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An integrated perspective on the interactions between *Quercus cerris* fine roots and microbial community in top- and sub-layers of urban rhizosphere

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ABSTRACT

The rhizosphere in urban environments is crucial to plant health and ecosystem sustainability. However, little is known about the spatial variability of the rhizosphere in urban forest ecosystems, especially for holobionts based on large organisms like trees. Thus, we aimed to identify the variations of the fine root functional traits of *Quercus cerris* and of the microbial community composition and their reciprocal dependencies analyzing the rhizosphere of the top- and sub-layer soil of non-urban, peri-urban and urban sites. Our findings showed that in non-urban forests, fine roots are mostly shallow and interact with diverse microbial phyla in the topsoil influencing necromass and, thus, carbon cycling. In peri-urban forests, fine root traits vary by depth, leading to higher specific root length in the top-layer and contributing to possible microhabitat fragmentation. In urban areas, more interactions occur at greater depths, mainly influencing root length and biomass, though without significant changes possibly indicating environmental disturbance. Although it is impossible to dissect every single interaction between roots and microbial phyla, underrepresented microbial phyla were demonstrated to play significant roles in shaping the root-microbiome interplay. These preliminary observations need further investigation to provide more insights into the reciprocal interplay spatially occurring in the rhizosphere.

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Root systems; urban environments; rhizomicrobiome; rhizosphere interplay; plant-microbe interactions

1. Introduction

The rhizosphere is one of the Earth's most intricate ecosystems, comprising plant roots, soil, and consortia of microbes (Wierzbicka-Woś et al. 2019). While its size and shape cannot be defined (McNear Jr 2013), the rhizosphere functions as a balanced microhabitat where dynamic interactions among components ensure ecosystem stability and sustainability (Hakim et al. 2021). Additionally, the association among plant roots and microorganisms is that intimate to induce their consideration as a meta-organism named holobiont (Vandenkoornhuyse et al. 2015). In the rhizosphere, plant roots influence microbial communities by releasing a cocktail of compounds serving as energy source and as attractant or recalcitrant to specific microbes (Ahkami et al. 2017). Conversely, recruited microorganisms play crucial roles in nutrient cycling, and in producing metabolites triggering plant functioning (Singh et al. 2023). Additionally, the rhizosphere microbes alter soil characteristics (Naz et al. 2022), directly or indirectly impacting root biology. Thus, the linkages within the rhizosphere reflect mutual feedback which may vary from positive to negative or even neutral, by a continuous plastic adaptation crucial to plant growth and resistance/resilience to biotic and abiotic stressors (Solomon et al. 2024).

Within the root system, fine roots serve as the primary interface between plants and their underground environment. Unlike coarse roots, they are highly dynamic in nature (Sheng et al. 2015) and play key roles in the belowground carbon allocation (Freschet and Roumet 2017). The fine root system is a key determinant of a tree's functioning at both individual and ecosystem levels. They play a crucial role in regulating the movement of water and nutrients through essential processes such as uptake, respiration, exudation, and turnover (Freschet et al. 2013; Philpott et al. 2018;

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Kumar et al. 2019). Furthermore, their variations in morphology and functions reflect plant adaptation to environmental changes (Freschet and Roumet 2017; Iversen et al. 2017). As reviewed by Freschet et al. (2021), root functional traits can be analyzed to obtain knowledge about resource acquisition, and element cycling of plants and they can reveal plant resilience and adaptation mechanisms to disturbance (Montagnoli et al. 2023). In particular, morphological traits of fine roots, such as length, biomass, diameter and specific root length (SRL), are commonly measured to characterize root size and construction. These traits are often linked to foraging strategies, as they reflect the roots' acquisitive capacity: root length is typically associated with resource acquisition (i.e. benefit), while root mass corresponds to the construction and maintenance costs of fine roots (Ostonen et al. 2007; Wang et al. 2021). For example, under drier soil conditions, plants modulate the production of longer and finer roots (smaller diameter classes), resulting in a relatively greater length per unit mass, which leads to an increase in SRL for the thinnest root populations (Montagnoli et al. 2012). Additionally, root activity varies with root diameter and their position in the branching hierarchy (root order), and the biomass of fine root orders adjust to different species, local climatic conditions and site fertility (Mucha et al. 2020). Therefore, the use of only one diameter category (< 2 mm) does not allow for inferring processes related to nutrient and water absorption and transport. Although segregating fine roots by diameter subclasses alone may not correspond to a functional class, especially when comparing different species (Mucha et al. 2020), their use could facilitate a more accurate functional interpretation of the data. Indeed, as previously observed by other authors (Mucha et al. 2020; Montagnoli et al. 2023), the diameter class ≤ 0.5 mm might be closely related to the absorptive roots as a whole (Ostonen et al. 2007). In addition, fine root modifications help to stabilize and to improve the soil physicochemical properties (Hao et al. 2020). Plants in urban environments need to deal with inputs strongly differing from those of rural/natural areas and include a wider, heterogenous and changing pattern of biotic and abiotic stress factors (Czaja et al. 2020). In urban contexts, the arrangement of fine root masses across soil layers is crucial to guarantee healthy and functional plants (Xie et al. 2020). Thus, acquiring more knowledge about fine root development of urban trees is crucial to understand and improve their ecosystems (Zhang et al. 2020), also in urban contexts. The ability of the plant-based holobiont to dynamically adapt to environmental changes strongly depends on the rhizosphere, which is pivotal to the functioning of the urban forest ecosystem (Day et al. 2010). Urban tree rhizosphere is suggested as a treasure trove of biodiversity (Mao et al. 2024), which can alleviate the stress factors that trees are exposed to (Czaja et al. 2020). Despite the growing interest over the past decade for ecosystem services delivered in urban forests, the soil environment—along with its intricate web of roots-microbiota interactions, which underpins multiple layers of biodiversity—remains understudied (Masson et al. 2025). Several studies highlighted that plant growth stage and genotype influence the dynamics of plant-soil interactions (Singhal et al. 2016; Ruan et al. 2019). Additionally, temporal shifts in microbial community composition were linked to the accumulation of root exudates (Chaparro et al. 2014) and to alterations in the nitrogen-to-phosphorus contents (Ren et al. 2017). However, the total variation observed is only partially explained by each holobiont aspect (Zancarini et al. 2021). The urban structure generates a combination of factors impacting plant growth which require new multidirectional studies contemplating also their interactions. So far, most of the available knowledge is based on experiments in controlled conditions or considering only one or two variables (Czaja et al. 2020). Only a few studies focused on the combined and reciprocal effects of soil factors, fine root functional traits, and microbial composition of the rhizosphere (Liu et al. 2020; Deng et al. 2022), and this type of knowledge is lacking with respect to different urban settings. Indeed, rhizosphere interactions remain a poorly investigated focus of urban forest ecosystems (Monaco et al. 2024).

Due to changing impacts in urban settings, the species to be used in afforestation and reforestation programs must meet the criteria of the specific location, of growth conditions, stress resistance/resilience, and also planned function (Czaja et al. 2020). In this regard, several tree species have been deemed adapt for urban environments (Italian Ministry of Ecological Transition, 2021a, 2021b), but little is known about their root systems (Fantozzi et al. 2024), which are crucial for tree performance in terms of resource acquisition and anchoring (Day et al. 2010; Dumroese et al. 2019; Montagnoli et al. 2019), the latter potentially revealing the safety of forests in both urban and non-urban (van Haaften et al. 2021). Among the species identified for afforestation/reforestation programs, *Quercus cerris* L. (Turkey oak) is an emerging candidate (Frigerio et al. 2023) due to its ability to resist extreme climatic and dry conditions (Šimková et al. 2023). This species is widely spread at the European level and predominant in mixed forests under natural and urban

settings in the Balkan and Italian peninsulas (Bagnoli et al. 2016; Caudullo et al. 2017; Quaranta et al. 2025).

The root system of *Q. cerris* has been extensively studied in natural environments, with its seasonal dynamics—particularly variations in fine root mass and length—well documented (Di Iorio et al. 2007; Montagnoli et al. 2012). However, the factors linking the plasticity of *Q. cerris* fine roots to overall plant performance, especially in relation to environmental conditions and holobiont interactions, remain poorly understood (Mausolf et al. 2018; Brunner et al. 2019). Thus, exploring the rhizosphere dynamics of *Q. cerris* under different urban conditions and environmental pressures could significantly enhance our understanding of the interactions within its holobiont system. Such insights would not only contribute to modeling plant-microbe interactions but also to support sustainable urban greening strategies.

