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Interspecific interactions alter functionality and promote the keystone species in a synthetic four-species community

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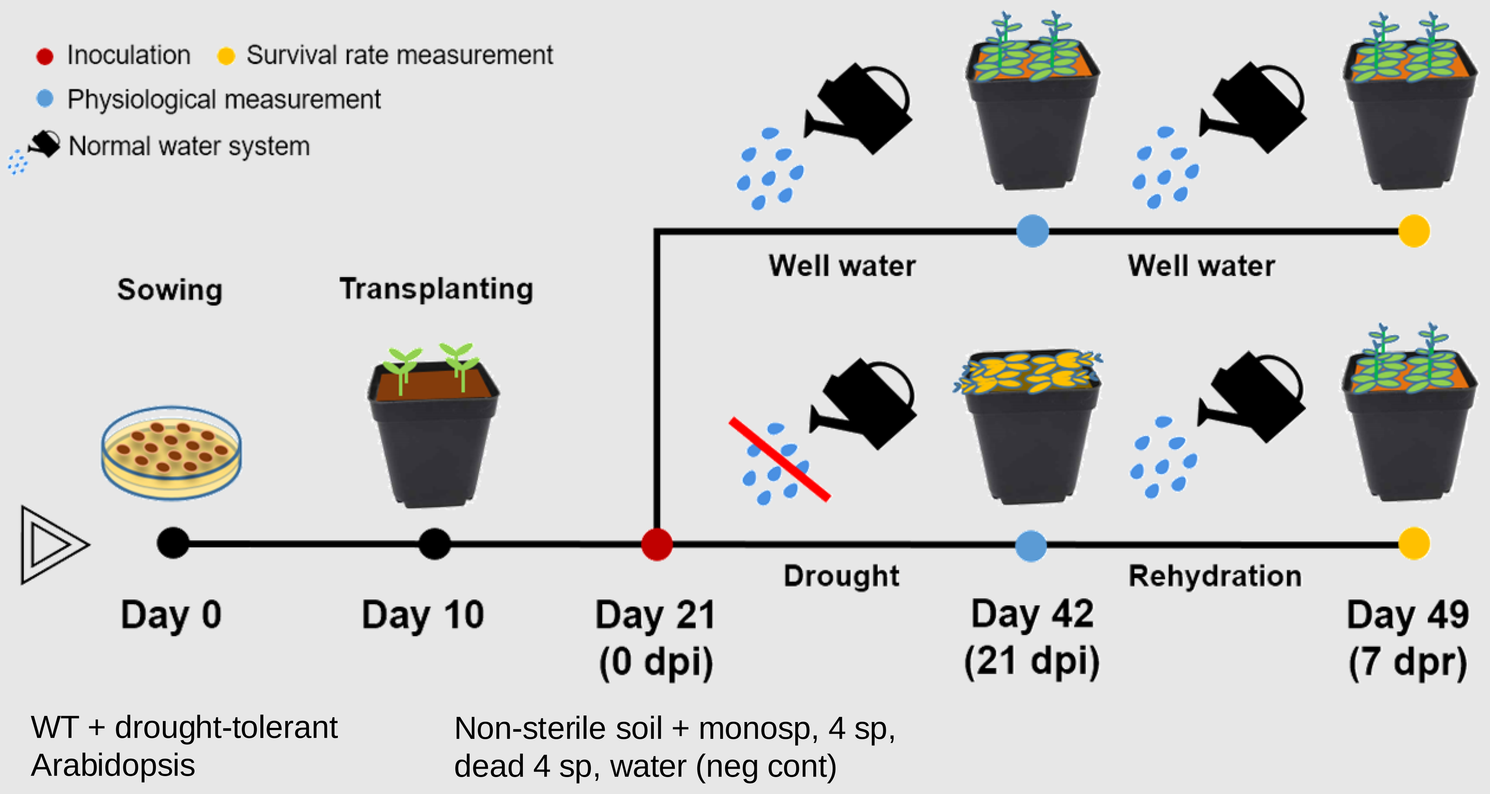
Introduction

