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REGULAR ARTICLE

Improved scaling of minirhizotron data using an empirically-derived depth of field and correcting for the underestimation of root diameters

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Abstract

Background and aims Accurate data on the standing crop, production, and turnover of fine roots is essential to our understanding of major terrestrial ecological processes. Minirhizotrons offer a unique opportunity to study the dynamic processes of root systems, but are susceptible to several measurement biases.

Methods We use roots extracted from minirhizotron tube surfaces to calculate the depth of field of a minirhizotron image and present a model to correct for the underestimation of root diameters obscured by soil in minirhizotron images.

Results Non-linear regression analysis resulted in an estimated depth of field of 0.78 mm for minirhizotron images. Unadjusted minirhizotron data underestimated root net primary production and fine root standing crop by 61 % when compared to adjusted data using our depth of field and root diameter corrections. Changes in depth of field accounted for >99 % of standing crop adjustments with root diameter corrections accounting for <1 %.

Conclusions Our results represent the first effort to empirically derive depth of field for minirhizotron images. This work may explain the commonly reported underestimation of fine roots using minirhizotrons, and stands to improve the ability of researchers to accurately scale minirhizotron data to large soil volumes.

Keywords Depth of field · Fine root · Minirhizotron · Root diameter · Root method · Root net primary production · Standing crop

Introduction

Estimates of fine root biomass and the turnover of this biomass continue to be one of the most poorly understood aspects of terrestrial carbon (C) cycling. It is estimated that up to 1/3 of net primary productivity (NPP) is allocated to fine roots, and that the amount of C and nitrogen (N) entering the soil from fine root decomposition is at least equivalent to that of aboveground litterfall (Cox et al. 1978; Arthur and Fahey 1992; Jackson et al. 1997). Until recent decades, our understanding of fine root turnover has been hampered by our inability to view the dynamic processes of roots over time. Destructive methods of measuring root systems, such as sequential coring and ingrowth cores, yield only snapshots of root standing crop at sparse time intervals, but development of minirhizotron technology now allows researchers to follow sets of individual roots through time to monitor dynamic processes such as production, mortality, and decomposition (Waddington 1971; Boehm 1974).

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Minirhizotrons have now become the preferred method for directly studying the dynamic properties of fine roots (Yuan and Chen 2012), and so our understanding of important processes such as resource allocation to fine roots and the movement of C and N into the soil via fine roots is largely dependent on the accuracy of data obtained by this method. Following the development of suitable camera technology for the widespread implementation of this method in the 1980's (Upchurch and Ritchie 1983), there has been a dramatic increase in publications using minirhizotrons, with 109 such studies reported by Web of Science in the past 5 years alone (Supplemental Figure 1).

Due to the increased influence of minirhizotrons on our understanding of fine root primary productivity, standing crop, and turnover, a full understanding of how to implement this method is critical to accurately characterize forest C and N cycling (McMichael and Taylor 1987; Majdi 1996; Johnson et al. 2001; Milchunas 2012; Vamerali et al. 2012). Many practical aspects of the minirhizotron technique have been addressed including soil compaction during tube installation, uncertainties about root length density at the tube surface vs. the bulk soil, images obscured by soil, and frame shift complications (O'Connell et al. 2003; Hendricks et al. 2006; Iversen et al. 2011; Rytter and Rytter 2012). Bernier and Robitaille (2004) also note that the influence of the tube surface on root growth patterns may effect root length estimates. The ability of minirhizotron images to accurately represent the roots they encounter is critical to estimates of standing crop, production, and turnover, as well as the ability to scale these estimates to larger soil volumes. However, empirical data for how accurately minirhizotron images portray the roots physically colonizing tube surfaces is absent from the literature.

A major step in calculating fine root biomass from minirhizotrons is the conversion of two-dimensional minirhizotron images to a three-dimensional estimate of root and soil volume, which requires an estimate of "depth of field," i.e. how far the minirhizotron observer can see into the soil (Johnson et al. 2001; Rewald and Ephrath 2013). The most commonly used values for depth of field range between 2 and 3 mm, which were originally established in studies that provide little to no support for the accuracy of these values (Taylor et al. 1970; Sanders and Brown 1978). Depth of field is an important value not only for the calculation and scaling of total soil volume sampled by a minirhizotron image, but also for the physical dimensions of the individual roots within the image.

