



Root estimation accuracy and sampling representativeness in relation to sample size in a subtropical evergreen broad-leaved forest: comparison between soil core and minirhizotron method

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Received: 9 April 2021 / Accepted: 2 September 2021 / Published online: 17 September 2021
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Abstract

Optimal sampling strategy is vital in root studies because of the high degree of variability found in the belowground ecosystems. To inform the appropriate sample size, ninety-six cores and minirhizotrons in a subtropical evergreen broadleaved forest were used to examine diameter-related variation in the distribution of root length and volume, and assess the effects of sample size on estimation accuracy and sample representativeness. The variation coefficients of root traits estimated by the minirhizotron method were much higher than those estimated by the soil core method. Furthermore, minirhizotrons observed higher proportions of root length and volume in small diameter classes, reflecting thinner roots than those estimated by the soil core method. The Monte Carlo simulation analysis shows that, for the 0–5 mm diameter class, under a confidence level of 90% and estimation accuracy of 80%, the sample size for soil cores required to estimate the root length and volume are 5 and 12, respectively, and for minirhizotrons being 54 and over 96, respectively. The cumulative root diameter class curve analysis shows that the sample sizes required to encounter 80%, 90% and 95% of all diameter classes for soil cores are 3, 6 and 10, respectively, and for minirhizotrons being 38, 61 and 77, respectively. We conclude that due to the fractal distribution of root diameter, and the difference in sampling volume between different methods, larger sample sizes are required for minirhizotrons or larger-diameter roots than for soil cores or smaller-diameter roots to achieve better estimation accuracy and sampling representativeness.

Keywords Distribution variation · Minirhizotron · Roots · Sample representativeness · Sample size · Soil core

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Introduction

Roots take up water and nutrients, anchor plant above-ground parts, consume photosynthetic products through turnover and respiration, and continuously transfer organic matter into the soil (Wang and Guo 2008; Dumroese et al. 2019; Montagnoli et al. 2020). These different functions are well reflected by root thickness. Generally, fine roots (e.g., < 2 mm in diameter) are the most active part of the root system mainly responsible for the acquisition of soil resources (Nadelhoffer 2000), with a short lifespan, a fast turnover rate and a low cost: benefit ratio (Montagnoli et al. 2021); while coarse roots (e.g., > 2 mm in diameter) develop a large root extension in soils mainly responsible for transportation, storage and plant anchorage, with a longer lifespan and secondary growth (Guo et al. 2008; McCormack et al. 2015; Dumroese et al. 2019; Montagnoli et al. 2020, 2021). Root turnover, especially those of fine roots, represent 22% of terrestrial net primary productivity (NPP) globally (McCormack et al. 2015), and is the major soil carbon (C) source (Adamczyk et al. 2019; Mayer et al. 2020). Thus, the accuracy in estimating root turnover is crucial to understand how terrestrial ecosystems respond to global climate change (Norby and Jackson 2000), which has long been a concern in terrestrial ecosystem studies (Guo et al. 2008).

The determination of an appropriate root sample size is a prerequisite for an accurate estimation of root turnover. Metcalfe et al. (2008) stressed that roots exhibit high variation at both large and small scales. Such variation reduces the predictability of root distributions and increases the difficulty of selecting sampling location, which together with non-normal distribution of root diameters (Tatsumi et al. 1989) make root studies prone to errors caused by under-sampling (Casper and Jackson 1997). Moreover, the branching process of root growth also results in fractal distribution of root diameter, and the physiological and ecological functions of roots with different diameters are different, which renders small-diameter roots more common and large ones relatively rare (Tatsumi et al. 1989; Eshel 1998; Halley et al. 2004; Mao et al. 2015; Montagnoli et al. 2021). Thus, if sample size or volume is insufficient, it may result in poor representation of the root pool. For example, a small sample volume may possibly omit larger diameter roots, resulting in measurements such as average diameter, root length, and root volume to be artifacts of the sample size. Therefore, a reliable assessment of root sample size necessitates a comprehensive consideration of both spatial variation of root distribution and fractal distribution of root diameter. Theoretically, accuracy of estimation increases with sample size (Bengough et al. 2000), which is accompanied by an increase in cost (labor and funding) (Metcalfe et al. 2007). As a result, researchers often determine a sampling plan based on the limitations of time and funding, which often leads to a small sample size and makes it impossible to guarantee root estimation accuracy and sampling representativeness (Pierret et al. 2005).

Optimal root sampling strategies have been explored by several studies on the basis of cost effect analysis in terms of method, sample size, and volume, which are mostly conducted in temperate and tropical forests (Metcalfe et al. 2008; Berhongaray et al. 2013; Garten et al. 2007). To determine an appropriate sample size for forests with high degree of variation in root distribution, the Monte Carlo method was introduced, and was particularly efficient at estimating non-normal root distribution (Xiong et al. 2016). Species accumulation curves, which are generally used to demonstrate the effect of sample size on species diversity (Maughan et al. 2012), can also be utilized to test the effect of sample size on root sample representativeness (e.g., Taylor et al. 2013). However, although the variation in root spatial distribution has been taken into account in previous studies on optimal sampling

strategy, the fractal distribution of root diameter has rarely been assessed. Moreover, few studies have been conducted in humid subtropical forests, where there is a complex forest structure with high species diversity and highly variable root distribution.

Soil coring has long been considered to be reliable in measuring root biomass, although it is a destructive, labor intensive method, and being inaccurate in assessing root mortality and decomposition. In contrast, the minirhizotron is an in situ, non-destructive method known to be effective in evaluating root growth and lifespan (Hendricks et al. 2006). Data such as root length and diameter observed in minirhizotrons can be converted to root biomass and production by developing correlations with soil coring data (Majdi et al. 2005). However, with an order of magnitude difference in individual sample volume between the soil core and minirhizotron methods (Taylor et al. 2013), there may exist discrepancies in the estimation accuracy and sample representativeness between these two methods, which has not been reported yet, so far.

