ECOLOGY LETTERS

Ecology Letters, (2013) doi: 10.1111/ele.12119

LETTER

Sampling volume in root studies: the pitfalls of under-sampling exposed using accumulation curves

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Abstract

Root systems are important for global models of below-ground carbon and nutrient cycling. Notoriously difficult sampling methods and the fractal distribution of root diameters in the soil make data being used in these models especially susceptible to error resulting from under-sampling. We applied the concept of species accumulation curves to root data to quantify the extent of under-sampling inherent to minirhizotron and soil coring sampling for both root uptake and carbon content studies. Based on differences in sample size alone, minirhizotron sampling missed approximately one third of the root diameters observed by soil core sampling. Sample volumes needed to encounter 90% of root diameters averaged 2481 cm^3 for uptake studies and 5878 cm^3 for root carbon content studies. These results show that small sample volumes encounter a non-representative sample of the overall root pool, and provide future guidelines for determining optimal sample volumes in root studies.

Keywords

Fractals, minirhizotron, monolith, roots, sample volume, scaling, soil core, species accumulation curves.

Ecology Letters (2013)

INTRODUCTION

Roots mediate plant competition, transfer carbon (C) into soil organic matter pools, acquire nutrients and water, and influence the structure and function of soil food webs. Production of fine roots alone is responsible for as much as one third of global net primary productivity (Jackson *et al.* 1997) and accounts for a substantial amount of the carbon cycled in many forests (Vogt *et al.* 1996). Because of this, our understanding of root systems is critical to creating global budgets for C and nitrogen (N; Iversen *et al.* 2008). To date, information on root systems has been constrained by difficult and time-consuming methods (Vogt *et al.* 1998; Rodrigues de Sousa & Gehring 2010). These methodological difficulties often result in researchers basing sampling effort largely on time and financial limitations rather than taking proper steps to ensure that samples accurately reflect the root pool present (Pierret *et al.* 2005).

The importance of accurate data for use in modelling is ever increasing as greater effort is being committed to expanding local estimates of root systems to global scales. Indeed, as data on root systems are scaled up to apply to large geographic areas, any errors associated with those data are similarly scaled. The substantial impact of root systems on calculations of carbon, nutrient and water budgets via global models makes the accuracy of empirically derived root data critical to our understanding of how terrestrial systems will respond to changing climatic conditions (Norby & Jackson 2000). With a host of disparate methods used to study root systems, it has been proposed that the lack of consensus on important inputs to global models, such as root production, biomass and turnover, is largely due to discrepancies in methods (Hendricks *et al.* 2006; Guo *et al.* 2008; Strand *et al.* 2008).

Studies of root systems are particularly vulnerable to errors from under-sampling due to the high spatial heterogeneity (Casper & Jackson 1997) and non-normal distribution of root diameters in the soil (Tatsumi *et al.* 1989). Branching processes like those giving rise to root systems are one mechanism to generate fractal characteristics in ecological systems whereby the smallest diameter roots are very common and larger diameter roots are increasingly rare (Tatsumi *et al.* 1989; Berntson 1996; Eshel 1998; Halley *et al.* 2004; Walk *et al.* 2004). One major consequence of a fractally distributed system is that parameter estimates (such as sample mean) are directly related to sampling intensity (Leibovitch 1998). In the context of root research, if a small sample volume results in the omission of the rarest roots, which by nature are the largest roots, then measures such as mean diameter, root biomass and mean specific root length (SRL) are likely to be an artefact of sample size rather than an accurate representation of the root pool present. Due to the self-similarity of fractal systems at multiple spatial scales (Hutchinson 1981), the issue of sample size in studies of fractally distributed roots is an important one whether one is concerned with the entire pool of roots or is focused on only a small range of root diameters.

Given the increased hazards of under-sampling in fractal systems such as plant roots, there has been surprisingly little attention paid to sampling volume within the literature, and a consensus on what constitutes an adequate sample volume is entirely absent (Ping *et al.* 2010; Rodrigues de Sousa & Gehring 2010). The issue of sample volume is further complicated in the field of root biology by methods (i.e. minirhizotrons, soil cores, soil monoliths) that vary by more than an order of magnitude with respect to soil volume sampled (Table 1). The soil volume of a minirhizotron image is based on assumptions of depth of view into the soil environment, which is typically only 1 to 3 mm. Based on this assumption, even large minirhizotron studies sample soil volumes that are dwarfed by typical soil core and monolith sampling efforts (Table 1). The extremely small soil volume sampled by a minirhizotron tube will likely

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Table 1 Soil volumes sampled (in cm^3 per experimental plot) for six studies employing at least 2 of the methods: minirhizotron imaging, soil cores and soil monoliths. Soil volumes for minirhizotrons were calculated using the depth of view stated in the study or 2 mm where not stated

