



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 (MRS�), ranged from 2.34 to 8.38 mm per root segment for both production and mortality. Most treatments did not alter MRS� substantially, the exceptions being CO₂ 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 re-

peatedly 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 disap-

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pearance) 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. (2001) studied roots of nine different trees 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., 2001). 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 elliotii*, *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. elliotii* 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 (Schroeder et al., 1999). Treatments consisted of controls and artificial gaps (37.5 m radius). The second *P. elliotii* 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
<i>Populus tremuloides</i>	Pellston, MI	Na ^a	NA	NA	NA	NA		Two levels of CO ₂ and two levels of nitrogen in a factorial design
<i>Pinus elliotii</i> ^b	Upper coastal plain, GA	Arenic, Plinthic, Kandiudults	1225	14	20–25, 40–49	<i>Prunus serotina</i> , <i>Quercus phellos</i> , <i>Quercus nigra</i>	18	Controls and artificial gaps (37.5 m radius).
<i>Pinus elliotii</i>	Bryceville, Callahan, and Yulee, FL	Typic, Haplanods			20	<i>Ilex glabra</i> , <i>Aristida stricta</i> , <i>Serenoa repens</i>	11	Control, fertilized, herbicide, fertilized + herbicide plots.
<i>Acer saccharum</i>	Northern lower peninsula, MI	Alfic/Entic, Typic, Haplorhods	810–850	5.8–7.6	74–78	<i>Acer rubrum</i> , <i>Quercus rubra</i> , <i>F. grandifolia</i>	18	Two sites separated by a north–south distance of 80 km
<i>Liquidambar styraciflua</i>	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
<i>Pinus glauca</i>	Tanana river floodplain, AK	Typic, Cryofluvents	269	-4.3	150–250	<i>Alnus spp.</i> , <i>P. balsamifera</i> , <i>Betula papyrifera</i>		Three mature forests; no manipulative treatments
<i>Salix spp.</i>	Tanana river floodplain, AK	Histic, Pergelic, Cryaquepts	269	-3.3	10	<i>Alnus tenuifolia</i> , <i>P. balsamifera</i> , <i>Picea glauca</i>	24	Three browsed and three non-browsed plots (30 m × 50 m × 5 m)

^a Not applicable, growth chamber study.

^b *Pinus elliotii* gap study (Schroeder, 1999).

136 dynamics. Treatments consisted of control, fertilizer
137 (280 kg ha⁻¹ di-ammonium phosphate, 280 kg ha⁻¹
138 urea, and 228 kg ha⁻¹ KCl), herbicide (3% solution
139 of Roundup prior to site preparation), and fertilizer
140 plus herbicide treatments (Shan et al., 2001).

141 An *A. saccharum* study, located in the northern
142 lower peninsula of Michigan (USA), was established
143 to understand the spatial and temporal dynamics of
144 roots in two forests separated by a north–south dis-
145 tance of 80 km. There were no experimental manipu-
146 lations applied in this study (Hendrick and Pregitzer,
147 1993a,b, 1996b).

148 Minirhizotrons were also used to quantify fine
149 root production, mortality (rate of disappearance)
150 and standing root crop dynamics in an intensively-
151 managed *L. styraciflua* coppice stand located in
152 the middle coastal plain of Georgia (USA). Treat-
153 ments consisted of two levels of fertilizer (19:9:19
154 NPK): a low (560 kg ha⁻¹ per year) and a high level
155 (1120 kg ha⁻¹ year) application (Price and Hendrick,
156 1998).

157 Two of the datasets are from the boreal forest of in-
158 terior Alaska, USA. In one, the fine root demography
159 of *P. glauca* fine roots was quantified in three mature
160 forests located on the Tanana river floodplain in inte-
161 rior Alaska, USA. Again, no experimental manipula-
162 tions were applied to the selected sites in this study. In
163 a related study on early successional *Salix* spp. com-
164 munities, minirhizotrons were used to characterize the
165 effects of aboveground mammalian browsing on rates
166 of fine root production, mortality, and decomposition.
167 Three large enclosures (30 m × 50 m × 5 m) were paired
168 with unfenced plots of the same size to create both
169 browsed and non-browsed treatments (Ruess et al.,
170 1998).

171 The final data are from a study conducted in
172 Michigan (USA) that was designed to examine the
173 effects of atmospheric CO₂ and nitrogen fertiliza-
174 tion on root production and mortality in *P. tremu-
175 loides*. Two levels of CO₂ and two levels of nitrogen
176 were examined in a factorial design (Pregitzer et al.,
177 2000).

