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METHODS PAPER

Optimizing soil core fine root collection and characterization: significant time reduction with a sub-sampling approach

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Abstract

Background and aims The quantification of plant roots from soil represents a pivotal step in many studies in plant ecology and soil science. However, the substantial time investment required for this process often represents a considerable impediment to research progress. The objective of this study is to evaluate and propose a time-saving method to minimize the time required for collecting roots without compromising data integrity compared to traditional approaches.

Methods The proposed Sub-sample Approach (SA) requires collecting fine roots from a sub-sample and subsequently leading calculations to estimate total root traits (mass, length, and length distribution among diameters) within the sampled soil core. A comparative analysis was carried out on root harvesting time between meticulous sample cleaning (Conventional Approach, CA) and SA. Moreover, these methods were assessed across different sites including grassland, oak forest, and olive orchard.

Results The analysis conducted across many sites resulted in high heterogeneity of processing time when employing the CA (ranging from 2.6 to 27.6 h per sample). Conversely, the adoption of SA reduced processing time and resulted in less variation between samples (ranging from 37 to 112 min per sample). Remarkably, root trait data obtained using SA showed similarity to those obtained through the CA.

Conclusion The SA offers a remarkable advantage over the CA by significantly reducing the time needed for root collection from soil core samples. Moreover, SA exhibits lower variability among different collection sites, while maintaining consistency in qualitative and quantitative data compared to the CA.

Keywords Fine root traits · Fine root biomass · Soil core analysis · Root quantification · Time-saving method · City of Bari

Abbreviations

CA	Conventional approach
SA	Sub-sample approach
OF	Organic fraction

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FOF	Fine organic fraction
r-FOF	Remaining fine organic fraction
sr-FOF	Sub-sample remaining fine organic fraction
c-RM	Coarse root mass
f-RM	Root fragments mass
sf-RM	Sub-sample root fragments mass

Introduction

Urban forestry has been considered an impactful method for reducing atmospheric CO_2 and urban heat "island effect", improving air and water quality and providing several social and psychological benefits for inhabitants (McHale et al. 2007; Sanesi et al. 2018). In addition, plants intercept rainfall, enhance soil water infiltration, increase soil organic matter content, and mitigate soil erosion (Wang et al. 2022). Natural vegetation restoration is also a management approach that increases carbon sequestration rates in soil (Sun et al. 2019). In this context, the study and deepening of the root systems are indicated as one of the most suitable methods to support the objectives of this kind of project. However, the comprehensive assessment of belowground site changes through the investigation of the plant root network is hindered by the time-consuming nature of such studies. This sheds light on the necessity to develop faster yet scientifically equivalent approaches.

Accounting for 16% to 63% of the total plant biomass (Poorter et al. 2012), root systems are the main link between soil and plants. They not only facilitate the uptake and transport of nutrients but also improve the physical and chemical properties of the soil, promoting the development and stability of soil aggregates, also contributing to soil and water conservation (Janssens et al. 2002; Li et al. 2020; Peng et al. 2019; Pérès et al. 2013; Zhao et al. 2017).

In almost all root systems, it is possible to distinguish a fine root system. Fine roots were commonly defined as a single pool of roots characterized by their ephemeral nature, having a diameter below 2 mm. In recent years, the robustness of this definition has diminished as several authors have pointed out that the fine root system may encompass varying dimensional ranges, potentially smaller or even larger, depending on plant species (Liu et al. 2016; McCormack et al. 2015; Pregitzer et al. 2002). More recently, Montagnoli et al. (2018) introduced a novel methodology that categorizes fine roots into new diameter sub-classes, closely aligned with their functional roles and/or architectural hierarchy. In consideration of the functional diversity among fine roots, numerous studies have implemented a classification system that differentiates fine roots into absorptive/fibrous fine roots and transport/pioneer fine roots (Montagnoli et al. 2021; Polverigiani et al. 2011; Zadworny and Eissenstat 2011). Fibrous fine roots represent the most distal roots that extend close to the soil surface, involved primarily in the acquisition and uptake of soil resources, whereas pioneer fine roots occur higher in the branching hierarchy and serve primarily structural and transport functions with some additional capacity for storage (McCormack et al. 2015).

Within the fibrous root network, finer roots significantly promote adhesion between soil particles and contribute to the stability of soil aggregates (Kohler et al. 2009). For instance, roots with diameters between 0.5–1 mm were found to enhance the stability of topsoil, whereas roots with a diameter greater than 2 mm affect the stability of deeper soil aggregates enhancing shear strength (Kang et al. 2023; Sun et al. 2019). These thicker fibrous roots may have a branched architecture that stabilizes the soil to a certain extent; however, they do not prevent surface erosion (Wang et al. 2018). Fine roots play a crucial role in plant's water and nutrient uptake, and the vertical distribution in the soil profile is an essential factor. Therefore, the morphological traits and the distribution of fine roots can effectively reflect the ability of plants to use soil resources (Kang et al. 2023). Different plant species exhibit varying patterns of fine root distribution, influenced by both genetic factors and environmental conditions, including soil type, water availability, and nutrient distribution. These factors have an overall impact on the ability to access water and nutrients (Tan et al. 2023). Consequently, plants may strategically concentrate their fine roots in specific soil layers that offer enhanced access to water or nutrients, thereby optimizing their absorption efficiency. Typically, root systems of herbaceous plants exhibit a greater dispersion, or a more fibrous nature compared to those of woody plants. However, bulbs, tuberous roots, and storage roots occur frequently in herbaceous plants. Overall, fibrous root systems tend to have a higher abundance of finer roots than the root systems of woody species (Stokes et al. 2009).

An important fine root trait is given by the "root turnover" (Brunner et al. 2013), which plays a crucial role in nutrient cycling and organic matter dynamics within ecosystems, since the nutrients released by dead roots during decomposition will then be available for uptake by other plants and soil organisms (Cheng et al. 2023; Saha et al. 2023).

