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USE OF NEXT GENERATION SEQUENCING IN SOIL MICROBIOM ANALYSIS

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Presentation Preference: Poster only

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Microbiome is a complex of bacteria, archaea, viruses and fungi in particular environmental niche. The complexity of microbiome investigation refers to interpretation of biological interactions and technical limitations. Biological interactions within microbiome community or with other organisms and environment are crucial for understanding microbiome influence on agriculture and potential benefits from it. One of the benefits could be the estimation of sample origin and pollution by microbiome composition convergence assessment. However, less than 2% of bacteria can be cultured in laboratory and this technical limitation calls into a question the representation of microbiome and taxon abundance assessment. This technical limitation can hide changes in microbe diversity which consequently hinders biotechnological development and application in agriculture as well as in many other scientific fields. The last major breach in technical limitations came by introduction of massive parallel sequencing technology known as next generation sequencing. This technology overcomes culturing limitations and provides direct assessment of microbiome diversity, taxon identification and quantification. In bacteria, sequencing and analysis of variable domain in conservative genes enables bacteria taxon identification and quantification. Therefore, we assessed deep sequencing approach using three different sample types, from unpolluted soil, polluted soil, and *Pyrus communis* cultivar 'Williams' leaf. DNA was isolated directly from samples using commercial silicate columns (The ZymoBIOMICS DNA Kits, Zymo Research, Irvine, U.S.A.). Highly variable V3-V4 region of rDNA was amplified with universal primers 341F and 805R. Amplicons were pair-end sequenced (2x300bp) on MiSeq V3 platform after 600 cycles. Average sequence coverage was approximately 100 000 reads per sample. Raw data were processed according to DADA2 pipeline in following order: quality control, filtering and trimming, pair ends merging, sequence table construction, taxonomy assignment. Our results showed distinct bacterial community composition between samples, as well as specific composition fingerprint of each sample type. The most abundant in plant sample were bacteria from Sphingomonadales and Pseudomonadales orders, while Enterobacteriales and Clostridiales orders were highly present in polluted samples. In conclusion, the massive parallel sequencing technology provides striking technological opportunity to investigate microbiome composition and identify bacteria from different sample types, but also to estimate sample origin by bacterial composition

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