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A comparison between minirhizotron and monolith sampling methods for measuring root growth of maize (*Zea mays* L.)

HOOSHANG MAJDI[1], ALVIN J.M. SMUCKER[2] and HANS PERSSON[1]
[1]*Department of Ecology and Environmental Research, Swedish University of Agricultural Sciences, P.O. Box 7072, S-75007, Uppsala, Sweden and* [2]*Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 488242, USA*

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Abstract

Transparent plastic minirhizotron tubes have been used to evaluate spatial and temporal growth activities of plant root systems. Root number was estimated from video recordings of roots intersecting minirhizotron tubes and of washed roots extracted from monoliths of the same soil profiles at the physiological maturity stage of a maize (*Zea mays* L.) crop. Root length was measured by the line intercept (LI) and computer image processing (CIP) methods from the monolith samples.

There was a slight significant correlation ($r = 0.28$, $p < 0.005$) between the number of roots measured by minirhizotron and root lengths measured by the LI method, however, no correlation was found with the CIP method. Using a single regression line, root number was underestimated by the minirhizotron method at depths between 0–7.6 cm. A correlation was found between root length estimated by LI and CIP. The slope of estimated RLD was significant with depth for these two methods. Root length density (RLD) measured by CIP showed a more erratic decline with distance from the plant row and soil surface than the LI method.

Introduction

The need for more accurate and reproducible methods to quantify root growth and activity has led to the development of several new approaches in recent years. The minirhizotron tube and associated micro video camera technology reported by Upchurch and Ritchie (1983) is one new and promising method. The monolith sampling method has furthermore been improved by separating the roots from soil by hydropneumatic elutriation (Smucker et al., 1982), and then using either the line intercept (LI) method or video recording of the roots followed by computer image processing (CIP) for measuring root length.

Minirhizotrons can be used to visually observe and photograph plant roots intersecting plastic tubes. This method has among other things been used to measure root numbers, branching frequencies as well as mesofaunal activity in the rhizosphere (Box et al., 1989; Ferguson and Smucker, 1989; Snider et al., 1990). Its main advantage is that production, development, ageing and mortality of roots can be followed continuously throughout the year at the same place in the soil profile. However, it is not clear how the plastic tubes affect the root growth observed.

Sampling of roots in field soils by the hand auger or the monolith sampling method provides washed root samples from which the root surface area, biomass, necromass, width, length and other morphological variables can be estimated

(Smucker et al., 1982, 1987; Srivastava et al., 1982; Vogt and Persson, 1991). Roots, rhizosphere and bulk soil samples can also be collected for chemical analyses.

All these methods of root investigations have their specific applications to the plant-root-soil interface and are, except for the minirhizotron method, very time-consuming. The purpose of this paper is a comparison of measurements of similar root systems by minirhizotron and monolith sampling methods.

Material and methods

This study was carried out at the Kellog Biological Station in Michigan. The soil type is a highly stratified glacial Kalamazoo loam soil (fine loamy, mixed, mesic, typic Hapludalf). Root growth was studied in four different treatments: conventional tillage, no tillage and fertilizer treatments of 0 and 150 kg N ha^{-1} during one year of the project. The experimental design was replicated 4 times. Each plot was 35×28 m in size. Maize (*Zea mays* L.) was planted on May 10. The monolith samples were taken in early September and minirhizotron observations were completed in late August 1987. The data derived from all replicates was considered as a general sample without regarding treatments. Statistical analyses were carried out using the SAS, GLM procedure (Student's t-test with accounting for missing values).

Minirhizotron measurements

In each plot, three minirhizotron tubes (183 cm length, 5.1 cm i.d., 5.7 cm o.d., made from butyrate) were installed into the soil beneath the plants. The tubes intersected the soil at a depth equivalent to position 7 in sample profile A (Fig. 1). Profile B, with the same subsample dimensions as profile A, was sampled between the plant rows at 22.8 to 38.0 cm from the plant row. This double-profile sampling scheme provided data for root distribution patterns for one half the distance between the plant rows (Fig. 1).

Soil cores (5.5 cm in dimeter) were removed at an angle of 45° to the soil surface, to depths of 119 cm using a Giddings hydraulic power auger soil sampler. The machined cutting points of the probe, which replaced conventional cutting points, resulted in compressing of the displaced soil toward the interior of the sampling tube (Box et al., 1990). Soil on the walls of the hole which had been oriented by the sampling probe, was removed by using a 5.5 cm fine circular steel brush.

