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REGULAR ARTICLE

An optimized fine root sampling methodology balancing accuracy and time investment

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Abstract
Aims Tree roots are spatially highly heterogeneous and it thus requires large numbers of samples to detect statistically significant changes in root biomass. The objectives of this study were to understand and quantify the sources of error in the assessment of fine root biomass (<2 mm) during the second year of a high-density *Populus* plantation.
Methods Soil cores were collected in winter (n=35) and in summer (n=20), and fine roots were picked by hand for varying lengths of time: 1, 2, 5, 20, 40, and 60 min. The root biomass data were used to identify the best combination of the time spent for root picking and the number of samples collected, that minimizes the overall uncertainty (i.e. the combination of the spatial error due to the incomplete sampling and the temporal error due to the incomplete core processing).
Results On average, 25 min was enough time to pick 90 % of the fine root biomass in winter, while in summer only 10 min were needed. In winter fewer samples were needed, but more time for picking was necessary as compared to summer when root biomass was higher.
Conclusions Fine root sampling can be optimized by minimizing the uncertainty of the biomass estimates and simultaneously decreasing root sampling time investment.

Keywords Auger sampling · Sampling time · Root picking time · Spatial error · Temporal error

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Introduction

For 250 years various techniques and methods have been developed for studying roots (Evelyn 1662; Noehden 1824), but all methods have their limitations (Jackson et al. 1996; Lauenroth 2000; Nadelhoffer and Raich 1992). Fine roots represent only a small fraction of the total root biomass in forest ecosystems (Jackson et al. 1997). But in comparison with their small contribution to the standing root biomass, fine root dynamics play a large role in biomass production and allocation, in plant-soil interactions, and in carbon cycling (Nadelhoffer and Raich 1992; Ostonen et al. 2005; Tufekcioglu et al. 1998). Fine root turnover represents a major carbon cost to the tree (Janssens et al. 2002) and a large carbon input to the soil (Ruess et al. 1996). Within the framework of the changing climate and the increasing demand for ecosystem

services provided by forests, the ability to accurately quantify fine root dynamics remains a daunting, but essential challenge that must be overcome (Brunner and Godbold 2007).

Over time a considerable number of methods has been developed to assess fine root biomass and fine root turnover (Böhm 1979; Mancuso 2011; Persson 1980; Publicover and Vogt 1993; Stokes 2000; Waisel et al. 2002). These methods include allometric techniques (e.g. root:shoot or other ratios), the direct excavation of the root system, core sampling, as well as in situ imaging methods (Mancuso 2011; Vogt and Persson 1991). Each of these methods has several sources of error. The analysis of data obtained from root sampling is constrained by the experimental design and by the associated statistical properties of the population of roots sampled. In a comparative study of different techniques for the assessment of biomass of fine and medium-sized roots, soil core sampling provided the same accuracy and was more cost effective than entire tree excavations (Jourdan et al. 2011). However, there is still no "uniform standard approach" for the assessment of fine root biomass, partly because each ecological setting requires a sampling procedure tailored to the specific situation. Therefore, an approach to optimize fine root sampling using soil cores that specifically accounts for the major sources of error would be of great help in forest ecological studies.

Fine root biomass is spatially and temporally highly variable (Metcalfe et al. 2008). In the core sampling method volumetric soil samples are taken manually in the field and washed in the lab to separate roots from the soil (Oliveira et al. 2000). The researcher chooses the number of samples to be taken (normally ranging from 8 to 30), and this decreases the error around the mean (Vogt and Persson 1991). Temporal changes in root biomass can only be detected if the assessments at different points in time are statistically different (Publicover and Vogt 1993). It is thus crucial to minimize the standard deviation of the mean. The power of the assessment thus increases with increasing sample size (Bengough et al. 2000). As root sampling is time consuming, the time and cost associated with increasing sample numbers rapidly increase and often become unrealistic (Metcalfe et al. 2007). For a given time available, the spatial sampling error declines with higher numbers of samples, but comes at the expense of the time that remains available for root picking in the lab (temporal error). The objectives of this study were (i) to understand two sources of error on the root biomass assessments (spatial and temporal), and (ii) to use experimental data to develop a statistically robust method of minimizing both the spatial and the temporal errors while at the same time decreasing the root sampling time costs. Other minor errors and difficulties are associated with root sampling, as vitality (live/dead), species recognition, loss fractions while picking or through sieves, losses through prolonged storage, soil texture and humidity, etc. But these are not being considered in the present study.

