Fine root heterogeneity by branch order: exploring the discrepancy in root turnover estimates between minirhizotron and carbon isotopic methods

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Summary

• Fine roots constitute a large and dynamic component of the carbon cycles of terrestrial ecosystems. The reported fivefold discrepancy in turnover estimates between median longevity (ML) from minirhizotrons and mean residence time (MRT) using carbon isotopes may have global consequences.

• Here, a root branch order-based model and a simulated factorial experiment were used to examine four sources of error.

• Inherent differences between ML, a number-based measure, and MRT, a mass-based measure, and the inability of the MRT method to account for multiple replacements of rapidly cycling roots were the two sources of error that contributed more to the disparity than did the improper choice of root age distribution models and sampling bias. Sensitivity analysis showed that the rate at which root longevity increases as order increases was the most important factor influencing the disparity between ML and MRT.

• Assessing root populations for each branch order may substantially reduce the errors in longevity estimates of the fine root guild. Our results point to the need to acquire longevity estimates of different orders, particularly those of higher orders.

Key words: carbon isotope, ecosystem carbon balance, fine roots, minirhizotron, root branch order, root longevity, root turnover.

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Introduction

Fine root turnover has been reported to transfer > 30% of net primary productivity to the soil in terrestrial ecosystems at the global scale (Jackson *et al.*, 1997). Despite the importance of fine root turnover, conceptual problems, such as determining what constitutes a fine root (Pregitzer, 2002), what assumptions may be made regarding their dynamics (Trumbore & Gaudinski, 2003), and how these dynamics are best measured (Tierney & Fahey, 2002; Hendricks *et al.*, 2006) have hindered the understanding of root dynamics at the ecosystem scale. Specifically, published estimates of fine root longevity (or turnover time) differ more than fivefold (Table 1). Since fine roots are increasingly recognized as a key to balancing whole-tree and ecosystem carbon (C) budgets (Norby & Jackson, 2000; Matamala *et al.*, 2003; Norby *et al.*, 2004), such large discrepancies in root longevity and turnover estimates lead to uncertainty in assessing

Table 1 Comparison of fine root longevity (turnover time) estimates derived from the minirhizotron (MR) and C isotope (bomb ^{14}C and free-air CO_2 enrichment (FACE) ^{13}C) methods

Species/ecosystem	Live fine root size (mm)	Method	Calculation approach	Longevity distribution	Longevity (yr)	Sources[d]
Pinus taeda	< 1	MR	Length production	Not estimated	0.5	1
	< 1	FACE ^{13}C	MRT	Exponential (assumed)	4.2	2
	1–2	MR	Length production	Not estimated	0.8	1
	1–2	FACE ^{13}C	MRT	Exponential (assumed)	5.7	2
Picea abies	< 1	MR	Length production	Not estimated	1.1	3
	< 1	MR	ML[a]	Not estimated	1.0	3
	< 2	MR	ML	Not estimated	0.8	4
Northern hardwoods	< 0.5	MR	Length production	Not estimated	1.5	5
at Hubbard Brook	< 0.5	MR	ML	Not estimated	1.9	5
Experimental Forest	< 0.5	MR	Parametric regression	Lognormal	3.3[b]	5
	< 0.5	Bomb ^{14}C	Bomb ^{14}C age	Normal (assumed)	4.5[c]	5
Various temperate forests	< 2	MR	ML	Not estimated	0.04–1	6, 7
Temperate forests at	< 0.5	Bomb ^{14}C	MRT	Normal (assumed)	3–5	8
Harvard Forest	0.5–3	Bomb ^{14}C	MRT	Normal (assumed)	22–32	8
Amazonian forests	< 2	Bomb ^{14}C	MRT	Normal (assumed)	7–11	9

ML, median longevity; MRT, mean residence time.
[a]This median longevity is length-based, not number-based as are others in this table.
[b]This value was derived from long-term (> 5 yr) MR observations by using parametric regression.
[c]This value was estimated from the roots of known age (3.3 yr) (see note 'b').
[d]Sources: 1, King *et al*. (2002); 2, Matamala *et al*. (2003); 3, Majdi & Andersson (2005); 4, Majdi & Kangas (1997); 5, Tierney & Fahey (2002); 6, Eissenstat & Yanai (1997); 7, Clark *et al*. (2001); 8, Gaudinski *et al*. (2001); 9, Trumbore *et al*. (2006).

terrestrial C cycles (Trumbore & Gaudinski, 2003; Högberg & Read, 2006).

While the large discrepancy among fine root turnover estimates may be related to variations in climate (Tierney *et al*., 2003), resource availability (Burton *et al*., 2000), and species (Matamala *et al*., 2003), the disparity among estimates may also be the result of differences in methods (Tierney & Fahey, 2002). Minirhizotron (MR) studies have found that fine roots (≤ 2 mm) live for 1 yr (Table 1), whereas studies based on C isotope approaches, such as ^{14}C dilution (emanating from bomb detonations; Gaudinski *et al*., 2001) and ^{13}C enrichment (as part of the free-air CO_2 enrichment (FACE); Matamala *et al*., 2003) have reported considerably longer (e.g. > 5 yr; Table 1) root C residence times, which have been interpreted as long turnover times. Thus, it is critical to evaluate the efficacy of the MR and C isotope techniques for measuring fine root turnover at the ecosystem scale (Tierney & Fahey, 2002; Luo, 2003).

Recent literature suggests that at least four factors contribute to the disparity among root longevity and turnover estimates between MR and C isotope methods. First, fine roots, conceptually treated as a homogenous pool previously, are now found to be a mixture of highly heterogeneous 'populations' (Wells & Eissenstat, 2001; Pregitzer *et al*., 2002; Tierney & Fahey, 2002; Guo *et al*., 2004). Even within fine roots < 0.5 mm, a 0.1 mm increase in diameter can lead to 43% increase in lifespan (Wells & Eissenstat, 2001; Tierney & Fahey, 2002), and one unit increase in root order can result in > 100% increase in root lifespan (Table 2). In a theoretical study of tropical tree stem turnover rates (i.e. mortality and recruitment), Sheil & May (1996) showed that heterogeneity in individual stem turnover rates may create artifacts in mean turnover estimates, and suggested that similar problems would influence any turnover estimation procedure that did not account for all the rate variation within a study population. It is increasingly recognized that fine roots ≤ 2 mm contain an extremely short-lived group and a long-lived group such that a single-pool model is not sufficient for characterizing fine root turnover (Högberg & Read, 2006; Joslin *et al*., 2006). Yet how the heterogeneity within the fine root guild influences root turnover estimates has not been thoroughly analyzed in either MR (Tierney & Fahey, 2002; Majdi *et al*., 2005) or bomb ^{14}C and FACE ^{13}C labeling experiments (Giardina *et al*., 2005).

