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A quick method to determine root biomass distribution in diameter classes

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Abstract Describing root biomass distribution in diameter classes is a fundamental way to understand the relation between a plant and its surrounding soil. Current methods used for its measurement are not well adapted to large root systems. A new quick method is proposed for the measurement of diameter distribution in large root systems. It is based on the one used in pedology to assess soil granulometry. Roots are dried, cut in a mixer and placed on a sieve column; biomass distribution according to root diameter is assessed by weighting the biomass recovered in each sieve. The validity of the method was tested by comparing the sieving method results with those obtained on dried root systems with a digital image analysing system. A sensitivity analysis showed that the optimal rotation speed of the mixer was 2,000 rpm and the optimal sieving time was 22 min. The actual diameter distribution of artificial root mixtures of known root diameter distribution was closely correlated with the root biomass distribution measured by the sieving method (r^2 = 0.87). Its application to four identical root systems resulted in values of biomass per diameter class with small standard errors. It is the first method allowing directly to measure biomass (and not length) distribution in diameter classes. It is quick, cheap and does not require root system sub-sampling; consequently, large root systems which were almost never studied can now be analysed. This method is thus adequate for repeated measurements of root diameter distribution in agronomical or ecological research.

Keywords Herbaceous species · Root biomass · Root diameter distribution · Root system structure · Sieving method · WinRHIZO

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Introduction

Root systems are fundamental components of terrestrial ecosystems since Belowground Net Primary Productivity ranges from 40 to 85% of the total NPP (Fogel 1985; Jackson et al. 1996; Scurlock and Olson 2001). However, methodological and analytical problems affect all root studies. One of the present challenges in root study is to improve techniques and methods to

assess root system structure. Here, we propose a quick and cheap method to assess the root diameter distribution of dried root systems.

Information on root diameter distribution is essential for a good understanding of root system and soil functioning. At the plant level, large-diameter roots represent most of the root system biomass and form the long-distance transport pathways that conduct water and nutrients. They also serve to store resources, to anchorage and to support lateral roots. Smaller-diameter roots make up most of the surface area of the root system and form the exchange site between plant and soil responsible for water and nutrient uptake (Eissenstat and Yanai 2002; Waisel and Eshel 2002). Root diameter distribution, which reflects the proportion of fine lateral roots, is related to plant capacity to uptake water and nutrients, particularly in cases where it is in competition with neighbours (Hodge et al. 1999; Robinson 2001). Root diameter distribution is also important at the ecosystem level. As they grow, plant roots contribute to soil porosity (Goss 1991), with root diameter controlling the size of pores. These pores, which have specific physical and chemical properties (Blanchart et al. 1999; Jegou et al. 2001; Read et al. 2003), are used as micro-habitats by the non-burrowing meso- and micro-faunas, as well as by specific microbial communities (Lavelle et al. 2004; Loranger et al. 1998). Root diameter distribution has been shown to modify ''consumer–resource'' interactions. For example, it influences the size of plant-parasitic nematodes (Jonz et al. 2001) as well as plant tolerance to parasitic nematodes (Rodriguez-Kabana et al. 1978).

In spite of its great interest, root diameter distribution is rarely measured especially on large root systems. Manual measurements, which are usually applied for length or mean diameter assessments, can in theory be used to estimate root diameter distribution. Root diameter is determined on small root sub-samples under binocular magnifying glass, using a graduated eyepiece. The length of the root segment used to compute an average diameter is however arbitrary and can lead to coarse estimates. The ''line intersect method'' (Newman 1966; Tennant 1975), commonly used to measure root length has been adapted (Kirchhof 1992) to determine root thicknesses ranging from 0.15 to 0.7 mm. However, positioning roots on the grid and counting intersections become laborious as the size and root density increased. The stereological method has also been used to measure root diameter distribution (Wulfsohn et al. 1999). It can be computed using ''total vertical projections,'' obtained by rotating a linear structure around an arbitrary vertical axis and projecting the linear structure onto a plane parallel to the axis of rotation. The great advantage of this method is to be non-destructive, allowing successive measurements on a single root system. The main problem is the necessity of growing plants in a transparent medium, which excludes all natural soils. Computerized image analysis is the most commonly used method, partly due to the important number of parameters which can be estimated. Root systems are spread on a plane and scanned. Images are then analysed with specific software. Recent studies however demonstrate that the estimation of root length and root diameter distribution is extremely sensitive to the scanning protocol: staining, maximum root density, scanner light used, scanning resolution and transformation threshold can all influence the results (Bouma et al. 2000; Himmelbauer et al. 2004; Pan and Bolton 1991; Smit et al. 1994). This method is accurate for small root samples but very time-consuming for most complete root systems. As an example, a total root length of 622.8 km has been reported for a single rye (*Secale cereale* L.) plant grown in a 4.3–l container (Dittmer 1937). Measuring such root systems by image analysis would require a very long time to spread all the roots without exceeding the maximum root density, or to apply sub-sampling of the root system as proposed by Costa et al. (2000).

