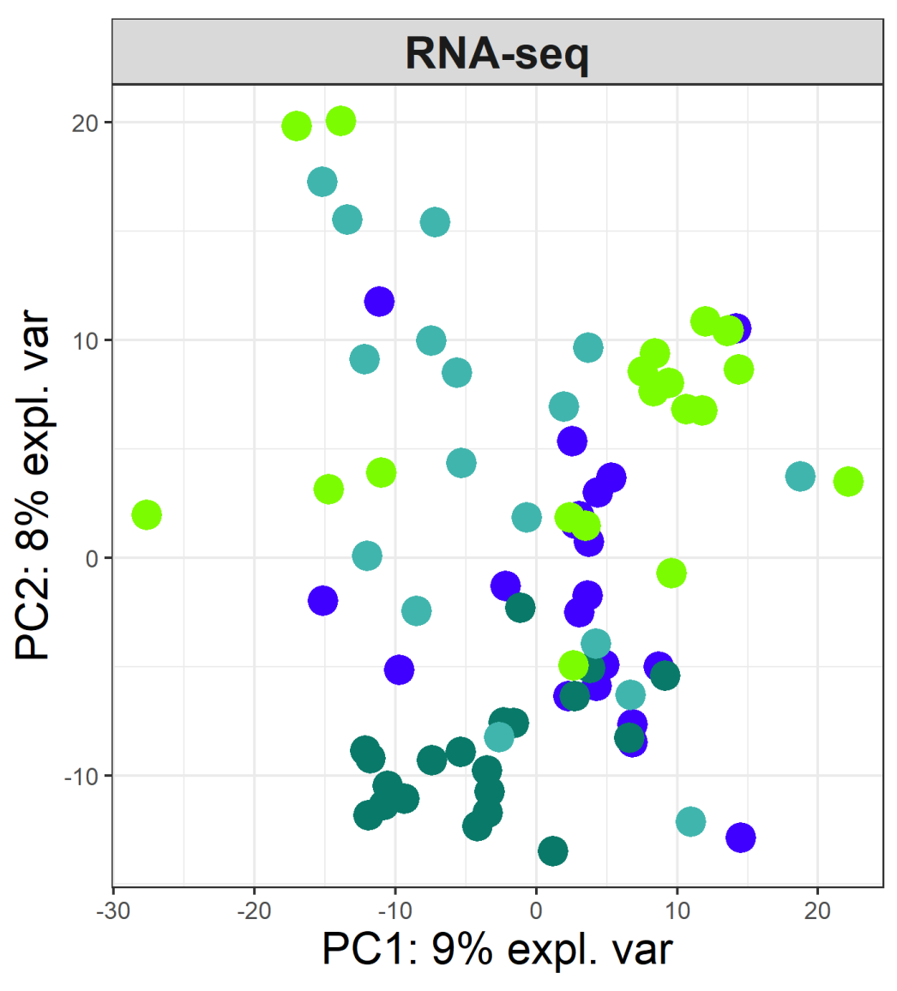

Figure 2 – Principal component analysis (PCA) of pre-processed of RNA sequencing.

Leaf bacteria related with plant response to stimulus and plant development

Data integration shows two distinct cluster of *A. thaliana* genes that are correlated with two different clusters of bacterial ASVs.