Second, the MR and C isotope methods calculate turnover differently (Table 1). In MR studies, the inverse of median longevity is often used to represent turnover (Eissenstat & Yanai, 1997; Fahey *et al*., 1999). Median longevity (ML) is a number-based measure and is dominated by first-order roots (Pregitzer *et al*., 2002; Wang *et al*., 2006). In bomb ^{14}C and FACE ^{13}C labeling studies, mean residence time (MRT) of C, a mass-based measure, has been used to define root age or time needed for a group of fine roots (< 1 or 2 mm) to turnover once (Matamala *et al*., 2003; Majdi *et al*., 2005). Depending on the actual root mass distribution among root orders, large differences between ML and MRT may occur simply because

Table 2 Summary of studies that estimated root longevity by branch order – all these studies show that longevity of a lower order is less than half of an immediately higher order

Plant species/ community	Order (diameter)	No. roots analyzed	Longevity[a] (d)	Survival distribution	Observation length (months)	Sampling interval (wk)	Method	Sources[b]
Acer saccharum	First order (< 0.25 mm)	N/A	339	Right-skewed	24	4	MR	1
	Second and third order (> 0.25 mm)	N/A	775	N/A				
Picea abies	First order	400	400	N/A	48	4	MR	2
	Second order	80	980	N/A				
Prunus persica	First order	N/A	105	Right-skewed	12	2–4	MR	3
	Second and third order	N/A	226	Right-skewed				
Actinidia deliciosa	First order	763	< 28	Right-skewed	23	2	Rhizotron	4
	Higher order	47	< 112	Poly modal				
Temperate forest	Lateral roots (< 1 mm)	N/A	1461 (4 yr)	N/A	N/A	N/A	Bomb ^{14}C	5
	Main stem roots (< 1 mm)	N/A	4019 (11 yr)	N/A	N/A	N/A		

[a]Longevity estimates from minirhizotron (MR) and rhizotron were median longevity.
[b]Sources: 1, Wells (1999); 2, Majdi *et al.* (2001); 3, Wells *et al.* (2002); 4, Reid *et al.* (1993); 5, Gaudinski *et al.* (2001).

of the difference between a number-based and a mass-based measure.

Third, the age structure of fine roots must be ascertained to accurately predict root turnover (Trumbore & Gaudinski, 2003; Trumbore *et al.*, 2006). The MR method directly assesses the age structure of the sampled populations, and a positively skewed longevity distribution is typical (Tierney & Fahey, 2002). In contrast, the C isotope method must assume a probability distribution for fine root age structure. Both normal (i.e. the highest probability of death in mid-aged roots; Meeker & Escobar, 1998) and exponential (i.e. the same probability of death for all roots; Meeker & Escobar, 1998) distributions have been used (Gaudinski *et al.*, 2001; Matamala *et al.*, 2003). Although different root age distribution models (e.g. positively skewed vs exponential) can lead to a 50% difference in estimated root longevity based on isotopic signals (Luo, 2003), the extent to which root age (longevity) distribution models influence MR vs C isotope turnover estimates remains undetermined.

Fourth, the MR and C isotope methods may not adequately sample the full range of populations within the fine root guild (Trumbore & Gaudinski, 2003). The MR method is more likely to sample the smaller and more dynamic lower-order roots (especially the distal first-order root tips), whereas soil cores used in C isotope studies may miss the smaller and more fragile lower-order roots, which are extremely difficult to extract from soil cores, and preferentially sample large and slow-cycling roots (Gaudinski *et al.*, 2001; Pregitzer, 2002). The potential effects of such sampling biases on root turnover estimates have yet to be quantified.

The primary objective of this study was to determine the potential effects of fine root heterogeneity, turnover calculation approaches, longevity distribution models, and sampling biases on the divergence in fine root longevity and turnover estimates between the MR and C isotope methods, and, specifically, between ML and MRT. A simulation model of fine root turnover, root turnover simulator (RTS), was developed specifically to evaluate the potential influences of these four factors that may account for the discrepancies in fine root turnover estimates.

Materials and Methods

Model structure

Root turnover simulator is a statistical model developed to simulate fine root populations and calculate measures of fine root longevity and turnover as used by MR and C isotopes. All equations and associated parameters used in RTS are explicitly defined in Table 3 (Eqns 1–9). All parameters in RTS are biologically based, and can be determined by field observations.

The RTS model was developed on the premise that the architecture or branching of fine root systems may be

Table 3 Root turnover simulator (RTS) model: definitions and equations of variables and parameters

Equation number	Variable name	Equation	Parameter name	Parameter equation (or symbol)
1	Root number by order	$N_k = N_1 \cdot S_N^{k-1}$	(a) Total number of fine roots	N
			(b) Branch order	k
			(c) Number scaling exponent	S_N
			(d) Root number in the first order	$N_1 = N \Big/ \left(1 + \sum_{k=2}^{D} S_N^{k-1}\right)$
			(e) Total number of branch order	D
2	Average individual root biomass by order	$B_k = B_1 \cdot S_B^{k-1}$	(a) Biomass scaling exponent	S_B
			(b) Average first-order root biomass	B_1
3	Average root longevity by order	$\bar{L}_k = \bar{L}_1 \cdot S_L^{k-1}$	(a) Longevity scaling exponent	S_L
			(b) Average first-order root longevity	$\bar{L}_1$
4	Root longevity SD by order	$\sigma_k = \sigma_1 \cdot S_L^{k-1}$	(a) The standard deviation (SD) of root longevity in the first order	σ_1
5	Longevity distribution of individual roots	$L_{ki} = \{L \mid L \sim \text{pdf}(t; \vec{p}_k)\}$	(a) Longevity probability density function (pdf)	$\text{pdf}(t; \vec{p}_k)$
			(b) Parameters of longevity probability distribution by order	$\vec{p}_k$
			(c) Time of death	t
6	Estimated average root longevity	$\bar{L}$	(a) ML in MR	$L_{\text{median}} = \text{MEDIAN}\{L_{ki}\}$
			(b) Biomass weighted mean longevity by C isotopes (BWML or MRT)	$\text{BWML} = \sum_{k=1}^{D}\sum_{i=1}^{N_k} B_{ki} \cdot L_{ki} \Big/ \sum_{k=1}^{D}\sum_{i=1}^{N_k} B_{ki}$
7	Estimated biomass mortality	$\hat{M} = B_{\text{total}}/\bar{L}$	(a) Total fine root biomass	B_{total}
8	Actual biomass mortality[a]	$M = \sum_{k=1}^{D}\sum_{i=1}^{N_k} B_{ki} \cdot \frac{1}{L_{ki}}$	(a) Branch order	k
			(b) Individual roots in each order	i
			(c) Total number of branch orders	D
9	Actual average longevity	$L^* = M/B_{\text{total}}$		

