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Review

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Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes

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Summary

Fine roots acquire essential soil resources and mediate biogeochemical cycling in terrestrial ecosystems. Estimates of carbon and nutrient allocation to build and maintain these structures remain uncertain because of the challenges of consistently measuring and interpreting fine-root systems. Traditionally, fine roots have been defined as all roots ≤ 2 mm in diameter, yet it is now recognized that this approach fails to capture the diversity of form and function observed among fine-root orders. Here, we demonstrate how order-based and functional classification frameworks improve our understanding of dynamic root processes in ecosystems dominated by perennial plants. In these frameworks, fine roots are either separated into individual root orders or functionally defined into a shorter-lived absorptive pool and a longer-lived transport fine-root pool. Using these frameworks, we estimate that fine-root production and turnover represent 22% of terrestrial net primary production globally – a *c.* 30% reduction from previous estimates assuming a single fine-root pool. Future work developing tools to rapidly differentiate functional fine-root classes, explicit incorporation of mycorrhizal fungi into fine-root studies, and wider adoption of a two-pool approach to model fine roots provide opportunities to better understand below-ground processes in the terrestrial biosphere.

I. Introduction

Fine roots play an important role in the cycling of water, nutrients, and carbon (C) in terrestrial ecosystems. Over evolutionary timescales, the expansion of plants from aquatic ecosystems onto land has been contingent upon the development of effective strategies for root acquisition of soil resources (Beerling, 2005), which ensure adequate supplies of water and nutrients for plant photosynthesis, growth, and maintenance. After millennia of evolution, the production of fine roots now comprises an important investment of plant photosynthate and resources. Fine roots also interact with and modify the surrounding soil environment through the exudation of labile C from living roots and the turnover of dead roots, which together stimulate microbial activity and mediate the dynamics of short- and long-term pools of soil organic C (Rasse *et al.*, 2005; Schmidt *et al.*, 2011; Phillips *et al.*, 2012; Tefs & Gleixner, 2012). Through both direct root processes and indirect effects mediated by root-associated microbial communities, the relatively small pool of fine-root biomass plays a disproportionately large role in terrestrial biogeochemical cycles (Silver & Miya, 2001; Rasse *et al.*, 2005; Tefs & Gleixner, 2012; Clemmensen *et al.*, 2013).

Despite the importance of fine roots to evolutionary and ecological processes, estimates of fine-root biomass and function are poorly constrained both within and among ecosystems. This is in stark contrast to studies of stems, branches, and leaves, which have yielded a more robust quantitative understanding of the relationships among plant traits and important processes such as photosynthesis and competition for light (Wright *et al.*, 2004; Chave *et al.*, 2009; Moles *et al.*, 2009). The resulting imbalance in mechanistic and predictive science between above-ground and below-ground dynamics calls for rapid development of greater theoretical and mechanistic understanding of fine-root processes. Moreover, this knowledge gap has hindered the representation of root processes in terrestrial biosphere models and limits their ability to predict ecosystem responses to changing environments (Jackson *et al.*, 2000; Smithwick *et al.*, 2014; Warren *et al.*, 2015). Future progress is contingent upon new conceptual frameworks in observations and in models that account for linkages between root form and function (Iversen, 2014).

To maximize the value of below-ground observations, it is critical that measures of fine-root traits are carried out consistently and precisely. Unfortunately, this has not always been the case. For example, published estimates of specific root length ($\mathrm{m\,g^{-1}}$) in fine roots of *Quercus alba* vary by an order of magnitude (Comas *et al.*, 2002; Yanai *et al.*, 2009; McCormack *et al.*, 2012). Among these studies and many others, the portion of the fine-root pool on which measurements are made ranges from individual root tips to small root branches or to all roots below a fixed diameter cutoff (Comas *et al.*, 2002; Pregitzer *et al.*, 2002; Guo *et al.*, 2008b; Holdaway *et al.*, 2011). The variable manner in which fine roots have been classified limits meaningful comparisons among studies but also represents a tractable area where future research can be improved through consistent treatment and definition of fine roots.

In this review, we highlight several ways in which traditionally defined fine roots (≤ 2 mm in diameter) differ in form and function, and describe the consequences of such differences in ecosystems dominated by perennial plants. As an alternative to the prevailing single-pool, diameter-based classification, we suggest that the broadly defined fine-root group should be split into two distinct classes or pools: absorptive fine roots and transport fine roots. Absorptive fine roots represent the most distal roots involved primarily in the acquisition and uptake of soil resources, whereas transport fine roots occur higher in the branching hierarchy and serve primarily structural and transport functions with some additional capacity for storage. Compared with their closest above-ground analogs, functional divergence between absorptive and transport fine roots would be equivalent to that observed between leaves and twigs. To account for these functional differences, we discuss two ways to move forward, using an order-based classification that allows for more standardized comparisons of root traits, especially for woody perennial species, and a functional classification that provides useful and generalizable information based on differences in absorptive and transport function among fine roots (Fig. 1). Each approach has potential advantages based on tradeoffs in precision and ease of sample processing (Table 1). With both approaches, we advocate for the explicit assessment of mycorrhizal fungi in conjunction with fine roots in an effort to develop a more complete understanding of nutrient and C cycles in terrestrial ecosystems.

II. Ordered variation in fine-root traits – different functions of different roots

Researchers have long sought approaches to classify and understand diversity within complex root systems (Cannon, 1949; Böhm, 1979; Lyford, 1980; Sutton & Tinus, 1983; Pagés & Kervella, 1990; Hishi, 2007). Historically, fine roots have most often been defined as a single pool according to a diameter-based cutoff, commonly ≤ 2 mm, below which roots are implicitly assumed to have similar function (Fig. 1a). More recently, some studies have assigned increasingly smaller diameter cutoffs (e.g. 1.0 or 0.5 mm) in an effort to explicitly emphasize more absorptive fine roots. However, because root morphology varies greatly among species,