Transparent minirhizotron tubes were manually forced into the holes. After installation, the 15 cm of the tube which remained above ground was painted black and then white to exclude light and reduce solar warming. The bottom and top openings of each tube were sealed by rubber stoppers. Tubes were labelled with an identification number and an index reference notch was installed 5 cm from the top end. After inserting the tubes in the holes, the soil surface was compressed around them to reduce water and light penetration along the tube surface. On each measurement occasion a Circon color video camera, was lowered into each minirhizotron tube by a square aluminium handle, containing registration holes 1.2 cm from center to center as described by Ferguson and Smucker (1989). Camera depth was controlled to ± 0.1 mm by a plastic drawer-carriage wheel which stopped at each registration hole in the handle. Four incandescent light bulbs, 3 watts each, placed around the lens, provided uniform, diffuse light at each 'window' of the minirhizotron tube. Computer generated bar codes were recorded on video tapes in the field to identify the date, time, minirhizotron number and depth to the deepest root. Root images were video recorded on standard 1.9 cm VHS tapes and catalogued for further analysis in the laboratory. Root counts were measured from the videotape by using one monitor for displaying each recorded image and another monitor for registration of the root count, depth, time and date of recording.

Two large profiles were extracted in the area of each minirhizotron tube (Fig. 1). The monoliths were extracted by the profile sampling technique described by Srivastava et al. (1982). Each profile was fractionated into 18 subsamples (each 439 cm^3), labeled and stored at 3°C.