In this study, we utilized data from 96 cores and minirhizotrons obtained during the pre-treatment of a large-scale nitrogen deposition experiment in a subtropical evergreen broad-leaved forest (Fan et al. 2019), to explore the optimal sampling strategies both for soil core and minirhizotron methods. Since root length relates to root functions such as nutrient and water absorption abilities (Ostonen et al. 2011; Kou et al. 2015) and root volume can be used to calculate root biomass, both of which are pivotal in root studies, we will focus on these two root traits in this study. Our aims were to: (1) reveal the variability and the diameter-related distribution pattern of root length and root volume density estimated by the soil core and minirhizotron method; and (2) explore the estimation accuracy and sample representativeness of these two root traits in relation to sampling size for both soil core and minirhizotron methods. We hypothesize that: (1) the variability of root traits measured by minirhizotron are higher than by soil core method; (2) the roots observed by minirhizotron are thinner than by soil core method; and (3) the sample size required is smaller for the soil core method or smaller-diameter (0–2 mm) roots than for the minirhizotron method or larger-diameter (2–5 mm) roots, to meet the requirements of estimation accuracy and sample representativeness.

Materials and methods

Site description

The study site is located in the Gesikao Nature Reserve (26°11'N, 117°28'E), Sanming City, Fujian Province, China. This region has a typical subtropical monsoon climate, with an average annual temperature of 19.5 °C and an average annual precipitation of 1700 mm (mainly from March to August). This experiment was conducted in a natural evergreen broad-leaved forest, which has been undisturbed for more than 200 years, and has a stem density of 1955 stems ha⁻¹, canopy coverage of 89%, mean tree height of 11.9 m, and mean tree diameter at breast height of 20 cm (Fan et al. 2019). The soil is formed from sandstone and classified as red soil according to the Chinese soil classification (Oxisols in USDA Soil Taxonomy). The soil texture is sandy clay, with 66.6% of sand (0.05–2 mm), 18.2% of silt (0.002–0.05 mm) and 15.1% of clay (<0.002 mm) in the 0–10 cm soil layer. At this depth, soil pH was 3.98, and the concentration of total organic C, total nitrogen and total phosphorus was 38.2, 1.34, and 0.48 g kg⁻¹, respectively (Guo et al. 2016). The dominant overstory species are *Castanopsis carlesii*, *Pinus massoniana*, and *Schima superba*,

while the understory vegetation mainly comprise of shrubs, herbs, and lianas such as *Clauseria excavate*, *Ilex cornuta*, *Tricalysia dubia*, *Adiantum capillus*, *Sarcandra glabra*, *Smilax china*, *Millettia dielsiana*, *Alyxia fascicularis*, and *Schisandra chinensis*.

Soil coring and minirhizotron installation

In July 2011, twelve 20 m×20 m plots were set up along the same northeast-facing slope with intervals of approximately 10 m to implement a large-scale nitrogen addition experiment in the above natural forest (Fan et al. 2019). For the convenience of minirhizotron installation, 8 sampling locations were randomly selected for soil coring in each 20 m×20 m plot (96 in total). At each sampling location, one soil core of 0–40 cm (vertical depth, converted by a 45° angle) was collected at an angle of 45° using a homemade cylindrical steel corer with an inner diameter of 4.05 cm, an outer diameter of 5.2 cm, and a length of 100 cm. These samplings were done by consecutively coring the soil to the depths of 0–10 cm, 10–20 cm and 20–40 cm to minimize soil shrinking. The surface organic horizons were removed before collection. The cores were separately placed in plastic bags, labeled, and taken to the laboratory. Following soil core collection, 96 transparent cylindrical tubes (minirhizotrons) made of PVC (polyvinyl chloride) materials (Bartz Technology, Carpinteria, CA, USA) were installed into the holes caused by soil cores, with an inner diameter of 5 cm, a length of 90 cm, and an installation angle of 45° (to ensure that most roots were captured). The vertical depth of the tubes in the soil was 40 cm, and the exposed parts of the tubes above the ground were wrapped with black tape, and the orifices were covered with special sleeves to prevent light, rain, litter, and small animals from entering the tubes.

Analysis of roots in soil cores

The soil cores were washed in running water with soil sieves of 0.5 mm pore size until they were cleaned. The living and dead roots were distinguished according to their appearance, color, elasticity, and the difficulty of separating the epidermis from the steles (McClougherty et al. 1982); dead roots were discarded. The living roots were divided into two diameter classes of 0–2 mm and 2–5 mm and then scanned with an Epson scanner (Expression 10000XL110, resolution 400 dpi). Root morphology was analyzed using WinRHIZO Pro2005b software (Regent Instruments Inc., Quebec, QC, Canada). Both length and volume of roots < 5 mm in diameter were grouped into 100 diameter bins at intervals of 0.05 mm. Roots of different tree species were pooled in this experiment because of the difficulty of identification. The calculation formulas for the root indices are as follows:

$$\text{Root length density (mm cm}^{-3}\text{)} = \text{total root length (mm)}/\text{soil core volume (cm}^3, \pi R^2 \times L\text{)}$$

$$\text{Root volume density (mm}^3 \text{cm}^{-3}\text{)} = \text{total root volume (mm}^3\text{)}/\text{soil core volume (cm}^3, \pi R^2 \times L\text{)}$$

where R (cm) and L (cm) denote the inner diameter and total length of the soil core, respectively.