Study	Minirhizotron	Soil Core	Monolith
Heeraman & Juma (1993)	15.84	1508	157 500
Samson & Sinclair (1994)	81	1838	–
Brown *et al.* (2009)	74.41	1923	–
Rodrigues de Sousa & Gehring (2010)	–	9817	250 000
Noguchi *et al.* (2011)	129.6	4341	–
Levillain *et al.* (2011)	–	4522	31 250

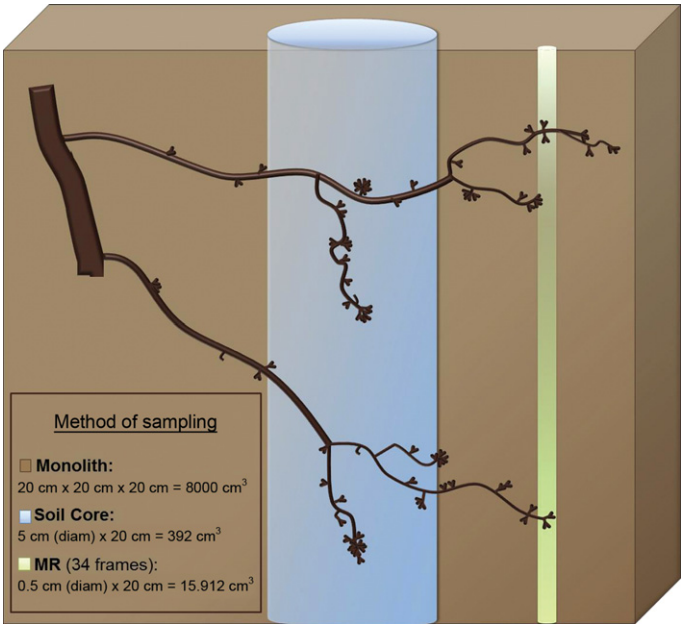

Figure 1 Diagram of example soil volumes from the three most common methods in root studies. The block represents a single 8000 cm^3 soil monolith, the centre column represents the volume of soil sampled by a 5 cm diameter × 20 cm deep soil core (392 cm^3), and the right-hand column represents the soil volume sampled by the most common minirhizotron tube (56 mm internal diameter) and imaging system (Bartz Technologies, Inc; 34 frames measuring 18 × 13 × 2 mm; 16 cm^3). Recently developed scanning minirhizotron cameras (CID Bio-Science Inc., Camas, WA, USA) sample volumes in the range of the soil core depicted here. Root orders are drawn to scale based on average proportions of total root length presented in (Pregitzer & DeForest 2002).

encounter only the most common first and second order roots (Fig. 1). Furthermore, it often takes several years following minirhizotron tube installation for root length densities on the tube/soil interface to reach an equilibrium state (Joslin & Wolfe 1999; Strand *et al.* 2008). In addition, it is likely that roots that first colonise the tube surface may be the smallest and most dynamic of the fine root pool (i.e. the smallest and most distal root orders). Conversely, large structural roots are relatively rare and are likely to be encountered only by large sampling efforts such as soil monoliths or trench sampling (Levillain *et al.* 2011). The effect of these methodological discrepancies in sampling volume can be seen in Jackson *et al.* (2009), in which an overall treatment effect of CO_2 fumigation on coarse root biomass was undetectable using soil cores, but was highly significant when analysing soil monoliths.

For several decades, studies investigating species diversity have used species accumulation curves as a statistical tool not only to visualise the distribution of species within a pool of individuals but also as a way to ensure adequate sampling effort (Gotelli & Colwell 2001; Ugland *et al.* 2003; Thompson *et al.* 2007). These accumulation curves plot the number of unique species encountered as a function of sampling effort. Given that in most communities a few species are very common and many species are rare, the nature of these curves is such that they rise steeply as common species are rapidly accumulated, but then reach an asymptote as additional sampling reveals relatively few novel species. Asymptotic flattening of a species accumulation curve indicates the point at which the majority of species present have been encountered, and traditionally serves as an indication of adequate sampling effort (Soberon & Llorente 1993; Colwell & Coddington 1994; Thompson *et al.* 2007).

Parallels between the distributions of species in a community and root diameters in the soil allow us to apply the species accumulation curve function to model the accumulation of novel root diameters with increased sampling effort in root studies. These curves highlight the danger of sampling small volumes of soil in root studies, especially when the aim is to provide accurate estimates of root biomass and C storage.