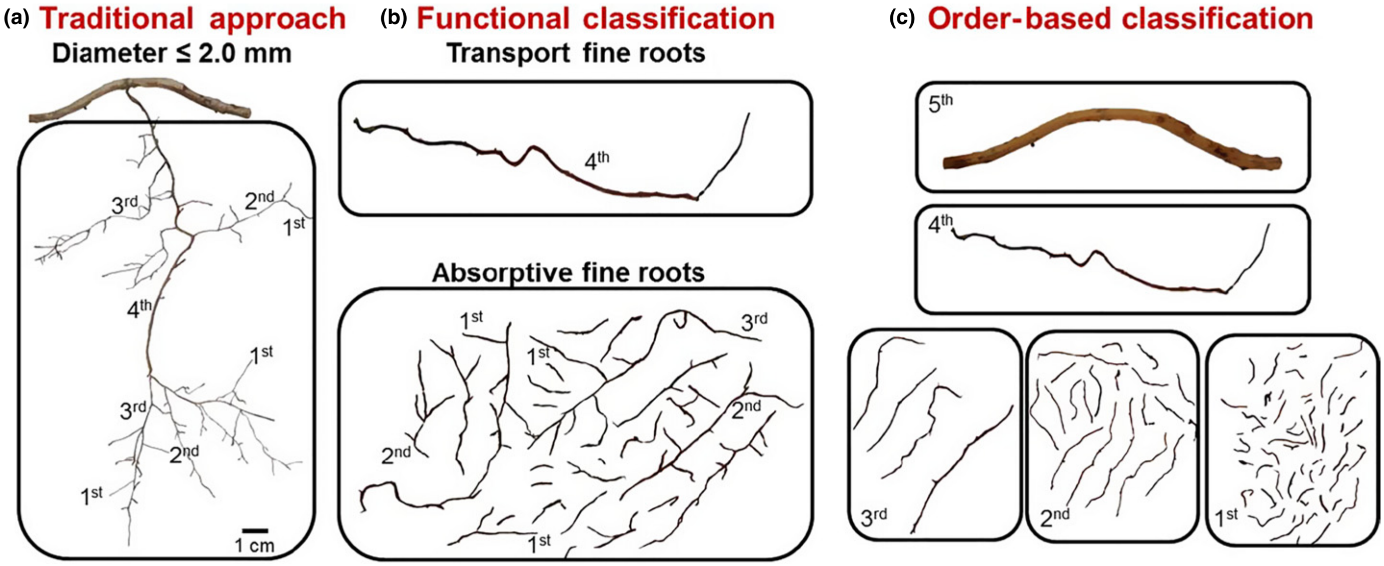


Fig. 1 (a) Picture displaying an intact root branch of *Liriodendron tulipifera* with all fine roots traditionally defined as those having diameters ≤ 2.0 mm; (b) the same branch as in (a) separated into transport fine root, and absorptive fine roots according to the functional classification; (c) the same branch as in (a) separated into individual root orders. In addition to showing all roots dissected to order in (c), examples of individual orders are marked (first (1st) through fifth (5th)) and are also labeled in (a) and (b).

Table 1 Advantages and disadvantages of the traditional fine-root classification, the order-based classification, and the functional classification approaches

Approach	Description	Processing time	Advantage	Disadvantage
Traditional classification	Roots ≤ 2 mm in diameter grouped together	Fast	Requires no prior knowledge of site or species	Root trait and biomass data are difficult to interpret and compare across species and sites
Order-based classification	Roots ≤ 2 mm in diameter separated into individual root orders	Slow	Consistent and accurate comparisons of root traits across species and sites	Labor-intensive and time-consuming
Functional classification	Roots ≤ 2 mm in diameter separated into absorptive and transport fine roots	Moderately fast	Enables comparisons among functionally similar roots, faster than order-based	Might require *a priori* assessment of root traits to determine functional divisions within branching hierarchy

applying a single diameter cutoff across species can be problematic (Fig. 2).

Now, over a decade since Pregitzer *et al.* (2002) demonstrated clear architectural diversity among fine roots and three decades since Fitter (1982) characterized and compared perennial root systems using a topological approach, efforts measuring and describing fine roots have advanced tremendously. Following these studies, fine roots are increasingly categorized using stream-order descriptions, where the most distal, unbranched roots are first order and where second-order roots begin at the junction of two first-order roots and so on (Pregitzer *et al.*, 2002) (Fig. 1). Numerous reports have now highlighted striking differences in many fine-root traits within the root branching hierarchy following the order-based classification (Fig. 3). Importantly, many traits that vary among root orders are often considered to be important for terrestrial cycles of C, water, and nutrients. For example, root life span, diameter, and secondary development increase with root order (Pregitzer *et al.*, 2002; Guo *et al.*, 2008c; Valenzuela-Estrada *et al.*, 2008; Espeleta *et al.*, 2009; Xia *et al.*, 2010; Rewald *et al.*, 2012), while root turnover, respiration, nitrogen and metal content, and mycorrhizal colonization tend to decrease (Figs 3, 4) (Gill & Jackson, 2000; Guo *et al.*, 2008b, 2013; Valenzuela-Estrada *et al.*, 2008; Helmisaari *et al.*, 2009; Makita *et al.*, 2009; Jia *et al.*, 2011; Sun & Mao, 2011; Burton *et al.*, 2012; Rewald *et al.*, 2012; Ouimette *et al.*, 2013). Root decomposition rates also differ strongly among root orders, probably in part because of differences in tissue substrate chemistry (Fan & Guo, 2010; Goebel *et al.*, 2011; Xiong *et al.*, 2013), although the specific factors have not been elucidated. Whereas some traits may increase or decrease in a roughly linearly fashion, it is also important to note that in many cases these relationships can be strongly nonlinear and may reflect thresholds in root form and function (Fig. 3).

Resource uptake and transport also vary consistently among fine-root orders. In particular, uptake capacity declines with increasing root order, while transport capacity increases. This transition is associated with ontogeny and secondary development within the root, as primary tissues, including the cortex, senesce while a cork periderm develops, cell walls are thickened, and additional suberin is deposited within the root. Together, these changes reduce movement of water and ions radially across the root (Table 2; Fig. 4) (Steudle & Peterson, 1998; Gambetta *et al.*, 2013). Furthermore, secondary development in higher-order roots reduces the ability of

