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Spatial variation in tree root distribution and growth associated with minirhizotrons

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Abstract

Four minirhizotrons were installed in each of three replicate plots in a deciduous forest dominated by *Acer saccharum* Marsh. The length growth of tree roots along the surface of the minirhizotrons was measured for a period of one year, and the resulting data were analyzed in nested, averaged and pooled arrangements. The analyses of nested data showed that spatial variation in root growth and abundance among minirhizotrons within plots was greater than variation among plots. Averaging data from minirhizotrons within plots prior to analysis reduced variation about plot means, but extensive intraplot variation invalidates this approach on statistical grounds. Both nested and averaged data failed to account for the contribution of individual roots to the mean, and root production rates were consequently overestimated. Pooling the data from the four minirhizotrons reduced variation about the means, and resulted in a more representative estimate of root production rates. The analysis of composited data can be used to incorporate small-scale variation into a single replicate sample in those circumstances where the activity of the root systems of plant communities is the object of study.

Introduction

Recent advances in microvideo technology have made minirhizotrons popular research tools in both agronomic (e.g. Cheng et al., 1990; Hansson and Andren, 1987; Keng, 1988; Upchurch and Ritchie, 1983) and natural plant communities (e.g. Eissenstat and Caldwell, 1988; Hendrick and Pregitzer, 1992). During these and other research efforts, several analytical and statistical problems with minirhizotron data have been identified. High minirhizotron-to-minirhizotron (i.e. spatial) variability in root density and distribution has proven to be especially problematic. Coefficients of variation range to as much as several hundred percent (Merrill et al., 1987; Upchurch and Ritchie, 1983; Upchurch, 1987), often making treatment differences difficult to detect. Quantifying and controlling spatial variation is necessary to properly execute minirhizotron experiments focused on root systems at the level of the plant community or ecosystem, but information on the extent of variation among minirhizotrons at various levels within the hierarchy of an experimental design is generally lacking in the literature.

Individual minirhizotrons are often the sampling units upon which measurements of roots are made, and several minirhizotrons are typically nested within replicate experimental units, usually a field plot or greenhouse pot (e.g. Beyrouty et al., 1987; Box and Johnson, 1987; Cheng et al., 1990). Alternatively, minirhizotrons are sometimes treated as experimental units, with measurements of root numbers or length made along several different transects

(sampling units) within the minirhizotron (Eissenstat and Caldwell, 1988; Gregory, 1979; Meyer and Barrs, 1985; Sanders and Brown, 1978). Rarely, however, has the full extent of among-sampling unit and among-experimental unit variability in the data been reported. Instead, sampling unit averages are the typical data upon which statistical analyses are made.

As an alternative to analyzing sampling unit averages as primary data, a few investigators have performed statistical analyses on composited data from multiple minirhizotrons (sampling units) within replicate experimental plots (Hendrick and Pregitzer, 1992) or from multiple transects (sampling units) within replicate minirhizotrons (Hansson and Andren, 1987). Pooling minirhizotron data incorporates spatial variation in root abundance and activity into a single aggregate sample. Multiple samples within experimental units are commonly pooled in studies of soil and plant characteristics, but the assumptions and implications of this practice in minirhizotron research have not been discussed.

Our objectives in this paper are: 1) to more fully describe the extent of spatial variation in tree root system attributes; 2) to discuss the impact of analyzing the same minirhizotron data under different experimental arrangements; and 3) to suggest some ways to better control spatial variability through analytical means. We have extensive minirhizotron data on individual tree roots from multiple minirhizotrons (sample units) within replicated plots (experimental units) and, hence, the ability to analyze minirhizotron data in a variety of experimental arrangements.

Methods

Our study site is a second-growth northern hardwoods stand dominated by sugar maple (*Acer Saccharum* Marsh), and is located in the northern lower peninsula of Michigan (Manistee County, 40° 42′ N, 85° 43′ W), USA. The soil is a Typic Haplorthod of the Blue Lake and Kalkaska series. Three 30-m by 30-m analogous plots were established at the site in 1987 (Burton et al., 1991). In June 1988, 4 randomly located minirhizotrons (2 m long × 5.08 cm inside diameter) were permanently installed in each plot at a 45° angle to the soil surface, and to a depth of 165 cm along their length (110 cm in vertical depth). The portion of the minirhizotron extending above the soil surface was painted and capped with a rubber stopper to prevent rainfall and light from entering. Numbered image frames were scribed onto the exterior surface of each minirhizotron prior to installation so that we could return to the same location within the minirhizotrons at all sampling dates. A total of 130 frames were scribed, one every 1.2 cm. The image frames were oriented vertically during installation. Minirhizotron images were collected on VHS videotape with a Circon Microvideo 9011 Color Agricultural Camera (Circon Co., Santa Barbara CA). Each minirhizotron was imaged throughout the 1989 growing season, and imaging was resumed in the spring of 1990 after snowmelt. The data presented in this report were collected on 27/4, 11/6, 22/6, 18/7, 18/8, 16/9 and 14/10 in 1989, and on 24/4 and 23/5 in 1990.