[a]Actual number mortality can be calculated by removing the biomass term (B_{ki}) from the equation.

represented as self-similar fractals of root orders (Fig. 1; West *et al.*, 1999; Sismilich *et al.*, 2003). Branching pattern was chosen as the core of RTS because order can effectively categorize the heterogeneous fine root pool into more homogeneous subpopulations in a systematic manner, thereby facilitating comparisons across species of different root architectures (Pregitzer *et al.*, 2002; Withington *et al.*, 2006). Model simulations were primarily controlled by three allometric scaling functions: the root number scaling exponent (S_N in Eqn 1, Table 3), or the rate at which total root number of each order increases as order increases; the root biomass scaling exponent (S_B in Eqn 2, Table 3), or the rate at which average individual root biomass increases as order increases; and the longevity scaling exponent (S_L in Eqn 3, Table 3), or the rate at which average longevity in each order increases as order increases. The scaling exponents were based on empirical data of root characteristics across orders (Reid *et al.*, 1993; Wells, 1999; Gaudinski *et al.*, 2001; Majdi *et al.*, 2001; Pregitzer *et al.*, 2002; Wells *et al.*, 2002; Guo *et al.*, 2004; Wang *et al.*, 2006), and the relationships derived

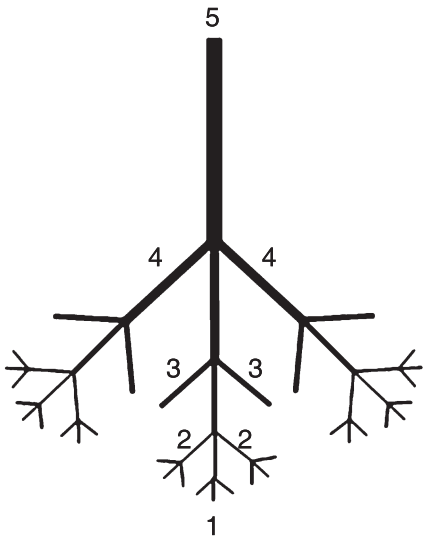

Fig. 1 A schematic branching root system consisting of five root orders (lines in different thickness, labeled as 1, 2, 3, 4, 5) with a number scaling exponent (or the ratio between root number of a higher order to root number of one order lower) of 0.33 between successive orders. The ordering scheme follows the conventional Strahler's stream ordering system used in a recent paper on fine root architecture (Pregitzer *et al*., 2002). The ordering rules are: (i) all roots without branches are the first order, or the most distal portions of root system; (ii) where two roots of order *i* join, they both terminate and give rise to a root of order *i* + 1; and (iii) where two roots of differing order meet, the lower-order root terminates and the higher-order root continues.

from allometry theory of vascular plant systems (hereafter referred to as WBE theory) (West *et al*., 1999). In addition to the scaling functions, theoretical probability distributions (PD) were used to characterize individual root longevity within each order (L_{ki} in Eqn 5, Table 3).

Based on simulated root population data, RTS calculated longevity distribution parameters (e.g. mean, median, skewness), biomass weighted mean longevity (BWML or MRT; yr), total standing biomass (g), root number mortality (no. yr^{-1}), biomass mortality (g yr^{-1}), and mortality-related variables such as survival and hazard rates for each order and the entire fine root pool. The output variables were defined as follows (Table 3). The estimated average root longevity was characterized by different measures of population longevity ($\bar{L}$ in Eqn 6, Table 3); median longevity (ML) was used for the MR method (L_{median} in Eqn 6a, Table 3), and the biomass weighted mean longevity for the C isotope approach (BWML or MRT in Eqn 6b, Table 3 – note that BWML is equivalent to MRT, Supplementary material, Appendix S1). The estimated biomass mortality was calculated by dividing the total standing biomass by estimated average root longevity ($\hat{M}$ in Eqn 7, Table 3). The actual biomass mortality was the total amount of actual individual root biomass mortality for the entire fine root population, defined as the sum of the product of the biomass and the turnover of all individual roots (M in Eqn 8, Table 3). It should be noted that actual biomass mortality was calculated based on the assumption that roots within each order are in a steady state (i.e. the death of an individual root will be immediately followed by the birth of an identical root). The actual average longevity was calculated by dividing the actual biomass mortality with the total standing biomass (L^* in Eqn 9, Table 3). The 'estimated' values were those approximated based on summary statistics (i.e. median longevity, or MRT), whereas the 'actual' values were those calculated directly from all the individual roots of the simulated root populations.

Experimental design

To evaluate potential sources of error in estimates of fine root longevity and turnover, the RTS model was used to conduct a factorial experiment with three factors (Table 4): number scaling exponent (S_N; three levels), longevity scaling exponent (S_L; three levels), and probability distribution (PD; four levels). Three levels of scaling exponents were used for both S_N and S_L, representing possible variability in these two scaling exponents (Table 4). For PD, root longevity was characterized by one of the four theoretical probability distributions (Table 4; Meeker & Escobar, 1998). For simplicity, all root orders or subpopulations had the same PD within a simulation. Thus, in Monte Carlo simulations, the longevity of individual roots was determined by Eqn 5 in Table 3 with the distributional parameters of shape, scale, and threshold being defined primarily by the mean and variance of root longevity (Meeker & Escobar, 1998). The experiment had 36 ($3 \times 3 \times 4$) treatments with 10 replicates per treatment, yielding a total of 360 simulations.

Other key model parameters were set at controlled values. The number of branch orders was set at five based on empirical data for fine roots ≤ 2 mm in diameter (Guo *et al*., 2004; Wang *et al*., 2006). The total number of individual roots was controlled at 10 000, a value large enough to allow the numbers of individual roots in all five orders to be sufficient for statistical analyses. The mean longevity of the first-order roots was set at 0.7 yr (Ruess *et al*., 2003; Withington *et al*., 2006), while longevity variability in each order was characterized by a coefficient of variation (CV) of 100% (Reid *et al*., 1993; Wells, 1999; Majdi *et al*., 2001; D. L. Guo, unpublished). Biomass scaling exponent was not considered as a factor explicitly, but was set to follow S_N based on a theoretical relation defined by the pipe model (i.e. $S_B = (S_N)^{4/3}$), which was the inverse of the relationship used by West *et al*. (1999) because we defined root branching hierarchy differently (i.e. from distal branches toward the main trunk). The pipe model uses an area-preserving principle and assumes a simple linear relationship between the biomass of individual roots and root volume (West *et al*., 1999). Biomass of individual roots was determined by assuming each to have the same mass in the same order, because of a lack of data relating root mass with