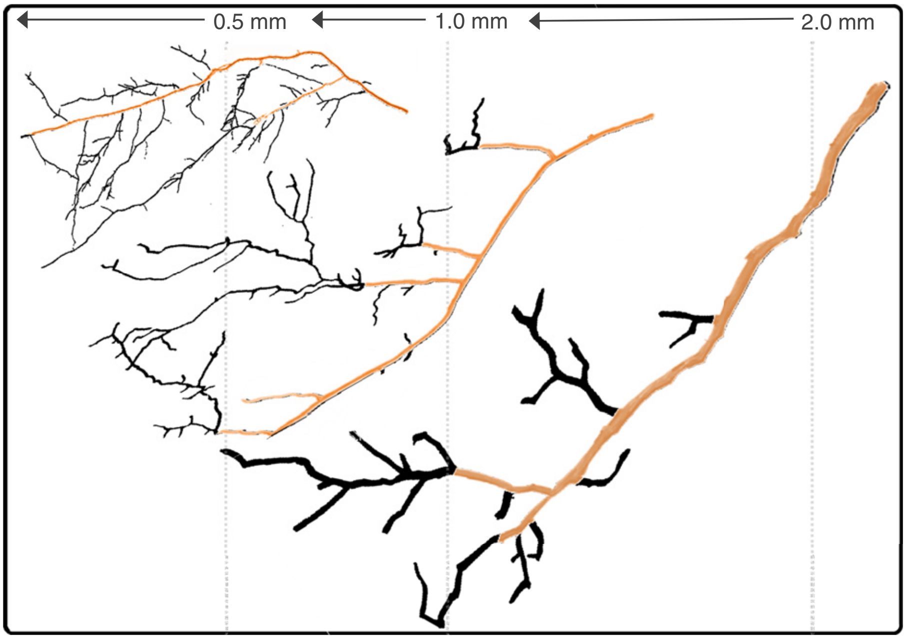


Fig. 2 Root branches of three architecturally diverse, co-occurring subtropical species, demonstrating the variable number of root orders that fall below different diameter classifications (common cutoffs of 0.5, 1.0, and 2.0 mm shown along top): *Schima superba* at the top left with five root orders ≤ 0.5 mm in diameter, including both absorptive and transport fine roots, *Choerospondias axillaris* in the middle with three root orders ≤ 0.5 mm in diameter and only including absorptive fine roots, and *Cinnamomum austrosinense* at the bottom right with no roots ≤ 0.5 mm in diameter. Black, absorptive fine roots; tan, transport fine roots.

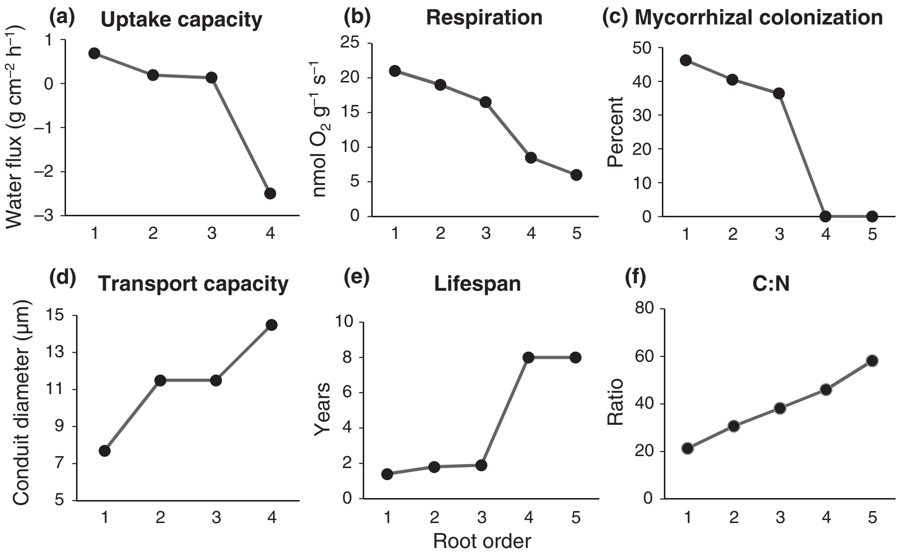


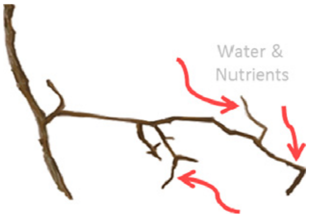
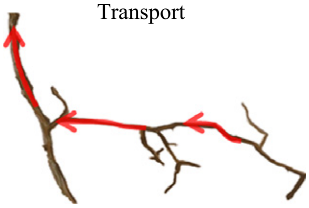
Fig. 3 Common patterns of root trait variation across distal fine-root orders. (a–f) Actual data highlighting trends for uptake capacity (water flux density) (a), respiration rate (b), mycorrhizal colonization (c), transport capacity (hydraulically weighted conduit diameter) (d), root life span (e), and carbon : nitrogen (C : N) ratio (f). Data are from Rewald *et al.* (2012) (a, d), Xia *et al.* (2010) (b, c, e), and M. Zadworny (unpublished) (f).

mycorrhizal fungi to colonize the root, which limits the transfer of soil resources to plants (Guo *et al.*, 2008b; Valenzuela-Estrada *et al.*, 2008). At the same time, as secondary development increases, transport capacity increases, owing to construction of larger-diameter conduits within the secondary xylem (Table 2; Figs 3, 4) (Steudle & Peterson, 1998; Hishi, 2007; Bagniewska-Zadworna *et al.*, 2012; Long *et al.*, 2013). Together, the greater uptake potential and increased mycorrhizal colonization of lower-order roots mark them as primarily absorptive roots. In many cases, these roots also form ephemeral root modules characterized by relatively short life span (Xia *et al.*, 2010). By contrast, greater secondary development and increased rates of maximum conductance of higher-order roots indicate more important roles in resource transport. While the general function of transport fine roots is similar to that of coarse roots, the former represent a discrete biomass pool owing largely to their periodic turnover and replacement (*c.* every 5–10 yr in woody species), whereas coarse roots turn over infrequently, barring disturbance or plant death.

III. Pitfalls and platforms – understanding bias and improving estimates of root processes

Formalizing the conceptual framework of fine roots with an appreciation of the diversity in fine-root traits and functions offers an opportunity to re-evaluate commonly held ecological assumptions. This framework reveals substantial differences in both ecosystem- and global-scale estimates of terrestrial C and nutrient cycling when compared with previous estimates. This perspective also sheds light on long-standing discrepancies among methods used to measure key root processes and provides a tractable

Table 2 Common traits associated with uptake and absorption of soil water and nutrients by fine roots or with increased capacity to efficiently transport resources within the root to more proximal plant tissues

Root function	Traits associated with root function
Uptake/absorption  	• High surface area to volume ratio • High mycorrhizal colonization • High root hair density • Little to no secondary development (cortex intact, lower suberization and absence cork periderm)
Transport 	• High stele to diameter ratio • Well-developed xylem conduits • Secondary development (loss of cortex and development of cork periderm, high suberization, increasing lignification)

approach for terrestrial biosphere models to better incorporate fine-root processes.

1. Improved understanding of root processes at local and global scales

Estimating and interpreting fine-root processes is difficult in natural systems. Order-based and functional classifications offer ways to more accurately characterize variation in key root processes that differ among absorptive and transport fine roots. The following examples highlight how these classification frameworks can improve understanding of seasonal differences in resource allocation within a root system or ecosystem and improve quantification of fine-root turnover and its consequences for terrestrial biogeochemistry at local and global scales.

