

using net CO₂ flux measurements alone. However, because PEPC fractionates by 5.7‰ in favour of ¹³C, the carbon assimilated into root organic matter should become enriched relative to its inorganic CO₂ substrate, thus causing the remaining CO₂ measured in the soil atmosphere to become more depleted. These isotopic fingerprints of PEPC, and the results from a thorough mass balance, led Bathellier *et al.* to hypothesize that PEPC activity could be responsible for the ¹³C enrichment observed in organic matter in whole plants (see their Fig. 3) and the corresponding ¹³C-depleted CO₂ signals during the heterotrophic growth phase.

Impact of divergent isotopic signals at larger scales

The above metabolic processes could also help explain the diurnal and seasonal variability in the δ¹³C signals measured at the ecosystem scale. For example, in a Sitka spruce forest, depleted values as low as −32‰ were observed from the soil (Wingate, unpublished), whilst δ¹³C signals from respiring whole branches in the dark were more enriched (around −26‰ to −28‰; Wingate *et al.*, 2007). However, these differences in the carbon isotopic signatures from leaves and soils in the dark were observed during canopy budburst in spring only. At other times of the growing season, the δ¹³C of soil-respired CO₂ was not as depleted and similar to the δ¹³C of bulk soil organic matter, around −28‰. Some other processes linked to important transitions in canopy carbon allocation might also be responsible for these observations. More studies on the phenology of δ¹³C signals using chamber measurements on branches, woody stems and soils should allow us to identify when and where metabolic switches occur in forest ecosystems and whether these are linked to transitions in heterotrophic/autotrophic status of the trees. Studying the intra-annual isotopic signals archived in root and stem cellulose should also prove useful in confirming some of these hypotheses, as these high-resolution isotope signals in tree rings imprint such metabolic switches dramatically during the transitions between heterotrophic and autotrophic activity, during budburst and leaf senescence of deciduous trees (Helle & Schleser, 2004).

The work of Bathellier *et al.* brings some timely, highly novel insights into the respiratory isotopic divergence observed between plant organs and stages of development. Such an understanding is needed to model dark respiration and the associated carbon isotopic signals more mechanistically and help us interpret intriguing isotope signals observed at larger scales caused by plant respiration.

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Can you believe what you see? Reconciling minirhizotron and isotopically derived estimates of fine root longevity

Despite their small size, fine roots (i.e. < 2 mm in diameter) have a disproportionate importance in uptake of soil resources. By virtue of their intimate and extensive contact with soil organisms, they link plant metabolism to soil nutrient cycles. Fine roots are ephemeral and must be frequently replaced. Development of new fine roots to replace dying or dead ones (a process called root turnover) is metabolically expensive and may account for 30% of global terrestrial net primary production (NPP) (Jackson *et al.*, 1997). Their

role in the soil environment often draws comparisons with the role of leaves in the aerial environment (Tjoelker *et al.*, 2005). Yet, we know little about basic fine root biology, including how long they live. It is exciting, therefore, to hear about the development of a method for quantifying longevity of fine roots using carbon isotopes. On pages 443–456 of this issue, Guo *et al.* review this new approach and develop a statistical model that explains why estimates of root longevity derived using this new isotope approach do not match previous estimates derived from direct root observations from minirhizotrons.

'If fine roots live many years, as these studies suggest, then the proportion of NPP allocated to support fine root turnover must be substantially less than previously thought'

Choose your method

The most popular approaches for quantifying fine root turnover include sequential soil coring, mass balance approaches, such as the N budget and C budget techniques, and minirhizotrons. There are four variations on the sequential soil coring approach (reviewed in Vogt *et al.*, 1998). These include the max-min method in which production is assumed to equal the difference between the maximum and minimum standing crop observed within a given year. In a related approach, the differences in fine root biomass between sequential harvests are summed throughout the year to estimate production. The third method, referred to as the compartment flow model, is similar but it also incorporates changes in dead root pools and compensates for loss of dead roots by explicitly accounting for decomposition. The last of the traditional soil coring methods is based on sequential coring of root free in-growth cores. Nitrogen and carbon budget approaches are less direct, and less popular than sequential soil coring methods. The N budget technique estimates fine root production from N mineralization rates and the C budget approaches require accurate inventories of C fluxes at the ecosystem scale, which can be challenging and expensive to obtain (Vogt *et al.*, 1998; Nadelhoffer, 2000). All of these methods have their own advantages, but all are subject to large errors because they rely on multiple tenuous assumptions (Vogt *et al.*, 1998; Nadelhoffer, 2000).

