Impacts of Fine-roots on

Terrestrial Net Primary Productivity and

Soil Nutrient Cycling

BY

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THESIS

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Contribution of Authors

Chapter 1 is a review of the current state of knowledge regarding lifespan of fine-roots and places my research in the context of other recent literature. **Chapter 2** is an unpublished manuscript describing a carbon allocation model that optimizes above- and below-ground biomass production simultaneously at the ecosystem scale. **Chapter 3** is a published manuscript for which I was primary author. All cited co-authors contributed to experimental design and writing of the manuscript. **Chapters 4 and 5** are unpublished experiments continuing the work from chapter 3. In tandem, they attempt a more functional approach to the traditional study of fine-roots after separation by fine-root branching order, as opposed to an arbitrary diameter classification. **Chapter 6** synthesizes my research and summarizes conclusions from the entire body of work.

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SUMMARY

Fine-roots are an important component of terrestrial ecosystems. For an individual plant, fine-roots assist in uptake of water and essential nutrients. Fine-roots serve an additional role as a main conduit of carbon from the atmosphere to soils, providing a significant portion of organic matter in soils. Despite these known roles of fine-roots, the ecology of fine-roots remains largely unexplored, particularly relative to knowledge of above-ground plant organs. Of particular interest, is the magnitude of the role of fine-roots in the terrestrial carbon cycle. Longevity of fine-roots remains the largest uncertain component of the terrestrial carbon budget. Short-lived fine-roots would indicate a large role for fine-roots in the carbon cycle. On the other hand, long-lived roots would reduce the amount of carbon that is transferred to soils from roots.

One contribution from my thesis is the exploration of the total amount of carbon that is allocated below-ground by plants. In order to do this, a dynamic vegetation model was creaeted that optimizes both above- and below-ground biomass allocation with respect to changing environmental conditions. The need for such a model was derived from the fact that many large-scale modeling Earth System Models (ESM) lack dynamic vegetation response to changing environmental conditions. However, the ultimate goal of such models is to predict scenarios under potential future environmental conditions, such as increased temperatures. Results from empirical research, including that done in my previous chapters, conclude that biomass allocation is plastic with respect to abiotic conditions. Thus, in order for Earth System Models to be truly diagnostic, a dynamic vegetation model must be incorporated. A major goal of the model developed here is to make progress towards more diagnostic Earth Systems Models.

The majority of my research involved empirical study of roots in situ to explore the amount of carbon involved in fine-root activities in a single species at a local scale. Study of fine-roots is methodologically difficult, with technologies only recently becoming widely available. In my dissertation, I utilized a carbon isotope tracer applied for 12 growing seasons at a long-term Free-Air CO_2 Enrichment (FACE) experiment in a *Liquidambar styraciflua* plantation in Tennessee to examine properties of fine-roots including longevity and sources of carbon for growth and respiration.

With the cessation of CO_2 fumigation in 2009, new carbon assimilated starting in spring of 2010 was isotopically distinct from carbon in existing biomass. Sequential intact soil cores of fine-roots were taken periodically for two growing seasons. Additionally, in-growth sampling bags were utilized to examine isotopic composition of newly produced roots. Fine-roots were extracted from soil and separated by diameter; samples included roots < 1 mm diameter and those > 1 mm and < 2 mm diameter. Extracted fine-roots were also incubated for the capture (and isotopic analysis) of respired CO_2. In this study, newly produced roots utilized exclusively new photosynthate, rather than using storage carbon from previous years. In contrast to new root growth, respired CO2 was a mixture of storage carbon (25%) and recent assimilate (75%). In the fine-root population, fine-root carbon was replaced fairly slowly, with about half of the carbon remaining after two full growing seasons. An exponential decay model applied to the data found that at least two turnover rates for carbon occur in the fine-root population, with 10% of carbon quickly being turned over (< 3 months) and 90% turning over more slowly (> 2 years).

Results from the initial study strongly indicated heterogeneity in the longevity of

fine-roots. However, that study was unable to examine in detail which roots would live longer than others. A relatively recent paradigm shift in studying fine-roots has undertaken analysis not by diameter classification, but by separation by root branching order. In a follow-up study, I utilized this potentially more functional approach by separating roots by branching order sampled two and three growing seasons following CO_2 fumigation cessation. Results indicate that branching order does correlate with root longevity. However, our analyses indicate that root nitrogen concentration is a potential explanatory variable for root longevity. Thus, simple traits such as nitrogen concentration may help elucidate root longevity and contribution to NPP in different species or at larger spatial scales in future studies

Knowledge of the longevity of fine-roots is essential for quantifying fine-root contribution to terrestrial NPP and forest nutrient cycling. However, another critical trait for modeling fine-root turnover is the relative biomass found within fine-root longevities. An extensive literature review was conducted to examine fine-root biomass within branching orders, revealing just nine previous reports of relative biomass in the five most distal branching orders. Even with this sparse data-set, it is clear that environmental conditions such as nutrient and water availability impact fine-root biomass distribution. Increased studies quantifying the amount of biomass in roots by branching order will be needed to fully calculate fine-root contribution to terrestrial carbon and nutrient cycling.

1. Introduction

A significant portion of plant biomass is found below-ground. Large differences exist between biomes, species, and individuals, with below-ground allocation constituting 13 – 67% of total net primary productivity (NPP, Chapin et al., 2002). Coarse roots are generally long-lived and act to stabilize plants in the soil and serve as conduits of water from soil to leaves and sugars from leaves to metabolically active fine-roots and sometimes, soils. Smaller diameter fine-roots are actively involved in the uptake of water and essential nutrients required by above-ground components. In addition to these critical roles for the plant, roots serve an additional vital role at the ecosystem-scale as a major source of carbon for soil organic matter accrual and soil microbial activity (Rasse et al., 2005). Despite these critical roles of fine-roots roles at both plant and ecosystem scales, research into below-ground carbon and biomass allocation is lacking compared to research on above-ground components.

One component of my dissertation involved the exploration and quantification of total carbon allocated below-ground to roots at the ecosystem scale. Allocation patterns are dynamic, with response to changing environmental conditions including resource availability (Litton et al., 2007). A theoretical biomass production model was developed that captures the dynamic nature of plant growth that occurs (Chapter 2). It is a simple, conceptually straight-forward model based on a long-used production theory of economics (Cobb & Douglas, 1928). This model framework is potentially useful in predicting the response of plants to a changing environment. As vegetation response to climate change remains highly uncertain, this model potentially has utility incorporated into larger scale biomass and climate models.

The majority of my research involved analysis of fine-root mediated carbon cycling in situ at a small scale in a single species. Methodological difficulties are a major reason for a lack

of knowledge on plant-mediated below-ground carbon and nutrient cycling. Due to their location below the soil, roots are difficult to monitor, especially without introducing a disturbance (Trumbore and Gaudinski, 2003). However, in recent decades, technological progress has provided multiple ways to assess carbon dynamics in roots. Two common methods include (1) long-term deployment of minirhizotrons, where sequential images of roots are taken that can track root production and mortality (e.g. Cheng et al., 1991) and (2) isotope tracers that are incorporated into plant biomass and tracked through the ecosystem (e.g. Matamala et al., 2003). Through these methods, the last decade of research has revealed much about the lives of roots.

Still, much remains to be learned about the role of fine-roots in net primary productivity and their importance in terrestrial nutrient cycling. I will briefly describe the current state of knowledge with respect to root-mediated nutrient dynamics and how my work, along with other recent studies, is changing the paradigm with which we view fine-roots, the active portion of the below-ground biomass.

Fine-roots are often considered analogous to leaves. Like leaves, individual fine roots senesce and then decompose, providing a major source of carbon (C) for soils (Rasse et al., 2005). Production of new fine roots replaces those roots that die, and provide ways for a plant to explore new areas of the soil, increasing water and nutrient extraction. Typically, the leaf analogy is also applied to root life-span, where the majority of the fine-root population is replaced annually. If true, fine-roots would constitute a significant component of net primary production (NPP) in terrestrial ecosystems, possibly up to 33% of NPP (Jackson et al., 1997). Alternatively, if some portion of the fine-root population survives for multiple growing seasons, fine-root proportion of NPP would be reduced.

No current technology allows real-time monitoring of root production and senescence in

a completely natural setting without significant disturbance. Comparisons between root sampling methods reveal large differences in longevity of fine roots, with estimates between minirhizotrons and isotopic tracer methods at the same site varying more than 5-fold (Strand et al., 2008). At least part of this difference is due to fundamentally different measurements (i.e., minirhizotrons measure longevity of individual roots while isotope tracers measure residence time of carbon in the root system). A major barrier to improved understanding of fine roots is a lack of a universally accepted paradigm for the fundamental unit of turnover. In contrast, for above-ground ephemeral biomass, it is relatively easy to identify and distinguish between individual leaves. Categorization of an individual root is not as straight-forward. Consequently, the most often used definition of a fine-root is any root less than an arbitrary diameter cut-off (e.g. < 1 mm or 2 mm diameter). An alternative approach to defining fine-roots has been utilization of the branching structure of roots (Pregitzer et al., 2002). This approach has yielded new concepts, including the possibility that lower order roots are produced and senesce together in 'ephemeral root modules' (Xia et al., 2010).

For my thesis, an initial study used a long-term isotope tracer applied for 12 growing seasons in a mature deciduous plantation (chapter 3, Lynch et al., 2013). Results included finding a small amount of root biomass that is short-lived (< 3 months) and a majority living for multiple growing seasons. However, that study did not examine individual roots or determine which root traits can be linked to short- or long-lived roots. However, recent research has found that with increased branching order, nitrogen content and respiration rate decreases (Guo et al., 2008), while diameter and non-structural carbohydrate content increases (Guo et al., 2004). Additional research at the ORNL FACE site utilized a branching order approach (chapter 4). Potentially important traits that may be linked to longevity, root diameter and root [N] concentration were

quantified and examined with respect to residence time of carbon in roots. Importantly, root [N] explains the largest amount of variation in root lifespan, possibly providing an easily measurable trait in future studies.

Compared to leaves, the number of studies analyzing traits of individual roots is miniscule. In fact, global leaf trait data-sets allowed the determination of specific traits common to either short-lived or long-lived leaves, or a leaf economics spectrum (Wright et al., 2004). Data for root traits is only available from a limited number of sites and species. Here, a literature review of published studies reporting relative biomass in the lowest five root branching orders revealed just 10 papers, including data from the ORNL site (chapter 5). Increasing the number of studies that examine root traits could eventually lead to the creation of a root economics spectrum that would make significant progress towards understanding of fine-root longevity. Another important unresolved question regarding fine roots is the rate at which fine root carbon is transferred to soils. Root chemistry seems to play an important role in soil organic matter stabilization (e.g. O'Brien et al., 2010). Currently, models (e.g. CENTURY) assume 50% of root litter accumulates as soil organic matter. Little empirical evidence exists to support this rate (see Lynch et al., 2013), and further studies validating it should be a high research priority.

A goal for researchers studying below-ground plant-mediated nutrient cycling should be to achieve the same high level of understanding as that of above-ground. It is clear that combining all roots below a single diameter class does not adequately capture the dynamics involved in hierarchical fine-root systems (Hishi et al., 2007). The number of research studies examining roots continues to increase as the importance of root systems in the terrestrial carbon cycle is recognized. Only with larger numbers of quality data-sets can we hope to understand root contributions to global C cycles.

In summary, my research has explored fine-root dynamics at a very detailed level. To do this, the cessation of CO_2 fumigation and resulting relaxation of a long-term isotope tracer was utilized to track the replacement of C molecules in the fine-root system of a deciduous plantation stand. The research presented here provides ample evidence of the complex structural, chemical, and importantly functional relationships within fine-roots, or those roots actively involved in water and nutrient uptake.

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2. A Cobb-Douglas optimization model for predicting dynamic above- and below-ground biomass production

2.1 Introduction

Earth systems models (ESMs) provide a major tool for integrating physical and biological processes at a global scale by coupling the interactions between the atmosphere, ocean, land, and ice to understand present and future climatic changes. The critical role of land-atmosphere coupling (Seneviratne et al., 2006) has led to the steadily increasing complexity of the land model components of ESMs to include characteristics of vegetation, biophysics, hydrology, biogeochemistry, land use change, land management, and more (Table 1). As such, ESMs have been used to explore impacts of land cover change on climate (Kumar et al., 2013; Lawrence, 2013; Brovkin et al., 2013), climate influences on the hydrology cycle (Saronjini et al., 2012; Seveviratne et al., 2013; Zhang et al., 2014) and changes in extreme temperature and precipitation (Kharin et al., 2013; Sillmann et al., 2013). However, the land components of ESMs often lack the dynamic responses of vegetation to a changing environment, thus limiting their predictive power (Scheiter *et al.*, 2013).

The carbon (C) allocation in ESMs generally varies by plant functional type (PFT) and is either a fixed ratio (does not vary seasonally or with water or resource availability) or dynamic (does vary seasonally considering changes in very few resources, Table 1). Most algorithms contain parameters that are either fixed or user-prescribed for a certain PFT that become static with changes in environmental conditions (*i.e*. climate change) and ignore plant competition (Dybzinski et al., 2014). Some ESMs (CESM/CLM4.0, ORCHIDEE, TEM; (Friedlingstein et al. (1998), Krinner *et al.*, 2005; Thornton *et al.*, 2007; Zaehle & Friend, 2010) now include dynamic response variables for photosynthesis with respect to changes in CO_2 or temperature, but little to

no attention has been paid to changes in respiration, and the resulting dynamic allocation of C and resources to different plant parts (Smith & Dukes, 2013). This model restriction results in the use of rule-based constant allocation of assimilated C for growth of new plant components after plant respiratory demands are met. For the widely used CLM4.5, belowground allocation is a user-prescribed static ratio (*i.e.* root-to-shoot ratio) that does not change seasonally or over the life of the organism or with changing resource availabilities (Thornton *et al.,* 2007). Furthermore, when N is included (and even less often, phosphorus), the carbon allocation is dependent on fulfilling C:N ratios – insufficient nitrogen to meet the stoichiometric C:N requirements results in a downscaling of photosynthesis.

Despite the lack of inclusion in models, clear empirical evidence demonstrates that vegetation biomass distribution is dynamic across environmental conditions. Plant biomass strategies are highly plastic, depending on the availability of both above- (Givnish 1982; Reynolds and Thornley 1982) and below-ground resources (Rastetter and Shaver 1992; Craine 2006). The importance of co-limitation of plant productivity is supported by experimental data, as in some elevated atmospheric CO_2 experiments where enhanced productivity was not sustained over time due to progressive nitrogen limitation (PNL) (Norby et al., 2010). The lack of sustained enhanced productivity with PNL highlights linkages between above- and below-ground resource harvesting and the dynamic nature of resource allocation. In order to maximize productivity, plants must properly trade-off biomass production in above-ground organs (for maximization of carbon gain) with biomass production in roots (for maximization of uptake of essential nutrients, Chapin and others 2002). Plants are expected to allocate to roots and shoots in order to optimize growth and reproductive success (Bloom et al., 1985) by allocating to organs that acquire the most limiting resource (Reynolds and Thornley 1982). Knowledge regarding

changes in plant resource allocation has been captured by detailed plant growth models with multiple parameters, which limits their applicability to ESMs and associated atmospheric, land and ocean coupled sub-models.

Dynamic vegetation models do not agree on the correct way to capture dynamic partitioning of photosynthate to biomass (Franklin et al., 2012). For example, in many terrestrial ecosystem models, biomass for a single plant compartment (typically leaves) is optimized, and biomass in other compartments (*i.e*. roots and wood/stems) is achieved assuming constant linear allometric relationships (Thornton and Zimmerman 2007; Fisher et al., 2010; Oleson et al., 2010; Wang et al., 2011). Optimizing only one plant organ restricts predictive capabilities (Scheiter et al., 2013), particularly with respect to changing resource availability. Ideally, modeling schemes for plant biomass production should permit plasticity in biomass ratios as a result of the co-limitation of carbon, water, and mineral nutrients (Dybzinksi et al., 2011; Norby and Zak 2011). To our knowledge, no simple modeling scheme has been developed to dynamically link biomass production for maximization of plant productivity at the plant or ecosystem scales.

Recognizing that a new dynamic modeling scheme in ESMs should have low computational demands and ease of integration, we propose the Cobb-Douglas production function (Cobb and Douglas 1928) as an alternative. The Cobb-Douglas production function was originally developed by economists to maximize production of a single output based on multiple inputs and forms the basis for numerous economics studies (*e.g*. Goldberger 1968; Douglas 1976; Felipe and Adams 2005). The Cobb-Douglas production function is rarely applied in ecological applications (but see Jorge et al., 2012), despite the parallels drawn between economics and plant growth (Bloom et al., 1985; McNickle and Dybzinski 2013). We applied the Cobb-Douglas production function in the context of production of shoot and root biomass

production, and explore the dynamic nature of plant growth using a simple optimization model. Our main objective is to explore the capability of the Cobb-Douglas production function to predict plant biomass gain. As such, we show initial results from a general model with no attempt to parameterize the model for a specific application (e.g. single plant, monoculture, ecosystem, grid cell). Qualitative results of the model are discussed with respect to empirical data. Specifically, we examine the impacts of (1) differences in stoichiometric requirements between plant functional types, (2) differences in per-unit costs of tissue production, and (3) changes in above- and below-ground resource availability on total biomass and distribution of new biomass between roots and shoots. Additionally, we will discuss how future implementations of the model can incorporate mechanistic process-based equations, and competition between plants with different biomass production strategies (e.g. between plant functional types).

2.2 Model Description

Here, we imagine an ecosystem of uniform plant functional type that maintains and produces biomass to maximize biomass production. For the current implementation of the model, we determine optimum biomass growth in above- and below-ground organs for a given set of constraints (e.g. nutrient demand, tissue cost resource availability). Above-ground biomass produces surplus carbon and below-ground growth contributes surplus nitrogen and other minerals. Total plant biomass production is a weighted product of surplus carbon and surplus nitrogen. Combining surplus carbon and nitrogen as a product recognizes the multiplicative nature of co-limitation, and it is a weighted product because carbon and nitrogen are not required in equal proportions. As a Cobb-Douglas production function (Goldberger 1968), vegetation maximizes a single output, total biomass production, based on these inputs (carbon harvested

aboveground, nitrogen harvested belowground) that are required in a vector-valued strategy. The stoichiometry of vegetation (represented by the Cobb-Douglas parameters) impacts the effects of surplus nutrients on biomass production, the nutrient costs of shoot or root biomass determine the burden of growing and maintaining both above- and below-biomass, and resource availabilities determine harvest opportunities. Combined, these factors influence growth and biomass distribution above- and below-ground. Similar to Ågren and Franklin (2003), the current implementation of this model will only consider production of total above-ground (shoot) and total below-ground (root) biomass. Straightforward equations are used for resource harvest and tissue costs for roots and shoots, but the Cobb-Douglas approach is general, and future implementation could incorporate process-based equations.

Above-ground, let π_C represent the net harvest of carbon (C) as a function of total photosynthetic capacity; π_C is in units of mass of carbon accumulated per area per time. Below-ground, let $\pi_N \ldots \pi_x$ represent the net harvest of each soil resource of interest (from nitrogen (N) up to element "*x*") as a function of the total uptake capacity of the root system in units of mass of nitrogen accumulated per area per time. In this implementation, the Cobb-Douglas production function maximizes NPP or total biomass production (F, i.e. remaining biomass produced after u_s and u_r are optimized) as a function of above- and below-ground production (u_s and u_r respectively, units of biomass per area per time) through the harvest of a combination of uniquely acquired above- and below-ground resources (i.e. C, N …. x),

$$F(u_s, u_r) = \pi_C{}^{\alpha} \pi_N{}^{\beta} \ldots \pi_x{}^{\lambda}, \qquad \text{Eqn 1}$$

where α represents relative demand for carbon, β represents relative demand for nitrogen and λ represents the demand for mineral resource *x*. The exponents represent the approximate stoichiometric ratio for each nutrient or resource of the target vegetation type, and as proportions

they are assumed to sum to unity ($\alpha+\beta+\ldots+\lambda=1$, and $\alpha, \beta, \lambda > 0$). In plants, the demand ratio for carbon to nitrogen will ensure that $\alpha > \beta$, and nitrogen is typically more limiting to growth than other mineral nutrients meaning more generally, $\alpha > \beta > \ldots > \lambda$. Since the exponents weight the contribution of each resource according to stoichiometric demand, the impact of each resource on NPP is not equal, but is weighted by these exponents. A key requirement is that for the group of plants to have any growth, there must be a positive net harvest of each nutrient included in the model. The Cobb-Douglas production function assumes that all elements included in the model (e.g. carbon, nitrogen … element x) are essential or complementary resources for growth (Tilman 1982). In other words, each element is required for growth and increased harvest of one element diminishes its value and enhances the value of all other resources. However, as described below, substitutable forms (Tilman 1982) of each element may also be included (e.g. nitrogen as ammonium and nitrate).

Let the net harvest of C, N … *x* be a function of total harvest minus costs needed to construct and maintain all biomass pools,

$$\pi_C = H_C(u_s) - c_C(u_s) - c_C(u_r), \qquad \text{Eqn 2a}$$

$$\pi_N = \sum_{j=1}^{m} H_{jN}(u_r) - c_N(u_s) - c_N(u_r), \qquad \text{Eqn 2b}$$

…

$$\pi_x = \sum_{k=1}^{l} H_{kx}(u_r) - c_x(u_s) - c_x(u_r), \qquad \text{Eqn 2c}$$

where $H_C(u_s)$ describes the total harvest of C based on light levels, CO_2 concentration, the total photosynthetic capacity and other environmental variables of interest (Fig. 2.5) that influence C uptake rates; $H_{jN}(u_r)$ … $H_{kx}(u_r)$ describe the harvest of nitrogen in chemical forms *j* to *m*, and element *x* in chemical forms *k* to *l* as a function of each chemical's availability and the uptake

capacity of the root system. In this manner, any number of substitutable forms of mineral nutrients in soil (e.g. ammonium, or nitrate) can also be included if desirable. However, each substitutable form included would add at least one more parameter to the model, and increasing model complexity should be done with caution. Above- and below-ground resource harvest functions, $H(u)$, can take any form so long as they increase at a diminishing rate with above- (for carbon) or below-ground (for mineral nutrients) biomass investment (Fig. 1). Diminishing returns for resource capture represent known physiological processes such as self shading (Dybzinksi et al., 2011), or root density limitations to nutrient uptake (McNickle and Brown 2012), among others. Let $c_i(u_s)$ and $c_i(u_r)$ represent cost functions in units of each element i required to grow and maintain shoots and roots respectively. These monotonically increasing functions may be linear, accelerating (increasing costs to scale), or decelerating (economy to scale). The shape of these functions is likely vegetation and environment specific, and their exact form does not influence the qualitative predictions of the model as long as they are monotonically increasing. Cost functions can incorporate any number of important physiological processes (Fig. 2.5, e.g., respiration; DeLucia et al., 2007, Gonzalez-Meler et al., 2004).TTT

Simultaneous optimization of the net harvest equations (Eqn 2a-c) with respect to shoot and root investment provide the solution for estimating portions of NPP allocated above- and below-ground. The first order necessary conditions for optimizing for shoot and root biomass are given by taking the derivative of Eqn 1 with respect to shoot biomass (u_s) and also with respect to root biomass (u_r),

$$\frac{\partial F_{(u_s,u_r)}}{\partial u_s} = \alpha\left(\frac{\partial \pi_C}{\partial u_s}\right)^{\alpha-1}\pi_N{}^{\beta} \ldots \pi_x{}^{\lambda} + \pi_C{}^{\alpha}\beta\left(\frac{\partial \pi_N}{\partial u_s}\right)^{\beta-1} \ldots \pi_x{}^{\lambda} + \cdots + \pi_C{}^{\alpha}\pi_N{}^{\beta} \ldots \lambda\left(\frac{\partial \pi_x}{\partial u_s}\right)^{\lambda-1}, \quad \text{Eqn 3a}$$

$$\frac{\partial F_{(u_s,u_r)}}{\partial u_r} = \alpha\left(\frac{\partial \pi_C}{\partial u_r}\right)^{\alpha-1}\pi_N{}^{\beta} \ldots \pi_x{}^{\lambda} + \pi_C{}^{\alpha}\beta\left(\frac{\partial \pi_N}{\partial u_r}\right)^{\beta-1} \ldots \pi_x{}^{\lambda} + \cdots + \pi_C{}^{\alpha}\pi_N{}^{\beta} \ldots \lambda\left(\frac{\partial \pi_x}{\partial u_r}\right)^{\lambda-1}. \quad \text{Eqn 3b}$$

Assuming that harvest functions have diminishing returns, at the maximum biomass production,

root (u_r) and shoot (u_s) production satisfy,

$$\frac{\partial F_{(u_s,u_r)}}{\partial u_s} = \frac{\partial F_{(u_s,u_r)}}{\partial u_r} = 0 \quad . \quad \text{Eqn 4}$$

The following paragraphs and equations above provide all of the equations used to generate the figures in the results section. The model applied here is meant to demonstrate the utility of the Cobb-Douglas production function using the simplest possible harvest equations (after McNickle & Brown 2012, McNickle and Brown 2014). More mechanistic process based equations for harvest and costs of these resources can easily be exchanged in this model format to incorporate physiological processes of interest to specific applications.

The functions that represent gross harvest of carbon (π_c) and nitrogen (π_N) follows a diminishing returns function (Fig 2.1), where H is the total harvest of the resource, C_{avail} is the maximum amount of carbon that can be fixed by photosynthesis per square meter per year, N_{avail} is the amount of total nitrogen available to the plant per square meter per year in the environment, and u_r, and u_s are the current 'strategies' of the plant in terms of root or shoot biomass respectively.

$$H_C = C_{avail}(1 - e^{-u_s}) \quad (5)$$

$$H_N = N_{avail}(1 - e^{-u_r}) \quad (6)$$

In order to maximize biomass production and solve numerically for shoot and root allocation strategies, the derivatives of the net harvest equations for shoot and root, respectively (Eqn. 2a-b) must be solved. Note that we only include carbon and nitrogen (thus only requiring Eqn 2a and 2b), and nitrogen is in only a single chemical form, removing the summation component of Eqn 2b, and each additional essential element included would add a row to the table below. With this in mind the derivatives are,

	Derivative with respect to u_s	Derivative with respect to u_r

Net above-ground harvest (Eqn. 2a)	$\frac{\partial \pi_C}{\partial u_s} = \frac{\partial H_C}{\partial u_s} - \frac{\partial c_C(u_s)}{\partial u_s}$ (7)	$\frac{\partial \pi_C}{\partial u_r} = - \frac{\partial c_C(u_r)}{\partial u_r}$ (8)
Net below-ground harvest (Eqn. 2b)	$\frac{\partial \pi_N}{\partial u_s} = - \frac{\partial c_N(u_s)}{\partial u_s}$ (9)	$\frac{\partial \pi_N}{\partial u_r} = \frac{\partial H_N}{\partial u_r} - \frac{\partial c_N(u_r)}{\partial u_r}$ (10)

The cost per biomass for C and N respectively must also be known for both above and below-ground biomass. For our model, we used simple linear cost functions per unit of biomass, where c_{xy} is a number to define costs of nutrient y to build tissue x

	Shoot Costs	Root costs
Carbon costs	$c_C(u_s) = c_{sc} * u_s$ (11)	$c_C(u_r) = c_{rc} * u_r$ (12)
Nitrogen costs	$c_N(u_s) = c_{sn} * u_s$ (13)	$c_N(u_r) = c_{rn} * u_r$ (14)

The derivatives of the net harvest equations (eq. 7 through 10) can be numerically solved by plugging in derivates of equations 5 and 6 for gross harvest and 11 through 14 for costs:

	Derivative respect to u_s	Derivative respect to u_r
Net above-ground harvest (Eqn. 2a)	$\frac{\partial \pi_C}{\partial u_s} = C_{avail}(e^{-u_s}) - c_{sc}$ (15)	$\frac{\partial \pi_C}{\partial u_r} = - c_{rc}$ (16)
Net below-ground harvest (Eqn. 2b)	$\frac{\partial \pi_N}{\partial u_s} = - c_{sn}$ (17)	$\frac{\partial \pi_N}{\partial u_r} = N_{avail}(e^{-u_r}) - c_{rn}$ (18)

Above, Eqn 1-4 represent a general Cobb-Douglas approach to dynamically linking shoot and root growth. To examine the basic behavior of this approach for capturing dynamic vegetation we focus on just two elements, carbon and nitrogen. We assume that production of

roots and shoots is adjusted to maximize biomass production. We then use the Cobb-Douglas production function to predict total NPP and proportion of NPP for roots and shoots with respect to differences in: a) relative resource demand or stoichiometric requirements (i.e. Cobb-Douglas exponents); b) tissue production/maintenance costs and; c) resource availabilities.

