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## Methods for estimating root biomass and production in forest and woodland ecosystem carbon studies: A review

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### ABSTRACT

Fine and coarse roots are key contributors to belowground net primary productivity, and play critical roles in the biogeochemical cycling of forest and woodland ecosystems. Despite their critical roles, roots have been understudied mainly due to methodological challenges. There is currently no consensus on which methods are most suitable to accurately study root biomass and production. Critical evaluation of the assumptions, strengths and inherent limitations of methods to study root biomass and production are necessary to help investigators decide which method is best for their purposes. This synthesis compares existing methods for root biomass and production estimation based on relevant criteria that include cost, labor requirements, time efficiency and accuracy and, also compares fine- and coarse-root biomass and production estimates from different methods measured at the same sites. Root excavation and soil-pit methods are commonly used to estimate coarse-root biomass, despite the high cost and labor required. Ground-Penetrating Radar is a very promising indirect approach to estimate coarse-root biomass, but may not be suitable for ecosystems with dense understory and soils with high organic matter and ion contents. Soil-core remains the most preferred method to estimate fine-root biomass. Empirical models are accepted as fast and cost-effective indirect approach to predict fine- and coarse-root biomass and production. Fine-root production is usually estimated with the (mini) rhizotrons, sequential-coring and ingrowth-core methods. Coarse-root biomass estimates were not significantly different between soil-pit and soil-core methods. There was a significant positive correlation ( $r^2 = 0.91$ ,  $p < 0.0001$ ) between fine-root biomass estimates obtained from soil-pit and soil-core methods. Fine-root production estimates were lower in the ingrowth-core ( $2.06 \pm 0.23 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ) compared to the (mini) rhizotrons ( $3.81 \pm 0.46 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ) and sequential-coring ( $3.84 \pm 0.93 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ) methods. Based on the reviewed literature and comparative analysis we propose that (mini) rhizotrons should be preferred over the others in estimating fine-root production. In situations where cost and site conditions preclude their use, the sequential-coring and ingrowth-core methods are suitable. The ingrowth-core should be used with caution in sites where root growth is slow and root biomass may be influenced by strong seasonal fluctuations. Multiple methods are still recommended for yielding realistic estimates of fine- and coarse-root production, and more comparative studies of different methods should be conducted on the same sites.

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### 1. Introduction

Fine and coarse roots are major contributors to the total biomass pools of forest and woodland ecosystems, and play critical roles in the cycling and allocation of carbon (C) and nutrients

(Clark et al., 2001; Brunner and Godbold, 2007; Malhi et al., 2011; Smyth et al., 2013; Raich et al., 2014). A significant fraction of C assimilated by plants through photosynthesis is transferred to roots and their symbionts (Litton et al., 2007; McCormack et al., 2015); this may even exceed the amount allocated to aboveground components (e.g. Moser et al., 2011). The carbon transferred belowground is estimated to account for 22–63% of the total gross primary productivity of forests (Litton et al., 2007). This large flux of C exerts a profound influence on the regulation of major soil processes that affect productivity and biogeochemical cycling in

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these ecosystems (Prescott, 2010; Clemmensen et al., 2013; Raich et al., 2014; Zhang and Wang, 2015). Despite these critical roles, roots have been understudied, and are poorly represented in many process-based ecosystem models, limiting the models ability to predict ecosystem responses to environmental changes and management practices (Smithwick et al., 2014; Warren et al., 2015). The uncertainty about root dynamics also hampers efforts to accurately estimate pool size for C accounting and climate mitigation measures such as the Reduced Emissions from Deforestation and Forest Degradation (REDD+) (Smyth et al., 2013; Yuen et al., 2013). This knowledge gap is partly attributable to methodological challenges in sampling roots to estimate biomass production and turnover (Vogt et al., 1998; Bledsoe et al., 1999; Makkonen and Helmisaari, 1999).

Estimation of fine and coarse root biomass and production can be grouped into direct and indirect methods. Fine-root biomass and production have been estimated with direct methods that include soil-core/sequential-coring (Makkonen and Helmisaari, 1999; Lauenroth, 2000), monolith (Bledsoe et al., 1999; Makita et al., 2011), soil-pit (Millikin and Bledsoe, 1999; Park et al., 2007), ingrowth-core (Persson, 1979; Vogt et al., 1998) and (mini) rhizotrons (Taylor et al., 1990; Madji, 1996), and indirectly through the use of empirical models (Shinozaki et al., 1964b; Kurz et al., 1996). For coarse roots, direct methods include root excavation (Bledsoe et al., 1999; Niyama et al., 2010), soil-pit/soil-pit ingrowth (Lawson et al., 1970; Kangas, 1992), wall or trench profiles and soil-core (van Noordwijk et al., 2000; Achat et al., 2008; Major et al., 2012), while the indirect methods include, but are not limited to, size-mass allometric equations (Whittaker et al., 1974; Kenzo et al., 2009; Brassard et al., 2011a), root-shoot or belowground-aboveground ratio (Keith et al., 2000; Levigne and Krasowski, 2007; Malhi et al., 2009), Ground-Penetrating Radar (GPR) (Butnor et al., 2001; Samuelson et al., 2015), and root biomass increment or difference (Steele et al., 1997; Kajimoto et al., 1999) as well as root radial increment (Zach et al., 2010; Moser et al., 2011).

