

# Uncertainties in interpretation of isotope signals for estimation of fine root longevity: theoretical considerations

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## Abstract

This paper examines uncertainties in the interpretation of isotope signals when estimating fine root longevity, particularly in forests. The isotope signals are depleted  $\delta^{13}\text{C}$  values from elevated  $\text{CO}_2$  experiments and enriched  $\Delta^{14}\text{C}$  values from bomb  $^{14}\text{C}$  in atmospheric  $\text{CO}_2$ . For the  $\text{CO}_2$  experiments, I explored the effects of six root mortality patterns (on–off, proportional, constant, normal, left skew, and right skew distributions), five levels of nonstructural carbohydrate (NSC) reserves, and increased root growth on root  $\delta^{13}\text{C}$  values after  $\text{CO}_2$  fumigation. My analysis indicates that fitting a linear equation to  $\delta^{13}\text{C}$  data provides unbiased estimates of longevity only if root mortality follows an on–off model, without dilution of isotope signals by pretreatment NSC reserves, and under a steady state between growth and death. If root mortality follows the other patterns, the linear extrapolation considerably overestimates root longevity. In contrast, fitting an exponential equation to  $\delta^{13}\text{C}$  data underestimates longevity with all the mortality patterns except the proportional one. With either linear or exponential extrapolation, dilution of isotope signals by pretreatment NSC reserves could result in overestimation of root longevity by several-fold. Root longevity is underestimated if elevated  $\text{CO}_2$  stimulates fine root growth. For the bomb  $^{14}\text{C}$  approach, I examined the effects of four mortality patterns (on–off, proportional, constant, and normal distribution) on root  $\Delta^{14}\text{C}$  values. For a given  $\Delta^{14}\text{C}$  value, the proportional pattern usually provides a shorter estimate of root longevity than the other patterns. Overall, we have to improve our understanding of root growth and mortality patterns and to measure NSC reserves in order to reduce uncertainties in estimated fine root longevity from isotope data.

*Keywords:* age structure, ecosystem carbon balance, forest, global change, mortality, root longevity, root turnover

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## Introduction

The use of isotopes as tracers has become a powerful tool in ecological research. The isotopes of carbon,  $^{13}\text{C}$  and  $^{14}\text{C}$ , have, for example, been used to track the fate of carbon through different pathways in terrestrial ecosystems (Johnson *et al.*, 2002), and to quantify longevity of fine roots (Gaudinski *et al.*, 2001), turnover of microbes (Gregorich *et al.* 2000), and residence times of carbon in various soil pools (Connin *et al.*, 1997; Cheng & Johnson, 1998; Bol *et al.*, 1999). In particular, bomb  $^{14}\text{C}$  in

atmospheric  $\text{CO}_2$  has been used to estimate mean ages of fine roots in the deciduous and coniferous forests of the eastern United States, which were found to be 3–18 years old (Gaudinski *et al.*, 2001). Similarly, depleted isotope  $^{13}\text{C}$  in elevated  $\text{CO}_2$  experiments has also been used to estimate fine root longevity in trees, which was found to be 4–9 years in a loblolly pine forest (R. Matamala & W.H. Schlesinger, pers. comm.; also presented by Pataki *et al.*, 2003 & Pendall, 2002). In general, the estimated longevity of fine roots using the isotope methods is far longer than those estimated by conventional methods (a few months to 1–2 years), such as mass balance and root minirhizotrons (Gill & Jackson, 2000; Tingey *et al.*, 2000).

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The discrepancy in estimated root longevity between the isotope and conventional methods may partly result from our poor understanding of the dynamics of fine root populations during their life cycles (Tierney & Fahey, 2002). Life spans of individual roots in an ecosystem are heterogeneous, varying with species and growth environments. As a consequence, mortality patterns and age structures of root populations are diverse (Wells & Eissenstat, 2001). The isotope methods associated with soil coring of fine roots, however, usually assume that all roots live for the same amount of time before they all die at the same time if a linear model is applied to extrapolate isotope data. In addition, the isotope methods likely quantify ages of the roots that persist longest in soil (Gaudinski *et al.*, 2001), whereas the minirhizotron method provides direct measurements but tends to emphasize the rapidly cycling roots. Therefore, it is imperative to examine how heterogeneity in fine root life spans and the dynamics of root populations affect the estimation of root longevity.

The discrepancy between the conventional and isotope methods could also be due to the dilution of isotope signals by nonstructural carbohydrate (NSC) reserves. Nonstructural carbohydrate reserves in various tissues play a critical role in regulating plant growth and maintenance under fluctuating and/or stress environments (Gower *et al.*, 1995). Pretreatment NSC reserves in CO<sub>2</sub> experiments have been showed to dilute isotope signals in fine root tissues grown at elevated CO<sub>2</sub> (Langley *et al.*, 2002). In comparison to herbaceous plants, trees particularly have a large amount of perennial NSC reserves (Kozlowski, 1992) that can substantially dilute isotope signals and consequently affect estimation of root longevity. In addition, fine root growth and death are usually not in a steady state over seasons and along vertical soil profiles (Luo *et al.*, 1995; Pritchard & Rogers, 2000). The nonsteady state between growth and death is particularly obvious in CO<sub>2</sub> experiments where fine root growth is usually stimulated by elevated CO<sub>2</sub> (Arnone III *et al.*, 2000; Matamala & Schlesinger, 2000; Tingey *et al.*, 2000; Pritchard *et al.*, 2001). Nonsteady state conditions for root growth and death have the potential to complicate interpretation of isotope signals.

This paper was designed to examine uncertainties in the interpretation of isotope signals, particularly in reference to  $\delta^{13}\text{C}$  data in fine roots from CO<sub>2</sub> experiments with depleted  $^{13}\text{C}$  signals and root  $\Delta^{14}\text{C}$  data derived from bomb  $^{14}\text{C}$  in atmospheric CO<sub>2</sub>. Through this theoretical analysis, I examined how dynamics of root  $\delta^{13}\text{C}$  values from CO<sub>2</sub> experiments vary with three variables: (i) six patterns of fine root mortality rates; (ii) five levels of NSC reserves in plants; (iii) a CO<sub>2</sub>-induced increase in fine root growth rates. In addition, I used model-generated data to estimate sampling errors in association with the

three variables. In addition, I analyzed how estimated longevity from root  $\Delta^{14}\text{C}$  values varies with root mortality patterns. Fine roots in this study are referred to roots with < 2 mm diameter as in other studies (Gaudinski *et al.*, 2001; Langley *et al.*, 2002).