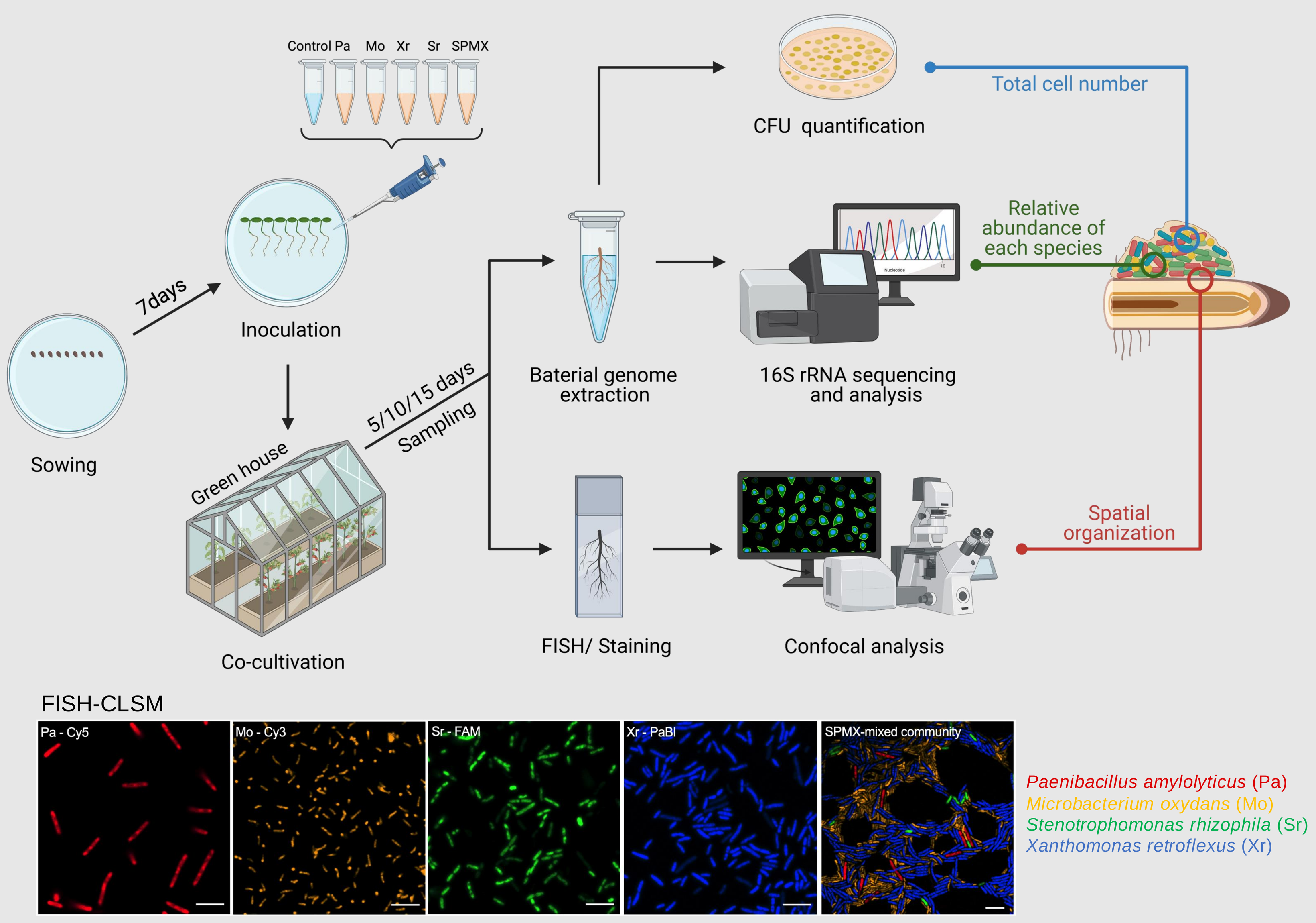
- **Biofilms** are highly diverse, harbouring multiple, interacting species. Such interspecies interactions lead to emergent properties unique to the community setting impacting composition, stability and functionality [1].
- **Biofilm formation** is an important process for many soil bacterial species in natural environments to colonize plant roots, interact with plants, and ultimately affect plant growth and health [2][3].
- Here, we aimed to study the how interspecific interactions shaped community dynamics and functionality of a synthetic four-species biofilm community.
- We studied a four-species consortium **(SPMX)**, composed of *Stenotrophomonas rhizophila* (Sr), *Xanthomonas retroflexus* (Xr), *Microbacterium oxydans* (Mo) and *Paenibacillus amylolyticus* (Pa), previously shown to interact synergistically in biofilm formation. We used *Arabidopsis thaliana* plants as hosts to evaluate bacterial impacts. The bacterial community was studied using molecular analyses (quantitative PCR and amplicon sequencing) and fluorescence *in situ* hybridization (FISH-CLSM) for analysis of spatial organization.
- The study's **hypotheses** are that **(1)** the four strains form a biofilm on the root, retain the water and protect the plant from drought , and **(2)** emergent properties within the four stains impact bacterial root colonization and functionality.

