

## **Colonization fitness governs bacterial invasion and drought rescue in a synthetic root microbiome**

Plant-associated microbiomes can buffer hosts against drought stress, but the traits that determine which bacterial taxa provide this benefit within a complex community remain poorly defined. Here, using a 34-member synthetic bacterial community (SC34) representative of the native *Arabidopsis thaliana* root microbiome, we show that SC34 confers substantial drought rescue and that certain taxa become differentially enriched under drought. Differential abundance among community members in seedlings shortly after inoculation, relative to the starting inoculum, correlated with both colonization efficiency and drought rescue capacity measured in mono-association. However, this relationship did not hold for enrichment patterns measured later, in shoots and roots under prolonged drought stress, indicating that early competitive success within the community—but not later community composition—reflects intrinsic bacterial fitness. To identify the determinants of this early colonization advantage, we used the model rhizobacterium *Pseudomonas simiae* WCS417 as a genetically tractable system and performed forward and reverse genetic screens, identifying amino acid auxotrophs (in methionine, tryptophan, and branched-chain amino acid biosynthesis) with impaired drought rescue. These mutants showed reduced fitness and colonization at both early and late timepoints, and restoring bacterial titer at the time of stress onset was sufficient to restore rescue, demonstrating that colonization fitness—not amino acid biosynthesis per se—is causally required for host benefit. Finally, using these genetically defined high- and low-fitness strains, we show that successful invasion of WCS417 into a non-rescuer synthetic community, and the resulting drought rescue, is dosage-dependent. Together, these findings identify early bacterial colonization fitness as a unifying determinant of community establishment, invasion, and functional outcome in a synthetic root microbiome, and show that enrichment patterns under prolonged stress alone are insufficient to predict which community members drive host stress tolerance.