Given their pivotal role in ecosystems, a plethora of methods have been developed to estimate both the quantitative and qualitative features of fine roots in ecological research. These methods encompass allometric techniques (e.g., root-to-shoot relationships), soil core sampling, mesh-based techniques, and in situ imaging methods (Addo-Danso et al. 2016; Freschet et al. 2021; Li et al. 2013; Rahman et al. 2020). It is important to acknowledge that each of these methods introduces potential sources of error that may derive from different factors including experimental design, statistical properties of the sampled root population, and ecological context variations (Berhongaray et al. 2013; Mancuso 2012). Among these methods, the sampling of soil cores and subsequent root collection through washing and hand-sorting is the most employed technique (Brunner et al. 2013; Freschet et al. 2021; McGowan et al. 1983; Persson and Ahlström 1990; Vogt and Persson 1991). This preference is attributable to its methodological simplicity and closer adherence to reality. The method allows researchers to assess various fine root characteristics, such as root biomass, root distribution, root length, root morphology, and functional traits (McCormack et al. 2015). Typically, the number of soil samples taken to characterize the fine root population traits ranged from 6 to 30 (Fahey et al. 2017). However, the process of collecting all root fragments from a single core sample is time-consuming, often requiring as much as 4 to 24 h (Benjamin and Nielsen 2004; Bernier et al. 2005; Rodrigues de Sousa and Gehring 2010; Persson and Ahlström 1990; Vogt and Persson 1991). Therefore, this translates into a substantial amount of time dedicated to this task delaying downstream analyses. Different methodologies were explored in attempts to address this issue. The method proposed by Schroth and Kolbe (1994) involved the combining and homogenizing of several soil cores from one plot before sub-sampling for root collection. This method offers advantages in terms of time-saving, particularly when dealing with large-scale assessments of root parameters. However, it may not be suitable for investigation requiring information about small-scale root variability, and in studies with a statistical comparison of root data measured at different times in a single plot, for which complementary techniques or individual core treatments may be necessary. In alternative approaches, some authors attempted to minimize root collection time by implementing temporal prediction techniques directly in the field, without soil particles removal and the collection of visible roots (Metcalfe et al. 2007; Silva et al. 2022), or by estimating root traits (but excluding root mass data) using image analysis while retaining a significant portion of soil sample debris, predominantly organic matter fragments (Benjamin and Nielsen 2004).

In light of our active role in the establishment of an urban forest in the municipality of Bari (Italy) aimed at studying its environmental impact in terms of biodiversity and ecosystem services through the use of native tree and shrub species (Gargano et al. 2021; Pardi et al. 2023), this work aims to assess and propose a time-saving method of root collection. The primary objective is to minimize the time required for root trait analysis. Precisely, the study compares the time taken for root harvesting between a thorough cleaning of samples and our novel method. Data obtained from both approaches were compared in terms of quantitative and qualitative values.

Materials and methods

Sample sites

To evaluate the proposed method under heterogeneous conditions, root trait characterization was carried out across distinct Mediterranean ecosystems in the Apulia region, South Italy. In particular, three sampling sites were selected: 1) a holm oak forest located in the municipality of Monopoli (40°53′46.0″N, 17°16′21.1″E; elevation 250 m.a.s.l.), which overstory is dominated by *Quercus ilex* L. (canopy cover > 75%) forming a high forest stand (hereafter referred to as the oak forest); 2) an unmanaged grassland field located in the municipality of Bari, characterized by annual and perennial herbaceous species (41°05′17.1″N 16°51′39.0″ E; elevation 40 m.a.s.l) (hereafter referred to as the grassland); 3) an olive orchard

located in the municipality of Bari (41°05′21.0″N 16°51′33.4″; elevation 33 m.a.s.l), with 50-years-old trees spaced with plant-row spacing of 4.0 m and 4.5 m and few scattered herbaceous understory species (hereafter referred to as the olive orchard).

Root collection

The root samples were harvested by soil coring. Twenty 15-cm-deep soil cores (15 cm diameter) were randomly collected in each site using a manual core sampler. In the presence of trees, soil cores were collected between 0.5–1.5 m from the trunk. The grassland was sampled in May 2023 while the oak forest in September 2023, and the olive orchard in June 2023. Samples were stored in plastic bags at 4 °C until processed.

Two methods were used to collect root from the samples, defined hereafter as Conventional Approach (CA, Fig. 1) and Sub-sample Approach (SA, Fig. 2). In order to better compare them, both qualitatively and quantitatively, during the soil cleaning process high attention was addressed to collect all the roots, up to fragments of less than a few millimetres in length using a stereomicroscope. The initial cleaning process of each sample was the same for both approaches (Figs. 1A-B and 2A-B) carried out as follows. The first step for roots collection consisted of dividing the organic fraction (OF), composed of roots and litter fragments, from the soil mineral fraction. This was achieved by taking advantage of the specific weight difference between the two fractions (Fig. 1A). The soil sample was soaked in a tray filled with water and OF was removed and collected in a sieve (mesh size of 1 mm) by panning-like action (separation of soil and/or gravels by washing in a pan with water). OF was then soaked back in water in a clean tray and all coarse objects were removed from the tray (long, big or branched roots, chunks of coal or wood, leaves, seeds, etc.). Roots were stored in a 70% ethanol solution while the rest of the coarse litter was discharged. The removal of coarse objects was intended to leave inside the tray a substrate composed of a homogeneous mixture of fine particles (FOF—fine litter and root fragments, see Figs. 1B and 2B). From this stage, the two methods diverged, as described below.

Conventional approach

According to the CA method, all the root fragments inside the FOF were collected by hand using a stereomicroscope and stored in a 70% ethanol solution until processed (within 30 days from collection) (Fig. 1C). The remaining litter was discharged. All roots (coarse roots and root fragments) were scanned submerged in water at a resolution of 800 dpi with a calibrated flatbed scanner coupled to a transparency unit for image acquisition (Epson Expression 10,000 XL) and analyzed by using WinRhizo Pro V. 2007d (Regent Instruments Inc., Quebec, Canada) by micrometric image analysis as described in Montagnoli et al. (2018). This method analyses each image with a progressive root diameter increment of 50 µm. Samples were then oven-dried at 80 °C until constant weight to obtain mass data (coarse root mass, c-RM; root fragments mass, f-RM, Fig. 1D).

Sub-sample approach

According to the SA method, the FOF was grouped in a sieve and transferred into a Petri dish. FOF was mixed by hand to homogenize its composition. The homogenization is crucial to avoid any kind of bias in the next sub-sampling. Indeed, inside the sieve some stratification may occur (e.g. sand on the bottom and roots on the top), as well as in the Petri dish if sub-sampling is delayed (e.g. free water tends to move to the bottom). Then, a small portion of the FOF (around 1 cm^2) was cut with scissors and collected. It is extremely important to cut the border of the sub-sample in order to prevent collecting longer root fragments outside the 1 cm^2 selected. After oven-drying at 80 °C, the dry weight of the remaining FOF (r-FOF) was measured (Fig. 2C) and then the FOF was discharged. Thereafter, the sub-sample was placed under a stereomicroscope and all the root fragments inside were collected. The remaining litter was then oven-dried at 80 °C, weighed (sr-FOF) and discharged (Fig. 2D). All roots (coarse roots and sub-sample root fragments) were scanned and analyzed as already described for CA. Samples were then oven-dried at 80 °C until constant weight to obtain mass data (coarse root mass, c-RM; sub-sample root fragments mass, sf-RM, Fig. 2E).

