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# Substituting root numbers for length: improving the use of minirhizotrons to study fine root dynamics

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**Abstract**

Minirhizotrons provide a unique way to repeatedly measure the production and fate of individual root segments, while minimizing soil disturbance and the confounding of spatial–temporal variation. However, the time associated with processing videotaped minirhizotron images limits the amount of data that can be extracted in a reasonable amount of time. We found that this limitation can be minimized using a more easily measured variable $r$ (i.e. root numbers) as a substitute of root length. Linear regression models were fitted between root length versus root number for production and mortality of seven sample datasets of varying tree species and treatments. The resulting $r^2$ values ranged from 0.79 to 0.99, suggesting that changes in root numbers can be used to predict root length dynamics reliably. Slope values, representing the mean root segment length (MRSL), ranged from 2.34 to 8.38 mm per root segment for both production and mortality. Most treatments did not alter MRSL substantially, the exceptions being $CO_2$ treatments and a girdling treatment that altered plant community composition and, consequently, root morphology. The high $r^2$ values demonstrated a robust relationship between variables irrespective of species or treatments. Once the quantitative relationship between root lengths and numbers has been established for a particular species–treatment combination, quantifying changes in root number through time should substantially decrease the time required to quantify root dynamics.
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## 1. Introduction

Minirhizotrons are an effective tool to observe and quantify root system dynamics, providing a unique method by which individual root segments can be repeatedly measured over multiple time intervals. Moreover, because they are less destructive than coring, minirhizotrons enable researchers to minimize soil disturbance as well as the confounding of spatial and temporal variation associated with other root research methods such as core collection (Bohm, 1979) or mesh in-growth bags (Persson, 1980; Steen, 1991; Ludovici and Morris, 1996). Most importantly, minirhizotrons allow the production and mortality (rate of

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disappearance) of fine roots to be measured as separate processes and, thus, provide direct observations of these two parameters independently (Hendrick and Pregitzer, 1996a). Alternative methods that do not account for simultaneous production and mortality can underestimate these values (Kurz and Kimmins, 1987).

While the benefits of minirhizotrons are now widely recognized, they are not as frequently utilized as some other methods. Hendrick and Pregitzer (1996a) reviewed the main limitations and possible reasons for this infrequent use, including the time and difficulty associated with the extraction of root data from videotaped images within a reasonable period of time. Manual image analysis is a time-intensive process that typically requires every root segment in each minirhizotron to be digitized for length and diameter (or width). This can represent a substantial time investment and research cost; analysis times per minirhizotron tube often range from 30 min to 8 h per sampling period in our experience.

Various alternative techniques have been devised for extracting data from minirhizotron images, but most fail to follow the fate of individual roots, negating the primary advantage of minirhizotrons over other methods. For example, variations of the grid intersection method originally proposed by Newman (1966) have been utilized to convert root-line intersections to root lengths or root length densities, but these yield only net changes in total root lengths. Counts of roots in contact with the minirhizotron surface can be used for conversions to root length densities (Upchurch, 1987), but this approach has not been used on an individual root basis. Automated image analysis shows potential for expediting data extraction but, again, currently available software does not facilitate the tracking of individual roots.

Other approaches involve direct and repeated manual tracing of individual roots using PC-based digitizer software. Hendrick and Pregitzer (1996a) cite some of the current image analysis programs that do facilitate the tracking of individual roots. These include an interactive PC-based program, ROOTS (Michigan State University, E. Lansing, MI, USA), a Macintosh and PC-based RooTracker program (Duke University Phytotron, Durham, NC, USA), and a Macintosh based NIH-image program (Smit and Zuin, 1996).