Materials & Methods

Drought protection [4]



Spatial organization and host impacts [5]



Results

➤ Four-species community enhanced plant drought tolerance when inoculated co-culture compared to monocultures [4]

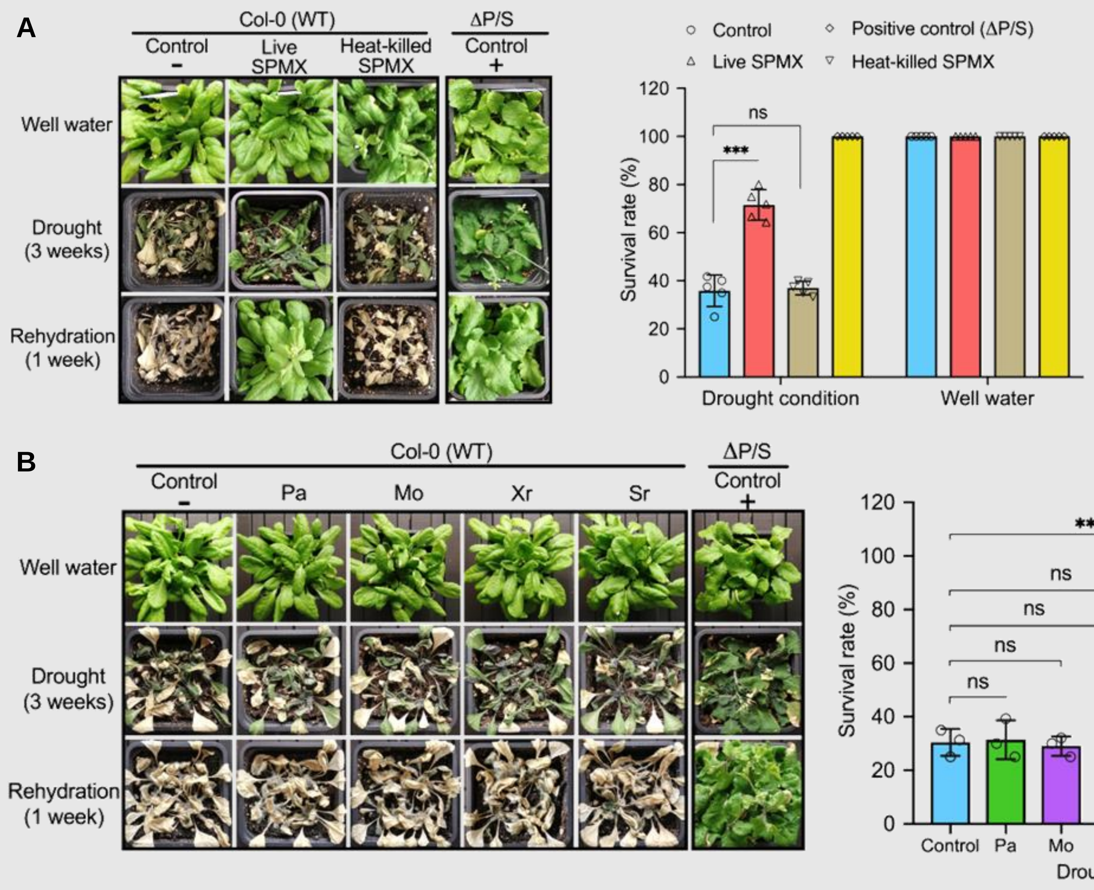


Fig.1. (A) Growth phenotype and survival rats of *Arabidopsis* plants inoculated with live/dead SPMX co-culture under drought/normal conditions after 21 dpi and 7 dpr. Col-0 (WT) and drought-resistant mutant P/S plants were treated with equal volumes of saline solution as negative control (−) and positive control (+), respectively. (B) Growth phenotype and survival rats of *Arabidopsis* plants inoculated with live SPMX monocultures under drought/normal conditions after 21 dpi and 7 dpr. Col-0 (WT) and drought-resistant