Treating apple replant disease with beneficial microbes: Effects of *Bacillus velezensis* FB01 and *Pseudomonas sp*. RU47 on the microbial community

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Apple replant disease (ARD) is a phenomenon occurring in apple production areas all over the world. Replanting apple on the same site leads to serious growth suppressions, a decline in yield and poor fruit quality. Even though ARD is a long-studied phenomenon and symptoms have been observed in apple-production areas worldwide, its etiology still remains unknown. However, actions to overcome ARD or at least reduce disease symptoms are urgently needed.

In the study presented, we aimed to test the common biocontrol agent *Bacilus velezensis* FB01 and the biostimulating strain *Pseudomonas sp.* RU47 as potential soil management strategies for ARD-affected soils. Both strains, FB01 and RU47 were tested in a greenhouse-trial where they were amended to ARD or healthy grass soil and planted with apple rootstock M26. Plants treated with sterile water served as control. Twenty-eight days after inoculation, samples from the microhabitats rhizoplane and bulk soil were harvested and analyzed by cultivation-dependent and –independent methods to gain knowledge about the establishment of the inoculants and their impact on the microbial community composition. The plant response was investigated three, 16 and 28 days after inoculation by measuring the regulation of selected ARD marker genes and the production of phytoalexins.

By selective plating of FB01 and RU47 on respective media, we showed that both strains had a good persistence in soil and rhizoplane as they were detectable in high abundance even four weeks after application. 16S rRNA-amplicon- and ITS-amplicon-sequencing of total community DNA extracted from both microhabitats showed that treating the plants resulted in shifts in the microbial community composition. The application of RU47 and FB01 had significant influence on alpha- and beta-diversity. For instance, treating plants with the beneficials lead to a clear increase of the species richness in bulk soil compared to untreated samples. Three days after inoculation, a significant short-term upregulation of the investigated ARD marker genes and only low amounts of phytoalexins were observed. After 16 and 28 days however, the abundance of these marker genes decreased to low levels.

With the results of this study, we hope to get one step closer to treat ARD in an environmentally friendly and sustainable way by the application of beneficial microorganisms.