Fine Root Dynamics in a *Pinus palustris* Mill. Ecosystem: The Role of Sampling Interval and the Soil Environment

by

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ABSTRACT

We examined the impact of sampling interval on fine root production and mortality estimates by comparing data from a weekly minirhizotron sampling regimen to subsets of the same data representing biweekly, monthly, bimonthly, and quarterly sampling regimens. We also investigated possible sources of error involved in the root tracing technique and estimated root herbivory using the full weekly sampling regimen. Data were collected for eleven months from a *Pinus palustris* Miller woodland in southwest Georgia. As sampling interval increased, estimates of production and mortality declined, while estimates of mean fine root lifespan increased. Annual production values ranged from a maximum of 1.26 mm/cm^2 for weekly sampling to 0.83 mm/cm^2 for quarterly sampling. Total mortality varied from 0.97 mm/cm^2 to 0.53 mm/cm^2. Bias increased at a decreasing rate when sample interval was increased from weekly to monthly. The root tracing protocol added some small, random error to growth measurements; re-measuring roots returned values 0.16% smaller than initial measures. We also observed a root mortality and regrowth phenomenon that may be measurement error or short-term fluctuation in root length. Herbivory accounted for greater than 20% of fine root biomass produced. Our study suggests that increases in sampling frequency from monthly to weekly can provide substantial gains in accuracy for estimates of root dynamics.

Key Words: Fine roots, minirhizotron, *Pinus palustris*, root herbivory, root demography, root production

INTRODUCTION

Fine roots account for a large proportion of total NPP and perform critical functions in forest ecosystems (McClaugherty et al. 1982, Aber et al. 1985, Nadelhoffer et al. 1985, Santantonio and Grace 1987, Gower et al. 1994). Among the methods that have been devised to sample roots *in situ* and measure fine root demography, the minirhizotron technique has become the most recent standard (Eissenstat and Caldwell 1988, Kosola et al. 1995, Hendrick and Pregitzer 1992, 1993, 1996, 1997, Steele et al. 1997, Ruess et al. 1998). Minirhizotron studies use the cohort life table approach. A cohort of roots is marked at the first sampling interval. At subsequent intervals, the growth or mortality of individual roots within the cohort are measured, and new cohorts are marked. Technological improvements, such as an indexing handle to provide repeated frame registration and direct capture of digital images in the field, have improved the efficiency of the technique. Overall, the minirhizotron is a powerful tool that can provide a great deal of information on patterns of fine root production, lifespan, and mortality.

One of the critical aspects of any demography study is the choice of sampling interval (Harper 1977). While this is particularly true in minirhizotron studies, sampling interval selection has not always been based on the interval most appropriate to the organism in question. Minirhizotron studies are time intensive: field studies require transport of video and computer equipment to often widely spaced plots, and substantial amounts of time are required to hand trace fine roots on each of many images. The selection of sampling interval, therefore, often hinges on labor availability, and most minirhizotron studies sample on either a monthly or bimonthly basis.

As sampling interval increases, increasing numbers of roots may appear and disappear between samples. These roots will not be counted. Since young roots are particularly vulnerable to herbivory or mortality (Graham 1995, Eissenstat and Yanai 1997), this missing fraction may be relatively large if long sampling intervals are used.

Intervals that are too fine-scaled may also introduce a bias if measurement error causes over- or underestimation. For example, we have observed that roots occasionally decline in length, only to recover the following week. This phenomenon may be actual decline and subsequent regrowth, or it may be related to short-term changes in soil moisture. If the former explanation is true, repeated sampling increases the accuracy of root growth estimates; in the latter case, repeated sampling may artificially inflate growth estimates and therefore introduce a

bias. Similarly, if a consistent measurement error occurred in the root tracing process (either an over- or underestimate), such an error would be magnified in short-term sampling efforts due to the increased number of measurements involved.

Finally, the specific sampling interval chosen for a study has an impact on the ability to detect herbivory. At monthly or longer intervals, all disappearances of fine root tissue are counted as mortality and decomposition. However, some of the roots may have disappeared due to herbivory, which can be substantial. Estimates of root tissue consumed by functional groups of herbivores (i.e., nematodes, insects, or rodents) range from 6 to 30% or more of belowground net primary production (Andersen 1987), and belowground herbivores can have measurable impacts on aboveground allocation (Ueckert 1979, Karban 1980, Vogel and Kindler 1980, Ingham and Detling 1990). Roots can disappear very rapidly in minirhizotron studies, often within a few weeks of sampling (Hendrick and Pregitzer 1992, 1996; Pregitzer et al. 1993). Previous studies, however, have not attributed these losses to herbivory, possibly because sample intervals were not short enough for the researchers to differentiate causes of mortality with confidence. We suggest that fine root herbivory has been underestimated or overlooked because previous studies have not used sufficiently short sampling intervals.

In this paper we examine minirhizotron sample intervals ranging between weekly and quarterly to determine how the missing fraction and re-measurement error relate to sampling interval, and to apportion fine root losses into classes representing important processes such as herbivory and 'standard' mortality and decay. We hypothesize that: (1) shorter sampling intervals capture higher levels of fine root production and mortality and therefore provide shorter estimates of mean fine root lifespan; (2) shorter intervals magnify biases associated with root shrinkage and tracing errors; and (3) herbivory accounts for a substantial quantity of fine root mortality.

METHODS

Experimental Design

Our research was conducted at the Joseph W. Jones Ecological Research Center in southwest Georgia, USA (31°N, 84°W) within a 140-hectare woodland dominated by 60- to 70-year-old *Pinus palustris* Miller (longleaf pine) and *Aristida stricta* Michaux. (wiregrass). The climate is humid subtropical (Bailey 1998); mean daily temperatures range between 11 and 27

degrees Celsius, and precipitation averages 132 cm per year. Soils are Orangeburg series sands over karst limestone bedrock.

Twelve study plots (each approximately 2.5 ha) were installed in October 1997 to examine the influence of several silvicultural techniques on plant resources and *P. palustris* regeneration. Our study was conducted in six of the twelve plots in the study area, representing two treatments: a no-harvest control and a partial harvest resulting in canopy gaps 35 m in radius. Basal area was 14.8 - 18.4 m^2/ha in the control plots and 11.2 - 15.4 m^2/ha in the harvested plots after gaps were created.

Six minirhizotron sampling stations were located within each plot (36 sampling stations total) using a stratified random approach. Plots were divided into a 5 x 5 m grid; at each grid intersection > 15 m from the edge of the plot, tree basal area was summed within 5 m, 10 m, and 15 m. Each sum was then divided by distance and the three resultant values were added together. The full range of this distance weighted overstory abundance index within each plot was then separated into 5 equal percentiles. One intensive sampling station was randomly located at a gridpoint within each percentile. A sixth station was placed in the lowest overstory percentile (the most open conditions) in an effort to focus on dynamics within canopy gaps (for reasons unrelated to this study). Sampling stations were spaced a minimum of 10 m apart.

The entire stand was burned in November 1997 after delineation of plots and sampling stations. Sampling stations included 3 x 3 m herbicided quadrats in which all understory vegetation was removed. Removal was initiated when the stand was burned (November 1997); thereafter, herbaceous vegetation in the sampling stations was controlled by monthly application of 4% glyphosphate solution to emergent herbaceous vegetation at monthly intervals. The original intent of herbicide application was to test for influences of non-pine roots on pine regeneration. In this study, herbicided stations afford the opportunity to examine fine root dynamics of a single species (*P. palustris*).

In January 1998, a single CAB minirhizotron tube (5 cm inside diameter x 80 cm length) was installed in the herbicided portion of each sampling station. A total of 36 tubes were installed at a 45-degree angle to the soil surface to approximately 40 cm vertical depth. Two tubes had no roots throughout the study period and were subsequently discarded from analyses. All tubes were etched at 1 cm intervals. Holes were drilled in the tops of the tubes to allow for proper registration of the indexing handle between samplings. The tubes were positioned so that

the captured frames were oriented upward at approximately zero degrees from vertical. Tubes were anchored in place (to minimize rotation and environmental-related heaving) by attaching them with wire and hose clamps to rebar stakes that had been driven into the ground to approximately one meter depth.

Root Measurements

Minirhizotron sampling was conducted at fairly constant 7-day intervals (mean sampling interval 6.7 days) beginning June 24, 1998 and continuing until May 26, 1999. A total of 49 samplings were conducted. Images were collected in the field either by use of a Hi-8 camcorder (June 1998 – February 1999) or by direct digital capture on a laptop computer (February – July 1999) using software and a minirhizotron camera system manufactured by Bartz Technology Company, Santa Barbara, CA. Individual fine roots were counted if they were partially or wholly within the 1.4 x 1.8 cm sampling image. If an individual root formed a branch, the branch was counted as a new individual root. ARCOS root tracing software (Graphic Equations Inc., Houston, TX) was used to measure the length of each fine root at each sample date. Birth and death of individual fine roots were tracked by hand. Roots were considered dead on the first date that they either (1) disappeared entirely from the frame, or (2) appeared to be dead, due to change in color (to gray or black) or apparent decomposition. Reduction in root length did not result in our classifying a root as dead.

Since understory vegetation was controlled for the duration of the study, our observations were primarily fine roots of *P. palustris* and fungal rhizomorphs. We attempted to eliminate rhizomorphs from the data set using the following criteria: rhizomorphs were very thin, constantly white, and exhibited diffuse outer edges and a fairly transparent appearance, while fine roots were typically much larger in diameter, often turned red or brown soon after appearance, and exhibited distinct tissue boundaries. Structures that exhibited distinctly fungal characteristics were eliminated from the data set.

To estimate the error associated with re-measurement of the same root, we re-measured 100 roots randomly selected from the 932 roots in the full data set. These second measurements were compared to the initials to detect any tendency to inflate or deflate estimates during re-measurement.

The two major growth parameters measured were production and mortality. Production was considered to be any increase in fine root length from week x to week x+1. This could include new roots plus growth of existing roots. Mortality was defined as any decrease in length from week x to week x+1. Mortality was further classified into specific categories that included shrinkage, herbivory, and senescence as defined below.

We observed that roots occasionally declined in length only to recover the following week. This was either the result of short-term changes in soil moisture or of actual decline and subsequent production. We termed this phenomenon root shrinkage and recorded it when any root decreased in length from week x to week x+1, only to increase in length in week x+2. The shrinkage length was recorded as that tissue that was added from week x+1 to x+2, up to a maximum of the decline between week x and week x+1. In the discussion below, we consider this shrinkage measurement to be a portion of fine root mortality.

We also observed rapid disappearance of fine root tissues, and assume that at least some of this disappearance is caused by root herbivory. We used two methods to estimate root losses due to herbivory. The first we considered to be the more conservative: any root present in week x, increased in length in week x+1, and then not present in week x+2 was considered eaten. Our less conservative estimate of herbivory considered any root that was present in week x, of equal or greater length in week x+1, and then not present in week x+2 to have been eaten. A root that declined in length from week x to week x+1 and then disappeared in week x+2 was never considered to have been eaten; such losses would be counted as senescence (see below). Herbivory estimates were produced using the weekly sampling because we assume that one week is substantially less than the mean decomposition period for pine roots.

Senescence included any mortality that did not fall into the categories of shrinkage or herbivory. Examples include roots that became progressively smaller before disappearing and decline in length that was not recovered the following week.

Root lengths obtained using the ARCOS tracing software provided length measurements in pixels. Conversion from pixels to centimeters was conducted using the following steps. First, the dimensions of the minirhizotron image provided by the camera system were determined by imaging a metric grid at the same focal length used for field estimates. These metric dimensions were then compared to ARCOS pixel counts for the same length and width dimensions, allowing a root length conversion factor to be determined. Fine root lengths (growth and mortality) were

ultimately converted to per-tube estimates (mm root/cm^2 soil) based on the area of the minirhizotron images (1.4 x 1.8 cm) and the number of frames imaged per tube (40).