mutant P/S plants were treated with equal volumes of saline solution as negative control (−) and positive control (+), respectively.

➤ Comparison of root colonization by the four-species co-culture and monocultures [5]

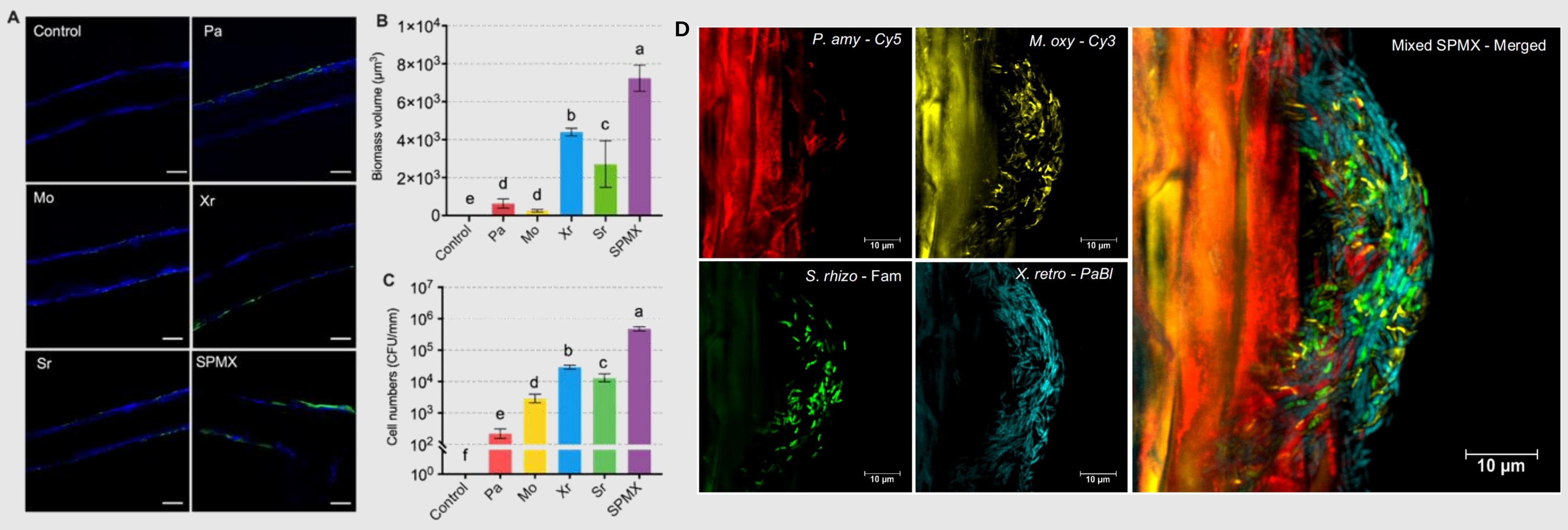


Fig.2. (A) Root colonization by SPMX co-culture and monocultures, captured by confocal laser scanning microscopy (CLSM) at five days (D5); the bacterial colonization was visualized by staining with SYTO9; the root was stained with calcofluor white (CFW) (scale bar = 50 μm); (B) and (C) quantification of bacterial colonization on the roots by 3D quantitative analysis and colony forming units (CFU); data were normalized by average root length (mm); (D) SPMX biofilm formation visualized by FISH on the root surfaces.