Because an observer must be able to see into the soil at least the distance of the root's radius to view the root's diameter, a paradox exists between an assumed depth of field and the ability to view a root with a diameter more than twice that depth of field. For example, by assuming a 2 mm depth of field, it would be impossible to accurately measure a root > 4 mm diameter because the root's diameter would be beyond the view of the observer. Although not common, minirhizotron studies (especially in forest systems) do encounter roots measuring well over 6 mm in diameter (Taylor, unpublished data). This paradox can be resolved one of two ways: 1) the assumed depth of field is increased to match the radius of the largest root encountered (Brown et al. 2009), or 2) the true diameter of many roots is hidden behind opaque soil, and the diameter seen in the minirhizotron image is an underestimation of the full root diameter (Fig. 1). Here, we will provide evidence for the second resolution to this paradox by calculating values for both depth of field and the underestimation of root diameters in minirhizotron images.

Assuming a cylindrically shaped root with a circular cross section, we can apply an established geometric relationship that exists between a circle's diameter (the true diameter of the root), a chord of the circle (the perceived diameter viewed in a minirhizotron image), and the height of the chord (depth of field). Equation 1 describes the nature of this relationship,

$$D = \frac{\left(f^2 + 1/4p^2\right)}{f} \tag{1}$$

in which "D" is the actual diameter of the root, "p" is the perceived diameter of the root in the minirhizotron image, and "f" is the depth of field of the minirhizotron image.

By measuring the discrepancy between root diameters in minirhizotron images and the true diameters of these same roots in the soil, it is possible to quantify the underestimation of diameters using minirhizotrons and provide a correction factor for this underestimation. We hypothesize that minirhizotron images will underestimate the true diameter of roots, and that this bias will increase, disproportionately, with root diameter. Once the degree of this bias is established, it is then possible to calculate the actual depth of field of a minirhizotron image. Due to the opacity of soil, we hypothesize that the calculated minirhizotron

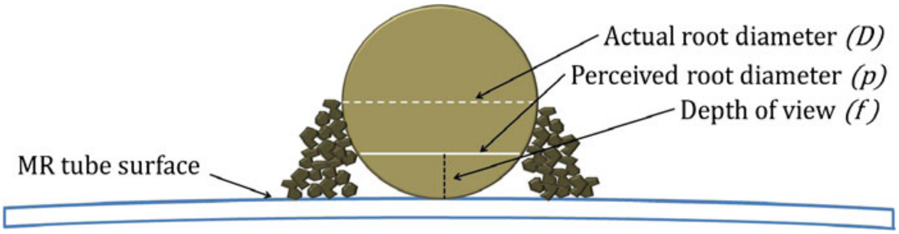

Fig. 1 Illustration representing a root colonizing the surface of a minirhizotron (MR) tube. This figure shows the manner in which a researcher would perceive a smaller diameter (*p*) than the true diameter of the root (*D*) if the root's radius is larger than the depth of field (*f*) of the minirhizotron image. The mathematical nature of this relationship is presented in Eq. 1

depth of field will be smaller than the currently used values of 2–3 mm. Finally, we hypothesize that the scaling of minirhizotron data using the corrected depth of field and root diameters will more closely reflect complimentary destructive sampling methods. If so, the application of these correction factors will improve biomass estimates from minirhizotron studies and provide an estimate for depth of field that will improve the ability of researchers to accurately scale minirhizotron data to larger soil volumes.

Methods

Site description

Data were obtained following the decommissioning of two minirhizotron studies in warm temperate loblolly pine (*Pinus taeda*, L.) plantations; one located at the Duke FACE site near Durham, NC (35° 58′ N, 79° 06′ W) and the other located near Cross, SC (33° 16′ N, 80° 10′ W). Site and minirhizotron installation descriptions can be found in Pritchard et al. (2008) and Pritchard et al. (2010), respectively. Minirhizotron tubes were installed at the NC in 1998 and at the SC site in 2005. These two sites differ markedly in soil types, with soils at the NC location being dominated by clay-loam of the Enon Series (McCarthy et al. 2010) and soils at the SC location being predominantly loamy-sand (Lynchburg/Ocilla/Seagate USDS Soil Classification).

