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A scanner-based approach to soil profile-wall mapping of root distribution

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Abstract: Root distribution sampling techniques are often inaccurate, time consuming and costly. We present an inexpensive approach to soil profile-wall mapping using a desktop scanner that allowed us to spend reduced time in the field. The scanner was pressed onto the vertical surface of a 1 x 1 m soil pit and images of the roots were taken *in situ*. In a common garden planting of eleven, 30-year-old conifer and hardwood tree species in Poland, we compared root counts (number of roots cm^{-2}) obtained by this method with independent measurements of root length density (RLD) obtained from soil cores. We found a positive correlation (Spearman rank correlation $r=0.93$; $P<0.001$) suggesting general agreement of the two approaches in ranking among the species. Soil coring as well as grid mapping with plastic overlays took a longer total time for quantifying root distribution than the scanning procedure. The desktop scanner approach we developed is an inexpensive, time efficient and accurate way of quantifying root distribution and abundance that allows a unique coupling of root data to soil properties.

Additional key words: methods, soil profile-wall mapping, root distribution, scanner

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Introduction

Root distribution in soil plays a major role in water and nutrient acquisition, plant competition, soil development and the composition and distribution of soil biota. Yet procedures for quantifying root distribution are often inaccurate, time consuming and costly. Most methods involve digging a trench or pit and recording roots by counting on a sampling grid, or marking positions on plastic overlays (van Noordwijk et al. 2000). Alternatively, soil cores can be taken and root length or mass quantified in each core. However, these techniques have disadvantages: sampling on a grid or plastic over-lay requires extensive time spent in the field marking each root in a grid, and substantial time quantifying the root distribution from the overlays. Root analyses conducted using soil cores are also time-costly and do not readily allow a comparison of root densities with soil genetic horizons.

We present here a novel approach to profile-wall mapping that allowed us to spend reduced time in the field. We used a desktop scanner that captures images of roots on a vertical soil surface to determine number of roots visible in a vertical plane (root count cm^{-2}) of different soil horizons. This method was described in brief in Dauer et al. 2007, however, the purpose of this paper is to 1) validate this new method by comparing it to independent measurements of root length density (RLD) obtained from soil cores taken at the same site and 2) compare advantages and disadvantages of the desk-top scanner technique to classic techniques of profile-wall mapping and soil core methods.

Methods

Study site

The study area was located in a common garden in the Siemianice Experimental National Forest in central Poland (51°14.87'N, 18°06.35'E, altitude: 150 m). Soils were nutrient poor with a plowed A-horizon, and the soil texture averaged 80% sand and 15% silt. Climate of the region is transitional between maritime and continental, and the mean annual precipitation was 591 mm. Mean temperature was 8.2°C with a mean growing season of ≈213 d, calculated as the number of days with a mean temperature =5°C.

Six conifer species (*Abies alba*, *Picea abies* [L.] Karst., *Pinus sylvestris* L., *P. nigra* Arn., *Larix decidua* Mill., *Pseudotsuga menziesii* Franco) and eight hardwood species (*Acer platanoides* L., *A. pseudoplatanus* L., *Betula pendula* Roth., *Carpinus betulus* L., *Fagus sylvatica* L., *Quercus robur* L., *Q. rubra* L., *Tilia cordata* Mill.) were planted in 1970 and 1971 in 1 × 1 m spacing in two adjacent plantings. Each planting had nine of the species, replicated three times, in a total of 27, 20 × 20 m monospecific plots. Details of the experimental area were presented elsewhere (Szymanski 1982, Withington et al. 2003, 2006; Reich et al. 2005; Hobbie et al. 2006, 2007; Knight et al. 2008; Przybyl et al. 2008). Due to the high tree density, few understory plants were present.

Image collection

During one week in 2002, pits 1 m wide, 1.8 m long and 2 m deep [soil pit dimensions differed from those of scanning windows] were excavated in each plot (from 3–6 plots per species, 53 pits in total). Because of the time constraints of working at a distant field site and the requirement to work ahead of soil scientists who were sampling soil profiles in each pit, it was not possible to map roots with the conventional plastic over-lay method, which may take half a day for each pit (Table 1). Dong et al. (2003) described a method of monitoring root growth of apple trees growing in greenhouses by pressing a scanner to a transparent acrylic sheet on one side of a root box. We modified this scanner-based approach from image acquisition of small areas in controlled indoor environment to documenting large area of roots growing in soil in field conditions. We used a scanner to capture images of our soil pits in Poland, which allowed us to map root distribution of a pit face of approximately 1 m^2 in about one hour. In each monoculture plot at our field site, the faces of each pit were prepared by smoothing the soil with a flat shovel and cement knife. Soil was brushed away to expose the roots, particularly in the organic horizon. All roots were clipped to <3 cm in length. The face was sprayed with water to increase the color contrast between roots and soil, wash soil from the roots and stabilize the sandy soil on the side of the pit. The face was grid-marked in squares 30.5 × 22.8 cm (fit to scanner window) to cover the area of the pit face (1 × 1 meter). The grid was marked with pins. Images of each square were taken at 200 dpi using a desktop scanner Epson Perfection 1250 (Seiko Epson Corporation) and a Mac PowerPC G3 computer with a 700 Mhz processor and 384 RAM. The scanner had a depth of field of approximately 3.5 cm. At 200 dpi, roots were magnified 2.2 times. The lid of the scanner was re-