Seasonal patterns of carbohydrate and nutrient concentrations in stems and leaves are well documented across many plant species. In many cases, resorption and translocation of plant resources can be important strategies for perennial plants to maximize resource use across multiple growing seasons (Freschet *et al.*, 2010; Richardson *et al.*, 2013; Dietze *et al.*, 2014). Below ground, such patterns are unclear, probably because measurements often combined short-lived absorptive fine roots with long-lived transport fine roots. This could be likened to pooling leaves together with twigs to quantify rates of nutrient resorption. However, recent studies that explicitly separated fine roots into individual orders revealed striking differences in seasonal patterns of tissue chemistry between absorptive and transport fine roots (Xia *et al.*, 2010; Jia *et al.*, 2011; Zadworny *et al.*, in press): nitrogen concentrations of absorptive fine roots of *Fraxinus mandshurica* and *Larix gmelinii* and *Quercus robur* peaked early in the growing season and then declined later in the growing season. By contrast, nitrogen concentrations in higher-order, transport fine roots were relatively stable through time. Importantly, if all roots ≤ 2 mm were grouped together, these seasonal differences would be muted and largely undetectable. Future estimates of ecosystem-level nutrient fluxes through fine roots can therefore be improved by consideration of the different concentrations of nitrogen and other elements found among absorptive and transport fine roots, as well as their differing seasonal dynamics.

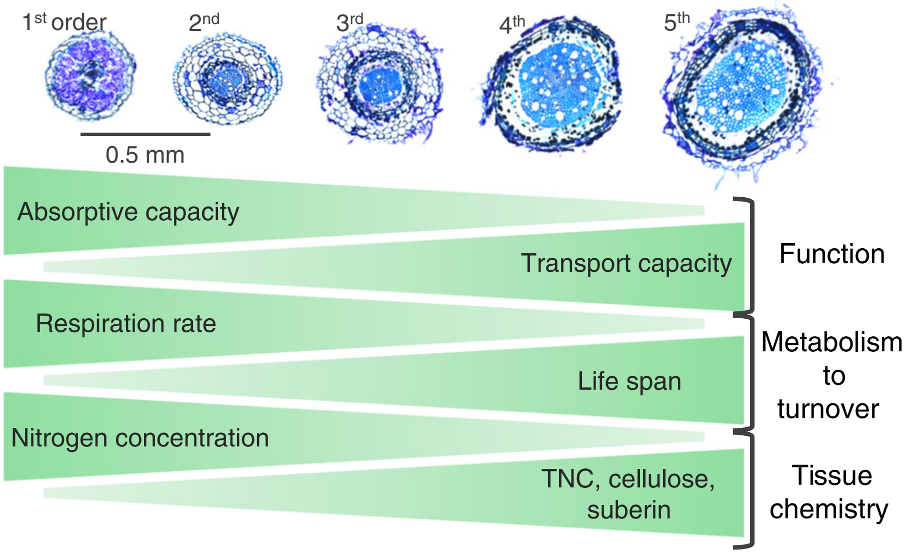


Fig. 4 Root cross-sections of *Acer plantanoides* show a typical pattern of increasing root diameter and secondary development with increasing root order. Notice that first- and second-order roots have little or no secondary development and first- to third-order roots still possess intact root cortical cells while fourth- and fifth-order roots have lost all cortex and instead have secondary xylem developed within the stele. Triangles depict simplified patterns of root function (absorptive and transport capacity) and root traits (respiration rate g^{-1} root, life span, total nonstructural carbohydrates (TNC) and other aspects of tissue chemistry) with increasing root order. Depending on the trait or species, the changes with root order may be nonlinear. It is also worth noting that despite their recognized importance to root function, many aspects of tissue chemistry, including cellulose, suberin, and phenolic content, are not well studied and patterns with root order may vary across species.

Estimates of root productivity and turnover are critical for understanding whole-plant C allocation and terrestrial biogeochemistry and for parameterizing terrestrial biosphere models. To provide broadly relevant and scalable estimates of root biomass and productivity, Jackson *et al.* (1997) synthesized root biomass estimates across 11 biomes and estimated that fine-root production and turnover account for *c.* 33% of global terrestrial net primary productivity (NPP). This estimate has since become an important benchmark for interpreting differences in fine-root productivity among species and study systems. A key assumption in this estimate was that the entire fine-root biomass pool (defined in their analysis as all roots ≤ 2 mm in diameter, i.e. traditional classification) would turn over once per yr (turnover rate = 1.0 yr^{-1}). In reality, only the distal, absorptive fine roots turn over this quickly (likely range of *c.* 0.5–2.0 yr^{-1}, depending on species and ecosystem). Transport fine roots, which often comprise over half of the total biomass of roots ≤ 2 mm in diameter, turn over on timescales closer to a decade and require a much lower proportion of annual NPP for their production and replacement (Table 3). As such, the 33% benchmark probably overestimates global terrestrial NPP allocated to fine-root production.

Here, we use the benchmark estimate of 33% provided by Jackson *et al.* (1997) as a case study to illustrate the important difference in interpretation of fine-root processes based on root function rather than diameter class. We updated the original estimate using known differences in turnover rates and observed patterns of biomass partitioning among separate pools of absorptive and transport fine roots (Tables 3, 4; Supporting Information, Tables S1, S2; Notes S1a). Based on these data, we estimated that global terrestrial NPP allocated to all roots ≤ 2 mm in diameter is *c.* 22% (Fig. 5; Notes S1a). Furthermore, because woody plants allocate a greater proportion of their fine-root C to long-lived, transport fine roots than do herbaceous plants, the reduction in the estimate of annual NPP allocation would be greater in biomes dominated by woody rather than herbaceous species (Tables S3, S4). The two-pool model (i.e. functional classification) of fine roots enables a more accurate characterization of root processes. While this case study analysis was conducted at the global scale, it is important to note that the same approach is relevant at the level of individual plants and ecosystems. Following a similar approach as described above we estimated that the annual requirement of annual NPP in longleaf pine (*Pinus palustris*) would be reduced from *c.* 18 to 11% using a one-pool vs a two-pool model (Notes S1b;

Table 3 Estimated proportions of fine-root biomass allocated to absorptive vs transport pools among different species or sites

