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Full Length Article

Variability of Root Length Density as Measured by Auger Core and Soil Profile Sampling Methods

Hongyu Wang, Zhengguo Cui, Zhenming Yang, Muhammad Rehman Naseer, Qiuzhu Li* and Jinhu Cui*
[1]*College of Plant Science, Jilin University, Changchun 130012, China*
Correspondence author: cuijinhu@163.com; liqz@jlu.edu.cn

Abstract

Root sampling methods, destructive excavation of monoliths (as reference method) and soil coring using auger, were compared for correlations and mean differences in root length density (RLD). Significant correlations between auger and monoliths methods were observed for RLD of maize roots in each depth interval (0.5198<=r<=0.7443, $P < 0.01$ at each depth), and when depths were pooled (r=0.692, $P < 0.01$). Linear relationships were also observed for faba within the 20 to 80 cm depth intervals. In contrast, there were no significant correlations observed in RLD between methods for barley or wheat at any depth. Overall, the average RLD from the soil depth of 100 cm for maize was 0.56 cm cm^{-3} (0–4.48 cm cm^{-3} range) in auger core, but only 0.37 cm cm^{-3} (0 to 1.32 cm cm^{-3} range) in monolith samples (F=20.08, P=0.0464). This indicates that RLD estimates will be higher when maize roots are sampled by the auger method than when monoliths are dug, particularly in the top 40 cm of soil. Average RLD did not vary between methods for wheat or barley (F=10.53, P=0.0833). It was, therefore, concluded that auger and monolith methods both yield reliable RLD data for fine root systems (*e.g*., barley and wheat). In contrast, RLDs of crops with coarser and taproots (*e.g*., maize and faba bean) were likely overestimated by the auger core method. Thus, the monolith method is likely more suitable for crops with coarser and taproot systems. These results are partly benefited for field researchers in optimizing root sampling methods.

Key words: Monolith; Auger; Maize; Faba; Barley; Wheat

Introduction

Root research has been an integral part of agro‐ecosystem experiments for many years. The root density in the soil determines the ability of a plant to acquire the water and nutrients necessary for sustaining crop growth (Craine *et al.*, 2002). Although important for the acquisition of water and nutrients, and, ultimately, grain yield, the RLD is hard to measure in the field. Since tedious, time–consuming, and costly procedures are required to expose and measure root systems, the number of studies on the growth of under‐ground root systems on field conditions is few compared with those on above–ground shoot systems (Gregory, 2006). Despite this importance only few studies have attempted to quantify fine root dynamics in agro‐ecosystems, mainly due to difficulties associated with root sampling. In one such study, the mean RLD and the RLD in the soil depth layer from 15 cm to 30 cm had significant positive effects on chickpea seed yield in field trials (Kashiwagi *et al.*, 2006). Moreover, root length estimates vary widely depending on differences in sampling methods, crop species, soil types, or rate of fertilizer application (Gregory, 2006). In one notable recent development, the ratio between root dry biomass and root fresh biomass, which is relatively easy to measure, has been recommended as an alternative of fine root tissue density (Birouste *et al.*, 2014).

Many methods have been used to study plant roots, especially in natural field experiments (Böhm, 1979). However, there is little information available on the comparison of two or more of these methods (Böhm *et al.*, 1977; Samson and Sinclair, 1994; Kücke *et al.*, 1995; Nissen *et al.*, 2008). These comparisons are not sufficiently comprehensive to guide decisions on the selection of suitable methods for root investigations. Questions remain on how to obtain reliable data and select an appropriate method for assessing root growth, distribution and activity. To date, no one technique has proven capable of solving all of the dilemmas associated with studying roots in field situations.

Methods employed in root research are generally high labor intensity and cause substantial destruction to study sites. In addition, each method has inherent advantages and disadvantages. One method is to collect soil cores using hand–operated or machine–equipped augers. Alternatively, the monolith (profile wall) method entails digging trenches to expose a soil profile along the face of a trench. The auger method consumes far less time and can be applied on easily penetrable soils by using augers driven to the desired

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sampling depth without obvious destructive to the crops to be conducted. Therefore, auger sampling is widely used, but at the cost of requiring a high number of replications and large diameter cores (Böhm, 1976; Buczko *et al*., 2009). The soil monolith method is advantageous for studying root distribution, but is labor intensive and technically demanding while, unfortunately, being destructive to the crops being sampled (Böhm, 1979). Beyond these considerations, the question remains as to whether the auger method can substitute for the monolith method. Understanding which differences in root morphological parameters are observable between the sampling methods in the same soil profile is essential information for choosing an appropriate method of studying root systems in agro‐ecosystems, as well as in crop populations in field systems. There have been insufficient reports detailing correlations and differences between the monolith and auger root sampling methods for crops. Furthermore, root lengths change as crops grow. A difficult problem, therefore, is to compare root parameters from different research and different locations where different methods have been applied and the results are often expressed in different ways.