It seemed likely that considerable time savings would result if we could identify a variable other than root length that still accounted for individual roots, was easy to extract from minirhizotron images, and demonstrated a high correlation to individual root segment length. Pregitzer et al. (2002) studied roots of nine different tree species from across North America and reported that the majority of fine root length was accounted for by short lateral branches only a few millimeters in length, and that lateral root branches appear to be deciduous. The lengths of individual roots of a given order did not vary significantly within a given species (Pregitzer et al., 2002). These results led us to hypothesize that there should be a strong relationship between root lengths and numbers in the temperate and boreal tree species we have studied previously. Likewise, Persson (1978) reported a close relationship between fine root lengths and changes in the number of fine root tips (roots $<1$ mm in diameter) of another species, *Pinus sylvestris*, using soil cores and the Newman grid intersection method. The objective of the present study was to determine if we could use root numbers to predict fine root length. We utilized datasets from several different biomes across North America to study variability in the relationships among fine root length and number.

## 2. Materials and methods

### 2.1. Datasets

Seven existing minirhizotron datasets were selected to represent a wide variety of tree species (*Acer saccharum*, *Liquidambar styraciflua*, *Pinus elliottii*, *Picea glauca*, *Salix* spp., *Populus tremuloides*) and a variety of study treatments (Table 1). Each study is described briefly below.

We used data from two *P. elliottii* experiments in our analyses. The first study, located in the upper Coastal Plain of Georgia (USA), examined the responses of fine root production and mortality to artificial gap formation similar to those created by the southern pine beetle (Schroeer et al., 1999). Treatments consisted of controls and artificial gaps (37.5 m radius). The second *P. elliottii* study, located in Florida (USA), examined the effects of fertilization and complete competition control on carbon storage and root

Table 1
Stand, soil, and climatic characteristics of seven minirhizotron study sites

| Study | Location | Soil type | Mean annual precipitation (mm) | Mean annual temperature (°C) | Overstory age (years) | Understory and other vegetation | Study duration (months) | Experimental treatments |
|---|---|---|---|---|---|---|---|---|
| *Populus tremuloides* | Pellston, MI | Na[a] | NA | NA | NA | NA | | Two levels of $CO_2$ and two levels of nitrogen in a factorial design |
| *Pinus elliottii*[b] | Upper coastal plain, GA | Arenic, Plinthic, Kandiudults | 1225 | 14 | 20–25, 40–49 | *Prunus serotina*, *Quercus phellos*, *Quercus nigra* | 18 | Controls and artificial gaps (37.5 m radius). |
| *Pinus elliottii* | Bryceville, Callahan, and Yulee, FL | Typic, Haplanods | | | 20 | *Ilex glabra*, *Aristida stricta*, *Serenoa repens* | 11 | Control, fertilized, herbicide, fertilized + herbicide plots. |
| *Acer saccharum* | Northern lower peninsula, MI | Alfic/Entic, Typic, Haplorthods | 810–850 | 5.8–7.6 | 74–78 | *Acer rubrum*, *Quercus rubra*, *F. grandifolia* | 18 | Two sites separated by a north–south distance of 80 km |
| *Liquidambar styraciflua* | Middle coastal plain, GA | Plinthic, Paleudults, Kandiudults | 1150 | 19.1 | 20 | | 12 | Two levels of fertilizer (19:9:19 NPK), 560 and 1120 kg $ha^{-1}$ per year |
| *Pinus glauca* | Tanana river floodplain, AK | Typic, Cryofluvents | 269 | −4.3 | 150–250 | *Alnus spp.*, *P. balsamifera*, *Betula papyrifera* | | Three mature forests; no manipulative treatments |
| *Salix* spp. | Tanana river floodplain, AK | Histic, Pergelic, Cryaquepts | 269 | −3.3 | 10 | *Alnus tenuifolia*, *P. balsamifera*, *Picea glauca* | 24 | Three browsed and three non-browsed plots (30 m × 50 m × 5 m) |

[a] Not applicable, growth chamber study.
[b] *Pinus elliottii* gap study (Schroeer, 1999).

dynamics. Treatments consisted of control, fertilizer (280 kg ha$^{-1}$ di-ammonium phosphate, 280 kg ha$^{-1}$ urea, and 228 kg ha$^{-1}$ KCl), herbicide (3% solution of Roundup® prior to site preparation), and fertilizer plus herbicide treatments (Shan et al., 2001).