Demographic Analyses

All statistical analyses were conducted using Statistical Analysis Software (Version 7, SAS Institute, Cary, NC). Fine root production and mortality were measured for each tube. Since there was no significant impact of harvest treatment on production (ANOVA, P=0.31) or mortality (P=0.07), treatments were ignored and each tube was considered an individual and independent experimental unit. The decision to count each tube as an experimental unit is justified by the fact that all sampling stations were separated by at least 10 m. Our estimates of measurement error were produced by comparing initial to remeasured lengths using a paired t-test.

To determine the effect of sampling interval on production and mortality estimates, we compared the results from the weekly sampling regimen to those calculated using semi-weekly, monthly, semi-monthly and quarterly sampling regimens. All regimens began and finished on the same sampling date; i.e. 49 sample dates and 48 weeks from beginning to end. Since datasets for the various regimens were highly correlated, differences in productivity and mortality estimates between sampling regimens were compared using bootstrap simulations in which 20,000 iterations were produced for each possible regimen combination (weekly vs. biweekly, biweekly vs. monthly, etc.). The distribution of the 20,000 differences in each test was inspected to determine the probability that the difference in growth or mortality estimates was zero. Because fifteen combinations of sampling regimens were examined, a Bonferroni correction was applied to the probability estimates to maintain an appropriate experiment-wide type I error rate.

Estimates of fine root lifespan under the different sampling regimens were produced using failure time analysis (Proc Lifetest in SAS). Since our data were right-censored (not all roots died by the end of the study), a Wilcoxon test was used to determine the impact of sampling interval on lifespan estimates. We did not attempt to make any distinction based on root order (i.e. primary, secondary, tertiary branching). Although it is fairly simple to assign branching order to those roots that branch after they are seen at the surface of the tube, it is impossible to determine the order of a root when it first appears. This does complicate interpretations of lifespan results to the extent that primary root systems have a reduced risk of

mortality after producing branches; however, this question is beyond the scope of the current experiment.

RESULTS

Increasing the frequency of sampling resulted in larger estimates of fine root production and mortality (Figure 1.1). Annual production values ranged from a maximum of 1.26 mm/cm^2 for weekly sampling to 0.83 mm/cm^2 for quarterly sampling. Total mortality varied similarly, from 0.97 mm/cm^2 to 0.53 mm/cm^2. If weekly sampling is assumed to capture all production and mortality, then monthly sampling (a fairly common interval) captured only 85% of the annual production and 79% of annual mortality in our study sites.

The downward trend in fine root production and mortality estimates as sampling interval increased was not constant (Fig. 1.1). For example, the largest per-week decrease occurred going from weekly to biweekly sampling intervals (an 8% decline in production estimates for a one-week increase in sampling interval). Monthly sampling resulted in a 15% decline in productivity estimates compared to the weekly sampling regimen, a 5% decrease per week. Comparatively little information is lost extending from bimonthly to quarterly sampling, where a four-week increase in sampling interval results in only a 6% further decrease in the estimate.

Sampling interval significantly impacted estimates of mean fine root lifespan (Wilcoxon test, $p < 0.0001$). Longer intervals resulted in greater estimates of lifespan (Fig. 1.2).

Re-measuring individual observations of more than 10% of the fine roots in our data set indicated that the first and second measures were not significantly different (P=0.88). The second measurement was 0.16% smaller than the first on average.

Using estimates derived from the weekly sampling interval and our conservative approach for estimating mortality, we estimated that shrinkage (losses regained the following week) accounted for 3.5% of total fine root mortality; herbivory represented another 24% (Fig. 1.3). The remaining 72.5% was classified as 'standard' mortality, which represents all mortality that was not regrown within one week or was not the rapid disappearance of actively growing root tissue. In our more relaxed estimations, herbivory accounted for 36.6% of total fine root mortality, while 'standard' mortality was reduced to 60%.

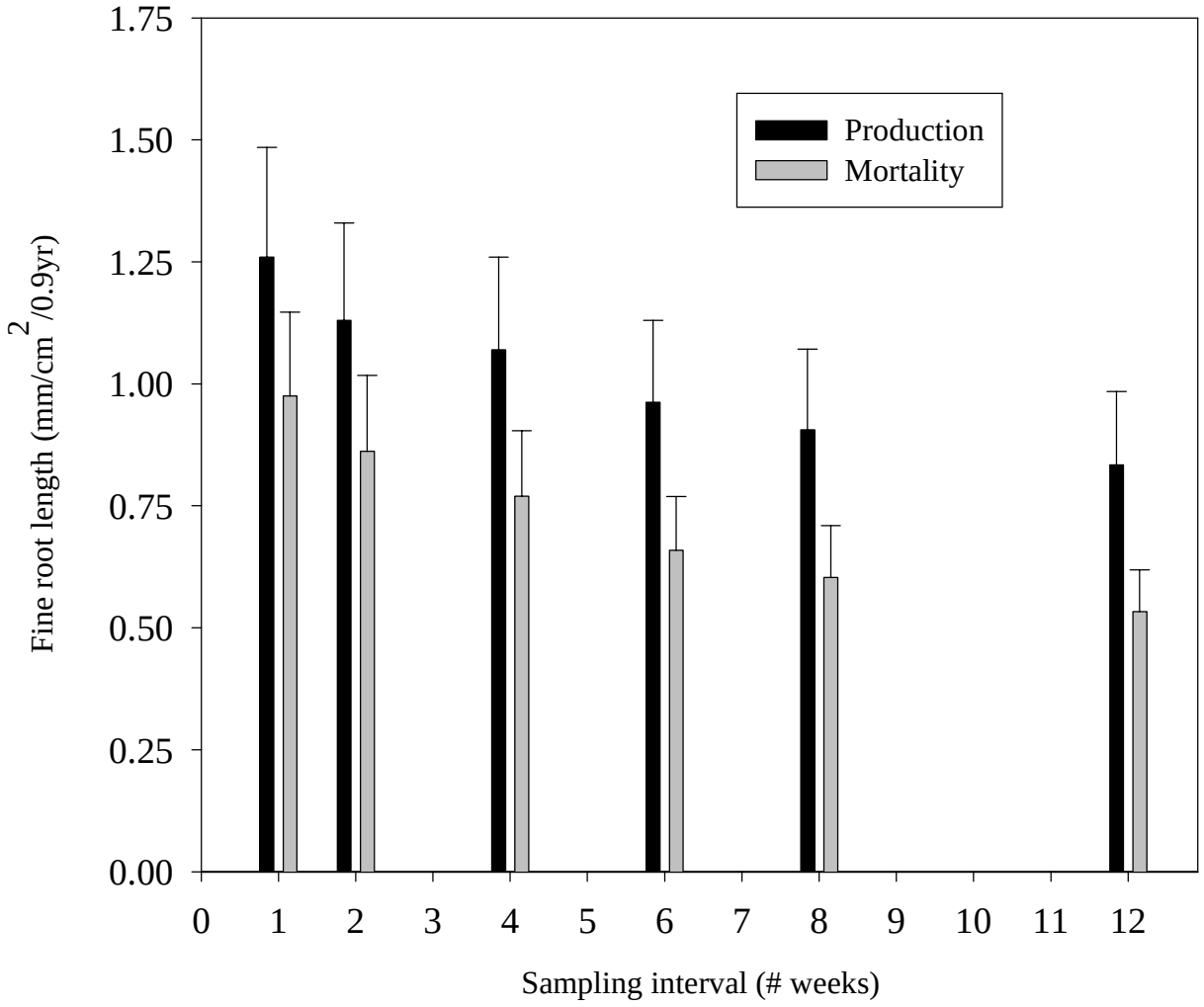

Figure 1.1 Estimates of production and mortality of *Pinus palustris* fine roots derived from a weekly sampling interval data set, with subsets representing sampling intervals of two, four, six, eight, and twelve weeks. Bars represent mean values + 1 se.

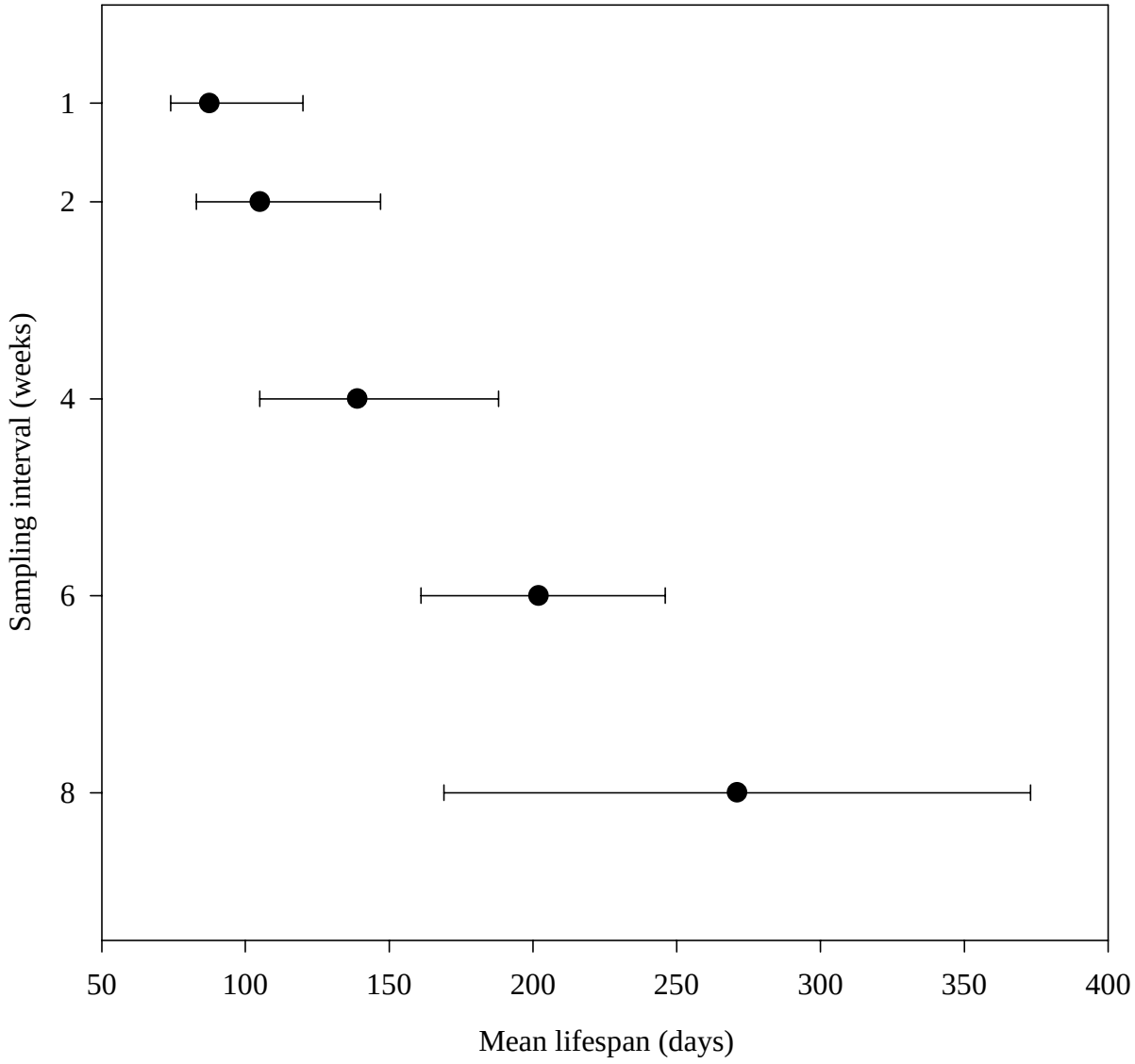

Figure 1.2 The impact of sampling regime on estimates of mean fine root lifespan. Plotted values are mean values +/- 1 se.