In this study, we investigated the functional traits of fine roots and the composition of the rhizomicrobiota—the microbial community within the rhizosphere—along with their interdependencies. These were analyzed at varying soil depths across non-urban, peri-urban, and urban sites. Rhizosphere samples were collected using a nondestructive method, followed by measurements of fine root traits and high-throughput sequencing of the 16S rRNA gene to assess microbial community composition. Correlation-based analyses and machine learning approaches were employed to unravel the complex interactions within the rhizosphere and to identify the dominant factors shaping its dynamics across the urbanization gradient.

We hypothesized that fine root traits, such as biomass and length, as well as rhizomicrobiota composition, would vary in response to the non-urban to urban gradient, with this gradient serving as a stronger determinant than soil depth. Furthermore, we expected that the mutual interactions between root functional traits and microbial communities would also reflect this urbanization-driven trend.

2. Materials and methods

2.1. Study sites, tree selection, stand characteristics, fine root and microbial sampling procedure

Three mixed forest stands, with a well-documented presence of *Quercus* species (Quaranta et al. 2022), were selected within the urban area of Campobasso (Molise administrative region, central-southern Italy) according to a previously described protocol (Varricchione et al. 2024; Dondina et al. 2025). Briefly, the three sites known as Bosco Faiete (BF; 41.55386986394396, 14.61640697891846), San Giovanni in Golfo (SG; 41.58205965765106, 14.691131315095594) and Villino Correra (VC; 41.54796033559403, 14.6614111009349) representing non-urban, peri-urban and urban forest (Quaranta et al. 2025), respectively were chosen. The soil chemical characteristics of the three sites are reported in Gillini et al. (2025).

Within each of these forest sites, we randomly selected seven *Q. cerris* trees separated by a mean distance of at least 50 m, equal to 2.4 times the tree mean height (Table 1). Each tree was considered an independent replicate.

At the end of May 2023 for each selected tree, two soil cores were randomly collected at 70–100 cm distance from the stem using a motor-driven portable soil corer. Before processing, from each soil core (5 cm diameter x 30 cm depth) was withdrawn the 0–10 cm layer (top-layer), and the 20–30 cm (sub-layer). Specifically, each of the soil layers (7 trees, x 3 areas x 2 soil cores x 2 depths) was stored in plastic bags at 4 °C until processing (within 20–40 days from collection) for fine root analysis. The sampling for microbial profiling was analogously executed by collecting, with a flame-sterilized spoon, a small amount of rhizospheric soil within the top- and sub-layers. The samples (7 trees x 3 areas x 2 depths) were stored at 4 °C into sterile tubes until the arrival at the lab for DNA extractions (within hours).

Additionally, the belowground presence of species other than the target one was assessed by collecting fine root samples that were carefully dogged and tracked to each species based on appearance, morphology, and anatomy to distinguish the fine root precisely and to create a catalog of the fine roots of cohabitant species. When doubtful, transverse sections of fresh root material were made to establish precisely the plant species to which the fine roots belonged.

2.2. Analysis of fine root morpho-functional traits

Each of the soil layers was enclosed in a nylon bag (300 µm mesh) and washed with cold water in a laundry washing machine until the soil was sieved (only roots and small rocks remained). Fine roots (diameter ≤ 2 mm) were removed with tweezers and, with the aid of a stereomicroscope (Nikon SMZ 800) and the support of the

Table 1. Tree characteristics in stands.

Stand	Forest type	Tree dbh (cm)	Tree height (m)	Tree mean distance (m)
Bosco Faiete	Non-urban	34 ± 6	21 ± 4	52 ± 19
San Giovanni in Golfo	Peri-urban	27 ± 13	17 ± 1	169 ± 84
Villino Correra	Urban	41 ± 8	24 ± 3	53 ± 25

Mean and standard error of replicates. *Dbh*: diameter at breast height.

fine root catalog of cohabitant species, divided into two main groups: *Q. cerris* and other species. As already mentioned, when root origin was in doubt, a transversal cut on fresh root material was used to establish the plant species' fine roots accurately.

Later, *Q. cerris* fine roots were segregated into live (biomass) and dead (necromass) by color, texture, turgor, and shape. We scanned roots submerged in water at 800 dpi using a calibrated flatbed scanner and a lighting system for image acquisition (Epson Expression 10,000 XL). Subsequently, images were analyzed using software (WinRhizo Pro V. 2007d, Regent Instruments Inc. Quebec, Canada) to separate roots into fine (< 2 mm) and coarse (> 2 mm) diameter categories. Live and dead roots were oven-dried at 70 °C up to constant weight to obtain their biomass and necromass, respectively.

Fine root morpho-functional traits such as length (mm) and biomass (g) were measured within three diameter size sub-classes ($d < 0.5$ mm; 0.5 mm $< d < 1.0$ mm; 1 mm $< d <$ 2 mm) and are expressed per m^2 and a defined soil depth. Finally, specific root length (SRL) was calculated as the root length to dry mass ratio.

2.3. Microbial profiling

According to the manufacturer's instructions, total genomic DNA was extracted from the soil samples using the DNeasy PowerSoil Pro Kit (Qiagen). Next Generation Sequencing (NGS) was performed at BMR Genomics (Padova, Italy), following the protocol previously reported (Monaco et al. 2020), with some modifications. In detail, the V3–V4 regions of the 16S rRNA gene were amplified with the primer pair Pro341F (5′-CCTACGGGNBGCASCAG-3′) and Pro805R (5′-GACTACNVGGGTATCTAATCC-3′), modified with universal tails (Takahashi et al. 2014). PCR products were purified with Thermolabile Exonuclease I (New England Biolabs), diluted 1:2, and amplified with Nextera XT Index in a second PCR step. Amplicons were normalized with SequalPrep (Thermo Fisher) and multiplexed. The pool was purified with Agencourt XP 1X magnetic beads. Lastly, the library was run on the Illumina MiSeq and sequenced with V3 chemistry – 300PE strategy.

2.4. Data analysis and processing

The 14 measurements of the fine root traits (length, biomass and specific root length) were averaged according to soil depth and, when needed, diameter class per each site. Data were normally distributed. The multiple comparisons between soil depths and sites, and also along the diameter classes were performed by Dunn's test, using the "dunn.test" function with the "holm" correction from "dunn.test" package (Dinno 2017) in R (R Core Team 2021).

For microbial profiling, starting from the obtained fastq files, QIIME2 tool (version 2023.7, Bokulich et al. 2018; Caporaso et al. 2010) was applied. The reads were cleaned by Cutadapt (v. 2023.7) and then processed with the denoised-paired plugin of the DADA2 software (Callahan et al. 2016). Briefly, sequences were trimmed at the 3′ end (forward: 270 bp; reverse: 215 bp), filtered by quality and length, dereplicated, and merged to obtain unique sequences. Lastly, chimeras were eliminated. The Amplicon Sequence Variants (ASVs) were filtered by length (thus eliminating potential "contaminant sequences") and by frequency (0.001%) to remove poorly represented sequences and balance the ASV number across the analyzed samples. All reads were classified to the lowest possible taxonomic rank using a reference dataset from the SILVA database (version 138).

Alpha-diversity was calculated with the Chao1 index, and the Kruskal-Wallis test was applied to assess the significance (p-value < 0.01) of comparisons between experimental conditions. Beta-diversity analyses were performed using the Bray-Curtis metric, while the statistical comparisons between experimental conditions were conducted using the PERMANOVA test (p-value < 0.01).

To infer the associations between the microbial community (at phylum level) and the fine root functional traits, the Spearman correlation analysis was performed using the "rcorr" function ("Hmisc" package version 5.2.2, Harrell 2025) of R (R Core Team 2021) in Rstudio (Posit team 2024). Significant p-value lower than 0.05, and a cutoff of 0.7 of correlation were adopted. Analogously, a script based on the "caret" package (version 7.0.1, Kuhn 2008) in R was implemented to apply a random forest approach to the microbial community (at phylum level) and the fine root functional traits. The training dataset was created by randomly selecting 80% of the whole, and the remaining 20% was used as test data. No missing values were detected. The importance of the top 10 predictor variables from the microbial phyla and those of the fine root functional traits were plotted by "ggdotchart" ("ggpubr" package, Kassambara 2023).

3. Results

3.1. Belowground biodiversity and fine root morpho-functional traits of Q. cerris

The fine roots from each soil layer were analyzed in order to assign them to a specific species, thereby

creating a catalog of *Q. cerris* cohabitants in the three sites. Specifically, 11 cohabitant plant species were identified in the non-urban site (BF), 16 cohabitants in the peri-urban site (SG) and 5 cohabitants in the urban site (VC) (Table 2).

Fine root traits from the top- and sub-layers analyzed differed significantly among sites (Figure 1). The fine root length in the top-layer (0–10 cm depth) of BF and VC exhibited the highest and the lowest values, respectively, and SG intermediate values (Figure 1a). SG showed the highest fine root length at the sub-layer (20–30 cm depth), and VC and BF were equally the lowest. Fine root length in the BF site significantly decreased along the soil depth, with the lowest values in the sub-layer (Figure 1a). On the contrary, both SG and VC sites did not show significant differences along soil depth (Figure 1a).