➤ *P. amylolyticus* affected spatial organization of the four-species biofilm community on plant roots [5]

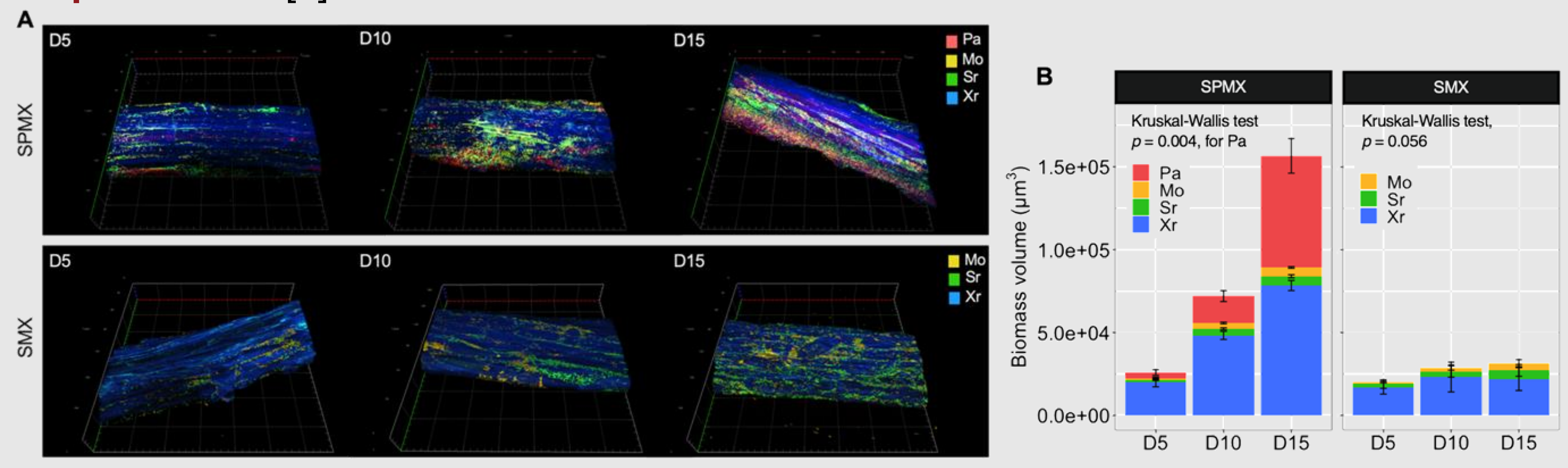


Fig.3. (A) Differences in spatial organization of the four-(SPMX) and three-species (SMX) biofilm formed on the roots over time to 15 days. (B) biomass quantification (μm³, bio-volume) of each species colonizing the roots throughout the SPMX and SMX biofilm at D5, D10, and D15.

➤ Functional differences between four- and three-species biofilm communities on plant growth over time [5]