178 Although production measurements refer to the oc-
179 currence of new roots as well as the growth of existing
180 roots in the minirhizotron tubes, mortality measure-
181 ments primarily track the rates of disappearance of the
182 fine roots, because it is difficult to distinguish dead
183 roots from those that are living. All of the studies we

utilized reported a rapid root disappearance rate, so it 184
is possible that some mortality is missed. In all stud- 185
ies, 80–85% of all roots measured were <0.5 mm in 186
diameter. 187

2.2. Modification of dBase program 188

In the past, we have used a dBase program (Ruess, 189
2001) to calculate the production and mortality of 190
roots based upon complete root segment length mea- 191
surements. To calculate the production and mortal- 192
ity of both root lengths and root numbers between 193
sample dates a count function was added to the 194
original program. The new program was used for 195
each minirhizotron file of the seven sample datasets. 196
Output files, within each dataset, were collated into 197
their respective treatments. The resulting output pro- 198
vided values of root numbers and the correspond- 199
ing root lengths for both production and mortality 200
data. 201

2.3. Linear regression models 202

For these analyses we fitted linear regression mod- 203
els of the form $y = bx$, where y = root segment 204
length and the independent variable (x) was the 205
number of roots. Models were constructed for each 206
species–treatment combination, and included length– 207
number relationships for both production (b_p) and 208
mortality (b_m). The models were restricted to force 209
the intercept through zero. The resulting slope value 210
(b) represents the mean root segment length (MRSL) 211
for each species and treatment. Once the regres- 212
sion analysis of each species- and treatment-specific 213
dataset was completed, we compared the slopes (i.e. 214
MRSL) of the regressions to determine if any of the 215
within-dataset treatments had altered the slope. Dif- 216
ferences in regression slopes were determined using 217
paired t -tests ($\alpha = 0.05$). 218

To determine if there were any significant temporal 219
changes in MRSLs (among image dates), the *P. elliotii* 220
intensive management study was selected for further 221
examination. We chose these data because of the large 222
number of treatments and because its b and r^2 values 223
fell in the mid-range of all data points. A repeated 224
measures analysis of minirhizotron image dates ($\alpha =$ 225
0.05) was done with and without treatments for both 226
production and mortality. 227

228 **3. Results and discussion**229 *3.1. An overview of minirhizotron data extraction*
230 *from recorded images*

231 The ease with which minirhizotron video tapped/
232 digital images are collected belies the difficulties as-
233 sociated with data extraction. Numerous techniques
234 have been devised to extract meaningful data for such
235 variables as rooting depths, root length densities,
236 root morphologies, and root dynamics. Root length,
237 for instance, has often been estimated using some
238 variation of the Newman grid intersection method
239 (Newman, 1966; Tennant, 1975). Manually counting
240 the intersections of roots with etched lines on the
241 minirhizotron surface, either random or fixed tran-
242 sects (McMicheal and Taylor, 1987), allows the user
243 to convert to root lengths with Newman's method
244 but requires a fine grid system with many intercep-
245 tions to obtain realistic data (Smucker et al., 1987).
246 Aside from intersection methods, a count of roots in
247 contact with the tube surface within a specified area
248 has also been utilized, by then converting counts to

length densities via regression against washed root 249
lengths. 250

Now that videotape and digital recording are com- 251
monly used, images are often manually processed by 252
tracing and recording the lengths (and sometimes di- 253
ameters) of root segments in contact with the minirhi- 254
zotron tube using PC-based computer software. This 255
is an effective way to extract length data quantifying 256
the development and fate of individual roots. How- 257
ever, manual image processing is time consuming and 258
laborious, hindering the usefulness of minirhizotrons. 259
Counting roots is much less time-intensive, and sub- 260
stituting root number for length could substantially in- 261
crease the rate at which minirhizotron images could 262
be analyzed. The results of our attempts to make this 263
substitution were rather encouraging. Linear regres- 264
sions derived from these datasets generally had high 265
coefficients of determinations (r^2). The calculated r^2 266
values fell in the range of 0.79–0.99 (Table 2) for both 267
production and mortality (rate of disappearance), sug- 268
gesting that root counts can be used to estimate root 269
lengths routinely. The *A. saccharum* study was the 270
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
<i>Populus tremuloides</i>	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
<i>Pinus elliotii</i>	Control	287	0.91	4.10b	0.91	4.28b
	Gap	287	0.87	2.34a	0.92	3.03a
<i>Pinus elliotii</i>	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
<i>Acer saccharum</i>	South site	428	0.81	3.69a	0.79	3.62a
	North site	467	0.88	4.57b	0.82	3.94b
<i>Liquidambar styraciflua</i>	Low N	83	0.96	3.45a	0.99	4.20a
	High N	82	0.95	3.37a	0.99	4.22a
<i>Pinus glauca</i>	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
<i>Salix</i> 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 (MRS�) in millimeters per root segment.

