

## Measuring root turnover using the minirhizotron technique

Weixin Cheng<sup>1\*</sup>, David C. Coleman<sup>1</sup> and James E. Box Jr.<sup>2</sup>

<sup>1</sup>*Institute of Ecology, University of Georgia, Athens, GA 30602 (U.S.A.)*

<sup>2</sup>*USDA ARS 1420 Experiment Station Road, P.O. Box 555, Watkinsville, GA 30677 (U.S.A.)*

(Accepted for publication 5 July 1990)

### ABSTRACT

Cheng, W.-X., Coleman, D.C. and Box, J.E., Jr., 1991. Measuring root turnover using the minirhizotron technique. *Agric. Ecosystems Environ.*, 34: 261–267.

Measurement of root turnover has been one of the most difficult problems in terrestrial ecosystem studies owing to the lack of an appropriate method. In this paper, a method for measuring intra-seasonal root turnover is described. The recently developed minirhizotron system was adapted and combined with hand tracing of the minirhizotron-produced video tapes. The basic components of a minirhizotron system are briefly introduced. A hand-tracing procedure and the subsequent data analyses are described.

### INTRODUCTION

It is widely recognized that root growth, death and subsequent decomposition of the dead roots, known as root turnover, are very important processes in all terrestrial ecosystems (Dalhman, 1968; Coleman, 1976, 1985; Milchunas et al., 1985). Root turnover is not only a path of energy input into the soil but is also related to other ecological processes, i.e. environmental adaptation of plants, nutrient acquisition, plant competition, interactions of plants with soil organisms, and soil structure and formation. Research on plant root systems under field conditions is difficult because soil limits their accessibility for observation (McMichael and Taylor, 1987). Measurement of root turnover has been one of the most intractable problems (Singh and Coleman, 1977; Fogel, 1985).

Radio-tracer techniques have been used to measure root turnover. Dalhman (1968) reported that the average annual root turnover rate in a grassland ecosystem was near 25% of the total below-ground biomass using <sup>14</sup>C-labelling techniques. Warembourg and Paul (1977) calculated an annual root turnover rate in a Canadian grassland of 9.5% using <sup>14</sup>C-tracer. Milchunas et al. (1985) demonstrated in a pot study using <sup>14</sup>C-tracer that root turnover

---

\*Present address: Systems Ecology Research Group, Department of Biology, San Diego State University, San Diego, CA 92182, U.S.A.

estimates produced by the  $^{14}\text{C}$  dilution method are more accurate than by soil core sampling. Singh and Coleman (1973, 1974, 1977) developed a  $^{14}\text{C}$ -tracer technique to distinguish functional roots from nonfunctional roots in Colorado short grass prairie. There are some problems associated with the radio-tracer method. Firstly, it has a safety problem owing to the use of radioactive materials. Secondly, introduction of radioactive material into plants (or labelling) is technically complicated and problematic. Thirdly, this method requires destructive sampling.

The use of glass wall rhizotrons has considerably accelerated progress in many fields of root ecology (Böhm, 1979) and root turnover can be studied using these methods (Ares, 1976; Atkinson, 1985). The rhizotron method has several advantages over most other root study methods when extensive measurements are required. Successive measurements are made at the same place, and estimates of root growth and death at the glass-soil interface are obtained rapidly (Huck and Taylor, 1982). This method also has certain disadvantages. First, since the roots are not growing in completely natural surroundings, the results produced using this method may differ substantially from those of field conditions. Second, there is a significant construction cost if a rhizotron facility is built.

The recently improved minirhizotron technique (Taylor, 1987) is very suitable for root turnover studies. It is non-destructive and relatively less labor intensive. Frequent measurements can be done *in situ* with very little disturbance to the natural environment using this technique.

In this paper, a hand-tracing method is adapted to the recently developed minirhizotron technique in order to study intra-seasonal root turnover. Each component of the minirhizotron system is briefly introduced. The tracing procedure and the subsequent calculation of the data are described in some detail. Some basic assumptions and practical tips are discussed.