Table 4 Levels and parameter values used in the simulation experiment

Component	Parameter	Levels	Value	Sources[a]
Architecture	Number scaling exponent (S_N)	3	Level 1 = 0.5	1
			Level 2 = 0.33	2
			Level 3 = 0.25	3
Longevity	Longevity scaling exponent (S_L)	3	Level 1 = 1.5	
			Level 2 = 2.0	4
			Level 3 = 2.5	
Longevity	Probability distributions (PD)	4	Level 1 = normal	5
			Level 2 = lognormal	6
			Level 3 = Weibull	7
			Level 4 = exponential	8

[a]Sources: 1, West *et al*. (1999); 2, Sismilich *et al*. (2003), Pregitzer *et al*. (2002), Wang *et al*. (2006); 3, D. L. Guo, unpublished data on > 40 Chinese temperate tree species; 4, all references listed in Table 2, which showed a S_L value of 2.0 for the first two orders; we assumed other orders follow the same S_L; to reduce the uncertainty, we allowed S_L to vary from 1.5 and 2.5; 5, Gaudinski *et al*. (2001); 6, Tierney & Fahey (2002); 7, Black *et al*. (1998); 8, Matamala *et al*. (2003).

longevity, and by setting the average biomass of the first-order roots at 0.1 mg (Wang *et al*., 2006).

As previously described, four potential sources of error in root longevity or turnover estimates with the MR and C isotope methods were examined: root heterogeneity; turnover calculation method; age structure assumptions; and sampling bias. In the case of sampling bias, we used a simplified assumption that MR samples only root orders 1–3, whereas C isotope sampling includes only root orders 2–5. While this simple approach may overestimate sampling bias, as not all roots in the omitted orders would actually be missed in field sampling, it can quantify to what degree the sampling bias of assumed magnitude influences root longevity and turnover estimates.

Sensitivity analysis

Sensitivity analysis was performed for the RTS model to determine the effects of changing input parameters on output variables (Katz, 2002; Li & Wu, 2006). All six input parameters were examined as independent factors, including the probability distribution as a nominal variable. It should be noted that the biomass scaling exponent was treated as a function of the number scaling exponent in the simulation experiment but evaluated independently in sensitivity analysis. The expected values for the input parameters were based on values from the literature or our unpublished data: (i) 0.33 for S_N (Pregitzer *et al*., 2002; Wang *et al*., 2006); (ii) 3.5 for S_B (Wang *et al*., 2006; D. L. Guo, unpublished); (iii) 2.0 for S_L (all references listed in Table 2); (iv) 1.0 for CV (D. L. Guo & R. J. Mitchell, unpublished); (v) normal for PD; and (vi) 0.7 yr for average longevity of the first order (Ruess *et al*., 2003; Withington *et al*., 2006). The effects of the six input parameters were assessed for eight output variables: (i) actual average longevity; (ii) median longevity (ML); (iii) MRT; (iv) the difference between ML and MRT (%); (v) error of ML; (vi) error of MRT; (vii) actual root number mortality; and (viii) actual root biomass mortality.

Results

The five fine root branch orders differed markedly in the total root number and root number mortality (Table 5). Across all 36 treatments, first-order roots consistently accounted for > 50% of total root number and > 60% of total root number mortality of the fine root pool (Table 5). The first order also contributed more than other orders to the biomass mortality of the total fine root pool in the majority of treatments (Table 6). The contribution by the first order to the total fine root biomass mortality was > 30% in 26 out of 36 treatments (detailed data not shown). Except for one case ($S_N = 0.25$, $S_L = 1.5$, longevity distribution = Weibull), the contribution of the first order to the total fine root biomass mortality was consistently equal to or greater than that of any other order.

The calculation approaches for the MR and C isotope methods yielded different estimates of root longevity for the same fine root populations and the difference varied as the scaling exponents were varied (Table 7). Across all treatments, ML was consistently lower than MRT, and the difference between the two longevity measures varied from 129% ($S_N = 0.5$ and $S_L = 1.5$) to 1647% ($S_N = 0.25$ and $S_L = 2.5$) (Table 7). Notably, the variation in the difference between ML and MRT was mainly caused by changes in MRT, not ML. Both S_N and S_L influenced MRT, but the influence by S_L was much greater (Table 7).

The discrepancy in longevity estimates between the MR and the C isotope techniques translated into substantial differences in biomass mortality estimates (Table 8). Compared with actual average longevity, ML overestimated root biomass mortality when its inverse was used as turnover rate, whereas

Table 5 Root number and root number mortality among the five branch orders under different number scaling exponent (S_N), longevity scaling exponent (S_L), and the longevity distributions

Root order	Root number	Normal (no. yr^{-1})	Weibull (no. yr^{-1})	Lognormal (no. yr^{-1})	Exponential (no. yr^{-1})
$S_N = 0.5$, $S_L = 1.5$					
1	5161 (52)	10 959 (72.5)	13 348 (61.7)	9202 (69.9)	8753 (69.3)
2	2581 (26)	2916 (19.3)	5342 (24.7)	2726 (20.7)	2664 (21.1)
3	1290 (13)	867 (5.7)	2045 (9.5)	858 (6.5)	851 (6.7)
4	645 (6.5)	280 (1.9)	722 (3.3)	279 (2.1)	278 (2.2)
5	323 (3.2)	92 (0.6)	181 (0.8)	91 (0.7)	92 (0.7)
Sum	10 000 (100)	15 114 (100)	21 638 (100)	13 156 (100)	12 638 (100)
$S_N = 0.33$, $S_L = 2$					
1	6726 (67)	14 089 (87.4)	17 531 (80.4)	12 070 (85.7)	11 501 (85.3)
2	2220 (22)	1715 (10.6)	3610 (16.6)	1695 (12.0)	1661 (12.3)
3	732 (7.3)	265 (1.6)	547 (2.5)	263 (1.9)	264 (2.0)
4	242 (2.4)	43 (0.3)	98 (0.5)	43 (0.3)	44 (0.3)
5	80 (0.8)	7 (0.04)	13 (0.1)	7 (0.1)	7 (0.1)
Sum	10 000 (100)	16 120 (100)	21 799 (100)	14 079 (100)	13 477 (100)
$S_N = 0.25$, $S_L = 2.5$					
1	7508 (75)	15 447 (92.6)	19 346 (87.1)	13 309 (91.5)	12 761 (91.2)
2	1877 (19)	1124 (6.7)	2607 (11.7)	1117 (7.7)	1103 (7.9)
3	469 (4.7)	108 (0.65)	210 (0.95)	108 (0.74)	107 (0.77)
4	117 (1.2)	11 (0.07)	36 (0.16)	11 (0.07)	11 (0.08)
5	29 (0.3)	1 (0.01)	3 (0.02)	1 (0.01)	1 (0.01)
Sum	10 000 (100)	16 691 (100)	22 202 (100)	14 544 (100)	13 984 (100)

Values in parenthesis are percentages represented by each order.
Notes: only 12 out of 36 S_N and S_L treatment combinations (three levels of S_N × three levels of S_L × four longevity distributions) are presented here to represent the lowest ($S_N = 0.5$ and $S_L = 1.5$), middle ($S_N = 0.33$ and $S_L = 2$), and highest ($S_N = 0.25$ and $S_L = 2.5$) proportions accounted for by the first order in both total root number and root number mortality.

