

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

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5 modifications from resource availability
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36 **Running title:** Inherent and resource controls on fine-roots
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40 **Keywords:** fertilization; fine-root biomass; intrinsic and extrinsic factors; irrigation; hazard
41 analysis; phenology; *Pinus taeda*; *Populus deltoides*; short rotation woody crops.
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1 **Abstract**

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

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

For Peer Review

28 Introduction

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

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44 Intrinsic factors such as root diameter, rooting depth, species and phenology, exert dominant
45 controls on fine-root dynamics. Lifespan consistently increases with root diameter and rooting
46 depth (Chen and Brassard 2013). Species differences, such as evergreen vs. conifer, or growth
47 rate, also consistently affect fine-root dynamics (Eissenstat and Yanai 1997; McCormack et al.
48 2014). Temporal factors occur on intra- or inter-annual time scales; however, we know more
49 about the former than the latter primarily because it is easier to make consistent measurements
50 over seasonal time scales. On intra-annual time scales, root production is maximum in spring

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3 51 and summer, while root mortality occurs mainly in fall and winter (Brassard et al. 2009). This
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5 52 pattern may be disrupted in climates with predominate summer droughts, where a sharp peak of
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7 53 root production occurs in spring (Misson et al. 2006), or bimodal peaks may occur in spring and
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9 54 fall (Atkinson 1980; Comas et al. 2005; Santantonio and Hermann 1985). Perhaps the least well-
10
11 55 understood intrinsic factor influencing fine-root dynamics is stand development. Inter-annual
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13 56 studies over time scales relevant to forest stand development are rare because of difficulties in
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15 57 sustaining observations over requisite observation times, or in finding comparable stands for
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17 58 chronosequence studies. Available studies indicate that as young forest stands establish and
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19 59 inter-tree competition increases, fine-root biomass becomes relatively constant and may even
20
21 60 decline thereafter (Brassard et al. 2009; Schoonmaker et al. 2016); suggesting turnover also
22
23 61 increases during stand development. However, there is little information on the progression of
24
25 62 fine-root dynamics observed in the same stand through different forest developmental stages
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27 63 (Borja et al. 2008; Brassard et al. 2009; Yuan and Chen 2010). Early stand development appears
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29 64 to show the most dynamic changes followed by stable or slow declines in fine-root standing crop
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31 65 as canopies differentiate through stem exclusion. These synthesis reports provide some insight
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33 66 into the net result of fine-root dynamics, but we still have a very poor understanding of how the
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35 67 individual processes of production and mortality change through stand development.
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44 69 Soil resources that have potential to influence fine-root production primarily include soil
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46 70 moisture and nutrient availability. We currently have an inadequate understanding of how
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48 71 resource availability influences fine-root production and mortality compared to root diameter,
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50 72 rooting depth, species and phenology. Investigators study the influence of soil moisture on fine-
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52 73 root biomass, production and lifespan using drought, irrigation, or flooding in both manipulated
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3 74 and natural-gradient studies with inconsistent and contradictory results. For example,
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5 75 manipulative studies report that higher relative soil moisture either: (1) decreases both fine-root
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7 76 mortality and production simultaneously (Gaul et al. 2008); (2) increases both production and
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9 77 mortality (Meier and Leuschner 2008; Olesinski et al. 2011); (3) increases production, not
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11 78 mortality (Bauerle et al. 2008; Katterer et al. 1995; Majdi and Andersson 2005; Rytter 2013); or
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13 79 (4) has no influence on fine-root dynamics (Joslin et al. 2001; King et al. 2002; Rytter 2013).
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15 80 Consequently, a meta-analysis found only a slight positive effect of increased soil moisture on
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17 81 root lifespan (Chen and Brassard 2013). Results from natural precipitation gradients have been
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19 82 equally inconclusive (Finer et al. 2011b; Gill and Jackson 2000; Hertel et al. 2013; Yuan and
20
21 83 Chen 2010). Various approaches have also yielded inconsistent and contradictory results that do
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23 84 not provide a clear understanding of the magnitude or even the direction of fine-root responses to
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25 85 nutrient availability. For example, reviews considering soil nutrients find that fine-root
26
27 86 production and mortality increase and decrease in response to nitrogen (N) and phosphorus
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29 87 additions, and they suggest that lack of consensus among reports is due to methodological
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31 88 differences or site variation (Brassard et al. 2009; Chen and Brassard 2013; Eissenstat et al.
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33 89 2013; Hodge 2004; Nadelhoffer 2000; Norby and Jackson 2000). Some reports suggest that we
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35 90 consider other factors, including intrinsic controls, to understand the response of fine-root
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37 91 production and turnover to resource availability. For example, Joslin et al. (2001) show that the
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39 92 response to favorable moisture availability was greater early in the growing season compared to
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41 93 later and conclude that to understand the response to water availability, it is necessary to account
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43 94 for plant phenology. Similarly, it was necessary for Kern et al. (2004) to consider only smaller
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45 95 diameter roots at the surface to show effects of N amendments on fine-root production.
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3 97 Here we empirically evaluate fundamental controls of fine-root production and lifespan using
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5 98 long-term observations of deciduous hardwood and evergreen conifer fine roots grown with
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7 99 water and nutrient amendments. Our approach is unique in that we observe fine-root dynamics in
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10 100 short-rotation woody crop plantations beginning at stand establishment and continuing past the
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12 101 point of full root occupation (root closure) as inter-tree competition increases and crown
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14 102 differentiation begins. Short-rotation forests mature rapidly and therefore are practical models for
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16 103 observing stand development over relatively short periods. We selected a site with low soil
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18 104 moisture and nutrient availability so that applied resource amendment treatments created a range
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20 105 in site quality. In addition, we determined the response of fine-root production, lifespan, and
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22 106 standing crop to soil resources and to various intrinsic factors known to control fine-root
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24 107 dynamics such as stand development, depth, root diameter, species, and phenology. Our
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26 108 objective was to determine the relative control that soil resources have on fine-root dynamics
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28 109 compared with intrinsic factors. We took advantage of our long-term observations to understand
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30 110 how fine-root dynamics change through stand development. Specifically, we hypothesized that
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32 111 (1) fine-root production would be highest, and mortality would be lowest, early in stand
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34 112 development; (2) as development continued, production would decrease and mortality would
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36 113 increase until the two processes reach an equilibrium where the standing crop remains somewhat
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38 114 constant; and (3) it would be necessary to control for dominant intrinsic factors to define
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40 115 accurately the subtle effects of resource availability on fine-root dynamics.
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49 117 **Materials and Methods**

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3 118 This research is part of a long-term forest productivity study designed to evaluate above- and
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5 119 belowground growth responses of several fast growing tree species. Coleman et al. (2004)
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7 120 describe in more detail the site, plant materials, and experimental design.
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12 122 Site Description and Preparation
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14 123 We conducted the experiment at U.S. Department of Energy Savannah River Site, a National
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16 124 Environmental Research Park near Aiken, SC in the Carolina Sand Hill physiographic region (lat
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18 125 33.387°, lon -81.676 °). The soil is predominately a Blanton sand (thermic Grossarenic
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20 126 Paleudults) with loamy subsoil at 120 to 200 cm depth (Rogers, 1990). Previous vegetation was
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22 127 plantation pine with an oak understory. We removed slash >15-cm diameter and pulverized
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24 128 stumps and remaining debris to less than 5cm diameter pieces, and incorporated biomass to 30
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26 129 cm (RS-500 Reclaimer/Stabilizer, CMI Corp., Oklahoma City, USA). Additional preparation
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28 130 consisted of disking and lime amendments.
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33 131 34 35 132 Plant Material and competition control

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37 133 The two species included in this study were eastern cottonwood (CW, *Populus deltoides* Bartr.,
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39 134 cv. ST66: Issaquena County, MS (Eckenwalder 2001)) and loblolly pine (LP, *Pinus taeda* L., cv.
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41 135 7-56, Williamsburg County, SC (Magbanua et al. 2011)). Bare-root LP seedlings were planted
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43 136 February 2000. We collected CW cuttings from stool beds during the previous winter and
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45 137 planted them April 2000. Rigorous and continuous weed control eliminated understory
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47 138 competition, so we could be certain live roots were solely plantation trees.
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53 140 Experimental Design
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3 141 Twenty-four plots included two species, four treatments and three replicate blocks (Fig. S1).
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5 142 Each 0.22 ha treatment plot had a central 0.04 ha measurement plot with 54 trees arranged in 2.5
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8 143 × 3 m spacing. There were at least four treated border rows (12 m) surrounding measurement
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10 144 plots. Treatments consisted of control (*C*), irrigation (*I*), fertilization (*F*) and irrigation +
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12 145 fertilization (*IF*). Within each of three block, the four treatment plots of a given species were
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14 146 grouped together to minimize within-block site gradients. We used drip irrigation to apply up to
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16 147 5 mm d⁻¹ between April and October to meet evaporative demand and ensure favorable soil
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18 148 moisture. The quantity equals average regional evaporation during those months and was
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20 149 designed to assure favorable soil moisture. During the study, average annual rainfall was 809
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22 150 mm. Irrigation supplied an average of 551 mm y⁻¹. We applied fertilizer at rates of 40 kg N ha⁻¹
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24 151 yr⁻¹ in 2000, 80 in 2001, and 120 in 2002-2005. Fertilizer increased annually to correspond with
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26 152 demand of growing trees based on estimated N mineralization rate, expected productivity, and
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28 153 tree nutrient content (Coleman et al. 2004). We split annual fertilizer treatments among 26
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30 154 weekly applications and applied them with drip irrigation. Fertilizer application supplied enough
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32 155 water to deliver liquid fertilizer and flush drip tubes (5 mm wk⁻¹). Control plots received 5 mm
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34 156 water wk⁻¹ to maintain experimental control. Thus, non-irrigated plots (i.e. *C* and *F*) received 130
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36 157 mm yr⁻¹ of irrigation in addition to annual precipitation.
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44 159 Stand inventory included annual measurements of stem diameter for all plot trees as described in
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46 160 (Coyle et al. 2016). We expressed diameter values as stand basal area and determined stem
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48 161 biomass using treatment- and species-specific allometric equations developed on several
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50 162 occasions. The progression of basal area and stem biomass over time quantitatively and
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3 163 continuously described stand development. These measures of stand development represent
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5 164 ontogenetic progression and substitute for time in analytical models.
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10 166 Each November, we determined fine-root biomass (< 5 mm diameter) from five random
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12 167 locations per plot as detailed by Coleman (2007). Briefly, we removed soil cores (5 cm diameter)
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14 168 from 0-15, 15-45, and 45-105 cm depths. Five cores were taken from each plot at both shallow
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16 169 depths for a total of 15 cores for each amendment-by-species treatment combination. A single
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18 170 core per plot was taken below 45 cm for a total of three per treatment combination. We
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20 171 separated fine roots into two classes based on diameter: <1 mm and 1 to 5 mm. November
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22 172 sampling corresponded with late autumn peak root length from a previous study with similar
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24 173 species (Coleman et al. 2000). We collected two samples along the irrigation drip line and two
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26 174 perpendicular to the drip line at points one-half and one-quarter the distance between trees, and
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28 175 collected a fifth sample at the center between four trees. These relative sample locations captured
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30 176 expected spatial heterogeneity in fine-root biomass resulting from drip application (Coleman
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32 177 2007). Sampling in each year occurred at five different randomly selected trees per plot with one
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34 178 of the five relative sample locations assigned to each. Double sampling did not occur from any
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36 179 one location. We washed roots by elutriation, manually separated live roots from dead organic
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38 180 matter, oven dried cleaned roots (60 °C), and weighed them to the nearest 0.01 mg.
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46 47 182 Minirhizotron observation tubes

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49 183 We measured fine-root production and lifespan using minirhizotron observation tubes (MROT).
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51 184 Tubes (5 cm acrylic) were installed at a 45° angle to a depth of 105 cm in May 2000. We painted
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53 185 the exposed aboveground portion of MROT black to limit light penetration and then white to
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3 186 limit heat absorption, and plugged the end with foam pipe insulation. The foam penetrated below
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5 187 ground line within MROT's to limit transfer of aboveground temperature. The upper end of the
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7 188 foam insulation was glued to the inside bottom of a topless aluminum beverage can so that it
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9 189 covered the tube when in place to limit entry of dirt and moisture. Plumber test plugs stoppered
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11 190 underground ends of each tube. A 5 cm pipe hanger secured the aboveground end to a 1.3 cm
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13 191 metal conduit driven 45 cm into the ground. We placed five MROT's per plot, or 15 tubes for
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15 192 each amendment-by-species treatment combination. Coleman et al. (2004) illustrate the
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17 193 locations, which were the same relative to trees as described above for soil samples. As with soil
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19 194 core samples, we randomly selected the five-reference trees and assigned one of the five relative
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21 195 locations to each. Soil coring for root biomass never occurred near MROT's.
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28 197 We installed MROT's in bare ground prior to substantial expansion of seedling roots to avoid
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30 198 installation effects cautioned by previous authors (Coleman et al. 2000; Joslin and Wolfe 1999;
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32 199 Krasowski et al. 2010; Olesinski et al. 2011). Previous observations of mrot installation impacts
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34 200 on fine root dynamics occur in established forests where installation prunes existing roots and
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36 201 alters production and mortality for one to two years as roots recover. Bare-field installation
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38 202 avoids those impacts because placement occurs before roots reach soil viewed by MROT's. There
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40 203 may also be disturbance impacts of tube installation on nutrient release (Johnson et al. 2001);
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42 204 however, this would be minimal in our study because site preparation activities disturbed the
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44 205 surface 30 cm where fine roots typically grow during seedling establishment. Consequently, it is
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46 206 reasonable to assume that observation directly following bare-field installation are free of
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48 207 installation artifacts.
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3 209 We captured digital images every 5 weeks starting September 2000 through June 2005 at precise
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5 210 locations along the upper MROT surface using a digital camera equipped with an indexing
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7 211 handle (BTC2; Bartz Technology, Santa Barbara, CA). To process images, operators used
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9 212 Rootracker (Duke University, Durham, NC, USA) image analysis software to tally observations
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11 213 of root length, width, branch order and condition. We could not positively identify branch order
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13 214 for all observed roots. We identified branch order for first-order root tips that appeared in the
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15 215 field of vision and for higher order roots subtending those tips. We categorized roots into three
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17 216 condition classes: new, previously observed, and missing, which allowed us to determine root
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19 217 lifespan. We did not include a condition class for dead roots due to the subjectivity in
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21 218 determining their viability. Consequently, we only considered roots to be dead when missing,
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23 219 which undoubtedly overestimated root longevity.
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31 Data analysis

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33 222 We calculated fine-root production and mortality from measurements collected during image
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35 223 analysis. Incremental fine-root length production was the sum of new root length between
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37 224 consecutive imaging dates, while mortality was the sum of missing root length. Cumulative
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39 225 production and mortality were the sum of all previous incremental production and mortality.
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41 226 Live-root length was the difference between cumulative production and cumulative mortality up
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43 227 to that observation date. Annual increment of fine-root length production and mortality was the
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45 228 sum of increments for that year's 10 observation dates. Annual cumulative production and
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47 229 mortality was the difference of that accumulated or missing between final observation dates of
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50 230 consecutive years. Annual live-root length, or annual standing crop, was the average crop of all
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3 231 observation dates that year. Equations for each of these values are in the supplemental
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5 232 information.
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10 234 We used repeated-measures analysis to assess fine-root biomass and MROT treatment responses
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12 235 over time. Data analysis evaluated all dependent variables using averaged within-plot
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14 236 measurements (n=3 replicate plots). Fixed factors included fertilization (*F*), irrigation (*I*), and
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16 237 species (*S*). Year (*Y*) was the repeated factor with plot being the random subject factor. Proc
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18 238 Mixed (SAS Institute Inc., Cary, NC) performed the analysis with $\alpha=0.05$. The Kenward-Roger
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20 239 method calculated denominator degrees of freedom. Lowest corrected Akaike's information
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22 240 criterion (AICC) (Littell et al. 2006, p.183) identified the best covariate structure for each
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24 241 dependent variable from a subset of appropriate structures. Tukey's HSD test compared
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26 242 treatment means. Temporal traces of fine-root variables measured with MROT were compared
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28 243 between species using the Kolmogorov- Smirnov two-sample test (SAS Proc NPar1Way) with
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30 244 $\alpha=0.05$.

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37 246 Survival analysis

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40 247 We achieve greater precision on the fate of fine-roots observed with MROT's using survival
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42 248 analysis compared with the repeated measures analysis (Coleman et al. 2000; Kern et al. 2004).
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44 249 The life-table method produced survival curves (Proc Lifetest, SAS Institute Inc., Cary, NC).
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46 250 Right-censored roots were those that had not disappeared by the end of the experiment. Log rank
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48 251 and Wilcoxon test compared survival curves. We estimated root lifespan from survival functions
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50 252 as the time of median root survival. Several fine-root populations of interest did not reach
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52 253 median survival, so it was not possible to determine lifespan universally using observed median
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3 254 root survival. Consequently, Cox' model estimated survival functions with corresponding
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5 255 median root lifespan.

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10 257 Cox' proportional hazards model estimates fine-root hazard ratios. Cox' model uses the partial
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12 258 likelihood function to estimate parameters associated with each of the covariates (Allison 2010;
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14 259 Savarese and Patetta 2010; Wells and Eissenstat 2001). It is a log-transformed exponential model

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19 261 $\log h_i(t) = \log \lambda_0(t) + \beta_1 x_{i1} + \dots + \beta_k x_{ik}$ Equation 1

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24 263 where $h_i(t)$ is the hazard of mortality for root i at time t , $\lambda_0(t)$ is an unknown and unspecified
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26 264 positive function, and β is the coefficient for each of k covariates. The SAS implementation of
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28 265 Cox' regression (Proc Phreg, SAS Institute Inc., Cary, NC) uses the partial likelihood function to
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30 266 eliminate $\lambda_0(t)$, assist development of the best model, calculate hazard ratios, estimate survival
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32 267 functions, and predict median lifespan (Allison 2010). Exponentiation of β_k model estimates
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34 268 calculates hazard ratios and provides odds ratios for the risk of root mortality given specified
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36 269 conditions as compared with a reference condition (Allison 2010). Mortality (M) based on the
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38 270 odds ratio was calculated as $M = 100 \cdot (e^\beta - 1)$, where β is the model coefficient for a factor
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40 271 expressed as a percentage of the reference condition.

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47 273 The selected Cox model included main effects of intrinsic covariates that stratified various fine-
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49 274 root conditions, as well as main effects and interactions of the experimental factors of S , F and I .
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51 275 The fully parameterized model—including all interactions (up to seven-way) among covariates

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3 276 and experimental factors—was difficult to interpret, and only improved the model fit (AIC) by
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5 277 0.5%. Therefore, we simplified the model to include main effects of covariates, experimental
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8 278 factors, and interactions among experimental factors.
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12 280 We compared stand age, or year of measurement to other variables that might better represent
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14 281 stand development as an independent variable in repeated measures models testing fine-root
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16 282 biomass, length and dynamics, and in Cox' hazard model. The stand development variables
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18 283 tested included stand basal area, stem biomass, stem volume and total stand biomass. We
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20 284 selected the best fitting models based on lowest Akaike's information criterion (AIC).
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26 286 **Results**

31 288 Fine-root biomass and length

33 289 Root biomass distribution compared favorably with the frequency of roots appearing in MROT, with
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35 290 some exceptions. The majority of fine-root biomass was ≤ 1 mm diameter. Roots ≤ 1 mm
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37 291 diameter composed 61% of CW biomass and 51% of LP biomass, with the remainder of fine-
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39 292 root biomass comprised of 1 to 5 mm diameter roots. In contrast, over 98.9% of CW and 97.0%
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41 293 of LP roots appearing in MROT were < 1 mm diameter (Fig. S2). When considering roots at the
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43 294 end of their lifespan, we found an almost identical diameter distribution, indicating observed fine
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45 295 roots did not increase in diameter. There was an obvious change in the slope of the diameter
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47 296 distribution curve for roots ≤ 1 mm diameter at appearance compared to those > 1 mm diameter;
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49 297 showing a rapid decline in the frequency of the finest roots and a more even distribution of roots
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51 298 1–5 mm diameter. Roots in biomass cores were often several centimeters long. Roots > 1 mm
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3 299 diameter were stiff and woody, which resulted in proportionally larger biomass relative to those
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5 300 <1 mm. In contrast, roots appearing in MROT's averaged 3.0 ± 3.2 mm in length (maximum 27
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7 301 mm), and the lengths were not disproportionately weighted based on diameter.
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12 303 Annual fine-root biomass responded to stand age, species and experimental factors. We selected
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14 304 stand age (i.e. year) as the best independent variable to represent stand development based on
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16 305 lowest AIC in models testing treatment responses of annual fine-root variables such as biomass,
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18 306 live-root length, production and mortality. The greatest effects on fine-root biomass were due to
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20 307 year of measurement and species (Fig. 1, greatest f -value for Y and S in Table 1). Species
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22 308 differences occurred in all years but 2000 ($S\times Y$ interactions in Table 1). Fine-root biomass
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24 309 generally increased through time. However, due to variation in the data, there was not always a
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26 310 stepwise annual increase. Any biomass response to F occurred between 2002 and 2004, whereas
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28 311 biomass did not respond to F in 2000 or 2005 ($F\times Y$ interactions in Table 1). The effects of F and
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30 312 I were both positive, but the F response was stronger (greater f -value in Table 1). For example,
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32 313 the maximum average annual increase in biomass due to F compared to C was 99%, whereas the
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34 314 maximum average annual increase due to I was only 42%
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42 316 The factors influencing fine-root length observed in MROT's were generally consistent with
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44 317 biomass response. For example, the order of influence on length was $Y > S > F > I$ (Table 1).
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46 318 Peak root length occurred in 2003 for both species in all treatments (Fig. 1). This peak in root
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48 319 length four years post-establishment was distinct from root biomass, which generally increased
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50 320 through time. Differences in fine-root length between species increased over time ($S\times Y$
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52 321 interaction). Root length consistently responded to treatments. For example, compared with C ,
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3 322 root length was consistently smaller with I and larger with F and IF (I , F and $I \times F$ effects in
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5 323 Table 1), especially in CW. In LP, F effects were not apparent ($F \times S$ interaction). Consistent
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7 324 treatment differences over time for root length was also distinct from root biomass where
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9 325 differences converged toward the end of observations.