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

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. elliotii* 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 (Schroeder 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. elliotii* 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. elliotii* 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. elliotii* 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

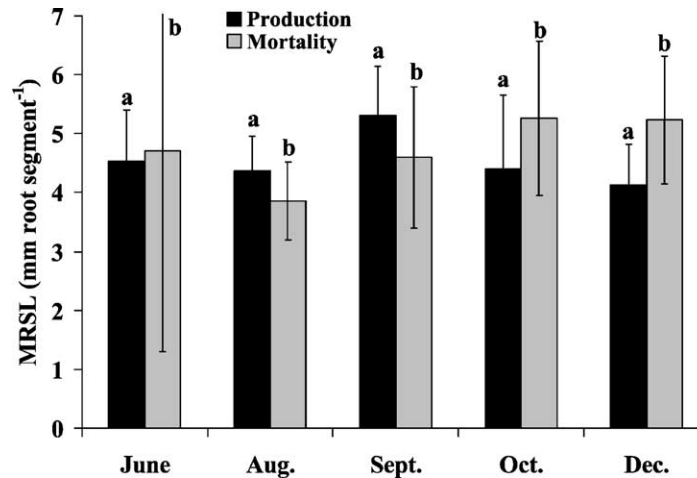


Fig. 1. Temporal analysis of mean root segment lengths (MRSLS, in millimeters per root segment) + L.S.D., for the *Pinus elliotii* intensive management study. Comparisons are between bars of similar color (i.e. productivity vs. productivity). No significant temporal differences were found ($\alpha =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

365 mortality and the corresponding birth or loss of root
366 numbers.

367 Assuming that both regressions yielded acceptable
368 coefficients of determination, images on most subse-
369 quent dates could be digitized for root counts only. In
370 practice, this means that the appearance of new roots
371 and the mortality of existing roots can be quantified by
372 procedures that are easily and quickly implemented.
373 However, it is possible that the relationships between
374 root lengths and root numbers (i.e. the magnitude of
375 b_p or b_m) could change over time if roots of substan-
376 tially different lengths were being produced or dying
377 at different times (e.g. phenological differences in root
378 morphology). Therefore, error checks should be made
379 on one or more subsequent dates (at least two sequen-
380 tial dates to account for root mortality) to confirm that
381 b_p or b_m did not differ significantly among dates. We
382 confirmed that there were no significant temporal dif-
383 ferences in either b_p or b_m for our intensively-managed
384 *P. elliotii* study (Shan et al., 2001), but this might not
385 necessarily be true for other species, treatments, or ge-
386 ographic locations. We suggest that periods of known
387 or suspected episodes of high root production and mor-
388 tality (e.g. spring and winter, respectively) might prove
389 most useful for error checking.

390 For all datasets except the *A. saccharum* and *P.*
391 *tremuloides*, MRSLS of dead roots for a given treat-
392 ment group were from 2 to 20% greater than their

393 corresponding production MRSLS (i.e. $b_m > b_p$). The
394 most likely explanation for this difference is root seg-
395 ment length (i.e. extension) growth between the time
396 of first appearance and death. The magnitude of ex-
397 tension growth was not consistent in or among our
398 datasets, and may be related to species, treatment or
399 temporal effects. However, assuming that extension
400 growth is a constant proportion of total root produc-
401 tion in any given species–treatment combination, its
402 magnitude can be quantified as the difference between
403 b_p and b_m . This difference in slope, if any, can then
404 be added to b_p to calculate total, rather than just new,
405 root length production for any time period.

4. Summary 406

407 The minirhizotron is clearly one of the most ef-
408 fective systems for quantifying below ground dynam-
409 ics (Hendrick and Pregitzer, 1996a). Despite its ob-
410 vious advantages, however, the minirhizotron is not
411 used as frequently as some other methods. Lengthy
412 analysis times, 30 min to 8 h per sampling period per
413 each minirhizotron, discourage its use in studies that
414 would otherwise benefit from an improved understand-
415 ing of fine root dynamics. Reducing image analysis
416 time will help ameliorate the main limitation of this
417 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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References