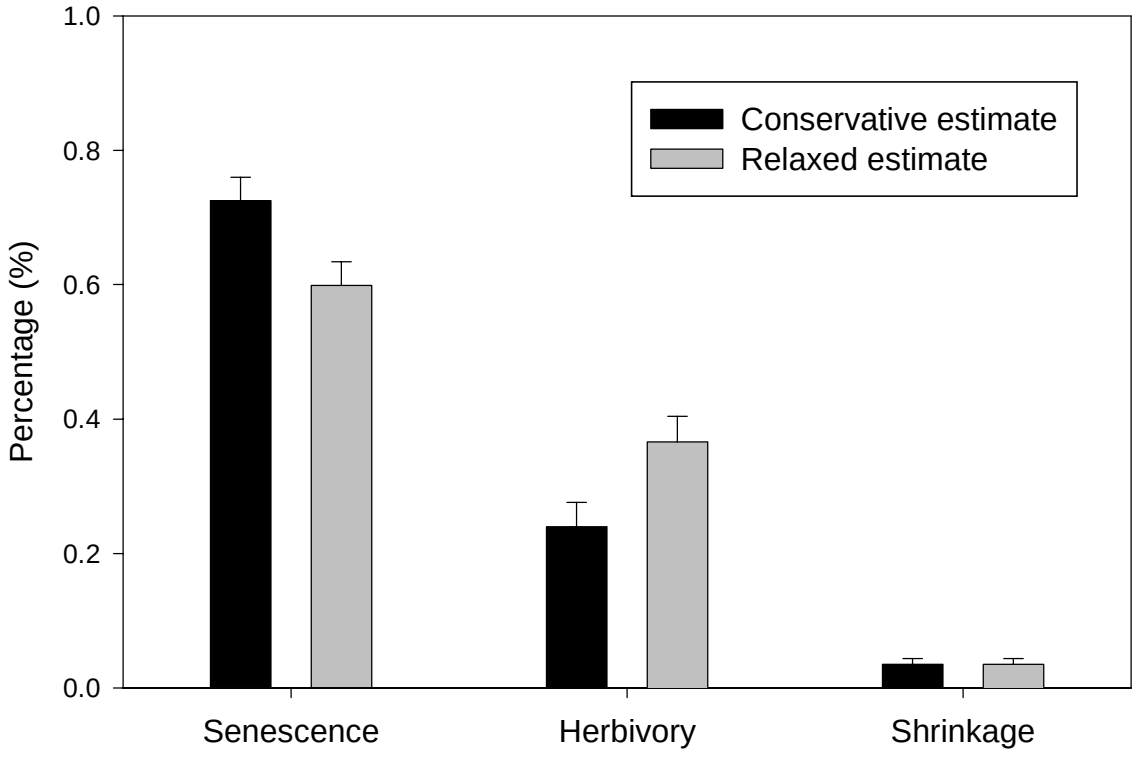

Figure 1.3. The proportion of *Pinus palustris* fine root mortality attributable to senescence, herbivory, and shrinkage. Values are based on a conservative herbivory estimate (length in week x+1 > week x, gone in week x+2) and a more relaxed herbivory estimate (week x+1 ≥ week x [i.e., not necessarily growing] and gone in week x+2). Plotted values represent means of 34 tubes + 1 se.

DISCUSSION

Sampling interval had a large impact on our estimates of fine root production, mortality, and lifespan. As the sampling interval increased, mortality and production estimates decreased while lifespan increased. The impact was particularly strong for lifespan estimates, which were 87.5 days for the weekly sampling regimen and 270 days for the quarterly. These levels of bias may have serious repercussions for ecosystem studies that use fine root demography to estimate belowground litter inputs.

Re-measurement error did not contribute significantly to the difference between frequent and infrequent sampling. We could, however, attribute a small portion of this difference (4%) to shrinkage, a temporary reduction in root length that is either the result of short-term changes in soil moisture or actual temporary decline. Longer sampling intervals are less likely to detect this shrinkage.

Lengthening sampling interval by a fixed amount, a week for example, does not always have the same impact on estimates of demographic parameters. Extending sampling intervals from weekly to monthly (a fairly common sampling interval in minirhizotron studies, and a three-week increase in sampling interval) resulted in a 20% reduction in estimates of production and mortality. The degree of underestimation increased when intervals were extended from four weeks to any of the longer intervals, but never so severely as when intervals were changed from weekly to biweekly, or weekly to monthly. Indeed, the loss in resolution between eight- and twelve-week sampling (a 6% loss in resolution for a 33% reduction in sampling effort) may be regarded as acceptable by labor-conscious researchers; i.e. if you are limited to sampling every two months, you might as well sample every three.

The results from this study indicate that herbivory may be an important factor in belowground carbon cycling patterns. Our conservative estimate of herbivory suggested that 24% of root length lost was consumed by root predators. Production was greater than mortality during our study; as a result, predation only represented 20 percent of belowground net primary production (BNPP). This twenty percent value is within the range but toward the upper end of previous BNPP estimates (Anderson 1987). It is also relatively large compared to aboveground herbivory, which varies from 10% to 20% of aboveground NPP across a range of terrestrial ecosystems (Crawley 1997). We have two reasons to believe that our high estimates are accurate. First, most previous herbivory studies were focused on single root herbivore functional

groups or a small subsample of the herbivore population. Examples include the work of Ausmus et al. (1978), who estimated consumption by phytophagous nematodes (8.5% of BNPP) and periodic cicadas (1.4%), and the work of Magnusson and Sohlenius (1980), who estimated consumption by phytophagous nematodes at 0.3% of BNPP. Such focused studies (which comprise the majority of belowground herbivory research) do not examine the impact of the entire community of belowground herbivores; their estimates of herbivory must therefore be considered low (Hunter 2001). Second, studies of fine root dynamics have not had sufficient sampling frequency to differentiate between decay and herbivory. Rapid disappearance of fine roots has been previously reported in fine root research in forested systems. Fahey and Hughes (1994) observed disappearance of between 17 and 23% of roots intersecting screens over a two-month interval, while Hendrick and Pregitzer (1992) observed a substantial number of roots that disappeared between approximately monthly sampling intervals without first showing evidence of mortality. The authors suggested that herbivory played a minor role in this disappearance and that mortality and decay must be very rapid by default. However, sampling intervals in these studies were not sufficiently short to determine whether roots that disappeared actually passed through a rapid decay phase or disappeared when in a seemingly 'healthy' state.

The weekly sampling regimen in this study allowed us to more accurately track the process of fine root decay and disappearance. Although many roots went through a period of decline before disappearing, some roots that appeared healthy did not. Given current knowledge of fine root decay rates, we feel that it is unlikely that an apparently healthy, growing root would die and fully decay between weekly sampling intervals. While it is an assumption to attribute these losses to herbivory, the large number of fossorial herbivores in our study system (Andersen 1987) suggests that the impact of fine root herbivory in belowground carbon cycling may indeed be as significant as, or even more significant than, our conservative estimates. Our estimates of belowground carbon losses to herbivory may have been even greater if we could have incorporated estimates of non-structural carbon (such as that lost to root-sucking nematodes or insects) rather than our simple estimates of structural carbon losses.

Indeed, the significance of the belowground herbivory appears to have been largely overlooked (Hunter 2001), primarily due to difficulties quantifying biomass of and flows to belowground herbivores. Ultimately, until direct observations of fine root tissue removal over

time are possible, herbivory estimates will necessarily be based on such circumstantial evidence, and the question of attributing disappearance to decay versus herbivory may remain unresolved.

It is unclear what the shrinkage and regrowth phenomenon we observed truly represents. We presume that this pattern of loss-and-recovery is a result of short-term changes in soil and/or tissue moisture that impact root elongation. The diameter if individual fine roots of cotton decreased significantly when conditions aboveground changed from cloudy to sunny (Dr. Hugo Rogers, USDA Soil Dynamics Lab, personal communication), similar changes in root length might also result from short-term changes in soil moisture without necessarily causing root death. It is also possible that the shrinkage represents some alteration in the position of the root, due to a reorientation of the growing tip away from the tube surface. In either case, shrinkage losses would represent a measurement error rather than changes in carbon allocation, and 'smoothing' root length data to eliminate such fluctuations may be appropriate at some stage in tracing or analysis. However, if the shrinkage we observed is not a measurement error, it could represent decline followed by root growth. While this is difficult to reconcile with root growth anatomy, if root decline followed by growth does occur, measuring shrinkage is important. Roots of corn seedlings were able to recover (and produce new tissue) from moisture stress that induced cortical collapse (Stasovski and Peterson 1991), suggesting that roots can regrow from damaged tissue. Tissues that shrink and then recover between sampling intervals may actually represent a 'double dip,' in that both the decline and the subsequent recovery represent an otherwise ignored carbon sink.

The subtropical climate at our site may be an important factor in interpreting our estimates of mortality and herbivory. A great deal of previous minirhizotron research on forested systems has been conducted in the northern United States, where cold winters and factors such as the timing of leaf expansion impact many fine root processes (Hendrick and Pregitzer 1993, Fahey and Hughes 1994, Steele et al. 1997, Ruess et al. 1998). Comparisons between these studies and our data may be misleading. It is possible that the populations of fine root herbivores may be larger or have more impact without severe winters. This may result in a higher percentage of short-term mortality due to herbivory in our system than would be typical for colder climates. In addition, the higher mean temperatures on our site may accelerate the decomposition process to such a degree that 'standard' root decomposition rates are inaccurate

comparisons, and the higher amounts of rapid decomposition may be merely a function of climate.

Our production estimates were lower than those published for minirhizotron studies in other forests. This is likely an impact of the fairly open canopy of *Pinus palustris* woodlands, where 'dense' stands have basal areas in the range of 20-30 m^2/ha and most stands have much lower values. A second factor that could have led to low estimates was our choice to limit fine root observations to *P. palustris* roots only. The application of herbicide to the sampling stations was performed to focus attention on the dynamics of *P. palustris* overstory roots in the absence of roots of understory species. However, root ingrowth core data from our study site indicated that the application of herbicide on our plots resulted in significantly increased pine root production (ANOVA, $p < 0.01$, unpublished data), so the absence of understory roots was partially compensated. Finally, two severe droughts before and during the study period (one in the spring of 1998, the other in spring of 1999) may have further limited production.

Overall, our study demonstrates that substantial biases may be introduced into minirhizotron investigations based on sampling interval. Intervals longer than weekly decrease accuracy of growth, mortality, and lifespan estimates. Weekly intervals increase accuracy and permit estimates of herbivory. Further short-interval sampling investigations in other systems will be necessary to clarify the impact of sampling interval on previous estimates of fine root production and mortality and to determine if herbivory and/or rapid disappearance play a major role in all systems.

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Chapter 2: Influences of Soil Environment on Fine-scale Root Dynamics

ABSTRACT

We examined the impact of soil environmental variables (soil temperature, moisture, and available nitrate (NO_3^-) and ammonium (NH_4^+)) on the production, mortality, standing crop, turnover, and lifespan of *Pinus palustris* Miller fine roots using the minirhizotron technique. Data were collected for a full year from a *P. palustris* woodland in southwest Georgia. Mean soil temperatures appeared to have little influence on root processes, while temperature variance had a strong effect. More thermally variable microsites had increased root turnover and reduced root lifespans. Soil resources had a significant impact on demography; in particular, soil moisture and nitrate stimulated production, mortality, and turnover. High levels of soil resource availability also significantly reduced lifespan. Root lifespan was variable among individual roots based on root width, depth in the soil volume, and season of root production. Soil moisture had the strongest overall influence on root demography. This may result from the nature of our ecosystem (deep sands and subtropical climate); in addition, severe drought during our study may have enhanced the role of soil moisture, allowing environmental controls to increase in strength relative to within-plant controls on root demography.

