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# Stand development and other intrinsic factors largely control fine-root dynamics with only subtle modifications from resource availability

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**Running title:** Inherent and resource controls on fine-roots

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## Abstract

Forest productivity depends on resource acquisition by ephemeral roots and leaves. A combination of intrinsic and environmental factors influence ephemeral organs; however, difficulties in studying belowground organs impede mechanistic understanding of fine-root production and turnover. To quantify factors controlling fine-root dynamics, we grew a deciduous hardwood (*Populus deltoides* Bartr.) and an evergreen conifer (*Pinus taeda* L.) with distinct soil moisture and nutrient availability treatments. We monitored fine-root dynamics with minirhizotrons for six years during early stand development and expressed results on a root length, biomass and mortality-risk basis. Stand development and other intrinsic factors consistently influenced both species in the same direction and by similar magnitude. Live-root length increased to a peak during establishment and slowly declined after roots of neighboring trees overlapped. Root longevity was highest during establishment and decreased thereafter. Root longevity consistently increased with depth of appearance and initial root diameter. Season of appearance affected root longevity in the following order spring > summer > fall > winter. The influence of soil resource availability on fine-root dynamics was inconsistent between species, and ranked below that of rooting depth, initial diameter, stand development and phenology. Fine-root biomass either increased or was unaffected by greater resource availability. Fine-root production and live root length decreased with irrigation for both species, and increased with fertilization only for poplar. Fine-root mortality risk both increased and decreased depending on species and amendment treatment. Differing responses to soil moisture and nutrient availability between species suggests we should carefully evaluate generalizations about the response of fine-root dynamics to resource availability. While attempting to describe and explain carbon allocation to fine-root production and turnover, modelers and physiologists

should first consider consistent patterns of allocation caused by different depth, diameter, stand development, phenology and species before considering allocation due to soil resource availability.

## Introduction

Forest productivity is constrained by acquisition of above- and belowground resources. Such processes depend on the area and activity of ephemeral organs. Aboveground, leaf characteristics, such as leaf area, leaf duration, and leaf nitrogen status, largely control energy capture and carbon assimilation. Belowground, fine-root characteristics such as surface area, specific root length, production, and turnover, largely control uptake of soil resources. The magnitude of annual carbon investment that trees allocate to production of these ephemeral fine roots (26-56%, Gill and Jackson 2000; Vogt et al. 1996; Yuan and Chen 2010) indicates the priority placed on maintaining high resource acquisition capacity. Ultimately, our understanding of - and ability to model - forest nutrient and carbon dynamics are limited by our understanding of the dynamics and controls of ephemeral tissues. While leaf dynamics are relatively easy to observe, and therefore understand, root dynamics are more difficult to observe and measure in their native environment. As with leaf dynamics, a combination of factors influence fine-root dynamics; however, we currently understand more about how some factors control fine-root dynamics than others.

Intrinsic factors such as root diameter, rooting depth, species and phenology, exert dominant controls on fine-root dynamics. Lifespan consistently increases with root diameter and rooting depth (Chen and Brassard 2013). Species differences, such as evergreen vs. conifer, or growth rate, also consistently affect fine-root dynamics (Eissenstat and Yanai 1997; McCormack et al. 2014). Temporal factors occur on intra- or inter-annual time scales; however, we know more about the former than the latter primarily because it is easier to make consistent measurements over seasonal time scales. On intra-annual time scales, root production is maximum in spring and summer, while root mortality occurs mainly in fall and winter (Brassard et al. 2009). This pattern may be disrupted in climates with predominate summer droughts, where a sharp peak of root production occurs in spring (Misson et al. 2006), or biomodal peaks may occur in spring and fall (Atkinson 1980; Comas et al. 2005; Santantonio and Hermann 1985). Perhaps the least well-understood intrinsic factor influencing fine-root dynamics is stand development. Inter-annual studies over time scales relevant to forest stand development are rare because of difficulties in sustaining observations over requisite observation times, or in finding comparable stands for chronosequence studies. Available studies indicate that as young forest stands establish and inter-tree competition increases, fine-root biomass becomes relatively constant and may even decline thereafter (Brassard et al. 2009; Schoonmaker et al. 2016); suggesting turnover also increases during stand development. However, there is little information on the progression of fine-root dynamics observed in the same stand through different forest developmental stages (Borja et al. 2008; Brassard et al. 2009; Yuan and Chen 2010). Early stand development appears to show the most dynamic changes followed by stable or slow declines in fine-root standing crop as canopies differentiate through stem exclusion. These synthesis reports provide some insight into the net result of fine-root dynamics, but we still have a very poor understanding of how the individual processes of production and mortality change through stand development.

Soil resources that have potential to influence fine-root production primarily include soil moisture and nutrient availability. We currently have an inadequate understanding of how resource availability influences fine-root production and mortality compared to root diameter, rooting depth, species and phenology. Investigators study the influence of soil moisture on fine-root biomass, production and lifespan using drought, irrigation, or flooding in both manipulated and natural-gradient studies with inconsistent and contradictory results. For example, manipulative studies report that higher relative soil moisture either: (1) decreases both fine-root mortality and production simultaneously (Gaul et al. 2008); (2) increases both production and mortality (Meier and Leuschner 2008; Olesinski et al. 2011); (3) increases production, not mortality (Bauerle et al. 2008; Katterer et al. 1995; Majdi and Andersson 2005; Rytter 2013); or (4) has no influence on fine-root dynamics (Joslin et al. 2001; King et al. 2002; Rytter 2013). Consequently, a meta-analysis found only a slight positive effect of increased soil moisture on root lifespan (Chen and Brassard 2013). Results from natural precipitation gradients have been equally inconclusive (Finer et al. 2011b; Gill and Jackson 2000; Hertel et al. 2013; Yuan and Chen 2010). Various approaches have also yielded inconsistent and contradictory results that do not provide a clear understanding of the magnitude or even the direction of fine-root responses to nutrient availability. For example, reviews considering soil nutrients find that fine-root production and mortality increase and decrease in response to nitrogen (N) and phosphorus additions, and they suggest that lack of consensus among reports is due to methodological differences or site variation (Brassard et al. 2009; Chen and Brassard 2013; Eissenstat et al. 2013; Hodge 2004; Nadelhoffer 2000; Norby and Jackson 2000). Some reports suggest that we consider other factors, including intrinsic controls, to understand the response of fine-root production and turnover to resource availability. For example, Joslin et al. (2001) show that the response to favorable moisture availability was greater early in the growing season compared to later and conclude that to understand the response to water availability, it is necessary to account for plant phenology. Similarly, it was necessary for Kern et al. (2004) to consider only smaller diameter roots at the surface to show effects of N amendments on fine-root production.

Here we empirically evaluate fundamental controls of fine-root production and lifespan using long-term observations of deciduous hardwood and evergreen conifer fine roots grown with water and nutrient amendments. Our approach is unique in that we observe fine-root dynamics in short-rotation woody crop plantations beginning at stand establishment and continuing past the point of full root occupation (root closure) as inter-tree competition increases and crown differentiation begins. Short-rotation forests mature rapidly and therefore are practical models for observing stand development over relatively short periods. We selected a site with low soil moisture and nutrient availability so that applied resource amendment treatments created a range in site quality. In addition, we determined the response of fine-root production, lifespan, and standing crop to soil resources and to various intrinsic factors known to control fine-root dynamics such as stand development, depth, root diameter, species, and phenology. Our objective was to determine the relative control that soil resources have on fine-root dynamics compared with intrinsic factors. We took advantage of our long-term observations to understand how fine-root dynamics change through stand development. Specifically, we hypothesized that (1) fine-root production would be highest, and mortality would be lowest, early in stand development; (2) as development continued, production would decrease and mortality would increase until the two processes reach an equilibrium where the standing crop remains somewhat constant; and (3) it would be necessary to control for dominant intrinsic factors to define accurately the subtle effects of resource availability on fine-root dynamics.

## Materials and Methods

This research is part of a long-term forest productivity study designed to evaluate above- and belowground growth responses of several fast growing tree species. Coleman et al. (2004) describe in more detail the site, plant materials, and experimental design.

## Site Description and Preparation

We conducted the experiment at U.S. Department of Energy Savannah River Site, a National Environmental Research Park near Aiken, SC in the Carolina Sand Hill physiographic region (lat 33.387°, lon -81.676 °). The soil is predominately a Blanton sand (thermic Grossarenic Paleudults) with loamy subsoil at 120 to 200 cm depth (Rogers, 1990). Previous vegetation was plantation pine with an oak understory. We removed slash >15-cm diameter and pulverized stumps and remaining debris to less than 5cm diameter pieces, and incorporated biomass to 30 cm (RS-500 Reclaimer/Stabilizer, CMI Corp., Oklahoma City, USA). Additional preparation consisted of disking and lime amendments.

## Plant Material and competition control

The two species included in this study were eastern cottonwood (CW, *Populus deltoides* Bartr., cv. ST66: Issaquena County, MS (Eckenwalder 2001)) and loblolly pine (LP, *Pinus taeda* L., cv. 7-56, Williamsburg County, SC (Magbanua et al. 2011)). Bare-root LP seedlings were planted February 2000. We collected CW cuttings from stool beds during the previous winter and planted them April 2000. Rigorous and continuous weed control eliminated understory competition, so we could be certain live roots were solely plantation trees.

## Experimental Design

Twenty-four plots included two species, four treatments and three replicate blocks (Fig. S1). Each 0.22 ha treatment plot had a central 0.04 ha measurement plot with 54 trees arranged in 2.5 × 3 m spacing. There were at least four treated border rows (12 m) surrounding measurement plots. Treatments consisted of control (*C*), irrigation (*I*), fertilization (*F*) and irrigation + fertilization (*IF*). Within each of three block, the four treatment plots of a given species were grouped together to minimize within-block site gradients. We used drip irrigation to apply up to 5 mm $d^{-1}$ between April and October to meet evaporative demand and ensure favorable soil moisture. The quantity equals average regional evaporation during those months and was designed to assure favorable soil moisture. During the study, average annual rainfall was 809 mm. Irrigation supplied an average of 551 mm $y^{-1}$. We applied fertilizer at rates of 40 kg N $ha^{-1}$ $yr^{-1}$ in 2000, 80 in 2001, and 120 in 2002-2005. Fertilizer increased annually to correspond with demand of growing trees based on estimated N mineralization rate, expected productivity, and tree nutrient content (Coleman et al. 2004). We split annual fertilizer treatments among 26 weekly applications and applied them with drip irrigation. Fertilizer application supplied enough water to deliver liquid fertilizer and flush drip tubes (5 mm $wk^{-1}$). Control plots received 5 mm water $wk^{-1}$ to maintain experimental control. Thus, non-irrigated plots (i.e. *C* and *F*) received 130 mm $yr^{-1}$ of irrigation in addition to annual precipitation.

Stand inventory included annual measurements of stem diameter for all plot trees as described in (Coyle et al. 2016). We expressed diameter values as stand basal area and determined stem biomass using treatment- and species-specific allometric equations developed on several occasions. The progression of basal area and stem biomass over time quantitatively and continuously described stand development. These measures of stand development represent ontogenetic progression and substitute for time in analytical models.

Each November, we determined fine-root biomass (< 5 mm diameter) from five random locations per plot as detailed by Coleman (2007). Briefly, we removed soil cores (5 cm diameter) from 0-15, 15-45, and 45-105 cm depths. Five cores were taken from each plot at both shallow depths for a total of 15 cores for each amendment-by-species treatment combination. A single core per plot was taken below 45 cm for a total of three per treatment combination. We separated fine roots into two classes based on diameter: <1 mm and 1 to 5 mm. November sampling corresponded with late autumn peak root length from a previous study with similar species (Coleman et al. 2000). We collected two samples along the irrigation drip line and two perpendicular to the drip line at points one-half and one-quarter the distance between trees, and collected a fifth sample at the center between four trees. These relative sample locations captured expected spatial heterogeneity in fine-root biomass resulting from drip application (Coleman 2007). Sampling in each year occurred at five different randomly selected trees per plot with one of the five relative sample locations assigned to each. Double sampling did not occur from any one location. We washed roots by elutriation, manually separated live roots from dead organic matter, oven dried cleaned roots (60 °C), and weighed them to the nearest 0.01 mg.

Minirhizotron observation tubes

We measured fine-root production and lifespan using minirhizotron observation tubes (MROTs). Tubes (5 cm acrylic) were installed at a 45° angle to a depth of 105 cm in May 2000. We painted the exposed aboveground portion of MROTs black to limit light penetration and then white to limit heat absorption, and plugged the end with foam pipe insulation. The foam penetrated below ground line within MROTs to limit transfer of aboveground temperature. The upper end of the foam insulation was glued to the inside bottom of a topless aluminum beverage can so that it covered the tube when in place to limit entry of dirt and moisture. Plumber test plugs stoppered underground ends of each tube. A 5 cm pipe hanger secured the aboveground end to a 1.3 cm metal conduit driven 45 cm into the ground. We placed five MROTs per plot, or 15 tubes for each amendment-by-species treatment combination. Coleman et al. (2004) illustrate the locations, which were the same relative to trees as described above for soil samples. As with soil core samples, we randomly selected the five-reference trees and assigned one of the five relative locations to each. Soil coring for root biomass never occurred near MROTs.

We installed MROTs in bare ground prior to substantial expansion of seedling roots to avoid installation effects cautioned by previous authors (Coleman et al. 2000; Joslin and Wolfe 1999; Krasowski et al. 2010; Olesinski et al. 2011). Previous observations of mrot installation impacts on fine root dynamics occur in established forests where installation prunes existing roots and alters production and mortality for one to two years as roots recover. Bare-field installation avoids those impacts because placement occurs before roots reach soil viewed by MROTs. There may also be disturbance impacts of tube installation on nutrient release (Johnson et al. 2001); however, this would be minimal in our study because site preparation activities disturbed the surface 30 cm where fine roots typically grow during seedling establishment. Consequently, it is reasonable to assume that observation directly following bare-field installation are free of installation artifacts.

We captured digital images every 5 weeks starting September 2000 through June 2005 at precise locations along the upper MROT surface using a digital camera equipped with an indexing handle (BTC2; Bartz Technology, Santa Barbara, CA). To process images, operators used Rootracker (Duke University, Durham, NC, USA) image analysis software to tally observations of root length, width, branch order and condition. We could not positively identify branch order for all observed roots. We identified branch order for first-order root tips that appeared in the field of vision and for higher order roots subtending those tips. We categorized roots into three condition classes: new, previously observed, and missing, which allowed us to determine root lifespan. We did not include a condition class for dead roots due to the subjectivity in determining their viability. Consequently, we only considered roots to be dead when missing, which undoubtedly overestimated root longevity.

### Data analysis

We calculated fine-root production and mortality from measurements collected during image analysis. Incremental fine-root length production was the sum of new root length between consecutive imaging dates, while mortality was the sum of missing root length. Cumulative production and mortality were the sum of all previous incremental production and mortality. Live-root length was the difference between cumulative production and cumulative mortality up to that observation date. Annual increment of fine-root length production and mortality was the sum of increments for that year's 10 observation dates. Annual cumulative production and mortality was the difference of that accumulated or missing between final observation dates of consecutive years. Annual live-root length, or annual standing crop, was the average crop of all observation dates that year. Equations for each of these values are in the supplemental information.

