Monitoring root systems and their abiotic and biotic interactions: novel non-invasive approaches for exploring urban biodiversity

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Root systems are essential for sustaining terrestrial ecosystem functions and supporting human well-being. As dynamic and stress-responsive organs, roots interact extensively with both abiotic and biotic soil components, regulating plant growth and influencing overall ecosystem functioning.

However, their characterization remains challenging due to their inaccessibility and soil heterogeneity. Thus, the necessity of uncovering belowground biodiversity and disentangling the links with the environmental components meet the need of innovative and non-disruptive techniques, especially in disturbed and anthropized environments where belowground dynamics influence ecosystem sustainability. In this scenario, we developed and tested innovative methods for root system study and monitoring focusing on rhizosphere interactions, root mapping and species identification by molecular methods.

First, root-microbe interactions between *Quercus cerris* fine root functional traits and microbial community occurred mostly in topsoil at non-urban sites, while in urban areas these interactions predominately occurred at greater depths. Moreover, biochar amendment significantly affected fine roots-environment interactions in rhizosphere and rhizoplane across non-urban and peri-urban soils, enhancing root and microbial functional stability. Second, non-destructive Electrical Resistivity Tomography proved to be a reliable approach for urban tree root system mapping and long-term monitoring, while an artificial intelligence-assisted mapping tool based on image analysis provided a fast and highly accurate species identification and vitality assessment of *Q. cerris* and *Fagus sylvatica* fine roots in urban soils. Third, a multi-locus barcode dataset based on the Italian Flora Portal was generated as reference to drive PCR- or sequencing-based plant species identification specially from complex root/soil samples. Accordingly, a PCR-based method was applied for identifying *Q. cerris* roots from soil bulk samples and further moved quantitative for estimating root density across non-urban-to-urban gradients and soil depths.

Concluding, these high-resolution approaches, while contributing to root system monitoring and ecosystem deciphering, being non-disruptive enable their application to biodiversity characterization also in complex and urban settings.