## Methods

### *Age distribution of fine root biomass and mortality*

To explore relationships between root population structure and isotope dynamics, this study first mathematically defined six root mortality patterns. The six patterns are on-off, proportional, constant, normal, left skew, and right skew distributions. Among the six patterns, the proportional one represents the most spread in age distribution of fine root populations and corresponds to the exponential model that is frequently used to extrapolate isotope data for estimation of root longevity (Gaudinski *et al.*, 2001). The on-off pattern characterizes the most concentrated age distribution and is related to the linear model for extrapolating isotope data to estimate root longevity (Saarinen, 1996). The constant mortality pattern has been documented in the literature (Wells & Eissenstat, 2001). The normal distribution of root mortality may be conceptually realistic whereas the left and right skew distributions provide variants of possible root mortality patterns. Overall, the six patterns encompass all possible cases of root mortality.

Except for the on-off pattern, I assumed that root mortality follows a continuous function of age. Each of the mortality patterns is described below.

*On-off mortality* This mortality pattern assumes that all roots, once initiated, live to the same age (i.e. mean age) before all die abruptly together. The pattern is expressed by:

$$X_i = \begin{cases} X_0 & \text{when } t \leq \mu \\ 0 & \text{when } t > \mu \end{cases} \quad (1)$$

where  $t$  is time,  $X_i$  is fine root biomass at time  $t = i$ ,  $X_0$  is an initial value of fine roots at time  $t = 0$ , and  $\mu$  is the mean age (i.e. longevity) of fine roots.  $X_0$  was set at 100 g in all the case studies.

*Proportional mortality* This pattern assumes that the mortality at a particular age is proportional to root biomass in that age cohort as:

$$\frac{dX_i}{dt} = \beta X_i \quad (2)$$

where  $\beta$  is relative mortality. Equation (2) is a first-order differential equation and generates an exponential decay of fine root biomass. Theoretically, fine roots initiated at

time  $t = 0$  can have a fraction that extends to an infinite future.

**Constant mortality** This pattern assumes that the amount of root biomass that dies ( $d_c$ ) is a constant fraction of the initial fine root biomass. That is:

$$\frac{dX_i}{dt} = d_c X_0 \quad (3)$$

where  $d_c$  is a constant death rate. Equation (3) generates an age distribution of root biomass that linearly decreases with time.

**Normal distribution of root mortality** This pattern assumes that the amount of fine roots that dies ( $d_n$ ) follow a normal distribution as:

$$d_{n,t} = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-(\tau-\mu)^2/\sigma^2} \quad (4)$$

where  $d_{n,t}$  is mortality at time  $t$ ,  $\tau$  is root age,  $\mu$  is the mean age,  $\sigma$  is the standard deviation, and  $\pi$  is 3.14. Live biomass at a particular time  $t$  is estimated by

$$X_i = \left(1 - \sum_0^t d_{n,t}\right) X_0 \quad (5)$$

**Left skew distribution of root mortality** This pattern assumes that the majority of mortality occurs when fine roots are young and a small fraction of roots lives for a long time. Mortality is estimated by:

$$d_{ls,t} = c_1 \tau^{c_2} e^{c_3 \tau} \quad (6)$$

where  $d_{ls,t}$  is mortality at time  $t$  that follows a left skew distribution, and  $c_1$ ,  $c_2$ , and  $c_3$  are coefficients. Live biomass is estimated using Eqn (5) with substitution of  $d_{n,t}$  by  $d_{ls,t}$ .

**Right skew distribution of root mortality** This pattern assumes that when fine roots are young, their mortality is lower than that in the normal distribution and thus the majority of death occurs when roots are old. The mortality is described by:

$$d_{rs,t} = k_1 \tau^{k_2} (1 - k_3 e^{k_4 \tau}) \quad (7)$$

where  $d_{rs,t}$  is mortality at time  $t$  that follows the right skew distribution, and  $k_1$ – $k_4$  are coefficients. Similarly, live biomass was estimated using Eqn (5) with substitution of  $d_{n,t}$  by  $d_{rs,t}$ .

When the six models were applied to analysis of  $\delta^{13}\text{C}$  signals from  $\text{CO}_2$  experiments, I set the mean longevity of fine roots ( $\mu$ ) to be 12 months for convenience of comparison. To generate an age distribution of fine roots with a mean age of 12 months,  $\beta$  in Eqn (2) was set at  $1/12$

( $1/\mu$ );  $d_c$  in Eqn (3) was set at  $1/24$  ( $1/2\mu$ ); coefficients  $c_1$ ,  $c_2$ , and  $c_3$  in Eqn (6) are 0.05112, 0.50, and  $-0.1262$ , respectively; and coefficients  $k_1$ – $k_4$  in Eqn (7) are 0.00003052, 3.59, 0.0385, and 0.195, respectively. I also set  $\sigma$  in Eqn (4) to be 3.6 months ( $0.3\mu$ ). When the first four models were applied to analysis of root  $\Delta^{14}\text{C}$  signals derived from atmospheric  $^{14}\text{CO}_2$ ,  $\mu$  varies from 1 to 30 years, with other coefficients varying accordingly.

#### Theoretical isotope $\delta^{13}\text{C}$ curves in $\text{CO}_2$ experiments

Carbon dioxide experiments usually release  $\text{CO}_2$  with depleted  $^{13}\text{C}$ , which results in  $\delta^{13}\text{C}$  values of approximately  $-39.5\%$  in newly synthesized carbohydrate at elevated  $\text{CO}_2$  in comparison with  $-27.5\%$  at ambient  $\text{CO}_2$  (Leavitt *et al.*, 2001). Thus, the total roots in an ecosystem can be divided into two groups: new vs old roots. The roots that grow before the  $\text{CO}_2$  experiment are 'old' and those that grow at elevated  $\text{CO}_2$  are considered 'new'. The old roots have  $^{13}\text{C}$  signals that reflect the ambient  $\text{CO}_2$  environment (i.e.  $\delta^{13}\text{C}_{or} = -27.5\%$ ). Based on the assumptions made about new root growth and its carbon sources, I defined three cases for estimating  $\delta^{13}\text{C}$  values of fine root populations. In case 1, I assumed that the growth rates of new and old roots are equal and the carbon for the new root production comes exclusively from current photosynthesis. Thus, the new roots have  $^{13}\text{C}$  signals that reflect the depleted  $^{13}\text{C}$  inputs of the fossil fuel  $\text{CO}_2$  (i.e.  $\delta^{13}\text{C}_{nr} = -39.5\%$ ). Accordingly, a sample of fine roots from an ecosystem at time  $t$  after the initiation of the  $\text{CO}_2$  experiment has an isotope signal

$$\delta^{13}\text{C}_{s,t} = \frac{(\delta^{13}\text{C}_{or} X_{o,t} + \delta^{13}\text{C}_{nr} X_{n,t})}{X_T} \quad (8)$$

where  $\delta^{13}\text{C}_{s,t}$  is the  $\delta^{13}\text{C}$  value in a root sample at time  $t$ ,  $X_{o,t}$  is old root biomass at time  $t$ ,  $X_{n,t}$  is new root biomass at time  $t$ , and  $X_T$  is the total root biomass. The value of  $X_{n,t}$  is the sum of fine root biomass in cohorts from age 0 to  $t_F$ , the time since  $\text{CO}_2$  fumigation, as:

$$X_{n,t} = \sum_{i=0}^{t_F} X_i \quad (9)$$

where  $X_i$  is the root biomass in age cohort  $i$ , which is defined in the six different mortality models. The old root biomass is the difference between the total and the new root biomass.