Table 1. Advantages and disadvantages of root distribution methods based on scanning and coring events in Poland (Withington et al. 2006), profile wall mapping in a grape vineyard in New York (Rick Dunst 2004 personal communication) and digitizing profile-wall maps (van Noordwijk, et al. 2000)

Technique	Field person-hours	Lab person-hours	Total hrs per plot	Other unique advantages
Profile-wall scanning images	1.2 per soil pit taking images	2.3 per soil pit analyzing images	3.5	Can quantify roots by soil location, data is in digital form
Profile-wall grid mapping with plastic over-lay	4.0 per soil pit	12.0 per soil pit digitizing profile-wall maps	16.0	Can quantify roots by soil location
Soil cores	3.0 per plot (based on 3 cores per plot [156 cores total] taken in Poland)	9.0 per plot (based on cleaning and sorting 3 cores per plot [156 cores total] in Poland)	12.0	Can group roots by order, analyze root biomass, architecture and anatomy

moved and the surface was covered with acetate and tape to protect it from being scratched. After the images were obtained, soil horizons in each pit were characterized and sampled for chemical analysis.

Analyzing images

Scanner images of the soil profile were used to determine the number of roots intersecting a given area of soil in each soil horizon. In Adobe Photoshop 7.0 (Adobe Systems Incorporated), a new image indicating roots and horizons was created using a digital transparent layer added to each scanned image. One dot indicated a root each time it transected an imaginary vertical plane (Fig. 1). Horizons were outlined, and labeled on each image. ImageJ 1.28 (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA) software was used to analyze the number of root intersections (dots) in the image for a given area (or number of roots in a given horizon).

Soil cores

Only eleven of the fourteen species were sampled for soil cores and used in this comparison. Soil cores were randomly collected from previously undisturbed soil in July 2001 using 15-cm long, 4.8 cm diameter soil core sampler (Arts Mfg. & Supply, American Falls, Idaho, USA). Cores were taken from the same hole at two consecutive depths: 0–15, 16–30 cm (Withington et al. 2006) with three reps per plot. Roots were cleaned and sorted into root order and scanned on a desktop scanner using WinRHIZO software at 400 dpi (Regents Instruments Inc., Quebec, Canada) to obtain root length density (RLD), the length of roots in a volume of soil (cm cm^{-3}). RLD was averaged over the two depths for comparison of root distribution at the top 30 cm of soil.