Key words: Fine roots, root turnover, root lifespan, root demography, *P. palustris*, soil environment

INTRODUCTION

Trees allocate large percentages of their fixed carbon belowground to fine roots (McClaugherty et al. 1982, Caldwell 1987, Santantonio and Grace 1987, Gower et al. 1994). Environmental factors that impact root demography may have important influences on plant carbon balance. If such factors result in premature root mortality or increased root turnover, they will impact root efficiency (nutrient uptake per unit C allocated, per Eissenstat and Yanai 1997) and may influence root-shoot carbon allocation or plant-level nutrient balances. Clarifying the influence of the soil environment on fine root dynamics in forest ecosystems is also a key to understanding the impact of climate change on both local and global carbon cycles (Atkin et al. 2000, BassiriRad 2000, Eissenstat et al. 2000, Gill and Jackson 2000, Nadelhoffer 2000, Pregitzer et al. 2000).

Despite the wide range of studies that have examined root response under varying conditions, no consensus has developed on the direction of root response to soil environmental changes. Reasons for this shortcoming include variation among and within studies in species composition, experimental approaches, and response factors analyzed (Ostertag 2001). An additional problem that has been overlooked relates to soil heterogeneity. Rather than being homogeneous throughout a stand, soil resource availability and environmental conditions are heterogeneous, and forest trees adjust carbon allocation patterns in response to this often fine-scaled patchy environment. To improve stand-level models of root response, therefore, it may be useful to measure microsite root responses to the soil environment and thereby attribute otherwise 'random' variations in root response to fluctuations in soil resources. If these localized root responses are coupled with measures of soil heterogeneity, responses could be scaled up to the stand level.

Three particularly critical abiotic factors that influence root response in forests are soil temperature, soil moisture, and nutrient availability. Changes in these soil factors may influence one or more aspects of fine root demography, including fine root production, mortality, standing crop, turnover, and lifespan. Fine root production and mortality are often stimulated by increases in soil resources such as nitrogen and soil moisture (Pregitzer et al. 1993, Hendrick and Pregitzer 1997, Ostertag 2001), while extremes of temperature may reduce production and increase mortality due to increased respiratory costs or interactions with soil moisture (Eissenstat and Yanai 1997).

Direct root observation (through rhizotrons or minirhizotrons) allows fine root response to be tracked at the level of individual roots from a single population or community. Minirhizotrons have the added benefit of being easily spread throughout an ecosystem to capture a range of local resource availability relevant to individual organisms or communities. Minirhizotrons, when sampled with sufficient frequency, allow a direct examination of multiple aspects of root demography. Thus, it is possible to determine whether differences in root standing crop and turnover are reflected in changes in lifespan, birth rates or mortality. In addition, the ability to track individual roots allows us to clarify relationships between demography and root diameter, depth in the soil volume, season of birth, and other root attributes.

In this paper we examine relationships between fine root demographic traits and soil environmental variables in a *Pinus palustris* Miller (longleaf pine) woodland in southwestern Georgia. Natural variation in soil temperature and resource availability (nitrogen and water) was augmented by a spatially variable tree harvest. We examine how this variation impacted fine root production, mortality, standing crop, turnover, and lifespan. Our hypotheses were that: (1) extremes in temperature would increase mortality and reduce production, turnover, standing crop, and lifespan; (2) increased soil resources would increase fine root production, mortality, and turnover, and decrease standing crop and lifespan; and (3) root lifespan is variable within subpopulations of roots representing different diameters, depth classes, and seasons, with deeper, wider roots tending to live longer.

METHODS

Experimental Design

This experiment was conducted at the Joseph W. Jones Ecological Research Center in southwestern Georgia, USA (31° N, 84° W) in a 60- to 70-year-old *P. palustris* stand. The climate is subtropical, with mean daily temperatures ranging between 11 and 27 degrees Celsius, and annual precipitation of 132 cm. The understory is dominated by *Aristida stricta* Michaux. (wiregrass). Soils are Orangeburg series sands over karst limestone bedrock.

Twelve study plots (each approximately 2.5 ha) were established in October 1997 to examine the influence of several silvicultural techniques on plant resources and *P. palustris* regeneration. The diameter at 1.4 m height (dbh) and location (x-y coordinates) of each tree

within each of the twelve plots was measured prior to treatment installation. Plots were randomly assigned to one of four treatments: control (not harvested); single tree harvest that resulted in a uniform residual tree distribution; small gap harvest (35 m diameter gaps); and large gap harvest (70 m diameter gaps).

Because of logistical limitations, we conducted our study in only six of the twelve plots, representing two treatments: the control and the large gap harvest. Since we anticipated the use of regression analyses, we chose two treatments that had substantial overlap in local soil conditions, and yet provided some extreme values with dense or sparse overstories. Plot basal area was 14.8 - 18.4 m^2/ha in the control plots and 11.2 - 15.4 m^2/ha in the harvested plots after gaps were created.

Each plot covered a wide range of overstory conditions (from dense clusters to open areas). Sampling stations were located within each plot using a stratified random approach. Plots were overlaid with a 5 x 5 m grid; at each intersection, an overstory abundance index was measured that accounted for the basal area of all trees within 15 m. In each plot, the range of these overstory density measures was separated into 5 equivalent percentiles. One sampling station was randomly located at a gridpoint within each percentile, and a sixth station was placed in the lowest overstory percentile (the most open conditions) to assure adequate representation of the variety of conditions that can occur in canopy gaps. All sampling stations were a minimum of 10 m apart, with most being much farther apart than this minimum.

Sampling stations included 3 x 3 m measurement quadrats in which al understory vegetation was removed. Initial removal occurred when the understory was burned in November 1997. Thereafter, herbaceous vegetation in the sampling stations was controlled by monthly application of glyphosphate. The original intent of herbicide application was to test for influences of understory removal on soil resources and pine regeneration. In this study, herbicided stations afforded the opportunity to test for the responses of a single species (*P. palustris*) to soil resource levels.

One minirhizotron tube (5 cm inside diameter x 80 cm length) was installed in each sampling station in January 1998. Thirty-six tubes were installed at a 45-degree angle to the soil surface to approximately 40 cm vertical depth. The tubes were positioned so that the captured frames were oriented at approximately zero vertical degrees. Prior to installation, tubes were etched at 1 cm intervals; registration holes were drilled in the tops of the tubes to allow the

camera to return to the same spot in the tube between samplings. Tubes were anchored in place (to minimize rotation and environmental-related heaving) by attaching them with wire and hose clamps to rebar stakes that had been driven one meter into the ground.

Ten one-year-old *P. palustris* seedlings were planted in each station in March of 1998. Above- and belowground portions of all surviving seedlings were harvested in October of 1998. While it is possible that these seedlings produced roots that reached the surface of the minirhizotron tubes, we are fairly certain that their influence was minimal. Southwest Georgia experienced a severe drought in the spring of 1998. As a likely result, 85% of the seedlings in the herbicided stations died during the summer; those that survived grew very little (unpublished data).

Root Measurements

Minirhizotron sampling was conducted weekly from June 24, 1998 to July 7, 1999, for a total of 55 sampling dates. Images were collected in the field by Hi-8 camcorder (June 1998 – February 1999) or by direct digital capture (February – July 1999) using software and a minirhizotron camera system (Bartz ICAP, Bartz Technology Company, Santa Barbara, CA). Individual fine roots (< 2 mm) were counted if they were partially or wholly within the 1.4 x 1.8 cm sampling image. If an individual root formed a branch, the branch was counted as a new individual root. Roots were considered 'born' on the first date they were seen. Roots were considered dead on the first date that they either (1) disappeared entirely from the frame, or (2) appeared to be dead, due to change in color (to gray or black) or apparent decomposition. Reduction in root length did not result in classifying the root as dead.

A root tracing software package (ARCOS, Graphics Equations Inc, Houston TX) was used to measure the length and diameter of each fine root at each sampling date. The diameter of each fine root at its maximum length and its depth in the soil volume were also recorded.

Because understory vegetation was controlled in our sampling stations, most observations of root-like structures were *P. palustris* roots or fungal hyphae. We attempted to remove hyphae from our analysis using the following qualitative criteria: fungal hyphae were very thin, always white and/or translucent, and exhibited diffuse edges, while fine roots were typically greater in diameter, often changed color to red or brown, and exhibited distinct edges. Structures that exhibited distinctly fungal characteristics were excluded from the data set.

Five fine root responses were estimated using data obtained from ARCOS length measurements and birth and death dates: production, mortality, standing crop, turnover rates, and lifespan. All responses except for lifespan were summed for ANOVA and regression analyses; these were summed for each tube or by depth class within each tube depending on the specific analysis. Total fine root production was any increase in fine root length from week x to week x+1 summed over the entire study period. In any week, this measure included the production of new roots and/or the growth of existing roots. Total fine root mortality was defined as any decrease in length from week x to week x+1 summed over the entire study period. Weekly fine root standing crop was measured as all live fine root length present at a given sample date. Mean weekly fine root standing crop was determined using all 55 sample dates. Fine root turnover rates were determined by summing the total production and mortality over the sample period, and dividing that total by the mean fine root standing crop (Norby and Jackson 2000). Fine root lifespan in days was measured for each root that died during the sample period; those roots that did not die during the experiment were maintained in the data set, but were considered right-censored for lifespan analyses (see *lifespan analysis* below).

Environmental Measurements

In each sampling station, we installed copper-constantan soil temperature thermocouples at depths of 10, 20, 30, and 40 cm (one each per depth). Soil temperature was measured at weekly intervals using an Omega handheld thermocouple meter (Omega HH21, Omega Engineering, Inc, Stamford, CT). Data from the four depths were averaged to determine weekly mean temperature over the top 40 cm. Annual mean soil temperature (hereafter mean temperature) on a per-station basis was determined using these weekly measures. In addition to simple temperature means, we measured temperature variance over the 55-week measurement period at each of the stations. Our presumption here was that more thermally variable stations are more likely to have extremes in temperature that will influence root response.

Nitrogen mineralization was measured at each of the sampling stations using *in situ* buried bag incubations (Eno 1960). At monthly intervals, six 2.5 cm diameter soil cores were collected from the 0-10 cm horizon, combined in the field, sieved in the laboratory, and subsampled to determine initial ammonium and nitrate concentrations. Two 35 g subsamples were placed in a gas-permeable plastic bag and then reburied within 24 hours. These were

incubated *in situ* for approximately one month, then removed from the soil, composited by sampling station, and subsampled to determine ammonium and nitrate concentrations. Samples were extracted using 2N KCl solution, and concentrations of nitrate and ammonium were measured using a Lachat QuikChem 8000 ion analyzer (Lachat Instruments, Milwaukee, WI). The net mineralization for each sampling interval was calculated on a per-station basis by subtracting the initial available ammonium or nitrate from the final availability. For each station, total soil ammonium and nitrate mineralization from March to November 1998 were calculated by summing values for each period; these total values were used in all analyses.

Paired stainless steel rods were installed to 40 cm for time domain reflectometry measures of soil moisture (Topp et al. 1980). Moisture data were collected weekly using a cable tester (Tektronix-2A, Tektronix Inc, Beaverton, OR). In-lab calibrations were used to convert cable test readings to soil moisture. Time domain reflectometry results in an estimate of soil moisture integrated over the length of the stainless steel rods (rather than the point estimate returned from the temperature thermocouples). Measurements were taken weekly at each sampling station over the period concurrent with minirhizotron sampling (June 1998 to July 1999). Annual mean soil moisture on a per-station basis was determined using these weekly 40 cm depth moisture measures.