METHODS

Root extraction

Fifteen soil monoliths measuring 20 cm × 20 cm × 20 cm (8000 cm^3) each were extracted from the Duke Forest near Durham, NC. This site is comprised of an unmanaged loblolly pine (*Pinus taeda*, L.) plantation with a mixed hardwood understory. *Pinus taeda* makes up > 90% of the basal area at this site (Matamala & Schlesinger 2000) and soils consist of predominantly clay-loam of the Enon series (McCarthy *et al.* 2010). Each monolith consisted of the O horizon and mineral soil to a depth of 20 cm, which has been shown to hold > 90% of the root biomass at this site (Matamala & Schlesinger 2000). Sampling sites were selected to be equidistant from all surrounding *P. taeda* trees, which are evenly planted at a 2.4 m × 2.4 m spacing. Monoliths remained frozen at −10 °C until processing.

Individual roots were extracted by carefully washing each monolith in its entirety over a series of 3 screens with mesh sizes of 3 mm, 1 mm and 0.5 mm. Root fragments were exhaustively removed from the 3 mm screen by hand using forceps. To account for the smallest root fragments that often separate from the root system during washing (Pierret *et al.* 2005), 5 mL subsamples of soil and root material caught by the 1 mm and 0.5 mm screens were searched for all root fragments present using a dissecting microscope at 10× (SMZ-1, Nikon Inc., Melville, NY, USA). Root data obtained from each of these 5 mL subsamples were then multiplied by the total volume of material caught by each respective screen to estimate all root fragments caught by that screen. These small root fragments represent the most dynamic part of root systems, thus great care was taken to ensure their accurate representation.

Once taken from the monolith, each root segment was individually cleaned of all remaining rhizosphere soil and fungal mycelia using forceps under a 3× magnifying lens. Digital images of cleaned roots were created using an Epson expression 10000 XL scanner at 400 dpi. These scanned images were then analysed using WinRhizo

root analysis software (Regent Instruments, Quebec, Canada) to obtain length and diameter data for each root. Due to the limited scanner size, multiple scans were needed to image all of the roots of an individual monolith. The number of scans needed per monolith depended on the total length and architecture of the roots in the monolith. Because total root length in each scan was variable, scans were normalised for scan size by dividing root length for each diameter class in a scan by the scan's total root length.

Diameter-bin classification

Due to the continuous nature of diameter data obtained by WinRhizo, diameter bins were used to separate roots into the distinct groups needed to construct accumulation curves. To provide a biologically relevant set of diameter bin classifications, two sets of non-uniform diameter bins were created, one based on changes in [N] and the other based on changes in root volume (a proxy for biomass and C content). These two sets of diameter bins addressed two different goals of root research. Given the established correlation between [N] and root function (Pregitzer *et al.* 1998; Atkin *et al.* 2000; Makita *et al.* 2009), accumulation curves using diameter bins based on [N] were used to estimate the sampling effort required for studies focusing on root uptake function or for studies attempting to quantify fine root turnover. Accumulation curves using diameter bins based on root volume were constructed to quantify the required sample volumes for studies attempting to quantify root biomass and the contribution of root standing crop to soil carbon pools.

Nitrogen-based diameter bins were defined as the range of diameters that represented a 10% change in [N]. Because [N] is relatively sensitive to changes in root diameter in small roots but much less so in larger roots (Makita *et al.* 2009), N-based diameter bins were relatively narrow for small-diameter roots and grew progressively wider for large-diameter roots. The relationship between root diameter and [N] was based on data of *P. taeda* roots ranging in diameter from 0.28 mm to 41.67 mm taken from trenches surrounding the monoliths dug during the process of monolith extraction. These roots were washed and digitised using WinRhizo software as described above for monolith roots, dried to constant mass and ground using a Wig-L-Bug grinding mill (REFLEX Analytical Corp., Ridgewood, NJ, USA). Ground samples were analysed for N content using a PDZ Europa ANCA-GSL elemental analyser (Sercon Ltd., Cheshire, UK) and a power function was determined relating [N] to diameter ($[N] = 0.0079 \times \text{diameter}^{-0.373}$; $r^2 = 0.71$).

Volume-based diameter bins were designed to emphasise the relatively large contribution made by large-diameter roots to root C storage, and so were defined as the range of diameters that equalled a 0.5% reduction in the volume of our largest diameter class (50 mm). This resulted in diameter bins that were relatively wide for small-diameter roots and grew progressively narrower for large-diameter roots. The 0.5% reduction in volume was chosen to provide a similar number of N-based and C-based diameter bins.

Diameter-class accumulation curve formation

Root diameter-class accumulation curves were constructed using the `specaccum` function in the vegan package of R statistical software (Oksanen *et al.* 2012). This function sequentially samples random individuals from a data set and measures the number of novel species encountered as additional individuals are sampled. Here, we used the 'random' method, which encounters sites in random order and samples individuals without replacement. For our data, root length data were broken up into 1-cm segments and each 1-cm segment of root length was treated as an 'individual.' Diameter bins were treated as 'species,' and each WinRhizo scan was normalised for total root length and treated as a 'site.' The soil volume represented by individual normalised scans in each monolith was calculated by dividing the total monolith volume (8000 cm^3) by the number of scans made for that monolith. Subsampling of the monolith data to represent minirhizotron and soil-core sample volumes was done by randomly sampling data from the number of WinRhizo scans ('sites') needed to represent the typical volume sampled by each of these methods. Individual curves were constructed for each of the 15 soil monoliths using both N-based and volume-based diameter bins. Each curve was plotted as the average of 1000 permutations of the `specaccum` function.