Minirhizotron analysis

Image collection of minirhizotron was started in March 2012 and the image data obtained in July 2012 were utilized, one year just after minirhizotron installation when it is assumed the regrowth of roots had reached equilibrium (Joslin and Wolfe 1999; Strand et al. 2008). While nitrogen deposition experiment was just commenced in October 2012. A BTC-100 camera (Bartz Technology, Carpinteria, CA, USA) was used for image collection at the end of each month. The actual size of the observation image was 18 mm×13.5 mm, and about 40 images were collected for each tube (about 3840 images in total per month). The collected images were processed by using Rootfly, a root image analysis software, to obtain data such as the root diameter and root length. As done for the soil core data, the observed roots (<5 mm) from minirhizotron at the vertical depth of 0–40 cm were divided into 100 diameter bins at intervals of 0.05 mm. Only living roots were studied (dead roots were defined as blackened and shrunken roots). The formulas for calculating the root indices of minirhizotrons are as follows:

$$\text{Root length density (mm cm}^{-2}\text{)} = \text{total root length (mm)}/A(\text{cm}^2)$$

$$\text{Root volume density (mm}^3\text{ cm}^{-2}\text{)} = \text{total root volume (mm}^3\text{)}/A(\text{cm}^2)$$

where A denotes the observation window area.

Statistical analysis

In this study, root length and volume density observed by the soil core and minirhizotron method were analyzed using descriptive statistics for the maximum, minimum, median, mean, standard deviation, coefficient of variation (CV), skewness, and kurtosis for different diameter classes. Root traits were considered to have weak variability with $CV \leq 10\%$, medium variability with $10\% < CV < 100\%$, and strong variability with $CV \geq 100\%$ (Xiong et al. 2016). The Shapiro–Wilk test was used to test normal distribution at a significance level of 0.05. The Monte Carlo method was used to detect changes in estimation accuracy with increasing sample size at different probability levels. During each evaluation of a given sample size, 1000 random samplings were conducted with replacement from the samples that were composed of 96 soil cores or 96 minirhizotron tubes; the mean value of the 1000 samples was calculated, and the probability that the mean value fell into the specific accuracy requirement was counted. The function `specaccum` in the `vegan` package was used to construct the cumulative root diameter class curves with increasing sample size for both soil cores and minirhizotrons. The R 4.0.5 software program was used to program the entire calculation process.

Results

Root distribution variation

The distribution characteristics of both root length density and root volume density varied with diameter class (Table 1). For both the soil core or minirhizotron method, the mean values of root length and root volume density decreased with the increase of root diameter,

and the Shapiro–Wilk test indicated right-skewed distribution for the root length density and volume density of all diameter classes (Table 1, Figs. 1 and 2).

The CV values of root traits measured by the minirhizotron method were greater than those measured by the soil core method. For example, the CV values of root length density measured by soil core ranged from 28.2 to 69.1% across diameter classes, while those measured by minirhizotron ranged from 90.3 to 345.9%. For root volume density measured by soil core, the CV values ranged from 32.9 to 81.0% across diameter classes, while those measured by minirhizotron ranged from 154.5 to 382.7%. In addition, for both root traits measured by both methods, the CV values were the smallest in 0–2 mm diameter class, belonging to medium variability, while the CV values measured by minirhizotron exceed 300% for diameter classes of 2–5 mm, which were 4.0–5.0 times higher than those measured by the soil core, indicating high variability.

Diameter distribution in root length and volume

There was a mismatch between root length and volume observed by the soil core and minirhizotron method. The proportion of total root length in the finest diameters observed by the minirhizotron method was larger than that obtained using the soil core method (Fig. 3a). For the soil core method, 20.2% of the total root length was contributed by roots ≤ 0.5 mm diameter class, 70.2% in roots ≤ 1 mm diameter class, 94.0% in roots ≤ 2 mm diameter class, and 6.0% in roots > 2 mm diameter class. In contrast, for the minirhizotron method, 80.3% of the total root length was contributed by roots ≤ 0.5 mm diameter class, 95.0% in roots ≤ 1 mm diameter class, 99.2% in roots ≤ 2 mm diameter class, and less than 1% in roots > 2 mm diameter class.

The proportion of total root volume in the finest roots observed by the minirhizotron method was also larger than that by the soil core method (Fig. 3b). For the soil core method, 1.4% of the total root volume was contributed by roots ≤ 0.5 mm in diameter class, 21.5% in roots ≤ 1 mm diameter class, 55.3% in roots ≤ 2 mm diameter class, and 44.7% in roots > 2 mm diameter class. In contrast, for the minirhizotron method, 23.7% of the total root volume was contributed by roots ≤ 0.5 mm diameter class, 51.2% in roots ≤ 1 mm diameter class, 78.3% in roots ≤ 2 mm diameter class, and 21.7% in roots > 2 mm diameter class.

Estimation accuracy in relation to sample size

The probability value with each root trait fell into the specific estimation accuracy (e.g., 80%, 90%, 95%) increased with increasing sample size, while decreased with increasing estimation accuracy under the same sample size (Fig. 4). In addition, under the same sample size and estimation accuracy, the probability values of both root length and volume density obtained by the soil core method were higher than those obtained by the minirhizotron method. The probability values of root length density obtained by either method were higher than those of root volume density. In other words, under the same confidence level and estimation accuracy, the sample size required to accurately estimate the root length density was smaller than that required to estimate the root volume density, both for the soil core and minirhizotron method.