2.3 Results

2.3.1 Plant stoichiometric resource demand (Cobb-Douglas exponents, α, β)

The Cobb-Douglas exponents represent relative demand for different resources by a given plant functional type, and the target stoichiometric composition of the vegetation of interest. A fractional increase in targeted carbon demand (increase in α) results in a corresponding decline in proportional nitrogen demand (and vice-versa). A system of plant functional type with a 9 to 1 target for carbon and nitrogen would have exponents α=0.9, β=0.1. We explored the effect of different values for α while holding all other parameter values constant (for parameter values see Table 2.3) on root and shoot biomass production. The model predicts that higher α (i.e. C demand) increases biomass in the above-ground carbon harvesting organs (Fig. 2.2a) but also decreases biomass in the below-ground harvesting organs (Fig. 2.2b). This is an intuitive result, as constructing tissues to increase C uptake would meet the new C demand requirements. As a result, across the entire range of values for α, relative production of roots versus shoots ranges from 0.5 to > 3 (Fig. 2.2c).

Plant C:N ratios are significantly greater than 1; that is, plant bodies are primarily composed of carbon by dry weight. Consequently, probable ranges for α are at minimum 0.8, and likely higher than 0.9 (shaded box in Fig. 2.2). Over this interval, slight shifts in resource demand, for example a shift in stoichiometery between PFTs can result in the largest shifts in biomass. The model predicts root production to shoot production ratio of < 1 indicating a

greater investment of biomass in the above-ground carbon harvesting organs. Additionally, the model predicts an increase in total biomass as α, or fractional demand for carbon, increases.

2.3.2 Tissue costs

In all cases, an increase in the per-unit cost of tissue production and maintenance leads to a decrease in biomass production of that tissue (for parameter values see Table 2.3). Increased costs consume a higher percentage of gross production, leading to reduce net production, though each cost has subtly different effects on the shoot and root biomass productions (Fig 2.3). For carbon (Fig. 2.3a-c), increased carbon costs per-unit of shoot or root tissue leads to a decreasing ability to produce shoots due to high costs of either tissue (Fig. 2.3a). The costs of shoot production have a sharper effect compared to the costs of root production. Yet, higher costs of either tissue contribute to lower shoot production (Fig. 2.3a). Below-ground, increased carbon costs per unit of shoot or root result in less root production. In comparison to shoots, the costs of root production have a sharper effect compared to the costs of shoot production (Fig. 2.3b). The basic result is that high root costs primarily limit root production and high shoot costs primarily limit shoot production but they interact. The resulting root and shoot production ratio shows that changes in root biomass production dominate whole plant and ecosystem responses (Fig. 2.3c) to changes in root or shoot costs. High demand for carbon (i.e. $\alpha>\beta$) means that plants should be more willing to forgo production of roots than shoots – the C harvesting organs.

For per-unit tissue nitrogen costs, the results are broadly similar: higher N costs for a tissue results in lower biomass production of that tissue (Fig. 2.3d, e). Changes in total shoot costs for nitrogen dominate vegetation responses (Fig. 2.3f), because for a given C demand by vegetation (i.e. $\alpha>\beta$) there is a correspondent N demand from root systems. In comparing shifting carbon costs and shifting nitrogen costs, larger shifts in biomass occur when nitrogen costs

change (Fig. 2.3c,f), indicating that changes in the costs of acquisition of the resource with less demand (in the case of plants, nitrogen) can drive shifts in the optimal above- and below-ground biomass ratios of vegetation.

Interestingly, the costs of the resource acquired by the organ itself (i.e. carbon for shoots, nitrogen for roots), does not appear to have large influences on biomass. Instead plants preferentially allocate to an organ when: (i) it is inexpensive to construct that organ leading to high marginal benefits for relatively low marginal costs (e.g. if any resource harvesting tissue is cheap to produce, then produce a lot of that tissue), or (ii) alternatively when one organ is prohibitively costly to produce this will limit the plant's ability to increase biomass (e.g. if shoots have very high carbon costs this will limit shoot production and as a result relative below-ground biomass will be high because of diminished shoot production not because of increased root growth), or (iii) a resource harvested by one organ is required in large amounts by other tissues then biomass will increase in the harvesting organ (e.g. if leaves demand high amounts of nitrogen, then more roots must be constructed to harvest the nitrogen).

5.3.3 Resource availability

As expected, NPP increases with increasing availability of either resource, with interactions leading to co-limitation (Fig. 2.4, for parameter values see Table 2.3). The isolines generated by the model reflect the fact that both carbon and nitrogen are essential plant resources. As a particular resource becomes more available, acquisition of the other resource becomes progressively more limiting, resulting in the curved isolines (Fig. 2.4a and 2.4b). That is, basic stoichiometry dictates that one when one resource is en excess, NPP and growth will still be limited. In terms of biomass ratios, the optimum strategy is driven by the most limiting resource, adhering to Liebig's law of the minimum. Relative biomass in roots is high when

nitrogen availability is low, and decreases with increasing nitrogen. Similarly, relative biomass in shoots is high when C availability is low, and decreases with increasing C availability (Fig. 2.4c). Interactions between carbon and nitrogen occur, and NPP increases as both C and N uptake increase.

2.4 Discussion

Accurate estimation of biomass distribution into multiple plant organs (e.g. above- and below-ground biomass) is an important goal for prognostic Earth Systems Modeling frameworks. A major objective of this paper was exploring the potential of the Cobb-Douglas production function to dynamically link root and shoot biomass production by plant functional types under different stoichiometric and environmental scenarios. In this framework, plant biomass in multiple tissues can be optimized for a plant co-limited by multiple resources. Below we discuss results of the simple version of the model applied here, potential advantages of this modeling approach in predictive ecosystem models, and future applications of the model framework.

Dynamic plant growth depends on stoichiometric demand for multiple resources (represented in this model by the Cobb-Douglas exponents). Increases in whole plant demand for a given resource resulted in an increase in biomass of the organ that harvests that resource (Fig. 2.2). In the realistic portion of the resource demand curve ($\alpha > 0.8$; Thornton and Zimmerman 2007), shoot biomass production was greater than root biomass production in agreement with standing biomass from a majority of biomes (Chapin et al., 2002). Importantly, the model predicts that the greatest shifts in relative biomass production between plant organs occurs in the portion of the resource demand curve that likely reflects real-world vegetation ($\alpha > 0.8$). Consequently, the observed different biomass ratios between groups of plants and plant functional types (Poorter et al., 2012) may be explained by difference in relative resource

demand.

Generally, higher per-unit costs for construction and maintenance of a given plant part leads to reduced biomass production in all other plant pools, with a few notable exceptions (Fig. 2.3). Specifically, higher carbon costs for shoots has only a weak negative effect on root production, while higher nitrogen costs of shoots actually has a weak positive effect on root biomass (Fig. 2.3e). However, the response to changing tissue costs is largely below-ground (Fig. 2.3f), as the model predicts that plants will more aggressively reduce net production of roots as opposed to shoots . This is likely a result of high stoichiometric demand for carbon relative to mineral nutrients in plants. Model results also highlight the linked nature of resource acquisition in plants. Predicted biomass changes were larger with respect to costs of the resource acquired elsewhere in the plant, as both NPP and relative production above- and below-ground were substantially impacted by cost for nitrogen acquisition in shoots and for carbon in roots. In support of our model predictions, soil warming experiments (which may lead to increased metabolic costs per unit of root biomass) have led to reduced total root mass and below-ground NPP (Burton et al., 2008; Melillo et al., 2011). However, root respiration may acclimate partially or entirely to changes in temperature, eliminating the increased metabolic cost (Atkin et al., 2008; Taneva and Gonzalez-Meler 2011). Therefore, other factors such as increased N mineralization may be contributing to plant and ecosystem responses to soil temperature in reducing root NPP (Melillo et al., 2011; Hopkins et al., 2013). In order to test these model predictions more specifically, experiments will need to directly analyze per unit tissue costs (e.g., by partitioning the cost function into its functional components, such as maintenance and growth respiration). Storage is also an important component in understanding above- and belowground vegetation responses to biotic (Flower and Gonzalez-Meler, 2015) or abiotic stress (Gonzalez-

Meler et al., 2014) as, for instance, defoliators have been shown to decrease NPP but increase the storage component under drought (Palacio et al., 2008; Wiley et al., 2013).

Additionally, model predictions were examined over a range of resource availabilities (Fig. 2.4). The model predicts an increase in total biomass production with increasing resource availability, consistent with empirical data (Litton et al., 2007; Nie et al., 2013) and other optimization models (Mäkelä et al., 2008; Valentine and Mäkelä 2012). In our model, biomass production shifted to harvest the most limiting resource, also in agreement with field observations (Wang and Taub 2010; Nie et al., 2013) and other models (King et al., 1993; Franklin et al., 2009; Dybzinski et al., 2011). Our model predicts a smooth transition between limiting resources, supporting a hypothesis that vegetation adjusts biomass production so as to be co-limited by multiple resources (Bloom et al., 1985; Ågren et al., 2012). This outcome is of particular utility when studying plant response to anthropogenic activities, where increasing CO_2 concentrations may lead to progressive N-limitation (Luo et al., 2004, Norby et al., 2011).

Despite the need for prognostic land modeling schemes incorporating vegetation, methodologies for dynamically linking above and below-ground processes are not obvious (e.g. Hopkins et al., 2013). In comparison with previous models, the Cobb-Douglas approach provides two distinct advantages. First, it simultaneously optimizes both shoot biomass production (u_s) and root biomass production (u_r) (Eqn. 1) with respect to relative resource demand for carbon and nitrogen. Dynamic linkage between above-ground and below-ground components in vegetation is not possible from a single trait optimization model framework. Second, the Cobb-Douglas function requires few parameters (Table 2.2), reducing the risk of over-parameterization, a genuine concern in ecological applications (Ginzburg and Jensen 2004). For these reasons, the Cobb-Douglas production function has potential for incorporation into existing

terrestrial ecosystem model frameworks.

Future applications of the general model presented here can extract processes of interest (e.g. respiration, nutrient uptake, herbivory) from the integrative parameters (Fig. 2.5). For example, fine-root turnover, which may constitute a significant portion of NPP (Lynch et al., 2013), can be investigated in this model framework by using specific terms in the harvest functions, as faster biomass turnover would require increased harvesting to construct new tissues. In fact, differences in root turnover in two forest stands potentially explain the difference in soil N availability following long-term elevated CO_2 treatments (Franklin et al., 2009). Additionally, the model as currently implemented does not resolve between species in mixed vegetation stands. Competition for resources within plant pools appears to be critical in determining plant above- and below-ground biomass (Farrar and Jones 2000), and could be included as a game theoretic optimization criterion (Anten et al., 2011; McNickle and Dybzinski 2013, McNickle and Brown 2014). For the present model we did not examine stems/wood separately from leaves (or fine roots from coarse roots), however in a competitive context plant investment into leaves and woody tissue would become important, producing a game theoretic Cobb-Douglas production function with competitive ability as the optimization criterion (Dybzinksi et al., 2011; Farrior et al., 2013). A game theoretic Cobb-Douglass production function could also address changes in resource availability over time; both short-term shifts during the growing season, and long-term trends such as climate induced nutrient limitation. This approach would be ideal to improve ESM future predictions of plant productivity.

2.5 Conclusions

The simple and generalized model presented here provides an analytical tool to estimate relative above- and below-ground biomass production of vegetation, based on co-limitation of C

and N through a Cobb-Douglas production function. The model can incorporate any number of substitutable or essential resources, and can be adapted to a wide variety of process-based functional responses of plants. Our novel approach simultaneously optimizes two important plant traits, production of shoot biomass and root biomass, and captures the qualitative behavior of plants in response to shifting resource demands. Additional benefits of the Cobb-Douglas function include few parameters, easy conceptualization and interpretation, minimal computational requirements, and applicability at multiple spatial and temporal scales. A key result of the model is the influence of relative resource availability on optimum above- and below-ground biomass production. Thus, prognostic spatial-scale models should incorporate dynamic response in both above and below-ground vegetation. Without this dynamic shift in nutrient allocation, current ESMs have a limited capacity to predict changes in future vegetation productivity. We conclude that the Cobb-Douglas function provides a useful framework for modeling production of above- and below-ground biomass.

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Table 2.1 Growth and allocation approaches of land surface models in the ESMs that participated in CMIP5. Note only land models that have carbon cycling are included. N or P denotes that the model also has Nitrogen or Phosphorus.

Parent ESM, **Land Model**	*Allocation*, **Growth Limiting Factors**	**Reference**
ACCESS **CABLE (NP)**	Allocation: coefficients vary with growth phase and plant type. Nutrient limitations: GPP downscaled from nitrogen or phosphorus limitation.	Wang et al., 2010
CAN-ESM1 **CTEM**	Allocation: weighted based on competition for light and water Nutrient limitation: nitrogen (parameterized)	Arora et al., 2005; Arora et al., 2009
CESM **CLM (N)**	Allocation: fixed, coefficients vary with plant type Nutrient Limitation: GPP downscaled based on nitrogen limitation	Oleson et al., 2013
FIO **CLM3.5**	Allocation: fixed or dynamic, based on water, light, and parameterized nitrogen Nutrient limitation: GPP downscaled based on belowground competition	Qiao et al., 2013
GFDL **LM3 (N)**	Allocation: fixed Nutrient limitation: photosynthesis downregulated based on plant nitrogen status	Shevliakova et al., 2009; Gerber et al., 2010
HADCM3 **TRIFFID (N)**	Allocation: fixed Nutrient limitation: none, but does allow competition with neighbors	Clark et al., 2011
ISPL-CM5 **ORCHIDEE (N)**	Allocation: weighted based on competition for light and water Nutrient limitation: nitrogen (parameterized)	Krinner et al., 2005
MIROC-ESM **SEIB-DGVM**	Allocation: based on semi-emperical models Nutrient limitation: none	Sato et al., 2007
MPI-MET **JSBACH (NP)**	Allocation: fixed Nutrient Limitation: GPP downscaled based on N and P limitation	Goll et al., 2012; Parida et al., 2010
Nor-ESMI-M (ME) **CLM4 (N)**	Allocation: fixed Nutrient Limitation: GPP downscaled based on decomposer competition	Tjiputra et al., 2013

Table 2.2. Model parameters

Symbol	Possible Values	Description
Inputs		
Cobb-Douglas exponents:		
α	0-1	Relative plant demand for above-ground resource (i.e. carbon); varies among plant functional types
β	0-1	Relative plant demand for below-ground resource (i.e. nitrogen); varies among plant functional types
Resources:		
C_{avail}	Any	Maximum carbon that can be fixed by photosynthesis per square meter per year.
N_{avail}	Any	Available nitrogen in the soil per square meter per year.
Costs:		
c_{sc}	Any	Cost of shoot carbon: relative amount of carbon units required to build and maintain above-ground tissue
c_{sn}	Any	Cost of shoot nitrogen: relative amount of nitrogen units required to build above-ground tissue
c_{rc}	Any	Cost of root carbon: relative amount of nitrogen units required to build and maintain below-ground tissue
c_{rn}	Any	Cost of root nitrogen: relative amount of nitrogen units required to build below-ground tissue
Outputs production:		
u_s	Any	Relative biomass of above-ground component
u_r	Any	Relative biomass of below-ground component

Table 2.3. Model parameter values used to generate each of the figures in the manuscript.

Parameter	Values for Fig. 2.2	Values for Fig. 2.3a-c	Values for Fig. 2.3d-f	Values for Fig. 2.4
Cobb-Douglas parameters:				
α	variable	0.962	0.962	0.962
β	(1-α)	0.038	0.038	0.038
Resources:				
C_{avail}	300	300	300	variable
N_{avail}	10	10	10	variable
Costs:				
c_{sc}	2	variable	2	2
c_{sn}	1.5	1.5	variable	1.5
c_{rc}	1	variable	1	1
c_{rn}	1	1	variable	1

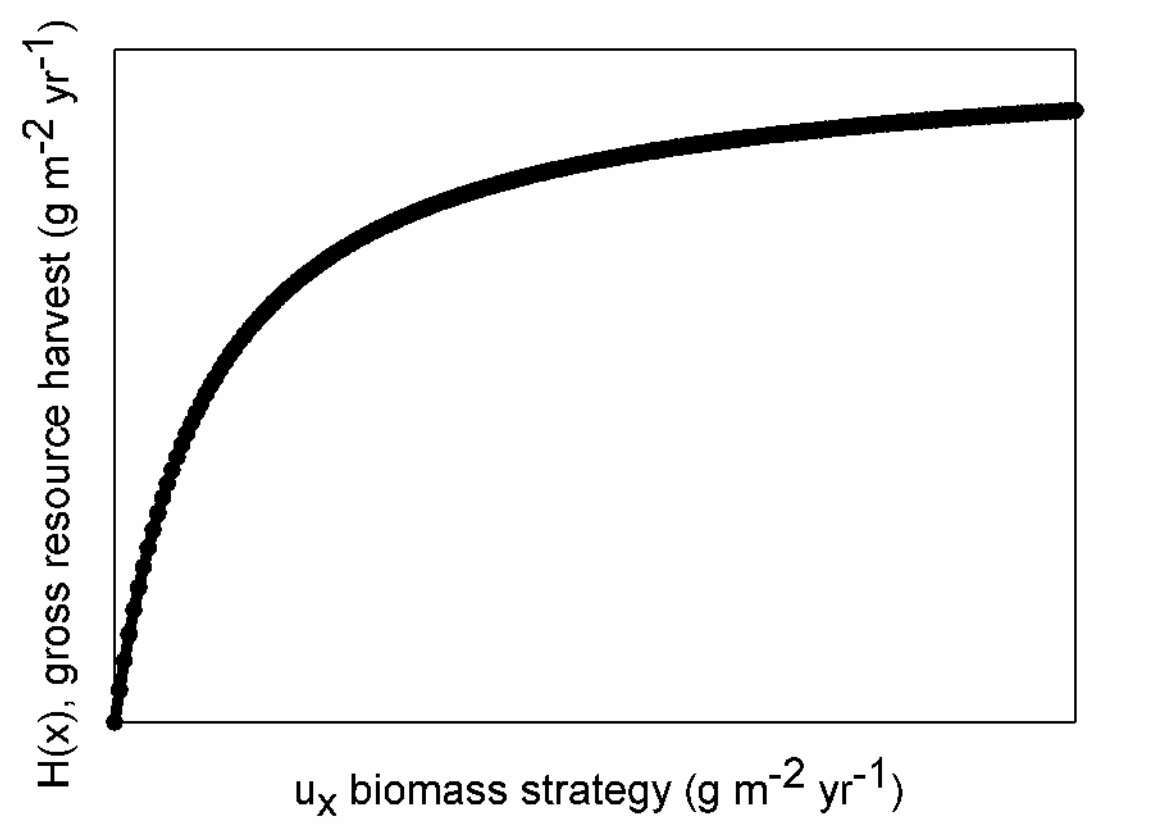

Fig. 2.1. Gross harvest of an essential plant resource plotted as a function of the biomass of the plant organ harvesting the resource. Harvest functions can take multiple forms but must follow the law of diminishing returns, where a maximum gross harvest is attained.

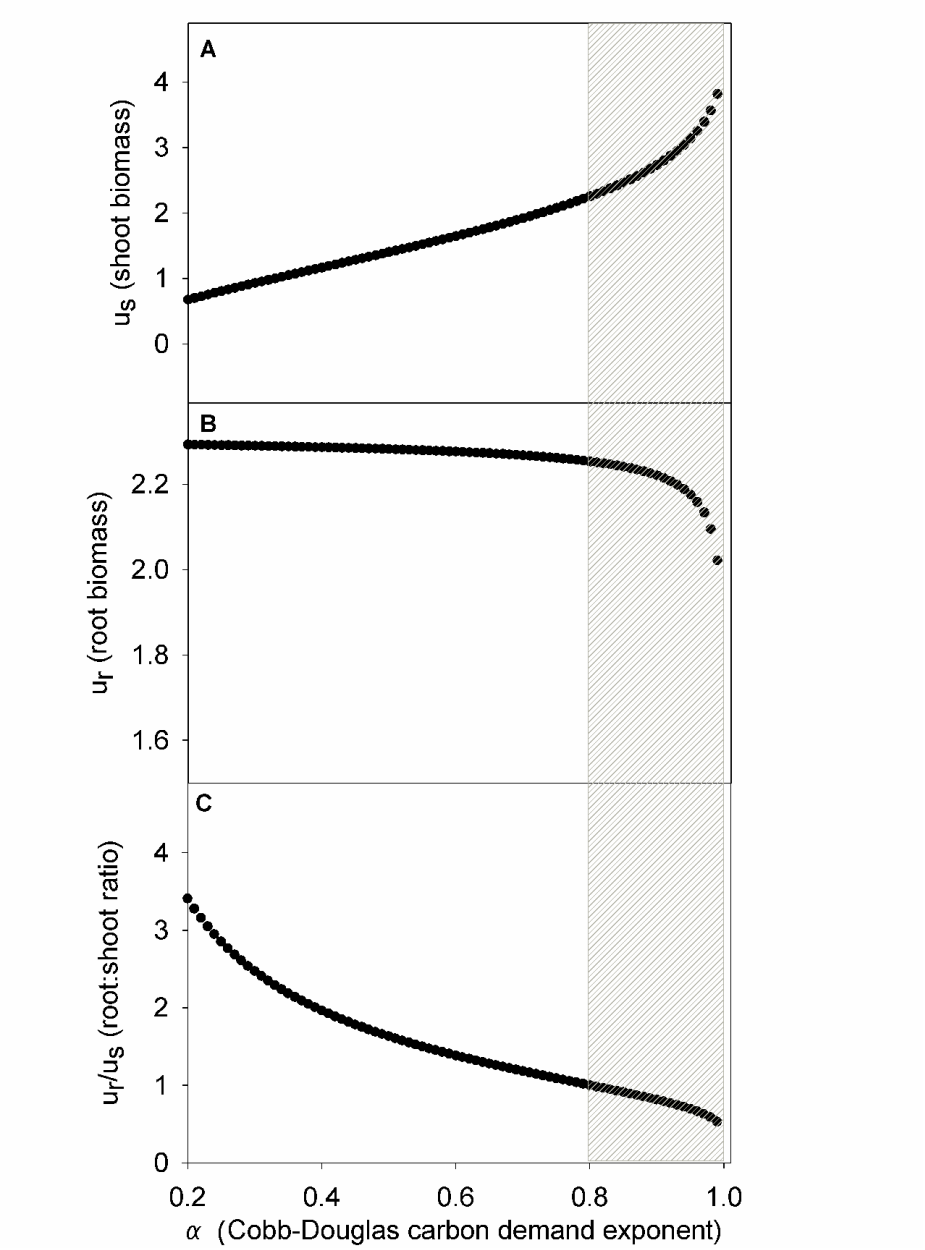

Fig. 2.2. Vegetation biomass production and ratio as a function of the Cobb-Douglas exponents which represent the relative resource demand. Here, β is always equal to 1-α. The shaded section (α > 0.8) indicates the likely range of parameters for real plants. (a) Shoot biomass increases as plant demand for nitrogen decreases. (b) Root biomass decreases as plant demand for nitrogen decreases. (c) The ratio of production of root biomass to shoot biomass decreases as plant demand for nitrogen decreases, and plants allocate more carbon to shoots relative to roots.

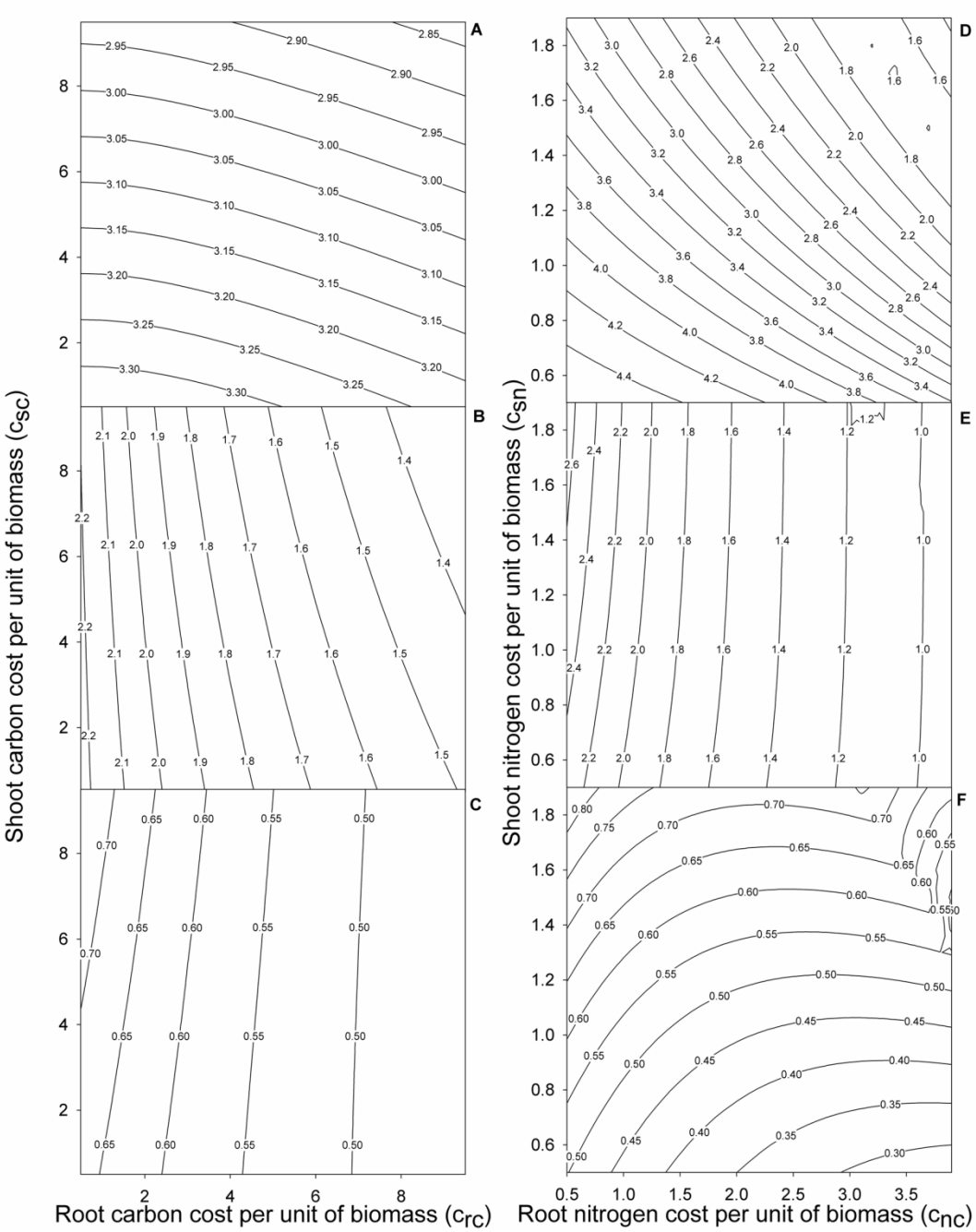

Fig.2. 3. Contour plots of biomass production and ratio as a function of costs for tissue production and maintenance. (a-c) Carbon costs for shoots (y-axis) and for roots (x-axis) with contours of (a) shoot biomass, (b), root biomass, and (c) relative production ratio. As costs to produce each organ increase, production decreases, with changes in carbon costs for roots driving changes in relative above- and below-ground production. (d-f) Nitrogen costs for shoots (y-axis) and for roots (x-axis) with contours of (d) shoot biomass, (e) root biomass and (f) relative production ratio. Similar to carbon, increased nitrogen costs for a given organ reduce biomass of that organ. In contrast to carbon, changes in shoot nitrogen costs drive changes in relative biomass production.

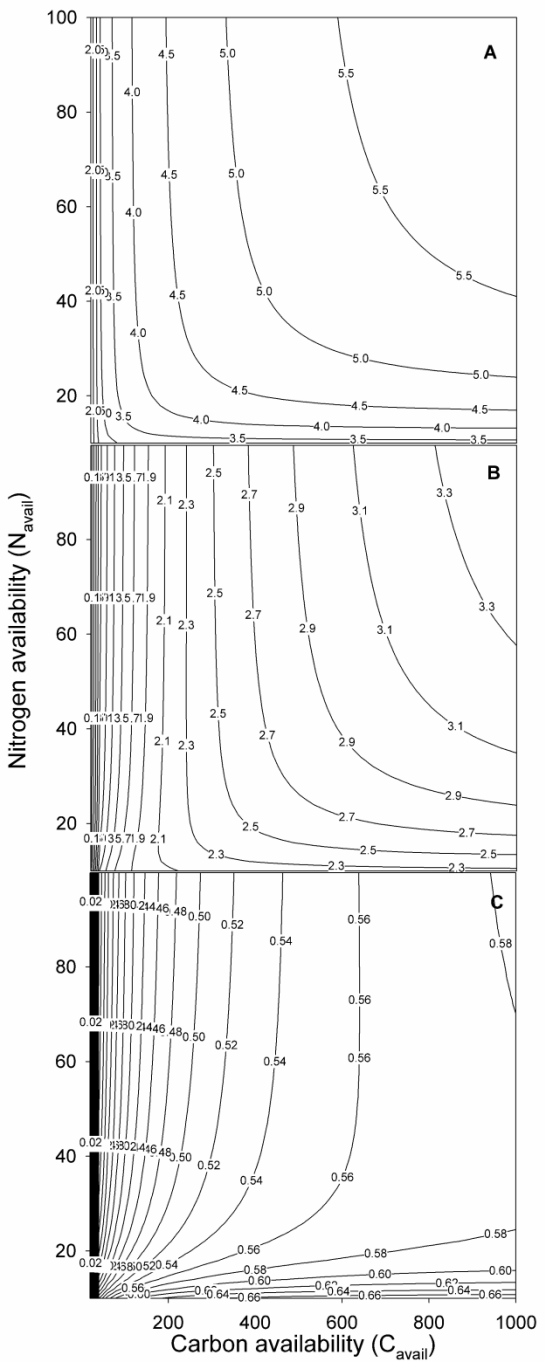

Fig. 2.4. Contour plots of biomass production and ratio as a function of nitrogen availability on the y-axis and carbon availability on the x-axis with contours of (a) shoot biomass, (b) root biomass, and (c) relative above- and below-ground production. Increased resource availability leads to an increase in biomass production. For a majority of resource space, relative production of roots and shoots is driven by C availability.