Materials and methods

Experimental field site

A high-density, short-rotation *Populus* plantation served as the experimental field site to provide the data. Fine root data for the current study were collected within the framework of a large-scale bioenergy research project (POPFULL; Broeckx et al. 2012; webh01.ua.ac.be/popfull). The experimental site was located in Lochristi, Belgium (51°06′N, 03°51′E) and consisted of a high-density poplar (*Populus spp.*) plantation. The long-term average annual temperature at the site is 9.5 °C and the average annual precipitation is 726 mm (Royal Meteorological Institute of Belgium). The soil has a sandy texture with a clay-enriched deeper soil layer, but a marginal profile development because of frequent deep tillage. The soil carbon (measured in February–March 2010 prior to planting) in the first 15 cm of the soil was on average 1.73±0.41 (%), the carbon:nitrogen (C:N) ratio 11.6±1.8 (n=110) and the bulk density was 1.361±0.13 g cm^{-3} (see Broeckx et al. (2012) for more details). Soil pH in the first 30 cm averaged 5.29±0.49 (n=42 locations distributed over the site).

After initial soil sampling and site preparation, 12 poplar clones were planted in monoclonal blocks in a double-row planting scheme on 7–10 April 2010. The distance between the narrow rows was 75 cm and that of the wide rows was 150 cm. The distance between trees within a row was 110 cm, yielding an overall density of 8,000 trees per ha. A total of 14.5 ha were planted. Manual and chemical weed control were applied during the first and the second year. No fertilization or irrigation was applied during the experiment.

Quantification of root biomass and of duration of root picking

Core sampling was used to assess fine root biomass dynamics during the second year of the plantation. Root biomass was estimated from soil samples collected up to 15 cm depth using an 8 cm diameter× 15 cm deep hand-driven corer (Eijkelkamp, The Netherlands) (Oliveira et al. 2000). 35 samples collected in winter (February–March 2011) and 20 samples collected in summer (July–August 2011), fine roots (<2 mm) were picked manually in the laboratory for 1, 2, 5, 20, 40 or 60 min. The time intervals were shorter at the beginning in order to capture the increments of root biomass at early phases of the root picking. Roots from weeds were separated from poplar roots and ignored from here on. At each time roots were washed in a plastic cuvette and weighed to determine the root biomass picked. Fresh biomass collected at each picking duration was later transformed to the proportion picked (see below). After the fresh biomass had been determined, roots were put into paper bags. Roots were dried at 70 °C to constant mass and expressed in dry matter (DM, g). Root biomass was scaled to $g\,m^{-2}$. We carefully quantified the time necessary for each step in the process: (1) the transport to the field site (60 km one way), (2) the collection of the samples in the field, (3) the return transport of the samples to the laboratory, (4) the logistic into the laboratory (incl. handling in and out the freezer, from storage to laboratory, and preparation of the materials for root picking), (5) the root picking at each time, (6) the washing and weighing of the sorted roots. For this purpose a chronometer was used. The time for ten individual random samples was measured in steps 2, 4, 5 and 6. The transport time in steps 1 and 3 was measured three times.

Picking duration error and ecosystem scale spatial error

The accumulated fresh root biomass at any given duration of picking was expressed as a fraction of the total fresh root biomass at the maximum time (i.e. 60 min of root picking). It was not possible to use a linear model to relate the accumulated proportion of fresh root biomass with the duration of root picking because the residuals did not have the same variance along the distribution, thus failing to support the assumption of homoscedasticity. Therefore Richard's equation was fitted to the transformed data:

$$y = a\left(1 - e^{-bx}\right)^{c} \quad (1)$$

where y = the proportion of roots picked, x = the duration of root picking, a = the parameter that describes the maximum of the function, b = the parameter that describes the curvature of the function, c = the parameter that describes the lag phase of the function, and e = the base of the natural logarithm (Causton and Venus 1981). The fitted equation was used to estimate the amount of roots picked at all other times. Overlapping of the confidence limits (95 %) for each parameter and an ANCOVA of the residuals (with root picking duration as covarying factor) were used to test for differences between the curves fitted to winter and summer samples.