The time required and the constraints specific to each method explain that root diameter distribution is not measured routinely. There is thus a need to design a quick and cheap method to accurately assess root diameter distribution on whole root systems. Here we propose a new method to determine root diameter distribution. It is fundamentally different from other methods in that it deals with large and dry root systems. It is based on the method used in pedology to assess

the distribution of soil particle size. Roots are dried, cut and placed on a sieve column; biomass distribution according to root diameter is assessed by weighting the biomass recovered in each sieve. This method is described; optimal parameters in cutting and sieving processes are determined with sensitivity analysis. The reliability of the method is then tested using roots of known diameter distribution. Finally, results obtained by applying our method to large herbaceous root systems are exposed.

Materials and methods

Method description

Roots are first harvested and coarsely separated from the bulk soil using a fine stream of water, on two stacked sieves (0.5 and 0.2 mm mesh screen) placed above a sedimentation vat. Detached roots remaining in the water of the sedimentation vat are retrieved with forceps. Root systems are dried at 50°C for 2 days. They are then cut in small fragments using a mixer (Polymix PX-MFC, Kinematica AG, Littau-Luzern, Switzerland) equipped with a speedometer. Root pieces escape the mixer through a 2-mm grid. It could be necessary to adapt the mixer grid and speed, e.g. with tree root systems. If the speed is too high or mixer grid too small, some root pieces are cut excessively, possibly affecting diameter and allowing pieces to cross sieves with smaller mesh size than their actual diameter. If the speed is too low or mixer grid too large, some root pieces keep their ramifications and stop in sieves with larger mesh size than their actual diameter. In the present study, the herbaceous species used had a maximal dry root diameter lower than 1 mm; a 2-mm mesh size was thus chosen to ensure that roots will not be cut in too small pieces.

To separate root fragments from remaining soil particles, differential sedimentation (Fenwick 1940), also called elutriation (Smucker et al. 1982), was applied. A slight water flux flowing out of a recipient carried away root pieces while soil particles stay at the bottom of the recipient due to their higher density. Root pieces are recovered on filter paper, and dried for 2 days at 50°C. They are then placed on a sieve column commonly used in pedology to assess soil granulometry (Rivière 1977). Sieve mesh sizes are 800, 630, 500, 400, 315, 250, 200, 160, 125, 100 and 80 μm (standard AFNOR norm). The number and mesh size of the sieves can be adapted to study requirements. The sieve column was placed on a sieve shaker (AS 200 Digit, Retsch, Haan, Germany) at continuous agitation. The content of the 11 sieves as well as the fraction recovered below the 80-μm sieve was collected and weighed. The proportion of root biomass in the 12 diameter classes was calculated as the ratio between biomass in each diameter class and the sum of the biomass recovered in the 12 diameter classes. Root diameter homogeneity in each class was checked by binocular observations of root fragments recovered in each sieve.

Sensitivity analysis

The ''sieving method'' allows the determination of the dry root biomass distribution in several predefined diameter classes. However, as mentioned above, different factors could impact the final biomass distribution: (1) the mixer speed, (2) the amount of root material lost during the cutting and sieving processes and (3) the sieving time. We therefore conducted sensitivity analyses for these three parameters to test their effect on the root biomass distribution among diameter classes.

We test the validity of the ''sieving method,'' by comparing the sieving method results with those obtained on the same root systems with a digital image analysing system (WinRHIZO, version 2003b, Regent Instrument, Quebec, Canada). Since WinRHIZO gives *length* distribution and the sieving method gives *biomass* distribution among diameter classes, we transformed biomass distribution in length distribution, using the specific root length (SRL, i.e. root length/root dry weight) of each class diameter. The SRL was determined using 35 short root segments of *Bromus erectus* Huds (1–2 cm length); longer root fragments would have present variation in root diameter along the axis. Each fresh root segment was scanned at 16 p mm^{-1} (400 dpi), dried at 50°C overnight and scanned again.