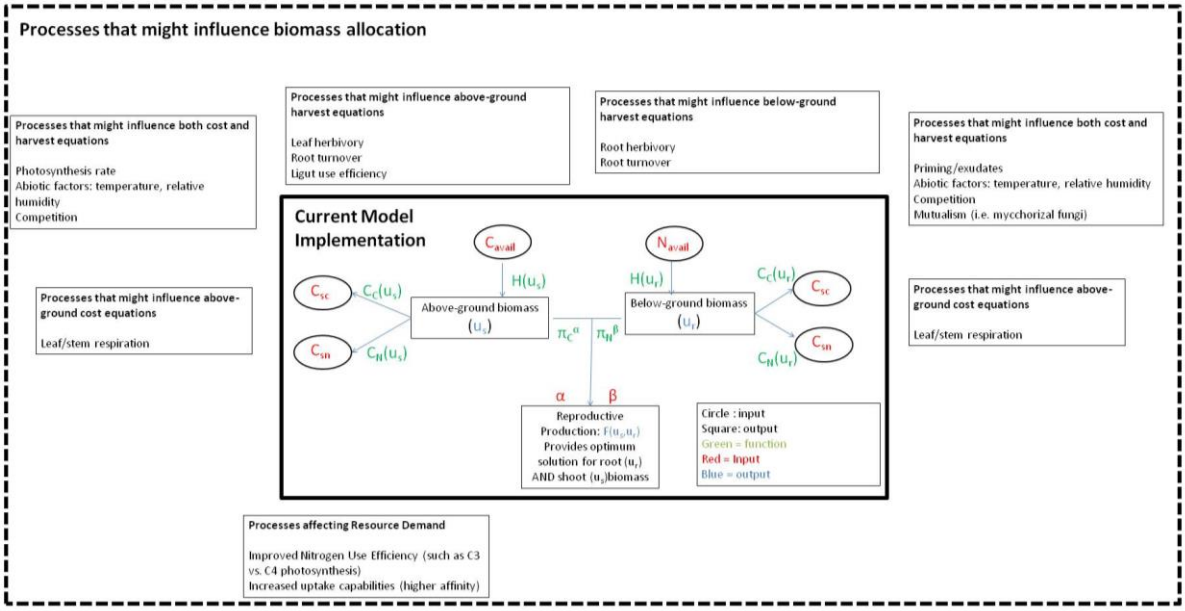

Figure 2.5. A summary flowchart for the model analyzed here with possible mechanisms to explore in future versions. This figure demonstrates the framework of the model presented here, and provides a partial list of physiological processes that may inflence biomass and root-shoot ratios. Each of these processes could be extracted from the integrated parameters and explored within the model framework.

3. Stored carbon partly fuels fine-root respiration but is not used for production of new fine roots

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3.1 Introduction

Fine roots, typically defined as roots less than 2 mm in diameter, are a significant component of net primary production in terrestrial ecosystems (Jackson *et al*., 1997). The respiration of CO_2 from the growth and maintenance of fine roots is an important component of the terrestrial C cycle, and may account for as much as half of soil CO_2 efflux (Taneva *et al*., 2006, Brüggemann *et al*., 2011) and up to 40% of total ecosystem respiration (Davidson *et al*., 2006).

Although we are beginning to understand environmental drivers for rates of root respiration at the ecosystem level (Tang *et al*., 2005; Trueman & Gonzalez-Meler, 2005; Drake *et al*., 2008; Taneva & Gonzalez-Meler, 2011), our understanding of the sources of C fueling root respiration (i.e., recent photosynthate compared with C stored in the plant) is less clear (Körner 2003, Trumbore 2006). New photosynthate was thought to be the main source of C for fine-root respiration (Högberg *et al*., 2001, Trueman & Gonzalez-Meler, 2005), but studies using a radiocarbon tracer found a significant contribution of stored C to the root-respired CO_2 (Czimczik *et al*., 2006, Schuur & Trumbore, 2006). The amount of stored C used in root respiration may differ between species or vary seasonally (Kuptz *et al*., 2011). While new evidence indicates that respired CO_2 may be a mixture of recent photosynthate and stored C, quantitative studies assessing the contribution from these pools to respired CO_2 are lacking.

The ability for plants to move C quickly to roots for respiration contrasts with the observations that at least some C in fine roots is multiple years old (e.g. Matamala *et al*., 2003, Gaudinski *et al*., 2010). The presence of older C in fine root mass may indicate that stored C was used in the production of new roots. However, stored C was not used in production of new fine roots in coniferous (Matamala *et al*., 2003) and deciduous tree plantations (Trueman & Gonzalez-Meler, 2005) exposed to elevated levels of atmospheric CO_2. However, as much as 55% of C used for the production of new roots was from a storage C pool in temperate and sub-tropical oak forests (Langley *et al*., 2002; Gaudinski *et al*., 2009). Furthermore, there appears to be differences in the contribution of stored C depending on root diameter. In *Pinus sylvestris*, roots < 0.5 mm diameter were produced from recent C, while roots 0.5 – 2 mm in diameter were produced using C that was up to 10 years old (Sah *et al*., 2011). Significant amounts of stored C were also used for the growth of new fine roots in a diverse forest in Switzerland (Bader *et al*., 2009).

The presence of older C in fine roots may also be due to multiple pools of C in the fine-root population that have different turnover rates (e.g. structural vs. non-structural C). While fine roots are an important source of C inputs to the soil (Rasse *et al*., 2005), the rate at which fine-root C is input to the soil system is not resolved. Theoretical modeling (Luo 2003, Guo *et al.*, 2008) and isotopic approaches (Trueman & Gonzalez-Meler, 2005; Riley *et al*., 2009, Gaudinski *et al.*, 2010, Keel *et al.*, 2012) have recently demonstrated heterogeneity in the turnover of C in fine roots in some cases (but see Matamala *et al*., 2003; 2004), with some C turning over relatively quickly (on the order of months), and other C having a multiple-year lifespan. The relative magnitude of the C

pool sizes, and the turnover rates for the 'fast' and 'slow' C pools is less clear. Gaudinski *et al*. (2010) placed 20% of fine root C into a fast (~1 – 3 year) pool and 80% into a slow (decadal) pool. However, in a temperate *Pinus taeda* forest with one fine-root C pool there was a general good agreement for at least three methods deployed where isotopes (^{13}C and ^{14}C, Matamala *et al*., 2003), minirhizotrons (Pritchard *et al*., 2008) and sequential coring methods (Matamala & Schlesinger, 2000) converged into similar root production estimates. Current models of the belowground C cycle often represent fine roots as a single pool with a fixed turnover rate (e.g. Parton *et al.*, 1987, Thornton *et al*., 2007). The relative distribution of fine roots into 'slow' and 'fast' turnover pools can affect C cycling in ecosystems, as mass and functionality of roots likely differ between roots of different ages (Gaudinski *et al*., 2010). Thus, if models are to accurately portray the productivity of ecosystems belowground and the contribution of roots to soil C, characterization of heterogeneity in fine roots is paramount to constraining terrestrial C cycles.

Here, we took advantage of a unique opportunity afforded by the conclusion of a long-term free-air CO_2 enrichment (FACE) experiment in a *Liquidambar styraciflua* (sweetgum) plantation at Oak Ridge National Laboratory to track rapid movements of C in the root system. The C in trees and soil that was assimilated during 12 years of CO_2 fumigation had a depleted isotopic C signal, and the relaxation of that signal as the trees assimilated less depleted C after cessation of CO_2 fumigation provided a means of tracking C cycling processes (as in Trueman *et al.*, 2009). We measured changes in the isotopic composition of newly produced fine roots and root-respired CO_2, and monitored the dilution of the depleted isotope tracer in the fine-root pool over two growing seasons

following the cessation of CO_2 fumigation. Our primary objectives were two-fold: (1) quantify the relative use of new photosynthate and a storage C pool for new root growth and root respiration, and (2) determine the relative size and turnover rate for fine-root C pools.

3.2 Materials and Methods

3.2.1 Site Description

This study was performed at the Oak Ridge National Laboratory (ORNL) free-air CO_2 enrichment (FACE) experiment, located in a sweetgum (*Liquidambar styraciflua* L.) plantation in eastern Tennessee, USA. The ORNL FACE experiment has been described in detail elsewhere (Norby *et al.*, 2001, 2002, 2004). Briefly, the experiment had four 25-m diameter FACE rings, two of which were fumigated with elevated [CO_2] to c. 550 ppm for 12 years, from 1998 to September, 2009. The other two rings were maintained at current, ambient [CO_2], which ranged from 384 to 405 ppm during the course of the experiment. A fifth control ring without a FACE apparatus was not used in this experiment. The CO_2 used in the experiment had a ^{13}C signature of c. -51‰, which resulted in the carbon isotope composition of the atmosphere in the elevated [CO_2] treatment to be -21‰ during fumigation (Matamala *et al*., 2003), compared to the ambient atmospheric value of about -8‰. The CO_2 fumigation was terminated in September 2009 after the leaves dropped.

3.2.2 Fine-root sampling from in-growth cores

To determine the sources of C used for new root production, root in-growth bags were sequentially placed and extracted for a full growing season following the cessation of CO_2 fumigation in the elevated [CO_2] plots. From October, 2009, through October,

2010, root in-growth bags (5-cm diameter by 10-cm depth), consisting of fiberglass 1x1 mm screen mesh filled with a sand and perlite mixture, were placed in pre-made holes in the elevated [CO_2] treatment and retrieved after different incubation periods as described below. The two elevated [CO_2] plots received 24 in-growth bags in October, 2009; shortly after CO_2 fumigation was terminated. Eight of these bags were extracted from each plot prior to leaf-out (March 2010), eight bags were extracted just after leaf-out (late April, 2010), and the remaining eight bags were extracted after a full year of incubation *in situ* (October, 2010). Also, beginning in March 2010, 16 bags were inserted into the soil each month except in June as indicated above; eight of these bags were extracted after 4 weeks and the other eight bags were extracted after 12 weeks of being placed in the soil. The differential in-growth bag incubation times (4 or 12 weeks) were to ensure that enough root material was retrieved for isotopic analysis, particularly for larger diameter roots during slower root growth periods. A final set of eight root in-growth bags was placed in the soil in August, 2010 and extracted in October, 2010. In total, 96 root in-growth bags were placed in the soil; a timeline depicting in-growth bag placement and extraction is depicted in Fig. 3.1. The extracted in-growth bags were transported to the laboratory on blue ice, and upon return to the laboratory were frozen at -20°C prior to shipment to the University of Illinois at Chicago, where root retrieval and isotopic analyses were completed.

3.2.3 Fine-root sampling from intact cores

To monitor turnover of C in the fine root pool and examine sources of C utilized for fine-root respiration, intact cores were extracted at regular intervals from the plots previously receiving elevated [CO_2] for two growing seasons following cessation of CO_2

fumigation. Intact soil cores (5-cm diameter) were taken to 10 cm depth from the elevated [CO_2] treatment at the time of extraction of in-growth cores in 2010 (see Fig. 3.1). Additional intact cores were extracted in June, August, September and October, 2011, and a final set of cores was collected in February, 2012. Intact soil cores were also extracted from the ambient [CO_2] treatment plots (i.e., plots never receiving fumigation with elevated [CO_2]) in May, 2010, August, 2011 and September, 2011. During sampling periods in 2010 and early 2011, eight cores were taken in each plot. Starting in August, 2011, the number of cores was three per plot. The extracted intact soil cores were transported to the laboratory on ice, and upon return to the laboratory were frozen at -20°C prior to shipment to the University of Illinois at Chicago, where root retrieval and isotopic analyses were completed.

3.2.4 Fine-root separation and respiration C source measurements

For both in-growth cores and intact soil cores, samples were first thawed in a 4°C refrigerator for 4 hours. Roots were separated from thawed soil and washed with deionized water. We focused here on the roots of sweetgum, as the trees were the main target of the elevated [CO_2] treatment. Live roots were separated using tensile strength (very few dead roots were found), and herbaceous roots, which were very distinct from tree roots were visually identified and removed. Roots were separated into two diameter classes; roots less than 1 mm diameter and roots greater than 1 mm but less than 2 mm diameter. Roots greater than 2 mm diameter were not used for analysis.

Roots extracted from intact cores from several sampling periods were incubated to capture CO_2 respired from fine roots for isotopic analysis and C source determination (as in Gomez-Casanovas *et al*., 2012). From the plots previously receiving elevated

[CO_2], roots from intact cores extracted in May and October, 2010, and in May, August, and September, 2011, were incubated for respired CO_2. All roots extracted from ambient [CO_2] plots (May, 2010, August, 2011 and September, 2011) were incubated for capture of respired CO_2. Roots less than 1 mm in diameter were separated from the soil and incubated using a system similar to that described in Taneva & Gonzalez-Meler (2011) and Gomez-Casanovas *et al*., (2012). Fresh, washed roots were placed into a 140 cm^3 PVC chamber with a moist tissue to prevent drying. The chamber was then flushed with CO_2-free air and sealed for 1 to 2 hours at 25°C. Following incubation, the CO_2 was collected in a gas flask and stored for less than 1 week prior to analysis for $^{13}CO_2$.

Roots collected from the in-growth cores and the intact soil cores were oven-dried at 65°C for 48 hours minimum and ground to a fine powder for ^{13}C analysis of the bulk root tissue (henceforth referred to as "structural root tissue").

3.2.5 Stable C isotope analysis

All gas samples and structural root tissues were analyzed for ^{13}C at the University of Illinois at Chicago (UIC) stable isotope laboratory. Gas samples were purified by cryogenic distillation, and pure CO_2 samples were analyzed for ^{13}C with a Gas Bench II (Thermo Finnigan, Bremen, Germany) coupled to a Finnigan Deltaplus XL isotope ratio mass spectrometer (IRMS, Thermo Finnigan, Bremen, Germany). Structural root samples were run on a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA) coupled to the same IRMS. The $\delta^{13}C$ values are reported relative to the standard VPDB following the equation:

$$\delta^{13}C_{sample} = \left[\frac{\frac{^{13}C}{^{12}C}sample}{\frac{^{13}C}{^{12}C}std} - 1\right] * 1000 \qquad \text{Eqn 1}$$

Partitioning of C sources

In order to differentiate between C fixed in the elevated [CO_2] treatment plots during fumigation (i.e. 'treatment' C that was isotopically-depleted) and C fixed after fumigation ceased at the conclusion of the ORNL FACE experiment (i.e., 'post-treatment' C, normal air), we applied a two-end-member mixing model to our data for both root-respired CO_2 and structural root C (Matamala *et al*., 2003; Taneva *et al*., 2006). For root-respired CO_2, we determined the end-member for the 'treatment' C by correcting the structural root C end-member by adding 4.5‰ , reflecting the consistent 4.5‰ enrichment in root-respired CO_2 with respect to structural root tissue (Fig. 3.3) also seen in other studies (discussed below). The post-treatment C end-member was determined by averaging the isotopic composition of respired CO_2 from all roots collected from intact cores in the ambient [CO_2] plots that never received [CO_2] fumigation. For structural root C, the 'treatment' C end-member was determined by roots sampled from intact cores in March, 2010 that was prior to leaf-out (i.e., roots that were produced using only C produced during CO_2 fumigation) and the 'post-treatment' C end-member was determined by averaging the isotopic composition of all roots collected from intact cores from both growing seasons in the plots that never received [CO_2] fumigation.

The amount of 'treatment' C (i.e., isotopically-depleted C fixed under elevated [CO_2]) in a sample (F_t) in % can be calculated from

$$F_t(\%) = \left[\frac{\delta^{13}C_{sample} - \delta^{13}C_{post-treatment}}{\delta^{13}C_{treatment} - \delta^{13}C_{post-treatment}}\right] * 100 \qquad \text{Eqn 2}$$

where $\delta^{13}C_{sample}$ is the $\delta^{13}C$ of the harvested roots (or the root-respired CO_2), $\delta^{13}C_{post-}$

treatment is the $\delta^{13}C$ of C incorporated after fumigation ceased and $\delta^{13}C_{treatment}$ is the $\delta^{13}C$ of C incorporated during fumigation with isotopically-depleted, elevated [CO_2].

3.2.6 Estimation of C turnover

We determined whether there was heterogeneity in the turnover rate of C in the fine-root population through a linearization approach, using a least-squares method to identify different turnover times in up to three different pools (as in Taneva *et al*., 2006). We also fit our data to both one-pool and two-pool exponential decay models and determined the best model fit using the model R^2 (Keel *et al*., 2012). In the exponential decay model, $F(t) = a_1 e^{-k_1 t} + a_2 e^{-k_2 t}$, where *F(t)* is the percent of 'treatment' C remaining, *a* is the initial amount of 'treatment' C and *k* is the decay rate of 'treatment' C for each respective pool. In the one-pool model, a_2 and k_2 are equal to zero.

3.2.7 Statistical Analysis

All statistical analyses were performed with *R* statistical analysis software, version 2.15.1 (R Development Core Team, 2012). Analysis of variance (ANOVA) models with sampling time as a replicate were utilized to analyze changes in sources of C for new root growth and for root respiration throughout the growing season with samples from the historical ambient [CO_2] plots combined and treated as one sampling period. An ANOVA model was also utilized to compare C isotopic composition between structural C and C in respired CO_2 in the historical ambient [CO_2] plots with sampling time as a replicate. Tukey's HSD (Honestly significant difference) tests were performed on ANOVA models to compare different sampling periods and treatment types. The rate of turnover and the pool size of C in fine roots were analyzed by the fitting of non-linear models (see above section).

3.3 Results

3.3.1 C sources for new root growth

The first set of root in-growth bags extracted in March, 2010 contained no sweetgum roots. For the population of roots less than 1 mm diameter, all subsequent sets of in-growth cores contained enough root mass for isotopic analysis. Initially, new roots extracted from in-growth cores (April, 2010) had a $\delta^{13}C$ in value of approximately -35 ‰, which is 5.6 ‰ more depleted than that of roots grown under ambient CO_2 conditions (average -29.4 ± 0.4 ‰). During the remainder of the growing season roots were less depleted (approximately -30 ‰) and similar to never-fumigated roots (Fig.3.2). The isotopic composition of the newly produced fine roots differed significantly between sampling periods, ($P < 0.001$, see Table 3.2 for ANOVA table), but only the two sets of cores extracted in April, 2010 significantly differed isotopically from the 'post-treatment' C end-member (Fig. 3.2, $P < 0.05$). After April 2010, very little isotopically-depleted 'treatment' C was found in newly produced fine roots.

Despite the 12 week incubation time for in-growth root bags, larger roots with a diameter between 1 and 2 mm were only grown in eight in-growth cores over five sampling periods (out of a total of 96 in-growth cores, Fig. 3.8). The isotopic composition of these few samples was consistent with the smaller-diameter roots collected on the same date, indicating that new roots from both diameter classes were derived mostly with C fixed by the trees 'post-treatment' (i.e., after fumigation with elevated CO_2 had ended).

3.3.2 C sources for root-respired CO_2

We found a significant enrichment in the $\delta^{13}C$ of root-respired CO_2 compared

with structural root tissue. The $\delta^{13}C$ of root-respired CO_2 from roots sampled from the ambient [CO_2] treatment was -24.9 ± 0.8 ‰ (mean ± SE, Fig. 3.3), which was on average 4.5 ‰ enriched compared to structural root tissue from the same plots ($P < 0.01$, Table 3.3). In the plots previously receiving elevated [CO_2], $\delta^{13}C$ of respired CO_2 ranged from -27.6 to -25.8‰ (Fig. 3.4). Sampling time had significant effects on $\delta^{13}C$ of respired CO_2 ($P < 0.05$, Table 3.4). After application of a two end-member mixing model (Eqn 2, note that end-members for C source mixing-model are enriched by 4.5 ‰ compared to end-members for structural root C in order to account for the observed post-carboxylation fractionation), approximately 24 and 10% of C in respired CO_2 was derived from 'treatment' C in 2010 and 2011, respectively (Fig. 3.5).

3.3.3 C turnover in fine roots

Roots sampled from intact soil cores, which represented the entire population of fine roots, including newly produced roots and older roots, had a more depleted isotopic signature than new roots alone. For roots less than 1 mm in diameter, the isotopic composition changed over the course of the study from -40.5 ‰ in March, 2010 to -33.6 ‰ in February, 2012 (Fig. 3.6). By February, 2012, approximately 40% of C in the population of roots less than 1 mm in diameter remained from the elevated CO_2 treatment that ended in September, 2009 (Fig. 3.6). While visual examination of the $\delta^{13}C$ data shows an initial step change by April, 2010 (Fig. 3.6), a linearity approach (e.g., Taneva *et al*., 2006) did not detect multiple C turnover pools in the population of roots less than 1 mm in diameter, though we had less than half of the required 25 data points for this method (Friedlander *et al*., 1981, Fig. 3.9). Data were also fit to both one-pool and two-pool exponential decay models (see Table 3.1 for model parameters). The R^2 of the

exponential decay model was 0.93 for the two-pool model and 0.92 for the one-pool model (Table 3.1). The two-pool model detected a 'fast' turnover root C pool comprising 9 ± 2.5% of total C, and a 'slow' turnover root C pool comprising 91 ± 2.4% of total C (Fig. 3.7 and Fig. 3.10). However, in the two-pool model, only the 'slow' pool parameters were statistically significant ($P < 0.001$). The fast root C pool detected by the two-pool model is consistent in magnitude (~9%) with the initial step change seen in the isotopic composition of roots after cessation of CO_2 fumigation (Fig. 3.6). The mean residence times (MRT = -1/k) of root C derived from the two-pool model were 0.2 and 2.7 years for the 'fast' and 'slow' turnover pools, respectively (Table 3.1).

For roots with a diameter between 1 and 2 mm, the isotopic composition changed over the course of our study from -40.3 ‰ in March, 2010, to -36.2 ‰ in February, 2012 (Fig. 3.6). By February, 2012, nearly 60% of C in the population of roots between 1 and 2 mm in diameter remained from the CO_2 fumigation treatment that ended in September, 2009 (Fig. 3.6). Similar to smaller diameter roots, an initial step change occurred in early spring 2010. A linearity approach did not detect multiple C turnover pools in our data (Fig. 3.9). In a one vs. two-pool exponential decay model comparison, the model R^2 was higher in the two-pool model (0.89) compared to the one-pool model (0.68) (Table 3.1). The two-pool model indicated a 'fast' turnover pool comprising 9.5 ± 4.0% of total root C, and a 'slow' turnover pool comprising 87 ± 3.1% of total root C (Fig. 3.7). Like the smaller-diameter roots, only the 'slow' pool parameters were statistically significant ($P < 0.001$). Mean residence time (MRT) of C derived from the two-pool model were 0.1 and 6.3 years for the 'fast' and 'slow' pools, respectively.

3.4 Discussion

We took advantage of the unique opportunity afforded by the end of a long-term FACE experiment in a mature stand of *L. styraciflua*, where the isotopic composition of C fixed during [CO_2] fumigation was different from C fixed after the FACE experiment was concluded in September, 2009. In contrast to experimental designs using a one-time pulse labeling of an isotopic tracer, the dilution of labeled C incorporated into the sweetgum biomass over the previous 12 years by the newly fixed, unlabeled C into plant biomass (i.e., the 'relaxation' of the isotopically-depleted ^{13}C signature of C fixed during fumigation with CO_2) allowed us to quantify a significant (~24%) use of a storage C pool fueling root respiration and a lack of storage C for new root growth. Additionally, our results indicate a small (10% of total biomass) 'fast' turnover pool and a large (90% of total biomass) 'slow' turnover pool in fine roots.

3.4.1 Post-carboxylation carbon isotope fractionation

Post-carboxylation isotope fractionation needs to be taken into account to properly constrain the C sources used for fine-root respiration (Werner *et al*., 2011). Respired CO_2 isotopic data from plots not receiving CO_2 fumigation indicate a substantial and consistent 4.5‰ enrichment in respired CO_2 relative to the root biomass (Fig. 3.3). This value is similar to that of other woody species including *Fagus sylvatica* (5‰, Formanek & Ambus, 2004) and somewhat larger than that seen in *Eucalyptus delegetenis* (0.7 to 3.1‰, Gessler *et al*., 2007). Respired CO_2 in herbaceous plants, on the other hand, has mostly been found to be depleted in ^{13}C with respect to root substrate or biomass (Bowling *et al*., 2008, Werth & Kuzyakov, 2010, Zhu & Cheng, 2011).

The mechanisms creating isotopic depletion or enrichment in respired CO_2 with

respect to root substrate in plants are not well understood (Bowling *et al*., 2008), but may include the use of different biochemical pathways during primary C metabolism (Gessler *et al*., 2009). Without considering any post-carboxylation fractionation in our study, we would have wrongly concluded that no storage C is used for fine root respiration. This highlights the importance of understanding plant processes that create post-carboxylation fractionation when performing isotope tracer studies. Isotope techniques are often employed to separate autotrophic and heterotrophic respiration components (e.g. Lin *et al*., 1999). However, these studies often estimate the isotopic composition of the respiration components based on the value of the bulk substrate (Zhu & Cheng, 2011), while others have used the isotopic value of respired CO_2 from different sources (Carbone & Trumbore, 2007; Taneva & Gonzalez-Meler, 2011; Gomez-Casanovas *et al*., 2012). Accurate partitioning of components of ecosystem fluxes requires the incorporation of isotope effects during transport and metabolism. Quantifying isotopic effects during transport and metabolism also allowed us to determine amounts of storage C used in root respiration.

3.4.2 C sources fueling fine-root respiration

Despite the availability of C that is just a few days old for respired CO_2 (Högberg *et al*., 2008; Gomez-Casanovas *et al*., 2012), roots may combine recent photosynthate with a storage C pool to fuel metabolic activity (Schuur & Trumbore, 2006). Our data show that a significant portion of C utilized for fine-root respiration is derived from a storage pool (Fig. 3.4). In the first year following cessation of fumigation (2010), ~24% of C was derived from storage in both spring and fall (Fig. 3.5). In 2011 (two years after the end of treatment), the isotopic composition of root-respired CO_2 from intact roots

indicated ~10% of C derived from storage fixed in 2009 (or earlier) throughout the growing season. These results from the 2nd year following fumigation cessation (2011) are more difficult to interpret, as C incorporated during 2010 is isotopically indistinguishable to C incorporated during 2011, even though it is a one-year old storage pool. Thus, the 10% from a storage pool is C that is at minimum 2 years old. Possibly, the difference in storage contribution to root respired CO_2 (~14%) between 2010 (one year after cessation of fumigation) and 2011 (two years after fumigation) represents a storage contribution to root respiration that is about ~1 year old, the rest (~10%) being from storage fixed in 2009 or earlier and therefore at least 2 years old (though this assumes a constant use of 24% storage C as found in 2010). Regardless of age, our data are in agreement with studies using radiocarbon tracers where a significant portion of root respired C is derived from a storage pool (Czimczik *et al*., 2006, Schuur & Trumbore, 2006).

3.4.3 C sources for new root growth

Uncertainties remain in understanding the C sources used to produce fine roots. In-growth cores placed in soils during the dormant season (October, 2009 to March, 2010) contained no *L. styraciflua* roots, indicating no significant new fine-root production when no new photosynthate is being made. In our study, new roots were produced using photosynthate made during the current year for the majority of the growing season (Fig. 3.2). Some stored C was used to produce new roots from in-growth cores extracted in late April 2010, shortly following leaf initiation (Fig. 3.2). All subsequent root production was derived exclusively from current year photosynthate and occurred following 50% leaf-out which occurs in Mid-May (Norby *et al*., 2003). A set of

cores in place for a full year (October 2009 to October 2010) were also not isotopically different from current year photosynthate, indicating a majority of fine root growth occurs during the growing season and comes from new photosynthate (i.e., the biomass of roots grown between October, 2009, and April, 2010, was small, and the isotopic signature of these roots was diluted by the large biomass of roots grown from April, 2010, to October, 2010, that had a strong signal of current-year photosynthate; Fig. 3.2). These results are consistent with studies showing little or no storage used for production of new fine roots (Matamala *et al*., 2003; Trueman & Gonzalez-Meler, 2005). However, other studies have reported up to 55% of new fine root production comes from a C storage pool (Bader *et al*., 2009, Gaudinski *et al*., 2009). For larger fine roots (> 1 mm diameter), few in-growth cores contained roots of this size, but the samples that did are in agreement with new root growth utilizing new C during the growing season (Fig. 3.8). In contrast with our results, a recent study found contribution of storage C in large diameter fine roots in mature boreal forests (Sah *et al*., 2011). Therefore, the use of a storage C pool for root production is not consistent between species or ecosystems, and mechanisms that may account for these differences are not known.