Table 6 Root biomass and mortality among the five branch orders under different number scaling exponent (S_N), longevity scaling exponent (S_L), and the longevity distributions

Root order	Root biomass (g)	Normal (g yr^{-1})	Weibull (g yr^{-1})	Lognormal (g yr^{-1})	Exponential (g yr^{-1})
$S_N = 0.25$, $S_L = 1.5$					
1	0.7508 (6.5)	1.565 (22.7)	1.951 (15.4)	1.339 (20.4)	1.281 (20.0)
2	1.1917 (10.3)	1.431 (20.7)	2.671 (21.1)	1.269 (19.4)	1.226 (19.2)
3	1.8907 (16.4)	1.304 (18.9)	2.740 (21.6)	1.260 (19.2)	1.246 (19.5)
4	2.9948 (26.0)	1.286 (18.6)	2.832 (22.3)	1.314 (20.6)	1.294 (20.3)
5	4.7131 (40.8)	1.316 (19.1)	2.493 (19.7)	1.369 (20.9)	1.344 (21.1)
Sum	11.5411 (100)	6.902 (100)	12.687 (100)	6.551 (100)	6.391 (100)
$S_N = 0.33$, $S_L = 2$					
1	0.6726 (8.4)	1.409 (42.7)	1.753 (30.8)	1.207 (39.1)	1.150 (38.1)
2	0.9735 (12.1)	0.752 (22.8)	1.583 (27.9)	0.743 (24.1)	0.729 (24.2)
3	1.4075 (17.5)	0.510 (15.5)	1.052 (18.5)	0.507 (16.4)	0.509 (16.9)
4	2.0404 (25.3)	0.365 (11.1)	0.826 (14.5)	0.365 (11.8)	0.366 (12.1)
5	2.9577 (36.7)	0.263 (8.0)	0.470 (8.3)	0.265 (8.6)	0.265 (8.8)
Sum	8.0517 (100)	3.299 (100)	5.684 (100)	3.087 (100)	3.019 (100)
$S_N = 0.5$, $S_L = 2.5$					
1	0.5161 (11.9)	1.048 (59.3)	1.352 (45.1)	0.908 (55.8)	0.880 (55.2)
2	0.6503 (15.1)	0.390 (22.1)	0.900 (30.0)	0.387 (23.8)	0.383 (24.0)
3	0.8191 (19.0)	0.188 (10.6)	0.441 (14.7)	0.189 (11.6)	0.188 (11.8)
4	1.0319 (23.9)	0.094 (5.3)	0.205 (6.8)	0.094 (5.8)	0.094 (5.9)
5	1.3021 (30.2)	0.048 (2.7)	0.103 (3.4)	0.048 (3.0)	0.048 (3.0)
Sum	4.3195 (100)	1.768 (100)	3.001 (100)	1.626 (100)	1.593 (100)

Values in parenthesis are percentages represented by each order.
Notes: only 12 out of 36 are presented to represent the lowest ($S_N = 0.25$ and $S_L = 1.5$), middle ($S_N = 0.33$ and $S_L = 2$), and highest ($S_N = 0.5$ and $S_L = 2.5$) proportions accounted for by the first order in both total root biomass and root biomass mortality.

Table 7 Different measures of root longevity under different root number scaling exponent (S_N) and longevity scaling exponent (S_L)

Number/longevity scaling exponent	Average longevity of first order (yr)	Average longevity of fifth order (yr)	ML (yr)	MRT (yr)	Difference %[a]
$S_N = 0.5$					
$S_L = 1.5$	0.72	3.56	0.95	2.18	129
$S_L = 2.0$	0.73	11.22	1.11	5.55	402
$S_L = 2.5$	0.72	27.32	1.24	12.03	872
$S_N = 0.33$					
$S_L = 1.5$	0.72	3.49	0.85	2.35	178
$S_L = 2.0$	0.72	11.26	0.90	6.28	598
$S_L = 2.5$	0.72	27.42	0.93	13.88	1397
$S_N = 0.25$					
$S_L = 1.5$	0.72	3.60	0.80	2.49	212
$S_L = 2.0$	0.72	11.30	0.84	6.62	687
$S_L = 2.5$	0.72	27.38	0.86	14.96	1647

[a]Difference % is the relative difference between median longevity (ML) and mean residence time (MRT) of the total fine root pool and was calculated as 100% · (MRT–ML)/ML. Root longevity distribution was assumed to be Normal (longevity distribution had only slight influences on the difference% as indicated by a CV of < 15% among four distributions). Only the average longevities of the first and the fifth order were provided, given that other orders can be calculated from the average longevity of the first order and the scaling exponent S_L.

MRT underestimated it (Table 8). The degree of error in root biomass mortality estimates was similar for ML and MRT; the error ranged from 141% ($S_N = 0.5$ and $S_L = 1.5$) to 438% ($S_N = 0.25$ and $S_L = 2.5$) for ML, and from 188% ($S_N = 0.5$ and $S_L = 1.5$) to 502% ($S_N = 0.25$ and $S_L = 2.5$) for MRT (detailed data now shown). Longevity scaling exponent, S_L, which determines the degree of demographic heterogeneity in the fine root population, had a strong impact on the difference between ML and MRT (Table 7), and on the magnitude of error in longevity and biomass mortality estimates by ML and MRT (Table 8).

Longevity distribution models also influenced biomass mortality estimates. Compared with the normal model, the actual biomass mortality was 72–88% higher for the Weibull model, but 7% lower for the lognormal model and 10% lower for the exponential model (calculated from data in Table 8).

Simulated bias in sampling the five fine root orders yielded moderate error in the actual biomass mortality and actual average longevity estimates for both methods (Table 9). The actual biomass mortality was underestimated by 9–41% with the MR method (i.e. excluding the fourth- and fifth-order roots) and by 20–54% with the C isotope soil core methods (i.e. excluding the first-order roots) (Table 9). By contrast, sampling biases led to underestimation of actual average root longevity by 14–48% in the MR method, but overestimation by 16–93% in C isotope methods (Table 9). For both variables, the errors caused by sampling biases of the two methods varied with the number and the longevity scaling exponent (Table 9).