Prior to this research, little information was available describing differences and correlations in root morphology estimates based upon disparate root sampling methods, probably due to the time consuming and laborious nature of such experiments (Jose *et al.*, 2001). When different roots types are considered, such results are rather scarce (Millikin and Bledsoe, 1999). Kücke *et al*. (1995) compared to those focusing on one of the four commonly used field methods for measuring root dry matter (*i.e*., core, core–break, root–extraction and trench–profile wall methods). More recently, four methods, auger, full Voronoi trench, half Voronoi trench and monolith, were compared for measuring under–ground dry matter in Eucalyptus forest plantations (Levillain *et al*., 2011). The present study contributes to this knowledge while focusing on the RLD of crops in field conditions.

The objective of this research was to assess methods for studying crop roots by comparing root length densities obtained using the auger and monolith methods for maize, faba bean, wheat and barley roots in soil sampled at depths of up to 100 cm below the soil surface from specific locations relative to the crop plants. If consistent variation between methods was identified, then auger core sampling can be applied under field conditions to reliably study roots.

Materials and Methods

Research Region

The experimental site was located at the Baiyun village, Wuwei city, northwest China (38°37′N, 102°40′ E). Annual rainfall amounts to about 150‐200 mm. Climate is typically arid. The soil was classified as Aridisol (FAO), with a significantly higher pH of 8.08, organic matter of 2.026%, total nitrogen (N) of 0.098%, Olsen P of 2.186 mg 100 g^{-1}, exchangeable potassium (K) 12.57 mg 100 g^{-1} average soil layer 0–25 cm.

Experimental Design

The experimental design was a single factor field experiment with three replicates, comprising 4 monocultures (barley, faba, maize and wheat) and 3 intercropping systems: maize (*Zea mays*)/barley (*Hordeum vulgare*), maize/faba (*Vicia faba*) and maize/wheat (*Triticum aestivum*). Barley, faba and wheat were seeded on 20 March 2015 and maize was seeded on 18 April 2015.

One experimental unit was 7.0 m length. Maize/barley and maize/wheat intercropping combinations included two rows of maize (at an inter‐row spacing of 39 cm) and six rows of barley or wheat (at an inter–row spacing of 12 cm). The distance between maize and the nearest barley or wheat row was 25.5 cm. Maize/faba intercropping included two rows of maize (at an inter‐row spacing of 40 cm) and two rows of faba (at an inter‐row spacing of 20 cm). Within each row, interplant distance was 20 cm for faba and 30 cm for maize in all plots. The distance between maize and the nearest faba row was 30 cm. Each intercropping plot consisted of three strips, one of which was a sample collection strip. In maize/barley and maize/wheat plots, maize occupied 52%, while barley or wheat occupied the remaining 48% of the intercropped area. In maize/faba plots, 2/3 of each intercropped area was occupied by maize and 1/3 by faba. Plant densities for each crop in intercropping plots were identical to those in monoculture plots.

Each plot received identical applications of 75 kg ha^{-1} P supplied as triple superphosphate and 225 kg ha^{-1} N as Urea. Half of the N and all of the P fertilizer was uniformly broadcast on the soil surface and incorporated into the soil layer 0‐20 cm using a disc harrow before sowing. The remaining half of N fertilizer was averaged into two parts and applied to plots during the elongation and pre–tasseling stage of maize. Harvests were conducted on the 20th of July for barley and wheat, the 1st of August for faba, and the 2nd of October for maize.

Root Sampling

Roots samples were got by the hand–operated auger method and the monolith method (Böhm, 1976, 1979). All plots were irrigated two or three days before sampling in order to decrease the difficulty of sampling. Samples were collected from all plots on the 7th of June (46 days after germination of maize) for maize, barley, faba and wheat, and on the 27th of August (116 days after germination of maize) for maize alone.

Hand–operated Auger Sampling

Root samples were collected from root auger cores

(diameter 5.5 cm, length 20 cm) to a depth of 100 cm in 20 cm increments. Six cores were collected and bulked per intercropping plot, and two were collected and bulked from each monoculture plot (Fig. 1). Samples were collected within and between rows in barley and wheat strips, and those from the same depth intervals were mixed uniformly for each plot. For maize and faba, one plant was cut level to the soil surface and the stem cross section was encircled by an auger for one sampling site, and the other site was the diagonal intersection of four plants. The auger was pulled out of the ground when the desired depth had been achieved. Soil cores were collected into plastic bags that were immediately sealed. As with the other crops, cores from faba and maize were pooled by 20 cm depth intervals to a maximum depth of 100 cm. A complication specific was that it was difficult to precisely determine 100 cm depths across monoliths due to an 8% slope in the field (Levillain *et al*., 2011).