3.4.4 Fine-root C turnover estimates

While newly fixed photosynthate and storage fuel root respiration, new root growth is mostly supported by recent photosynthate. The turnover rate of C used to produce fine roots was measured by tracking disappearance of the 12 year treatment C isotopic tracer in the intact fine- root pool over two full growing seasons (Fig. 3.6). In contrast to the in-growth cores, roots collected from intact cores represented both old roots produced during CO_2 fumigation and new roots produced following fumigation

cessation and therefore the rate of C replacement represents the C turnover of a given root pool ((Table 3.1, Figs 3.10, 3.11; Matamala *et al*., 2003). There was an initial step change in the isotopic composition of fine-roots (first few weeks; Fig. 3.6) suggesting the existence of a fast turnover root pool representing about 9% of the total root biomass. Although data linearization (Taneva *et al*., 2006) did not reveal a fast and a slow pool , the R^2 was higher for a two-pool model compared to the one-pool model(Keel *et al*., 2012) for both diameter classes (though the difference was much small in the < 1 mm diameter class). Fine roots appear to have a small 'fast' pool with turnover times of a few months (~10% of total root C) and a larger 'slow' pool with turnover times of multiple years (~90% of total C).

Mean residence time of C in fine roots estimated at the onset of CO_2 fumigation at this FACE site (Matamala *et al*., 2003) was less than estimates from this study (Table 3.1). Part of the difference can be attributed to the identification of a fast root pool in this study, as MRT of root C in the 'slow' pool is increased after removing the 'fast' pool. Part of the difference between the two studies may also be due to an effect of elevated [CO_2] on root turnover, as root turnover appeared to be longer under elevated [CO_2] conditions at the site (Iversen *et al*., 2008) which may have influenced previous estimates of root C turnover. Despite the differences, C residence times in fine roots measured at the onset and cessation of fumigation with isotope tracers are in agreement with each and longer than minirhizotron estimates of root turnover at the same ORNL site, which was less than 2 years for fine roots < 2 mm in diameter from 2001 through 2006 at elevated CO_2 and less than 1 year at ambient CO_2 (Iversen *et al*., 2008).

Disparities between minirhizotron and isotope tracer studies on root (C) turnover

and longevity may stem from several independent reasons. While minirhizotron approaches are based on direct observations of production and senescence of individual root structures in situ, isotope tracers quantify residence time of C in root systems. Any re-absorption of root C after senescence of existing roots will increase longevity of C compared to root structures. Minirhizotron installations also seem to promote turnover of roots due to soil disturbance, and it may take 3-5 years for the system to stabilize (Pritchard *et al*., 2008). Other potential reasons for differences between isotopic tracer experiments and minirhizotron studies are that soil coring and extraction can miss the smallest roots when separating from the soil matrix while minirhizotrons mostly observe the finest roots (Majdi *et al*., 2005). Recent evidence suggests that fine-root production and mortality may occur in clusters of low order roots that differ in their function and structure (Xia *et al*., 2010), but only a fraction of the fine roots turnover rapidly (Guo *et al*., 2008). It is clear from this and previous isotope tracer studies that some C persists in fine roots for multiple years. While a large portion of C in fine roots remains for multiple years, a small amount is turned over very quickly, in a few weeks or months. This 'fast' turnover pool may include 10% of total fine-root C, as found here or up to 20% as found elsewhere (Gaudinski *et al*., 2010). Fine roots are an important component of forest NPP, but uncertainties currently remain in quantifying fine root contribution to forest NPP. If the smaller 'fast' pool is replaced multiple times during a growing season, the smaller pool may have larger effects on NPP than their size suggests.

3.4.5 Implications for C cycle models

Studies quantifying both C allocation for various plant processes and C turnover in plant organs (particularly belowground) are important in understanding plant

physiology (Bond-Lamberty & Thomson, 2010) and for incorporation into terrestrial C cycle models at various scales (Iversen 2010). Most ecosystem-scale or larger-scale models currently incorporate a single C pool for root turnover (Fisher *et al*., 2010; Gaudinski *et al*., 2010). The fumigation of trees with elevated atmospheric CO_2 at the FACE sites introduced into the ecosystem a C tracer that can help to reevaluate current model assumptions, particularly those for the belowground processes. If fine roots exist in two root turnover pools, as this study indicates, then it is possible to calculate the contributions of roots to soil organic C (SOC) and soil organic N (SON) pools and validate those contributions with observed soil accruals at the site. For example, given fine root C and N contents of 80 g C m^{-2} and 2 g N m^{-2} at 10-cm soil depth (Iversen *et al*., 2012), respectively, if 10% of the fine root mass has a 0.2-yr turnover (replacing the biomass 5 times in a year) and 90% of the fine root mass is replaced at a rate of 0.37 yr^{-1}, then the fast root turnover will produce about 20 g C m^{-2} yr^{-1} and 1 g N m^{-2} yr^{-1}, and the slower-turnover roots will produce about 13 g C m^{-2} yr^{-1} and 0.7 g N m^{-2} yr^{-1}, if we assume that 50% of the root litter stays in the soil as soil organic matter (Parton *et al*., 1987). The ORNL FACE showed increases in SOC (44 g C m^{-2} yr^{-1}) and SON (3.2 g N m^{-2} yr^{-1}) during the first 6 yr of CO_2 fumigation (Jastrow *et al*., 2005). These increases continued, although at a slight slower rate, until the end of the CO_2 fumigation experiment at this depth (Jastrow, JD., personal communication). Thus, fine root C and N turnover represents about 80% of the SOC accrual and about 76% of the SON accrual observed in this experiment, with the remainder produced by coarser root turnover and leaf litter decomposition. Such an agreement shows that the belowground C cycle can be modeled properly with two heterogeneous pools for fine roots (Gaudinski *et al*., 2010).

Better representation of ecological and physiological properties of fine-root activities in models may improve understanding of the belowground C flow, nutrient acquisition by plants and soil C sequestration in terrestrial ecosystems.

3.5 Conclusions

In this study we monitored the relaxant of a C isotope tracer following the conclusion of the long-term FACE experiment. Our results have confirmed relatively long turnover times for fine root C (on the order of years) determined by previous isotope studies. Additionally, we have provided evidence for heterogeneity in C turnover in fine roots, as suggested by previous studies (Guo *et al*., 2008, Gaudinski *et al*., 2010). Our results indicate a small pool with fast (<< 1 year) turnover and a large pool with slow (multiple year) turnover. Our turnover time estimates were not confounded by use of storage C for new root growth as almost all C incorporated into new roots was from current year photosynthate. We did find a substantial use of storage C for fine root respiration (~24% of total C), which appears to be consistent throughout the growing season. Additionally, a 4.5‰ post-carboxylation enrichment in respired CO_2 relative to the root substrate must be considered in interpretation of studies utilizing isotopic tracers to examine root respiration.

3.6 Acknowledgements

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Table 3.1 Model parameters for exponential decay models explaining turnover of C in fine roots.

Roots < 1 mm diameter

		Slow Pool				Fast Pool (if applicable)				
Source	**Model Type**	**a_1**	**k_1**	**MRT (yrs)**	**95% Turnover Time (yrs)**	**a_2**	**k_2**	**MRT (years)**	**95% Turnover Time (yrs)**	**R^2**
Current Study	one pool	0.99 ± 0.022	-0.44 ± 0.027	2.0 - 2.6	7		N/A			0.92
Current study	two pool	0.91 ± 0.024	-0.37 ± 0.026	2.4 - 3.2	8	0.09 ± 0.025	-4.15 ± 12.5	0.2	0.2	0.93
Matamala *et al* 2003	one pool	0.99	-0.8356	1.1 - 1.4	4		N/A			0.99

Roots 1- 2 mm diameter

		Slow Pool				Fast Pool (if applicable)				
Source	**Model Type**	**a_1**	**k_1**	**MRT (yrs)**	**95% Turnover Time (yrs)**	**a_2**	**k_2**	**MRT (years)**	**95% Turnover Time (yrs)**	**R^2**
Current Study	one pool	0.99 ± 0.023	-0.26 ± 0.026	3.2 - 4.73	11.4		N/A			0.68
Current study	two pool	0.87 ± 0.031	-0.16 ± 0.028	4.8-9	17.8	0.095 ± 0.04	-10 ± 13.7	0.1	0.1	0.89
Matamala *et al* 2003	one pool	1	-0.3333	2.7 - 3.3	9		N/A			0.98

Table 3.2: ANOVA results for bulk δ13C of fine roots < 1 mm diameter from in-growth cores as a function of sampling period. Sampling time is significant. A Tukeys HSD (honestly significant difference) test on the model demonstrates that only in two sampling periods does the root isotopic composition differ from the 'new' C end-member (see text for details).

Variation	Df	SS	MS	F-value	Pr(>F)
Sampling time	11	82.22	7.475	8.362	4.99E-09
Residuals	67	59.89	0.894		

Table 3.3: ANOVA results for comparing bulk root δ13C and fine root respired CO2 in ambient [CO2] rings across sampling time. Both sampling time and sampling type are significant. A Tukeys HSD (honestly significant difference) test on the model indicates that at all time periods, bulk root $\delta^{13}C$ differs significantly from $\delta^{13}C$ of respired CO_2.

Variation	Df	SS	MS	F-value	Pr(>F)
sampling time	3	23.43	7.81	7.116	3.76E-04
C source[1]	1	238.2	238.2	217.039	2E-16
sampling time * C source	3	3.47	1.16	1.054	0.375912
Residuals	58	63.66	1.1		

Notes: [1]C source is either bulk root C or respired CO_2

Table 3.4: ANOVA results for comparing δ13C of fine root respired CO2 in the elevated [CO2] rings. C source does not significantly differ across sampling periods. A Tukeys HSD (honestly significant difference) test on the model does not indicate a significant difference between $\delta^{13}C$ of respired CO_2 at any time period and the ambient end-member.

Variation	Df	SS	MS	F-value	Pr(>F)
Sampling time	6	56.82	9.469	3.198	.0134
Residuals	34	100.68	2.961		

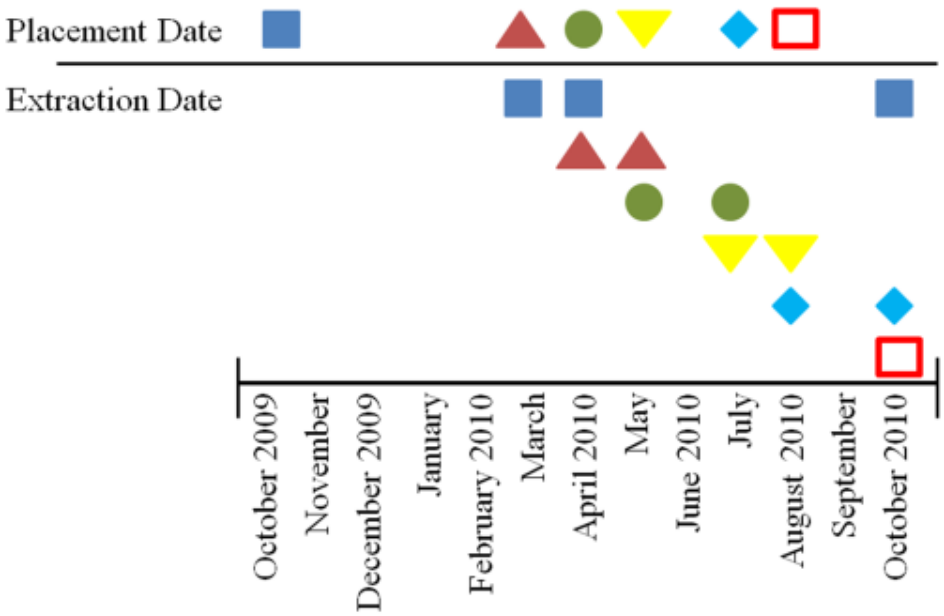

Fig. 3.1 A timeline of placement and extraction of in-growth cores used to analyze sources of C for new fine-root growth. CO_2 fumigation ceased in September, 2009.

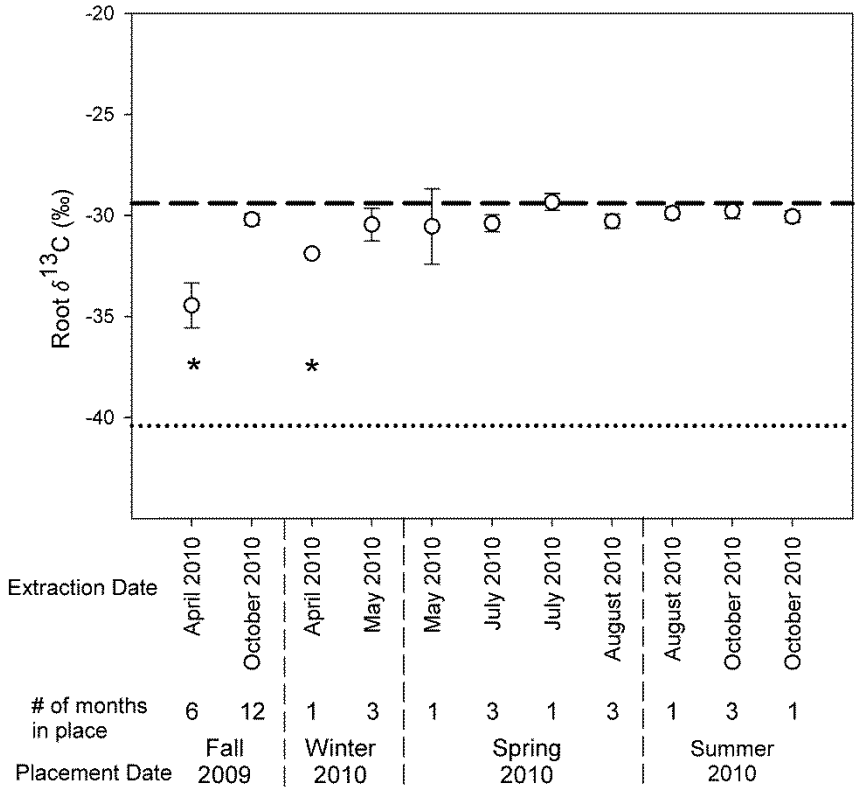

Fig. 3.2 Bulk root δ13C for fine roots < 1 mm from in-growth cores in 2010. Data shown are means (± 1 SE). The dotted line indicates the isotopic composition of C incorporated during fumigation (treatment) and the dashed line the isotopic composition of C incorporated after fumigation (post-treatment). The * indicates significant difference from post-treatment end-member (dashed line) from Tukey's HSD tests on ANOVA model ($P < 0.01$).

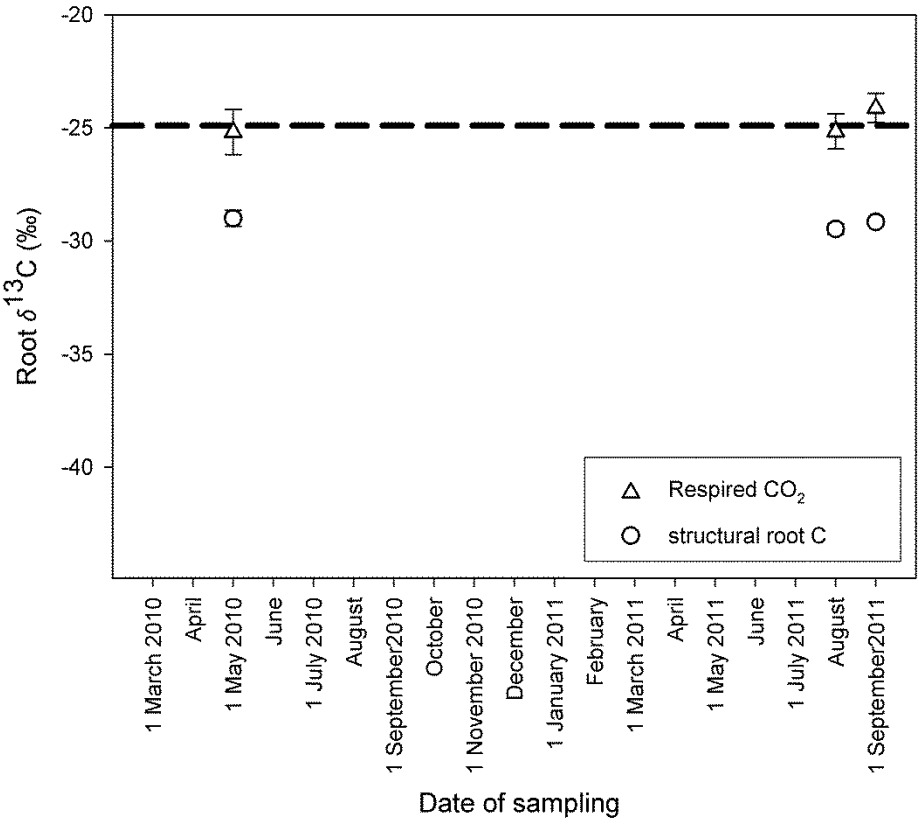

Fig. 3.3 Structural root δ13C and respired CO2 δ13C for fine roots < 1 mm from intact cores in the ambient [CO2] treatment. Data shown are means (± 1 SE). Dashed line indicates the mean for all respired CO_2 $\delta^{13}C$ samples. Tukey's HSD on an analysis of variance model indicates that bulk root $\delta^{13}C$ significantly differs from respired CO_2 $\delta^{13}C$ in all cases.

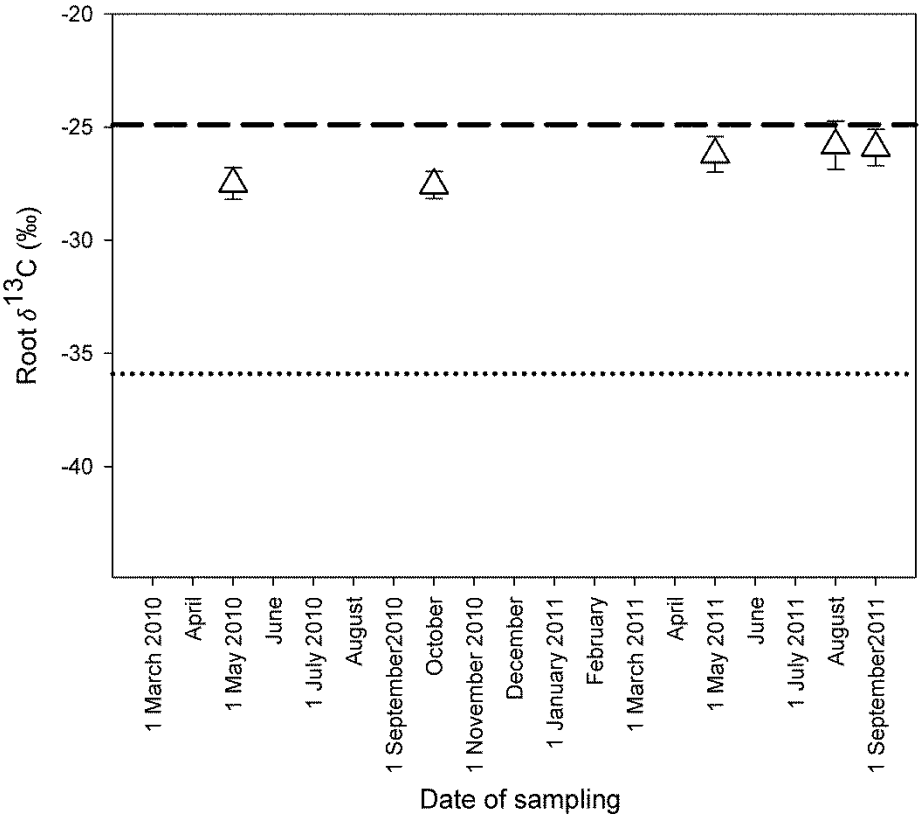

Fig. 3.4 Respired CO2 δ13C for fine roots < 1 mm from intact cores in the elevated [CO2] treatment. Data shown are means (± 1 SE). The dotted line indicates the isotopic composition of C incorporated during fumigation (treatment) and the dashed line the isotopic composition of C incorporated after fumigation (post-treatment). Tukey's HSD on an analysis of variance model does not indicate a significant difference between respired CO_2 $\delta^{13}C$ and respired CO_2 incorporated after cessation of fumigation (dashed line).

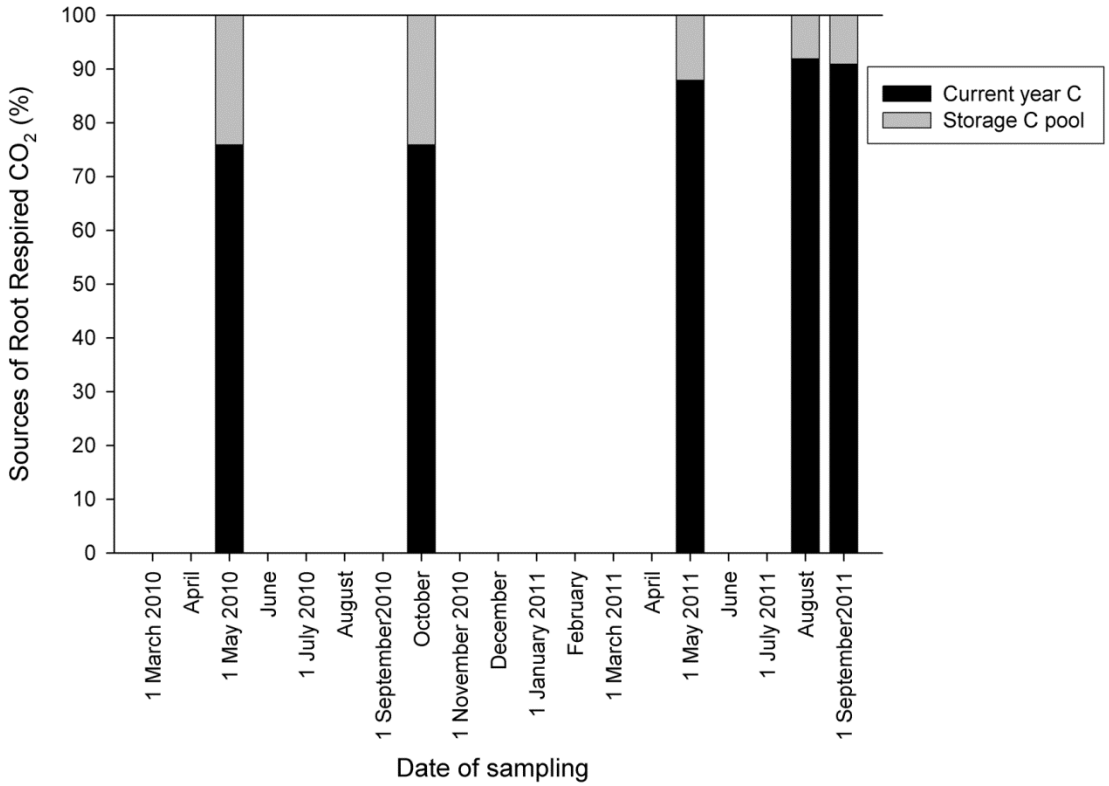

Fig. 3.5 Sources of C for respired CO2 for fine roots < 1 mm from intact cores in the elevated [CO2] treatment after applying a two end-member mixing model. Approximately 24% and 10% of C respired from fine roots is storage C in 2010 and 2011, respectively.

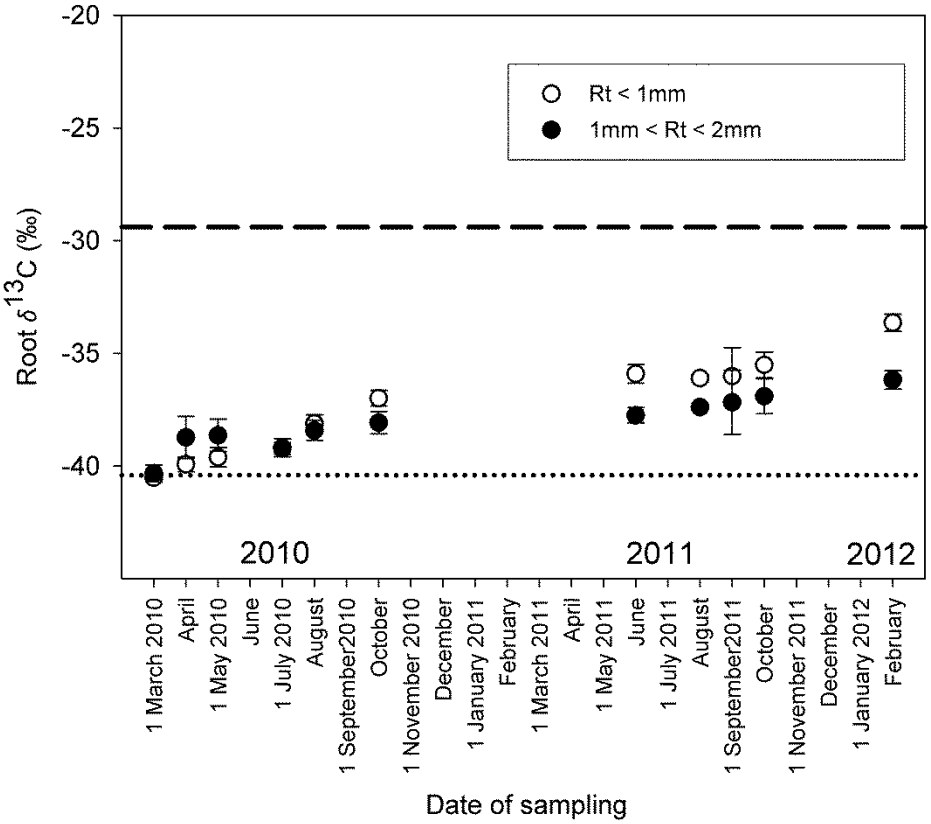

Fig. 3.6 Bulk root δ13C for fine roots < 1 mm (open symbols) and 1 – 2 mm (closed symbols) from intact cores. Data shown are means (± 1 SE). The dotted line indicates the isotopic composition of C incorporated during fumigation (treatment) and the dashed line the isotopic composition of C incorporated after fumigation (post-treatment).

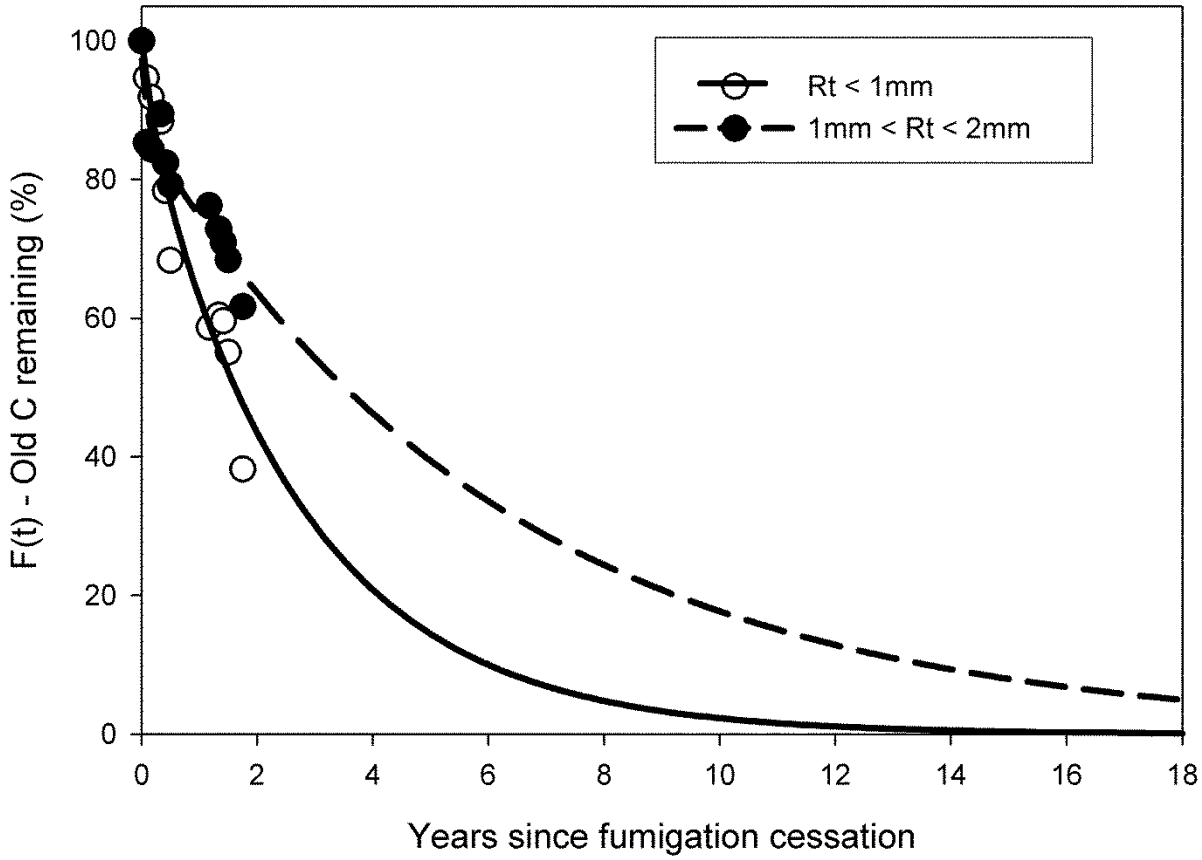

Fig. 3.7 Bulk root δ13C for fine roots < 1 mm (open symbols) and 1 – 2 mm (closed symbols) from intact cores presented as % Old C after applying a two end-member mixing model. Each diameter class has been fit to a two-pool exponential decay model $F(t) = a_1e^{-k1t} + a_2e^{-k2t}$ where F(t) is % fumigation C remaining, a is initial amount of fumigation C and k is the decay rate of fumigation C for each respective pool. For < 1 mm roots, $F(t) = 0.91e^{-0.368t} + 0.09e^{-4.15t}$ and for 1 – 2 mm roots, $F(t) = 0.87e^{-0.16t} + 0.095e^{-10t}$. Mean residence time (MRT) = -1/k and is 2.7 and 0.2 years for the < 1mm larger and smaller pools, respectively and is 6.3 and 0.1 years for the 1 – 2 mm larger and smaller pools, respectively.