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14 327 We observed a positive linear relationship between fine-root biomass and fine-root length (Fig.
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16 328 2), indicating that dynamics observed in MROT measurements were representative of variation
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18 329 in fine-root biomass (cf. Johnson et al. 2001; Majdi 1996). Based on this assumption, the
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20 330 regression equation presented in Fig. 2 was used to translate MROT root length values to
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22 331 biomass on a per unit land area basis.

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27 28 333 Fine-root dynamics

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30 334 Species and year predominately controlled fine-root dynamics (S and Y effects in Table 1).
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32 335 Cottonwood cumulative production and mortality observed in MROTs were consistently greater
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34 336 than LP. Cumulative root length production showed annual growth cycles, with rapid early-
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36 337 season growth that decreased later in the season and remained low throughout dormancy (Fig.
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38 338 3a). Annual growth patterns were most evident in 1- to 3-year old CW and they dampened with
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40 339 age.

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46 341 Fine-root mortality was minimal for the first three years and increased thereafter (Y effect in
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48 342 Table 1). Cumulative fine-root mortality was not distinct between species until 2004 (Fig. 3b).
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50 343 Annual oscillations in cumulative mortality were less evident than that observed in cumulative
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52 344 production.

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5 346 The length of live fine roots present at each observation (standing crop) resembled cumulative
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7 347 production during the first three growing seasons and reached a peak in the fourth growing
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9 348 season (Fig. 3c). Cottonwood live-root length was twice that of LP. Relatively stable live-root
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11 349 length occurred during the last five observation dates with values averaging 6.5 ± 0.8 mm cm⁻² for
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13 350 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.
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15 351 2).

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21 353 Fine-root production increments were low in year 1, peaked in years 2-4 and then progressively
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23 354 decreased in years 5-6 (Fig. S3a) for both CW and LP. Fine-root mortality increment peaked
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25 355 during the fourth year (Fig. S3b). Cottonwood typically had greater root mortality than LP;
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27 356 however, this difference was only significant in 2003 and 2004. Seasonal patterns were more
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29 357 distinct for fine-root production increments compared with mortality increments. Production
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31 358 increment during dormancy was a fraction of that observed during the growing season (Fig. S3a).
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33 359 Mortality increments were not seasonally consistent (Fig. S3b).

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38 361 Fertilizer effects on annual fine-root dynamics depended on species. Cottonwood live-root length
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40 362 increased in response to fertilization, while LP live-root length did not (Fig. 4c, $F \times S$ interaction
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42 363 in Table 1). Production and mortality increment response to fertilization also depended on
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44 364 species although the effect was not as strong as that seen for live-root length (Fig. 4a&b).

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50 366 Annual fine-root production increment and live-root length decreased with I , but were not
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52 367 influenced by F or IF (Fig. 4d, $I \times F$ interaction in Table 1). A similar but weaker response

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3 368 occurred for annual mortality increment. The influence of I on annual fine-root production
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5 369 increment occurred only in the third and fourth years ($I \times Y$ interaction).
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10 371 Fine-root survival analysis
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12 372 Soil depth predominately controlled the risk of fine-root mortality followed by diameter, total
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14 373 stem biomass, season, treatments, and species (chi-square, Table 2). Fine-root survival increased
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16 374 with depth for both species (Fig. S4). Based on Cox' regression parameter estimates, the risk of
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18 375 mortality was 19% higher for LP than CW, causing lifespan to increase 3.7 d for every cm depth
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20 376 in CW, and 2.8 d for every cm depth in LP. Lifespan increased with diameter and decreased as
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22 377 stands developed (Fig. S5). Risk of mortality decreased 5.1% for every 0.1 mm increase in root
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24 378 diameter and increased 2.3% for every Mg increase in stem biomass. Fine-root lifespan appeared
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26 379 to approach an asymptote as stands developed (Figs. 5 and S5). Fine roots appearing in winter
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28 380 had the highest risk of mortality (Fig. 6a) and the shortest lifespan (268 days).
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35 382 Several stand development variables explained risk of fine-root mortality. We selected stem
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37 383 biomass (Fig. S6) to represent stand development for evaluating fine-root dynamics because it
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39 384 best fit the data. Other measures of stand development like year (AIC = 1,107,336), stand basal
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41 385 area (AIC = 1,107,169), total stem volume (AIC = 1,107,098) or total stand biomass (AIC =
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43 386 1,107,058) did not fit as well as stem biomass (AIC = 1,107,019). Yet they all adequately
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45 387 represent development because each ranked third (chi-square) behind soil depth and root
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47 388 diameter, and always ranked higher than other factors tested in Cox' regression model. For the
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49 389 purpose of our study, stem biomass best represented stand development in determining risk of
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3 390 fine-root mortality (Table 2). The risk of mortality increased 2.3% for every Mg increase in stem
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5 391 biomass per ha, meaning that fine-root lifespan decreased four days per $\text{Mg}\cdot\text{ha}^{-1}$ (Figs. 5 and S5).
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10 393 Irrigation influenced the risk of mortality differently between species, but the response to
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12 394 fertilization was similar for both species (Fig. 6b). For CW, the risk of mortality decreased with
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14 395 *I*, causing median lifespan to increase from 268 d for *C* to 322 d for *I* at the reference condition
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16 396 (depth=1 cm, stem biomass = 5 $\text{Mg}\cdot\text{ha}^{-1}$, season=winter, diameter=0.5 mm). For LP, the risk of
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18 397 mortality increased with *I*, so median lifespan decreased from 231 d for *C* to 210 d for *I*. The risk
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20 398 of mortality decreased the same percentage for both species when grown with *F*, causing median
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22 399 lifespan to increase 40 d in CW and 46 d in LP. The risk of mortality decreased for both species
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24 400 when grown with *IF*, but the magnitude was distinct. Median lifespan increased by 113 days for
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26 401 CW grown with *IF* and it increased only 7 days for LP. Treatment responses in CW became
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28 402 more distinct with depth based on Cox' regression model estimates (Fig. S7). In this case,
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30 403 estimated lifespan increased 42% in *IF* relative to *C* at 1 cm depth compared to 61% at 90 cm. A
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32 404 similar increasing response did not occur in LP, where lifespan in *F* was greatest relative to *C*,
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34 405 but the increase was 20% at both 1 cm and 90 cm depth.
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407 Discussion

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

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3 413 MROT occurred in bare soil prior to root colonization, thus avoiding many of the confounding
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5 414 effects associated with installation artefacts reported in other MROT studies (see methods for
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7 415 details). Overall, we found consistent root production and turnover responses to stand
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9 416 development, phenology, rooting depth, initial root diameter, and species (Table 2). Only after
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11 417 considering such dominant controls, could we most accurately assess the more subtle fine-root
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13 418 responses to nutrient and moisture availability. Specifically, Cox' multivariate hazard analysis
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15 419 simultaneously accounted for variation caused by dominant controlling factors to estimate
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17 420 accurately risk of root mortality in response to resource availability. Among dominant control
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19 421 factors, we focus on stand development as a unique contribution of this study. For example, had
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21 422 we not accounted for a 10 Mg ha⁻¹ increase in stem biomass, which was the difference in stem
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23 423 biomass between *C* and *F* in 2005 (Fig. S6), it would have negated the 40 d increase in fine-root
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25 424 lifespan that we observed in response to *F* in both species (Fig. 5 and S7). Similarly, had we not
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27 425 accounted for other intrinsic factors such as root depth, diameter, or season, it would have altered
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29 426 both direction and magnitude of fine-root mortality risk in response to resource availability.
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38 Stand development effects on fine-root dynamics

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40 429 Although, stand age (year) best explained annual fine-root parameters (Table 1, Fig. 1), stem
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42 430 biomass (Fig. S6) better explained the fate of fine roots in Cox' hazard models because stem
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44 431 biomass increased differentially over time in response to resource availability treatments. Indeed,
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46 432 LP stem biomass production at this site was three times that of CW, and there were important
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48 433 distinctions between species in treatment response. Including stand biomass or basal area in the
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50 434 hazard analysis captured these effects. While we are unaware of studies directly investigating
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53 435 fine-root dynamics as a function of stand development, there are some reports where considering

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3 436 stand development might be useful for explaining observed results. For example, one study
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5 437 concluded that stem diameter increment at a common age controlled fine-root lifespan
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7 438 (McCormack et al. 2012; McCormack et al. 2014). These authors were uncertain about the
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10 439 reason for a negative correlation between stem diameter growth rate and fine-root lifespan, yet
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12 440 our results suggest that their observation may have been predominantly due to differences in
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14 441 stand development, since tree size is an important measure of stand development.
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19 443 Our approach considers the dynamic stages of early stand development. Short-rotation
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21 444 intensively managed forests represent model systems for natural stand development that rapidly
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23 445 progress through stand establishment and increased inter-tree competition. Few other reports
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25 446 addressing temporal effects on fine roots consider time scales relevant to questions of stand
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27 447 development (Borja et al. 2008; Brassard et al. 2009). Studies that consider fine-root responses to
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29 448 stand development typically compare neighboring stands of different ages. Such chronosequence
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31 449 studies show that fine-root biomass increases during establishment and then remains constant or
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33 450 declines as stands age (Borja et al. 2008; Brassard et al. 2009; Yuan and Chen 2010), which
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35 451 confirms the peak we observed after four years (Fig. 3c). This peak built up as production
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37 452 initially exceeded mortality, and then declined after year 4 when mortality equaled or exceeded
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39 453 production (Fig. S3). The peak corresponded with root closure observed with biomass cores
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41 454 (Coleman 2007). Fine-root biomass continued to increase after site occupation, while fine-root
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43 455 length stabilized (Fig. 1). This may result from a different proportion of functional classes
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45 456 observed in root biomass and MROT approaches. (cf. Guo et al. 2008; Trumbore and Gaudinski
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47 457 2003). Our observed maximum fine-root length represents fine roots <1 mm diameter (Fig. S2).
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49 458 The diameter distribution at initial appearance in MROT was indistinguishable from the
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3 459 diameter distribution of older roots, which suggests that MROT's did not monitor roots with
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5 460 secondary thickening; however, high tensile and flexural strength in roots found in biomass
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7 461 samples indicated thickening, which suggests that mass distribution does not show the same
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9 462 pattern as fine-root length seen in MROT's.
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14 464 We are only aware of three other MROT studies of similar duration, each of which began in
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16 465 established stands. Norby et al.'s (2004) observations in *Liquidambar styraciflua* L. started in a
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18 466 9-yr-old stand with a basal area of 28 m² ha⁻¹. They found that fine-root standing crop increased
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20 467 to a plateau after 4 yr. The plateau is consistent with the peak standing crop we observed in year
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22 468 4 (i.e. 3-yr-old); although their plateau was significantly delayed in comparison and it does not
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24 469 appear to decline. The *L. styraciflua* stands in our study developed more slowly than did CW or
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26 470 LP (Coyle et al. 2016), which might help explain the delayed peak; however, these patterns may
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28 471 also be artefacts of installing MROT's after root closure actually occurred. Krasowski et al.
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30 472 (2010) report a similar increase in fine-root standing crop in the first years of observation and
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32 473 attributed that to artefacts of tube-installation. In their case, standing crop reached a peak after 3
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34 474 to 6 years where it remained constant or slightly declined depending on study location. Pritchard
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36 475 et al.'s (2008) observations occurred in 17-yr-old LP starting at a basal area of 17 – 25 m² ha⁻¹
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38 476 (Ellsworth et al. 1995) where they found a steady temporal decline in fine-root standing crop.
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40 477 That decline appears to be free of installation artefacts and is consistent with our observed
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42 478 declines occurring after peak standing crop. Consequently, each of these studies in established
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44 479 stands report constant or declining standing crop following any initial post-installation surge in
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46 480 production.
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3 482 Belowground site occupation appears to be similar to that aboveground where leaf area index
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5 483 increases to a maximum and then remains constant or declines (Schoonmaker et al. 2016; White
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7 484 et al. 2010; Yuan and Chen 2010). These parallel root and leaf developmental patterns suggest
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10 485 that established stands regulate production and mortality of roots and leaves to maintain
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12 486 appropriate surface area for resource acquisition. Positive correlations between stand growth,
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14 487 root biomass and leaf area in response to resource availability (Coyle et al. 2016; Martin and
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16 488 Jokela 2004; Vose and Allen 1988) also suggests that regulation of absorption surface are inter-
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18 489 dependent processes of stand growth and development (cf. Litton et al. 2007). Our results
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20 490 demonstrate this inter-dependence based on the large and significant chi-square for stem biomass
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22 491 in Cox' regression model (Table 2), which shows that whole-tree growth responses influence
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24 492 fine-root dynamics. Production and turnover of leaves and fine-roots appear to regulate leaf and
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26 493 root surface area presentation during early stand development.
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33 495 Although the literature contains many examples of resource availability and stand development
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35 496 influencing maximum leaf area and canopy closure (Carlyle 1998; Landsberg and Waring 1997;
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37 497 Martin and Jokela 2004; Vose and Allen 1988; White et al. 2010), relatively little is known about
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39 498 similar influences on fine-root standing crop and root closure. Based on what we know about leaf
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41 499 area dynamics through stand development, we expected fine-root standing crop to peak at an
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43 500 early age, and then remain relatively constant or decline. When considering fine-root length, the
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45 501 time required to achieve peak fine-root standing crop was independent of resource availability,
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47 502 but maximum fine-root standing crop was not (Fig. 1). Thus, this study leads to questions of
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49 503 timing and extent of root and canopy closure, and site carrying capacity for resource acquisition
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53 504 surface both above and belowground. It also emphasizes the need to account for stand

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3 505 development (i.e. ontogeny) when comparing the balance between those surfaces among stands
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5 506 grown with different levels of resource availability.
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10 508 The large allocation of carbon to fine-root production in young stands suggest that establishing
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12 509 root systems is a priority. The proportion of fine root production (FRP) to total net primary
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14 510 productivity (NPP) typically ranges from 26% to 56% (Gill and Jackson 2000; Vogt et al. 1996;
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16 511 Yuan and Chen 2010) depending upon species, climate and site interactions, but there are few
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18 512 reports regarding shifts in allocation with stand development. We found that FRP:NPP declined
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20 513 from 66% in year 1 to less than 3% in year 6 in LP, whereas it reached a minimum of 14% in
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22 514 year 2 and then it rose to an average of 81% between years 4 and 6 for CW. During this
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24 515 substantial developmental change, FRP:NPP was not affected by irrigation, yet the ratio for
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26 516 fertilized plots was consistently half that of non-fertilized. The variation in FRP:NPP observed
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28 517 with these species suggests that species have adopted different allocation strategies which may
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30 518 be related to functional type and seral stage (Grime 1979). Peak total NPP in our study occurred
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32 519 about the time of maximum leaf area (Coyle et al. 2016; Coyle and Coleman 2005; Coyle et al.
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34 520 2008), which is consistent with other observations (Ryan et al. 2004). In addition, annual fine
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36 521 root production was correlated with total NPP, but only for CW ($P<0.001$, $r^2=0.40$) because LP
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38 522 ($P=0.89$) fine root production peaked at least one year before total NPP. While belowground
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40 523 carbon allocation is often directly related to total NPP and other autotrophic processes (Högberg
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42 524 et al. 2001; Irvine et al. 2005), our results show that autotrophic components of belowground
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44 525 carbon allocation are not always in phase with total NPP. This might explain why correlations
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46 526 between belowground carbon allocation and total NPP are not always evident (Litton et al.
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48 527 2007). The eventual increase in FRP:NPP observed with CW is consistent with the increasing
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3 528 proportion of total belowground carbon allocation relative to gross primary productivity (Ryan et
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5 529 al. 2004) and increasing soil CO₂ efflux response with stand age (Wiseman and Seiler 2004; Yan
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7 530 et al. 2011), considering autotrophic respiration can account for half of soil CO₂ efflux (Hanson
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9 531 et al. 2000). In contrast, the decline in FRP:NPP found with LP is consistent with decreased soil
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11 532 CO₂ efflux with stand age (Klopatek 2002; Saiz et al. 2006). The links between fine-root
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13 533 dynamics, total NPP and soil CO₂ efflux remain an open question for understanding components
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15 534 of belowground carbon allocation, yet here we demonstrate that stand development is a critical
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17 535 control factor.
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537 Resource availability effects on fine-root dynamics

538 Resource availability had subtle and inconsistent influence on fine-root variables compared to
539 the predictable responses observed to root depth, initial root diameter, phenology or stand
540 development. Consequently, it was not possible to generalize about even the direction of
541 resource availability influence on fine-root dynamics. Fine-root biomass either increased relative
542 to *C* or was unaffected by *I*, *F*, or *IF* (Fig. 1). Fine-root production and root length standing crop
543 decreased with *I* relative to *C*; however, these variables increased with fertilization for CW and
544 had no effect on LP (Fig. 4). The risk of mortality both increased and decreased relative to *C*
545 depending on species and amendment treatment (Fig. 6b). Since risk of mortality is also
546 expressed as root longevity, it also serves as a surrogate for fine-root turnover, since turnover is
547 the inverse of root longevity. Based on this relationship we might conclude that carbon
548 allocation to turnover in response to resource availability is equally inconsistent. Literature
549 reports describe wide-ranging fine-root responses to soil moisture and nutrient availability,
550 which supports the variable results we observe between species on fine-root production and

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3 551 turnover in response to experimental resource availability treatments. Previous studies reporting
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5 552 fine-root mortality and production responses to increased water availability demonstrate
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8 553 decreases (Gaul et al. 2008), increases (Meier and Leuschner 2008; Olesinski et al. 2011), or no
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10 554 effect (Joslin et al. 2001; King et al. 2002; Rytter 2013). Responses to nutrient availability are
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12 555 equally inconclusive (Brassard et al. 2009; Chen and Brassard 2013; Eissenstat et al. 2013;
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14 556 Hodge 2004; Nadelhoffer 2000; Norby and Jackson 2000). However, most of these studies
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17 557 compare chronological age rather than normalizing with a measure of stand development like
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19 558 stem biomass or stand basal area. Yet as described above, risk of fine-root mortality consistently
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21 559 responds to stand development, and other intrinsic factors. Accounting for these factors has
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24 560 important consequences for understanding responses to resource availability.

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28 562 These inconsistencies contradict the concept that greater carbon or biomass allocation will be
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31 563 directed toward roots when soil resources are limited compared to when they are abundant
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33 564 (Axelsson and Axelsson 1986; Brassard et al. 2009; Brouwer 1983; Hunt and Lloyd 1987; Keyes
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35 565 and Grier 1981; Lambers 1983; Reynolds and Thornley 1982). Thus, our results combined with
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38 566 those of others considering fine-root dynamics do not support the general concept that lower
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40 567 amounts of growth-limiting resources will increase allocation to roots because that directional
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42 568 shift is observed in some, but not all cases. Crucially, compared to the dominant and consistent
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44 569 influence of stand development, rooting depth, diameter, phenology and species, the minor and
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47 570 directionally inconsistent responses to resource availability do not warrant the attention they
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49 571 receive. In fact, resource responses are so subtle that accounting for them never contradicts our
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51 572 inference that intrinsic factors primarily control fine-root dynamics (e.g., see Figs. 5, 7 and S7).

