Urbanisation shapes the root microbiota of *Celtis australis*

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Urban trees can act as reservoirs for microbes adapted to disturbed environments. Our research aims to study the root microbiota of *Celtis australis* L., a resilient Mediterranean-endemic tree widely used in greenspaces and forestry, by combining culture-dependent and -independent (DNA meta-barcoding) approaches in urban environments. In autumn 2024, roots of seventy *C. australis* plants were collected across an urbanisation gradient in Turin (Northern Italy) considering two distinct growth areas, urban parks and avenues. The rhizosphere and endosphere compartments were separated and endospheric DNA was extracted to perform 16S/ITS amplicon sequencing to profile bacterial and fungal communities. In addition, a bacterial strain collection was obtained from both compartments through culturomics totaling 250 strains which were taxonomically identified by MALDI-TOF. In parallel physico-chemical parameters, microbial respiration and enzymatic activities were measured in the bulk soil surrounding each tree. The meta-barcoding analysis revealed that both urbanisation and tree growth areas significantly shaped microbial community composition in *C. australis* roots, without affecting alpha diversity. Bacterial taxa such as Actinobacteriota (e.g. *Jatrophihabitans*) increased with urbanisation level, suggesting adaptation to stressful conditions, while *Bacillus* declined. Fungal communities in highly urbanised sites were enriched in opportunistic and stress-associated taxa, particularly within Helotiales. These shifts were consistent with soil conditions, where increased urbanisation led to metal accumulation (Fe, Zn, Cd, Pb) and acidification, although measured enzymatic activities remained stable. Only β-glucosidase activity increased in avenue soils, indicating enhanced carbon turnover. Predictive metagenomics indicated functional shifts between growth areas, with increased carbohydrate metabolism in avenue-associated microbiota. Culturomics confirmed a diverse but uneven microbiota, dominated by *Acinetobacter* and *Pseudomonas*, with isolates known for plant growth-promoting traits. Preliminary in vivo assays suggested a positive effect of *Rhizophagus irregularis*, enhanced by co-inoculation with *Bacillus cereus*, highlighting potential applications for improving plant resilience in urban environments.

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