WinRHIZO was used to determine the length and diameter of each fresh and dry segment. We analysed both fresh and dried roots in order to test the effect of drying on the root length and diameter. The SRL was calculated per segment and per diameter class (Fig. 1). The diameter classes correspond to the one of the sieve mesh sizes available except for the lowest classes. As it was difficult to get fragments which diameter were lower than 200 μm (DM basis), we combined (1) the classes 0–80 and 80–100 μm in one class 0–100 μm and (2) the classes 100–125, 125–160 and 160–200 μm in one class 100–200 μm. Figure 1 showed a marked decrease in SRL with increasing root diameter.

Mixer speed and percentage of biomass lost during the ''sieving method''

Root systems of *B. erectus* (about 500 mg DM each) were stained with methylene blue (5 g l^{-1}) to increase contrast during scanning. They were rinsed, spread out in water onto a mesh tray, and finally transferred onto transparent acetate sheet. To obtain 16 root systems of 500 mg DM each, it was necessary to spread roots on more than 100 acetate sheets. The sheets with roots were led in an oven and dried at 50°C overnight. To determine root length and diameter, dry roots were scanned and analysed using WinRHIZO, with the scanning protocol proposed by Bouma et al. (2000): a resolution of 16 p mm^{-1} (400 dpi), a root density less than 0.5 mm root per mm^2 surface and the automatic threshold option. Diameter class limits were fixed in order to correspond to the sieve mesh sizes used with the sieving method (i.e. 2,000–800, 800–630, 630–500, 500–400, 400–315, 315–250, 250–200, 200–160, 160–125, 125–100, 100–80 and 80–0; standard AFNOR norm). After scanning the 18 root systems were recovered, weighted and submitted to the sieving method, using four different mixer rotation speeds: 2,000, 4,000, 6,000 and 8,000 rpm (4 root systems per mixer speed). They were then sieved during 22 min and the content of each sieve was weighted. The sum of the root biomass recovered in each of the 12 sieves was compared to the root biomass measured before the cutting process, in order to estimate the percentage of biomass lost during the ''sieving method.'' Root biomass distribution was transformed in root length distribution (see above) and compared with WinRHIZO results. The optimal mixer speed was determined according to cumulated differences between the root length proportions obtained by image analysis and those obtained with the ''sieving method.''

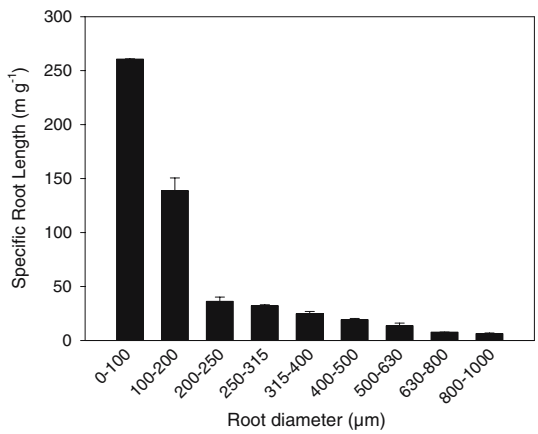


Fig. 1 Mean and standard error of specific root length (*SRL*) for root fragments of *Bromus erectus* Huds from different diameter classes

Length of sieving

To test the effect of the sieving time on root diameter distribution, a single root system of *B. erectus* (600 mg DM) was used and cut at 2,000 rpm. Ten sieving times were performed: 0.25, 0.5, 2, 5, 15, 30, 45, 60, 90 and 120 min. Randomly, we first applied a sieving period of 0.5 min and weighed the content of each sieve. Root fragments from all sieves were retrieved, mixed together, placed in the sieve column and sieved during 5 min. This procedure was renewed for 60, 30, 15, 2, 120, 0.25, 90 and 45 min. We then determined for each diameter class, how long it took to obtain 95 or 99% of the biomass recovered after the longest sieving time (120 min).