We used repeated-measures analysis to assess fine-root biomass and MROT treatment responses over time. Data analysis evaluated all dependent variables using averaged within-plot measurements (n=3 replicate plots). Fixed factors included fertilization (*F*), irrigation (*I*), and species (*S*). Year (*Y*) was the repeated factor with plot being the random subject factor. Proc Mixed (SAS Institute Inc., Cary, NC) performed the analysis with $\alpha$=0.05. The Kenward-Roger method calculated denominator degrees of freedom. Lowest corrected Akaike's information criterion (AICC) (Littell et al. 2006, p.183) identified the best covariate structure for each dependent variable from a subset of appropriate structures. Tukey's HSD test compared treatment means. Temporal traces of fine-root variables measured with MROT were compared between species using the Kolmogorov- Smirnov two-sample test (SAS Proc NPar1Way) with $\alpha$=0.05.

<u>Survival analysis</u>

We achieve greater precision on the fate of fine-roots observed with MROTs using survival analysis compared with the repeated measures analysis (Coleman et al. 2000; Kern et al. 2004). The life-table method produced survival curves (Proc Lifetest, SAS Institute Inc., Cary, NC). Right-censored roots were those that had not disappeared by the end of the experiment. Log rank and Wilcoxon test compared survival curves. We estimated root lifespan from survival functions as the time of median root survival. Several fine-root populations of interest did not reach median survival, so it was not possible to determine lifespan universally using observed median root survival. Consequently, Cox' model estimated survival functions with corresponding median root lifespan.

Cox' proportional hazards model estimates fine-root hazard ratios. Cox' model uses the partial likelihood function to estimate parameters associated with each of the covariates (Allison 2010; Savarese and Patetta 2010; Wells and Eissenstat 2001). It is a log-transformed exponential model

$$\log h_i(t) = \log \lambda_0(t) + \beta_{1xi1} + \ldots + \beta_k x_{ik} \qquad \text{Equation 1}$$

where $h_i(t)$ is the hazard of mortality for root $i$ at time $t$, $\lambda_0(t)$ is an unknown and unspecified positive function, and $\beta$ is the coefficient for each of $k$ covariates. The SAS implementation of Cox' regression (Proc Phreg, SAS Institute Inc., Cary, NC) uses the partial likelihood function to eliminate $\lambda_0(t)$, assist development of the best model, calculate hazard ratios, estimate survival functions, and predict median lifespan (Allison 2010). Exponentiation of $\beta_k$ model estimates calculates hazard ratios and provides odds ratios for the risk of root mortality given specified conditions as compared with a reference condition (Allison 2010). Mortality ($M$) based on the odds ratio was calculated as $M$=100·($e^{\beta}$ – 1), where $\beta$ is the model coefficient for a factor expressed as a percentage of the reference condition.

The selected Cox model included main effects of intrinsic covariates that stratified various fine-root conditions, as well as main effects and interactions of the experimental factors of $S$, $F$ and $I$. The fully parameterized model—including all interactions (up to seven-way) among covariates and experimental factors—was difficult to interpret, and only improved the model fit (AIC) by 0.5%. Therefore, we simplified the model to include main effects of covariates, experimental factors, and interactions among experimental factors.

We compared stand age, or year of measurement to other variables that might better represent stand development as an independent variable in repeated measures models testing fine-root biomass, length and dynamics, and in Cox' hazard model. The stand development variables tested included stand basal area, stem biomass, stem volume and total stand biomass. We selected the best fitting models based on lowest Akaike's information criterion (AIC).

## Results

### Fine-root biomass and length

Root biomass distribution compared favorably with the frequency of roots appearing in MROTs, with some exceptions. The majority of fine-root biomass was ≤1 mm diameter. Roots ≤ 1 mm diameter composed 61% of CW biomass and 51% of LP biomass, with the remainder of fine-root biomass comprised of 1 to 5 mm diameter roots. In contrast, over 98.9% of CW and 97.0% of LP roots appearing in MROTs were <1 mm diameter (Fig. S2). When considering roots at the end of their lifespan, we found an almost identical diameter distribution, indicating observed fine roots did not increase in diameter. There was an obvious change in the slope of the diameter distribution curve for roots ≤ 1 mm diameter at appearance compared to those > 1mm diameter; showing a rapid decline in the frequency of the finest roots and a more even distribution of roots 1–5 mm diameter. Roots in biomass cores were often several centimeters long. Roots >1 mm diameter were stiff and woody, which resulted in proportionally larger biomass relative to those <1 mm. In contrast, roots appearing in MROTs averaged 3.0±3.2 mm in length (maximum 27 mm), and the lengths were not disproportionally weighted based on diameter.

Annual fine-root biomass responded to stand age, species and experimental factors. We selected stand age (i.e. year) as the best independent variable to represent stand development based on lowest AIC in models testing treatment responses of annual fine-root variables such as biomass, live-root length, production and mortality. The greatest effects on fine-root biomass were due to year of measurement and species (Fig. 1, greatest *f*-value for *Y* and *S* in Table 1). Species differences occurred in all years but 2000 (*S*×*Y* interactions in Table 1). Fine-root biomass generally increased through time. However, due to variation in the data, there was not always a stepwise annual increase. Any biomass response to *F* occurred between 2002 and 2004, whereas biomass did not respond to *F* in 2000 or 2005 (*F*×*Y* interactions in Table 1). The effects of *F* and *I* were both positive, but the *F* response was stronger (greater *f*-value in Table 1). For example, the maximum average annual increase in biomass due to *F* compared to *C* was 99%, whereas the maximum average annual increase due to *I* was only 42%

The factors influencing fine-root length observed in MROTs were generally consistent with biomass response. For example, the order of influence on length was $Y > S > F > I$ (Table 1). Peak root length occurred in 2003 for both species in all treatments (Fig. 1). This peak in root length four years post-establishment was distinct from root biomass, which generally increased through time. Differences in fine-root length between species increased over time (*S*×*Y* interaction). Root length consistently responded to treatments. For example, compared with *C*, root length was consistently smaller with *I* and larger with *F* and *IF* (*I*, *F* and *I*×*F* effects in Table 1), especially in CW. In LP, *F* effects were not apparent (*FxS* interaction). Consistent treatment differences over time for root length was also distinct from root biomass where differences converged toward the end of observations.

We observed a positive linear relationship between fine-root biomass and fine-root length (Fig. 2), indicating that dynamics observed in MROT measurements were representative of variation in fine-root biomass (cf. Johnson et al. 2001; Majdi 1996). Based on this assumption, the regression equation presented in Fig. 2 was used to translate MROT root length values to biomass on a per unit land area basis.

<u>Fine-root dynamics</u>

Species and year predominately controlled fine-root dynamics (*S* and *Y* effects in Table 1). Cottonwood cumulative production and mortality observed in MROTs were consistently greater than LP. Cumulative root length production showed annual growth cycles, with rapid early-season growth that decreased later in the season and remained low throughout dormancy (Fig. 3a). Annual growth patterns were most evident in 1- to 3-year old CW and they dampened with age.

Fine-root mortality was minimal for the first three years and increased thereafter (*Y* effect in Table 1). Cumulative fine-root mortality was not distinct between species until 2004 (Fig. 3b). Annual oscillations in cumulative mortality were less evident than that observed in cumulative production.

The length of live fine roots present at each observation (standing crop) resembled cumulative production during the first three growing seasons and reached a peak in the fourth growing season (Fig. 3c). Cottonwood live-root length was twice that of LP. Relatively stable live-root length occurred during the last five observation dates with values averaging 6.5±0.8 mm $cm^{-2}$ for CW and 3.2±0.5 for LP. This corresponds to 1.4±0.1 Mg $ha^{-1}$ for CW and 0.7±0.1 for LP (Fig. 2).

Fine-root production increments were low in year 1, peaked in years 2-4 and then progressively decreased in years 5-6 (Fig. S3a) for both CW and LP. Fine-root mortality increment peaked during the fourth year (Fig. S3b). Cottonwood typically had greater root mortality than LP; however, this difference was only significant in 2003 and 2004. Seasonal patterns were more distinct for fine-root production increments compared with mortality increments. Production increment during dormancy was a fraction of that observed during the growing season (Fig. S3a). Mortality increments were not seasonally consistent (Fig. S3b).

Fertilizer effects on annual fine-root dynamics depended on species. Cottonwood live-root length increased in response to fertilization, while LP live-root length did not (Fig. 4c, $F \times S$ interaction in Table 1). Production and mortality increment response to fertilization also depended on species although the effect was not as strong as that seen for live-root length (Fig. 4a&b).

Annual fine-root production increment and live-root length decreased with $I$, but were not influenced by $F$ or $IF$ (Fig. 4d, $I \times F$ interaction in Table 1). A similar but weaker response occurred for annual mortality increment. The influence of *I* on annual fine-root production increment occurred only in the third and fourth years (*I*×*Y* interaction).

Fine-root survival analysis

Soil depth predominately controlled the risk of fine-root mortality followed by diameter, total stem biomass, season, treatments, and species (chi-square, Table 2). Fine-root survival increased with depth for both species (Fig. S4). Based on Cox' regression parameter estimates, the risk of mortality was 19% higher for LP than CW, causing lifespan to increase 3.7 d for every cm depth in CW, and 2.8 d for every cm depth in LP. Lifespan increased with diameter and decreased as stands developed (Fig. S5). Risk of mortality decreased 5.1% for every 0.1 mm increase in root diameter and increased 2.3% for every Mg increase in stem biomass. Fine-root lifespan appeared to approach an asymptote as stands developed (Figs. 5 and S5). Fine roots appearing in winter had the highest risk of mortality (Fig. 6a) and the shortest lifespan (268 days).

Several stand development variables explained risk of fine-root mortality. We selected stem biomass (Fig. S6) to represent stand development for evaluating fine-root dynamics because it best fit the data. Other measures of stand development like year (AIC = 1,107,336), stand basal area (AIC = 1,107,169), total stem volume (AIC = 1,107,098) or total stand biomass (AIC = 1,107,058) did not fit as well as stem biomass (AIC = 1,107,019). Yet they all adequately represent development because each ranked third (chi-square) behind soil depth and root diameter, and always ranked higher than other factors tested in Cox' regression model. For the purpose of our study, stem biomass best represented stand development in determining risk of fine-root mortality (Table 2). The risk of mortality increased 2.3% for every Mg increase in stem biomass per ha, meaning that fine-root lifespan decreased four days per Mg-ha$^{-1}$ (Figs. 5 and S5).

Irrigation influenced the risk of mortality differently between species, but the response to fertilization was similar for both species (Fig. 6b). For CW, the risk of mortality decreased with *I*, causing median lifespan to increase from 268 d for *C* to 322 d for *I* at the reference condition (depth=1 cm, stem biomass = 5 Mg ha$^{-1}$, season=winter, diameter=0.5 mm). For LP, the risk of mortality increased with *I*, so median lifespan decreased from 231 d for *C* to 210 d for *I*. The risk of mortality decreased the same percentage for both species when grown with *F*, causing median lifespan to increase 40 d in CW and 46 d in LP. The risk of mortality decreased for both species when grown with *IF*, but the magnitude was distinct. Median lifespan increased by 113 days for CW grown with *IF* and it increased only 7 days for LP. Treatment responses in CW became more distinct with depth based on Cox' regression model estimates (Fig. S7). In this case, estimated lifespan increased 42% in *IF* relative to *C* at 1 cm depth compared to 61% at 90 cm. A similar increasing response did not occur in LP, where lifespan in *F* was greatest relative to *C*, but the increase was 20% at both 1 cm and 90 cm depth.

## Discussion

Our results demonstrate that stand development and other intrinsic factors largely determine fine-root production and turnover, with subtle modifications by resource availability. These results are unique in that we considered two functionally distinct tree species (Aubrey et al. 2012) receiving soil resource amendments and directly observed their fine-root dynamics through several early stand development stages. Moreover, our approach was robust in that installation of

MROTs occurred in bare soil prior to root colonization, thus avoiding many of the confounding effects associated with installation artefacts reported in other MROT studies (see methods for details). Overall, we found consistent root production and turnover responses to stand development, phenology, rooting depth, initial root diameter, and species (Table 2). Only after considering such dominant controls, could we most accurately assess the more subtle fine-root responses to nutrient and moisture availability. Specifically, Cox' multivariate hazard analysis simultaneously accounted for variation caused by dominant controlling factors to estimate accurately risk of root mortality in response to resource availability. Among dominant control factors, we focus on stand development as a unique contribution of this study. For example, had we not accounted for a 10 Mg ha$^{-1}$ increase in stem biomass, which was the difference in stem biomass between *C* and *F* in 2005 (Fig. S6), it would have negated the 40 d increase in fine-root lifespan that we observed in response to *F* in both species (Fig. 5 and S7). Similarly, had we not accounted for other intrinsic factors such as root depth, diameter, or season, it would have altered both direction and magnitude of fine-root mortality risk in response to resource availability.

## Stand development effects on fine-root dynamics

Although, stand age (year) best explained annual fine-root parameters (Table 1, Fig. 1), stem biomass (Fig. S6) better explained the fate of fine roots in Cox' hazard models because stem biomass increased differentially over time in response to resource availability treatments. Indeed, LP stem biomass production at this site was three times that of CW, and there were important distinctions between species in treatment response. Including stand biomass or basal area in the hazard analysis captured these effects. While we are unaware of studies directly investigating fine-root dynamics as a function of stand development, there are some reports where considering stand development might be useful for explaining observed results. For example, one study concluded that stem diameter increment at a common age controlled fine-root lifespan (McCormack et al. 2012; McCormack et al. 2014). These authors were uncertain about the reason for a negative correlation between stem diameter growth rate and fine-root lifespan, yet our results suggest that their observation may have been predominantly due to differences in stand development, since tree size is an important measure of stand development.

Our approach considers the dynamic stages of early stand development. Short-rotation intensively managed forests represent model systems for natural stand development that rapidly progress through stand establishment and increased inter-tree competition. Few other reports addressing temporal effects on fine roots consider time scales relevant to questions of stand development (Borja et al. 2008; Brassard et al. 2009). Studies that consider fine-root responses to stand development typically compare neighboring stands of different ages. Such chronosequence studies show that fine-root biomass increases during establishment and then remains constant or declines as stands age (Borja et al. 2008; Brassard et al. 2009; Yuan and Chen 2010), which confirms the peak we observed after four years (Fig. 3c). This peak built up as production initially exceeded mortality, and then declined after year 4 when mortality equaled or exceeded production (Fig. S3). The peak corresponded with root closure observed with biomass cores (Coleman 2007). Fine-root biomass continued to increase after site occupation, while fine-root length stabilized (Fig. 1). This may result from a different proportion of functional classes observed in root biomass and MROT approaches. (cf. Guo et al. 2008; Trumbore and Gaudinski 2003). Our observed maximum fine-root length represents fine roots <1 mm diameter (Fig. S2). The diameter distribution at initial appearance in MROTs was indistinguishable from the diameter distribution of older roots, which suggests that MROTs did not monitor roots with secondary thickening; however, high tensile and flexural strength in roots found in biomass samples indicated thickening, which suggests that mass distribution does not show the same pattern as fine-root length seen in MROTs.