For the 0–5 mm diameter class, under 0.9 probability (i.e., 90% confidence level) and 80% estimation accuracy, the sample sizes required to measure the root length density and volume density by the soil core method were 5 and 12, respectively, while 54

Table 1 Statistical characteristics of roots with different diameter classes observed by the soil core and minirhizotron method (n = 96) in the soil vertical depth of 0–40 cm

Root trait	Diameter class (mm)	Method	Maximum	Minimum	Median	Mean	SD ^a	CV ^b (%)	Skewness	Kurtosis	p ^c	Distribution
Root length density	0–5	Soil core (mm cm ⁻³)	8.58	2.08	4.26	4.31	1.17	27.3	0.61	0.84	0.031	Right-skewed
		Minirhizotron (mm cm ⁻²)	7.99	0.00	1.37	1.94	1.75	90.0	1.51	2.21	<0.001	Right-skewed
	0–2	Soil core (mm cm ⁻³)	8.39	1.98	4.06	4.09	1.15	28.2	0.66	1.05	0.017	Right-skewed
Root volume density	2–5	Minirhizotron (mm cm ⁻²)	7.99	0.00	1.37	1.92	1.74	90.3	1.49	2.09	<0.001	Right-skewed
		Soil core (mm cm ⁻³)	0.66	0.00	0.20	0.21	0.15	69.1	0.55	0.00	0.008	Right-skewed
	0–5	Minirhizotron (mm ³ cm ⁻³)	12.24	1.26	4.39	4.71	1.97	41.8	0.98	11.38	<0.001	Right-skewed
Root volume density	0–2	Soil core (mm ³ cm ⁻²)	3.55	0.00	0.17	0.39	0.63	161.1	3.23	11.62	<0.001	Right-skewed
		Minirhizotron (mm ³ cm ⁻³)	6.25	0.93	2.92	2.87	0.94	32.9	0.69	1.39	0.006	Right-skewed
	2–5	Soil core (mm ³ cm ⁻³)	8.19	0.00	1.70	1.84	1.49	81.0	1.21	2.59	<0.001	Right-skewed
		Minirhizotron (mm ³ cm ⁻²)	2.29	0.00	0.00	0.08	0.32	382.7	4.69	25.18	<0.001	Right-skewed

^aSD, standard deviation; ^bCV, coefficient of variation; ^cp value for test of normal distribution by Shapiro–Wilk test

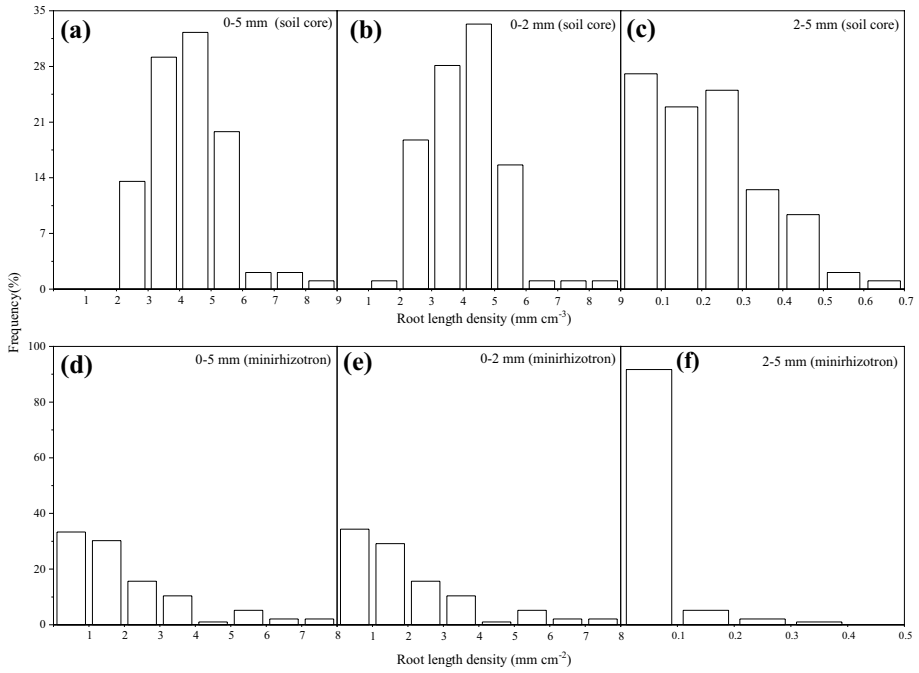


Fig. 1 Frequency distribution of root length density of root diameter classes with 0–5, 0–2 and 2–5 mm for **a–c** soil cores and **d–f** minirhizotrons

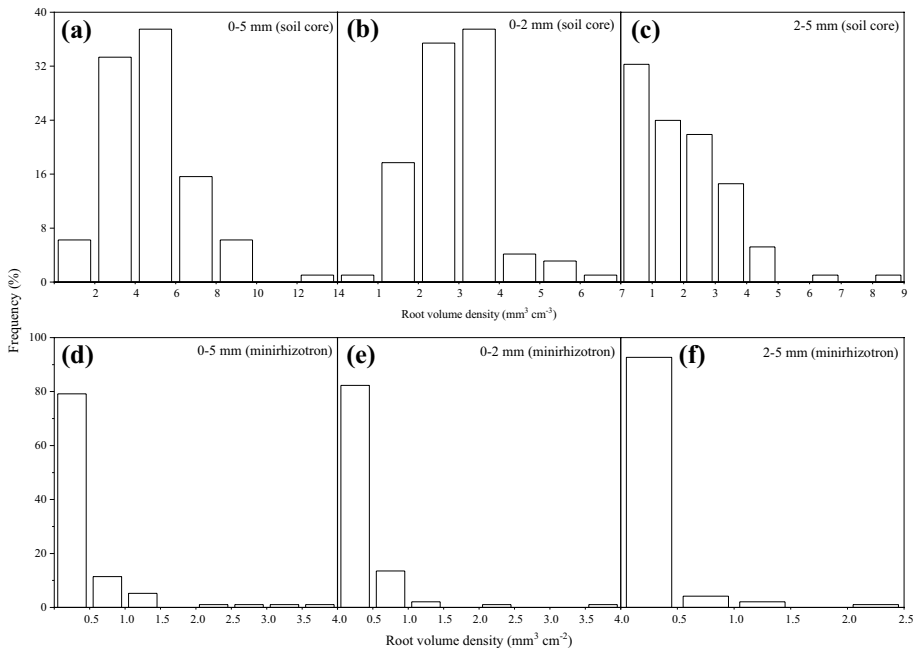


Fig. 2 Frequency distribution of root volume density of root diameter classes with 0–5, 0–2 and 2–5 mm for **a–c** soil cores and **d–f** minirhizotrons