Validation of the sieving method with artificial root systems of known length distribution

The accuracy of the sieving method was also tested on nine artificial root mixtures of known root diameter distribution: six mixtures of

Arrhenatherum elatius (L.) Beauv. and three mixtures of *B. erectus*. Root systems of both species were harvested and carefully washed under a fine water stream to remove soil and organic matter particles. For each species, different root axes were selected, spread on filter paper and dried horizontally at 50°C overnight. Ramifications emerging from the main root axes were removed with a scalpel and axes were then coarsely sorted in diameter classes. Because it was a very laborious process, only three diameter (D) classes were considered: $D < 160$ μm; 160 μm $< D <$ 315 μm; $D >$ 315 μm. Each class limit corresponded to one of the sieve mesh sizes available (standard AFNOR norm). In order to eliminate improperly classed roots, the roots of each class were scanned with WinRHIZO using the root diameter distribution option with the class limits set at 160 and 315 μm. Three homogeneous diameter classes were thus constituted. Nine artificial root systems or ''mixtures'' (six from *A. elatius* and three from *B. erectus*; each 90 mg DM) were prepared by mixing known weights of axes from the three diameter classes; the percentage of root biomass in each class was calculated for each mixture (''actual percentage''). Roots of the three mixtures were broken up at 2,000 rpm during 22 min. Two sieves with a mesh size of 160 and 315 μm were used to separate the root fragments into the three classes defined in WinRHIZO. Roots of each diameter class were weighted and expressed as a percentage of the total root biomass recovered (''measured percentage''). The actual percentage of root biomass in each diameter class was compared to the measured percentage in a regression analysis. The method can be judged accurate if the intercept does not differ from 0 and if the slope does not differ from 1. All statistical analyses were performed with SAS (SAS 1989).

Example of application

The ''sieving method'' was applied to determine the root diameter distribution of three entire root systems of *Oryza sativa* L. cv. Moroberekan. Plants were sown in pots filled with 1 kg of pure sand from Fontainebleau (France). They were grown for 3 months under artificial light (600 μ mol photons m^{-2} s^{-1}) at 28°C/day and 24°C/night temperatures and at 75 ± 5% humidity and watered with a modified Hoagland nutritive solution. The average root system dry mass was 3.2 g; it was broken up at 2,000 rpm during 22 min and placed in the sieve column equipped with 11 sieves.