An *A. saccharum* study, located in the northern lower peninsula of Michigan (USA), was established to understand the spatial and temporal dynamics of roots in two forests separated by a north–south distance of 80 km. There were no experimental manipulations applied in this study (Hendrick and Pregitzer, 1993a,b, 1996b).

Minirhizotrons were also used to quantify fine root production, mortality (rate of disappearance) and standing root crop dynamics in an intensively-managed *L. styraciflua* coppice stand located in the middle Coastal Plain of Georgia (USA). Treatments consisted of two levels of fertilizer (19:9:19 NPK): a low (560 kg ha$^{-1}$ per year) and a high level (1120 kg ha$^{-1}$ year) application (Price and Hendrick, 1998).

Two of the datasets are from the boreal forest of interior Alaska, USA. In one, the fine root demography of *P. glauca* fine roots was quantified in three mature forests located on the Tanana river floodplain in interior Alaska, USA. Again, no experimental manipulations were applied to the selected sites in this study. In a related study on early successional *Salix* spp. communities, minirhizotrons were used to characterize the effects of aboveground mammalian browsing on rates of fine root production, mortality, and decomposition. Three large enclosures (30 m × 50 m × 5 m) were paired with unfenced plots of the same size to create both browsed and non-browsed treatments (Ruess et al., 1998).

The final data are from a study conducted in Michigan (USA) that was designed to examine the effects of atmospheric $CO_2$ and nitrogen fertilization on root production and mortality in *P. tremuloides*. Two levels of $CO_2$ and two levels of nitrogen were examined in a factorial design (Pregitzer et al., 2000).

Although production measurements refer to the occurrence of new roots as well as the growth of existing roots in the minirhizotron tubes, mortality measurements primarily track the rates of disappearance of the fine roots, because it is difficult to distinguish dead roots from those that are living. All of the studies we utilized reported a rapid root disappearance rate, so it is possible that some mortality is missed. In all studies, 80–85% of all roots measured were <0.5 mm in diameter.

### 2.2. Modification of dBase program

In the past, we have used a dBase program (Ruess, 2001) to calculate the production and mortality of roots based upon complete root segment length measurements. To calculate the production and mortality of both root lengths and root numbers between sample dates a count function was added to the original program. The new program was used for each minirhizotron file of the seven sample datasets. Output files, within each dataset, were collated into their respective treatments. The resulting output provided values of root numbers and the corresponding root lengths for both production and mortality data.

### 2.3. Linear regression models

For these analyses we fitted linear regression models of the form $y = bx$, where $y$ = root segment length and the independent variable ($x$) was the number of roots. Models were constructed for each species–treatment combination, and included length–number relationships for both production ($b_p$) and mortality ($b_m$). The models were restricted to force the intercept through zero. The resulting slope value ($b$) represents the mean root segment length (MRSL) for each species and treatment. Once the regression analysis of each species- and treatment-specific dataset was completed, we compared the slopes (i.e. MRSL) of the regressions to determine if any of the within-dataset treatments had altered the slope. Differences in regression slopes were determined using paired $t$-tests ($\alpha = 0.05$).

To determine if there were any significant temporal changes in MRSLs (among image dates), the *P. elliottii* intensive management study was selected for further examination. We chose these data because of the large number of treatments and because its $b$ and $r^2$ values fell in the mid-range of all data points. A repeated measures analysis of minirhizotron image dates ($\alpha = 0.05$) was done with and without treatments for both production and mortality.

## 3. Results and discussion

### 3.1. An overview of minirhizotron data extraction from recorded images

The ease with which minirhizotron video taped/digital images are collected belies the difficulties associated with data extraction. Numerous techniques have been devised to extract meaningful data for such variables as rooting depths, root length densities, root morphologies, and root dynamics. Root length, for instance, has often been estimated using some variation of the Newman grid intersection method (Newman, 1966; Tennant, 1975). Manually counting the intersections of roots with etched lines on the minirhizotron surface, either random or fixed transects (McMicheal and Taylor, 1987), allows the user to convert to root lengths with Newman's method but requires a fine grid system with many interceptions to obtain realistic data (Smucker et al., 1987). Aside from intersection methods, a count of roots in contact with the tube surface within a specified area has also been utilized, by then converting counts to length densities via regression against washed root lengths.