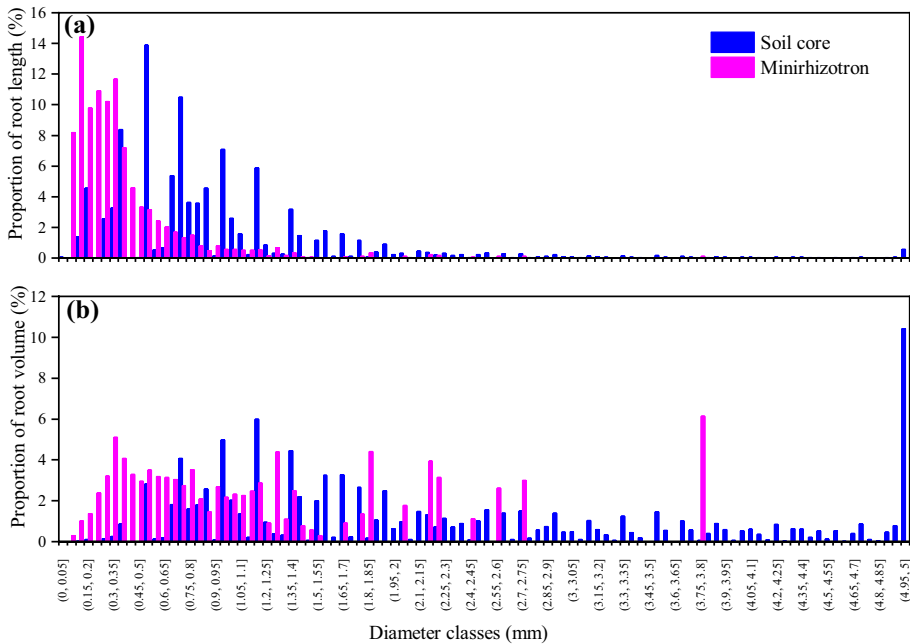


Fig. 3 The proportion of **a** root length and **b** root volume contained in diameter classes encountered for soil cores and minirhizotrons. The diameter classes increase in 0.05 mm increments up to 5 mm

and > 96 were required, respectively, by the minirhizotron method (Fig. 4a). Under a 90% confidence level and 90% estimation accuracy, the sample sizes required to measure the root length density and volume density by the soil core method were 20 and 45, respectively, whereas 96 minirhizotrons could not meet the corresponding requirement (Fig. 4b). Under a 90% confidence level and 95% estimation accuracy, the sample size required to measure the root length density by the soil core method was 78, while 96 minirhizotrons failed also to meet the corresponding requirements (Fig. 4c).

When roots were further divided into the 0–2 mm and 2–5 mm diameter classes, the results were similar in order to meet a specific accuracy: larger sample size is needed for the minirhizotron method than for the soil core method, and for measuring root volume density than for measuring root length density (Fig. 4d–i). However, the sample size needed for measuring any root trait of the 2–5 mm diameter class is much larger than for measuring that of the 0–2 mm diameter class, given the same method and the accuracy requirement (Fig. 4d–i).

Sampling representativeness in relation to sample size

The cumulative curves of the root diameter class showed an asymptotic pattern with the increase in sample size for both methods, but flatten with much smaller sample size for the soil core method than for the minirhizotron method (Fig. 5). For the 0–5 mm diameter class, the sample sizes required to encounter 80%, 90% and 95% of diameter classes for soil cores were 3, 6 and 10, respectively, whereas 38, 61 and 77 were required for minirhizotrons (Fig. 5a). For the 0–2 mm diameter class, the sample sizes required to encounter 80%, 90%

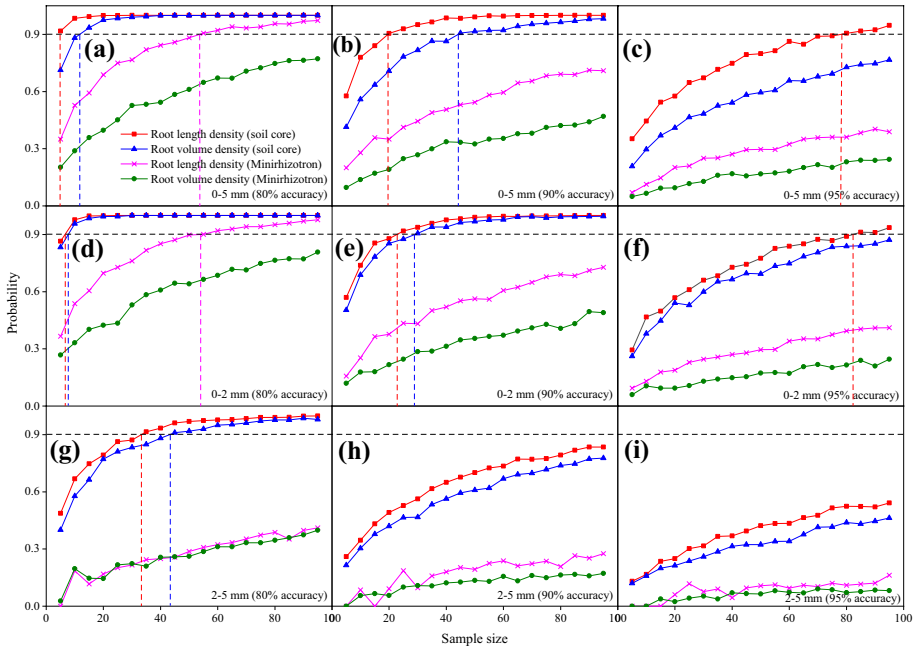


Fig. 4 The relationship between probabilities and sample sizes of soil cores and minirhizotrons of **a–c** 0–5 mm, **d–f** 0–2 mm and **e–i** 2–5 mm root diameter classes with 80%, 90% and 95% accuracy under Monte Carlo simulations. The black horizontal dashed lines represent 90% probability, while the red, blue, and magenta vertical lines represent the sample sizes required to measure root length density (soil core), root volume density (soil core), and root length density (minirhizotron), respectively, for a given accuracy at 90% probability