We are only aware of three other MROT studies of similar duration, each of which began in established stands. Norby et al.'s (2004) observations in *Liquidambar styraciflua* L. started in a 9-yr-old stand with a basal area of 28 $m^2$ $ha^{-1}$. They found that fine-root standing crop increased to a plateau after 4 yr. The plateau is consistent with the peak standing crop we observed in year 4 (i.e. 3-yr-old); although their plateau was significantly delayed in comparison and it does not appear to decline. The *L. styraciflua* stands in our study developed more slowly than did CW or LP (Coyle et al. 2016), which might help explain the delayed peak; however, these patterns may also be artefacts of installing MROTs after root closure actually occurred. Krasowski et al. (2010) report a similar increase in fine-root standing crop in the first years of observation and attributed that to artefacts of tube-installation. In their case, standing crop reached a peak after 3 to 6 years where it remained constant or slightly declined depending on study location. Pritchard et al.'s (2008) observations occurred in 17-yr-old LP starting at a basal area of 17 – 25 $m^2$ $ha^{-1}$ (Ellsworth et al. 1995) where they found a steady temporal decline in fine-root standing crop. That decline appears to be free of installation artefacts and is consistent with our observed declines occurring after peak standing crop. Consequently, each of these studies in established stands report constant or declining standing crop following any initial post-installation surge in production.

Belowground site occupation appears to be similar to that aboveground where leaf area index increases to a maximum and then remains constant or declines (Schoonmaker et al. 2016; White et al. 2010; Yuan and Chen 2010). These parallel root and leaf developmental patterns suggest that established stands regulate production and mortality of roots and leaves to maintain appropriate surface area for resource acquisition. Positive correlations between stand growth, root biomass and leaf area in response to resource availability (Coyle et al. 2016; Martin and Jokela 2004; Vose and Allen 1988) also suggests that regulation of absorption surface are inter-dependent processes of stand growth and development (cf. Litton et al. 2007). Our results demonstrate this inter-dependence based on the large and significant chi-square for stem biomass in Cox' regression model (Table 2), which shows that whole-tree growth responses influence fine-root dynamics. Production and turnover of leaves and fine-roots appear to regulate leaf and root surface area presentation during early stand development.

Although the literature contains many examples of resource availability and stand development influencing maximum leaf area and canopy closure (Carlyle 1998; Landsberg and Waring 1997; Martin and Jokela 2004; Vose and Allen 1988; White et al. 2010), relatively little is known about similar influences on fine-root standing crop and root closure. Based on what we know about leaf area dynamics through stand development, we expected fine-root standing crop to peak at an early age, and then remain relatively constant or decline. When considering fine-root length, the time required to achieve peak fine-root standing crop was independent of resource availability, but maximum fine-root standing crop was not (Fig. 1). Thus, this study leads to questions of timing and extent of root and canopy closure, and site carrying capacity for resource acquisition surface both above and belowground. It also emphasizes the need to account for stand development (i.e. ontogeny) when comparing the balance between those surfaces among stands grown with different levels of resource availability.

The large allocation of carbon to fine-root production in young stands suggest that establishing root systems is a priority. The proportion of fine root production (FRP) to total net primary productivity (NPP) typically ranges from 26% to 56% (Gill and Jackson 2000; Vogt et al. 1996; Yuan and Chen 2010) depending upon species, climate and site interactions, but there are few reports regarding shifts in allocation with stand development. We found that FRP:NPP declined from 66% in year 1 to less than 3% in year 6 in LP, whereas it reached a minimum of 14% in year 2 and then it rose to an average of 81% between years 4 and 6 for CW. During this substantial developmental change, FRP:NPP was not affected by irrigation, yet the ratio for fertilized plots was consistently half that of non-fertilized. The variation in FRP:NPP observed with these species suggests that species have adopted different allocation strategies which may be related to functional type and seral stage (Grime 1979). Peak total NPP in our study occurred about the time of maximum leaf area (Coyle et al. 2016; Coyle and Coleman 2005; Coyle et al. 2008), which is consistent with other observations (Ryan et al. 2004). In addition, annual fine root production was correlated with total NPP, but only for CW ($P<0.001$, $r^2=0.40$) because LP ($P=0.89$) fine root production peaked at least one year before total NPP. While belowground carbon allocation is often directly related to total NPP and other autotrophic processes (Högberg et al. 2001; Irvine et al. 2005), our results show that autotrophic components of belowground carbon allocation are not always in phase with total NPP. This might explain why correlations between belowground carbon allocation and total NPP are not always evident (Litton et al. 2007). The eventual increase in FRP:NPP observed with CW is consistent with the increasing proportion of total belowground carbon allocation relative to gross primary productivity (Ryan et al. 2004) and increasing soil $CO_2$ efflux response with stand age (Wiseman and Seiler 2004; Yan et al. 2011), considering autotrophic respiration can account for half of soil $CO_2$ efflux (Hanson et al. 2000). In contrast, the decline in FRP:NPP found with LP is consistent with decreased soil $CO_2$ efflux with stand age (Klopatek 2002; Saiz et al. 2006). The links between fine-root dynamics, total NPP and soil $CO_2$ efflux remain an open question for understanding components of belowground carbon allocation, yet here we demonstrate that stand development is a critical control factor.

## Resource availability effects on fine-root dynamics

Resource availability had subtle and inconsistent influence on fine-root variables compared to the predictable responses observed to root depth, initial root diameter, phenology or stand development. Consequently, it was not possible to generalize about even the direction of resource availability influence on fine-root dynamics. Fine-root biomass either increased relative to *C* or was unaffected by *I*, *F*, or *IF* (Fig. 1). Fine-root production and root length standing crop decreased with *I* relative to *C*; however, these variables increased with fertilization for CW and had no effect on LP (Fig. 4). The risk of mortality both increased and decreased relative to *C* depending on species and amendment treatment (Fig. 6b). Since risk of mortality is also expressed as root longevity, it also serves as a surrogate for fine-root turnover, since turnover is the inverse of root longevity. Based on this relationship we might conclude that carbon allocation to turnover in response to resource availability is equally inconsistent. Literature reports describe wide-ranging fine-root responses to soil moisture and nutrient availability, which supports the variable results we observe between species on fine-root production and turnover in response to experimental resource availability treatments. Previous studies reporting fine-root mortality and production responses to increased water availability demonstrate decreases (Gaul et al. 2008), increases (Meier and Leuschner 2008; Olesinski et al. 2011), or no effect (Joslin et al. 2001; King et al. 2002; Rytter 2013). Responses to nutrient availability are equally inconclusive (Brassard et al. 2009; Chen and Brassard 2013; Eissenstat et al. 2013; Hodge 2004; Nadelhoffer 2000; Norby and Jackson 2000). However, most of these studies compare chronological age rather than normalizing with a measure of stand development like stem biomass or stand basal area. Yet as described above, risk of fine-root mortality consistently responds to stand development, and other intrinsic factors. Accounting for these factors has important consequences for understanding responses to resource availability.

These inconsistencies contradict the concept that greater carbon or biomass allocation will be directed toward roots when soil resources are limited compared to when they are abundant (Axelsson and Axelsson 1986; Brassard et al. 2009; Brouwer 1983; Hunt and Lloyd 1987; Keyes and Grier 1981; Lambers 1983; Reynolds and Thornley 1982). Thus, our results combined with those of others considering fine-root dynamics do not support the general concept that lower amounts of growth-limiting resources will increase allocation to roots because that directional shift is observed in some, but not all cases. Crucially, compared to the dominant and consistent influence of stand development, rooting depth, diameter, phenology and species, the minor and directionally inconsistent responses to resource availability do not warrant the attention they receive. In fact, resource responses are so subtle that accounting for them never contradicts our inference that intrinsic factors primarily control fine-root dynamics (e.g., see Figs. 5, 7 and S7).

Other fine-root control factors

Soil depth, root diameter, branch order, phenology and species exerted consistent influence on fine-root turnover as measured through mortality risk. Figure 7 illustrates the relative magnitude of factors controlling mortality risk observed for the given species based on chi-square values in Table 1. Soil depth was especially dominate in our study as an important intrinsic factor affecting mortality risk. Authors consistently report that roots growing in deeper soil have increased root longevity (Baddeley and Watson 2005; Coleman et al. 2000; Kern et al. 2004; Peek et al. 2006; Stover et al. 2010; Wells et al. 2002) and decreased rooting density (Jackson et al. 1996). In our study, soil depth explained the most variation of any factor for risk of root mortality (Table 2, Fig. S4) and biomass distribution (Coleman 2007). Not only were there fewer roots at depth, but deeper roots had longer lifespans. Considering the very different chemical and physical environment at depth (Fang and Moncrieff 2005), deeper roots are expected to be functionally distinct from those growing near the surface (Brassard et al. 2009; Chen and Brassard 2013; Iversen 2010; Pregitzer et al. 1998).

Root diameter was second to soil depth in explaining mortality risk. We measured root diameter using a continuous scale and conclude that the risk of mortality decreased 5.1% for every 0.1 mm increase in diameter. This confirms other reports that conclude fine-root lifespan increases with increasing root diameter class (Chen and Brassard 2013; Coleman et al. 2000; Kern et al. 2004; Wells and Eissenstat 2001). We chose to use root diameter as a co-factor over root branch order for both effectiveness and practicality. Root branch order is considered to be an important criteria for evaluating root function and lifespan (Chen and Brassard 2013; Comas et al. 2002; Fitter 1985; Fitter 1992; Jia et al. 2013; Majdi et al. 2001; McCormack et al. 2015; Pregitzer et al. 1997; Wells et al. 2002). Branch order explained a significant amount of variation in fine-root lifespan in our study for those roots where it was available; however, the branch order of most roots observed via MROTs was uncertain (86% not declared) because we could only confirm root order of the most distal roots with obvious root tips and those subtending. In contrast, we precisely measured root diameter on all roots. Diameter and root order are strongly correlated within a species (Comas et al. 2002; Guo et al. 2004; Jia et al. 2013; Pregitzer et al. 2002). Consequently, diameter measured on a continuous scale was a more practical and effective measurement than branch order when controlling for root morphology.

Phenology and species also influenced fine-root turnover and therefore are influential co-factors. We observed distinct annual oscillations of fine-root production with maxima during the growing season and minima during dormancy. Numerous reports describe similar phenological patterns of fine-root production and turnover (Baddeley and Watson 2005; Coleman et al. 2000; Joslin et al. 2001; Kern et al. 2004; King et al. 2002; McCormack et al. 2014; Rytter 2013; Tierney et al. 2003). The magnitude of fine-root production, mortality and live-root standing crop differed between tree species.  Differences in root traits occur for species with different leaf habits (Vogt et al. 1996), relative growth rate (Comas et al. 2002; Comas and Eissenstat 2004; McCormack et al. 2012; McCormack et al. 2014; Wright and Westoby 1999), and among species from different biomes (Finer et al. 2011a; Finer et al. 2011b; Vogt et al. 1996). Thus, controlling for phenology and comparing functionally distinct species is also vital for assessing responses of fine-root dynamics to soil resources. Due to the variety of species distinction, Figure 7 does not attempt to rank the influence of species among other factors controlling fine-root dynamics.  Rather, the figure seeks to demonstrate within a given species the relatively minor influence of resource availability among other factors, based on data presented in Table 2.

## Conclusion

Our study identified stand development as a major factor explaining the variation in fine-root dynamics. Furthermore, to determine accurately the response of fine-root dynamics to resource availability, we conclude that it is necessary to consider the influence of intrinsic factors, including stand development, rooting depth, initial root diameter, phenology and species. Multivariate analysis using Cox' regression demonstrates a useful approach to control for the influence of intrinsic factors when attempting to measure the relatively minor effects of resource availability. The variation we observed among species and resource availability treatments and the dependence upon measures of stand development demonstrates that generalizations about the directional response of fine-root dynamics to resource availability require reevaluation.

Our results have important implications for understanding and modeling factors controlling carbon allocation to fine roots. We understand with certainty the direction if not magnitude of several intrinsic factors controlling fine-root turnover like depth, root diameter, and phenology. Here we quantified the relative impact of stand development, which also appears to exert consistent controls on fine-root turnover. Predictive models should include turnover functions that vary by depth, root diameter and stand development to predict more accurately carbon flux from live roots to soil organic matter. Models that include intra-annual time steps should include phenological shifts from production in spring to mortality in autumn. However, there is a necessity to revisit those models that include generalized increases in carbon allocation to fine roots especially when soil moisture is limiting. Such functions exist in many prominent ecosystem process models (Landsberg and Waring 1997; Parton et al. 2010; Running and Gower 1991), some of which are now used in land models to predict continental- and global-scale responses to environmental change (Lawrence et al. 2011; Smithwick et al. 2014). Based on our results, and those of other researchers, the modeling community should incorporate details introduced by intrinsic factors controlling fine roots (cf. Franklin et al. 2012) and suspend the use of simplistic resource availability controls to describe allocation to fine roots.

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## Figure legends

Figure 1. Annual biomass and length (standing crop) of cottonwood (CW) and loblolly pine (LP) fine roots <1 mm in diameter in response to soil nutrient and water availability treatments. The coring approach measured biomass and the MROT approach measured length. Treatments included un-amended control (*C*), irrigated only (*I*) fertilized only (*F*) and irrigated plus fertilizer (*IF*). Samples were collected to a depth of 105 cm from replicate plots (n=3) for six years following establishment. Error bars represent standard error of the mean.

Figure 2. Relationship between live fine-root biomass and live-root length for cottonwood (CW) and loblolly pine (LP). This compares biomass sampling in November to the nearest MROT imaging date. Each point is the treatment mean (n=3) for an observation year. Natural-log transformed data included roots 1 mm diameter or less to a depth of 105 cm. Regression lines between species or among treatments were not significantly different. The pooled regression equation is $y=1.10\,x - 1.69$ ($P<0.001$, $r^2=0.73$).

Figure 3. Cumulative fine-root-length production (a) and cumulative root-length mortality (b) for cottonwood (CW) and loblolly pine (LP) during the first six years following planting. The length of live fine roots (c) is the difference between cumulative production and cumulative mortality. Points are the average of all treatments. Shaded vertical bars represent the growing season that started April 1st and ended October 1st. The right biomass axis converts from length values using

regression equation from Fig. 2. Error bars are standard errors (n=12). The P-values are for Kolmogorov-Smirnov two-sample non-parametric tests comparing lines within each panel.

Figure 4. Average annual production increment (a), annual mortality increment (b) and live-root length (crop) (c) for cottonwood (CW) and loblolly pine (LP) grown with (Fert) or without (No Fert) fertilization treatments and averaged for irrigation treatments. Average annual production increment, annual mortality increment and live-root length (crop) (d) grown with irrigation (*I*), fertilization (*F*), or their combination (*IF*) compared with untreated control (*C*) and averaged for species. Error bars are standard errors (n=36). Bars with the same letter within each variable are not significantly different (Tukey's HSD, α=0.10).

Figure 5. Cox regression model estimates of fine-root lifespan versus stem biomass for cottonwood (CW) and loblolly pine (LP) grown with irrigated (*I*), fertilized (*F*), or the combined (*IF*) treatments relative to the untreated control (*C*). Lifespan is median root survival predicted using Equation 1 and coefficients shown in Table 2. Error bars are 95% confidence intervals. We maintained cofactors at the following references levels: season = winter, soil depth = 1 cm and root diameter = 0.5.