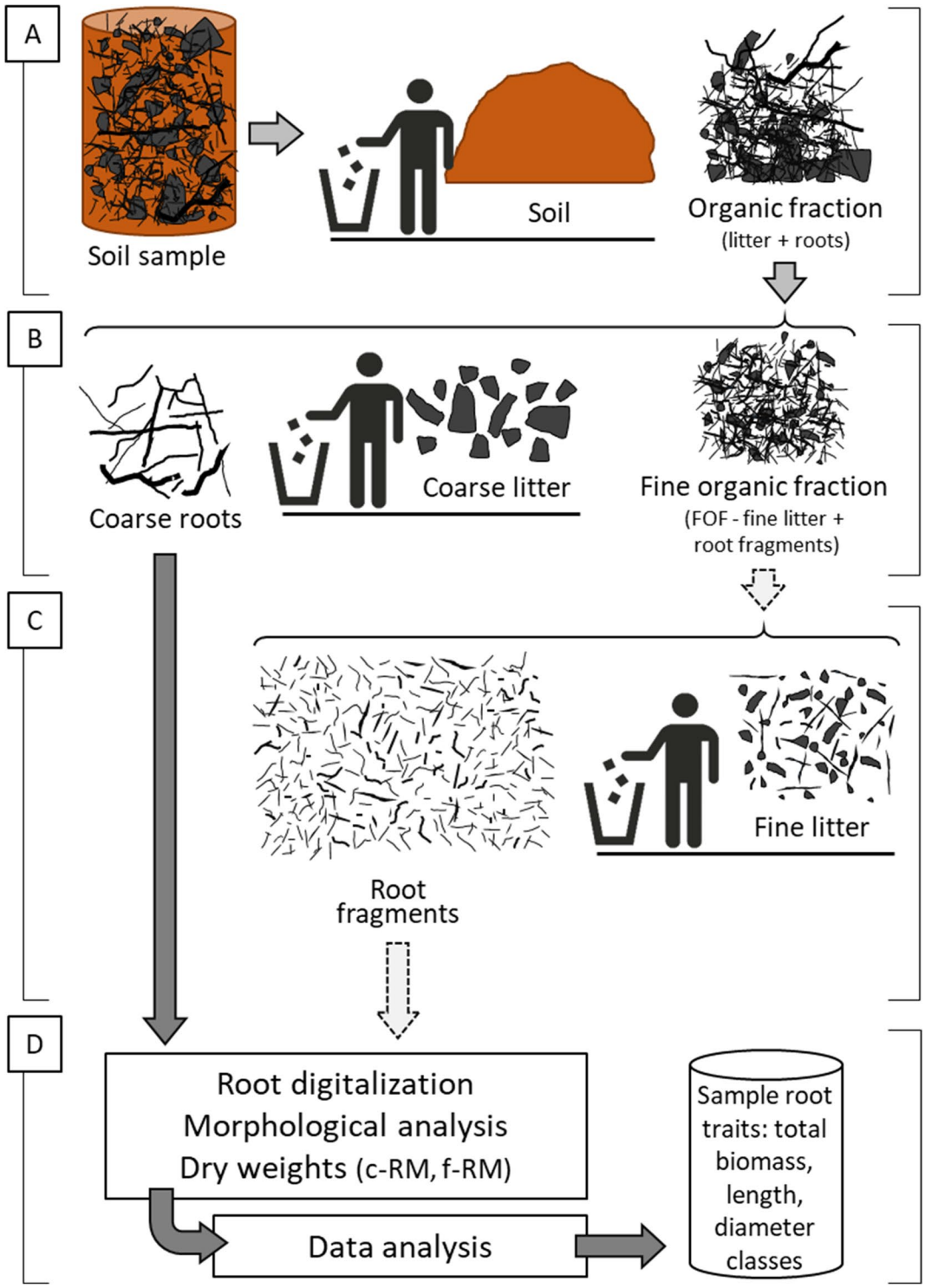

Fig. 1 Conventional approach (CA): root collection steps

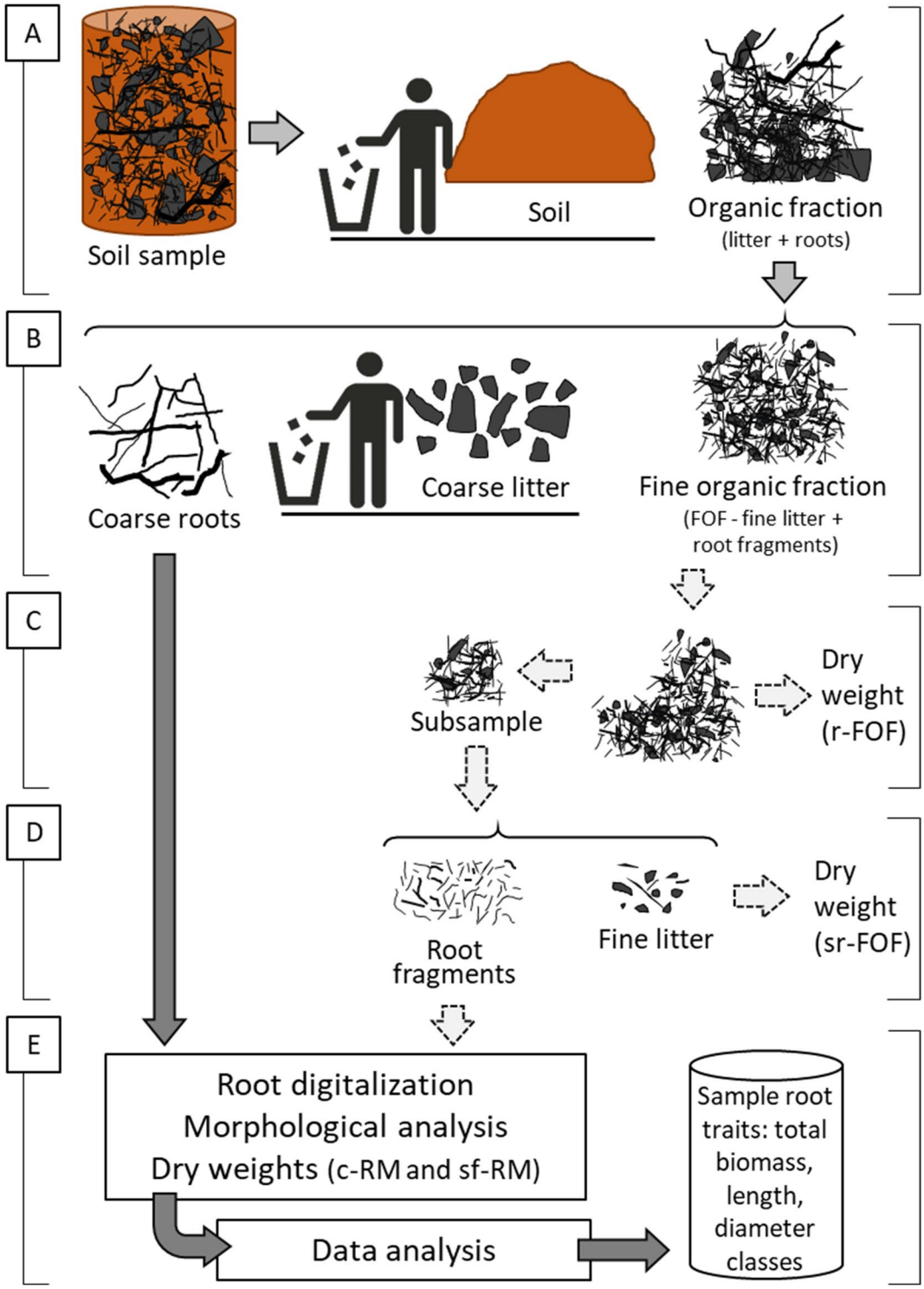

Fig. 2 Sub-sample approach (SA): root collection steps

In order to obtain the total root fragments traits (i.e. total root mass and length in the FOF), a multiplicative factor (c) was calculated to be applied to the sub-sample data, as follows:

$$c = (FOF\ dry\ mass)/(sub - sample\ dry\ mass)$$
$$= (r - FOF + sr - FOF + sf - RM)/(sr - FOF + sf - RM)$$

Then, c was used to calculate the root trait of all root fragments inside the FOF, as follows:

$$Total\ fragment\ root\ trait = c * sub - sample\ trait.$$

As example, Total fragment root mass = c * *sf-RM*. Similarly to biomass, the multiplicative factor was used to transform the root length data obtained from sub-sample roots analyzed with WinRhizo.