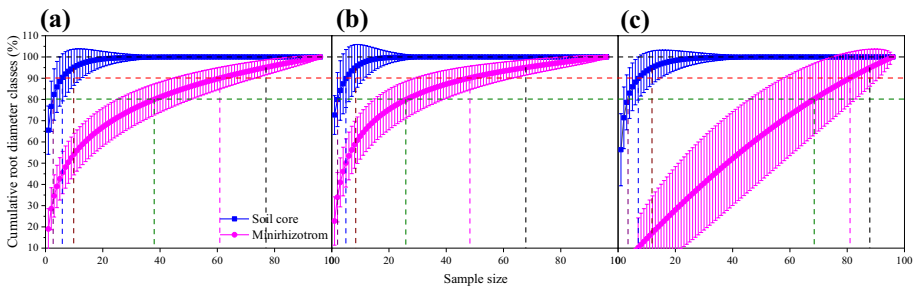


Fig. 5 The cumulative root diameter class curves of **a** 0–5 mm, **b** 0–2 mm and **c** 2–5 mm observed by the soil core and minirhizotron methods. The olive, red, and black horizontal dashed lines represent 80%, 90% and 95% of diameter classes encountered, respectively. The purple, blue, and wine vertical lines represent the sample sizes required to encounter 80%, 90% and 95% of diameter classes by the soil core method, respectively, while the olive, magenta, and black vertical lines represent the sample sizes required to encounter 80%, 90% and 95% of diameter classes by the minirhizotron method, respectively

and 95% of diameter classes for soil cores were 2, 5 and 9, respectively, whereas 26, 48 and 68 were required for minirhizotrons (Fig. 5b). For the 2–5 mm diameter class, the sample sizes required to encounter 80%, 90%, and 95% of diameter classes for soil cores were 4, 7 and 12, respectively, whereas 68, 81, and 88 were required for minirhizotrons (Fig. 5c).

Discussion

Root distribution variation

Although a couple of studies have reported heterogeneous distribution of root biomass (Metcalf et al. 2008; Xiong et al. 2016), studies that report the distribution of other root traits are relatively scarce (but see Montagnolia et al. 2018). This study showed a high degree of variation in root length and volume density distribution (Table 1). This variation may be closely related to in situ site conditions, such as uneven soil water and nutrient availability (Liu et al. 2017; Ochoa-Hueso et al. 2020), which is shaped by a variety of biotic and abiotic factors. For example, the forest canopy can redistribute the resources such as input of precipitation and the associated dissolved organic matter, temperature, and light intensity, which tremendously change the soil physiochemical properties under the canopy and around the trunk, consequently changing the root distribution pattern (Olsthoorn et al. 1999, Montagnolia et al. 2014a).

A medium or strong variability was found for the root length density and volume density in different diameter classes, with the coefficient of variation (CV) values being higher in roots with diameter > 2 mm than in those with diameter ≤ 2 mm (Table 1). This may be due to the fact that smaller diameter roots are low in carbon cost, few secondary growth, and mainly responsible for water and nutrient uptake and fully occupy the soil space. In contrast, the larger diameter roots provide transport, storage and mechanic stability of the trees, and are more concentrated around the main roots and trunks, resulting in higher variation (Ostonen et al. 2007; Polverigiani et al. 2011; Montagnoli et al. 2021).

To some extent, the CV values depend on the method and equipment used. For the root length density or volume density, the CV values obtained from minirhizotrons method were higher than those from soil cores method, probably owing to the sampling volume (Heeraman and Juma 1993). Large sampling volume in the soil core can reduce the errors caused by under-sampling (Heeraman and Juma 1993; Kücke et al. 1995). In this study, the total sample volume by the soil core method (96 samples) was approximately 30 times as much as that by the minirhizotron method, resulting in a lower CV value for the soil core method, which supports our first hypothesis. Ping et al. (2010) evaluated the effects of sample volume (10 cm, 3.8 cm diameter soil core and 1 m², 0.25 m² monolith) on the measurement accuracy of root biomass in a temperate grassland in Inner Mongolia and reported that the CV value of root biomass showed a logarithmic decrease with an increase in sample volume.

Soil conditions (e.g. soil texture) are also one of the important factors affecting CV value. Metcalfe et al. (2008) used soil cores (14 cm in diameter) to measure root mass at a 0–30 cm depth in a tropical rainforest, and found that CV values in the sandy soil plots, clay soil plots and fertile soil plots were 63.3%, 27.6% and 33.8%, respectively, while the CV values of root growth observed with rhizotrons (30 cm in length and 21 cm in width) in these plots were 39.9%, 20.6% and 22.1%, respectively.

In this study, with a sample size of 96 for both minirhizotron and soil core method, the Shapiro–Wilk test showed that the root length density and volume density of all diameter classes measured by both methods were right-skewed in distribution (Table 1). The right-skewed distribution was closely related to the root sample size and method, which resulted in serious underestimation of the mean value of both root traits. This non-normal distribution of roots (especially the larger diameter roots, such as 2–5 mm diameter class) might be related to the variation in root distribution, that is, it might be affected by the size and

location of trees (Chen et al. 2005). Due to the variation in root distribution, sufficient samples and random sampling are necessary to obtain samples with normal distribution in field sampling.