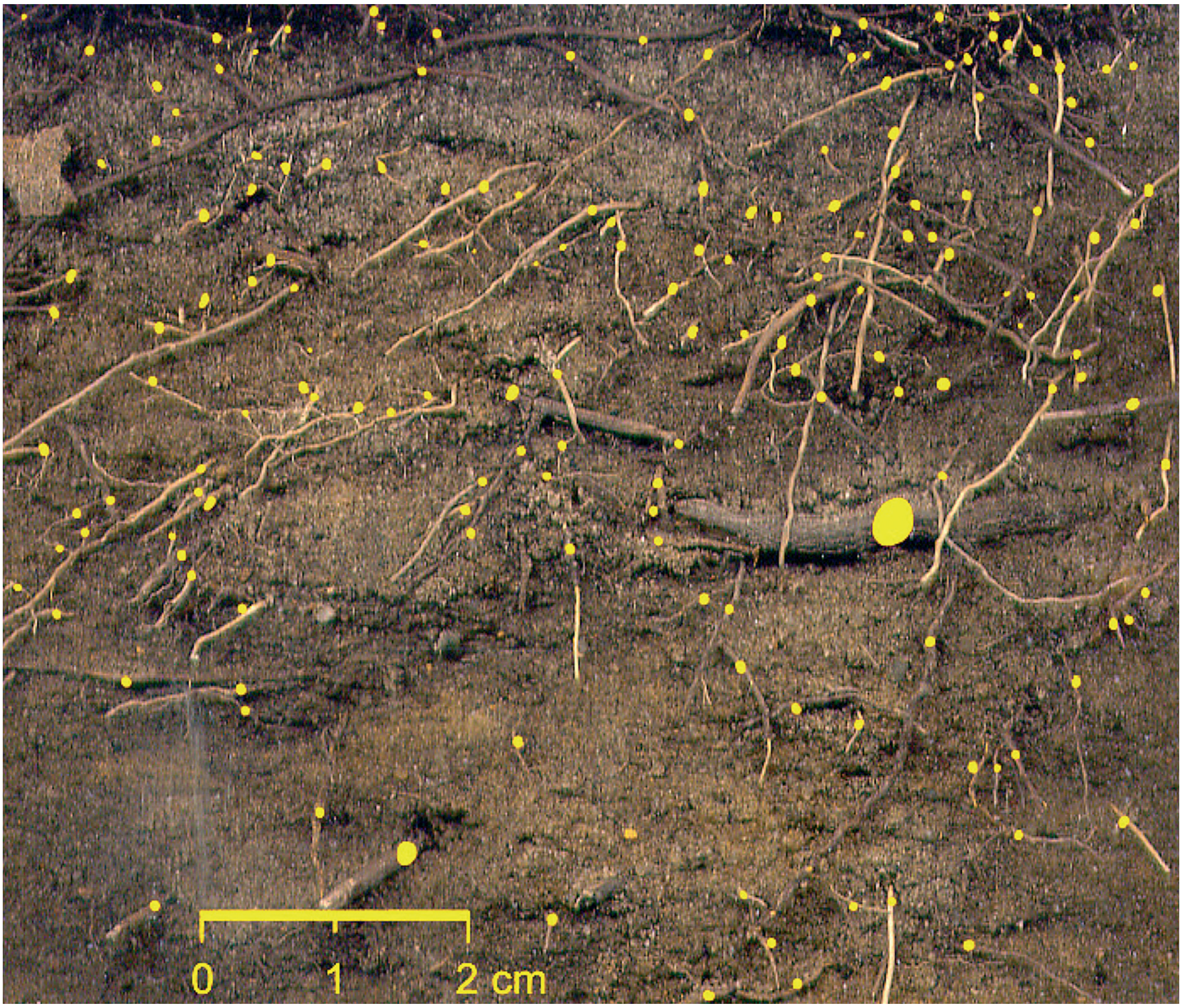

Fig. 1. *Carpinus betulus* roots *in situ*, image taken with a scanner and enlarged to show roots < 1 mm. Each root was marked with one dot indicating each time a root transected an imaginary vertical plane

Results and Discussion

Because scanner data were delineated by horizon and soil core data were grouped by depth, we used only data with median horizon depth < 30 cm in order to compare the two methods. Root numbers from scanned images were positively correlated with RLD from soil cores (Fig. 2; $r=0.93$; $P<0.001$), suggesting general agreement of the two approaches in ranking root density among the species. Potential errors in the scanner approach include underestimates in root number in areas where, due to uneven soil surfaces, the image was dark or unfocused or where the roots were too small to detect, and overestimates due to inconsistency in clipping roots at 3 cm from the soil surface.

Both methods indicate that *Acer platanoides* and *Acer pseudoplatanus* ranked highest among the species examined in the number of roots in a given vertical plane or volume of soil (Fig. 2). The scanning method showed both *Acer* species had twice the number of roots per unit area of profile-wall than the other species. With the exception of *Tilia cordata*, the hardwood trees had higher root count (roots m^{-2}) and higher root length density (RLD, cm cm^{-3}) than the conifer trees (Fig. 2).

Both soil coring in Poland, as well as grid mapping and analysis in a grape vineyard in New York (Rick Dunst 2004, personal communication) took longer total time for quantifying root distribution than the scanning procedure (Table 1). The time required for each scan depends on the resolution of the image. A higher dpi would allow a greater magnification of the images but at the cost of longer duration for image acquisition. At 200 dpi we were able to detect roots with a diameter of 1 mm and take one 30.5 × 22.8 cm image in approximately 30 seconds. These time constraints may be mitigated by faster computers and faster scanners than those used in this study. Time required for image acquisition may increase if the soils are rocky, requiring more time to smooth the soil surface.

