

Bioremediation and restoration of a beneficial microbiota in a walnut grove through the input of different biofertilizers

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Walnut (*Juglans regia*) is a high-value crop of growing importance for Europe; this tree species is valued not only for its nutritious fruits, but also to produce premium timber. Walnut, although adaptable to several agroforestry systems, is conveniently grown in loose, well-aerated soil, which promotes deep root growth, optimal aeration, and proper drainage, thus preventing root rot by fungal pathogens and tree physiological decay. In the eastern part of Emilia-Romagna, several hundreds of hectares have been planted in the last two decades, in some cases in areas repeatedly subjected to flooding: in such areas, young orchards are showing symptoms of reduced growth, premature phylloptosis, scarce fruit set, root decay and increased susceptibility to heat and water stress.

In a walnut grove (approx. 160 ha), soil samples were taken for metagenomic analyses from areas where the trees are suffering from progressive decline (DW), together with soil samples from fields with healthy and productive trees (HW). Metagenomic analyses of healthy and diseased walnut soils revealed that, although both shared a conserved core microbiota at the genus level, their relative abundances differed markedly. Focusing on the top 20 most abundant genera, DW soils were enriched in *Pelobacter*, *Burkholderia*, *Rhodospirillum*, *Azoracis*, and *Desulfuromonas*, whereas HW soils favored *Geobacter*, *Sorangium*, *Phycoccus*, *Friedmanniella*, and *Pirellula*, reflecting higher alpha-diversity and microbial evenness. Species-level biomarker analysis highlighted *Geobacter metallireducens* and *Sorangium cellulosum* as potential contributors to nutrient cycling and pathogen suppression. Bray–Curtis PCoA confirmed a clear structural separation between the communities. Additionally, healthy soil contained a remarkable amount of *Klebsiella* spp., notoriously bacteria with diazotrophic metabolism: *Klebsiella* spp. were absent in diseased soil, which, conversely, contained a significant higher number of myxobacteria. These findings reveal soil dysbiosis associated with walnut decline and define candidate microbial indicators for soil health, providing a scientific foundation for targeted interventions, biofertilizer applications, and sustainable orchard management in European agroecosystems.

To restore the soil physical and chemical structure and support the development of a beneficial soil microbial community, the following commercial biofertilizers were used: 1) municipal organic waste compost and 2) compost from agri-food chain scraps. First results confirmed the increase of microbial activity after both treatments, compared to untreated soil, with a better performance in soil amended with the agri-food waste, supporting the hypothesis of an increase of the microbial biomass (Baldi et al., 2026). Additionally, metagenomic analyses are done to assess the virome in the compost to quantify phages that may affect the structure of soil microbiota. A three-year monitoring of soil structure, chemistry and microbiota will lead to a better understanding of the possibility to restore a beneficial soil microbiota, together with crop productivity, and the role of biofertilizer inputs on the microbial population structure.

References

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