#### COMPONENTS OF THE MINIRHIZOTRON SYSTEM

The modern minirhizotron system usually consists of: (1) a transparent observation tube installed into the soil at each sampling position; and (2) a video camera system which includes a miniature video camera, a TV monitor, a video cassette recorder (VCR) and other accessories.

Observation tubes can be made of glass, acrylic, lexan, plexiglas, polybutyrate or polycarbonate. None of these materials seems to interfere with the observation (Brown and Upchurch, 1987). The shape of the tubes can be round, square or other shapes depending on the purpose of the experiment. The advantages of round tubes over square ones are that they are usually available from stock at plastic supply companies, can be installed with conventional soil augers or probes, and the viewing camera can be rotated  $360^\circ$  in the tubes. The disadvantage of the round tubes is that uniform lighting is more difficult

to obtain because of the curved surface. The size of the tubes can range from 12 to 80 mm in diameter depending on the experimental requirements and the equipment available. The installation of the tubes is a very critical step since it determines how well the soil-tube interface can be maintained. For a realistic measurement of roots in situ, tubes inserted at angles less than 90° seem to be more desirable (Bragg et al., 1983). Tools involved in the tube installation can range from a hand-operated auger to heavy vehicle-mounted hydraulic systems (Bragg et al., 1983; Upchurch and Ritche, 1983; Box and Johnson, 1987). The criteria here are to maintain the natural soil surface for interfacing the tube and to keep the disturbance as low as possible. The part of the tube above the soil surface should be capped, wrapped and sheltered to keep the light from entering the tube (Levan et al., 1987). To study root distribution along soil profiles the tubes should be marked incrementally before installation.

Color and black and white video cameras have been specially designed for use in minirhizotrons (Circon Corporation, Santa Barbara, CA). They are available now in different shapes and magnifications. Bartz Technology (Bartz Technology Co., Santa Barbara, CA) supplies cameras with ultraviolet and incandescent lighting. The camera and the optics system can be lowered into a tube and transmit a video image to a monitor (TV) and recording (VCR) equipment above-ground simultaneously. The image can be recorded in video tapes for later analysis. The system can be powered by batteries and assembled on a back pack for one person to carry during field sampling, or loaded on a wheel cart (Box and Johnson, 1987; Brown and Upchurch, 1987; Box et al., 1989; Cheng et al., 1990). During recording, the moving speed of the camera should be approximately 3 mm s<sup>-1</sup> or less to facilitate subsequent observing, counting and tracing of the root image. Smucker et al. (1987) have reported a self-designed automatic device to move a camera in a minirhizotron tube one screen distance at a time. An image recording of a root picture strip can be obtained after each time of sampling.

#### ANALYSIS OF THE RECORDED IMAGE FOR ROOT TURNOVER

In this step, a high quality VCR with four or more heads and a small TV set with a flat and square-shaped screen are needed for the analysis of the resulting tapes of root image in a laboratory. In this section, the hand drawing approach by Bates (1937) and Waddington (1971) is modified and adapted to analysis of the root image produced from the modern minirhizotron system. After field observation and image recording, the resulting tapes are ready for analysis. Each screen of the live root picture strip from the first sampling is fixed on the screen though the pause function of the VCR and then the live root is traced on a sheet of clear plastic. A consecutive root picture strip from the first sampling date is obtained by displaying and tracing sequential and

continuous root images onto a clear polyethylene sheet for each minirhizotron tube. For all the following sampling dates, the same sheet is overlain on the TV screen, and the root picture already on the sheet is compared with that on the screen. Root growth and death incurred during each sampling interval are identified and traced or marked onto the same sheet. Newly grown roots are recognized by not being present on the previous sampling date. Criteria that can be used in identifying dead roots are: previously recorded roots that either turn dark (black or brown) or disappear from the picture. Different color pens can be used to designate different sampling dates. A planimeter is used to measure root length. Root length density can be calculated using the equation:

$$RLD = L / (A * D)$$

where *RLD* is root length density (cm cm<sup>-3</sup>), *L* is the length reading from the map measure, *A* is the area of the tube wall measured (cm<sup>2</sup>), and *D* is the distance from the outside surface of the tube to the depth of the soil in which roots can be observed in the camera, the actual value of *D* is variable depending on the types of soil and plants (Huck and Taylor, 1982; Atkinson, 1985). The measured root growth and death can be recorded in a matrix structured data table with both the rows and columns designated for the periods of samplings (Fig. 1). The following parameters of root dynamics can be calculated using the data in the table:

$$D_j = \sum_{j-1}^{i=1} D_{ij} \quad (i = 1, 2, 3, j - 1; j = 1, 2, 3, \dots);$$

Plot:                      Minirhizotron #:                      Depth:

RL \ j	TIME PERIOD											
	1	2	3	,	,	j	,	,	n			
TIME PERIOD \ i	1	G <sub>11</sub>	d <sub>12</sub>	d <sub>13</sub>			d <sub>1j</sub>			d <sub>1n</sub>		
	2		G <sub>22</sub>	d <sub>23</sub>			d <sub>2j</sub>			d <sub>2n</sub>		
	3			G <sub>33</sub>	d <sub>3,</sub>		d <sub>3j</sub>			d <sub>3n</sub>		
	,				G <sub>,,</sub>	d <sub>,,</sub>				d <sub>,n</sub>		
	i					G <sub>ii</sub>	d <sub>ij</sub>			d <sub>in</sub>		
	,						G <sub>,</sub>	d <sub>,,</sub>		d <sub>,,</sub>		
	m							G <sub>mm</sub>		d <sub>mn</sub>		

Fig. 1. A sample data sheet for analysis of recorded video tapes of root image from minirhizotrons. Note: *i* and *j* are both designated for time period between two recordings; *G<sub>ii</sub>*=newly grown roots during the time period *i*; *d<sub>ij</sub>*=roots which appeared during time period *i* died during time period *j*.

$$LR_j = \sum_j^{j=1} (G_{jj} - D_j) \quad (j=1, 2, 3, \dots);$$

$$DR_j = \sum_j^{j=1} D_j \quad (j=1, 2, 3, \dots);$$

$$TP_j = \sum_j^{j=1} G_{jj} \quad (j=1, 2, 3, \dots);$$

$$sGr_j = (G_j/t_j) / [(LR_{j-1}) + LR_j] / 2 \quad (j=1, 2, 3, \dots)$$

$$sDr_j = (D_j/t_j) / [(LR_{j-1}) + LR_j] / 2 \quad (j=1, 2, 3, \dots)$$

$$TI_j = (sGr_j + sDr_j) / 2 \quad j=1, 2, 3, \dots)$$

where  $G_{jj}$  is the root grown during the time period  $j$  recorded in those diagonal cells of Fig. 1;  $D_{ij}$  designates the roots which appeared during time period  $i$  and died during time period  $j$  recorded in Fig. 1;  $LR_j$  is the standing live root length density during time  $j$ ;  $DR_j$  is the total dead roots produced by time period  $j$ ;  $TP_j$  is the total root production until time  $j$  from the beginning;  $t_j$  is the time length during period  $j$ ;  $sGr_j$  is the specific root growth rate during time period  $j$ ;  $sDr_j$  is the specific root death rate during time period  $j$ ; and  $TI_j$  is the root turnover index during time period  $j$ .