Monolith (Trench Profile Wall) Sampling

Root systems were also collected using the monolith method (Smit *et al*., 2000). Trenches were dug with hand in each unit. The length of each trench was vertical to the crop row and consisted of half of one strip (about 80 cm for maize/wheat or maize/barley, and about 60 cm for faba) of an intercropped two crop species, or at least two rows for mono–cropped units (about 20 cm for faba, wheat and barley, about 40 cm for maize). The length, depth and width of each trench were 80 cm × 100 cm × 80 cm for maize/barley and maize/wheat, 60 cm × 100 cm × 80 cm for maize/faba, 20 cm × 100 cm × 20 cm for pure barley, faba and wheat crops, and 40 cm × 100 cm × 40 cm for pure maize. The wall of the soil profile, or the working face was smoothened and loose soil was removed from the bottom of the trench after the trenches had been finished. Then, the wall was marked with 10 cm × 10 cm grid lines. Soil monoliths of 10 cm in length, 10 cm in width and 10 cm in depth were collected from the smoothened wall by pressing a broad metal knife and sharp–edged steel boxes perpendicularly into the soil surface. Each box was open at the top, and the dimensions were 10 cm in length, 10 cm in width, and 10 cm in depth, which obtained soil monoliths of those dimensions. The thickness of the steel was at least 2 mm. The box was inserted into the soil with a heavy hammer, with hard wood placed on top of the box for protection while hammering. Boxes were driven vertically into the soil. A sharp broad metal knife was used to cut the soil under the box, and then the monolith was finished. With this technique, precise volumes of soil could be sampled. There were 80 monoliths (8 vertical × 10 horizontal) collected from each maize/wheat and barley/maize plot, 60 monoliths (6 vertical × 10 horizontal) from each maize/faba plot, 40 monoliths (4 vertical × 10 horizontal) collected from mono‐cultured maize, and 20 monoliths–2 vertical × 10 horizontal) collected from mono‐cultured faba, wheat and barley (Fig. 2). In total, with three replicates, 1920 and 720 samples were collected at the first and second (maize only) sampling times respectively. Each monolith was placed in a marked plastic bag.

The distance between the locations of auger– and monolith–samplings was 1m in the same unit, in order to reduce the effects by heterogeneity of root distribution or variation of plant growth around individual sampling locations. The soil blocks in planting rows were collected in the same way with auger and monolith methods.

Washing and Storing Root Samples

Soil core samples were stored for a maximum of 3 days in polyethylene bags at 4°C prior to washing. All soil–root samples were processed through 0.5 mm sieves using tap water in order to collect roots for further cleaning. All soils samples were soaked for approximately one hour with continuous hand rotation of the sieves at the water surface until roots were washed free from the soil. Roots were then collected from the sieve by hand using tweezers. Rubbles, weeds, and dead roots were sorted by hand from the 'live' roots during washing, based on the visual observation of 'live' roots appearing lighter in color. Extracted root samples were sealed in plastic bags, frozen and stored at about –20°C until length determination was performed.

Distinguishing Crop Roots

In maize/barley, maize/faba, and maize/wheat intercropping systems, some monoliths contained two crops roots. In order to assign root lengths to each crop, roots had to be distinguished from each other based on visible features. Roots of faba were quickly oxidized and blackened upon exposure to air. The diameter of maize roots was usually larger than the diameter of wheat and barley roots, and fine maize roots were slightly yellow, fragile and had visible nodes. Barley and wheat roots were all fine, brown and flexible, so they could be distinguished from maize roots, whilst not being present together in the same monolith.

Determination of RLD (Root Length Density) by Scanning Root Samples

Each crop root sample was put in a waterproof and transparent rectangular dish (250 mm × 290 mm) with a layer of deionized water about 4‐5 mm deep included to assist in the untangling of roots and thereby minimizing the crossing and overlapping of roots. When necessary, root samples were divided into several sub‐samples that were each placed into the rectangle dish until the sample had been completely scanned. This was common for roots from the second sampling time, when roots were often extensive. Roots were scanned (Epson Perfection 4990) at 600 dpi, and images were saved. Scanned root images were analyzed using the software Win–RHIZO Root Analyzer System