Statistical analysis

Root data were square-rooted or log-transformed to ensure normal distributions and equal variances to allow the use of parametric statistics. A one-way ANOVA was carried out to compare the timing across different sampling sites (oak forest, grassland, olive orchard) and root collection approaches (CA, SA). Subsequently, post hoc Bonferroni test was used to discern overall differences between data obtained with the two approaches or among sampling sites. Mann Whitney U test was applied to compare SA and CA root length data of the micrometric diameter increments. Analyses were applied to a 95% significance level. Statistical analysis was carried out using statistical software package SPSS 17.0 (SPSS Inc., Chicago, IL).

Results

Processing time

The sub-sample approach (SA) showed a substantial reduction in root collection time when compared to the conventional approach (CA), as illustrated in Fig. 3. In particular, the processing time of CA varied depending on the site: the grassland and the olive orchard showed much longer time than the oak forest (570 ± 93 min, 635 ± 67 min and 227 ± 14 min, respectively). Regarding SA, the grassland processing time (56 ± 5 min) was lower than the other two sites (oak forest 68 ± 2 min, olive orchard 74 ± 3 min) (Fig. 3). Table 1 shows the temporal requirements for the processing steps associated with each approach. The total fine root collection was the most time-demanding step of the CA, irrespective of the study site (81–85% of the total processing time), with the oak forest showing the lowest value compared to the other two sites. In the SA, the fine root collection time was lower for the olive orchard than the oak forest, while coarse root digitization took longer in the olive orchard than in the other two sites. Furthermore, both fine root collection time and fine root digitization time were significantly higher in the CA compared to the SA.

Quantitative root trait comparisons

Figure 4 provides a comparison of the quantitative root traits between CA and SA. In terms of total root biomass, length, and volume, the two approaches reveal similar results. However, the SA multiplicative factor shows a tendency to overestimate the CA data of all parameters by 8.8 ± 3.6% (mean ± standard deviation).

Biomass data showed higher mean values for the olive orchard (CA 560 ± 48 g m^{-2}; SA 598 ± 52 g m^{-2}) than the oak forest (CA 285 ± 24 g m^{-2}; SA 314 ± 25 g m^{-2}) and the grassland (CA 119 ± 29 g m^{-2}; SA 129 ± 30 g m^{-2}). In terms of length, the olive orchard showed higher mean values (CA 13.9 ± 1.3 km m^{-2}; SA 14.9 ± 1.4 km m^{-2}) than the grassland (CA 8.3 ± 1.3 km m^{-2}; SA 9.1 ± 1.4 km m^{-2}) and the oak forest (CA 2.2 ± 0.1 km m^{-2}; SA 2.4 ± 0.1 km m^{-2}). Finally, the volume showed higher mean values for the olive orchard (CA 2124 ± 185 cm^3 m^{-2}; SA 2271 ± 197 cm^3 m^{-2}) compared to the oak forest (CA 683 ± 51 cm^3 m^{-2}; SA 756 ± 57 cm^3 m^{-2}) and the grassland (CA 647 ± 168 cm^3 m^{-2}; SA 698 ± 174 cm^3 m^{-2}).

Qualitative root traits comparison

The distribution of the root length among the diameter classes provided qualitative insights into the root data. The comparison between CA and SA (Fig. 5) showed similar patterns in root length distributions, irrespective of the origin of the sample site. No significant differences were found.

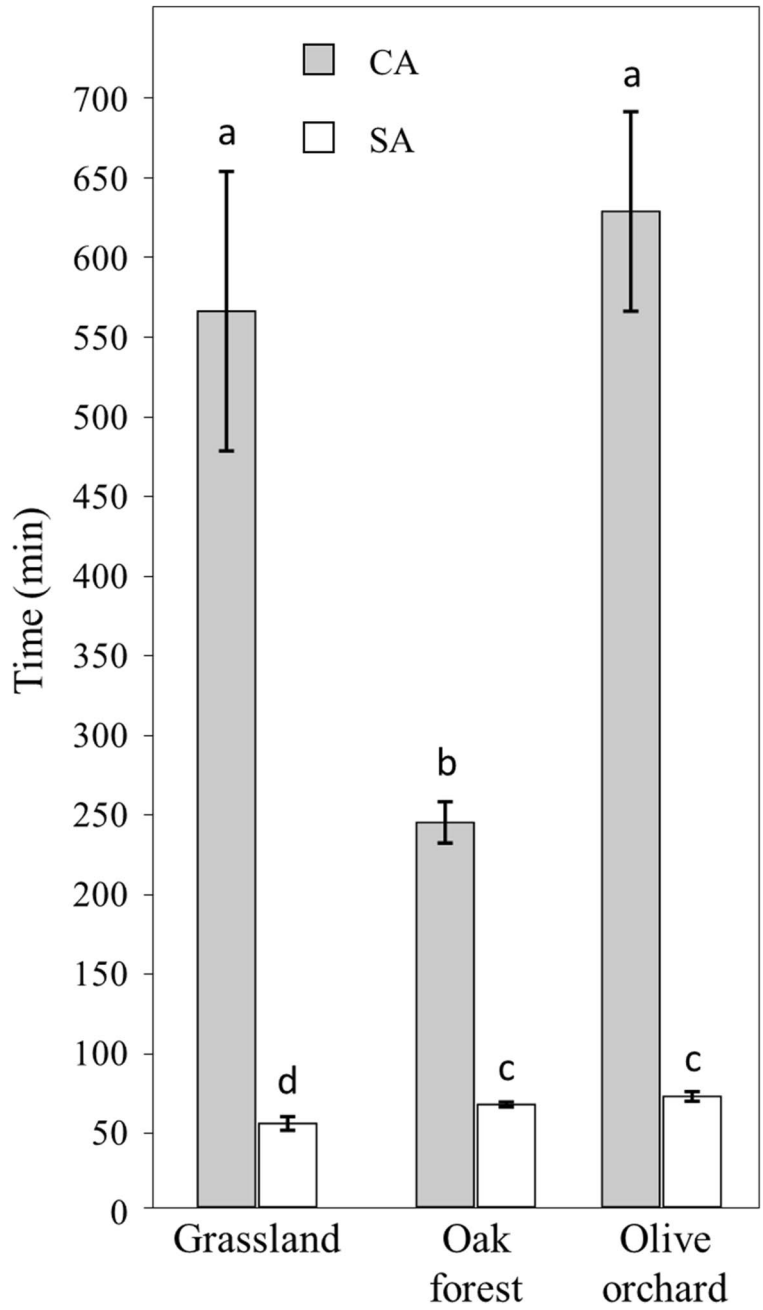

Fig. 3 Total sample processing time required by both methods for each study site. **a**-**d** indicate significant differences among sites and applied approach. Post hoc Bonferroni test ($p < 0.05$). Error bars are standard deviations

In the grassland site, more than 80% of the root length was observed in diameter classes ranging from 0 to 0.25 mm. In the same diameter range, the olive orchard and the oak forest showed respectively 19 and 38% of the total root length. In order to reach at least 80% of the total root length, for the oak forest and the olive orchard all roots with a diameter lower than 0.55 mm should be considered.