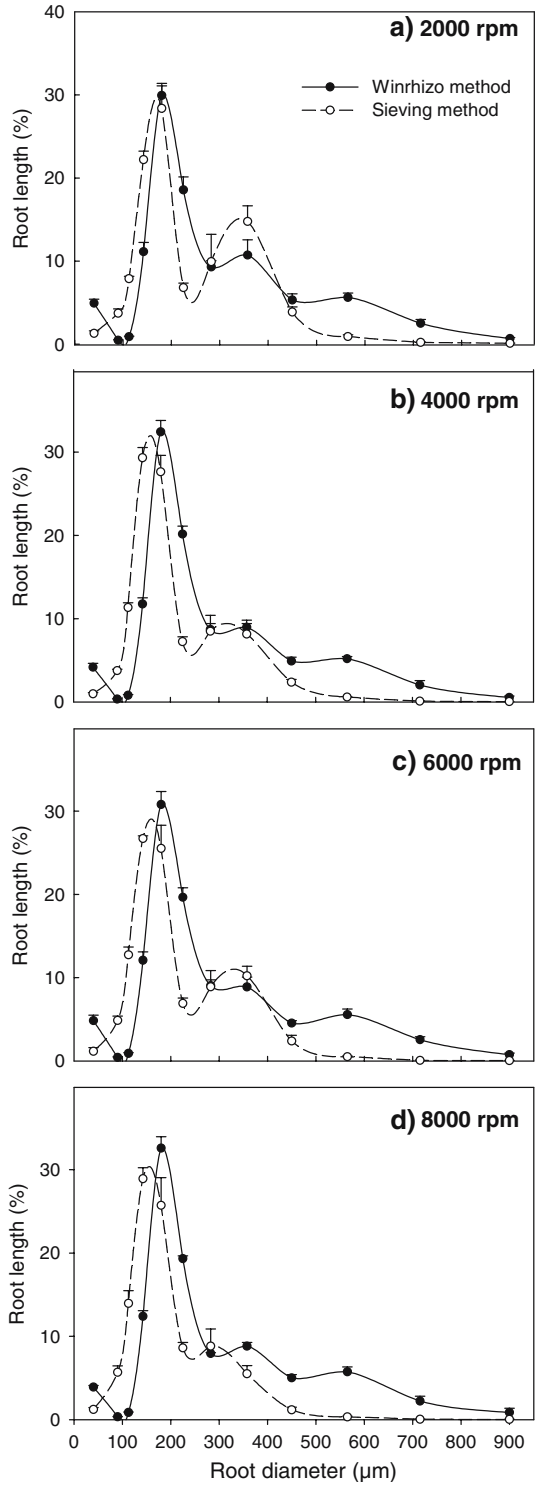
Results

Loss of biomass during sieving method application

The proportion of material lost during the sieving method application, was dependant on the mixer speed. At 2,000 rpm, 4.5 ± 0.3% of the root biomass was lost during cutting, differential sedimentation and drying (mean ± S.E., $n = 4$); losses reached 6.9 ± 0.2, 8.4 ± 0.4 and 10 ± 0.5% for a cutting speed set at 4,000, 6,000 and 8,000 rpm, respectively. In addition, an average 1.1 ± 0.7% of root biomass was lost during sieving and weighting (mean ± S.E., $n = 10$). Altogether, about 6–11% of the root biomass was lost during method application according to the mixer speed. The following results are thus expressed as percentage of the root biomass recovered in the different sieves.

Effect of mixer speed on diameter distribution

Figure 2 showed length diameter distribution measured either with WinRHIZO or the sieving method, for four different mixer speeds. The length distribution measured with the two methods showed rather similar pattern. There was however a small shift between methods: the sieving method overestimates the length of roots with diameter ranging from 80 to 160 μm and underestimates the length of roots with diameter ranging from 160 to 250 μm and from 400 to 800 μm. This shift cannot be attributed to a modification of root diameter due to root drying, since both methods were applied on dry roots. However, it could be due to a lack of resolution of WinRHIZO at 16 p mm^{-1} (400 dpi): the size of a pixel being too important to distinguish correctly for the smallest diameter classes. Thus some very fine roots (<125 μm) were probably not considered in the right diameter class. Another



◀**Fig. 2** Effect of mixer rotation speed on the root length diameter distribution of *Bromus erectus* Huds. The root length diameter distribution obtained with the "sieving method" (○) was compared with that obtained with the image analysis using WinRHIZO (●). For the sieving method, four mixer speeds were tested: **a** 2,000 rpm, **b** 4,000 rpm, **c** 6,000 rpm, **d** 8,000 rpm. A sieving time of 22 min was applied to obtain biomass distribution in 11 sieves (AFNOR norm). Root diameter refers to the average diameter of each diameter class. Diameter distribution was determined on four root samples per mixer speed and per method

explanation could be that the cutting process modified roots with large diameter.

By comparing global biomass distributions obtained at 2,000, 4,000, 6,000 and 8,000 rpm, we found no significant differences (MANOVA, Wilks' Lambda test, $P = 0.31$). However, as we wanted to estimate the mixer speed at which the sieving method results were the closest to WinRHIZO results, we summed the absolute differences between the image analysis and the sieving methods for each diameter class. The best speed was 2,000 rpm, and then, in decreasing order, 4,000, 6,000 and 8,000 rpm. Data not presented here suggested that the optimal speed was slightly higher than 2,000 rpm.

Sieving time

Biomass percentage in each class was reaching a plateau when the sieving time was increasing (Fig. 3a, b). Consequently, the best regression model was an inverse function ($y = a/x + b$). For the finest diameter classes, i.e. diameter < 160 µm (Fig. 3a), the biomass percentage significantly increased ($a > 0$) with the sieving time, while it did not changed for the two middle classes, i.e. 160–200 and 200–250 µm (Fig. 3a). For the thickest roots, i.e. diameter > 250 µm (Fig. 3b), an increasing sieving time reduced the biomass percentage ($a < 0$). These results suggested that root fragments cross large meshes when the