Means and standard deviations were calculated for the proportion of fresh root biomass collected at each duration of picking (1, 2, 5, 20, 40 and 60 min). Using Eq. 2, we then estimated the number of samples that could be processed within a given amount of time invested, i.e. 100, 300, 600, 1,200 and 2,400 min. The total time invested was divided by the time necessary to process one sample (sample + logistic + duration of root picking + sorting, washing & weighing) to obtain the number of samples that could be processed:

$$n(\text{samples}) = \frac{Time\ invested(\text{min})}{Time\ processing(\text{min}/\text{sample})} \quad (2)$$

The standard error for each picking duration was obtained by dividing the standard deviation by the square root of the number of samples obtained from Eq. 2. This standard error was then divided by the mean to obtain the relative standard error, defined as the picking duration error (PDE).

From the mean and the standard deviation of the fresh root biomass collected after 60 min of picking, we estimated the ecosystem scale spatial error (ESSE) for different numbers of samples, both for winter and summer samples. The standard error was divided by the mean to obtain the ESSE for all numbers of samples. The different relative standard errors for different numbers of samples were used to assess the spatial variation in the field. More details of the calculation could be found in the Appendix 1.

For a different number of samples collected in the field we thus calculated a PDE and an ESSE. Both standard errors were summed to obtain the total relative standard error (TRSE). The PDE and ESSE were plotted against the number of samples, and the minimum TRSE was selected as the optimal number of samples collected for a given time period (e.g. winter, summer).

Results

Fine root biomass varied significantly among sampling periods. For the subset used for the error analysis (winter n=35, summer n=20), total fresh root biomass at 15 cm depth was 62.4 gm^{-2} (14.0 g DM m^{-2}) in winter versus 320 gm^{-2} (75.4 g DM m^{-2}) in summer.

Fresh root biomass increased and PDE decreased with increasing duration of root picking (Fig. 1). The recovery of roots was faster at the beginning of the picking as there were still more roots in the sample. The increments of the proportion of roots picked decreased with increasing duration of picking. In general 30 % of all fine roots were picked after the first minute. Root picking for 60 min instead of 40 min only increased the recovered root biomass by 2 %. On average, 25 min of root picking was enough to pick 90 % of the root biomass in winter, while 10 min sufficed for the same proportion of roots in summer. The proportion of roots picked after a certain period was proportional to the root biomass in the sample. The time necessary to pick 90 % of the fine root biomass decreased with increasing root biomass in the sample.

Part of the time devoted to process one sample was variable while another part required a constant amount of time (Table 1). The time spent per sample in the field and handling in the laboratory was constant. Also the time needed to collect a sample in the field and to transport it to the laboratory was constant. So, these durations were similar for each sample and independent of the duration of root picking. In contrast, the time needed for washing and weighing increased with the duration of root picking, because more roots were retrieved that needed to be washed and weighed. By far most of the time spent for each sample was devoted to manually separating the roots from the soil, i.e. the root picking. An overview of the time cost of a sample collection campaign and the concomitant analysis is shown in Table 2. Transport (i.e. the driving time) to the field site was independent of the amount of samples. When only a few samples were taken, the time spent in transport represented a high proportion of the total time invested (including transport). With more than 30 samples the transport represented only 3–5 % of the total time cost. The time spent in root picking, sorting, washing and weighing represented 84–93 % of the total time needed to process the samples. Tripling the picking duration (from 20 to 60 min) only doubled the total time needed.

An increase in the duration of root picking was accompanied by a reduction in the uncertainty of the