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3 574 Other fine-root control factors
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5 575 Soil depth, root diameter, branch order, phenology and species exerted consistent influence on
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7 576 fine-root turnover as measured through mortality risk. Figure 7 illustrates the relative magnitude
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10 577 of factors controlling mortality risk observed for the given species based on chi-square values in
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12 578 Table 1. Soil depth was especially dominate in our study as an important intrinsic factor
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14 579 affecting mortality risk. Authors consistently report that roots growing in deeper soil have
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16 580 increased root longevity (Baddeley and Watson 2005; Coleman et al. 2000; Kern et al. 2004;
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18 581 Peek et al. 2006; Stover et al. 2010; Wells et al. 2002) and decreased rooting density (Jackson et
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20 582 al. 1996). In our study, soil depth explained the most variation of any factor for risk of root
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22 583 mortality (Table 2, Fig. S4) and biomass distribution (Coleman 2007). Not only were there fewer
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24 584 roots at depth, but deeper roots had longer lifespans. Considering the very different chemical and
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26 585 physical environment at depth (Fang and Moncrieff 2005), deeper roots are expected to be
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28 586 functionally distinct from those growing near the surface (Brassard et al. 2009; Chen and
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30 587 Brassard 2013; Iversen 2010; Pregitzer et al. 1998).
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589 Root diameter was second to soil depth in explaining mortality risk. We measured root diameter
590 using a continuous scale and conclude that the risk of mortality decreased 5.1% for every 0.1 mm
591 increase in diameter. This confirms other reports that conclude fine-root lifespan increases with
592 increasing root diameter class (Chen and Brassard 2013; Coleman et al. 2000; Kern et al. 2004;
593 Wells and Eissenstat 2001). We chose to use root diameter as a co-factor over root branch order
594 for both effectiveness and practicality. Root branch order is considered to be an important
595 criteria for evaluating root function and lifespan (Chen and Brassard 2013; Comas et al. 2002;
596 Fitter 1985; Fitter 1992; Jia et al. 2013; Majdi et al. 2001; McCormack et al. 2015; Pregitzer et

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3 597 al. 1997; Wells et al. 2002). Branch order explained a significant amount of variation in fine-root
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5 598 lifespan in our study for those roots where it was available; however, the branch order of most
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7 599 roots observed via MROTs was uncertain (86% not declared) because we could only confirm
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9 600 root order of the most distal roots with obvious root tips and those subtending. In contrast, we
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11 601 precisely measured root diameter on all roots. Diameter and root order are strongly correlated
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13 602 within a species (Comas et al. 2002; Guo et al. 2004; Jia et al. 2013; Pregitzer et al. 2002).
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15 603 Consequently, diameter measured on a continuous scale was a more practical and effective
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17 604 measurement than branch order when controlling for root morphology.
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24 606 Phenology and species also influenced fine-root turnover and therefore are influential co-factors.
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26 607 We observed distinct annual oscillations of fine-root production with maxima during the growing
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28 608 season and minima during dormancy. Numerous reports describe similar phenological patterns of
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30 609 fine-root production and turnover (Baddeley and Watson 2005; Coleman et al. 2000; Joslin et al.
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32 610 2001; Kern et al. 2004; King et al. 2002; McCormack et al. 2014; Rytter 2013; Tierney et al.
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34 611 2003). The magnitude of fine-root production, mortality and live-root standing crop differed
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36 612 between tree species. Differences in root traits occur for species with different leaf habits (Vogt
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38 613 et al. 1996), relative growth rate (Comas et al. 2002; Comas and Eissenstat 2004; McCormack et
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40 614 al. 2012; McCormack et al. 2014; Wright and Westoby 1999), and among species from different
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42 615 biomes (Finer et al. 2011a; Finer et al. 2011b; Vogt et al. 1996). Thus, controlling for phenology
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44 616 and comparing functionally distinct species is also vital for assessing responses of fine-root
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46 617 dynamics to soil resources. Due to the variety of species distinction, Figure 7 does not attempt to
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48 618 rank the influence of species among other factors controlling fine-root dynamics. Rather, the
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3 619 figure seeks to demonstrate within a given species the relatively minor influence of resource
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5 620 availability among other factors, based on data presented in Table 2.
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10 622 **Conclusion**

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12 623 Our study identified stand development as a major factor explaining the variation in fine-root
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14 624 dynamics. Furthermore, to determine accurately the response of fine-root dynamics to resource
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16 625 availability, we conclude that it is necessary to consider the influence of intrinsic factors,
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18 626 including stand development, rooting depth, initial root diameter, phenology and species.
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20 627 Multivariate analysis using Cox' regression demonstrates a useful approach to control for the
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22 628 influence of intrinsic factors when attempting to measure the relatively minor effects of resource
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24 629 availability. The variation we observed among species and resource availability treatments and
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26 630 the dependence upon measures of stand development demonstrates that generalizations about the
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28 631 directional response of fine-root dynamics to resource availability require reevaluation.
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35 633 Our results have important implications for understanding and modeling factors controlling
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37 634 carbon allocation to fine roots. We understand with certainty the direction if not magnitude of
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39 635 several intrinsic factors controlling fine-root turnover like depth, root diameter, and phenology.
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41 636 Here we quantified the relative impact of stand development, which also appears to exert
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43 637 consistent controls on fine-root turnover. Predictive models should include turnover functions
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45 638 that vary by depth, root diameter and stand development to predict more accurately carbon flux
46
47 639 from live roots to soil organic matter. Models that include intra-annual time steps should include
48
49 640 phenological shifts from production in spring to mortality in autumn. However, there is a
50
51 641 necessity to revisit those models that include generalized increases in carbon allocation to fine
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3 642 roots especially when soil moisture is limiting. Such functions exist in many prominent
4
5 643 ecosystem process models (Landsberg and Waring 1997; Parton et al. 2010; Running and Gower
6
7 644 1991), some of which are now used in land models to predict continental- and global-scale
8
9
10 645 responses to environmental change (Lawrence et al. 2011; Smithwick et al. 2014). Based on our
11
12 646 results, and those of other researchers, the modeling community should incorporate details
13
14 647 introduced by intrinsic factors controlling fine roots (cf. Franklin et al. 2012) and suspend the use
15
16
17 648 of simplistic resource availability controls to describe allocation to fine roots.
18

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43

44 660

45 46 47 661 **References**

48
49 662 Allison PD (2010) Survival Analysis Using SAS: A Practical Guide, 2nd edn. Sas Press, Cary,
50
51 663 NC.

- 1
2
3 664 Atkinson D (1980) The Distribution and Effectiveness of the Roots of Tree Crops. Horticultural
4
5 665 Reviews. 2:424-490.
6
7
8 666 Aubrey D, Coyle D, Coleman M (2012) Functional groups show distinct differences in nitrogen
9
10 667 cycling during early stand development: implications for forest management. Plant Soil.
11
12 668 351:219-236.
13
14
15 669 Axelsson E, Axelsson B (1986) Changes in carbon allocation patterns in spruce and pine trees
16
17 670 following irrigation and fertilization. Tree Physiology. 2:189-204.
18
19 671 Baddeley J, Watson C (2005) Influences of Root Diameter, Tree Age, Soil Depth and Season on
20
21 672 Fine Root Survivorship in *Prunus avium*. Plant Soil. 276:15-22.
22
23
24 673 Bauerle TL, Richards JH, Smart DR, Eissenstat DM (2008) Importance of internal hydraulic
25
26 674 redistribution for prolonging the lifespan of roots in dry soil. Plant Cell and Environment.
27
28 675 31:177-186.
29
30
31 676 Borja I, De Wit HA, Steffenrem A, Majdi H (2008) Stand age and fine root biomass, distribution
32
33 677 and morphology in a Norway spruce chronosequence in southeast Norway. Tree
34
35 678 Physiology. 28:773-784.
36
37
38 679 Brassard BW, Chen HYH, Bergeron Y (2009) Influence of Environmental Variability on Root
39
40 680 Dynamics in Northern Forests. Crit Rev Plant Sci. 28:179-197.
41
42
43 681 Brouwer R (1983) Functional equilibrium: sense or nonsense? Netherlands Journal of
44
45 682 Agricultural Science. 31:335-348.
46
47
48 683 Carlyle JC (1998) Relationships between nitrogen uptake, leaf area, water status and growth in
49
50 684 an 11-year-old *Pinus radiata* plantation in response to thinning, thinning residue, and
51
52 685 nitrogen fertiliser. For Ecol Manage. 108:41-55.
53
54
55
56
57
58
59
60

- 1
2
3 686 Chen HYH, Brassard BW (2013) Intrinsic and Extrinsic Controls of Fine Root Life Span. Crit
4
5 687 Rev Plant Sci. 32:151-161.
6
7
8 688 Coleman M (2007) Spatial and temporal patterns of root distribution in developing stands of four
9
10 689 woody crop species grown with drip irrigation and fertilization. Plant Soil. 299:195-213.
11
12 690 Coleman MD, Coyle DR, Blake J, Britton K, Buford M, Campbell RG, Cox J, Cregg B, Daniels
13
14 691 D, Jacobson M, Johnson K, McDonald T, McLeod K, Nelson E, Robison D, Rummer R,
15
16 692 Sanchez F, Stanturf J, Stokes B, Trettin C, Tuskan J, Wright L, Wullschlegel S. 2004.
17
18 693 Production of Short Rotation Woody Crops Grown with a Range of Nutrient and Water
19
20 694 Availability: Establishment Report and First-Year Responses. USDA Forest Service,
21
22 695 Southern Research Station, Asheville, NC, USA, p 21.
23
24
25
26 696 Coleman MD, Dickson RE, Isebrands JG (2000) Contrasting fine-root production, survival and
27
28 697 soil CO₂ efflux in pine and poplar plantations. Plant Soil. 225:129-139.
29
30
31 698 Comas LH, Anderson LJ, Dunst RM, Lakso AN, Eissenstat DM (2005) Canopy and
32
33 699 environmental control of root dynamics in a long-term study of Concord grape. New
34
35 700 Phytol. 167:829-840.
36
37
38 701 Comas LH, Bouma TJ, Eissenstat DM (2002) Linking root traits to potential growth rate in six
39
40 702 temperate tree species. Oecologia. 132:34-43.
41
42
43 703 Comas LH, Eissenstat DM (2004) Linking fine root traits to maximum potential growth rate
44
45 704 among 11 mature temperate tree species. Funct Ecol. 18:388-397.
46
47 705 Coyle DR, Aubrey DP, Coleman MD (2016) Growth responses of narrow or broad site adapted
48
49 706 tree species to a range of resource availability treatments after a full harvest rotation. For
50
51 707 Ecol Manage. 362:107-119.
52
53
54
55
56
57
58
59
60

- 1
2
3 708 Coyle DR, Coleman MD (2005) Forest production responses to irrigation and fertilization are not
4
5 709 explained by shifts in allocation. For Ecol Manage. 208:137–152.
6
7
8 710 Coyle DR, Coleman MD, Aubrey D (2008) Above- and below-ground biomass accumulation,
9
10 711 production and distribution of sweetgum and loblolly pine grown with irrigation and
11
12 712 fertilization. Can J For Res. 38:1335-1348.
13
14
15 713 Eckenwalder JE (2001) Descriptions of clonal characteristics. In: Dickmann DI, Isebrands JG,
16
17 714 Eckenwalder JE, Richardson J (eds) Poplar Culture in North America. National Research
18
19 715 Council of Canada, NRC Press, Ottawa, pp 336-382.
20
21
22 716 Eissenstat DM, McCormack ML, Du QY (2013) Global Change and Root Lifespan. Plant Roots:
23
24 717 The Hidden Half, 4th Edition.
25
26 718 Eissenstat DM, Yanai RD (1997) The ecology of root lifespan. Adv Ecol Res. 27:1-60.
27
28
29 719 Ellsworth DS, Oren R, Huaun C, Phillips N, Hendrey GR (1995) Leaf and canopy responses to
30
31 720 elevated CO₂ in a pine forest under free-air CO₂ enrichment. Oecologia. 104:139-146.
32
33 721 Fang C, Moncrieff J (2005) The variation of soil microbial respiration with depth in relation to
34
35 722 soil carbon composition. Plant Soil. 268:243-253.
36
37
38 723 Finer L, Ohashi M, Noguchi K, Hirano Y (2011a) Factors causing variation in fine root biomass
39
40 724 in forest ecosystems. For Ecol Manage. 261:265-277.
41
42
43 725 Finer L, Ohashi M, Noguchi K, Hirano Y (2011b) Fine root production and turnover in forest
44
45 726 ecosystems in relation to stand and environmental characteristics. For Ecol Manage.
46
47 727 262:2008-2023.
48
49 728 Fitter AH (1985) Functional significance of root morphology and root system architecture. In:
50
51 729 Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soil.
52
53 730 Blackwell Scientific Publication, Oxford, pp 87-106.
54
55
56
57
58
59
60

- 1
2
3 731 Fitter AH (1992) Architecture and biomass allocation as components of the plastic response of
4
5 732 roots to soil heterogeneity. In: Caldwell MM, Pearcy RW (eds) Exploitation of
6
7 733 environmental heterogeneity by plants. Academic Press.
8
9
10 734 Franklin O, Johansson J, Dewar RC, Dieckmann U, McMurtrie RE, Brännström Å, Dybzinski R
11
12 735 (2012) Modeling carbon allocation in trees: a search for principles. *Tree Physiology*.
13
14 736 32:648-666.
15
16
17 737 Gaul D, Hertel D, Borken W, Matzner E, Leuschner C (2008) Effects of experimental drought on
18
19 738 the fine root system of mature Norway spruce. *For Ecol Manage*. 256:1151-1159.
20
21 739 Gill RA, Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. *New*
22
23 740 *Phytol*. 147:13-31.
24
25
26 741 Grime JP (1979) *Plant strategies and vegetation processes*. John Wiley, Chichester.
27
28 742 Guo DL, Li H, Mitchell RJ, Han WX, Hendricks JJ, Fahey TJ, Hendrick RL (2008) Fine root
29
30 743 heterogeneity by branch order: exploring the discrepancy in root turnover estimates
31
32 744 between minirhizotron and carbon isotopic methods. *New Phytol*. 177:443-456.
33
34
35 745 Guo DL, Mitchell RJ, Hendricks JJ (2004) Fine root branch orders respond differentially to
36
37 746 carbon source-sink manipulations in a longleaf pine forest. *Oecologia*. 140:450-457.
38
39
40 747 Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial
41
42 748 contributions to soil respiration: A review of methods and observations.
43
44 749 *Biogeochemistry*. 48:115-146.
45
46
47 750 Hertel D, Strecker T, Muller-Haubold H, Leuschner C (2013) Fine root biomass and dynamics in
48
49 751 beech forests across a precipitation gradient - is optimal resource partitioning theory
50
51 752 applicable to water-limited mature trees? *J Ecol*. 101
52
53
54
55
56
57
58
59
60

- 1
2
3 753 Hodge A (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. *New*
4
5 754 *Phytol.* 162:9-24.
6
7
8 755 Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G,
9
10 756 Ottosson-Lofvenius M, Read DJ (2001) Large-scale forest girdling shows that current
11
12 757 photosynthesis drives soil respiration. *Nature.* 411:789-792.
13
14
15 758 Hunt R, Lloyd PS (1987) Growth and partitioning. *New Phytol.* 106:235-249.
16
17 759 Irvine J, Law BE, Kurpius MR (2005) Coupling of canopy gas exchange with root and
18
19 760 rhizosphere respiration in a semi-arid forest. *Biogeochemistry.* 73:271-282.
20
21 761 Iversen CM (2010) Digging deeper: fine-root responses to rising atmospheric CO₂ concentration
22
23 762 in forested ecosystems. *New Phytol.* 186:346-357.
24
25
26 763 Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED (1996) A global
27
28 764 analysis of root distributions for terrestrial biomes. *Oecologia.* 108:389-411.
29
30
31 765 Jia S, McLaughlin NB, Gu J, Li X, Wang Z (2013) Relationships between root respiration rate
32
33 766 and root morphology, chemistry and anatomy in *Larix gmelinii* and *Fraxinus*
34
35 767 *mandshurica*. *Tree Physiology.* 33:579-589.
36
37
38 768 Johnson MG, Tingey DT, Phillips DL, Storm MJ (2001) Advancing fine root research with
39
40 769 minirhizotrons. *Environ Exp Bot.* 45:263; 289.
41
42
43 770 Joslin JD, Wolfe MH (1999) Disturbances during minirhizotron installation can affect
44
45 771 observation data. *Soil Sci Soc Am J.* 63:218-221.
46
47 772 Joslin JD, Wolfe MH, Hanson PJ (2001) Factors controlling the timing of root elongation
48
49 773 intensity in a mature upland oak stand. *Plant Soil.* 228:201; 212.
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 774 Katterer T, Fabiao A, Madeira M, Ribeiro C, Steen E (1995) Fine-Root Dynamics, Soil-Moisture
4
5 775 and Soil Carbon Content in a Eucalyptus-Globulus Plantation under Different Irrigation
6
7 776 and Fertilization Regimes. For Ecol Manage. 74:1-12.
8
9
10 777 Kern CC, Friend AL, Johnson JM-F, Coleman MD (2004) Fine-root dynamics in a developing
11
12 778 *Populus deltoides* plantation. Tree Physiology. 24:651-660.
13
14
15 779 Keyes MR, Grier CC (1981) Above- and below-ground net production in 40-year-old Douglas-
16
17 780 fir stands on low and high productivity sites. Can J For Res. 11:599-605.
18
19 781 King JS, Albaugh TJ, Allen HL, Buford M, Strain BR, Dougherty P (2002) Below-ground
20
21 782 carbon input to soil is controlled by nutrient availability and fine root dynamics in
22
23 783 loblolly pine. New Phytol. 154:389-398.
24
25
26 784 Klopatek JM (2002) Belowground carbon pools and processes in different age stands of
27
28 785 Douglas-fir. Tree Physiology. 22:197-204.
29
30
31 786 Krasowski MJ, Lavigne MB, Olesinski J, Bernier PY (2010) Advantages of long-term
32
33 787 measurement of fine root demographics with a minirhizotron at two balsam fir sites. Can
34
35 788 J For Res. 40:1128-1135.
36
37
38 789 Lambers H (1983) "The functional equilibrium," nibbling on the edges of a paradigm.
39
40 790 Netherlands Journal of Agricultural Science. 31:305-311.
41
42
43 791 Landsberg JJ, Waring RH (1997) A generalised model of forest productivity using simplified
44
45 792 concepts of radiation-use efficiency, carbon balance and partitioning. For Ecol Manage.
46
47 793 95:209-228.
48
49 794 Lawrence DM, Oleson KW, Flanner MG, Thornton PE, Swenson SC, Lawrence PJ, Zeng XB,
50
51 795 Yang ZL, Levis S, Sakaguchi K, Bonan GB, Slater AG (2011) Parameterization
52
53
54
55
56
57
58
59
60

- 1
2
3 796 Improvements and Functional and Structural Advances in Version 4 of the Community
4
5 797 Land Model. *J Adv Mod Earth Sys.* 3
6
7
8 798 Littell RC, Milliken GA, Stroup WW, Wolfinger RD (2006) SAS for Mixed Models, 2nd edn.
9
10 799 SAS Institute, Inc., Cary, NC, USA.
11
12 800 Litton CM, Raich JW, Ryan MG (2007) Carbon allocation in forest ecosystems. *Global Change*
13
14 801 *Biol.* 13:2089-2109.
15
16
17 802 Magbanua ZV, Ozkan S, Bartlett BD, Chouvarine P, Saski CA, Liston A, Cronn RC, Nelson CD,
18
19 803 Peterson DG (2011) Adventures in the Enormous: A 1.8 Million Clone BAC Library for
20
21 804 the 21.7 Gb Genome of Loblolly Pine. *PLoS ONE.* 6:e16214.
22
23
24 805 Majdi H (1996) Root sampling methods - applications and limitations of the minirhizotron
25
26 806 technique. *Plant Soil.* 185:255-258.
27
28
29 807 Majdi H, Andersson P (2005) Fine root production and turnover in a Norway spruce stand in
30
31 808 northern Sweden: Effects of nitrogen and water manipulation. *Ecosystems.* 8:191-199.
32
33
34 809 Majdi H, Damm E, Nylund JE (2001) Longevity of mycorrhizal roots depends on branching
35
36 810 order and nutrient availability. *New Phytol.* 150:195-202.
37
38 811 Martin TA, Jokela EJ (2004) Stand development and production dynamics of loblolly pine under
39
40 812 a range of cultural treatments in north-central Florida USA. *For Ecol Manage.* 192:39-58.
41
42
43 813 McCormack ML, Adams TS, Smithwick EAH, Eissenstat DM (2012) Predicting fine root
44
45 814 lifespan from plant functional traits in temperate trees. *New Phytol.* 195:823-831.
46
47 815 McCormack ML, Adams TS, Smithwick EAH, Eissenstat DM (2014) Variability in root
48
49 816 production, phenology, and turnover rate among 12 temperate tree species. *Ecology.*
50
51 817 95:2224-2235.
52
53
54
55
56
57
58
59
60

- 1
2
3 818 McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari H-S,
4
5 819 Hobbie EA, Iversen CM, Jackson RB, Leppälammii-Kujansuu J, Norby RJ, Phillips RP,
6
7 820 Pregitzer KS, Pritchard SG, Rewald B, Zadworny M (2015) Redefining fine roots
8
9
10 821 improves understanding of below-ground contributions to terrestrial biosphere processes.
11
12 822 New Phytol. 207:505-518.
- 14 823 Meier IC, Leuschner C (2008) Genotypic variation and phenotypic plasticity in the drought
15
16 824 response of fine roots of European beech. *Tree Physiology*. 28:297-309.
- 19 825 Misson L, Gershenson A, Tang JW, McKay M, Cheng WX, Goldstein A (2006) Influences of
20
21 826 canopy photosynthesis and summer rain pulses on root dynamics and soil respiration in a
22
23 827 young ponderosa pine forest. *Tree Physiology*. 26:833-844.
- 26 828 Nadelhoffer KJ (2000) The potential effects of nitrogen deposition on fine-root production in
27
28 829 forest ecosystems. *New Phytol*. 147:131; 139.
- 31 830 Norby RJ, Jackson RB (2000) Root dynamics and global change: seeking an ecosystem
32
33 831 perspective. *New Phytol*. 147:3-12.
- 35 832 Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG (2004) Fine-root production dominates
36
37 833 response of a deciduous forest to atmospheric CO₂ enrichment. *Proceedings of the*
38
39 834 *National Academy of Sciences of the United States of America*. 101:9689-9693.
- 42 835 Olesinski J, Lavigne MB, Krasowski MJ (2011) Effects of soil moisture manipulations on fine
43
44 836 root dynamics in a mature balsam fir (*Abies balsamea* L. Mill.) forest. *Tree Physiology*.
45
46 837 31:339-348.
- 49 838 Parton WJ, Hanson PJ, Swanston C, Torn M, Trumbore SE, Riley W, Kelly R (2010) ForCent
50
51 839 model development and testing using the Enriched Background Isotope Study
52
53 840 experiment. *Journal of Geophysical Research: Biogeosciences*. 115:G04001.