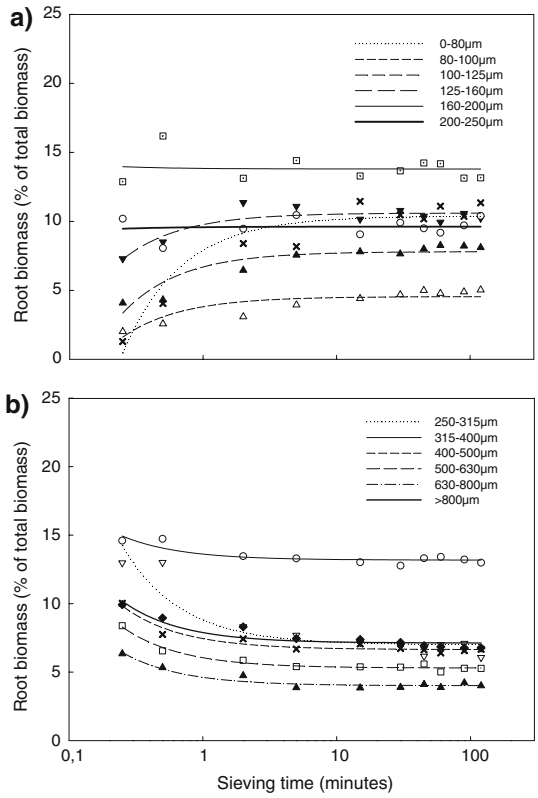


Fig. 3 Effect of the sieving time on the biomass diameter distribution of a single root system of *Bromus erectus* Huds. **a** Diameter classes from 0 to 250 µm: biomass percentage was increasing with the sieving time. **b** Diameter classes from 250 up to 800 µm: biomass percentage was decreasing with the sieving time. The sieving method was applied with mixer rotation speed at 2,000 rpm and with ten different sieving times: 0.25, 0.5, 2, 5, 15, 30, 45, 60, 90 and 120 min. Diameter classes corresponded to the mesh size of 11 sieves (AFNOR norm). *Lines* corresponded to non-linear regressions ($y = a/x + b$, $n = 10$)

sieving is long, and thus fall on the smallest sieve meshes. The two middle classes are globally earning as much root weight from the upper classes as they are loosing towards the lower classes. From regression equations, we determined that all classes presented a biomass proportion higher or equal to 95% of the biomass recovered at 120 min (taken as the plateau value) after 5 min of sieving. It was necessary to sieve during 22 min to get at least 99% in each class. This last sieving time was thus used in the following analyses.

Quality of the roots in each biomass diameter class

Root fragments recovered in each sieve after application of the sieving method with a mixer rotation speed at 2,000 rpm and a sieving time of 22 min were observed with a binocular. Root fragments were homogeneous within each class diameter, the mean diameter of each class was increasing according to the sieve mesh and there were few remaining lateral bases attached to the main axes. However, some roots of the middle class (250–400 µm) were only stele without cortex. These roots were probably larger than 400 µm before method application, but their cortex has probably been removed during the cutting process. Nevertheless, very little cortex dust was found in the thinner classes, suggesting that these light particles of cortex were loss during method application.

Validation of the sieving method with artificial root systems of known biomass distribution

When artificial root systems of known biomass distribution were cut at 2,000 rpm and sieved for 22 min using two sieves, the analysis showed that the biomass proportion measured with the sieving method were close to the actual ones (Fig. 4). The linear regression line between actual and measured biomass proportions was close to the bisecting line. The equation of the bisecting line is $y = x$; the one between actual *versus* measured biomass proportions was $y = 0.89x + 3.67$, with $r^2 = 0.87$ ($n = 27$). The slope was not significantly different from 1 ($P = 0.12$), and the ordinate at origin was not significantly different from 0 ($P = 0.21$).

Method application and repeatability

The distribution of root biomass in diameter classes was measured with the sieving method for three rice root systems. The distribution presented three peaks at 160–200, 400–500 and 630–800 µm (Fig. 5). These peaks probably corresponded to branch orders of the ramified root system: axes, first and second order laterals. Root system developmental classification (Rose 1983)

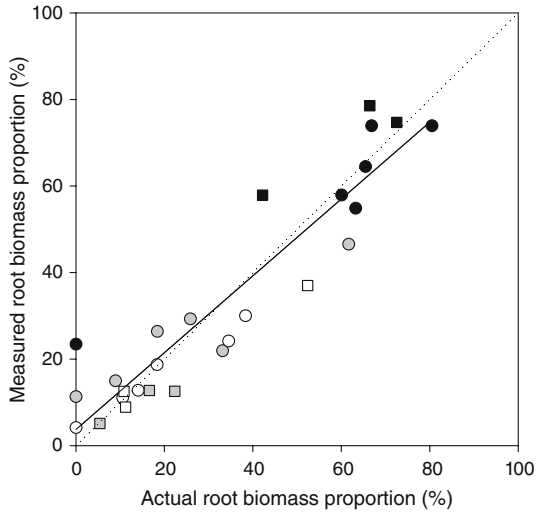


Fig. 4 Actual *versus* measured root biomass proportion in three diameter classes for nine artificial root mixtures of *Arrhenatherum elatius* (L.) Beauv. (○; six root systems) and *Bromus erectus* (□; three root systems). ○,□: diameter (D) < 160 µm; ●,■: 160 µm < D < 315 µm; ●,■: D > 315 µm. Actual biomass diameter distribution was obtained using WinRHIZO; measured biomass diameter distribution was obtained using the "sieving method" applied with mixer rotation speed at 2,000 rpm and sieving time of 22 min. The *dashed line* represents the 1:1 line. The *solid line* denotes a significant regression ($y = 0.89x + 3.67$, $r^2 = 0.87$, $n = 27$)