Statistical Analyses

The scale of this experiment should be considered the patch or micro-site rather than stand or plot-level because we are comparing dynamics between fairly small volumes of soil. All statistical analyses were conducted using Statistical Analysis Software (Version 8.01, SAS Institute, Cary, NC). Data for environmental and response variables were natural-log transformed where such transformations resulted in sample distribution no longer statistically different from a normal distribution, as measured by Kolmogrov-Smirnov tests of normality (SAS Proc UNIVARIATE). Fine root production, mortality, standing crop, and turnover were measured for each tube. Because tubes were spaced a minimum of 10 m apart (and were typically much farther), each tube was considered an independent observation unit. We observed no production or mortality in two of the 36 tubes, and removed them from consideration in the experiment.

An analysis of covariance was conducted to determine whether there were any significant effects of harvest treatment on fine root response to the soil environment. This involved sixteen models (four root responses, four environmental variables per response) in which we examined the impact of the environmental variable and harvest treatment on root response. We observed significant harvest treatment effects only in the case of soil moisture. Upon closer inspection, we determined that two sampling stations located in control treatment plots were extreme outliers in soil moisture vs. fine root response graphs. These stations were located approximately 20 m apart in an area with a significant clay layer beginning approximately 10 cm below the surface and extending beyond the depth of the minirhizotron tubes (> 40 cm vertical depth). As a result, these sampling stations were submerged during periods of heavy rainfall, and often remained submerged for several days (*pers. obs.*). Because these sampling stations had a soil environment that was markedly different from the rest of the stations, they were removed from the analysis. After their removal, there was no significant impact of harvest treatment on fine root response.

We used Pearson's correlation coefficients (SAS, Proc CORR) to determine individual pairwise relationships among soil environmental variables and among all fine root response parameters *except* fine root lifespan. We used simple linear regressions (SAS, Proc REG) to determine relationships between fine root response parameters and soil environmental variables. We used multiple linear regression (SAS, Proc REG) to develop explanatory models of the influence of soil environmental variables on these same fine root responses. First, we selected the model with maximum adjusted R-square. The adjusted R-square statistic minimizes residual (or error) mean square and guards against model overspecification (Montgomery and Peck 1992). Next, we used a stepwise selection procedure, in which variables are entered into the model if they meet a significance level for entry of alpha = 0.10 or less. Variables may then be retained in the model or dropped as their significance level drops below 0.10 due to the addition of other variables. Stepwise models include only variables that are significant at this 0.10 level, rather than including statistically insignificant factors that may play a detectable role in reducing the mean square error or improving model fit. Eigenvalues and variance inflation factors in all final models were inspected for possible multicollineraity concerns.

We used analysis of variance (ANOVA; SAS proc GLM) to test for depth-related trends in fine root production, mortality, standing crop, and turnover. Our 0-40 cm sampling range was

divided into four equal 10 cm intervals; analysis was then conducted to determine whether any of the four response variables varied significantly with depth. Due to zero values in depth classes for some tubes, non-transformed data were used for this analysis. The influence of depth on root lifespan was examined in a separate analysis.

Fine root lifespan data were analyzed using a proportional hazards model (Cox's proportional hazards model, SAS Proc PHREG). Proportional hazards models are appropriate in this study because not all roots died by the end of sampling (Fox 1993). They are preferable to accelerated failure-time models (the alternate form of analysis for censored data) because periods of high hazard (here, periods of stressful environmental variables) are not created by the roots themselves; rather, roots respond to environmental variables to varying degrees (Fox 1993). Individual variables examined in this model included soil temperature, net nitrate mineralization, net ammonium mineralization, soil moisture, depth in the soil volume, and root diameter. Season effects on fine root lifespan were examined by comparing two models. In one model, roots were stratified based upon the season they were produced; this model allowed survivorship curves to vary between strata. The results from this model were compared to those from a non-stratified model in which all roots were considered to have the same survivorship curve. The difference between log likelihood statistics (-2 log likelihood) for the stratified and non-stratified models was compared to a chi-square distribution to determine whether allowing hazard functions to vary between seasons led to a significantly improved model of the soil environment's impact on fine root lifespan (i.e., whether season of production had a significant effect on root lifespan). Individual pairwise regressions and model selection using stepwise model selection procedures were conducted in a fashion similar to that described above for multiple linear regression.

RESULTS

Correlations between variables

None of our soil resource variables (nitrate, ammonium, and moisture) were significantly correlated with each other at the 0.10 level (Figure 2.1). Our two temperature related variables (mean temperature and temperature variance) were significantly and positively correlated ($p < 0.01$) but the correlation coefficient was only 0.56, suggesting that they provide different

information. Both of the temperature variables had significant positive correlations with nitrate mineralization.

Significant positive correlations were observed between fine root production, mortality, and standing crop (all $p < 0.05$, Figure 2.2). Fine root turnover was significantly and positively correlated with production and mortality ($p < 0.05$) and significantly and inversely correlated with standing crop ($p < 0.05$, Figure 2.2); however, such correlations are to be expected given that these values were all included in the formula we used to calculate turnover.

Regression models

Production. Simple linear regressions indicated a significant positive relationship between fine root production and soil moisture ($p < 0.01$) and a weaker positive relationship between production and available nitrate ($p = 0.06$) (Table 2.1). Model selection procedures based on maximizing the adjusted R^2 values suggested that a three-variable model containing soil moisture, nitrate mineralization, and ammonium mineralization was the 'best' model. Model selection based on the stepwise procedure returned a model containing only soil moisture (Table 2.2).

Mortality. Simple linear regressions indicated that soil moisture ($p < 0.01$) and available nitrate ($p = 0.07$) were positively related to fine root mortality (Table 2.1). Multiple regression that maximized adjusted R^2 values returned a four-variable model containing soil moisture, nitrate mineralization, ammonium mineralization, and mean temperature. The stepwise procedure identified a two-variable model containing soil moisture and nitrate mineralization (Table 2.2).

Standing Crop. Simple linear regressions indicated that ammonium mineralization had the strongest relationship with standing crop (Table 2.1). The two variables were positively correlated, but not significantly so ($p = 0.09$). The adjusted R^2 procedure suggested that the 'best' multiple regression model contained soil moisture, ammonium, and temperature variance. The stepwise procedure, however, included only ammonium (Table 2.2).

Turnover. Simple linear regressions indicated that nitrate ($p < 0.001$), temperature variance ($p < 0.01$) and soil moisture ($p = 0.07$) were positively related to fine root turnover (Table 2.1). The best adjusted R^2 model contained all environmental variables. The stepwise model, however, contained only nitrate mineralization (Table 2.2).

Impact of Depth

There was a significant impact of depth on fine root production and mortality (ANOVA, $p < 0.05$ for both, Figure 2.3) while standing crop and turnover showed no significant depth-based variability. Mortality was greatest in the 0-10 cm class. This surface class also had the largest mean production, although production in the 0-10 cm class was only significantly greater than that in the 21-30 cm class.

The lack of depth response in turnover despite apparently greater growth and mortality in the surface depth class was puzzling. Closer inspection revealed 'pulses' of production and rapid mortality in some tubes; roots produced in these pulses were sometimes very short lived (1-4 weeks). In lower depth classes, where mean standing crop was sometimes very small, these pulses sometimes resulted in turnover estimates of 100+, where more typical turnover values were near 10. This resulted in the inflation of turnover estimates in lower classes. The impact of such pulses was diluted in the 0-10 cm class and in whole-tube estimates by greater standing crop values.

Lifespan Analysis

Fine root lifespan varied significantly depending on the season of root production. Allowing survivorship curves to vary by season produced an improved model of fine root lifespan (log-likelihood estimate comparison, Chi-Square test, $p < 0.001$). To provide the best possible test of other factors, models were stratified by season. Simple pairwise proportional hazards model regressions indicated that lifespan was negatively associated with temperature variance, nitrate mineralization, and soil moisture, and positively associated with soil depth and root width (Table 2.3). Stepwise selection returned a three-variable model that included depth, width, and temperature variance ($p < 0.0001$).

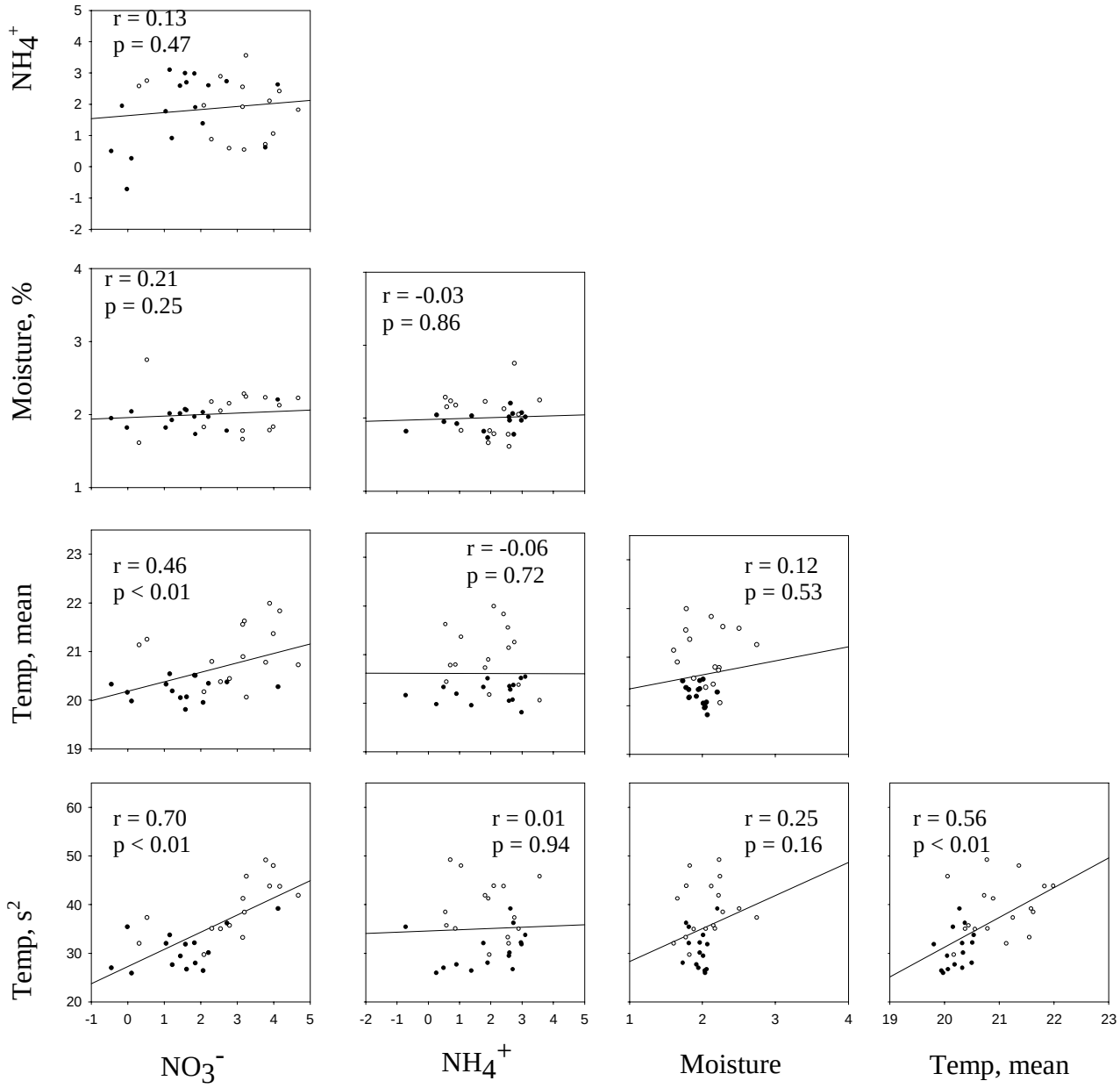

Figure 2.1 Correlations between soil environmental variables. Closed circles represent stations in control plots; open circles represent stations in harvested plots. N=32, 17 stations in harvested treatment, 15 in control. Ammonium and Nitrate variables are in kg/ha, natural log transformed. Soil moisture units are percentage soil moisture, natural log transformed. Mean temperature and temperature variance units are Celsius degrees, non-transformed.