Figure 6. Risk of fine-root mortality for (a) three seasons of the year relative to that observed in winter, and (b) for cottonwood (CW) and loblolly pine (LP) grown with irrigated (*I*), fertilized (*F*), or the combined (*IF*) treatments relative to the untreated control (*C*). Seasons were designated base on solstice and equinox dates. Percentages were calculated from model

coefficients as % Mort = $100 \cdot (e^{\beta s} - 1)$, where $\beta$ is the parameter estimate from Table 2 for season $s$.

Figure 7. Conceptual model illustrating the relative magnitude of different controls on fine-root dynamics. The width of the pyramid and shading reflects the relative magnitude of each factor. As the width narrows and shading decreases, the magnitude of the response decreases. Likewise, as the width narrows and shading decreases, the consistency and direction of the response across species decreases.

# Stand development and other intrinsic factors largely control fine-root dynamics with only subtle modifications from resource availability

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**Running title:** Inherent and resource controls on fine-roots

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## Abstract

Forest productivity depends on resource acquisition by ephemeral roots and leaves. A combination of intrinsic and environmental factors influence ephemeral organs; however, difficulties in studying belowground organs impede mechanistic understanding of fine-root production and turnover. To quantify factors controlling fine-root dynamics, we grew a deciduous hardwood (*Populus deltoides* Bartr.) and an evergreen conifer (*Pinus taeda* L.) with distinct soil moisture and nutrient availability treatments. We monitored fine-root dynamics with minirhizotrons for six years during early stand development and expressed results on a root length, biomass and mortality-risk basis. Stand development and other intrinsic factors consistently influenced both species in the same direction and by similar magnitude. Live-root length increased to a peak during establishment and slowly declined after roots of neighboring trees overlapped. Root longevity was highest during establishment and decreased thereafter. Root longevity consistently increased with depth of appearance and initial root diameter. Season of appearance affected root longevity in the following order spring > summer > fall > winter. The influence of soil resource availability on fine-root dynamics was inconsistent between species, and ranked below that of rooting depth, initial diameter, stand development and phenology. Fine-root biomass either increased or was unaffected by greater resource availability. Fine-root production and live root length decreased with irrigation for both species, and increased with fertilization only for poplar. Fine-root mortality risk both increased and decreased depending on species and amendment treatment. Differing responses to soil moisture and nutrient availability between species suggests we should carefully evaluate generalizations about the response of fine-root dynamics to resource availability. While attempting to describe and explain carbon allocation to fine-root production and turnover, modelers and physiologists

should first consider consistent patterns of allocation caused by different depth, diameter, stand development, phenology and species before considering allocation due to soil resource availability.

## Introduction

Forest productivity is constrained by acquisition of above- and belowground resources. Such processes depend on the area and activity of ephemeral organs. Aboveground, leaf characteristics, such as leaf area, leaf duration, and leaf nitrogen status, largely control energy capture and carbon assimilation. Belowground, fine-root characteristics such as surface area, specific root length, production, and turnover, largely control uptake of soil resources. The magnitude of annual carbon investment that trees allocate to production of these ephemeral fine roots (26-56%, Gill and Jackson 2000; Vogt et al. 1996; Yuan and Chen 2010) indicates the priority placed on maintaining high resource acquisition capacity. Ultimately, our understanding of - and ability to model - forest nutrient and carbon dynamics are limited by our understanding of the dynamics and controls of ephemeral tissues. While leaf dynamics are relatively easy to observe, and therefore understand, root dynamics are more difficult to observe and measure in their native environment. As with leaf dynamics, a combination of factors influence fine-root dynamics; however, we currently understand more about how some factors control fine-root dynamics than others.

Intrinsic factors such as root diameter, rooting depth, species and phenology, exert dominant controls on fine-root dynamics. Lifespan consistently increases with root diameter and rooting depth (Chen and Brassard 2013). Species differences, such as evergreen vs. conifer, or growth rate, also consistently affect fine-root dynamics (Eissenstat and Yanai 1997; McCormack et al. 2014). Temporal factors occur on intra- or inter-annual time scales; however, we know more about the former than the latter primarily because it is easier to make consistent measurements over seasonal time scales. On intra-annual time scales, root production is maximum in spring and summer, while root mortality occurs mainly in fall and winter (Brassard et al. 2009). This pattern may be disrupted in climates with predominate summer droughts, where a sharp peak of root production occurs in spring (Misson et al. 2006), or biomodal peaks may occur in spring and fall (Atkinson 1980; Comas et al. 2005; Santantonio and Hermann 1985). Perhaps the least well-understood intrinsic factor influencing fine-root dynamics is stand development. Inter-annual studies over time scales relevant to forest stand development are rare because of difficulties in sustaining observations over requisite observation times, or in finding comparable stands for chronosequence studies. Available studies indicate that as young forest stands establish and inter-tree competition increases, fine-root biomass becomes relatively constant and may even decline thereafter (Brassard et al. 2009; Schoonmaker et al. 2016); suggesting turnover also increases during stand development. However, there is little information on the progression of fine-root dynamics observed in the same stand through different forest developmental stages (Borja et al. 2008; Brassard et al. 2009; Yuan and Chen 2010). Early stand development appears to show the most dynamic changes followed by stable or slow declines in fine-root standing crop as canopies differentiate through stem exclusion. These synthesis reports provide some insight into the net result of fine-root dynamics, but we still have a very poor understanding of how the individual processes of production and mortality change through stand development.

Soil resources that have potential to influence fine-root production primarily include soil moisture and nutrient availability. We currently have an inadequate understanding of how resource availability influences fine-root production and mortality compared to root diameter, rooting depth, species and phenology. Investigators study the influence of soil moisture on fine-root biomass, production and lifespan using drought, irrigation, or flooding in both manipulated and natural-gradient studies with inconsistent and contradictory results. For example, manipulative studies report that higher relative soil moisture either: (1) decreases both fine-root mortality and production simultaneously (Gaul et al. 2008); (2) increases both production and mortality (Meier and Leuschner 2008; Olesinski et al. 2011); (3) increases production, not mortality (Bauerle et al. 2008; Katterer et al. 1995; Majdi and Andersson 2005; Rytter 2013); or (4) has no influence on fine-root dynamics (Joslin et al. 2001; King et al. 2002; Rytter 2013). Consequently, a meta-analysis found only a slight positive effect of increased soil moisture on root lifespan (Chen and Brassard 2013). Results from natural precipitation gradients have been equally inconclusive (Finer et al. 2011b; Gill and Jackson 2000; Hertel et al. 2013; Yuan and Chen 2010). Various approaches have also yielded inconsistent and contradictory results that do not provide a clear understanding of the magnitude or even the direction of fine-root responses to nutrient availability. For example, reviews considering soil nutrients find that fine-root production and mortality increase and decrease in response to nitrogen (N) and phosphorus additions, and they suggest that lack of consensus among reports is due to methodological differences or site variation (Brassard et al. 2009; Chen and Brassard 2013; Eissenstat et al. 2013; Hodge 2004; Nadelhoffer 2000; Norby and Jackson 2000). Some reports suggest that we consider other factors, including intrinsic controls, to understand the response of fine-root production and turnover to resource availability. For example, Joslin et al. (2001) show that the response to favorable moisture availability was greater early in the growing season compared to later and conclude that to understand the response to water availability, it is necessary to account for plant phenology. Similarly, it was necessary for Kern et al. (2004) to consider only smaller diameter roots at the surface to show effects of N amendments on fine-root production.

Here we empirically evaluate fundamental controls of fine-root production and lifespan using long-term observations of deciduous hardwood and evergreen conifer fine roots grown with water and nutrient amendments. Our approach is unique in that we observe fine-root dynamics in short-rotation woody crop plantations beginning at stand establishment and continuing past the point of full root occupation (root closure) as inter-tree competition increases and crown differentiation begins. Short-rotation forests mature rapidly and therefore are practical models for observing stand development over relatively short periods. We selected a site with low soil moisture and nutrient availability so that applied resource amendment treatments created a range in site quality. In addition, we determined the response of fine-root production, lifespan, and standing crop to soil resources and to various intrinsic factors known to control fine-root dynamics such as stand development, depth, root diameter, species, and phenology. Our objective was to determine the relative control that soil resources have on fine-root dynamics compared with intrinsic factors. We took advantage of our long-term observations to understand how fine-root dynamics change through stand development. Specifically, we hypothesized that (~~I~~1) fine-root production would be highest, and mortality would be lowest, early in stand development; (~~II~~2) as development continued, production would decrease and mortality would increase until the two processes reach an equilibrium where the standing crop remains somewhat constant; and (~~III~~3) it would be necessary to control for dominant intrinsic factors to define accurately the subtle effects of resource availability on fine-root dynamics.

## Materials and Methods

This research is part of a long-term forest productivity study designed to evaluate above- and belowground growth responses of several fast growing tree species. Coleman et al. (2004) describe in more detail the site, plant materials, and experimental design.

Site Description and Preparation

We conducted the experiment at U.S. Department of Energy Savannah River Site, a National Environmental Research Park near Aiken, SC in the Carolina Sand Hill physiographic region (lat 33.387°, lon -81.676 °). The soil is predominately a Blanton sand (thermic Grossarenic Paleudults) with loamy subsoil at 120 to 200 cm depth (Rogers, 1990). Previous vegetation was plantation pine with an oak understory. We removed slash >15-cm diameter and pulverized stumps and remaining debris to less than 5cm diameter pieces, and incorporated biomass to 30 cm (RS-500 Reclaimer/Stabilizer, CMI Corp., Oklahoma City, USA). Additional preparation consisted of disking and lime amendments.

Plant Material and competition control

The two species included in this study were eastern cottonwood (CW, *Populus deltoides* Bartr., cv. ST66: Issaquena County, MS (Eckenwalder 2001)) and loblolly pine (LP, *Pinus taeda* L., cv. 7-56, Williamsburg County, SC (Magbanua et al. 2011)). Bare-root LP seedlings were planted February 2000. We collected CW cuttings from stool beds during the previous winter and planted them April 2000. Rigorous and continuous weed control eliminated understory competition, so we could be certain live roots were solely plantation trees.

Experimental Design

Twenty-four plots included two species, four treatments and three replicate blocks (Fig. S1). Each 0.22 ha treatment plot had a central 0.04 ha measurement plot with 54 trees arranged in 2.5 × 3 m spacing. There were at least four treated border rows (12 m) surrounding measurement plots. Treatments consisted of control (*C*), irrigation (*I*), fertilization (*F*) and irrigation + fertilization (*IF*). Within each of three block, the four treatment plots of a given species were grouped together to minimize within-block site gradients. We used drip irrigation to apply up to 5 mm $d^{-1}$ between April and October to meet evaporative demand and ensure favorable soil moisture. The quantity equals average regional evaporation during those months and was designed to assure favorable soil moisture. During the study, average annual rainfall was 809 mm. Irrigation supplied an average of 551 mm $y^{-1}$. We applied fertilizer at rates of 40 kg N $ha^{-1}$ $yr^{-1}$ in 2000, 80 in 2001, and 120 in 2002-2005. Fertilizer increased annually to correspond with demand of growing trees based on estimated N mineralization rate, expected productivity, and tree nutrient content (Coleman et al. 2004). We split annual fertilizer treatments among 26 weekly applications and applied them with drip irrigation. Fertilizer application supplied enough water to deliver liquid fertilizer and flush drip tubes (5 mm $wk^{-1}$). Control plots received 5 mm water $wk^{-1}$ to maintain experimental control. Thus, non-irrigated plots (i.e. *C* and *F*) received 130 mm $yr^{-1}$ of irrigation in addition to annual precipitation.

Stand inventory included annual measurements of stem diameter for all plot trees as described in (Coyle et al. 2016). We expressed diameter values as stand basal area and determined stem biomass using treatment- and species-specific allometric equations developed on several occasions. The progression of basal area and stem biomass over time quantitatively and continuously described stand development. These measures of stand development represent ontogenetic progression and substitute for time in analytical models.

Each November, we determined fine-root biomass (< 5 mm diameter) from five random locations per plot as detailed by Coleman (2007). Briefly, we removed soil cores (5 cm diameter) from 0-15, 15-45, and 45-105 cm depths. Five cores were taken from each plot at both shallow depths for a total of 15 cores for each amendment-by-species treatment combination. A single core per plot was taken below 45 cm for a total of three per treatment combination. We separated fine roots into two classes based on diameter: <1 mm and 1 to 5 mm. November sampling corresponded with late autumn peak root length from a previous study with similar species (Coleman et al. 2000). We collected two samples along the irrigation drip line and two perpendicular to the drip line at points one-half and one-quarter the distance between trees, and collected a fifth sample at the center between four trees. These relative sample locations captured expected spatial heterogeneity in fine-root biomass resulting from drip application (Coleman 2007). Sampling in each year occurred at five different randomly selected trees per plot with one of the five relative sample locations assigned to each. Double sampling did not occur from any one location. We washed roots by elutriation, manually separated live roots from dead organic matter, oven dried cleaned roots (60 $^{o}$C), and weighed them to the nearest 0.01 mg.

Minirhizotron observation tubes

We measured fine-root production and lifespan using minirhizotron observation tubes (MROTs). Tubes (5 cm acrylic) were installed at a 45$^{o}$ angle to a depth of 105 cm in May 2000. We painted the exposed aboveground portion of MROTs black to limit light penetration and then white to limit heat absorption, and plugged the end with foam pipe insulation. The foam penetrated below ground line within MROTs to limit transfer of aboveground temperature. The upper end of the foam insulation was glued to the inside bottom of a topless aluminum beverage can so that it covered the tube when in place to limit entry of dirt and moisture. Plumber test plugs stoppered underground ends of each tube. A 5 cm pipe hanger secured the aboveground end to a 1.3 cm metal conduit driven 45 cm into the ground. We placed five MROTs per plot, or 15 tubes for each amendment-by-species treatment combination. Coleman et al. (2004) illustrate the locations, which were the same relative to trees as described above for soil samples. As with soil core samples, we randomly selected the five-reference trees and assigned one of the five relative locations to each. Soil coring for root biomass never occurred near MROTs.

We installed MROTs in bare ground prior to substantial expansion of seedling roots to avoid installation effects cautioned by previous authors (Coleman et al. 2000; Joslin and Wolfe 1999; Krasowski et al. 2010; Olesinski et al. 2011). Previous observations of mrot installation impacts on fine root dynamics occur in established forests where installation prunes existing roots and alters production and mortality for one to two years as roots recover. Bare-field installation avoids those impacts because placement occurs before roots reach soil viewed by MROTs. There may also be disturbance impacts of tube installation on nutrient release (Johnson et al. 2001); however, this would be minimal in our study because site preparation activities disturbed the surface 30 cm where fine roots typically grow during seedling establishment. Consequently, it is reasonable to assume that observation directly following bare-field installation are free of installation artifacts.