RESULTS

A total of 120 000 cm^3 of soil was analysed containing 649 139 cm of root length with an average of 43 276 cm of root length contained in an individual monolith. Root diameters encountered ranged from 0.025 mm to 42.55 mm, with 99% of the total root length encountered having a diameter < 2 mm (Fig. 2b).

Nitrogen-based accumulation curves reached asymptotic flattening for each individual monolith (Fig. 3a, b), however, for volume-based accumulation curves, the volume of one soil monolith was often insufficient to achieve flattening (Fig. 3c, d). The average soil volume needed to encounter 90% of the N-based diameter classes,

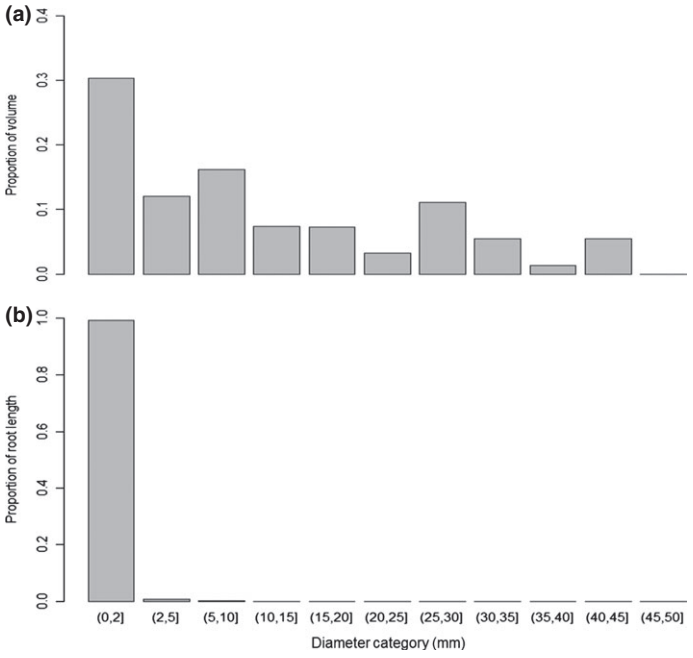

Figure 2 The proportion of (a) root volume and (b) root length contained in diameter categories encountered for all study monoliths combined. The first diameter category represents traditionally defined fine roots (< 2 mm). Subsequent diameter categories are 2–5 mm, and increase in 5 mm increments up to 50 mm.