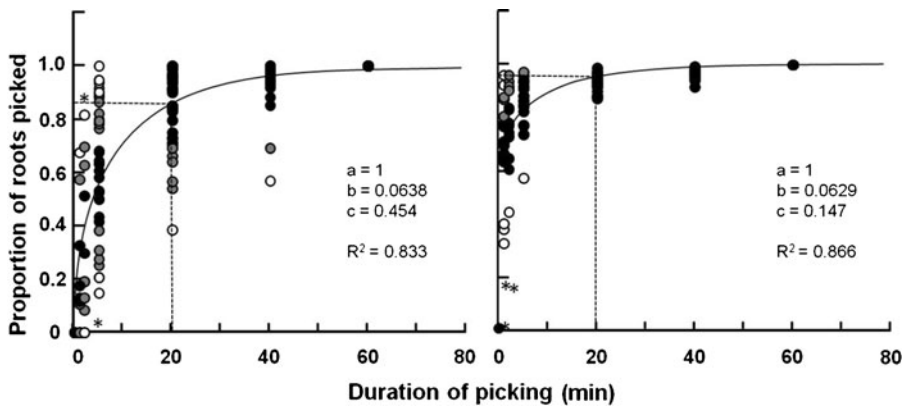


Fig. 1 Increments in the proportion of fresh fine root biomass picked as a function of the duration of root picking in winter (*left*) and summer (*right panel*). Proportions are relative to the maximum root biomass picked after 60 min. Richard's equation $\left(y = a\left(1 - e^{-bx}\right)^{c}\right)$ was fitted through the data points. *Black dots* are at <1 SD, *grey symbols* are at <2 SD, *empty symbols* are at <3 SD and *asterisks* are at >3 SD. The *dotted line* represents the proportion of root picked at a picking duration of 20 min. SD = standard deviation

Table 1 Time per sample devoted to drive to the field; to collect the samples in the field; to transport samples to the laboratory; to store and to handle the samples in the laboratory; to pick, to sort, to wash and to weigh the roots. The handling in the laboratory includes the transport from the car to the storage room, in and out of the freezers and the oven, and from the storage room to the laboratory. The times to drive to the field, to collect the sample, to transport the samples to the laboratory and to handle in the laboratory were the same for any duration of root picking. All values were rounded to the nearest entire number and are all given in min

Duration of picking	Sample in the field	Handle in the laboratory	Sort, wash & weigh	Total time in lab	Transport to the site	Total time including transport
1	4	3	8	16	120	136
2	4	3	10	18	120	138
5	4	3	11	23	120	143
20	4	3	14	41	120	161
40	4	3	15	62	120	182
60	4	3	16	83	120	203

fine root estimation (Fig. 1). By increasing the duration of root picking by four (from 5 to 20 min) we gained 41 % in accuracy. The fitted Richard's equation and the associated PDE significantly differed between the sampling periods (p=<0.001; 95 % confidence limits of the parameter c for winter: 0.340–0.566 and summer: 0.089–0.204). The ANCOVA further indicated that this difference was not affected by root picking duration (p=0.59). In winter it took 15 min to pick 80 % of the roots while in summer it took only 4 min for the same proportion of fine roots. Overall, a higher root biomass and a better accuracy were obtained in summer than in winter. Thus, sampling periods greatly affected the time needed to retrieve the root biomass from each sample.

The duration of root picking and the associated PDE played an important role in determining the optimal number of samples to collect (Fig. 2). For a given total time invested, there was a trade off between the time spent in sampling and in root picking; when we collected more samples, the time available for root picking per sample decreased. Reducing the time of root picking increased the PDE (Fig. 1). By definition, the PDE was zero for the maximum time devoted to picking (60 min) and was largest at the minimum time devoted to root picking (1 min). However, as the PDE increased with larger numbers of samples, the ESSE decreased. The minimum ESSE was obtained with the maximum number of samples.

The magnitude and the importance of the two sources of error were different for sampling periods. The ESSE was similar in both seasons, but in summer the PDE was lower (Fig. 1) and the minimum TRSE was reached at a higher number of samples than in winter

Table 2 Time devoted to pick, to sort, to wash and to weigh the roots; to collect the samples in the field; to bring to and to store in the laboratory; and to drive to the field site for different combinations of picking time and number of samples. All values have been derived from Table 1. The time to transport (driving time) to the field site was the same for any amount of samples or time picking. All values were rounded to the nearest entire value and are given in min

Number of samples	Duration of picking per sample	Total time picking	Sample in the field	Handle in the laboratory	Sort, wash & weigh	Total time in lab	Transport to site	Total time including transport
1	20	20	4	3	14	41	120	161
	60	60	4	3	16	83	120	203
10	20	200	35	30	140	405	120	525
	60	600	35	30	161	826	120	946
30	20	600	105	90	420	1215	120	1335
	60	1800	105	90	483	2478	120	2598
50	20	1000	175	150	700	2025	120	2145
	60	3000	175	150	805	4130	120	4250