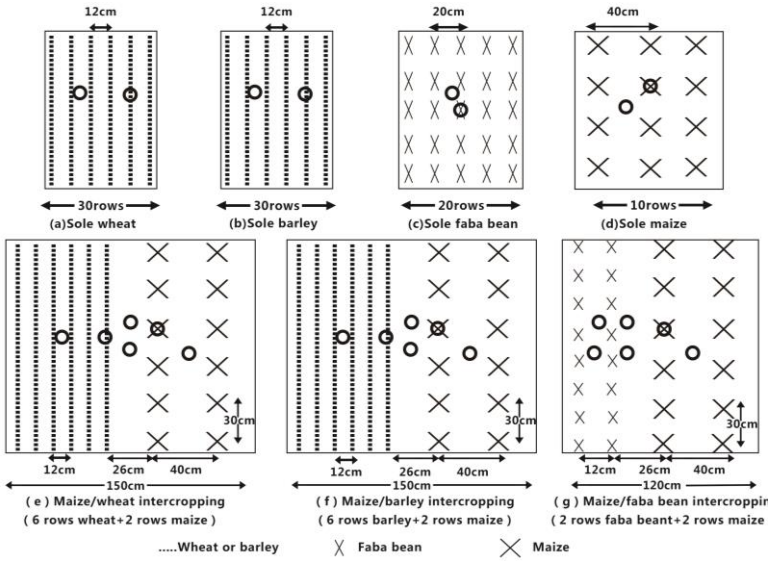

Fig. 1: Schematic diagram of field plots sampled by auger coring. Circles mark sampling sites

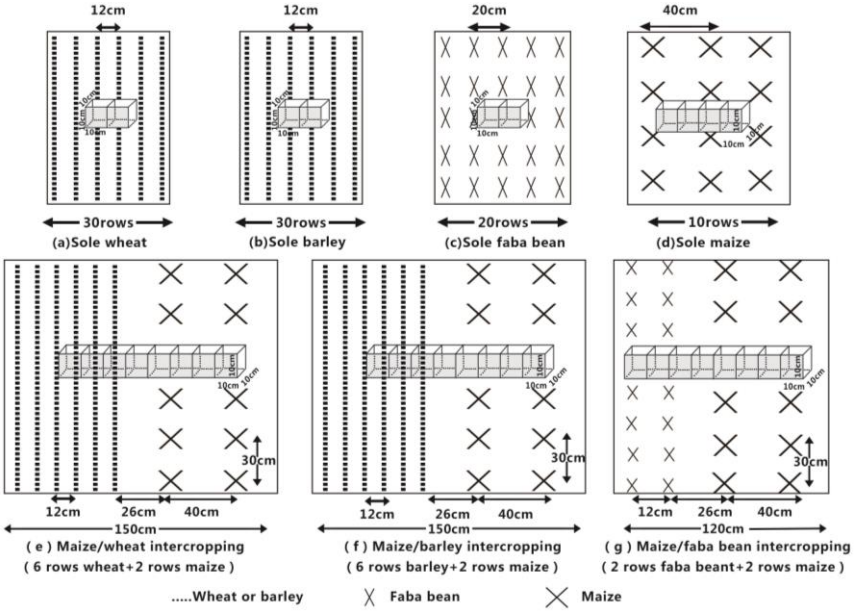

Fig. 2: Schematic diagram of field plots sampled by monolith trenching. Cubes mark sampling sites

version 5.0 (Regent Instruments Inc., Quebec City, Canada) to get root morphological parameters, including root length density and average diameter. Root length density was calculated as RLD (cm cm $^{-3}$) = root length (cm)/soil V (cm^3).

Statistical Analysis

Linear regression and Analysis of variance (ANOVA) with mean separation by the least significant difference (LSD) method were conducted for multiple comparisons using the SAS statistical package version 8.2 (SAS Institute, 2001).

Results

The Correlation between Monolith and Auger Sampling Methods for Measuring RLD

There was a significant linear relationship of RLD measurements for maize between the two sampling methods within each 20 cm interval down to 100 cm of depth (Table 1). Similar relationships were also observed in faba for the three depth intervals from 20 cm to 80 cm soil depths (Table 1). In contrast, there were no significant correlations in RLD for barley or wheat between the two methods within any 20 cm soil depth interval (Table 1).

RLD of Maize Measured by Monolith and Auger Methods

Maize RLD in the soil layer 0–20 cm was significantly higher in auger core samples than in monolith samples, regardless of the cropping system (Table 2). While there was a decreasing trend of RLD from 20 to 100 cm in soil depth layer for both barley and wheat in both cropping systems, there were no correlations between sampling methods observed for either plant in any soil layer (Table 1 and Table 3).

The RLD means were 0.184, 0.3784, 0.6438, 0.4299, and 0.4007 for the 0–20, 20–40, 40–60, 60–80 and 80–100 cm soil layers, respectively (Fig. 3 and Table 1).