In both soil layers analyzed (0–10, 20–30 cm), BF and VC exhibited the highest and the lowest values of fine root biomass, respectively, and SG intermediate values (Figure 1b). Also, no significant variation in biomass distribution was observed within each site across the different soil depths (Figure 1b).

Table 2. Quercus cerris cohabitant species per sites.

	BF	SG	VC
Acer campestre	X	X	
Acer opalus	X		
Carpinus betulus	X		
Carpinus orientalis		X	
Cornus sp.	X	X	
Crataegus monogyna	X	X	
Euonymus sp.		X	
Fraxinus ornus	X		
Hedera helix	X	X	X
Ligustrum sp.		X	
Prunus avium		X	
Prunus spinosa		X	
Quercus frainetto		X	
Quercus pubescens		X	X
Rosa canina		X	
Rubus macrophyllus	X	X	X
Ruscus aculeatus	X	X	X
Sorbus domestica	X		X
Torminalis glaberrima	X		
Ulmus minor		X	
Viburnum sp.		X	

Fine root necromass showed a low pronounced difference across sites with values of one-fourth of those of the fine root biomass (Figure 1c). Fine root necromass did not differ among the sites in the top-layer (0–10 cm). On the contrary, in the sub-layer, BF and VC were characterized by the highest and the lowest values, respectively. Also, fine root necromass for the three sites did not show significant differences along the soil depth (Figure 1c).

Specific root lengths (SRL) did not differ significantly among sites in either of the analyzed soil layers (Figure 1d). Furthermore, the only significant variation was identified comparing the top- and sub-layer samples in SG (Figure 1d).

Fine root traits varied significantly across diameter classes and sites, and this variation differed depending on the soil layer analyzed (Figure 2). Fine root length was inversely related to the diameter classes, regardless of soil depth (Figure 2a and b). In the top-layer (0–10 cm depth), fine root length did not differ among sites across the three diameter classes, except for the thinnest roots (< 0.5 mm), which showed similar values in BF and SG and significantly higher values in VC (Figure 2a). At the deeper soil depth (sub-layer at 20–30 cm), fine root length for the < 0.5 and 0.5–1 mm diameter classes was the highest in SG and the lowest in VC, with intermediate values in BF (Figure 2b). The thickest fine root diameter class (1–2 mm) did not show differences among sites.

Fine root biomass remained similar across diameter classes independently of the soil depth analyzed with slight differences detected (Figure 2c and d). In particular, in the top-layer (0–10 cm), the biomass of the thinnest fine roots (< 0.5 mm) was the highest and the

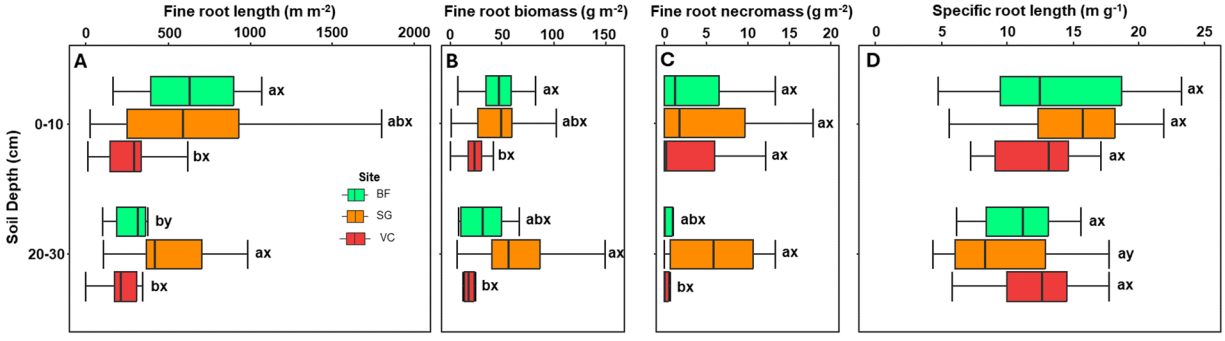

Figure 1. Comparison of fine root traits across the three sites (BF for Bosco Faiete, SG for San Giovanni in Golfo, and VC for Villino Correra) and for top-layer (0–10 cm) and sub-layer soil (20–30 cm) depths. The boxplot indicates the median (line in the middle of the boxes), and 1.5 times the interquartile range (whiskers). Letters a, b, and c indicate significant differences ($p < 0.05$) among sites. Letters x, and y indicate significant differences ($p < 0.05$) between soil depths. Number of observations = 14.

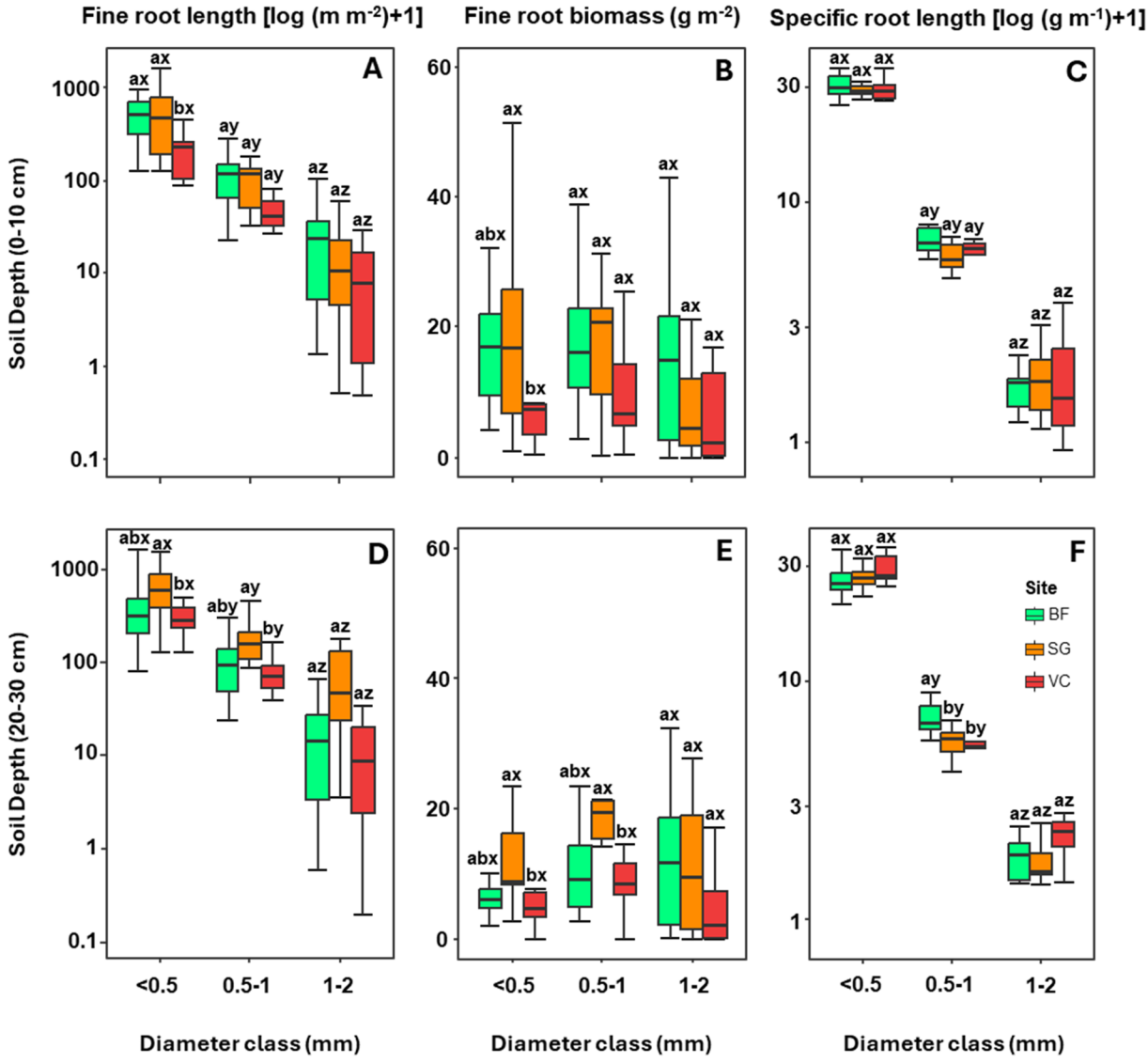

Figure 2. Comparison of fine root traits across the three sites (BF for Bosco Faiete, SG for San Giovanni in Golfo and VC for Villino Correra) and across three different root diameter classes for each soil depth layer. Fine root length and specific root length are plotted as logarithmic only to visualize smaller values. The boxplot indicates the median (line in the middle of the boxes), and 1.5 times the interquartile range (whiskers). Letters a, b, and c indicate significant differences ($p < 0.05$) among sites. Letters x, y, and z indicate significant differences ($p < 0.05$) between fine root diameters. Number of observations = 14.

lowest in SG and VC sites, while thicker roots, both 0.5–1 mm and 1–2 mm diameter classes, did not differ across sites (Figure 2c). In the sub-layer (20–30 cm), the < 0.5 mm and 0.5–1 mm diameter classes also showed the highest and the lowest biomass in SG and VC sites, respectively, while the thickest fine root class (1–2 mm) did not differ across sites (Figure 2d).