Total soil core root length and processing time

The relationship between the total root length and the processing time of each sample is illustrated in Fig. 6. Both methods showed an increase in the processing time related to the increase of the total root length. However, whereas for the CA the intra- and inter-site diversity entailed highly variable processing

Table 1 Processing time (min) required for each step of conventional (CA) or sub-sample (SA) approach

Study site	Method	Step				
		Sub-sample preparation	Fine root collection	Fine root digitalization	Coarse root collection	Coarse root digitalization
Grassland	CA	––	475.8 ± 78.3 ax	77.3 ± 13.5 ax	7.4 ± 0.6 a	9.2 ± 0.4 b
	SA	10.0 ± 0.3 a*	19.4 ± 3.0 aby	9.8 ± 1.7 ay		
Oak forest	CA	––	180.4 ± 11.7 bx	18.2 ± 1.2 bx	10.7 ± 0.7 a	10.8 ± 0.6 b
	SA	10.4 ± 0.4 a	24.6 ± 1.2 ay	12.1 ± 0.2 ay		
Olive orchard	CA	––	509.7 ± 56.9 ax	92.0 ± 9.4 ax	8.9 ± 0.7 a	25.1 ± 2.4 a
	SA	10.2 ± 0.3 a	18.3 ± 1.1 by	11.4 ± 0.2 ay		

Values are the mean of 20 measurements (± standard deviation). The sub-sample preparation step refers only to the SA. Coarse root collection and digitalization steps are the same for both methods

* a,b indicate significant difference among study site within the same method. x,y indicate significant difference between methods within the same study site. Post hoc Bonferroni test ($p < 0.05$)

time (from 2.6 to 27.6 h for one sample), for the SA differences in terms of tens of minutes were observed (from 37 to 112 min for one sample). Furthermore, even if a small amount of roots were found, SA still takes less time than CA. In addition, regardless the approach applied, when a similar amount of roots were detected, the oak forest showed higher processing time than the grassland and the olive orchard sites.

Discussion

The substantial time needed for the collection process of all roots from a single soil core sample is a well-known issue (Rodrigues de Sousa and Gehring 2010; Metcalfe et al. 2007; Persson and Ahlström 1990; Vogt and Persson 1991). The study aimed to introduce an innovative approach in fine root research to accelerate the labor-intensive process of selecting and processing fine roots from a soil sample. This time-saving method, named Sub-sample Approach (SA), succeeded in reducing fine-root collection time while maintaining similar results, in terms of qualitative and quantitative data, compared to the Conventional Approach (CA).

It is interesting to note that the process timing of each approach differed according to the specific ecological context. In particular, the olive orchard showed a very high amount of roots, despite displaying homogeneous root traits that facilitated root collection. The grassland not only displayed an abundance of roots, but also finer roots that were more difficult to separate as a result. On the other hand, the oak forest samples were characterized by a high amount of organic matter fragments making the root collection more challenging. This site diversity resulted in increased processing time when using the CA for both the grassland and the olive orchard sites. On the contrary, the presence of high organic matter in the oak forest or the abundance of coarse roots in the olive orchard affected the processing time when employing the SA method. However, SA maintained a significantly lower processing time when compared to CA, keeping the analysis to just a few tens of minutes instead of several hours. Thus, the SA method consistently resulted in a more stable processing time regardless of the number of roots in the sample, demonstrating its methodological efficacy across varying root quantities. However, it was observed a slight overestimation in the data obtained with SA, which is likely to be linked to the sub-sampling step. Therefore, for the method to be as representative and objective as possible, it is essential to adopt a standard area of the fine organic fraction sub-sample (1 cm^2). It is worth noting that the proposed method represents the culmination of a preliminary refinement process. This resulted in a reduction of overestimation from 31.3 ± 15.0% when calculating the multiplication factor using fresh weights, to 16.1 ± 5.4% when using dry weights but not paying sufficient attention to sub-sample extraction, thereby including some longer root fragments pulled out entirely from the fine organic fraction.

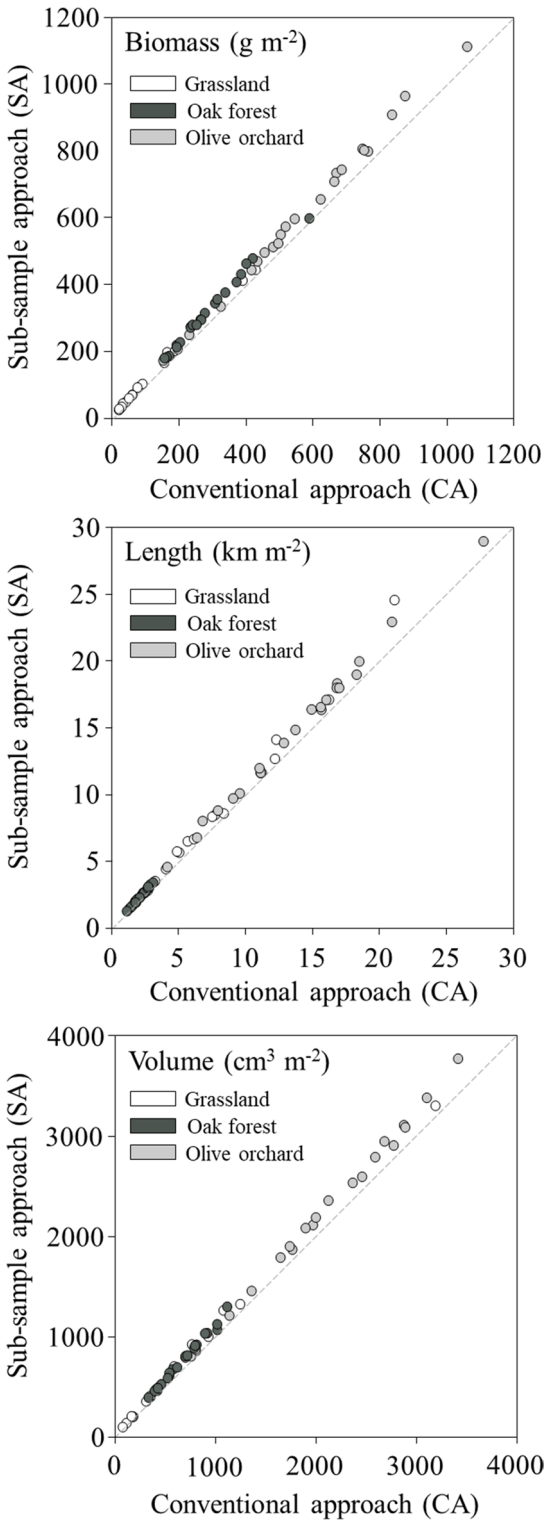

Fig. 4 Comparison of data obtained by the two methods. Different colors indicate each sample site. The dashed line represents the 1:1 line