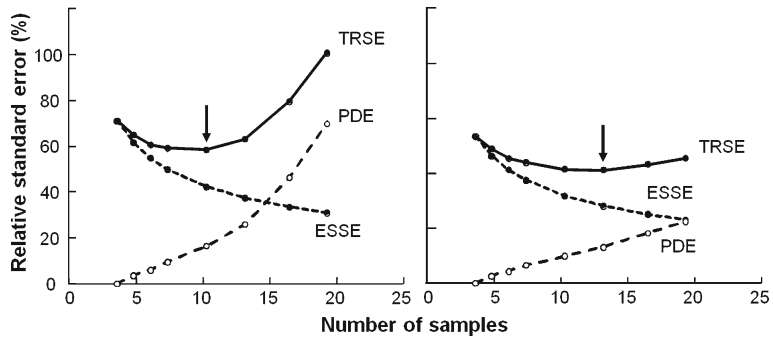


Fig. 2 Total relative standard errors (TRSE) as a function of the number of samples analyzed. Samples were collected in winter (*left*) and in summer (*right panel*) for a total time invested of 300 min. The *dotted line with open symbols* represents the picking duration error (PDE) for a given time available. The *dotted line with solid points* represents the ecosystem scale spatial error (ESSE). This is the standard error around the mean given the standard deviation of the different soil cores collected in the field. The *solid line* represents the sum of both relative standard errors (TRSE = PDE + ESSE). The *arrow marks* the minimum TRSE

(Fig. 2). Consequently, the optimal number of samples differed between sampling periods. More samples were necessary to reach the minimum TRSE in summer than in winter.

The optimal number of samples — defined by the minimum TRSE — not only varied with sampling periods (summer versus winter), but also with the total time invested (i.e. 100, 300, 600, 1,200 and 2,400 min). For the same number of samples, the TRSE was reduced by increasing the time invested. An increment of time invested induced an increment in the optimal number of samples. It was, however, less crucial to be very close to the optimum when the total time devoted increased, because TRSE became much less sensitive to changes in the number of samples (Fig. 3). With more time invested, more samples were needed, but the much smaller sensitivity of the TRSE to changes in the number of samples also allowed a large reduction in the number of samples to be analysed. The optimal number of samples linearly increased with the time invested (Fig. 4, top panel). When TRSE was increased by 10 % the number of samples could be reduced by 40 % in winter, and by 46 % in summer (Fig. 4, top panel, dotted lines). The reduction in the number of samples held regardless of the amount of time invested, because the shorter duration for the collection of the samples was counterbalanced by the longer root picking time.

The TRSE decreased exponentially with the time invested (Fig. 4, lower panel). Decreases in the TRSE were around 30–40 % when the time invested was doubled. These decreases were more important when increasing from 300 to 600 min than when going to 1,200 min. The TRSE was always lower in the summer samplings than in the winter samplings. The smaller TRSE was associated with more time invested because more time implied more samples, and the number of samples is the denominator in the calculation of PDE and ESSE. By definition the sum of PDE and ESSE equalled the TRSE. The dotted lines (Fig. 4, lower panel) show the small increment that represents an increase of the uncertainty by 10 %. The number of samples could be significantly decreased with only a small increase in the uncertainty (Fig. 4).

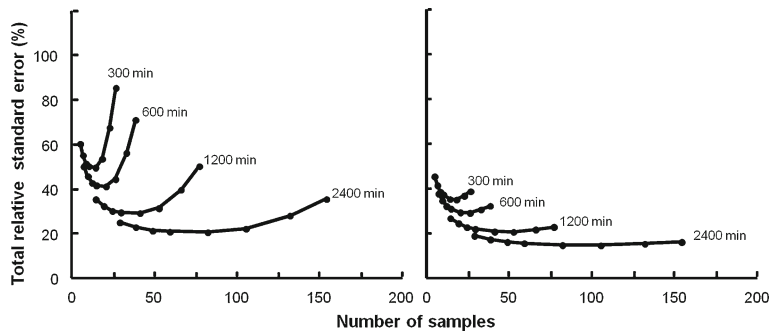


Fig. 3 Total relative standard errors (TRSE) as a function of the number of samples for different time investments (300, 600, 1,200 and 2,400 min) for samples collected in winter (*left*) and in summer (*right panel*). The *lines* represent the total (spatial + temporal) relative standard error. Investing more time reduces the TRSE