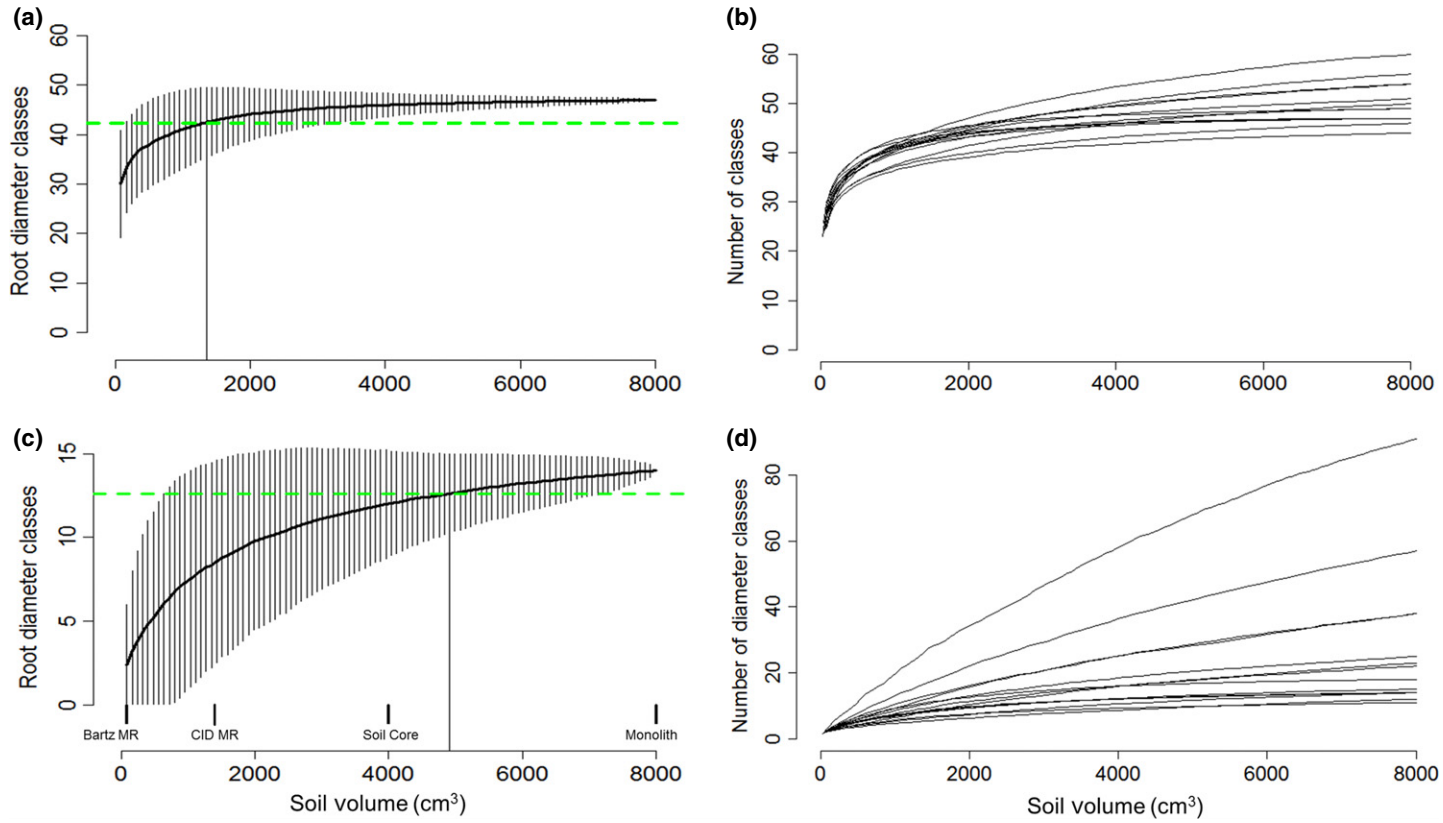

Figure 3 (a) Representative diameter-class accumulation curve constructed using N-based diameter classes. The horizontal dashed line represents 90% of all diameter classes encountered, and the black vertical line represents the sample volume needed to encounter 90% of the diameter classes. Typical sampling volumes for Bartz minirhizotron, CID minirhizotron, soil core and monolith methods are indicated; (b) N-based accumulation curves for each of the 15 study monoliths; (c) Representative accumulation curve using volume-based diameter classes; and (d) Volume-based accumulation curves for each of the 15 study monoliths.

Table 2 Total root length measured and the soil volume (cm^3) needed to encounter 90% and 95% of nitrogen-based and volume-based diameter bins, respectively, for each study monolith

Monolith	Total root length	Volume needed for 90% N-bins	Volume needed for 95% N-bins	Volume needed for 90% volume- bins	Volume needed for 95% volume- bins
1	30794.88	1346.57	2455.51	5069.44	6336.80
2	31794.50	4150.35	5654.10	6616.65	7218.00
3	55313.09	1556.64	3070.04	5188.80	6399.52
4	45999.30	3692.34	5538.51	6974.42	7589.81
5	61042.46	1451.83	2532.98	4695.28	5776.43
6	33670.74	4121.14	5696.87	6787.76	7393.81
7	42777.37	2615.28	4615.20	6384.36	7153.56
8	68073.32	3595.20	5302.92	6785.94	7370.16
9	34729.88	2353.00	3953.04	6117.80	7153.12
10	24912.36	852.81	1665.01	5279.30	6538.21
11	38974.35	3532.60	5298.90	6649.60	7273.00
12	21842.52	1425.78	2376.30	4594.18	6019.96
13	67360.83	1408.00	2752.00	4288.00	5568.00
14	67372.39	2472.82	4291.07	6253.92	7126.56
15	24480.88	2646.60	4631.55	6496.20	7218.00

and 90% of the volume-based diameter classes was 2481 cm^3 and 5878 cm^3 respectively (Table 2).

To illustrate the importance of both the common fine roots and the rare coarse roots in the total biomass of the root system, we examined the distribution of root volume across the range of root diameters as compared to total root length encountered in this study (Fig. 2). At this site, 30.3% of all root volume encountered was contained in fine roots < 2 mm in diameter. An additional 23.5% of the total root volume was held in roots with a

diameter > 25 mm, of which there were only four individuals encountered.

Monolith data were randomly subsampled to show the contrasting distributions of root volume encountered by minirhizotron, core and monolith sampling (Fig. 4a–c). For roots < 2 mm in diameter, generalised additive models (Hastie & Tibshirani 1990) were used to compare the relationship between diameter and the proportion of root volume encountered by minirhizotrons and larger sampling volumes (soil cores and monoliths). Likelihood ratio tests indicated that the distribution of fine roots encountered by minirhizotrons is significantly different than that encountered by the other two methods ($G = 10.9$, $P = 0.011$; Fig. 4d).