- Bohm, W., 1979. Methods of Studying Root Systems. Ecol. Stud., vol. 33. Springer, Heidelberg.
- Burton, A.J., Pregitzer, K.S., Hendrick, R.L., 2000. Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. *Oecologia* 128, 389–399.
- Eissenstat, D.M., 1991. On the relationship between specific root length and the rate of root proliferation: a field study using citrus rootstock. *New Phytol.* 118, 63–68.
- Hendrick, R.L., Pregitzer, K.S., 1993a. The dynamics of fine root length, biomass and nitrogen content in two northern hardwood ecosystems. *Can. J. For. Res.* 23, 2507–2520.
- Hendrick, R.L., Pregitzer, K.S., 1993b. Patterns of fine root mortality in two sugar maple forests. *Nature* 361, 59–61.
- Hendrick, R.L., Pregitzer, K.S., 1996a. Applications of minirhizotrons to understand root function in forests and other natural ecosystems. *Plant Soil* 185, 293–304.
- Hendrick, R.L., Pregitzer, K.S., 1996b. Temporal and depth-related patterns of fine root dynamics in northern hardwood forests. *J. Ecol.* 84, 167–176.
- Jackson, R.B., Caldwell, M.M., 1989. The timing and degree of root proliferation in fertile-soil microsites for three cold-desert perennials. *Oecologia* 81, 149–153.
- Kurz, W.A., Kimmins, J.P., 1987. Analysis of error in methods used to determine fine root production in forest ecosystems: a simulation approach. *Can. J. For. Res.* 17, 909–912.
- Ludovici, K.H., Morris, L.A., 1996. Responses of loblolly pine, sweet gum and grass roots to localized increases in nitrogen in two watering regimes. *Tree Phys.* 16, 933–939.
- McMicheal, B.L., Taylor, H.M., 1987. Applications and limitations of rhizotrons and minirhizotrons. In: Taylor, H.M. (Ed.), *Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics*. American Society of Agronomy Special Publication Number 50, Madison, WI, USA, pp. 1–13.
- Mou, P., Mitchell, R.J., Jones, R.H., 1995. Root distributions of two species under heterogeneous nutrient environments. *J. Appl. Ecol.* 34, 645–656.
- Newman, E.L., 1966. A method of estimating the total length of roots in a sample. *J. Appl. Ecol.* 3, 139–145.
- Persson, H., 1978. Root dynamics in a young Scots pine stand in central Sweden. *Oikos* 30, 508–519.
- Persson, H., 1980. Fine-root production, mortality and decomposition in forest ecosystems. *Vegetation* 41 (2), 101–109.
- Pregitzer, K.S., Hendrick, R.L., Fogel, R., 1993. The demography of fine roots in response to patches of water and nitrogen. *New Phytol.* 125, 575–580.
- Pregitzer, K.S., Zak, D.R., Maziasz, J., DeForest, J., Curtis, P.S., Lussenhop, J., 2000. Interactive effects of atmospheric CO₂ and soil-N availability on fine roots of *Populus tremuloides*. *Ecol. Appl.* 10 (1), 18–33.
- Pregitzer, K.S., DeForest, J.L., Burton, A.J., Allen, M.F., Ruess, R.W., Hendrick, R.L., 2001. Fine root length, diameter, specific root length and nitrogen concentration of nine tree species across four North American biomes. *Ecology* 72, 293–309.
- Price, J.S., Hendrick, R.L., 1998. Fine root length production, mortality and standing root crop dynamics in an intensively managed sweetgum (*Liquidambar styraciflua* L.) coppice. *Plant Soil* 205, 193–201.
- Ruess, R.W., Hendrick, R.L., Bryant, J.P., 1998. Regulation of fine root dynamics by mammalian browsers in early successional Alaskan taiga forests. *Ecology* 79 (8), 2706–2720.
- Ruess, R.W., 2001. <http://mercury.bio.uaf.edu/~rruess.faculty/Programs.htm>.
- Schroeder, A.E., Hendrick, R.L., Harrington, T.B., 1999. Root ground cover and litterfall dynamics within canopy gaps in a slash pine (*Pinus elliotii* Engelm.) dominated forest. *Ecoscience* 6 (4), 548–555.
- Shan, J.P., Morris, L.A., Hendrick, R.L., 2001. The effects of management on soil and plant carbon sequestration in slash pine plantations. *J. Appl. Ecol.* 38 (5), 932–941.
- Smit, A.L., Zuin, A., 1996. Root growth dynamics of Brussel sprouts (*Brassica olearacea*, var. *gemmifera*) and leeks (*Allium porrum* L.) as reflected by root length, root colour, and UV fluorescence. *Plant Soil* 185, 269–278.
- Smucker, A.J.M., Ferguson, J.C., DeBruyn, W.P., Belford, R.K., Ritchie, J.T., 1987. Image analysis of video recorded plant root systems. In: Taylor, H.M. (Ed.), *Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere*

- 521 Dynamics. American Society of Agronomy Special Publication
522 Number 50, Madison, WI, USA, pp. 67–80.
- 523 Steen, E., 1991. Usefulness of the mesh bag method in quantitative
524 root studies. *Swed. J. Agric. Res.* 14, 93–97.
- 525 Tennant, D., 1975. A test of a modified line intersect method of
526 estimating root length. *J. Ecol.* 3, 995–1001.
- Upchurch, D.R., 1987. Conversion of minirhizotron–root
intersections to root length density. In: Taylor, H.M. (Ed),
Minirhizotron Observation Tubes: Methods and Applications
for Measuring Rhizosphere Dynamics. American Society of
Agronomy Special Publication Number 50, Madison, WI, USA,
pp. 51–65.

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