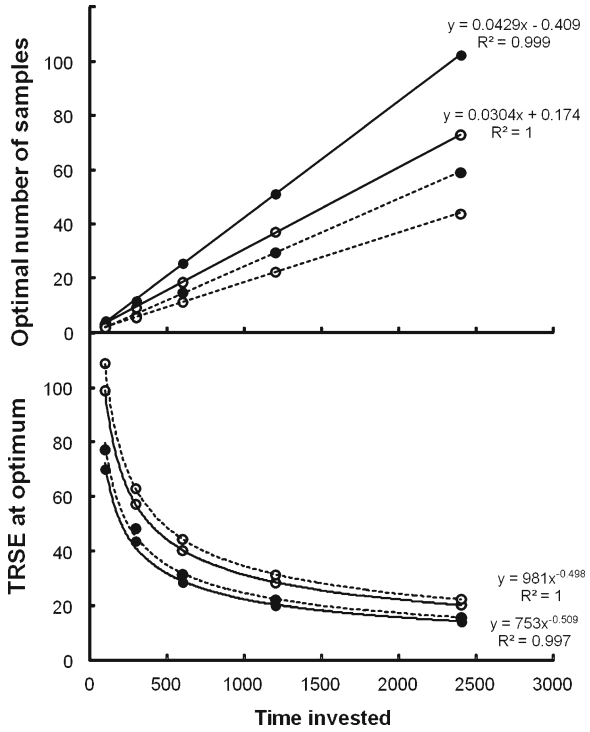


Fig. 4 Optimal number of samples (*top panel*) in relation to the total time invested in the root analysis. *Filled symbols* represent summer samples and *open symbols* represent winter samples. The *solid lines* are the optimal number given by the minimum total relative standard error (TRSE). The *dotted lines* represent the number of samples given by increasing the minimum TRSE by 10 %. Total relative standard error (TRSE) at the optimum (*lower panel*). *Filled symbols* represent summer samples and *open symbols* represent winter samples. The *solid line* represents the TRSE at the optimal number of samples; the *dotted lines* represent increments of the minimum TRSE by 10 %

Discussion

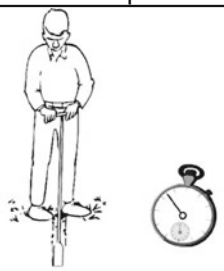
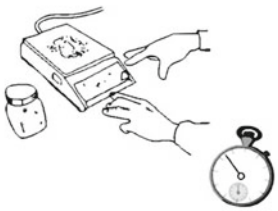
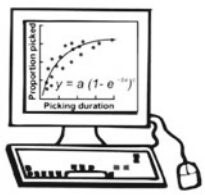
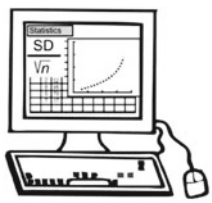
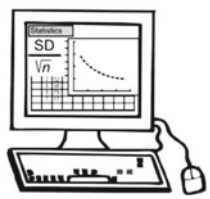
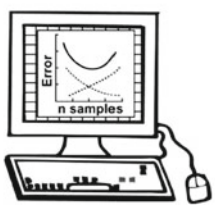
The main objectives of the current study were to understand the sources of error in the estimation of fine root biomass in a young, high-density *Populus* plantation, and to develop a quantitative methodology for optimizing fine root biomass sampling to increase accuracy and decrease time investment costs. Several studies have tried to determine the sample size by only accounting for spatial variation of root biomass distribution (Garten et al. 2007; Liski 1995; Metcalfe et al. 2008). The present study improves this technique by optimizing, for a given time investment, the combination of the picking duration and the spatial errors associated with root sampling. The main message extracted from the study is that sampling effort and time investment processing each core could be minimized in root studies, specially taking into account that after 25 min up to 90 % of the roots were already picked. This is an interesting result as most root researchers often pass a lot more time processing cores of similar size. This has been obtained through a statistically robust methodology that is defined by the specific conditions of the experimental design and the ecological conditions of the tree plantation (Table 3).

The large time investment, and the resulting financial (i.e. personnel) cost, is the primary limiting factor for field sampling of root biomass. As a consequence, several researchers have tried to decrease the time invested in root sampling and analysis (Benjamin and Nielsen 2004; Levillain et al. 2011; Metcalfe et al. 2007). The time needed for washing and weighing, together with the duration of root picking, represented most of the time spent per sample. The time to collect the sample in the field and to transport it to the laboratory was constant for any duration of root picking and only represented a small proportion of the total time, especially in comparison with root excavations (Rodrigues de Sousa and Gehring 2010). The time to drive to the field site is only applicable for the specific situation of this study, but it gives an idea of the proportion of time that was needed for a campaign of root sampling in the field. All this information could be useful to estimate the time cost (in amount of work hours, Table 2), to optimally design a field campaign for root sampling. The required amounts of time and the associated cost of the research are very relevant for realistic project proposals.