We captured digital images every 5 weeks starting September 2000 through June 2005 at precise locations along the upper MROT surface using a digital camera equipped with an indexing handle (BTC2; Bartz Technology, Santa Barbara, CA). To process images, operators used Rootracker (Duke University, Durham, NC, USA) image analysis software to tally observations of root length, width, branch order and condition. We could not positively identify branch order for all observed roots. We identified branch order for first-order root tips that appeared in the field of vision and for higher order roots subtending those tips. We categorized roots into three condition classes: new, previously observed, and missing, which allowed us to determine root lifespan. We did not include a condition class for dead roots due to the subjectivity in determining their viability. Consequently, we only considered roots to be dead when missing, which undoubtedly overestimated root longevity.

### Data analysis

We calculated fine-root production and mortality from measurements collected during image analysis. Incremental fine-root length production was the sum of new root length between consecutive imaging dates, while mortality was the sum of missing root length. Cumulative production and mortality were the sum of all previous incremental production and mortality. Live-root length was the difference between cumulative production and cumulative mortality up to that observation date. Annual increment of fine-root length production and mortality was the sum of increments for that year's 10 observation dates. Annual cumulative production and mortality was the difference of that accumulated or missing between final observation dates of consecutive years. Annual live-root length, or annual standing crop, was the average crop of all observation dates that year. Equations for each of these values are in the supplemental information.

We used repeated-measures analysis to assess fine-root biomass and MROT treatment responses over time. Data analysis evaluated all dependent variables using averaged within-plot measurements (n=3 replicate plots). Fixed factors included fertilization (*F*), irrigation (*I*), and species (*S*). Year (*Y*) was the repeated factor with plot being the random subject factor. Proc Mixed (SAS Institute Inc., Cary, NC) performed the analysis with $\alpha$=0.05. The Kenward-Roger method calculated denominator degrees of freedom. Lowest corrected Akaike's information criterion (AICC) (Littell et al. 2006, p.183) identified the best covariate structure for each dependent variable from a subset of appropriate structures. Tukey's HSD test compared treatment means. Temporal traces of fine-root variables measured with MROT were compared between species using the Kolmogorov- Smirnov two-sample test (SAS Proc NPar1Way) with $\alpha$=0.05.

Survival analysis

We achieve greater precision on the fate of fine-roots observed with MROTs using survival analysis compared with the repeated measures analysis (Coleman et al. 2000; Kern et al. 2004). The life-table method produced survival curves (Proc Lifetest, SAS Institute Inc., Cary, NC). Right-censored roots were those that had not disappeared by the end of the experiment. Log rank and Wilcoxon test compared survival curves. We estimated root lifespan from survival functions as the time of median root survival. Several fine-root populations of interest did not reach median survival, so it was not possible to determine lifespan universally using observed median root survival. Consequently, Cox' model estimated survival functions with corresponding median root lifespan.

Cox' proportional hazards model estimates fine-root hazard ratios. Cox' model uses the partial likelihood function to estimate parameters associated with each of the covariates (Allison 2010; Savarese and Patetta 2010; Wells and Eissenstat 2001). It is a log-transformed exponential model

$$\log h_i(t)=\log \lambda_0(t) + \beta_{1xi1} +\ldots+ \beta_k x_{ik} \qquad \text{Equation 1}$$

where $h_i(t)$ is the hazard of mortality for root $i$ at time $t$, $\lambda_0(t)$ is an unknown and unspecified positive function, and $\beta$ is the coefficient for each of $k$ covariates. The SAS implementation of Cox' regression (Proc Phreg, SAS Institute Inc., Cary, NC) uses the partial likelihood function to eliminate $\lambda_0(t)$, assist development of the best model, calculate hazard ratios, estimate survival functions, and predict median lifespan (Allison 2010). Exponentiation of $\beta_k$ model estimates calculates hazard ratios and provides odds ratios for the risk of root mortality given specified conditions as compared with a reference condition (Allison 2010). Mortality ($M$) based on the odds ratio was calculated as $M=100\cdot(e^{\beta}-1)$, where $\beta$ is the model coefficient for a factor expressed as a percentage of the reference condition.

The selected Cox model included main effects of intrinsic covariates that stratified various fine-root conditions, as well as main effects and interactions of the experimental factors of $S$, $F$ and $I$. The fully parameterized model—including all interactions (up to seven-way) among covariates and experimental factors—was difficult to interpret, and only improved the model fit (AIC) by 0.5%. Therefore, we simplified the model to include main effects of covariates, experimental factors, and interactions among experimental factors.

We compared stand age, or year of measurement to other variables that might better represent stand development as an independent variable in repeated measures models testing fine-root biomass, length and dynamics, and in Cox' hazard model. The stand development variables tested included stand basal area, stem biomass, stem volume and total stand biomass. We selected the best fitting models based on lowest Akaike's information criterion (AIC).

## Results

### Fine-root biomass and length

Root biomass distribution compared favorably with the frequency of roots appearing in MROTs, with some exceptions. The majority of fine-root biomass was ≤1 mm diameter. Roots ≤ 1 mm diameter composed 61% of CW biomass and 51% of LP biomass, with the remainder of fine-root biomass comprised of 1 to 5 mm diameter roots. In contrast, over 98.9% of CW and 97.0% of LP roots appearing in MROTs were <1 mm diameter (Fig. S2). When considering roots at the end of their lifespan, we found an almost identical diameter distribution, indicating observed fine roots did not increase in diameter. There was an obvious change in the slope of the diameter distribution curve for roots ≤ 1 mm diameter at appearance compared to those > 1mm diameter; showing a rapid decline in the frequency of the finest roots and a more even distribution of roots 1–5 mm diameter. Roots in biomass cores were often several centimeters long. Roots >1 mm diameter were stiff and woody, which resulted in proportionally larger biomass relative to those <1 mm. In contrast, roots appearing in MROTs averaged 3.0±3.2 mm in length (maximum 27 mm), and the lengths were not disproportionally weighted based on diameter.

Annual fine-root biomass responded to stand age, species and experimental factors. We selected stand age (i.e. year) as the best independent variable to represent stand development based on lowest AIC in models testing treatment responses of annual fine-root variables such as biomass, live-root length, production and mortality. The greatest effects on fine-root biomass were due to year of measurement and species (Fig. 1, greatest *f*-value for *Y* and *S* in Table 1). Species differences occurred in all years but 2000 ($S \times Y$ interactions in Table 1). Fine-root biomass generally increased through time. However, due to variation in the data, there was not always a stepwise annual increase. Any biomass response to *F* occurred between 2002 and 2004, whereas biomass did not respond to *F* in 2000 or 2005 ($F \times Y$ interactions in Table 1). The effects of *F* and *I* were both positive, but the *F* response was stronger (greater *f*-value in Table 1). For example, the maximum average annual increase in biomass due to *F* compared to *C* was 99%, whereas the maximum average annual increase due to *I* was only 42%

The factors influencing fine-root length observed in MROTs were generally consistent with biomass response. For example, the order of influence on length was $Y > S > F > I$ (Table 1). Peak root length occurred in 2003 for both species in all treatments (Fig. 1). This peak in root length four years post-establishment was distinct from root biomass, which generally increased through time. Differences in fine-root length between species increased over time ($S \times Y$ interaction). Root length consistently responded to treatments. For example, compared with *C*, root length was consistently smaller with *I* and larger with *F* and *IF* (*I*, *F* and *I*×*F* effects in Table 1), especially in CW. In LP, *F* effects were not apparent (*FxS* interaction). Consistent treatment differences over time for root length was also distinct from root biomass where differences converged toward the end of observations.

We observed a positive linear relationship between fine-root biomass and fine-root length (Fig. 2), indicating that dynamics observed in MROT measurements were representative of variation in fine-root biomass (cf. Johnson et al. 2001; Majdi 1996). Based on this assumption, the regression equation presented in Fig. 2 was used to translate MROT root length values to biomass on a per unit land area basis.

<u>Fine-root dynamics</u>

Species and year predominately controlled fine-root dynamics (*S* and *Y* effects in Table 1). Cottonwood cumulative production and mortality observed in MROTs were consistently greater than LP. Cumulative root length production showed annual growth cycles, with rapid early-season growth that decreased later in the season and remained low throughout dormancy (Fig. 3a). Annual growth patterns were most evident in 1- to 3-year old CW and they dampened with age.

Fine-root mortality was minimal for the first three years and increased thereafter (*Y* effect in Table 1). Cumulative fine-root mortality was not distinct between species until 2004 (Fig. 3b). Annual oscillations in cumulative mortality were less evident than that observed in cumulative production.

The length of live fine roots present at each observation (standing crop) resembled cumulative production during the first three growing seasons and reached a peak in the fourth growing season (Fig. 3c). Cottonwood live-root length was twice that of LP. Relatively stable live-root length occurred during the last five observation dates with values averaging 6.5±0.8 mm $cm^{-2}$ for CW and 3.2±0.5 for LP. This corresponds to 1.4±0.1 Mg $ha^{-1}$ for CW and 0.7±0.1 for LP (Fig. 2).

Fine-root production increments were low in year 1, peaked in years 2-4 and then progressively decreased in years 5-6 (Fig. S3a) for both CW and LP. Fine-root mortality increment peaked during the fourth year (Fig. S3b). Cottonwood typically had greater root mortality than LP; however, this difference was only significant in 2003 and 2004. Seasonal patterns were more distinct for fine-root production increments compared with mortality increments. Production increment during dormancy was a fraction of that observed during the growing season (Fig. S3a). Mortality increments were not seasonally consistent (Fig. S3b).

Fertilizer effects on annual fine-root dynamics depended on species. Cottonwood live-root length increased in response to fertilization, while LP live-root length did not (Fig. 4c, $F \times S$ interaction in Table 1). Production and mortality increment response to fertilization also depended on species although the effect was not as strong as that seen for live-root length (Fig. 4a&b).

Annual fine-root production increment and live-root length decreased with *I*, but were not influenced by *F* or *IF* (Fig. 4d, $I \times F$ interaction in Table 1). A similar but weaker response occurred for annual mortality increment. The influence of *I* on annual fine-root production increment occurred only in the third and fourth years ($I \times Y$ interaction).

Fine-root survival analysis

Soil depth predominately controlled the risk of fine-root mortality followed by diameter, total stem biomass, season, treatments, and species (chi-square, Table 2). Fine-root survival increased with depth for both species (Fig. S4). Based on Cox' regression parameter estimates, the risk of mortality was 19% higher for LP than CW, causing lifespan to increase 3.7 d for every cm depth in CW, and 2.8 d for every cm depth in LP. Lifespan increased with diameter and decreased as stands developed (Fig. S5). Risk of mortality decreased 5.1% for every 0.1 mm increase in root diameter and increased 2.3% for every Mg increase in stem biomass. Fine-root lifespan appeared to approach an asymptote as stands developed (Figs. 5 and S5). Fine roots appearing in winter had the highest risk of mortality (Fig. 6a) and the shortest lifespan (268 days).

Several stand development variables explained risk of fine-root mortality. We selected stem biomass (Fig. S6) to represent stand development for evaluating fine-root dynamics because it best fit the data. Other measures of stand development like year (AIC = 1,107,336), stand basal area (AIC = 1,107,169), total stem volume (AIC = 1,107,098) or total stand biomass (AIC = 1,107,058) did not fit as well as stem biomass (AIC = 1,107,019). Yet they all adequately represent development because each ranked third (chi-square) behind soil depth and root diameter, and always ranked higher than other factors tested in Cox' regression model. For the purpose of our study, stem biomass best represented stand development in determining risk of fine-root mortality (Table 2). The risk of mortality increased 2.3% for every Mg increase in stem biomass per ha, meaning that fine-root lifespan decreased four days per Mg-ha$^{-1}$ (Figs. 5 and S5).

Irrigation influenced the risk of mortality differently between species, but the response to fertilization was similar for both species (Fig. 6b). For CW, the risk of mortality decreased with *I*, causing median lifespan to increase from 268 d for *C* to 322 d for *I* at the reference condition (depth=1 cm, stem biomass = 5 Mg ha$^{-1}$, season=winter, diameter=0.5 mm). For LP, the risk of mortality increased with *I*, so median lifespan decreased from 231 d for *C* to 210 d for *I*. The risk of mortality decreased the same percentage for both species when grown with *F*, causing median lifespan to increase 40 d in CW and 46 d in LP. The risk of mortality decreased for both species when grown with *IF*, but the magnitude was distinct. Median lifespan increased by 113 days for CW grown with *IF* and it increased only 7 days for LP. Treatment responses in CW became more distinct with depth based on Cox' regression model estimates (Fig. S7). In this case, estimated lifespan increased 42% in *IF* relative to *C* at 1 cm depth compared to 61% at 90 cm. A similar increasing response did not occur in LP, where lifespan in *F* was greatest relative to *C*, but the increase was 20% at both 1 cm and 90 cm depth.

## Discussion

Our results demonstrate that stand development and other intrinsic factors largely determine fine-root production and turnover, with subtle modifications by resource availability. These results are unique in that we considered two functionally distinct tree species (Aubrey et al. 2012) receiving soil resource amendments and directly observed their fine-root dynamics through several early stand development stages. Moreover, our approach was robust in that installation of

MROTs occurred in bare soil prior to root colonization, thus avoiding many of the confounding effects associated with installation artefacts reported in other MROT studies (see methods for details). Overall, we found consistent root production and turnover responses to stand development, phenology, rooting depth, initial root diameter, and species (Table 2). Only after considering such dominant controls, could we most accurately assess the more subtle fine-root responses to nutrient and moisture availability. Specifically, Cox' multivariate hazard analysis simultaneously accounted for variation caused by dominant controlling factors to estimate accurately risk of root mortality in response to resource availability. Among dominant control factors, we focus on stand development as a unique contribution of this study. For example, had we not accounted for a 10 Mg ha$^{-1}$ increase in stem biomass, which was the difference in stem biomass between *C* and *F* in 2005 (Fig. S6), it would have negated the 40 d increase in fine-root lifespan that we observed in response to *F* in both species (Fig. 5 and S7). Similarly, had we not accounted for other intrinsic factors such as root depth, diameter, or season, it would have altered both direction and magnitude of fine-root mortality risk in response to resource availability.

## Stand development effects on fine-root dynamics

Although, stand age (year) best explained annual fine-root parameters (Table 1, Fig. 1), stem biomass (Fig. S6) better explained the fate of fine roots in Cox' hazard models because stem biomass increased differentially over time in response to resource availability treatments. Indeed, LP stem biomass production at this site was three times that of CW, and there were important distinctions between species in treatment response. Including stand biomass or basal area in the hazard analysis captured these effects. While we are unaware of studies directly investigating fine-root dynamics as a function of stand development, there are some reports where considering stand development might be useful for explaining observed results. For example, one study concluded that stem diameter increment at a common age controlled fine-root lifespan (McCormack et al. 2012; McCormack et al. 2014). These authors were uncertain about the reason for a negative correlation between stem diameter growth rate and fine-root lifespan, yet our results suggest that their observation may have been predominantly due to differences in stand development, since tree size is an important measure of stand development.