Nevertheless, the error encountered (around 8.8%) was considered entirely acceptable, given the significant time saved and the significantly more detailed data that SA yields compared to alternative methods proposed in literature. Previous authors tried to reduce the collecting time of roots by applying temporal prediction methods directly in the field by collecting roots without removing soil particles (Metcalfe et al. 2007; Silva et al. 2022) or estimating root traits with image analysis without removing soil sample debris (mainly organic matter fragments, Benjamin and Nielsen 2004). These methods were shown to be effective in reducing the amount of time needed to estimate fine roots that are "bigger" (diameter > 0.3 mm), well visible, not fragmented and so easily to collect. However, the smallest and most fragmented roots may be highly underestimated by these methods. That was evident when samples were observed under the stereomicroscope. Indeed, regardless of the type of soil sample, numerous tiny root fragments (live or dead) within the fine organic fraction were found. The loss of this highly dynamic component of the root system, which is strictly related to plant growth behavior and environmental factors (Montagnoli et al. 2012; Saha et al. 2023), may compromise or weaken the successful outcome of any scientific study.

Regarding the root data obtained, our results were coherent with other published values. In particular, this study confirmed that grasslands typically exhibit higher root length compared to Mediterranean forest ecosystems (Montagnoli et al. 2019; Moreno et al. 2005; Kaneda et al. 2022). Moreover, similar values in terms of biomass and length were observed for the olive orchard (Searles et al. 2009; Soda et al. 2017). From a qualitative point of view, roots were classified into different classes according to their diameter, and the distribution of roots in these classes was analyzed. The diameter classes between 0 and 0.25 mm showed a predominance for the grassland roots in terms of length compared to both the oak forest and the olive orchard, meanwhile, larger roots were absent. Grasslands are mainly composed of annual herbaceous species with ephemeral root systems that do not undergo secondary growth. In contrast, in the olive orchard or in the oak forest, even when considering only fine roots (diameter < 2 mm), it is common to find bigger lignified roots (starting from diameters around 0.30 mm) due to the presence of shrubs and trees.

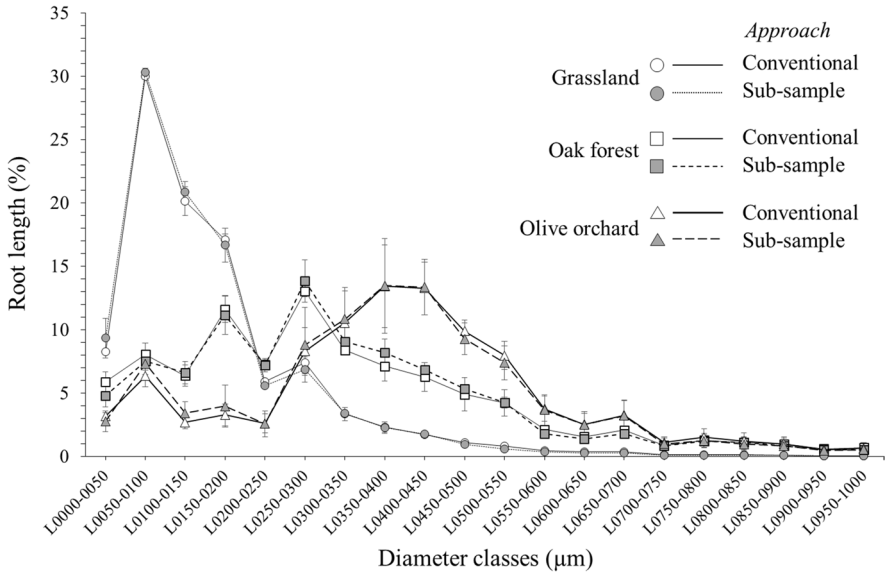

Fig. 5 Percentage of the total root length of both approaches tested for each micrometric diameter class analyzed. Different symbols indicate each sample site. Values are the mean of 20 samples ± standard deviation. No significant differences between CA and SA were detected (Mann Whitney U test, $p < 0.05$)

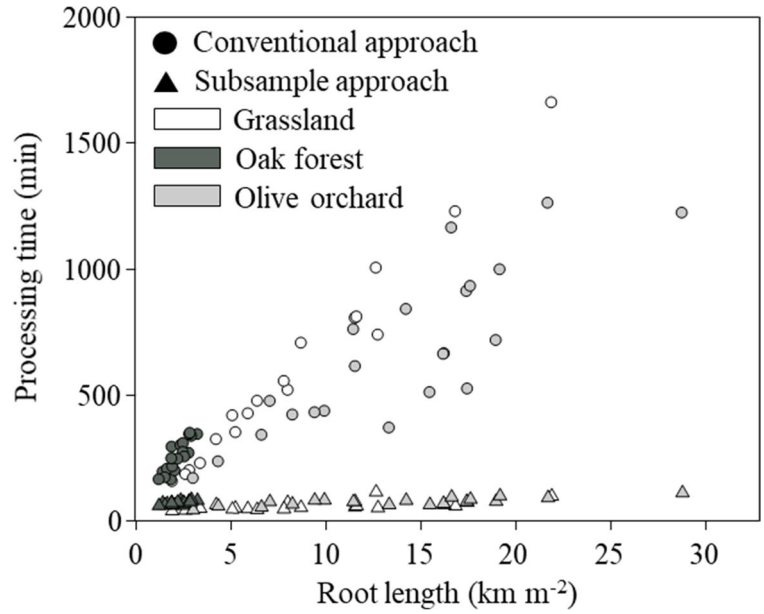

Fig. 6 Relationship between the total sample root length and the processing timing of each tested method. Colors indicate different sample sites, while dot shapes represent different methodological approaches

The proposed method may pose initial challenges, particularly for those newly engaging in root studies, as identifying root fragments can be tedious, difficult, and require initial training. For instance, many organic fibrous materials, e.g. common in grasslands, can easily be mistaken by an inexperienced eye, potentially leading to significant overestimations if not correctly identified. Moreover, extended periods spent at the stereomicroscope may become fatiguing. In addition, operators must exercise great care and attention throughout all steps to avoid losing root fragments or organic compounds during collection or weighing. Lastly, the method has been applied to a limited number of ecosystems sampled by soil coring, and future studies may provide further insights into its effectiveness. Although developed for roots originating from soil cores, we are confident that the method could be applicable in all cases where a substantial and homogeneous quantity of fine roots needs to be characterized, whether they come from pot

experiments, different substrates, or even hydroponic setups.