Independently of the site and the soil depth analyzed, the SRL significantly decreased with increasing diameter size (Figure 2e and f). Similarly, SRL showed no significant differences among sites or across diameter classes (Figure 2e and f), except for the 0.5–1 mm class of the sub-layer, where BF exhibited significantly higher values compared to SG and VC, which were similar (Figure 2f).

3.2. Microbial diversity analysis

Sequencing analyses provided information on the rhizomicrobiota across the layers of the investigated sites of Campobasso city. The total number of reads varied between 19,538 and 66,557. The sequences generated in the present study have been deposited in the NCBI Sequence Read Archive (SRA) under the accession number PRJNA1238894.

The majority of the microbial species retrieved in the rhizosphere belonged to the *Bacteria* domain, with *Archaea* poorly represented. In fact, they exceeded 0.5% only in a few samples, specifically from SG, where the *Crenarchaeota* phylum reached relative abundance values as high as 1.97%.

To obtain an overall view of the taxonomic composition of the rhizomicrobiota at each layer within the analyzed *Q. cerris* forests, the relative abundance values of the taxa found at different depths were averaged. The microbial phyla with an average relative abundance greater than 1% in at least one of the samples were predominantly composed of *Acidobacteriota*, *Actinobacteriota*, *Bacteroidota*, *Chloroflexi*, *Firmicutes*, *Myxococcota*, *Proteobacteria*, and *Verrucomicrobiota* (Figure 3a).

Differences in the rhizomicrobiota structure were observed based on the study site and depth. In the top-layer, most of the rhizomicrobiota was compsed of *Acidobacteriota*, *Actinobacteriota*, *Proteobacteria* and *Verrucomicrobiota* that showed mean relative abundances higher than 10%. In contrast, *Bacteroidota*, *Firmicutes*, *Chloroflexi*, *Myxococcota*, *RCP2-54* contributed less to the rhizomicrobiota composition of the top-layer (Figure 3a).

In detail, in the top-layer of BF, the species belonging to the *Acidobacteriota* phylum were the most represented with an average relative abundance of 34.69%, followed by *Verrucomicrobiota* (25.63%) and *Proteobacteria* (22.87%), while the other phyla were represented in relative abundances lower than 10%. In the top-layer of SG, the most abundant were the species belonging to *Proteobacteria* (24.67%), then *Verrucomicrobiota* (21.25%) and *Acidobacteriota* (20.13%) and *Actinobacteriota* (12.31%), while other phyla were represented by abundances lower than 10%. In the top-layer of VC, the most abundant species belonged to *Proteobacteria* (26.61%), *Verrucomicrobiota* (22.95%), *Acidobacteriota* (18.20%) and *Actinobacteriota* (15.08%) with other phyla represented with abundances lower than 10% (Figure 3a).

In the sub-layer, an analogous situation was found with *Acidobacteriota*, *Actinobacteriota*, *Proteobacteria* and *Verrucomicrobiota* being the phyla mostly represented together with *Firmicutes*, while *Bacteroidota*, *Chloroflexi*, *Myxococcota*, *RCP2-54* contributed to the rhizomicrobiota composition with percentages lower than 10 (Figure 3a). Specifically, in BF sub-layer, the most abundant species belonged to the phylum of *Verrucomicrobiota* (35.15%), *Acidobacteriota* (27.83%) and *Proteobacteria* (20.79%). All the other phyla were represented with abundances lower than 10%. In the sub-layer of SG, *Verrucomicrobiota* (24.67%) is the most represented phylum followed by *Proteobacteria* (21.62%), *Acidobacteriota* (16.16%), *Actinobacteriota* (12.66%) and *Firmicutes* (11.94%), with all the other phyla represented in percentages lower than 10. And finally, in the sub-layer of VC, the species belonging to the *Verrucomicrobiota* phylum were the most represented with an average relative abundance of 35.30%, followed by *Acidobacteriota* (22.04%) *Proteobacteria* (18.83%) and *Actinobacteriota* (12.09%), while the species belonging to other phyla were on average present by relative abundances lower than 10% (Figure 3a).

Beta-diversity analyses were conducted using the Bray-Curtis metric to compare the microbial communities of *Q. cerris* rhizosphere across the examined layers and sites (Figure 3b). Although some outliers were present, indicating a degree of cluster heterogeneity, the Principal Coordinate Analysis (PCoA) revealed a distinction in the rhizomicrobiota based on the study area, with significant differences between the microbial communities of BF and those found in VC and in SG (Figure 3b and Table 3). Specifically, top-layer community of BF was significantly differing from top-layer community of VC (F-value = 5.67; *p*-value = 0.002; r^2 =0.321) and sub-layer community of SG (F-value = 7; *p*-value = 0.003; r^2 = 0.378). Additionally, the sub-layer community of BF was significantly differing from both the top-layer communities of SG (F-value = 9; *p*-value = 0.004; r^2 =0.431) and VC (F-value = 9; *p*-value = 0.001; r^2 =0.428) and from the sub-layer communities of SG (F-value = 9; *p*-value = 0.004; r^2 =0.427) and VC (F-value = 3; *p*-value = 0.008; r^2 =0.206) (Table 3).

The rarefaction curves generated using the Chao1 index (Figure 3c and Table 4) revealed lower microbial alpha-diversity in BF. In contrast, the microbial communities from the SG and VC showed the higher alpha-diversity with highest values for the top-layer community of VC. Overall, as depth increased, a reduction of the alpha-diversity was observed for both VC and SG communities. An opposite trend was observed, instead, in the site BF.

3.3. An integrated perspective on fine roots and microbial communities

The associations and reciprocal influence among fine root functional traits and microbial phyla were investigated based on Spearman correlation (Figure 4) and random forest analysis (Figure 5).

Spearman correlation analysis (Figure 4) revealed eleven associations (six positive and five negative) in the top-layer of the non-urban site (BF): fine root biomass was positively associated with *Eisenbacteria* ($r = 0.76$, $p = 0.049$); fine root length was positively associated with *Eisenbacteria* ($r = 0.80$, $p = 0.030$); fine root necromass was positively associated with *Firmicutes_D* ($r = 0.86$, $p = 0.014$), *Gemmatimonadota* ($r = 0.90$, $p = 0.0056$), *Methylomirabilota* ($r = 0.96$, $p = 0.00047$) and *Verrucomicrobiota* ($r = 0.96$, $p = 0.00045$) and negatively