Both soil core methods and mass balance approaches yield indirect estimates of fine root production; neither directly measures longevity of the individual roots. Fine root longevity

is equal to the reciprocal of root turnover. Root turnover is calculated by dividing either annual below-ground production or mortality by either maximum or mean yearly fine root standing crop (Gill & Jackson, 2000; Norby and Jackson, 2000). By the time production and standing crop are measured by the approaches described above, and fine root longevity is calculated, considerable error can result even when sampling the same experimental plots (Hendricks *et al.*, 2005).

In recent years, the minirhizotron has become the favorite method for characterizing fine root lifespan because it directly measures individual root longevity from repeated video or digital images (Vogt *et al.*, 1998; Majdi *et al.*, 2005). Recent advances in automating the analysis of minirhizotron images, reported in this issue by Zeng *et al.* (pp. 549–557), could lead to increased use of this method of root analysis. Based largely on minirhizotron experiments, it is now widely accepted that fine roots live an average of 1 yr or less (Guo *et al.*, this issue). Can we believe what we see? Recent estimates of longevity derived from carbon isotopic techniques suggest we cannot.

The controversy

Several recent studies based on changes in carbon isotopic ratios through time, such as bomb ^{14}C techniques, have reported that turnover time of fine roots is between 4.2 and 32 yr (Gaudinski *et al.*, 2001; Tierney & Fahey, 2002; Johnson *et al.*, 2005; Trumbore *et al.*, 2006). In one recent report based on isotope tracer experiments at Duke University and Oak Ridge National Laboratory (ORNL) free air CO_2 -enrichment sites (FACE), mean residence time (MRT) of fine root carbon, also referred to as fine root turnover (Trumbore & Gaudinski, 2003), was estimated to be 1.3–3 yr (deciduous forest) and 4.2–5.7 yr (pine forest) (Matamala *et al.*, 2003). These estimates were made possible by fumigating large forest plots with CO_2 possessing a carbon isotopic signature distinct from ambient CO_2 . Sequential cores were then harvested and the rate of depletion of old carbon in fine root biomass pools was determined. The rate of disappearance of old carbon was fitted to an exponential decay model to calculate the mean residence time of fine roots.

If fine roots live many years, as these studies suggest, then the proportion of NPP allocated to support fine root turnover must be substantially less than previously thought. The implications of this finding for how we think about root structure and function, soil microbiology, tree nutrition, and carbon cycling are significant. How can such disparate estimates of fine root longevity be reconciled?

The reconciliation

Guo *et al.* developed a statistical model to explore the potential effects of fine root heterogeneity, turnover calculation methods, longevity distribution models, and sampling bias on root longevity estimates. Their model simulations indicated

that median longevity estimates, as commonly obtained with minirhizotrons, always underestimated actual longevity, whereas simulated mean residence time of carbon, as would be obtained from isotopic studies, always overestimated longevity. Their results suggest that heterogeneity in the fine root pool accounts for the majority of this error.

Longevity distributions of fine roots are typically positively skewed, indicating the presence of multiple root pools with inherently different longevities (Fig. 1; Tierney & Fahey, 2002; Trumbore *et al.*, 2006; Joslin *et al.*, 2006). The model of Guo *et al.* accounts for this heterogeneity by assuming that the most dynamic pool is dominated by the smallest first- and second-order roots, while the longer-lived root pool is dominated by larger fourth- and fifth-order roots (Eissenstat *et al.*, 2000; Wells *et al.*, 2002; Guo *et al.*, 2004). Because isotopic studies are based on residence time of mass, they are biased by the larger, fourth- and fifth-order fine roots that are less numerous but contain more carbon and live longer than first- and second-order roots. On the other hand, because minirhizotron estimates are number (not mass)-based, they are biased by the first- and second-order roots, which are most numerous, turn over the fastest, but contain less carbon than higher-order roots. Our data from an 8 yr minirhizotron study at the Duke FACE site are consistent with results obtained by Guo *et al.* We observed a positively skewed longevity distribution for fine roots at the Duke FACE site, indicating the presence of both short- and long-lived fine roots (Fig. 1). We also observed considerable heterogeneity that could not be explained by depth or diameter (Fig. 2) and suggest that branch order might account for much of this variation.