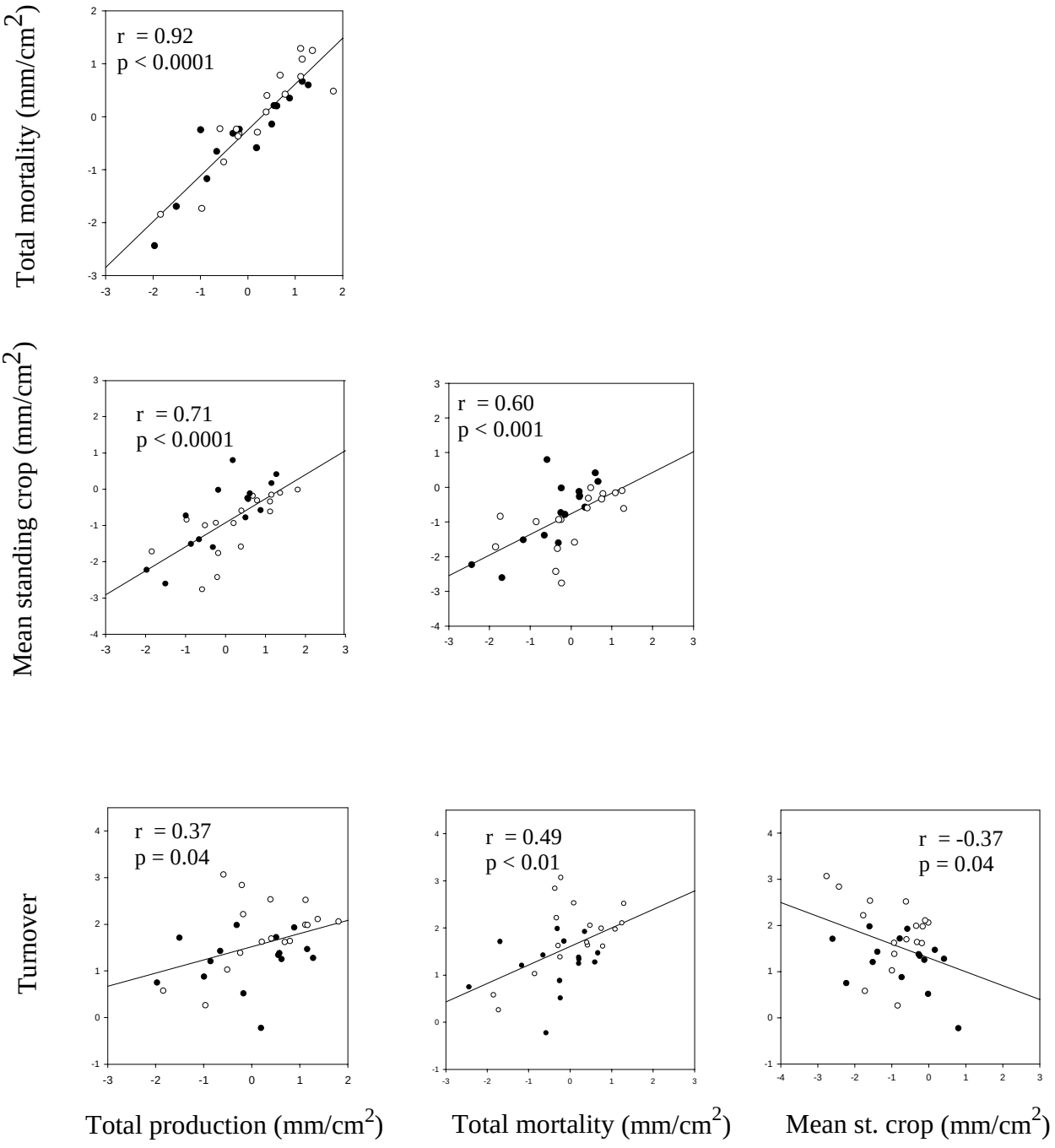

Figure 2.2 Correlations between fine root response variables. Closed circles represent stations in control plots; open circles represent stations in harvested plots. N=32, 17 stations in harvest treatment, 15 in control. All variables were log transformed (natural log) prior to analysis. No significant effect of harvest treatment on root response was observed.

Table 2.1 Pairwise linear regressions between root response parameters and environmental variables. Response variables natural log transformed prior to analysis; environmental variables transformed where noted.

Root response	Variable	F score	p > F	R^2
Production	Soil moisture (log)	8.40	< 0.01	0.22
	NO3- (log)	3.77	0.05	0.11
	NH4+ (log)	1.92	0.17	0.06
	Temperature variance	1.27	0.27	0.04
	Mean temperature	0.09	0.77	0.00
Mortality	Soil moisture (log)	9.54	< 0.01	0.24
	NO3- (log)	5.43	0.03	0.15
	Temperature variance	2.37	0.13	0.07
	NH4+ (log)	1.48	0.23	0.05
	Mean temperature	0.02	0.88	0.00
Standing Crop	NH4+ (log)	3.01	0.09	0.09
	Soil moisture (log)	1.82	0.18	0.06
	Temperature variance	1.06	0.31	0.03
	NO3- (log)	0.45	0.51	0.02
	Mean temperature	0.08	0.78	0.00
Turnover	NO3- (log)	17.40	< 0.001	0.37
	Temperature variance	11.08	< 0.01	0.27
	Soil moisture (log)	3.62	0.07	0.11
	Mean temperature	0.43	0.52	0.01
	NH4+ (log)	0.24	0.63	0.01

Table 2.2. Summary of multiple regression models relating fine root response to soil environmental variables (soil moisture, nitrate mineralization, ammonium mineralization, mean temperature, and temperature variance).

Response	Model	Variables in Model	MSE	Adj. R^2	p > F
Production	adjusted R^2	Soil H_2O, NO_3^-, NH_4^+	0.68	0.26	0.01
	Stepwise	Soil H_2O	0.74	0.19	0.01
Mortality	adjusted R^2	Soil H_2O, NO_3^-, NH_4^+, mean temp	0.57	0.30	0.01
	Stepwise	Soil H_2O, NO_3^-	0.58	0.28	< 0.01
Standing Crop	adjusted R^2	Soil H_2O, NH_4^+, temp. variance	0.69	0.14	.09
	Stepwise	NH_4^+	0.75	0.06	.09
Turnover	adjusted R^2	All	0.31	0.42	< 0.01
	Stepwise	NO_3^-	0.34	0.35	< 0.01

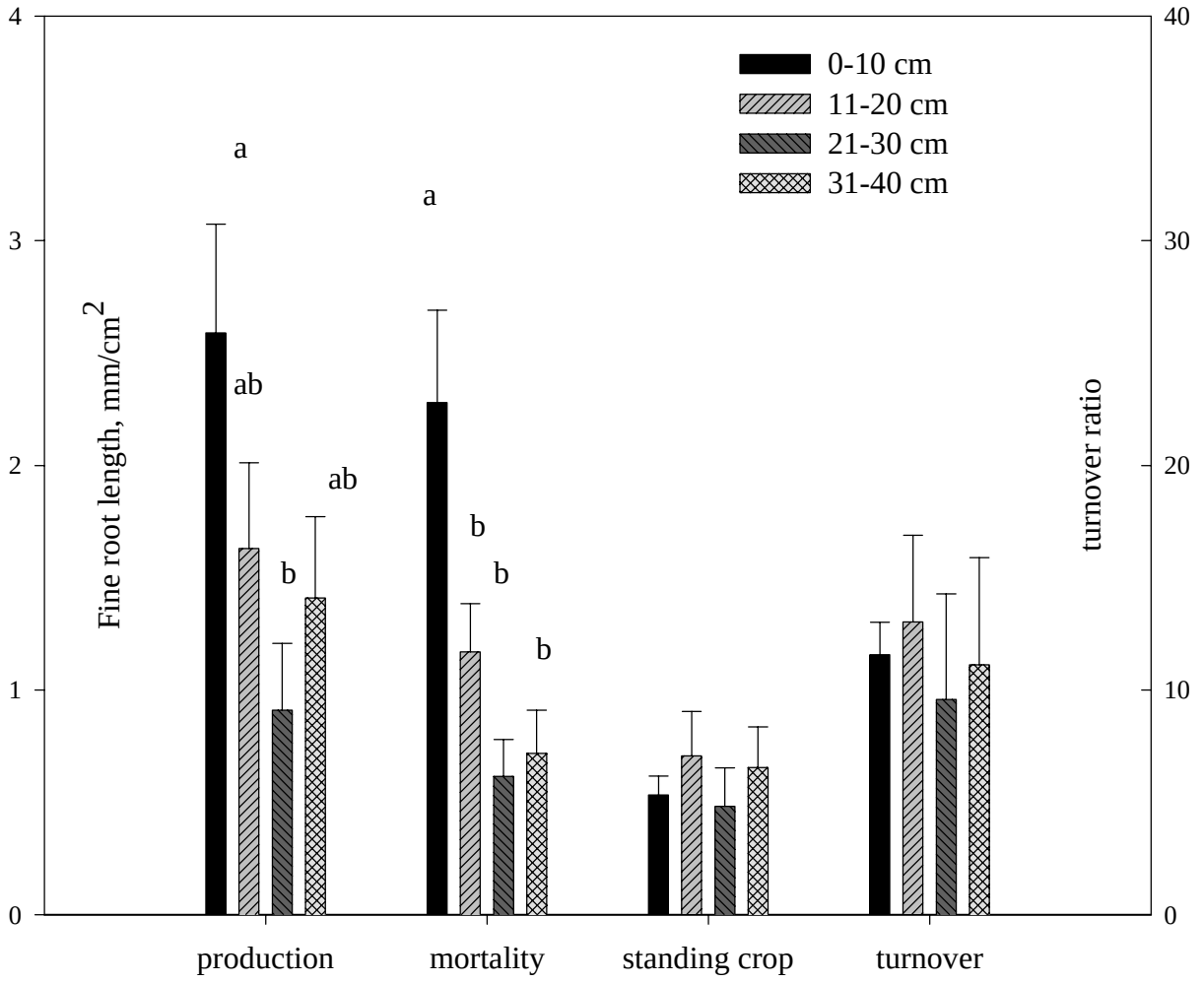

Figure 2.3 Mean (+1 SE) root response by depth class. Each bar represents the mean of 32 tubes. Within response types, different letters indicate significant depth differences (ANOVA, $p < 0.05$) followed by a Tukey multiple range test. Production and mortality means represent total length in mm/cm^2 over the 12-mo study; standing crop means represent mean weekly standing crop in mm/cm^2. Mean turnover calculated by averaging per-tube turnover ((production +mortality)/mean standing crop).

Table 2.3. Summary of pairwise proportional hazards model regressions between fine root lifespan and listed variables (natural log transformed where noted). N = 654.