could thus be assessed with our method. Standard errors of average biomass proportion of each root class were very small (<1%, excepted for three classes) (Fig. 5). Because standard errors reflected both the variability due to differences between the three root systems and the variability due to the method itself, this suggests that the method itself did not introduce variability and is thus repeatable.

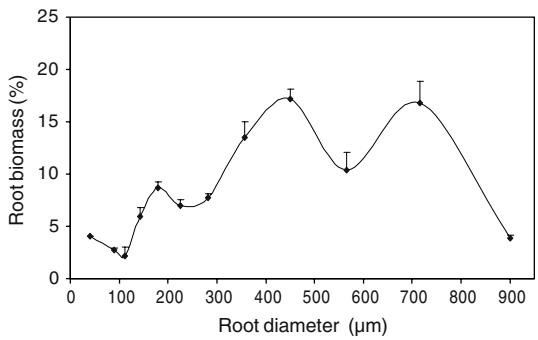


Fig. 5 Root diameter distribution of *Oryza sativa* L. measured with the "sieving method" applied with a mixer rotation speed at 2,000 rpm and a sieving time of 22 min. Diameters refers to the average diameter of each diameter class defined by the mesh size of 11 sieves (AFNOR norm) (mean ± S.E.; $n = 3$)

Discussion

We described a new method, the "sieving method," to determine the distribution of root biomass in diameter classes. The patterns of root diameter distribution obtained with the sieving method and by image analysis with WinRHIZO were very close (Fig. 2) and no significant difference was observed between the two methods (Fig. 4). There was however, a small shift between distributions obtained using WinRHIZO and the "sieving method" (Fig. 2); the proportion of large-diameter roots was underestimated with the sieving method. Two explanations can be proposed. First, the validation of our method by a comparison with results obtained by image analysis asks the question of the reliability of WinRHIZO to measure root length distribution in diameter classes. As every method, this one has its own bias, especially related to the resolution level (Bouma et al. 2000; Himmelbauer et al. 2004). Thus, the distribution taken as reference could slightly differ from the actual distribution. If we assume that the referent distribution is the actual one, the underestimation of large-diameter roots proportion could otherwise be explained by large roots decortication during the cutting process. After drying, the cortex of the thickest roots became easily breakable and during cutting, it was sometimes torn off by the flail of the mixer. Some roots with a diameter > 500 µm were changed in decorticated roots (with smaller diameter) and fragments of cortex. Consequently, there was as a general tendency a biomass deficit in the diameter classes >500 µm and a biomass surplus in the diameter classes <500 µm. This hypothesis was confirmed by binocular observations which showed few decorticated roots among roots from the middle classes. These tendencies are increased when the mixer speed is increased. Sensitivity analyses showed that for the two grass species used in this study, the more accurate results are obtained with mixer speed set at

2,000 rpm and with a sieving time of 22 min. For other species with contrasted root morphology, preliminary tests are recommended to determine the optimal mixer speed. For root systems with large proportion of thick and fragile roots, the cutting process could be made with a knife mixer instead of a flail mixer: in fact, a flail mixer beats root fragments, causing damage to the more fragile, whereas a knife mixer would only slice root fragments. This required further analyses.

Some fundamental differences with other methods can be seen as advantages or drawbacks, according to the aim of the study. First, our method is the only one allowing determination of *biomass* distribution among diameter classes, whereas image analysis methods deal with *length* distribution among diameter classes. The conversion from length to biomass is possible when the SRL is known. We showed, as Pregitzer et al. (1997), that SRL decreased rapidly as root diameter increased (Fig. 1). The SRL of root with a diameter lower than 200 µm was difficult to estimate (Sect. ''Sensitivity analysis''), whereas it is extremely sensitive in this specific diameter range (Fig. 1). As small changes in SRL estimates induced important errors in the conversion of length to biomass, it seems that this conversion has to be avoided. So, image analysis and the sieving method are two complementary methods, which should be used to measure respectively length and biomass.