Root extraction

Following the final image collection for each set of minirhizotron tubes, individual roots monitored by these tubes were physically extracted from the soil by digging along the surface of the tube. Extraction of roots at the NC and SC sites took place in January and February of 2013, respectively. Images of each targeted root were collected at the time of extraction using the BTC-2 ICAP system (Bartz Technology Corp., Carpinteria, CA, USA) and image analysis was done using Rootfly software (Wells and Birchfield, Clemson University, SC, USA). Each minirhizotron image was analyzed for root diameter at the point along the image of the root that provided the most clear and complete sight of the diameter of the root. Once extracted from the soil, the physical roots were frozen using liquid nitrogen to preserve root tissue. The true diameters (*D*) of the frozen roots were then measured using electronic calipers. Diameters were also measured for a subset of roots immediately following removal from the tube surface to test for effects of freezing on root diameter measurements. A Pearson's correlation test produced a correlation factor of 0.99 ($P<0.0001$) indicating no significant effect of freezing on measured diameter.

Image calibration

To ensure accuracy of measurements made using the Bartz camera - Rootfly software combination, distances viewed in a minirhizotron image were calibrated using a standard metric ruler. A minirhizotron image was taken of a ruler fixed to the outer surface of a minirhizotron tube, and known distances in this image (shown by ruler markings) were measured using Rootfly software. This method was used to calibrate both length and diameter measurements, and resulted in a conversion factor of 37 pixels per mm.

Model

We used non-linear regression to apply Eq. 1 to our data of perceived and measured diameters of roots excavated from minirhizotron tube surfaces. This analysis allowed us to both estimate depth of field (*f*) for minrhizotrons and to test the ability of Eq. 1 to model empirical data. These analyses were done using the nls function in the base package of R (R Core Team 2012). This and all other analyses were performed using R statistical software.

Data application

The relationship between actual diameter and perceived diameter in minirhizotron images was used to create a conversion factor to adjust diameters viewed in minirhizotrons to the actual diameter of the root. This conversion was then applied to data obtained from the minirhizotron tubes at our NC site over a 5 year period from 2005 to 2010. Because an observer only needs to see one half of the root in order to view the full diameter (Fig. 1), it was assumed that all roots with a diameter less than twice the depth of field had equivalent perceived and actual diameters. The maximum diameter for which the perceived and actual diameters are equivalent was assumed to be twice the depth of field calculated in this study, so only roots greater than this diameter were adjusted (Fig. 2). Root net primary production (RNPP) and fine root standing crop were calculated using both adjusted and non-adjusted root diameters.

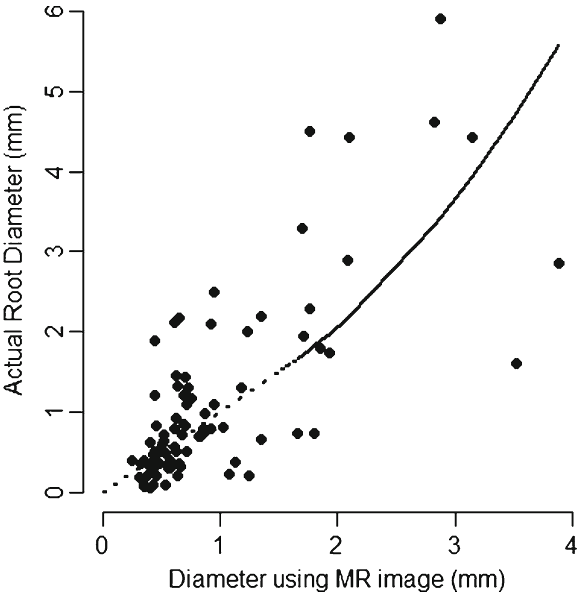

Fig. 2 The relationship between the perceived diameter measured using a minirhizotron image (*p*) and the actual measured diameter of the root (*D*). The solid portion of the line represents the model presented in Eq. 2 using a depth of field of 0.7848 mm (R^2=0.43). The dashed portion of the line represents the 1:1 relationship between perceived and actual diameter for roots <1.57 mm