Variable	Chi Sq	p > Chi Sq	Impact on Lifespan
Depth	50.87	< 0.0001	Positive
Width	6.06	0.01	Positive
Temperature variance	42.76	< 0.0001	Negative
NO_3^- (log)	27.73	< 0.0001	Negative
Soil moisture (log)	4.10	0.04	Negative
NH_4^+ (log)	2.64	0.10	n/s
Mean temperature	0.39	0.52	n/s

DISCUSSION

We originally hypothesized that temperature fluctuations would increase mortality and decrease production, standing crop, and lifespan; that increased soil resources would increase fine root production, mortality, and turnover, and decrease standing crop and lifespan; and that root lifespan would be influenced by root diameter, depth in the soil, and season of production. Fine root response to resources and temperature often differed from our original hypotheses. For the sake of clarity, the influence of each variable is discussed separately below, followed by a synthesis. In all cases, there was no difference in fine root response between harvest treatments (see Figs. 2.1 and 2.2); this likely resulted from the distribution of sampling stations in each plot over the full range of overstory conditions (dense to open conditions).

The role of temperature

Our data generally support the hypothesis that extremes in temperature will reduce production and enhance mortality. Temperature variability did not directly impact mortality and production (Tables 2.1 and 2.2); however, our finding that increased temperature variability reduced lifespans and increased turnover provides evidence that our hypothesis is correct.

Temperature variability was a more important variable than mean temperature. Indeed, temperature variance was the only environmental variable to make it into our stepwise selection model of root lifespan (although nitrate and soil moisture were significant in pairwise analyses), suggesting that temperature variance captured much of the information provided by these other environmental measures.

Microsites with wider annual temperature variation were likely more exposed sites, perhaps undergoing more severe daily fluctuations or more severe fluctuations in moisture. If this assumption is true, these more variable microsites would have less time when the soil environment is suitable to proliferation, and therefore reduced production. Any roots produced during favorable periods would be more likely to be exposed to hazardous conditions, and would have reduced lifespan as a result.

The fact that mean temperature had no significant effect was not particularly surprising. A similar response was found by Hendrick and Pregitzer (1997), who observed little influence of temperature differences between microsites on root dynamics. Furthermore, the minimum temperature for root growth (0 to 5 degrees Celsius, Kuhns et al. 1985) is well outside the normal

range for our system. Mean temperature effects on roots might be more likely observed in comparisons of ecosystems at different latitudes or elevations (*sensu* Hendrick and Pregitzer 1992, 1993, Steele 1997) that show differences in growing degree days rather than at our within-stand comparison. In addition, Pregitzer et al. (2000) state that the positive relationship between temperature and root growth generally only operates under circumstances where other factors (such as soil moisture or nutrient availability) are not limiting. Such an assumption would not be accurate for our system particularly given the drought during the spring of 1999 and strong root responses to soil moisture levels (see *the role of soil moisture* below).

Forest root dynamics can show strong seasonal responses that are likely based on mean temperature (Deans 1979; Hendrick and Pregitzer 1992, 1996; Fahey and Hughes 1994). However, our calculations of mean temperature integrated all measurements for the entire year, and this potential seasonal impact might have been obscured. To check for the possibility of seasonal influences, we entered mean summer and mean winter variables into our regressions. We found that summer mean temperatures were positively related to turnover ($p = 0.05$), and that winter mean temperatures were negatively related to mortality ($p = 0.10$) and turnover ($p = 0.01$). These values correspond with the fact that we observed a significant influence of birth season on root lifespan. The seasonal relationships we observed may be related directly to temperature, they may reflect the influence of seasonal root predators or other seasonally variable factors such as soil moisture (see *intra-population variability* discussion below), or they may reflect seasonal fluctuations in carbon allocation determined by photoperiod.

The role of ammonium

The overall response to ammonium availability was limited and weak; significant effects were detected only on fine root standing crop (at the 0.10 level) and lifespan (at the 0.05 level). As hypothesized, standing crop increased (although only slightly) on higher ammonium microsites, and roots on these sites had reduced lifespan.

The lack of response to ammonium is surprising, particularly as researchers have observed significant responses to ammonium additions. Trends in response to ammonium, however, have not been uniform between systems. For example, Alexander and Fairley (1985) observed increased longevity, reduced production, and reduced mortality in response to ammonium in Sitka spruce stands, while Cuevas and Medina (1988) observed increased root

growth in ammonium fertilized soil cores in forests in Venezuela. Such conflicting results suggest that response to ammonium may be highly variable between ecosystems.

The role of nitrate

In accord with our predictions, there were significant increases in production, turnover, and mortality on high-nitrate microsites. However, the stimulation of production was balanced by the increase in mortality and turnover, and thus, standing crop was seemingly unaffected.

Contrary to our results, increased standing crop of fine roots in nitrogen-rich microsites has been reported often (Drew and Saker 1975, Jackson and Caldwell 1989, Pregitzer et al 1993, Robinson 1994, Mou et al. 1995, Einsmann et al. 1999). What has not always been clear, however, is whether this increase is due to increased lifespan or production, or decreased mortality. Evidence for increases in mortality and decreased lifespan have been uncovered (Gross et al. 1993, Pregitzer et al. 1995); however, so have some conflicting trends (Pregitzer et al. 1993, Fahey and Hughes 1994). We observed both increased mortality and reduced lifespan on our more fertile microsites; the relationship with lifespan was likely clearer because our fine root lifespan regressions were based on individual roots, which gives a much larger sample size (N = 654) compared to our analyses of mortality, which relied on tube totals (N = 32). The reduced lifespan on nitrate-rich microsites agrees with Campbell and Grime (1989), who suggested plants adapted to less-fertile soils with short-lived nutrient pulses would have long-lived roots, while fertile soils would result in more short-lived roots. Our results suggest an increase in fine root production and mortality on nitrate-rich sites resulting in a larger population of younger roots. Because young roots have increased potential for nutrient uptake (Gross et al. 1993, Eissenstat and Yanai 1997), this could represent an adaptive mechanism of *P. palustris* to maximize uptake in favorable sites.

The role of soil moisture

All of our results regarding soil moisture corresponded well with our hypotheses. Indeed, soil moisture appears to be the dominant soil environmental variable driving fine root dynamics in our system. Wetter microsites showed clear increases in fine root production and mortality relative to drier sites; this observation is consistent with literature results (Gower et al. 1992, DeVisser et al. 1994). The influence of moisture on lifespan was less clear but still

significant ($p = 0.05$); roots on the wetter microsites did not live as long as on drier sites. We did not observe a change in standing crop on wetter sites. Joslin et al. (2000) observed no change in standing crop between wet and dry microsites despite apparent moisture effects on production; in their study, wet sites had a fairly consistent standing crop, and dry microsites had a high degree of variance (low during dry periods, very high during wet periods).

Increased root mortality on wetter sites does not correspond well with some literature results: Hendrick and Pregitzer (1997) observed that high soil moisture was negatively correlated with mortality, and a 40-day increase in soil moisture availability decreased root mortality in a northern hardwood forest (Pregitzer et al. 1993).

Southwest Georgia experienced several periods of low rainfall during our study that may have played a major role in regulating root response to soil moisture. In particular, little or no rain fell on our sampling sites for seven weeks from March to May of 1999, producing corresponding decreases in soil moisture (Figure 2.4). A similarly severe drought occurred in the spring of 1998 (data not shown); in addition, rainfall levels were very low during the fall of 1998. Such severe conditions may have acted to increase the relative strength of environmental controls relative to internal regulation of root dynamics by the plants themselves, and may have magnified the differences between wetter and drier sites, making moisture effects easier to discern.

Our regressions suggest that micro sites with higher average soil moisture levels represented important resource patches. More fine roots were produced in these patches, and these roots died faster than in drier sites. This may reflect developmental plasticity in root production. Younger, finer, and more absorptive roots are more efficient at water uptake than older and thicker roots (Yanai et al. 1995, Eissenstat and Yanai 1997); overstory *P. palustris* trees may preferentially slough older roots on moist microsites, replacing them with younger roots to maintain a standing crop in this efficient state. More tolerant, slower growing roots may be produced on drier microsites. Desert succulents undergo rapid root production after rainfall; these roots are shed quickly when soil moisture levels drop (Huang and Nobel 1992). Rootstocks of citrus reduce maintenance respiration costs during drought, making them more 'economical' to maintain (Eissenstat and Yanai 1997).

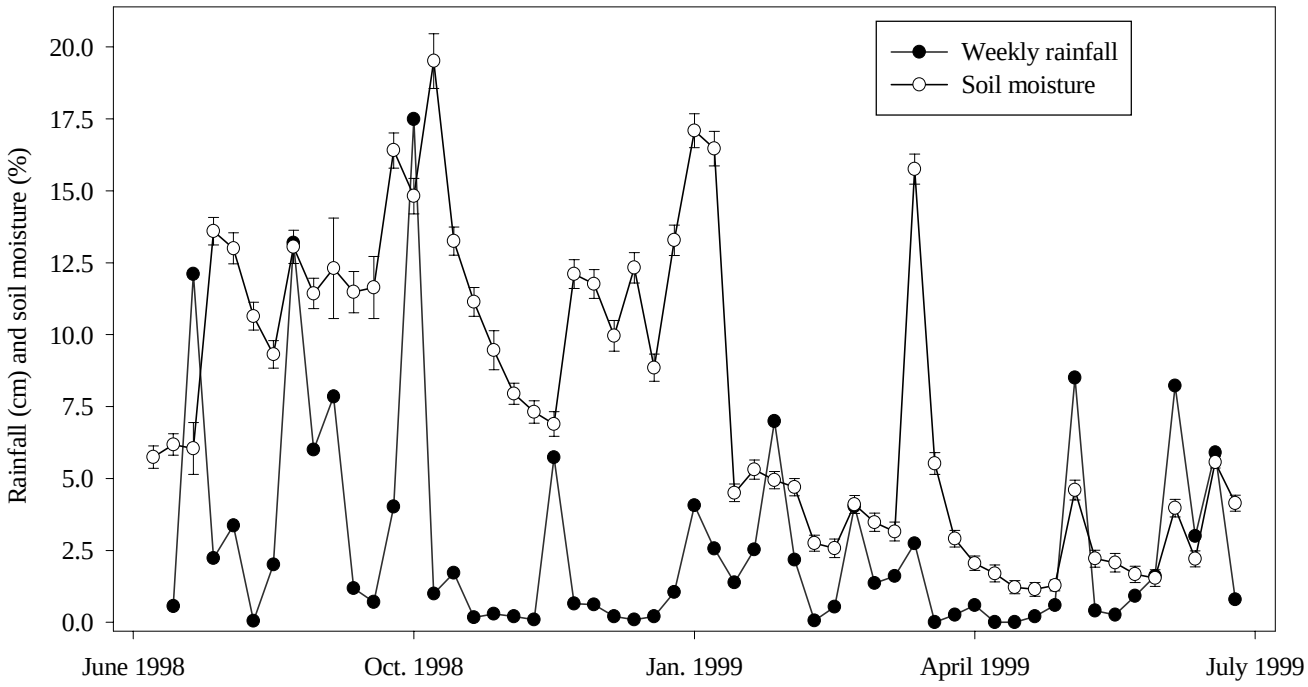

Figure 2.4 Weekly rainfall and soil moisture measurements during the study period. Soil moisture measurements represent mean soil moisture +/- 1 se (32 stations) in the upper 40cm of soil.

The role of intra-population variability (diameter, depth, and season)

Our hypotheses regarding root diameter, depth, and season were all supported by the data we collected. These factors appear to play a large role in fine root demography.