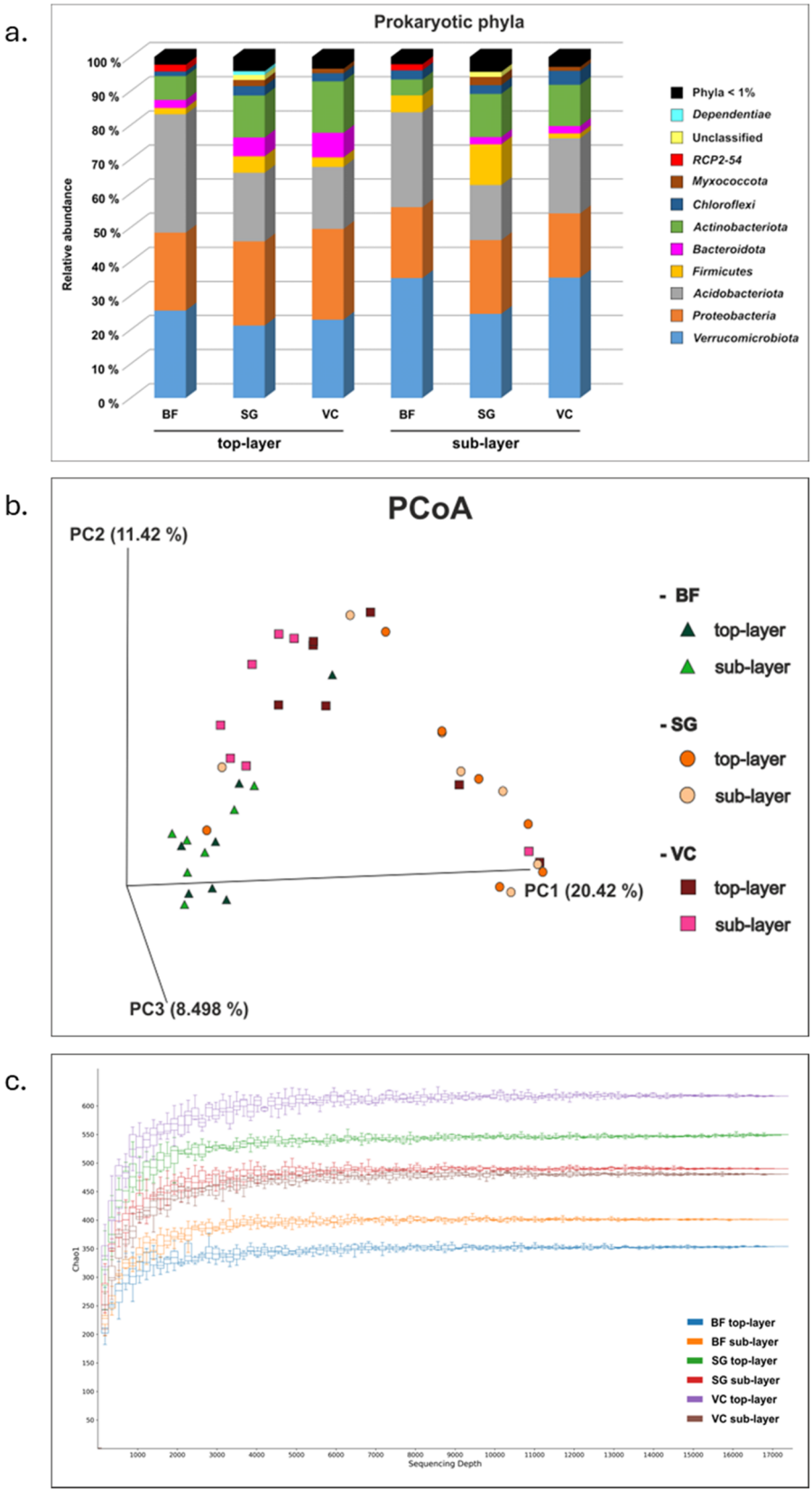

Figure 3. (a) Stacked bar chart showing prokaryotic phyla with average relative abundance values greater than 1% in at least one of the six experimental groups. BF for Bosco Faiete, SG for San Giovanni in Golfo and VC for Villino Correra. (b) Plot of the principal coordinate analysis (PCoA) based on Bray-Curtis metric showing the rhizosphere microbial communities. Colors trace sampling sites and depths. (c) Rarefaction curves showing alpha-diversity based on Chao1 index within rhizosphere microbial communities in the three study sites (BF: Bosco Faiete; SG: San Giovanni in Golfo; VC: Villino Correra) at the top-layer and Sub-layer depths.

Table 3. Beta-diversity summary.

Pair	F-value	R-squared	*P*-value
BF10 vs BF30	2	0.14003	0.118
BF10 vs SG10	6	0.34446	0.01
BF10 vs SG30	**7**	**0.37844**	**0.003**
BF10 vs VC10	**5.67**	**0.32088**	**0.002**
BF10 vs VC30	2	0.1662	0.057
BF30 vs SG10	**9**	**0.43112**	**0.004**
BF30 vs SG30	**9**	**0.42678**	**0.004**
BF30 vs VC10	**9**	**0.42809**	**0.001**
BF30 vs VC30	**3**	**0.20619**	**0.008**
SG10 vs SG30	0.78735	0.061573	0.607
SG10 vs VC10	2	0.11306	0.127
SG10 vs VC30	3	0.21524	0.036
SG30 vs VC10	2	0.166	0.026
SG30 vs VC30	3	0.21508	0.023
VC10 vs VC30	2	0.1637	0.053

Bold for significant values.

Table 4. Alpha-diversity summary.

Pair	Statistic	*P*-value
BF10 vs BF30	24	1
BF10 vs SG10	12	0.12437
BF10 vs SG30	15	0.24911
BF10 vs VC10	4	0.010518
BF10 vs VC30	17	0.37057
BF30 vs SG10	6	0.021308
BF30 vs SG30	5	0.015082
BF30 vs VC10	**0**	**0.00058275**
BF30 vs VC30	16	0.3176
SG10 vs SG30	35	0.20034
SG10 vs VC10	11	0.096331
SG10 vs VC30	26	0.89822
SG30 vs VC10	**2**	**0.0048906**
SG30 vs VC30	22	0.79808
VC10 vs VC30	36	0.17923

Bold for significant values.

associated with *Bdellovibrionota_E* (r = −0.79, $p = 0.035$), *Elusimicrobiota* (r = −0.87, $p = 0.011$), *Patescibacteria* (r = −0.80, $p = 0.030$), *Planctomycetota* (r = −0.86, $p = 0.014$); SRL was negatively associated with *Chloroflexota* (r = −0.79, $p = 0.036$). The sub-layer of BF showed only a positive association between fine root necromass and *Desulfobacterota_B* ($r = 0.76$, $p = 0.049$).

In the peri-urban site (SG) six associations were identified (one positive and 5 negative) in the top-layer and nine associations (four positive and five negative) in the sub-layer (Figure 4). Specifically, in the top-layer, fine root biomass was negatively associated with *Firmicutes_B_370539* (r = −0.76, $p = 0.049$) and *Firmicutes_D* (r = −0.89, $p = 0.0068$), while fine root length was negatively associated only with *Firmicutes_B_370539* (r = −0.76, $p = 0.049$). Fine root necromass, instead, was positively associated with *Verrucomicrobiota* ($r = 0.89$, $p = 0.0068$) and negatively associated with *Eisenbacteria* (r = −0.79, $p = 0.036$) and *Gemmatimonadota* (r = −0.86, $p = 0.014$). Instead, in the sub-layer, fine root biomass was positively associated with *Dependentiae* ($r = 0.80$, $p = 0.031$) and negatively associated with *Firmicutes_D* (r = −0.79, $p = 0.036$) and *Krumholzibacteriota* (r = −0.81, $p = 0.028$). Fine root length was positively associated with *Dependentiae* ($r = 0.76$, $p = 0.049$), fine root necromass was positively associated with *Verrucomicrobiota* ($r = 0.79$, $p = 0.36$) and negatively associated with *Actinobacteriota* (r = −0.86, $p = 0.014$), *Gemmatimonadota* (r = −0.86, $p = 0.014$) and *Thermoproteota* (r = −0.95, $p = 0.00080$), while SRL was positively associated with *Firmicutes_A* ($r = 0.79$, $p = 0.036$).

In the urban site (VC) we identified five associations (two positive and three negative) in the top-layer and seventeen associations (ten positive and seven negative) in the sub-layer (Figure 4). Specifically, in the top-layer, fine root biomass was negatively associated with *Methylomirabilota* (r = −0.79, $p = 0.033$) and *Nitrospirota_A_437815* (r = −0.77, $p = 0.043$), fine root length was positively associated with *Proteobacteria* ($r = 0.86$, $p = 0.014$) and negatively associated with *Firmicutes_D* (r = −0.79, $p = 0.036$), no associations emerged with fine root necromass while SRL was positively associated with *Desulfobacterota_G_459546* ($r = 0.77$, $p = 0.044$). In the sub-layer of VC, fine root biomass was positively associated with *Bdellovibrionota_E* ($r = 0.80$, $p = 0.030$), *Cyanobacteria* ($r = 0.76$, $p = 0.049$), *Elusimicrobiota* ($r = 0.80$, $p = 0.030$), *Eremiobacterota* ($r = 0.80$, $p = 0.030$) and negatively associated with *Firmicutes_D* (r = −0.89, $p = 0.0068$), *Gemmatimonadota* (r = −0.79, $p = 0.033$) and *Myxococcota_A_473307* (r = −0.79, $p = 0.036$). The fine root length was positively associated with *Bdellovibrionota_E* ($r = 0.76$, $p = 0.049$), *Cyanobacteria* ($r = 0.80$, $p = 0.030$), *Elusimicrobiota* ($r = 0.76$, $p = 0.049$) and *Eremiobacterota* ($r = 0.76$, $p = 0.049$) and negatively associated with *Desulfobacterota_B* (r = −0.93, $p = 0.0025$), *Firmicutes_D* (r = −0.86, $p = 0.014$), *Gemmatimonadota* (r = −0.83, $p = 0.021$) and *Methylomirabilota* (r = −0.81, $p = 0.027$). The fine root necromass was positively associated with *Firmicutes_B_370539* ($r = 0.99$, $p = 0.000015$) and *Myxococcota_A_473307* ($r = 0.89$, $p = 0.0068$). No associations emerged in the case of SRL.