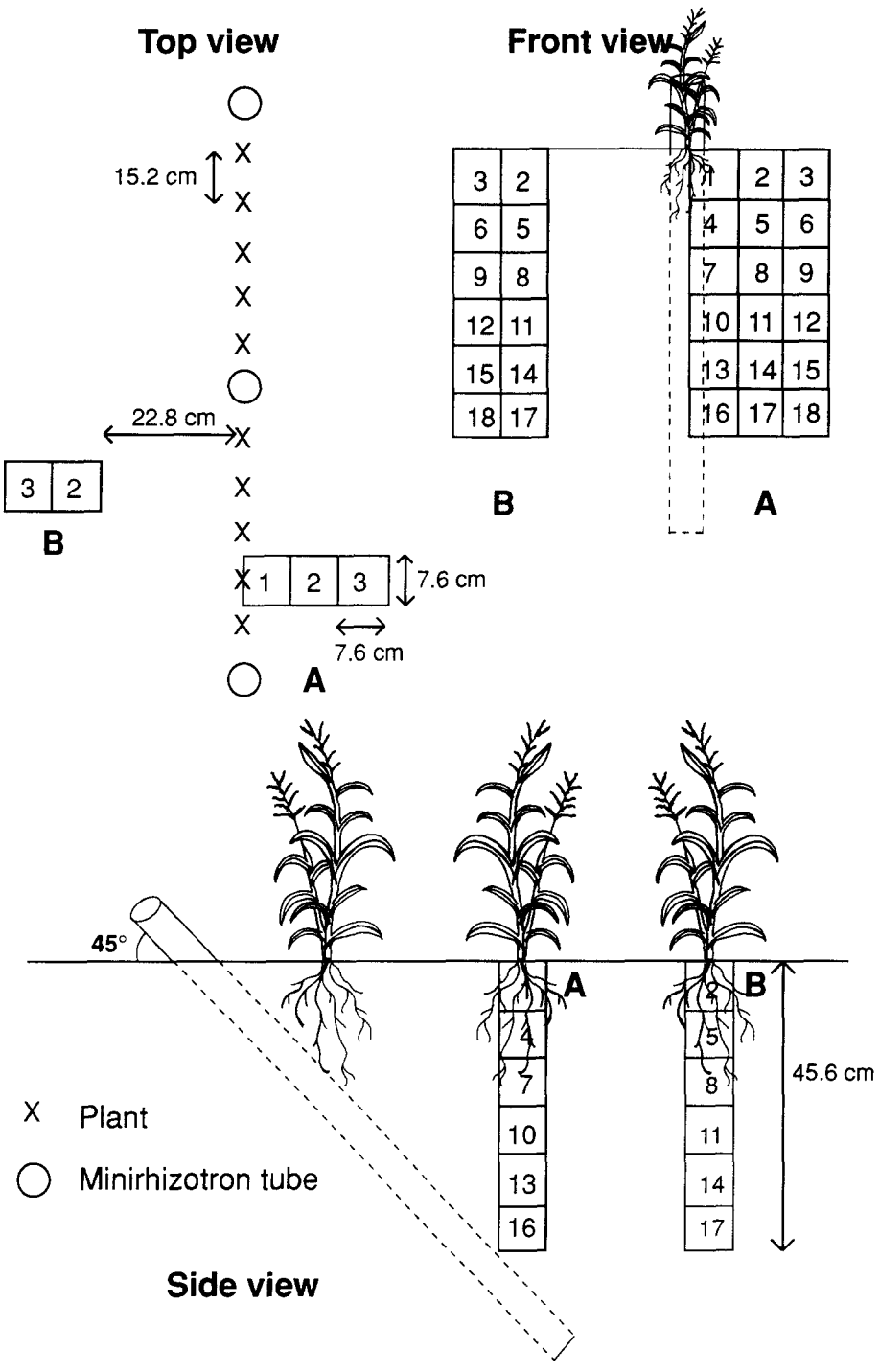

Fig. 1. Diagram of relative locations of the monolith profile samples (**A** and **B**) and the minirhizotron tubes with respect to the plant row.

Computer image processing

Roots were washed from the soil by the hydropneumatic elutriation method (cf Smucker et al., 1982). Washed roots were processed immediately or stored in plastic bags containing 100–200 mL 10–15% methanol solution and stored at 3°C. Roots were dyed with Malachite green oxalate 24–48 h before video recording, by injecting 5–10 mL of 1% dye into the plastic storage bag. Stained roots were rinsed with distilled water on an ultra fine (25 μm) nylon screen and placed uniformly into a clear glass tray where the roots were covered with a thin water film of approximately 1 mm above the root samples. The glass tray was illuminated from below by a translucent light table and video recorded by a computer controlled robotic camera which recorded 64% of the area on a VHS video tape (Smucker, 1990).

The video images were transferred to the Vicom image analysis system (Smucker et al., 1987; Smucker, 1990) and digitized for measurement of root length and diameter classes. Video recorded root images (64 per tray samples from each 439 cm^3 of soil) were analyzed by the Vicom pipeline and parallel image processor. Processing time for each image varied between from 1.6 to 2.5 minutes, depending on the quantities of non-root residues in each sample. The amount of residue in each sample ranged from zero, for the deepest samples to 17% for the surface samples. The Vicom computer algorithms are designed to measure root lengths and diameters with a 3% error for roots ranging in diameter from 0 to 3 mm when residue contents are less than 20%. Samples with more than 20% residues result in greater errors.

Line intercept method

Manual measurements of washed maize root length were obtained by the line intercept method (Tennant, 1975). Each tray of root samples, which had previously been video recorded, was counted for a 4 cm grid after uniformly redistribution the roots across the tray. The same technician manually counted the roots of all samples to minimize errors which may occur among several operators (Bland et al., 1988).

Results and discussion

Figure 2 indicates a correlation between the LI and CIP methods of estimating root length ($r = 0.782$, $p < 0.001$). However, the manual LI method (LSD/least significant difference = 0.05; $p < 0.05$) yielded values higher than the CIP, (LSD = 0.03; $p < 0.05$), at root lengths larger than 11 meters and lower values at short root length (Fig. 2). The higher estimate of root length by LI was probably caused by an overestimation of the actual intersections (Tennant, 1975). However, the image processor may also discard many small root fragments, which are similar in size and shape to organic debris (Smucker et al., 1987).

Root length densities (RLD) decreased with soil depth and also with distance from the plant at all soil depths when estimated by both LI and CIP (Fig. 3 and 4) and this decline was more erratic for CIP than it was for LI. Since RLD obtained by the monolith sampling methods (LI and CIP) was larger beneath plants and the minirhizotron tubes were installed parallel to the plant row, a comparison between these two methods of root measurement was limited to the sampling locations immediately beneath the

