

From the root's point of view: abiotic environment as a strong determinant to the beneficial action of growth promoting bacteria

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Environmental pollution and ongoing climate change require solutions that reduce fertilizer use, and improve plant performance at changing temperature.

To understand the potential and mode of action of plant growth promoting bacteria (PGPB), we have used the model cereal *Brachypodium* with the non-invasive root phenotyping platform GrowScreen Agar [1], and EcoFab microcosms [2]. The latter was adapted and used with: low cost non-invasive root and shoot phenotyping [3], N uptake, and root architecture changes, over time. We consistently find that shoot and root phenotype, nutrient contents, and molecular responses vary depending on time, plant age and developmental stage. Time series studies are allowing us to pin-point the time-window when growth promotion takes effect. However, plant responses to PGPB depend on the abiotic conditions too.

Brachypodium plants inoculated with a potential N-fixing PGPB at two N conditions showed increased N content in low N which led to improved overall plant performance. Plants grown under sufficient N were inhibited in growth upon inoculation with the same strain. Phenotyping and proteomics shed light on the systemic response of the plants. A different N-fixing bacterial inoculum seems to promote plants after a hormonal adjustment at low nitrogen, based on transcript profiling. In accordance, increased primary root length is found in these plants, resulting in increased total root length. Interestingly, when sufficient N is present in the medium, a mass balance calculation shows that inoculation with this second strain leads to presence of additional N in the plant - on top of N available in the media, pointing to N-fixation. Thus various interaction pathways are being utilized by the same partners, depending on the abiotic conditions.

Additionally, the plant response can be very specific and time/temperature dependent. *Arabidopsis* plants subjected to elevated temperature and inoculated with a PGPB that improves thermo-tolerance, show the largest increase on a higher order lateral roots, at specific time post inoculation. In this case the PGPB is beneficial in both ambient and elevated temperature, but the extent of growth promotion varies.

We argue that for successful use of beneficial, plant-associated microbes we need to account for: time in context of plant developmental stage [4], the organism genotypes, and the growing environment.