Finally, the random forest machine learning analysis identified the most predictive variables among the root functional traits and microbial phyla discriminating the top-layer from the sub-layer (Figure 5a) and the study sites (Figure 5b). The most distinguishable root functional trait contributing to the diversity of the rhizosphere between the top-layer and the sub-layer (Figure 5a) are SRL (19.70%), followed by fine root length (12.20%), fine root necromass (2.68%) and fine root biomass (1.45%), while the most distinguishable phyla are *Bacteroidota* (37.88%), *Firmicutes_D* (5.34%), *Chloroflexota* (3.57%), *Methylomirabilota* (3.36%), *Myxococcota_A_473307* (2.83%), *Eremiobacterota* (2.76%), *Verrucomicrobiota* (2.60%), *Cyanobacteria*

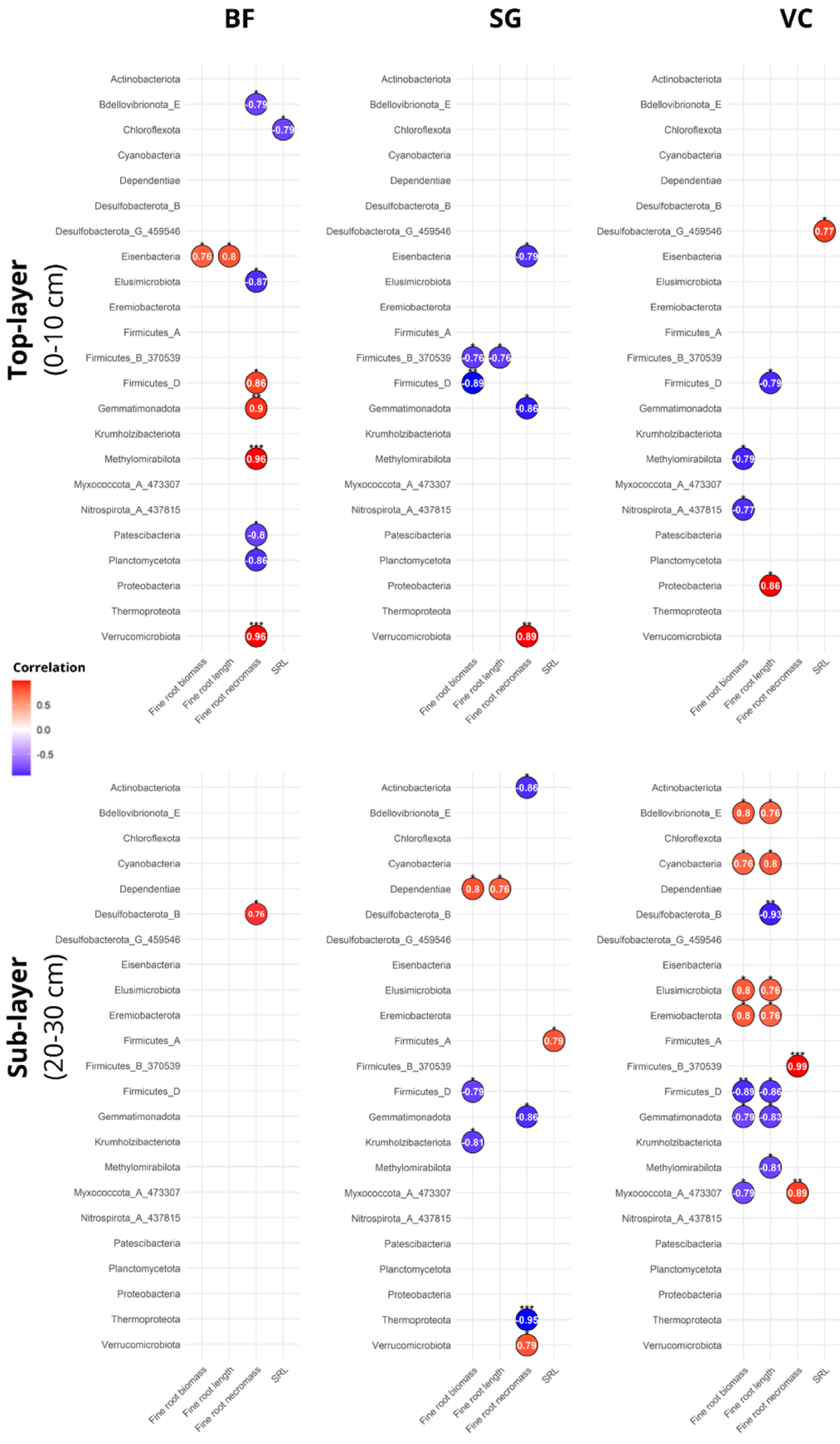

Figure 4. Correlations between fine root functional traits and microbial phyla for top-layer and sub-layer of Bosco Faiete (BF), San Giovanni in Golfo (SG), and Villino Correra (VC). SRL for specific root length. Asterisks to mark significances (*for p-value < 0,05; **for p-value < 0,01; ***for p-value < 0,001).

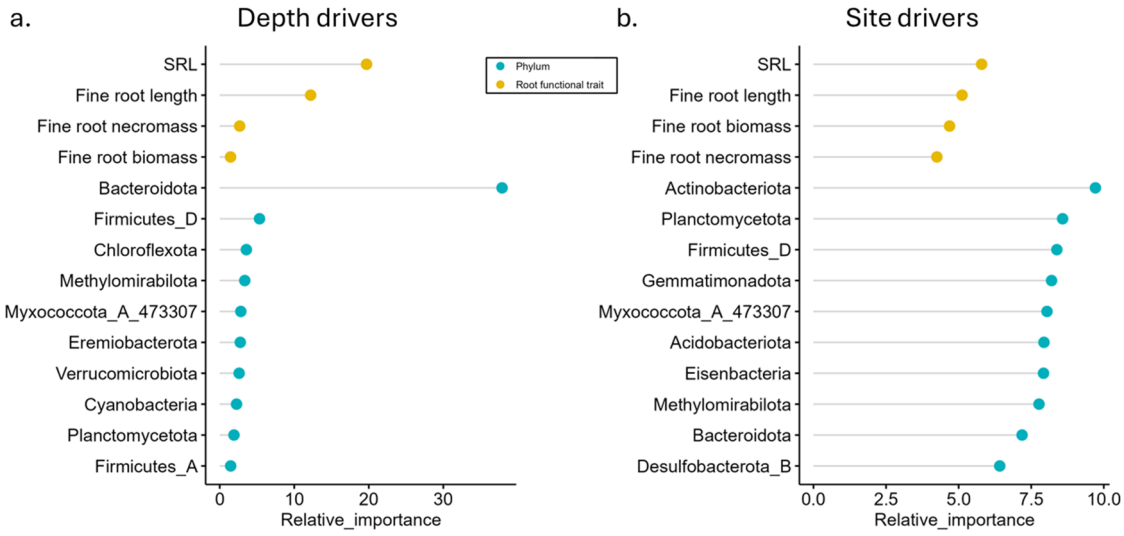

Figure 5. Fine root functional traits and phyla with their relative importance as drivers in defining the diversity of the rhizosphere among top-and sub-layer depths (a) and urban sites (b). SRL for specific root length.

(2.25%), *Planctomycetota* (1.91%) and *Firmicutes_A* (1.47%).

Among the variables contributing to the diversification of the study sites (Figure 5b), SRL contributed for 5.79%, fine root length for 5.12%, fine root biomass for 4.69% and fine root necromass for 4.24%, while the most distinguishable phyla were *Actinobacteriota* (9.71%), *Planctomycetota* (8.58%), Firmicutes_B (8.38%), *Gemmatimonadota* (8.20%), *Myxococcota_A_473307* (8.04%), *Acidobacteriota* (7.94%), *Eisenbacteria* (7.92%), *Methylomirabilota* (7.77%), *Bacteroidota* (7.18%) and *Desulfobacterota_B* (6.41%).