DISCUSSION

The most commonly measured properties of roots, such as SRL, elemental composition, metabolism, biomass, lifespan and soil symbionts, all vary with root diameter, creating the need for a sampling scheme that accurately pairs the root pool of interest with the appropriate sample volume to obtain accurate measurements for these properties. As data on root properties are taken from study sites and extrapolated to regional and global scales, measurement errors due to insufficient sampling stand to be greatly magnified. The application of the diameter class accumulation curves presented here is potentially a useful tool to ensure that the majority of root diameters present are accounted for, reducing the risk of under-sampling and helping to assure the accuracy of data used in large-scale models.

Attempts to characterise root structure and function are complicated by the fractal distribution of root diameters in the soil, requiring relatively large sample volumes to account for rare, large-diameter roots. Small sample sizes are an issue of concern in all scientific studies, yet it is important to keep in mind that the relationship between small samples and the overall population is very different for fractal distributions than for the Gaussian distributions that biologists are traditionally more familiar with (Leibovitch 1998). In fractally distributed systems such as roots, a small sample is not simply a scaled-down version of the larger population. As shown in Fig. 4, the relative proportions of root diameters encountered with minirhizotron-scale sample volumes are not a representative subset of the overall root pool but a sample of only the smallest, most common roots.

The soil volumes presented in Table 2 represent the volume needed to encounter 90% or 95% of all root diameters present in our samples using either N-based or volume-based diameter classes, respectively, and should serve as examples for target sampling volumes at this site. These sample volumes serve both as an assurance of thorough sampling but also as a means to avoid the time and expense of excess sampling. It is important to note, however, that before estimating a target sample volume from a diameter class accumulation curve, the curve must have reached a point of flattening (Fig. 3a). This point of flattening will vary depending on the number and relative distribution of root diameters at a site. The absence of flattening in an accumulation curves indicates that a significant number of diameter classes have not been encountered in that sample volume and additional sampling is needed to obtain accurate parameter estimates (Fig. 3d).

Due to the ever-decreasing slope of a diameter class accumulation curve, the soil volume needed to obtain 95% of the diameter classes present is much greater, proportionally, than the volume required to obtain 90% of the diameter classes. Indeed, in many of the accumulation curves presented here, the volume required to sample 90% of all diameter classes must be approximately doubled to obtain an additional 5% of the diameter classes (Table 2). The diminishing returns of additional sampling in root studies requires each researcher to balance available resources against sampling intensity. Diameter class accumulation curves provide a valuable resource in this decision-making process.

The distribution of root volume across a range of diameters indicates the necessity of sampling large soil volumes to account for large roots when estimating the amount of belowground carbon held in root systems (Fig. 2a). Roots < 2 mm in diameter accounted for over 30% of the total root volume. However, the 23% of the total root volume that was contained in roots > 25 mm came from 36.7 cm of root length, which represents a mere 0.01% of the total root length in this study. This highlights the extreme rarity of large-diameter roots in the soil, and indicates that studies reporting coarse root biomass sampled using soil cores (e.g. Dhyani & Tripathi 2000; Soethe *et al.* 2007; Major *et al.* 2012) may be systematically underestimating coarse root biomass.

Increasing attention is being paid to the importance of plant roots to belowground nutrient and C cycling from ecosystem to global scales. In recent years, minirhizotrons have been used to provide much of the data on which our global estimates of fine root systems are based. The minirhizotron method provides a unique opportunity to directly measure the dynamic properties of fine root systems, yet because even large minirhizotron studies sample a relatively small volume of soil, this method is particularly vulnerable to inaccuracies in estimates of physical properties of roots due to under-sampling. If implemented into models covering large spatial scales, these inaccuracies stand to be greatly magnified, potentially misleading our estimates of the role of roots in global nutrient cycles.

The manner in which fractally distributed root data responds to sample size is similar whether one examines the entire root system or only the fine root pool. The tendency for small sample volumes to overestimate the proportions of very fine roots and underestimate the proportions of larger roots is found even for roots < 2 mm (Fig. 4d). Although minirhizotrons do sample roots up to 2 mm in diameter, their small sample volumes bias relative proportions of fine roots towards the most common, finest roots. Notably, sample volumes for which our N-based accumulation curves have reached flattening (core and monolith sampling; Fig. 3 a,b) closely agree in their representation of the fine root pool, whereas minirhizotron sample volumes for which our N-based curves are still rising differ in their representation of the relative proportions of fine roots (Fig. 4d). This represents a problem when turnover rates obtained from minirhizotrons are multiplied by fine root standing crop biomass estimates obtained from soil cores, as is often done to calculate fine root net primary productivity (e.g. Pritchard *et al.* 2008). By assuming that these two methods are sampling the same pool of roots, a researcher will likely overestimate fine root biomass production and turnover because minirhizotrons are inherently biased towards small, short-lived roots compared to the biomass pool sampled by cores.