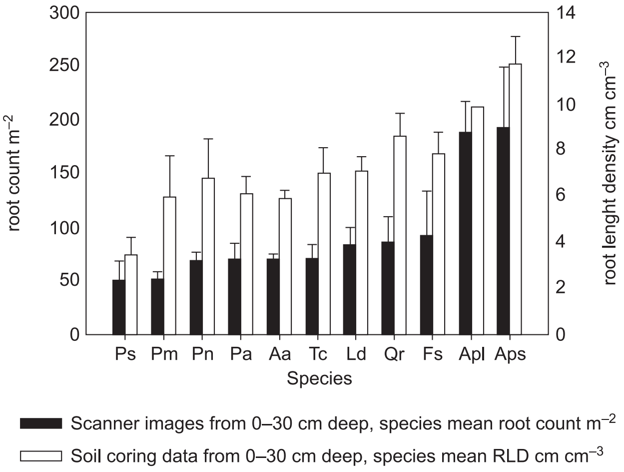

Fig. 2. Eleven species of forest trees ranked by increasing root count m^{-2} obtained by scanner images. There is a significant correlation of root count m^{-2} with root length density (RLD) obtained by soil cores taken at the same site. Spearman rank correlation $r= 0.93$; $P<0.001$. The abbreviation Ps = *Pinus sylvestris*, Pm = *Pseudotsuga menziesii*, Pn = *Pinus nigra*, Pa = *Picea abies*, Aa = *Abies alba*, Tc = *Tilia cordata*, Qr = *Quercus rubra*, Fs = *Fagus sylvatica*, Ld = *Larix decidua*, Apl = *Acer platanoides*, Aps = *Acer pseudoplatanus*

Sorting roots from soil after coring can be a long process. It took 150 hours in the field and 480 hours in the lab to sort roots from 156 soil cores (Table 1). The hours spent in this example are typical of such root work, but also include sorting roots into size classes, which inflated the hours to some extent. Alternatively, the soil core-break method does not require roots to be sorted from the soil, and instead involves estimating the number of roots by breaking the soil core in half and counting the number of roots sticking out of both soil surfaces (Escamilla et al. 1991, van Noordwijk et al. 2000). Due to sources of error, including effects of preferential root orientation, the break being unrepresentative, random variation of numbers of roots intersecting the plane of observation, and counting errors, this technique can be of low precision (Bland 1989, 1991). In general, soil cores are disadvantageous in directly relating local root density with other soil characteristics observable from soil pits. Additionally, because of low root densities deeper in the soil, obtaining accurate estimates of root distribution at deeper depths with soil coring may require a very large number of cores. However, soil cores have the advantage of being less destructive than large soil profiling pits.

A distinct advantage of the scanner technique over using a plastic sheet to map roots on profile-walls is the short amount of time spent in the field acquiring data (Table 1). The scanner may also save processing time because the images are already in a digital format and there is no extra time logging coordinates into the computer. Other studies have captured *in situ* root images digitally by photography (Schmid and Kazda 2002), but photographs require complicated image rectification to account for camera angle and uneven light sources.

A disadvantage of both soil cores and profile-wall mapping, is the possibility of missing the finest roots in the soil. Minirhizotrons are a root quantification technique that can capture the smallest roots by lowering a camera through a clear plastic tube that resides in the soil. We found that minirhizotron root length intensity (cm root length cm^{-2} window viewing area) data taken from the same plots did not correlate well with either soil core RLD data or scanner root count data (unpublished data). Minirhizotrons have been known to distort distribution of roots, giving an inaccurate underestimation of root intensity in the upper soil layers and an overestimate of roots in the deeper layers (Bragg et al. 1983, Hansson and Andren 1987, Parker et al. 1991, Heeraman and Juma 1993, Samson and Sinclair 1994, Pages and Bengough 1997, Ephrath et al. 1999, Smit 2000). Detecting tiny roots using the scanner approach may be possible in the future as increasingly faster computers, data transfer and scanner image acquisition should decrease collection time of high-resolution images allowing greater magnification of images.

The scanner technique may be less expensive than other approaches as it saves time in labor wages. Also, it required very inexpensive equipment. The cost of the scanner was $100, while soil corers typically cost from $100–$1000 (Giddings Machine Co., Windsor, Colorado), and a minirhizotron camera can cost $20,000. Image processing software with simple analyzing capabilities was readily available in the public domain (ImageJ). The greatest strength of the scanner approach was its ability to couple root distribution with soil characteristics. For example, root data from this site will be coupled to soil solution and exchangeable ion data by horizon to address questions about species-specific local accessibility to nutrients. We were able to examine roots in the soil habitat in which they reside without destroying genetic horizons.

Conclusion

The approach we developed using a desktop scanner is a time efficient, inexpensive and apparently accurate way of quantifying root distribution and abundance. Quantifying root numbers by soil horizon allows a unique coupling with soil properties. The scanner technique may be a useful tool in studying a range of ecological questions including nutrient cycling, resource partitioning and biogeochemistry.

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