Most of the time spent was invested in separating the fine roots from the soil (Table 1). Generally, the amount of root biomass retrieved from a soil sample increases with the duration of the root picking time, while the error decreases (Metcalfe et al. 2007). In our case, the proportion of roots retrieved did increase with the time invested in root picking, but the relationship differed greatly between the sampling periods. This has implications for optimizing the root sampling design. The reason for the easier root picking in summer was probably the higher connectivity or clumping of a larger root biomass.

Differences in the proportion of root biomass picked and the PDE with the duration of picking, defined the optimal number of samples for each season. The optimal number of samples was defined by the error of the estimation of the correct root mass and the time needed to separate the roots from soil. For a given

Table 3 Description of the step-by-step procedure to reproduce the proposed methodological approach. SD = standard deviation, PDE = picking duration error, ESSE = ecosystem scale spatial error, TRSE = total relative standard error

Step	Procedure
	1. Collect samples in the field and quantify the time needed to collect, to transport and to handle each sample.
	2. For each sample pick the roots manually for different durations of time (i.e. 1, 2, 5, 20... min). Sort, wash and weigh the roots after each time duration. Quantify the time for washing, sorting and weighing at each picking duration. Sum the time needed to collect and to handle (step 1) with the time for washing, sorting and weighing (step 2) for each picking duration. Use this information (time) in step 4 (Eq. 2, time processing).
	3. For each sample, convert the biomass picked at each interval as a proportion of the root biomass picked at the maximum duration. Using data from all the samples, fit Eq. 1 ($y = a\ (1- e^{-bx})^c$) to the increments in the proportion of roots picked with time, and obtain the mean and SD for each picking duration.
$\frac{\text{Time invested (min)}}{\text{Time processing (min/sample)}} = n\ \text{(samples)}$	4. Set an available time (time invested) for the root sampling and calculate the number of samples possible for each different root picking duration (step 2) using the Eq. 2.
	5. *Estimate the picking duration error (PDE):* For each picking duration, divide the SD (step 2) by the square root of the number of samples (step 4). Plot PDE versus the number of samples prescribed by root picking duration and the available time (Fig. 2, dotted line).
	6. *Estimate the ecosystem scale spatial error (ESSE):* Take SD of root biomass picked at the maximum picking duration (i.e. 60 min) and divide by the square root of the number of samples (step 4) and then by the mean of the biomass picked at the maximum picking duration to get the ESSE. Plot ESSE versus the number of samples (Fig. 2 dashed line)
	7. Sum ESSE and PDE to obtain the total relative standard error (TRSE). Plot TRSE versus the number of samples (Fig. 2, solid line). Get the optimum sample number with the minimum uncertainty. If the uncertainty is above your expectation, return to step 4 and increase the time available. If the uncertainty is lower than your expectation reduce the time invested in step 3.

distribution the precision of a statistical estimator increases with an increasing number of replicate samples (Underwood 1997). In the present study more samples decreased the TRSE, but this also meant that there was less time available to process the roots. Most of the time spent with the samples was devoted to manually separating/picking roots from the soil (Table 1), in line with recent observations of Rodrigues de Sousa and Gehring (2010). On the other hand the optimal number of samples increased linearly when we had more time available to determining root biomass (Fig. 4, above panel). If we had chosen 600 min instead of 300 min to process the samples, the optimal number of samples would have more than doubled.

The second source of error examined in the current work was the ESSE, i.e. the random error associated with the spatial variation in root biomass distribution. This error has received particular attention from many authors (Metcalfe et al. 2008; Publicover and Vogt 1993). The present analysis demonstrates that if we increased TRSE by an acceptably small amount (by increasing ESSE and reducing PDE, Fig. 2), the number of samples collected could be decreased significantly (Fig. 4). An increase of 10 % of the TRSE allowed us to decrease the number of samples by more than 40 % in both seasons. The decreases in numbers of samples held regardless of the amount of time invested, because the time reduced to take samples was employed in longer pickings. Although this reduction in the number of samples collected does not necessarily mean a reduction in the total time invested in studying roots, it means a reduction in the amount of time spent in the field, in the number of samples to carry/transport, in the storage capacity needed in the laboratory, and in the amount of data management. All of these time durations translate directly into decreased costs, potentially freeing up resources that could be devoted to other aspects of the research.

