Apple Replant Disease soil effects on the microbial community: a split-root experiment.

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Apple Replant Disease (ARD) occurs when young apple trees of the same or closely related species are planted repeatedly at the same site leading to decreased growth and root browning with subsequent losses in fruit yield and quality. Causal agents of ARD have yet not been defined, but biotic factors are presumably responsible.

A split-root experiment was performed to give insights into the causal agents of ARD and to determine how ARD soil shapes the apple rhizosphere microbiota. Therefore, young apple trees were grown in rhizoboxes with two separated compartments consisting of different combinations of ARD soil (+ARD), gamma treated ARD soil (-ARD) and soil never planted with apple (Control). Plants were grown in a climate chamber for five weeks with continuous root growth monitoring. Samples were taken from several spheres (rhizoplane, rhizosphere and bulk soil) and total microbial community DNA was extracted. Microbial community structure and function was studied by quantitative PCR, DNA fingerprinting by DGGE (Denaturing Gradient Gel Electrophoresis) and Southern blot hybridization.

Plant root growth was substantially lower in +ARD soil than in -ARD and control soils. DGGE of bacterial 16S rRNA and fungal ITS (internal transcribed spacer) amplicons exhibited several responders to ARD. Permutation tests based on similarities of DGGE fingerprints showed significant differences in the bacterial and fungal community structure between +ARD vs. control, control vs. -ARD and +ARD vs. -ARD soils at all three spheres, especially at the rhizoplane. Gamma-irradiation led to severe shifts in bacterial and fungal communities in rhizosphere and bulk soil. The ARD effect was most pronounced in the bulk soil and rhizoplane compared to control soil. By group-specific PCR-DGGE fingerprinting, strongest responders to ARD could be affiliated to *Actinobacteria*, *Alphaproteobacteria* and *Betaproteobacteria*. Southern blot hybridization of functional genes and of plasmids (IncP-9, IncP-1) involved in degradation of aromatic compounds, which are common exudates of apple roots, displayed differences between soils, treatments and within spheres, with higher abundance of IncP-9 plasmids in the rhizoplane and rhizosphere in all soil treatments whilst IncP-1 was only present in the rhizoplane of the control and gamma-treated soils.

In summary, our results indicate a strong effect of ARD soil on root growth and plant performance accompanied by shifts in the microbial community composition which might contribute to ARD. To which extent microorganisms are causal agents or responders to plant symptoms is currently studied by Illumina MiSeq sequencing of 16S rRNA gene and ITS amplicons.