An interactive PC-based software program (ROOTS) that we have developed was used to analyze the minirhizotron video images, A TARGA (Truevision Inc., Indianapolis IN) video board was used in conjunction with ROOTS to digitize the images from VHS tape, project them onto a computer monitor and temporarily save them on-screen for processing. The length of all roots present in each image was traced using a mouse and written to a database file (dBASE III+, Ashton-Tate, Torrance, CA) by ROOTS. Individual roots are defined here as segments, or branches from segments, present and visible within the minirhizotron images. The tracings of each root were permanently saved by ROOTS on a separate file. Each root was classified as live or dead, based upon its appearance. Dead roots were distinguished from live roots by one or more of the following characteristics: very dark brown or black color, partial decay of the existing root and/or the appearance and growth of fungal mycelia around the root. Roots that completely decayed and disappeared were classified as 'missing'.

An identification code was assigned to each root and written to the database. The code number was derived from the order in which a root was traced within an image; the frame, mini-

rhizotron and plot in which it was located; and the date on which it was imaged. The same code (except for the collection date) was used for each root during the analysis of subsequent images. We were able to identify the same roots at successive dates by using ROOTS to recall and overlay the tracings and identification numbers from an image at time t-1 when analyzing time t images. Complete records were kept for each root throughout its development, even after it died and/or disappeared. After the images from all sampling dates had been processed for each minirhizotron, the records for the entire year were combined. The final database for each minirhizotron was a time series of lengths and condition (live, dead or missing) for each root. Data presented here are from the upper 30 cm of the soil only.

To calculate root production, the total live root length present on the first imaging date (27/4/89, $t = 0$) was summed for each minirhizotron. Root length production was followed for the next 363 days (24/4/90). The production of new root length was calculated for the intervals t to $t + 1$ by summing the lengths of new live or dead roots present at time $t + 1$ that were not present at time t, and then adding the length growth of existing roots. Root production thus includes both growth of existing roots and production of new roots for a given time interval. Annual root length production was calculated by summing the production values for each observation period. The annual production rate, expressed as a percentage of initial root length, was calculated by dividing total annual length production by the initial (27/4/89) root lengths.

The data were analyzed under three different experimental arrangements: 1) a nested design with individual minirhizotrons as subsamples with in replicate plots ($s = 4$, $r = 3$, $n = 12$); 2) minirhizotrons as duplicates within replicated plots, with averages of the duplicates treated as the primary data ($n = 3$); and 3) data from each minirhizotron pooled within replicate plots ($n = 3$). To determine the extent to which root length production rates were related to the initial amount of root length present in each minirhizotron, a correlation of total annual length production with initial root length was made ($n = 12$).

Results

A summary of the statistical analyses (Table 1) shows that the same data, analyzed under different experimental arrangements, can lead to different levels of variation around estimates of the mean. The mean initial root length densities ($mm\ cm^{-2}$) and root length production values ($mm\ cm^{-2}\ yr$) are the same, regardless of the manner in which the data were arranged prior to analysis. However, the variation around the mean differs widely among the three analyses. When data from individual minirhizotrons are treated as samples within the replicated plots, coefficients of variation (CV's) for both initial root length density and annual root length production are around 50%. (The standard deviation in this arrangement was calculated from the experimental, i.e. among-plot, error mean

Table 1. Analysis of initial root length, annual length production, and annual production rate (annual length production as a percentage of initial root length), under three experimental arrangements. Initial length and length production are expressed in mm of root length per cm^2 of minirhizotron viewing surface. Data shown are means, standard deviations and coefficients of variation. Standard deviation for minirhizotrons as subsamples derived from experimental (among-plot) error mean square