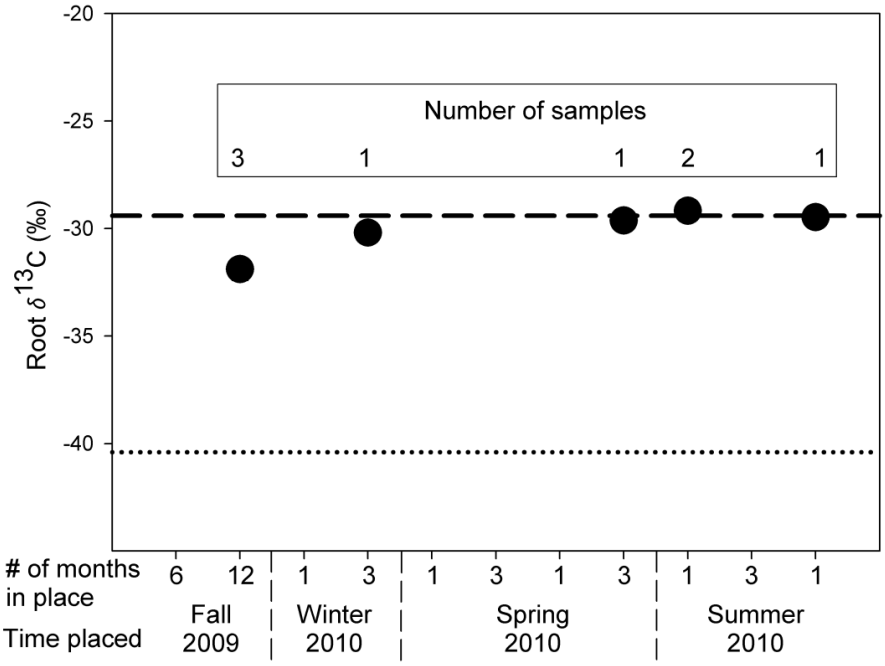

Fig. 3.8 Bulk root δ13C from diameter class 1 - 2 mm from in-growth cores. Very few samples contained roots this large (number of samples for each period is in the figure). However, these data are consistent with the Rt < 1 mm diameter class, in that C for new root growth is new photosynthate as soon as available.

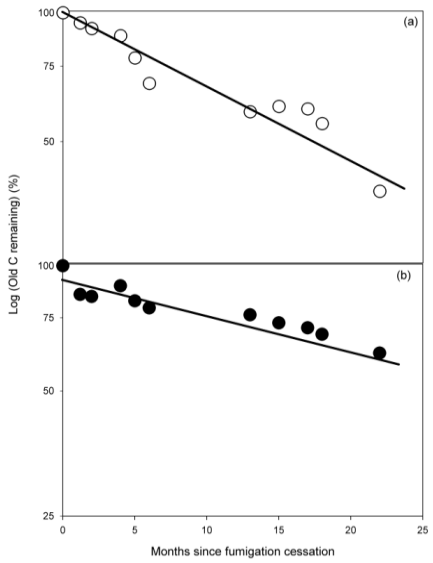

Fig. 3.9 Log(% Old C remaining) vs time after CO2 fumigation cessation in months for (A) roots < 1 mm and (B) roots 1 – 2 mm. This linearity approach followed the methodology of Taneva et al. (2006) in an attempt to separate C pools with different turnover rates. For our data, this approach was unable to distinguish multiple turnover pools. However, our data set is less than half of the required 25 data points for this method (Friedlander et al., 1981).

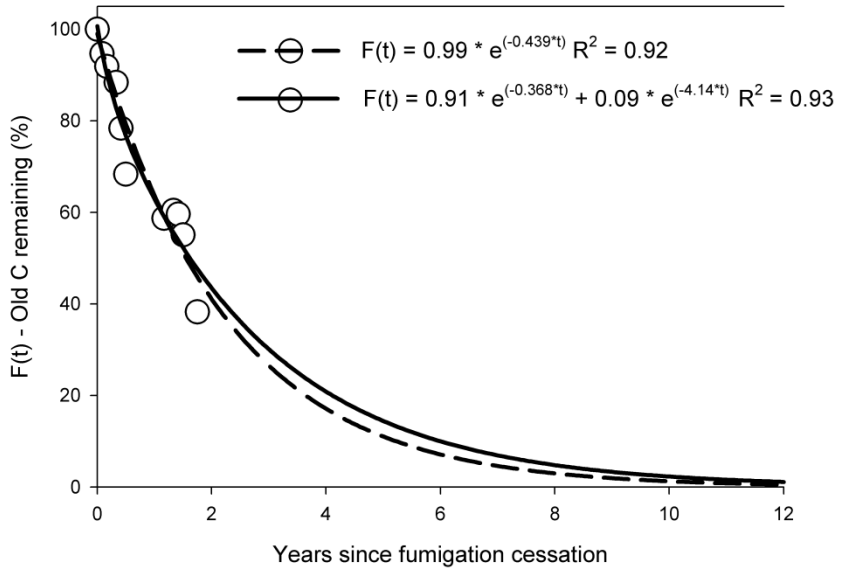

Fig. 3.10 A comparison of one pool and two pool exponential decay functions for Rt < 1 mm fine roots.

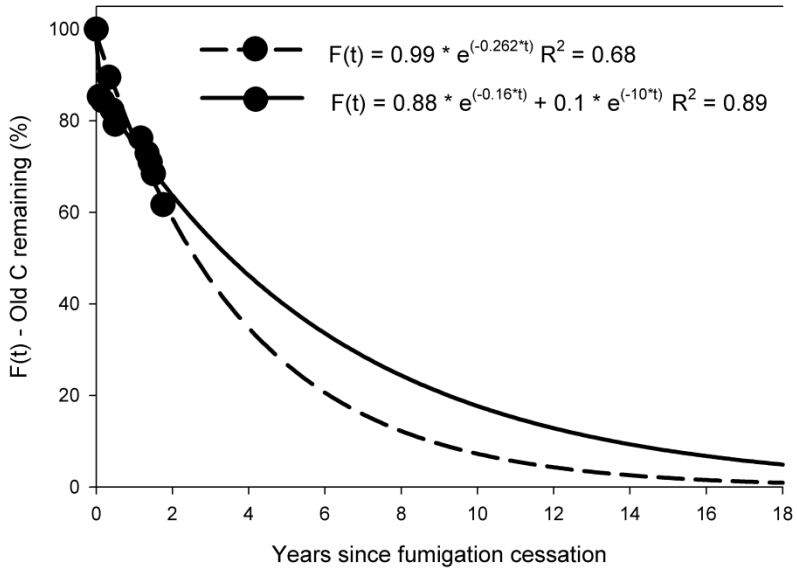

Fig. 3.11 A comparison of one pool and two pool exponential decay functions for Rt 1 – 2 mm fine roots.

4. Root N concentration as a proxy for longevity of different root branching orders

4.1 Introduction

Fine roots play an important role in nutrient and water uptake. In addition to this important role for individual plants, fine roots function at the ecosystem-scale as a major conduit for carbon from the atmosphere to soils (Rasse et al., 2005). Despite these known important functions, much uncertainty remains regarding fine-root dynamics. For example, a classification system for analysis of fine roots is currently lacking. Historically, the definition of fine roots has been an arbitrary diameter size cut-off, most often < 2 mm. This approach assumes that all roots within a given diameter cohort are homogenous, both anatomically and physiologically. However, recent studies have demonstrated a wide variety of heterogenous morphological characteristics within the traditionally defined fine-root system (Hishi 2007). A different, perhaps more functional approach for fine-root analysis is the use of hierarchical branching that occurs in fine roots (Pregitzer et al., 2002). With increased branching order, roots from multiple species increase in length and diameter, while decreasing in number and nitrogen (N) concentration (Pregitzer et al., 2002). Increased branching order also results in reduced respiration rate (Guo et al., 2008), and increased non-structural carbohydrate content (i.e. storage, Guo et al., 2004), indicating different functionality for roots of different branching orders.

The branching order approach to studying root dynamics may reveal physiological information that is lost when consolidating all roots below an arbitrary diameter classification (Hishi 2007, Pregitzer et al., 2002). While potentially more physiologically appropriate, dissection of roots by branching order requires substantially more time than separating into diameter classes, as well as potentially increased analytical costs for chemical measurements.

Thus, depending upon the goal of the investigation, a simpler diameter approach may be more appropriate. Despite the increased sampling and processing time, some questions regarding fine-root dynamics likely benefit from a branching order technique.

One such long-standing question regarding fine roots is the longevity of the fine-root population (Eisensttat and Yanai, 1997). Initial measurements found a high rate of fine-root turnover and thus a high (up to 33%) contribution to NPP (Jackson et al., 1997). A major assumption of this approach is an equal probability of mortality for all fine roots. Recent studies have found that modeling the rate of turnover of fine roots is improved when using two carbon pools, allowing heterogeneity in lifespan within fine roots (Gaudinski et al., 2010, Lynch et al., 2013). It is increasingly clear that lifespan in fine roots is heterogeneous, yet it remains unknown what controls the lifespan of an individual root. As measurement of lifespan remains methodologically challenging (Trumbore and Gaudinski 2003, Strand et al., 2008), identification of traits that can be linked to lifespan is an important topic of research (McCormack et al., 2012). Analysis of root lifespan by branching order may reveal important information regarding heterogeneity in fine roots. In fact, one such study using minirhizotrons concluded that ephemeral lower order (1 – 3) roots die in clusters, with perennial higher (4 – 5) order roots living much longer (Xia et al., 2010). Few studies to date have investigated carbon residence time in fine roots separated by root branching order using a long-term isotope tracer.

Here, we utilize the unique opportunity afforded by the cessation of CO_2 fumigation of a long-term free-air CO_2 enrichment (FACE) experiment in a *Liquidambar styraciflua* plantation at Oak Ridge National Laboratory, TN, USA. Carbon assimilated during the 12 yr of the experiment was isotopically depleted with respect to carbon assimilated following the conclusion of the experiment. We measured isotopic composition of fine roots of the five lowest branching

orders in addition to morphological and chemical parameters from roots sampled two and three years following the cessation of CO_2 fumigation. Our primary objectives were to determine the relationship between root branching order and mean carbon residence time in fine roots and also to explore potential relationships between mean carbon residence time (and thus longevity) in fine roots and root morphological and chemical traits.

4.2 Materials and Methods

4.2.1 Site Description

The study site was a sweetgum (*Liquidambar styraciflua* L.) plantation in eastern Tennessee, USA, at the Oak Ridge National Laboratory (ORNL) free-air CO_2 enrichment (FACE) experiment, described in detail elsewhere (Norby *et al.*, 2001; 2002; 2004). The experiment was comprised of four 25-m diameter FACE rings, two of which were fumigated with elevated [CO_2] to c. 550 ppm for 12 years, from 1998 to September, 2009. The remaining FACE rings were at current, ambient [CO_2], approximately 384 to 405 ppm. Fumigation CO_2 had a consistent ^{13}C signature of c. -51 ‰ with the elevated [CO_2] atmosphere -21 ‰ during fumigation (Matamala *et al*., 2003). Background ambient atmospheric ^{13}C signature was about -8 ‰. Elevated [CO_2] treatment produced leaf litter averaging -40.0 ‰ ± 0.4 compared to -29.4 ‰ ± 0.2 in ambient [CO_2] conditions (Norby RJ, unpublished; Garten *et al*., 2011).

Experiments that utilize the few available ecosystem isotopic composition manipulations that are both long-term and at ecosystem-scale, such as the ORNL-FACE site are important for improving understanding of ecosystem C cycling, particularly carbon residence time in different compartments (Epron *et al.*, 2012). One limitation of combined labeling and fumigation experiments (e.g., Keel *et al.*, 2006) is label results are confounded with the effects of elevated CO_2 on C allocation. Consuquently, results may not represent C cycling in current, ambient CO_2

(Epron *et al.* 2012). At this experiment, this potential limitation was addressed through several means (see Lynch et al., 2013 for a complete discussion). Briefly, the effects were limited by 1) sampling following the cessation of CO_2 fumigation, eliminating direct effects of elevated [CO_2] and 2) multiple lines of evidence indicate that for the final 2 years of CO_2-enrichment, minimal differences in above- and below-ground C cycling occurred between the experimental and control rings.

4.2.2 Sampling of intact root sections

Sampling was conducted in late winter of 2012 and 2013. Sampling was performed in the dormant season, when fine-root production is low (Norby et al., 2004), in order to avoid a sampling bias towards new roots with very short lifespan (i.e. < 3 months, Lynch et al., 2013). During each sampling year, two soil blocks of 10 x 20 cm were extracted to 10 cm depth from each FACE ring (both treatment and control) for a total of eight soil blocks. In 2012, intact root sections were extracted from the soil matrix on-site, while in 2013 soil blocks were frozen and brought to the laboratory. The extracted intact soil blocks or root sections were transported to the laboratory on ice, and frozen at -20°C prior to shipment to the University of Illinois at Chicago, where root retrieval, dissection, morphological and chemical analyses were completed.

4.2.3 Fine-root separation by root branching order

In total, 4,364 individual roots were excised from 49 intact root sections, averaging approximately 89 roots per intact root section. Prior to dissection, intact root sections were carefully teased from the soil matrix, using great care to avoid any breakage. Once teased from the soil, intact root sections were washed thoroughly with deionized water kept at 1°C. To ensure no soil particles remained on the roots, intact roots were examined under a microscope prior to, and in most cases again after, dissection. Root separation by root branching order followed the

protocol described in Pregitzer et al. (2002). All dissection of the lower orders occurred using a dissecting microscope with 12.5x magnification (Leitz), with the dissection of higher order roots completed using a magni-focuser (Edroy Products Model 113). The processing time for an individual root section varied depending on size, but roots were kept moist with 1°C deionized water for the duration of the processing. Individual roots were dissected starting with the most distal order one roots, and proceeding to the highest order, in most cases order five or six. Dissected roots were temporarily stored in a petri dish in 1°C deionized water (a separate dish for each root order), where digital images were taken for root diameter analysis, followed by oven-drying and chemical analysis (see following section).

4.2.4 Fine-root morphological and chemical measurements

For root diameter, digital images of freshly dissected roots were taken while roots were kept moist in 1°C deionized water in a petri dish. Petri dishes were placed on a scanner (ImageRUNNER 5070, Canon), and a high resolution (600 dpi) black and white image was taken. Images were analyzed using SmartRoot software, developed specifically for quantification of root architecture (Lobet et al., 2011), a freeware module interface developed for ImageJ (Rasband 1997-2008). Wires of known diameter were included with the root samples, to provide a quality control for diameter measurements. In all cases, diameter measurements of control wires were within 2% of known diameter. For diameter measurements, each individual root was measured and averaged within a sample prior to data analysis.

After digital images were taken, all roots from a given order in each sample were pooled for subsequent extractions and chemical measurements $\delta^{13}C$ and N concentrations, in order to obtain sufficient mass for chemical analysis. Roots were oven-dried at 65°C for at least 48 hours, and ground to a fine powder for ^{13}C and N concentration analysis of the bulk root tissue. An

aliquot of each root tissue sample was analyzed for ^{13}C and [N] at the University of Illinois at Chicago (UIC) stable isotope laboratory, using a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA) coupled to a Finnigan Deltaplus XL isotope ratio mass spectrometer (IRMS, Thermo Finnigan). The $\delta^{13}C$ values are reported relative to the standard VPDB following the equation:

$$\delta^{13}C_{sample} = \left[\frac{\frac{^{13}C}{^{12}C}sample}{\frac{^{13}C}{^{12}C}std} - 1\right] * 1000 \qquad \text{Eqn 1}$$

4.2.5 Non- structural carbohydrates determination

In order to account for changes in N concentration from changes in non-structural carbohydrates (Drake et al., 1997), the samples were also analyzed for non-structural carbohydrates as physiologically important carbon storage compounds. Due to limited mass, attempts to extract starch were unsuccessful, and only sugar concentrations are reported.

In the root samples used for morphological assessment, the determination of total non-structural carbohydrates concentration were obtained after the addition of glucose, fructose, sucrose and sorbitol concentrations. For NSC extraction, 5-15 mg of sample was added to 1 mL of distilled water, vortexed and incubated for 10 min at 65 ˚C and centrifuged for 6 min at 12000 g. Pipette extracted supernatant was stored on ice water extraction repeated. For some samples a dilution with bi-distilled water (1:5 or 1:10) was used prior to high/pressure liquid chromatography pulse amperometric detection (HPLC-PAD) on a ion chromatography system (Dionex ICS 3000) equipped with an autosampler (Raessler *et al.*, 2010). An aliquot (200 µl) of the sugar extracts was pipetted twice into tin cups and oven-dried at 45°C over night. Extracts $\delta^{13}C$ were measured with a Finnigan MAT DeltaPlus XL EA-IRMS (ThermoFinnigan GmbH, Bremen, Germany) at

the Max Planck Institute for Biogeochemistry, Germany.

4.2.6 Determination of C age in fine roots

In order to distinguish between amounts of C fixed during fumigation and C fixed after fumigation ceased (i.e. post-treatment C, normal air), a two-end-member mixing model was applied (Matamala *et al*., 2003; Taneva *et al*., 2006). For each root order, the post-treatment C end-member was determined by the average value obtained from the roots from the ambient [CO_2] rings that never received fumigation. The treatment C end-member for each root branching order was not directly measured, as no roots dissected by branching order during the time of fumigation were available. The isotopic composition of fine roots separated by diameter class was measured in previous study (Lynch et al., 2013). Using this data and the fractionation measured in the ambient [CO_2] rings, treatment C end-members were calculated and a small depletion in higher branching orders was applied.

The amount of istopically-depleted treatment C in a given sample (F_t) in % can then be calculated from

$$F_t(\%) = \left[\frac{\delta^{13}C_{sample} - \delta^{13}C_{post-treatment}}{\delta^{13}C_{treatment} - \delta^{13}C_{post-treatment}}\right] * 100 \qquad \text{Eqn 2}$$

where $\delta^{13}C_{sample}$ is the δ^{13}C of the harvested roots (or the root-respired CO_2), $\delta^{13}C_{post\text{-}treatment}$ is the δ^{13}C of C incorporated after fumigation ceased and $\delta^{13}C_{treatment}$ is the δ^{13}C of C incorporated during fumigation with isotopically-depleted, elevated [CO_2].

4.2.7 Statistical Analysis

All statistical analyses were performed with *R* statistical analysis software, version 2.15.1 (R Development Core Team, 2012). We used the nonlinear least squares procedure to fit a power

function to the relationship between root N concentration and root diameter. Analysis of variance (ANOVA) models to examine differences in C and N concentrations, diameter and amount of post-treatment C between root orders. Tukey's HSD (Honestly significant difference) tests were performed on ANOVA models for post-hoc comparisons of ANOVA models. Linear models were utilized to explore the relationship between morphological and chemical parameters and the amount of post-treatment C in roots. Model fits were compared through stepwise regression and the Akaike Information Criteria (AIC). Differences were considered significant at $P < 0.05$.

4.3 Results

Almost all of roots in the 1st through 5th orders are less than 2 mm in diameter, falling into a common definition of fine roots (Fig 4.1). Root diameter significantly changed within the lowest five branching orders ($P < 0.05$, Table 4.2), but did not differ between the previously elevated [CO_2] and control treatments ($P > 0.05$, data not shown). Root diameter for the two lowest branching orders did not differ, but there was a significant increase in diameter with every additional increase in root branching order after that (Fig 4.1).

As shown in Fig. 4.2, higher branching orders demonstrate a small but significant depletion in the ambient rings never receiving CO_2 fumigation. This difference was applied in the post-treatment C end-members in our mixing model. However, the end-members for the treatment C likely differ in a similar manner. As no roots were available for measurement of the treatment end-member, we used the value for all roots < 1 mm diameter of -40.5 ‰ from Lynch et al (2013). As the roots < 1 mm diameter represent the lowest 4 branching orders, most of the biomass will be orders 2 -4, which is ~-0.6 ‰ depleted with respect to order 1. Consequently, for our mixing model, we applied a 0.6 ‰ enrichment to the order 1 end-member, and used the -40.5 ‰ value for all subsequent branching orders.

Root non-structural carbohydrate concentration ranged from 8.81 ug/mg in order 1 roots to 24.5 ug/mg in order 5 roots (Table 4.1). There were no significant differences in concentration between roots from the elevated and ambient [CO2] treatment (data not shown). Carbon isotope composition results were most enriched in order 1 roots (-29.8 ‰) becoming more depleted with increasing root order to -35.6 ‰ in order 5 roots (Table 4.1).

Root [C] did not differ between branching orders or treatment types (data not shown, $P > 0.05$), averaging 44.6 ± 0.16 % (mean ± SE), similar to previous results from the site (Iversen et al., 2008). This value for fine-root [C] falls within the expected range for root tissue, providing evidence that the analyzed roots were free of soil contamination (Ouimette et al., 2012). As has been found previously (Guo et al., 2004), root N concentration was highest in lower order roots (Fig. 4.3), and significantly changed within the five lowest orders (Table 4.3, $P < 0.05$). In contrast to diameter, root N concentration did significantly decrease between branching order 1 and 2 (Fig 4.3). Additionally, the highest branching orders quantified, orders 4 and 5, did not differ in root N concentration.

A clear relationship between root diameter and root N concentration was found (Fig. 4.4), with N concentration decreasing with increasing diameter following a negative power function ($R^2 = 0.48$, $P < 0.001$). The relationship did not differ between the previously elevated [CO_2] and control treatments ($P > 0.05$) and had similar parameters to previous results at this site where roots were separated by diameter class as opposed to root branching order (Iversen et al., 2008).

The amount of old carbon remaining in fine roots did significantly change between root orders (Fig. 4.5, Table 4.4, $P < 0.05$). A significant difference was found in the amount of older carbon remaining between order 1 and order 2 roots, with no difference in roots of orders 3 through 5. The median amount of old carbon remaining in the most distal order 1 roots was 10%,

indicating that some of even the smallest roots survive for multiple years, though in many of the samples all of the carbon from order 1 roots was fixed after fumigation cessation. Large variation was found in the amount of old carbon remaining in all root branching orders.

In order to investigate differences within samples, and potentially remove the large variation found between samples with respect to the age of carbon (Fig. 4.5), values were normalized to the 1st most distal branching order. Root diameter, root N concentration, and carbon age, as inferred by the two end-member mixing model, were compared between the different branching orders (Table 4.5 –4. 7). For each of these parameters, the higher branching orders were normalized to the most distal 1st order roots, in order to determine the relationships between the branching orders (Fig. 4.6). General trends were towards increasing amounts of old C and diameter and decreasing root N concentration with increasing branching order. However, all 5 branching orders differed with respect to root N concentration, but orders 1 and 2 did not differ with respect to diameter.

Measured parameters root branching order, root diameter and root N concentration were used to explore their impact on fine-root lifespan, as inferred by the amount of 'old' fumigation C remaining in fine roots. Models based on lowest AIC scores included only root N concentration or root N with root branching order, but not diameter with an R^2 of ~0.4 (Fig. 4.7, Table 4.8). This result held for both for the entire data-set and when examining sampling periods individually. In a model with root branching order and root N concentration as explanatory variables, a hierarchical partitioning approach assigns approximately 50% of the variation in amount of old C remaining to root N concentration and to branching order respectively. Thus, root branching order does potentially explain a significant portion of the variation in carbon residence time. However, the model including branching order and root N concentration explains

just 1 % more of the variation than a model with N concentration alone.

4.4 Discussion

Here, we present the first measurements of mean carbon residence time in fine roots dissected by root branching order following a long-term labeling experiment. In *L. styraciflua*, the traditional classification of fine roots (< 2 mm diameter) is comprised of 5 or sometimes 6 branching orders, with considerable heterogeneity in morphology, chemistry and potentially function. Similar to previous studies, with increasing root order, fine roots increase in diameter and decrease in N concentration. A negative power-law function describes the relationship between root diameter and root N concentration, with model parameters closely matching previous study at this site with root dissection into small diameter classes (Iversen et al., 2008). The carbon isotope composition of roots sampled two and three years following the cessation of CO_2 fumigation provides a proxy for the age of the carbon in individual root orders. We found considerable variation in mean carbon age between samples (Fig. 4.5), which indicates spatial heterogeneity in root longevity at the tree to landscape scale. However, when examining the difference in carbon age between root orders within a sample (Fig. 4.6a), carbon residence times increase with each increase in root branching order. Together, these results imply that while some roots may die in clusters of lower order roots, or ephemeral root modules (Xia et al., 2010), other factors also influence production and mortality of individual lower order roots. Finally, a multi-variate analysis demonstrates that of the parameters measured here, root N concentration provides an easily measurable proxy for root longevity.

4.4.1 Heterogeneity in fine-root lifespan

In a majority of modeling schemes at landscape to global scales, fine root C is stored in a single pool with uniform turnover (Fisher et al., 2010, Gaudinski et al., 2010). The framework of

a single C pool has been questioned by both theoretical approaches (Luo, 2003, Guo et al., 2008), and empirical evidence using isotopic tracers (Trueman & Gonzalez-Meler, 2005; Riley et al., 2009; Gaudinski et al., 2010; Keel et al., 2012). In fact, using a two-pool approach to root turnover, soil organic C and soil organic N accrual can be validated (Lynch et al., 2013). Despite the increasing evidence that not all fine roots have the same lifespan, it remains unclear which proportion of fine-root biomass has a short lifespan versus a long lifespan. Additionally, precise measurements of lifespan for both short-lived roots and long-lived roots are lacking. If below-ground carbon allocation and fine-root proportion to NPP are to be accurately quantified, then relative pool sizes and turnover rates need better constrained.

Published two-pool models using isotope tracers at ecosystem scale have placed 10 (Lynch et al., 2013) to 20 % (Gaudinski et al., 2010) of total fine-root biomass into a 'fast' turnover pool. While potentially useful for modeling schemes, these approaches lack a functional approach to understanding longevity of individual roots. An alternative approach examining longevity of individual roots using rhizotrons concluded that lower order (1 – 3) roots rapidly die in clusters, termed 'ephemeral root modules', while higher order (4 – 5) roots live much longer, termed perennial roots (Xia et al., 2010). Here, we quantified persistence of carbon in roots of differeng branching orders using a long-term isotope tracer, and find that carbon residence times are lower for lower order, more distal roots relative to higher order roots (Fig. 4.5). In our data-set, large differences were found between samples for all root branching orders (Fig. 4.5). This large variability potentially indicates spatial heterogeneity in root lifespan, as roots were sampled from multiple soil blocks and not necessarily from the same tree. Previous short-term isotope dilution studies also find large spatial variation (Fahey et al., 2012).

However, when comparing branching orders within a sample by normalizing to the

lowest order roots, we find that with each increase in root branching order, less ‘new’ carbon has been incorporated into the root population (Fig. 4.6a). Thus, we do not find evidence that lower order roots die in clusters. Instead, it appears that other factors control mortality of some of the finest order 1 and order 2 roots, though certainly mortality of a higher order root would result in mortality of all lower order roots on that branch. Order 4 and 5 roots sampled in 2013 do maintain on average more than 60 % carbon that is over 3 years old, supporting the idea that higher order orders are longer lived. Our data generally support previous findings of heterogeneity in the longevity of fine-roots, though root branching order alone does not provide enough information to separate roots into short- and long-lived pools.

4.4.2 Identifying specific root traits that relate to lifespan

If root branching order alone cannot explain the heterogeneity in fine-root lifespan, then perhaps other root traits can be useful as proxies for lifespan. As the measurement of root longevity remains methodologically challenging, identification of proxies for root lifespan would greatly assist comparisons of root longevity between species and ecosystems and along environmental gradients. Yet to date, linking plant traits to lifespan of roots has received little attention, particularly when compared to work linking traits to leaf lifespan, particularly the development of the leaf economics spectrum (Wright et al., 2004). Much of the work linking specific traits to root longevity has been done in containers or did not directly involve root observations (McCormack et al., 2012). A common garden experiment performed analysis of multiple root traits in 12 species and found that species differences in root longevity is best explained by plant growth rate, root diameter and potentially root N:C ratio, at least for or first- and second-order roots (McCormack et al., 2012).