- 1
2
3 841 Peek MS, Leffler AJ, Hipps L, Ivans S, Ryel RJ, Caldwell MM (2006) Root turnover and
4
5 842 relocation in the soil profile in response to seasonal soil water variation in a natural stand
6
7 843 of Utah juniper (*Juniperus osteosperma*). *Tree Physiology*. 26:1469-1476.
8
9
10 844 Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL (2002) Fine root
11
12 845 architecture of nine North American trees. *Ecol Monogr*. 72:293-309.
13
14 846 Pregitzer KS, Kubiske ME, Yu CK, Hendrick RL (1997) Relationships among root branch order,
15
16 847 carbon, and nitrogen in four temperate species. *Oecologia*. 111:302-308.
17
18
19 848 Pregitzer KS, Laskowski MJ, Burton AJ, Lessard VC, Zak DR (1998) Variation in sugar maple
20
21 849 respiration with root diameter and soil depth. *Tree Physiology*. 18:665-670.
22
23
24 850 Pritchard SG, Strand AE, McCormack ML, Davis MA, Finz AC, Jackson RB, Matamala R,
25
26 851 Rogers HH, Oren R (2008) Fine root dynamics in a loblolly pine forest are influenced by
27
28 852 free-air-CO₂-enrichment: a six-year-minirhizotron study. *Global Change Biol*. 14:588-
29
30 853 602.
31
32
33 854 Reynolds JF, Thornley JHM (1982) A shoot:root partitioning model. *Ann Bot*. 49:585-597.
34
35 855 Running SW, Gower ST (1991) FOREST-BGC, A general model of forest ecosystem processes
36
37 856 for regional applications. II. Dynamic carbon allocation and nitrogen budgets. *Tree*
38
39 857 *Physiology*. 9:147-160.
40
41
42 858 Ryan MG, Binkley D, Fownes JH, Giardina CP, Senock RS (2004) An experimental test of the
43
44 859 causes of forest growth decline with stand age. *Ecol Monogr*. 74:393-414.
45
46
47 860 Rytter RM (2013) The effect of limited availability of N or water on C allocation to fine roots
48
49 861 and annual fine root turnover in *Alnus incana* and *Salix viminalis*. *Tree Physiology*.
50
51 862 33:924-939.
52
53
54
55
56
57
58
59
60

- 1
2
3 863 Saiz G, Byrne KA, Butterbach-Bahl K, Kiese R, Blujdea V, Farrell EP (2006) Stand age-related
4
5 864 effects on soil respiration in a first rotation Sitka spruce chronosequence in central
6
7 865 Ireland. *Global Change Biol.* 12:1007-1020.
8
9
10 866 Santantonio D, Hermann RK (1985) Standing crop, production and turnover of fine roots on dry,
11
12 867 moderate, and wet sites of mature Douglas-fir in western Oregon. *Annales des Sciences*
13
14 868 *Forestieres.* 42:113-142.
15
16
17 869 Savarese PT, Patetta MJ. 2010. An Overview of the CLASS, CONTRAST, and
18
19 870 HAZARDRATIO Statements in the SAS® 9.2 PHREG Procedure. SAS Institute Inc.,
20
21 871 Cary, NC, p 23.
22
23
24 872 Schoonmaker AS, Liefers VJ, Landhäusser SM (2016) Viewing forests from below: fine root
25
26 873 mass declines relative to leaf area in aging lodgepole pine stands. *Oecologia.* 181:733-
27
28 874 747.
29
30
31 875 Smithwick EAH, Lucash MS, McCormack ML, Sivandran G (2014) Improving the
32
33 876 representation of roots in terrestrial models. *Ecol Model.* 291:193-204.
34
35
36 877 Stover DB, Day FP, Drake BG, Hinkle CR (2010) The long-term effects of CO2 enrichment on
37
38 878 fine root productivity, mortality, and survivorship in a scrub-oak ecosystem at Kennedy
39
40 879 Space Center, Florida, USA. *Environ Exp Bot.* 69:214-222.
41
42
43 880 Tierney GL, Fahey TJ, Groffman PM, Hardy JP, Fitzhugh RD, Driscoll CT, Yavitt JB (2003)
44
45 881 Environmental control of fine root dynamics in a northern hardwood forest. *Global*
46
47 882 *Change Biol.* 9:670-679.
48
49 883 Trumbore SE, Gaudinski JB (2003) The secret lives of roots. *Science.* 302:1344-1345.
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 884 Vogt KA, Vogt DJ, Palmiotto PA, Boon P, O'Hara J, Asbjornsen H (1996) Review of root
4
5 885 dynamics in forest ecosystems grouped by climate, climatic forest type and species. *Plant*
6
7 886 *Soil.* 187:159-219.
8
9
10 887 Vose JA, Allen HL (1988) Leaf area, stemwood growth, and nutrition relationships in loblolly
11
12 888 pine. *For Sci.* 34:547-563.
13
14 889 Wells CE, Eissenstat DM (2001) Marked differences in survivorship among apple roots of
15
16 890 different diameters. *Ecology.* 82:882-892.
17
18 891 Wells CE, Glenn DM, Eissenstat DM (2002) Changes in the risk of fine-root mortality with age:
19
20 892 A case study in peach, *Prunus persica* (Rosaceae). *Am J Bot.* 89:79-87.
21
22 893 White DA, Battaglia M, Mendham DS, Crombie DS, Kinal J, McGrath JF (2010) Observed and
23
24 894 modelled leaf area index in *Eucalyptus globulus* plantations: tests of optimality and
25
26 895 equilibrium hypotheses. *Tree Physiology.* 30:831-844.
27
28 896 Wiseman PE, Seiler JR (2004) Soil CO₂ efflux across four age classes of plantation loblolly pine
29
30 897 (*Pinus taeda* L.) on the Virginia Piedmont. *For Ecol Manage.* 192:297-311.
31
32 898 Wright IJ, Westoby M (1999) Differences in seedling growth behaviour among species: trait
33
34 899 correlations across species, and trait shifts along nutrient compared to rainfall gradients. *J*
35
36 900 *Ecol.* 87:85-97.
37
38 901 Yan MF, Zhang XS, Jiang Y, Zhou GS (2011) Effects of irrigation and plowing on soil carbon
39
40 902 dioxide efflux in a poplar plantation chronosequence in northwest China. *Soil Sci Plant*
41
42 903 *Nutr.* 57:466-474.
43
44 904 Yuan ZY, Chen HYH (2010) Fine Root Biomass, Production, Turnover Rates, and Nutrient
45
46 905 Contents in Boreal Forest Ecosystems in Relation to Species, Climate, Fertility, and
47
48 906 Stand Age: Literature Review and Meta-Analyses. *Crit Rev Plant Sci.* 29:204-221.
49
50
51
52
53
54
55
56
57
58
59
60

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908 **Figure legends**

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

915

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

922

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

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2
3 928 regression equation from Fig. 2. Error bars are standard errors (n=12). The P-values are for
4
5 929 Kolmogorov-Smirnov two-sample non-parametric tests comparing lines within each panel.
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8 930
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10 931 Figure 4. Average annual production increment (a), annual mortality increment (b) and live-root
11
12 932 length (crop) (c) for cottonwood (CW) and loblolly pine (LP) grown with (Fert) or without (No
13
14 933 Fert) fertilization treatments and averaged for irrigation treatments. Average annual production
15
16 934 increment, annual mortality increment and live-root length (crop) (d) grown with irrigation (*I*),
17
18 935 fertilization (*F*), or their combination (*IF*) compared with untreated control (*C*) and averaged for
19
20 936 species. Error bars are standard errors (n=36). Bars with the same letter within each variable are
21
22 937 not significantly different (Tukey's HSD, $\alpha=0.10$).
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26 938
27
28 939 Figure 5. Cox regression model estimates of fine-root lifespan versus stem biomass for
29
30 940 cottonwood (CW) and loblolly pine (LP) grown with irrigated (*I*), fertilized (*F*), or the combined
31
32 941 (*IF*) treatments relative to the untreated control (*C*). Lifespan is median root survival predicted
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34 942 using Equation 1 and coefficients shown in Table 2. Error bars are 95% confidence intervals. We
35
36 943 maintained cofactors at the following references levels: season = winter, soil depth = 1 cm and
37
38 944 root diameter = 0.5.
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42 945
43
44 946 Figure 6. Risk of fine-root mortality for (a) three seasons of the year relative to that observed in
45
46 947 winter, and (b) for cottonwood (CW) and loblolly pine (LP) grown with irrigated (*I*), fertilized
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48 948 (*F*), or the combined (*IF*) treatments relative to the untreated control (*C*). Seasons were
49
50 949 designated base on solstice and equinox dates. Percentages were calculated from model
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3 950 coefficients as $\% \text{Mort} = 100 \cdot (e^{\beta s} - 1)$, where β is the parameter estimate from Table 2 for season
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6 951 *s*.

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10 953 Figure 7. Conceptual model illustrating the relative magnitude of different controls on fine-root
11
12 954 dynamics. The width of the pyramid and shading reflects the relative magnitude of each factor.

13
14 955 As the width narrows and shading decreases, the magnitude of the response decreases. Likewise,
15
16
17 956 as the width narrows and shading decreases, the consistency and direction of the response across
18
19 957 species decreases.
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3 Stand development and other intrinsic factors largely control fine-root dynamics with only subtle
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5 modifications from resource availability
6

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36 **Running title:** Inherent and resource controls on fine-roots
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40 **Keywords:** fertilization; fine-root biomass; intrinsic and extrinsic factors; irrigation; hazard
41 | analysis; phenology; *Pinus taeda*; *Populus deltoides*; short rotation woody crops-
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1 Abstract

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

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3 24 should first consider consistent patterns of allocation caused by different depth, diameter, stand
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5 25 development, phenology and species before considering allocation due to soil resource
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8 26 availability.
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28 Introduction

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

43
44 Intrinsic factors such as root diameter, rooting depth, species and phenology, exert dominant
45 controls on fine-root dynamics. Lifespan consistently increases with root diameter and rooting
46 depth (Chen and Brassard 2013). Species differences, such as evergreen vs. conifer, or growth
47 rate, also consistently affect fine-root dynamics (Eissenstat and Yanai 1997; McCormack et al.
48 2014). Temporal factors occur on intra- or inter-annual time scales; however, we know more
49 about the former than the latter primarily because it is easier to make consistent measurements
50 over seasonal time scales. On intra-annual time scales, root production is maximum in spring

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3 51 and summer, while root mortality occurs mainly in fall and winter (Brassard et al. 2009). This
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5 52 pattern may be disrupted in climates with predominate summer droughts, where a sharp peak of
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7 53 root production occurs in spring (Misson et al. 2006), or bimodal peaks may occur in spring and
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9 54 fall (Atkinson 1980; Comas et al. 2005; Santantonio and Hermann 1985). Perhaps the least well-
10
11 55 understood intrinsic factor influencing fine-root dynamics is stand development. Inter-annual
12
13 56 studies over time scales relevant to forest stand development are rare because of difficulties in
14
15 57 sustaining observations over requisite observation times, or in finding comparable stands for
16
17 58 chronosequence studies. Available studies indicate that as young forest stands establish and
18
19 59 inter-tree competition increases, fine-root biomass becomes relatively constant and may even
20
21 60 decline thereafter (Brassard et al. 2009; Schoonmaker et al. 2016); suggesting turnover also
22
23 61 increases during stand development. However, there is little information on the progression of
24
25 62 fine-root dynamics observed in the same stand through different forest developmental stages
26
27 63 (Borja et al. 2008; Brassard et al. 2009; Yuan and Chen 2010). Early stand development appears
28
29 64 to show the most dynamic changes followed by stable or slow declines in fine-root standing crop
30
31 65 as canopies differentiate through stem exclusion. These synthesis reports provide some insight
32
33 66 into the net result of fine-root dynamics, but we still have a very poor understanding of how the
34
35 67 individual processes of production and mortality change through stand development.
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44 69 Soil resources that have potential to influence fine-root production primarily include soil
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46 70 moisture and nutrient availability. We currently have an inadequate understanding of how
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48 71 resource availability influences fine-root production and mortality compared to root diameter,
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50 72 rooting depth, species and phenology. Investigators study the influence of soil moisture on fine-
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52 73 root biomass, production and lifespan using drought, irrigation, or flooding in both manipulated
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3 74 and natural-gradient studies with inconsistent and contradictory results. For example,
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5 75 manipulative studies report that higher relative soil moisture either: (1) decreases both fine-root
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7 76 mortality and production simultaneously (Gaul et al. 2008); (2) increases both production and
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10 77 mortality (Meier and Leuschner 2008; Olesinski et al. 2011); (3) increases production, not
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12 78 mortality (Bauerle et al. 2008; Katterer et al. 1995; Majdi and Andersson 2005; Rytter 2013); or
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14
15 79 (4) has no influence on fine-root dynamics (Joslin et al. 2001; King et al. 2002; Rytter 2013).
16
17 80 Consequently, a meta-analysis found only a slight positive effect of increased soil moisture on
18
19 81 root lifespan (Chen and Brassard 2013). Results from natural precipitation gradients have been
20
21 82 equally inconclusive (Finer et al. 2011b; Gill and Jackson 2000; Hertel et al. 2013; Yuan and
22
23
24 83 Chen 2010). Various approaches have also yielded inconsistent and contradictory results that do
25
26 84 not provide a clear understanding of the magnitude or even the direction of fine-root responses to
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28 85 nutrient availability. For example, reviews considering soil nutrients find that fine-root
29
30 86 production and mortality increase and decrease in response to nitrogen (N) and phosphorus
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33 87 additions, and they suggest that lack of consensus among reports is due to methodological
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35 88 differences or site variation (Brassard et al. 2009; Chen and Brassard 2013; Eissenstat et al.
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38 89 2013; Hodge 2004; Nadelhoffer 2000; Norby and Jackson 2000). Some reports suggest that we
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40 90 consider other factors, including intrinsic controls, to understand the response of fine-root
41
42 91 production and turnover to resource availability. For example, Joslin et al. (2001) show that the
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45 92 response to favorable moisture availability was greater early in the growing season compared to
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47 93 later and conclude that to understand the response to water availability, it is necessary to account
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49 94 for plant phenology. Similarly, it was necessary for Kern et al. (2004) to consider only smaller
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51 95 diameter roots at the surface to show effects of N amendments on fine-root production.
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3 97 Here we empirically evaluate fundamental controls of fine-root production and lifespan using
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5 98 long-term observations of deciduous hardwood and evergreen conifer fine roots grown with
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8 99 water and nutrient amendments. Our approach is unique in that we observe fine-root dynamics in
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10 100 short-rotation woody crop plantations beginning at stand establishment and continuing past the
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12 101 point of full root occupation (root closure) as inter-tree competition increases and crown
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14 102 differentiation begins. Short-rotation forests mature rapidly and therefore are practical models for
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16 103 observing stand development over relatively short periods. We selected a site with low soil
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18 104 moisture and nutrient availability so **that** applied resource amendment treatments created a range
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21 105 in site quality. In addition, we determined the response of fine-root production, lifespan, and
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23 106 standing crop to soil resources and to various intrinsic factors known to control fine-root
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25 107 dynamics such as stand development, depth, root diameter, species, and phenology. Our
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27 108 objective was to determine the relative control that soil resources have on fine-root dynamics
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29 109 compared with intrinsic factors. We took advantage of our long-term observations to understand
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31 110 how fine-root dynamics change through stand development. Specifically, we hypothesized that
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33 111 **(H1)** fine-root production would be highest, and mortality would be lowest, early in stand
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36 112 development; **(H2)** as development continued, production would decrease and mortality would
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39 113 increase until the two processes reach an equilibrium where the standing crop remains somewhat
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41
42 114 constant; and **(H3)** it would be necessary to control for dominant intrinsic factors to define
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45 115 accurately the subtle effects of resource availability on fine-root dynamics.

116

117 **Materials and Methods**

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3 118 This research is part of a long-term forest productivity study designed to evaluate above- and
4
5 119 belowground growth responses of several fast growing tree species. Coleman et al. (2004)
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7
8 120 describe in more detail the site, plant materials, and experimental design.
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12 122 Site Description and Preparation

14 123 We conducted the experiment at U.S. Department of Energy Savannah River Site, a National
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16
17 124 Environmental Research Park near Aiken, SC in the Carolina Sand Hill physiographic region (lat
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19 125 33.387°, lon -81.676 °). The soil is predominately a Blanton sand (thermic Grossarenic
20
21 126 Paleudults) with loamy subsoil at 120 to 200 cm depth (Rogers, 1990). Previous vegetation was
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23 127 plantation pine with an oak understory. We removed slash >15-cm diameter and pulverized
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26 128 stumps and remaining debris to less than 5cm diameter pieces, and incorporated biomass to 30
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28 129 cm (RS-500 Reclaimer/Stabilizer, CMI Corp., Oklahoma City, USA). Additional preparation
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30
31 130 consisted of disking and lime amendments.
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33 131

35 132 Plant Material and competition control

37 133 The two species included in this study were eastern cottonwood (CW, *Populus deltoides* Bartr.,
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39 134 cv. ST66: Issaquena County, MS (Eckenwalder 2001)) and loblolly pine (LP, *Pinus taeda* L., cv.
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41
42 135 7-56, Williamsburg County, SC (Magbanua et al. 2011)). Bare-root LP seedlings were planted
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44 136 February 2000. We collected CW cuttings from stool beds during the previous winter and
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46
47 137 planted them April 2000. Rigorous and continuous weed control eliminated understory
48
49 138 competition, so we could be certain live roots were solely plantation trees.
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54 140 Experimental Design

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3 141 Twenty-four plots included two species, four treatments and three replicate blocks (Fig. S1).
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5 142 Each 0.22 ha treatment plot had a central 0.04 ha measurement plot with 54 trees arranged in 2.5
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8 143 $\times 3$ m spacing. There were at least four treated border rows (12 m) surrounding measurement
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10 144 plots. Treatments consisted of control (*C*), irrigation (*I*), fertilization (*F*) and irrigation +
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12 145 fertilization (*IF*). Within each of three block, the four treatment plots of a given species were
13
14 146 grouped together to minimize within-block site gradients. We used drip irrigation to apply up to
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16 147 5 mm d⁻¹ between April and October to meet evaporative demand and ensure favorable soil
17
18 148 moisture. The quantity equals average regional evaporation during those months and was
19
20 149 designed to assure favorable soil moisture. During the study, average annual rainfall was 809
21
22 150 mm. Irrigation supplied an average of 551 mm y⁻¹. We applied fertilizer at rates of 40 kg N ha⁻¹
23
24 151 yr⁻¹ in 2000, 80 in 2001, and 120 in 2002-2005. Fertilizer increased annually to correspond with
25
26 152 demand of growing trees based on estimated N mineralization rate, expected productivity, and
27
28 153 tree nutrient content (Coleman et al. 2004). We split annual fertilizer treatments among 26
29
30 154 weekly applications and applied them with drip irrigation. Fertilizer application supplied enough
31
32 155 water to deliver liquid fertilizer and flush drip tubes (5 mm wk⁻¹). Control plots received 5 mm
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34 156 water wk⁻¹ to maintain experimental control. Thus, non-irrigated plots (i.e. *C* and *F*) received 130
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36 157 mm yr⁻¹ of irrigation in addition to annual precipitation.
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44 159 Stand inventory included annual measurements of stem diameter for all plot trees as described in
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46 160 (Coyle et al. 2016). We expressed diameter values as stand basal area and determined stem
47
48 161 biomass using treatment- and species-specific allometric equations developed on several
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50 162 occasions. The progression of basal area and stem biomass over time quantitatively and
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3 163 continuously described stand development. These measures of stand development represent
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5 164 ontogenetic progression and substitute for time in analytical models.
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10 166 Each November, we determined fine-root biomass (< 5 mm diameter) from five random
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12 167 locations per plot as detailed by Coleman (2007). Briefly, we removed soil cores (5 cm diameter)
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14 168 from 0-15, 15-45, and 45-105 cm depths. Five cores were taken from each plot at both shallow
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16
17 169 depths for a total of 15 cores for each amendment-by-species treatment combination. A single
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19 170 core per plot was taken below 45 cm for a total of three per treatment combination. We
20
21 171 separated fine roots into two classes based on diameter: <1 mm and 1 to 5 mm. November
22
23 172 sampling corresponded with late autumn peak root length from a previous study with similar
24
25 173 species (Coleman et al. 2000). We collected two samples along the irrigation drip line and two
26
27 174 perpendicular to the drip line at points one-half and one-quarter the distance between trees, and
28
29
30 175 collected a fifth sample at the center between four trees. These relative sample locations captured
31
32 176 expected spatial heterogeneity in fine-root biomass resulting from drip application (Coleman
33
34 177 2007). Sampling in each year occurred at five different randomly selected trees per plot with one
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37 178 of the five relative sample locations assigned to each. Double sampling did not occur from any
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39 179 one location. We washed roots by elutriation, manually separated live roots from dead organic
40
41 180 matter, oven dried cleaned roots (60 °C), and weighed them to the nearest 0.01 mg.
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45 46 47 182 Minirhizotron observation tubes

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49 183 We measured fine-root production and lifespan using minirhizotron observation tubes (MROT).
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51 184 Tubes (5 cm acrylic) were installed at a 45° angle to a depth of 105 cm in May 2000. We painted
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54 185 the exposed aboveground portion of MROT black to limit light penetration and then white to