	Mean	SD	CV%
Minirhizotron data as subsamples within plots ($s = 4$, $n = 3$)			
Initial length ($mm\ cm^{-2}$)	3.57	2.02	56
Annual production ($mm\ cm^{-2}$)	3.83	1.83	48
Annual production rate ($\%\ yr^{-1}$)	131	76	58
Minirhizotron data averaged within plots ($n = 3$)			
Initial length ($mm\ cm^{-2}$)	3.57	0.40	11
Annual production ($mm\ cm^{-2}$)	3.83	0.80	21
Annual production rate ($\%\ yr^{-1}$)	131	38	29
Minirhizotron data pooled within plots ($n = 3$)			
Initial length ($mm\ cm^{-2}$)	3.57	0.40	11
Annual production ($mm\ cm^{-2}$)	3.83	0.80	21
Annual production rate ($\%\ yr^{-1}$)	106	11	10

square of the nested ANOVA; Steel and Torrie, 1980.) Alternatively, when the data from each minirhizotron are averaged by plot and then analyzed, CV's for initial length and length production are 11 and 21%, respectively, a considerable reduction in variation. This improvement is not unexpected, as one source of variation (among minirhizotrons within plots) has been removed in this arrangement of the data. When minirhizotron data are pooled by plot prior to analysis, CV's for initial length and annual production are the same as those where the data are averaged over the tubes in each plot. Again, within-plot variation among minirhizotrons has been removed (combined into one sample) in this arrangement of the data. The means of percentage production rates differ among the three arrangements of the data (Table 1). The means for the nested and averaged data were the same. This was expected to be the case. The grand mean of n plot means, each derived from m minirhizotrons, will be the same as the overall mean of the nm minirhizotrons, provided that the number of minirhizotrons in each experimental plot is the same.

The production rate derived from the pooled data (106%) is considerably lower than the rate derived via the other two arrangements of the data (131%). High production rates were not consistently associated with high initial root lengths. There was no apparent relationship between the initial amount of root length present in a minirhizotron and the amount of root length produced along its surface (Fig. 1). The Pearson product moment coefficient between initial root length and annual production was very low (0.008) and not significantly different from zero (alpha = 0.05).

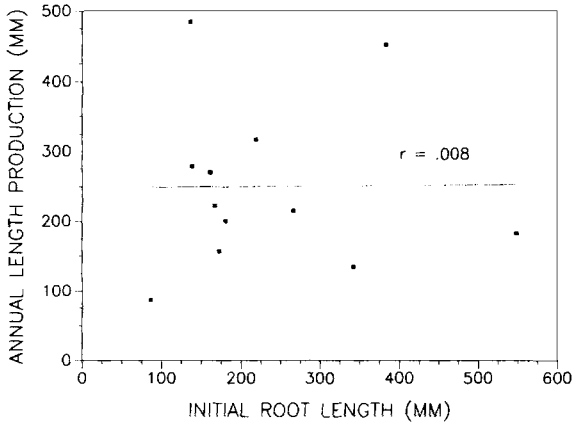

Fig. 1. Relationship between initial root length (27/4/89) in the upper 30 cm of soil and total production during the following year, as viewed with the minirhizotrons. Data shown are from 12 minirhizotrons, four within each of three replicate plots.

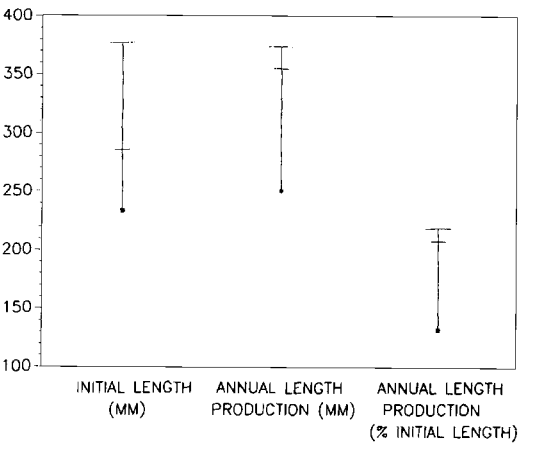

Fig. 2. Plot means, sampling (outer, wide bars) and experimental (inner, narrow bars) error for initial root length, annual length growth and annual production as a percentage of initial length. Units for Y-axis are in parentheses below X-axis label for each estimate.