	Absorptive/transport			
Species or site	Growth form	Fine roots	Notes	References
Oak Ridge Free-Air CO$_2$ Enrichment	Mixed forest	10/90	Based on two-pool model of carbon turnover from fine roots in mixed forest	Lynch *et al.* (2013)
Picea abies	Woody tree	11/89	Based on proportion of ectomycorrhizal short roots compared with all roots ≤ 2 mm in diameter from lower latitude sites	Ostonen *et al.* (2011)
Blodgett	Mixed forest	16/84	Proportional biomass estimated based on two-pool model of fine-root mean residence times in mixed forests	Gaudinski *et al.* (2010)
Harvard	Mixed forest	22/78		
Knottåsen	Mixed forest	23/77		
Pinus sylvestris	Woody tree	29/71	Based on proportion of ectomycorrhizal short roots compared with all roots ≤ 2 mm in diameter	Helmisaari *et al.* (2007, 2009)
Picea abies	Woody tree	30/70	Based on proportion of ectomycorrhizal short roots compared with all roots ≤ 2 mm in diameter from higher latitude sites	Ostonen *et al.* (2011)
Cunninghamia lanceolata	Woody tree	38/62	Biomass measured on all roots to a depth of 40 cm, separated into short-lived and long-lived roots based or root order	Liao *et al.* (2014)
Pinus palustris	Woody tree	48/52	Biomass determined for each of five root orders ≤ 2 mm in diameter	Guo *et al.* (2004)
Larix gmelinii	Woody tree	49/51	Biomass determined for each of five root orders ≤ 2 mm in diameter sampled from 0 to 10 cm depth in July	Wang *et al.* (2006)
Fraxinus manshurica	Woody tree	58/42		
Geum aleppicum	Forb	60/40	Whole root systems harvested and dissected to determine biomass of individual root orders	K. Sun *et al.* (unpublished)*
Rubus saxatilis	Shrub	60/40		
Tephroseris kirilowii	Forb	70/30		
Roegneria hondae	Grass	80/20		
Agrimonia pilosa	Forb	100/0		
Thalictrum petaloideum	Forb	100/0		
Valeriana officianalis	Forb	100/0		

Data are collected from studies that report biomass for all root orders with diameters ≤ 2 mm. In studies where the proportion of absorptive vs transport fine roots was not explicitly reported, the proportions estimated here assume that, in woody systems, the first-, second-, and third-order roots constituted the absorptive roots and the higher-order roots represented the transport fine roots.

*Herbaceous roots were classified as absorptive or transport fine roots based on observed differences in root life span.

Table 4 Proportional allocation of fine-root biomass to absorptive and transport fine roots

	Ratio of biomass allocated to absorptive/transport fine roots		
Biome or plant type	Scenario 1: minimum absorptive	Scenario 2: average	Scenario 3: maximum absorptive
Woody*	10/90	33/67	60/40
Herbaceous†	50/50	81/19	90/10
Cultivated annual‡	100/0	100/0	100/0

Three scenarios represent the range of estimates identified from studies reported in Table 3. Scenario 1 is the minimum fraction of biomass allocated to absorptive fine roots and scenario 3 is the maximum, while Scenario 2 represents the average for woody and herbaceous species.

*Estimates for woody plants did not include studies based on ectomycorrhizal short roots, as they discount the potentially absorptive capacity of second- + third-order roots without prominent mycorrhizal colonization. However, in boreal systems, the biomass of ectomycorrhizal short roots probably captures the majority of absorptive capacity and these estimates fall within the range found in other studies.

†*Rhubus saxatilis* was considered as herbaceous in the calculations, as it experiences complete annual replacement above ground similar to other herbaceous species.

‡All roots classified as absorptive, assuming that no roots in cultivated annual systems persist through multiple years.

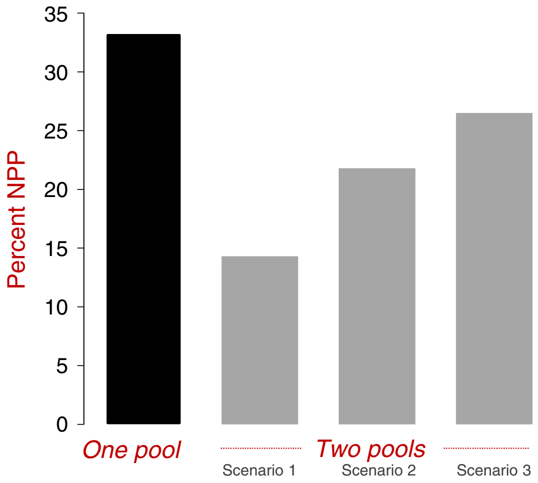


Fig. 5 Percent of global terrestrial net primary productivity (NPP) allocated to fine-root production and turnover. Scenario 2 is based on the average estimated biomass proportions of absorptive and transport fine-root pools in woody and herbaceous biomes, while scenarios 1 and 3 represent the minimum and maximum estimates of reported allocation to absorptive roots (see Tables 3 and 4 and Supporting Information Notes S1a).

Fig. S1). As uncertainty regarding root production and turnover is reduced at both global and local scales, more attention can be paid to other key below-ground activities, including root respiration, root exudation, and fluxes through mycorrhizal fungi, all of which play important roles in nutrient cycling and can also account for a substantial portion of annual NPP.

2. Embracing functional diversity to measure and model fine-root processes

Uncertainty regarding pools and fluxes of root C has become an important factor in limiting improvements in model performance at both ecosystem and global scales (Ostle *et al.*, 2009). As shown earlier, a relatively simple adjustment to the definition and treatment of fine roots lowered the estimated global NPP allocated to this pool by about one-third. Broader application of a two-pool, functional classification to describe fine roots in models will better constrain model estimates of allocation to fine-root biomass and turnover. Indeed, some models have already adopted a two-pool approach to better capture observed patterns of root production and turnover (Gaudinski *et al.*, 2010; Parton *et al.*, 2010). Patterns of fine-root biomass replacement also have important implications for biomass partitioning and resource allocation among plant components (e.g. foliage, wood, coarse roots, fine roots, and respiration from each) (McCormack *et al.*, 2015), further emphasizing the need to better constrain estimates at the root level to improve estimates of C flux, nutrient and water uptake, and soil CO_2 efflux at the whole-ecosystem level. This is particularly true in systems where tradeoffs between fine roots and other plant components (e.g. wood) have previously been identified and changes in root biomass are associated with changes in total plant and ecosystem productivity (Litton *et al.*, 2007; Dybzinski *et al.*, 2011; Malhi *et al.*, 2011). Furthermore, as descriptions of fine-root productivity are improved in models, they can be combined with independent measures of total below-ground C allocation to better constrain estimates of C allocated to mycorrhizal fungi (Litton *et al.*, 2007).