However, data examining traits to root longevity within the branching system of

individual species are lacking. We applied stepwise regression to determine traits that can explain residence time of carbon in roots (as inferred by the amount of fumigation carbon remaining in fine roots). Specifically, root branching order, root diameter, and root N concentration were included as potential explanatory variables. In *L. styraciflua*, root carbon age is explained by root N concentration and root branching order (Table 4.8) though a model with just root N explains almost as much of the variation (Fig. 4.7). In this species, root diameter does not significantly explain the variation in carbon residence time, likely because of the significant difference between first- and second-order roots in old C remaining, but no difference in diameter (Fig. 4.6a, c). Future studies that quantify N concentration and root branching order will provide increased quantification on the distribution of fine-root biomass between slow and fast turnover pools. Root diameter does provide an analytically easier and cheaper method, which can potentially be linked to N concentration (Fig. 4.4), and potentially serve as a proxy for root longevity.

4.5 Conclusions

To our knowledge, this is the first report utilizing a root branching order approach to examine mean carbon residence time in a long-term labeling experiment. We found considerable variation in carbon residence time between samples for all branching orders. This considerable variation indicates heterogeneity at the landscape scale in root production and mortality. However, analysis within each sample indicates an increase in carbon residence time with increasing branching order. Roots of higher branching order hold a higher probability of a longer residence time for carbon and thus a longer lifespan. Our data is in agreement with several recent studies finding heterogeneity in lifespan within roots < 2 mm diameter, one commonly used

definition of fine roots. Finally, of the parameters measured here, root N concentration provides a relationship with root carbon residence time, and is potentially useful as a proxy for root longevity. A likely species-specific relationship between diameter and root N concentration, once established, may provide a relatively simple parameter for assessment of root longevity in future studies.

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Table 4.1 Non-structural carbohydrates concentration (includes all sugars only), isotopic composition and the number of samples obtained for each root branching order. Data shown for the elevated rings only.

	Root branching order				
	1	2	3	4	5
Non-structural carbohydrate concentration (ug/mg)	8.81 (0.99)	15.71 (2.10)	20.02 (3.11)	23.77 (4.31)	24.5 (5.01)
isotopic composition (‰)	-29.8 (0.9)	-32.5 (2.6)	-33.2 (2.6)	-34.5 (2.8)	-35.6 (3.2)
number of samples	3	5	6	6	6

Table 4.2: ANOVA results for root diameter for each root order. Diameter does differ by root order. Tukeys HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 3.1.

Variation	**Df**	**SS**	**MS**	**F-value**	**Pr(>F)**
Root Order	4	23.541	5.885	112.7	<2e-16 ***
Residuals	219	8.301	0.052		

Table 4.3: ANOVA results for root N concentration for each root order. N concentration does differ by root order. Tukeys HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 4.3.

Variation	**Df**	**SS**	**MS**	**F-value**	**Pr(>F)**
Root Order	4	24.491	6.123	231.6	<2e-16 ***
Residuals	219	5.764	0.026		

Table 4.4: ANOVA results for % Old C remaining for each root order. The amount of old C remaining does differ by root order. Tukeys HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 4.3.

Variation	**Df**	**SS**	**MS**	**F-value**	**Pr(>F)**
Root Order	4	55631	13908	24.81	5.15e-16
Residuals	162	90243	561		

Table 4.5: ANOVA results for differences between order 1 roots and higher order roots in amount of new C incorporated. Tukeys HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 4.6a.

Variation	Df	SS	MS	F-value	Pr(>F)
ΔRoot Order	3	16880	5627	13.69	8.75e-08
Residuals	131	51794	411		

Table 4.6: ANOVA results for differences between order 1 roots and higher order roots in root N concentration. HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 6a.

Variation	Df	SS	MS	F-value	Pr(>F)
ΔRoot Order	3	4.875	1.62	42.36	<2e-16
Residuals	131	6.52	0.0384		

Table 4.7: ANOVA results for differences between order 1 roots and higher order roots in root diameter. HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 4.6a.

Variation	Df	SS	MS	F-value	Pr(>F)
ΔRoot Order	3	19.34	6.448	104.2	<2e-16
Residuals	131	7.67	0.062		

Table 4.8**:** Model information for predicting the amount of old C remaining in fine roots, ranked by lowest to highest AICc score.

Model	ΔAIC	R^2
[N]	0	0.40
[N], order	0.336	0.41
Order	1.81	0.37
Root diameter	16.52	0.22

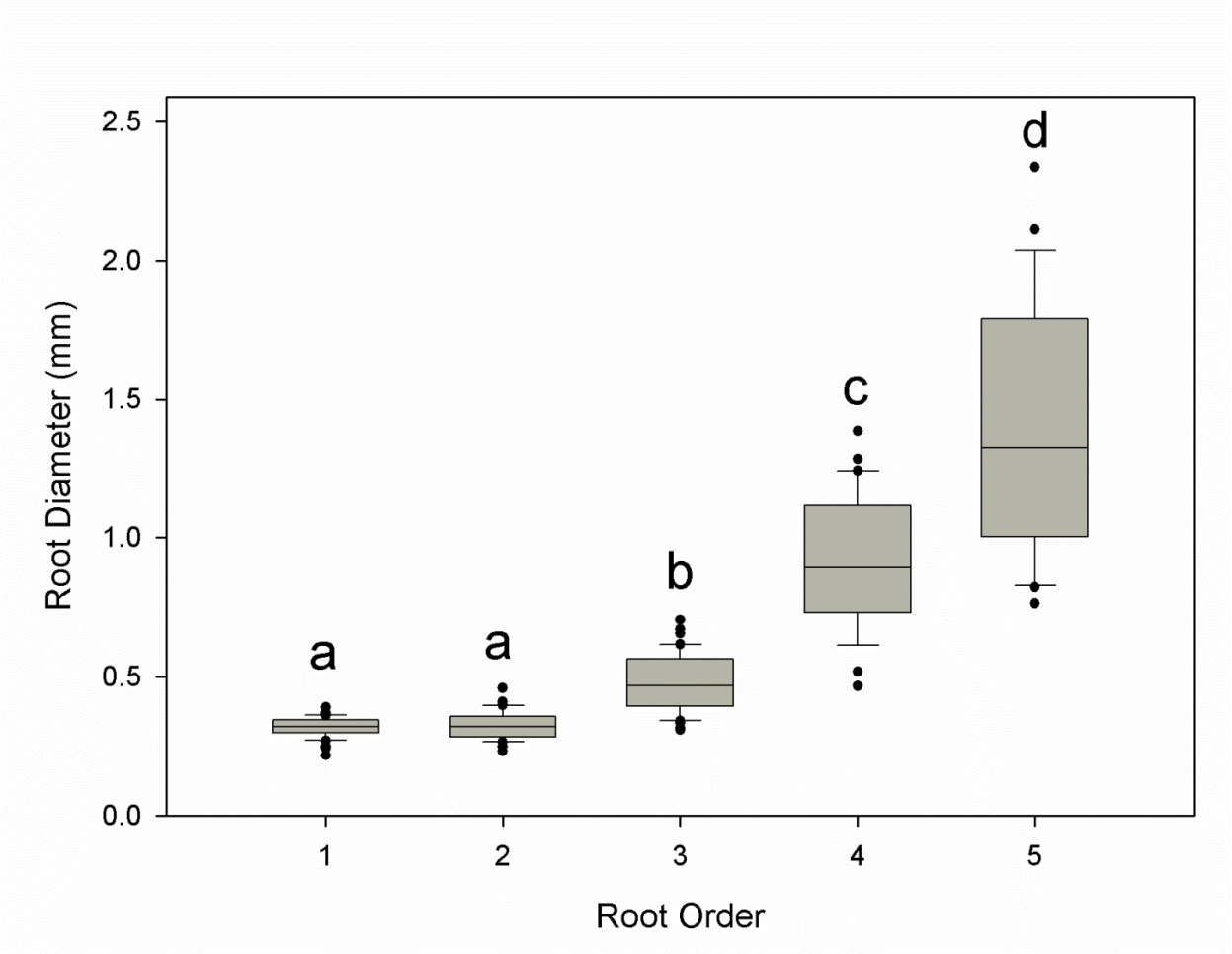

Fig. 4.1 Root diameters for the first five branching orders from L. styraciflua. Data are pooled across years and treatment. Box plot boundary is 25th and 7th percentiles, line within the box indicates the median, and whiskers indicate 10th and 90th percentiles. Lowercase letters that differ indicate significant ($P < 0.05$) differences between branching orders.

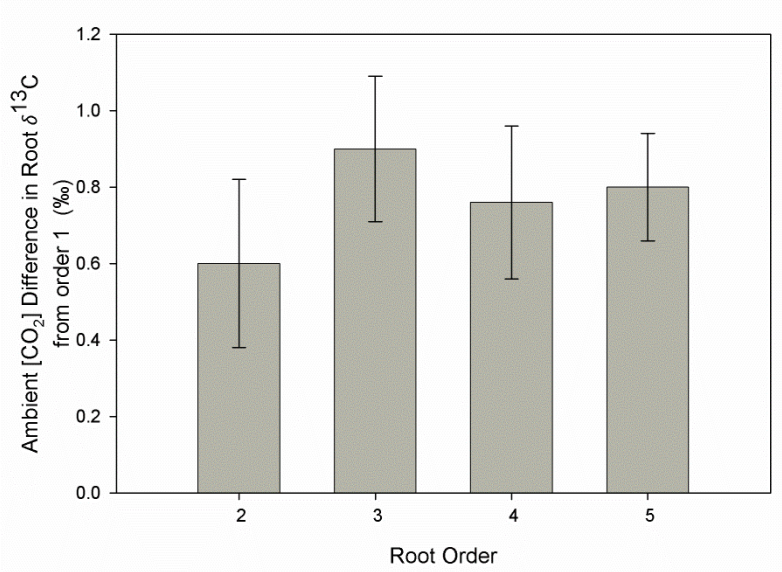

Fig 4.2 For higher root orders (2 – 5), the absolute value of the difference from the 1st branching order in δ13C in the non-treatment rings. In all higher order roots, there is a significant depletion relative to the lowest order 1 root. For order 2, the depletion is ~ -0.6 ‰, and in orders 3, 4, and 5 the depletion is ~ -0.8 ‰.

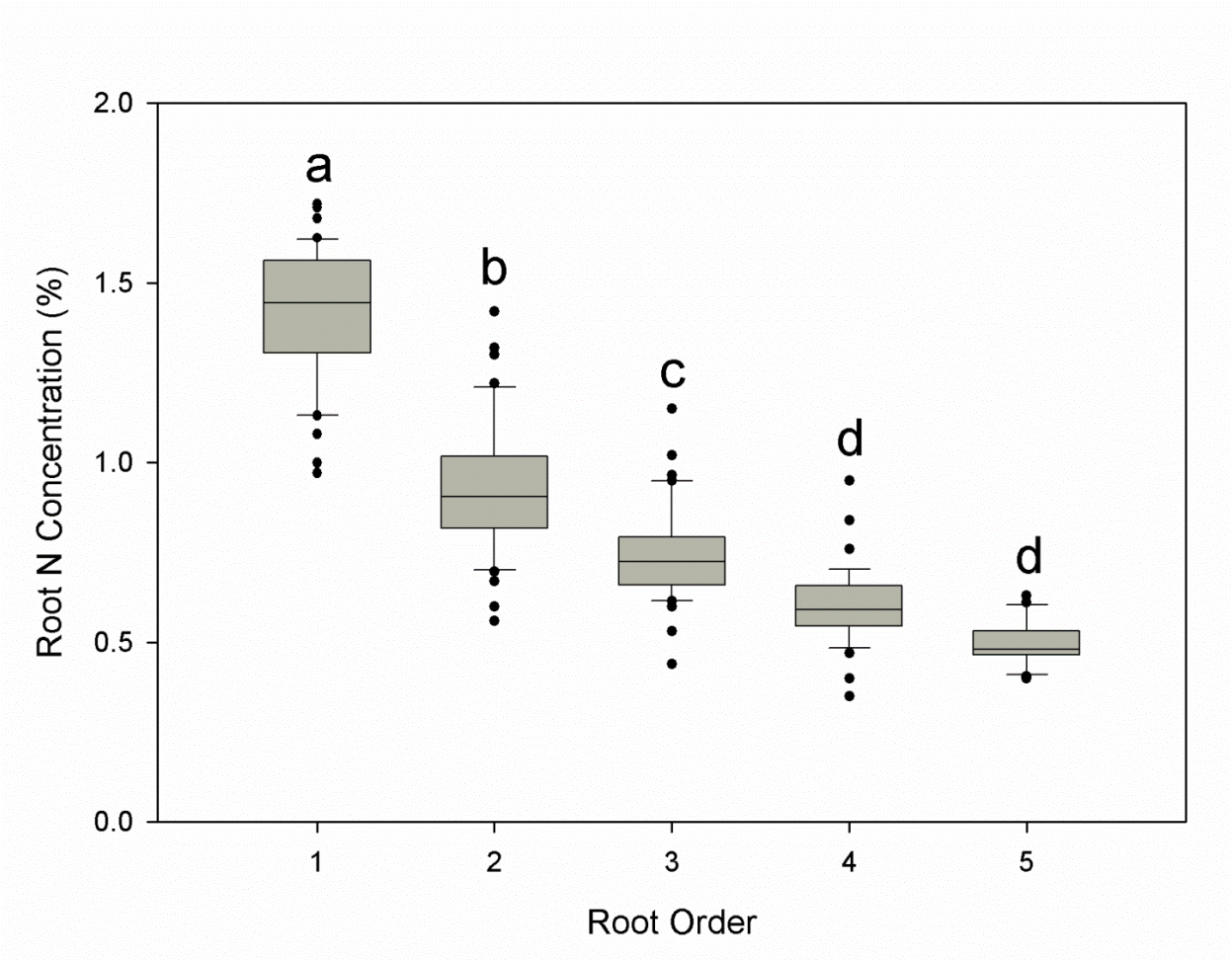

Fig. 4.3 Root N concentrations for the first five branching orders from L. styraciflua. Data are pooled across years and treatment. Box plot boundary is 25th and 7th percentiles, line within the box indicates the median, and whiskers indicate 10th and 90th percentiles. Lowercase letters that differ indicate significant ($P < 0.05$) differences between branching orders.

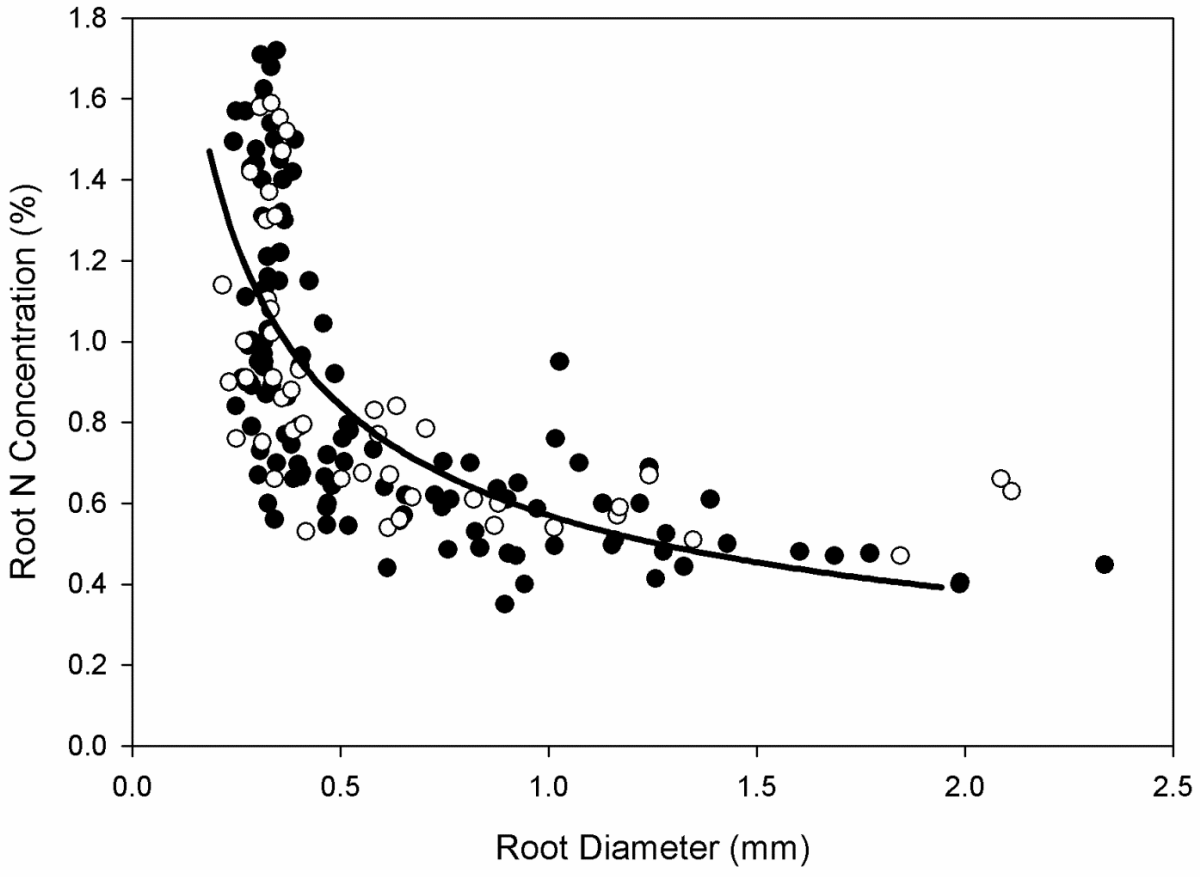

Fig. 4.4 Relationship between root diameter and root nitrogen (N) concentration follows a negative power function that does not differ between control and rings previously receiving [CO2] fumigation. The relationship is [N] = 0.57 * Diameter$^{-0.56}$. Filled circles are from samples that previously received [CO_2] fumigation, open circles are samples from control plots.

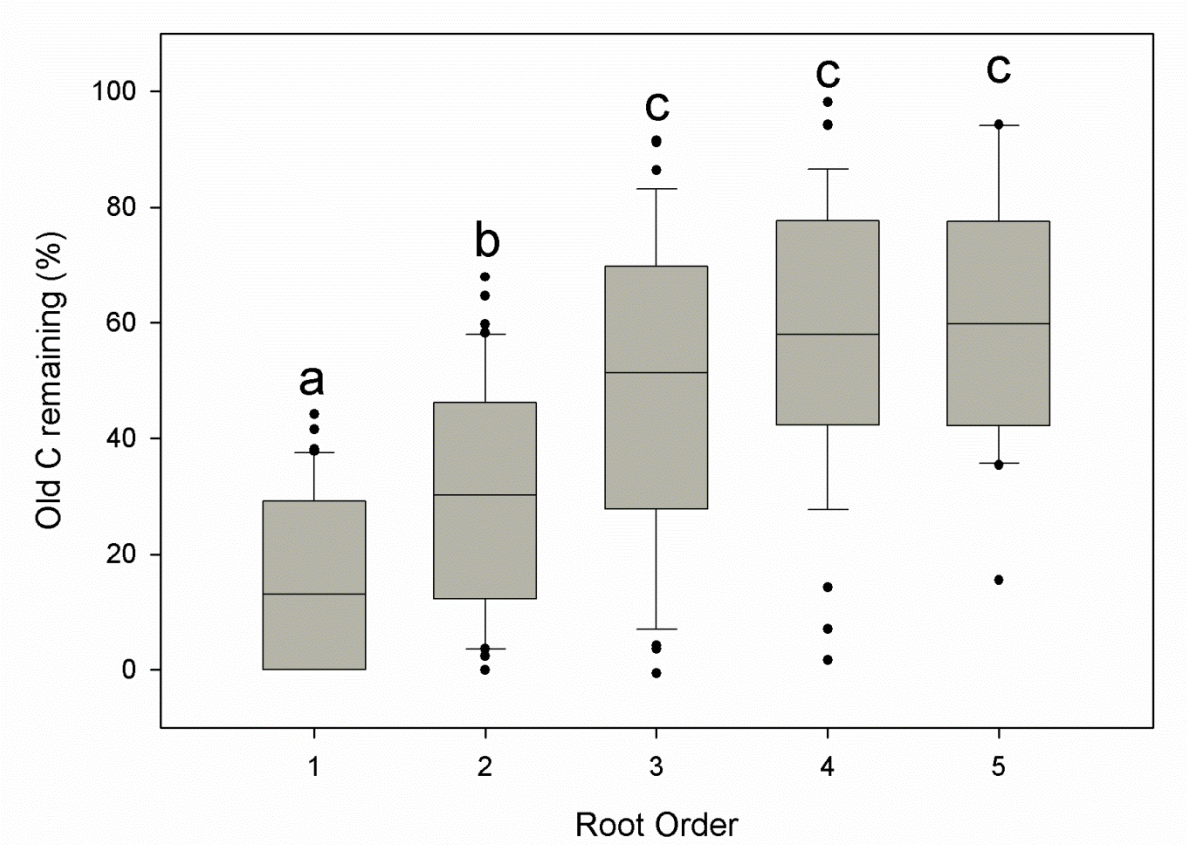

Fig. 4.5 Amount of old C remaining for the first five branching orders from L. styraciflua. Data are pooled for both years. Box plot boundary is 25th and 7th percentiles, line within the box indicates the median, and whiskers indicate 10th and 90th percentiles. Lowercase letters that differ indicate significant ($P < 0.05$) differences between branching orders.

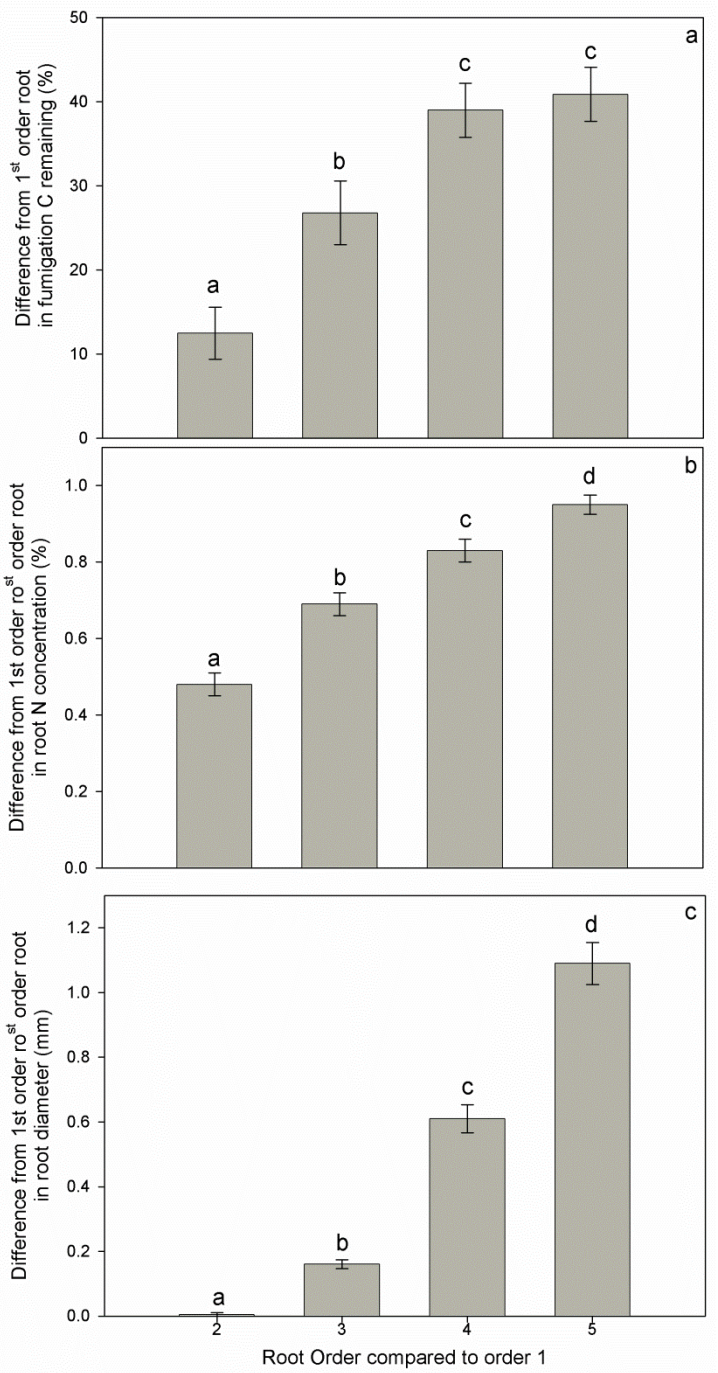

Fig 4.6 For higher root orders (2 – 5), the absolute value of the difference from the 1st branching order in the amount of fumigation C remaining (a), root N concentration (b) and root diameter (c). Data shown is from the rings previously receiving [CO_2] fumigation only. (a) In each of the lowest four branching orders, the amount of old C remaining increases with branching order. (b) Nitrogen concentration significantly decreases with each root branching order. (c) Root diameter does not differ between the lowest 2 branching orders, but increases with subsequent branching orders. In all panels, lowercase letters indicate significant ($P < 0.05$) differences between branching orders.

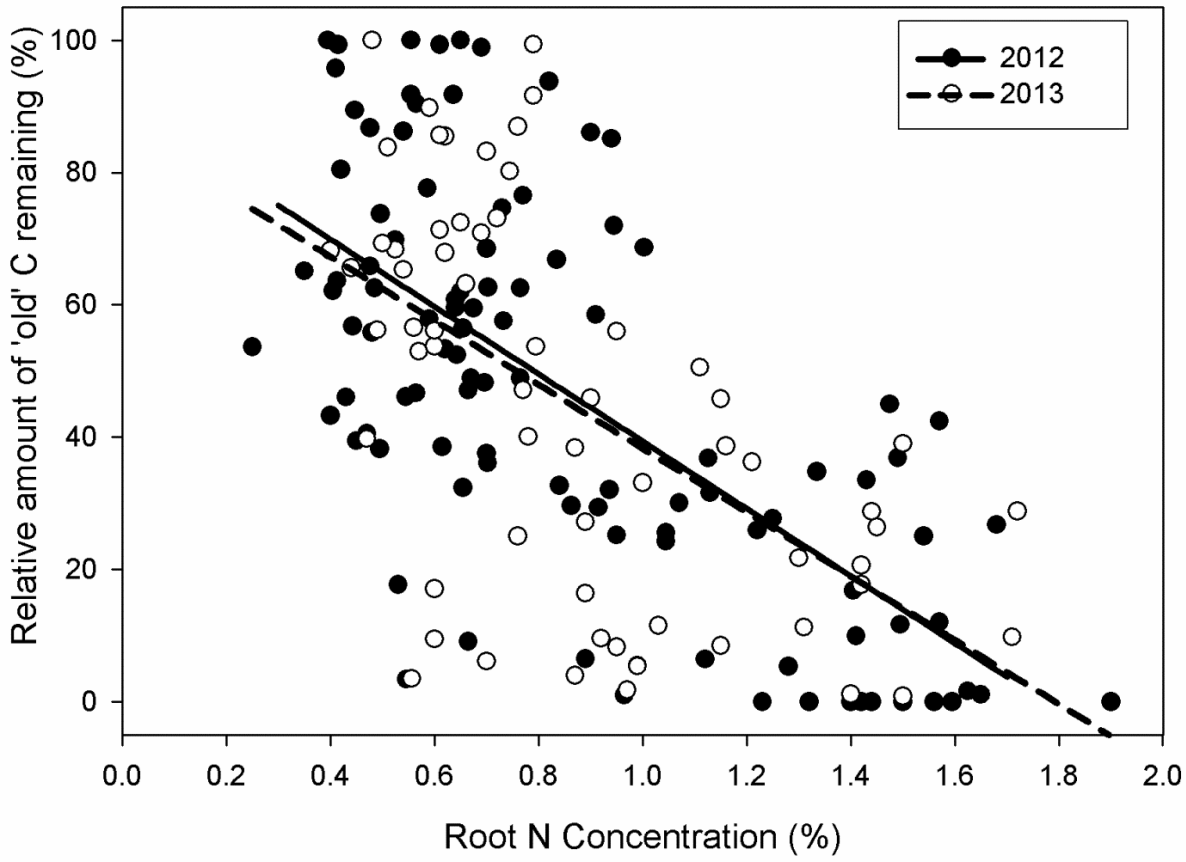

Fig 4.7 The relationship between the amount of old, isotopically-labeled fumigation C remaining in roots and root N concentration for each year of sampling. In both years, N concentration is significantly related to the amount of fumigation C remaining ($P < 0.0001$). In 2012, the amount of old C remaining = -50.9 * [N] + 90.3, $R^2 = 0.44$; in 2013, the amount of old C remaining = -48.3 * [N] + 86.6, $R^2 = 0.31$.