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3 186 limit heat absorption, and plugged the end with foam pipe insulation. The foam penetrated below
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5 187 ground line within MROTs to limit transfer of aboveground temperature. The upper end of the
6
7 188 foam insulation was glued to the inside bottom of a topless aluminum beverage can so that it
8
9 189 covered the tube when in place to limit entry of dirt and moisture. Plumber test plugs stoppered
10
11 190 underground ends of each tube. A 5 cm pipe hanger secured the aboveground end to a 1.3 cm
12
13 191 metal conduit driven 45 cm into the ground. We placed five MROTs per plot, or 15 tubes for
14
15 192 each amendment-by-species treatment combination. Coleman et al. (2004) illustrate the
16
17 193 locations, which were the same relative to trees as described above for soil samples. As with soil
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19 194 core samples, we randomly selected the five-reference trees and assigned one of the five relative
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21 195 locations to each. Soil coring for root biomass never occurred near MROTs.
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27
28 197 We installed MROTs in bare ground prior to substantial expansion of seedling roots to avoid
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30 198 installation effects cautioned by previous authors (Coleman et al. 2000; Joslin and Wolfe 1999;
31
32 199 Krasowski et al. 2010; Olesinski et al. 2011). Previous observations of mrot installation impacts
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34 200 on fine root dynamics occur in established forests where installation prunes existing roots and
35
36 201 alters production and mortality for one to two years as roots recover. Bare-field installation
37
38 202 avoids those impacts because placement occurs before roots reach soil viewed by MROTs. There
39
40 203 may also be disturbance impacts of tube installation on nutrient release (Johnson et al. 2001);
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42 204 however, this would be minimal in our study because site preparation activities disturbed the
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44 205 surface 30 cm where fine roots typically grow during seedling establishment. Consequently, it is
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46 206 reasonable to assume that observation directly following bare-field installation are free of
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48 207 installation artifacts.
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3 209 We captured digital images every 5 weeks starting September 2000 through June 2005 at precise
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5 210 locations along the upper MROT surface using a digital camera equipped with an indexing
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7 211 handle (BTC2; Bartz Technology, Santa Barbara, CA). To process images, operators used
8
9 212 Rootracker (Duke University, Durham, NC, USA) image analysis software to tally observations
10
11 213 of root length, width, branch order and condition. We could not positively identify branch order
12
13 214 for all observed roots. We identified branch order for first-order root tips that appeared in the
14
15 215 field of vision and for higher order roots subtending those tips. We categorized roots into three
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17 216 condition classes: new, previously observed, and missing, which allowed us to determine root
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19 217 lifespan. We did not include a condition class for dead roots due to the subjectivity in
20
21 218 determining their viability. Consequently, we only considered roots to be dead when missing,
22
23 219 which undoubtedly overestimated root longevity.
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221 Data analysis

222 We calculated fine-root production and mortality from measurements collected during image
223 analysis. Incremental fine-root length production was the sum of new root length between
224 consecutive imaging dates, while mortality was the sum of missing root length. Cumulative
225 production and mortality were the sum of all previous incremental production and mortality.
226 Live-root length was the difference between cumulative production and cumulative mortality up
227 to that observation date. Annual increment of fine-root length production and mortality was the
228 sum of increments for that year's 10 observation dates. Annual cumulative production and
229 mortality was the difference of that accumulated or missing between final observation dates of
230 consecutive years. Annual live-root length, or annual standing crop, was the average crop of all

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3 231 observation dates that year. Equations for each of these values are in the supplemental
4
5 232 information.
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9
10 234 We used repeated-measures analysis to assess fine-root biomass and MROT treatment responses
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12 235 over time. Data analysis evaluated all dependent variables using averaged within-plot
13
14 236 measurements (n=3 replicate plots). Fixed factors included fertilization (*F*), irrigation (*I*), and
15
16 237 species (*S*). Year (*Y*) was the repeated factor with plot being the random subject factor. Proc
17
18 238 Mixed (SAS Institute Inc., Cary, NC) performed the analysis with $\alpha=0.05$. The Kenward-Roger
19
20 239 method calculated denominator degrees of freedom. Lowest corrected Akaike's information
21
22 240 criterion (AICC) (Littell et al. 2006, p.183) identified the best covariate structure for each
23
24 241 dependent variable from a subset of appropriate structures. Tukey's HSD test compared
25
26 242 treatment means. Temporal traces of fine-root variables measured with MROT were compared
27
28 243 between species using the Kolmogorov- Smirnov two-sample test (SAS Proc NPar1Way) with
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30 244 $\alpha=0.05$.
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37 246 Survival analysis

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40 247 We achieve greater precision on the fate of fine-roots observed with MROT's using survival
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42 248 analysis compared with the repeated measures analysis (Coleman et al. 2000; Kern et al. 2004).
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44 249 The life-table method produced survival curves (Proc Lifetest, SAS Institute Inc., Cary, NC).
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46
47 250 Right-censored roots were those that had not disappeared by the end of the experiment. Log rank
48
49 251 and Wilcoxon test compared survival curves. We estimated root lifespan from survival functions
50
51 252 as the time of median root survival. Several fine-root populations of interest did not reach
52
53
54 253 median survival, so it was not possible to determine lifespan universally using observed median
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1
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3 254 root survival. Consequently, Cox' model estimated survival functions with corresponding
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5 255 median root lifespan.

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10 257 Cox' proportional hazards model estimates fine-root hazard ratios. Cox' model uses the partial
11
12 258 likelihood function to estimate parameters associated with each of the covariates (Allison 2010;
13
14 259 Savarese and Patetta 2010; Wells and Eissenstat 2001). It is a log-transformed exponential model
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18
19 261 $\log h_i(t) = \log \lambda_0(t) + \beta_1 x_{i1} + \dots + \beta_k x_{ik}$ Equation 1
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21

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23
24 263 where $h_i(t)$ is the hazard of mortality for root i at time t , $\lambda_0(t)$ is an unknown and unspecified
25
26 264 positive function, and β is the coefficient for each of k covariates. The SAS implementation of
27
28 265 Cox' regression (Proc Phreg, SAS Institute Inc., Cary, NC) uses the partial likelihood function to
29
30 266 eliminate $\lambda_0(t)$, assist development of the best model, calculate hazard ratios, estimate survival
31
32 267 functions, and predict median lifespan (Allison 2010). Exponentiation of β_k model estimates
33
34 268 calculates hazard ratios and provides odds ratios for the risk of root mortality given specified
35
36 269 conditions as compared with a reference condition (Allison 2010). Mortality (M) based on the
37
38 270 odds ratio was calculated as $M = 100 \cdot (e^\beta - 1)$, where β is the model coefficient for a factor
39
40 271 expressed as a percentage of the reference condition.
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47 273 The selected Cox model included main effects of intrinsic covariates that stratified various fine-
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49 274 root conditions, as well as main effects and interactions of the experimental factors of S , F and I .
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51 275 The fully parameterized model—including all interactions (up to seven-way) among covariates
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3 276 and experimental factors—was difficult to interpret, and only improved the model fit (AIC) by
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5 277 0.5%. Therefore, we simplified the model to include main effects of covariates, experimental
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7 278 factors, and interactions among experimental factors.
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12 280 We compared stand age, or year of measurement to other variables that might better represent
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14 281 stand development as an independent variable in repeated measures models testing fine-root
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16 282 biomass, length and dynamics, and in Cox' hazard model. The stand development variables
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18 283 tested included stand basal area, stem biomass, stem volume and total stand biomass. We
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20 284 selected the best fitting models based on lowest Akaike's information criterion (AIC).
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26 286 **Results**

31 288 Fine-root biomass and length

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33 289 Root biomass distribution compared favorably with the frequency of roots appearing in MROT, with
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35 290 some exceptions. The majority of fine-root biomass was ≤ 1 mm diameter. Roots ≤ 1 mm
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37 291 diameter composed 61% of CW biomass and 51% of LP biomass, with the remainder of fine-
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39 292 root biomass comprised of 1 to 5 mm diameter roots. In contrast, over 98.9% of CW and 97.0%
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41 293 of LP roots appearing in MROT were < 1 mm diameter (Fig. S2). When considering roots at the
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43 294 end of their lifespan, we found an almost identical diameter distribution, indicating observed fine
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45 295 roots did not increase in diameter. There was an obvious change in the slope of the diameter
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47 296 distribution curve for roots ≤ 1 mm diameter at appearance compared to those > 1 mm diameter;
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49 297 showing a rapid decline in the frequency of the finest roots and a more even distribution of roots
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51 298 1–5 mm diameter. Roots in biomass cores were often several centimeters long. Roots > 1 mm
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3 299 diameter were stiff and woody, which resulted in proportionally larger biomass relative to those
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5 300 <1 mm. In contrast, roots appearing in MROT's averaged 3.0 ± 3.2 mm in length (maximum 27
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7 301 mm), and the lengths were not disproportionately weighted based on diameter.
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12 303 Annual fine-root biomass responded to stand age, species and experimental factors. We selected
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14 304 stand age (i.e. year) as the best independent variable to represent stand development based on
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16 305 lowest AIC in models testing treatment responses of annual fine-root variables such as biomass,
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18 306 live-root length, production and mortality. The greatest effects on fine-root biomass were due to
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20 307 year of measurement and species (Fig. 1, greatest f -value for Y and S in Table 1). Species
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22 308 differences occurred in all years but 2000 ($S\times Y$ interactions in Table 1). Fine-root biomass
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24 309 generally increased through time. However, due to variation in the data, there was not always a
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26 310 stepwise annual increase. Any biomass response to F occurred between 2002 and 2004, whereas
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28 311 biomass did not respond to F in 2000 or 2005 ($F\times Y$ interactions in Table 1). The effects of F and
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30 312 I were both positive, but the F response was stronger (greater f -value in Table 1). For example,
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32 313 the maximum average annual increase in biomass due to F compared to C was 99%, whereas the
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34 314 maximum average annual increase due to I was only 42%
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42 316 The factors influencing fine-root length observed in MROT's were generally consistent with
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44 317 biomass response. For example, the order of influence on length was $Y > S > F > I$ (Table 1).
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46 318 Peak root length occurred in 2003 for both species in all treatments (Fig. 1). This peak in root
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48 319 length four years post-establishment was distinct from root biomass, which generally increased
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50 320 through time. Differences in fine-root length between species increased over time ($S\times Y$
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52 321 interaction). Root length consistently responded to treatments. For example, compared with C ,
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3 322 root length was consistently smaller with *I* and larger with *F* and *IF* (*I*, *F* and *I*×*F* effects in
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5 323 Table 1), especially in CW. In LP, *F* effects were not apparent (*F*×*S* interaction). Consistent
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7 324 treatment differences over time for root length was also distinct from root biomass where
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9 325 differences converged toward the end of observations.

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14 327 We observed a positive linear relationship between fine-root biomass and fine-root length (Fig.
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16 328 2), indicating that dynamics observed in MROT measurements were representative of variation
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18 329 in fine-root biomass (cf. Johnson et al. 2001; Majdi 1996). Based on this assumption, the
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20 330 regression equation presented in Fig. 2 was used to translate MROT root length values to
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22 331 biomass on a per unit land area basis.

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27 28 333 Fine-root dynamics

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30 334 Species and year predominately controlled fine-root dynamics (*S* and *Y* effects in Table 1).
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32 335 Cottonwood cumulative production and mortality observed in MROTs were consistently greater
33
34 336 than LP. Cumulative root length production showed annual growth cycles, with rapid early-
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36 337 season growth that decreased later in the season and remained low throughout dormancy (Fig.
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38 338 3a). Annual growth patterns were most evident in 1- to 3-year old CW and they dampened with
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40 339 age.

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46 341 Fine-root mortality was minimal for the first three years and increased thereafter (*Y* effect in
47
48 342 Table 1). Cumulative fine-root mortality was not distinct between species until 2004 (Fig. 3b).
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50 343 Annual oscillations in cumulative mortality were less evident than that observed in cumulative
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52 344 production.

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5 346 The length of live fine roots present at each observation (standing crop) resembled cumulative
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7 347 production during the first three growing seasons and reached a peak in the fourth growing
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9 348 season (Fig. 3c). Cottonwood live-root length was twice that of LP. Relatively stable live-root
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11 349 length occurred during the last five observation dates with values averaging 6.5 ± 0.8 mm cm⁻² for
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13 350 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.
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15 351 2).

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21 353 Fine-root production increments were low in year 1, peaked in years 2-4 and then progressively
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23 354 decreased in years 5-6 (Fig. S3a) for both CW and LP. Fine-root mortality increment peaked
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25 355 during the fourth year (Fig. S3b). Cottonwood typically had greater root mortality than LP;
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27 356 however, this difference was only significant in 2003 and 2004. Seasonal patterns were more
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29 357 distinct for fine-root production increments compared with mortality increments. Production
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31 358 increment during dormancy was a fraction of that observed during the growing season (Fig. S3a).
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33 359 Mortality increments were not seasonally consistent (Fig. S3b).
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40 361 Fertilizer effects on annual fine-root dynamics depended on species. Cottonwood live-root length
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42 362 increased in response to fertilization, while LP live-root length did not (Fig. 4c, $F \times S$ interaction
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44 363 in Table 1). Production and mortality increment response to fertilization also depended on
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46 364 species although the effect was not as strong as that seen for live-root length (Fig. 4a&b).
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52 366 Annual fine-root production increment and live-root length decreased with I , but were not
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54 367 influenced by F or IF (Fig. 4d, $I \times F$ interaction in Table 1). A similar but weaker response

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3 368 occurred for annual mortality increment. The influence of I on annual fine-root production
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5 369 increment occurred only in the third and fourth years ($I \times Y$ interaction).
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10 371 Fine-root survival analysis
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12 372 Soil depth predominately controlled the risk of fine-root mortality followed by diameter, total
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14 373 stem biomass, season, treatments, and species (chi-square, Table 2). Fine-root survival increased
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16 374 with depth for both species (Fig. S4). Based on Cox' regression parameter estimates, the risk of
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18 375 mortality was 19% higher for LP than CW, causing lifespan to increase 3.7 d for every cm depth
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20 376 in CW, and 2.8 d for every cm depth in LP. Lifespan increased with diameter and decreased as
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22 377 stands developed (Fig. S5). Risk of mortality decreased 5.1% for every 0.1 mm increase in root
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24 378 diameter and increased 2.3% for every Mg increase in stem biomass. Fine-root lifespan appeared
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26 379 to approach an asymptote as stands developed (Figs. 5 and S5). Fine roots appearing in winter
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28 380 had the highest risk of mortality (Fig. 6a) and the shortest lifespan (268 days).
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35 382 Several stand development variables explained risk of fine-root mortality. We selected stem
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37 383 biomass (Fig. S6) to represent stand development for evaluating fine-root dynamics because it
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39 384 best fit the data. Other measures of stand development like year (AIC = 1,107,336), stand basal
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41 385 area (AIC = 1,107,169), total stem volume (AIC = 1,107,098) or total stand biomass (AIC =
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43 386 1,107,058) did not fit as well as stem biomass (AIC = 1,107,019). Yet they all adequately
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45 387 represent development because each ranked third (chi-square) behind soil depth and root
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47 388 diameter, and always ranked higher than other factors tested in Cox' regression model. For the
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49 389 purpose of our study, stem biomass best represented stand development in determining risk of
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3 390 fine-root mortality (Table 2). The risk of mortality increased 2.3% for every Mg increase in stem
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5 391 biomass per ha, meaning that fine-root lifespan decreased four days per $\text{Mg}\cdot\text{ha}^{-1}$ (Figs. 5 and S5).
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10 393 Irrigation influenced the risk of mortality differently between species, but the response to
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12 394 fertilization was similar for both species (Fig. 6b). For CW, the risk of mortality decreased with
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14 395 *I*, causing median lifespan to increase from 268 d for *C* to 322 d for *I* at the reference condition
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16 396 (depth=1 cm, stem biomass = 5 $\text{Mg}\cdot\text{ha}^{-1}$, season=winter, diameter=0.5 mm). For LP, the risk of
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18 397 mortality increased with *I*, so median lifespan decreased from 231 d for *C* to 210 d for *I*. The risk
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20 398 of mortality decreased the same percentage for both species when grown with *F*, causing median
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22 399 lifespan to increase 40 d in CW and 46 d in LP. The risk of mortality decreased for both species
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24 400 when grown with *IF*, but the magnitude was distinct. Median lifespan increased by 113 days for
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26 401 CW grown with *IF* and it increased only 7 days for LP. Treatment responses in CW became
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28 402 more distinct with depth based on Cox' regression model estimates (Fig. S7). In this case,
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30 403 estimated lifespan increased 42% in *IF* relative to *C* at 1 cm depth compared to 61% at 90 cm. A
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32 404 similar increasing response did not occur in LP, where lifespan in *F* was greatest relative to *C*,
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34 405 but the increase was 20% at both 1 cm and 90 cm depth.
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407 **Discussion**

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

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3 413 MROT occurred in bare soil prior to root colonization, thus avoiding many of the confounding
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5 414 effects associated with installation artefacts reported in other MROT studies (see methods for
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7 415 details). Overall, we found consistent root production and turnover responses to stand
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9 416 development, phenology, rooting depth, initial root diameter, and species (Table 2). Only after
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11 417 considering such dominant controls, could we most accurately assess the more subtle fine-root
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13 418 responses to nutrient and moisture availability. Specifically, Cox' multivariate hazard analysis
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15 419 simultaneously accounted for variation caused by dominant controlling factors to estimate
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17 420 accurately risk of root mortality in response to resource availability. Among dominant control
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19 421 factors, we focus on stand development as a unique contribution of this study. For example, had
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21 422 we not accounted for a 10 Mg ha⁻¹ increase in stem biomass, which was the difference in stem
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23 423 biomass between *C* and *F* in 2005 (Fig. S6), it would have negated the 40 d increase in fine-root
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25 424 lifespan that we observed in response to *F* in both species (Fig. 5 and S7). Similarly, had we not
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27 425 accounted for other intrinsic factors such as root depth, diameter, or season, it would have altered
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29 426 both direction and magnitude of fine-root mortality risk in response to resource availability.
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38 Stand development effects on fine-root dynamics

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40 429 Although, stand age (year) best explained annual fine-root parameters (Table 1, Fig. 1), stem
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42 430 biomass (Fig. S6) better explained the fate of fine roots in Cox' hazard models because stem
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44 431 biomass increased differentially over time in response to resource availability treatments. Indeed,
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46 432 LP stem biomass production at this site was three times that of CW, and there were important
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48 433 distinctions between species in treatment response. Including stand biomass or basal area in the
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50 434 hazard analysis captured these effects. While we are unaware of studies directly investigating
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53 435 fine-root dynamics as a function of stand development, there are some reports where considering