Sensitivity analysis

Sensitivity analysis revealed relationships between model input parameters and output variables (Fig. 2). For measures of root longevity, positive relationships with input parameters were observed. MRT was more sensitive to changes in input parameters than the actual average longevity, which in turn was more sensitive than ML (Fig. 2a–c). The difference between ML and MRT was most sensitive to S_L, the factor to which MRT was also most sensitive (Fig. 2c,d). All three scaling exponents produced strong responses in error of ML, whereas S_L was the only scaling exponent that caused a large change in error of MRT (Fig. 2e,f). For measures of root mortality, the actual biomass mortality showed greater responses to input parameters with both positive (S_N and S_B) and negative (S_L and L_1) relationships (Fig. 2h), while the actual root number mortality was primarily affected by L_1 with a negative relationship (Fig. 2g).

Different individual model parameters affected a different set of output variables (Fig. 2). S_N caused most change in the actual average longevity, had some effects on error of ML and the difference between ML and MRT, but little effect on error of MRT. S_B was the most critical parameter to both the actual average longevity and error of ML. S_L strongly affected more output variables than any other input parameters, and the output variables being influenced including MRT, error of MRT, the difference between ML and MRT, and the actual biomass mortality. However, S_L showed little effect on ML. L_1 caused negative effects of similar magnitude on the two mortality measures, produced positive responses in ML, but showed little influence on error of ML, error of MRT, and the difference between ML and MRT. CV of root longevity within order had little effect on output parameters. Because of its nominal scale, the results of sensitivity analysis by PD are not shown in Fig. 2. In general, PD had little effect on output variables, except for the Weibull distribution, which affected error of MRT and of ML, the

Table 8 Measures of root longevity, relative errors, and corresponding biomass mortality under different number scaling exponents (S_N), longevity scaling exponents (S_L), and the longevity distributions

Number/ longevity scaling exponent	Longevity distribution	Actual average longevity (yr)	MR mean longevity (yr)	ML (yr)	Relative error ML (%)[a]	MRT (yr)	Relative error MRT (%)[a]	Standing biomass (g)	Actual biomass mortality (g yr^{-1})	Estimated biomass mortality ML (g yr^{-1})	Estimated biomass mortality MRT (g yr^{-1})
$S_N = 0.5$, $S_L = 1.5$											
	Normal	1.35	1.11	0.95	–29.48	2.18	61.48	4.32	3.20	4.54	1.98
	Weibull	0.74	1.11	0.82	11.67	2.19	196.84	4.32	5.86	5.25	1.98
	Lognormal	1.46	1.10	0.89	–38.60	2.18	49.64	4.32	2.97	4.83	1.98
	Exponential	1.49	1.11	0.88	–41.02	2.18	46.18	4.32	2.90	4.92	1.99
$S_N = 0.33$, $S_L = 2.0$											
	Normal	2.44	1.23	0.90	–63.15	6.28	157.24	8.05	3.30	8.96	1.28
	Weibull	1.42	1.21	0.78	–44.81	6.04	326.57	8.05	5.69	10.30	1.33
	Lognormal	2.61	1.21	0.83	–68.25	6.25	139.51	8.05	3.09	9.72	1.29
	Exponential	2.67	1.21	0.82	–69.42	6.24	133.93	8.05	3.02	9.87	1.29
$S_N = 0.25$, $S_L = 2.5$											
	Normal	3.68	1.28	0.86	–76.71	14.96	306.98	11.54	3.14	13.48	0.77
	Weibull	1.95	1.29	0.76	–61.05	16.68	754.94	11.54	5.92	15.19	0.69
	Lognormal	3.95	1.27	0.76	–80.70	14.92	277.53	11.54	2.92	15.15	0.77
	Exponential	4.04	1.27	0.74	–81.78	14.98	270.88	11.54	2.86	15.68	0.77

MR, minirhizotron; ML, median longevity; MRT, mean residence time.
Only 12 out of 36 are presented to represent the low ($S_N = 0.5$ and $S_L = 1.5$), middle ($S_N = 0.33$ and $S_L = 2$), and high ($S_N = 0.25$ and $S_L = 2.5$) degrees of error for both ML and MRT.
[a]Relative error percentage for ML and MRT was calculated as 100% × (ML(MRT) – actual average longevity)/actual average longevity. The equations for calculating other parameters are listed in Table 3.

Table 9 Relative errors in biomass mortality and actual average longevity caused by sampling bias under different number scaling exponents (S_N) and longevity scaling exponents (S_L)

	MR		C isotope	
Number/longevity scaling exponent	Biomass mortality	Actual average longevity	Biomass mortality	Actual average longevity
$S_N = 0.5$				
$S_L = 1.5$	–28%	–20%	–30%	25%
$S_L = 2.0$	–16%	–36%	–43%	57%
$S_L = 2.5$	–9%	–48%	–54%	93%
$S_N = 0.33$				
$S_L = 1.5$	–36%	–17%	–24%	20%
$S_L = 2.0$	–21%	–32%	–38%	48%
$S_L = 2.5$	–13%	–43%	–48%	77%
$S_N = 0.25$				
$S_L = 1.5$	–41%	–14%	–20%	16%
$S_L = 2.0$	–25%	–27%	–32%	38%
$S_L = 2.5$	–18%	–39%	–43%	66%

MR, minirhizotron.
Relative errors were calculated by 100% × (values with sampling bias – values without sampling bias)/values without sampling bias. Values presented here were averages across four longevity distributions, which had only a slight impact on these values.