5. Biomass in fine-root branching orders can provide insight into fine-root contribution to soil nutrient cycling

5.1 Introduction

The dynamics of fine roots remain a highly uncertain component of terrestrial ecosystem nutrient cycles. Fine roots have historically been contrasted with coarse roots by function, with fine roots active in water and nutrient uptake (e.g. Vogt et al., 1996). Coarse roots, on the other hand, are not directly involved in nutrient uptake, have undergone secondary thickening, are metabolically less active and have longer lifespan relative to fine roots. Despite these definitions, researchers currently lack a clear way to separate roots into their respective category, i.e. a way to distinguish between fine and coarse roots in a natural setting. Consequently, fine roots have been treated as roots less than an arbitrarily chosen diameter, most commonly < 2 mm or < 5 mm diameter (Vogt et al., 1996, Xia et al., 2010 and refs therein). However, the last decade of research has revealed a host of anatomical, morphological and physiological traits that vary within roots < 2 mm diameter (reviewed in Hishi 2007). These differences question the current paradigm of an arbitrarily chosen diameter for defining fine roots. A more functional approach to studying fine roots may be dissection by root branching order, which allows for researchers to more precisely consider differences within fine roots (Pregitzer at al., 2002). Advantages of the root order approach may include more refined characterization of root anatomy, morphology and physiology, with potential to provide new insights into fine-root mediated nutrient cycling and allow for more appropriate comparison between species and ecosystems.

A key parameter that may benefit from branching order analysis is root turnover rates (i.e. longevity of the fine-root population). A majority of current modeling approaches utilize a single, approximately annual, lifespan for all fine roots (Gaudinski et al., 2010). Empirical

evidence increasingly demonstrates heterogeneity in lifespan within fine roots (Gaudinski et al., 2010; Xia et al., 2010; Lynch et al., 2013). Consequently, for improved understanding of the role of fine roots in terrestrial carbon and nutrient cycling, improved estimates of root longevity within fine roots are necessary. Direct measurement of root longevity remains methodologically challenging, with current methods including sequential minirhizotron images (Xia et al., 2010), and isotopic approaches (Matamala et al., 2003; Gaudinski et al., 2010; Lynch et al., 2013). Because of difficulties in directly measuring root lifespan, attempts to find root traits that can serve as proxies for root longevity may prove more useful. In particular, root N concentration appears to be a promising candidate for a root trait that links to root longevity both between and within species (McCormack et al., 2012, Lynch et al., in prep). Additionally, root diameter can be used as a proxy for root N concentration (Iversen et al., 2008, Lynch et al., in prep) and may provide a relatively cost-effective and easily measureable proxy for longevity. If simple to measure root traits can provide utility for gaining insight into root longevity, these types of measurements can be performed at large spatial scales and in multiple species to improve knowledge of root turnover at plant and ecosystem scales.

The ability to measure simple parameters as proxies for root longevity may be especially important with respect to changing environmental conditions. An individual plant experiencing changes in water and nutrient availability may alter the allocation of resources for the production and maintenance of fine roots. For example, N-fertilization reduced relative biomass of the lowest root branching orders most active in nutrient uptake, in addition to reducing total fine-root biomass (Jia et al., 2010). As significant heterogeneity in many root traits occurs within fine roots, a shift in relative biomass between root branching orders may have consequences for resource allocation to roots at the plant scale, and fine-root mediated carbon and nutrient cycling

at ecosystem scales.

Several studies have examined root traits after dissection by root branching order, but to our knowledge, results have not been summarized. Here we provide the first summary of reports of relative biomass and N concentration (a potentially useful proxy for fine-root longevity) in fine root branching orders. Additionally, we will utilize relative biomass and carbon isotope composition in root branching orders and compare and contrast results to a diameter class study performed at the same site, a long-term FACE study, using an isotope tracer to track carbon replacement in the fine-root population (Lynch et al., 2013). Our objectives are to synthesize the existing literature regarding fine-root biomass allocation by branching order and discuss the value of a root branching order approach to studying fine-root dynamics compared to diameter class separation.

5.2 Materials and Methods

5.2.1 Root biomass by root order collection

Data collection for this study occurred at the Oak Ridge National Laboratory (ORNL) free-air CO_2 enrichment (FACE) experiment, located in a sweetgum (*Liquidambar styraciflua* L.) plantation in eastern Tennessee, USA, described in detail elsewhere (Norby *et al.,* 2001; 2002; 2004). The plantation consisted of one-year old seedlings planted at a spacing of 2.3m x 1.2 m in 1988 (Norby et al., 2004). Thus, sampling of *L. styraciflua* roots occurred in mature c. 25 year old trees. The experiment consisted of four 25-m diameter FACE rings, two of which received CO2 fumigation to c. 550 ppm for 12 years, beginning in 1998 and concluding in September, 2009. A fifth control ring without FACE infrastructure was not utilized in this study.

Fine-root data presented here was collected in conjunction with other work utilizing the

long-term isotope tracer applied during fumigation to investigate carbon turnover rates in fine root branching orders (Lynch et al., in prep). In each of the four FACE rings, two soil blocks of 10 x 20 cm were extracted to 10 cm depth. Paired with each soil block, one intact soil core (5-cm diameter) was also extracted at the same time. Sampling was conducted in late winter 2012 and again in late winter 2013. In 2012, intact root sections were carefully teased out of the soil matrix in the field, while in 2013 soil blocks were frozen intact. In both years, samples were immediately transported to the laboratory on ice, and frozen at -20°C, then shipped to the University of Illinois at Chicago for analysis.

In total, 49 intact root sections were dissected (Lynch et al., in prep). Intact root sections were teased out of the soil matrix, using great caution to avoid breaking off the most distal roots prior to dissection (Pregitzer et al., 2002). During all stages of the root dissection process, roots were kept moist with deionized water at 1°C. Roots were dissected by branching order, following the method in Pregitzer et al (2002). During the dissection of each intact root section, all dissected roots of each branch order were kept moist in 1°C deionized water. Prior to and following dissection, roots were inspected under 12.5x microscope (Leitz) for adhered soil particles, and all particles were washed off of the roots. Consistent values for root C concentrations of root tissue confirmed a minimal amount of soil contamination in samples (Lynch et al., in prep). Upon completion of the dissection of an entire sample, roots were digitally scanned for diameter analysis, then oven-dried at 65°C for a minimum of 48 hours. Following oven-drying, dry mass was quantified for each root order on a digital scale, and roots were then ground to a fine powder for chemical analysis.

5.2.2 Literature review of root biomass by root order

In order to compare the relative biomass of fine roots contained in each of the lowest five

branching orders, a literature review was conducted in an attempt to summarize current knowledge of the proportion of biomass in the five lower branching orders. An initial review was conducted on November 13, 2013 using the ISI web of science tool. The search attempted to find all indexed, published papers that measured fine-root biomass of different branching orders and therefore contained searched a variety strings. Search strings included Topic=("fine root" morphology) OR Topic=("fine root" biomass) OR Topic=("fine root" architecture) OR Topic=("fine root" lifespan) OR Topic=("fine root" order) OR Topic=("fine root" branch order) OR Topic=("fine root" branching order). On November 15, 2013, the search yielded 1527 papers. The same search was conducted again on April 22, 2014, yielding an additional 20 papers. All 1547 papers were investigated for the desired data, with the result that just nine previous studies to date have reported biomass in fine roots by branching order, with a minimum requirement that the 5 lowest branching orders be included (Table 5.1). Five branching orders was selected as a minimum as this constitutes what is typically considered fine roots, or < 2 mm diameter, at least in *L. styraciflua* (Lynch et al., in prep). Further information regarding N concentration of fine roots per branching order was gathered from the nine papers, with six of the nine also reporting N concentration (Table 5.1). Several additional papers that report fine-root biomass by branching order but do not include five branching orders were excluded here.

5.2.3 Comparison of root order and diameter approaches

Information regarding the relative biomass in individual branching orders in conjunction with knowledge of N concentration in each branching order or the turnover rate for each branching order can be used to calculate expected N concentration or turnover rate incorporated in the total fine root population. We utilize the stable isotope tracer employed at the ORNL FACE site to track the amount of 'new' C incorporated after the cessation of CO_2 fumigation (Lynch et al., in

prep), along with relative biomass per branching order to determine amount of 'new' C incorporated into the total fine-root population:

$$Fine-root\ 'new'C = \sum_{O=1}^{n} 'new'C * relative\ biomass \quad \text{Eqn. 1}$$

$$Fine-root\ [N] = \sum_{O=1}^{n} [N] * relative\ biomass \quad \text{Eqn. 2}$$

where O = root branching order, n = the highest order root included in the calculation, Fine-root 'new' C is the % of total carbon replaced, and fine-root N is average N concentration in the fine-root population. With this approach, a comparison to previous study examining fine-root turnover by root diameter classes can be made (Lynch et al., 2013). In that study, roots were separated into roots < 1 mm diameter, and roots greater than 1 but < 2 mm diameter. In our root order dissections, all roots from order 1 -3 and a majority of order 4 roots were < 1 mm diameter (Lynch et al., in prep). Thus, in order to compare carbon residence time and root N concentration between the methods, we compare roots < 1 mm diameter to roots from the lowest 4 branching orders. In both 2012 and 2013, roots were sampled both as intact root sections and from soil cores for traditional diameter class separation, allowing direct comparison between root separation methods. Additionally, the measured amount of 'new' C incorporated can be compared to calculated values from a two-pool exponential decay model fit to data on mean C residence time (Lynch et al., 2013).

5.2.4 Calculations of fine-root contribution to soil organic carbon(SOC) and nitrogen (SON) matter

Data collected from this study was also used to calculate the contribution from fine-roots to soil organic carbon (SOC) accrual and soil organic nitrogen (SON) accrual at the ORNL FACE site. During the elevated CO_2 experiment, SOC accrual in the elevated rings was 48 g C m^{-2} y^{-1} and SON accrual was 2.4 g N m^{-2} y^{-1} (Jastrow, unpublished data?).

Here, we use our data regarding relative biomass in root branching orders and turnover rates per branching order fit by an exponential decay model to estimate fine-root calculation to SOC and SON accrual. Results will be compared to SOC and SON accrual using the two-pool model from Lynch et al., 2013). The 1st step is to calculate the amount of standing biomass per pool (in root branching order calculations there are five pools, and two pools in the approach using root diameter only).

$$Standing\ C(N)\ per\ pool\ (g\ C(N)\ m^{-2}) = Total\ standing\ C(N) * \%\ biomass\ in\ pool$$

Eqn 3

where total standing C or N is taken from the literature and % biomass is the relative amount of total biomass that is in a given pool. Next, annual turnover of C or N per pool is calculated:

$$Annual\ C(N) turnover\ per\ pool\ (g\ C(N)\ m^{-2}) = Total\ standing\ C(N) per\ pool * pool\ turnover\ rate$$

Eqn 4

where total standing C or N per pool is from equation 3 and pool turnover rates are derived as MRT^{-1} from exponential decay models fit to the replacement of carbon in the root pool (see

Lynch et al., 2013 for details). Next, total transfer to the soil by fine roots is calculated from the sum of transfer rates of all pools multiplied by the transfer rate to soils

$$Root\ transfer\ to\ soil\ (g\ C(N)\ m^{-2}) = (\sum_1^n Annual\ turnover\ per\ pool\ ) * C(N) transfer\ rate \quad \text{Eqn 5}$$

where n = 2 in the two-pool model and n = 5 in the root branching order approach. Transfer rates of C or N are taken from the literature and constitute a major source of uncertainty in these calculations. Finally, the percentage of total SOC or SON accrual that can be attributed to fine-roots is derived from

$$Total\ SOC(SON) accrual\ from\ roots\ (\%) = \frac{Root\ transfer\ to\ soil\ (g\ C(N)\ m^{-2})}{Measured\ SOC(SON) accrual} * 100 \quad \text{Eqn 6}$$

where measured SOC and SON accrual are taken from the literature and root contribution is from equation 5.

Table 5.3 contains a summary of inputs required for these calculations and the sources for values used here.

5.2.5 Statistical analysis

All statistical analyses were performed with *R* statistical analysis software, version 2.15.1 (R Development Core Team, 2012). Analysis of variance (ANOVA) models to examine differences in relative biomass, N concentration and amount of post-treatment C between root orders. Tukey's HSD (Honestly significant difference) tests were performed on ANOVA models for post-hoc comparisons of root orders.

5.3 Results

5.3.1 Relative biomass

For measurement of relative biomass in fine-roots from the ORNL FACE site, only intact root samples that included a 6th order root were included in the analysis to ensure that 5th order mass was not under-estimated. Consequently, 16 samples were available for analysis, 12 from the rings previously receiving CO_2 fumigation and 4 from the control rings never receiving fumigation. In *L. styraciflua*, relative biomass per branching order is highest in the 5th order roots, followed by 4th and 1st order roots, respectively. The only statistically significant difference is that 5th order roots maintain more biomass than all other branching orders (Fig. 5.1). There is no difference in relative biomass detected between the rings previously receiving CO_2 fumigation and the control rings (data not shown).

In the literature review keyword search for fine-root biomass by branching order, 54 unique biomass measurements were reported from just seven species (Table 5.1). Within those samples, 1st and 5th order roots maintain statistically significantly more biomass than the other orders (Fig. 5.1). However, within the data-set, several experimental treatments were applied that may impact relative biomass between root branching orders, including N fertilization and insecticide (Table 5.1). For this reason, caution should be employed when interpreting these results, as the sample size is very small (33 replicates with no treatment, 11 receiving nitrogen fertilization, and 8 where insecticide was applied, and 2 were excluded from treatment analysis, 1 where thinning occurred and 1 where treatments were pooled). When analyzed within the experimental treatments, there is no treatment effect on relative biomass by branching order, but there is a significant treatment x root order interaction (Table 5.5). N-fertilization decreased 1st and 5th order biomass, while insecticide application led to increased biomass in lower order (1st

and 2nd) roots, with a concurrent reduction in relative 5th order biomass (Fig. 5.2).

5.3.2 N concentration

N concentration in fine roots at the ORNL FACE site decreased significantly with branching order from a maximum of 1.5% in 1st order roots to ~0.5% in 4th and 5th order roots (Fig. 5.3). Similar to results from relative biomass, no statistical difference between the control rings and those previously receiving CO_2 fumigation was detected (data not shown).

In the literature review, six studies reported N concentration along with relative biomass from four species (Table 5.1). A reduction in N concentration occurs with increased branching order, though no statistical difference is found between the 1st and 2nd order roots (Fig. 5.3). For all branching orders, published values in the literature for N concentration are higher than those measured for *L. styraciflua* in this study. Species-specific differences in N concentration have been found previously in the lowest three branching orders, with values here within previously reported overall ranges (Pregitzer et al., 2002). In some of the studies reporting root branching order N concentration, experimental N-fertilization was applied (Table 5.1). In all root branching orders, N fertilization increases root N concentration (Fig. 5.4), which also may partially account for higher N concentration in the literature compared to results here from *L. styraciflua*.

5.3.3 Comparison of root order and diameter approaches

In both sampling years, the smallest amount of 'new' C incorporated into fine-roots was found in sampled roots < 1 mm diameter (Fig. 5.5). Using eqn 1 to calculate amount of 'new' C in the fine-root population resulted in slightly more 'new' C incorporated into fine-roots. The two-pool exponential decay models, both that calculated from previous sampling (Lynch et al., 2013) and an updated version using the additional sample periods collected for this study, over-estimate the amount of 'new' C incorporated into fine-roots in both sampling years (Fig. 5.1).

For N concentration in fine-roots, roots < 1 mm diameter are compared to a calculated N concentration in the fine-root pool using equation 2. In both sampling years, mean N concentration determined from the root order calculation is higher than in that from diameter sampling (Fig. 5.6). Similar to the 'new' C calculations, only the mean N concentration for root order was applied in eqn. 2, thus no error is associated with this calculation. Reduced N concentration in the diameter sampling is potentially a reflection of the loss of a portion of the finest roots during sample preparation (Luo 2003).

5.3.4 Plasticity in relative biomass by root branching order

As both N concentration and C mean residence time of fine-roots changes between branching orders (Lynch et al., in prep), a shift in relative biomass between branching orders may have large consequences for fine-root contribution to C and N cycling. In order to examine the potential impacts of a shift in relative biomass between the lowest branching orders, we applied equations 1 and 2 to a range of values derived from our data and the literature (Table 5.2). The maximum amount of 'new' C incorporated and highest N concentration occurred using the maximum order 1 biomass derived from the literature (Table 5.2). The minimum amount of 'new' C incorporated and the lowest N concentration occurred when the minimum order 1 biomass derived in the literature is used, also equivalent to the N concentration derived from the diameter method. In the range of biomass values examined, mean root N concentration in the fine-root population ranged from 0.8 to 1.1 %, and the amount of 'new' C incorporated after two growing season, serving as a proxy for mean C residence time, ranged from 50 to 65% (Table 5.2). Thus, the relative biomass occurring in different branching orders may have significant consequences for total N and C cycling mediated by fine roots.

5.3.5 Calculations of fine-root contribution to soil organic carbon(SOC) and nitrogen (SON) matter

Values used from the literature for these calculations can be found in Table 5.3. Using the values presented in that table for maximum SOC accrual and data collected here for root biomass and turnover rates, fine-roots account for 75 % or 24% of total SOC accrual using the two-pool approach or root branching order approach respectively. For SON accrual using the same inputs but assuming transfer rate of 1 (i.e. all N in roots remains in soils), fine-roots account for 76% or 24% of total SON accrual using the two-pool approach or root branching order approach respectively.

5.4 Discussion

We measured fine-root biomass in each of the lowest five branching orders from intact root sections of *L. styraciflua*. Roots were sampled two and three years following the cessation of CO_2 fumigation at a long-term FACE experiment, where a unique isotope tracer was applied. Relative biomass per root order, in conjunction with the isotope composition of roots as a measurement of carbon residence time in fine roots (Lynch et al., in prep), was used to calculate mean carbon residence time in the fine-root population. Additionally, results were compared to sampling of fine roots by diameter class, sampled at the same time as intact root sections. In both sampling years dissection of roots by root branching order had a larger percentage of 'new' carbon, compared to the diameter method, but the differences were quite small. A literature search for data on relative biomass and N concentration in the five most distal branching orders reveal a very small number of reported results. Within the most distal branching orders, the most relative biomass is in the largest 5^{th} order, followed by the most distal 1^{st} order roots. Absolute N concentration varies between species and experimental design, but consistently decreases with

increasing root branching order.

Based on our literature search, just nine other studies to date have reported either absolute or relative biomass in root branching orders for each of the lowest 5 branching orders (Table 5.1). Several of those nine studies were conducted on the same species, and relative biomass data is available for a total of seven species, and almost exclusively from temperate forest species. While caution should be used in making and conclusions from such a small sample size, generally a significant fraction of fine-root biomass (greater than 50%) is in the largest 4^{th} and 5^{th} order roots (Fig. 5.1). Several of the data-sets reporting relative biomass involved manipulative experiments that may alter proportion of fine-root biomass between branching orders. Fertilization with nitrogen decreases the lowest 1^{st} order biomass and increases 5^{th} order biomass (Fig. 5.4). Insecticide application had the opposite effect, resulting in increased relative biomass in the lowest order roots, with a decrease in 5^{th} order biomass (Fig. 5.4). Even with the limited data available, it is clear that environmental factors influence the proportion of biomass in different root branching orders.

If relative biomass apportioned within fine roots does change significantly, there may be profound influence on fine-root mediated nutrient cycling. Recent evidence indicates significant heterogeneity in turnover of fine roots (Gaudinski et al., 2010, Lynch et al., 2013), with potentially a distinction between ‘ephemeral’ lower order roots and ‘perennial’ higher order roots (Xia et al., 2010), although there may be an increase in longevity for each branching order (Lynch et al., in prep). Consequently, a shift in biomass between the branching orders would also shift the total amount of C, N and other nutrients involved in supporting fine roots. In fact, analysis over a range of relative biomass values demonstates significant influence on carbon

turnover and N concentration in the fine-root population (Table 5.2). Based on measured root N concentrations (Fig. 5.5), a shift in relative biomass between branching alters N concentration from 0.8 to 1.1 % (Table 5.2). A shift in root biomass towards lower branching orders results in higher N concentrations and decreased root longevity. Thus, measurements of fine-root biomass by branching order should be a research priority as it may provide additional insight into fine-root contribution to NPP, and nutrient cycling.

Historically, fine root dynamics have been measured on all roots less than an arbitrary diameter. A previous study of *L. styraciflua* used this approach to extensively sample fine roots immediately following the cessation of CO_2 fumigation (Lynch et al., 2013). Lynch et al. (2013) found that a two-pool exponential decay model fit carbon turnover in roots better than a single-pool model, with 10% of the total biomass rapidly being turned over (months) and 90% more slowly (years). However, it was not possible to determine longevity for individual roots. Dissection by root branching order may help separate characteristics of individual roots that can identify which roots belong into the short- and long-lived pools. In both sampling years of the present study, the amount of 'new' carbon incorporated into fine roots and N concentration based on diameter class was lower than that measured by root order. Together, these results are likely a result of a bias against the smallest roots when sampling by diameter class, a concern when using stable isotope tracers (Luo 2003). Results for 'new' C incorporated into different branch orders were also compared with predicted results from the two-pool exponential decay model (Lynch et al., 2013), using both the model produced in that paper, and newly calculated model parameters with the additional sampling points measured here (Fig. 5.5, model parameters in Table 5.10). The two-pool models do over-estimate incorporation of 'new' carbon compared to measurements using either a diameter class or root order approach. Exponential decay models likely over-

estimate turnover of carbon in the total fine-root population, but inclusion of two carbon pools represents a significant improvement over single pool models (Lynch et al., 2013). The root order approach can provide higher level of accuracy regarding fine-root mediated nutrient cycling as compared to diameter approach, particularly with respect to nitrogen. However, separation of root branching orders did not necessarily improve the estimation of C and N transferred to soil from fine root turnover. It is possible that errors associated to biomass per turnover pool contributed to this result.

5.5 Conclusions

Fine-root biomass by root branching order potentially reveals considerable information regarding nutrient cycling in terrestrial ecosystems. To date, just ten studies (including this one) have reported either absolute or relative fine-root biomass for the five most distal root branching orders. Even with the minimal data available, we reveal potentially significant environmental influence on fine-root biomass allocation to different branching orders. Nitrogen fertilization leads to a decrease in biomass in the finest roots, with a higher amount of biomass in higher order, longer-lived roots. In contrast, insecticide application leads to a larger amount of biomass in lower order roots, likely due to decreased herbivory. Changes in relative biomass between branching orders may influence plant-mediated carbon and nutrient cycling. We demonstrate that knowledge of total fine-root biomass based on an arbitrary diameter classification is not sufficient to understand the fine-root contribution to NPP and terrestrial nutrient cycling. We conclude that measurement of fine-root biomass in branching orders, along with other root traits, such as N concentration and diameter, will allow for improved understanding of the contribution of fine roots to terrestrial carbon and nutrient cycling.

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Table 5.1**.** A list of the references found during the literature review that contained biomass and N concentration information for at least five root branching orders.

Summary of studies reporting relative biomass per branching order for the finest 5 branching orders

Species	Treatment	[N] Reported	Sources[a]
Pinus palustris	Pooled N-fertilization, foliar removal	Y	1
Fraxinus mandshurica			
Larix gmelinii	season, depth	Y[b]	2
Vaccinium corymbosum	none	N	3
Acer rubrum[c]	none	N	4
Fraxinus mandshurica			
Larix gmelinii	season, N-fertilization	Y	5
Fraxinus mandshurica	none	Y	6
Fraxinus mandshurica			
Larix gmelinii	Insecticide	Y	7
Pinus tabuliformis	N-fertilization	N	8
Cunninghamia lanceolata	thinning	Y	9

[a]sources : 1, Guo et al., 2004; 2, Wang et al., 2006; 3, Valenzuela-Estrada et al., 2008; 4, Guadinski et al., 2010; 5, Jia et al., 2010; 6, Xia et al., 2010; 7, Sun et al., 2011; 8, Wang et al., 2013a; 9, Wang et al., 2013b

[b][N] associated with sampling conducted for source 2 is reported in Ouimette et al., 2012

[c]two other species reported, but data from order 2 are absent

Table 5.2. Indication of the amount of change in the calculated value for C residence time with respect to changes in relative biomass per root branching order. Analysis is constrained by comparing roots from the lowest 4 branching orders, or all roots < 1 mm diameter in order to compare to measured values from Lynch et al., 2013 (new C measured at 55% and [N] at 0.8% for all roots < 1 mm diameter.

	Relative biomass per branching order (%)					
condition	1	2	3	4	New C incorporated (%, per 2012 $\delta^{13}C$ measurements)	Root [N] (%)
Measured results (Fig. 5.1)	27.8	18.8	22.7	30.7	57.5	0.9
Match < 1 mm new C %[a]	24	17	17	42	54.3	0.9
Match < 1 mm [N][b]	15	16	17	52	49.9	0.8
Literature review mean values	33.2	20.5	20.4	25.7	60.1	1.0
Literature review max order 1	48.7	17.9	15.4	17.9	65.7	1.1
Literature review min order 1	10.8	17.6	32.4	39.2	50.9	0.8

[a] Relative biomass values were adjusted to obtain the amount of new C incorporated equal to that measured from roots < 1 mm diameter sampled from soil cores

[b] Relative biomass values were adjusted to obtain [N] equal to that measured from roots < 1 mm diameter from soil cores

Table 5.3 Variables and sources for values used for calculation fine-root contribution to SOC and SON accrual. Parameters highlighted in grey are taken from the literature, while other values are from data collected for this study (or Lynch et al., 2013) at the ORNL site. Note that for the parameters collected in this study, branching order data contains 5 pools, while the diameter approach contains two pools.

	SOC accrual				SON accrual		
Parameter	**Value**	**Unit**	**Source**	**Parameter**	**Value**	**Unit**	**Source**
standing root C	80	g C m-2	Iversen et al. 2012	standing root N	2	g N m-2	Iversen et al. 2012
SOC accrual max	44	g C m-2	Jastrow, pers comm	SON accrual max	2.2	g N m-2	Jastrow, pers comm
SOC accrual min	22.2	g C m-2	Jastrow, pers comm	SON accrual min	1.05	g N m-2	Jastrow, pers comm
C transfer rate	0.5		?	N transfer rate	0.5		?
biomass per pool		%	my data	biomass per pool		%	my data
turnover rate per pool		yr^{-1}	fit to my data	turnover rate per pool		yr^{-1}	fit to my data

Table 5.4: ANOVA results for root Relative biomass in the 1st five branching orders from the literature review. Biomass does differ by root order. Tukeys HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 5.1 of the manuscript.

Variation	Df	SS	MS	F-value	Pr(>F)
Root Order	4	9147	2287	46.7	<2e-16 ***
Residuals	265	12977	49		

Table 5.5: ANOVA results for root Relative biomass in the 1st five branching orders from the literature review. Root order as well as a root order x treatment interaction differ by root order. Tukeys HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 5.3.

Variation	Df	SS	MS	F-value	Pr(>F)
Root Order	4	8297	2074.2	47.019	<2e-16 ***
Treatment	2	0	0	0	1
Root Order x Treatment	8	922	2.612	2.612	0.00926
Residuals	245	10808	44.1	44.1	

Table 5.6: ANOVA results for root Relative biomass in the 1st five branching orders from the ORNL L. styraciflua roots. Biomass does differ by root order. Tukeys HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 5.1 of the manuscript.

Variation	Df	SS	MS	F-value	Pr(>F)
Root Order	4	3628	907	11.1	4.02e-07
Residuals	75	6131	81.7		

Table 5.7: ANOVA results for root N concentration in the 1st five branching orders from the literature review. N concentration does differ by root order. Tukeys HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 2 of the manuscript.

Variation	Df	SS	MS	F-value	Pr(>F)
Root Order	4	25.3	6.325	33.54	<2e-16 ***
Residuals	95	17.91	0.189		

Table 5.8: ANOVA results for root N concentration in the 1st five branching orders from the literature review. Root order as well as treatment differ by root order. Tukeys HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 5.4.

Variation	Df	SS	MS	F-value	Pr(>F)
Root Order	4	25.414	6.353	55.346	<2e-16 ***
Treatment	1	1.914	1.914	16.676	0.000104
Root Order x Treatment	4	0.359	0.09	0.782	0.540159
Residuals	80	9.184	0.115	44.1	

Table 5.9: ANOVA results for root N concentration in the 1st five branching orders from the ORNL L. styraciflua roots. N concentration does differ by root order. Tukeys HSD (honestly significant difference) test on the model differences shown by lowercase letters in Fig 5.2 of the manuscript.

Variation	Df	SS	MS	F-value	Pr(>F)
Root Order	4	24.291	6.123	231.6	<2e-16
Residuals	219	5.764	0.026		

Table 5.10: Parameters from the two-pool exponential decay models applied to calculating the amount of 'new' C incorporated in Figure 5.3. The only difference between the model from Lynch et al., 2013 and the updated model is the inclusion of the additional sampling period conducted in 2013 for this study. In each case, roots < 1 mm diameter have been fit to a two-pool exponential decay model $F(t) = a_1e^{-k1t} + a_2e^{-k2t}$ where F(t) is % fumigation C remaining, a is initial amount of fumigation C and k is the decay rate of fumigation C for each respective pool.