Case 2 assumes that the carbon for the new root production comes from a mixture of current photosynthesis and pretreatment NSC reserves in a  $\text{CO}_2$  experiment, while the growth rate of new roots equals that of old roots. Mixing pretreatment NSC reserves with current photosynthesis dilutes  $\delta^{13}\text{C}$  values of newly constructed tissue of fine roots after  $\text{CO}_2$  fumigation. Thus, the  $\delta^{13}\text{C}_{nr}$

signal of new roots at elevated CO<sub>2</sub> is no longer a fixed number (−39.5‰), but varies with time as:

$$\delta^{13}C_{nr,t} = \frac{(m-1)\delta^{13}C_{nr,t-1} + (-39.5)}{m} \quad (10)$$

where  $\delta^{13}C_{nr,t}$  and  $\delta^{13}C_{nr,t-1}$  are isotope signals in newly constructed roots at time  $t$  and  $t-1$ , respectively (in monthly time steps), and  $m$  is the residence time of the pretreatment NSC at five levels (i.e. 6, 12, 18, 24, and 36 months). The assumed residence time from 6 to 36 months is based on the following considerations: A residence time of 6 months means that biomass C content of a tree is roughly 2.5 times the annual C fixation by photosynthesis. If we assume that the crown of a tree occupies 1 m<sup>2</sup> and that the annual photosynthetic carbon fixation of the tree crown is 1000 g C m<sup>-2</sup>, the tree that has 2500 g C (~5 kg dry weight) with an NSC content of 20% can have a NSC residence time of 6 months. Similarly, a residence time of 36 months means that a tree has a size of 30 kg dry weight with 20% NSC. This assumption on NSC residence times is also supported by experimental evidence (Langley *et al.*, 2002).

Case 3 assumes that the growth rate of new roots is 40% higher than that of old roots while the carbon for the new root production comes exclusively from current photosynthesis. Fine root growth is usually stimulated at elevated CO<sub>2</sub> in comparison to that at ambient CO<sub>2</sub> (Matamala & Schlesinger, 2000; Tingey *et al.*, 2000). The assumed 40% increase in the fine root growth rate at elevated CO<sub>2</sub> is incorporated into Eqn (8) to examine isotope dynamics.

#### Relative errors in estimated fine root longevity in CO<sub>2</sub> experiments

In practice, a linear or an exponential equation usually fits isotope data from a field CO<sub>2</sub> experiment to estimate root longevity (Saarinen, 1996). To fit the exponential equation, isotope values are first linearized by  $\ln [(-39.5 - \delta^{13}C_o)/(-12)]$ , where −39.5 is the equilibrated  $\delta^{13}C$  value at elevated CO<sub>2</sub>, −12 is the difference in the  $\delta^{13}C$  values between ambient and elevated CO<sub>2</sub> treatments, and  $\delta^{13}C_o$  is an observed  $\delta^{13}C$  data point. From a slope coefficient of the fitted linear or linearized exponential equation, a mean longevity of fine roots is estimated. The estimated root longevity by the linear or exponential extrapolation of isotope data may or may not equal the 'true' longevity, depending on inherent root mortality patterns, pretreatment NSC, fine root growth rates, and sampling duration. To evaluate the accuracy of the linear and exponential extrapolations, I used modeled isotope values in the first 6 and 12 months to estimate the mean longevity, which was compared with a 'true' longevity set up in the model to compute a relative error ( $\epsilon$ ) as

$$\epsilon(\%) = \frac{\tau_e - \tau_t}{\tau_t} \times 100 \quad (11)$$

where  $\tau_e$  is the estimated age and  $\tau_t$  is the 'true' age (12 months) designated in the model.

#### Theoretical isotope $\Delta^{14}C$ curves in fine roots derived from atmospheric $^{14}CO_2$

The isotopic value of a fine root sample in a given year ( $\Delta^{14}C_{s,yr}$ ) reflects a mass-weighted integration of roots with varying  $^{14}C$  values over the period that their ages span. Since atmospheric  $^{14}C$  of CO<sub>2</sub> is changing over time,  $\Delta^{14}C_{s,yr}$  is estimated by:

$$\Delta^{14}C_{s,yr} = \frac{\sum_{t=yr-6\mu}^{yr} \Delta^{14}C_t R_t}{R_T} \quad (12)$$

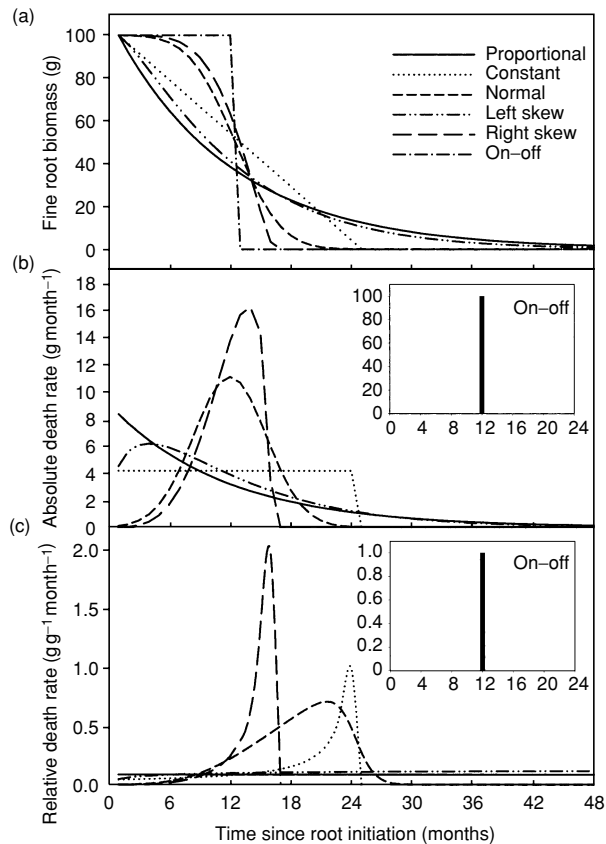
where  $\Delta^{14}C_t$  is the  $\Delta^{14}C$  value in the atmospheric CO<sub>2</sub> at year  $t$  and  $R_t$  is root biomass at an age cohort  $t$  along an age distribution from 0 to  $6\mu$  years old. The root biomass was estimated from the four root mortality models (on-off, proportional, constant, normal distribution).