The results of the nested ANOVA of total root length, with minirhizotrons as subsamples, are shown graphically (Fig. 2). Variation in initial root length among minirhizotrons within a plot (sampling error) was greater than variation among plots (experimental error). These data suggest that the spatial distribution of tree roots in the forest is highly variable over quite small areas; distances between minirhizotrons within plots vary from one to several meters, while plots are up to several 10's of meters apart. Length production and production rates show different spatial patterns. Large-scale (among-plot) variation in these variables is of approximately the same magnitude as small-scale (within-plot) variation.

Discussion

There is no right way to analyze all types of minirhizotron data. Different experimental designs dictate different statistical analyses, as do the hypotheses and questions being addressed.

However, general statistical principles should guide the process of analyzing data from an experiment in which the data are arranged in a nested or hierarchical fashion. For example if more than one minirhizotron is sampled within each replicate experimental unit, or if more than one transect is sampled in each minirhizotron, the data should not be averaged prior to analysis. Averaging data from transects within minirhizotrons or minirhizotrons within plots is undesirable unless variability among sampling units is effectively zero. However, our data (Fig. 2) show that variability among the minirhizotrons within our plots is considerable, greater in fact than variation among plots. We suspect that this may be true in other studies and experimental scenarios as well. The loss of information on among-sampling unit variability resulting from the analysis of averaged data leads to a more conservative estimate of experimental error and a greater risk of making a Type I error.

Instead of averaging, statistical analyses should be performed on either nested or pooled data, or perhaps both. A nested ANOVA should always be performed first to ascertain the extent of variation at both the experimental and sampling unit levels. If sample unit variation is low, relative to variation among experimental units, only a nested ANOVA should be performed; compositing or pooling the data will have little effect on estimates of means and variation. In these circumstances, the contribution of each minirhizotron should be weighted by the number of roots or amount of root length growing along its surface. If sampling error is large relative to experimental error, other options are available. It is possible to reduce intraplot variation by computational means (without increased replication) through an analysis of covariance (ANCOVA) in a nested design if root abundance and activity are related in a systematic fashion. However, abundance and growth are rather uncoupled in the root system we studied (Fig. 1).

Increased replication or an adjustment in the number of sampling units can also be employed. When sampling error is larger than experimental error, standard analyses of design efficiency are precluded and determining the proper number of experimental units and the allocation of samplings units among them can be difficult. It is apparent from our data that little gain in precision would be made by increasing the number of minirhizotrons within plots without a concomitant increase in the number of plots. The formulas presented by Sokal and Rolf (1981, pp. 309–310) indicate that to achieve a mean standard error of 20% for length growth at our study site, a minimum of 5 replicate plots, each with 10 minirhizotrons, is required. This number is not excessively high from a logistical standpoint, but might be financially prohibitive if the cost of establishing replicate plots is high (as was our experience) or if a large number of experimental treatments are to be applied.

If deemed appropriate, data from multiple subsamples can be pooled prior to analysis to reduce variation among experimental units. Data should not be pooled as a matter of standard practice. Since small-scale variability is incorporated into the estimate when compositing data, this approach is not appropriate if quantifying the extent of spatial variation in root distribution and activity is of interest. However, when the root systems of plant communities are the object of study, it is probably best to have as much of the root system as possible in each replicate sample.

One of the primary advantages of analyzing pooled data is that the effects of heterogeneous distributions of roots among minirhizotrons are removed from estimates of production and other means. We feel that a serious problem with analyses of both averaged and non-weighted nested data is the failure to consider the relative contribution of each minirhizotron to the overall mean. Heterogeneous distributions of roots among minirhizotrons can result from many causes, including chance, various physical, biological or chemical soil factors, differences in species abundance and root morphology, inconsistencies in the minirhizotron-soil interface, and possibly others. In analyses of averaged or nested data, the result is that minirhizotrons with few roots or little root length present contribute proportionately more information to an estimate than do minirhizotrons with a larger number of roots or root length. This can be seen in our root production data (Table 1). The higher production rates associated with the nested and averaged data resulted from the presence of at least

one minirhizotron per plot with a low initial root length exhibiting a large relative amount of root production during the year. The lower estimate of annual production derived from the pooled data is likely to be a more representative reflection of actual root system activity than the averaged or nested estimates, since each root is given full consideration in the pooled analysis.

In summary, it is important that we keep in mind the object(s) of interest when planning minirhizotron experiments and subsequently analyzing the data. It is the acquisition of better information on the average root, not the average minirhizotron, that will help us answer our questions about roots and root systems.

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