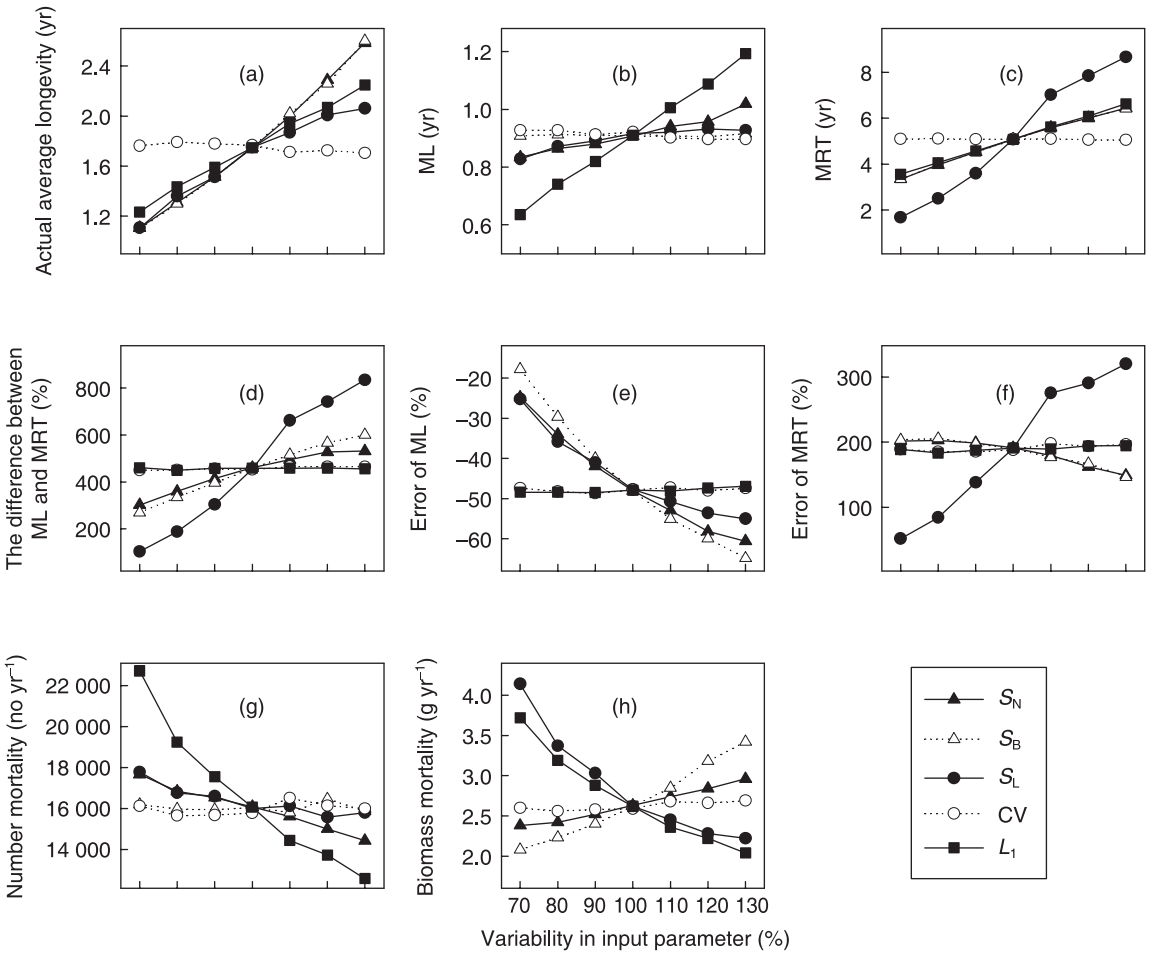

Fig. 2 Sensitivity of the root turnover simulator (RTS) model output variables to changing input parameters. All panels share the same *x*-axis, and panels (a–h) represent different output variables on the *y*-axis. ML, median longevity; MRT, mean residence time; S_N, number scaling exponent; S_B, biomass scaling exponent; S_L, longevity scaling exponent; CV, coefficient of variation of root longevity in each order; L_1, average longevity of the first order roots.

actual number and biomass mortality, and the actual average longevity.

Discussion

Large discrepancies exist in fine root longevity and turnover estimates obtained by the MR and the C isotope (bomb ^{14}C and FACE ^{13}C) methods (Table 1). The RTS model indicated that heterogeneity among fine roots and the differences in turnover calculation methods contributed more to the discrepancy among MR and C isotope fine root turnover estimates than did longevity distributions and sampling biases (Tables 8, 9). In particular, the MR ML calculation method consistently (with only one exception out of 36 treatments) and substantially underestimated the actual average longevity, whereas the C isotope MRT calculation method consistently overestimated the actual average longevity. Underestimation of root longevity by the ML method results from the skewness in root longevity distributions and from the fact that ML is a number-based measure while the actual average longevity is a biomass-based measure. For a positively skewed root longevity distribution commonly observed in the field and represented in all simulations of this study, ML overemphasizes the importance of rapidly cycling roots, but discounts the effect of less dynamic roots within the fine root guild (Tierney & Fahey, 2002). By contrast, MRT overestimates root longevity and underestimates biomass mortality mainly because it discounts the multiple replacements of rapidly cycling labeled roots (which are most likely to be the lower-order roots) during the sampling period used to measure isotope depletion curves (Table 8; Appendix S2).

Our simulations are consistent with the prediction based on the theory of Sheil & May (1996) that average turnover estimates (e.g. by MRT) will overestimate root longevity and underestimate turnover when root populations are highly heterogeneous in turnover rates. In Appendix S2, we show mathematically that the underestimation of root biomass mortality based on MRT increases with increasing heterogeneity in fine root longevity. The overestimation of root longevity and underestimation of biomass mortality by MRT can be eliminated only when all roots have identical longevity (Appendix S3), which is the homogeneity assumption used to define fine roots, but an unlikely condition in nature.

The overestimation of root longevity by MRT as a result of its inability to account for multiple replacements of labeled roots may provide a critical explanation for the large difference between the bomb ^{14}C age of forest fine roots (7–11 yr) and the turnover time estimates derived from biomass production methods (1–3 yr) reported by Trumbore *et al.* (2006). Certainly, other factors, including long life of some roots in the root sample, stored C, and the difficulty of separating live and dead roots, may also be responsible for the discrepancy observed, as noted by the authors (Trumbore *et al.*, 2006).

Evaluation of model assumptions and literature comparisons

Our assumption that root longevity scales with order at rates of 1.5, 2, and 2.5 seems to represent reasonable rates of change in longevity as order increases. At a first-order longevity of 0.7 yr (a common value among temperate trees, Ruess *et al.*, 2003; Withington *et al.*, 2006), assuming a longevity scaling exponent of 1.5 yielded a fifth-order root longevity of 3.56 yr (Table 7), a value far lower than the upper bound of root longevity (*c.* 20 yr) reported by bomb ^{14}C studies (Gaudinski *et al.*, 2001) and FACE ^{13}C depletion experiments (Matamala *et al.*, 2003). When assuming a longevity scaling exponent of 2.5, the longevity of the fifth-order roots was *c.* 27 yr, higher than the upper bound root longevity reported by C isotope studies. Therefore, our assumptions on the root longevity scaling exponent allowed us to encompass the reported longevity values for the different populations of fine roots observed by different methods.