For the sake of simplicity, this study did not consider the two other mortality patterns. Nor did I examine the effects of pretreatment NSC reserves or nonsteady-state conditions on root  $\Delta^{14}C$  dynamics because relevant results on  $\delta^{13}C$  are applicable. This paper does not analyze potential deviation in estimated root longevity caused by sampling bias along soil profiles, or over different growing seasons. To focus this study on the general dynamic nature of  $\delta^{13}C$  or  $\Delta^{14}C$  in fine roots, this study did not consider diurnal and yearly variations in environmental factors and growth phenology.

## Results

### Age distributions of fine root biomass and mortality

Age distributions of fine roots vary with mortality patterns. The on-off pattern has a constant fine root biomass of 100 g until the age of 12 months and then all the roots die at once right after 12 months (Fig. 1a). With the proportional pattern, fine root biomass exponentially declines with age and is 35.2, 12.4, and 4.4 g at months 12, 24, and 36, respectively. The constant pattern results in fine root biomass that linearly declines to 0 at month 24. In the normal distribution of root mortality, the biomass has a reverse S-shaped curve. The left skew distribution of root mortality has the age distribution of root biomass between the normal distribution and the proportional pattern, whereas the right skew distribution has the age distribution between the normal distribution and the on-off pattern. Among all the root mortality patterns,



**Fig. 1** Age distributions of fine root biomass (a), absolute mortality rate (b), and relative mortality rate (c) for the six mortality patterns. The insert figures in panel (b) and (c) show absolute and relative death rates, respectively, for the on-off mortality pattern. Among the six patterns, the on-off and proportional ones generate two extreme (i.e. the most concentrated and the most spread, respectively) age distributions of root biomass. Age distributions of root biomass have great implications for isotope dynamics.

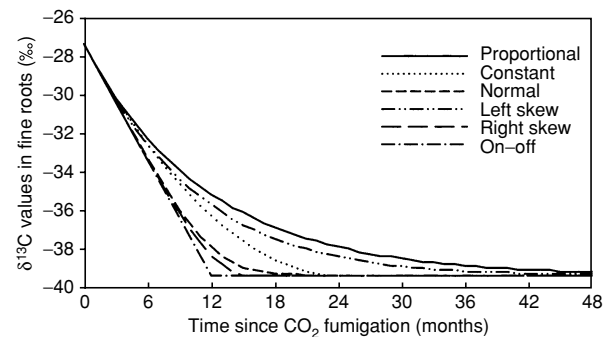
the proportional and left skew patterns have root ages that span several-fold older than the mean age.

The six mortality patterns have different absolute and relative rates of mortality (Fig 1b, c). The on-off pattern has an absolute mortality of  $100 \text{ g month}^{-1}$  and a relative mortality of  $1 \text{ g g}^{-1} \text{ month}^{-1}$  (i.e., g dead root per g live root per month) at month 13. The proportional pattern has a constant relative mortality of  $0.087 \text{ g g}^{-1} \text{ month}^{-1}$  in the entire simulation period but the absolute mortality decreases from  $8.33 \text{ g g}^{-1} \text{ month}^{-1}$  at month 1 to nearly 0 in 48 months. The constant pattern has a constant absolute mortality of  $4.167 \text{ g g}^{-1} \text{ month}^{-1}$  in the first 24 months. The relative mortality gradually increases to  $1 \text{ g g}^{-1} \text{ month}^{-1}$  at month 23 and drops to 0 at month 24. The normal, left, and right skew distributions of mortality are defined based on their absolute mortalities. All their relative mortalities are skewed toward older ages.

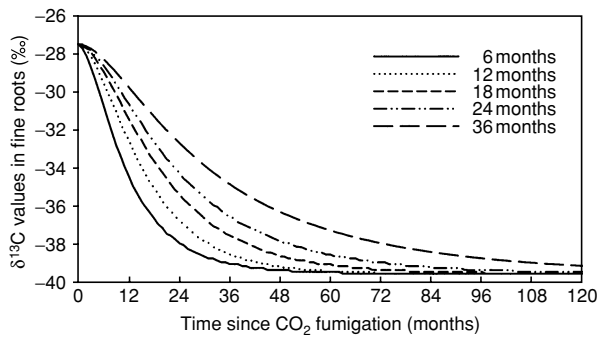
### Dynamics of isotope signals in fine roots in $\text{CO}_2$ experiments

As a consequence of different age distributions of root biomass, isotope signals in fine root populations vary among the mortality patterns even with identical mean root longevity (Fig. 2). The on-off pattern results in a linear decline in  $\delta^{13}\text{C}$  to  $-39.5\%$  in 12 months. The proportional pattern leads to an exponential decline in  $\delta^{13}\text{C}$  that approaches  $-39.5\%$  in 48 months. The value of root sample  $\delta^{13}\text{C}$  for the left skew distribution declines slightly faster than that for the proportional pattern. The constant pattern results in a decrease in the  $\delta^{13}\text{C}$  value to  $-39.5\%$  in 24 months. The  $\delta^{13}\text{C}$  values for the right skew and normally distributed mortality patterns are close to that for the on-off pattern. With an identical mean age for all fine roots, different mortality patterns lead to diverse dynamics of isotope signals in fine root populations in  $\text{CO}_2$  experiments. Due to the wide spread in age distribution generated by the proportional root mortality pattern, it takes several-fold longer than the mean age before the root  $\delta^{13}\text{C}$  value declines to the depleted level at elevated  $\text{CO}_2$ .

The curves in Fig. 2 represent dynamics of the  $\delta^{13}\text{C}$  values of root populations in which all the new root material is made of current photosynthesis. However, the root  $\delta^{13}\text{C}$  value at elevated  $\text{CO}_2$  could be greatly influenced by pretreatment NSC reserves (Fig. 3). If the



**Fig. 2** The theoretical  $\delta^{13}\text{C}$  values in fine roots from elevated  $\text{CO}_2$  experiments with the six root mortality patterns. All the mortality patterns have the same mean root age but lead to different isotope dynamics. The on-off mortality pattern results in a linear decline in the  $\delta^{13}\text{C}$  values from  $-27.5$  to  $-39.5\%$  whereas the proportional mortality pattern causes a slow decline in the  $\delta^{13}\text{C}$  values. Fitting a linear equation to the isotope data yield a unbiased estimate of root longevity only if fine root population mortality follows the on-off pattern but produces substantial overestimations for the other mortality patterns (Table 1). In contrast, fitting an exponential equation to the isotope data yield a unbiased estimate of root longevity only if fine root mortality follows the proportional pattern but produces substantial underestimations for the other mortality patterns (Table 1).