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3 436 stand development might be useful for explaining observed results. For example, one study
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5 437 concluded that stem diameter increment at a common age controlled fine-root lifespan
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7 438 (McCormack et al. 2012; McCormack et al. 2014). These authors were uncertain about the
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10 439 reason for a negative correlation between stem diameter growth rate and fine-root lifespan, yet
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12 440 our results suggest that their observation may have been predominantly due to differences in
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14 441 stand development, since tree size is an important measure of stand development.
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19 443 Our approach considers the dynamic stages of early stand development. Short-rotation
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21 444 intensively managed forests represent model systems for natural stand development that rapidly
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23 445 progress through stand establishment and increased inter-tree competition. Few other reports
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25 446 addressing temporal effects on fine roots consider time scales relevant to questions of stand
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27 447 development (Borja et al. 2008; Brassard et al. 2009). Studies that consider fine-root responses to
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29 448 stand development typically compare neighboring stands of different ages. Such chronosequence
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31 449 studies show that fine-root biomass increases during establishment and then remains constant or
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33 450 declines as stands age (Borja et al. 2008; Brassard et al. 2009; Yuan and Chen 2010), which
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35 451 confirms the peak we observed after four years (Fig. 3c). This peak built up as production
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37 452 initially exceeded mortality, and then declined after year 4 when mortality equaled or exceeded
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39 453 production (Fig. S3). The peak corresponded with root closure observed with biomass cores
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41 454 (Coleman 2007). Fine-root biomass continued to increase after site occupation, while fine-root
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43 455 length stabilized (Fig. 1). This may result from a different proportion of functional classes
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45 456 observed in root biomass and MROT approaches. (cf. Guo et al. 2008; Trumbore and Gaudinski
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47 457 2003). Our observed maximum fine-root length represents fine roots <1 mm diameter (Fig. S2).
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49 458 The diameter distribution at initial appearance in MROT was indistinguishable from the
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3 459 diameter distribution of older roots, which suggests that MROT's did not monitor roots with
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5 460 secondary thickening; however, high tensile and flexural strength in roots found in biomass
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7 461 samples indicated thickening, which suggests that mass distribution does not show the same
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9 462 pattern as fine-root length seen in MROT's.
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14 464 We are only aware of three other MROT studies of similar duration, each of which began in
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16 465 established stands. Norby et al.'s (2004) observations in *Liquidambar styraciflua* L. started in a
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18 466 9-yr-old stand with a basal area of 28 m² ha⁻¹. They found that fine-root standing crop increased
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20 467 to a plateau after 4 yr. The plateau is consistent with the peak standing crop we observed in year
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22 468 4 (i.e. 3-yr-old); although their plateau was significantly delayed in comparison and it does not
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24 469 appear to decline. The *L. styraciflua* stands in our study developed more slowly than did CW or
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26 470 LP (Coyle et al. 2016), which might help explain the delayed peak; however, these patterns may
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28 471 also be artefacts of installing MROT's after root closure actually occurred. Krasowski et al.
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30 472 (2010) report a similar increase in fine-root standing crop in the first years of observation and
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32 473 attributed that to artefacts of tube-installation. In their case, standing crop reached a peak after 3
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34 474 to 6 years where it remained constant or slightly declined depending on study location. Pritchard
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36 475 et al.'s (2008) observations occurred in 17-yr-old LP starting at a basal area of 17 – 25 m² ha⁻¹
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38 476 (Ellsworth et al. 1995) where they found a steady temporal decline in fine-root standing crop.
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40 477 That decline appears to be free of installation artefacts and is consistent with our observed
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42 478 declines occurring after peak standing crop. Consequently, each of these studies in established
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44 479 stands report constant or declining standing crop following any initial post-installation surge in
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46 480 production.
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3 482 Belowground site occupation appears to be similar to that aboveground where leaf area index
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5 483 increases to a maximum and then remains constant or declines (Schoonmaker et al. 2016; White
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7 484 et al. 2010; Yuan and Chen 2010). These parallel root and leaf developmental patterns suggest
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10 485 that established stands regulate production and mortality of roots and leaves to maintain
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12 486 appropriate surface area for resource acquisition. Positive correlations between stand growth,
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14 487 root biomass and leaf area in response to resource availability (Coyle et al. 2016; Martin and
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16 488 Jokela 2004; Vose and Allen 1988) also suggests that regulation of absorption surface are inter-
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18 489 dependent processes of stand growth and development (cf. Litton et al. 2007). Our results
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20 490 demonstrate this inter-dependence based on the large and significant chi-square for stem biomass
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22 491 in Cox' regression model (Table 2), which shows that whole-tree growth responses influence
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24 492 fine-root dynamics. Production and turnover of leaves and fine-roots appear to regulate leaf and
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26 493 root surface area presentation during early stand development.
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33 495 Although the literature contains many examples of resource availability and stand development
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35 496 influencing maximum leaf area and canopy closure (Carlyle 1998; Landsberg and Waring 1997;
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37 497 Martin and Jokela 2004; Vose and Allen 1988; White et al. 2010), relatively little is known about
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39 498 similar influences on fine-root standing crop and root closure. Based on what we know about leaf
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41 499 area dynamics through stand development, we expected fine-root standing crop to peak at an
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43 500 early age, and then remain relatively constant or decline. When considering fine-root length, the
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45 501 time required to achieve peak fine-root standing crop was independent of resource availability,
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47 502 but maximum fine-root standing crop was not (Fig. 1). Thus, this study leads to questions of
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49 503 timing and extent of root and canopy closure, and site carrying capacity for resource acquisition
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52 504 surface both above and belowground. It also emphasizes the need to account for stand
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3 505 development (i.e. ontogeny) when comparing the balance between those surfaces among stands
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5 506 grown with different levels of resource availability.
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10 508 The large allocation of carbon to fine-root production in young stands suggest that establishing
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12 509 root systems is a priority. The proportion of fine root production (FRP) to total net primary
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14 510 productivity (NPP) typically ranges from 26% to 56% (Gill and Jackson 2000; Vogt et al. 1996;
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16 511 Yuan and Chen 2010) depending upon species, climate and site interactions, but there are few
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18 512 reports regarding shifts in allocation with stand development. We found that FRP:NPP declined
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20 513 from 66% in year 1 to less than 3% in year 6 in LP, whereas it reached a minimum of 14% in
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22 514 year 2 and then it rose to an average of 81% between years 4 and 6 for CW. During this
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24 515 substantial developmental change, FRP:NPP was not affected by irrigation, yet the ratio for
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26 516 fertilized plots was consistently half that of non-fertilized. The variation in FRP:NPP observed
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28 517 with these species suggests that species have adopted different allocation strategies which may
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30 518 be related to functional type and seral stage (Grime 1979). Peak total NPP in our study occurred
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32 519 about the time of maximum leaf area (Coyle et al. 2016; Coyle and Coleman 2005; Coyle et al.
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34 520 2008), which is consistent with other observations (Ryan et al. 2004). In addition, annual fine
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36 521 root production was correlated with total NPP, but only for CW ($P<0.001$, $r^2=0.40$) because LP
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38 522 ($P=0.89$) fine root production peaked at least one year before total NPP. While belowground
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40 523 carbon allocation is often directly related to total NPP and other autotrophic processes (Högberg
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42 524 et al. 2001; Irvine et al. 2005), our results show that autotrophic components of belowground
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44 525 carbon allocation are not always in phase with total NPP. This might explain why correlations
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46 526 between belowground carbon allocation and total NPP are not always evident (Litton et al.
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48 527 2007). The eventual increase in FRP:NPP observed with CW is consistent with the increasing
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3 528 [proportion of total belowground carbon allocation relative to gross primary productivity](#) (Ryan et
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5 529 al. 2004) [and increasing soil CO₂ efflux response with stand age](#) (Wiseman and Seiler 2004; Yan
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7 530 et al. 2011), [considering autotrophic respiration can account for half of soil CO₂ efflux](#) (Hanson
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9 531 et al. 2000). [In contrast, the decline in FRP:NPP found with LP is consistent with decreased soil](#)
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11 532 [CO₂ efflux with stand age](#) (Klopatek 2002; Saiz et al. 2006). [The links between fine-root](#)
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13 533 [dynamics, total NPP and soil CO₂ efflux remain an open question for understanding components](#)
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15 534 [of belowground carbon allocation, yet here we demonstrate that stand development is a critical](#)
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17 535 [control factor.](#)
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23 24 537 Resource availability effects on fine-root dynamics

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26 538 Resource availability had subtle and inconsistent influence on fine-root variables compared to
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28 539 the predictable responses observed to root depth, initial root diameter, phenology or stand
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30 540 development. Consequently, it was not possible to generalize about even the direction of
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32 541 resource availability influence on fine-root dynamics. Fine-root biomass either increased relative
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34 542 to *C* or was unaffected by *I*, *F*, or *IF* (Fig. 1). Fine-root production and root length standing crop
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36 543 decreased with *I* relative to *C*; however, these variables increased with fertilization for CW and
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38 544 had no effect on LP (Fig. 4). The risk of mortality both increased and decreased relative to *C*
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40 545 depending on species and amendment treatment (Fig. 6b). Since risk of mortality is; also
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42 546 expressed as root longevity, it also serves as a surrogate for fine-root turnover, since turnover is
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44 547 the inverse of root longevity. Based on this relationship we might conclude that carbon
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46 548 allocation to turnover in response to resource availability is equally inconsistent. Literature
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48 549 reports describe wide-ranging fine-root responses to soil moisture and nutrient availability,
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50 550 which supports the variable results we observe between species on fine-root production and
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3 551 turnover in response to experimental resource availability treatments. Previous studies reporting
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5 552 fine-root mortality and production responses to increased water availability demonstrate
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7 553 decreases (Gaul et al. 2008), increases (Meier and Leuschner 2008; Olesinski et al. 2011), or no
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9 554 effect (Joslin et al. 2001; King et al. 2002; Rytter 2013). Responses to nutrient availability are
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11 555 equally inconclusive (Brassard et al. 2009; Chen and Brassard 2013; Eissenstat et al. 2013;
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13 556 Hodge 2004; Nadelhoffer 2000; Norby and Jackson 2000). However, most of these studies
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15 557 compare chronological age rather than normalizing with a measure of stand development like
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17 558 stem biomass or stand basal area. Yet as described above, risk of fine-root mortality consistently
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19 559 responds to stand development, and other intrinsic factors. Accounting for these factors has
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21 560 important consequences for understanding responses to resource availability.
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28 562 These inconsistencies contradict the concept that greater carbon or biomass allocation will be
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30 563 directed toward roots when soil resources are limited compared to when they are abundant
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32 564 (Axelsson and Axelsson 1986; Brassard et al. 2009; Brouwer 1983; Hunt and Lloyd 1987; Keyes
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34 565 and Grier 1981; Lambers 1983; Reynolds and Thornley 1982). Thus, our results combined with
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36 566 those of others considering fine-root dynamics do not support the general concept that lower
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38 567 amounts of growth-limiting resources will increase allocation to roots because that directional
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40 568 shift is observed in some, but not all cases. Crucially, compared to the dominant and consistent
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42 569 influence of stand development, rooting depth, diameter, phenology and species, the minor and
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44 570 directionally inconsistent responses to resource availability do not warrant the attention they
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46 571 receive. In fact, resource responses are so subtle that accounting for them never contradicts our
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48 572 inference that intrinsic factors primarily control fine-root dynamics (e.g., see Figs. 5, 7 and S7).
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3 574 Other fine-root control factors
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5 575 Soil depth, root diameter, branch order, phenology and species exerted consistent influence on
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7 576 fine-root turnover as measured through mortality risk. Figure 7 illustrates the relative magnitude
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10 577 of factors controlling mortality risk observed for the given species based on chi-square values in
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12 578 Table 1. Soil depth was especially dominate in our study as an important intrinsic factor
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14 579 affecting mortality risk. Authors consistently report that roots growing in deeper soil have
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16 580 increased root longevity (Baddeley and Watson 2005; Coleman et al. 2000; Kern et al. 2004;
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18 581 Peek et al. 2006; Stover et al. 2010; Wells et al. 2002) and decreased root^{ing} density (Jackson et
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20 al. 1996). In our study, soil depth explained the most variation of any factor for risk of root
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22 582 mortality (Table 2, Fig. S4) and biomass distribution (Coleman 2007). Not only were there fewer
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24 583 roots at depth, but deeper roots had longer lifespans. Considering the very different chemical and
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26 584 physical environment at depth (Fang and Moncrieff 2005), deeper roots are expected to be
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28 585 functionally distinct from those growing near the surface (Brassard et al. 2009; Chen and
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30 586 Brassard 2013; Iversen 2010; Pregitzer et al. 1998).
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38 589 Root diameter was second to soil depth in explaining mortality risk. We measured root diameter
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40 590 using a continuous scale and conclude that the risk of mortality decreased 5.1% for every 0.1 mm
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42 591 increase in diameter. This confirms other reports that conclude fine-root lifespan increases with
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44 592 increasing root diameter class (Chen and Brassard 2013; Coleman et al. 2000; Kern et al. 2004;
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46 593 Wells and Eissenstat 2001). We chose to use root diameter as a co-factor over root branch order
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48 594 for both effectiveness and practicality. Root branch order is considered to be an important
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50 595 criteria for evaluating root function and lifespan (Chen and Brassard 2013; Comas et al. 2002;
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52 596 Fitter 1985; Fitter 1992; Jia et al. 2013; Majdi et al. 2001; McCormack et al. 2015; Pregitzer et
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3 597 al. 1997; Wells et al. 2002). Branch order explained a significant amount of variation in fine-root
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5 598 lifespan in our study for those roots where it was available; however, the branch order of most
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7 599 roots observed via MROT's was uncertain (86% not declared) because we could only confirm
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9 600 root order of the most distal roots with obvious root tips and those subtending. In contrast, we
10
11 601 precisely measured root diameter on all roots. Diameter and root order are strongly correlated
12
13 602 within a species (Comas et al. 2002; Guo et al. 2004; Jia et al. 2013; Pregitzer et al. 2002).
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15 603 Consequently, diameter measured on a continuous scale was a more practical and effective
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17 604 measurement than branch order when controlling for root morphology.
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24 606 Phenology and species also influenced fine-root turnover and therefore are influential co-factors.
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26 607 We observed distinct annual oscillations of fine-root production with maxima during the growing
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28 608 season and minima during dormancy. Numerous reports describe similar phenological patterns of
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30 609 fine-root production and turnover (Baddeley and Watson 2005; Coleman et al. 2000; Joslin et al.
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32 610 2001; Kern et al. 2004; King et al. 2002; McCormack et al. 2014; Rytter 2013; Tierney et al.
33
34 611 2003). The magnitude of fine-root production, mortality and live-root standing crop differed
35
36 612 between tree species. Differences in root traits occur for species with different leaf habits (Vogt
37
38 613 et al. 1996), relative growth rate (Comas et al. 2002; Comas and Eissenstat 2004; McCormack et
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40 614 al. 2012; McCormack et al. 2014; Wright and Westoby 1999), and among species from different
41
42 615 biomes (Finer et al. 2011a; Finer et al. 2011b; Vogt et al. 1996). Thus, controlling for phenology
43
44 616 and comparing functionally distinct species is also vital for assessing responses of fine-root
45
46 617 dynamics to soil resources. Due to the variety of species distinction, Figure 7 does not attempt to
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48 618 rank the influence of species among other factors controlling fine-root dynamics. Rather, the
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3 619 figure seeks to demonstrate within a given species the relatively minor influence of resource
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5 620 availability among other factors, based on data presented in Table 2.
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10 622 **Conclusion**

11
12 623 Our study identified stand development as a major factor explaining the variation in fine-root
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14 624 dynamics. Furthermore, to determine accurately the response of fine-root dynamics to resource
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16 625 availability, we conclude that it is necessary to consider the influence of intrinsic factors,
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18 626 including stand development, rooting depth, initial root diameter, phenology and species.
19
20 627 Multivariate analysis using Cox' regression demonstrates a useful approach to control for the
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22 628 influence of intrinsic factors when attempting to measure the relatively minor effects of resource
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24 629 availability. The variation we observed among species and resource availability treatments and
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26 630 the dependence upon measures of stand development demonstrates that generalizations about the
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28 631 directional response of fine-root dynamics to resource availability require reevaluation.
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35 633 Our results have important implications for understanding and modeling factors controlling
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37 634 carbon allocation to fine roots. We understand with certainty the direction if not magnitude of
38
39 635 several intrinsic factors controlling fine-root turnover like depth, root diameter, and phenology.
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41 636 Here we quantified the relative impact of stand development, which also appears to exert
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43 637 consistent controls on fine-root turnover. Predictive models should include turnover functions
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45 638 that vary by depth, root diameter and stand development to predict more accurately carbon flux
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47 639 from live roots to soil organic matter. Models that include intra-annual time steps should include
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49 640 phenological shifts from production in spring to mortality in autumn. However, there is a
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51 641 necessity to revisit those models that include generalized increases in carbon allocation to fine
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3 642 | roots especially when ~~water and nutrients are~~ soil moisture is limiting. Such functions exist in
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5 643 | many prominent ecosystem process models (Landsberg and Waring 1997; Parton et al. 2010;
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7 644 | Running and Gower 1991), some of which are now used in land models to predict continental-
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9 645 | and global-scale responses to environmental change (Lawrence et al. 2011; Smithwick et al.
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11 646 | 2014). Based on our results, and those of other researchers, the modeling community should
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13 647 | incorporate details introduced by intrinsic factors controlling fine roots (cf. Franklin et al. 2012)
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15 648 | and suspend the use of simplistic resource availability controls to describe allocation to fine
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17 649 | roots.
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45
46
47

48 662 **References**

49
50
51 663 | Allison PD (2010) Survival Analysis Using SAS: A Practical Guide, 2nd edn. Sas Press, Cary,
52
53 664 | NC.
54
55
56
57
58
59
60

- 1
2
3 665 Atkinson D (1980) The Distribution and Effectiveness of the Roots of Tree Crops. Horticultural
4
5 666 Reviews. 2:424-490.
6
7
8 667 Aubrey D, Coyle D, Coleman M (2012) Functional groups show distinct differences in nitrogen
9
10 668 cycling during early stand development: implications for forest management. Plant Soil.
11
12 669 351:219-236.
13
14
15 670 Axelsson E, Axelsson B (1986) Changes in carbon allocation patterns in spruce and pine trees
16
17 671 following irrigation and fertilization. Tree Physiology. 2:189-204.
18
19 672 Baddeley J, Watson C (2005) Influences of Root Diameter, Tree Age, Soil Depth and Season on
20
21 673 Fine Root Survivorship in *Prunus avium*. Plant Soil. 276:15-22.
22
23
24 674 Bauerle TL, Richards JH, Smart DR, Eissenstat DM (2008) Importance of internal hydraulic
25
26 675 redistribution for prolonging the lifespan of roots in dry soil. Plant Cell and Environment.
27
28 676 31:177-186.
29
30
31 677 Borja I, De Wit HA, Steffenrem A, Majdi H (2008) Stand age and fine root biomass, distribution
32
33 678 and morphology in a Norway spruce chronosequence in southeast Norway. Tree
34
35 679 Physiology. 28:773-784.
36
37
38 680 Brassard BW, Chen HYH, Bergeron Y (2009) Influence of Environmental Variability on Root
39
40 681 Dynamics in Northern Forests. Crit Rev Plant Sci. 28:179-197.
41
42
43 682 Brouwer R (1983) Functional equilibrium: sense or nonsense? Netherlands Journal of
44
45 683 Agricultural Science. 31:335-348.
46
47 684 Carlyle JC (1998) Relationships between nitrogen uptake, leaf area, water status and growth in
48
49 685 an 11-year-old *Pinus radiata* plantation in response to thinning, thinning residue, and
50
51 686 nitrogen fertiliser. For Ecol Manage. 108:41-55.
52
53
54
55
56
57
58
59
60

- 1
2
3 687 Chen HYH, Brassard BW (2013) Intrinsic and Extrinsic Controls of Fine Root Life Span. Crit
4
5 688 Rev Plant Sci. 32:151-161.
6
7
8 689 Coleman M (2007) Spatial and temporal patterns of root distribution in developing stands of four
9
10 690 woody crop species grown with drip irrigation and fertilization. Plant Soil. 299:195-213.
11
12 691 Coleman MD, Coyle DR, Blake J, Britton K, Buford M, Campbell RG, Cox J, Cregg B, Daniels
13
14 692 D, Jacobson M, Johnson K, McDonald T, McLeod K, Nelson E, Robison D, Rummer R,
15
16 693 Sanchez F, Stanturf J, Stokes B, Trettin C, Tuskan J, Wright L, Wullschleger S. 2004.
17
18 694 Production of Short Rotation Woody Crops Grown with a Range of Nutrient and Water
19
20 695 Availability: Establishment Report and First-Year Responses. USDA Forest Service,
21
22 696 Southern Research Station, Asheville, NC, USA, p 21.
23
24
25
26 697 Coleman MD, Dickson RE, Isebrands JG (2000) Contrasting fine-root production, survival and
27
28 698 soil CO₂ efflux in pine and poplar plantations. Plant Soil. 225:129-139.
29
30
31 699 Comas LH, Anderson LJ, Dunst RM, Lakso AN, Eissenstat DM (2005) Canopy and
32
33 700 environmental control of root dynamics in a long-term study of Concord grape. New
34
35 701 Phytol. 167:829-840.
36
37
38 702 Comas LH, Bouma TJ, Eissenstat DM (2002) Linking root traits to potential growth rate in six
39
40 703 temperate tree species. Oecologia. 132:34-43.
41
42
43 704 Comas LH, Eissenstat DM (2004) Linking fine root traits to maximum potential growth rate
44
45 705 among 11 mature temperate tree species. Funct Ecol. 18:388-397.
46
47 706 Coyle DR, Aubrey DP, Coleman MD (2016) Growth responses of narrow or broad site adapted
48
49 707 tree species to a range of resource availability treatments after a full harvest rotation. For
50
51 708 Ecol Manage. 362:107-119.
52
53
54
55
56
57
58
59
60

- 1
2
3 709 Coyle DR, Coleman MD (2005) Forest production responses to irrigation and fertilization are not
4
5 710 explained by shifts in allocation. For Ecol Manage. 208:137–152.
6
7
8 711 Coyle DR, Coleman MD, Aubrey D (2008) Above- and below-ground biomass accumulation,
9
10 712 production and distribution of sweetgum and loblolly pine grown with irrigation and
11
12 713 fertilization. Can J For Res. 38:1335-1348.
13
14
15 714 Eckenwalder JE (2001) Descriptions of clonal characteristics. In: Dickmann DI, Isebrands JG,
16
17 715 Eckenwalder JE, Richardson J (eds) Poplar Culture in North America. National Research
18
19 716 Council of Canada, NRC Press, Ottawa, pp 336-382.
20
21
22 717 Eissenstat DM, McCormack ML, Du QY (2013) Global Change and Root Lifespan. Plant Roots:
23
24 718 The Hidden Half, 4th Edition.
25
26 719 Eissenstat DM, Yanai RD (1997) The ecology of root lifespan. Adv Ecol Res. 27:1-60.
27
28
29 720 Ellsworth DS, Oren R, Huaun C, Phillips N, Hendrey GR (1995) Leaf and canopy responses to
30
31 721 elevated CO₂ in a pine forest under free-air CO₂ enrichment. Oecologia. 104:139-146.
32
33 722 Fang C, Moncrieff J (2005) The variation of soil microbial respiration with depth in relation to
34
35 723 soil carbon composition. Plant Soil. 268:243-253.
36
37
38 724 Finer L, Ohashi M, Noguchi K, Hirano Y (2011a) Factors causing variation in fine root biomass
39
40 725 in forest ecosystems. For Ecol Manage. 261:265-277.
41
42
43 726 Finer L, Ohashi M, Noguchi K, Hirano Y (2011b) Fine root production and turnover in forest
44
45 727 ecosystems in relation to stand and environmental characteristics. For Ecol Manage.
46
47 728 262:2008-2023.
48
49 729 Fitter AH (1985) Functional significance of root morphology and root system architecture. In:
50
51 730 Fitter AH, Atkinson D, Read DJ, Usher MB (eds) Ecological interactions in soil.
52
53 731 Blackwell Scientific Publication, Oxford, pp 87-106.
54
55
56
57
58
59
60

- 1
2
3 732 Fitter AH (1992) Architecture and biomass allocation as components of the plastic response of
4
5 733 roots to soil heterogeneity. In: Caldwell MM, Pearcy RW (eds) Exploitation of
6
7 734 environmental heterogeneity by plants. Academic Press.
8
9
10 735 Franklin O, Johansson J, Dewar RC, Dieckmann U, McMurtrie RE, Brännström Å, Dybzinski R
11
12 736 (2012) Modeling carbon allocation in trees: a search for principles. *Tree Physiology*.
13
14 737 32:648-666.
15
16
17 738 Gaul D, Hertel D, Borken W, Matzner E, Leuschner C (2008) Effects of experimental drought on
18
19 739 the fine root system of mature Norway spruce. *For Ecol Manage*. 256:1151-1159.
20
21 740 Gill RA, Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. *New*
22
23 741 *Phytol*. 147:13-31.
24
25
26 742 Grime JP (1979) *Plant strategies and vegetation processes*. John Wiley, Chichester.
27
28 743 Guo DL, Li H, Mitchell RJ, Han WX, Hendricks JJ, Fahey TJ, Hendrick RL (2008) Fine root
29
30 744 heterogeneity by branch order: exploring the discrepancy in root turnover estimates
31
32 745 between minirhizotron and carbon isotopic methods. *New Phytol*. 177:443-456.
33
34
35 746 Guo DL, Mitchell RJ, Hendricks JJ (2004) Fine root branch orders respond differentially to
36
37 747 carbon source-sink manipulations in a longleaf pine forest. *Oecologia*. 140:450-457.
38
39
40 748 Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial
41
42 749 contributions to soil respiration: A review of methods and observations.
43
44 750 *Biogeochemistry*. 48:115-146.
45
46
47 751 Hertel D, Strecker T, Muller-Haubold H, Leuschner C (2013) Fine root biomass and dynamics in
48
49 752 beech forests across a precipitation gradient - is optimal resource partitioning theory
50
51 753 applicable to water-limited mature trees? *J Ecol*. 101
52
53
54
55
56
57
58
59
60