Under our model assumptions, first-order roots played an important role in the dynamics of the fine root pool, accounting for at least 20% of the total fine root biomass mortality in 35 out of the 36 treatments, and for more than 30% of total fine root biomass mortality in 26 out of 36 treatments (Table 6). These results were obtained by assuming that first-order roots represented a small fraction of the total fine root biomass (6.5–11.9%, see Table 6). However, for all three temperate tree species on which ecosystem-scale biomass estimates for five fine root branch orders have been made to date, first-order roots represented 17% (*P. palustris*; Guo *et al.*, 2004), 19% (*L. gmelinii*; Wang *et al.*, 2006), and 30% (*F. mandshurica*; Wang *et al.*, 2006) of the total fine root biomass. Accordingly, first-order roots may account for a greater proportion of total fine root biomass mortality than the results of our simulations.

As noted earlier, the longevity model for root population age structure may have a significant impact on biomass mortality estimates. In the most extreme case, for the same set of order-specific mean longevity and biomass parameters, simulations using the Weibull and exponential longevity models differed by $\leq 80\%$ in actual biomass mortality and actual average longevity (based on calculations from Table 8 using values of actual biomass mortality and actual average longevity). This degree of difference is comparable to that reported in a previous simulation assessing longevity distribution effects (50% between normal and exponential longevity distribution models; Luo, 2003). In contrast, the normal, lognormal, and exponential models yielded similar estimates of the root longevity and biomass mortality (Table 8).

Sampling bias as assumed leads to errors in both root mortality and actual average root longevity, but to different directions in different methods. When fourth- and fifth-order roots were excluded to mimic the MR sampling bias, both the actual biomass mortality and actual average root longevity were underestimated (Table 9). When first-order roots were

excluded to mimic the sampling bias of soil cores used in the C isotope methods, the actual biomass mortality was underestimated but the actual average root longevity was overestimated (Table 9). Because first-order roots generally contribute more than other orders to the total fine root biomass mortality (Table 6), missing first-order roots leads to greater error than omitting fourth- and fifth-order roots.

It was suggested that the discrepancy between MR and C isotope methods may, in part, be because the two methods sample different populations of fine roots (Gaudinski *et al.*, 2001; Trumbore & Gaudinski, 2003). The current study suggests that such a sampling bias, if present, is a minor source of the discrepancy in turnover estimates reported so far. There is some evidence that MR may not observe higher-order roots. Although only limited studies have reported longevity estimates by order using the MR method (Reid *et al.*, 1993; Majdi *et al.*, 2001; Wells *et al.*, 2002), none has reported observations of fourth- or higher-order roots. However, this bias may be overcome by longer observation periods (S. Pritchard, personal communication), whose efficacy needs be tested in future work. For the soil core method used in C isotope studies, limited evidence suggests that root losses can be substantial during excavation and sorting unless special measures are taken (Caldwell & Virginia, 1989; Friend *et al.*, 1991; Le Goff & Ottorini, 2001). Choosing smaller sieve size and sampling intact branches (Pregitzer *et al.*, 2002; Guo *et al.*, 2004) will likely reduce losses of lower-order roots.

Implications, uncertainties, and gaps in data collection in fine root turnover assessments

Previous studies (Joslin *et al.*, 2006; Högberg & Read, 2006) and the results of this work suggest that the one-pool model used in fine root turnover is flawed. Joslin *et al.* (2006) showed that fine roots contain both a short-lived and a long-lived pool, and thus fine root turnover should be characterized by a two-pool model. Högberg & Read (2006) also suggested that, to reconcile the notion of fast root turnover based on MR (or root production methods) and the slow turnover based on C isotope evidence, we must assume a considerable dichotomy within the < 2-mm-diameter class between an extremely short-lived group and a long-lived group. However, Joslin *et al.* (2006) acknowledged that the two-pool model used in their study may still be an oversimplification of fine root turnover dynamics. In practice, this two-pool model may be difficult to apply in the field unless linked to root structure. We suggest that separating roots into branch orders is both a practical and an effective means by which a heterogeneous fine root population can be classified into functionally similar groups (Pregitzer *et al.*, 2002; Wells *et al.*, 2002).

We recognize that large uncertainty exists in how root longevity scales with branch order. In principle, root longevity must increase with branch order because the death of a higher-order root will entail the death of all its lower-order laterals. However, the rate of increase is far from clear. As already discussed, the assumptions in the present study about how root longevity scales with root order encompassed the reported variability among different orders, and thus our results may be used to bound errors for different methods used to estimate fine root turnover. However, as sensitivity analysis showed, the difference between ML and MRT is highly sensitive to the rate at which root longevity scales with branch order (i.e. S_L) (Fig. 2d). Better estimates of the heterogeneity in fine root longevity are needed to resolve more definitively the difference between ML and MRT.

Minirhizotron and C isotope approaches may be modified to better assess turnover dynamics of different branch orders. Currently, the MR appears a better approach for lower orders (e.g. one to three), whereas the C isotope methods (particularly the bomb ^{14}C method, which is less expensive and more widely applicable than the FACE ^{13}C method; Luo, 2003) are better suited for higher orders. Thus, the two techniques could be coupled to obtain more comprehensive and reliable longevity and turnover estimates for the entire fine root system.

Another uncertainty of our results lies with the calculation of actual biomass mortality and actual average longevity, which was based on the assumption that roots within each order are in a steady state (i.e. the mortality of an individual root will be immediately followed by the birth of an identical root). If there is a time lag between the death of a root and the growth of its replacement, much the same as the time lag between leaf fall in one year and the leaf growth in the next in deciduous forests with a relatively long winter, then the actual biomass mortality would be smaller, and the actual average longevity would be greater, than the simulated results presented in this work, all else being equal. Therefore, the error of ML (or MRT) presented here, which was calculated as the percentage difference between ML (or MRT) and actual average longevity, should be considered as the upper-bound estimates of the possible errors.

This study was not designed to show the necessity of adopting an order-centric view, but to test the potential limitations of using traditional arbitrary diameter classes to scale root turnover in ecosystem C cycles. Choosing a more narrow diameter class such as 0–0.5 mm may reduce the heterogeneity in a root sample and improve the accuracy of turnover estimates, but cannot eliminate problems associated with heterogeneity in scaling root turnover, and still suffers from an inability to compare across species (at the diameter class of < 0.5 mm, some species have three or more orders, while others have none; Pregitzer *et al.*, 2002). Adopting a root-order-based approach to replace diameter-defined classes in future root sampling requires further empirical tests and method development but shows promise in resolving some of the past difficulties in below-ground ecosystem ecology.

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Supplementary material

The following supplementary material is available for this article online:

Appendix S1 Mean residence time (MRT) and biomass weighted mean longevity (BWML) are mathematically equivalent.

Appendix S2 Underestimation of turnover by mean residence time (MRT) increases with increasing variability in individual root longevity.

Appendix S3 Mean residence time (MRT) underestimates root turnover unless all individual fine roots have the same longevity.

Fig. S1 Relationships between the underestimation of root C mortality and the range of longevity variations in a root sample.

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