Future improvements in ecosystem and global-scale models will also include more mechanistic descriptions of soil resource uptake as functions of fine root biomass, length, or physiology. Recent modeling efforts linking nitrogen uptake to root biomass highlight the need to better quantify and constrain effective uptake rates of fine roots in natural systems (Zaehle & Friend, 2010; McMurtrie *et al.*, 2012; Thomas & Williams, 2014). Understanding and incorporating functional divisions within the fine-root hierarchy provide a means to more accurately model complex fine-root systems in a tractable manner. Applying a two-pool approach (i.e. absorptive and transport fine roots) to model fine roots enables better estimates of C flux through terrestrial systems and will help to constrain estimates of resource uptake by plants.

3. Understanding bias and appropriate use of different methods

Many different methods and approaches for measuring fine-root processes have been developed in an effort to overcome difficulties of below-ground observation. While each technique has strengths, all methods also have some associated weakness or artifact. As a consequence, different methods can yield dissimilar results even when examining the same processes within the same system (Strand *et al.*, 2008; Yuan & Chen, 2012). Some of the discrepancies may be partially explained by methodological bias towards absorptive vs transport fine roots.

Previous reports have demonstrated a systematic bias between isotope-based and minirhizotron-based measures of fine-root C turnover, which can result in misleading estimates of fine-root turnover rates (Tierney & Fahey, 2002; Guo *et al.*, 2008a; Strand *et al.*, 2008). For example, owing to disturbance during installation and the relatively low volume of soil sampled by minirhizotron tubes, the probability of observing longer-lived, transport fine roots is low, even after long equilibration periods (Taylor *et al.*, 2013; Leppälammi-Kujansuu *et al.*, 2014). As such, minirhizotrons are only effective for monitoring the dynamics of the absorptive fine-root population. Conversely, when fine roots are analyzed as a single pool, isotopic techniques are biased towards higher-order roots with greater mass (Guo *et al.*, 2008a). Furthermore, the mixing of stored carbohydrates within plants and limited temporal resolution of the bomb ^{14}C approach hinder application of isotope techniques to characterize the life span of the short-lived, absorptive fine-root pool (Adams & Eissenstat, 2014). Separately, these two methods are unlikely to accurately describe turnover of the entire fine-root population (i.e. absorptive plus transport fine roots) in perennial vegetation. However, by recognizing the biases and strengths of each method, it is possible to better constrain turnover estimates for surveys, experiments and model parameterization (Gaudinski *et al.*, 2010; Lynch *et al.*, 2013; Ahrens *et al.*, 2014).

Most methods associated with the study of fine roots disturb the litter layer and soil (e.g. ingrowth cores, minirhizotron tubes, root boxes, root bags, and soil probes). While alterations to the local soil environment may be unavoidable, it is important that researchers recognize how these disturbances influence the root population that will be sampled later. For example, severing roots in soil during sampling and installations (e.g. with ingrowth cores) often elicits a wounding response. While the strength of the wounding responses varies by species and time of year, it can result in a large and atypical pulse of root growth. Furthermore, the roots that first emerge may not be representative of the fine-root population; in some species this can be biased towards dense proliferation of absorptive fine roots, while in other species, larger pioneer roots, which are morphologically and anatomically distinct from absorptive fine roots, may be the first to emerge (Zadworny & Eissenstat, 2011). In any case, it will be important for researchers to recognize ways in which different methods may bias observations and sampling towards either absorptive or transport (and pioneer) fine roots to ensure that results are interpreted correctly.

IV. Moving forward using root orders and functional classifications

Future below-ground research should be designed carefully to maximize value and broader comparability of fine-root data, and the identification of different functional categories of fine roots should be employed whenever feasible or appropriate. Moving forward, there are three likely approaches to advance more rigorous descriptions of perennial fine-root systems. One approach is to use increasingly smaller diameter cutoffs (e.g. ≤ 1.0 or ≤ 0.5 mm) to limit observations to a single functional group. Within species, diameter cutoffs can be useful as they often mirror differences among orders (e.g. Wells & Eissenstat, 2001; Burton *et al.*, 2012). However, when a single diameter class is applied across species that differ significantly in their root morphology, a single size threshold will not reasonably and equitably differentiate functional classes of roots from one species to another. For example, a diameter cutoff of 0.5 mm would include roughly three to four root orders for many *Acer* and *Pinus* species, up to five orders for *Quercus* species, and more than seven root orders for *Vaccinium* species. At the same time, a 0.5 mm cutoff would effectively exclude all roots from more coarsely rooted species, including many in the Lauraceae and the Magnoliaceae (Valenzuela-Estrada *et al.*, 2008; McCormack *et al.*, 2012; Kong *et al.*, 2014). These difficulties are not limited to temperate forests as in the example here, but will arise in any ecosystem where species with relatively thin roots regularly coincide with more species with relatively thick roots (Fig. 2).

While diameter cutoffs are difficult to apply uniformly across species, the order-based classification, in which the distal, terminal root segments are considered first-order, is widely applicable and can be used effectively to compare key aspects of root anatomy, morphology, and chemistry across species (Pregitzer *et al.*, 2002) (Fig. 1c). This approach, sometimes referred to as the morphometric or centripetal approach, is especially useful for the large, complex root systems common in woody plant species. Order-based approaches that count from the stem outwards (i.e. developmental or centrifugal approach) have also been widely used for smaller, simpler root systems often found in herbaceous plants. However, because the developmental approach cannot be readily applied to most mature woody species as a result of their spatially extensive root systems, we encourage broad and more consistent use of the morphometric approach for perennial root systems. That is, all aspects of morphology, anatomy, chemistry, and other root traits should be measured and compared on an order-specific basis, beginning with the most distal root orders whenever possible. Following wider adoption of a standardized unit for root trait comparisons, it will be increasingly important to develop and contribute these data to appropriate databases, enhancing empirical understanding of global root traits as well as providing robust datasets for use in modeling applications (Kattge *et al.*, 2011; Iversen, 2014).

Unfortunately, dissecting roots to individual root orders is time-consuming and not always logistically feasible (Table 1). In these situations, a third approach, the functional classification, separating roots ≤ 2 mm in diameter into two functional classes – absorptive fine roots and transport fine roots – may be more appropriate (Fig. 1b). In the functional classification, absorptive fine roots principally responsible for resource acquisition are separated from transport fine roots, which have reduced capacity for resource uptake and function principally to conduct or transport resources towards the stem and above-ground tissues.

In most cases, the functional classification reduces sample processing time compared with the order-based approach by avoiding the need to separate all individual root orders, but still allows for meaningful comparisons of biomass and key root processes (e.g. respiration, exudation, lifespan and turnover) across treatments, species, and sites.