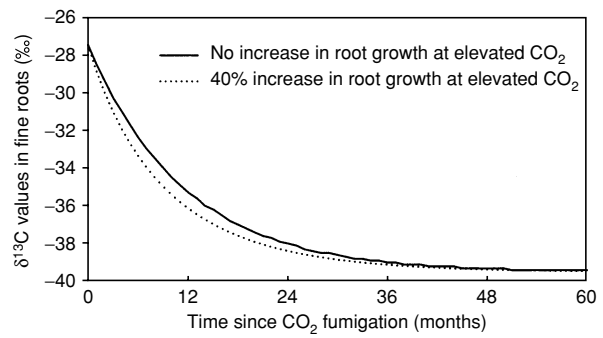
**Fig. 3** The theoretical  $\delta^{13}\text{C}$  values in fine roots from elevated  $\text{CO}_2$  experiments as affected by NSC dilution. Months indicate residence times of NSC reserves. The dilution by pretreatment NSC reserves considerably slows down depletion processes of isotope in fine roots, resulting in tremendous overestimations of fine root longevity (Table 1). The proportional mortality is used as the baseline pattern for this analysis.

NSC reserves in trees have a residence time of 6 months, the decrease in the  $\delta^{13}\text{C}$  values after the  $\text{CO}_2$  fumigation is delayed by several months. As the residence time of NSC reserves increases, its dilution effect on isotope signals becomes stronger and the  $\delta^{13}\text{C}$  value declines progressively slower. With the residence time of 36 months and the designated mean root longevity of 12 months, the  $\delta^{13}\text{C}$  value decreases from  $-27.5\text{‰}$  in the beginning of a  $\text{CO}_2$  experiment to  $-29.7$ ,  $-37.2$ , and  $-39.1\text{‰}$ , respectively, after 12, 60, and 120 months of  $\text{CO}_2$  fumigation (Fig. 3).

In contrast to the dilution effects of pretreatment NSC reserves, a  $\text{CO}_2$ -stimulated fine root growth accelerates the decline in the root  $\delta^{13}\text{C}$  value in comparison to that without  $\text{CO}_2$  stimulation (Fig. 4). A 40% increase in fine root growth at elevated  $\text{CO}_2$  in comparison to that at ambient  $\text{CO}_2$  results in the  $\delta^{13}\text{C}$  values of  $-33.4$  and  $-36.2\text{‰}$  after 6 and 12 months of  $\text{CO}_2$  fumigation, respectively, with the proportional mortality. The corresponding  $\delta^{13}\text{C}$  values are  $-32.4$  and  $-35.3\text{‰}$  without growth stimulation of fine roots by elevated  $\text{CO}_2$ .

#### *Potential errors in interpretation of isotope signals in $\text{CO}_2$ experiments*

Variations in isotope signals with root mortality patterns (Fig. 2), pretreatment NSC (Fig. 3), and growth rates (Fig. 4) are potential sources of uncertainties in the estimation of root longevity. To examine the uncertainties associated with those variables, I truncated modeled  $\delta^{13}\text{C}$  values as in Figs 2–4 at 6 and 12 months and fitted linear and exponential equations to the truncated data to estimate root longevity. The estimated root longevity was compared to that set in the model (i.e., 12 months) to



**Fig. 4** The theoretical  $\delta^{13}\text{C}$  values in fine roots from  $\text{CO}_2$  fumigated experiments as affected by  $\text{CO}_2$ -induced increase in fine root growth. An increase in fine root growth at elevated  $\text{CO}_2$  accelerates depletion of  $^{13}\text{C}$  and potentially leads to underestimation of root longevity. The proportional mortality is used as the baseline pattern for this analysis.

calculate relative errors according to Eqn 1. If fine root mortality follows the on–off pattern, the root  $\delta^{13}\text{C}$  value declines linearly with time (Fig. 2). The longevity estimated with the linear equation is equal to the ‘true’ value. In this case, the interpretation of isotope signals in  $\text{CO}_2$  experiments yields a perfect estimate of root longevity without any error (Table 1). Fitting a linear equation to  $\delta^{13}\text{C}$  data generated by the other mortality patterns overestimates root longevity by up to 39%. Because the right skew and normal distributions of root mortality generate root age distributions and  $\delta^{13}\text{C}$  dynamics that are close to those of the on–off pattern, the linear interpretation yields small errors for the two mortality patterns. If the root mortality follows the proportional pattern, the potential interpretation errors incurred from using the linear model are very high.

When the exponential equation was used to interpret isotope data, the estimated longevity is equal to the ‘true’ value if fine root mortality follows the proportional pattern (Table 1). With the other root mortality patterns, fitting the exponential equation to isotope data underestimates root longevity by up to 54.3%. Since the mortality patterns of left skew, linear, normal, right skew, and linear distributions generate  $\delta^{13}\text{C}$  dynamic curves that become, respectively, further away from the proportional curve, the magnitude of underestimation for root longevity accordingly increases with those mortality patterns. Given that the linear and exponential extrapolations of isotope data represent two extreme estimates (overestimation and underestimation), fitting both equations to one single data set at least brackets the longevity regardless of root mortality patterns.

In addition to influences of root mortality patterns, pretreatment NSC reserves in trees can substantially dilute isotope signals, resulting in an overestimation of

**Table 1** Relative errors in estimated fine root longevity by linear and exponential extrapolations of 6- and 12-months model output

Scenarios	6 month data		12 month data	
	Linear	Exponential	Linear	Exponential
<i>Mortality pattern</i>				
Proportional	17.3	0.0	39.0	0.0
On-off	0.0	-22.6	0.0	-54.3
Constant	12.6	-9.0	25.8	-20.0
Normal distribution	1.2	-21.2	7.4	-42.8
Left skew distribution	10.7	-11.0	30.4	-13.8
Right skew distribution	0.0	-22.6	4.5	-47.6
<i>Dilution by NSC reserves with residence time</i>				
6 months	72.7	84.4	25.2	31.4
12 months	193.5	226.8	82.6	113.7
18 months	325.2	370.8	145.1	201.9
24 months	423.3	512.7	206.3	293.1
36 months	686.8	805.8	334.8	474.7
<i>Nonsteady state between root growth and death</i>				
40% increase in growth at elevated CO <sub>2</sub>	-17.1	-26.7	-6.7	-23.3

Positive values indicate overestimation and negative underestimation. See Eqn (11) for the definition of relative error. Note: To avoid confounding error estimations, I used the on-off root mortality pattern for the linear extrapolation and the proportional mortality pattern for the exponential extrapolation for the five scenarios with dilution of isotope signals by NSC reserves and the scenario with increased growth.