Ideally, the root sampling methodology should be determined by the objectives of the study, by the experimental design, and by biological characteristics of the root systems being studied. Fine root biomass varies seasonally, normally peaking in summer (Lukac et al. 2003; Santanantonio and Santanantonio 1987). Therefore, some authors have suggested to decrease sampling intensity during periods of expected high root biomass (Vogt et al. 1998). Our study clearly shows that more samples were needed in summer when root biomass was high compared to winter (Fig. 2). The ESSE for both seasons was virtually the same, and therefore the difference resulted mainly from the PDE. Summer samples had more root biomass and inter-connections between roots, resulting in a shorter duration for root picking and in more time available for sampling. These results suggest that it is necessary to vary the sampling intensity, not only in the number of samples, but also in the duration of picking (i.e. separating roots from soil).

Root sampling should also follow the spatial variation in root distribution. The current study focused on the top layer of the soil only, and on specific times during the growing season. Root biomass tends to decrease with depth (Jackson et al. 1996) while the ESSE increases (Trumbore et al. 2006). Fine root depth profiles differ between tree species (De Baets et al. 2007), between clones (Al Afas et al. 2008), and even for the same clone with differences in management (Mulia and Dupraz 2006). Furthermore root biomass and composition (diameter, species, etc.) change during the year, and differently for top layers and deeper soil layers (Burke and Raynal 1994; Janssens et al. 2002; Santanantonio and Santanantonio 1987). These factors have to be considered in order to calculate the number of samples throughout the year (Vogt et al. 1986). By minimizing the combined spatial and temporal errors, our methodology maximizes the efficiency of root sampling allowing a more effective allocation of resources to account for the myriad of factors that must be considered in the design of accurate, cost-effective studies of fine root dynamics.

Conclusion

In conclusion, most of the roots were retrieved in the first minutes of the picking. But, more time to pick roots per sample was needed during the winter, where lower root biomass was present, than during the summer sampling periods. In the sampling made in winter, the minimum total relative standard error (TRSE) occurred at a smaller number of samples than in the summer sampling. In winter, the smallest error was achieved by taking fewer samples, but picking them a bit longer. In summer, with a larger biomass, taking more samples and picking them faster provided the smallest error. Our understanding of the sources of error allowed us to optimize the time invested in root sampling, processing and analysis.

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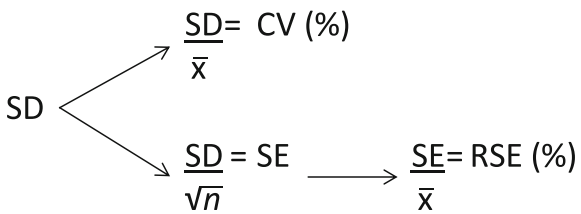
Appendix 1

1) Definition of the relative standard error used in the calculations.



Explanatory note: the relative standard error (RSE) is calculated from the standard deviation (SD), the number of samples (n) and the mean ($\overline{x}$). The RSE is an alternative option for the coefficient of variation (CV) that varies with the number of samples used.

2) The total relative standard error (TRSE) is the sum of the defined two contributors to uncertainty in fine root biomass: the picking duration (picking duration error = PDE) and spatial distribution (ecosystem scale standard error = ESSE).

PDE is a RSE that is calculated for each duration of picking, using the SD and the proportion of root picked ($\overline{x}$) at each duration of picking, and n from Eq. 2 presented in Materials and methods section.

$$\mathrm{PDE} = \frac{\mathrm{SD}}{\frac{\overline{x}}{\sqrt{n}}}$$

Explanatory example for the calculation of the PDE:

Duration of picking (min)	Proportion of roots	SD	Total time processing (min)	Time invested (min)	n (Eq. 2)	PDE
1	0.50	1.0	10	300	30	0.365
2	0.70	0.8	12	300	25	0.229
3	0.80	0.7	14	300	21	0.189
5	0.90	0.6	16	300	19	0.154
20	0.95	0.5	18	300	17	0.129
40	0.98	0.4	20	300	15	0.105
60	0.99	0.3	22	300	14	0.082

ESSE is a RSE that is calculated using only the SD and the mean ($\overline{x}$) from the absolute value at the maximum picking duration and varying n obtained from Eq. 2 presented in Materials and methods section.

$$\mathrm{ESSE} = \frac{\mathrm{SD}}{\frac{\overline{x}}{\sqrt{n}}}$$

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