Key to successful application of the functional classification is the identification of functional breaks among absorptive and transport fine roots within the root branching hierarchy. For most studies, grouping first- to third-order roots together and separating these distal branches from higher-order roots probably serves as a reasonable approximation for absorptive fine roots (Comas *et al.*, 2002; Holdaway *et al.*, 2011; Liao *et al.*, 2014). However, whenever possible, close inspection of functional root traits within a subset of roots before full sample collection can be used to formally define functional breaks within the branching order based on morphological and anatomical differences. In some cases, the transition from absorptive to transport roots may occur gradually (Figs 3, 4) and the functional unit is likely to vary among species and across biomes (Holdaway *et al.*, 2011). For example, the 'short roots' identified by Salguero-Gómez & Casper (2011) in the desert species *Cryptantha flava* take a very different form than those of the temperate tree *Fraxinus mandshurica* studied by Xia *et al.* (2010). Yet, both species displayed consistent and functionally distinct modules of roots that could be readily identified and used to simplify categorization and quantification of root processes in soils.

Once breakpoints are defined, they may be translated to more generic diameter cutoffs for individual species or sites (e.g. ≤ 0.5 mm for *Acer* spp.). These can be particularly useful when collecting samples from smaller diameter soil cores (e.g. 5 cm diameter) as the relatively small sample volume limits the number of intact root branches likely to be encountered. Increased use of larger diameter cores (> 10 cm diameter) may help to better capture intact root branches and allow for more consistent checks comparing observed branching hierarchy with the empirically established diameter cutoff, although this amount of soil disturbance may be untenable in long-term experiments.

Useful comparisons with order-based and functional approaches can still be made with older studies reporting biomass for a single fine-root pool. For example, total fine-root biomass measured in ecosystems for all roots ≤ 2 mm diameter can be compared with newer studies by summing absorptive and transport fine-root pools together. Previous studies that explicitly assessed diameter distributions within the fine-root pool (i.e. ≤ 2 mm) can also be compared with order-based studies if species-specific relationships between root diameter and order are known or measured. Additionally, estimates partitioning biomass into absorptive and transport fine-root pools provided in Tables 3 and 4 can be used to derive first approximations of biomass among these two fine-root pools from older estimates reported across all roots ≤ 2 mm in diameter. Further work refining these partitioning estimates using more species from different biomes will be valuable. In these ways, future work can continue to build on previous research while moving towards a better understanding of fine roots and their contributions to ecosystem processes.

Throughout this review, absorptive and transport roots have generally been referred to as subdivisions of the fine-root system, to draw a clear connection between this and previous 'fine-root' research. Moving forward, the terms absorptive fine roots and transport fine roots may possibly be shortened simply to absorptive roots and transport roots (both separate from coarse roots), provided that researchers clearly designate the specific pool of roots being considered. But continued work to refine these definitions and methods of fine-root study is still needed. Currently, because direct measures of root uptake rates are rare, divisions between absorptive and transport fine roots can be drawn most clearly from differences in root anatomy as well as differences in root chemistry, metabolic activity, and morphology. The development of additional approaches to differentiate functionally distinct root classes will be important, and adapting existing genomic techniques to identify meaningful differences in gene expression associated with distinct functional biochemical pathways (e.g. nutrient uptake) may prove useful (Weston *et al.*, 2008; Leakey *et al.*, 2009). While some rapid techniques have been proposed (Kong & Ma, 2014), further development of simple, consistent, and field-ready assays targeting aspects of root function, chemistry, morphology, or appearance that reveal consistent differences in root activity will also be important. For example, root color, which often changes with root age, could be a useful indicator of function in some species and could help to further segregate the most physiologically active, absorptive roots from other roots in some species (Rewald *et al.*, 2014). Similarly, development of more accurate methods to rapidly and confidently differentiate live from functionally dead roots is also needed.

V. Can we integrate mycorrhizal fungi with root classifications?

Absorptive fine roots of most perennial plants form symbiotic associations with mycorrhizal fungi. Beyond simply increasing the total absorptive area available to roots, mycorrhizal fungi also increase nutrient availability to, and uptake by, the plant by exploring small pore spaces, enhancing microbial decomposition (Hodge & Storer, 2015) and, in the case of ectomycorrhiza and ericoid mycorrhizas, exuding enzymes that actively degrade and solubilize organic compounds (Smith & Read, 2008). Focusing on only one portion of the symbiosis (the roots) at the expense of the other (the fungal hyphae and rhizomorphs in soil) may lead to incomplete or inaccurate interpretations of plant strategies for resource acquisition or ecosystem responses to environmental change (Rillig *et al.*, 1999; McCormack *et al.*, 2010).

How, then, can mycorrhizal fungi be integrated into new approaches for fine-root study? Focusing specifically on the root–fungal interface, estimates of colonization rates by root order can be a simple but important first step, particularly for arbuscular mycorrhizal symbioses (Brundrett *et al.*, 1996). As with many other traits, mycorrhizal colonization varies greatly among absorptive and transport fine roots (Fig. 3) (Guo *et al.*, 2008b). Therefore, estimates of colonization rates should be done separately for individual root orders to ensure meaningful cross-taxa comparisons of roots that are capable of being mycorrhizal (typically one to three orders for arbuscular symbioses) from roots that serve transport functions.

For ectomycorrhizal plants, assessments based on root order present interesting challenges. Most ectomycorrhizal plants have heterorhizic roots, with ectomycorrhizas forming on short, determinate roots. These ectomycorrhizas range from unbranched (monopodial) to highly branched structures, and even to compact, convoluted 'tuberculate' forms with up to thousands of root tips and where branching can no longer be clearly distinguished (Trappe, 1965). The degree of branching is determined by both plant and fungal identity. For example, the fungus *Cenococcum geophilum* typically produces unbranched or lightly branched ectomycorrhizas across all plant species, while the formation of bifurcate, Y-shaped ectomycorrhizas is only observed in gymnosperms. We suggest that branching within an ectomycorrhizal cluster should not be included in order classification, as it is impossible to count branching orders in some ectomycorrhiza (e.g. tuberculate); it would make little sense to consider a supporting fine root as a high-order root (potentially fifth-order or higher) simply because of branching within an ectomycorrhizal cluster on that root; and branching within the ectomycorrhizal cluster appears to be largely under fungal control.