RLD measurements of maize pooled over all sampling depths averaged 0.56 cm cm^{-3} (0–4.48 cm cm^{-3} range) in auger core samples and 0.37 cm cm^{-3} (0–1.32 cm cm^{-3}) in monolith samples (Fig. 4A. F=20.08, P=0.0464). The correlation coefficient was 0.692 between auger core and monolith profile methods (n=240, $P < 0.01$), which indicated that the monolith method is suitable for crops such as maize, with coarse roots systems.

RLD of Faba Measured by Monolith and Auger Methods

RLD of faba pooled over all depths averaged 0.22 cm cm^{-3} (0–1.86 cm cm^{-3} range) in auger core samples and 0.11 cm cm^{-3} (0–1.19 cm cm^{-3}) when sampled by the monolith method (Table 1). There was a significant linear relationship between the two methods, with a correlation coefficient of 0.2596 (n=60, $P < 0.05$) with all samples tested together (Fig. 4B). Within soil layers, correlation was significant between the two methods for the three layers from 20–80 cm in soil depth, and it was marginally significant (r=0.5186, n=12, p=0.05) in the soil layer 0–20 cm (Table 1).

RLD of Wheat and Barley Measured by Monolith and Auger Methods

Average RLD did not vary between methods for wheat or barley (F=10.53, P=0.0833). The correlation coefficients were 0.6284 and 0.78 (n=60, $P < 0.01$) between the auger and the monolith methods for RLD of wheat and barley, respectively, in the combined soil depths of 0–100 cm (Fig. 4C, D). However, RLD of wheat and barley were not significantly correlated within any 20 cm soil layer (Table 1). Correlation between methods was largely consistent for maize and faba, regardless of whether studied within soil layers or when combining soil layers. Correlations for wheat and barley depended on whether soil layers were observed separately or together.

The range of RLD varied among the four studied crops. Mean RLD was much higher for barley than for any of the other three crops (Fig. 4).

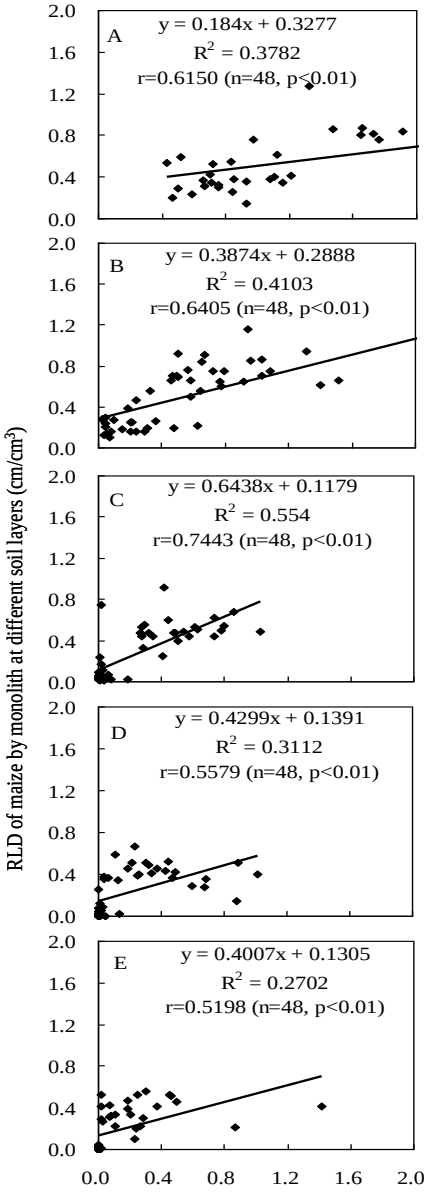

Fig. 3: Correlation of RLD measured by the auger method versus the monolith method for maize (**A**), faba bean (**B**), wheat (**C**) and barley (**D**) roots in soil depths of 0-100 cm

Discussion

The results showed that RLD of maize was greater when obtained from auger cores than from monoliths, especially in the 0–40 cm layers (Table 2). A likely explanation is that the volume of the samples is smaller for auger cores than those obtained from the monolith methods. A more likely explanation is that the samples were not equivalent in terms of distance from the plant. Half of the soil cores were taken where the plant was seeded, and half were taken between plants. Much less than