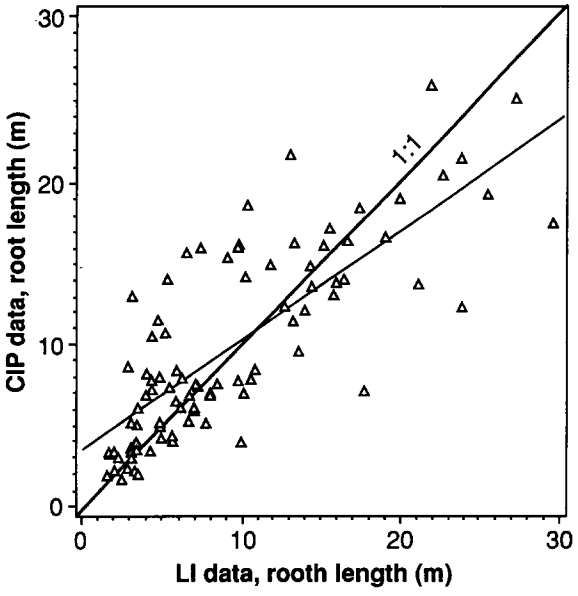

Fig. 2. Correlation of root length data evaluated by the line intercept (LI) and computer image processing (CIP) methods.; $r = 0.781$; $p < 0.001$.

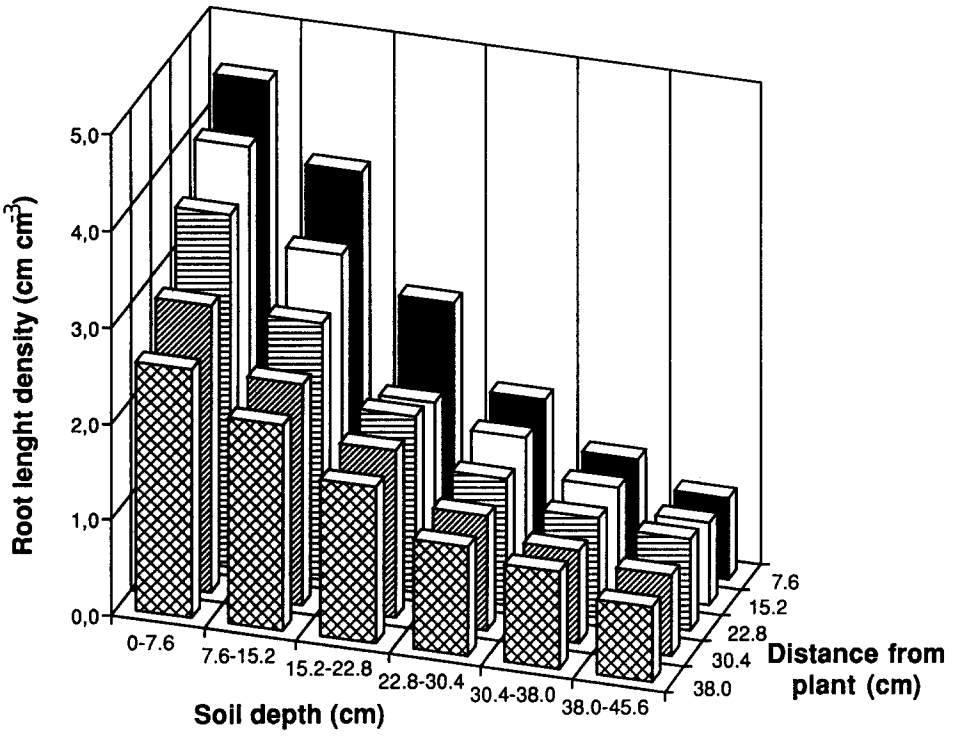

Fig. 3. Root length density distributions with soil depth and distance from the plant. Data represents average of 4 replicates obtained by the monolith sampling method and line intercept estimates (LI) of washed roots.