The considerable debate over the differences in fine root turnover estimates obtained from minirhizotrons vs. those obtained using isotopic tracer techniques serves as a prime example of how methodological discrepancies can influence important inputs to global C

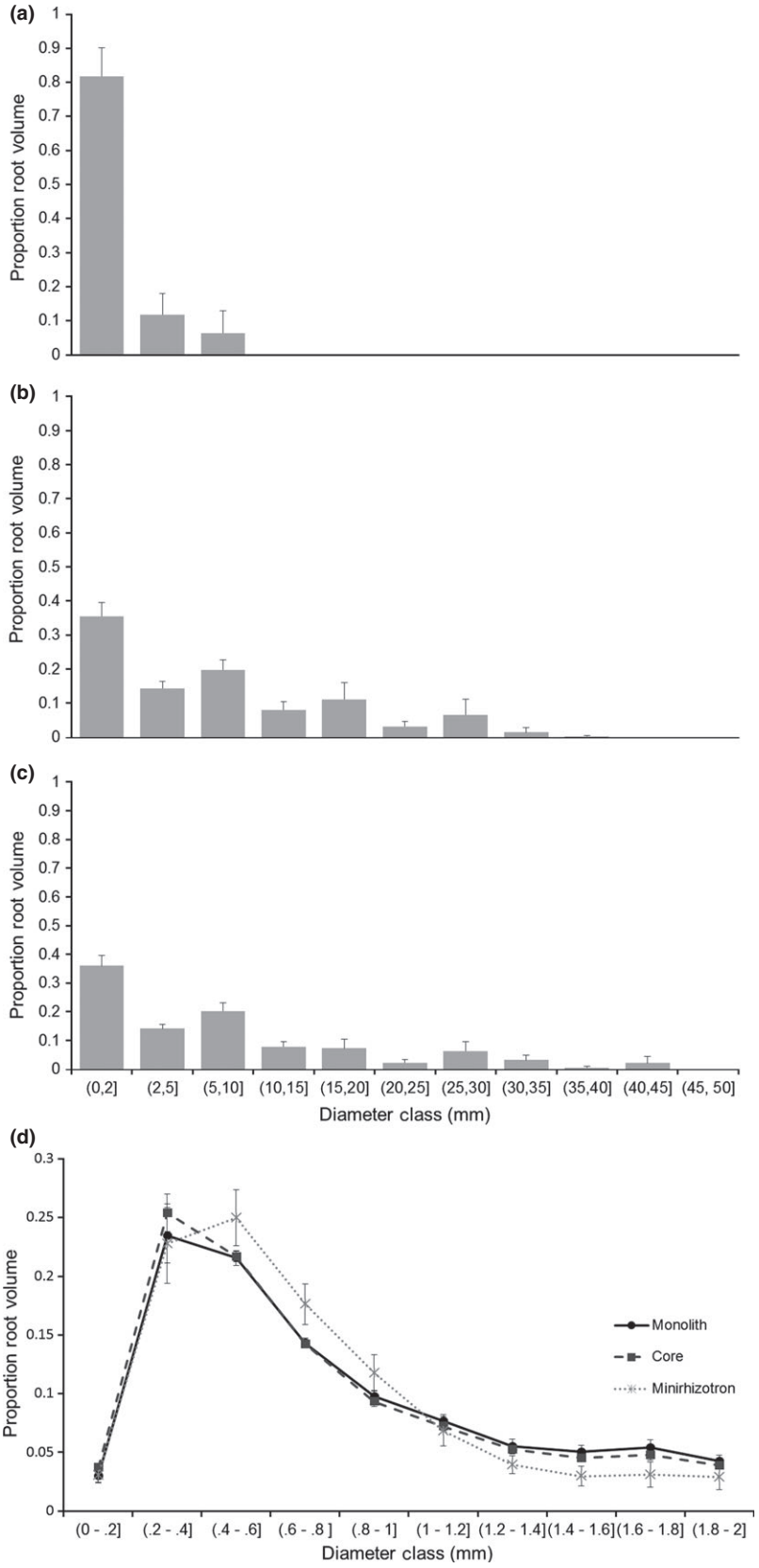

Figure 4 The proportion of root volume distributed by diameter category for three different sample volumes randomly subsampled from each of the 15 study monoliths. Subsamples of data from each monolith represent (a) sample volume of a typical minirhizotron study (~100 cm^3), (b) volume of a typical soil core study (4000 cm^3), and (c) the entire soil monolith (8000 cm^3). (d) The distribution of proportion of root length for diameter classes 0–2 mm sampled by monolith, core and minirhizotron sample volumes. Error bars represent standard error (n = 15).