Diameter distribution in root length and volume

It was found in this study that the root length of the thinner roots accounted for most of the total root length; this is consistent with previous studies. For example, Pregitzer et al. (2002) found using the soil block method that for 8 of 9 tree species in North America, more than 75% of the total root length was accounted for by roots <0.5 mm diameter. A study conducted in a *Liquidambar styraciflua* forest, by using the minirhizotron method, showed that about 80% of the root length was distributed in <0.5 mm roots, while less than 5% was distributed in >1 mm roots (Norby et al. 2004). In this study, soil core and minirhizotron methods indicated that roots with ≤ 1 mm diameter accounted for 70.2% and 95.0%, respectively, of the total root length, but contributed only 21.5% and 51.2% to the total root volume (Fig. 3). In contrast, roots with >2 mm diameter contributed to only 6.0% and 0.8% of the total root length, but accounted for 44.7% and 21.7% of the total root volume (Fig. 3), as determined using the soil core and minirhizotron methods, respectively, indicating the rarity of large-diameter roots in soil. This asymmetric relationship between root lengths and root volumes reflects the functional difference between thin and thick roots (Mei et al. 2006).

The proportion of total root length and volume in the smallest diameter class observed by minirhizotron is larger than that observed by the soil core method (Fig. 3), which supports our second hypothesis. The main reasons are as follows: (1) There exists a limited observation scope of the minirhizotron method (Chen et al. 2018). The soil volume of a minirhizotron image is based on the assumed soil depth that can be viewed, which is generally 2–3 mm. In this study, the total sample volume of minirhizotrons was only 2333 cm³ for a sample size of 96, which was significantly smaller than that of the soil core (69,949 cm³ for the same sample size). In addition, owing to the fractal root distribution, minirhizotrons can only encounter the first- and second-order roots with smaller diameters, while roots with larger diameters are relatively rare (Eshel 1998; Halley et al. 2004; Mao et al. 2015). (2) The root diameter is underestimated by the minirhizotron method. Since roots are below ground, the obscured root margin and the limited soil depth observed by the minirhizotron may lead to the underestimation of the large diameter roots (Taylor et al. 2014). (3) Small diameter roots are more likely to grow around minirhizotrons. It takes weeks to months or one year or more for root growth to reach an equilibrium state at the tube-soil interface following the minirhizotron installation (Price and Hendrick 1998; Weber and Day 1996; Wells et al. 2002; Joslin and Wolfe 1999; Strand et al. 2008). Generally, the most active part of the root pool, such as the smallest and the most distal roots, grow first on the surface of minirhizotrons. However, we noticed that the time interval from the installation of minirhizotrons to image collection was only one year. If a longer time is allowed, the root population around the tube surface would increase to be more similar to the root population in natural soil, increasing the probability of encountering larger roots. (4) Minirhizotron can identify the small-diameter roots with relatively high accuracy due to the high-resolution minirhizotron images (Chen et al. 2018). In contrast, the finer roots are much easier to be unnoticed than the larger ones observed by soil core method, during the process of root picking and washing (Heerman and Juma 1993; Sierra et al. 2003). It's worth noting that damage to the roots due to soil coring during the installation of

minirhizotron would lead to an over-production of fine roots, because the root apex might be stocked or wounded (Montagnolia et al. 2014b). The study by Freschet and Roumet (2017) also demonstrated that different sampling methods might capture different functional parts of roots.

The soil core method is considered to be an accurate method for assessing root biomass, though it is destructive and labor consuming (Kücke et al. 1995; Machado et al. 2003). In contrast, the minirhizotron is the most direct and reliable method for studying fine root dynamics and estimating fine root turnover (Yuan and Chen 2012; Hendricks et al. 2006; Chen et al. 2018). Fine root biomass measured using the soil core method, integrated with the lifespan observed by the minirhizotron method, is recommended as an efficient way to calculate fine root turnover (production and mortality) in temperate and boreal forests (Majdi et al. 2005). However, in the present study, we found a mismatch between the fine root populations observed by the soil core and minirhizotron methods (Fig. 3). The minirhizotron method is prone to underestimate the thicker roots owing to insufficient sample volume, while the soil core method tends to underestimate the thinner roots owing to losses during root processing with a 0.5 mm sieve. The under-representativeness of thin roots in the soil core method may cause only minor errors in estimating fine root biomass, because thin roots contribute a minor proportion to total root biomass (as seen in Fig. 3b). In contrast, in the minirhizotron method, thick roots generally have a long lifespan and account for a larger proportion of fine root biomass, hence, their under-representativeness could cause a substantial overestimation of root turnover rate. If fine roots are traditionally defined as a single fine-root pool (e.g., <2 mm in diameter), then the fine root turnover estimated by this integrated method may be overestimated (McCormack et al. 2015). Therefore, researchers must ensure accurate matching of soil core and minirhizotron data. For example, when fine root turnover is estimated, fine roots should be separated into individual diameter classes, individual root orders, or functionally defined into shorter-lived absorptive and longer-lived transport root pools (McCormack et al. 2015).

Estimation accuracy and sample representativeness in relation to sample size

Our results show that, compared with the minirhizotron method, the sample sizes required to meet the sampling accuracy and representativeness are smaller when the soil core method is used to measure root traits. Furthermore, the sample sizes required to measure roots of the 2–5 mm diameter classes are larger than those measuring roots of 0–2 mm diameter class no matter soil cores or minirhizotrons were used (Fig. 5b, c), which supports our third hypothesis. The smaller sample sizes required for soil core is mainly due to its higher sampling soil volume which reduce the CVs of root traits and increase the opportunity of capturing more diameter bin, while larger sample sizes required for thicker roots is due to the branching process of root growth which makes thicker roots rarer and more heterogeneously distributed (Eshel 1998; Halley et al. 2004).