Conclusion

The method here proposed involves sub-sampling the organic matter remaining after washing a soil core and the collection of coarse roots. This is followed by collecting all root fragments in the sub-sample by using a stereomicroscope and leading subsequent calculations to estimate the total root traits (mass, length and length distribution among diameters) within the sampled soil core. The results obtained using the sub-sample method were compared with root traits determined through meticulous collection, once again under a stereomicroscope, of all roots included in the soil sample. Regardless of the magnitude of root traits within the soil core, the sub-sampling method consistently provided accurate estimates of the root population, both quantitatively and qualitatively. Moreover, this method required only tens of minutes to process each sample instead of several hours needed for a complete root collection. Similar results are expected for any kind of soil sample, even with higher root amounts than those tested, and additional studies will be needed to gain supplementary insights into the effectiveness of method. All of this will result in improvements in all types of fine root studies, increasing the accuracy of the methodology and drastically reducing the amount of time invested, thus giving rise to projects that potentially would have not been initiated due to the high investment of temporal resources. However, it is undeniable that the study of fine root and the appropriate skill acquisition necessarily require a period of training for the operator. The method we propose, also requires a willingness to work for long periods with a stereomicroscope. However, by drastically reducing and standardizing sample processing time, the SA method enables improved time management, thus diminishing potential discouragement.

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Author contributions Mattia Terzaghi conceived the research project, proposed the methodological approach and was responsible for the experimental process and data collection. Mattia Terzaghi and Raimondo Pardi dealt with soil core sampling, processing, and data arrangement. Mattia Terzaghi and Raimondo Pardi performed the data analysis and provided chart visualization. Raimondo Pardi wrote the manuscript draft. Maria Letizia Gargano, Cecilia Lasorella and Mattia Terzaghi dealt with the draft revision process. Maria Letizia Gargano, Cecilia Lasorella, Raimondo Pardi and Mattia Terzaghi finalized the manuscript. Maria Letizia Gargano act as supervisor of the manuscript. All authors contributed to the article and approved the submitted version.

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Declarations

Conflicts of interest The authors declare no conflict of interest.

Competing interests The authors have no relevant financial interests to disclose.

References

Addo-Danso SD, Prescott CE, Smith AR (2016) Methods for estimating root biomass and production in forest and woodland ecosystem carbon studies: a review. For Ecol Manag 359:332–351. https://doi.org/10.1016/j.foreco.2015.08.015

Benjamin JG, Nielsen DC (2004) A method to separate plant roots from soil and analyze root surface area. Plant Soil 267:225–234. https://doi.org/10.1007/s11104-005-4887-3

Berhongaray G, King JS, Janssens IA, Ceulemans R (2013) An optimized fine root sampling methodology balancing accuracy and time investment. Plant Soil 366:351–361. https://doi.org/10.1007/s11104-012-1438-6

Bernier PY, Robitaille G, Rioux D (2005) Estimating the mass density of fine roots of trees for minirhizotron-based estimates of productivity. Can J For Res 35:1708–1713. https://doi.org/10.1139/x05-09

Brunner I, Bakker M, Björk R et al (2013) Fine-root turnover rates of European forests revisited: an analysis of data from sequential coring and ingrowth cores. Plant Soil 362:357–372. https://doi.org/10.1007/s11104-012-1313-5

Cheng X, Jiang S, El-Naggar A, Tang Y, Liu X, Zhang J (2023) Dynamics of fine root decomposition in different vegetation types: investigating the impact of soil fungal communities and enzyme activities. Forests 14(7):1321. https://doi.org/10.3390/f14071321

Fahey TJ, Yanai RD, Gonzales KE, Lombardi JA (2017) Sampling and processing roots from rocky forest soils. Ecosphere 8:e01863. https://doi.org/10.1002/ecs2.1863

Freschet GT, Pagès L, Iversen CM et al (2021) A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. New Phytol 232(3):973–1122. https://doi.org/10.1111/nph.17572

Gargano ML, Di Gristina E, Domina G, Venturella G (2021) Trees and shrubs in the city of Bari (Italy). Fl Medit 31:23–30. https://doi.org/10.7320/FlMedit31.023

Janssens IA, Sampson DA, Curiel-Yuste J, Carrara A, Ceulemans R (2002) The carbon cost of fine root turnover in a Scots pine forest. For Ecol Manag 168(1–3):231–240. https://doi.org/10.1016/S0378-1127(01)00755-1

Kaneda S, Zedníková P, Frouz J (2022) Herbaceous and woody root biomass, seasonal changes in root turnover, and arbuscular mycorrhizal and ectomycorrhizal colonization during primary succession in post-mining sites. Diversity 14(8):644. https://doi.org/10.3390/d14080644

Kang Z, Lou G, Guo Y et al (2023) How does fine root influence aggregates? Comparing root diameter distribution of two herbaceous plants: a pot experiment. J Soil Sci Plant Nutr 23:6377–6391. https://doi.org/10.1007/s42729-023-01491-1

Kohler J, Caravaca F, Roldán A (2009) Effect of drought on the stability of rhizosphere soil aggregates of Lactuca sativa grown in a degraded soil inoculated with PGPR and AM fungi. Appl Soil Ecol 42(2):160–165. https://doi.org/10.1016/j.apsoil.2009.03.007

Li X, Zhu J, Lange H, Han S (2013) A modified ingrowth core method for measuring fine root production, mortality and decomposition in forests. Tree Physiol 33:18–25. https://doi.org/10.1093/treephys/tps124

Li J, Yuan X, Ge L et al (2020) Rhizosphere effects promote soil aggregate stability and associated organic carbon sequestration in rocky areas of desertification. Agric Ecosyst Environ 304:107126. https://doi.org/10.1016/j.agee.2020.107126

Liu B, He J, Zeng F, Lei J, Arndt SK (2016) Life span and structure of ephemeral root modules of different functional groups from a desert system. New Phytol 211:103–112. https://doi.org/10.1111/nph.13880

Mancuso S (2012) Measuring roots, an updated approach. Springer-Verlag, Dordrecht London, New York. https://doi.org/10.1007/978-3-642-22067-8

McCormack ML, Dickie IA, Eissenstat DM et al (2015) Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytol 207(3):505–518. https://doi.org/10.1111/nph.13363

McHale MR, McPherson EG, Burke IC (2007) The potential of urban tree plantings to be cost effective in carbon credit markets. Urban For Urban Green 6(1):49–60. https://doi.org/10.1016/j.ufug.2007.01.001

McGowan M, Armstrong MJ, Corrie JA (1983) A rapid fluorescent-dye technique for measuring root length. Exp Agric 19(3):209–216. https://doi.org/10.1017/S0014479700022730

Metcalfe DB, Williams M, Aragão LEOC et al (2007) A method for extracting plant roots from soil which facilitates rapid sample processing without compromising measurement accuracy. New Phytol 174(3):697–703. https://doi.org/10.1111/j.1469-8137.2007.02032.x