4. Discussion

4.1. Fine root morpho-functional traits differ along the urban gradient

Fine root morpho-functional traits of *Q. cerris* were analyzed at two soil depths across three sites representing distinct urbanization gradients: non-urban (Bosco Faiete, BF), peri-urban (San Giovanni in Golfo, SG), and urban (Villino Correra, VC). A study by Gillini et al. (2025) characterized the soil properties of these sites revealing higher pH, total organic carbon, total and ammoniacal nitrogen levels in peri-urban and urban compared to non-urban soil. Additionally, both SG and VC were characterized by higher concentrations of certain metal(loid)s (such as Co, As, Ni, Pb) and elements (Cl, K, Na) than BF, mainly attributed to anthropogenic activities. These characteristics could contribute to influence the observed changes in fine root length, biomass, and necromass, revealed also in other urban forests composed by the same and similar species (Suseela et al. 2020; Tran et al. 2024). Overall, the trend of these traits showed higher values in BF, followed by SG and VC in the top-layer of the rhizosphere while in the sub-layer higher values in SG than BF and VC. Fine roots actively expand to take up water and nutrients (Jackson et al. 1997) and are an integral component of the net primary productivity (NPP), reflecting the amount of carbon the plant gains through photosynthesis (Van Do et al. 2015). In forests, fine roots contribute to NPP with percentages up to 60% (Jackson et al. 1997), and their distribution patterns usually depend on wide varieties of factors, also including soil properties, biotic cohabitants, proximity to infrastructures/barriers (Jackson et al. 1997) and depth (Addo-Danso et al. 2020). In the woody species *Pinus densiflora*, Sakashita et al. (2024) observed a change in fine-root exudation rate according to soil depth with no significant variations in the fine root traits and, in other species (*Chamaecyparis obtuse* and *Camelia japonica)*, instead, fine root traits, like SRL, were higher in the top-layer suggesting this soil portion to be richer in nutrients.

Although, we did not observe a consistent and significant variation in root functional traits with soil depth across all sites, fine root length and SRL were found as key traits in BF and SG, respectively. These findings may be explained with a greater features heterogeneity between soil layers in BF and SG, whereas the VC site appears to be more homogeneous.

Fine roots have been shown to exhibit species-specific changes in response to depth mainly as a response to available resources and soil microclimate changes (Makita et al. 2011; Addo-Danso et al. 2020). However, most studies focusing on fine roots

are biased by the analysis of only the top-layer rhizosphere (Callesen et al. 2016). Intrestingly, Coleman and Aubrey (2018) observed that resource availability is not the main driver of the fine root changes and that intrinsic factors and stand developmental features control the process. Thus, an integrated evaluation of root traits according to depths variation and other variables, like urbanization, may reveal specific peculiarities. Also changes in modified environments and landscapes such as urban settings (Tran et al. 2024) and agroecosystems (Ryadin et al. 2022) may impact fine roots. Concerning this, the study sites were originally part of a continuous natural forest that was gradually fragmented by urbanization and agricultural expansion, with no signs of planting, thinning or recreational disturbance (Quaranta et al. 2025). However, we observed higher belowground biodiversity in SG, where *Q. cerris* coexisted with the greatest number of other plant species. This suggests other factors possibly affecting fine root development. Indeed, plant species mixtures tend to increase the fine root length respect single species dominated-stands (Huang et al. 2023) and sites under intermediate disturbances, such as peri-urban areas, often feature more diverse habitats forming mosaics of distinct ecological niches with more cohabitant species (Mayor et al. 2012). Accordingly, in the most root biodiverse site (SG) we observed higher values of fine root length in the sub-layer soil. However, the links between root functional traits and community/population dynamics remain unexplored since available knowledge regards only the aboveground parts (Caplan et al. 2019), especially in urban contexts.

4.2. Changes in the rhizosphere microbial community composition with soil depth and urbanization

A total of 1013 microbial species, belonging to 34 diverse phyla, were comprehensively identified in the non-urban (BF), peri-urban (SG), and urban sites (VC). Overall, we identified communities predominantly composed by *Acidobacteriota*, *Actinobacteriota*, *Proteobacteria*, and *Verrucomicrobiota* with less represented phyla such as *Bacteroidota*, *Chloroflexi*, *Firmicutes* and *Myxococcota*. Our results align with previous studies that identified these phyla as the main bacterial taxa in rhizospheric soils (Monaco et al. 2024). By comparing data from approximately 60 cities worldwide, Danko et al. (2021) identified a total of 4246 microbial species, 31 of which were consistently found across all analyzed cities. The same authors also reported that a significant proportion of the urban microbiome likely represents previously unobserved diversity which is usually mentioned as unclassified in microbiome studies (Hsu et al. 2016). Our results evidenced that both soil depth and characteristics (non-urban, peri-urban, and urban) affect the rhizosphere microbial composition. Thus, the identified species represent an extended investigation of microbial diversity not only based on urbanization but also moving deeper into the soil. In both soil layers and sites, most of the rhizomicrobiota was composed of *Acidobacteriota*, *Actinobacteriota*, *Proteobacteria* and *Verrucomicrobiota* while the *Bacteroidota*, *Chloroflexi*, *Myxococcota*, *RCP2-54* contributed less. Taxa dominating urban soils are coherent with phyla detected in non-urban soils (Lysak and Lapygina 2018) though their abundance shifts along the non-urban-to-urban gradient (Tan et al. 2019; Stoma et al. 2020), significantly affecting community functioning (Nugent and Allison 2022). Urban and peri-urban areas derived from land use changes, with peri-urban sites acting as buffers into the rural-to-urban gradient and exhibiting greater heterogeneity and more diversified niches (Binelli et al. 2000; Mayor et al. 2012). In general, human activities continuously impact urban and peri-urban soils shaping higher belowground biodiversity respect to rural ones (Li et al. 2023).

Indeed, analyzing in greater detail the phyla percentage among sites and depth, in the top-layer soil, the non-urban site was dominated by *Acidobacteriota,* whereas the peri-urban and urban sites were dominated by *Actinobacteriota*. In the sub-layer, the *Verrucomicrobiota* was the most represented phylum in all three sites, while *Firmicutes* abundantly characterized the peri-urban site.

Actinobacteria and *Acidobacteria* have remarkable ecological roles in plant-soil ecosystems by actively engaging in nutrient cycling, thereby enhancing beneficial plant-soil interactions, especially in urban environments (Kalam et al. 2020; Bao et al. 2021).

Oligotrophic microbes, like *Verrucomicrobiota*, are generally slow-growing and well adapted to soils with low nutrient concentrations (Bergmann et al. 2011). *Firmicutes* appear more abundant under anthropogenic activities (Al et al. 2022) by a trend associated with urbanization (Rosier et al. 2021). However, *Firmicutes* resulted more abundant in peri-urban than in urban or rural soils (Li et al. 2023). Additionally, along with *Actinobacteria*, *Firmicutes* are recognized as human-associated bacteria, showing high relative abundance in residential green spaces (Zhang et al. 2024b) and shaping also indoor microbiomes (Dockx et al. 2021). Since specific taxa may serve as habitat biomarkers (Parelho et al. 2016), if we consider *Firmicutes* as markers of anthropogenic activities, the

peri-urban site resulted more impacted with respect to the proper urban area. Peri-urban areas are typically situated between urban core and rural sites with a hypothetical intermediate disturbance (Binelli et al. 2000), which creates a more diversified habitat with a higher degree of species cohabitation and more independent ecological niches (Mayor et al. 2012). Coherently our results showed the alpha-diversity in SG was significantly higher than in the other sites.

Also the *Proteobacteria* were abundant in both layers of all the study sites. This phylum was positively associated with tree canopy (Maestre et al. 2024); however, we cannot make correlations with the aboveground traits, which will be desirable in future studies. Anyway, *Proteobacteria*, together with *Acidobacteriota* and *Chloroflexi*, which were found in all study sites, do not seem to be peculiar to forest types, locations, or tree species (Rosier et al. 2021). Thus, unlike the *Firmicutes*, they may not serve as markers of ecological niches. It is reported that the abundance of *Verrucomicrobiota*, whose optimum pH is close to 5.5 (Zhou et al. 2024), diminishes with increasing soil fertility (Navarrete et al. 2015). In agreement, we observed a lower abundance of this phylum in SG which has a feldspathic quartz sandstone lithology with a pH (= 5.8) higher than 5.6 which is the pH of the other two study sites (BF and VC) characterized by sandstone and conglomerates lithology (Quaranta et al. 2025). Also, *Bacteroidota* and *Actinobacteriota* have pH preferences that, however, variate within subgroups (Zhou et al. 2024). A higher presence of *Myxococcota* phylum was observed in SG and VC, which showed higher alpha-diversity values compared to the rhizomicrobiota of BF. Interestingly, various environmental factors can modulate *Myxococcota* predatory activity, shaping the community to higher diversity (Zhou et al. 2020), though the direct effects of the abiotic components on the process remain unclear (Phillips et al. 2022). In general, the composition and diversity of bacterial communities depends on soil properties, climate and biotic factors, with soil pH explaining more variation than any other environmental factor (Zhou et al. 2024). In the study sites the pH varied and showed higher values in the peri-urban (8.07 ± 0.05) and urban (7.3 ± 0.08) sites compared to the non-urban one (6.18 ± 0.06) (Gillini et al. 2025). The peri-urban and urban sites were also characterized by higher concentrations of certain metal(loid)s and elements (Gillini et al. 2025). Anthropogenic activities are the main sources of these elements which may induce shifts in soil pH, consequently affecting the microbial community composition and diversity, soil compaction and possibly root growth (Angon et al. 2024). In the future, experiments – also in controlled conditions – may provide greater insights on the preferential pH niche of each bacterial phylum in relation to the plant species present and other soil and environmental parameters. This will be crucial for a comprehensive analysis of the links between bacterial distribution patterns in the rhizosphere and the various plant species following an urbanization gradient as already done for biomes (Zhou et al. 2024) or for other urban settings (Danko et al. 2021).