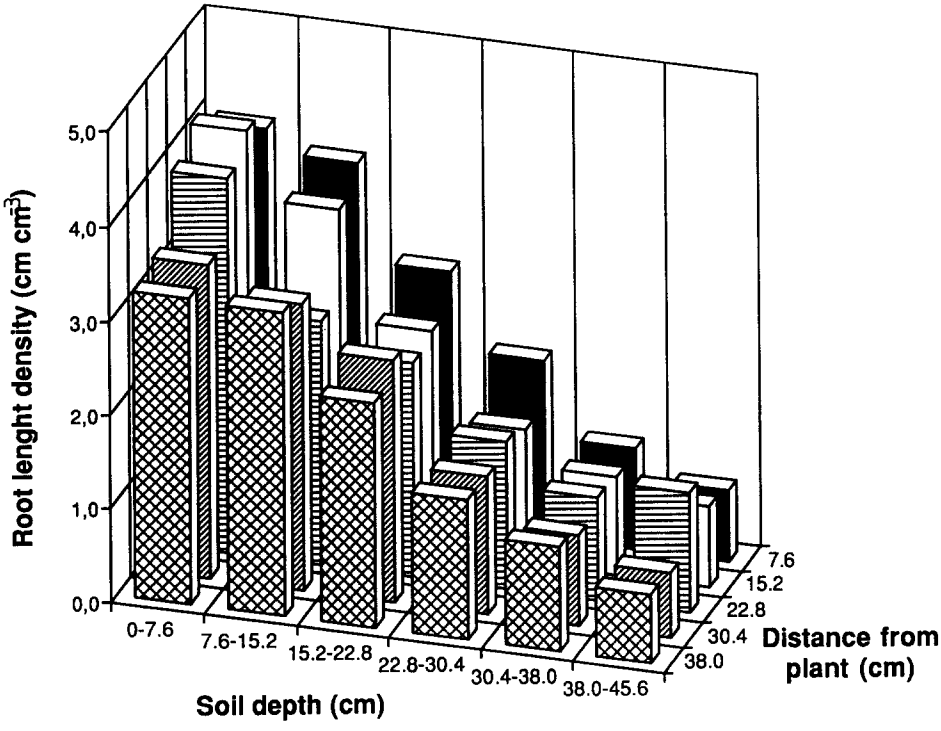

Fig. 4. Root length density distribution with soil depth and distance from the plant. Data represents average of 4 replicates obtained by the monolith sampling method and computer image processing estimates (CIP) of washed roots.

plant for all depths (cf Fig. 1, profile A locations 1, 4, 7, 10, 13 and 16). The minirhizotrons were furthermore installed at an angle of 45°, in order to give better estimates of root distribution (cf Bragg et al., 1983).

Minirhizotron measurements indicated fewer roots at the 0–7.6 cm than at the 7.6–15.2 cm depth (Fig. 5). In contrast to the minirhizotron data, RLD measured by LI and CIP, showed the highest quantities of roots in the 0–7.6 cm portion of the soil profile and showed a significant slope with depth. Values of RLD measured by LI method declined (Student's t-test, $p < 0.001$) steadily to the deepest depth with a significant decline in root length in the plow pan region of the soil from 15.2 to 22.8 cm depth. Discrepancies between root quantities in the surface horizons of soils have been reported by other investigators using similar methods (Bragg et al., 1983; Upchurch and Ritchie, 1983; van Noordwijk et al., 1985). This could be a result of soil compaction above the minirhizotron tubes (McMichael and Taylor, 1987), reducing root growth in these regions of the soil. On the other hand, since more plant residues occur in the shallow horizons, this could also be the result of larger root lengths measured using the LI and CIP methods, since it is impossible to exclude all residue content from the root samples. Furthermore, several authors have reported that poor soil/tube contact can influence the minirhizotron measurements, due to reduced visibility of roots growing in the gap between the minirhizotron wall and the soil (Gijsman et al., 1991, Parker et al., 1991).

There was only a slight, but statistically significant correlation ($r = 0.28$, $p < 0.005$) between the number of roots measured by minirhizotron and RLD measured by the LI method (Fig. 6). No significant correlation was found between RLD obtained by CIP and minirhizotron measurements. Bland and Dugas (1988) also reported a correlation ($r = 0.44$) between number of sorghum roots counted in minirhizotrons and root lengths measured by the line intercept method. In the latter case, the data, however, according to the latter authors, had been corrected for measurement errors. The reliability of