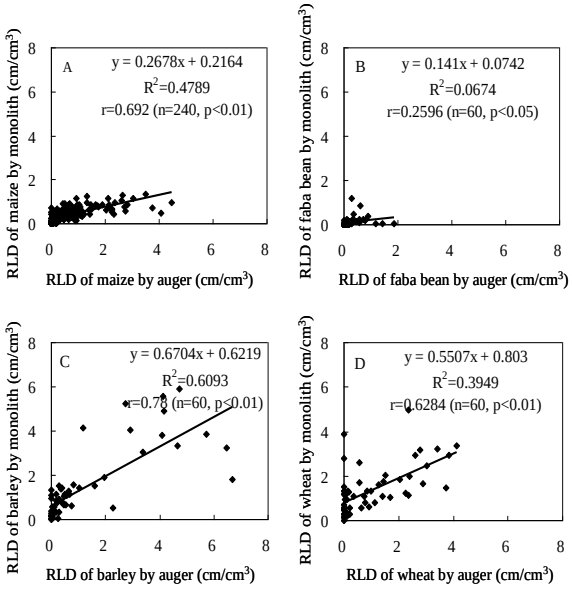

Fig. 4: Correlation of RLD measured by the auger method versus the monolith method for maize in soil layers at 0-20 cm (**A**), 20-40 cm (**B**), 40-60 cm (**C**), 60-80 cm (**D**) and 80-100 cm (E) depths

Table 1: Pearson's correlation coefficients and P values for RLD measured by the auger method versus the monolith method for maize, barley, wheat and faba bean roots in 20 cm soil layers down to 100 cm soil deep

Species	Depth (cm)	N	Equation	r	p
Maize	0-20	48	y=0.184x+0.3277	0.6149	<0.01
	20-40	48	y=0.3784x+0.2888	0.6405	<0.01
	40-60	48	y=0.6438x+0.1179	0.7443	<0.01
	60-80	48	y=0.4299x+0.1319	0.5579	<0.01
	80-100	48	y=0.4007x+0.1305	0.5198	<0.01
Faba bean	0-20	12	y=-0.4146x+0.6426	0.5186	n.s.
	20-40	12	y=0.6298x+0.047	0.6147	<0.05
	40-60	12	y=0.3174x+0.0194	0.6375	<0.05
	60-80	12	y=1.3812x-0.0079	0.8754	<0.01
	80-100	12	y=0.1554x+0.0008	0.2217	n.s.
Barley	0-20	12	y=-0.3013x+5.3487	0.4109	n.s.
	20-40	12	y=0.0124x+1.2007	0.0200	n.s.
	40-60	12	y=0.2176x+0.89	0.2121	n.s.
	60-80	12	y=0.4166x+0.4084	0.1664	n.s.
	80-100	12	y=1.1388x+0.1361	0.4581	n.s.
Wheat	0-20	12	y=-0.1876x+3.3947	0.2606	n.s.
	20-40	12	y=-0.1211x+1.6893	0.1800	n.s.
	40-60	12	y=-0.0866x+1.0239	0.1122	n.s.
	60-80	12	y=-0.1999x+0.759	0.0985	n.s.
	80-100	12	y=-1.8319x+0.404	0.2252	n.s.

Note: N is the number of root samples, n.s. indicates no significance in correlation between auger core and monolith profile methods within the given 20 cm soil depth interval. x=auger core method (cm roots cm^{-3}); y=monolith profile method (cm roots cm^{-3})

half of the monolith samples were taken at the location of the crop stem. For this reason, it is reasonable to conclude that auger cores overestimated RLD in the present study. In future studies, more care should be taken to ensure that samples are compared at comparable average distances to the plant stems.

When soil layers were examined separately there were no correlations for wheat or barley. Possible explanations are that there were relatively few samples taken for these crops (n=12) and these roots were sample only once during growth period. Taken at face value, the results indicate that correlations between the root sampling methods is weak for fine roots, such as barley and wheat, yet averages were similar. This indicates that averaging replicates was better than scatter plots of individual replicates.

In the present field experiment, a significant linear relationship in crop RLD was observed between auger and monolith root sampling methods, which was consist with previous studies in an alley cropping system with maize, black walnut and northern red oak (Jose *et al*., 2001).

This research documented that the auger and monolith methods used for sampling roots were both reliable for RLD estimates for barley and wheat with fine root systems (Table 3). On the other hand, RLD estimates for maize and faba obtained using the auger core method was significantly higher than those obtained using the monolith profile method (Fig. 3 and Table 2). For reasons explained above, RLD may have been overestimated for auger cores as sampled in the present study. Nevertheless, auger cores are still a valuable tool for estimating RLD in field experiments. The volume of the samples obtained from augers is generally smaller than those from monolith samples. Also, when using augers, a 10–cm core diameter is seldom exceeded, which minimizes impacts on growing plants. Still, there is little information available on how to choose an appropriate method for root sampling. Pierret *et al.* (2005) suggested that fine roots are the major component of root systems of most (if not all) annual and perennial plants, and traditional methodology and measurements underestimated fine root length and biomass, which devalues their contributions to the root system and entire plant.