Now that videotape and digital recording are commonly used, images are often manually processed by tracing and recording the lengths (and sometimes diameters) of root segments in contact with the minirhizotron tube using PC-based computer software. This is an effective way to extract length data quantifying the development and fate of individual roots. However, manual image processing is time consuming and laborious, hindering the usefulness of minirhizotrons. Counting roots is much less time-intensive, and substituting root number for length could substantially increase the rate at which minirhizotron images could be analyzed. The results of our attempts to make this substitution were rather encouraging. Linear regressions derived from these datasets generally had high coefficients of determinations ($r^2$). The calculated $r^2$ values fell in the range of 0.79–0.99 (Table 2) for both production and mortality (rate of disappearance), suggesting that root counts can be used to estimate root lengths routinely. The *A. saccharum* study was the only dataset in which $r^2$ values were consistently be-

Table 2
Root length versus number regressions for seven minirhizotron datasets

| Study | Treatment | d.f. | Production | | Mortality | |
|---|---|---|---|---|---|---|
| | | | $r^2$ | $b_p$ | $r^2$ | $b_m$ |
| *Populus tremuloides* | Amb $CO_2$ + low N | 65 | 0.98 | 8.29c | 0.86 | 6.59b |
| | Amb $CO_2$ + high N | 89 | 0.95 | 7.04a | 0.92 | 5.41a |
| | Elev $CO_2$ + low N | 83 | 0.97 | 7.59b | 0.94 | 8.38c |
| | Elev $CO_2$ + high N | 95 | 0.96 | 7.13a | 0.87 | 5.31a |
| *Pinus elliottii* | Control | 287 | 0.91 | 4.10b | 0.91 | 4.28b |
| | Gap | 287 | 0.87 | 2.34a | 0.92 | 3.03a |
| *Pinus elliottii* | Control (C) | 179 | 0.91 | 4.46a | 0.89 | 4.79a |
| | Fertilizer (F) | 178 | 0.90 | 4.76b | 0.93 | 4.84b |
| | Herbicide (H) | 155 | 0.94 | 4.54ab | 0.83 | 4.39a |
| | F + H | 179 | 0.92 | 4.54ab | 0.89 | 4.63a |
| *Acer saccharum* | South site | 428 | 0.81 | 3.69a | 0.79 | 3.62a |
| | North site | 467 | 0.88 | 4.57b | 0.82 | 3.94b |
| *Liquidambar styraciflua* | Low N | 83 | 0.96 | 3.45a | 0.99 | 4.20a |
| | High N | 82 | 0.95 | 3.37a | 0.99 | 4.22a |
| *Pinus glauca* | Site A | 119 | 0.88 | 3.01a | 0.90 | 3.42a |
| | Site B | 119 | 0.89 | 3.39b | 0.89 | 3.66b |
| | Site C | 119 | 0.91 | 3.73c | 0.94 | 4.36c |
| *Salix* spp. | Control | 80 | 0.95 | 4.99b | 0.97 | 5.48a |
| | Browsed | 80 | 0.94 | 4.72a | 0.95 | 5.27a |

Different values (a–c) indicate coefficients of determination ($r^2$) and significance ($\alpha = 0.05$) of slopes ($b$) within each dataset. Slope values represent the mean root segment length (MRSL) in millimeters per root segment.

low 0.90, while the best fit was found in the *L. styraciflua* study ($r^2 = 0.95$–$0.99$).