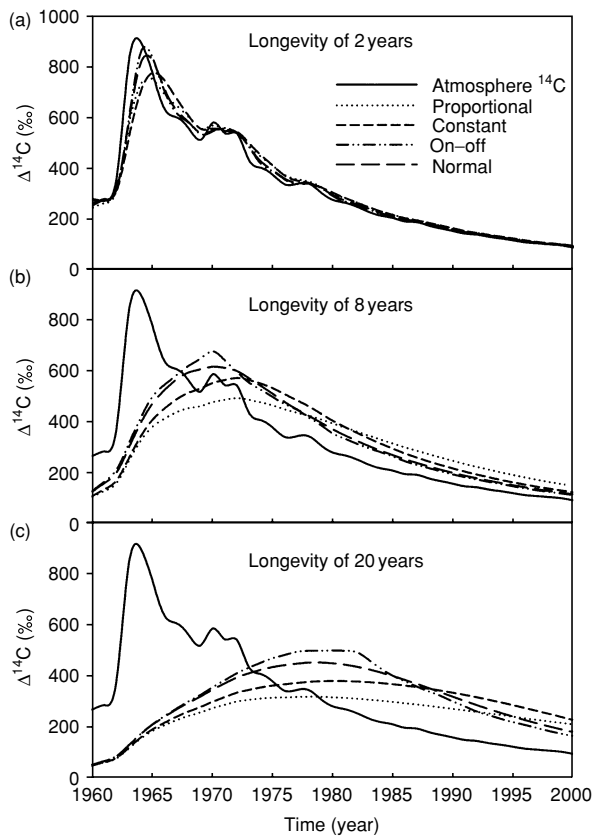
root longevity (Table 1). If the NSC reserves have a residence time of 6 months, a linear extrapolation of 6-month  $\delta^{13}\text{C}$  data yields a 73% overestimation of root longevity. The linear extrapolation of 12-month  $\delta^{13}\text{C}$  data overestimates root longevity by 25%. Similarly, fitting an exponential equation to 6- and 12-month isotope data overestimates root longevity by 84 and 31%, respectively. The magnitude of the overestimation increases with residence times of the NSC reserves. In the case of NSC reserves with a 36-month residence time, the potential errors in the linear extrapolations of 6- and 12-month  $\delta^{13}\text{C}$  data can reach as high as 687 and 334%, respectively. The exponential extrapolations of 6- and 12-month  $\delta^{13}\text{C}$  data overestimate root longevity by 806 and 475%, respectively. The longer time series of isotope data leads to decreased relative errors in association with dilution effects of the NSC reserves. Increased root growth at elevated CO<sub>2</sub>, however, causes underestimation of longevity no matter whether the linear or exponential approach is used to analyze isotope data (Table 1).

#### *Dynamics of root $\Delta^{14}\text{C}$ values resulting from bomb $^{14}\text{C}$ in atmospheric CO<sub>2</sub>*

To examine uncertainties in estimated root longevity from root  $\Delta^{14}\text{C}$  data, I plotted theoretical root isotope values together with  $\Delta^{14}\text{C}$  in atmospheric CO<sub>2</sub> in Fig. 5.

With a root longevity of 2 years, the  $\Delta^{14}\text{C}$  values in fine roots lag slightly behind those in the atmosphere. Since the proportional pattern yields the greatest spread in age distribution of fine roots (Fig. 1), the root  $\Delta^{14}\text{C}$  values reflect integrated atmospheric  $\Delta^{14}\text{C}$  values over more years and, thus, less closely track the change in the atmospheric  $^{14}\text{C}$  than in the other patterns. As the root longevity increases up to 20 years, root  $\Delta^{14}\text{C}$  values become smooth curves over the period from 1960 to 2000 and differ substantially among the four mortality patterns.

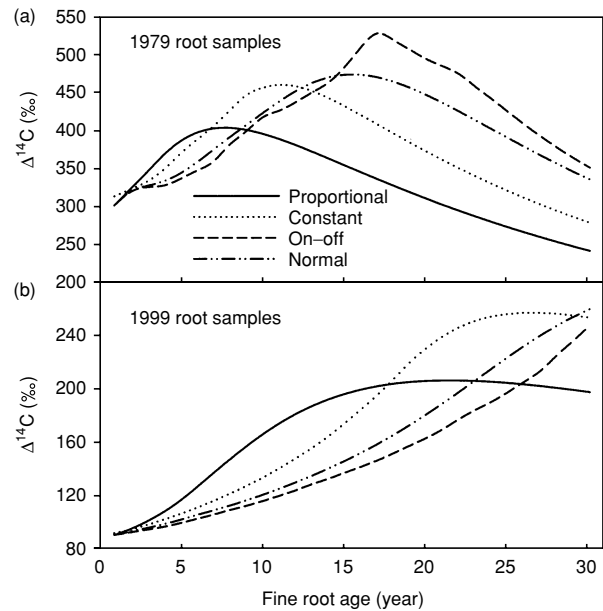
To further illustrate potential variation in  $^{14}\text{C}$ -estimated root longevity caused by different mortality patterns, I plotted root  $\Delta^{14}\text{C}$  values against root ages for samples from 1979 and 1999 (Fig. 6). The curve in the 1999 samples for the proportional pattern is identical to that in Fig. 3 of Gaudinski *et al.* (2001). The maximal  $\Delta^{14}\text{C}$  value from the 1979 root samples that can be used to estimate root longevity is 400.5, 425.2, 469.4, and 523.7‰ for the proportional, constant, normal, and on-off patterns, respectively. Any  $\Delta^{14}\text{C}$  value from 1979 root samples that is between 400.5‰ and the contemporary atmospheric  $\Delta^{14}\text{C}$  value (i.e. 299.4‰ in 1979) has no unique solution to estimation of root longevity with any of the four mortality patterns (Fig. 6a). Usually, the proportional pattern gives a shorter estimate of root longevity than the other patterns. If an observed  $\Delta^{14}\text{C}$  value is



**Fig. 5** The theoretical  $\Delta^{14}\text{C}$  values in fine roots derived from bomb  $^{14}\text{C}$  in atmospheric  $\text{CO}_2$  as affected by root mortality patterns with root longevity of 2 (a), 8 (b), and 20 years (c). Root  $\Delta^{14}\text{C}$  values with the on-off mortality pattern are most closely tracking atmospheric  $^{14}\text{C}$  among the four patterns examined. The longer the root longevity is, the more the root mortality patterns matter.

377‰, for example, the estimated shortest root longevity is 5, 6, 8, or 7 years with the proportional, constant, on-off, or normally distributed patterns, respectively.