The present study showed that auger cores may not be suitable for measuring coarse–root length. This is consistent with previous researches on eucalyptus and others plants (Resh *et al.*, 2003; Macinnis–Ng *et al.*, 2010). Although the auger core method for estimating fine ‑ root length (*e.g.*, barley and wheat herein) offers an attractive alternative to destructive root sampling, it has also been shown to underestimate RLD in comparison to monolith sampling in 20 ‑ 100 cm deep soil layers (Table 3).

In contrast, the results presented here provide evidence that the auger method is suitable for estimating RLD for plants, such as barley and wheat, with fine roots evenly distributed in the soil. Meanwhile, the monolith method appears to be more appropriate for maize and faba and other plants with a taproot or coarse roots, which can be unevenly distributed around the plant (Macinnis–Ng *et al.*, 2010). This result can be explained to some extent by the bigger soil volume sampled by soil monoliths (1000 cm^3) compared to auger cores (475 cm^3). Another explanation is

Table 2: Values of maize RLD (cm cm^{-3}) when intercropped with barley, faba bean, or wheat, along with RLD in monoculture determined in 20 cm layers up to 100 cm in depth using auger and monolith sampling methods

Sampling Date	Soil Depth (cm)	Maize Inter-cropped with						Sole Maize	
		Barley		Faba bean		Wheat			
		Auger†	Monolith†	Auger	Monolith	Auger	Monolith	Auger	Monolith
2015.6.17	0-20	0.93 ± 0.17[a]	0.35 ± 0.05[b]	0.91 ± 0.19[a]	0.40 ± 0.19[b]	0.55 ± 0.15[a]	0.39 ± 0.13[a]	1.16 ± 0.32[a]	0.58 ± 0.26[a]
	20-40	0.08 ± 0.01[a]	0.18 ± 0.09[a]	0.21 ± 0.07[a]	0.24 ± 0.12[a]	0.10 ± 0.09[a]	0.21 ± 0.07[a]	0.20 ± 0.17[a]	0.24 ± 0.07[a]
	40-60	0.00 ± 0.00[a]	0.05 ± 0.03[a]	0.02 ± 0.01[a]	0.07 ± 0.03[a]	0.02 ± 0.02[a]	0.04 ± 0.01[a]	0.04 ± 0.04[a]	0.03 ± 0.01[a]
	60-80	0.00 ± 0.00[a]	0.03 ± 0.04[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.01 ± 0.01[a]	0.02 ± 0.01[a]	0.04 ± 0.07[a]	0.01 ± 0.01[a]
	80-100	0.00 ± 0.00[a]	0.02 ± 0.02[a]	0.00 ± 0.00[a]	0.00 ± 0.01[a]	0.01 ± 0.01[a]	0.02 ± 0.01[a]	0.00 ± 0.00[a]	0.00 ± 0.01[a]
2015.8.27	0-20	2.22 ± 0.52[a]	0.72 ± 0.13[a]	2.58 ± 1.61[a]	0.87 ± 0.23[a]	2.59 ± 1.30[a]	0.65 ± 0.21[a]	2.28 ± 0.48[a]	0.96 ± 0.17[a]
	20-40	0.59 ± 0.16[a]	0.67 ± 0.06[a]	1.00 ± 0.22[a]	0.71 ± 0.13[a]	0.86 ± 0.20[a]	0.64 ± 0.08[a]	0.70 ± 0.25[a]	0.82 ± 0.10[a]
	40-60	0.40 ± 0.12[a]	0.46 ± 0.03[a]	0.61 ± 0.13[a]	0.54 ± 0.05[a]	0.46 ± 0.05[a]	0.40 ± 0.13[a]	0.60 ± 0.28[a]	0.44 ± 0.11[a]
	60-80	0.39 ± 0.31[a]	0.38 ± 0.04[a]	0.46 ± 0.07[a]	0.38 ± 0.10[a]	0.67 ± 0.22[a]	0.32 ± 0.19[a]	0.19 ± 0.27[a]	0.38 ± 0.04[a]
	80-100	0.59 ± 0.72[a]	0.34 ± 0.06[a]	0.42 ± 0.23[a]	0.31 ± 0.09[a]	0.28 ± 0.08[a]	0.24 ± 0.16[a]	0.12 ± 0.11[a]	0.38 ± 0.13[a]

Note: †Average ± standard deviation. Mean root length densities are expressed cm cm^{-3}. Means with three replicates are compared by method; the values with the same superscripted letters are not significantly different (ANVOA's 1 test, P, 0.05)

Table 3: Values of RLD (cm cm^{-3}) for barley, faba bean, and wheat when intercropped with maize, as well as, in corresponding monocultures as measured by auger core and monolith methods in 20 cm soil layers up to 100 cm in soil depth