## DISCUSSION

The method described above has been used with some success in root turnover studies in agroecosystems (Cheng et al., 1990). One significant advantage of this method is that root turnover measurements produced using this method are direct observations instead of indirect estimates and that the experimental conditions achieved by using minirhizotrons are more natural than with big rhizotrons. As is widely known, this method is nondestructive and less labor intensive than the conventional soil coring. It makes multiple replications in the field possible (Brown and Upchurch, 1987; McMichael and Taylor, 1987). However, this method is based on one critical assumption; that is, the root dynamics observed from the soil-tube interface are representative of those in the bulk soil. The validity of this assumption has been discussed by Atkinson (1985) and Huck and Taylor (1982) in regard to rhizotrons. Calibration studies of minirhizotron methods in this aspect are very scarce. Vos and Groenwold (1987) have demonstrated that minirhizotron observations can be related quantitatively to soil core analyses with some degree of confidence. It has been reported that light leaks through the soil-tube interface (Levan et al., 1987), root damage during late installation (Bragg et al., 1983), and gaps existing between the soil and the minirhizotron wall (van Noordwijk et al., 1985) might result in an underestimate of root density by

the minirhizotron technique at the upper surface soil layer. However, this problem can be partially solved by using light-shielding precautions (Levan et al., 1987) and proper installation procedures (Box and Johnson, 1987).

The hand-tracing technique involved in this method is relatively simple and easy to learn, but also tedious and labor intensive. Hopefully this part of the work will be replaced by computers in the near future as the techniques of image analysis are advanced (Smucker et al., 1987; Neufeld et al., 1989).

## REFERENCES

- Ares, J., 1976. Dynamics of the root system of blue grama. *J. Range Manage.*, 29: 208–213.
- Atkinson, D., 1985. Spatial and temporal aspects of root distribution as indicated by the use of root observation laboratory. In: A.H. Fitter, D. Atkinson, D.J. Read and M.B. Usher (Editors), *Ecological Interactions in Soil* Special Publication Number 4 of the British Ecological Society. Blackwell Scientific Publications, Oxford, pp. 43–65.
- Bates, G.H., 1937. A device for the observation of root growth in the soil. *Nature (London)*, 139: 966–967.
- Böhm, W., 1979. *Methods of Studying Root Systems*. Ecological Studies 33. Springer, Berlin.
- Box, J.E., Jr. and Johnson, J.W., 1987. Minirhizotron rooting comparisons of three wheat cultivars. In: H.M. Taylor (Editor), *Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics*. American Society of Agronomy Special Publication Number 50, Madison, WI, pp. 123–130.
- Box, J.E., Jr., Smucker, A.J.M. and Ritchie, J.T., 1989. Minirhizotron installation techniques for investigating root responses to drought and oxygen stress. *Soil Sci. Soc. Am. J.*, 53: 115–118.
- Bragg, P.J., Govi, G. and Cannell, R.Q., 1983. A comparison of methods, including angled and vertical minirhizotrons, for studying root growth and distribution in a spring oat crop. *Plant Soil*, 73: 435–440.
- Brown, D.A. and Upchurch, D.R., 1987. Minirhizotrons: a summary of methods and instruments in current use. In: H.M. Taylor (Editor), *Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics*. American Society of Agronomy Special Publication Number 50, Madison, WI, pp. 15–30.
- Cheng, W.X., Coleman, D.C. and Box, J.E., Jr., 1990. Root dynamics, production and distribution in agroecosystems on the Georgia Piedmont using minirhizotrons. *J. Appl. Ecol.*, 27: 592–604.
- Coleman, D.C., 1976. A review of root production processes and their influence on soil biota in terrestrial ecosystems. In: J.M. Anderson and A. Macfadyen (Editors), *The role of terrestrial and aquatic organisms in decomposition processes*. Blackwell Scientific Publications, Oxford, pp. 417–433.
- Coleman, D.C., 1985. Through a ped darkly: an ecological assessment of root–soil–microbial–faunal interactions. In: A.H. Fitter, D. Atkinson, D.J. Read and M.B. Usher (Editors), *Ecological Interactions in Soil* Special Publication Number 4 of the British Ecological Society. Blackwell Scientific Publications, Oxford, pp. 1–21.
- Dalhman, R.C., 1968. Root productivity and turnover of carbon in the root–soil matrix of a grassland ecosystem. In: M.S. Ghilarov (Editor), *Methods of Productivity Studies in Root Systems and Rhizosphere Organisms*. Nauka, Leningrad, pp. 11–21.
- Fogel, R., 1985. Roots as primary producers in below-ground ecosystems. In: A.H. Fitter, D. Atkinson, D.J. Read and M.B. Usher (Editors), *Ecological Interactions in Soil* Special