Biomass and standing crop estimates were calculated using a relationship between diameter and specific root length (SRL) established for loblolly pine roots at our NC site. Specific root length was determined by dividing root total length by dry mass for roots obtained from the trenches dug surrounding a set of soil monoliths during their extraction in November 2010. *Pinus taeda* roots obtained from these samples were separated into up to 14 coarse diameter categories ranging in diameter between 0.3 and 39 mm. Each category of roots was scanned and analyzed for total length and average diameter using WinRhizo root analysis software (Regent Instruments Inc., Quebec, Canada). These roots were then dried to a constant mass at 65 °C and weighed. Specific root length was calculated as the total root length of a category divided by the dry mass of the category, resulting in a relationship between SRL and diameter represented by the equation $\mathrm{SRL}=355.72 \times \mathrm{diameter}^{-2.029}$.

Estimates for RNPP and standing crop were scaled up from the soil volume sampled by the minirhizotron tubes to a ground surface area of 1 m^2. Non-adjusted diameters were scaled using a minirhizotron sample volume based on the traditional 2 mm depth of field, and adjusted diameters were scaled using a minirhizotron sample volume based on the depth of field calculated in this study (presented in the results). Scaling calculations were done to represent an area of 1 m^2 to a soil depth of 16 cm in order to compare our estimates with published data on fine root biomass at our NC site (Jackson et al. 2009).

Results

A total of 92 roots were extracted from tube surfaces at both sites ranging in actual diameters from 0.06 mm to 6.00 mm. Non-linear least squares analysis resulted in an estimated depth of field for minirhizotron images of 0.7848 mm. When applying this depth of field to Eq. 1, the resulting relationship between perceived diameter (*p*) and actual diameter (*D*) is shown in Eq. 2.

$$D = \frac{\left(.7848^2 + 1/4p^2\right)}{.7848} \tag{2}$$

This model fit our empirical data with an R^2=0.430 (Fig. 2). A likelihood ratio test showed no significant difference in the fit of this model when adding site as a parameter, indicating no difference in how our model fits data from our NC and SC sampling sites ($G=0.45$, $p=0.50$).

Adjustments to RNPP and standing crop numbers were calculated based on the volume of a single minirhizotron image being 0.442 cm^3 (13 mm × 17 mm × 2 mm) and 0.173 cm^3 (13 mm × 17 mm × 0.78 mm) for non-adjusted and adjusted depths of field, respectively. The use of adjusted root diameters paired with a 0.7848 mm depth of field resulted in an average RNPP of 170.3 (±37.38 SE) g/m^2/year, whereas unadjusted root diameters and a traditional 2 mm depth of field yielded an average RNPP of 66.6 (±14.59) g/m^2/year—an underestimation of 61 %, which was consistent across the 5 years of this study (Fig. 3a). Average fine root standing crop estimates for the top 16 cm of soil using unadjusted and adjusted root diameters and depth of view were 126.24 g/m^2 and 322.49 g/m^2, respectively. The use of unadjusted minirhizotron data also consistently underestimated fine root standing crop obtained from soil cores (Jackson et al. 2009) at the NC site, while data using adjusted diameters and a 0.7848 mm depth of field generally agreed with these destructive sampling estimates (Fig. 3b).

Discussion

The model presented in Eq. 2 represents a mathematical correction that can be applied to minirhizotron data to correct for the underestimation of root diameters in minirhizotron images resulting from root margins obscured by soil. The nature of this relationship is such that the discrepancy between perceived and actual root diameter increases with increasing diameter (Fig. 2). Points falling below the 1:1 line in Fig. 2 (indicating a larger diameter using minirhizotron images than using calipers) are likely due to occasions when rhizosphere soil attached to the root surface resulted in an overestimation of root diameter in minirhizotron images. Three large roots (> 20 mm) were extracted from tube surfaces during this study, and although these roots did conform to the model described in Eq. 2, they were excluded from all analyses due to the highly variable nature of monitoring coarse roots using minirhizotrons (Taylor et al. 2013). The average discrepancy between perceived and actual diameter for these three largest roots (23 mm–26 mm actual diameter) was 15.32 mm.