Within the age range from 1 to 30 years for the 1999 root samples, the maximal  $\Delta^{14}\text{C}$  value that can be explained by at least one of the mortality patterns is 259‰. If an observed  $\Delta^{14}\text{C}$  value is larger than 259‰, the root samples could be either older than 30 years or made of NSC reserves that carried over richer isotope  $^{14}\text{C}$ . The estimated root longevity is shorter with the proportional pattern than with the other patterns when the  $\Delta^{14}\text{C}$  value in the 1999 root samples is less than 203‰. A  $\Delta^{14}\text{C}$  value of 197‰, for example, gives the estimated root longevity of 6, 10, 14, and 12 years with the proportional, constant, on-off, and normally distributed patterns, respectively. If we assume that the 'true' root mortality pattern is the constant one, fitting a linear



**Fig. 6** The theoretical  $\Delta^{14}\text{C}$  values in 1979 (a) and 1999 (b) fine root samples that vary with root ages and mortality patterns. With the age range from 1 to 30 years, a root  $\Delta^{14}\text{C}$  value cannot be explained by any of the mortality patterns if it is larger than 524‰ in the 1979 samples and 295‰ in 1999 samples. The proportional mortality pattern offers the shortest estimate of root longevity if root  $\Delta^{14}\text{C}$  value is smaller than 395‰ in the 1979 samples and 110‰ in the 1999 samples.

model (corresponding to the on-off pattern) to  $\Delta^{14}\text{C}$  data would overestimate root longevity by 40% whereas using an exponential model (corresponding to the proportional pattern) would result in a 40% underestimation.

While this study did not explicitly investigate how NSC reserves and nonsteady states between growth and death influence root  $\Delta^{14}\text{C}$  dynamics, their effects can be reasonably inferred. Since NSC carries over past isotope signals as shown for  $\delta^{13}\text{C}$  values in Fig. 3, it is expected that a root sample constructed partly from NSC reserves integrates atmospheric  $^{14}\text{C}$  over a longer period than does a sample without NSC reserves. Accordingly, the root longevity would be overestimated for a given value of  $\Delta^{14}\text{C}$  should NSC reserves be used in root growth but not appropriately considered in the analysis. An increase in root biomass growth in a developing stand should reflect more recent atmospheric  $^{14}\text{C}$  signals than decreased root growth in a declining stand. For a given value of  $\Delta^{14}\text{C}$ , therefore, the root longevity would be underestimated for the developing stand and overestimated for the declining stand, if the nonsteady state between growth and death is not considered in the analysis.



## Discussion

Uncertainties are often associated with interpretation of isotope data (Schwarcz, 1991; Phillips & Gregg, 2001; Phillips & Koch, 2002; Wang & Hsieh, 2002). When a simple linear mixing model is used for source partitioning in animal dietary studies, for example, uncertainties may originate from unknown dietary sources, multiple (> 3) dietary sources, and small differences but high variances in isotopic signatures among dietary sources (Phillips & Koch, 2002). In this study, I showed that root mortality patterns and NSC reserves could be major origins of uncertainty in the interpretation of isotope data when estimating fine root longevity. In particular, the dilution of depleted  $^{13}\text{C}$  signals by pretreatment NSC reserves in  $\text{CO}_2$  experiments potentially results in enormous overestimation of root longevity that can adequately account for the substantial discrepancy between the isotope and conventional methods. Although the nonsteady state between root growth and death has been suspected to be a major source of uncertainty in the interpretation of isotope signals (Gaudinski *et al.*, 2001), my analysis indicates that a 40% increase in root growth causes a relatively minor error in the estimated root longevity (Table 1). While this study primarily examined assumptions underlying the interpretation of isotope signals, conventional methods also involve uncertainties in estimated root longevity (Tierney & Fahey, 2002).

### *NSC reserves and uncertainties in estimated root longevity*

Plant tissues usually contain NSC reserves that range from 10 to 20% of their dry weight (Lewis *et al.*, 1996; Myers *et al.*, 1999) and can reach as high as 30% of dry weight in fine roots (Ericsson & Persson, 1980). Gaudinski *et al.* (2001) considered the possible dilution of isotope signals by NSC reserves but did not detect any with their technique that has a resolution of 2 years or longer. Thus, the residence time of NSC in their study sites, the eastern US forests, must be less than 2 years if at all. Langley *et al.* (2002) have clearly documented the dilution of isotope signals by pretreatment NSC reserves in a  $\text{CO}_2$  experiment in a regenerating scrub oak ecosystem in Florida, USA. In their study, a controlled burn was executed in the spring of 1996 before the  $\text{CO}_2$  experiment and the placement of ingrowth bags. Two and a half years later, measured  $\delta^{13}\text{C}$  values from roots in the ingrowth bags averaged  $-38.4$ ,  $5.5\text{‰}$  higher than that in foliage. Using a mixing model, they estimated that over a third of the C in the newly formed ingrowth roots was derived from a source (i.e., NSC reserves) other than recent photosynthesis (Langley *et al.*, 2002), indicating that stored NSC can have a residence time of 3 years or longer in that ecosystem.

Dilution of isotope signals by NSC reserves, if not accounted for, has the potential to result in overwhelming errors in the interpretation of root  $\delta^{13}\text{C}$  data from  $\text{CO}_2$  experiments (Fig. 3, Table 1). The dilution of isotope signals by NSC can result in overestimation of fine root longevity by 84% with a 6-month residence time and by eightfold with a 36-month residence time. To help appreciate the implications of the overestimation, I assume a case in which the pretreatment NSC reserves have a mean residence time of 18 months. Without considering dilution effects of the NSC reserves, root longevity was assumed to be 6 years as estimated from a root  $\delta^{13}\text{C}$  data set. In fact, root longevity could be overestimated by 145–371% due to dilution by NSC reserves with 18 months of residence time (Table 1). If the dilution effects were accounted for in the interpretation of the same root  $\delta^{13}\text{C}$  data set, the estimated root longevity could only be 1.27–2.45 years rather than 6 years. The longevity values of 1.27–2.45 years are comparable with estimates using conventional approaches. Thus, documenting NSC in plant tissues is crucial for the correct interpretation of isotope signals.

### *Root mortality patterns and interpretation of isotope data*

Root mortality patterns are crucial for choosing correct models to interpret isotope data as well as for understanding potential errors in estimated root longevity. A linear model is often used to interpret isotope signals in estimating turnover rates of bacteria (Bååth, 1998), fine roots (Saarinen, 1996), and soil carbon (Balesdent *et al.*, 1988; Van Kessel *et al.*, 2000; Leavitt *et al.*, 2001). This analysis shows that fitting a linear equation to root  $\delta^{13}\text{C}$  data from  $\text{CO}_2$  experiments is valid to estimate longevity only if fine root mortality follows an on–off pattern. That is, all fine roots, once initiated, live to the same age and then all die together at the same time. If root mortality follows the other patterns, the linear extrapolation usually overestimates root longevity (Table 1). For example, the application of the linear model to a  $\delta^{13}\text{C}$  data set, which results from the proportional mortality pattern, overestimate longevity by 17 and 39% if 6- and 12-month data were analyzed, respectively. In contrast, fitting an exponential equation to  $\delta^{13}\text{C}$  data offers an unbiased estimate of longevity if root mortality follows the proportional pattern but underestimates root longevity by up to 54% in the other mortality patterns.