	Slow Pool		Fast Pool	
Source	**a_1**	**k_1**	**a_2**	**k_2**
Lynch et al., 2013	0.91	-0.37	0.09	-4.15
Updated model	0.89	-0.33	0.1	-3.87

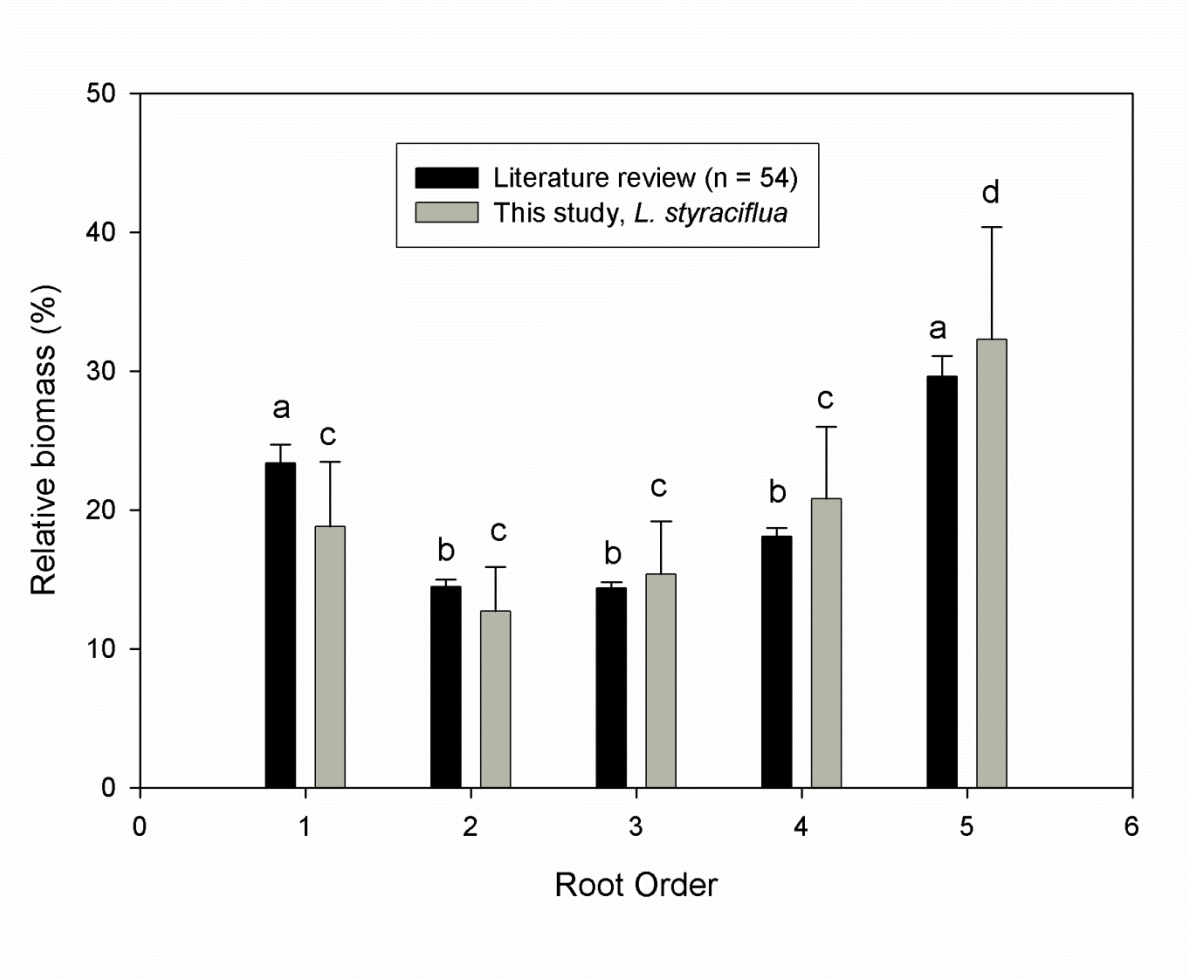

Fig. 5.1 Relative biomass in each of the five lowest branching orders from the literature review and from this study in L. styraciflua (total biomass equals 100% in all cases). For data from this study, data are pooled across years. Data-sets were statistically analyzed separately, and lowercase letters indicate significant ($P < 0.05$) differences between branching orders.

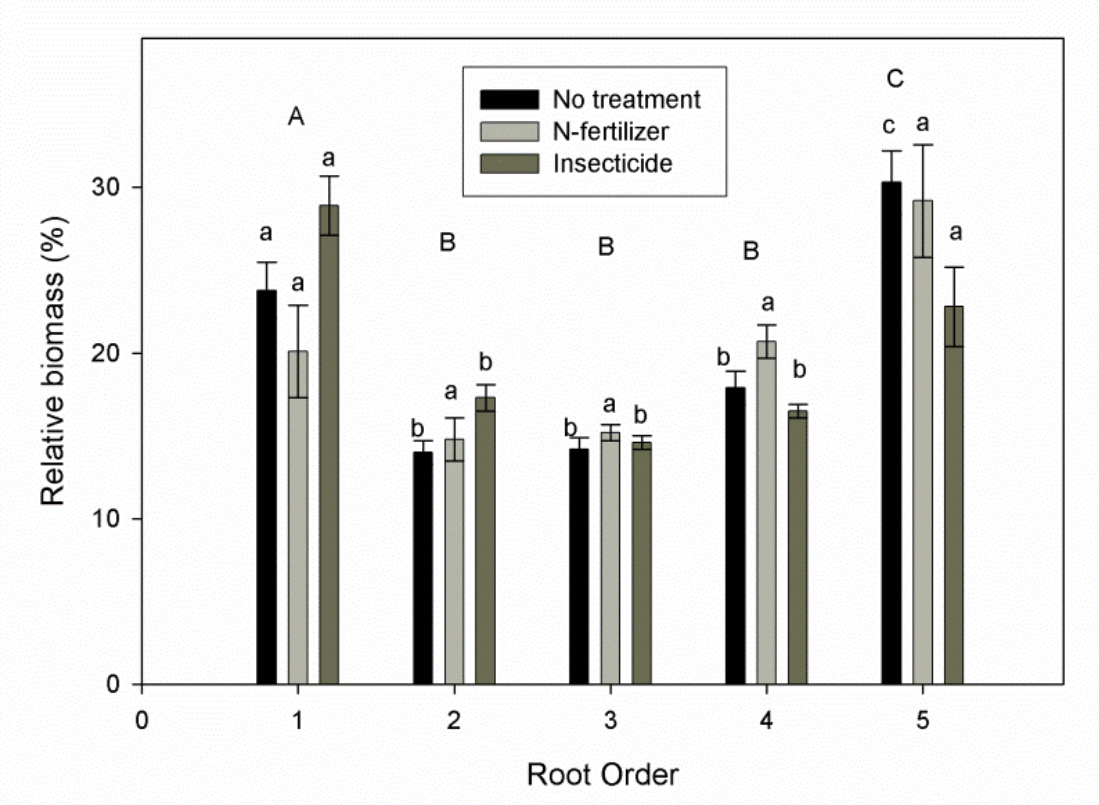

Fig 5.2 Relative biomass in the 1st five branching orders from the literature review. Capital letters indicate significant differences between branching order, lower-case letters indicate significant differences within treatment.

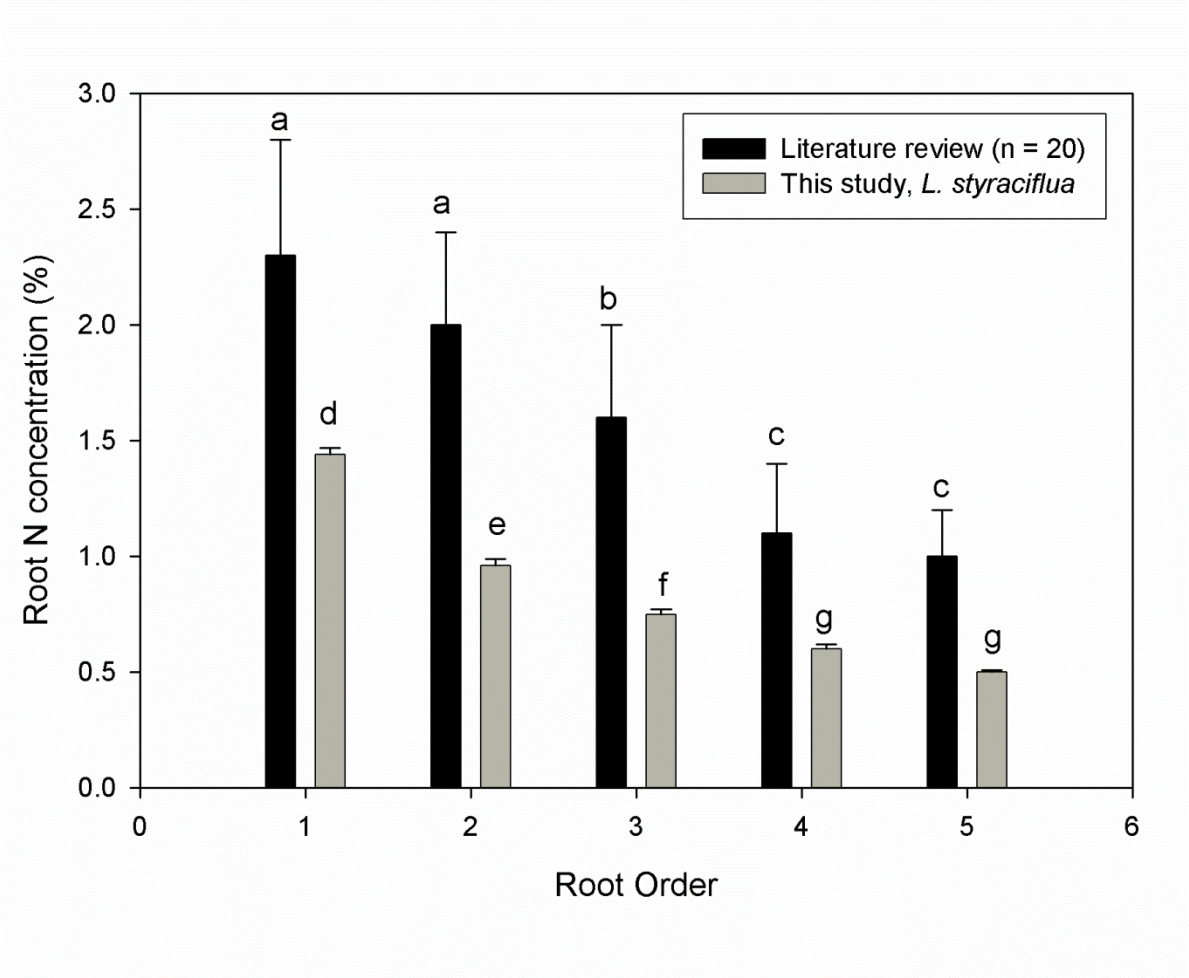

Fig. 5.3 N concentration in each of the five lowest branching orders from the literature review and from this study in L. styraciflua. For data from this study, data are pooled across years. Data-sets were statistically analyzed separately, and lowercase letters indicate significant ($P < 0.05$) differences between branching orders.

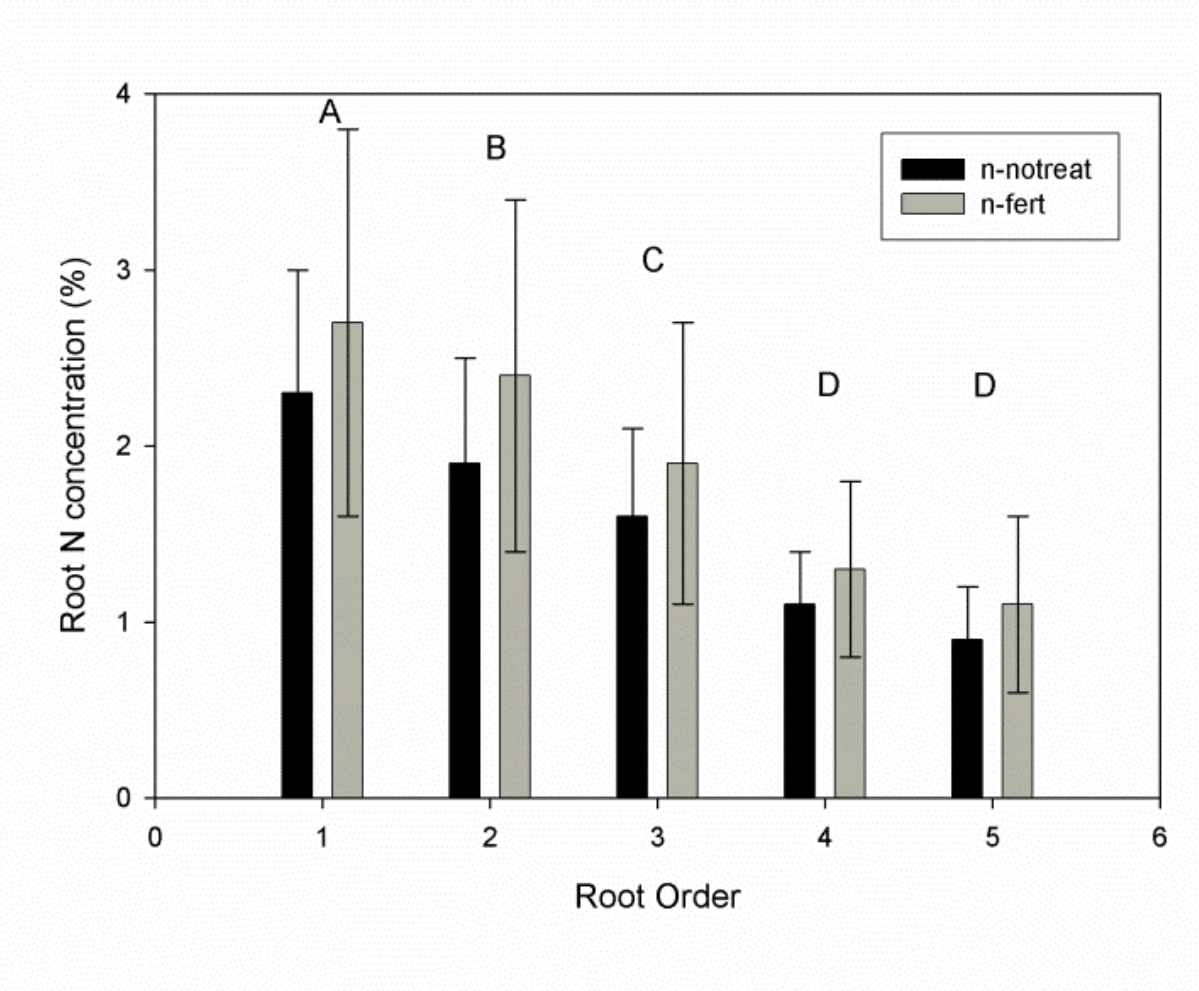

Fig 5.4 Relative N concentration in the 1st five branching orders from the literature review. Capital letters indicate significant differences between branching order for the whole data-set, and for within each treatment. In all cases orders 1 through 3 are not different and 4 and 5 are not different.

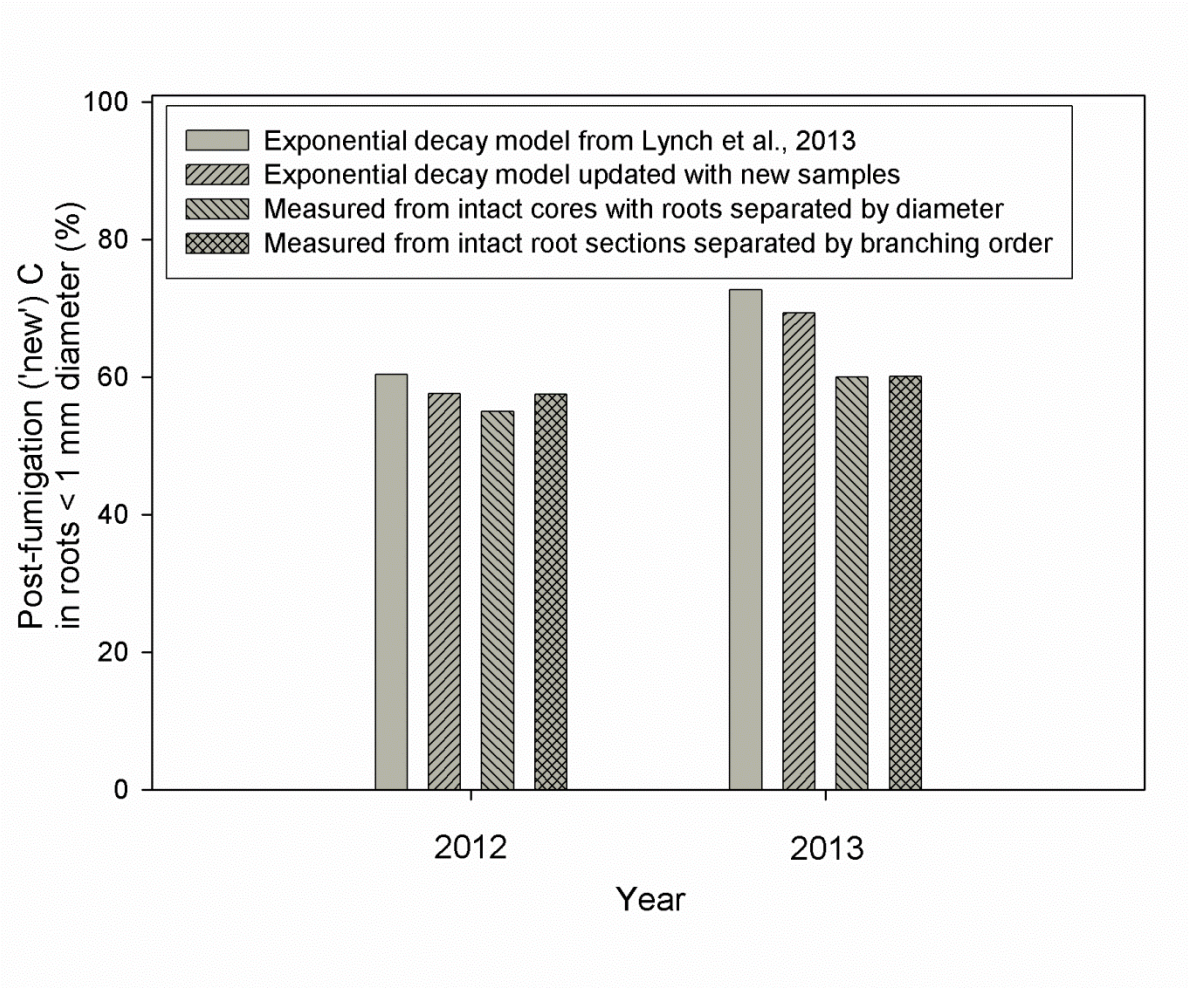

Fig. 5.5 Comparison of multiple methods for determining amount of 'new' C that has been incorporated into fine-roots. Plain grey bars are calculated from two-pool exponential decay model from Lynch et al., 2013. Rising cross-bars are calculated from an updated two-pool exponential decay model. Decreasing bars are measured from 5-cm diameter cores and roots separated by root branching order. Hatched bars are measured from intact root sections separated by root branching order.

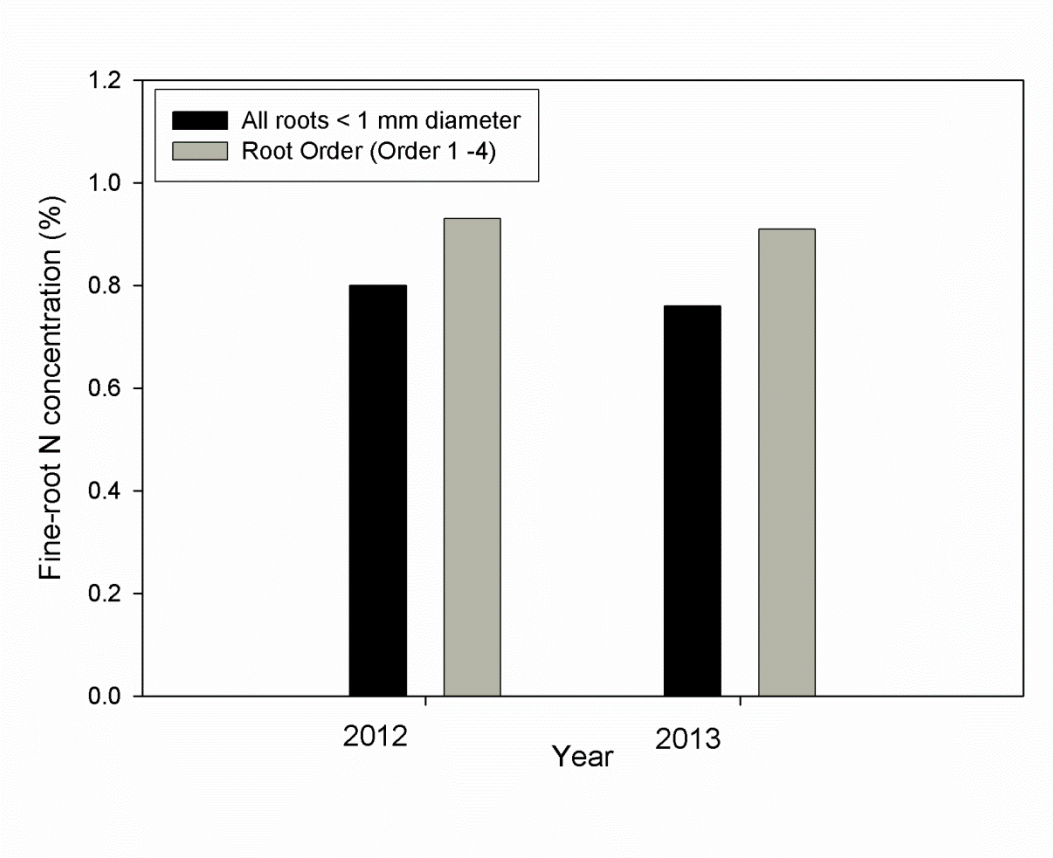

Fig. 5.6 Comparison of methods for measuring N concentration in fine-roots. Black bars are measured from 5-cm diameter cores and roots separated by root branching order. Grey bars are measured from intact root sections separated by root branching order.

6. Broader Impacts

Results presented here corroborate with other recent studies finding heterogeneity in the lifespan of fine-roots, or those roots most active in water and nutrient uptake. Specifically, we found in chapter 3 that only a portion of the fine-root population is turned over annually, with much of the carbon stored in roots remaining for multiple years. Current modeling schemes that use a single, often annual, lifespan for all fine-root biomass likely over-estimate the impacts of fine-roots on nutrient cycling in terrestrial ecosystems. However, sampling of roots less than a given diameter class, as was performed in the initial study, does not provide a functional basis for separating roots. Consequently, identification of which roots have a shorter lifespan, or identification of root traits that are linked to lifespan is not possible to answer with that approach. A different approach to separation of roots is separation by root branching order, pooling only those roots that are equivelantly far from the main coarse rooting system. Using this approach in chapter 4, we found that the most distal root branches are smaller in diameter and tend to have a shorter lifespan. Importantly, the most important trait examined in correlating with root lifespan is root nitrogen concentration. As root [N] may have species-specific relationship with root diameter, diameter analyses may provide a an inexpensive easily measurable trait for assessing root lifespan across spatial or temporal gradients. In order to scale up findings of root longevity using these traits, it is important to know relative biomass distribution amongst the branching orders. A literature review of this topic (chapter 5) revealed a paucity of reports of fine-root biomass by branching order. In order to more thoroughly understand fine-root contribution to terrestrial nutrient cycling, more research measuring biomass distributions is needed. Additionally, a theoretical biomass allocation model highlights the dynamic nature of plant growth, both above- and below-ground (chapter 2). The modeling approach, demonstrated here, potentially provides a

way to integrate dynamic vegetation response into large-scale Earth System Models (ESMs), which currently lack a dynamic modeling scheme.

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Appendix A. Permission to use chapter 3 from Wiley

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Thank you for getting back to me. No, that is not the correct paper. The paper I would like to re-use for my thesis is titled: **Stored carbon partly fuels fine-root respiration but is not used for production of new fine roots**

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Professional Experience

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Peer-reviewed publications

Lynch DJ, Matamala R, Iversen CM, Norby RJ, Gonzalez-Meler MA. 2013. Stored carbon partly fuels fine-root respiration but is not used for production of new fine roots. *New Phytologist*. 199: 420-430.

Gonzalez-Meler MA, **Lynch DJ**, Blanc-Betes E. 2013. Hidden Challenges in Ecosystem Responses to Climate Change. *JSM Environ Science & Ecology*. 1(2): 1006.

Hopkins, F, Gonzalez-Meler MA, Flower CE, **Lynch DJ**, Czimczik C, Tang J, Subke J. 2013. Ecosystem-level controls on root-rhizosphere respiration. *New Phytologist.* 199: 339-351.

Lynch DJ, McInerney FA, Kouwenberg LR, Gonzalez-Meler MA. 2012. Plasticity in bundle sheath extensions in canopy and under-story species. *American Journal of Botany.* 99(7): 1197-1206.

Belaire, JA, Dribin AK, Johnston DP, **Lynch DJ**, Minor ES. 2011. Mapping stewardship networks in urban ecosystems. *Conservation Letters.* 4: 464-473.

.

Other Publications

Flower CE, Gonzalez-Meler MA, **Lynch DJ**. 2012. Global Change: natural and human induced drivers. In Craig RK, Nagle JC, Pardy B, Schmitz O, & Smith W (Eds.), *The Encyclopedia of Sustainability*, Vol. 5: *Ecosystem Management and Sustainability*, pp. 162-167. Great Barrington, MA: Berkshire Publishing.

Oral Presentations

Lynch DJ, Matamala R, Iversen CM , Norby RJ, Gonzalez-Meler MA. *Liquidambar styraciflua* fine-root replacement occurs in clusters of lower order roots. AGU Fall Meeting, San Fransisco, CA. December 7, 2012.

Lynch DJ, Matamala R, Iversen CM , Norby RJ, Gonzalez-Meler MA. Quantifying fine root respiration carbon sources using ^{13}C tracer at the conclusion of a long-term FACE experiment. **Scaling Root Processes: Global Impacts Workshop, Arlington, VA. March 7, 2012.**

Posters

*Under-graduate student presenter

*Genev I, **Lynch DJ**, Gonzalez-Meler MA. Carbon Sources for Root Respiration Following Girdling Using a Stable Isotope Ecosystem Tracer. UIC Student Research Forum, Chicago, IL. April, 2013.

Lynch DJ, Gonzalez-Meler MA. Impacts of long-term air pollution on trees through analysis of tree ring chemistry. 2012 IGERT online poster competition. May, 2012. http://posterhall.org/igert2012/posters/319

*Wagner M, **Lynch DJ**, Gonzalez-Meler MA. Using stable isotopes to examine diet in Chicago. Pre-Dental Undergraduate Research Symposium. Chicago, IL. April, 2011.

Lynch DJ, Matamala R, Norby RJ, Iversen CM, Gonzalez-Meler MA. Belowground carbon sources and fine root turnover times using ^{13}C tracer at the conclusion of a long-term FACE experiment. AGU Fall Meeting. San Francisco, CA. December, 2011.

*Quigley K, **Lynch DJ**, Smith FA. Bundle sheath extension density along a light gradient. Chicago Botanic Garden Research Experience for Undergraduates Poster competition. Glencoe, IL. 2008.

Teaching Experience

Courses

Homeostasis, BIOS 240. University of Illinois at Chicago. Spring 2014.

Guest Lectures

Homeostasis, BIOS 240. University of Illinois at Chicago. “Phloem Translocation” Spring 2013.
Homeostasis, BIOS 240. University of Illinois at Chicago. “Plant Water Relations” Summer

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Teaching Assistantships
University of Illinois at Chicago, Department of Biological Sciences 2011-2013.
General Ecology Laboratory, BIOS 331, Fall 2013
Homeostasis**,** BIOS 240, Summer 2013
Biology of cells and organisms, BIOS 100, Spring 2013
Introduction to Populations and Communities, BIOS 101, Fall 2011, 2012

Northwestern University, Department of Biological Sciences 2008.
Field Ecology, BIOL_SCI 346, Spring quarter 2008

Undergraduate Mentoring

Joy Tepple, UIC undergraduate volunteer 2013-Present
Ivo Genev, UIC undergraduate volunteer and honors college project coordinator, 2012-2013
Elizabeth Sande, UIC Research Experience for Undergraduates student, 2012
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Grants and Awards

UIC Department of Biological Sciences Research Achievement Award, 2013 ($200)
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Elmer Hadley Research Award, 2011 ($2429)
NSF IGERT Fellow (Landscape, Ecological & Anthropogenic Processes), 2009-2011
Plant Biology and Conservation Award (Northwestern University), 2008 ($1500)

Service, Outreach and Professional Development

Sensors Workshop, Argonne National Laboratory. 2013.
Invited attendee of workshop featuring discussions on sensor needs in terrestrial ecosystem ecology.

Improvisation Techniques for Scientific Communication workshop, UIC. April-May 2012.
With IGERT students, organized 6-week workshop on improving communication of scientific ideas for UIC graduate students.

Dendroecology Course, University of Arizona. 2011.
Attended summer short course on dendroecology offered at the University of Arizona's Laboratory for Tree Ring Research.

Chicago Botanic Garden Research Experience for Undergraduates, 2009.
Served as judge of undergraduate poster competition.

Peer Reviewer for internationally recognized journals including: *Tree Physiology* (2012),

Arboriculture and Urban Forestry (2013), *Southern Forests* (2013), *PLOS ONE* (2014)