Not only were the relationships between the root counts and root segment lengths quite strong within each dataset, the manipulative treatments and site differences appeared to have little absolute effect on the magnitude of within-species MRSLs, despite several statistically significant treatment effects. The most dramatic effects occurred in the *P. elliottii* study (Table 2), where the gap treatment reduced MRSLs of live roots by 77% ($b_p$ of 2.34 versus 4.10) and dead MRSLs by 29% ($b_m$ of 3.03 versus 4.28). This effect predominantly appears to be due to altered root morphology, in that the gaps were rapidly colonized by invading herbaceous plants, whose roots quickly dominated the minirhizotron images (Schroeer et al., 1999).

With respect to other possible treatment effects, there may have been a small overall positive effect of increased nutrient availability on MRSL. For example, in the intensively-managed *P. elliottii* stands the greatest live MRSLs ($b_p = 4.76$ mm per root segment) were found in the fertilizer inclusive treatments, while the lowest live MRSLs were in the control (4.39 mm per root segment) and herbicide plots (4.46 mm per root segment). Similarly, the shortest *A. saccharum* MRSLs (e.g. slopes $b_p$ and $b_m$) were found at the southern site, while MRSLs were significantly greater at the northern site (3.69 mm versus 4.57 mm per root segment and 3.62 mm versus 3.94 mm per root segment for root production and mortality, respectively). Although differences in root dynamics between these two sites were originally attributed to warmer soils at the southern site (Hendrick and Pregitzer, 1993b), they were subsequently proposed to be due to greater nitrogen availability at the northern site (Burton et al., 2000). Increases in total root length in response to increased nutrient availability have been widely observed (e.g. Eissenstat, 1991; Jackson and Caldwell, 1989; Mou et al., 1995; Price and Hendrick, 1998; Pregitzer et al., 1993), and these effects may be evident in the MRSL differences between fertilizer treatments, or among sites of different fertility in our datasets. However, we should note that although the MRSLs of *L. styraciflua* calculated in this study did not respond significantly to fertilization (Table 2), total root length in the minirhizotrons did (Price and Hendrick, 1998).

Browsing seemed to have a negative effect on *Salix* root length; the lowest MRSLs (i.e. shallowest slopes $b_p$ and $b_m$) occurred in the browsing treatments (4.72 and 5.27 mm per root segment, respectively), while the control plot MRSLs were significantly greater (Table 2). All of the *P. glauca* study sites were significantly different from one another (C > B > A for both $b_p$ and $b_m$, Table 2), even though we have not identified any substantial differences in soils or other factors among these sites. With respect to differences among the datasets we examined, the MRSLs for *P. tremuloides* were unusually high (5.31–8.38 mm per root segment) relative to all other species, especially in the elevated $CO_2$ treatments. Conversely, temporal analysis of the *P. elliottii* intensive management study indicated that there was no significant time effect on MRSL production or mortality over all treatments (Fig. 1).

Comparisons within and among the studies and datasets we analyzed suggest that the effects of many common experimental treatments, such as fertilization or irrigation, may not substantially alter MRSLs. Conversely, there seem to be some significant inherent differences between species (i.e. *P. tremuloides* versus all others), and among treatments that alter composition (e.g. gap colonization). In addition, changes in soil structure (e.g. compaction) that impact the ability of roots to grow unimpeded may alter MRSLs; however, our manipulations did not explicitly test these effects. If the results of the *P. elliottii* fertilization study are indicative of general trends, MRSLs seem to be rather consistent throughout the year for a particular species.

### 3.2. Using root numbers to predict root length

To implement this simplified approach to quantifying root length production and mortality using minirhizotrons, one would first need to completely digitize the length of root segments in the minirhizotron images from the first (or early) sample dates. On the subsequent sampling date, only new and newly dead roots (i.e. dead roots not existing in the previous time period) would be digitized for length (or substitute the last known live length for roots that have already disappeared). These data would then be used to establish the relationship between root length production and