4.3. The interplay among microbial community and root functional traits in peri-urban and urban environments is more complex and strongly based on neglected microbial species

We applied a combination of correlation-based and machine learning analysis to infer the associations among fine root functional traits and microbial phyla and their relative importance in defining the diversity between rhizosphere layers or sites. The correlation-based approaches can potentially reveal the reciprocal effects among rhizosphere components in modeling its diversity (Deng et al. 2022). Our results showed for the first time that soil depth and diverse non-urban (BF), peri-urban (SG) and urban (VC) environments affect not only root functional traits and microbial composition of *Q. cerris* rhizosphere, but also the pattern of their reciprocal interplay. Additionally, this approach allowed us to identify lowly represented microorganisms as key actors in this manifold environmental-depending interplay.

Root-associated microbial communities require spatial sampling to yield unique insights into factors structuring the interactions (Fleishman et al. 2023) and depth has been demonstrated as a crucial variable changing characteristics and interactions occurring in the rhizosphere (Wang et al. 2024a). In agreement, we identified the correlations among root traits and rhizomicrobiome phyla which showed different patterns depending on both soil depth and the site characteristics respecting urbanization. Additionally, in the rhizosphere, negative associations are more common than positive ones (Zhang et al. 2024a) indicating an inhibitory effect between root traits and microbial groups (Li et al. 2024). Intriguing, we inferred similar numbers of positive and negative correlations. However, interestingly, we observed higher positive correlations in the case of the sub-layer of VC and higher negative correlations in the case of the top-layer of SG. These observations must consider that available knowledge regards the correlations among fine root

traits and highly abundant microbial phyla (Zhang et al. 2024a). We included in the analysis also underrepresented microbial species whose interaction, in relation to fine root functional traits, is poorly characterized. Thus, our results may suggest different strategies of bacterial recruitment by fine roots. Indeed, King et al. (2023) in a recent work highlighted the complexity of the recruitment of the microbial species which is guided by fine root functions. Further investigations to deepen this reciprocal interplay should also include matrix characteristics and molecular and functional aspects of both fine root categories and microbial species.

Furthermore, here we report, for the first time, that several species involved in these significant associations belong to underrepresented rhizomicrobiota phyla such as *Bdellovibrionota*, *Cyanobacteria*, *Dependentiae*, *Desulfobacterota*, *Eisenbacteria*, *Elusimicrobiota*, *Ermiobacterota*, *Gemmatimonadota*, *Krumholzibacteriota*, *Methylomirabilota*, *Nitrospirota*, *Patescibacteria*, *Planctomycetota* and *Thermoproteota*. These need further investigation to elucidate their role in the interactions with fine roots.

Applying machine learning approach based on random forest we identified the functional traits and the phyla reciprocally influencing based on depth and on study sites. In both cases, SRL was the root trait mostly influencing microbial variability. On the other hand, *Bacteroidota* was the phylum predominantly influencing root functional traits according to depth, while *Actinobacteriota*, *Planctomycetota* and *Firmicutes_D* were the phyla mostly influencing root functional trait variability across the three sites. Greater SRL is associated with higher microbial diversity due to more niches provided (Nunez-Mir and McCary 2024). A positive association between SRL and the root exudate release was already reported as able to regulate soil biogeochemical processes and microbial activities around the roots, deemed comprehensively as "rhizosphere effect" (Wang et al. 2024b). In this regard, future studies would benefit from including in the analysis the plant growth rate potential (López et al. 2023). From microbial perspective, bacteria belonging to *Bacteroidota* phylum have been shown to be prevalent worldwide together with other phyla which comprise *Firmicutes* and *Chloroflexa*. Danko et al. (2021) attribute the difference in the community functioning to the variability in the abundances by which the main phyla are present in a microbiome and indicate soil depth as one of the variability drivers (Wang et al. 2024a). In this scenario, *Bacteroidota* and *Firmicutes* belong to the copiotrophic group, thus their presence is directly dependent on the carbon availability (Hu et al. 2018; Su et al. 2023), they are associated with efficient carbon mineralization (King et al. 2021) and may be selected in the rhizosphere of adsorptive roots (King et al. 2024). Instead, the *Chloroflexi* is a phylum of oligotroph bacteria which activities are coordinated by mutual interactions with copiotroph ones (Su et al. 2023). Our findings suggest a possible diverse balance among abundances of these phyla determining diverse microhabitats at different depths with effects on the fine root development/turnover. In this regard, specific analysis on the microbial communities associated with adsorptive or transportive fine roots would define the precise roles that the various patterns associated with the phyla abundances have regarding the overall performance of the diverse fine root categories, undecipherable in the current study.

Actinobacteriota, the phylum emerging as the main driver for diversity among study sites, is frequently dominant in the rhizosphere where it contributes to soil system, promotes plant growth (El-Tarabily et al. 2021), is crucial to fine root decomposition (Su et al. 2023) and with relative abundances driven by spatial factors (Fleishman et al. 2023).

Surprisingly, the second phylum emerging as a driver for fine root diversity among sites is *Planctomycetota* which has been described to be abundant in the rhizosphere at high altitudes (Xie et al. 2023), while in our dataset, it is among the underrepresented phyla. Microbiomes have a complex nature and require efforts to reveal their functioning (Falkowski et al. 2008). Usually, this task starts with identifying the most abundant or core microorganisms followed by analyzing their correlation to operational factors (Gao et al. 2019; Christel et al. 2023). However, low-abundance microorganisms are neglected by these approaches in favor of the high-abundance taxa (Han and Vaishnava 2023). Thus, neglected species, which play vital roles in maintaining relationships and shaping the community, need to be investigated as a comprehensive part of the ecosystem they belong to Han and Vaishnava (2023). Also *Firmicutes* emerged as driver for fine root diversity among sites. Some genera belonging to this phylum have been described as plant growing-promoting rhizobacteria (Ngalimat et al. 2021) inducing fine roots and root hair development via hormonal pattern variations (Erturk et al. 2010).

Overall, although we cannot dissect each single interaction among fine roots and microbial phyla, we can argue that *Q. cerris* fine root functional traits are tuned by a wide variety of phyla, some of which are underrepresented in the community, and that this tuning also works in reverse.

5. Conclusions

The analysis of *Q. cerris* rhizosphere at different depths in non-urban, peri-urban and urban sites within the area of Campobasso (Italy) allowed to decipher the interactions occurring between the fine root functional traits and the microbial community and, thus, provide meaningful knowledge on the rhizosphere complexity and key players. In less impacted environments, such as non-urban forests, the fine roots have a shallow distribution which can be a driver for interactions among phyla and root traits. Indeed, interactions are found in the top-layer where the roots are longer, and with diverse phyla modulating mainly the fine root necromass (and vice versa) and thus carbon cycling. In peri-urban forests the fine roots exhibit traits related to improving abilities of exploring soils. However, consistent with the potentially greater variety of microhabitats present at this site, these traits exhibit depth-specific adjustments, shaped by diverse interactions with different phyla. This leads to a higher specific root length in the top-layer compared to the sub-layer, which may in turn contribute to microhabitat fragmentation. In the urban site, instead, a high number of interactions occured at greater depths, possibly suggesting an intricate interplay primarily affecting fine root length and biomass, which, however, did not lead to significant trait variation. This highly interactive pattern could represent a characteristic feature of disturbed environments, such as urban ones. Additionally, our study revealed several underrepresented phyla having key roles, underscoring their importance as integral and active contributors to variations in the root functional traits in urban environments.

In summary, our study suggests each interaction as a part of multifaceted reciprocal crosstalk among fine roots, microbial community and other environmental components which need to be elucidated to characterize all the processes. Disentangling the factors that drive the presence and abundance of microbes colonizing the rhizosphere would advance management of root-microbial interactions and, in turn, promote plant growth. However, it requires an understanding of how plants exert their influence on different microbiomes, considering also the immense root spatial-functional heterogeneity within complex root systems. We hope that this information could represent a "rhizosphere fingerprint" useful as indicator of rhizosphere status.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

All data generated or analyzed during this study are included in this published article and, if not deposited in public databases, available under request to author Gabriella Sferra (gabriella.sferra@unimol.it).

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