Wider roots lived longer than narrow roots, even within the range of roots typically considered 'fine' (< 2 mm diameter). The increased longevity of wider roots is well supported in the literature (Yanai et al. 1995, Eissenstat and Yanai 1997, Eissenstat et al. 2000, Wells and Eissenstat 2001), but may reflect increased lifespan of wider 'stem' roots relative to 'branch' roots rather than (or in addition to) a simple difference based on diameter. We did not attempt to make any distinction based on root order (i.e. primary, secondary, tertiary branching). Although it is fairly simple to assign branching order to those roots that branch after they are seen at the surface of the tube, it is impossible to determine the order of a root when it first appears. This complicates interpretations of lifespan results to the extent that primary root systems have a reduced risk of mortality after producing branches; however, this question is beyond the scope of the current experiment. Demography studies of more enclosed root systems (i.e. in narrow observation boxes where nearly all roots can be seen) would provide more accurate information on the role of branching order in determining fine root lifespan.

Roots produced deeper in the soil volume lived significantly longer than shallow roots. However, both production and mortality appeared strongly concentrated in the upper 10 cm of the soil (Figure 2.3). Similar depth dependent trends in production and mortality have been previously observed (Joslin and Henderson 1987, Hendrick and Pregitzer 1996, Burch et al. 1997). The apparent tradeoff between increased lifespan versus decreased production and mortality with depth may reflect depth-related trends in soil conditions and resources. The combination of a fairly open canopy, low surface litter accumulation, and long, hot summers often result in very harsh conditions at the soil surface in *P. palustris* woodlands; this is reflected in the role of temperature variance (presumably higher on exposed sites) on lifespan and turnover. Roots at the surface experience more severe changes in the soil environment and die more rapidly as a result; however, during favorable conditions these surface areas likely have a high nutrient availability, stimulating production.

The phenomenon of changing root longevity based on season has been reported frequently for forests (Keyes and Grier 1981; McClaugherty et al. 1982; Hendrick and Pregitzer 1992, 1993; Fahey and Hughes 1994). However, seasonal patterns are not consistent between

ecosystems. Hendrick and Pregitzer (1996) observed that fine root production peaked in early spring, while fine root mortality remained constant throughout the year. Fine roots in northern hardwood forests had a seasonal distribution in lifespan; fine roots produced in fall and in spring lived longer than roots produced in the summer (Hendrick and Pregitzer 1992, 1993). This pattern was different for subtropical citrus roots (Kosola et al. 1995): those produced in the fall had shorter life spans than spring and summer root cohorts, likely due to increases in *Phytophthora nicotianae* in the fall (Eissenstat and Yanai 1997). In examinations of rootstocks of Carrizo orange, root cohorts produced in July had a median lifespan of 348 days, compared to the much shorter lifespan of May cohorts (141 days) (Eissenstat and Yanai 1997).

Fine root lifespan is highly variable, but can be influenced either directly by seasonal changes in temperature or moisture, or indirectly by increases in parasitic or predatory infestations brought about by the temperature or moisture changes. Given the strong influence of soil moisture on demography and the apparent seasonal patterns in both rainfall and soil moisture (Figure 2.4), we would suggest that the seasonal trends in lifespan we observed were caused by fluctuations in soil moisture. This is particularly likely given the long droughts that occurred in the spring of 1998 and 1999, with roots produced under more favorable conditions (i.e., moister periods in winter and fall) likely having much different life history and survivorship patterns. These patterns may reflect whole-plant responses to soil moisture, with reductions in both new root growth and maintenance of existing roots under drought conditions. Additionally, carbon allocation to root tissues likely varies significantly between seasons, following canopy photosynthesis patterns.

CONCLUSIONS

Overall, we have shown that the soil environment does have important influences on root demography (Table 2.4); such influences are observed both at the scale of the individual root (lifespan) and at the microsite scale (production, mortality, standing crop, and turnover).

Variation in temperature functioned as a stressor, reducing root lifespan and increasing turnover at thermally variable microsites. Little response to mean temperature was observed. Increased resource availability had the same effects as thermal variability; i.e., a reduction in lifespan and an increase in root turnover. However, unlike the response to thermal variability,

Table 2.4. Summary of study results. Proportional hazards regression results presented for all lifespan analyses. Remaining results determined using ANOVA (soil depth) and pairwise regression (all other variables). The impact of root width could only be determined for fine root lifespan. Relationship denoted by sign (+ = positive, - = negative, 0 = not significant); significance level denoted by number of characters (1 = 0.10, 2 =0.05, 3 = 0.01, 4 = 0.001 or less).

	Growth	Mortality	Standing Crop	Turnover	Lifespan
NO_3^-	+	++	0	+++	----
NH_4^+	0	0	+	0	--
Soil moisture	+++	+++	0	+	--
Mean temp.	0	0	0	0	0
Temp. variance	0	0	0	+++	----
Root width	n/a	n/a	n/a	n/a	++
Soil depth	++	++	0	0	++++

fine root production responded positively to resources. Fine root production was significantly greater on microsites with increased soil moisture or soil nitrate levels (Table 2.4), suggesting that the reduced lifespan and increased mortality observed on these sites represents more than a simple stress response. Lifespan decreases on these resource-rich sites might help to maximize resource uptake (by producing a population of younger roots, per Eissenstat and Yanai 1997). On the other hand, if decreases in lifespan result from increased predation of nutrient-rich tissues, then these resource patches represent hazardous microsites. Interestingly, substantial, rapid disappearance of actively growing root tissue was observed in a related aspect of this study (Chapter 1), which implies that the influence of root predation in this system may be high. Fine root responses likely reflect both efficiency concerns and predation influences.

We saw stronger growth and mortality responses to soil moisture than to nitrate (Table 2.4), a relationship that contrasts with previous research on these variables (Pregitzer et al. 1993); however, it may be that soil moisture is the more limiting factor in these stands. Severe drought during the study most certainly contributed to the importance of moisture-rich microsites; fine root mortality spiked during spring of 1999 concurrent with the drought and dropped after rainfall recovered; growth levels also decreased during drought (Figure 2.5). Roots responded much more strongly to availability of nitrate than to that of ammonium. The weak response we observed to ammonium is unexpected, particularly since ammonium was nearly as variable as nitrate on our microsites. Aber et al. (1985) suggested that this may be because nitrate is more mobile than ammonium. Interestingly, however, they suggested that forests will adjust fine root standing crop to nitrate availability, a process we did not observe. Indeed, standing crop appeared to be most strongly influenced by ammonium rather than nitrate (Table 2.4). It seems that in this system the less-mobile form of nitrogen (ammonium) plays a role in regulating microsite standing crop levels, while the more mobile ion (nitrate) influences root proliferation, turnover, and lifespan. Another mechanism for the differential response to N form may be strong microbial competition for ammonium uptake relative to nitrate (Nadelhoffer et al. 1999, Currie et al. 1999). In our study system, resource differentiation may be occurring between microbial and plant communities in form of N uptake.

This study helps to clarify root demographic response to the soil environment at the local scale. Past studies have been complicated by a number of factors, including variation in species composition between microsites, limited examination of the range of demographic parameters, or

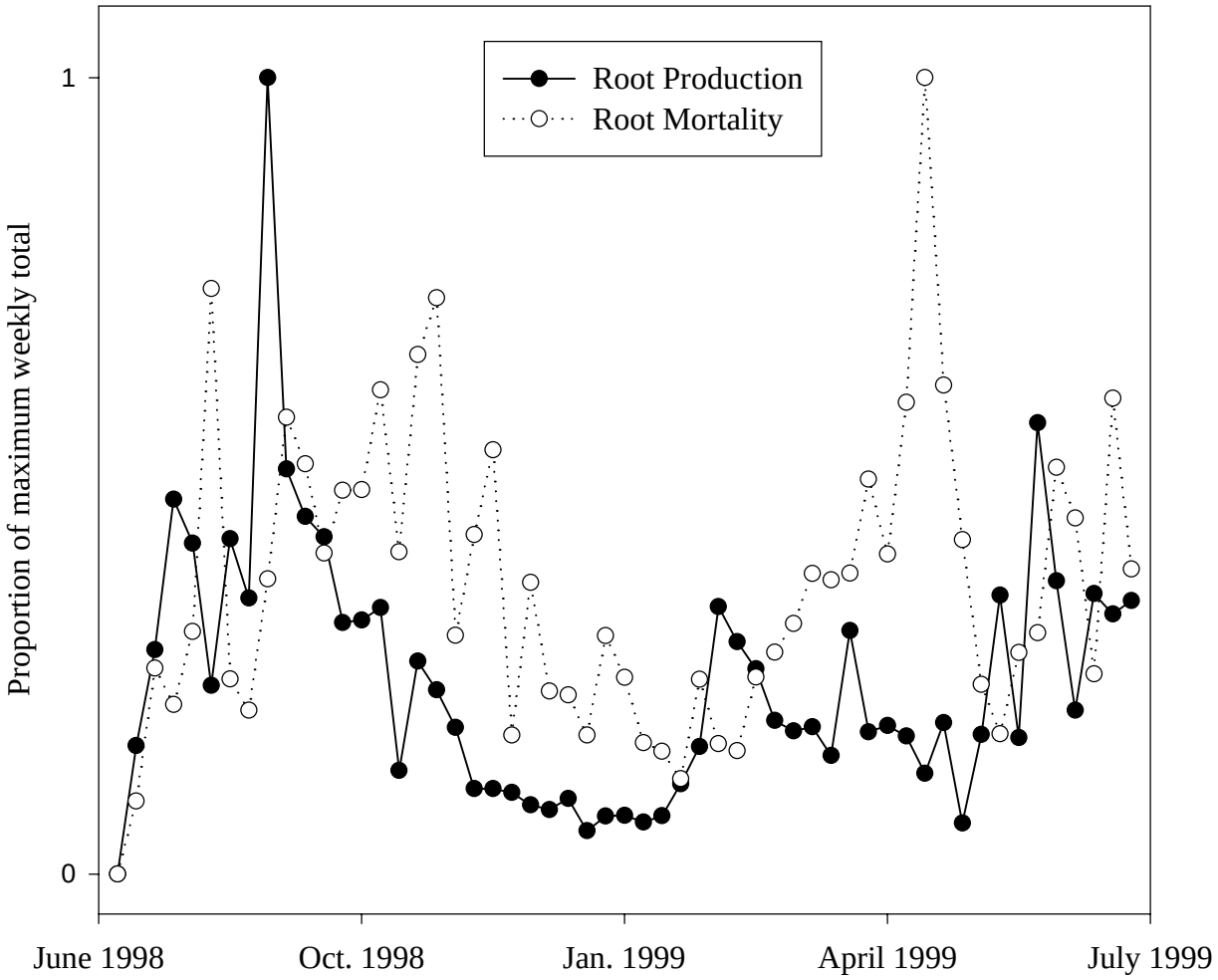

Figure 2.5. Annual trends in fine root growth and mortality from June 1998 to July 1999. Stand-level weekly production measures are shown as a percentage of the maximum weekly total.

differences in methodology (Ostertag 2001). In this study, we limited species composition to a single species and measured the full range of related demography parameters. The extent to which we perceived 'typical' resource levels in such conditions can be questioned, given the drought during the study. Perhaps our picture of resource impacts was strongly influenced by severe conditions. If so, such information may prove particularly important in predicting responses to changes in rainfall or temperature patterns related to climate change.

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LINKS TO RAW DATA

The following links are to separate files containing raw data on root length, soil temperature, and soil moisture. Also provided are links that allow this information to be combined by sampling station and sample date. Root length data are provided by tube number, while temperature and moisture data are provided by plot/station combinations. Both refer to unique sampling stations (i.e. Plot 4 Station 1 is Tube 1, Plot 1 Station 6 is Tube 7, etc.); this file can be used in SAS to combine the two files. Finally, a file is provided that relates sampling dates to the alphanumeric codes that are used to describe them in the root length data set.

Links:
Root length data set (text file, 401 KB)
Temperature and moisture data set (text file, 291 KB)
Plot/station to tube conversions (text file, 2 KB)
Date to alphanumeric conversions (text file, 2 KB)