Sampling bias inherent to different methods might also contribute to divergence of minirhizotron and isotopic results, although the modeling study by Guo *et al.* suggests

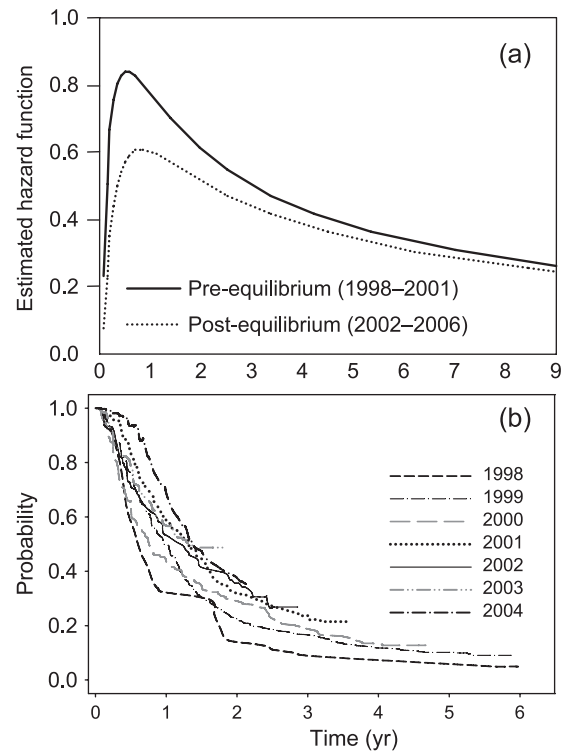
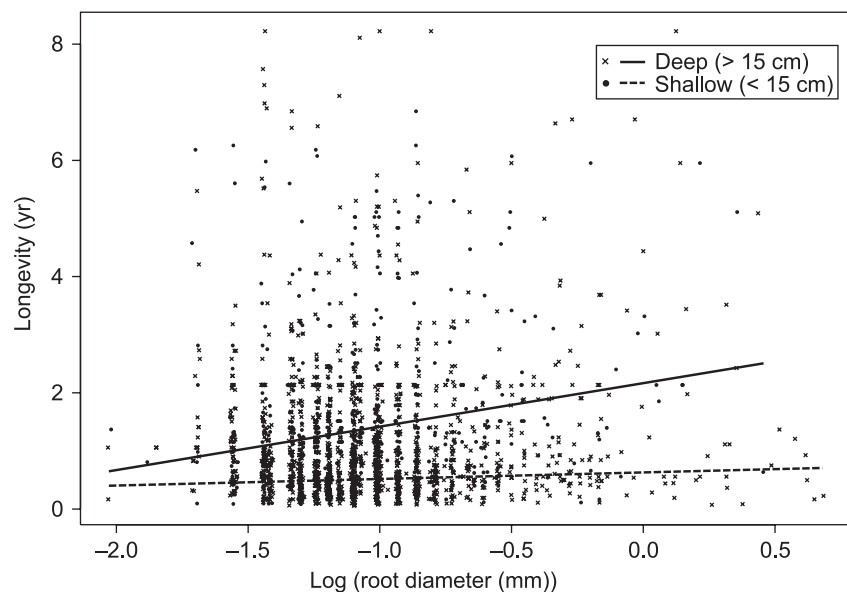


Fig. 1 (a) Estimated log-normal hazard function for all fine roots at the Duke FACE (free air CO₂ enrichment) site located in Durham, NC, USA. This hazard function illustrates that fine roots initiated during the first 3 yr of this minirhizotron experiment (i.e. pre-equilibrium) had a greater hazard of dying compared with roots that were initiated during the last 5 yr (i.e. post-equilibrium). The right-skewed nature of the hazard distribution suggests the presence of both long- and short-lived root pools. (b) Kaplan–Meier survivorship curves of fine roots initiated during each of the first 6 yr of the minirhizotron experiment. Median longevity of fine roots increased with increasing duration of the study.

Fig. 2 Relationship between root longevity (yr) and root diameter for roots produced in shallow (0–15 cm) and deep soil (15–30) at the Duke FACE site from Fall 1998 through January 2007. Longevity was determined based on visual observations of roots using minirhizotrons. Root longevity was not influenced by diameter in shallow soil, but increased with diameter in deeper soil. These data point out the heterogeneity in longevity of fine roots (< 2.0 mm in diameter).



that this effect is likely to be small. Results of isotopic and other core-based approaches are only accurate if all of the roots in a soil core are recovered and contribute to a sample mean; difficulties in recovering the finest, most ephemeral roots from soil cores are well known but not well quantified (Pierret *et al.*, 2005; Metcalfe *et al.*, 2007). There may also be sampling errors inherent to the minirhizotron technique that are not addressed in the current modeling study. Our data from Duke FACE indicate that the first roots to colonize the minirhizotron tube surface are the most dynamic and that it may take several years for the less dynamic, longer-lived roots to reach the surface and therefore for the population of roots sampled to reflect bulk soil (Fig. 1). This problem, although not explicitly addressed in the study by Guo *et al.*, likely contributes to the short turnover times reported in many minirhizotron studies of short duration (i.e. ≤ 2 yr).