Sampling Date	Soil Depth (cm)	Inter-cropped		Inter-cropped		Inter-cropped	
		Barley		Faba bean		Wheat	
		Auger†	Monolith†	Auger	Monolith	Auger	Monolith
2015.6.17	0-20	4.38 ± 1.50[a]	4.13 ± 0.98[a]	1.00 ± 0.74[a]	0.35 ± 0.47[a]	1.14 ± 1.97[a]	3.30 ± 0.56[a]
	20-40	0.77 ± 0.70[a]	1.43 ± 0.07[a]	0.12 ± 0.10[a]	0.09 ± 0.07[a]	1.67 ± 0.31[a]	1.87 ± 0.14[a]
	40-60	0.28 ± 0.19[a]	0.83 ± 0.28[a]	0.09 ± 0.08[a]	0.04 ± 0.06[a]	0.38 ± 0.39[a]	1.02 ± 0.20[a]
	60-80	0.04 ± 0.04[a]	0.26 ± 0.07[a]	0.03 ± 0.02[a]	0.00 ± 0.00[a]	0.22 ± 0.37[a]	0.58 ± 0.02[a]
	80-100	0.02 ± 0.02[a]	0.12 ± 0.06[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.05 ± 0.05[a]	0.35 ± 0.16[a]
		Sole Barley		Sole Faba bean		Sole Wheat	
	0-20	5.11 ± 1.22[a]	4.90 ± 1.45[a]	0.44 ± 0.14[a]	0.48 ± 0.67[a]	2.94 ± 0.80[a]	3.61 ± 1.16[a]
	20-40	1.61 ± 0.91[a]	1.18 ± 0.70[a]	0.18 ± 0.06[a]	0.18 ± 0.05[a]	1.40 ± 0.92[a]	1.80 ± 0.75[a]
	40-60	0.51 ± 0.47[a]	1.35 ± 0.20[a]	0.17 ± 0.19[a]	0.10 ± 0.10[a]	0.32 ± 0.53[a]	1.01 ± 0.46[a]
	60-80	0.20 ± 0.13[a]	0.41 ± 0.44[a]	0.06 ± 0.06[a]	0.10 ± 0.10[a]	0.04 ± 0.04[a]	0.89 ± 0.38[a]
	80-100	0.02 ± 0.03[a]	0.06 ± 0.07[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.04 ± 0.07[a]	0.21 ± 0.07[a]

Note: †Average ± standard deviation. Mean root length densities are expressed cm cm^{-3}. Means with three replicates are compared by method; the values with the same superscripted letters are not significantly different (ANVOAs test, $P < 0.05$)

that wheat and barley grow closer together than faba and maize. Therefore, results from faba and maize are more sensitive to how far away from plants samples are taken. If monoliths, on average, were taken further from maize and faba than auger cores, then a lower RLD is expected. Wheat and barley are grown at higher densities and, therefore, the distance from sampling sites to plants is similar for cores and trenches.

By increasing the sampled soil volume, the monolith profile method enables more thorough sampling of heterogeneously distributed coarse roots growing around a tap root. This method more effectively takes into account the uncertainty of coarse–root distribution and, therefore, reduces subsequent biases generated by normal auger–sampling methods (Macinnis-Ng *et al.,* 2010). The differences in these sample sizes might be less important than the placement of these samples with respect to the plant. The auger method requires less time than the monolith method and removes a much smaller soil sample (Böhm *et al.*, 1977).

Nonetheless, the monolith method provided the best representation of the general distribution of roots. This is consistent with the results of Levillain *et al.* (2011), who showed that the most effective method for estimating root biomasses was auger coring for fine–root systems (diameter < 2 mm), while half and full Voronoi trenches were the most appropriate methods for medium (diameter from 2 to 10 mm) and coarse (diameter > 10 mm) roots.

Conclusion

This present study has shown good correlations between auger sampling and monolith excavation for estimating maize and faba RLD, but poor correlations for barley and wheat. The auger core method promises to be reliable for RLD estimates for barley and wheat, while monolith profiles might be required for maize and faba. Each of the two methods has advantages and disadvantages. Due to differences in sampling locations relative to the sampled plants, RLD obtained from the auger core method was much higher than RLD obtained from the monolith method, and was likely overestimated, especially in the soil layer 0–20 cm for maize and faba. It is concluded from these results that the auger core method is suitable for estimating RLD in crops with fine root systems, *i.e.*, barley and wheat. For maize and faba, these results suggest that further research is warranted in terms of sample locations, replications and frequency. For

reliable RLD data, the choice of methods is an important consideration for each experiment, site and crop. This report contributes to available information that provides guidance in selecting a suitable method for root studies.

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