- 1
2
3 754 Hodge A (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. *New*
4
5 755 *Phytol.* 162:9-24.
6
7 756 Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G,
8
9
10 757 Ottosson-Lofvenius M, Read DJ (2001) Large-scale forest girdling shows that current
11
12 758 photosynthesis drives soil respiration. *Nature.* 411:789-792.
13
14 759 Hunt R, Lloyd PS (1987) Growth and partitioning. *New Phytol.* 106:235-249.
15
16 760 Irvine J, Law BE, Kurpius MR (2005) Coupling of canopy gas exchange with root and
17
18 761 rhizosphere respiration in a semi-arid forest. *Biogeochemistry.* 73:271-282.
19
20 762 Iversen CM (2010) Digging deeper: fine-root responses to rising atmospheric CO₂ concentration
21
22 763 in forested ecosystems. *New Phytol.* 186:346-357.
23
24 764 Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED (1996) A global
25
26 765 analysis of root distributions for terrestrial biomes. *Oecologia.* 108:389-411.
27
28 766 Jia S, McLaughlin NB, Gu J, Li X, Wang Z (2013) Relationships between root respiration rate
29
30 767 and root morphology, chemistry and anatomy in *Larix gmelinii* and *Fraxinus*
31
32 768 *mandshurica*. *Tree Physiology.* 33:579-589.
33
34 769 Johnson MG, Tingey DT, Phillips DL, Storm MJ (2001) Advancing fine root research with
35
36 770 minirhizotrons. *Environ Exp Bot.* 45:263; 289.
37
38 771 Joslin JD, Wolfe MH (1999) Disturbances during minirhizotron installation can affect
39
40 772 observation data. *Soil Sci Soc Am J.* 63:218-221.
41
42 773 Joslin JD, Wolfe MH, Hanson PJ (2001) Factors controlling the timing of root elongation
43
44 774 intensity in a mature upland oak stand. *Plant Soil.* 228:201; 212.
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 775 Katterer T, Fabiao A, Madeira M, Ribeiro C, Steen E (1995) Fine-Root Dynamics, Soil-Moisture
4
5 776 and Soil Carbon Content in a Eucalyptus-Globulus Plantation under Different Irrigation
6
7 777 and Fertilization Regimes. For Ecol Manage. 74:1-12.
8
9
10 778 Kern CC, Friend AL, Johnson JM-F, Coleman MD (2004) Fine-root dynamics in a developing
11
12 779 *Populus deltoides* plantation. Tree Physiology. 24:651-660.
13
14 780 Keyes MR, Grier CC (1981) Above- and below-ground net production in 40-year-old Douglas-
15
16 781 fir stands on low and high productivity sites. Can J For Res. 11:599-605.
17
18 782 King JS, Albaugh TJ, Allen HL, Buford M, Strain BR, Dougherty P (2002) Below-ground
19
20 783 carbon input to soil is controlled by nutrient availability and fine root dynamics in
21
22 784 loblolly pine. New Phytol. 154:389-398.
23
24 785 Klopatek JM (2002) Belowground carbon pools and processes in different age stands of
25
26 786 Douglas-fir. Tree Physiology. 22:197-204.
27
28 787 Krasowski MJ, Lavigne MB, Olesinski J, Bernier PY (2010) Advantages of long-term
29
30 788 measurement of fine root demographics with a minirhizotron at two balsam fir sites. Can
31
32 789 J For Res. 40:1128-1135.
33
34 790 Lambers H (1983) "The functional equilibrium," nibbling on the edges of a paradigm.
35
36 791 Netherlands Journal of Agricultural Science. 31:305-311.
37
38 792 Landsberg JJ, Waring RH (1997) A generalised model of forest productivity using simplified
39
40 793 concepts of radiation-use efficiency, carbon balance and partitioning. For Ecol Manage.
41
42 794 95:209-228.
43
44 795 Lawrence DM, Oleson KW, Flanner MG, Thornton PE, Swenson SC, Lawrence PJ, Zeng XB,
45
46 796 Yang ZL, Levis S, Sakaguchi K, Bonan GB, Slater AG (2011) Parameterization
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 797 Improvements and Functional and Structural Advances in Version 4 of the Community
4
5 798 Land Model. *J Adv Mod Earth Sys.* 3
6
7
8 799 Littell RC, Milliken GA, Stroup WW, Wolfinger RD (2006) SAS for Mixed Models, 2nd edn.
9
10 800 SAS Institute, Inc., Cary, NC, USA.
11
12 801 Litton CM, Raich JW, Ryan MG (2007) Carbon allocation in forest ecosystems. *Global Change*
13
14 802 *Biol.* 13:2089-2109.
15
16
17 803 Magbanua ZV, Ozkan S, Bartlett BD, Chouvarine P, Saski CA, Liston A, Cronn RC, Nelson CD,
18
19 804 Peterson DG (2011) Adventures in the Enormous: A 1.8 Million Clone BAC Library for
20
21 805 the 21.7 Gb Genome of Loblolly Pine. *PLoS ONE.* 6:e16214.
22
23
24 806 Majdi H (1996) Root sampling methods - applications and limitations of the minirhizotron
25
26 807 technique. *Plant Soil.* 185:255-258.
27
28
29 808 Majdi H, Andersson P (2005) Fine root production and turnover in a Norway spruce stand in
30
31 809 northern Sweden: Effects of nitrogen and water manipulation. *Ecosystems.* 8:191-199.
32
33
34 810 Majdi H, Damm E, Nylund JE (2001) Longevity of mycorrhizal roots depends on branching
35
36 811 order and nutrient availability. *New Phytol.* 150:195-202.
37
38 812 Martin TA, Jokela EJ (2004) Stand development and production dynamics of loblolly pine under
39
40 813 a range of cultural treatments in north-central Florida USA. *For Ecol Manage.* 192:39-58.
41
42
43 814 McCormack ML, Adams TS, Smithwick EAH, Eissenstat DM (2012) Predicting fine root
44
45 815 lifespan from plant functional traits in temperate trees. *New Phytol.* 195:823-831.
46
47 816 McCormack ML, Adams TS, Smithwick EAH, Eissenstat DM (2014) Variability in root
48
49 817 production, phenology, and turnover rate among 12 temperate tree species. *Ecology.*
50
51 818 95:2224-2235.
52
53
54
55
56
57
58
59
60

- 1
2
3 819 McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari H-S,
4
5 820 Hobbie EA, Iversen CM, Jackson RB, Leppälammı-Kujansuu J, Norby RJ, Phillips RP,
6
7 821 Pregitzer KS, Pritchard SG, Rewald B, Zadworny M (2015) Redefining fine roots
8
9 822 improves understanding of below-ground contributions to terrestrial biosphere processes.
10
11 823 New Phytol. 207:505-518.
- 12
13
14 824 Meier IC, Leuschner C (2008) Genotypic variation and phenotypic plasticity in the drought
15
16 825 response of fine roots of European beech. *Tree Physiology*. 28:297-309.
- 17
18
19 826 Misson L, Gershenson A, Tang JW, McKay M, Cheng WX, Goldstein A (2006) Influences of
20
21 827 canopy photosynthesis and summer rain pulses on root dynamics and soil respiration in a
22
23 828 young ponderosa pine forest. *Tree Physiology*. 26:833-844.
- 24
25
26 829 Nadelhoffer KJ (2000) The potential effects of nitrogen deposition on fine-root production in
27
28 830 forest ecosystems. *New Phytol*. 147:131; 139.
- 29
30
31 831 Norby RJ, Jackson RB (2000) Root dynamics and global change: seeking an ecosystem
32
33 832 perspective. *New Phytol*. 147:3-12.
- 34
35 833 Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG (2004) Fine-root production dominates
36
37 834 response of a deciduous forest to atmospheric CO₂ enrichment. *Proceedings of the*
38
39 835 *National Academy of Sciences of the United States of America*. 101:9689-9693.
- 40
41
42 836 Olesinski J, Lavigne MB, Krasowski MJ (2011) Effects of soil moisture manipulations on fine
43
44 837 root dynamics in a mature balsam fir (*Abies balsamea* L. Mill.) forest. *Tree Physiology*.
45
46 838 31:339-348.
- 47
48
49 839 Parton WJ, Hanson PJ, Swanston C, Torn M, Trumbore SE, Riley W, Kelly R (2010) ForCent
50
51 840 model development and testing using the Enriched Background Isotope Study
52
53 841 experiment. *Journal of Geophysical Research: Biogeosciences*. 115:G04001.

- 1
2
3 842 Peek MS, Leffler AJ, Hipps L, Ivans S, Ryel RJ, Caldwell MM (2006) Root turnover and
4
5 843 relocation in the soil profile in response to seasonal soil water variation in a natural stand
6
7 844 of Utah juniper (*Juniperus osteosperma*). *Tree Physiology*. 26:1469-1476.
8
9
10 845 Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL (2002) Fine root
11
12 846 architecture of nine North American trees. *Ecol Monogr*. 72:293-309.
13
14 847 Pregitzer KS, Kubiske ME, Yu CK, Hendrick RL (1997) Relationships among root branch order,
15
16 848 carbon, and nitrogen in four temperate species. *Oecologia*. 111:302-308.
17
18
19 849 Pregitzer KS, Laskowski MJ, Burton AJ, Lessard VC, Zak DR (1998) Variation in sugar maple
20
21 850 respiration with root diameter and soil depth. *Tree Physiology*. 18:665-670.
22
23
24 851 Pritchard SG, Strand AE, McCormack ML, Davis MA, Finz AC, Jackson RB, Matamala R,
25
26 852 Rogers HH, Oren R (2008) Fine root dynamics in a loblolly pine forest are influenced by
27
28 853 free-air-CO₂-enrichment: a six-year-minirhizotron study. *Global Change Biol*. 14:588-
29
30 854 602.
31
32
33 855 Reynolds JF, Thornley JHM (1982) A shoot:root partitioning model. *Ann Bot*. 49:585-597.
34
35 856 Running SW, Gower ST (1991) FOREST-BGC, A general model of forest ecosystem processes
36
37 857 for regional applications. II. Dynamic carbon allocation and nitrogen budgets. *Tree*
38
39 858 *Physiology*. 9:147-160.
40
41
42 859 Ryan MG, Binkley D, Fownes JH, Giardina CP, Senock RS (2004) An experimental test of the
43
44 860 causes of forest growth decline with stand age. *Ecol Monogr*. 74:393-414.
45
46
47 861 Rytter RM (2013) The effect of limited availability of N or water on C allocation to fine roots
48
49 862 and annual fine root turnover in *Alnus incana* and *Salix viminalis*. *Tree Physiology*.
50
51 863 33:924-939.
52
53
54
55
56
57
58
59
60

- 1
2
3 864 Saiz G, Byrne KA, Butterbach-Bahl K, Kiese R, Blujdea V, Farrell EP (2006) Stand age-related
4
5 865 effects on soil respiration in a first rotation Sitka spruce chronosequence in central
6
7 866 Ireland. *Global Change Biol.* 12:1007-1020.
- 8
9
10 867 Santantonio D, Hermann RK (1985) Standing crop, production and turnover of fine roots on dry,
11
12 868 moderate, and wet sites of mature Douglas-fir in western Oregon. *Annales des Sciences*
13
14 869 *Forestieres.* 42:113-142.
- 15
16
17 870 Savarese PT, Patetta MJ. 2010. An Overview of the CLASS, CONTRAST, and
18
19 871 HAZARDRATIO Statements in the SAS® 9.2 PHREG Procedure. SAS Institute Inc.,
20
21 872 Cary, NC, p 23.
- 22
23
24 873 Schoonmaker AS, Liefers VJ, Landhäuser SM (2016) Viewing forests from below: fine root
25
26 874 mass declines relative to leaf area in aging lodgepole pine stands. *Oecologia.* 181:733-
27
28 875 747.
- 29
30
31 876 Smithwick EAH, Lucash MS, McCormack ML, Sivandran G (2014) Improving the
32
33 877 representation of roots in terrestrial models. *Ecol Model.* 291:193-204.
- 34
35 878 Stover DB, Day FP, Drake BG, Hinkle CR (2010) The long-term effects of CO₂ enrichment on
36
37 879 fine root productivity, mortality, and survivorship in a scrub-oak ecosystem at Kennedy
38
39 880 Space Center, Florida, USA. *Environ Exp Bot.* 69:214-222.
- 40
41
42 881 Tierney GL, Fahey TJ, Groffman PM, Hardy JP, Fitzhugh RD, Driscoll CT, Yavitt JB (2003)
43
44 882 Environmental control of fine root dynamics in a northern hardwood forest. *Global*
45
46 883 *Change Biol.* 9:670-679.
- 47
48
49 884 Trumbore SE, Gaudinski JB (2003) The secret lives of roots. *Science.* 302:1344-1345.
50
51
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- 1
2
3 885 Vogt KA, Vogt DJ, Palmiotto PA, Boon P, O'Hara J, Asbjornsen H (1996) Review of root
4
5 886 dynamics in forest ecosystems grouped by climate, climatic forest type and species. *Plant*
6
7 887 *Soil.* 187:159-219.
- 8
9
10 888 Vose JA, Allen HL (1988) Leaf area, stemwood growth, and nutrition relationships in loblolly
11
12 889 pine. *For Sci.* 34:547-563.
- 13
14 890 Wells CE, Eissenstat DM (2001) Marked differences in survivorship among apple roots of
15
16 891 different diameters. *Ecology.* 82:882-892.
- 17
18
19 892 Wells CE, Glenn DM, Eissenstat DM (2002) Changes in the risk of fine-root mortality with age:
20
21 893 A case study in peach, *Prunus persica* (Rosaceae). *Am J Bot.* 89:79-87.
- 22
23 894 White DA, Battaglia M, Mendham DS, Crombie DS, Kinal J, McGrath JF (2010) Observed and
24
25 895 modelled leaf area index in *Eucalyptus globulus* plantations: tests of optimality and
26
27 896 equilibrium hypotheses. *Tree Physiology.* 30:831-844.
- 28
29
30 897 Wiseman PE, Seiler JR (2004) Soil CO₂ efflux across four age classes of plantation loblolly pine
31
32 898 (*Pinus taeda* L.) on the Virginia Piedmont. *For Ecol Manage.* 192:297-311.
- 33
34
35 899 Wright IJ, Westoby M (1999) Differences in seedling growth behaviour among species: trait
36
37 900 correlations across species, and trait shifts along nutrient compared to rainfall gradients. *J*
38
39 901 *Ecol.* 87:85-97.
- 40
41
42 902 Yan MF, Zhang XS, Jiang Y, Zhou GS (2011) Effects of irrigation and plowing on soil carbon
43
44 903 dioxide efflux in a poplar plantation chronosequence in northwest China. *Soil Sci Plant*
45
46 904 *Nutr.* 57:466-474.
- 47
48
49 905 Yuan ZY, Chen HYH (2010) Fine Root Biomass, Production, Turnover Rates, and Nutrient
50
51 906 Contents in Boreal Forest Ecosystems in Relation to Species, Climate, Fertility, and
52
53 907 Stand Age: Literature Review and Meta-Analyses. *Crit Rev Plant Sci.* 29:204-221.

908

909 **Figure legends**

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

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

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

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3 929 regression equation from Fig. 2. Error bars are standard errors (n=12). The P-values are for
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5 930 Kolmogorov-Smirnov two-sample non-parametric tests comparing lines within each panel.
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10 932 Figure 4. Average annual production increment (a), annual mortality increment (b) and live-root
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12 933 length (crop) (c) for cottonwood (CW) and loblolly pine (LP) grown with (Fert) or without (No
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14 934 Fert) fertilization treatments and averaged for irrigation treatments. Average annual production
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16 935 increment, annual mortality increment and live-root length (crop) (d) grown with irrigation (*I*),
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18 936 fertilization (*F*), or their combination (*IF*) compared with untreated control (*C*) and averaged for
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20 937 species. Error bars are standard errors (n=36). Bars with the same letter within each variable are
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22 938 not significantly different (Tukey's HSD, $\alpha=0.10$).
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28 940 Figure 5. Cox regression model estimates of fine-root lifespan versus stem biomass for
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30 941 cottonwood (CW) and loblolly pine (LP) grown with irrigated (*I*), fertilized (*F*), or the combined
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32 942 (*IF*) treatments relative to the untreated control (*C*). Lifespan is median root survival predicted
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34 943 using Equation 1 and coefficients shown in Table 2. Error bars are 95% confidence intervals. We
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36 944 maintained cofactors at the following references levels: season = winter, soil depth = 1 cm and
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38 945 root diameter = 0.5.
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44 947 Figure 6. Risk of fine-root mortality for (a) three seasons of the year relative to that observed in
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46 948 winter, and (b) for cottonwood (CW) and loblolly pine (LP) grown with irrigated (*I*), fertilized
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48 949 (*F*), or the combined (*IF*) treatments relative to the untreated control (*C*). Seasons were
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50 950 designated base on solstice and equinox dates. Percentages were calculated from model
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3 951 coefficients as $\% \text{Mort} = 100 \cdot (e^{\beta s} - 1)$, where β is the parameter estimate from Table 2 for season
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6 952 *s*.

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10 954 Figure 7. Conceptual model illustrating the relative magnitude of different controls on fine-root
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12 955 dynamics. The width of the pyramid and shading reflects the relative magnitude of each factor.

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14 956 As the width narrows and shading decreases, the magnitude of the response decreases. Likewise,
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17 957 as the width narrows and shading decreases, the consistency and direction of the response across
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19 958 species decreases.

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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 ($P \leq 0.05$) are in bold typeface, while those that are marginally significant ($p \leq 0.10$) 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	
	<i>f</i>	<i>p</i>	<i>f</i>	<i>p</i>	<i>f</i>	<i>p</i>	<i>f</i>	<i>p</i>	<i>f</i>	<i>p</i>	<i>f</i>	<i>p</i>
	<i>I</i>	5.1	0.03	2.9	<u>0.10</u>	2.6	0.12	1.5	0.24	2.2	0.15	2.2
<i>F</i>	15.8	<.01	4.3	0.05	1.3	0.26	0.2	0.71	0.7	0.41	0.4	0.55
<i>I</i> × <i>F</i>	0.3	0.57	3.4	<u>0.08</u>	3.4	<u>0.07</u>	2.3	0.15	4.6	0.04	3.3	<u>0.08</u>
<i>S</i>	45.1	<.01	25.3	<.01	10.7	<.01	2.4	0.14	13.1	<.01	5.1	0.03
<i>I</i> × <i>S</i>	1.4	0.24	1.2	0.28	0.3	0.58	0.0	0.90	0.1	0.73	0.0	0.85
<i>F</i> × <i>S</i>	1.5	0.24	9.1	<.01	5.4	0.03	2.4	0.14	5.2	0.03	4.0	<u>0.05</u>
<i>I</i> × <i>F</i> × <i>S</i>	0.9	0.36	0.6	0.44	0.4	0.54	0.2	0.66	0.7	0.40	0.5	0.48
<i>Y</i>	49.7	<.01	29.2	<.01	41.6	<.01	90.0	<.01	39.5	<.01	42.5	<.01
<i>I</i> × <i>Y</i>	1.1	0.37	1.5	0.24	1.6	0.18	1.1	0.39	2.2	<u>0.07</u>	1.2	0.33
<i>F</i> × <i>Y</i>	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>I</i> × <i>F</i> × <i>Y</i>	1.6	0.18	0.5	0.80	1.4	0.25	2.1	0.10	0.9	0.50	0.4	0.88
<i>S</i> × <i>Y</i>	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>I</i> × <i>S</i> × <i>Y</i>	0.6	0.73	0.4	0.82	0.9	0.51	0.5	0.80	0.7	0.67	0.5	0.77
<i>F</i> × <i>S</i> × <i>Y</i>	1.7	0.15	1.5	0.22	1.8	0.14	1.2	0.34	1.9	0.12	2.4	<u>0.05</u>
<i>I</i> × <i>F</i> × <i>S</i> × <i>Y</i>	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
$S \times I$	0.368	0.026	203	<.001
$S \times F$	-0.012	0.024	0.3	0.601
$F \times I$	0.028	0.024	1	0.252
$S \times F \times I$	0.057	0.036	2	0.118