Our approach considers the dynamic stages of early stand development. Short-rotation intensively managed forests represent model systems for natural stand development that rapidly progress through stand establishment and increased inter-tree competition. Few other reports addressing temporal effects on fine roots consider time scales relevant to questions of stand development (Borja et al. 2008; Brassard et al. 2009). Studies that consider fine-root responses to stand development typically compare neighboring stands of different ages. Such chronosequence studies show that fine-root biomass increases during establishment and then remains constant or declines as stands age (Borja et al. 2008; Brassard et al. 2009; Yuan and Chen 2010), which confirms the peak we observed after four years (Fig. 3c). This peak built up as production initially exceeded mortality, and then declined after year 4 when mortality equaled or exceeded production (Fig. S3). The peak corresponded with root closure observed with biomass cores (Coleman 2007). Fine-root biomass continued to increase after site occupation, while fine-root length stabilized (Fig. 1). This may result from a different proportion of functional classes observed in root biomass and MROT approaches. (cf. Guo et al. 2008; Trumbore and Gaudinski 2003). Our observed maximum fine-root length represents fine roots <1 mm diameter (Fig. S2). The diameter distribution at initial appearance in MROTs was indistinguishable from the diameter distribution of older roots, which suggests that MROTs did not monitor roots with secondary thickening; however, high tensile and flexural strength in roots found in biomass samples indicated thickening, which suggests that mass distribution does not show the same pattern as fine-root length seen in MROTs.

We are only aware of three other MROT studies of similar duration, each of which began in established stands. Norby et al.'s (2004) observations in *Liquidambar styraciflua* L. started in a 9-yr-old stand with a basal area of 28 $m^2$ $ha^{-1}$. They found that fine-root standing crop increased to a plateau after 4 yr. The plateau is consistent with the peak standing crop we observed in year 4 (i.e. 3-yr-old); although their plateau was significantly delayed in comparison and it does not appear to decline. The *L. styraciflua* stands in our study developed more slowly than did CW or LP (Coyle et al. 2016), which might help explain the delayed peak; however, these patterns may also be artefacts of installing MROTs after root closure actually occurred. Krasowski et al. (2010) report a similar increase in fine-root standing crop in the first years of observation and attributed that to artefacts of tube-installation. In their case, standing crop reached a peak after 3 to 6 years where it remained constant or slightly declined depending on study location. Pritchard et al.'s (2008) observations occurred in 17-yr-old LP starting at a basal area of 17 – 25 $m^2$ $ha^{-1}$ (Ellsworth et al. 1995) where they found a steady temporal decline in fine-root standing crop. That decline appears to be free of installation artefacts and is consistent with our observed declines occurring after peak standing crop. Consequently, each of these studies in established stands report constant or declining standing crop following any initial post-installation surge in production.

Belowground site occupation appears to be similar to that aboveground where leaf area index increases to a maximum and then remains constant or declines (Schoonmaker et al. 2016; White et al. 2010; Yuan and Chen 2010). These parallel root and leaf developmental patterns suggest that established stands regulate production and mortality of roots and leaves to maintain appropriate surface area for resource acquisition. Positive correlations between stand growth, root biomass and leaf area in response to resource availability (Coyle et al. 2016; Martin and Jokela 2004; Vose and Allen 1988) also suggests that regulation of absorption surface are inter-dependent processes of stand growth and development (cf. Litton et al. 2007). Our results demonstrate this inter-dependence based on the large and significant chi-square for stem biomass in Cox' regression model (Table 2), which shows that whole-tree growth responses influence fine-root dynamics. Production and turnover of leaves and fine-roots appear to regulate leaf and root surface area presentation during early stand development.

Although the literature contains many examples of resource availability and stand development influencing maximum leaf area and canopy closure (Carlyle 1998; Landsberg and Waring 1997; Martin and Jokela 2004; Vose and Allen 1988; White et al. 2010), relatively little is known about similar influences on fine-root standing crop and root closure. Based on what we know about leaf area dynamics through stand development, we expected fine-root standing crop to peak at an early age, and then remain relatively constant or decline. When considering fine-root length, the time required to achieve peak fine-root standing crop was independent of resource availability, but maximum fine-root standing crop was not (Fig. 1). Thus, this study leads to questions of timing and extent of root and canopy closure, and site carrying capacity for resource acquisition surface both above and belowground. It also emphasizes the need to account for stand development (i.e. ontogeny) when comparing the balance between those surfaces among stands grown with different levels of resource availability.

The large allocation of carbon to fine-root production in young stands suggest that establishing root systems is a priority. The proportion of fine root production (FRP) to total net primary productivity (NPP) typically ranges from 26% to 56% (Gill and Jackson 2000; Vogt et al. 1996; Yuan and Chen 2010) depending upon species, climate and site interactions, but there are few reports regarding shifts in allocation with stand development. We found that FRP:NPP declined from 66% in year 1 to less than 3% in year 6 in LP, whereas it reached a minimum of 14% in year 2 and then it rose to an average of 81% between years 4 and 6 for CW. During this substantial developmental change, FRP:NPP was not affected by irrigation, yet the ratio for fertilized plots was consistently half that of non-fertilized. The variation in FRP:NPP observed with these species suggests that species have adopted different allocation strategies which may be related to functional type and seral stage (Grime 1979). Peak total NPP in our study occurred about the time of maximum leaf area (Coyle et al. 2016; Coyle and Coleman 2005; Coyle et al. 2008), which is consistent with other observations (Ryan et al. 2004). In addition, annual fine root production was correlated with total NPP, but only for CW ($P<0.001$, $r^2=0.40$) because LP ($P=0.89$) fine root production peaked at least one year before total NPP. While belowground carbon allocation is often directly related to total NPP and other autotrophic processes (Högberg et al. 2001; Irvine et al. 2005), our results show that autotrophic components of belowground carbon allocation are not always in phase with total NPP. This might explain why correlations between belowground carbon allocation and total NPP are not always evident (Litton et al. 2007). The eventual increase in FRP:NPP observed with CW is consistent with the increasing proportion of total belowground carbon allocation relative to gross primary productivity (Ryan et al. 2004) and increasing soil $CO_2$ efflux response with stand age (Wiseman and Seiler 2004; Yan et al. 2011), considering autotrophic respiration can account for half of soil $CO_2$ efflux (Hanson et al. 2000). In contrast, the decline in FRP:NPP found with LP is consistent with decreased soil $CO_2$ efflux with stand age (Klopatek 2002; Saiz et al. 2006). The links between fine-root dynamics, total NPP and soil $CO_2$ efflux remain an open question for understanding components of belowground carbon allocation, yet here we demonstrate that stand development is a critical control factor.

Resource availability effects on fine-root dynamics

Resource availability had subtle and inconsistent influence on fine-root variables compared to the predictable responses observed to root depth, initial root diameter, phenology or stand development. Consequently, it was not possible to generalize about even the direction of resource availability influence on fine-root dynamics. Fine-root biomass either increased relative to *C* or was unaffected by *I*, *F*, or *IF* (Fig. 1). Fine-root production and root length standing crop decreased with *I* relative to *C*; however, these variables increased with fertilization for CW and had no effect on LP (Fig. 4). The risk of mortality both increased and decreased relative to *C* depending on species and amendment treatment (Fig. 6b). Since risk of mortality is~~,~~ also expressed as root longevity, it also serves as a surrogate for fine-root turnover, since turnover is the inverse of root longevity. Based on this relationship we might conclude that carbon allocation to turnover in response to resource availability is equally inconsistent. Literature reports describe wide-ranging fine-root responses to soil moisture and nutrient availability, which supports the variable results we observe between species on fine-root production and turnover in response to experimental resource availability treatments. Previous studies reporting fine-root mortality and production responses to increased water availability demonstrate decreases (Gaul et al. 2008), increases (Meier and Leuschner 2008; Olesinski et al. 2011), or no effect (Joslin et al. 2001; King et al. 2002; Rytter 2013). Responses to nutrient availability are equally inconclusive (Brassard et al. 2009; Chen and Brassard 2013; Eissenstat et al. 2013; Hodge 2004; Nadelhoffer 2000; Norby and Jackson 2000). However, most of these studies compare chronological age rather than normalizing with a measure of stand development like stem biomass or stand basal area. Yet as described above, risk of fine-root mortality consistently responds to stand development, and other intrinsic factors. Accounting for these factors has important consequences for understanding responses to resource availability.

These inconsistencies contradict the concept that greater carbon or biomass allocation will be directed toward roots when soil resources are limited compared to when they are abundant (Axelsson and Axelsson 1986; Brassard et al. 2009; Brouwer 1983; Hunt and Lloyd 1987; Keyes and Grier 1981; Lambers 1983; Reynolds and Thornley 1982). Thus, our results combined with those of others considering fine-root dynamics do not support the general concept that lower amounts of growth-limiting resources will increase allocation to roots because that directional shift is observed in some, but not all cases. Crucially, compared to the dominant and consistent influence of stand development, rooting depth, diameter, phenology and species, the minor and directionally inconsistent responses to resource availability do not warrant the attention they receive. In fact, resource responses are so subtle that accounting for them never contradicts our inference that intrinsic factors primarily control fine-root dynamics (e.g., see Figs. 5, 7 and S7).

## Other fine-root control factors

Soil depth, root diameter, branch order, phenology and species exerted consistent influence on fine-root turnover as measured through mortality risk. Figure 7 illustrates the relative magnitude of factors controlling mortality risk observed for the given species based on chi-square values in Table 1. Soil depth was especially dominate in our study as an important intrinsic factor affecting mortality risk. Authors consistently report that roots growing in deeper soil have increased root longevity (Baddeley and Watson 2005; Coleman et al. 2000; Kern et al. 2004; Peek et al. 2006; Stover et al. 2010; Wells et al. 2002) and decreased rooting density (Jackson et al. 1996). In our study, soil depth explained the most variation of any factor for risk of root mortality (Table 2, Fig. S4) and biomass distribution (Coleman 2007). Not only were there fewer roots at depth, but deeper roots had longer lifespans. Considering the very different chemical and physical environment at depth (Fang and Moncrieff 2005), deeper roots are expected to be functionally distinct from those growing near the surface (Brassard et al. 2009; Chen and Brassard 2013; Iversen 2010; Pregitzer et al. 1998).

Root diameter was second to soil depth in explaining mortality risk. We measured root diameter using a continuous scale and conclude that the risk of mortality decreased 5.1% for every 0.1 mm increase in diameter. This confirms other reports that conclude fine-root lifespan increases with increasing root diameter class (Chen and Brassard 2013; Coleman et al. 2000; Kern et al. 2004; Wells and Eissenstat 2001). We chose to use root diameter as a co-factor over root branch order for both effectiveness and practicality. Root branch order is considered to be an important criteria for evaluating root function and lifespan (Chen and Brassard 2013; Comas et al. 2002; Fitter 1985; Fitter 1992; Jia et al. 2013; Majdi et al. 2001; McCormack et al. 2015; Pregitzer et al. 1997; Wells et al. 2002). Branch order explained a significant amount of variation in fine-root lifespan in our study for those roots where it was available; however, the branch order of most roots observed via MROTs was uncertain (86% not declared) because we could only confirm root order of the most distal roots with obvious root tips and those subtending. In contrast, we precisely measured root diameter on all roots. Diameter and root order are strongly correlated within a species (Comas et al. 2002; Guo et al. 2004; Jia et al. 2013; Pregitzer et al. 2002). Consequently, diameter measured on a continuous scale was a more practical and effective measurement than branch order when controlling for root morphology.

Phenology and species also influenced fine-root turnover and therefore are influential co-factors. We observed distinct annual oscillations of fine-root production with maxima during the growing season and minima during dormancy. Numerous reports describe similar phenological patterns of fine-root production and turnover (Baddeley and Watson 2005; Coleman et al. 2000; Joslin et al. 2001; Kern et al. 2004; King et al. 2002; McCormack et al. 2014; Rytter 2013; Tierney et al. 2003). The magnitude of fine-root production, mortality and live-root standing crop differed between tree species. Differences in root traits occur for species with different leaf habits (Vogt et al. 1996), relative growth rate (Comas et al. 2002; Comas and Eissenstat 2004; McCormack et al. 2012; McCormack et al. 2014; Wright and Westoby 1999), and among species from different biomes (Finer et al. 2011a; Finer et al. 2011b; Vogt et al. 1996). Thus, controlling for phenology and comparing functionally distinct species is also vital for assessing responses of fine-root dynamics to soil resources. Due to the variety of species distinction, Figure 7 does not attempt to rank the influence of species among other factors controlling fine-root dynamics. Rather, the figure seeks to demonstrate within a given species the relatively minor influence of resource availability among other factors, based on data presented in Table 2.

## Conclusion

Our study identified stand development as a major factor explaining the variation in fine-root dynamics. Furthermore, to determine accurately the response of fine-root dynamics to resource availability, we conclude that it is necessary to consider the influence of intrinsic factors, including stand development, rooting depth, initial root diameter, phenology and species. Multivariate analysis using Cox' regression demonstrates a useful approach to control for the influence of intrinsic factors when attempting to measure the relatively minor effects of resource availability. The variation we observed among species and resource availability treatments and the dependence upon measures of stand development demonstrates that generalizations about the directional response of fine-root dynamics to resource availability require reevaluation.

Our results have important implications for understanding and modeling factors controlling carbon allocation to fine roots. We understand with certainty the direction if not magnitude of several intrinsic factors controlling fine-root turnover like depth, root diameter, and phenology. Here we quantified the relative impact of stand development, which also appears to exert consistent controls on fine-root turnover. Predictive models should include turnover functions that vary by depth, root diameter and stand development to predict more accurately carbon flux from live roots to soil organic matter. Models that include intra-annual time steps should include phenological shifts from production in spring to mortality in autumn. However, there is a necessity to revisit those models that include generalized increases in carbon allocation to fine roots especially when ~~water and nutrients are~~soil moisture is limiting. Such functions exist in many prominent ecosystem process models (Landsberg and Waring 1997; Parton et al. 2010; Running and Gower 1991), some of which are now used in land models to predict continental- and global-scale responses to environmental change (Lawrence et al. 2011; Smithwick et al. 2014). Based on our results, and those of other researchers, the modeling community should incorporate details introduced by intrinsic factors controlling fine roots (cf. Franklin et al. 2012) and suspend the use of simplistic resource availability controls to describe allocation to fine roots.

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## Figure legends

Figure 1. Annual biomass and length (standing crop) of cottonwood (CW) and loblolly pine (LP) fine roots <1 mm in diameter in response to soil nutrient and water availability treatments. The coring approach measured biomass and the MROT approach measured length. Treatments included un-amended control (*C*), irrigated only (*I*) fertilized only (*F*) and irrigated plus fertilizer (*IF*). Samples were collected to a depth of 105 cm from replicate plots (n=3) for six years following establishment. Error bars represent standard error of the mean.

Figure 2. Relationship between live fine-root biomass and live-root length for cottonwood (CW) and loblolly pine (LP). This compares biomass sampling in November to the nearest MROT imaging date. Each point is the treatment mean (n=3) for an observation year. Natural-log transformed data included roots 1 mm diameter or less to a depth of 105 cm. Regression lines between species or among treatments were not significantly different. The pooled regression equation is $y$=1.10 $x$ - 1.69 ($P$<0.001, $r^2$=0.73).