There is no consensus in the literature on how best to estimate root biomass, production and turnover (Vogt et al., 1998; Bledsoe et al., 1999; Levillain et al., 2011; Milchunas, 2012; Yuan and Chen, 2012a). For instance, in a global study that compared fine root production estimates for terrestrial ecosystems, Yuan and Chen (2012a) reported significantly higher fine-root production estimates from indirect than direct methods, which is contrary to other studies where no differences between direct and indirect methods were observed (e.g. Vogt et al., 1998; Finér et al., 2011). This uncertainty means that the choice of a method may be determined by considerations such as cost, labor availability, site constraints and individual preferences rather than accuracy and precision (Vogt et al., 1998; Levillain et al., 2011; Makita et al., 2011), with implications for modeling ecosystem C budget and allocation patterns. This lack of consensus therefore calls for critical evaluation of the assumptions, strengths and inherent limitations of the various methods to help investigators decide which method is best for their purposes.

It is often recommended to use multiple methods to quantify root dynamics (Vogt et al., 1998; Hendricks et al., 2006; Yuan and Chen, 2012a), but few studies compare methods at the same sites and at the same sampling time (e.g. Makkonen and Helmisaari, 1999; Hertel and Leuschner, 2002; Ostonen et al., 2005; Hendricks et al., 2006; Moser et al., 2010; Levillain et al., 2011; Girardin et al., 2013; Yuan and Chen, 2012a; Sun et al., 2015). This study builds on earlier reviews (e.g. Vogt et al., 1998), but with greater emphasis on coarse roots due to the present recognition of their important roles in ecosystem C budgets and allocation patterns (Clark et al., 2001; Smyth et al., 2013; Doughty et al., 2014; Varik et al., 2015; Zhang and Wang, 2015).

The objectives of this review were to (1) synthesize and compare existing methods for root biomass and production based on relevant criteria, and (2) compare fine and coarse root biomass and production estimates from different methods measured at the same sites.

## 2. Methods

### 2.1. Literature search and data compilation

Data was compiled through a literature search from journal platforms (Web of Science, Scirus, JSTOR and Google Scholar) and library sources using keywords and the terms 'fine root', 'coarse root', 'root biomass and production' and 'belowground biomass allocation'. All data are from studies conducted in forest and woodland ecosystems (as they contain more than 60% of terrestrial C (Dixon et al., 1994)), which should improve the clarity of the relationship between root biomass and production estimates provided by different methods. Stands of all ages were used, including managed (irrigated, thinned and fertilized) and unmanaged stands. With respect to root sampling, additional criteria were: (i) the study must have included the diameter used to define fine and coarse roots; (ii) roots were sampled by using the soil-pit and soil-core methods to quantify biomass; (iii) fine-roots were sampled using at least two of the direct methods (ingrowth-core, (mini) rhizotrons and sequential-coring) to estimate production; (iv) sampling for fine-root production should have lasted at least one vegetation season or 12 months; and (v) data were collected from a single site and within the same period.

Criteria used to identify fine and coarse roots are not uniform, and are usually defined based on arbitrary diameter classes (e.g. Nadelhoffer and Raich, 1992; Resh et al., 2003; Levigne and Krasowski, 2007; Finér et al., 2011). From the database, fine roots were defined as  $\leq 0.5$  mm,  $\leq 1$  mm,  $\leq 2$  mm and  $\leq 5$  mm in diameter. Coarse roots also ranged from  $> 2$  mm to  $> 50$  mm in diameter. However the majority of the studies defined fine and coarse roots as  $\leq 2$  mm and  $> 2$  mm in diameter (see Appendices A and B and references therein). These definitions will be used to broadly classify fine and coarse roots in the first part of this review. These classifications have also been used in other reviews (e.g. Yuan and Chen, 2012a; Zhang and Wang, 2015). In the analysis of root biomass and production, fine and coarse roots were not standardized to specific diameter classes (Finér et al., 2011), but were considered to be as defined in the original studies (Nadelhoffer and Raich, 1992).

For the first objective, the database was critically assessed to extract information on existing root biomass and production methods (including new and less known ones), their operational principles and strengths and limitations. From the information gathered a matrix was developed to compare methods based on criteria such as ease of field application, cost-effectiveness, labor requirements, time efficiency, accuracy and impact on the ecosystem (Tables 1 and 2). For this review, time efficiency is considered to be the person-hours required to complete field (set-up and sampling) and laboratory processing (Levillain et al., 2011), and accuracy is the capacity for a method to provide accurate estimates. For the second objective, root biomass data were compiled from studies that compared more than one method at the same site. For fine and coarse roots biomass data were compared for the soil-pit and soil-core methods. Nine observations were obtained for fine root biomass, while eleven observations of coarse root biomass were made from seven studies (Appendix A). The Voronoi trench was considered as a 'soil pit' since its field application is similar to the soil-pit method (Levillain et al., 2011). Moreover the study by Levillain et al. (2011) did not compare coarse root biomass ( $> 10$  cm in diameter) between soil pit and soil cores, but was done

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