- Publication Number 4 of the British Ecological Society. Blackwell Scientific Publications, Oxford, pp. 23–35.
- Huck, M.G. and Taylor, H.M., 1982. The rhizotron as a tool for root research. *Adv. Agron.*, 35: 1–35.
- Levan, M.A., Ycas, J.W. and Hummel, J.W., 1987. Light effects on near-surface soybean rooting observed with minirhizotrons. In: H.M. Taylor (Editor), *Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics*. American Society of Agronomy Special Publication Number 50, Madison, WI, pp. 89–98.
- McMichael, B.L. and Taylor, H.M., 1987. Applications and limitations of rhizotrons and minirhizotrons. In: H.M. Taylor (Editor), *Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics*. American Society of Agronomy Special Publication Number 50, Madison, WI, pp. 1–13.
- Milchunas, D.G., Lauenroth, W.K., Singh, J.S., Cole, C.V. and Hunt, H.W., 1985. Root turnover and production by  $^{14}\text{C}$  dilution: implications of carbon partitioning in plants. *Plant Soil*, 88: 353–365.
- Neufeld, H.S., Durall, D.M., Rich, P.M. and Tingey, D.T., 1989. A root box for quantitative observations on intact entire root systems. *Plant Soil*, 177: 295–298.
- Singh, J.S. and Coleman, D.C., 1973. A technique for evaluating functional root biomass in grassland ecosystem. *Can. J. Bot.*, 51: 1867–1870.
- Singh, J.S. and Coleman, D.C., 1974. Distribution of photo-assimilated  $^{14}\text{C}$  in the root system of a shortgrass prairie. *J. Ecol.*, 62: 359–365.
- Singh, J.S. and Coleman, D.C., 1977. Evaluation of functional root biomass and translocation of photoassimilated  $^{14}\text{C}$  in a shortgrass prairie ecosystems. In: J.K. Marshall (Editor), *A Synthesis of Plant-associated Processes*. Range Science Department Science Series Number 26, Colorado State University, Fort Collins, CO, pp. 123–131.
- Smucker, A.J.M., Ferguson, J.C., DeBruyn, W.P., Belford, R.K. and Ritchie, J.T., 1987. Image analysis of video-recorded plant root systems. In: H.M. Taylor (Editor), *Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics*. American Society of Agronomy Special Publication Number 50, Madison, WI, pp. 67–80.
- Taylor, H.M., 1987. *Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics*. American Society of Agronomy Special Publication Number 50, Madison, WI.
- Upchurch, D.R. and Ritchie, J.T., 1983. Root observations using a video recording system in mini-rhizotrons. *Agron. J.*, 73: 1009–1015.
- Van Noordwijk, M., de Jager, A. and Floris, J., 1985. A new dimension to observations in minirhizotrons: a stereoscopic view on root photographs. *Plant Soil*, 86: 447–453.
- Vos, J. and Groenwold, J., 1987. The relation between root growth along observation tubes and in bulk soil. In: H.M. Taylor (Editor), *Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics*. American Society of Agronomy Special Publication Number 50, Madison, WI, pp. 39–49.
- Waddington, J., 1971. Observation of plant roots in situ. *Can. J. Bot.*, 49: 1850–1852.
- Warembourg, F.R. and Paul, E.A., 1977. Seasonal transfers of assimilated  $^{14}\text{C}$  in grassland: plant production and turnover, soil and plant respiration. *Soil Biol. Biochem.*, 9: 295–301.