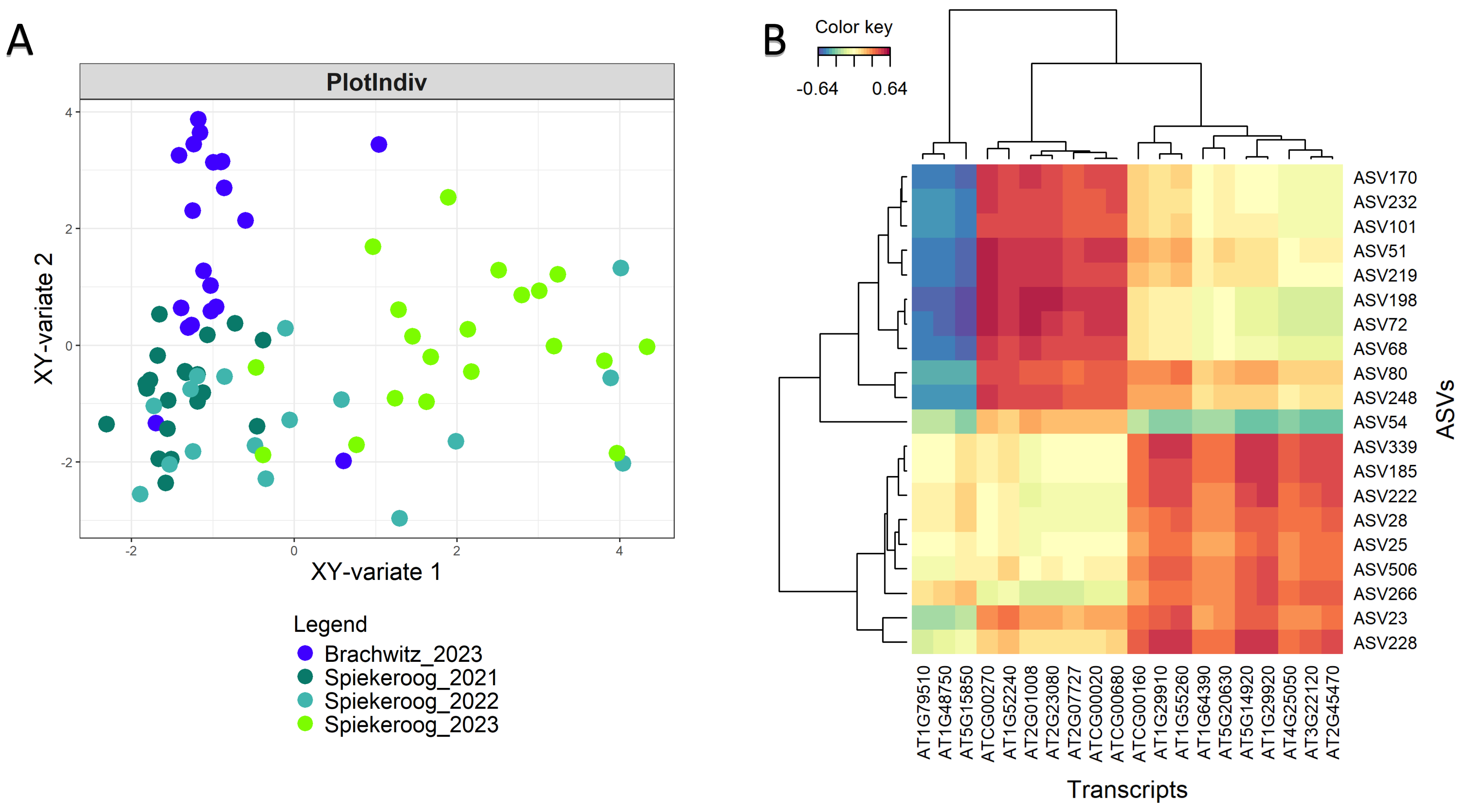

Figure 3 – sparse Projection to Latent Structures (sPLS)-unsupervised model analysis done with mixOmics. **(A)** Projection of samples into the space spanned by the averaged components of both datasets. **(B)** Heatmap with correlations bigger than 0.3.

Relative abundance of the ASVs capture on data integration shows that they represent up to 20% of the bacterial community composition in the plants.