Future directions

Guo *et al.* have illuminated some of the important drawbacks inherent to minirhizotron and isotopic techniques. It now seems clear that direct application of (mass-based) isotopic results to estimate fine root longevity is unwise. On the other hand, it is equally unwise to apply median root longevity values derived from short-duration minirhizotron studies to quantify flux of C and N through forest soils. We suggest that survival analysis conducted on units of root volume, instead of the individual roots themselves, could be used to better quantify turnover of fine root mass in minirhizotron studies. In longer-duration minirhizotron studies, a larger proportion of fine roots can be observed until death and therefore it is possible to estimate more accurately mean, rather than median, longevity.

The current study does not attempt to explain why fine root longevity estimates derived from sequential soil coring methods are also much lower than longevity estimates determined isotopically. Because both approaches are based on roots obtained with soil cores and since they both measure residence time of either carbon or biomass, they should be subject to similar sampling biases and should yield similar results. A review by Gill & Jackson (2000), based mainly on soil coring and ecosystem budgeting techniques, indicated that average longevity of tree fine roots is approx. 1.8 yr, far shorter than isotopic estimates. Indeed, a compartment flow study conducted at the Duke FACE experiment suggested that fine roots lived approx. 3 yr (Matamala & Schlesinger, 2000), whereas results from an isotopic study in the same plots reported root longevities of 4.2–5.7 yr (Matamala *et al.*, 2003).

The possibility that C spends a significant amount of time in storage pools, or that significant quantities of carbon-containing compounds are transported from senescing to nascent roots, needs to be further explored (Luo, 2003). Mobilization of old carbon to construct new roots could contribute to overestimation of fine root longevity estimates derived from isotopic studies (Luo *et al.*, 2004).

In the end, understanding the biology of the plant/soil continuum will require a better grasp on controls and sources of the considerable heterogeneity that characterizes the fine root pool (Fig. 2; Hishi, 2007). As Guo *et al.* have shown, branch order may account for a large proportion of variation in fine root longevity. Heterogeneity from root herbivory might also be important, considering that the smallest, most ephemeral roots are also the most nutritious (Pregitzer *et al.*, 2002). Proximity to carbohydrate sources, soil moisture, temperature, chemistry, microbiological activity, and depth are all likely to be important too. One can easily imagine the existence of fine root populations, even within a common branch order, that are adapted to function best within a particular location or at a particular time relative to tree ontogeny or the passing of the seasons. Some might argue that this heterogeneity is not important for ecosystem carbon and nitrogen budgeting exercises. But considering the physiological linkages and feedbacks between forest carbon and nitrogen dynamics and resource uptake through the finest, most ephemeral roots along with their fungal symbionts, such a view may be unwise.

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Key words: coring, fine root, isotope, longevity, minirhizotron, root turnover.

Letters

The endodermis: a horsetail's tale

Cui *et al.* (2007) provided a compelling explanation of the mechanism controlling endodermis specification in the root of *Arabidopsis*. The mechanism consists of a positive feedback loop that involves the transcription factors SHORTROOT (SHR) and SCARECROW (SCR). According to Cui *et al.*'s (2007) model, endodermal specification is achieved by export of SHR from the root stele into endodermis precursors that express SCR. There, SHR binds to SCR and together they promote production of more SCR, which traps all SHR exported from the stele so that none can escape beyond the endodermal layer to trigger the formation of additional endodermises. Interestingly, Cui *et al.* (2007) also identified potential functional homologs of SHR and SCR in the rice

root. Based on these findings, they suggested that an SHR-SCR feedback loop-type mechanism of endodermal specification might have been acquired early in land plant evolution and could be conserved across most vascular plants. If this were true, then Cui *et al.*'s (2007) model could apply to the development of the endodermis in most, if not all, tracheophytes. The identification, in *Pinus sylvestris* roots, of an SCR putative homolog expressed in the quiescent center equivalent (stele and root cap initials) and in the endodermis (Laajanen *et al.*, 2007) suggests that SCR has comparable functions (maintenance of root radial patterning and endodermal specification) in gymnosperms and angiosperms. This indicates that Cui *et al.*'s (2007) model could indeed apply at least to all seed plants, and provides additional impetus for the hypothesis of a conserved mechanism controlling endodermal specification across all vascular plants.

Discussing this exciting possibility, Dolan (2007) pointed out that there are some rare exceptions from the simple structure