The relationship between perceived and actual root diameter did not differ across sites, indicating that this model may be widely applicable as a correction factor for minirhizotron studies implemented in a variety of soil types. Our NC and SC sites represent near extremes of clay- and sand-dominated soils, respectively. Although this does provide confidence in the potential for this model and depth of field to be applied across a wide range of soil types, differences may arise when using minirhizotrons in systems such as wetlands (Iversen et al. 2011). Additionally, the method used to calibrate measurements made using Rootfly software to known distances ensures that this model will be useful as a correction factor regardless of the software used to analyze minirhizotron images. Taken together, our results indicate the 0.78 mm calculated depth of field should be applicable for minirhizotron studies using any image analysis software and in a wide range of soil types.

The constancy of depth of field across soil types and the fact that the majority of the roots in this study had a larger true diameter (D) than was indicated by the minirhizotron image provide support for the second resolution to the paradox presented in the introduction. In order for the paradox's first resolution to be appropriate, a researcher would assume that the full diameter of each root encountered is visible in the minirhizotron image and would adjust the depth of field to ½ the diameter of the largest root. The largest diameter measurement made using a minirhizotron image (p) at our NC site was 11.3 mm, meaning that the depth of field for that image would need to be 5.65 mm using the first resolution to the paradox. However, the true diameter (D) for this root was 26 mm, providing direct support for resolution 2 to our paradox of large diameter roots encountered by minirhizotron images.

Our calculation of minirhizotron depth of field is, to our knowledge, the first attempt to directly calculate a depth of field using empirical data. Notably, Tingey et al. (2005) did use varying depths of field (between 0.3 and 2 mm, mean = 1 mm), which were assigned so that their scaled fine root biomass data matched previous estimates at their site. Thus, the only two efforts to provide support for an estimate of depth of field generally agree on values that are substantially smaller than the 2 mm and 3 mm values traditionally used. This difference is an important one, as scaling of minirhizotron data is dependent on multiplying fine root measures by a factor based on the amount of soil that is assumed to have been sampled by the minirhizotron tubes (Johnson et al. 2001; Bernier and Robitaille 2004; Brown et al. 2009). Even a small difference in the depth of field can result in large differences to fine root estimates due to the multiplication of this difference when scaling. For our data, > 99 % of the difference between adjusted