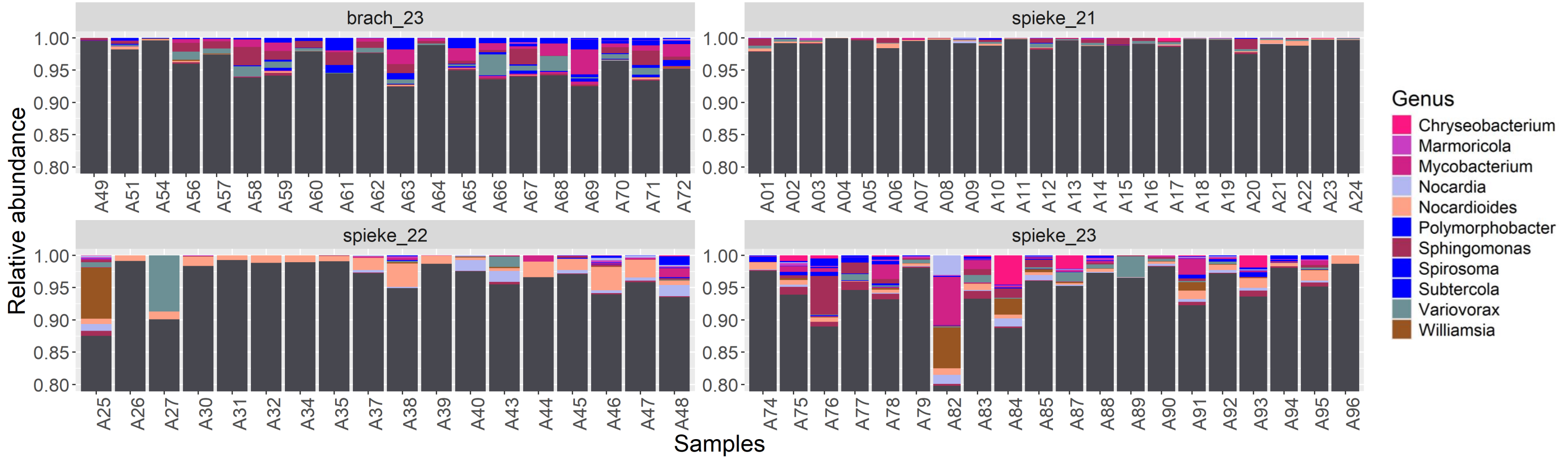

Figure 4 – Bacterial community composition of the ASVs from the data integration analysis (Fig.3). Color coding is the same as Fig.1, and genus with blue color correspond to those that did not appeared in Fig.1.

The two clusters harbor different bacterial community and functional profiles.

Bacterial taxa
*Pseudomonadota (3x), e.g., *Sphingomonas*;
*Bacteroidota (3x), only *Spirosoma*;
*Actinomycetota (3x), mainly *Mycobacterium*.

Function of transcripts (GO terms - BP)
*Response to stress;
*Response to external stimulus;
*Response to biotical stimulus.

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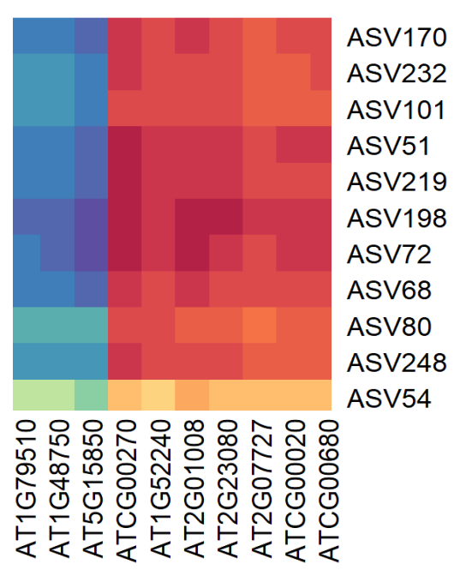

Bacterial taxa
Actinomycetota (6x), with *Nocadia* and *Nocardioides*;
*Pseudomonadota (3x), mainly *Sphingomonas;*
* Bacteroidota (2x), with *Spirosoma*

Function of transcripts (GO terms - BP)
*Multicellular organisms development;
*Anatomical structure development;
*Circadian rhythm.

Take-home messages

- Bacterial community composition reflects space and time of sampling, whereas *Sphingomonas* dominates them;
- Data integration shows clustering between different ASVs and transcripts, where two groups were found;
- Transcripts function point to response to stimulus and plant development in the two bacterial clusters;
- Next steps involve including in the data integration information about phenotypic plant traits and sample location.

References

Rohart, F. et al., "mixOmics: An R package for ‘omics feature selection and multiple data integration." PLoS computational biology 13.11 (2017): e1005752.
Love, M. I., et al. "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." *Genome biology* 15.12 (2014): 550.
Callahan, B. J., et al. "DADA2: High-resolution sample inference from Illumina amplicon data." Nature methods 13.7 (2016): 581-583.

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