For the minirhizotron method, Taylor et al. (1990) suggested that at least 8 minirhizotrons are needed to meet the accuracy requirement in estimating the root length density; however, in most studies, 5–8 minirhizotrons are used (Norby et al. 2004; Lee et al. 2016; King et al. 2002; Brown et al. 2009). The present study shows that to achieve 80% accuracy, the sample size of 54 minirhizotrons is minimum in estimating the root length density of 0–5 mm diameter class (Fig. 4a), indicating that the current sampling size is far from enough, at least in subtropical forests. The minirhizotron used for image collection in this study was the BTC-100 system (Bartz Technology, Carpinteria, CA, USA). The actual size

for an individual image was 18 mm × 13.5 mm. Thus, even if there is a large increase in sample size, the root estimation accuracy and sampling representativeness may still not be guaranteed. Minirhizotron systems with a much wider image scope or root boxes are therefore more suitable for estimating root turnover. CI-600, a plant root growth monitoring system with an actual size for an individual image of 216 mm × 196 mm, can observe a sample volume that is not significantly different from that of the soil core method (10-cm-diameter), if a field depth of 3 mm is assumed (Zhang et al. 2009). Therefore, besides increasing the sampling quantity, increasing the individual sample volume is an alternative to improve estimation accuracy and sample representativeness.

For the soil core method, many studies tend to use the soil core to estimate root biomass, with a sample size of 8–30 being common (Berhongeray et al. 2013); however, Olsthoorn (1991) thought that the sample size should be at least 10. Unfortunately, the present study demonstrates that the previously used sample sizes could be far from being enough for the soil core to achieve high estimation accuracy, such as 90% or 95%. However, the soil core method used in this study was relatively smaller (4.05 cm in diameter) compared with that in the literature (e.g., 14 cm in diameter in Metcalfe et al. 2008). It is worth noting that the soil core method can be applied to small-diameter roots, which are more evenly distributed, while for thick roots that are relatively few and unevenly distributed, methods such as the monolith and trench, with a large volume, should be adopted (Levillain et al. 2011; Macinis-Ng et al. 2010). Therefore, the difference in sampling methods and the diameter of the sampling core should be carefully considered when determining sample size.

The accumulation curves do not only serve as a guarantee for adequate sampling, but also reduce the labor and cost caused by over-sampling. Notably, a steep rise in the accumulation curve, which reveals that additional sampling is required, indicates that common diameter classes have been rapidly encountered (Taylor et al. 2013). As the curve becomes flat, it indicates the point at which majority of the diameter classes are encountered, and the sample size is sufficient for certain accuracy. As the slope of the curve gradually decreases, a much larger sample size is required to encounter additional diameter class. Taking the 0–5 mm diameter class as an example, in the present study, to elevate the encounter of root diameter class from 80% to 90%, the sample sizes for soil cores and minirhizotrons should be increased by 100% and 61%, respectively. A further 5% increase in diameter class encounter requires a sample size increase of 67% and 26% for soil cores and minirhizotrons, respectively (Fig. 5a). Therefore, the trade-off between available resources and sample size should be considered owing to the diminishing returns of additional sampling (Taylor et al. 2013).

The present study demonstrates that sample size is constrained by root distribution variation and sample representativeness and varies with the sampling method, the selection of which is not straightforward. Taking the 0–5 mm diameter class as an example, for the soil core method with a 90% confidence level and 80% estimation accuracy, the sample size ($n=5$) required to measure the root length density could only encounter 80% of all diameter classes, while the sample size ($n=12$) required to measure the root volume density could encounter 95% of all diameter classes. In contrast, for the minirhizotron method under the same confidence level and estimation accuracy, the sample size ($n=54$) required to measure the root length density could only encounter 80% of all diameter classes. As for the root volume density, the current maximum sampling size of 96 could not meet the confidence level and estimation accuracy required for root length density in the present study, although it could encounter 95% of all diameter classes. Therefore, the determination of sample size needs to compromise the requirements of root distribution variation and sample representativeness, which is also dependent on target root traits such as root

length and volume. In the present study, our findings suggest that a sample size of 12 could be appropriate for the soil core method to measure root length and volume density, which could meet 90% confidence level and 80% estimation accuracy and simultaneously encounter 95% of all diameter classes; however, for minirhizotron, a sample size of more than 96 is required.

It should be noted, however, that an oblique core sampling is uncommon for soil core method, but it would not affect the overall results of comparing soil core method to minirhizotron method, because all root trait data are calculated on a soil volume basis. In addition, the optimal sample size revealed in this study could not be directly used in other studies, because the optimal root sampling strategy depends on many factors, including the target root traits, the equipment used (related to the sampling volume), and the ecosystem type. For example, a study in a rainforest has shown that soil texture can affect the sample size (Metcalf et al. 2008). Instead, our study presents an example assessing simultaneously the effects of root spatial variation and root fractal distribution on the estimation accuracy and sample representativeness, which could serve as a reference for root sampling strategy.

Conclusions

Our study reveals a high variation in root distribution in a subtropical evergreen broad-leaved forest, and the variation is greater for minirhizotrons than soil cores. The roots observed by the minirhizotron are generally thinner than those observed by the soil core method, leading to a mismatch in the root population observed by the two methods. When the soil core method is used to measure root traits, the requirements for sampling accuracy and representativeness can be easily met with a relatively small sample size, while larger sample size is required for the minirhizotron method. Likewise, the sample size required for large-diameter roots is also larger than that for small-diameter roots. Apart from increasing the sample size, increasing the individual sample volume, such as increasing the diameter of the soil core or using a root window with a larger observation scope, is an alternative to increase the estimation accuracy and sample representativeness. Our study indicates that if root turnover is estimated by integrating minirhizotron with the soil core method, the fine root needs to be divided into different pools (such as absorptive roots and transport roots, or based on different diameter classes) to match each other between methods.

Acknowledgements The study was supported by the National Natural Science Foundation of China (Nos. 31830014, 31660192), and the Science and Technology Research Project of Education Department of Jiangxi Province (No. GJJ190974). We thank Fei Deng, Yunyu Chen, Weiwei Wang for helps in field and laboratory assistance.

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