Despite the critical importance of root mortality patterns in the estimation of root longevity, our knowledge about them is scarce. Fine roots have long been studied in ecology with a variety of methods, including mass balance (Gill & Jackson, 2000), seasonal sampling, and minirhizotrons (Tingey *et al.*, 2000; Wells & Eissenstat, 2001). Although the minirhizotron method is technically

capable of documenting root-age structure and demography, such data are still lacking due to labour and time constraints. Among the six root mortality patterns examined in this study, the on-off one is likely the least realistic representation of actual root population dynamics. However, this mortality pattern relates to the most commonly used, linear model in the interpretation of isotope signals. The proportional pattern generates exponential decay in the age distribution of fine root biomass and in  $\delta^{13}\text{C}$  values. The documented root demographic patterns for a 60-year-old-sugar maple stand (Wells & Eissenstat, 2001) appear to be similar to the constant mortality pattern. Tierney & Fahey (2002) observed a left skew distribution of mortality for fine roots in the organic horizon of a northern hardwood forest in New Hampshire. The normal and right skew distributions of fine root mortality may bear some reality but have yet to be verified by experimental data. Since we may not have enough root demography data to correctly interpret isotope signals, fitting both linear and exponential equations to the same isotope data sets can practically bracket root longevity.

#### *The $\delta^{13}\text{C}$ vs $\Delta^{14}\text{C}$ method for estimation of root longevity*

By comparison, the  $\delta^{13}\text{C}$  method in  $\text{CO}_2$  experiments has higher resolution than the  $\Delta^{14}\text{C}$  method for estimating root longevity. The  $\delta^{13}\text{C}$  method takes advantage of an abrupt depletion of approximately 12‰ in the beginning of the  $\text{CO}_2$  experiments (Leavitt *et al.*, 2001), which offers a strong contrast in  $\delta^{13}\text{C}$  values between ambient and elevated  $\text{CO}_2$  treatments. However, the  $\delta^{13}\text{C}$  method can be used only in the very beginning of a  $\text{CO}_2$  experiment for perennial species. The  $\Delta^{14}\text{C}$  method uses a slow decline in  $\Delta^{14}\text{C}$  in the atmospheric  $\text{CO}_2$ , which provides weak signals in the estimation of fine root longevity. The  $\Delta^{14}\text{C}$  value in atmospheric  $\text{CO}_2$  currently decreases at a rate of approximately  $4 \pm 2\%$ , which is smaller than a sampling error having one standard deviation of 6%. Thus, the  $\Delta^{14}\text{C}$  method can usually be used for roots that are more than 2 years old (Gaudinski *et al.*, 2001).

Among the four root mortality patterns, the proportional model is most likely to offer the shortest estimate of root longevity (Figs 5 and 6) when it is used to interpret  $\Delta^{14}\text{C}$  values in fine roots that derived from bomb  $^{14}\text{C}$  in atmospheric  $\text{CO}_2$  (Gaudinski *et al.*, 2001). Like the interpretation of the  $\delta^{13}\text{C}$  data in  $\text{CO}_2$  experiments, the root longevity would be underestimated from root  $\Delta^{14}\text{C}$  values by the exponential model and overestimated by the linear model if the 'true' root mortality pattern is the constant or normal distribution. The use of NSC reserves in fine root growth would result in an overestimation of

root longevity if not appropriately accounted for in the analysis of fine root  $\Delta^{14}\text{C}$  data.

#### *Analysis of partial isotope data sets and implications*

This paper primarily examines factors that could potentially cause major uncertainties in root longevity estimated from isotope data. Conversely, we might ask what kinds of models match isotope data so as to give accurate estimates of root longevity. As discussed above, the precise estimation of root longevity from isotope data requires knowledge of root mortality patterns, dilution of isotope signals by NSC reserves, and changes in growth rates at minimum. Without complete data sets of all those variables, the root longevity may be inferred by the use of inverse analysis. This approach has been applied to inferential selection of candidate models (Burnham & Anderson, 1998) and parameter estimation of carbon residence times (Luo *et al.*, 2003) at ambient and elevated  $\text{CO}_2$  in the Duke Forest. In ecological research, we rarely have all data sets necessary to fully solve an issue. In most cases, we only have partial data sets available to address an issue. Inverse analysis is an approach that exploits as much available data and as many information sources as possible to facilitate the selection of the best model and help reduce the uncertainty in parameter estimation. For the interpretation of isotope data, the candidate models are those associated with various root mortality patterns. The parameter values to be estimated are root longevity and residence time of NSC reserves.

The estimation of root longevity is not only important in its own right but also critical to understand the ecosystem carbon balance. Fine root turnover is one of the few major pathways that deliver carbon from plants to soil. If root longevity is long, the amount of carbon that is transferred from plants to soil via root turnover must be small. At the Duke Forest, for example, carbon content in soil from 0 to 30 cm in depth increased by nearly  $160 \text{ g m}^{-2}$  at ambient  $\text{CO}_2$  and nearly  $400 \text{ g m}^{-2}$  at elevated  $\text{CO}_2$  in the first 3 years of the FACE experiment from 1996 to 1999 (Schlesinger & Lichter, 2001). With root longevity of 4–9 years, carbon delivered through root turnover accounted for 37 and  $57 \text{ g m}^{-2}$  at ambient and elevated  $\text{CO}_2$ , respectively. Other rhizosphere processes, such as root exudation, must transfer a large amount of carbon from plants to soil in order to balance soil carbon content. However, analysis of time courses of soil respiration in response to elevated  $\text{CO}_2$  during the same period suggests that root exudation might play a minor role in the carbon transfer (Luo *et al.*, 2001). Therefore, the estimation of root longevity from isotope data has profound implications for balancing the ecosystem carbon budget and hence warrants further investigation.

## Conclusions

This study examined three variables – mortality patterns of fine root populations, nonstructural carbohydrate (NSC) reserves, and a nonsteady state between root growth and death – in relation to the estimation of root longevity from isotope data. By comparison, the nonsteady state between root growth and death causes relatively minor uncertainties in estimated root longevity. The dilution of isotope signals by NSC reserves, if not accounted for, could cause overestimation of root longevity by several-fold, which is large enough to account for the substantial discrepancy between the isotope and conventional methods. Among the six mortality patterns (proportional, on–off, constant, normal, left skew, and right skew distributions) examined in this study, the proportional one generates the greatest spread in age structure of root biomass and isotope signals over time but the on–off pattern gives the least spread in age structure and isotope signals. Fitting a linear equation to isotope data yields unbiased estimates of longevity only if root mortality follows the on–off pattern but overestimates longevity in the other mortality patterns. Fitting an exponential equation to isotope data offers unbiased estimates of longevity only if root mortality follows the proportional pattern but underestimates longevity in the other patterns. Due to the significant role that fine root turnover plays in ecosystem carbon balance, the substantial discrepancy in estimated root longevity between the isotope and traditional methods warrants further study with both experimental and theoretical approaches.

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