Secondly, the sieving method necessarily deals with *dry* roots due to the cutting and sieving processes, while other methods analyse the root diameter distribution of *fresh* roots. As far as accuracy is concerned, root diameter of fresh roots is closely linked to root water content, which varies greatly among sub-samples of a single root system (Costa et al. 2000). Root drying could thus be a way to avoid this variability source. If fresh root parameters are required, dry root parameters can be converted in fresh root parameters using equations linking fresh and dry diameters, lengths or weights. In this study fresh root diameter (FD) is closely related to dry root diameter ($DD = 0.65\ FD + 0.1$, $r^2 = 0.87$, $P < 0.001$, $n = 35$), fresh length (FL) to dry length ($DL = 0.89\ FL + 0.08$, $r^2 = 0.94$, $P < 0.001$, $n = 35$) and fresh weight (FW) to dry weight ($DW = 0.23\ FW + 0.009$, $r^2 = 0.79$, $P < 0.001$, $n = 35$).

Third, diameter classes are imposed by sieve meshes, whereas they can be chosen with image analysis. However, if AFNOR norm classes are not adequate, other sieve meshes can be found in other standard norms.

Finally, the ''sieving method'' focuses only on biomass distribution in diameter classes, it is thus adequate to estimate biomass allocation among diameter classes, while other methods are best suited to estimate architecture, since in addition to root length diameter distribution, they give information about a variety of root traits, e.g. root area, volume, number of tips, root topology.

In spite of these specificities, the sieving method presents some clear advantages over others. First, it can be use for entire, large root system. To solve problems linked to large root systems, most methods need sub-sampling of root systems (Bouma et al. 2000; Himmelbauer et al. 2004; Kimura and Yamasaki 2003). But root development is centrifugal and hierarchical, and diameters are heterogeneously represented in the environment. A sub-sampling representative of the entire root system is therefore difficult to obtain, and differences between treatments or plots could be missed if the variability due to sub-sampling is too high. To our knowledge, no one has addressed the question of whether sub-samples are representative of the entire root system, with the exception of Costa et al. (2000). To solve the sampling problem, it has recently been proposed to sample a homogeneous mix of a fragmented root system obtained using a mixing device (Costa et al. 2000; Pietola and Smucker 1998). A bootstrapping statistical procedure (Costa et al. 2000) makes it possible to determine the number and size of samples that should be studied to obtain a correct representation of the entire root system. Nevertheless, in new situations, i.e. with other species or environmental conditions, the number and size of samples has to be recalculated. Such a rigorous approach to root system sampling has not been used since Costa et al. (2000). Today, sub-sampling is used without testing the representativity of sub-samples in spite of a well-demonstrated effect on the results. Our method avoids this problem: entire root systems can be analysed, without sub-sampling.

Another advantage of this method is that it is less time-consuming than others. First, the root system can be washed very quickly, even coarsely; remaining soil particles or dead organic matter will be separated from root pieces after cutting, by differential sedimentation. This avoids the use of complex root/soil separation devices (Smucker et al. 1982; Benjamin and Nielsen 2004). Soil and root separation takes 20 min: washing coarsely (5 min), cutting (5 min) and differential sedimentation (10 min). This was shorter than with the automatic washing mechanism described by Benjamin and Nielsen (2004), which requires 90 min. However, this was longer than the hydropneumatic elutriation system which requires 3–10 min according to soil texture and plant species, but concerns only small soil cores between 115 and 825 cm^3 (Smucker et al. 1982). Secondly, roots did not need to be spread on the scanner, the most time-consuming step in image analysis. Excluding washing and cutting times, Costa et al. (2000) estimate that approximately 43 h would have been necessary to analyse an entire root system of *Zea mays* L. (about 130 g of fresh biomass) with WinRHIZO without sub-sampling, whereas they needed 6 h by applying the sub-sampling procedure described above. After the separation of soil and roots, our method requires only 40 min to sieve automatically (22 min) and to weight the content of the 12 sieves (20 min). Taking into account all manipulations except drying, 60 min were needed per root system, whatever its size. This saving of time opens new perspectives, making possible analyses of large and numerous root systems.

By avoiding fastidious sub-sampling and time-consuming approaches, this method allows biomass, or eventually length measurements on entire large root systems which were seldom analysed before now. Because it is accurate, repeatable, cheap and quick, it will be of great interest in agronomical and ecological research, where statistical analysis requires the comparison of many root systems in a variety of environmental conditions.

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