models (Matamala & Gonzalez-Meler 2003; Guo *et al.* 2008; Pritchard & Strand 2008; Strand *et al.* 2008). Turnover estimates derived from minirhizotrons suggest that roots turn over very quickly while turnover estimates of fine root C from isotopic methods indicate that fine roots turn over much more slowly. Several differences in the basic assumptions made by each of these approaches are outlined in Pritchard & Strand (2008); however, one potentially important discrepancy between these two methods is that of sample size. Guo *et al.* (2008) propose that much of the disagreement between these two methods arises from the heterogeneity of root orders (which vary by diameter), and the resulting heterogeneity of root turnover within the fine root pool. One consequence of this heterogeneity that has gone previously unmentioned is the effect of very small sample volumes, such as those observed by minirhizotrons, inherently missing many of the larger, high-order fine roots that are proposed to have a disproportionate impact on fine root C turnover estimates. Using the N-based accumulation curves presented above, we estimated that a traditional minirhizotron study sampling ~100 cm^3 of soil per plot will miss 34% of the root diameter classes that a soil core (used in C isotope techniques) study sampling ~4000 cm^3 would observe. When considering that this 34% discrepancy is likely made up of the larger, rarer roots that can heavily influence isotopic estimations, it is apparent that soil sample volumes stand to greatly impact some of the most important measures of fine root systems used in global C models.

Recent developments of minirhizotron camera systems capable of sampling much larger areas of the tube surface hold the potential to overcome some of the sampling issues of traditional minirhizotron cameras. The discrepancy in image dimensions between traditional camera systems such as those produced by Bartz Technology (Carpinteria, CA, USA) (18 mm × 13 mm) and new scanning minirhizotron cameras such as those produced by CID Bioscience Inc. (Camas, WA, USA) (216 mm × 196 mm) is substantial. Assuming a 2 mm image depth of view, studies using new CID minirhizotron systems can sample soil volumes comparable to soil core studies (e.g. Zhang *et al.* 2009). While these scanning minirhizotron systems hold great promise in the effort to accurately pair data between minirhizotrons and soil core studies, consideration should be given to the effect of additional image processing time on number of tubes able to be employed.

The accurate representation of both fine and coarse roots in C budgets are of great importance (Fig. 2a), but for different reasons. The biomass of live and decaying coarse roots, which equal an estimated 50% of stem biomass, represents a substantial and relatively stable pool of belowground C storage that is suggested to be largely underestimated (Albaugh *et al.* 2006; Robinson 2007; Wang *et al.* 2012). Conversely, the ~30% of root biomass held in fine roots represents a relatively dynamic source of C flow from plant biomass into the soil via turnover. Despite their relatively small sample volumes, minirhizotrons currently represent the most reliable method to assess fine root turnover. Because even fine root pools are susceptible to errors from insufficient sample size, however, all reasonable efforts should be made to ensure that dynamic data obtained by minirhizotrons accurately matches the fine root pool sampled by soil cores. We recommend a combination of monolith sampling and allometric calculations for estimating coarse root biomass, and a combination of soil cores with high-volume minirhizotrons for linking estimates of fine root biomass and turnover.

Here, we present the concept of root diameter-class accumulation curves to highlight the potential for data inaccuracies due to insufficient sampling. Potential also exists to use these accumulation curves to identify a target sample volume that will maximise sampling efficiency. The non-normal distribution of root diameters in the soil makes root studies especially susceptible to effects of sample size. Implementation of diameter class accumulation curves stands to greatly increase the reliability of root data being used in global models, improving our understanding of how root systems influence processes of plant growth and nutrient cycling on a global scale.

ACKNOWLEDGEMENTS

The authors thank R. Oren, and R. Nettles with assistance in obtaining our samples, N. Gotelli and W. Bridges for statistical advice, and three anonymous referees for comments that have greatly improved this study. This study was supported by the Office of Science (BER), US Department of Energy, Grant No. DE-FG02-95ER62083, and the National Science Foundation, award number DE-FC02-06ER64156. SGP thanks the participants of the COST FP 0803 meeting on Belowground Complexity for useful discussions on the topic of root sampling methods.

AUTHORSHIP

BT designed study, collected data, analysed data and prepared manuscript; KB processed data, performed image analysis and created figures; SP designed study and collected data; AS collected and analysed data; EC collected data and performed image analyses. All authors contributed to manuscript revisions.

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Editor, Aimee Classen
Manuscript received 20 February 2013
First decision made 21 March 2013
Manuscript accepted 2 April 2013