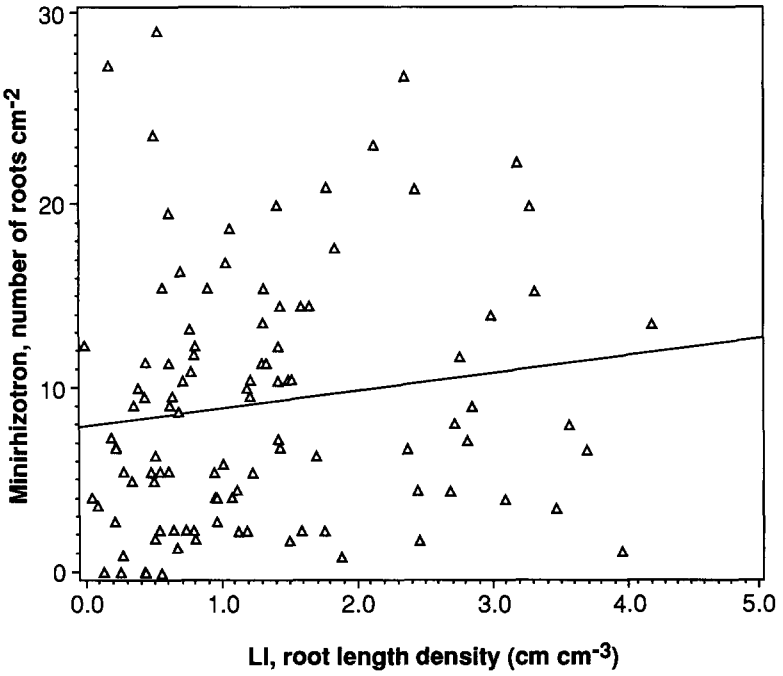

Fig. 5. Comparison of root numbers measured by the minirhizotron method and root length density (RLD) measured by the (CIP) and (LI) of washed root samples from monolith sampling throughout the upper 45.7 cm of soil. Each value is the average of 16 samples.

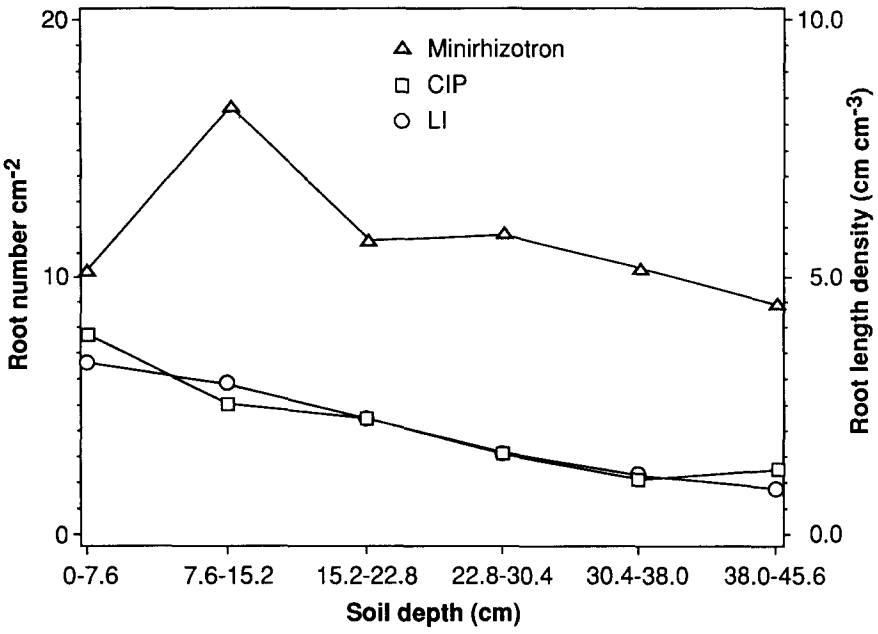

Fig. 6. Correlation of root number as measured by the minirhizotron and root length data obtained by the line intercept method (LI). $Y = 165X + 7.8$; $r = 0.28$, $p < 0.001$.

results obtained from minirhizotron measurements depends to a great extent on whether or not the root growth observed along the minirhizotron walls correspond to actual root dynamics in the soil. Our results indicate that there is no clearcut and simple relationship between the minirhizotron data and the data from the monolith methods since different kinds of measurement error may influence the results. The measurement errors will be large since the data must be derived from different soil volumes at different distances from the maize plant.

Conclusions

The number of roots measured by the minirhizotron technique and root length density measured by monolith sampling methods was not strongly correlated. Root length density measured by the CIP method was more erratic than it was for the LI method. Roots intersecting the minirhizotrons can be frequently observed in situ, while root samples obtained by destructive methods can be measured for length and width and weighted for biomass at occasional samplings. Further investigations to establish the adequacy of minirhizotron data and to determine which improvements to this technique are required. Root length densities (root length per unit volume) derived by monolith sampling methods and root intensities (root number per unit area) measured by minirhizotrons may be comparable, but we should remember that each provides estimates of distinctly different variables. Moreover, the relative pattern of root intensity with depth may differ from that of root length density.

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