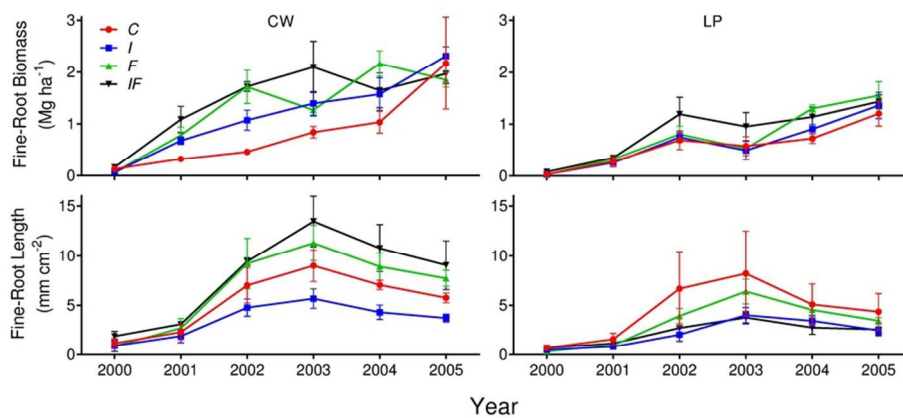


Figure 1

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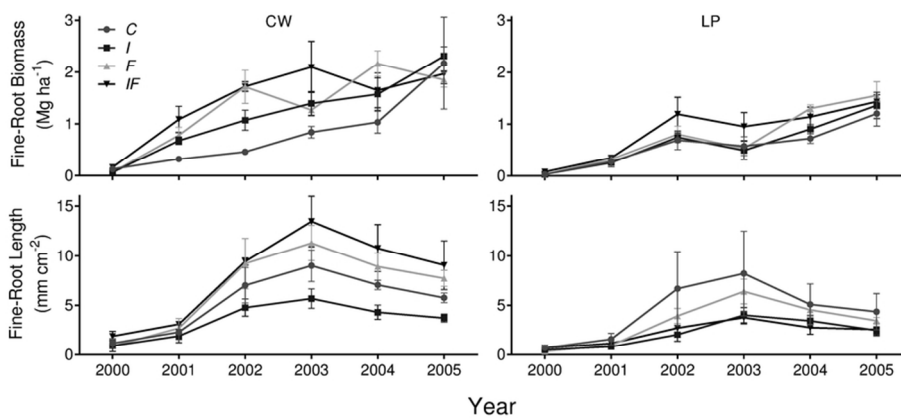


Figure 1

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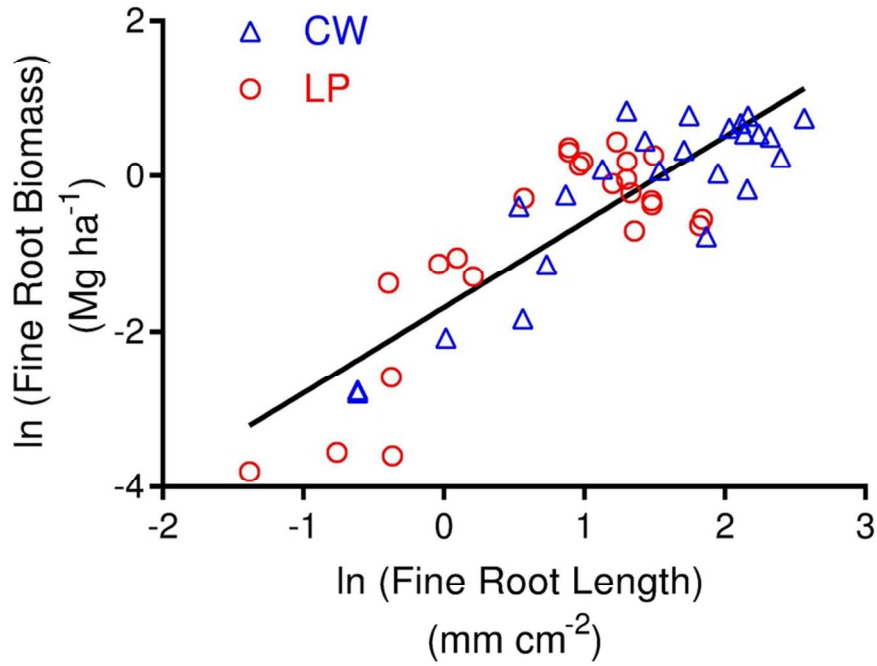


Figure 2

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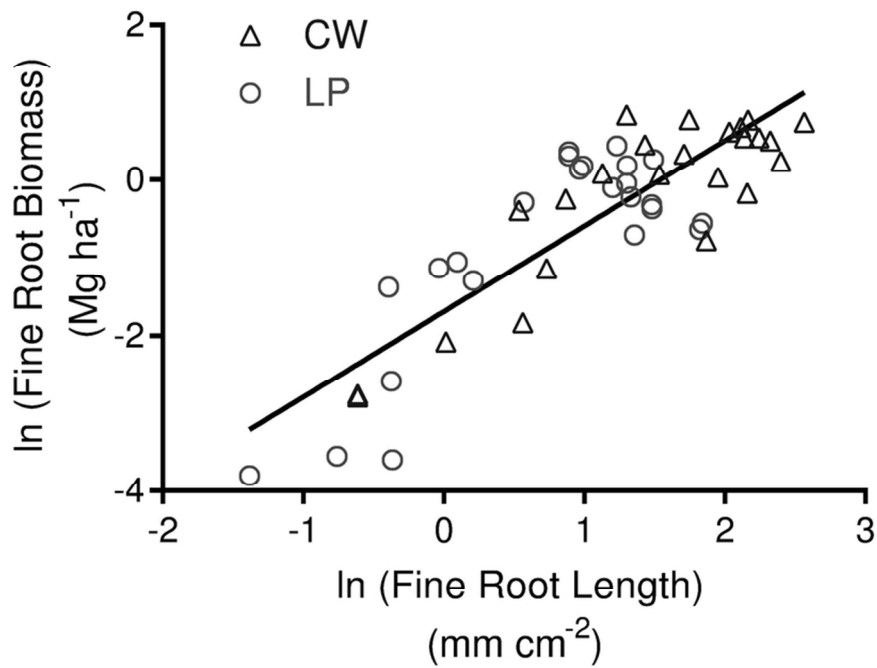


Figure 2

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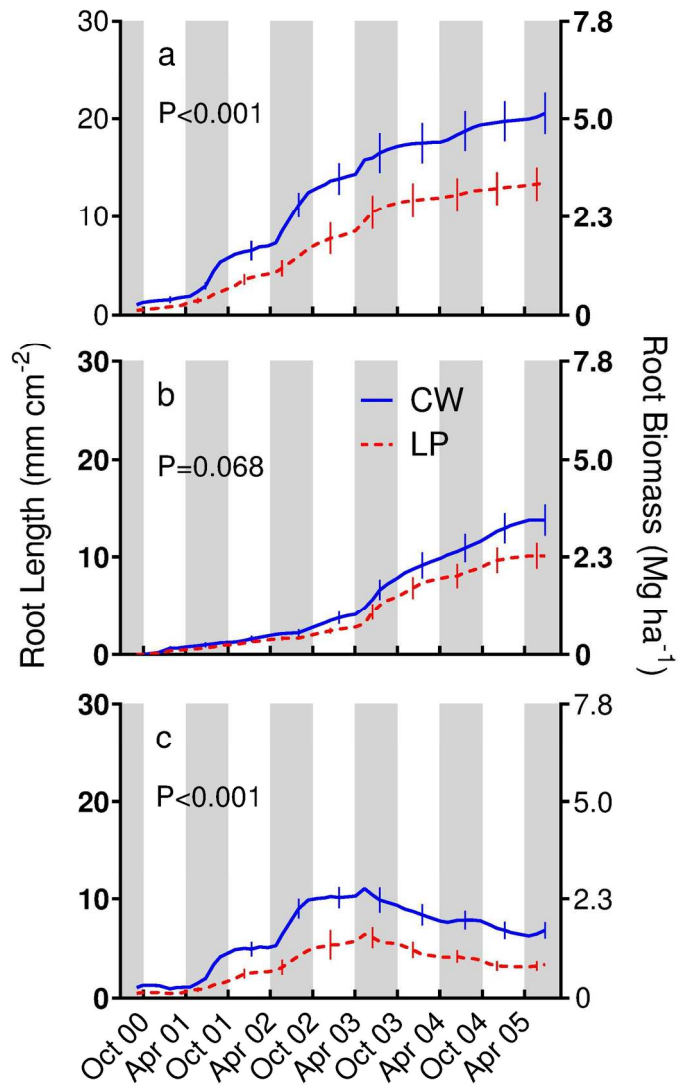


Figure 3

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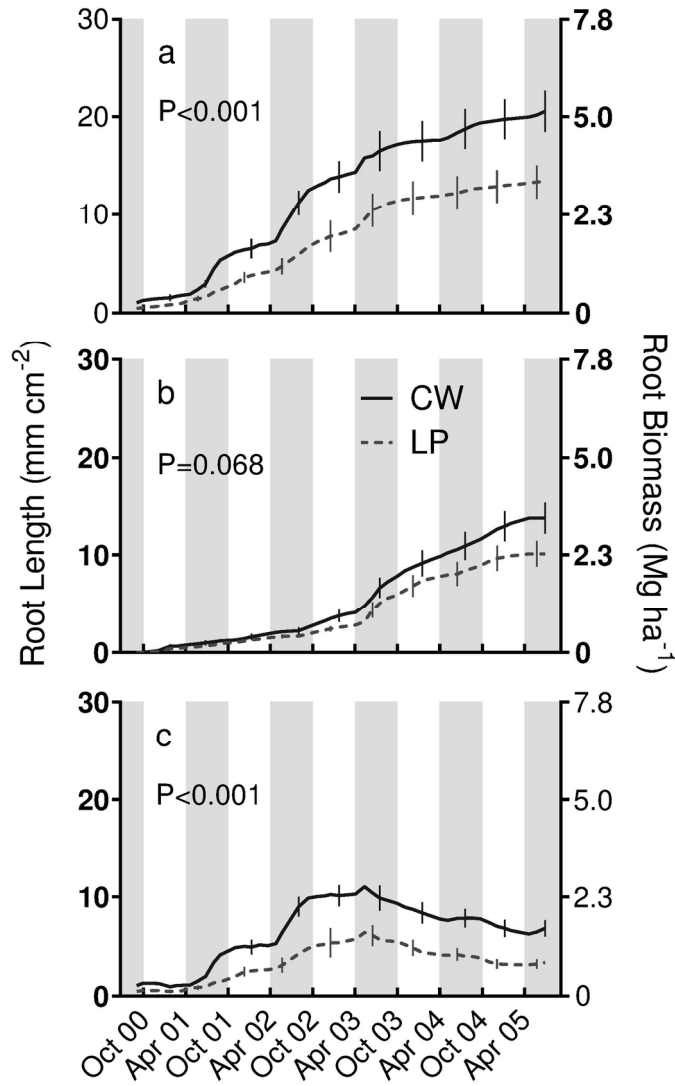


Figure 3

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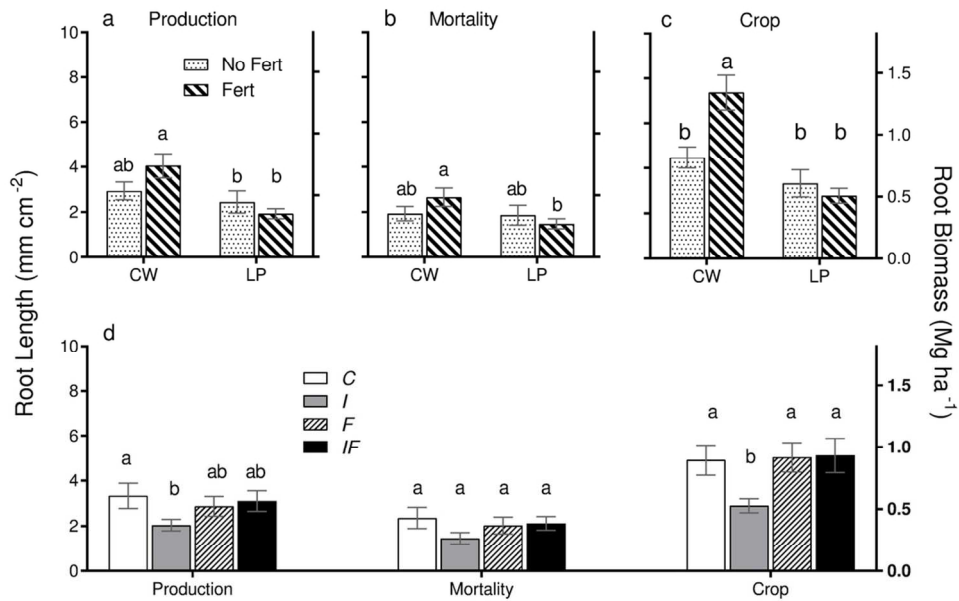


Figure 4

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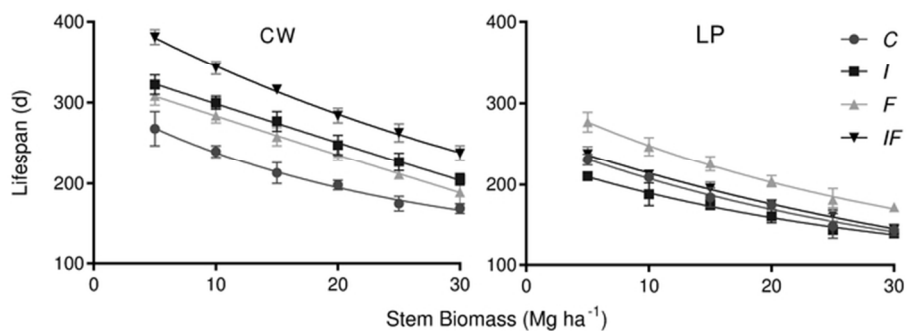


Figure 5

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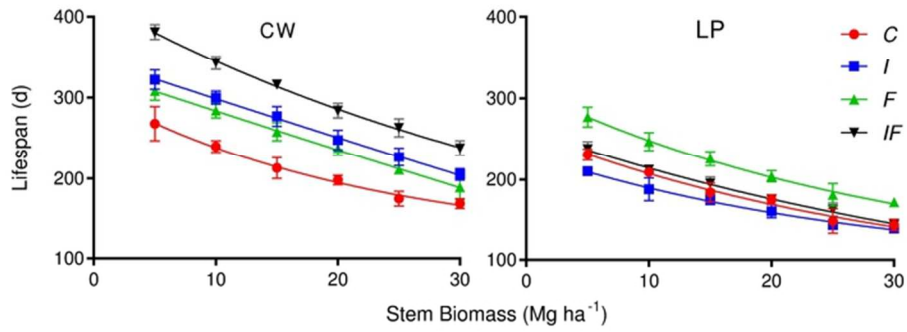


Figure 5

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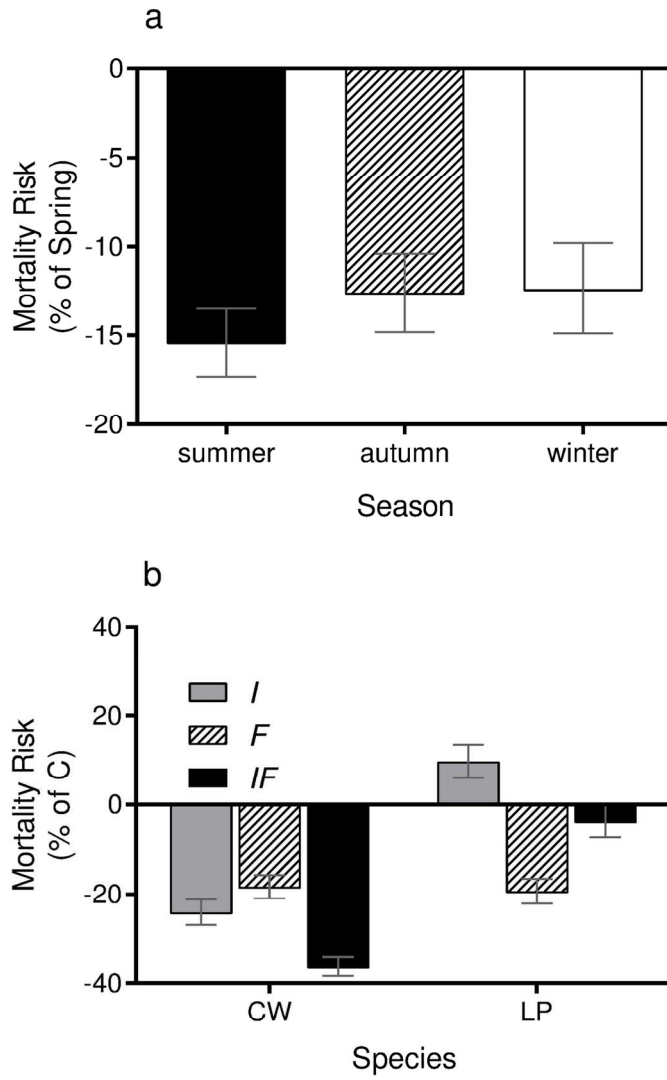


Figure 6

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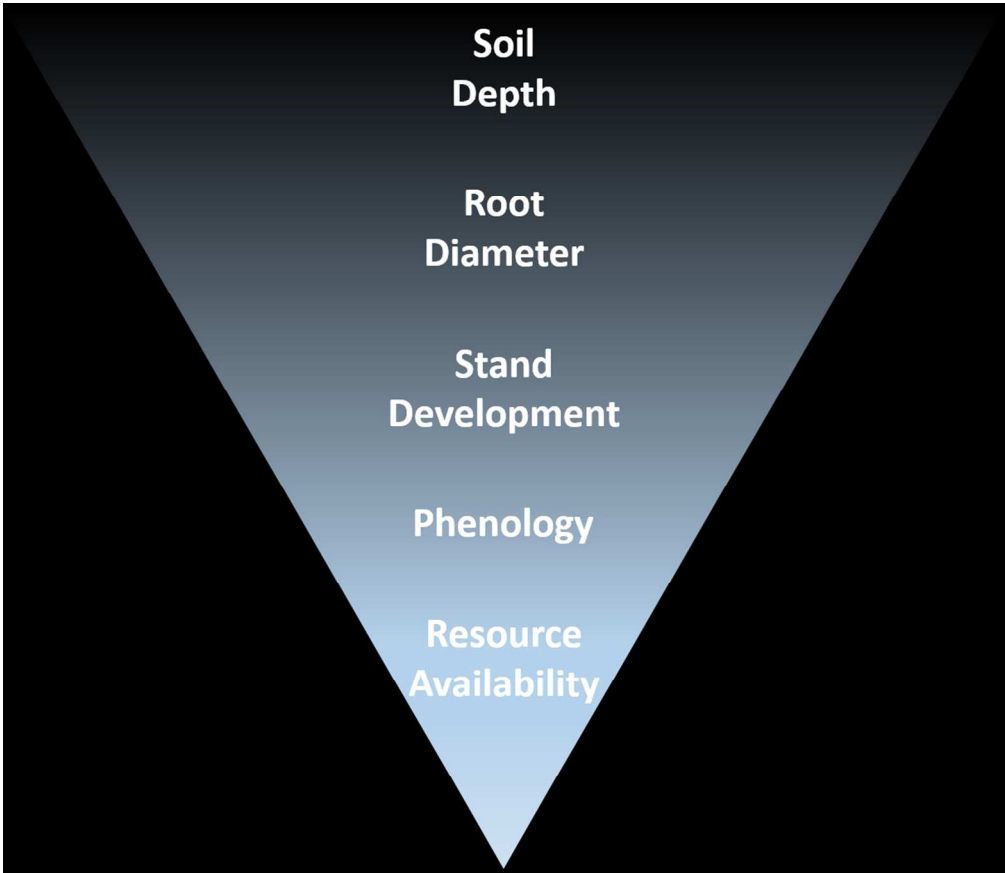


Figure 7

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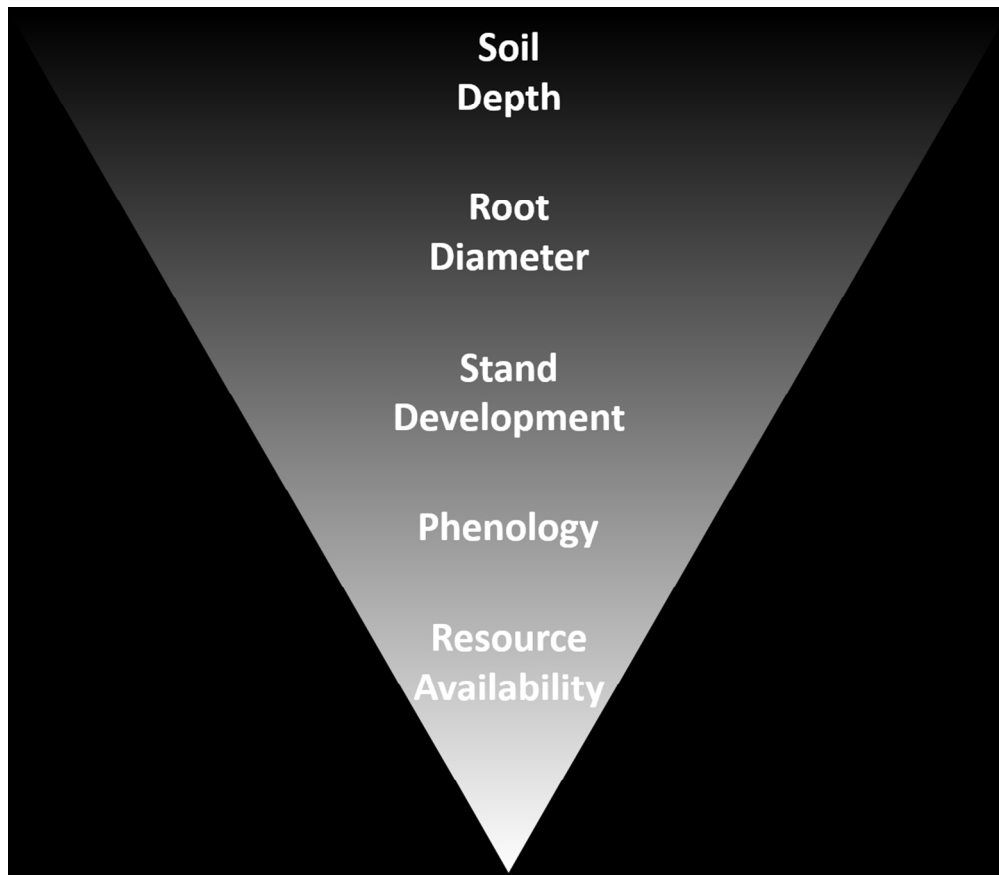


Figure 7

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