Even after classifying all root tips within a cluster as first-order, regardless of internal branching, it remains unclear if ectomycorrhizal root clusters should be included together with other first-order roots or placed into a separate group. Keeping these tips separate is preferable in many ways as their morphology, anatomy, life span, and chemistry differ from other roots. Furthermore, ectomycorrhizas may themselves represent a significant portion of ecosystem productivity and below-ground biomass (Taylor *et al.*, 2000; Ostonen *et al.*, 2005; Helmisaari *et al.*, 2009). Our general suggestion is that ectomycorrhizal root tips should be separated from other first-order roots for order-based assessments of morphology, chemistry, and anatomy, particularly in systems where ectomycorrhizal roots represent a dominant component of absorptive root biomass (e.g. boreal forests) (Ostonen & Lõhmus, 2003). However, we recognize that this may not be tractable for all studies and, as such, ectomycorrhizal root tips may need to be included with other absorptive fine roots for biomass assessments following the functional classification. Finally, we extend the recommendation to the short, determinate 'nodules' of the Podocarpaceae and related gymnosperms, which might be analogous structures to ectomycorrhizal root tips and might need to be measured independently of non-'nodule' first-order roots in special cases (Dickie & Holdaway, 2011).

Moving beyond the root tip and into the soil, it will be important to characterize biomass and turnover of mycorrhizal hyphae. Currently, several approaches exist to quantitatively estimate fungal biomass and activity in soils (Miller *et al.*, 1995; Hobbie, 2006; Allen & Kitajima, 2013; Wallander *et al.*, 2013). However, more work is needed to connect information gleaned from these and other methods to ecosystem processes, particularly in terms of fungal community composition as opposed to total fungal biomass. Classifications of fungal species by life-history traits (Chagnon *et al.*, 2013), hyphal exploration types (Agerer, 2001; Peay *et al.*, 2011), enzymatic capabilities (Courty *et al.*, 2005), or tissue chemistry, persistence and decomposition (Koide & Malcolm, 2009; Fernandez & Koide, 2012, 2013) is increasingly being utilized. Knowledge of how the abundance of species or groups of fungi vary could then help to explain broad patterns in soil, plant, and ecosystem processes (Hobbie & Agerer, 2010; Phillips *et al.*, 2013; Averill *et al.*, 2014) and enable broader incorporation of both fine-root and mycorrhizal activity into ecosystem- and global-scale models (Orwin *et al.*, 2011; Brzostek *et al.*, 2014).

VI. Conclusions and recommendations

Past research defining fine roots with simple diameter cutoffs has provided valuable information, but future progress is contingent on devising standard approaches that take root form and function into account (Iversen, 2014). The order-based and functional classification approaches enable meaningful comparisons of root traits and root contributions to ecosystem processes and should replace the use of arbitrary diameter cutoffs. We suggest the following priorities and goals to help guide future research in both field studies and model development:

(1) The traditional fine-root pool should be viewed as a heterogeneous group of roots that are best defined, measured, and modeled separately as absorptive fine roots and transport fine roots. Trait comparisons involving root morphology, anatomy, chemistry, and mycorrhizal colonization should be made on an order basis. Ectomycorrhizal roots should be separated when appropriate. The functional classification, which is intended to mirror the order-based system (i.e. absorptive fine roots approximately represent the first-, second-, and often third-order roots, while transport fine roots are those of higher developmental orders characterized by secondary development), should be tested and applied where logistical constraints prevent order-based assessments, as in extensive field campaigns measuring root biomass. This approach is also appropriate for processes that are difficult to measure on individual root orders, including respiration, resource uptake, life span, and turnover.

(2) Terrestrial biosphere models should incorporate two separate fine-root pools based on the functional classification (i.e. absorptive and transport fine roots) to model fine-root distribution, dynamics, and function. These pools should be parameterized separately and respond differently to changing environmental conditions. As more data become available, it will also be important to incorporate mycorrhizal biomass and function separately from roots, leading to more accurate characterizations of ecosystem processes.

(3) Wide participation among scientists to develop and submit order-based root and fungal trait data to trait databases is critical (e.g. TRY Plant Trait Database; Kattge *et al.*, 2011). This will also facilitate comparisons between above- and below-ground plant components and help to create more of a 'one-stop shop' for modeling applications. These data would then help to identify better order- and diameter-based breaks to separate absorptive and transport fine roots among different taxa and can be used to inform subsequent work targeting underrepresented biomes and phylogenies.

(4) Focused work is needed to understand variation in root function across species and across root orders within species. Current approaches to identify functional breaks among root orders rely on morphological, anatomical, and other physical cues. Future work developing novel approaches to rapidly and consistently identify functional breaks within the root-branch hierarchy will be important.
(5) Making joint assessments of root and mycorrhizal biomass the rule rather than the exception will be critical. In the near term this will probably be contingent on close collaboration among root and fungal research groups, and in the long term requires development of easier, cheaper, and more reliable approaches to observe and quantify mycorrhizal biomass and activity than are currently available.

Few would argue that separating leaves from twigs when quantifying above-ground plant function is unnecessary or too time-consuming. A similar, updated approach to study the below-ground environment is needed. Redefining fine roots – with explicit consideration of their function – enables greater fundamental understanding of below-ground processes, variation in root traits among species, and whole-plant responses to environmental change. Simple examples highlighted earlier use known differences among absorptive and transport fine roots to better quantify patterns of C allocation to fine roots and better appreciate seasonal patterns of nitrogen content among fine-root orders. These examples illustrate the large improvements that can be made in basic understanding of below-ground processes based on functional differences among roots occurring below a traditional diameter cutoff of 2 mm.

Fine roots are a challenging, yet integral component of the terrestrial biosphere. The order-based and functional classifications advocated here provide tractable and widely applicable approaches to better quantify and understand heterogeneous root systems. Focused efforts will enable direct comparisons of root traits across a wide range of species from around the globe. Improved efforts modeling fine-root processes together with better understanding of trait relationships within species and across phylogenies together represent significant advances in our appreciation of global below-ground diversity and biogeochemical processes in the terrestrial biosphere.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Percent of total NPP estimated to be allocated to fine-root production and turnover in longleaf pine grown in a typical, xeric site in central Georgia, in the United States.

Table S1 Estimates of turnover rate (or root longevity) of transport fine roots derived using isotopic techniques that return mean ages of C in fine root pools

Table S2 Percent of live fine root biomass allocated to absorptive and transport fine roots across 11 biomes in three different scenarios

Table S3 Calculated fine root biomass (FRB) in absorptive and transport fine roots and the annual turnover of fine root biomass based on a simple two-pool model of fine root turnover

Table S4 Percent of NPP allocated to fine roots in woody dominated biomes, herbaceous dominated biomes, and globally across all biomes based on a simple two-pool model of fine root turnover

Table S5 Standing biomass and estimated annual replacement for five root orders of longleaf pine (*Pinus palustris*) fine roots subdivided into absorptive and transport fine roots

Notes S1 (a) Rescaling estimates of global NPP allocated to fine-root production and turnover; (b) rescaling estimates of NPP allocated to fine-root production and turnover in a longleaf pine forest.

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