Figure 3. Cumulative fine-root-length production (a) and cumulative root-length mortality (b) for cottonwood (CW) and loblolly pine (LP) during the first six years following planting. The length of live fine roots (c) is the difference between cumulative production and cumulative mortality. Points are the average of all treatments. Shaded vertical bars represent the growing season that started April 1st and ended October 1st. The right biomass axis converts from length values using

regression equation from Fig. 2. Error bars are standard errors (n=12). The P-values are for Kolmogorov-Smirnov two-sample non-parametric tests comparing lines within each panel.

Figure 4. Average annual production increment (a), annual mortality increment (b) and live-root length (crop) (c) for cottonwood (CW) and loblolly pine (LP) grown with (Fert) or without (No Fert) fertilization treatments and averaged for irrigation treatments. Average annual production increment, annual mortality increment and live-root length (crop) (d) grown with irrigation (*I*), fertilization (*F*), or their combination (*IF*) compared with untreated control (*C*) and averaged for species. Error bars are standard errors (n=36). Bars with the same letter within each variable are not significantly different (Tukey's HSD, α=0.10).

Figure 5. Cox regression model estimates of fine-root lifespan versus stem biomass for cottonwood (CW) and loblolly pine (LP) grown with irrigated (*I*), fertilized (*F*), or the combined (*IF*) treatments relative to the untreated control (*C*). Lifespan is median root survival predicted using Equation 1 and coefficients shown in Table 2. Error bars are 95% confidence intervals. We maintained cofactors at the following references levels: season = winter, soil depth = 1 cm and root diameter = 0.5.

Figure 6. Risk of fine-root mortality for (a) three seasons of the year relative to that observed in winter, and (b) for cottonwood (CW) and loblolly pine (LP) grown with irrigated (*I*), fertilized (*F*), or the combined (*IF*) treatments relative to the untreated control (*C*). Seasons were designated base on solstice and equinox dates. Percentages were calculated from model

coefficients as % Mort = $100 \cdot (e^{\beta s} - 1)$, where $\beta$ is the parameter estimate from Table 2 for season $s$.

Figure 7. Conceptual model illustrating the relative magnitude of different controls on fine-root dynamics. The width of the pyramid and shading reflects the relative magnitude of each factor. As the width narrows and shading decreases, the magnitude of the response decreases. Likewise, as the width narrows and shading decreases, the consistency and direction of the response across species decreases.

Table 1. Repeated measures analyses for annual fine-root variables during six years following establishment. Effects evaluated include irrigation (*I*), fertilization (*F*), species (*S*), and year (*Y*). Roots 1 mm diameter or less to a depth of 105 cm were included. Analysis parameters shown include the *f*-test statistic (*f*) and *p*-values (*p*). Significant p-values ($P\leq0.05$) are in bold typeface, while those that are marginally significant ($p\leq0.10$) are underlined. Toeplitz with two bands was the covariate structure selected for biomass, whereas standard Toeplitz was selected for all MROT-derived data.

| Effect | Fine-root biomass | | MROT live-root length | | MROT cumulative production | | MROT cumulative mortality | | MROT production increment | | MROT mortality increment | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *f* | *p* | *f* | *p* | *f* | *p* | *f* | *p* | *f* | *p* | *f* | *p* |
| *I* | 5.1 | **0.03** | 2.9 | 0.10 | 2.6 | 0.12 | 1.5 | 0.24 | 2.2 | 0.15 | 2.2 | 0.15 |
| *F* | 15.8 | **<.01** | 4.3 | **0.05** | 1.3 | 0.26 | 0.2 | 0.71 | 0.7 | 0.41 | 0.4 | 0.55 |
| *I*×*F* | 0.3 | 0.57 | 3.4 | 0.08 | 3.4 | 0.07 | 2.3 | 0.15 | 4.6 | **0.04** | 3.3 | 0.08 |
| *S* | 45.1 | **<.01** | 25.3 | **<.01** | 10.7 | **<.01** | 2.4 | 0.14 | 13.1 | **<.01** | 5.1 | **0.03** |
| *I*×*S* | 1.4 | 0.24 | 1.2 | 0.28 | 0.3 | 0.58 | 0.0 | 0.90 | 0.1 | 0.73 | 0.0 | 0.85 |
| *F*×*S* | 1.5 | 0.24 | 9.1 | **<.01** | 5.4 | **0.03** | 2.4 | 0.14 | 5.2 | **0.03** | 4.0 | 0.05 |
| *I*×*F*×*S* | 0.9 | 0.36 | 0.6 | 0.44 | 0.4 | 0.54 | 0.2 | 0.66 | 0.7 | 0.40 | 0.5 | 0.48 |
| *Y* | 49.7 | **<.01** | 29.2 | **<.01** | 41.6 | **<.01** | 90.0 | **<.01** | 39.5 | **<.01** | 42.5 | **<.01** |
| *I*×*Y* | 1.1 | 0.37 | 1.5 | 0.24 | 1.6 | 0.18 | 1.1 | 0.39 | 2.2 | 0.07 | 1.2 | 0.33 |
| *F*×*Y* | 3.0 | **0.02** | 0.4 | 0.83 | 0.4 | 0.85 | 1.3 | 0.31 | 0.4 | 0.84 | 0.2 | 0.97 |
| *I*×*F*×*Y* | 1.6 | 0.18 | 0.5 | 0.80 | 1.4 | 0.25 | 2.1 | 0.10 | 0.9 | 0.50 | 0.4 | 0.88 |
| *S*×*Y* | 2.5 | **0.04** | 2.7 | **0.04** | 3.2 | **0.02** | 3.9 | **0.01** | 2.8 | **0.03** | 0.4 | 0.87 |
| *I*×*S*×*Y* | 0.6 | 0.73 | 0.4 | 0.82 | 0.9 | 0.51 | 0.5 | 0.80 | 0.7 | 0.67 | 0.5 | 0.77 |
| *F*×*S*×*Y* | 1.7 | 0.15 | 1.5 | 0.22 | 1.8 | 0.14 | 1.2 | 0.34 | 1.9 | 0.12 | 2.4 | 0.05 |
| *I*×*F*×*S*×*Y* | 0.6 | 0.68 | 0.7 | 0.64 | 0.5 | 0.75 | 1.8 | 0.16 | 0.5 | 0.79 | 0.6 | 0.71 |

Table 2. Analysis of maximum likelihood model parameter estimates for factors controlling fine-root mortality risk using Cox' regression. The chi-square and associated *P*-value (*p*) tests the null hypothesis that the parameter estimate is zero. Categorical variables are compared to reference values: Winter for season, CW for Species, untreated controls for irrigated (*I*) and fertilized (*F*).

| Factors | Estimate | Std Error | Chi-Square | *p* |
|---|---|---|---|---|
| Soil Depth (cm) | -0.014 | 0.0002 | 5633 | <.001 |
| Stem biomass (Mg ha$^{-1}$) | 0.023 | 0.001 | 737 | <.001 |
| Rt Dia @ appearance (mm) | -0.705 | 0.022 | 1012 | <.001 |
| Spring to winter | -0.167 | 0.012 | 211 | <.001 |
| Summer to winter | -0.135 | 0.013 | 109 | <.001 |
| Autumn to winter | -0.132 | 0.015 | 77 | <.001 |
| Species (*S*) | 0.173 | 0.016 | 111 | <.001 |
| Irrigation (*I*) | -0.276 | 0.019 | 209 | <.001 |
| Fertilization (*F*) | -0.203 | 0.016 | 157 | <.001 |
| *S* x *I* | 0.368 | 0.026 | 203 | <.001 |
| *S* x *F* | -0.012 | 0.024 | 0.3 | 0.601 |
| *F* x *I* | 0.028 | 0.024 | 1 | 0.252 |
| *S* x *F* x *I* | 0.057 | 0.036 | 2 | 0.118 |

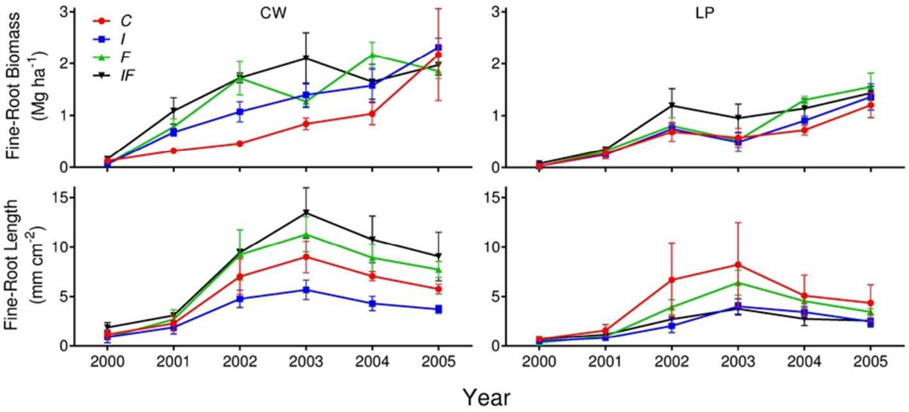

Figure 1

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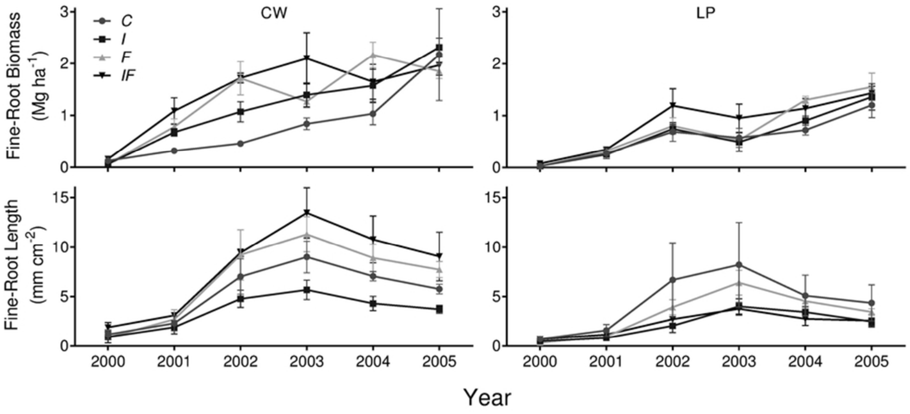

Figure 1

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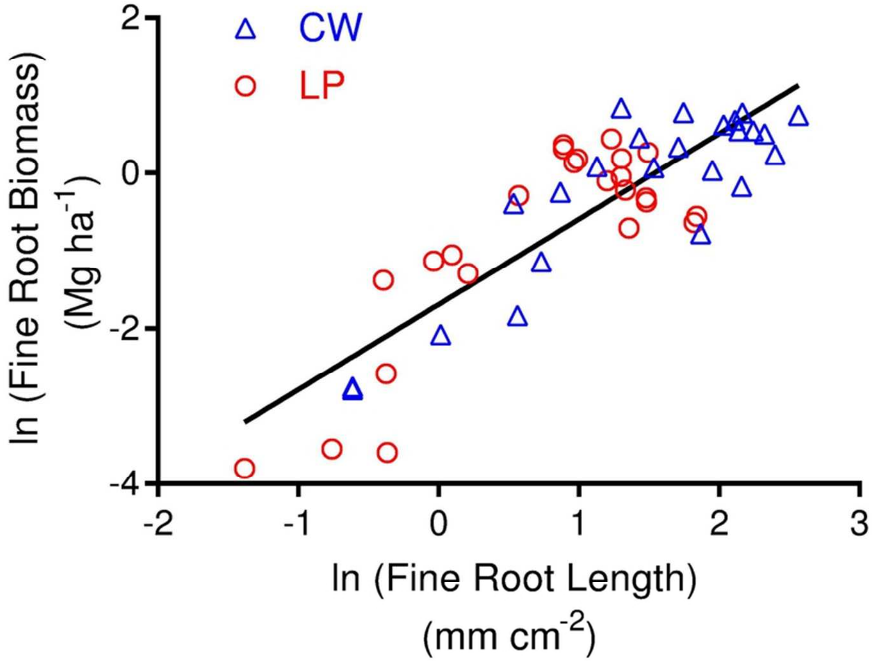

Figure 2

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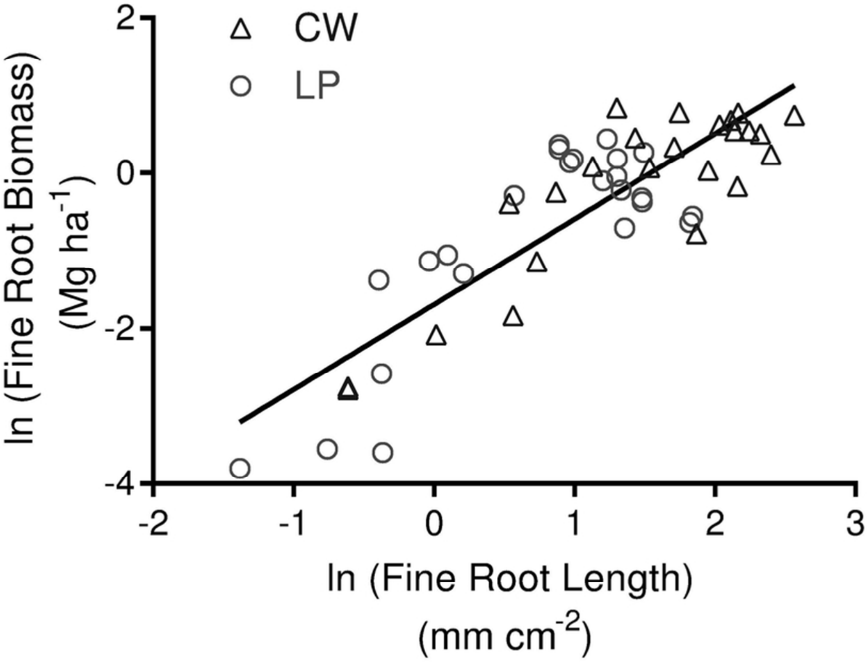

Figure 2

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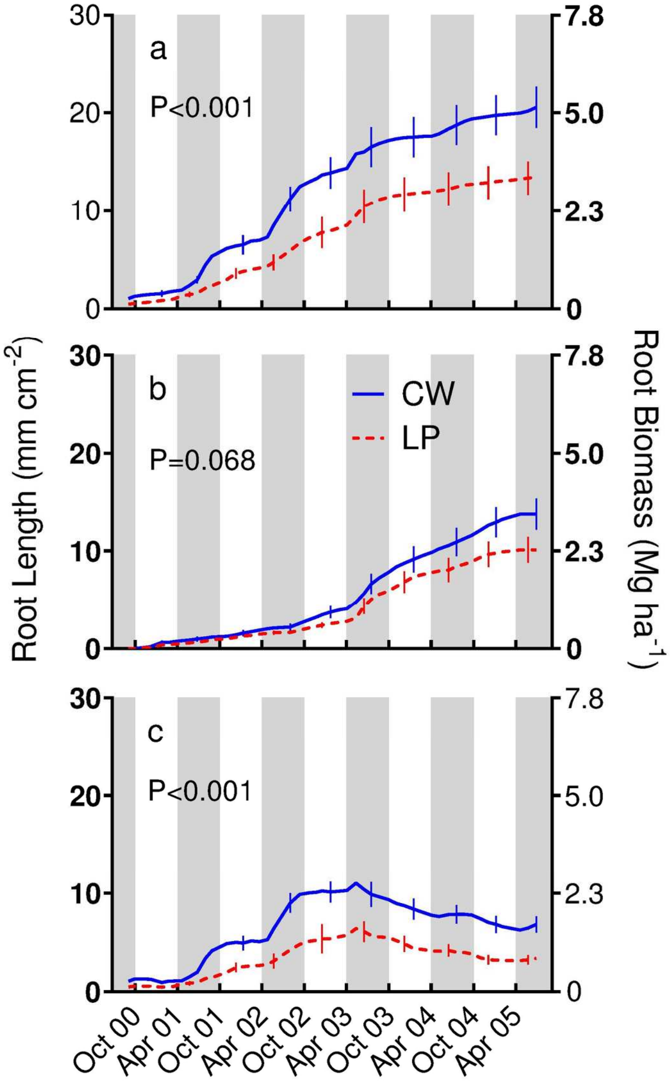