Montagnoli A, Terzaghi M, Di Iorio A, Scippa GS, Chiatante D (2012) Fine-root morphological and growth traits in a Turkey-oak stand in relation to seasonal changes in soil moisture in the Southern Apennines, Italy. Ecol Res 27:1015–1025. https://doi.org/10.1007/s11284-012-0981-1

Montagnoli A, Terzaghi M, Giussani B, Scippa GS, Chiatante D (2018) An integrated method for high-resolution definition of new diameter-based fine root sub-classes of Fagus sylvatica L. Ann For Sci 75:1–13. https://doi.org/10.1007/s13595-018-0758-y

Montagnoli A, Dumroese RK, Terzaghi M, Onelli E, Scippa GS, Chiatante D (2019) Seasonality of fine root dynamics and activity of root and shoot vascular cambium in a Quercus ilex L. forest (Italy). For Ecol Manag 431:26–34. https://doi.org/10.1016/j.foreco.2018.06.044

Montagnoli A, Baronti S, Alberto D, Chiatante D, Scippa GS, Terzaghi M (2021) Pioneer and fibrous root seasonal dynamics of Vitis vinifera L. are affected by biochar application to a low fertility soil: a rhizobox approach. Sci Total Environ 751:141455. https://doi.org/10.1016/j.scitotenv.2020.141455

Moreno G, Obrador JJ, Cubera E, Dupraz C (2005) Fine root distribution in Dehesas of Central-Western Spain. Plant Soil 277:153–162. https://doi.org/10.1007/s11104-005-6805-0

Pardi R, Venturella G, Domina G, Di Gristina E, Cirlincione F, Gargano ML (2023) Forest nurseries and the national recovery and resilience plan: the case of Sicily and Apulia (Italy). Italian Bot 15:49–63. https://doi.org/10.3897/italianbotanist.15.102133

Peng X, Tong X, Hao L, Wu F (2019) Applicability of biochar for limiting interrill erosion and organic carbon export of sloping cropland in a semi-arid area of China. Agric Ecosyst Environ 280:68–76. https://doi.org/10.1016/j.agee.2019.04.021

Pérès G, Cluzeau D, Menasseri S et al (2013) Mechanisms linking plant community properties to soil aggregate stability

in an experimental grassland diversity gradient. Plant Soil 373:285–299. https://doi.org/10.1007/s11104-013-1791-0

Persson H, Ahlström K (1990) The effects of forest liming on fertilization on fine-root growth. Water Air Soil Pollut 54:365–375. https://doi.org/10.1007/BF02385231

Polverigiani S, McCormack ML, Mueller CW, Eissenstat DM (2011) Growth and physiology of olive pioneer and fibrous roots exposed to soil moisture deficits. Tree Physiol 31(11):1228–1237. https://doi.org/10.1093/treephys/tpr110

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol 193(1):30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x

Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL (2002) Fine root architecture of nine North American trees. Ecol Monogr 72(2):293–309. https://doi.org/10.2307/3100029

Rahman G, Sohag H, Chowdhury R, Wahid KA, Dinh A, Arcand M, Vail S (2020) SoilCam: a fully automated minirhizotron using multispectral imaging for root activity monitoring. Sensors 20(3):787. https://doi.org/10.3390/s20030787

Rodrigues de Sousa JT, Gehring C (2010) Adequacy of contrasting sampling methods for root mass quantification in a slash-and-burn agroecosystem in the eastern periphery of Amazonia. Biol Fertil Soils 46:851–859. https://doi.org/10.1007/s00374-010-0490-8

Saha S, Huang L, Khoso MA, Wu H, Poudel TR, Li B et al (2023) Fine root decomposition in forest ecosystems: an ecological perspective. Front Plant Sci 14:1277510. https://doi.org/10.3389/fpls.2023.1277510

Sanesi G, Colangelo G, Lafortezza R, Calvo E, Davies C (2018) Urban green infrastructure and urban forests: a case study of the Metropolitan area of Milan. Landsc Res 42(2):164–175. https://doi.org/10.1080/01426397.2016.1173658

Schroth G, Kolbe D (1994) A method of processing soil core samples for root studies by subsampling. Biol Fertil Soils 18:60–62. https://doi.org/10.1007/BF00336446

Searles PS, Saravia DA, Rousseaux MC (2009) Root length density and soil water distribution in drip-irrigated olive orchards in Argentina under arid conditions. Crop Pasture Sci 60(3):280–288. https://doi.org/10.1071/CP08135

Silva CA, Londe V, D'Angioli AM et al (2022) Testing collection-time reduction in fine-root biomass estimation in Atlantic forests. Appl Veg Sci 25(1):e12638. https://doi.org/10.1111/avsc.12638

Soda N, Ephrath JE, Dag A, Beiersdorf I, Presnov E, Yermiyahu U, Ben-Gal A (2017) Root growth dynamics of olive (Olea europaea L.) affected by irrigation induced salinity. Plant Soil 411:305–318. https://doi.org/10.1007/s11104-016-3032-9

Stokes A, Atger C, Bengough AG, Fourcaud T, Sidle RC (2009) Desirable Plant root traits for protecting natural and engineered slopes against landslides. Plant Soil 324:1–30. https://doi.org/10.1007/s11104-009-0159-y

Sun L, He L, Wang G, Jing H, Liu G (2019) Natural vegetation restoration of Liaodong oak (Quercus liaotungensis Koidz.) forests rapidly increased the content and ratio of inert carbon in soil macroaggregates. J Arid Land 11:928–938. https://doi.org/10.1007/s40333-019-0004-y

Tan J, Yu W, Liu Y et al (2023) Response of fine-root traits of Populus tomentosa to drought in shallow and deep soil. Forests 14(5):951. https://doi.org/10.3390/f14050951

Vogt K, Persson H (1991) Measuring growth and development of roots. In: Lassoie JP, Hinckley TM (eds) Techniques and approaches in forest tree ecophysiology. CRC Press, Boca Raton, pp 477–502

Wang B, Zhang GH, Yang YF, Li PP, Liu JX (2018) Response of soil detachment capacity to plant root and soil properties in typical grasslands on the Loess Plateau. Agric Ecosyst Environ 266:68–75. https://doi.org/10.1016/j.agee.2018.07.016

Wang H, Zhu X, Zakari S, Chen C, Liu W, Jiang X-J (2022) Assessing the effects of plant roots on soil water infiltration using dyes and Hydrus-1D. Forests 13(7):1095. https://doi.org/10.3390/f13071095

Zadworny M, Eissenstat DM (2011) Contrasting the morphology, anatomy and fungal colonization of new pioneer and fibrous roots. New Phytol 190(1):213–221. https://doi.org/10.1111/j.1469-8137.2010.03598.x

Zhao D, Xu M, Liu G, Ma L, Zhang S, Xiao T, Peng G (2017) Effect of vegetation type on microstructure of soil aggregates on the Loess Plateau, China. Agric Ecosyst Environ 242:1–8. https://doi.org/10.1016/j.agee.2017.03.014