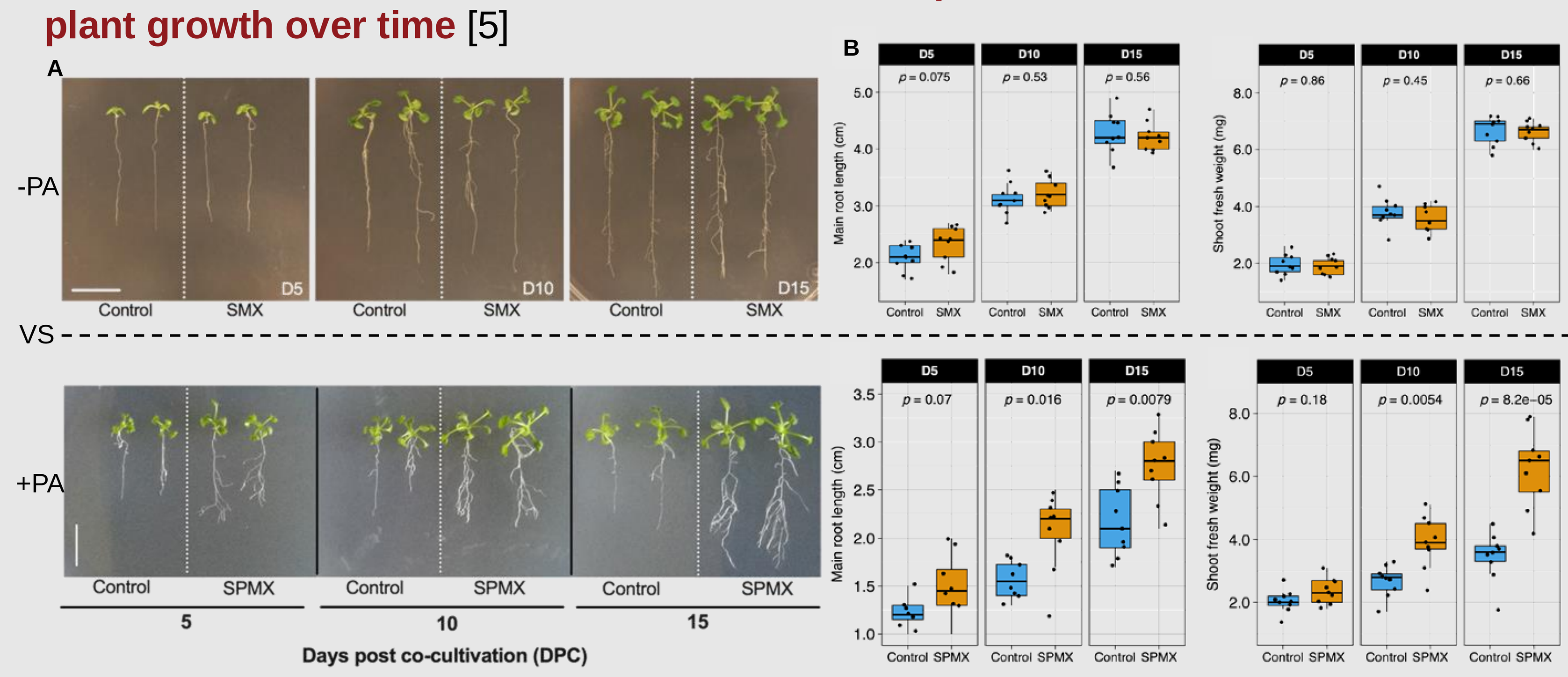


Fig.4. (A) Differences in growth phenotype of plants inoculated with four-species (SPMX) and three-species (SMX) co-cultures with removal of *P. amylolyticus* over time up to 15 days. (Scale bar = 1 cm) (B) main root length (cm) and shoot fresh weight (mg) of *Arabidopsis* seedlings co-cultivated with either four-species (SPMX) or three-species (SMX) co-cultures at D5, D10, and D15 (n = 9); data are based on nine biological replicates (n = 9) from three independent experiments.

Conclusion

Our results highlight the significance of **emergent properties** in multispecies biofilm communities for enhancing plant drought tolerance. Moreover, interspecific interactions are essential for the establishment of the **keystone species**, shaping community function to promote plant growth. Such bacterial interactions should be considered in the design of synthetic communities for biotechnological application.

References:

[1] Flemming, Hans-Curt, et al. "Biofilms: an emergent form of bacterial life." *Nature Reviews Microbiology* (2016).
[2] Trivedi, Pankaj, et al. "Plant–microbiome interactions: from community assembly to plant health." *Nature reviews microbiology* (2020).
[3] Liu, Yunpeng, et al. "Root colonization by beneficial rhizobacteria." *FEMS Microbiology Reviews* 48.1 (2024).
[4] Yang, Nan, et al. "Emergent bacterial community properties induce enhanced drought tolerance in Arabidopsis." *NPJ biofilms and microbiomes* (2021).
[5] Yang, Nan, et al. "Interspecific interactions facilitate keystone species in a multispecies biofilm that promotes plant growth." *The ISME Journal* (2024).

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