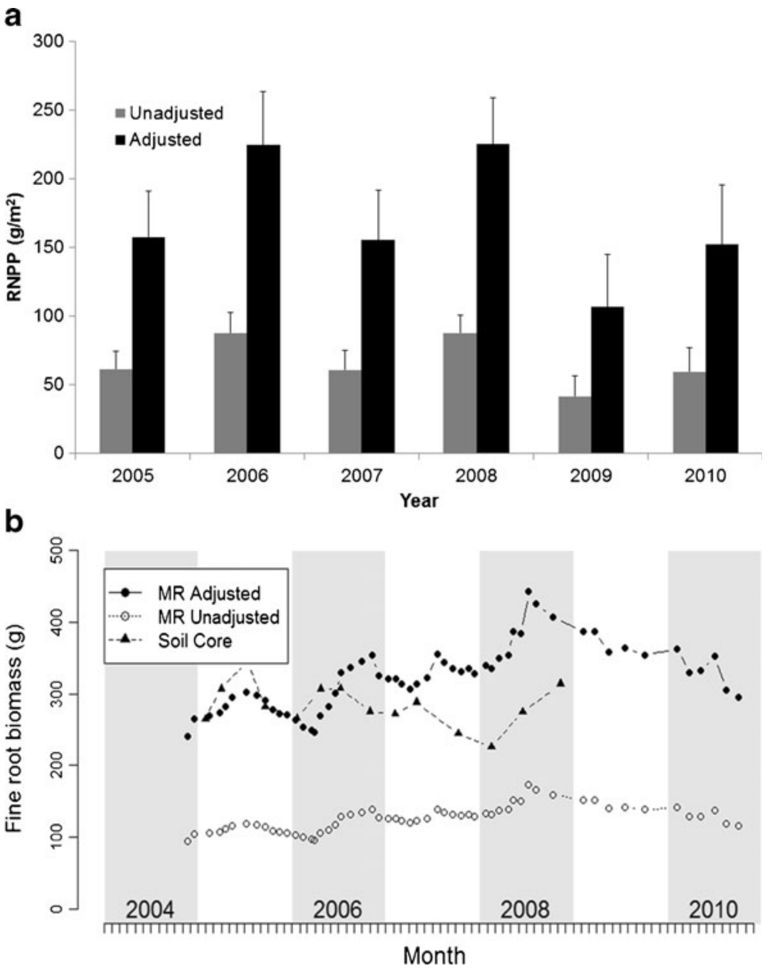

Fig. 3 **a** Fine root net primary production (RNPP) for each year between 2005 and 2010. RNPP numbers represent roots 0–2 mm in diameter and are scaled to a ground surface area of 1 m^2 to a depth of 16 cm. Grey bars represent production calculated using unadjusted root diameters and scaled using a minirhizotron depth of field of 2 mm. Black bars represent production calculated using diameters adjusted with Eq. 2 and scaled using a minirhizotron depth of field of 0.7848 mm. **b** Fine root standing crop estimates from minirhizotron (MR) tubes using both adjusted and unadjusted diameters and published soil core data, all sampled at our NC site. Soil core data is the average of ambient and elevated CO_2 treatments published in Jackson et al. (2009). Adjusted minirhizotron diameters were calculated using Eq. 2 and a depth of field of 0.7848 mm. Only data from the upper 16 cm of minirhizotron tubes was used to make these estimates comparable to the soil cores, which sampled to a depth of 15 cm

and unadjusted RNPP and standing crop was due to the change in depth of field, with <1 % of the adjustment resulting from adjusting root diameters. This is partially due to the small number of roots in this study with diameters between 1.57 mm and 2 mm. The effect of diameter adjustments may be significantly larger for studies investigating species with relatively thick fine roots (e.g. *Liriodendron tulipifera*, *Fraxinus americana*), but would likely always be much smaller than the effect of depth of field on RNPP and standing crop adjustments.

The use of the calculated depth of field and diameter correction model presented here may provide a solution to the consistent underestimation of fine roots by minirhizotrons in the upper layers of soil. Numerous studies have noted a significant underestimation of fine root biomass using minirhizotrons vs. destructive methods (e.g. Bragg et al. 1983; Parker et al. 1991; Samson and Sinclair 1994; Yuan and Chen 2012; Day et al. 2013). Likewise, the unadjusted data from minirhizotron tubes underestimated total fine root standing crop as compared to soil core data at our NC site (Fig. 3). Day et al. (2013) report similar underestimations of fine root biomass determined by minirhizotrons when compared to values derived using soil cores at the same site (33–41 %). It is possible that much of the underestimation seen in minirhizotron data throughout the literature is an artifact of the underestimation of root diameters and the overestimation of soil volume sampled by minirhizotron images. A recent study on the effects of sample size in root studies notes that discrepancies between minirhizotrons and destructive sampling are at least partially due to the volume of soil sampled (Taylor et al. 2013). Inaccuracies in minirhizotron data resulting from small samples and an inaccurate depth of field are separate but related methodological issues. We suggest that minirhizotron studies implementing both the largest soil volume possible and the correction factors presented here provide the best current method for accurately scaling minirhizotron data to larger soil volumes.

Minirhizotrons represent one of the most promising methods for the non-destructive study of roots *in situ*, yet a disconnect exists between roots viewed in minirhizotron images and those physically extracted via destructive sampling. The data presented here provide a simple method for adjusting root diameters underestimated by minirhizotron imaging. This correction factor, along with an empirically derived calculation for minirhizotron depth of field, will greatly improve the ability for researchers to apply dynamic root data obtained from minirhizotrons to estimate the entire root pool obtained by destructive methods, and should provide an improved method for scaling fine root biomass and production estimates obtained using minirhizotrons. Given the importance of fine roots in the movement of nutrients through the soil, the accuracy of data on the dynamic nature of these roots obtained by minirhizotrons is essential to a comprehensive understanding of terrestrial nutrient cycling at the ecosystems scale.

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