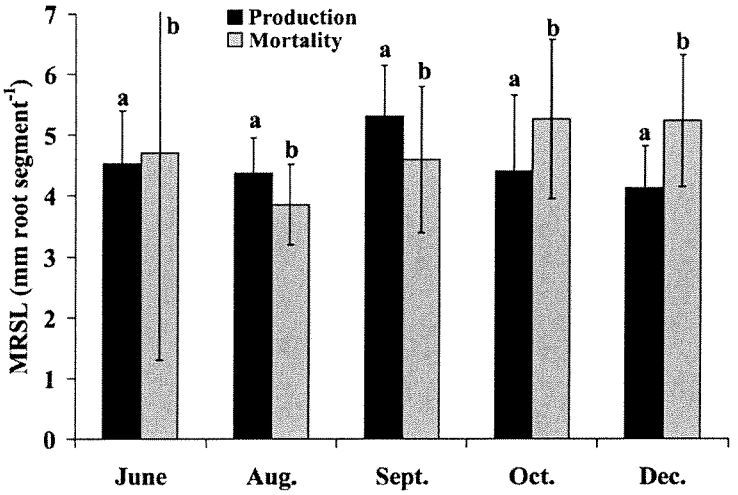


Fig. 1. Temporal analysis of mean root segment lengths (MRSL, in millimeters per root segment) + L.S.D., for the *Pinus elliottii* intensive management study. Comparisons are between bars of similar color (i.e. production vs. production). No significant temporal differences were found ($\alpha = 0.05$).

mortality and the corresponding birth or loss of root numbers.

Assuming that both regressions yielded acceptable coefficients of determination, images on most subsequent dates could be digitized for root counts only. In practice, this means that the appearance of new roots and the mortality of existing roots can be quantified by procedures that are easily and quickly implemented. However, it is possible that the relationships between root lengths and root numbers (i.e. the magnitude of $b_p$ or $b_m$) could change over time if roots of substantially different lengths were being produced or dying at different times (e.g. phenological differences in root morphology). Therefore, error checks should be made on one or more subsequent dates (at least two sequential dates to account for root mortality) to confirm that $b_p$ or $b_m$ did not differ significantly among dates. We confirmed that there were no significant temporal differences in either $b_p$ or $b_m$ for our intensively-managed *P. elliottii* study (Shan et al., 2001), but this might not necessarily be true for other species, treatments, or geographic locations. We suggest that periods of known or suspected episodes of high root production and mortality (e.g. spring and winter, respectively) might prove most useful for error checking.

For all datasets except the *A. saccharum* and *P. tremuloides*, MRSLs of dead roots for a given treatment group were from 2 to 20% greater than their corresponding production MRSLs (i.e. $b_m > b_p$). The most likely explanation for this difference is root segment length (i.e. extension) growth between the time of first appearance and death. The magnitude of extension growth was not consistent in or among our datasets, and may be related to species, treatment or temporal effects. However, assuming that extension growth is a constant proportion of total root production in any given species–treatment combination, its magnitude can be quantified as the difference between $b_p$ and $b_m$. This difference in slope, if any, can then be added to $b_p$ to calculate total, rather than just new, root length production for any time period.

## 4. Summary

The minirhizotron is clearly one of the most effective systems for quantifying below ground dynamics (Hendrick and Pregitzer, 1996a). Despite its obvious advantages, however, the minirhizotron is not used as frequently as some other methods. Lengthy analysis times, 30 min to 8 h per sampling period per each minirhizotron, discourage its use in studies that would otherwise benefit from an improved understanding of fine root dynamics. Reducing image analysis time will help ameliorate the main limitation of this method.

We hypothesized that a manual count of roots could be used to estimate root lengths accurately after quantitative relationships between the two parameters were established. Our results indicate that root numbers were indeed a good predictor of root lengths in all of our studies. This method could be applied to minirhizotrons to substantially reduce minirhizotron image analysis times. How much time can be saved by substituting root numbers for length? Much of it depends upon root segment densities within individual images, their average length, and individual digitizing abilities. Estimates from our own work suggest that this time savings may be in the range of 33–75%, meaning that image processing volume per unit time could potentially be increased as much as four-fold.

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