Figure 3

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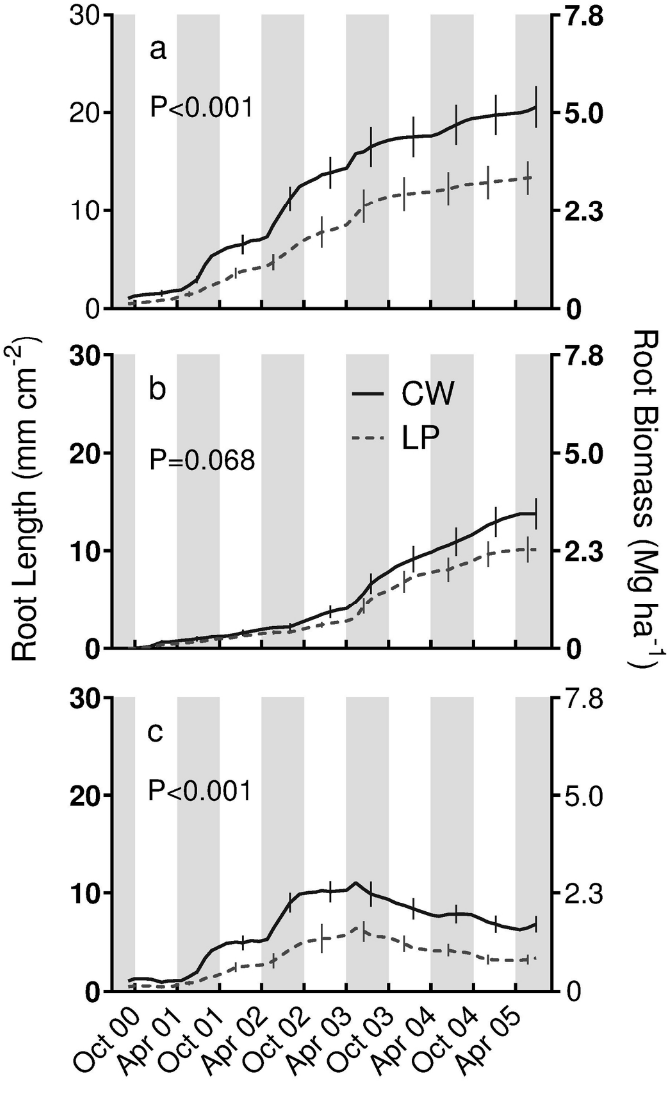

Figure 3

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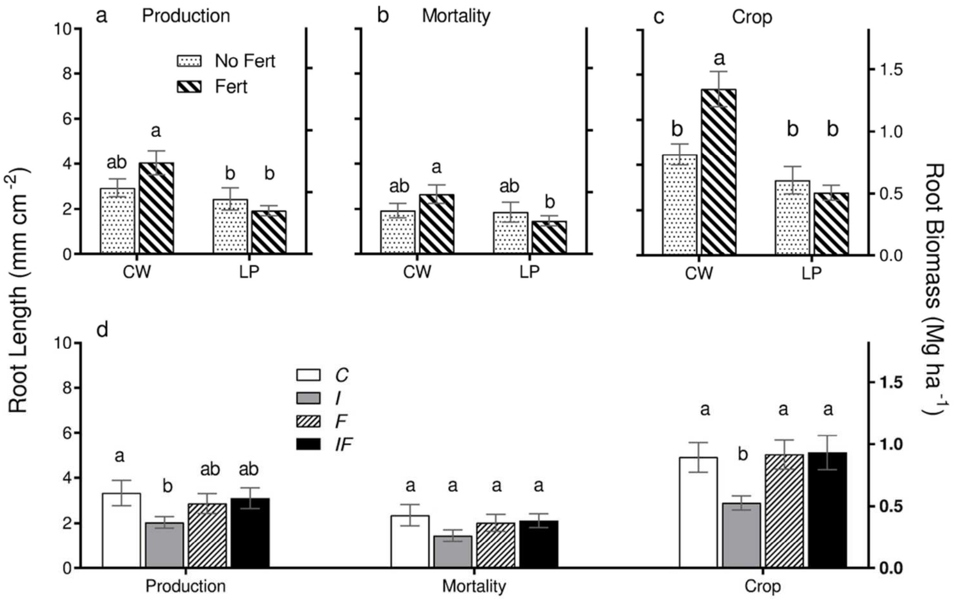

Figure 4

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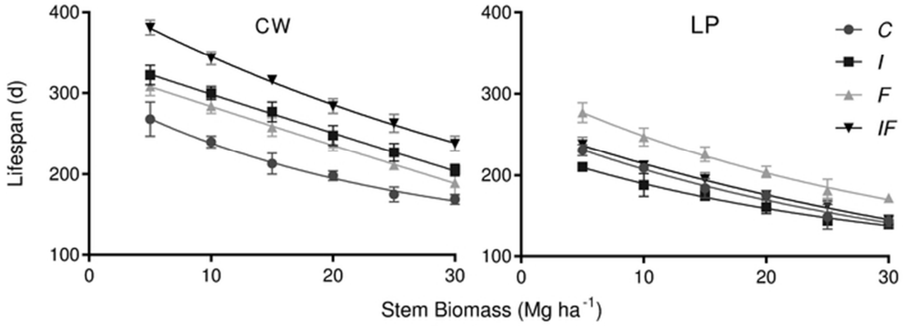

Figure 5

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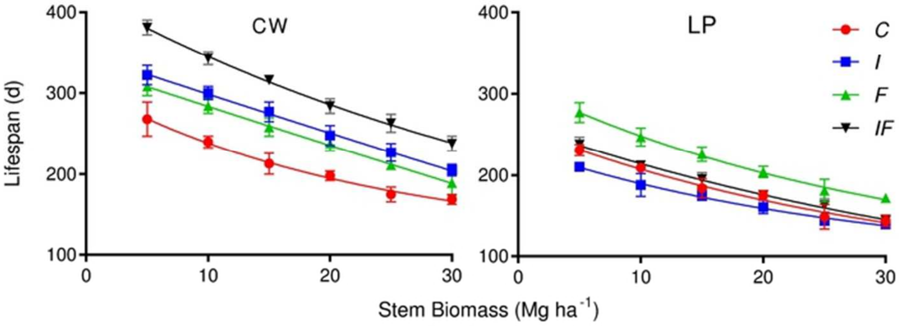

Figure 5

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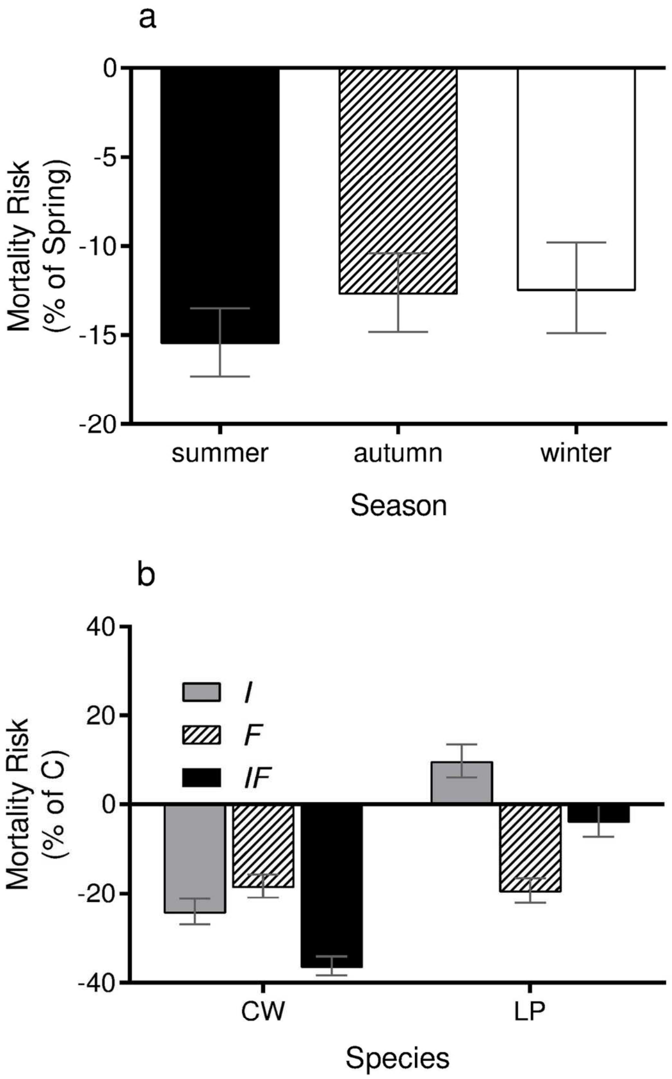

Figure 6

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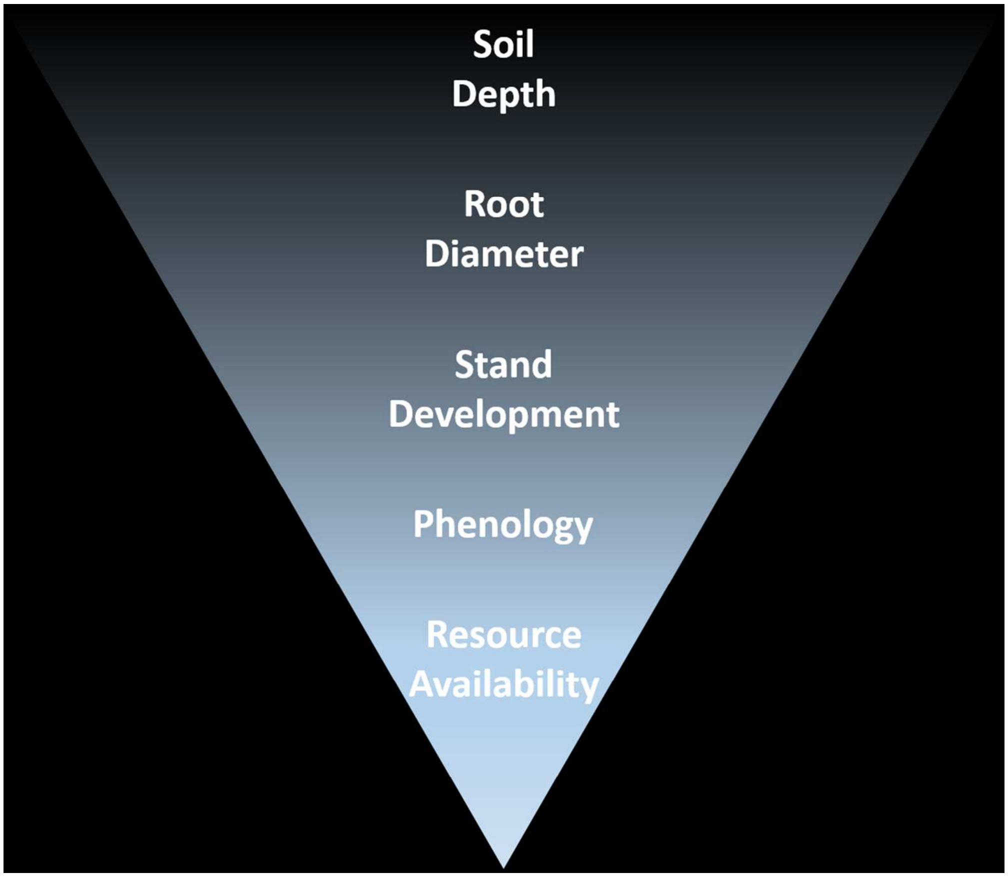
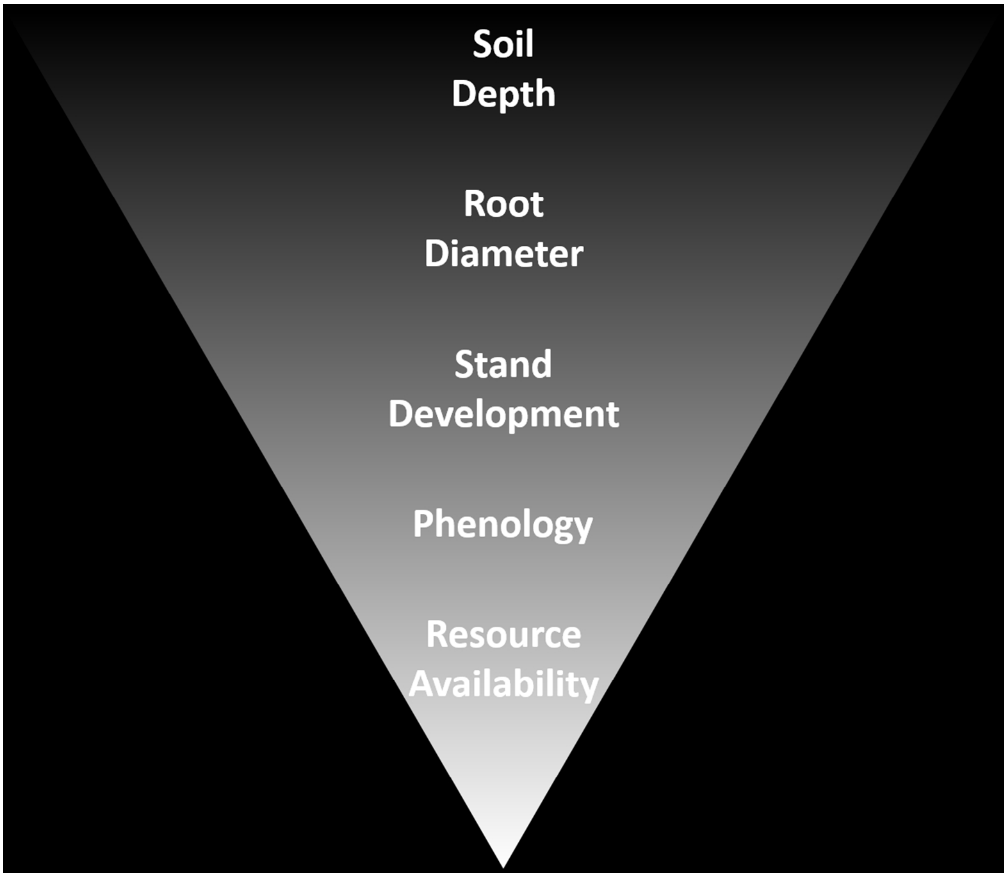

Soil Depth
Root Diameter
Stand Development
Phenology
Resource Availability

Figure 7


Soil
Depth
Root
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Development
Phenology
Resource
Availability

Figure 7